DESK-BASED STUDY OF CURRENT KNOWLEDGE ON VETERINARY MEDICINES IN DRINKING WATER AND ESTIMATION OF POTENTIAL LEVELS

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SUMMARY

Humans may potentially be exposed to veterinary medicines in the environment by a number of routes including the consumption of: 1) crops that have accumulated substances from soils as a result of exposure to contaminated manure and slurry; 2) livestock that have accumulated veterinary medicines from food material that has accumulated substances from contaminated soils or water; 3) fish exposed to treatments used in aquaculture; and 4) abstracted groundwater and surface waters containing veterinary medicines. Whilst veterinary medicines are routinely monitored in target food materials to ensure that concentrations are below the maximum residue limits, the magnitude of the exposure via many of the routes listed and the health impacts of such exposure have not been extensively quantified. While assessments for human medicines in the USA and UK indicates that consumption of humans medicines via drinking waters poses no appreciable risk to human health, our knowledge of the risks of veterinary medicines in drinking waters is much less developed. This project therefore addressed this knowledge gap by exploring the occurrence of veterinary medicines in raw and treated waters, assessing potential exposure for veterinary medicines in use in England and Wales and carrying out a desk-based assessment of the health risks.

In the first instance, a review was performed of the published and grey literature on the fate and occurrence of veterinary medicines in the environment. Following this review a systematic evaluation of the potential levels of contamination and health risks of veterinary medicines in use in England and Wales was performed. Data were obtained on the usage, treatment regimes, metabolism, environmental fate and toxicity of around 450 active ingredients in use in the UK. Simple modelling approaches were then used to identify those active ingredients that are likely to pose the greatest risk to human health.

Twenty six substances were identified of potential concern and these were then evaluated using more complex modelling approaches for estimating exposure levels in raw waters and for estimating removal in different drinking water treatment processes. The outputs from these exposure predictions were then combined with data on toxicity to assess potential risks to adults, toddlers and infants. The exposure modelling considered concentrations in waterbodies in close proximity to fields where veterinary medicines are applied. In reality, there would be significant dilution of the water between an area of veterinary medicine use and a drinking water abstraction point. The exposure predictions and subsequent risk assessments therefore provide a highly conservative assessment of risks of veterinary medicines to consumers.

Even though a conservative approach was used, with the exception of a few cases where more information on exposure, toxicokinetics or toxicology is required, the results of this risk assessment were judged to be highly reassuring. For 14 of the 26 selected priority veterinary medicines, the estimated intakes from conventional or advanced treated water were less than 10% of the Acceptable Daily Intake (ADI) for all sections of the population evaluated. It is concluded, therefore, that these 14 veterinary medicines — albendazole, amoxicillin, chlortetracycline, chlorsulon, cypermethrin, cyromazine, diazinon, enrofloxacin, eprinomectin, lasalocid, salinomycin, tiamulin, trimethoprim and tylosin — are not a potential risk to consumer health. Very minor exceedences of the guide value (equivalent to 10% of the ADI) in all populations assessed were found for a further two compounds: halofuginone and tilmicosin. However, these were not considered to be a potential risk to consumer health.

For the remaining 10 compounds (acetyl salicylic acid, altrenogest, apramycin, cefapirin, dicyclanil, florfenicol, lincomycin, luprostiol, monensin, sulfadiazine), the worst case predicted exposure levels, based on consumption of either raw (environmental) water or conventionally treated water were close to or exceeded ADI values. In some cases the predicted levels of exposure significantly exceeded ADI values. The highest exceedences of ADI values arose from exposure to water sourced from groundwater. There is some evidence that the groundwater model, that was used in the study, significantly over estimates actual concentrations in the real environment. In the advanced water treatment scenario, which is widely used in England and Wales, worst case predicted exposure estimates only exceeded the ADI value for four compounds (acetylsalicylic acid, florfenicol, lincomycin and luprostiol). All of these ADI exceedences were related to the groundwater scenario. Whilst concentrations above an ADI do not necessarily imply a risk to human health, a risk for these substances cannot currently be ruled out. As the approach used in this study was modelling-based, used a number of conservative assumptions and employed conservative defaults where model input data were not available for individual compounds, further work may be required on these compounds to better establish the potential risks.

1. INTRODUCTION

Medicines play an important role in the treatment and prevention of disease in humans and animals. Whilst the side effects on human and animal health have been widely documented, only recently have the occurrence and fate and effects of such medicines in the environment been considered. Veterinary medicines are widely used in livestock treatment, and will be released to land either directly in faeces or urine, or indirectly through the application of slurry and manure. A range of veterinary medicines, including hormones, antibiotics and parasiticides, have been detected in soils, surface waters and groundwaters. Whilst the reported concentrations are generally low (i.e. with the exception of a handful of substances, sub $\mu g\ l^{-1}$ levels being measured in surface waters around the world), the substances have been observed throughout the year across a variety of hydrological, climatic and land-use settings. Some substances (e.g. oxytetracycline) may also persist in the environment for over a year. As a result, questions have been raised by the scientific community over the potential impacts of veterinary medicines in the environment on human and animal health, such as the promotion of the spread of antibiotic resistance. As such it is prudent to carry out an initial assessment of potential risk.

Humans may be exposed to veterinary medicines in the environment by a number of routes including the consumption of: 1) crops that have accumulated substances from soils as a result of exposure to contaminated manure and slurry; 2) livestock that have accumulated veterinary medicines through the food chain; 3) fish exposed to treatments used in aquaculture; and 4) abstracted groundwater and surface waters containing veterinary medicines. Exposure may also occur via the inhalation of dust emitted from intensively reared livestock facilities or as a result of contact with contaminated fleeces from treated sheep. Whilst veterinary medicines are routinely monitored in target food materials to ensure that concentrations are below the maximum residue limits, the magnitude of the exposure via many of the routes listed and the health impacts of such exposure have not been extensively quantified. Studies have demonstrated the presence of medicines in water bodies and the accumulation of veterinary medicines from surface waters by fish, shellfish and crustacea. While assessments for human medicines in the USA and UK indicates that consumption of human medicines via drinking waters poses no appreciable risk to human health, our knowledge of the risks of veterinary medicines in drinking waters is much less developed.

This project therefore addressed this knowledge gap by exploring the occurrence of veterinary medicines in raw and treated waters, assessing potential exposure for veterinary medicines in use in England and Wales and carrying out a desk-based assessment of the health risks. In the first instance, a review was performed of the published and grey literature on the fate and occurrence of veterinary medicines in the environment.

Following this review a systematic evaluation of the potential occurence and health risks of veterinary medicines in use in England and Wales was performed. Data were obtained on the usage, treatment regimes, metabolism, environmental fate and toxicity of over 450 active ingredients in use in the UK. Simple modelling approaches were then used to identify those active ingredients that are likely to pose the greatest risk to human health.

Those substances that were predicted to pose the greatest risk were then evaluated using more complex modelling approaches for estimating exposure levels in raw waters and for estimating removal in different drinking water treatment processes.

2. FATE AND OCCURRENCE OF VETERINARY MEDICINES IN WATER BODIES

Once a veterinary medicine is released into the environment, its behaviour will be determined by its underlying physical properties (including water solubility, lipophilicity, volatility and sorption potential). In the following section information on the fate and transport of veterinary medicines in the environment is reviewed. This is a broad review of information on the fate and occurrence of veterinary medicines in the environment so includes information on veterinary medicines that have been used in the past as well as medicines that are used in other geographical regions.

2.1. Sorption in soil

The degree to which veterinary medicines may adsorb to particulates varies widely. Consequently, the mobility of different veterinary medicinal products also varies widely. Available data indicate that sulfonamide antibiotics and organophosphate parasiticides will be mobile in the environment whereas tetracycline, macrolide and fluoroquinolone antibiotics will exhibit low mobility. The sorption behaviour of individual veterinary medicines can also vary widely in different soil types and unlike many other classes of soil contaminant (e.g. hydrocarbons and many pesticides); the difference in sorption of a given compound in different soils cannot be explained by variations in soil organic carbon. These large differences in sorption behaviour are explained by the fact that many veterinary medicines are ionisable with dissociation coefficient (pKa) values in the pH range of natural soils. Medicines can therefore occur in the environment as negative, neutral, zwitterionic and positively charged species (e.g. Ter Laak et al. 2006a and b). Depending on the chemical species, interactions with soil can occur through electrostatic attraction, surface bridging, hydrogen bonding or hydrophobic interactions (Ter Laak et al. 2006b). The sorption behaviour is also influenced by the properties of the soil including pH, organic carbon content, metal oxide content, ionic strength and cationic exchange capacity (e.g. Jones et al. 2005; Sassman and Lee 2005; Strock et al. 2005; Ter Laak et al. 2006b). The complexity of the sorbate-sorbent interactions means that modelling approaches developed for predicting the sorption of other groups of chemicals (e.g. pesticides and neutral organics) are inappropriate for use on veterinary medicines. Manure and slurry may also alter the behaviour and transport of medicines. Studies have demonstrated that the addition of these matrices can affect the sorption behaviour of veterinary medicines and that they may affect persistence (e.g. Boxall et al. 2002; Thiele Bruhn and Aust 2004). These effects have been attributed to changes in pH or alterations in the nature of dissolved organic carbon in the soil/manure system.

2.2. Persistence in soil

The main route for degradation of veterinary medicines in soils is via aerobic soil biodegradation. Degradation of veterinary medicines is affected by environmental conditions such as temperature and pH and the presence of specific degrading bacteria that have developed to degrade groups of medicines (Gilbertson et al. 1990; Ingerslev and Halling-Sørensen 2001). As well as varying significantly between chemical classes, degradation rates for veterinary medicines also vary within a chemical class. When manure is combined with soil, degradation may be enhanced

for selected medicines. Depending on the nature of the chemical, other degradation and depletion mechanisms may occur, including soil photolysis and hydrolysis (e.g. Wolters and Steffens 2005). The degradation processes may well result in the formation of degradation products (e.g. Kolz et al. 2005). In some instances, these degradation products may be of greater environmental concern than the parent compound as some have similar or greater toxicity, some are more persistent and some are more mobile (Boxall et al. 2003). It is therefore important that the fate of the degradation products in soils is considered when assessing the impact of a veterinary medicine on the environment.

2.3. Transport in soil systems

Contaminants applied to soil can be transported to aquatic systems in surface runoff, subsurface flow and drainflow. The extent of transport via any of these processes is determined by a range of factors, including: the solubility, sorption behaviour and persistence of the contaminant; the physical structure, pH, organic carbon content and cation exchange capacity of the soil matrix, and climatic conditions such as temperature and rainfall volume and intensity. Most work to date on contaminant transport from agricultural fields has focused on pesticides, nutrients and bacteria, but recently a number of studies have explored the fate and transport of veterinary medicines. Lysimeter, field-plot and full-scale field studies have investigated the transport of veterinary medicines from the soil surface to field drains, ditches, streams, rivers and groundwater (e.g. Aga et al. 2003; Kay et al. 2004; 2005a, b and c; Burkhard et al. 2005; Hamscher et al. 2005; Kreuzig and Holtge 2005; Blackwell et al. 2007 and 2009). A range of experimental designs and sampling methodologies has been used. These investigations are described in more detail below.

2.3.1. Leaching to groundwater

The movement of sulfonamides and tetracyclines in soil profiles has been investigated at the field scale using suction probes (Hamscher et al. 2000a; Blackwell et al. 2007). In these studies, sulfonamides were found at depth but the tetracyclines were not, which is most likely due to the high potential for tetracyclines to sorb to soil. Carlson and Mabury (2006) reported that chlortetracycline applied to agricultural soil in manure was detected at soil depths of 25 and 35 cm, but monensin remained in the upper soil layers. There are only a few reports of veterinary medicines in groundwater (Hirsch et al. 1999; Hamscher et al. 2000a). In an extensive monitoring study conducted in Germany (Hirsch et al. 1999), while no antibiotics were detected in groundwater at most of the sites investigated, residues of sulfonamide antibiotics were detected at a few of the study sites. Contamination at two of these sites was attributed to irrigation of agricultural land with domestic sewage but the other sites were believed to have become contaminated due to the application of animal manures to the soil surface (Hirsch et al. 1999).

2.3.2. Runoff

Transport of veterinary medicines via runoff (i.e. overland flow) has been observed for tetracycline antibiotics (i.e. oxytetracycline) and sulfonamide antibiotics (sulfadiazine, sulfamethazine, sulfathiazole and sulfachloropyridazine) (Kay et al. 2005a; Kreuzig et al. 2005b). Just like leaching, the transport of these substances is influenced by the sorption behaviour of the compounds, the presence of manure in the soil matrix and the nature of the land to which the manure is applied. Runoff of highly sorptive substances, such as tetracyclines, was observed to be significantly

lower than the more mobile sulfonamides (Kay et al. 2005). However, even for the relatively water soluble sulfonamides, total mass losses to surface are small (between 0.04% and 0.6 % of the mass applied) under actual field conditions (Stoob et al. 2007). Manure and slurry has been shown to increase the transport of sulfonamides via runoff by 10-40 times in comparison to runoff following direct application of these medicines to soils (Burkhard et al. 2005). Possible explanations for this observation include physical "sealing" of the soil surface by the slurry and/or a change in pH as a result of manure addition that alters the speciation and fate of the medicines (Burkhard et al. 2005). It has been shown that overland transport from ploughed soils is significantly lower than runoff from grasslands (Kreuzig et al. 2005b).

2.3.3. Drain flow

The transport of a range of antibacterial substance (i.e. tetracyclines, macrolides, sulfonamides and trimethoprim) has been investigated using lysimeter and field-based studies in tile-drained clay soils (Kay et al. 2004; Boxall et al. 2006). Following application of pig slurry spiked with oxytetracycline and sulfachloropyridazine, the test compounds were detected in drainflow water (Kay et al. 2004). Concentrations of the sulfonamide were an order of magnitude higher than the tetracycline even though the spiking concentrations for the test compounds were similar; these differences are again likely to be due to differences in sorption behaviour. In a subsequent investigation at the same site (Kay et al. 2004), in which the soil was tilled, much lower concentrations were observed in the drainflow suggested that tillage may be a useful mitigation strategy in the event that a veterinary product is found to pose a risk to aquatic systems. While the pig slurry used in these studies was obtained from a pig farm where tylosin was used as a prophylactic treatment, this substance was not detected in any drainflow samples, possibly because it is not persistent in slurry (Loke et al. 2000).

2.3.4. Surface waters

In the water column, substances may be degraded abiotically via photodegradation and/or hydrolysis or biotically by aerobic or anaerobic organisms. Highly sorptive substances may partition to the bed sediment. For example, mesocosm studies using ivermectin show that when added to water, the compound dissipates quickly from the water column and that this dissipation is observed as an increase in the concentration of the compound in the bed sediment (e.g. Sanderson et al. 2007).

A significant amount of information is available on the fate and behaviour of many veterinary medicines in sediment due to their use as aquaculture treatments (Jacobsen and Berglind 1988; Samuelsen 1989; Bjorklund et al. 1990; Samuelsen et al. 1991, 1992a, 1994; Pouliquen et al. 1992; Coyne et al. 1994; Hektoen et al. 1995; Lai et al. 1995; Lunestad et al. 1995). While many compounds degrade very quickly (e.g. chloramphenicol, florfenicol, furazolidone and ormethoprim), others persist in the sediment for months to years (e.g. flumequine, ivermectin, oxolinic acid, oxytetracycline, sarafloxicin, sulfadiazine and trimethoprim).

2.3.5. Occurrence

Alongside the fate experiments described above, a series of studies have monitored concentrations of veterinary compounds in different matrices. Veterinary medicines have been measured in surface waters, groundwaters, sediments, slurry/manure and biota. Monitoring studies have focused on veterinary products used in sheep dips,

and as antibiotic treatments for livestock. Results for surface water and groundwater monitoring are summarised in Table 3.1.

Several veterinary drugs have been detected in soil that has been treated with animal manure. In three separate investigations in Germany, soil samples collected from regions with intensive livestock production were analysed for tetracyclines (Hamscher et al. 2000a, b and c). Concentrations of up to 41.8 µg kg^{-1} were detected in these samples. Elsewhere, American researchers detected trace amounts (approximately 0.1-2 µg kg^{-1}) of ivermectin in the top (0-3 inches) of soil in a cattle feedlot housing animals treated 28 days previously (200 µg kg^{-1} body weight) (Nessel et al. 1989). The authors suggest the concentrations detected in the soil are probably as a result of the faeces being trampled into the mud and subsequently being protected from light thus retarding degradation. In a recent monitoring study in the UK, oxytetracycline, lincomycin, sulfadiazine, trimethoprim, ivermectin and enrofloxacin (and its metabolite ciprofloxacin) were monitored in soils (Boxall et al. 2006). Concentrations of the antibacterials detected ranged from 0.5 µg kg^{-1} (trimethoprim) to 305 (oxytetracycline) µg kg^{-1}.

While monitoring sewage treatment work effluents and associated receiving surface waters for antibiotic substances in Germany, residues of chloramphenicol were detected at concentrations of 0.06 and 0.56 µg l^{-1} (Hirsch et al. 1999). As its use in human medicine is extremely limited, the authors of the paper suggested that the two positive detections were most likely from its sporadic veterinary use in fattening farms.

In a national monitoring study in the US (Kolpin et al. 2002) a wide range of medicines were monitored in watercourses. A number of substances that are used as veterinary medicines, including sulfonamides, fluoroquinolones, tetracyclines and macrolides were detected in the ng l^{-1} range. Many of these substances are also used as human medicines so the concentrations may result from a combination of inputs from both human and veterinary sources. Similar broad-scale monitoring studies have been done in other regions (including Europe and Asia) and show similar results.

The majority of surface monitoring studies involve grab sampling on a number of occasions across a variety of sites. As inputs of many veterinary medicines are likely to be intermittent, it is likely that concentrations reported in the studies are significantly lower than peak concentrations. To address this, a recent UK study used continuous monitoring of water and sediment, at farms where veterinary medicines (including oxytetracycline, lincomycin, sulfadiazine, trimethoprim, ivermectin and doramectin) were known to be in use, to determine typical exposure profiles for aquatic systems (Boxall et al. 2006). Maximum concentrations of antibacterials in stream water ranged from 0.02 µg l^{-1} (trimethoprim) to 21.1 (lincomycin) µg l^{-1}; the parasiticides (doramectin and ivermectin) were not detected. Concentrations of antibacterials in sediment were 0.5–813 µg kg^{-1} and those for doramectin and ivermectin were 2.7 and 4.9 µg kg^{-1} respectively.

There are only a few reports of veterinary medicines being detected in groundwater (Hirsch et al. 1999; Hamscher et al. 2000a). In an extensive monitoring study conducted in Germany, a large number of groundwater samples were collected from agricultural areas in order to determine the extent of contamination by antibiotics (Hirsch et al. 1999). The data show that in most areas with intensive livestock breeding, no antibiotics were present above the limit of detection (0.02-0.05 µg l-1). Sulfonamide residues were, however, detected in four samples. Whilst the source of contamination of two of these is considered to be attributable to irrigation with

sewage, the authors concluded that sulfamethazine, detected at concentrations of 0.08 and 0.16 µg l-1, could possibly have derived from veterinary applications, as it is not used in human medicine.

In the investigations of Hamscher et al. (2000a) soil water was collected and analysed from four separate areas of agricultural land, two belonging to livestock farms and treated with animal slurry and two where no animal manure had been applied for approximately five years. Chlortetracycline, oxtetracycline, tetracycline and tylosin were all found at the limit of detection (0.1-0.3 µg l-1) in water samples collected at 80 and 120 cm depth, independent of soil treatment. In addition, no biologically active residues could be detected with microbiological assays that had approximately five-fold higher detection limits.

Veterinary medicines are also known to leach from landfill sites. In Denmark, high concentrations (mg/l) of numerous sulfonamides were found in leachates close to a landfill site where a pharmaceutical manufacturer had previously disposed of large amounts of these drugs over a 45 year period (Holm et al. 1995). Concentrations dropped off significantly tens of metres down gradient, most probably due to microbial attenuation. Although this is recognised as a specific problem, in the UK the disposal of smaller quantities of veterinary medicines to landfill should nevertheless be considered a potential route for environmental contamination.

Table 2.1 Measured concentrations of veterinary medicines in waters

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
seepage water				
flubendazole	Germany	<0.5	pig farm, no treatment data, no soil data, limited climate data	Weiss et al., 2008
sulfamethazine	Germany	13	pig farm, no treatment data, no soil data, limited climate data	Weiss et al., 2008
surface waters				
carbadox	Canada	ND (0.002)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
chloramphenicol	China	0.002		Tong et al., 2009
chloramphenicol	Canada	ND (0.005)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
chlortetracycline	Canada	0.192	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
chlortetracycline	USA	0.21	monitoring agriculturally influenced area on Cache la Poudre River in Colorado; no treatment data or climatic information	Kim and Carlson, 2007
doramectin	UK	ND (<0.001)	monitoring of agricultural stream in field containing cattle treated with doramectin; treatment data and some site characteristic data available	Boxall et al., 2006
doxycycline	Canada	0.073	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
erythromycin	Canada	0.051	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
erythromycin	USA	0.45	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
ivermectin	UK	ND (0.0002)	monitoring of agricultural stream in field containing cattle treated with ivermectin; treatment data and some site characteristic data available	Boxall et al., 2006
lincomycin	Canada	0.355	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
lincomycin	UK	21.1	monitoring of agricultural stream adjacent to field receiving pig slurry; animal treatment data, soil data and manure application data available	

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
monensin	Canada	1.172	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
monensin	USA	0.036	monitoring of river downstream of agricultural land	Kim and Carlson, 2006
monensin	USA	0.05	monitoring of river downstream of agricultural land	Cha et al., 2005
monensin	Canada	0.220	surface waters from site receiving agricultural runoff	Hao et al., 2006
narasin	USA	0.038	monitoring of river downstream of agricultural land	Kim and Carlson, 2006
narasin	USA	0.06	monitoring of river downstream of agricultural land	Cha et al., 2005
oxytetracycline	Japan	68	river basin with cattle, swine and chicken farming; no treatment, soil or climatic data	Matsui et al., 2008
oxytetracycline	UK	4.49	monitoring of agricultural stream adjacent to field receiving pig slurry; animal treatment data, soil data and manure application data available	Boxall et al., 2006
oxytetracycline	Canada	ND (0.006)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
oxytetracycline	USA	1.21		Kim and Carlson, 2007
salinomycin	USA	0.007	monitoring of river downstream of agricultural land	Kim and Carlson, 2006
salinomycin	USA	0.04	monitoring of river downstream of agricultural land	Cha et al., 2005
sulfachloropyridazine	Canada	0.007	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
sulfachloropyridazine	USA	0.03	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
sulfadiazine	UK	4.13	monitoring of agricultural stream adjacent to field receiving pig slurry; animal treatment data, soil data and manure application data available	Boxall et al., 2006
sulfadimethoxine	Canada	0.056	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
sulfadimethoxine	USA	0.04	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
sulfamerazine	Canada	ND (0.0003)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
sulfamerazine	USA	0.06	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
sulfamethazine	Canada	0.408	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
sulfamethazine	USA	0.02	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
sulfamethoxazole	Canada	0.009	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
sulfamethoxazole	USA	0.32	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
sulfathiazole	Canada	0.016	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
sulfathiazole	USA	0.03	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
tetracycline	Canada	ND (0.060)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
tetraccycline	USA	0.03	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
trimethoprim	Canada	0.015	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
trimethoprim	UK	0.02	monitoring of agricultural stream adjacent to field receiving pig slurry; animal treatment data, soil data and manure application data available	Boxall et al., 2006
tylosin	Canada	trace (10)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
tylosin	USA	0.05	monitoring of river downstream of agricultural land	Kim and Carlson, 2007
virginiamycin	Canada	ND (30)	monitoring of a watershed in Ontario; cattle, swine and poultry	Lissemore et al., 2006
groundwaters				
carbodox	USA	ND (0.1)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
chloramphenicol	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
chortetracycline	Germany	ND (0.05)	groundwater from sites from agricultural areas	Hirsch et al., 1999
chlortetracycline	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
ciprofloxacin	USA	ND (0.02)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
clarithromycin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
cloxacillin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
dicloxacillin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
doxycycline	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
doxycycline	USA	ND (0.1)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
enrofloxacin	USA	ND (0.02)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
erythromycin-H_2O	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
erythromycin-H2O	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
lincomycin	USA	0.32	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
methicillin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
monensin	USA	0.39	groundwater sampling at dairy farms in California	Watanabe et al., 2007
nafcillin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
norfloxacin	USA	ND (0.02)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
oxacillin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
oxytetracycline	Germany	ND (0.05)	groundwater from sites from agricultural areas	Hirsch et al., 1999
oxytetracycline	USA	ND (0.1)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
penicillin G	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
penicillin V	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
roxithromycin	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
roxithromycin	USA	ND (0.03)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
sarafloxicin	USA	ND (0.02)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
sulfadimethoxine	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
sulfamerazine	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
sulfamethazine	Germany	0.16	groundwater from sites from agricultural areas	Hirsch et al., 1999
sulfamethazine	USA	0.36	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
sulfamethizole	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
sulfamethoxazole	Germany	0.47	groundwater from sites from agricultural areas	Hirsch et al., 1999
sulfamethoxazole	USA	1.11	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
sulfathiazole	USA	ND (0.1)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
tetracycline	Germany	ND (0.05)	groundwater from sites from agricultural areas	Hirsch et al., 1999
tetracycline	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
trimethoprim	Germany	ND (0.02)	groundwater from sites from agricultural areas	Hirsch et al., 1999
trimethoprim	USA	ND (0.014)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
tylosin	USA	ND (0.05)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
virginiamycin	USA	ND (0.1)	national reconnaissance of pharmaceuticals at sites thought to be susceptible to contamination by agricultural or human wastewaters	Barnes et al., 2008
untreated drinking water sources				
azithromycin	USA	0.029	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
carbodox	USA	ND (0.1)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
chlortetracycline	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
ciprofloxacin	USA	0.03	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
demeclocycline	USA	ND (0.02)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
doxycycline	USA	ND (0.1)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
enrofloxacin	USA	0.04	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
erythromycin-H2O	USA	0.3	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
lincomycin	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
methotrexate	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
minocycline	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
norfloxacin	USA	ND (0.02)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
oxytetracycline	USA	ND (0.1)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
roxithromycin	USA	ND (0.03)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
sarafloxicin	USA	0.02 (0.02)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
sulfadimethoxine	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
sulfamerazine	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
sulfamethazine	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
sulfamethizole	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
sulfamethoxazole	USA	ND (0.023)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
sulfathiazole	USA	ND (0.1)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
tetracycline	USA	ND (0.05	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008

Compound	Location	Maximum concentration (μg/l)	Notes	Reference
trimethoprim	USA	0.02	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
tylosin	USA	ND (0.05)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008
virginiamycin	USA	ND (0.1)	monitoring of untreated sources of drinking waters from 74 sites (both groundwater and surface water) across the US; sites covered a variety of land uses	Focazio et al., 2008

ND = not detected. In instances where compounds are not detected, analytical limits of detection are provided in parentheses.

3. SCREENING-LEVEL PRIORITISATION OF VETERINARY MEDICINES IN USE IN THE UK

3.1. Introduction

There are around 450 active ingredients that are used as veterinary medicines in the UK and from the previous Chapter it is clear that monitoring data are only available for a few of these and even less data are available on occurrence in the UK environment. It is therefore impractical to perform a detailed health risk assessment for all of the compounds in use. Therefore in the first instance, a simple pre-screening approach was used to identify those active ingredients that are likely to pose the greatest risk to human health. As so few monitoring data are available, this screening method used models for estimated exposure concentrations.

The pre-screening exercise used the lists of veterinary medicines in use in the UK, developed for previous environmental prioritisation projects (e.g. Boxall *et al.* 2002; Kools *et al*., 2008). These lists were reviewed and refined to develop an up-to-date list (Appendix 1). Information on the usage, fate and effects of each of the compounds on the new list was then used to estimate their concentrations in the main environmental compartments. The resulting concentrations were then compared to acceptable daily intakes (ADIs) to identify veterinary medicines that pose the greatest risk to sources of drinking water.

3.1.1. Compilation of active substance list and pre-screening

A list of active substances in use in the European Union was previously compiled by Kools et al., (2008). This list was compiled from a number of sources including the list for the centralized authorisation procedure developed by the European Medicines Agency (EMA); the list of compounds with maximum residue limits (MRLs) in food; and information on authorized veterinary medicines in Germany, the Netherlands and the UK. The overall compilation included 447 active substances.

This compilation of active substances was reviewed and the following classes of substance were removed prior to further assessment:

- compounds that are no longer in use in the UK
- homoeopathic medicines
- veterinary active substances that occur in the environment naturally (e.g. vitamins and minerals)
- veterinary medicinal products comprising complex and uncharacterised mixtures
- feed additives and growth promoters
- companion animal treatments (i.e. for cats, dogs, horses, pigeons etc.) and euthanasia products
- excipients and solvents
- products where no data could be found on treatment characteristics meaning that estimations of exposure concentrations were not possible.

A list of the removed active ingredients is provided in Appendix 1. Removal of these substances left around 130 active substances that were then taken forward for risk-based prioritisation (Appendix 2).

3.1.2. Calculation of environmental exposure

The simple model and scenarios described in the Technical Guidance Document for environmental risk assessment of veterinary medicines (CVMP, 2007) was used to estimate concentrations of amoxicillin in soil.

Concentrations were predicted using Equation 1. This equation does not account for either removal by metabolism in the treated animal or removal by degradation during manure or slurry storage. The animal scenarios used in the model are given in Table 3.1. In order to derive the concentrations in soil, data were required on representative treatment regimes (i.e. dosages, duration and frequency of treatment). These data were collated for each substance from a number of sources including Veterinary Applied Pharmacology and Therapeutics (Brander et al., 1991), the Veterinary Formulary (Debuf, 1991), Diseases of Poultry (Calneck et al., 1997); Veterinary Medicine (Radostis et al., 2000) and the Compendium of data sheets for Veterinary Products (NOAH, 2008).

$$PEC_{soil} = \left(\frac{D \text{ x } Ad \text{ x } BW \text{ x } P \text{ x } 170 \text{ x } Fh}{1500 \text{ x } 10000 \text{ x } 0.05 \text{ x } Ny \text{ x } H} \right) \text{x } 1000 \qquad \textbf{Eq 1}$$

Where:

PEC_{soil}	= predicted environmental concentration in soil (µg kg^{-1})
D	= daily dose of the active ingredient (mg kg bw^{-1} d^{-1})
Ad	= number of days of treatment (d)
BW	= animal body weight (kg bw)
P	= animal turnover rate per place per year (place y^{-1})
170	= EU Nitrogen spreading limit (kg N^{-1} ha^{-1})
Fh	= fraction of herd treated
1500	= bulk density of dry soil (kg m^{-3})
10000	= area of 1 hectare (m^2 ha^{-1})
0.05	= depth of penetration into soil (m)
Ny	= nitrogen produced in one year per place (kg N y^{-1})
H	= housing factor
1000	= conversion factor (mg to µg)

Table 3.1 Animal scenarios used in the soil exposure modelling (taken from CVMP, 2007)

Animal type	P (place y^{-1})	BW (kg)	Ny (kg N y^{-1})
broiler	9	1	0.23
laying hen [a]	na	na	na
replacement layer	2.6	0.8	0.24
broiler breeder	1	1.7	0.69
turkey	2.7	6.5	0.9
weaner pig	6.9	12.5	2.25
fattening pig	3	65	7.5
sow (with litter)	1	240	26

[a] - not for chickens laying eggs for human consumption

Concentrations in soil pore water were then estimated using methods described in the Technical Guidance Document (CVMP, 2007) using Equations 2-5. Where available, soil sorption data were taken from the literature. Sorption coefficients were also predicted using the USEPA's EPISuite v 4.00 estimation software. (USEPA, 2009). The software uses two approaches to estimate sorption coefficient: the first is based on the octanol-water partition coefficient for a compound and the second is based on the molecular connectivity indices for a compound. Estimations of pore water concentrations were obtained using both the experiment and predicted sorption data meaning that three concentrations were estimated for some compounds (one based on the experimental sorption data and two based on sorption data obtained using the two different predictive methods). In instances where a range of experimental sorption coefficients was available for a particular compound, the lowest value was used in the modelling.

$PEC_{groundwater} = (PEC_{soil} \times RHO_{soil})/(K_{soil\text{-}water} \times 1000)$ **Eq 2**

$K_{soil\text{-}water} = (F_{air\ in\ soil} \times K_{air\text{-}water}) + F_{water\ in\ soil} + (F_{solid\ in\ soil} \times (K_{psoil}/1000) \times RHO_{solid})$ **Eq 3**

$K_{air\text{-}water} = (VP \times MW)/(SOL \times R \times TEMP)$ **Eq 4**

$K_{psoil} = Foc_{soil} \times K_{oc}$ **Eq 5**

Where:
RHO_{soil} - bulk density of fresh soil (1700 kg m^{-3})
RHOsolid - density of soil solids (2500 kg m^{-3})
Fair in soil - fraction of air in soil (0.2 m^3 m^{-3})
Fwater in soil - fraction of water in soil (0.2 m^3 m^{-3})
Fsolid in soil - Fraction of solids in soil (0.6 m^3 m^{-3})
Foc soil - Weight fraction of organic carbon in soil (0.02 kg kg^{-1})
TEMP - temperature at air soil interface (283 K)
R - gas constant (8.314 Pa m^{-3} mol^{-1} K^{-1})
VP - vapour pressure (Pa)(for very low VPs a default of 0.5 is used)
MOLW - molar mass (g mol^{-1}) = 383.42
SOL - water solubility (mg L^{-1}) = 109.8
Ksoil-water - Partition coefficient between soil and water (m^3 m^{-3}) = 7.001
Kpsoil - partition coefficients between solid and water in soil (L kg^{-1}) = 4.534
Kair-water - partition coefficient between air and water in soil (m^3 m^{-3}) = 7.42 x10^{-4}
Koc - water-organic carbon distribution coefficient (L kg^{-1}) = 226.7
PECsoil - maximum predicted concentration (μg kg^{-1}) in soil assuming a mixing depth of 20 cm (i.e PECsoil-initial/4)

3.1.3. Risk-based prioritisation

Data on the acceptable daily intake (ADI) was obtained for each active substance. The ADI and pore water concentration predictions were then used to calculate a risk index (RI) using Equation 6.

$$RI = \frac{PEC_{groundwater}}{ADI}$$ **Eq 6**

Substances were then ranked based on the risk index to give three ranking lists: one based on pore water concentrations obtained using experimental sorption data and two lists based on pore water concentrations obtained using the two predictive methods for estimating sorption. The top compounds in each list are provided in Table 3.2. From these three lists, the following veterinary medicines were selected for more detailed health risk assessment:

Acetylsalicylic acid
Apramycin
Albendazole
Cefapirin
Clorsulon
Diazinon
Dicyclanil
Florfenicol
Enrofloxacin
Halofuginone
Lincomycin
Luprostiol
Tylosin
Monensin, sodium
Salinomycin sodium
Sulfadiazine
chlortetracycline
Tiamulin
Tilmicosin
Trimethoprim
Altrenogest
Amoxicillin
Cypermethrin
Cyromazine
Eprinomectin
Lasalocid

Table 3.2. Top ranked active ingredients in terms of potential risk to human health, based on either measured sorption data or predictions of sorption using two different methods

Ranking	Experimental Koc	Koc predicted based on MIC	Koc predicted based on Kow
1	Salinomycin sodium	Doxycycline, hyclate	Doxycycline, hyclate
2	Lincomycin	Acetylsalicylic acid	Chlortetracycline
3	Acetylsalicylic acid	Chlortetracycline	Monensin, sodium
4	Florfenicol	Spectinomycin	Lincomycin
5	Sulfadiazine	Monensin, sodium	Oxytetracycline
6	Clorsulon	Enrofloxacin	Acetylsalicylic acid
7	Tiamulin	Lincomycin	Spectinomycin
8	Trimethoprim	Gentamicin	Enrofloxacin
9	Diazinon	Oxytetracycline	Florfenicol
10	Fenbendazole	Apramycin	Gentamicin
11	Oxfendazole	Streptomycin	Apramycin
12	Tilmicosin	Florfenicol	Streptomycin
13	Tylosin	Neomycin	Difloxacin
14	Halofuginone	Dicyclanil	Neomycin
15	Erythromycin	Salinomycin sodium	Halofuginone

Ranking	Experimental Koc	Koc predicted based on MIC	Koc predicted based on Kow
16	Mebendazole	Halofuginone	Tetracycline
17	Albendazole, -oxide, -sulphoxide	Tetracycline	Luprostiol
18	Enrofloxacin	Sulfadiazine	Cefapirin
19	Ceftiofur	Cefapirin	Altrenogest
20	Apramycin	Lasalocid	Amoxicillin
21	Eprinomectin	Dihydrostreptomycin	Dicyclanil
22	Chlortetracycline	Amoxicillin	Salinomycin sodium
23	Doramectin	Cyromazine	Sulfadiazine
24	Moxidectin	Marbofloxacin	Trimethoprim
25	Oxytetracycline	Clorsulon	Lasalocid
26	Cypermethrin	Altrenogest	Dihydrostreptomycin
27	Danofloxacin	Difloxacin	Tylosin
28	Tetracycline	Clavulanic acid	Cefquinome
29	Pirlimycin	Trimethoprim	Cefalexin
30	Deltamethrin	Meloxicam	
31		Luprostiol	

4. DETAILED EXPOSURE ASSESSMENT OF PRIORITY COMPOUNDS

4.1. Introduction

The prioritisation method described in Chapter 3 used very simple models and assumptions to identify those veterinary medicines that are likely to pose the greatest risk to human health. This was done to reduce the number of active ingredients in use in the UK down to a manageable size for detailed evaluation. From Chapter 2, it is clear that veterinary medicines may enter water bodies via leaching, drainage systems and surface runoff. Therefore in this Chapter, more sophisticated models that describe these transport pathways are used to evaluate potential exposure. The potential for removal of the veterinary medicines in conventional and advanced drinking water treatment systems is also considered. The assessment employed models and scenarios developed by the Forum for Co-ordination of Pesticide Fate Models and their Use (FOCUS) for risk assessment of pesticides. The FOCUS approach is designed to estimate potential exposure of surface water and groundwaters to pesticides in the European agricultural landscape.

The key chemical inputs to the leaching, drainage and runoff models are the application rate, the soil sorption coefficient and the half life in soil. Application rates were obtained based on Equation 1 in Chapter 3. Sorption data were either obtained from the literature or predicted. Where available, degradation data were obtained from the literature but in instances where literature data were not available, simulations were done using DT50 values of 100 and 1000 days.

4.2. Prediction of concentrations in source waters

4.2.1. Groundwaters

The potential for the prioritised veterinary medicines to leach to groundwater was assessed and predicted environmental concentrations in groundwater (PEC_{GW}) under European conditions were determined. The mathematical model PEARL was used to predict leaching of the prioritised veterinary medicines for a range of soil and climate scenarios developed by the Forum for the Co-ordination of Pesticide Fate Models and their Use (FOCUS). The crop types selected for the modelling were maize and winter cereals. Soil / weather scenarios were selected in accordance with FOCUS (2000) for each crop.

The approach used followed standard methods for pesticide exposure assessment. The veterinary medicines were assumed to be applied in each of 26 successive years, with the final 20 years being considered for the assessment. The 80^{th} percentile annual average concentration of each veterinary medicine in leachate at 100-cm depth within the 20-year simulation period was then calculated. The PEARL model is used by industry and regulators across Europe to assess the risks of pesticides and veterinary medicines to groundwaters. The leachate concentration at 100 cm depth is used as the basis for these assessments; hence this endpoint was used in the current study to assess risks of veterinary medicines arising from consumption of treated groundwater.

Model

Leaching of the prioritised veterinary medicines was simulated using the model PEARL (FOCUS Version 2.2.2). PEARL is a deterministic model used to evaluate the leaching of pesticide to the groundwater in support of the Dutch and European pesticide registration procedures. The model is linked with the Soil Water Atmosphere Plant (SWAP) model, which simulates water flow according to the Richards' equation.

Application date for groundwater exposure simulations

Two application dates were selected to cover worst-case scenarios. At and after the chosen application dates precipitation is large and degradation is slow due to low temperatures. All simulations were run twice, once with the application on the 1st of February and once with applications on the 1st of November in each year.

FOCUS scenarios

Soil, weather and crop properties were based on standard FOCUS scenarios, selected for each crop type according to FOCUS (2000) guidelines. Weather and soil details for the scenarios used (selected as realistic worst-case scenarios representative of agriculture in the EU) are given in Table 4.1. For each simulation, leaching was to 100-cm depth.

Table 4.1. FOCUS scenario and crop details for groundwater exposure simulations

Location	Mean annual temperature (°C)	Annual rainfall (mm)	Topsoil	Organic matter (%)
Châteaudun	11.4	648 (I[a])	silty clay loam	2.4
Hamburg	9.2	786	sandy loam	2.6
Jokioinen	4.3	638	loamy sand	7.0
Kremsmünster	8.8	900	loam / silt loam	3.6
Okehampton	10.4	1038	loam	3.8
Piacenza	13.3	857 (I[a])	loam	1.7
Porto	14.8	1150	loam	6.6
Sevilla	18.1	493 (I[a])	silt loam	1.6
Thiva	16.2	500 (I[a])	loam	1.3

[a] Irrigation included in scenario

4.2.2. Surface waters

FOCUS surface water modelling was used to assess the potential for the prioritised veterinary medicines to reach surface water following application to winter cereals. The modelling simulations were carried out in accordance with the FOCUS surface water scenarios workgroup guidelines and as such represent realistic worst-case simulations for application to winter cereals within Europe (FOCUS, 2001). The veterinary medicines were assessed using the FOCUS Step 3 modelling approach using the FOCUS surface water models (PRZM_SW, MACRO and TOXSWA). The use pattern was a single application before emergence. The exact application date was determined for each scenario by the FOCUS application timer. The scenarios considered are summarised in Table 4.2.

Table 4.2. Inherent Agro-environmental characteristics of the Surface water scenarios (from FOCUS 2001)

Scenario	Mean spring & autumn temp.(°C)	Mean annual rainfall (mm)	Mean annual recharge (mm)	Slope (%)	Soil
D1	<6.6	600 – 800	100 – 200	0 – 0.5	Clay with shallow groundwater
D2	6.6 – 10	600 – 800	200 – 300	0.5 – 2	Clay over impermeable substrate
D3	6.6 – 10	600 – 800	200 – 300	0 – 0.5	Sand with shallow groundwater
D4	6.6 – 10	600 – 800	100 – 200	0.5 – 2	Light loam over slowly permeable substrate
D5	10 – 12.5	600 – 800	100 – 200	2 – 4	Medium loam with shallow groundwater
D6	>12.5	600 – 800	200 – 300	0 – 0.5	Heavy loam with shallow groundwater
R1	6.6 – 10	600 – 800	100 – 200	2 – 4	Light silt with small organic matter
R2	10 – 12.5	>1000	>300	10 – 15	Organic-rich light loam
R3	10 – 12.5	800 – 1000	>300	4 – 10	Heavy loam with small organic matter
R4	>12.5	600 – 800	100 – 200	4 – 10	Medium loam with small organic matter

Modelling approach

FOCUS has recommended a tiered approach to surface exposure assessment (FOCUS, 2001). The FOCUS surface water methodology at Step 3 has ten scenarios (Table 4.2) that together represent realistic worst-case combinations of soil, agriculture and weather across Europe (FOCUS, 2001). These scenarios are used in conjunction with a suite of models to generate predicted environmental concentrations (PECs) in surface water and sediment. The models included SWASH version 1.1, MACRO version 4.4.2, PRZM_SW version 1.1.1 and TOXSWA version 1.1.1. The scenarios D1 to D6 represent drained soil, where the dominating transport

processes to surface waters is via leaching to drainage systems. In the scenarios R1 to R4 the compound is transported to surface water via runoff.

Application

The models MACRO and PRZM use the Pesticide Application Timer (PAT) to internally calculate the actual date of application. A window of possible dates has to be provided. Dates of the application to winter cereals selected by PAT were between 8th September and 17th December depending on the scenario. The application method was set to soil incorporation with 5 cm incorporation depth.

4.2.3. Predicted concentrations

The maximum concentrations of each of the priority compounds obtained using the above models are summarised in Table 4.3. Surface water concentrations are shown for catchments where drainage and runoff are the main routes of input for the veterinary medicine. These concentrations correspond to levels that are likely to occur in streams and ditches within an agricultural system. Time weighted average (TWA) concentrations are also shown for a one week period. The TWA concentrations are used in the risk assessment work described in Chapter 5 as these concentrations are probably more representative of the levels that a consumer might be exposed to compared to the peak concentrations.

Groundwater concentrations represent the highest 80th percentile concentration at 100 cm depth for the 'worst case' agricultural scenario that was modelled. This value was selected as it is the standard endpoint used in the assessment of risks of pesticides to human health.

Table 4.3 Predicted concentrations (μg l^{-1}) in surface waters and groundwaters.

Compound	DT50	Surface water in drainage dominated systems	Surface water 7d TWA in drainage dominated system	Surface water in runoff dominated system	Surface water 7d TWA in runoff dominated system	Groundwater
acetylsalicylic acid	1000	193.6	193.3	105.2	3.35	1004
acetylsalicylic acid	100	114.6	114.4	97.9	3.12	581
albendazole	1000	<0.001	<0.001	0.03	0.003	<0.001
albendazole	100	<0.001	<0.001	0.08	0.01	<0.001
altrenogest	1000	0.002	0.003	0.351	0.02	0.839
altrenogest	100	0,020	<0.001	0.321	0.018	0.036
amoxicillin	0.29	0.002	<0.001	<0.001	<0.001	0.001
apramycin	1000	252	251.3	128	4.05	1270
cefapirin	180	2.571	2.559	1.588	0.05	11.61

Compound	DT50	Surface water in drainage dominated systems	Surface water 7d TWA in drainage dominated system	Surface water in runoff dominated system	Surface water 7d TWA in runoff dominated system	Groundwater
chlortetracycline	100	0.06	0.002	6.93	0.512	<0.001
chlortetracycline	1000	<0.001	<0.001	5.07	0.34	<0.001
clorsulon	19.6	0.012	0.004	0.302	0.005	0.004
cypermethrin	112	<0.001	<0.001	<0.001	<0.001	<0.001
cyromazine	142	3.33	3.32	3.084	0.099	16.25
diazinon	112	<0.001	<0.001	0.048	0.003	<0.001
dicyclanil	100	10.70	10.69	6.47	0.21	55.3
dicyclanil	1000	5.22	4.71	6.01	0.19	26.5
enrofloxacin	696	<0.001	<0.001	0.074	0.011	<0.001
eprinomectin	150	0.023	0.016	0.093	0.004	0.099
florfenicol	103	20.6	20.5	16.8	0.54	102.5
halofuginone	20	0.1	0.063	0.36	0.012	0.33
lasalocid	49	0.005	0.0003	12.89	0.78	<0.001
lincomycin	26	73.3	48.7	94.6	2.99	505

Compound	DT50	Surface water in drainage dominated systems	Surface water 7d TWA in drainage dominated system	Surface water in runoff dominated system	Surface water 7d TWA in runoff dominated system	Groundwater
luprostiol	1000	0.80	0.8	0.53	0.017	1.33
luprostiol	100	0.35	0.29	0.49	0.016	4.65
monensin	7.5	89.6	46.4	240	7.68	326
salinomycin	64	0.003	<0.001	32.5	2.15	<0.001
sulfadiazine	103	72.6	61.8	98.7	3.25	284
tiamulin	301	0.156	0.019	21.5	1.3	0.897
tilmicosin	1000	0.18	0.016	10.8	0.69	3.06
tilmicosin	100	0.005	<0.001	9.13	0.58	<0.001
trimethoprim	110	0.002	<0.001	1.50	0.092	<0.001
tylosin	8.1	<0.001	<0.001	1.83	0.062	<0.001

Evaluation of model outputs

The monitoring data reported in Chapter 2, offers a possibility to begin to evaluate the performance of the FOCUS surface water and leaching models for use in the exposure assessment of veterinary medicines. Reported ranges of measured concentrations in surface waters and groundwaters were therefore compared with concentration ranges obtained using the different models (Tables 4.4 and 4.5)

Table 4.4 Comparison of modelled concentrations with measured concentrations for surface waters

Compound	Measured concentrations ($\mu g/l^{-1}$)	Maximum modelled concentrations ($\mu g/l^{-1}$)	Maximum TWA concentrations ($\mu g/l^{-1}$)
Lincomycin	0.36 - 21.1	73.3 – 94.6	2.99 – 48.7
Monensin	0.05 – 1.17	89.6 - 240	7.68 – 46.4
Salinomycin	0.007 – 0.04	0.003 – 32.5	<0.001 – 2.15
Sulfadiazine	4.13	72.6 – 98.7	3.25 – 61.8
Trimethoprim	0.015 – 0.02	0.002 – 1.50	<0.001 – 0.092
Tylosin	0.05	<0.001 – 1.83	<0.001 – 0.062

Table 4.5 Comparison of modelled concentrations with measured concentrations for groundwaters

Compound	Measured concentrations ($\mu g/l^{-1}$)	Modelled concentrations ($\mu g/l^{-1}$)
Chlortetracycline	ND	<0.001
Enrofloxacin	ND	<0.001
Lincomycin	0.32	505
Monensin	0.39	326
Trimethoprim	ND	<0.001
Tylosin	ND	<0.001

The majority of measured surface water concentrations fall within the range of model predictions. In all cases, predicted maximum concentrations are significantly higher than the monitored levels whereas, in general, time weighted average concentrations were not dissimilar from the measured levels (Table 4.4).

The groundwater models seemed to work well in identifying compounds that do not leach. Veterinary medicines that were predicted to not leach were also not detected in the field monitoring studies. In instances where compounds were detected in groundwater in the field (i.e. monensin and lincomycin), the model did predict that the compounds would leach but predicted concentrations were significantly higher than concentrations seen in the field. This mismatch might be explained by differences in the scenario that is modelled (i.e. concentration at 100 cm depth) and systems where monitoring has been done, many of which are aquifers much deeper than 100 cm.

Overall the comparison indicates that the use of time weighted averages from the surface water models will provide an adequate estimate of potential exposure in the real environment. The groundwater predictions are however likely to be extremely conservative and may therefore greatly overestimate risks.

4.2.4. Predicted removal during water treatment processes

In this section the removal of selected veterinary pharmaceuticals by drinking water treatment processes (DWTP) is considered. There is a growing body of evidence for the removal of pharmaceuticals and pesticides during water treatment processes so where available published data on compound removal by DWTPs has been used and where not removal by specific treatment processes has been estimated based on the physical chemical properties of the veterinary medicines.

Two treatment scenarios were considered (i) the first was a 'conventional treatment' consisting of coagulation-flocculation followed by filtration and disinfection. The second is 'advanced treatment' consisting of coagulation, ozonation and activated carbon followed by disinfection. The drinking water modelling employed a similar strategy to that used in the parallel study on human pharmaceuticals (Watts et al., 2007).

In general coagulation-flocculation/filtration will only remove charged colloidal species from water (Parsons and Jefferson 2006). Species that are negatively charged (anionic) at pH 7 are more amenable to removal and this has been used to predict removal. Filtration is not expected to give any further removal as the remaining pesticide metabolites would be fully dissolved. Therefore the removal predicted using coagulation would be the same as the removal for conventional treatment. Default values of 7.5 or 10% removal were therefore used in modelling both the conventional and advanced plants to account for removal in coagulation-flocculation/filtration. Disinfection (e.g. chlorination) can remove compounds but there is evidence that, in some cases, the parent compound is not fully degraded but is converted to stable transformation products (Sedlak and Von Gunten, 2011). There is some concern that transformation products may also pose a risk to human health. To account for these concerns, we therefore assumed that disinfection chlorine did not remove the parent compound.

Adams et al. (2002) investigated the removal of seven common antibiotics compounds including trimethoprim in a range of water treatment processes including coagulation, adsorption and oxidation with UV light and ozone. They reported no removal of any of the seven compounds with either iron or aluminium coagulants but did show that a 20 g L^{-1} powdered activated carbon (PAC) dose was sufficient to give 80% removal of trimethoprim. 90% removal was possible with 1.3 minutes contact with 0.3 mg L^{-1} of ozone, well below normal dose and contact times at advanced water treatment works. Nakada et al. (2007) also reported >90% removal of trimethoprim with ozone.

Ozone is routinely used to oxidise pesticides in water treatment before removal by activated carbon (Parsons and Jefferson 2006). Ozone reacts with organic compounds either through the direct reaction with molecular ozone or through the formation of free radicals including the hydroxyl radical. Molecular ozone is a selective electrophile that reacts quickly with double bonds, activated aromatic systems and nonprotonated amines. These preferred reaction pathways allow an assessment of the relative reactivity of trace organic compounds with ozone (Drewes et al. 2007).

A number of other authors have shown how effective ozone can be for micropollutants. Okuda et al. (2008) in their study of 66 pharmaceuticals (including tetracycline) in wastewater effluents showed 99% removal was possible when using ozone but the combination of coagulation, sand filtration and chlorine alone was

insufficient to remove the pharmaceuticals. Wu et al. (2007) reported the oxidation of four pesticides including diazinon and cypermethrin and showed 61% and 99% removal was possible at a dose of 1.4 mg L^{-1} for 30 minutes. Dodd et al. (2006) reported the oxidation of a range of antibiotic compounds using ozone including enrofloxacin, lincomycin and trimethoprim and reported removals of 60, 75 and 75% respectively at realistic ozone doses. Dodd et al. (2009) later studies 13 compounds including tylosin, tetracycline and enrofloxacin, lincomycin and trimethoprim and showed that ozone was an effective treatment process when used to treat wastewater effluents at doses of 5-10 mg L^{-1}. Benitez et al. (2009) used ozone to transform four pharmaceutical compounds including amoxicillin. They found removal of amoxicillin increased from 50% with an ozone dose of 0.5 mg L^{-1} up to 85% at a dose of 1.5 mg L^{-1}.

To determine the reactivity of the veterinary medicines in ozonation, the four aspects of their structure that were considered were (Drewes et al. 2007):

1) Electron-donating groups (e.g., hydroxyl, amine, conjugated double bond, and sulfide) enhance reactivity with ozone, whereas electron-withdrawing groups (e.g., iodine, chlorine, fluorine, and nitro) reduce the reaction rate
2) Electron-donating groups enhance the reactivity of aromatic compounds toward ozone, while electron-withdrawing groups inhibit the reactivity
3) Phenolic compounds are highly amenable to an attack by ozone, whereas ketone groups decrease the reactivity of ozone with adjacent carbons on aromatic structures
4) Hydroxyl and ketone groups have an activating effect on the adjacent methylene groups of an aliphatic chain, though the oxidation rates are lower than those of corresponding aromatic structures

A score from 1 to 10 was assigned to each veterinary medicine based on its reactivity according to the categories listed above. The veterinary medicines were then separated into four categories to determine their removal during water treatment:

1) Good removal (>90%)
2) Intermediate removal (90-50%)
3) Moderate removal (50-25%)
4) Poor removal (<25%)

Activated carbon is a broad-scale adsorbent of dissolved substances. Dissolved, colloidal and particulate substances are attracted and attached to the surface of the carbon particles. It is used to remove taste and odour causing compounds as well as toxic organic chemicals. Precipitation and other chemical reactions also occur on the carbon surface. A variety of carbon adsorbers can be designed, including batch and continuous flow units. The adsorption capacity of the carbon is eventually exhausted. The carbon is regenerated by heating the carbon, which burns and volatilises the substances accumulated on it. The activated carbon can take the form of granules (granular activated carbon – GAC) or powder (powdered activated carbon – PAC). Adsorption of organics onto activated carbon is complex and can depend on the activated carbon characteristics such as surface area, pore size distribution, surface chemistry and ash content. It also depends on adsorptive characteristics such as molecular weight, polarity, pKa, molecular size and functional groups.

Domingues et al. (2007) reported how effective activated carbon can be for cypermetherin removal and showed that 95% removal was possible in granular activated carbon and reported the capacity to be 303 μg/g. Choi et al. (2008) also found GAC to be an effective treatment process for the removal of seven tetracycline

compounds including chlortetracycline and reported removal of >90% for all compounds with both coal and coconut based carbon. Otero et al. (2004) investigated the removal of *salicylic acid* on polymeric adsorbent and activated carbon (Filtrasorb F400) and showed it was readily removed on all adsorbent but more so on F400 (Q = 351 mg/g). Where available published data was used and if not each compound was put into one of the following categories based on the octanol/water coefficient (log K_{ow}) and the charge of compound at pH 7 (Drewes et al. 2007):

1) log K_{ow} > 4 (pH 7); uncharged
2) log K_{ow} = 0–4 (pH 7); uncharged
3) log K_{ow} < 0 (pH 7); uncharged
4) log K_{ow} = 0–1.5 (pH 7); protonated base
5) log K_{ow} < 0 (pH 7); protonated base
6) log K_{ow} = 0–2.5 (pH 7); deprotonated acid
7) log K_{ow} < 0 (pH 7); deprotonated acid

The removal for each category by activated carbon was therefore as follows:

1) >90% removal
2) 90-50% removal
3) 50-25% removal
4) 90-50% removal
5) 50-25% removal
6) 50-25% removal
7) <25% removal

The theoretical 'reasonable worst-case' indirect exposure of consumers though intake of water, resulting from the use of the 30 selected, priority veterinary medicines was estimated from predicted concentrations of these compounds in water arising from leaching (ground water), drainage and run-off. The results are summarised in Tables 4.6 and 4.7. These predictions are used in the consumer risk assessment work described in the next Chapter.

Table 4.6. Predicted concentrations (μg l^{-1}) of the priority veterinary medicines in drinking water from a conventional drinking water treatment plant for surface water dominated by drainage or runoff inputs or groundwater

Compound	DT50	% Removal	7d TWA drainage dominated	7d TWA runoff dominated	Groundwater
acetylsalicylic acid	1000	7.5	179	3.10	537
acetylsalicylic acid	100	7.5	106	2.89	929
albendazole	1000	7.5	<0.001	0.01	<0.001
albendazole	100	7.5	<0.001	0.003	<0.001
altrenogest	1000	10.0	0.003	0.018	0.76
altrenogest	100	10.0	<0.001	0.016	0.03
amoxicillin	0.29	7.5	<0.001	<0.001	0.001
apramycin	1000	10.0	226	3.65	1143
cefapirin	180	10.0	2.30	0.045	10.5

Compound	DT50	% Removal	TWA drainage dominated systems	TWA runoff dominated systems	Groundwater
chlortetracycline	100	10.0	0.002	0.46	<0.001
chlortetracycline	1000	10.0	<0.001	0.30	<0.001
clorsulon	19.6	10.0	<0.001	0.005	0.004
cypermethrin	112	10.0	<0.001	<0.001	<0.001
cyromazine	142	10.0	2.99	0.089	14.63
diazinon	112	10.0	<0.001	0.003	<0.001
dicyclanil	100	10.0	9.62	0.189	49.8
dicyclanil	1000	10.0	4.24	0.171	23.9
enrofloxacin	696	10.0	<0.001	0.01	<0.001
eprinomectin	150	10.0	0.01	0.004	0.089
florfenicol	103	7.5	18.96	0.500	94.8
halofuginone	20	10.0	0.06	0.011	0.30
lasalocid	49	10.0	<0.001	0.702	<0.001
lincomycin	26	10.0	42.8	2.69	417
luprostiol	1000	10.0	0.72	0.015	1.20

Compound	DT50	% Removal	TWA drainage dominated systems	TWA runoff dominated systems	Groundwater
luprostiol	100	10.0	0.26	0.014	4.18
monensin	7.5	10.0	41.8	6.91	293
salinomycin	64	10.0	<0.001	1.94	<0.001
sulfadiazine	103	10.0	55.6	2.93	256
tiamulin	301	10.0	0.02	1.17	0.89
tilmicosin	1000	10.0	0.014	0.62	2.75
tilmicosin	100	10.0	<0.001	0.52	<0.001
trimethoprim	110	10.0	<0.001	0.08	<0.001
tylosin	8.1	10.0	<0.001	0.06	<0.001

Table 4.7. Predicted concentrations of the priority veterinary medicines in drinking water from an advanced drinking water treatment plant for inputs surface waters with drainage and runoff dominated inputs and groundwater

Compound	DT50	% Removal	TWA drainage dominated systems	TWA runoff dominated systems	Groundwater
acetylsalicylic acid	1000	72	54.12	0.94	162.68
acetylsalicylic acid	100	72	32.03	0.87	281.12
albendazole	1000	99	<0.001	<0.001	<0.001
albendazole	100	99	<0.001	<0.001	<0.001
altrenogest	1000	81	<0.001	<0.001	0.16
altrenogest	100	81	<0.001	<0.001	0.007
amoxicillin	0.29	92	<0.001	<0.001	<0.001
apramycin	1000	93	17.59	0.28	88.90
cefapirin	180	93	0.18	<0.001	0.81
chlortetracycline	100	97	0.002	0.02	<0.001

Compound	DT50	% Removal	TWA drainage dominated systems	TWA runoff dominated systems	Groundwater
chlortetracycline	1000	97	<0.001	0.01	<0.001
clorsulon	19.6	100	<0.001	<0.001	0.00
cypermethrin	112	98	<0.001	<0.001	0.00
cyromazine	142	96	0.13	<0.001	0.65
diazinon	112	99	<0.001	<0.001	0.00
dicyclanil	100	96	0.43	0.01	2.21
dicyclanil	1000	96	0.19	0.01	1.06
enrofloxacin	696	70	<0.001	0.003	<0.001
eprinomectin	150	98	0.00	<0.001	0.00
florfenicol	103	63	7.59	0.20	37.93
halofuginone	20	81	0.01	<0.001	0.06
lasalocid	49	95	0.00	0.04	0.00
lincomycin	26	81	9.3	0.57	96
luprostiol	1000	63	0.30	0.01	0.49
luprostiol	100	63	0.11	0.01	1.72

Compound	DT50	% Removal	TWA drainage dominated systems	TWA runoff dominated systems	Groundwater
monensin	7.5	100	3.25	<0.001	<0.001
salinomycin	64	93	0.00	0.15	<0.001
sulfadiazine	103	75	15.45	0.81	71
tiamulin	301	93	0.00	0.09	0.06
tilmicosin	1000	98	0.004	0.01	0.06
tilmicosin	100	98	0.001	0.01	<0.001
trimethoprim	110	98	<0.001	0.002	<0.001
tylosin	8.1	81	<0.001	0.006	<0.001

5. DETAILED REVIEW OF TOXICOLOGICAL INFORMATION AND HEALTH RISK ASSESSMENT

5.1. Toxicological data search strategy

Following determination of the prioritised list of 26 veterinary medicines, toxicological profiles were developed for each compound. These profiles were based on a review of the most current evaluations published by authoritative organisations, such as the European Medicines Agency (EMEA) Committee on Veterinary Medicinal Products (CVMP), the World Health Organization/Food and Agriculture Organization (WHO/FAO) Joint Expert Committee on Food Additives (JECFA) and Joint Meeting on Pesticide Residues (JMPR). In many cases, these sources did not provide a complete dataset; therefore extensive literature searches were conducted to identify any other relevant evaluations or publications. The searches utilised biomedical, toxicological and environmental on-line databases (see Table 5.1).

Table 5.1. Search database summary

Database	Database producer	Coverage
Scopus	Elsevier B.V.	1960-present
Biological Sciences	ProQuest CSA	1982–present
Biology Digest	Plexus Publishing, Inc	1989–present
Toxline	U.S. National Library of Medicine	1998–present
Medline	National Library of Medicine	1999–present

A summary of the toxicity terms used is shown in Table 5.2. In all databases phrases and keywords (title, abstract, descriptors) were combined using the 'and' operator with synonyms and CAS numbers for the individual medicines.

Table 5.2 Summary of the search strategy

Database	**Operator**	**Keyword**
Veterinary medicine CAS number	AND[1]	Health effect* Adverse effect* Toxic* Carcinogen* Teratogen* Mutagen* Neurotoxic* Cytotoxic* Genotoxic* Poison*

[1] "AND" used to narrow search and retrieve records containing all of the words it separates
*truncation symbol searches any characters that follow the truncated word

The full toxicity profiles of the 26 veterinary medicines are given in Appendix 3.

5.2. Risks to human health

An assessment of the potential risk posed to the general population from indirect exposure to each of 26 selected priority veterinary medicines was carried out. The modelled concentrations of veterinary medicines in Chapter 4 were used as a basis for the risk assessment. As the modelling approach derived concentrations in water bodies in close proximity to manured fields, it is likely to greatly overestimate consumer risk as dilution would be anticipated before the compounds reached a drinking water abstraction point.

The risk assessment involved the following components:

- estimated daily intake of veterinary medicine through consumption of water from leaching, drainage or run-off sources;
- estimated daily intake of veterinary medicine following conventional or advanced water treatment;
- an evaluation of the proportion of the acceptable daily intake (ADI) represented by estimated daily intake for the general population, toddlers and infants; and
- consideration of the potential toxicological hazard of the veterinary medicine.

As described in Section 4, the average daily intake of each veterinary medicine was estimated for adults and toddlers, based on standard estimates of daily water consumption (Environment Agency, 2009). Estimated percentage contribution to ADIs are detailed in Tables 5.1-5.3.

The predicted reasonable worst-case daily intake for adults, toddlers and infants was compared with the ADI of each veterinary medicine for pre-treated water (Table 5.1), and following conventional (Table 5.2) and advanced (Tables 5.3) treatments. Where the total predicted daily intake from all untreated (environmental) water sources amounted to less than 10% of the ADI, it was considered that there was no appreciable risk (Section 5.1). However, where an exceedence of 10% of the ADI was estimated for a veterinary medicine in adults, toddlers or infants, a more detailed assessment of the extent and nature of the risk was undertaken; for this group, the impact of conventional and advanced water treatments on removal of the veterinary medicine was also assessed and daily intake values re-calculated.

In preparing the risk assessments, estimated exposure was considered in the light of the nature of the toxicological hazard of each of 26 selected priority veterinary medicines. Toxicological profiles were, therefore, prepared for each of them. The toxicological profiles were based on a review of the most current evaluations published by authoritative organisations, such as the European Medicines Agency (EMEA) Committee on Veterinary Medicinal Products (CVMP), the World Health Organization/Food and Agriculture Organization (WHO/FAO) Joint Expert Committee on Food Additives (JECFA) and Joint Meeting on Pesticide Residues (JMPR). In many cases, these sources did not provide a complete dataset; therefore extensive literature searches were conducted to identify any other relevant evaluations or published papers. Data on the toxicity profile of the 26 veterinary medicines has been summarised. The hazard assessments are given in Annexe 3.

Table 5.1 Calculated worst case intake from three untreated (environmental) water sources for the general adult population, toddlers (1-2 yrs) and infants (0–1 yrs) as percentage of the ADI

COMPOUND	ADI ug/kg bw/day	DT50	Drainage			Runoff			Leaching		
			Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI
Acetylsalicylic acid	8.30	1000	78	233	349	1	4	6	403	1210	1814
Acetylsalicylic acid	8.30	100	46	138	207	1	4	6	233	700	1050
Albendazole	5.00	1000	<1	<1	<1	<1	<1	<1	<1	<1	<1
Albendazole	5.00	100	<1	<1	<1	<1	<1	<1	<1	<1	<1
Altrenogest	0.04	1000	<1	1	1	2	5	8	70	210	315
Altrenogest	0.04	100	<1	<1	<1	2	5	7	3	9	14
Amoxicillin	0.50	0	<1	<1	<1	<1	<1	<1	<1	<1	<1
Apramycin	40.00	1000	21	63	94	<1	1	2	106	318	476
Cefapirin	2.54	180	3	10	15	<1	<1	<1	15	46	69
Chlortetracycline	3.00	1000	<1	<1	<1	1	2	3	<1	<1	<1
Chlortetracycline	3.00	100	<1	<1	<1	<1	1	2	<1	<1	<1
Clorsulon	2.00	20	<1	<1	<1	<1	<1	<1	<1	<1	<1

			Drainage			Runoff			Leaching		
COMPOUND	**ADI ug/kg bw/day**	**DT50**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**
Cypermethrin	15.00	112	<1	<1	<1	<1	<1	<1	<1	<1	<1
Cyromazine	60.00	142	<1	1	1	<1	<1	<1	1	3	4
Diazinon	2.00	112	<1	<1	<1	<1	<1	<1	<1	<1	<1
Dicyclanil	7.00	1000	5	15	23	<1	<1	<1	26	79	119
Dicyclanil	7.00	100	2	7	10	<1	<1	<1	13	38	57
Enrofloxacin	6.20	696	<1	<1	<1	<1	<1	<1	<1	<1	<1
Eprinomectin	5.00	150	<1	<1	<1	<1	<1	<1	<1	<1	<1
Florfenicol	3.00	103	23	68	103	1	2	3	114	342	513
Halofuginone	0.30	20	1	2	3	<1	<1	1	4	11	17
Lasalocid	2.50	49	<1	<1	<1	1	3	5	<1	<1	<1
Lincomycin	10.00	26	16	49	73	1	3	4	168	505	758
Luprostiol	0.20	1000	13	40	60	<1	1	1	22	67	100
Luprostiol	0.20	100	5	15	22	<1	1	1	77	232	349
Monensin, sodium	3.45	8	45	134	202	7	22	33	315	945	1417
Salinomycin	5.00	64	<1	<1	<1	1	4	6	<1	<1	<1
Sulfadiazine	20.00	103	10	31	46	1	2	2	47	142	213

COMPOUND	ADI ug/kg bw/day	DT50	Drainage			Runoff			Leaching		
			Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI
Tiamulin	30.00	301	<1	<1	<1	<1	<1	1	<1	<1	<1
Tilmicosin	4.00	1000	<1	<1	<1	1	2	3	3	8	11
Tilmicosin	4.00	100	<1	<1	<1	<1	1	2	<1	<1	<1
Trimethoprim	4.20	110	<1	<1	<1	<1	<1	<1	<1	<1	<1
Tylosin	6.00	8	<1	<1	<1	<1	<1	<1	<1	<1	<1

Table 5.2. Calculated worst case intake from three conventionally treated water sources for the general adult population, toddlers (1-2 yrs) and infants (0–1 yrs) as percentage of the ADI

COMPOUND	ADI ug/kg bw/day	DT50	Drainage			Runoff			Leaching		
			Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI
Acetylsalicylic acid	8.30	1000	72	215	323	1	4	6	373	1119	1678
Acetylsalicylic acid	8.30	100	42	127	191	1	3	5	216	648	971
Albendazole	5.00	1000	<1	<1	<1	<1	<1	<1	<1	<1	<1
Albendazole	5.00	100	<1	<1	<1	<1	<1	<1	<1	<1	<1
Altrenogest	0.04	1000	<1	1	1	2	5	7	63	189	283
Altrenogest	0.04	100	<1	<1	<1	1	4	6	3	8	12
Amoxicillin	0.50	0	<1	<1	<1	<1	<1	<1	<1	<1	<1
Apramycin	40.00	1000	19	57	85	<1	1	1	95	286	429
Cefapirin	2.54	180	3	9	14	<1	<1	<1	14	41	62
Chlortetracycline	3.00	1000	<1	<1	<1	1	2	2	<1	<1	<1
Chlortetracycline	3.00	100	<1	<1	<1	<1	1	2	<1	<1	<1
Clorsulon	2.00	20	<1	<1	<1	<1	<1	<1	<1	<1	<1

			Drainage			Runoff			Leaching		
COMPOUND	**ADI ug/kg bw/day**	**DT50**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**
Cypermethrin	15.00	112	<1	<1	<1	<1	<1	<1	<1	<1	<1
Cyromazine	60.00	142	<1	<1	1	<1	<1	<1	1	2	4
Diazinon	2.00	112	<1	<1	<1	<1	<1	<1	<1	<1	<1
Dicyclanil	7.00	1000	5	14	21	<1	<1	<1	24	71	107
Dicyclanil	7.00	100	2	6	9	<1	<1	<1	11	34	51
Enrofloxacin	6.20	696	<1	<1	<1	<1	<1	<1	<1	<1	<1
Eprinomectin	5.00	150	<1	<1	<1	<1	<1	<1	<1	<1	<1
Florfenicol	3.00	103	21	63	95	1	2	2	105	316	474
Halofuginone	0.30	20	1	2	3	<1	<1	1	3	10	15
Lasalocid	2.50	49	<1	<1	<1	1	3	4	<1	<1	<1
Lincomycin	10.00	26	15	44	66	1	3	4	152	455	682
Luprostiol	0.20	1000	12	36	54	<1	1	1	20	60	90
Luprostiol	0.20	100	4	13	20	<1	1	1	70	209	314
Monensin, sodium	3.45	8	41	124	187	7	21	31	291	874	1311
Salinomycin	5.00	64	<1	<1	<1	1	4	6	<1	<1	<1
Sulfadiazine	20.00	103	9	28	42	0	1	2	43	128	192

			Drainage			Runoff			Leaching		
COMPOUND	**ADI ug/kg bw/day**	**DT50**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**
Tiamulin	30.00	301	<1	<1	<1	<1	<1	1	<1	<1	<1
Tilmicosin	4.00	1000	<1	<1	<1	1	2	2	2	7	10
Tilmicosin	4.00	100	<1	<1	<1	<1	1	2	<1	<1	<1
Trimethoprim	4.20	110	<1	<1	<1	<1	<1	<1	<1	<1	<1
Tylosin	6.00	8	<1	<1	<1	<1	<1	<1	<1	<1	<1

Table 5.3. Calculated worst case intake from three advanced treated water sources for the general adult population, toddlers (1-2 yrs) and infants (0–1 yrs) as percentage of the ADI

COMPOUND	ADI ug/kg bw/day	DT50	Drainage			Runoff			Leaching		
			Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI	Adult % ADI	Toddler % ADI	Infant % ADI
Acetylsalicylic acid	8.30	1000	22	65	98	<1	1	2	113	339	508
Acetylsalicylic acid	8.30	100	13	39	58	<1	1	2	65	196	294
Albendazole	5.00	1000	<1	<1	<1	<1	<1	<1	<1	<1	<1
Albendazole	5.00	100	<1	<1	<1	<1	<1	<1	<1	<1	<1
Altrenogest	0.04	1000	<1	<1	<1	<1	1	1	13	40	60
Altrenogest	0.04	100	<1	<1	<1	<1	1	1	1	2	3
Amoxicillin	0.50	0	<1	<1	<1	<1	<1	<1	<1	<1	<1
Apramycin	40.00	1000	1	4	7	<1	<1	<1	7	22	33
Cefapirin	2.54	180	<1	1	1	<1	<1	<1	1	3	5
Chlortetracycline	3.00	1000	<1	<1	<1	<1	<1	<1	<1	<1	<1
Chlortetracycline	3.00	100	<1	<1	<1	<1	<1	<1	<1	<1	<1
Clorsulon	2.00	20	<1	<1	<1	<1	<1	<1	<1	<1	<1

			Drainage			Runoff			Leaching		
COMPOUND	**ADI ug/kg bw/day**	**DT50**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**
Cypermethrin	15.00	112	<1	<1	<1	<1	<1	<1	<1	<1	<1
Cyromazine	60.00	142	<1	<1	<1	<1	<1	<1	<1	<1	<1
Diazinon	2.00	112	<1	<1	<1	<1	<1	<1	<1	<1	<1
Dicyclanil	7.00	1000	<1	1	1	<1	<1	<1	1	3	5
Dicyclanil	7.00	100	<1	<1	<1	<1	<1	<1	1	2	2
Enrofloxacin	6.20	696	<1	<1	<1	<1	<1	<1	<1	<1	<1
Eprinomectin	5.00	150	<1	<1	<1	<1	<1	<1	<1	<1	<1
Florfenicol	3.00	103	8	25	38	<1	1	1	42	126	190
Halofuginone	0.30	20	<1	<1	1	<1	<1	<1	1	2	3
Lasalocid	2.50	49	<1	<1	<1	<1	<1	<1	<1	<1	<1
Lincomycin	10.00	26	3	9	14	<1	1	1	32	96	144
Luprostiol	0.20	1000	5	15	22	<1	<1	<1	8	25	37
Luprostiol	0.20	100	2	5	8	<1	<1	<1	29	86	129
Monensin, sodium	3.45	8	<1	<1	<1	<1	<1	<1	<1	<1	<1
Salinomycin	5.00	64	<1	<1	<1	<1	<1	<1	<1	<1	<1
Sulfadiazine	20.00	103	3	8	12	<1	<1	1	12	36	53

			Drainage			Runoff			Leaching		
COMPOUND	**ADI ug/kg bw/day**	**DT50**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**	**Adult % ADI**	**Toddler % ADI**	**Infant % ADI**
Tiamulin	30.00	301	<1	<1	<1	<1	<1	<1	<1	<1	<1
Tilmicosin	4.00	1000	<1	<1	<1	<1	<1	<1	<1	<1	<1
Tilmicosin	4.00	100	<1	<1	<1	<1	<1	<1	<1	<1	<1
Trimethoprim	4.20	110	<1	<1	<1	<1	<1	<1	<1	<1	<1
Tylosin	6.00	8	<1	<1	<1	<1	<1	<1	<1	<1	<1

Veterinary medicines for which modelled intakes from water sources are of no concern

For the selected veterinary medicines for which the total predicted daily intake from leaching, drainage or run-off water amounted to less than 10% of the ADI for all populations evaluated, there was not judged to be any appreciable risk to consumers. Thus, no further consideration was undertaken for the following veterinary medicines — albendazole, amoxicillin, apramycin, chlortetracycline, clorsulon, cypermethrin, cyromazine, diazinon, enrofloxacin, eprinomectin, halofuginone, tiamulin, trimethoprim and tylosin.

Veterinary medicines for which the estimated worst-case intake for adults and/or toddlers and/or infants exceeds 10% of the ADI

Raw (environmental) water

Where the predicted reasonable worst-case daily intake was above 10% of the ADI for adults, toddlers or infants, but did not exceed the ADI for that population, the margin of safety was considered to be adequate for all sources. Nonetheless, the nature of the chemical's hazard profile was taken into account in risk characterisation and to add additional reassurance to the assessment of overall risk.

For cases where the ADI was predicted to be equalled or even exceeded, it was deemed possible that the margin of safety that is normally provided by the established ADI could be eroded. In such cases a more detailed evaluation of the nature of the risk posed was undertaken. Assessments of the risks posed by individual veterinary medicines are presented below. These should also be considered in the context that they relate to raw water in the environment and not treated drinking water as supplied to consumers.

Acetylsalicylic acid

EMEA CVMP established a pharmacologically-based ADI of 8.3 µg/kg bw for acetylsalicylic acid determined from a lowest observed effect level (LOEL) of 0.167 mg/kg bw in humans based on prolongation of bleeding times. CVMP applied an uncertainty factor of 20 based on a default factor of 10 multiplied by 2 due to the use of a LOEL.

In groundwater, the modelled worst case total intakes of acetylsalicylic acid for adults, toddlers and infants were approximately 403% of the ADI for adults, 1210% for toddlers and 1814% for infants respectively. Although predicted intakes from surface water were less, acetylsalicylic acid values from surface water in drainage dominated systems exceeded 10% of the ADI for adults, toddlers and infants. However, as acetylsalicylic acid is a commonly used medicine in humans, the contribution to the intakes predicted here in comparison with therapeutic dose requires discussion.

A therapeutic dose of acetylsalicylic acid is considered to be a minimum of 300 mg/person every 4-6 hours; with a preventative dose of 50 mg/person/day being recommended in individuals with cardiovascular disease [recent research may change the cardio preventive role of acetylsalicylic acid in the near future]. The

highest predicted intake of acetylsalicylic acid from leachate water was determined for adults as 33.5 µg/kg bw/day, which is more than three orders of magnitude lower than the recommended preventative dose (based on 60kg adult). However, in children, exposure to salicylic acid has been shown to be related, in a dose-dependent manner, to the occurrence of Reye's syndrome. In a study reported by Pinsky *et al.,* Reye's syndrome patients were shown to have higher average blood sodium acetylsalicylate levels (median = 25.1 mg/kg) compared with controls (median = 14.5 mg/kg) and higher maximum daily doses of sodium acetylsalicylate (median = 33.0 mg/kg compared with control median = 19.0 mg/kg). Reye's syndrome patients were also found to have received elevated doses of sodium acetylsalicylate (median = 65.4 mg/kg) compared to controls (27.0 mg/kg) for the first four days preceding the development of antecedent respiratory or chicken pox illness; controls for the study were children affected with the same illnesses who had received lower doses of aspirin (Pinsky *et al.,* 1988).

Taking the average body weight of a toddler (aged 1-2 years) to be approximately 10 kg and of an infant (aged 0-1 years) to be 5 kg (WHO, 2008), the modelled intake from leachate water in this study was 100 µg/kg bw/day for toddlers and 150 µg/kg bw/day for infants. When compared with the median therapeutic dose shown to be associated with development of Reye's syndrome (25.1 mg/kg) in toddlers, the modelled intake is approximately 250 times less than this level; modelled intakes for an infant were 167 times less than this level. Whilst it is noted that healthy individuals should not receive therapeutic doses of any medicine in drinking water, this example is helpful to put into context the modelled levels of intake.

It is anticipated that the use of acetylsalicylic acid as a veterinary medicine will generally be low, which taken in conjunction with a lack of reported toxicity findings from human exposure studies, leads to the assessment that intake of this veterinary medicine through drinking water is unlikely to pose a risk to human health.

Altrenogest

EMEA CVMP has recommended a pharmacologically-based ADI of 0.04 µg/kg bw. This level was based on the absence of hormonal effects of altrenogest at a concentration of 4 µg/kg bw in monkeys and pigs, and application of an uncertainty factor of 100 to allow for inter- and intra-species differences

The modelled worst case total intakes of altrenogest for adults from groundwater were estimated to be 70 % of the ADI. In toddlers and infants, water from leaching sources gave rise to estimated intake values of 210 and 315 % of the ADI respectively. Concentrations of altrenogest in surface waters were below 10% of the ADI for adults.

This may be of concern, as sensitive individuals such as toddlers and infants may be at greater risk from the hormonal effects described following exposure to altrenogest. However, it should be noted that the established ADI has an inherent safety factor of 100 below the level at which no observed adverse effects were noted in monkeys and pigs. Although altrenogest is not used as a human medicine, it would seem prudent to investigate other potentially important environmental sources, so as to assess the overall magnitude of exposure across the population and in particular the juvenile population.

While this chemical is eliminated quite quickly by the body in a number of species, no human toxicokinetic or toxicodynamic data have been identified and, it is thus not clear whether the estimated 'worst-case' intake could represent a risk to developing

children. Hence, it would be prudent to investigate actual concentrations present in water and other potential sources of altrenogest intake, to confirm that the levels of exposure do not constitute an appreciable risk to children.

Cefapirin

EMEA CVMP has established a toxicologically-based ADI of 0.1 mg/kg bw based on a NOEL of 20 mg/kg/day from 3 month studies in rats and dogs; an uncertainty factor of 200 was applied as only one dose level was assessed. In the light of the microbiological activity of cefapirin, a microbiologically based ADI of 2.54 µg/kg bw has also been established, which being substantially lower than the toxicologically-based ADI, has been applied by EMEA CVMP. Where seen, exceedences of the ADI guide value of 10% estimated in this study were very minor and are considered not to pose a risk to adults. Minor exceedences only occurred in adults exposed to leached water sources (15.24 %) however, levels of exposure from groundwater sources were higher in toddlers and infants at 1.2 and 1.7 µg/kg bw/day respectively, equating to approximately 46% and 69% of the ADI respectively. Estimated levels in surface waters were below 10% of the ADI for adults. As the microbiologically-based ADI is approximately 39 times lower than the toxicologically-based ADI, application of the toxicologically-based ADI would result in modelled intakes being under 2% for both toddlers and infants exposed to groundwater.

In view of the large variation between the toxicological and microbiologically-based ADI values, investigation of actual intakes by the general population to confirm levels of exposure may be prudent to confirm that no appreciable risk to consumer health exists.

Dicyclanil

EMEA CVMP has established a toxicologically-based ADI of 7 µg/kg bw based on a NOEL of 0.7 mg/kg bw/day from a one year study in dogs with an uncertainty factor of 100 applied.

Modelled total intakes of dicyclanil were seen to vary between water sources, however, where estimated intakes exceeded the ADI guide value of 10% this was considered not to constitute an appreciable risk to consumer health.

From groundwater, minor exceedences of the ADI guide value of 10% were seen in adults (maximally 26.%). The ADI was exceeded from estimations of exposure in toddlers and infants exposed to leachate water, with intakes of approximately 5.5 and 8.3 µg/kg bw/day, or 79 and 119% of the ADI respectively. Estimated intakes in adults from surface waters were below 10% of the ADI. Modelled intakes from surface waters in drainage dominated systems were above the ADI guide value of 10% with intakes in toddlers and infants having a maximum of 15% and 23% of the ADI respectively. In toddlers and infants exposed to run-off water estimated intakes were below 10% of the ADI. Such levels of exposure would not be expected to result in any adverse health effects as they are considerably lower than the ADI, which has an inherent safety factor of 100 fold less than the NOEL.

Whilst there is depletion of the margin of ‘safety’ offered by the ADI, overall it would seem unlikely that significant risks to the health of children would occur, as no developmental toxicity has been reported for dicyclanil. As dicyclanil is not used in human medicine, it would seem prudent to investigate additional environmental sources, so as to fully assess exposure and corresponding risk across the juvenile population in particular.

Florfenicol

A toxicological ADI of10 µg/kg has been established by EMEA CVMP for florfenicol using a NOAEL of 1 mg/kg bw/day, based upon low pup viability index in rats; an uncertainty factor of 100 was applied. Additionally, a microbiological ADI of 3 µg/kg bw has been established, and has been utilised in this assessment. The predicted total intake of were well above the 10% value in groundwaters and surface waters in drained areas. However, as the microbiological ADI was applied, the exceedences seen may be spuriously high and application of the toxicological ADI may provide a more realistic basis for risk assessment purposes, A more detailed assessment of the exposure to and possible toxicity of this substance may be warranted, with particular focus on toddlers and infants.

Lincomycin

A toxicologically based ADI of 300 µg/kg bw has been derived from a teratogenicity study in the rat with an uncertainty factor of 100 applied to a NOEL of 30 mg/kg bw/day (IPCS, 2000). However, due to the activity of lincomycin on the human gastrointestinal flora and the excretion of the unchanged parent compound, the most sensitive endpoint was based on microbiological activity. Therefore a microbiological ADI of 10 µg/kg bw was established, using a NOEL of 0.1 mg/kg bw/day based on an antibiotic-associated colitis model in the Syrian hamster; an uncertainty factor of 10 was applied for inter-animal variation only. No correction factor was required for the extrapolation from animals to humans because of the sensitivity of the hamster model.

Estimated intakes of lincomycin from groundwater exceeded the ADI in all populations. Estimated intakes from surface waters were below the ADI. Lincomycin has been shown to pass through the placenta when given intramuscularly, though it is unclear whether orally-ingested lincomycin is able to pass through in the same manner. In human medicines, lincomycin is used to treat cervicitis or vaginitis of pregnant women at 2 g/person for up to seven days; no evidence of adverse effects has been reported in offspring that have been monitored for up to 7 years after birth following maternal treatment. Although the monitoring period of this study was of a long duration, the treatment period was relatively short in comparison to potential exposure durations achieved from water intake. Therefore more detailed assessment of the exposure to, and possible toxicity of, lincomycin may be prudent.

Luprostiol

The EMEA CVMP has established an ADI of 0.2 µg/kg bw based on a NOEL of 0.2 mg/kg bw/day from a developmental toxicity study in rabbits; an uncertainty factor of 100 was applied.

Modelled intakes for adults, toddlers and infants from groundwater and surface waters in drainage dominated areas were more than 10% of the ADI.

In veterinary medicine, luprostiol is used for the control of oestrus or induction of parturition; although it is not used in human medicines, it would seem likely that the most sensitive sub-population following exposure to luprostiol would be pregnant women. Adverse effects identified from animal studies are generally associated with the pharmacological activity of luprostiol, although decreased litter size was also observed. In light of the sensitivity of the toxicological end point and the estimated exceedence of the ADI guide value of 10% in adults, and the ADI in toddlers and infants from some water sources, it would seem prudent to investigate total exposure levels in order that risk to consumers can be more fully assessed.

Monensin

In establishing an ADI for monensin, the European Food Safety Authority (EFSA) identified a NOEL of 1.1 mg/kg bw/day from a 2 year carcinogenicity study in rats. However a NOEL of 0.345 mg/kg bw/day was also identified from an acute toxicity study following acute pharmacological effects. Therefore this lower value of 0.345 mg/kg bw/day was adopted by EFSA and an ADI of 3.45 µg/kg bw established with an uncertainty factor of 100 applied.

For groundwater, estimated intakes exceeded the ADI in adults and toddlers/infants respectively. Intakes from surface waters in drainage dominated systems were greater than the ADI for toddlers and infants. Monensin is not used in human medicines and, overall, little data is available on the toxicokinetic behaviour of this compound. Therefore although the ADI levels have been estimated to be approximately equalled or substantially exceeded in some water sources, it is not possible to determine actual levels of consumer exposure; this may warrant further investigation.

Sulfadiazine

The National Registration Authority (NRA) For Agricultural and Veterinary Chemicals review by the Australian Government established an ADI of 20 µg/kg bw for sulfadiazine. This was based on a NOEL of 37.5 mg/kg/day from a rat reproductive toxicity study with an uncertainty factor of 2000 applied; limited data was available for sulfadiazine and, as such, the rationale for application of such a high uncertainty factor is not known.

Modelled intake from groundwater for toddlers and infants exceeded the ADI. The use of sulfadiazine in human medicines as a broad spectrum antibiotic has been associated with idiosyncratic hypersensitivity reactions, however, no reports of human toxicity were identified. Therefore, using currently available toxicity data, the estimated intake levels are not thought to pose a risk to human health. It should be noted however, that as animal data for chronic exposure and carcinogenicity studies is unavailable, and in light of the reported induction of hypersensitivity reactions, it may be prudent to assess exposure from all potential sources of sulfadiazone, allowing the risk to juvenile consumers to be more fully assessed.

Tilmicosin

The EMEA CVMP has established a microbiological ADI of 4 µg/kg derived from a NOEL of 0.4 mg/kg bw/day established in an *in vivo* microbiological study in rats with an uncertainty factor of 100 applied (EMEA, 2000). WHO and the US FDA have also established a toxicologically-based ADI of 40 µg/kg bw based on a NOEL of 4 mg/kg bw/day from a 12 month study in dogs with an uncertainty factor of 100 applied (WHO. JECFA, 2008, FDA, 2009). Adopting a precautionary approach, the lower ADI of 4 µg/kg bw was utilised in this assessment.

From groundwaters, levels of intake for infants were very slightly higher than 10% of the ADI. As the exceedence of the ADI guide value with the more conservative estimate is slight, and well within the overall ADI, tilmicosin is not expected to pose a risk to human health at this level of exposure.

Conventional water treatment

Conventional water treatment was predicted to reduce the intake of every veterinary medicine by between 7.5 – 10% from each water source evaluated in this study,

including those of particular concern, which are discussed individually above. However, as the predicted intakes of these veterinary medicines from pre-treated water were generally well above 10% of the ADI, caution is still advised.

Advanced water treatment

Advanced water treatment had a significant impact on veterinary medicine levels. Of those veterinary medicines that were regarded to be of particular concern, as highlighted above, the total predicted daily intake from all water sources following advanced water treatment amounted to less than 10% of the ADI, for all populations considered, for the following: cefapirin, dicyclanil, lasalocid, monensin, tilmicosin.

6. DISCUSSION AND RECOMMENDATIONS

With the exception of a few cases where more information on exposure, toxicokinetics or toxicology is required, the results of this risk assessment investigating novel exposure pathways, taking into consideration water treatments, were judged to be generally reassuring.

For 15 of the 26 selected priority veterinary medicines, even when conservative predictions of water concentrations are used, the estimated intakes from conventional or advanced treated water were less than 10% of the ADI for all sections of the population evaluated. It is concluded, therefore, that these veterinary medicines do not present a potential risk to human health via drinking water.

Very minor exceedences of the ADI guide value in all populations assessed were found for halofuginone and tilmicosin. However, these were not considered to be a potential risk to human health.

Overall, where exceedences of the ADI guide value of 10% occurred, they were greatest (in order) from groundwater, surface water in drainage dominated areas and surface water in run-off dominated areas. The majority of the compounds identified as posing a potential risk (acetylsalicylic acid, altrenogest, apramycin, dicyclanil, luprostiol) had no experimental data available on their persistence. Conservative values for persistence were therefore used in the modelling. In addition, for many of these substances, no data were available on sorption so structure-property relationships were used to estimate potential sorption behaviour. It is known that these relationships tend to underestimate sorption coefficients. As a result estimates of exposure in the different water bodies are likely to be higher than if experimental data were available on the sorption and persistence of these substances. In addition, due to the complexities of catchment-scale modelling, the estimates did not take into account likely dilution of compounds in the environment prior to any abstraction for drinking water purposes. Predicted exposure concentrations, and hence risk estimates, for these compounds are therefore likely to be highly conservative.

The exposure modelling also did not take into account the relative usage of the different study compounds in the UK. It is known that many of the compounds that were assessed (e.g. many of the antibiotics and antiparasitic) compounds are widely used in the UK. However, limited or no data are available on the usage of other classes of substance (e.g. the anti-inflammatory compounds). It is therefore possible that some of the ‘high risk’ substances are only used in limited amounts meaning that the potential for water contamination is limited.

Conventional water treatment is expected to have little impact on the levels of veterinary medicines in water from all sources examined. However, advanced water treatment is likely to be effective, with only estimated intakes of acetylsalicylic acid, florfenicol, lincomycin and luprostiol, from treated groundwater, exceeding the ADI for toddlers and infants.

The principal key factors that influenced the above conclusions are:

1) exposure assessments were based on assumptions that will overestimate the true exposure levels for the majority of the population; this is not the case for the high-level consumer, who has not been included in this assessment
2) advanced water treatment was predicted to either completely or largely remove virtually all of the veterinary medicines of concern. Therefore,

following such treatment, the contribution from all water sources, with the exception of acetylsalicylic acid, lincomycin, luprostiol and monensin, would be expected to be negligible.

3) a cut-off level of 10% of the ADI was adopted in the risk assessment and veterinary medicines for which intake did not exceed 10% of the ADI were not discussed in detail. While the 10% level is arbitrary, it was thought to be reasonable as it allowed a substantial margin for possible exposures via other sources without the ADI being exceeded. It should also be noted that the actual levels of exposure of the selected veterinary medicines from water and other sources combined is uncertain and warrants further research as this factor could potentially impact the risk assessment of these veterinary medicines.

Based on these assessments, it is recommended that work focuses in the first instance on two main areas:

- For substances where data were not available on persistence or sorption, these data are obtained and the exposure assessments re-run to obtain a better indication of the distribution of the compounds in the environment. The information required for these assessments may well have been generated by industry as part of the regulatory environmental risk assessment process for veterinary medicines. By working with industry, it may be possible to gain access to the data and refine the model predictions.
- For the 'high' priority compounds, it is recommended that information is obtained on the level of use of these compounds in the UK. Compounds with low use can probably be discounted from further study.

In the longer term, a targeted monitoring study might be warranted that focuses on quantifying compounds that pose the highest risk.

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APPENDIX 1 Active ingredients eliminated in the pre-screening assessment

(2-Isopropoxyphenyl)(methylcarbamat)
2-Phenylphenol
8-Hydroxyquinoline
Abamectin
Acepromazine maleate
Acetic acid glacial
Aglepristone
Alfacalcidol
Alfadolone acetate
Aluminium acetate compounds
Aluminium salicylate, basic
Apomorphinhydrochlorid
Atipamezole hydrochloride
Avilamycin
Azagly-nafarelin
Bacitracin
Baquiloprim
Beclomethasone dipropionate
Benazepril hydrochloride
Bendiocarb
Benzoyl peroxide
Benzylchlorophenol
Boldenone-undecylenate
Bromide salts
Bromocriptin mesilate
Buprenorphine hydrochloride
Butafosfan
Cabergoline
Calcium inorganic compounds
Calcium organic compounds
Carazolol
Carnidazole
Cefacetrile
Cefazolin
Chloramine
Chloramphenicol
Chlormadinone
Chloroform
Chloroxylenol
Chlorphenamine, -maleat
Chlorpromazine
Choline
Chondroitinpolysulfat
Cimetidine
Cinchocaine hydrochloride
Cinchophen
Clazuril
Clindamycin, -hydrochloride
Clomipramine hydrochloride

Clotrimazole
Codeine phosphate
Corticotropin
Cortisone acetate
Coumafos
Cropropamide
Crotethamide
Cyclizine hydrochloride
Cyclosporin
Cyfluthrin
Cyhalothrin
Cymiazole
Cythioate
Dapsone
Delmadinone acetate
Denaverine hydrochloride
Dexmedetomidine hydrochloride
Dextran
Dextromethorphanhydrobromid
Dextromoramide tartrate
Diazepam
Dichlorophen
Dichlorvos
Diclofenac
Dicloxacillin
Diethanolamine fusidate
Diflubenzuron
Diltiazem hydrochloride
Dimethyl sulphoxide (DMSO)
Dimetridazole
Diphenhydramin hydrochlorid
Diprenorphine
Diprophylline
Eltenac
Embutramid
Emodepside
Enalapril maleate
Epinephrine
Epsiprantel
Ergometrine maleate
Estriol
Etamsylate
Ethyloestrenol
Etorphine hydrochloride
Febantel
Fenpipramide hydrochloride
Fentanyl citrate
Fenthion
Fenticlor
Fenticonazole
Fenvalerate
Fertirelin acetate
Firocoxib
Fipronil
Flavophospholipol

Fluanisone
Fluazuron
Fludrocortisoneacetate
Flumequine
Flumetason
Flumethrin
Follicle stimulating hormone (natural FSH from all species and their synthetic analogues)
Formic acid
Fumagillin bicyclohexylamine salt
Fusidic acid
Glutaraldehyde
Griseofulvin
Guaifenesin
Halothane
Heparin
Heptaminol
Hesperidin
Hexetidine
Histaminediphosphate-monohydrate
Human chorionic gonadotropin (natural HCG and its synthetic analogues), Human menopausal urinary gonadotrophin
Hyaluronic acid
Hydrochlorothiazide
Hydrocortisone
Hydrogen peroxide
Hydroxyethylsalicylate
Hyoscine butylbromide
Ibafloxacin
Ibuprofen
Imidacloprid
Imidapril hydrochloride
Imipramine hydrochloride
Iron inorganic compounds
Iron organic compounds
Isoxsuprine
Itraconazole
Josamycin
Kanamycin, -sulphate
Ketamine
Ketanserin tartrate
k-Strophanthin
Lactulose
Lecirelin
Levomethadone (= methadone)
Lidocaine
Lindan
Lobeline
Loperamide hydrochloride
Lufenuron
Luteinizing hormone (natural LH from all species and their synthetic analogues)
Lutropin
Magnesium inorganic compounds
Magnesium organic compounds
Manganese inorganic compounds

Manganese organic compounds
Mannitol
Mebezoniumiodid
Mecetroniumetilsulfat
Mecillinam
Meclofenamic acid
Medetomidine hydrochloride
Megestrol acetate
Menbutone
Mepivacaine
Mercaptamine hydrochloride
Metergolin
Methadon hydrochloride
Methenamine (=Hexamine)
Methoprene
Methyl benzoate
Methyl nicotinate
Methyl salicylate
Methyltestosterone
Metoclopramide hydrochloride-monohydrate
Metrifonat
Miconazole nitrate
Milbemycin oxime
Montanide
Morantel
Nafcillin
Naled
Nandrolone cyclohexyl propionate, -Laurate, -Phenylpropionate
Neostigmine
Nicergoline
Nickel gluconate, sulphate
Niclosamide
Nicoboxil
Nimesulide
Nitenpyram
Nitrofurans (including furazolidone)
Nitroscanate
Nonoxinol-Iod
Noradrenaline bitartrate
Norgestomet
Nystatin
Omeprazole
Orbifloxacin
Orgotein
Oxacillin
Oxibendazole
Oxolinic acid
Papain
Papaverine
Parconazole
Paromomycin
Pentobarbitalone sodium
Pentosan polysulphate sodium
Peracetic acid
Pethidine hydrochloride

Phenobarbital
Phenothrin
Phenoxyethanol
Phenylbutazone
Phenylpropanolamine hydrochloride
Phloroglucinol, trimethyl-
Phosphoethanolamine
Phoxim
Pilocarpine hydrochloride
Pimobendan
Policresulen
Polygeline
Polymyxin B sulphate
Polysulphated glycosaminoglycan
Povidone - iodine
Pregnant mare serum gonadotrophin (PMSG)
Primidone
Proligestone
Propentamphos
Propentofylline
Propofol
Propoxur
Pyrantel embonate
Pyriproxifen
Rafoxanide
Ramifenazone
Ramipril
Resocortol butyrate
Resorcinol
Riboflavin
Rifaximin
Romifidine
Ronidazole
Rosin
Sarafloxacin
Scopolaminebutylbromide
Selamectin
Selegiline hydrochloride
Selenium sulfide
Serotonin
Sevoflurane
Simethicone
Simeticon =simethicone
Somatosalm
Sulfachlorpyridazine-sodium
Sulfaclozin-sodium
Sulfadimethoxine sodium
Sulfamerazin
Sulfamethazine (Sulphadimidine)
Sulfamethoxazol
Sulfamethoxypyridazin
Sulfaquinoxaline sodium
Sulfasalazine
Sulfathiazol
Sulfogaiacol

Suxibuzone
Tau fluvalinate
Teflubenzuron
Tepoxalin
Testosterone, esters, isocaproate, phenylpropionate, decanoate
Tetracaine (=amethocaine)
Tetrachlorvinphos
Theobromine
Theophylline
Thiamazole (Methimazol)
Thiamphenicol
Thiamylal
Thiopental sodium
Thiostrepton
Tiaprost
Tiletaminhydrochlorid
Tiludronic acid disodium salt
Triamcinolone acetonide
Trichlormethiazide
Trilostane
Vedaprofen
Zinc organic compounds
Zolazepam hydrochloride

APPENDIX 2 Active ingredients considered in the initial prioritisation and predicted concentrations used in the prioritisation

Name	CAS	PEC porewater	PEC porewater	PEC porewater	ADI /MRL/PDV	Ratio PEC porewater/ADI	Ratio PEC porewater/ADI	Ratio PEC porewater/ADI
		Koc exp (µg/L)	Koc pred 2009 MIC (µg/L)	Koc pred 2009 Kow (µg/L)	µg/kg bw	Koc exp (µg/L)	Koc pred 2009 MIC (µg/L)	Koc pred 2009 Kow (µg/L)
Acetylsalicylic acid	50-78-2	1726.52	6462.11	7963.03	8.3	208.014	778.568	959.401
Albendazole, -oxide, -sulphoxide	54965-21-8	0.19	0.84	3.74	5	0.037	0.169	0.748
Altrenogest	850-52-2		0.19	2.40	0.04		4.683	60.021
Amoxicillin	26787-78-0		424.69	2801.91	50		8.494	56.038
Ampicillin	69-53-4		125.95	623.05	50		2.519	12.461
Apramycin	37321-09-8	0.35	7539.13	13193.48	40	0.009	188.478	329.837
Azaperone	1649-18-9		0.02	0.10	0.8		0.025	0.124
Bromhexine	3572-43-8		0.07	0.08	5		0.014	0.016
Carprofen	53716-49-7		0.10	0.26	10		0.010	0.026
Cefalexin	15686-71-2		104.64	770.95	54.4		1.924	14.172
Cefalonium	5575-21-3		0.39	4.15	15.3		0.026	0.271
Cefapirin	21593-23-7		42.35	179.77	2.54		16.673	70.776
Cefoperazone	62893-19-0		1.15	1.97	2.8		0.410	0.705
Cefquinome	84957-30-2		0.05	73.86	3.8		0.012	19.436
Ceftiofur	80370-57-6	0.33	2.63	67.09	20	0.017	0.132	3.354
Chlortetracycline	57-62-5	0.01	1234.29	6987.92	3	0.004	411.428	2329.306
Clavulanic acid	58001-44-8		208.08	363.32	50		4.162	7.266
Cloprostenol	40665-92-7		0.00	0.00	0.075		0.004	0.062
Clorsulon	60200-06-8	14.97	4.78	8.17	1	14.968	4.782	8.172
Closantel	57808-65-8		0.02	0.01	1800		0.000	0.000
Cloxacillin	61-72-3		1.56	33.93	300		0.005	0.113
Colistin	1066-17-7		0.00	23.08	5		0.000	4.616
Cypermethrin	52315-07-8	0.01	0.01	0.01	15	0.000	0.000	0.001
Cyromazine	66215-27-8		107.77	207.26	20		5.389	10.363
Danofloxacin	112398-08-0	0.00	33.80	63.71	24	0.000	1.408	2.655
Decoquinate	18507-89-6		0.03	0.03	75		0.000	0.000
Deltamethrin	52918-63-5	0.00	0.00	0.00	10	0.000	0.000	0.000

Name	CAS	PEC porewater Koc exp (µg/L)	PEC porewater Koc pred 2009 MIC (µg/L)	PEC porewater Koc pred 2009 Kow (µg/L)	ADI /MRL/PDV µg/kg bw	Ratio PEC porewater/ADI Koc exp (µg/L)	Ratio PEC porewater/ADI Koc pred 2009 MIC (µg/L)	Ratio PEC porewater/ADI Koc pred 2009 Kow (µg/L)
Dembrexine	83200-09-3		1.27	2.44	20		0.064	0.122
Detomidine	76631-46-4		0.00	0.00	0.3		0.001	0.003
Dexamethasone	50-02-2		0.02	0.12	0.015		1.295	7.849
Diazinon	333-41-5	0.84	0.25	0.34	2	0.419	0.124	0.172
Diclazuril	101831-37-2		0.03	0.19	30		0.001	0.006
Dicyclanil	112636-83-6		388.57	376.00	7		55.510	53.715
Difloxacin	98106-17-3		44.39	1395.57	10		4.439	139.557
Dihydrostreptomycin	128-46-1		243.48	553.92	25		9.739	22.157
Dinoprost, tromethamine	551-11-1		0.01	0.07	0.83		0.016	0.087
Doramectin	117704-25-3	0.00	0.00	0.76	1	0.004	0.000	0.756
Doxycycline, hyclate	564-25-0		1129.12	3539.22	0.003		376373.143	1179738.461
Enrofloxacin	93106-60-6	0.18	1778.31	3363.21	6.2	0.029	286.824	542.453
Eprinomectin	123997-26-2	0.03	0.00	2.03	5	0.005	0.000	0.406
Erythromycin	114-07-8	20.98	110.44	1650.62	300	0.070	0.368	5.502
Fenbendazole	43210-67-9	0.98	0.10	1.05	2.5	0.394	0.041	0.420
Florfenicol	73231-34-2	592.03	344.06	1499.09	3	197.343	114.686	499.696
Flubendazole	31430-15-6		21.92	93.79	12		1.827	7.816
Flugestone acetate	337-03-1		0.05	0.11	0.03		1.614	3.680
Flunixin	38677-85-9		0.66	0.36	6		0.109	0.060
Halofuginone	55837-20-2	0.02	15.12	34.64	0.3	0.074	50.409	115.483
Imidocarb	27885-92-3		0.03	0.81	10		0.003	0.081
Ketoprofen	22071-15-4		1.11	3.34	20		0.056	0.167
Lasalocid	25999-31-9		28.48	67.15	2.5		11.390	26.862
Levamisole	14769-73-4		0.66	27.91	6		0.109	4.652
Lincomycin	154-21-2	12398.43	2145.19	12209.12	10	1239.843	214.519	1220.912
Luprostiol	67110-79-6		0.63	17.03	0.2		3.160	85.145
Marbofloxacin	115550-35-1		211.02	359.14	40		5.275	8.978
Mebendazole	31431-39-7	0.56	0.86	2.54	12.5	0.045	0.069	0.203
Medroxyprogesterone acetate	520-85-4		0.01	0.02	0.01		0.916	1.604

Name	CAS	PEC porewater Koc exp (µg/L)	PEC porewater Koc pred 2009 MIC (µg/L)	PEC porewater Koc pred 2009 Kow (µg/L)	ADI /MRL/PDV µg/kg bw	Ratio PEC porewater/ADI Koc exp (µg/L)	Ratio PEC porewater/ADI Koc pred 2009 MIC (µg/L)	Ratio PEC porewater/ADI Koc pred 2009 Kow (µg/L)
Meloxicam	71125-38-7		4.26	0.74	1.25		3.411	0.594
Metamizole	68-89-3		105.51	184.44	150		0.703	1.230
Monensin, sodium	22373-78-0	2783.92	14001.48	7.6			366.306	1842.300
Moxidectin	113507-06-5	0.01	0.00	0.06	3	0.002	0.000	0.019
Neomycin (including framycetin)	1404-04-2		4523.48	7916.09	60		75.391	131.935
Novobiocin	303-81-1		1.16	6.61	1.25		0.926	5.287
Oxfendazole	53716-50-0	1.65	0.56	7.99	7	0.236	0.080	1.141
Oxyclozanide	2277-92-1		0.56	0.16	30		0.019	0.005
Oxytetracycline	79-57-2	0.01	566.05	3378.57	3	0.002	188.684	1126.190
Paracetamol	103-90-2		126.41	215.49	50		2.528	4.310
Penethamate	3689-73-4		4.18	173.73	50		0.084	3.475
Permethrin	52645-53-1		0.03	0.09	10		0.003	0.009
Pirlimycin	79548-73-5	0.00	0.13	0.49	6	0.000	0.022	0.081
Praziquantel	55268-74-1		0.12	2.74	170		0.001	0.016
Prednisolone	50-24-8		0.02	0.06	0.2		0.085	0.283
Procaine	59-46-1		0.22	0.56	40		0.005	0.014
Salinomycin sodium	53003-10-4	8553.80	129.93	126.47	2.5	3421.521	51.973	50.589
Spectinomycin	1695-77-8		15078.26	26258.71	40		376.957	656.468
Streptomycin	57-92-1		10770.19	18847.82	80		134.627	235.598
Sulfadiazine	68-35-9	1589.59	1720.41	3968.66	100	15.896	17.204	39.687
Sulfadoxine	2447-57-6		54.92	24.63	100		0.549	0.246
Sulfathiazole	72-14-0		13.93	96.67	180		0.077	0.537
Tetracycline	60-54-8	0.01	2625.81	10710.41	100	0.000	26.258	107.104

Name	CAS	PEC porewater Koc exp (µg/L)	PEC porewater Koc pred 2009 MIC (µg/L)	PEC porewater Koc pred 2009 Kow (µg/L)	ADI /MRL/PDV µg/kg bw	Ratio PEC porewater/ADI Koc exp (µg/L)	Ratio PEC porewater/ADI Koc pred 2009 MIC (µg/L)	Ratio PEC porewater/ADI Koc pred 2009 Kow (µg/L)
Tiamulin	55297-95-5	93.11	4.19	93.11	30	3.104	0.140	3.104
Tilmicosin	108050-54-0	41.73	10.79	2002.07	240	0.174	0.045	8.342
Tolfenamic acid	13710-19-5		0.49	0.14	10		0.049	0.014
Toltrazuril	69004-03-1		0.40	0.22	2		0.202	0.108
Triclabendazole	68786-66-3		0.05	0.10	1.5		0.032	0.069
Trimethoprim	738-70-5	8.56	17.16	136.66	4.2	2.038	4.086	32.539
Tylosin	1401-69-0	26.80	148.93	7571.27	360	0.074	0.414	21.031
Valnemulin	101312-92-9		0.96	68.70	7.95		0.121	8.641
Vetrabutine hydrochloride	3735-45-3		0.00	0.01	15		0.000	0.001

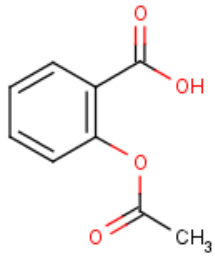
APPENDIX 3 Toxicity profiles for the priority study compounds

Acetylsalicylic acid

1 Introduction

Acetylsalicylic acid, commonly known as aspirin, (2-(acetyloxy)benzoic acid; molecular formula $C_9H_8O_4$; CAS no. 50-78-2; see Figure 1.1) is an anti-inflammatory, antipyretic and analgesic agent; it is one of the classic non-steroidal anti-inflammatory drugs (NSAID). Acetylsalicylic acid has many formulations (see Box 1) of which sodium acetylsalicylate, acetylsalicylic acid DL-lysine and carbasalate calcium are mainly used in veterinary medicine (EMEA, 1999)

Figure 1.1 Molecular structure of acetylsalicylic acid



From ChemID Plus

Box 1: Synonyms and trade names for acetylsalicylic acid

2-(Acetyloxy)benzoic acid; 2-Acetoxybenzoic acid; 2-Carboxyphenyl acetate; 4-10-00-00138 (Beilstein Handbook Reference); 8-hour Bayer; A.S.A.; A.S.A. empirin; AC 5230; AI3-02956; ASA; Acenterine; Acesal; Aceticyl; Acetilsalicilico; Acetilum acidulatum; Acetisal; Acetol; Acetol (VAN); Acetonyl; Acetophen; Acetosal; Acetosalic acid; Acetosalin; Acetylin; Acetylsal; Acetylsalicylate; Acetylsalicylsaure; Acetylsalicylsaure [German]; Acetylsalycilic acid; Acide acetylsalicylique; Acide acetylsalicylique [French]; Acido O-acetil-benzoico; Acido O-acetil-benzoico [Italian]; Acido acetilsalicilico; Acido acetilsalicilico [Italian]; Acidum acetylsalicylicum; Acimetten; Acisal; Acylpyrin; Adiro; Asagran; Aspec; Aspergum; Aspir-Mox; Aspirdrops; Aspirin; Aspirina 03; Aspirine; Aspro; Aspro Clear; Asteric; BRN 0779271; Bayer Aspirin 8 Hour; Bayer Buffered; Bayer Children's Aspirin; Bayer Enteric 325 mg Regular Strength; Bayer Enteric 500 mg Arthritis Strength; Bayer Enteric 81 mg Adult Low Strength; Bayer Extra Strength Aspirin for Migraine Pain; Bayer Plus; Benaspir; Benzoic acid, 2-(acetyloxy)-; Bi-prin; Bialpirina; Bialpirinia; Bufferin; CCRIS 3243; Caprin; Cemirit; Claradin; Clariprin; Colfarit; Contrheuma retard; Decaten; Delgesic; Dolean pH 8; Duramax; ECM; EINECS 200-064-1; Easprin; Ecolen; Ecotrin; Empirin; Endydol; Entericin; Enterophen; Enterosarein; Enterosarine; Entrophen; Extren; Globentyl; Globoid; HSDB 652; Helicon; Idragin; Istopirin; Kapsazal; Kyselina 2-acetoxybenzoova; Kyselina 2-acetoxybenzoova [Czech]; Kyselina acetylsalicylova; Kyselina acetylsalicylova [Czech]; Levius; Measurin; Medisyl; Micristin; NSC 27223; Neuronika; Novid; O-Acetylsalicylic acid; Pharmacin; Pirseal; Polopiryna; Premaspin; R16CO5Y76E [UNII]; Rheumin tabletten; Rheumintabletten; Rhodine; Rhonal; Ronal; S-211; SP 189; Salacetin; Salcetogen; Saletin; Salicylic acid acetate; Solfrin; Solprin acid; Solpyron; Spira-Dine; St. Joseph Aspirin for Adults; Temperal; Triple-sal; UNII R16CO5Y76E; UNII-R16CO5Y76E; UNII=R16CO5Y76E; Xaxa; Yasta; ZORprin; o-Acetoxybenzoic acid; o-Carboxyphenyl acetate.

From ChemID Plus

Acetylsalicylic acid is commonly used in human medicine. The dose at which it is administered is dependent on the indication, e.g. for anti-inflammatory, antipyretic and analgesic use, a concentration of 300 – 900 mg/person every 4 - 6 hours is advised, with a recommended maximum of 4000 mg/person/day (EMEA, 1999). In addition, long-term low dose regimes are used for prevention of cardiovascular diseases, where recommended doses start at 50-100 mg/person/day (Berger *et al.*, 2008).

In veterinary medicine, acetylsalicylic acid, largely as its sodium salt, is used orally in water and feed for pigs, calves and chickens at doses of 5 – 300 mg/kg/day for periods of no longer than 10 days. Acetylsalicylic acid DL-lysine is used in cattle (25 – 100 mg/kg/day) and pigs (25 – 50 mg/kg/day) for which it is administered twice daily by intravenous (i.v.), subcutaneous (s.c.) or intramuscular (i.m.) injection or orally to calves (at 200 mg/kg/day for 5 - 7 days). Carbasalate calcium is used as an oral aqueous solution in pigs, calves and chickens at doses of 40 – 130 mg/kg/day for five days. It may also be given in milk to calves (EMEA, 1999).

Acetylsalicylic acid is mainly bioactivated through the metabolism of the parent compound to a metabolite salicylic acid, which is formed by hydrolysis. Salicylic acid has inhibitory effects on cyclo-oxygenase and the synthesis of prostanoids from arachidonic acid. Inhibition of the prostanoids $PGF_{2\alpha}$ and PGE_2 from thrombin stimulated platelets and the synthesis of thromboxanes favour the production of prostacyclin (PGI_2) leading to inhibition of platelet aggregation and prolongation of bleeding time, hence its anti-thrombotic activity (EMEA, 1999, Rang *et al.*, 1996). This was illustrated in a clinical trial where acetylsalicylic acid administration led to significant prolongation of bleeding times, yielding a pharmacological LOEL of 10 mg/person, equivalent to 0.167 mg/kg/day (EMEA, 1999).

2 Toxicokinetics

In humans, acetylsalicylic acid is poorly soluble in the stomach following oral administration. However, following hydrolysis, salicylic acid and acetic acid are formed in the stomach and pass to the small intestine where the large surface area leads to rapid absorption at therapeutic doses. Under overdose conditions, absorption can be slow with blood levels rising for up to 24 hours after ingestion. The plasma half-life of salicylic acid at therapeutic doses is 2 - 4.5 hours. Approximately 50 - 80% of salicylic acid in the blood is bound in inactive form to proteins with the remainder being biologically active. Protein binding is concentration dependent and saturation of binding leads to more free salicylic acid and increased toxicity (IPCS, 1991).

In dogs, cats and pigs, acetylsalicylic acid is more readily absorbed from the gastro-intestinal tract, with a half life of absorption of approximately 0.6 hours, although in pigs a half life of 0.9 hours and a C_{max} of 64 µg/ml after 3.9 hours was noted. Absorption in horses and cattle is more comparable with that of humans, with a half-life of approximately 2.9 hours noted in cattle (EMEA, 1999). Following oral administration of acetylsalicylic acid DL-lysine to dogs and cattle (90 mg/kg), the maximum blood concentration was achieved after between 2 and 4 hours (t_{max}); the maximum concentration (C_{max}) in dogs was 104 µg/ml, which is approximately twice that in cows (64 µg/ml (EMEA, 1999). The area under the curve (AUC; no time specified) was 65,639 µg/min/ml for dogs and 4837 µg/min/ml in cattle, which further illustrates the different kinetic profiles of dogs and cattle.

In a study on male Wistar rats, animals were dosed by oral gavage with radio-labelled acetylsalicylic acid (^{14}C on the acetyl or carboxyl group, at 2µCi/1.67 mg) and radioactivity counted 10 min, 30 min, 1 hour and 3 hours after dosing. Results showed absorption to be mainly via the stomach wall followed by transport to the liver via the portal circulation.

Metabolism has been shown to be qualitatively similar in all species studied to date (with the exception of the rabbit; see below). It involves hydrolysis of the parent compound in plasma, liver and some other organs to salicylic acid. This is followed by the formation of salicyluric acid, salicyluric glucuronide, salicyl ester glucuronide, salicyl phenol glucuronide, gentisic acid and gentisuric acid (EMEA, 1999).

In humans at low doses, approximately 80% of the salicylic acid is metabolised in the liver. Conjugation with glycine forms salicyluric acid, and conjugation with glucuronic acid forms salicyl acyl glucuronide and salicyl phenolic glucuronide (Hutt *et al.*, 1986). Salicylic acid can also be hydroxylated to gentisic acid. Salicyluric acid and salicyl phenolic glucuronide are both formed following Michaelis Menten kinetics. Salicyl acyl glucuronide and gentisic acid are formed following first order kinetics. 300 mg of aspirin or less led to approximately 90% of the dose being excreted as salicyluric acid or salicyl phenolic glucuronide. Also, it was identified that plasma salicylic acid steady state concentrations increase with dose, steady state concentrations of salicyluric acid do not. After 3 g of aspirin, 50% was excreted as salicyluric acid, 20% as salicyl phenolic glucuronide, 14% as salicylic acid, 11% as salicyl acyl glucuronide and 3.1% as gentisuric acid (Needs & Brooks, 1985). At doses of less than 250 mg/person/day, the elimination half-life is 2 - 3 hours. However at above 4000 mg/person/day, the elimination half-life rises to between 15 - 30 hours due to saturation of metabolic pathways (IPCS, 1991).

The elimination half-life is approximately ten times shorter in cattle than dogs (36.5 min in cattle versus 368 min in dogs); this difference is considered to reflect the lower volume of distribution in cattle (0.35 l/kg), compared with dogs (0.64 l/kg). Therefore, unlike the dog, in cattle the kinetic parameters indicate a slow and more incomplete absorption, a low AUC and a low volume of distribution and rapid elimination (EMEA, 1999).

The toxicokinetics of acetylsalicylic acid in pigs was assessed for a single oral dose of commercial medicated premix (formulation details not stated in the EMEA summary report) at 50 mg acetylsalicylic acid/kg bw. A rapid absorption half-life of 0.9 hours and a C_{max} of 64 µg/ml after 3.9 hours and an elimination half-life of 4.2 hours were noted. Twelve hours after dosing, the plasma concentration was 21 µg/ml and after 24 hours, levels were below the limit of detection (0.8µg/ml). The volume of distribution was 0.5 L/kg (EMEA, 1999).

In male Wistar rats dosed by oral gavage with radio-labelled acetylsalicylic acid (^{14}C on the acetyl or carboxyl group, at 2µCi/1.67 mg), after absorption and transport to the liver via the portal circulation hydrolysis of acetylsalicylic acid was noted to occur widely including in the stomach, liver and lung (Hatori *et al.*, 1984).

In a study in goats, intravenous sodium salicylate (44 mg/kg) led to excretion of parent compound and salicyluric acid at 67.9% and 34.6% respectively, while oral dosing led to 30.2% and 71.7% parent compound and salicyluric acid respectively (Short *et al.*, 1990). Intravenous administration of sodium salicylate (44 mg/kg) to cattle led to lower excretion of the parent (54%) but more salicyluric acid (49.9%), an observation also noted following oral administration. In both goat and cattle, almost 90% of the drug excreted as sodium salicylate was in the urine (Short *et al.*, 1990).

In New Zealand White rabbits administered sodium salicylate (44 mg/kg) either orally or by i.v., over 50% of the dose was excreted in the urine as salicylic acid. In contrast to other species, salicyluric acid was only a minor metabolite in the rabbit. Following oral administration, the AUC was almost twice that of i.v. administration, for identical doses, suggesting that hepatic elimination of salicylate is easily saturable in the rabbit, a situation which is aggravated by oral administration where all the drug absorbed passes directly to the liver via the portal vein (Short *et al.*, 1991).

3 Toxicity profile

3.1 Acute toxicity

Accidental or deliberate oral ingestion is the most common route of overdose in humans. Mild poisoning (>150 mg/kg bw) symptoms include nausea, vomiting, lethargy, dizziness and tinnitus. Moderate poisoning (>250 mg/kg bw) symptoms include tachypnoea, hyperpyrexia, sweating, dehydration and ataxia. Severe poisoning (>500 mg/kg bw) symptoms include hypotension, metabolic acidosis, renal failure, coma and convulsions (Greene *et al.*, 2005). More unusually, cardiac dysrhythmias may occur due to hypokalaemia, and tetany and paraesthesias may develop due to low ionised (i.e. biologically active) calcium levels. In cases involving high intakes, hallucination, stupor, convulsions, papilloedema and coma may occur and a metabolic acidosis may develop. Hepatotoxicity is a common complication as is non-cardiogenic pulmonary oedema (IPCS, 1991).

In a sub-population of asthma patients, acetylsalicylic acid may also induce non-allergic hypersensitivity reactions. In these asthma patients, who tend to be characterised by the presence of nasal polyps and chronic hyperplastic eosinophilic sinusitis, ingestion of acetylsalicylic acid may lead to a variety of reactions including urticaria, rhinitis, paroxysmal bronchospasm, dyspnoea, hypotension, shock and syncope (IPCS, 1991, Stevenson, 2009).

Oral dosing studies with acetylsalicylic acid DL-lysine in mice and rats gave LD_{50} values of 2200 – 2600 mg/kg bw; clinical signs were non-specific and comprised nausea, restlessness, seizures, coma and respiratory alkalosis-induced respiratory stimulation (EMEA, 1999)

3.2 Repeat dose toxicity

Reflecting the widespread use of acetylsalicylic acid, many adverse events have been reported following chronic use in humans. Effects noted include heartburn, epigastric pain/discomfort, nausea and vomiting. These may lead to complications including oesophageal, gastric or duodenal erosion and ulceration due to the effects of acetylsalicylic acid on the gastrointestinal tract. Haemorrhagic stroke may also occur as a consequence of the anti-platelet activity of acetylsalicylic acid (Bjørklund *et al.*, 2009). Acetylsalicylic acid has also been linked with haemolytic anaemia in individuals with glucose-6P-dehydrogenase deficiency. Daily doses of 300 mg/person (5 mg/kg bw) or less may pose a risk of peptic ulcer bleeding and hypersensitivity reactions have also been noted. A dose-dependent tinnitus and hearing loss have been reported at serum levels of higher than 110 µg/ml and serum concentrations of higher than 150 µg/ml have been linked with reversible hepatotoxicity (EMEA, 1999)

Reye's syndrome has been associated with repeated ingestion of acetylsalicylic acid in children with viral infections such as varicella or influenza. This syndrome presents as acute encephalopathy and hepatic fatty degeneration. Following its recognition, acetylsalicylic acid is no longer generally indicated in children, except for juvenile rheumatoid arthritis in some countries. The occurrence and severity of the response seen in Reye's syndrome shows dose-dependency with salicylate. Data collected in the USA showed that Reye's syndrome patients had higher average (median = 25.1 vs. 14.5 mg/kg) and maximum daily doses of sodium acetylsalicylate (median = 33.0 vs. 19.0 mg/kg) and had received elevated doses of sodium acetylsalicylate (median = 65.4 vs. 27.0 mg/kg) for the first four days preceding the development of antecedent respiratory or chicken pox illness, compared with controls, where controls were children affected with the same illnesses but received lower doses of aspirin (Pinsky *et al.*, 1988). Following the change in medical advice on the use of aspirin in young children, the number of cases of the syndrome decreased sharply. In countries where sodium acetylsalicylate and other salicylates are used widely in the treatment of animals, elevated levels of the syndrome are not found and it is considered highly unlikely that ingestion of

track amounts of acetylsalicylate residues in food would elicit this effect, as recommended doses are considered safe up to a maximum of 4000 mg/person/day in adults (EMEA, 1999).

In an unpublished study reported in (EMEA, 1999), rats were given acetylsalicylic acid DL-lysine at 50 or 150 mg/kg bw or sodium acetylsalicylate at 500 mg/kg bw (equivalent to 0, 50, 150 or 500 mg acetylsalicylic acid/kg bw/day); details of the strain, duration of dosing and other experimental details were not included in the EMEA Summary report. At the highest dose, severe clinical signs and some deaths were reported. Although no clinical signs were observed at 150 mg/kg bw/day, congestion, petechiae, haemorrhages and punctiform lesions of the stomach were noted histopathologically at 150 and 500 mg/kg bw. Hepatomegaly and increased kidney weight were also noted in a dose-related manner in all male treated groups. Clinical chemistry parameters showed a dose-related decrease in serum globulin level that still attained statistical significance in low dose females (but not males) (EMEA, 1999) also report an unpublished study in dogs in which acetylsalicylic acid DL-lysine was given at levels of 0, 50, 150, 250 or 500 mg acetylsalicylic acid equivalents/kg/day for three months and sodium acetylsalicylate was administered at 0, 250 or 500 mg/kg/day. Details of strain of dog and other methodological details were not included in the EMEA summary report. At the highest dose, all animals died within two to seven days. Some deaths and vomiting were also noted at 150 and 250 mg/kg. Less frequent vomiting but no deaths was observed at 50 mg/kg. At 50 mg/kg/day, one (of six) dogs showed gastric striae and two dogs had focal atrophy of the mucosa with dedifferentiated epithelial lining and glandular epithelium at necropsy. In all treated animals, a slight decrease in heart rate was observed (EMEA, 1999).

Overall, it is not possible to derive a definitive no observed effect level (NOEL) for acetylsalicylic acid in animal studies since changes were apparent in the lowest doses in these studies, though target areas were liver, stomach and gastrointestinal tract.

3.3 Carcinogenicity and mutagenicity

Epidemiologic studies of the possible risks associated with chronic low dose acetylsalicylic acid (taken as aspirin) do not consistently support a cancer risk to humans (Friis *et al.*, 2003). Although an increased risk of renal cancer in elderly individuals on long-term therapy with acetylsalicylic acid has been suggested (Paganini-Hill *et al.*, 1989), this was not supported by other studies which indicated chronic (at least 9 years) low dose oral aspirin therapy to be associated with a lower incidence of colorectal cancer and colonic adenoma (Friis *et al.*, 2003, Dube *et al.*, 2007).

Experimentally, no studies on carcinogenicity were identified. However, studies have investigated the potential of acetylsalicylic acid to promote tumours initiated by another agent. In a study of the promotional activity of chemicals on gastric carcinogenesis (using *N*-methyl-*N'*-nitro-*N*-nitroso guanidine; MNNG to induce tumours) in the Sprague Dawley rat, acetylsalicylic acid (at 0.5 % in diet for up to 52 weeks) was associated with a significant increase in the incidence of gastric mucosal adenocarcinoma, compared with control animals (Newberne *et al.*, 1987). However 0.5 and 1% acetylsalicylic acid for 58 weeks had no effect on the incidence of liver tumours in rats pre-treated with a potent initiator of liver cancer (inducing agent not stated) (EMEA, 1999) . In Fischer 344 rats given 0.5% aspirin in the diet for six or sixty-one weeks, an inhibition of bladder carcinogenesis was noted with both an initiating agent (N-[4-(5-Nitro-2-furyl)-2-thiazolyl]formamide) and a promoting agent (sodium saccharin) of bladder carcinogenesis. Throughout this study, a group of rats received aspirin only, which did not have increased tumour incidence compared with the rats that received no drug treatment (Sakata *et al.*, 1986).

Study of the mutagenicity of acetylsalicylic acid in humans is limited. No evidence of chromosomal damage was noted in the lymphocytes of human volunteers given oral doses of 2.4 g per person/day of acetylsalicylic acid for 1 month (EMEA, 1999).

Bacterial gene mutation assays in the presence or absence of metabolic activation with sodium salicylate, were negative. *In vitro* DNA-repair tests in both bacteria and primary rat hepatocytes were also negative. Acetylsalicylic acid did not induce recessive lethal mutation in *Drosophila melanogaster*. Positive *in vitro* metaphase analyses results were obtained in fibroblast and lymphocyte cultures (species not stated) but findings were negative in V79 cells, with or without metabolic activation. A cell transformation assay in mouse embryo cells was also negative (EMEA, 1999).

While results are generally negative *in vitro*, an *in vivo* metaphase analysis in rat bone marrow was positive. However, *in vivo* chromosomal aberration assays in rat embryos and *in vivo* micronucleus tests in bone marrow from rats and mice were negative (EMEA, 1999).

Overall, the CVMP has concluded that acetylsalicylic acid is not genotoxic, and the EMEA summary report (EMEA, 1999) states that there is no evidence to suggest that concentrations of acetylsalicylic acid likely to occur as food residues cause tumour promotion.

3.4 Reproductive and developmental toxicity

No reproductive or developmental epidemiology studies have been identified in humans. Many studies have considered the safety of various doses of aspirin in pregnant women. Findings are inconsistent, with some showing possible associations with maternal and fetal effects, although a causal association has not been established (Østensen & Skomsvoll, 2004). However, aspirin is the most frequently ingested drug during pregnancy and some studies have suggested potential beneficial effects such as prevention of hypertension and premature birth (Østensen & Skomsvoll, 2004).

Experimentally, acetylsalicylic acid has been assessed for reproductive and developmental toxicity. In dogs (gestation days 15 to 21), mice (period of treatment not stated) and rats (gestation days 6 to 15) orally dosed with between 500 and 1200 mg/kg bw acetylsalicylic acid/day, a high incidence of still-born dogs and of resorptions in mice and rats were reported (EMEA, 1999). In rabbits, inseminated does given seven doses of 150 mg/kg bw of acetylsalicylic acid prior to implantation showed reduced fertility and abnormal blastocyst development. However, in pregnant Rhesus monkeys given 40 mg acetylsalicylic acid /kg bw/day from day 25 of gestation to term, no anomalies were induced (EMEA, 1999).

In a study in pregnant dogs orally dosed with 100 or 400 mg acetylsalicylic acid/kg bw (on gestation days 15 – 22 or days 23 - 30), no teratogenic or embryotoxic effects were observed at 100 mg/kg. However at doses of 400 mg/kg, increases in number of resorptions and of malformations of the pups (including cleft palate, micrognathia, anasarca, cardiovascular and tail abnormalities) were noted (Robertson *et al.*, 1979). Despite the lack of effects at 100 mg/kg, a NOEL for this study was not stated.

In a study in rats, animals were orally dosed with 30, 90 or 180 mg sodium salicylate/kg bw on days 6 – 15 of gestation. Significant dose-related reductions in fetal weight and a significant increase in delayed ossification of limbs and vertebrae were noted at 90 and 180 mg/kg bw/day. At 90 mg/kg bw/day, one fetus displayed anophthalmia and another showed generalised oedema and a malformed tail. At the highest dose, 30% of pups showed malformations of which the predominant (22.7%) was craniorachischisis (congenital malformation of the central nervous system and adjacent structures related to defective neural tube closure). An oral dose of 30 mg sodium salicylate/kg bw was considered to represent the NOEL for teratogenicity (an equivalent dose in terms of acetylsalicylic acid was not stated (EMEA, 1999).

More recent studies in Sprague-Dawley and Wistar rats dosed with acetylsalicylic acid during gestation days 6 – 17 at 0, 50 125 or 250 mg/kg/day, showed a significant decrease in

maternal body weight gain and food consumption at 125 mg/kg/day or above. An increased incidence of resorption, post implantation loss and lower placental weight was noted at 250 mg/kg/day and a decrease in uterine weight was noted at all doses, which was most pronounced at 250 mg/kg/day. Developmental variations noted at the high dose included the number of runts, exophthalmia and distended ureter. Abnormalities were only significantly increased at the high dose; effects included craniorachischisis, kinked tail and gastroschisis (Gupta *et al.*, 2003). Maternal or fetal NOEL values were not identified from this study.

A study in New Zealand White rabbits given acetylsalicylic acid at 0, 125, 250 or 350 mg/kg/day by oral gavage on gestation days 7 - 19 showed maternal deaths, decreased food consumption and body weight gain at the mid- and high-dose. Fetal body weight was significantly reduced only at 350 mg/kg/days. However, external, visceral and skeletal malformations were not affected by treatment (Cappon *et al.*, 2003). Although there were no treatment related toxicological effects reported at the low dose, a NOEL level was not stated by the authors.

4 Guidelines and standards

Although acetylsalicylic acid is widely used therapeutically in humans, a NOEL and therefore an ADI have not been established from the available animal studies. However a pharmacological ADI of 0.0083 mg/kg bw has been established, based on a lowest observed effect level (LOEL) of 0.167 mg/kg bw in humans following the effect of acetylsalicylic acid on prolongation of bleeding times. This ADI was derived by applying a safety factor of 20 based on the default safety factor of 10 multiplied by 2 since a LOEL not a NOEL was used (EMEA, 1999). This ADI value has been applied in the risk assessment process.

EMEA considered from residue depletion studies in cattle, pigs and chickens that the maximum amounts of acetylsalicylic acid likely to be ingested by the consumer are below the ADI for all scenarios considered. Thus, for example, 11% of the ADI would be obtained from consumption of a standard portion of bovine tissue from cattle killed only 12 hours after treatment, 33% ADI from porcine tissues from pigs killed 24 hours after treatment, and 36% ADI from chicken killed three days after treatment (EMEA, 1999).

The Committee for Medicinal Products for Veterinary Use (CVMP) recommendations on the inclusion of acetylsalicylic acid, sodium acetylsalicylate, acetylsalicylate DL-lysine and carbasalate calcium are given in Annex II of Council regulation (EEC) No 2377/90 for bovine, porcine and chicken species and are detailed in Table 1. No pharmacokinetic or metabolism data are available for fish but the substances have no therapeutic value in these species.

Table 4.1 Guidelines for acetylsalicylic acid and related substances

Pharmacologically active substance	Animal species	Other provisions
Acetylsalicylic acid	All food producing species except fish	Not for use in animals from which milk or eggs are produced for human consumption
Sodium acetylsalicylate	All food producing species except fish	Not for use in animals from which milk or eggs are produced for human consumption
Acetylsalicylic acid DL-lysine	All food producing species except fish	Not for use in animals from which milk or eggs are produced for human consumption

Carbasalate calcium	All food producing species except fish	Not for use in animals from which milk or eggs are produced for human consumption

EMEA update 1999-2003, applies to all bovine, porcine and chicken; animals producing milk or eggs for human consumption are exempt.

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Albendazole

1 Introduction

Albendazole (5-(propylthio)-2-carboxymethoxyaminobenzimidazol, molecular formula $C_{12}H_{15}N_3O_2S$, CAS No. 54965-21-8; Figure 1.1; Box 1) is an oral broad-spectrum benzimidazole anthelmintic, which is used in both human and veterinary medicine. Its mode of action involves binding with tubulin in the intestinal cells of nematodes, resulting in loss of absorptive function, with consequent starvation and death (Sharma & Abuzar, 1983, ChemID Plus).

Figure 1.1 Molecular structure of albendazole

From ChemID Plus

Box 1 Synonyms and trade names for albendazole

Albenza; Bilutac; DRG-0277; EINECS 259-414-7; Eskazole, Proftril; SK&F 6279; SKF 62979; Valbazen; Zental; Zentel; ((Propylthio)-5 1H-benzimidazolyl-2) carbamate de methyle; ((Propylthio)-5 1H-benzimidazolyl-2) carbamate de methyle [French]; (5-(Propylthio)-1H-benzimidazol-2-yl)carbamic acid methyl ester; 5-(Propylthio)-2-carbomethoxyaminobenzimidazole; Albendazol; Albendazole; Albendazolum; Carbamic acid, (5-(propylthio)-1H-benzimidazol-2-yl)-, methyl ester; HSDB 7444; Methyl 5-(propylthio)-2-benzimidazolecarbamate; NSC 220008; O-Methyl N-(5-(propylthio)-2-benzimidazolyl carbamate; Proftril; SK&F 62979; SKF 62979; UNII-F4216019LN; Carbamic acid, (5-(propylthio)-1H-benzimidazol-2-yl)-, methyl ester

From ChemID Plus

Albendazole is an oral broad-spectrum benzimidazole anthelmintic used in both human and veterinary medicine. The sulphoxide metabolite is considered to be probably responsible for the therapeutic activity (Dayan, 2003).

It was first approved for human use in 1982 (Dayan, 2003), and is used in over 80 countries to treat gastrointestinal parasitic infections in humans at a recommended dose of 400 mg/person (IPCS, 1990, EMEA). However, it is not recommended for use in pregnant women or those thought to be pregnant (Dayan, 2003).

In cattle and sheep, oral doses of 5–7.5 mg/kg bw are given to for the control of roundworm, lungworm and tapeworm, and doses of 7.5–10 mg/kg bw for treatment of flukes (*Fasciola hepatica*) (EMEA, 1997).

2 Toxicokinetics

The toxicokinetics of albendazole are generally similar for humans, rodents, cattle and sheep. Albendazole has low aqueous solubility and, in humans, is only very poorly absorbed from the gastrointestinal tract. However, in ruminants, albendazole can be readily absorbed from the gut when taken orally (EMEA, 2004). For example, studies in volunteers given albendazole orally have shown that only approximately 1% is absorbed, although this may

increase by approx. 4.6 times if ingested with a high fat content meal. Experimental studies suggest a somewhat higher level of absorption in some species e.g. cattle can absorb 50% of the oral dose (EMEA 2004). In male CD mice and female Sprague-Dawley rats administration of radiolabelled albendazole in 1% carboxymethylcellulose resulted in 20.5% and 31% respectively of the administered radioactivity being detected in the urine over a 72-hour period. Similarly, 47% of administered radioactivity was found in the urine of cattle over a 7-day period (Parish *et al.*, 1977). This could indicate albendazole to be less toxic to humans than other species (EMEA, 2004).

Once absorbed, plasma levels of the parent compound are negligible irrespective of species. This is attributable to rapid first-pass hepatic oxidation of the sulphide group to form a sulphoxide (which is believed to be the systemically active form) and then a sulphide metabolite, with albendazole sulphoxide showing a half-life in humans of the order of 11–17 hours. Metabolism depends upon cytochrome P450 oxidases and other flavin-containing oxidases (Dayan, 2003). The metabolites are progressively converted to the 2-amino sulphone and a number of other minor metabolites that are believed to be pharmacologically inactive. Deacetylation of the carbamate group results in the formation of an amine. For example, radioactivity was mainly present in the liver as the parent and the sulphoxide and sulphone metabolites, in calves given a single oral dose of 20 mg/kg of radiolabelled albendazole. The parent compound was no longer present by day 6, while sulphoxide and sulphones were progressively converted to the amino-sulphone and other metabolites over the following 6 days; a similar profile was noted in the kidneys (Kraeer *et al.*,, 1977). In sheep given a single oral dose of radiolabelled albendazole at 10 mg/kg, the hepatic content on day 1 was mainly as the sulphoxide and sulphone metabolites. Over the next 7 days, these were converted mainly to the 2-aminosulphone (Parish *et al.*, 1977, Colman *et al.*, 1977). Albendazole is also known to induce some hepatic enzymes, which may result in enhanced rates of degradation of the sulphoxide to sulphone following repeated exposure (Amri *et al.*, 1988). As noted above, radiotracer studies in a number of species have confirmed that the principal route of excretion is via the kidneys (IPCS, 1990, Rouini *et al.*, 2002). Irrespective of parent compound administered (netobimin, albendazole or albendazole sulphoxide), the types of metabolites formed was reported to be the same (EMEA, 2004). Netobimin is closely related to albendazole as the majority of the administered netobimin needs to be converted in the target animal which happens naturally in the gut, to albendazole, in order to be pharmacologically active. The safety data derived from the testing of albendazole and its metabolites (including albendazole oxide) are considered of relevance to the safety assessment of netobimin. However, it is also recognised that netobimin may have its own toxicological properties (EMEA, 2004).

A HPLC analytical method has been validated, and is capable of individual residual measurements for albendazole sulphoxide, albendazole sulphone and albendazole 2-amino sulphone in the edible tissues and milk of cattle and sheep. Quantification limits are 15 µg/kg for milk, 20 µg/kg for muscle and fat and 100 µg/kg for liver and kidney. The determined method can be applicable to other ruminant species (EMEA, 2004).

The pharmacokinetics of pregnancy have also been studied in sheep at 120–130 days of gestation given a single oral dose of 7.5 mg/kg and in Sprague-Dawley rats at gestation day 10 given a single oral dose of 8.5, 10 or 14 mg/kg, of a racemic mixture of albendazole sulphoxide. In the ewe, the area under the plasma concentration- time curve (AUC) and peak concentration (C_{max}) were approximately twice that of the fetus (AUC 60.0 and 27.4 µg.h/mℓ; C_{max} 2.5 and 1.2 µg/mℓ, respectively), while the time to reach peak level (T_{max} 11.0 and 9.3 h, respectively) and mean residency time (16.5 and 17.7 h, respectively) were similar. However, while no significant differences in pharmacokinetic profile was noted between enantiomers in the fetus, differences were apparent for the ewe with impairment of trans-placental transfer apparent with the (+)- form (Capece *et al.*, 2002). In contrast in the rat dams, the T_{max} was much shorter at 2.5 hours for each dosage. The C_{max} and AUC, although similar at the lower

dosages, showed a marked increase at the highest dosage (C_{max} 4.1, 4.2 and 8 µg/mℓ; AUC 23.7, 27.2 and 52.5 µg.h/mℓ; at dosages of 8.5, 10 and 14 mg/kg, respectively). Mean maternal plasma and embryo concentrations were similar at dosages of 8.5 mg/kg (1.5 µg/mℓ *versus* 1.3 µg/g, respectively). Differences in profiles were again noted for the various enantiomers, with the (-)- enantiomer showing a higher peak concentration in the dam and embryo than the (+) enantiomer (Capece *et al.*, 2003).

3 Toxicity profile

3.1 Acute toxicity

Common adverse effects of albendazole include nausea, vomiting, constipation, thirst, dizziness, headache, hair loss and pruritus (Choi et al., 2008). Similar effects were also reported in a survey of a sample population (2,319 persons) by means of a pre-tested questionnaire. Of the 268 adverse drug reactions that were reported, 34.7% was attributable to drowsiness, 23.1% were headaches, gastrointestinal symptoms (nausea, vomiting, abdominal pain or diarrhoea) were 18.7% and other effects such as dizziness and faintness, 11.9% (Gunawardena *et al.*, 2008). In another study (Choi *et al.*, 2008) the possibility of albendazole-induced hepatitis, was investigated and results showed potential for hepatitis to be induced although it could not be fully established due to lack of medical records. However, similar associations were also made in an earlier study (Morris & Smith, 1987) where adverse effects on the liver were reported for 6/40 patients receiving treatment for albendazole (Choi *et al.*, 2008). There appear to be no controls for comparison, as these results were obtained from observation in hospitals rather than studies.

In animals, albendazole is considered of low acute oral toxicity (EMEA). LD_{50} values (assumed to relate to the oral route) have been reported for a number of species: 1320 mg/kg (Walker, 1976d) or above in rats; 3000 mg/kg in mice (Macko *et al.*, 1975); >10 000 mg/kg in hamster (Walker, 1976b); 900 mg/kg in guinea-pig (Walker, 1976a); and 500–1250 mg/kg in rabbits (Walker, 1976c). The few clinical signs reported were considered to reflect non-specific toxicity (IPCS, 1990).

Furthermore, in dermal and eye irritancy tests on rabbits, albendazole was found to be non-irritant at 500 and 100 mg, respectively (Macko *et al.*, 1975). Although not considering any studies on the parent compound, the EMEA CVMP reported that albendazole sulphoxide did not show dermal or ocular irritancy in rabbits but, on the basis of a guinea-pig maximisation test, was a potential skin sensitizer (EMEA, 1997).

3.2 Repeat dose toxicity

In a single case, a 68 year old male presented to a hospital with scleral icterus (yellowing of the whites of the eye), facial spider angiomata, and bulging flanks. The patient also suffered with significant cirrhosis and oesophageal varices. In addition an echinococcal cyst (leaking) was also diagnosed. Treatment with albendazole for four weeks, 400mg twice per day, was given. Following sixteen days of treatment, the male returned to the hospital unwell. Nine days after hospital admission the patient died. It is hypothesised that significant hepatic dysfunction within the patient (due to cirrhosis) resulted in significant reduction in the conversion of albendazole to albendazole sulfoxide and sulfone, which led to increased albendazole levels in the circulation. This can increase the toxic effects on cellular functions (particularly microtubules) because albendazole is comparatively a more potent inhibitor of tubulin polymerization than the sulfoxide. This is supported by other case studies in humans and animal studies (Opatrny *et al.*, 2005)

A number of studies have assessed the general toxic properties of albendazole in rodents following repeated exposure. In a study in which Sprague-Dawley rats were given

albendazole by oral gavage at 4, 25, 48 or 168 mg/kg/day for 4 weeks, signs of frank toxicity (including weight loss and death of 39/40) were noted at 168 mg/kg/day. At 48 mg/kg/day, effects were still significant comprising impaired growth performance, slightly decreased food consumption, decreased red and white blood cells counts and, in 7/30, death before the completion of the treatment period. Pathological changes comprised increased adrenal size and, microscopically, hypoplasia of the testes, bone marrow, spleen and lymph nodes in rats given 48 or 168 mg/kg/day. In those rats given 48 mg/kg/day that survived for 4 weeks, atrophy of the testes were noted. No adverse effects were reported at 4 or 25 mg/kg/day, while the effects noted at 48 mg/kg/day were noted to be reversible following a 4-week period of withdrawal of treatment (Simon,1979a). No significant toxicity was reported for a study on Long-Evans rats in which albendazole was given in the diet at dosages of up to 30 mg/kg/day for 91 days (Killeen & Rapp, 1975).

Dietary administration at up to 5 mg/kg/day was also well tolerated by the parental (F_0) generation of CD rats of a multigenerational study. However, mortality was high in offspring when weaned onto a diet at a higher dosage of 45 mg/kg/day; a range of adverse effects were also observed in rats of the F_1 generation given 30 mg/kg/day although, apart from a persistent depression in the myeloid series, these effects showed good recovery following the reduction in dosage to 20 mg/kg/day at 26 weeks of age (Daly & Hogan, 1982).

In two studies on CD-1 mice fed diets containing albendazole at up to 1600 mg/kg bw/day for 90 days, growth performance was impaired at 1600 mg/kg/day, and food intake was reduced at 400 mg/kg/day or above. Ear lesions were also noted from Week 9 for some animals given 800 or 1600 mg/kg/day. Haematological changes comprised a decrease in haematocrit and erythrocyte levels and, in males only, decreased leucocyte counts at 800 mg/kg/day or above, while an increase in liver weight was also noted from 40 mg/kg/day in males and 80 mg/kg/day in females. Over the period of study, high mortality was noted at 1600 mg/kg/day (5/10 males, 10/10 females; (Daly & Rinehart, 1980a, Daly & Rinehart, 1980b)). In a 25-month study in CD-1 mice, erythrocyte and leucocyte counts were decreased and platelet count was increased following dietary administration at 400 mg/kg/day. Testicular atrophy was also noted at this dosage, and increased centrilobular hepatic vacuolation was noted at 100 or 400 mg/kg/day. Although the incidence of ocular opacity was elevated in some groups, this change was not attributed to treatment, and the NOEL was considered to be 25 mg/kg/day (based on two studies with two control groups; (Daly & Knezevich, 1987, Sauer, 1985 & 1987, Selwyn, 1987)).

In dogs given albendazole gavage dosages of up to 168 mg/kg/day for 4 weeks, marked toxicity (including diarrhoea, vomiting and cardiopulmonary disturbance) was noted at 48 mg/kg/day or above, with the death of 1/10 and 6/10 dogs at 48 and 168 mg/kg/day respectively. Leucocyte count was low at these dosages, while blood alkaline phosphatase was increased at 16 mg/kg/day or above. No effects were noted at the lowest dosage, 4 mg/kg/day, tested (Simon, 1979). In contrast, no treatment-related responses were observed in a study in which dogs were orally dosed at 2 to 39 mg/kg/day for 91 days (Killeen & Rapp, 1975). In another study in which dogs were dosed for 6 months at up to 60 mg/kg/day, impaired weight gain and food intake, and reduced red and white cell parameters and hypocellularity of the bone marrow, were noted at the high dosage. The weights of the testes, uterus, liver and kidneys were also affected in these animals. At 30 mg/kg/day, effects were restricted to a reduced leucocyte (particularly neutrophil) count, and no effects were observed at 5 mg/kg/day (Daly & Hogan, 1980).

The main noted effects of albendazole in repeat oral dose studies were hepatotoxicity and testicular toxicity caused by exposure to netobimin and albendazole. A NOEL of 7 mg/kg bw/day has been identified from the study which exposed rats to albendazole for over 60 days in one generation (including treatment during mating, gestation and the post-natal period), followed by exposure to their offspring for 24 months (EMEA, 2004).

3.3 Carcinogenicity and mutagenicity

Albendazole is considered not to be carcinogenic (EMEA). Among rats of the F1 generation, in the multigeneration study described in Sections 3.2 and 3.4, that were maintained on study to 24 months of age, higher incidences of endometrial/cervical tumours and skin histiocytic sarcomas were noted in some treatment groups, compared with the concurrent controls. However, statistical significance was not attained, and all frequencies were within the historical control range for the laboratory. Furthermore, no statistically significant increases in tumour incidences were noted at dosages of up to 400 mg/kg/day, with all frequencies falling within the historic control range (Daly & Knezevich, 1987, Sauer, 1985 & 1987, Selwyn, 1987).

Genotoxicity was evaluated in a study examining the effects on human lymphocytes *in vivo*. 14 children, 8 males and 6 females aged 6-13years, were administered 15mg/kg of albendazole daily for 28 days with a maximum dose of 400mg. The genotoxicity of albendazole was assessed by the frequency of sister chromatid exchange (SCE) and micronucleated cells (MN) in the patients' lymphocytes. The occurrence of SCE and MN were measured separately, before and after treatment. The frequency of SCE increased after drug administration and for MN the frequency increased significantly following treatment. The results were found to be 95% statistically significant, because albendazole has the ability to inhibit microtubule formulation during mitosis of parasites (Oztas *et al.*, 2007). This is also supported by similar studies (Moore, 2001, Leder & Weller, 2003, Ramírez *et al.*, 2001).

The mutagenic and genotoxic potential of albendazole has been investigated in a series of *in vitro* studies on *S. typhimurium*, CHO cells and BALB/3T3 mouse cells (with and without metabolic activation). No effect on bacterial mutation, chromosomal aberration or morphological transformation has been noted. Furthermore, the 2-aminosulphone metabolite is also known not to cause bacterial mutation. However, a positive result has been found in an *in vivo* mouse bone marrow micronucleus test suggesting that it is an *in vivo* aneugen, which may lead to a loss in chromosomes and altered gene expression (EMEA, 1997, IPCS, 1990, EMEA). While this might be taken to suggest that there would be no level at which no risk of mutagenic change could be established, it has more recently been proposed that aneuploidogenesis shows a threshold response, suggesting that normal therapeutic levels do not pose a risk of aneuploidy in humans (Dayan, 2003).

3.4 Reproductive and developmental toxicity

A study has been reported in which 17 pregnant women had been inadvertently given albendazole during the first trimester without adverse effects on mother or offspring (IPCS, 1990). However, another study observed the effects of ivermectin and albendazole, alone and combined, on soil-transmitted helminth infections (STHs) in the second trimester of pregnancy. In each group between 140 and 241 pregnant women were observed. In all, five stillbirths occurred in the albendazole group and three in the combined group. One abortion also occurred in the albendazole group. 21.2% and 22% of women administered with albendazole alone and combined, respectively, were found to have anaemia at the first antenatal care (ANC) visit, compared to the controlled group, 16%. There were no severe adverse events reported after administration, during the second trimester, however long term pharmacovigilance is advised to assess the safety of these medicines in pregnancy (Ndyomugyenyi *et al.*, 2008).

In a multigeneration study, treatment was well tolerated in parental (F_0) generation CD rats given albendazole in the diet at 1, 2.5 or 5 mg/kg/day. However, in the F_1 generation, which was fed a diet at 5, 30 or 45 mg/kg/day following weaning, marked signs of toxicity (comprising emaciation, dermal scabbing and swelling of the lymph nodes, paws and genital regions and haematological and blood chemical disturbance) were noted at the highest

dosage. By week 25 of age, of the F_1 animals given the high dosage, 92/100 males and 99/100 females had died; multiple organ pathology was noted at necropsy. Furthermore, increased urinary protein was noted from 3 months of age in rats given 30 mg/kg/day, with haematological and bone marrow abnormalities also being noted for this group by 6 months of age. Histopathological examination of a subset of animals given 30 mg/kg/day showed thymic hypocellularity and minor hepatic centrilobular swelling, vacuolation and necrosis. Continuation of treatment of the group given 30 mg/kg/day at a reduced dosage of 20 mg/kg/day showed a clear recovery within 4 weeks of adopting the lower dosage, apart from a persistent depression in the myeloid series in the bone marrow while animals given the lowest dosage of 5 mg/kg/day were unaffected throughout. The surviving animals were maintained on study to an age of 24 months to assess the carcinogenic potential of albendazole (see Section 3.3; IPCS, 1990).

In a study in which CD rats were orally given dosages of 0, 5, 20 or 40 mg/kg/day on gestation days 16–20, no evidence of maternal toxicity was noted. Although the intergroup differences in pup mortality lacked a consistent dosage-relationship, levels were particularly high at the high dosage of 40 mg/kg/day, a dosage at which small lungs and kidneys and anasarca were also noted. Although these changes were not unequivocally attributed to treatment, the NOEL was established as 20 mg/kg/day.

In a further series of studies on Long–Evans rats treated with albendazole (or, in one case, freeze dried liver from cattle previously given albendazole) at various dosages on gestation days 6–15, increased levels of foetal resorptions and reduction in size of surviving foetuses were noted at dosages of 10 mg/kg/day or above (maximum dose of 30 mg/kg bw/day). Although an apparent increase in foetal abnormalities were also initially reported a re-evaluation concluded that the differences were probably artifactual and not related to treatment. An overall NOEL of 5 mg/kg/day was established in the Long–Evans rats (IPCS, 1990).

In Sprague–Dawley rats given albendazole or its metabolites on gestation day 8–15, an increase in skeletal abnormalities (principally craniofacial and bone defects) were noted with albendazole at 6.62 mg/kg/day or above, and decreased fetal weight at 8.83 mg/kg/day. Similar findings were also noted with equimolar amounts of albendazole sulphoxide but not the other metabolites investigated; the embryotoxicity and developmental effects of the parent compound and the sulphoxide were almost completely suppressed by co-administration of the microsomal oxidation inhibitor, SKF-525-A. A NOEL of 6 mg/kg/day albendazole was established. A further study in this strain in which albendazole was given, either as albendazole, at dosages of 23, 24 or 36 mg/kg bw/day, or liver from cattle treated with albendazole (intake was calculated to represent 0.42 mg/kg bw/d albendazole equivalents) indicated a NOEL of 12 mg/kg/day (IPCS, 1990).

In a more recent study on Wistar rats given albendazole sulphoxide via the diet, at dosages of 0, 2.5, 5, 10, 20 or 30 mg/kg bw/day for days 6 to 15 of gestation, no overt signs of toxicity were noted in the dams. A significant increase in total numbers resorbed was noted at 20 and 30 mg/kg/day, with early resorptions showing an increase only at 30 mg/kg/day; abnormal and small placentas were also reported for these groups. Dosage-related decreases in foetal size and increases in incidence and extent of ossification were noted at 5 mg/kg/day or above. At the low dosage of 2.5 mg/kg/day, a low (but not statistically significant) incidence of late ossification of the metacarpals and metatarsals was noted. However, since this finding was not noted in the contemporaneous control group and no historic control data were reported, it is probably prudent to regard this as a LOAEL rather than a NOAEL (Teruel *et al.*, 2003). In a further study, focusing on the pharmacokinetics of pregnant rats given a single oral dosage of albendazole sulphoxide (Section 2), all litters were resorbed at the high dosage of 14 mg/kg and the numbers of external and skeletal malformations were significantly increased at 10 but not 8.5 mg/kg when compared with untreated controls (Capece *et al.*, 2003).

No maternal toxicity or evidence of foetotoxic or teratogenicity was noted in CD-1 mouse dams given albendazole at up to 30 mg/kg/day by gavage from gestation day 6 to 15 (IPCS, 1990).

In New Zealand White rabbits treated on gestation days 7–9, maternal toxicity and reduced numbers of implants and increased rates of resorption and ectrodactyly were observed at 30 mg/kg/day. Reduction in foetal size was apparent at 10 mg/kg/day. However, no effects were noted at 5 mg/kg/day (IPCS, 1990).

In two studies in ewes, premature delivery and increased post partum losses were noted at dosages of 15 mg/kg or above following a single oral dose on gestation day 17. Non-viable lambs showed increased incidences of pognathia, scoliosis, spina bifida and reduced tail at 20 mg/kg and renal abnormalities at 15 or 20 mg/kg. No effects were noted at 10 mg/kg (IPCS, 1990).

It has been suggested, based upon data from sheep and cattle, that there is a relationship between peak plasma level of albendazole sulphoxide and teratogenesis (IPCS, 1990).

In a comprehensive series of developmental studies carried out in mice, rats, rabbits, and sheep, albendazole was teratogenic. Observed malformations included visceral, craniofacial and bone defects (including shortened limbs). For any of the studies, the lowest NOEL for albendazole was 5 mg/kg bw/day, administered orally to rats or rabbits. Netobimin and albendazole sulphoxide were also teratogens with similar potency to albendazole (EMEA, 2004).

4 Guidelines and standards

The EMEA derived an ADI of 5 µg/kg bw, by applying a larger safety factor of 1000 to the NOEL for reproductive toxicity, 5 mg/kg/day. This was considered appropriate as teratogenicity, which was the principal end-point of concern, has been shown following comprehensive developmental studies and in the light of the evidence of mutagenicity *in vivo* (EMEA, EMEA, 1997, EMEA, 2004).

On the basis of the above, EMEA CVMP derived overall MRLs for the parent and major metabolites in muscle, fat and milk of 100 µg/kg (albendazole equivalent) for all ruminants; MRLs of 1000 µg/kg and 500 µg/kg were also established for liver and kidney, respectively. On the basis of these MRLs, the daily intake from food sources of extractable residues was estimated by EMEA to account for 103% of the ADI; however, this was not considered to represent a significant risk to consumers because at least 75% of the residues in tissues were not bioavailable (EMEA, 1997).

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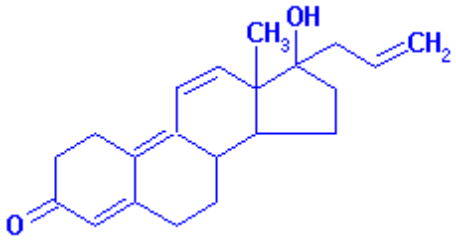
Altrenogest

1 Introduction

Altrenogest (allyltrenbolone, molecular formula $C_{21}H_{26}O_2$, CAS No. 850-52-2; Figure 1.1; Box 1) is a synthetic trienic C21-steroidal hormone used in veterinary medicine as an orally active progestomimetic (EMEA, 2002, ChemID Plus).

Figure 1.1 Molecular structure of altrenogest



From ChemID Plus

Box 1 Synonyms and trade names for altrenogest

17-alpha-Allyl-estratriene-4,9,11,17-beta-ol-3-one; 17alpha-Allyl-17-hydroxyestra-4,9,11-trien-3-one; A 35957; Allyltrenbolone; Altrenogest;Altrenogestum; DRC 6246; EINECS 212-703-1; R 2267; REGU-MATE; RU 2267; (17beta)-17-Hydroxy-17-(2-propenyl)estra-4,9,11-trien-3-one; Estra-4,9,11-trien-3-one, 17-alpha-allyl-17-hydroxy-; Estra-4,9,11-trien-3-one,17-hydroxy-17-(2-propenyl)-,(17-beta)-(9CI); Estra-4,9,11-trien-3-one, 17beta-hydroxy-17-(2-propenyl)-; UNII-2U0X0JA2NB

From ChemID Plus

Altrenogest is used for zootechnical purposes in veterinary medicine, mainly for estrus synchronisation in gilts (an immature female pig) and mares, to aid with estrus detection in preparation for natural or artificial insemination. The recommended therapeutic dose for gilts is 20 mg/animal/day for 18 consecutive days and for mares is 0.044 mg/kg bw/day for 10–15 days. As well as the progestomimetic effect, altrenogest has anti-gonadotrophic, weakly estrogenic, anabolic and androgenic activities. There is no evidence of the use of altrenogest in human medicine and consequently data on the toxicological effects in humans are lacking (EMEA, 2002).

2 Toxicokinetics

No human toxicokinetic data are available.

The analytical method for detection of altrenogest in kidney, skin plus fat , liver and muscle of pigs and liver, kidney and fat of horses is by HPLC with UV detection. The limit of quantification (LOQ) of this routine analytical method is 1.0 µg/kg in all tissues of both species except for the liver of pigs, for this the LOQ was 0.2µg/kg (EMEA, 2004). The LOQ for this method for horses is not yet fully validated because the LOQ in horse liver is above that of the MRL, for this target tissue. In the pig the LOQ is fine, therefore as the horse is a minor species the pigs validated data is accepted for the horses (EMEA, 2004). The method does not include a step to hydrolyse the conjugates of altrenogest and the quantified residue does not include the isobaric form (EMEA, 2002).

Absorption of orally administered, radiolabelled altrenogest in pigs and horses is rapid and peak levels are reached after 3–6 hours. The half-life of elimination in the plasma of pigs has been shown to be approximately 10 days but in both pigs and horses, the decline in plasma concentration is biphasic. In these species, distribution of altrenogest is mainly to the liver, followed by kidney, muscle and fat. Limited data indicate that the major metabolic pathway for altrenogest is conjugation; metabolites are less lipophilic and have lower hormonal activity than the parent compound. The dominant route of elimination in pigs is in the bile and faeces; approximately 20% is excreted via the urine. In the first 24 hours, horses excrete about 44% of the dose in urine and about 53% in faeces (EMEA, 2002).

A pharmacokinetics study in rats given a single oral dose of altrenogest reported (in the first 24 hours) approximately 60% of the excretion to be via the biliary route and 20% via the urinary route; the remainder was eliminated in the faeces (EMEA, 2002).

3 Toxicity profile

3.1 Acute toxicity

The LD_{50} values for intraperitoneally administered altrenogest in rats and mice are 176 and 233 mg/kg bw, respectively. However, studies in dogs have shown good tolerance to oral doses of altrenogest up to 400 mg/kg bw (EMEA, 2002).

3.2 Repeat dose toxicity

Repeat dose toxicity tests have been conducted in several species. A series of studies in rats involved giving altrenogest orally at 0, 0.5 or 2 mg/kg bw/day; 0, 1, 10, 100 mg/kg feed (equivalent to 0.06–7.82 mg/kg bw/day); or 0, 2, 10 or 50 mg/kg bw/day for 2 months, 13 weeks or 1 year, respectively. In a further study, dogs were given altrenogest orally at 0, 0.04, 0.2 or 1.0 mg/kg bw/day for 1 year. All these studies showed a reduction in the weights of hormone-dependent organs as well as histopathological changes. A LOEL of 0.04 mg/kg bw/day was determined for the study in dogs (EMEA, 2002).

In one of several tolerance studies in adult pigs, altrenogest was given orally at dosages of 4, 40 or 200 µg/kg bw/day for three months (EMEA, 2002). The main effects observed related to the hormonal activity of altrenogest, and comprised decreased organ weight and histopathological changes in the ovaries, uterus, mammary glands, prostate, testes and seminal vesicles; a no-hormonal-effect level was identified as 4 µg/kg bw/day. Similarly, a no-hormonal-effect level of 4 µg/kg bw/day was established for monkeys (species unspecified) given altrenogest (dosages unspecified) during three menstrual cycles.

3.3 Carcinogenicity and mutagenicity

No long-term carcinogenicity tests for altrenogest have been performed. In 1999, JECFA, the Scientific Committee on Veterinary Measures Relating to Public Health of the European Commission and the International Agency for Research on Cancer reviewed data for steroid hormones, which included 17β-estradiol, but not altrenogest. EMEA CVMP concluded that there were no *in vivo* data within the range of normal physiological concentrations that indicated genotoxic or carcinogenic effects and that any carcinogenic effect would only be expected to occur after prolonged exposure to abnormally high levels of such compounds (EMEA, 2002).

A number of tests for the mutagenicity of altrenogest have been reported including *in vitro* tests such as the Ames test, forward mutation test, chromosome aberration test and DNA repair tests, and an *in vivo* chromosome aberration test in rats. These have shown no genotoxic effects.

3.4 Reproductive and developmental toxicity

No studies on reproductive or developmental effects in humans are available.

Some alterations in reproductive functions indicative of a hormone-related mechanism have been reported in two studies in rats and one study in mares. However, no evidence of any teratogenic effects have been reported for a number of species. In a one-generation study, rats were given the drug via the diet at concentrations of 25, 50, and 100 mg/kg feed, and, in a two-generation study, at 0.4, 4 and 40 mg/kg feed (achieved dosages unspecified). Effects observed included reduced pregnancy rate, depressed spermatogenesis, decreased litter size and litter weight, and a decrease in weights of hormone-dependent organs of dams. A NOEL of 0.4 mg/kg feed was identified, which is equivalent to an achieved dosage of 0.03 mg/kg bw/day. In addition, in the two-generation study, offspring were examined for evidence of teratogenic effects but none was found. No evidence of teratogenicity was observed in a tolerance study in pigs given altrenogest at 20 mg/day on gestational days 28–112 (EMEA, 2002).

Nursing mares were given altrenogest at 0.44 mg/kg bw/day by daily gavage for 7 or 15 days postpartum. Following parturition, mares were assessed daily for estrus, and allowed to breed with stallions of known fertility then monitored for rate of pregnancy. Altrenogest suppressed estrus in the mares exposed to the drug during the first 15 days postpartum, but there was no effect on their reproductive performance. The serum chemistry, growth and development of suckling foals exposed via lactation were also normal when assessed at intervals between post-natal day 1–30 (Kesler & Lock, 1998).

Fertility and early embryonic development was studied in Landrace gilts treated for estrus-synchronisation with altrenogest via the diet at 20 mg/animal/day for 18 days. Gilts were checked for estrus and artificially inseminated twice at 12 hour intervals. No effects on fertility or early embryonic development were observed and no histopathological changes were detected in the ovaries and oviducts of the treated gilts (Lee *et al.*, 1992).

In a reproductive study the effects of altrenogest treatment were analysed for mares during late gestation until foaling, including neonatal adaptation and postnatal development. There were 6 mares in the treatment group and 7 in the control group, each received a daily dose of altrenogest (0.088mg/kg daily). For mares in the treatment group the gestational length tended to be shorter, the second stage of parturition was prolonged ($p<0.05$) and the foals had a significantly lower respiratory rate than control foals during their first 30 minutes after being born ($p<0.05$) and venous plasma pH was significantly higher than in control foals at 15 and 30 minutes after birth ($p<0.05$). Other noticeable effects in the treatment group included base excess (an estimate of the metabolic component of the acid-base balance) in foals was significantly higher than in control foals at 45 minutes and up to 12 hours after birth ($p<0.05$). There were significantly more problems in the perinatal period; one treated foal died within 30 minutes following delivery and a second foal was delivered by caesarean stillborn. Out of the remaining 4 foals one initially showed signs of severe respiratory depression, however it recovered in intensive care. In the control group no significant problems were observed. The study indicated that altrenogest treatment affected the foals ability to adapt to the extrauterine environment (Neuhauser *et al.*, 2008).

4 Guidelines and standards

EMEA CVMP has recommended a pharmacologically-based ADI of 0.04 µg/kg bw (2.4 µg/person) assuming a bodyweight of 60 kg. This level was based on the absence of hormonal effects of altrenogest at a concentration of 4 µg/kg bw in monkeys and pigs. A safety factor of 100 was employed to allow for inter- and intraspecies differences.

EMEA CVMP initially established a provisional MRL of 3 µg/kg for altrenogest residues in porcine skin and fat, and liver and kidney tissues, and an MRL of 3 µg/kg for altrenogest residues in equine fat, liver and kidney was also recommended. Since 2005 the MRLs for pigs and horses have been lowered to 1µg/kg for porcine skin and fat tissues and 0.4µg/kg for liver tissues. The recommendations for equidae fat tissues are 1µg/kg and for the liver tissues 0.9µg/kg (EMEA, 2004). EMEA estimated that the theoretical maximum daily intake would be equivalent to approximately 98% of the pharmacological ADI in horses and 93% in pigs (EMEA, 2004).

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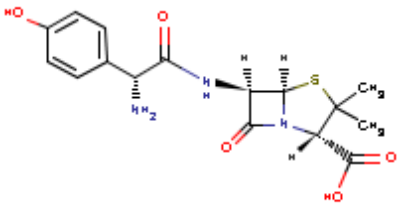
Amoxicillin

1 Introduction

The semi-synthetic β-lactam antibiotic amoxicillin ((2S,5R,6R)-6-[(R)-(-)-2-amino-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicycl [3.2.0]heptane-2-carboxylic acid trihydrate, molecular formula $C_{16}H_{19}N_3O_5S$, CAS No. 26787-78-0; Figure 1.1, Box 1) an analogue of ampicillin, has a broad spectrum of bactericidal activity against many Gram-positive and Gram-negative micro-organisms, and is a human and veterinary medicine (ChemID Plus, RxList, 2008). Like other penicillins, amoxicillin produces its bactericidal effect by inhibiting bacterial cell wall synthesis. These antibiotics must penetrate the cell wall to attach to specific proteins on the inner surface of the bacterial cell membrane. In actively growing cells, the binding of amoxicillin within the cell leads to interference with production of cell wall peptidoglycans and subsequent lysis of the cell in an iso-osmotic environment (US Pharmacopeia, 2007).

The toxicity data publicly available for this veterinary medicine are extremely limited, particularly for low level and long term exposures. The majority of the data presented in this assessment have been taken from websites.

Figure 1.1 Molecular structure of amoxicillin



From ChemID Plus

Box 1 Synonyms and trade names for amoxicillin

(-)-6-(2-Amino-2-(P-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-(3.2.0)heptane-2-carboxylic acid; 6-(D-(-)-alpha-Amino-p-hydroxyphenylacetamido)penicillanic acid; 6-(D-(-)-p-Hydroxy-alpha-aminobenzyl)penicillin; 6-(p-Hydroxy-alpha-aminophenylacetamido)penicillanic acid; AMPC; Amoclen; Amolin; Amopenixin; Amoxi; Amoxi-Mast; Amoxicillin; Amoxiden; Amoxil; Amoxivet; Amoxycillin; Ampy-Penyl; Anemolin; Aspenil; BLP 1410; Bristamox; Cemoxin; Clamoxyl; D-(-)-alpha-Amino-p-hydroxybenzylpenicillin; D-2-Amino-2-(4-hydroxyphenyl)acetamidopenicillanic acid; D-Amoxicillin; Delacillin; DisperMox; EINECS 248-003-8; Efpenix; Flemoxin; HSDB 3204; Hiconcil; Histocillin; Ibiamox; Imacillin; Metafarma capsules; Metifarma capsules; Moxacin; Moxal; NSC 277174; Piramox; Ro 10-8756; Robamox; Sawamox PM; Sumox; Unicillin; Vetramox; alpha-Amino-p-hydroxybenzylpenicillin; p-Hydroxyampicillin

From ChemID Plus

2 Toxicokinetics

Amoxicillin is stable in the presence of gastric acid and is rapidly absorbed after oral administration. Amoxicillin is distributed to most body tissues and fluids, with the exception of the brain and spinal fluid, unless the meninges are inflamed. The half-life of amoxicillin is 61.3 minutes, with most excreted unchanged in the urine within 6–8 hours. Amoxicillin is about 20% protein-bound in blood serum. Peak blood levels occur 1–2 hours following oral administration, with detectable serum levels up to 8 hours after administration (RxList Inc, 2008). However, the presence of food in the gastrointestinal tract results in a lower and delayed peak serum concentration of amoxicillin, but the total amount of drug absorbed does not appear to be affected. Penicillins are cleared by both filtration and secretion by the kidneys (HSDB, 2003). High blood levels occur most easily in patients with impaired renal function because of decreased clearance of the drug (RxList Inc, 2008).

Penicillins are excreted in human milk. Therefore use of amoxicillin by nursing mothers could lead to sensitisation of infants. As neonates and young infants have incompletely developed renal function, their ability to eliminate amoxicillin is reduced compared with adults (RxList , 2008).

The currently accepted method for measuring amoxicillin residues in food animals is by LCMS as it can accurately quantify the parent compound**.** In some instances fluorescence detection was the selected method, but while this method can detect the parent compound it also detects amoxicilloic acid. Both these compounds are converted to the same fluorescent derivative which can lead to apparently high levels and inaccurate conclusions, particularly for liver and kidney tissues, therefore fluorescence detection can lead to over-conservative results (EMEA, 2008). Hence results obtained following fluorescence detection only should be interpreted with caution

For the detection of amoxicillin in milk samples the use of the method *Bac, stearothermophilus* is employed, with detection limits ranging from 2-4µg/kg. In the case of edible tissues the four plate method is used, 30-60 4µg/kg is the limit of detection (EMEA, 2008)

3 Toxicity profile

3.1 Acute toxicity

The most common health effects are the side-effects of these antibiotics experienced following their therapeutic use. As with other penicillins the most serious of these effects is the hypersensitivity reaction. These reactions are most likely to occur in individuals who have a history of allergy, asthma, hay fever, or urticaria, and those who have previously demonstrated hypersensitivity to penicillins. The hypersensitivity reactions can take the form of anaphylactic shock, serum sickness-like reactions, erythematous maculopapular rashes, erthema multi-forme, Stevens–Johnson syndrome, exfoliative dermatitis, toxic epidermal necrolysis, acute generalized exanthematous pustulosis, hypersensitivity vasculitis and urticaria. These reactions can be controlled with antihistamines and where necessary, corticosteroids, but treatment should be discontinued unless the condition being treated is more life threatening (RxList, 2008).

Anaemia, including haemolytic anaemia, thrombocytopenia, thrombocytopenic purpura, eosinophilia, leukopenia, and granulocytosis has been reported during therapy with penicillins. The effects are reversible on discontinuation of therapy and are believed to be part of the hypersensitivity reaction (RxList, 2008).

Gastrointestinal effects are common and include nausea, vomiting, diarrhoea, and haemorrhagic/pseudomembranous colitis. Pseudomembranous colitis may range in severity from mild to life-threatening (HSDB, 2003; RxList, 2008).

Following treatment with amoxicillin a moderate rise in aspartate aminotransferase (AST) and/or alanine transaminase (ALT) has been noted in patients, but the significance of this change is not known. Hepatic dysfunction, including cholestatic jaundice, hepatic cholestasis and acute cytolytic hepatitis, has been reported (RxList , 2008).

Interstitial nephritis resulting in oliguric renal failure has been reported in a small number of patients after overdose with amoxicillin. Renal impairment appears to be reversible with cessation of drug administration[4]. Glomerulonephritis caused by drug allergy is usually observed as part of a serum sickness. Deposition of antigen–antibody complexes occurs non-specifically along the glomeruli (Haddad, 1990).

Effects on the central nervous system, such as reversible hyperactivity, agitation, anxiety, insomnia, confusion, convulsions, behavioural changes, and/or dizziness have been reported, but are rare (RxList, 2008).

A prospective study of 51 paediatric patients at a poison-control centre suggested that overdoses of less than 250 mg/kg of amoxicillin are not associated with significant clinical symptoms (RxList, 2008).

3.2 Repeat dose toxicity

Drug-related immunologic destruction of granulocytes usually develops after the second week of treatment with amoxicillin, but may be delayed and occur weeks or months into a course of therapy. It is characterised by a sudden fall in the peripheral neutrophil count and fever might be present. Absolute neutropenia can be severe and can place the patient at increased risk of infection. Neutropenia with prolonged high dose therapy is of unknown etiology (Haddad, 1990).

3.3 Carcinogenicity and mutagenicity

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of amoxicillin.

Studies to detect the mutagenic potential of this substance alone have not been conducted. However, there are data available for Augmentin, a 4:1 mixture of amoxicillin and potassium clavulanate. Augmentin was non-mutagenic in the Ames bacterial mutation assay but was weakly positive at toxic doses in the mouse lymphoma assay. Augmentin was also negative in the mouse micronucleus test, and in the dominant lethal assay in mice (RxList, 2008).

3.4 Reproductive and developmental toxicity

Amoxicillin administered to pregnant women had no reported adverse effects on the foetus. According to the USCDC recommendations, the use of amoxicillin in pregnancy for those with chlamydia infections is allowed (McEvoy, 1995).

A population-based study conducted by Jepsen *et al*. (2003) identified all primiparous women with live birth, or a stillbirth after the 28th gestational week, from 1 January 1991 until 31 December 2000 in the county of North Jutland in Denmark. Data on prescriptions for amoxicillin and outcome were obtained from population-based registries. Comparisons were made for pregnancy outcomes between women who had been prescribed amoxicillin during pregnancy and those who had not. Adjustments for potential confounding factors were made. A total of 401 women redeemed a prescription for amoxicillin during their pregnancy. The

control group consisted of 10 237 women who did not redeem any prescriptions for 3 months before pregnancy until the end of pregnancy. No effect on birth weight, preterm delivery, congenital malformation or spontaneous abortion was found. The authors concluded that more research was required.

In lactating women, findings showed peak milk amoxicillin levels occurred 4-5 hours later after an oral dose of amoxicillin (1gram). The average milk levels ranged from 0.46-0.88mg/L and 0.39-1.3 mg/L at 4 and 5 hours respectively after the dose. Conclusions of the study show that exclusively breastfed infants could receive a maximum daily dose of approximately 0.1 mg/kg of amoxicillin where the maternal dose is 500 mg 3 times daily. This amounts to between 0.25 and 0.5% of a typical infant amoxicillin dose (Kafetzis *et al.,* 1981).

In a telephone study 3/25 nursing mothers reported diarrhoea symptoms in the infant (Ito et al., 1993). In addition a small, controlled, prospective study had mothers observe their infants for symptoms of adverse effects (furring of the tongue, feeding difficulties, changes in stool frequency and consistency and rashes). Information regarding their weight change and the development of jaundice were also recorded. No statistical differences were found between the infants of the control mothers and those of mothers taking ampicillin or ampicillin-clavulanate (Campbell et al., 1991).

A controlled study (nursing mothers invited prior to administration of amoxicillin/clavulanic acid or cefuroxime) was undertaken to examine the effect of amoxicillin/clavulanic acid or cefuroxime on infants, via nursing mothers receiving treatment. Information is not available for the route of administration or the dose. Out of 156 infants, 16 infants had 1 adverse effect and 3 infants had more than 2 adverse effects. No severe adverse events were observed. Symptoms of the adverse effects included; constipation, rashes, diarrhoea, irritability and elevated liver enzymes (in one case only). Following cessation infants symptoms disappeared (Benyamini *et al.*, 2005).

In a multi-generation reproductive toxicity study in rats, no impairment of fertility or other adverse reproductive effects were observed at doses up to 500 mg/kg. This dose is approximately three times the therapeutic dose used in humans (RxList, 2008).

Reproductive toxicity studies have been performed in mice and rats at doses up to ten times the human therapeutic dose and have not revealed any evidence of impaired fertility or adverse effects on the fetus as a result of treatment with amoxicillin. There have been no well-controlled studies in humans (RxList, 2008).

4 Guidelines and standards

JECFA has recommended an ADI for residues of other β-lactam penicillins, benzylpenicillin and procaine benzylpenicillin of 0.03 mg of the penicillin moiety (see Annex on penicillins). This is equivalent to 0.5 µg/kg bw/day for a 60 kg person. The Committee concluded that in the absence of adequate data to establish a NOEL, the determining factor in the safety evaluation was allergy. The risk associated with the occurrence of mild hypersensitivity reactions at this level was considered to be insignificant (IPCS, 1991).

In 1995, the Australian Government set an ADI of 0.2mg/kg bw, based on a NOEL of 200 mg/kg bw, the study used in establishing this ADI was not discussed (Department of Health and Ageing. Australian Government, 2005).

In 2006 the Committee for Medicinal Products for Veterinary Use (CMVP) adopted the concluding opinion that the inclusion of amoxicillin in eggs cannot be recommended (EMEA, 2006a &b).

Furthermore, by consensus, marketing authorisations for Suramox 15% LA (amoxicillin) and the associated invented name, Stabox 15% LA have been suspended. The following reasons apply; inability to establish a withdrawal period for cattle and pigs based on available data, current withdrawal periods for cattle and pigs already established are unsuitable in ensuring the exceedance of the MRLs, inadequate withdrawal periods could create a health hazard to the consumer due to residues in foodstuffs obtained from the treated animal. This referral was initiated by Belgium under Article 35 of Directive 2001/82/EC. The CVMP will review the grounds for the suspension should new data on the establishment of withdrawal periods be submitted for evaluation (EMEA, 2006b). For the current assessment, the ADI of 0.5 µg/kg bw/day will be used.

Table 4.1 MRLs for amoxicillin

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissue	Comment
Amoxicillin	Amoxicillin	All food-producing species	50 µg/kg	Muscle	None
			50 µg/kg	Fat	
			50 µg/kg	Liver	
			50 µg/kg	Kidney	
			4 µg/kg	Milk	

From EMEA, 2006b

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Apramycin

1 Introduction

Apramycin (D-Streptamine, 4-O-((8R)-2-amino-O-(4-amino-4-deoxy-alpha-D-glucopyranosyl)-2,3,7-trideoxy-7-(methylamino)-D-glycero-alpha-D-allo-octodialdo-1,5:8,4-dipyranos-1-yl-2-deoxy; molecular formula $C_{21}H_{41}N_5O_{11}$, CAS No. 37321-09-8; (ChemID Plus); Figure 1.1; Box 1) is an aminoglycoside antibiotic.

Figure 1.1 Molecular structure of apramycin

No Structure

From ChemID Plus

Box 1 Synonyms and trade names for apramycin

4-O-((8R)-2-Amino-8-O-(4-amino-4-deoxy-alpha-D-glucopyranosyl)-2,3,7-trideoxy-7-(methylamino)-D-glycero-alpha-D-allo-octodialdo-1,5:8,4-dipyranos-1-yl)-2-deoxy-D-streptamine; -O-(3alpha-Amino-6alpha-((4-amino-4-deoxy-alpha-D-glucopyranosyl)oxy)-2,3,4,5abeta,6,7,8,8aalpha-octahydro-8beta-hydroxy-7beta-(methylamino)pyrano(3,2-b)pyran-2alpha-yl)-2-deoxy-D-streptamine; Ambylan; Apralan; Apramicina [INN-Spanish]; Apramycine [INN-French]; Apramycinum [INN-Latin]; D-Streptamine, 4-O-((8R)-2-amino-8-O-(4-amino-4-deoxy-alpha-D-glucopyranosyl)-2,3,7-trideoxy-7-(methylamino)-D-glycero-alpha-D-allo-octodialdo-1,5:8,4-dipyranos-1-yl)-2-deoxy-; EINECS 253-460-1; EL 857; EL-857/820; Nebramycin II; Nebramycin factor 2; UNII-388K3TR36Z

From ChemID Plus

Apramycin is used in the treatment of colibacillosis and calmonellosis in calves, bacterial enteritis in pigs, colibacillosis in lambs and *Escherichia coli* septicaemia in poultry. It also has applications in rabbits. Apramycin is not authorised for use in laying birds or in cattle or sheep producing milk for human consumption. Apramycin comes in many formulations including a sulphate used as an oral doser in neonatal pigs, a premix for incorporation into pig feed, a soluble powder for administration in drinking water or milk replacer for calves and as an injectible formulation for intramuscular administration to calves. Apramycin can also be given via drinking water or feed to rabbits (EMEA, 1999). Apramycin is not used in human medicine.

EMEA CVMP (EMEA, 1999) and FDA (FDA, 2009a) have produced summary reports for apramycin, which form the primary sources for this assessment. No other authoritative literature reviews have been identified.

2 Toxicokinetics

Oral administration of ^{14}C-apramycin (4 mg) to rats resulted in recovery of 99.5% and ~0.5% in the faeces and urine respectively. Following s.c. administration of ^{14}C-apramycin (4 mg) to rats, 93% and ~7% were recovered from faeces and urine respectively. Low levels of

radioactivity were noted in the kidney which, along with the residues in the urine consisted mostly of the parent compound (EMEA, 1999).

In the dogs after 6 month or 1 year, peak serum concentrations were directly proportional to dose and reached peak levels 1 – 2 hours after dosing and were below the limit of detection 24 hours after dosing (EMEA, 1999).

I.M injection of ^{14}C-labelled apramycin (dose not stated) were given to two pigs and a calf and peak radioactivity concentrations were detected in the blood 0.5 hours after administration and were at the limit of detection 24 hours after administration. Almost all the radiolabelled material in the blood and over 90% of material in the excreta appeared to be microbiologically active, unmetabolised apramycin. Urinary excretion accounted for only 0.3 – 10.5% of administered dose. In over 75% of the radiolabelled material in the liver and kidney was unmetabolised apramycin (EMEA, 1999). These data suggested that apramycin is poorly absorbed following all routes of administration that were examined (EMEA, 1999).

In residue depletion studies conducted in pigs, calves, lamb, chickens and rabbits using microbiological and HPLC assay methods, apramycin residues depleted rapidly. Approximately seven to fourteen days after treatment (with 10 -40 mg/kg day in pigs, lamb and calves for up to seven consecutive days) irrespective of routes of administration, apramycin residue levels fell significantly in pigs, broiler chickens and rabbits to below the limit of detection. In calves, residues showed a slower depletion (taking approximately 28 days in calves and over 30 days in lamb) to generally below the limit of detection in calves and lambs and also below the limit of quantification in a more recent lamb study (EMEA, 1999).

3 Toxicity profile

3.1 Acute toxicity

Acute oral toxicity has been evaluated in a number of species. LD_{50} values of > 1250 mg/kg bw were noted in Hartley guinea pigs, > 1600 mg/kg bw in New Zealand White rabbits, > 1000 mg/kg bw in Beagle dogs, > 8000 mg/kg bw in Wistar rats and > 10,000 mg/kg bw in ICR mice (EMEA, 1999). I.V. apramycin showed greater toxicity, with LD_{50}'s of 573 mg/kg in mice and 1640 mg/kg in rats (FDA, 2009a, FDA, 2009c).

Pharmacological studies on isolated smooth and cardiac muscle showed that apramycin slightly increased the contractile rate of guinea pig atria and slightly decreased the response of rat aorta to phenylephrine (10^{-5} M); no significant effects were observed on samples of ileum, trachea, vas deferens or uterus. I.V. administration to anaesthetised dogs induced increased blood pressure and heart rate at 10 and 25 mg/kg bw; this was considered to be due to sympathomimetic activity. Such effects were not noted at 5 mg/kg bw. No changes in electrocardiogram, cardiac output or respiratory function were observed at any dose tested.

Intra-venous (i.v.) dosing at 25 mg/kg bw or oral dosing at 250 or 400 mg/kg bw did not affect neuromuscular transmission in the rat. However, in the rat, oral administration of 50 mg/kg bw led to a statistically significant reduction in urine volume and increased electrolyte concentration, considered to be possibly attributable to increased antidiuretic hormone secretion or direct effects on the renal tubule. The overall pharmacological NOEL was considered to be 10 mg/kg bw, based on the effects on renal function observed in the rat (EMEA, 1999).

Dermal application of apramycin (1040 mg/kg) to intact or abraded rabbit skin followed by 14 days observation resulted in no deaths.

Instillation of 19 mg apramycin to one eye of a rabbit caused slight reversible conjunctival reddening in two out of six animals (FDA, 2009a, FDA, 2009c).

3.2 Repeat dose toxicity

Apramycin is not used in human medicine and as such, there are no data concerning the possible effects in man (EMEA, 1999).

In a series of unpublished repeat dose toxicity studies in rats of between three and six months duration, effects were generally limited to mild anaemia and diarrhoea (which may associate with the pharmacological effects of apramycin on gut flora). Evidence of nephrotoxicity was noted in one study, in three out of 15 males fed diet containing the highest level of 1% apramycin. Details of strain of rats, exact duration of exposure and other details were not presented in the EMEA Summary report. Overall, it was concluded that the NOEL in rats was 20.9 and 26.1 mg/kg/day, for males and females respectively (EMEA, 1999).

In a repeat dose toxicity study on Fischer 344 rats fed diet containing up to 5.0% apramycin for up to two years body weight gain and liver and kidney weights were significantly reduced at the highest dose. The NOEL was considered to be 0.25% diet, equivalent to 124 mg/kg/day in males and 154 mg/kg/day in females (EMEA, 1999).

In a repeat dose toxicity study, B6C3F1 mice were fed diets containing 0, 0.15, 0.5, 1.5 or 4.5% apramycin for up to two years; reduced body weight gain was noted at the 0.5, 1.5 and 4.5% level in females and 4.5% level in males. Slightly increased erythrocyte count, haemoglobin concentration and packed cell volume was observed at 4.5% apramycin. Clinical chemistry changes comprised decreased serum glucose concentration and increased blood urea nitrogen concentration at the 4.5% in males and females, and elevated serum alkaline phosphatase at 0.5, 1.5 and 4.5% in females only. Histopathological changes included punctuate cytoplasmic basophilia of the renal tubular epithelium in mice given 0.5% apramycin or above which presented in a dose-related manner and was more pronounced in females compared with males. The NOEL was 0.15% diet, equivalent to approximately 200 mg/kg/day (FDA, 2009a, FDA, 2009c).

In a one year repeat dose toxicity study in Beagle dogs (n = 4/sex/group), orally administration of apramycin at 0, 25, 50 or 100 mg/kg/day resulted in statistically significant changes in haematology and organ weights although no histopathological correlates were noted. The NOEL was considered to be 50 mg/kg/day (FDA, 2009a, FDA, 2009c).

In none of the oral studies considered was evidence of ototoxicity or nephrotoxicity reported. As both effects are commonly associated with aminoglycoside antibiotics this was suggested to possibly reflect the poor oral absorption of apramycin (Taber & Pasko, 2008, Harvey *et al.*, 2000).

In a repeat dose study specifically conducted to investigate ototoxicity in cats (n=2 males and 2 females), apramycin was administered s.c. at 100 mg/kg/day for up to 30 days. Righting reflex and duration of post-rotatory nystagmus were unaffected in three out of four animals. The other animal shows a rapid decline in post-rotatory nystagmus from day 22 to 25 (when it was killed); elevated clinical chemistry parameters were noted at this time. Severe nephrosis was noted in remaining animals at histopathological evaluation, and it was concluded that apramycin has a greater nephrotoxic potential when administered parenterally (EMEA, 1999, FDA, 2009b).

No evidence of dermal sensitisation was noted in guinea-pigs following three weekly applications of 29.2 mg apramycin followed by a dermal challenge two weeks later (FDA, 2009a, FDA, 2009c).

Tests in swine did not reveal any ototoxic effect; the most frequently observed effects were irritation at site of injection and diarrhoea at high doses (EMEA, 1999).

3.3 Carcinogenicity and mutagenicity

In a carcinogenicity study, B6C3F1 mice were fed diets containing 0, 0.15, 0.5, 1.5 or 4.5% apramycin for up to two years; no evidence of carcinogenicity was observed. The NOEL was 0.15% diet, equivalent to approximately 200 mg/kg/day due to changes unrelated to carcinogenesis (see repeat dose toxicity section;(FDA, 2009a, FDA, 2009c).

In a carcinogenicity study on Fischer 344 rats fed diet containing up to 5.0% apramycin for up to two years, no evidence of carcinogenicity was observed. The NOEL was considered to be 0.25% diet, equivalent to 124 mg/kg/day in males and 154 mg/kg/day in females due to changes unrelated to carcinogenesis (see repeat dose toxicity section; (EMEA, 1999).

No evidence of mutagenic potential was shown in three standard *in vitro* mutagenicity assays using *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538), mammalian cells (mouse lymphoma assay) or an unscheduled DNA synthesis assay in primary rat hepatocytes (EMEA, 1999).

3.4 Reproductive and developmental toxicity

In a four-generation study, Fischer 344 rats were fed diet containing 0, 0.25, 0.5 or 1.0% apramycin (equivalent to 0, 194, 388 or 785 mg/kg bodyweight/day, respectively). No significant adverse effects on parental mortality, body weight or clinical signs were noted. Reproductive performance (including fertility, litter size, gestation period and fetal survival and offspring survival, weight and sex distribution) was unaffected. A NOEL of 785 mg/kg/day, the highest dose tested, was established (EMEA, 1999, FDA, 2009a, FDA, 2009c).

In a teratogenicity study in pregnant Wistar rats given apramycin orally at (0, 250, 500 or 1000 mg/kg/day from days 6 – 15 of gestation, there was no evidence of maternal toxicity, fetotoxicity or teratogenicity at any dose (EMEA, 1999).

In a rabbit teratogenicity study in pregnant females given oral apramycin at 0, 2, 8 or 32 mg/kg/day from days 6 – 18 of gestation, dose-related reductions in maternal food consumption and body weight gain were seen and there was a dose-related increase in numbers of dams aborting. The incidence of resorption was also significantly increased at 32 mg/kg/day dose. Fetotoxicity was noted at all doses and a dose-related reduction in fetal weight was reported. One case of cleft palate and one case of omphalocele (exact details unspecified) were observed in offspring of the 32 mg/kg/day group. However, as only five dams bearing 24 fetuses in this dose group survived the scheduled treatment period, the significance of this finding is uncertain. A dose-related increase in incidence of supernumerary ribs was also observed, though only the highest dose group showed levels above historical controls. It has been suggested that the effects on the fetus are secondary to maternal toxicity, however, a NOEL was not established for either maternal or feto-toxicity. Rabbits are, however, highly susceptible to the effects of antibiotics on their gut flora and this species may therefore be an inappropriate model for assessment of the teratogenicity of antibiotics (EMEA, 1999).

4 Guidelines and standards

A toxicological ADI of 0.25 mg/kg bw was derived by applying a safety factor of 100 to the NOEL of 25 mg/kg/day identified in a six month repeat dose toxicity study in the dog.

Residue depletion studies have demonstrated residues are highest in neonatal calves, particularly when dosed parenterally, reflecting the greater absorption compared with oral administration. The maximum residue limit (MRL) was determined using residue distribution data. In all the other species examined, low apramycin residues were apparent. In pigs, residues were only detected within one day of treatment in porcine skin and fat and were not detectable in porcine muscle and liver at any time point. On this basis, it has been estimated that customer intake of microbiologically active apramycin residues from porcine tissues would only occur if animals were killed within one day of treatment; assuming this, even then the intake would only equate to less than 13% of ADI (EMEA, 1999).

Residues of apramycin were not detected in ovine or chicken muscle nor in ovine fat at any time point. Low levels were detected in some liver, chicken skin and fat at time points close to that of dosing. It was estimated that within one day of end of treatment, consumer intake of microbiologically active residues from ovine and total residues in poultry tissues would amount to less than 15% and 6% of ADI, respectively. Residues in rabbit tissues were below the limit of detection at all time points. Estimation of MRLs for pigs, sheep, rabbits and chickens were considered unnecessary (EMEA, 1999).

With respect to the microbiological ADI, *in vitro* minimum inhibitory concentration (MIC) data were evaluated for bacteria originating from both human and veterinary sources. Many strains were insensitive to apramycin, and therefore it was decided to establish a microbiological ADI using the MIC_{50} for *Escherichia coli* (EMEA, 1999) giving a value of 40 µg/kg bw (i.e. 2400 µg/person). Since pharmacokinetic and metabolism studies has shown that almost all the material excreted is unmetabolised apramycin and that, in tracer studies, greater than 75% of radiolabelled material present in the liver and kidney was unmetabolised apramycin in cattle and pigs, it was concluded that apramycin is an appropriate marker residue for these tissues.

The CVMP recommended the inclusion of apramycin in Annex I of council regulation (EEC) No 2377/90 in accordance with MRLs as shown in Table 1.

Table 4.1 MRLs for apramycin

Pharmacologically active substance	Marker residue	Animal species	MRLs (µg/kg)	Target tissues	Other provisions
Apramycin	Apramycin	Bovine	1,000 1,000 10,000 20,000	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption

From EMEA, 1999

Using these microbiologically-based MRLs, EMEA estimated that daily intake from food sources in humans will not exceed 97% of ADI (EMEA, 1999).

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Cefapirin

1 Introduction

Cefapirin (5-Thia-1-azabicyclo (4.2.0) oct-2-ene-2-carboxylic acid, 3-((acetyloxy) methyl)-8-oxo-7-(((4-pyridinylthio)acetyl)amino)-, (6R-trans)-, molecular formula $C_{17}H_{17}N_3O_6S_2$, CAS No. 21593-23-7; Figure 1.1; Box 1) is a first generation semi-synthetic cephalosporin which posses broad spectrum activity for both gram-positive and gram-negative bacteria *in vitro* (EMEA, 2001, ChemID Plus).

Figure 1.1 Molecular structure of cefapirin

From ChemID Plus

Box 1 Synonyms and trade names

(6R,7R)-3-(Acetoxymethyl)-8-oxo-7-(2-(4-pyridylthio)acetamido)-5-thia-1-azabicyclo(4.2.0)oct-2-en-2-carbonsaeure; 3-(Hydroxymethyl)-8-oxo-7-(2-(4-pyridylthio)acetamidol-5-thia-1-azabicyclo(4.2.0)oct-2-en-2carbonsaeure acetate; 7-(2-(4-Pyridylthio)acetamido)cephalosporanic acid; BRN 1095157; Cefapirin; Cefapirina; Cefapirina [INN-Spanish]; Cefapirine; Cefapirine [INN-French]; Cefapirinum; Cefapirinum [INN-Latin]; Cefaprin; Cephalosporanic acid, 7-(2-(4-pyridylthio)acetamido)-; Cephapirin; Cephapirine; EINECS 244-466-5; HSDB 3215; UNII-89B59H32VN

From ChemID Plus

Cefapirin has twice been evaluated by the CVMP (EMEA, 1996).

Cefapirin possesses bactericidal activity through inhibition of bacterial cell wall synthesis via binding to one or more Penicillin Binding Proteins, which are located under the cell wall of susceptible bacteria. This causes high osmotic pressure to develop in the cell causing rupture of the cytoplasmic membrane. This, the bactericidal activity of cefapirin is similar that of most penicillins and cefalosporins (EMEA, 2001).

In human medicine, cefapirin (and other cephalosporins) is used primarily as an alternative to penicillin (Gilman *et al.*, 1990). It has also been used as a prophylactic to prevent post-operative infection (Effler *et al.*, 1982).

In veterinary medicine, cefapirin is used for intra-mammary treatment of mastitis in dry and lactating cows as the benzathine and sodium salt form at 200 - 300 mg/quarter (i.e. one of four mammary glands of the udder). The benzathine salt is also used for intrauterine treatment of endometritis at 500 mg/cow (EMEA, 2001).

2 Toxicokinetics

There is a lack of information on the oral absorption of cefapirin in humans. However, in mice it appears that oral bioavailability is low, since a greater than a 20-fold increase in dose is needed when given orally to achieve a given serum cefapirin level by parenteral routes (EMEA, 2001).

There is some data available on the kinetics of elimination of cefapirin when given parenterally which suggest that it is rapidly eliminated from the body. Injection of ten healthy volunteers via the i.v. or i.m. routes with 1 g cefapirin, resulted in a peak serum level of 72.6 µg/ml 15 minutes after the i.v. dose and 24.2 µg/ml 30 minutes after i.m. dosing. By six hours after injection, 72% and 53% of the i.v. and i.m. doses respectively, had been excreted in the urine (Axelrod *et al.*, 1972). In a further study i.v. cefapirin (1 g) to humans, gave plasma half lives of 0.5 and 0.43 hours of cefapirin and desacetylcefapirin respectively (Cabana *et al.*, 1976). Desacetylcephapirin has approximately 50% of the antimicrobial activity of the parent (Goodman & Gilman, 1975). When administered i.v., the kidney is thought to have a role in the metabolism of parent to desacetylcefapirin, due to renal plasma flow exceeding renal clearance of desacetylcefapirin. The excretion of both compounds is also via the kidney (Cabana *et al.*, 1975). Six hours after injection (1 g), approximately 48.5% of the dose was excreted in urine as parent and 45% as desacetylcefapirin (Cabana *et al.*, 1976). I.M. injection of cefapirin (1 g), gave an absorption half life of 1.25 hours and 100% of the dose appeared in the plasma as unchanged parent. However 24 hours after dosing, equal percentages (43%) of the dose was excreted as parent and as desacetylcefapirin (Cabana *et al.*, 1975).

The main metabolite in mice, rats and dogs, is also desacetylcefapirin, with the rate and extent of metabolism being lower in dogs than rodents. Following i.v. administration to dogs (30 mg/kg), cefapirin was found to rapidly distribute throughout the body, with a plasma half-life of approximately 0.4 hours. Approximately 8 hours after dosing, 32% and 66% of the administered dose was excreted as parent and desacetylcefapirin, accounting for 98% of the administered dose (Cabana *et al.*, 1976).

3 Toxicity profile

3.1 Acute toxicity

Cefapirin is structurally similar to penicillin and other cephalosporins and, as such, individuals allergic to these agents and may also have a reaction with cefapirin. Such human hypersensitivity reactions commonly present as anaphylaxis, bronchspasm and urticaria (Gilman et al., 1990). There is also a published report of two cases of cephalosporin induced hepatotoxicity with associated jaundice. In these, symptoms developed shortly after i.v. cephalosporin therapy commenced. Both patients fully recovered from jaundice, and it was concluded that these reactions represented hypersensitivity reactions similar to that seen in penicillin-induced liver toxicity (Eggleston & Belandres, 1985).

Experimentally, the oral LD_{50} in male rats is 14,000 mg/kg bw, while the i.p. LD_{50} in this species is 8400 mg/kg bw. In the dog, the i.v. LD_{50} value is 2500 mg/kg bw . The main effects observed were ataxia and convulsions (EMEA, 2001).

3.2 Repeat dose toxicity

There is some information on the response of humans to repeated parenteral administration. In a study in healthy volunteers, cefapirin was given by i.v. infusion at 0.5 g every six hours for one day and then 1 gram every six hours for a further four days. Eight of the 10 subjects developed mild phlebitis from cefapirin infusion (Lane *et al.*, 1972). In a further study, ten patients (eight of whom were heroin addicts) with bacterial infective endocarditis were treated with i.v. cephapirin (2 g every four hours, for four to six weeks). In all cases, cefapirin was well tolerated following i.v. administration and high serum levels were reached without toxicity or bioaccumulation (Burch *et al.*, 1983). In a study where cefapirin (50 mg/kg) was administered for the prevention of haemodialysis associated infections, cefapirin was given during the initial and final 30 mins of 5.5 hour haemodialysis sessions in patients with infectious episodes for five consecutive haemodialysis sessions. In these patients, cefapirin was well tolerated, with reports of transient itching and nausea only (Berman *et al.*, 1978).

In three month studies in rats and dogs in which sodium cefapirin was given orally at 20 mg/kg/day, no clear treatment related adverse events were noted. Thus, the oral NOEL for repeat dosing was established as 20 mg/kg/day cefapirin (EMEA, 2001).

Data are also available on the effects of this compound when given parentrally, although it should be noted that, given the poor oral absorption of the substance, similar effects would only be expected to potentially occur at very high oral intakes. Rats were administered sodium cefapirin at 0, 200, 500 or 1000 mg/kg/day i.p. for nine weeks or six months. After nine weeks, male body weight was slightly increased in all treated groups although no dose relationship was observed. At six months, all females and the high dose males showed a lower body weight gain than their controls. Some animals showed a mild dose-related anaemia, associated with reticulocytosis (EMEA, 2001). No NOEL was stated in the EMEA report.

In a ten week study, dogs were administered i.m. with 0, 100, 200 or 400 mg/kg/day. Increased kidney weights were noted at 400 mg/kg/day but no effects were observed at the other doses. In a six month study in which the same treatment regimen was adopted, severe anaemia occurred in some animals of all treated groups. This was considered to be due to a direct effect of cefapirin on red blood cell membranes (EMEA, 2001). No NOEL was stated in the EMEA report.

3.3 Carcinogenicity and mutagenicity

The cefapirin molecule does not contain structural alerts suggestive of mutagenic or carcinogenic activity.

While no studies have been performed on the carcinogenicity of this compound, the available repeat dose studies have identified no effects suggestive of preneoplastic change. Furthermore, an *in vitro* Ames *S. typhimurium* test, *in vitro* mouse lymphoma assay and *in vivo* oral mouse micronucleus test have been conducted and showed no evidence of mutagenicity (EMEA, 2001). The currently available data set was therefore considered adequate and did not warrant specific carcinogenicity studies (EMEA, 2001).

Since the EMEA summary report, no carcinogenicity studies have been published.

3.4 Reproductive and developmental toxicity

The effect of sodium cefapirin treatment by s.c. injection at 0, 200 or 400 mg/kg/day was evaluated in rats and mice in a series of studies comprising classical segment I and III studies in rats and classical segment II studies in both rats and mice. No reproductive, fetotoxic or teratogenic effects were reported (EMEA, 2001).

4 Guidelines and standards

A toxicologically-based oral NOEL of 20 mg/kg/day was considered appropriate based on 3 month studies in rats and dogs. However, in these studies only one dose level was evaluated. Therefore a safety factor of 200 was applied to give an ADI of 0.1 mg/kg bw (equivalent to 6 mg/person, assuming average body weight of 60 kg).

A microbiologically-based ADI of 2.54 µg/kg bw (equivalent to 153 µg/person) was also derived based on the minimum inhibitory concentration required to inhibit the growth of 50% of organisms (MIC_{50}) *in vitro* values for ten genera of bacteria representative of human intestinal flora. Since this is substantially lower than the toxicologically-based ADI, it will be used as the initial basis of the safety assessment.

The CVMP recommended the inclusion of cefapirin in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the MRLs shown below (Table 4.1). The metabolite, desacetylcefapirin is also included with cefapirin in the marker residue limits because of a number of reasons including the synergistic effect that has been observed with metabolite and parent in a number of species of bacteria, though synergy was only complete in a minority of tested strains, and observations *in vitro* of degradation of parent to metabolite (EMEA, 2001).

Table 4.1 MRLs for cefapirin

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues
Cefapirin	Sum of cefapirin and desacetylcefapirin	Bovine	50 µg/kg 50 µg/kg 100 µg/kg 60 µg/kg	Muscle Fat Kidney Milk

From EMEA, 2001

Based on these MRL values, EMEA estimate the daily intake based on consumption of bovine tissues and milk, to amount to approximately 73% of the microbiologically-based ADI (EMEA, 2001).

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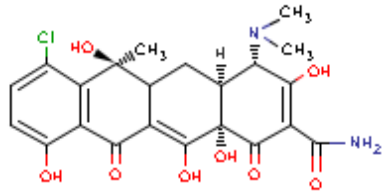
Chlortetracycline

1 Introduction

Chlortetracycline (Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, (4S,4aS,5aS,6S,12aS)-, molecular formula C22H23ClN2O8, CAS No. 57-62-5; Figure 1.1; Box 1 (ChemID Plus), is an antimicrobial drug with a long history of veterinary use, for the treatment and control of a wide range of bacterial infections. Chlortetracycline is administered via feed or water for prophylactic purposes. This drug has been extensively reviewed by JECFA and a number of pertinent publications are available. The tetracyclines, of which chlortetracycline is part of, are primarily bacteriostatic antibiotics that inhibit protein synthesis in susceptible bacterial strains (IPCS, 1996). They are active against a broad spectrum of Gram-positive and -negative microbial strains, as well as Mycoplasma and Chlamydia and some amoebae. The antimicrobial potencies of tetracyclines are similar, with minimal inhibitory concentrations (MICs) ranging from 0.1 to > 100 µg/mℓ for a number of clinical isolates.

Figure 1.1 Molecular structure of chlortetracycline



From ChemID Plus

Synonyms and trade names for chlortetracycline

7-Chlorotetracycline; Acronize; Aureocina; Aureomycin; Aureomycin A-377; Aureomykoin; Biomitsin; Biomycin; Biomycin a; CTC; CTC (abtibiotic); Caswell No. 219B; Chlortetracycline; Chrysomykine; Duomycin; EINECS 200-341-7; EPA Pesticide Chemical Code 006301; Flamycin; Trichlortetracycline; Uromycin

From ChemID Plus

2 Toxicokinetics

Detailed information concerning the absorption, distribution, metabolism and excretion of chlortetracycline are available (IPCS, 1996) and are summarised here. In humans, approximately 30% of the oral dose of chlortetracycline can be absorbed on an empty stomach. This occurred following oral administration of chlortetracycline (Sande & Mandell, 1990). Most absorption takes place from the stomach and upper small intestine and is greatest in the fasting state (Hardman *et al.*, 1996). Plasma half-lives for chlortetracycline are

reportedly 8–10 hours (Martindale, 1989). Tetracyclines have appeared in the milk of nursing mothers at approximately 60% of the plasma concentration of the antibiotic. In addition, tetracyclines can cross the placenta, reaching concentrations between 25 and 75% of the maternal plasma concentration (Martindale, 1989). The primary route of elimination for the tetracyclines is the kidney, although they can also be concentrated in the liver and excreted by bile into the intestine from where they are partially reabsorbed (Hardman *et al.,* 1996). Chlortetracycline is excreted in the urine and faeces in equal amounts. The major presumed metabolite of chlortetracycline in the dog and rat is 4-epichlortetracycline which is inactive in microbiological assays. However, there is uncertainty as to whether this is a true metabolite, or in fact a degradation product and artefact of the extraction protocol (Wulf & Eisner, 1961). Detectable levels of tetracyclines can persist in bone for more than 28 weeks after dosing. The volume of distribution of the tetracyclines is relatively larger than that of body water indicating that these drugs may be sequestered in body tissues (reticulendothelial cells of the liver, spleen and bone marrow, in bone and dentine and the enamel of unerupted teeth (Goodman & Gilman, 1975)).

Studies undertaken on the absorption of chlortetracyline are summarised as follows. In rats, ^{14}C-labelled chlortetracycline was orally administered at a dose of 60 mg/kg bw, i.p administered at a dose of 30mg/kg bw. Following oral administration, at 24, 48 and 72 hours radioactivity was measured in urine and faeces. Most of the radioactivity was detected in the faeces, 92% was excreted in the faeces within 72 hours. Approximately 5% of the radioactivity was recovered in urine. Following i.p administration, 33% of the radioactivity was excreted in the urine within the first 24 h, and 5% in the faeces. Between 24 and 72 h, 7% was excreted in the urine and 40% in the faeces (Wulf & Eisner, 1961).

Single oral doses of chlotetracycline were administered to groups of 6 rats. The administered dose was 75 mg/kg bw. The rats' plasma levels were 2.1 mg/l at 1 hour following administration, plasma levels declined to 0.8 mg/l at 6 hours following administration. The rats were killed at 1, 2, 3, 4 or 6 hours after treatment and residues were observed. The highest residue levels were found in the liver and kidneys in all cases. The maximum concentration occurred 2 hours following administration in the liver, and after 1 h in the kidney (Berté & Vandoni, 1962).

Oral doses of chlortetracycline were administered to female rats and male guinea pigs. The doses ranged from 6 to 800 mg/kg bw and no proportional increases in serum chlortetracycline concentrations were observed. Guinea-pigs receiving the same doses each day for 9 days had higher serum levels compared to levels found for the single doses. Serum chlortetracycline levels were found to increase following simultaneous administration of adjuvants, such as citric acid. This effect (observed in doses of chlortetracycline up to 200 mg/kg bw) occurred within 1 h following administration for up to 8 hours (Eisner *et al.*, 1953).

3 Toxicity profile

3.1 Acute toxicity

Severe toxicity following acute overdose is unlikely (HSDB, 2002). No toxic effects were observed in premature infants, children, adults or the elderly when administered with chlortetracycline. Some effects observed following short and long term exposure included an increase in body-weight gain, and prophylactic activity against acute and chronic infections and diarrhoea (Hines, 1956).

Because tetracycline drugs are poorly absorbed form the human intestine, high concentrations are readily achieved in the intestine within 48 hours of daily treatment (IPCS, 1998).

However, new data on the effects of tetracycline drugs on human intestinal microflora were used in a later evaluation of the effects of these residues in food (IPCS, 1996). These investigations showed that a dose equivalent to 2.5 mg/kg bw tetracycline in a chemostat culture method caused an initial increase in the number of resistant *E. coli* strains (from < 20 to >60% after 48 hours); however, the increase fell to approximately 35% by day 6 of exposure, despite continued treatment with the drug (IPCS, 1998).

Orally administered chlortetracycline has moderate acute oral toxicity in rats and mice. Acute oral LD_{50} values in rats or mice for chlortetracycline range from 2150 to >4000 mg/kg bw (IPCS, 1996).

Reports suggest that all of the tetracyclines (dose levels not specified) can cause phototoxicity. Skin reactions may vary from mild to severe on exposure to direct sunlight (Sande & Mandell, 1990, Schindel, 1965).

3.2 Repeat dose toxicity

Repeated dose toxicity tests of chlortetracycline in rats or mice did not cause any toxic effects (IPCS, 1996).

Following repeated parenteral administration of tetracyclines, liver effects such as fatty accumulation have been observed. No dose levels were specified. In patients with impaired renal functions, tetracyclines can aggravate uremia (urine in the blood) (Sande & Mandell, 1990, Pratt & Fekety, 1986, Friedman *et al.,* 1990).

3.3 Carcinogenicity and mutagenicity

No evidence for carcinogenicity of chlortetracycline was observed in long-term studies in mice or rats (IPCS, 1998). No evidence for carcinogenicity was observed in rats treated with chlortetracycline up to 1000 mg/kg bw/day for 52 weeks.

The genotoxicity of chlortetracycline was tested for in a range of *in vitro* and *in vivo* test systems. Chlortetracycline tested negative in all tests. It was concluded that chlortetracycline did not pose a genotoxic hazard (IPCS, 1996**)**.

3.4 Reproductive and developmental toxicity

All tetracyclines can form a stable calcium complex in bone-forming tissue, therefore they can cause permanent discolouration of the teeth, enamel hypoplasia and inhibition of skeletal growth in the foetus, infants and children up to 8 years of age; for these reasons they should not be used in these groups. Tetracyclines cross the human placenta and are distributed into breast milk (USP Convention. USPDI, 1997).

No effects of chlortetracycline on reproduction were observed in a two-generation study of rats treated with the drug at 500 mg/kg bw/day. Therefore it was concluded that chlortetracycline did not cause significant toxic effects on either reproduction or development (IPCS, 1996).

4 Guidelines and standards

JECFA had established an ADI of 0–30 µg/kg bw for the tetracyclines (chlortetracycline, oxytetracycline and tetracycline) separately or in combination. This was based on the NOEL of 2 mg/person/day for the effects of oxytetracycline on the gut flora in human volunteers, a safety factor of 10, and an average human body weight of 60 kg. The lowest NOEL for toxicological effects observed for any of these drugs was 100 mg/kg bw/day for

chlortetracycline in dogs. The ADI therefore provided an adequate margin of safety for both toxicological and microbiological effects (WHO. JECFA, 1999).

MRLs established by JECFA (WHO. JECFA, 2001) with chlortetracycline alone or in combination with oxytetracycline and tetracycline are summarised in Tables 4.1. This ADI value of 30 µg/kg bw has been used in the current assessment.

Table 4.1 MRLs for Chlortetracycline

Pharmacologically active substance(s)	Species	Target tissue	MRLs (µg/kg or µg/L)
Chlortetracycline	Bovine/Porcine/Ovine	Kidney	1200
		Liver	600
		Muscle	200
	Bovine/Ovine	Milk	100
	Poultry	Eggs	400

From WHO.JECFA, 2001

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Clorsulon

1 Introduction

Clorsulon (4-Amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide; molecular formula $C_8H_8Cl_3N_3O_4S_2$; CAS No. 60200-06-8; Figure 1.1; Box 1) belongs to the benzene sulphonamide family and is recommended for the control of adult liver flukes (*Fasciola hepatica* and *Fasciola gigantica*) in cattle (EMEA, 1998; ChemID Plus).

Figure 1.1 Molecular structure of clorsulon

From ChemID Plus

Box 1 Synonyms and trade names

1,3-Benzenedisulfonamide, 4-Amino-6-(trichloroethenyl)-; 4-Amino-6-(trichlorovinyl)-m-benzenedisulfonamide; BRN 2821757; Clorsulon [Spanish]; Clorsulone [French]; Clorsulonum [Latin]; Curatrem; EINECS 262-100-2; L 631529; L-631,529; MK 401; UNII-EG1ZDO6LRD

(From ChemID Plus)

Clorsulon can be administered as oral suspension or as an injectable formulation, at 7 mg/kg orally or 2 mg/kg subcutaneously. Clorsulon is often used with ivermectin – a broad spectrum anti-parasitic agent. Clorsulon is not used in human medicine (EMEA, 2008).

Studies *in vitro* have suggested clorsulon acts by direct inhibition of 3-phosphyglycerate kinase and phosphoglyceromutase, which blocks the glycolytic pathway of flukes. Clorsulon has efficacy in both mature and immature flukes in cattle and sheep (Chiu *et al.*, 1989).

Clorsulon has been evaluated by the EMEA CVMP on three occasions, most recently resulting in a change to the ADI due to the identification of discrepancies in the identity of the test substance used in some studies (i.e. related substances not clorsulon itself used; (EMEA, 2008). This latest EMEA report is the primary source used for this assessment, as no other authoritative literature reviews were identified for clorsulon.

2 Toxicokinetics

No toxicokinetics studies in humans have been identified.

In cattle, intraruminal dosing of ^{14}C-clorsulon at 10 mg/kg led to maximal plasma levels (~3,000 µg/L) 24 hours after dosing; this fell to 14 µg/L by day 21. The percentage of clorsulon to total residue was evaluated at 7, 14 and 21 days after administration a single

intraruminal dose ^{14}C-clorsulon at 10 mg/kg. After seven days, values were 75% in kidney, 55% in liver and 41% in muscle, while levels in fat were too low for a value to be established. Fourteen and 21 days after administration, levels were 67% and 74% in kidney and 47% and 61% in liver, respectively; levels in muscle were to low for a percentage to be established (EMEA, 2008).

After an intraruminal dose of 10 mg/kg ^{14}C-clorsulon was given to steers (castrated male cattle), approximately 80% of radioactive residues were extracted (by organic solvents) from the liver and kidney. Acid hydrolysis of the liver revealed two major metabolites, an acetaldehyde derivative (2.9%) and butyric acid derivative (6.2%). Several other metabolites were identified of which ten were less polar and three more polar entities. However, individually each of these compounds did not account for more than 5% of the total residue. In the kidney, the major component recovered was the parent with other components (five less and three more polar) accounting for no more than 5% of the total radioactivity (EMEA, 2008).

Following subcutaneous administration (therapeutic route) of ^{14}C-clorsulon at 2 or 3 mg/kg to cattle, maximum plasma concentrations of 1290 and 2500 µg/l respectively, were observed 6 hours after injection. Seven days after treatment, plasma concentrations were close to the limit of detection (10 µg/l). In another study, intraruminal administration of ^{35}S-clorsulon at 6.6 mg/kg or ^{14}C-clorsulon at 15 mg/kg led to approximately 90% of the dose being excreted within seven days of which approximately 70% was in faeces and approximately 30% in urine (EMEA, 2008).

In sheep and goats given 7 mg/kg clorsulon i.v or orally, peak plasma concentrations occurred at 14 and 15 hours, in the goats and sheep respectively. After i.v. administration, the elimination half lives were 17 and 12 hours in sheep and goats respectively. However absorption from the gastro-intestinal tract increased the elimination half life to 28 hours in sheep and 23 hours in goats after oral administration. In both species, approximately 50 % of the dose was recovered in the urine following i.v. dosing. Following oral administration, only 41% and 30% of the dose was recovered, from goats and sheep respectively. The elimination rate constant was twice as large in goats as in sheep; this could account for the reduced efficacy of clorsulon observed in goats (Sundlof & Whitlock, 1992).

3 Toxicity profile

3.1 Acute toxicity

Oral LD_{50} values were in excess of 10,000 mg/kg in mice and rats. The intraperitoneal clorsulon LD_{50} in mice and rats ranged from 678 – 938 mg/kg (EMEA, 2008).

Clorsulon has been reported to be extremely safe when given as intraruminal doses; up to 400 mg/kg did not induce any frank toxicity in sheep (Chiu *et al.*, 1989).

3.2 Repeat dose toxicity

In a thirteen week oral toxicity study in rats (n=10 rats/sex/group), clorsulon was added to the diet at 20, 150 or 425 mg/kg bw/day. Details of the strain of rats was not included in the EMEA summary report. Results showed increases in relative organ weights of thyroid, adrenal, brain, kidney, spleen and lung in high dose animals. Urinary bladder hyperplasia was noted in seven males and one female, and kidney pelvic epithelial hyperplasia was noted in one male and five females at the high dose at which thyroid follicular cell hyperplasia was noted in four males. At 150 mg/kg/day, a significant increase in relative thyroid weight was noted in males; this associated with three cases of thyroid follicular hyperplasia. Urinary bladder hyperplasia was noted in six males at this dose. At the low dose, a significant increase

in relative thyroid weight was observed in males, at a magnitude that mirrored the mid dose group. However, at the low dose, the increase in organ weight did not correlate with histopathological findings. A NOEL was not identified due to the findings in the thyroid organ weight changes (EMEA, 2008).

Dogs were orally dosed for 14 weeks at 0, 2, 8 or 32 mg/kg/day (details of the strain of dogs were not stated in the EMEA summary report). In the absence of any effect on thyroid weights, a NOEL was identified as 2 mg/kg/day (EMEA, 2008).

A 54 week oral toxicity study in rats was performed with acetazolamide, a carbonic anhydrase inhibitor (an activity that clorsulon also possesses). Details of the strain of rats and doses used were not stated in the EMEA summary report but results suggest that the bladder hyperplasia noted in the studies described above on clorsulon could be due to changes in urine composition following carbonic anhydrase inhibition rather than a direct action of clorsulon (EMEA, 2008).

3.3 Carcinogenicity and mutagenicity

Two carcinogenicity studies in mice were conducted at 44, 120 or 306 mg/kg/day for two years. However due to the poor survival rate (20%), the value of the study is limited. In a study in rats which included *in utero* exposure to clorsulon at 3.8, 12.6 or 48.8 mg/kg/day for 126 weeks and which showed an overall 50% survival rate, a conclusion was reached that clorsulon was not carcinogenic in this species (EMEA, 2008). The EMEA summary report does not state the strains used, routes of administration or details of the results obtained.

Three *in vitro* tests for mutagenicity, a *Salmonella*-microsomal assay, an unscheduled DNA synthesis assay in human MRL-90 fibroblasts and measurement of DNA single strand breaks by alkaline elution in human MRL-90 fibroblasts, were negative. The EMEA summary report did not indicate the doses used, strains or if metabolic activation was included. In contrast to these findings, two *in vivo* tests using a bone marrow micronucleus assay (oral doses of up to 2000 mg/kg in mice) and a chromosomal aberration test (oral doses of up to 500 mg/kg in mice) were positive (EMEA, 2008); strain of animal was not specified.

Although results were positive in *in vivo* mutagenicity studies and urinary bladder hyperplasia was noted in the 13 week repeat dose oral toxicity studies in the rat, clorsulon was suggested to have acted via an indirect mechanism; increasing urinary pH and urine composition. Further support for this mechanism comes from the observation that addition of 5% ammonium chloride (acidic) to test diets reduced incidence and severity of urothelial cell hyperplasia and the reversibility of the hyperplasia persisted despite continued treatment, thus confirming that the lesion was not preneoplastic (Lankas & Peter, 1992). EMEA therefore concluded that clorsulon is not carcinogenic (EMEA, 2008).

3.4 Reproductive and developmental toxicity

In a three-generation study in rats, oral doses of 0, 3, 30 or 300 mg/kg/day clorsulon significantly affected reproductive performance in females, and viability and growth of offspring in each generation, at 300 mg/kg/day; details of study design were not presented. Since there were no treatment-related effects on reproductive performance at 3 or 30 mg/kg/day, a NOEL of 30 mg/kg/day was established (EMEA, 2008).

In teratogenicity studies in mice and rabbits at oral doses of 0, 2, 10 or 50 mg/kg/day, the methodological details of which were not presented, negative findings were noted. In mice, there were no signs of maternotoxicity at any doses but a significant decrease in fetal weight was apparent at 50 mg/kg/day; the NOEL for fetotoxicity was 10 mg/kg/day. In rabbits, there

were signs of maternal and fetal toxicity (reduced body weights at 10 and 50 mg/kg/day). The NOEL for maternal and fetal toxicity were 2 and 10 mg/kg/day, respectively (EMEA, 2008).

4 Guidelines and standards

A toxicological-based ADI of 0.002 mg/kg (0.120 mg/person) was established based on applying a safety factor of 1000 to the NOEL of 2 mg/kg/day identified in a 14 weeks study in dogs. This safety factor was used based on the standard of 100 and the addition of a factor of 10 due to the positive results from the mutagenicity studies and the inadequacies within the carcinogenicity studies (EMEA, 2008).

In residue depletion studies, it was concluded that by seven days after intraruminal treatment of clorsulon, the parent compound represented approximately 75, 55 and 41 % of total residues in the kidney, liver and muscle respectively. Residues in the fat were low and level of the parent compound could not be established. Therefore fat was considered not to be a target tissue. Clorsulon itself was identified as the marker residue (EMEA, 2008).

The CVMP recommended the inclusion of clorsulon in Annex I of Council Regulation (EEC) No 2377/90 in accordance with MRL as shown in Table 4.1:

Table 4.1 MRLs for clorsulon

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues
Clorsulon	Clorsulon	Bovine	35 µg/kg 100 µg/kg 200 µg/kg	Muscle Liver Kidney

From EMEA, 2008

Based on these MRLs, EMEA estimate the daily intake of total residues (0.057 mg) to represent approximately 48% of the toxicological ADI (EMEA, 2008).

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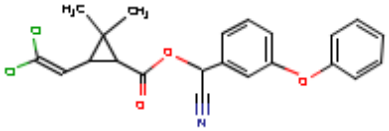
Cypermethrin

1 Introduction

Cypermethrin ((RS)-α-cyano-3-phenoxybenzyl,(1RS,3RS;1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate, molecular formula $C_{22}H_{19}Cl_2NO_3$, CAS No. 52315-07-8), is a type II synthetic pyrethroid insecticide (ChemID Plus). Cypermethrin, along with all synthetic pyrethroids, is a neuropoison that acts on the axons in the peripheral and central nervous systems by interacting with sodium channels in mammals and/or insects (IPCS, 1989).

Figure 1.1 Molecular structure of cypermethrin



From ChemID Plus

Synonyms and trade name for cypermethrin

(+)-alpha-Cyano-3-phenoxybenzyl-(+)-cis,trans-2,2-dichlorovinyl-2,2-dimethylcyclopropanecarboxylate; (+-)-alpha-Cyano-3-phenoxybenzyl 2,2-dimethyl-3-(2,2-dichlorovinyl)cyclopropane carboxylate; (+-)-alpha-Cyano-3-phenoxybenzyl-(+-)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate; (RS)-alpha-Cyano-3-phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; 3-(2,2-Dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic; acid cyano(3-phenoxyphenyl)-, methyl ester; AI3-29295; Agrothrin; Ambush C; Ambush CY; Ammo; Antiborer 3767; Ardap; Arrivo; Asymmethrin; BRN 2422506; Barricade; Barricade 10EC; Basathrin; Beta-cypermethrin; CCN 52; CCRIS 2499; Caswell No. 268AA; Chinmix; Colt; Creokhin; Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcycloptopanecarboxylate; Cyano(3-phenoxyphenyl)methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; Cymbush; Cympa-Ti; Cymperator; Cypercare; Cyperco; Cypercopal; Cyperil; Cyperkill; Cypermethrin; Cypermethrin-25EC; Cypermethrine; Cypersect; Cypor; Cyrux; Demon; Demon TC; Drago; Dysect; EINECS 257-842-9; EPA Pesticide Chemical Code 109702; EPA Pesticide Chemical Code 129064; EXP 5598; Ecofleece Sheep Dip (Non-OP); Ectomin; Ectopor; Excis; FMC 30980; FMC 45497; FMC 45806; Fenom; Flectron; Fury; HSDB 6600; Hilcyperin; JF 5705F; Kalif Super; Kordon; Kreokhin; Mustang; NRDC 149; Neramethrin; Neramethrin EC 50; Nurele; Nurelle; PP 383; Polytrin; RU 27998; Ripcord; Rycopel; SF 06646; Sherpa; Siperin; Supercypermethrin; Supercypermethrin forte; Topclip Parasol; Toppel; Ustaad; Vucht 424; WL 43467; WL 8517; Wrdc149; YT 305; Zeta-cypermethrin; beta-Cypermethrin; cis-Cypermethrin

From SIS NLM (2009)

Cypermethrin consists of a mixture of 4 *cis*- and 4 *trans*-isomers, the ratio of which (between 40:60 and 80:20) depends on the commercial product and its manufacturer (EMEA, 2001). The *cis*-isomers are more biologically active. α-Cypermethrin is a racemic mixture of the insecticidally most active enantiomer pair of the four *cis*-isomers of cypermethrin (>90%; (IPCS, 1996).

Since the toxicity of α-cypermethrin is four times greater than that of cypermethrin (IPCS, 1992), and the toxicity of the two products is well documented, both have been discussed,

where information is available, in this assessment. There are many publicly available data on the properties of cypermethrin and α-cypermethrin, including reports from EMEA CVMP, JECFA, JMPR and WHO, and these have all been used in preparing this report. Many of the toxicity studies for cypermethrin were conducted over 20 years ago so do not comply with current test guidelines or meet current standards of reporting.

Cypermethrin is used topically (dip, spray or pour-on) for the control of ectoparasites, such as ticks, fleas, lice and blowflies, in cattle, sheep, goats, pigs and poultry (EMEA, 1998). It is also used to treat sea lice in salmon and is widely used as an insecticide on crops (Tomlin, 1995).

2 Toxicokinetics

The toxicokinetics of cypermethrin has been studied extensively, and the kinetics of cypermethrin and α-cypermethrin are comparable. In addition, there does not appear to be any isomeric interconversion during metabolism (IPCS, 1996). All reviews are in agreement that cypermethrin is readily absorbed following oral administration in a number of species including rats, mice and humans (EMEA, 2004). Absorption from the gastrointestinal tract is most rapid for the *trans*-isomers (IPCS, 1998). Owing to its lipophilic nature, cypermethrin is largely distributed to fatty tissue, the liver and kidneys. The half-life of *cis*-cypermethrin in the adipose tissue of experimental rats is in the range of 12–19 days and that of the *trans*-isomer, 3-4 days. In mice, these half-lives are 13 days and 1 day, respectively (IPCS, 1989). The metabolic profile of cypermethrin is complex owing to the number of isomers. However, metabolic transformation is similar in the experimental animals studied and in humans. Differences relate to the rate of formation rather than to the nature of the metabolites formed (IPCS, 1989). All isomers undergo cleavage of the ester bond to produce cyclopropane carboxylic acid and a 3-phenoxybenzyl moiety, which is oxidised to form a phenol. The phenol is then almost totally conjugated to form the main metabolite (Crawford & Hutson, 1977). The type of conjugate varies in some species but humans and rats excrete the sulphate (IPCS, 1992). Cyclopropane carboxylic acid is the second most important metabolite. Other aryl metabolites comprise a small proportion of the total metabolites (Crawford & Hutson, 1977). Elimination of cyclopropane carboxylic acid in the rat, over a 7-day period, was in the range 40–60% in the urine and 30–50% in the faeces; elimination of the 3-phenoxybenzyl moiety was about 30% in the urine and 55–60% in the faeces. Biliary excretion is a minor route of elimination for the cyclopropane moiety and small amounts are exhaled as carbon dioxide. Following administration, cypermethrin is rapidly eliminated via the urine and faeces, with the largest part of the absorbed dose being excreted in the urine (Crawford *et al.*, 1981).

Following dermal exposure, absorption of cypermethrin was much slower than following oral administration, and the extent of metabolism was reduced (EMEA, 1998).

An analytical method based on GC-ECD has been used to determine cypermethrin in bovine and ovine tissues. Quantification limits are 10 µg/kg in muscle, liver and kidney and 100 µg/kg in fat tissue. This method has been validated in accordance with the European Union and should be applicable to other ruminant species, therefore extrapolation to the tissues and milk of others is possible (EMEA, 2004).

3 Toxicity profile

3.1 Acute toxicity

Moderate intoxication occurred in 45 humans after oral ingestion of cypermethrin of up to 140 mg/kg, all cases survived following gastric irrigation (Hayes Jr & Laws Jr., 1991). No further details were available.

Cypermethrin poisoning was reported in 5 employees of an accounting office where exposure occurred due to insect control within the office. Symptoms such as dizziness, headaches, nausea, & vertigo occurred immediately, even though workers entered the building two days after treatment and switching on the air conditioning increased the severity of their symptoms. Symptoms were still reported when employees, on several occasions, tried to re-enter the building. Wipe samples showed the maximum concentration of 4240 µg/sq ft to be on the carpet. Exact exposure levels of workers when the air conditioning was switched on is unknown (Lessenger, 1992).

The acute toxicity of cypermethrin is variable and dependent upon the ratio of *cis*- and *trans*-isomers, the age, sex, and strain of the test animal, nature of the solvent and the environmental conditions (EMEA, 1998). The *cis*-isomers are more acutely toxic than the *trans*-isomers (EMEA, 1998). Cypermethrin is moderately toxic, whereas α-cypermethrin is moderately to highly toxic (3–4 times more toxic than cypermethrin; IPCS, 1996). In rats and mice, the oral LD_{50} values were in the range 82 – 4000 mg/kg bw for cypermethrin, and 35 – >5000 mg/kg bw for α-cypermethrin (IPCS, 1996). The variations were due to the range of vehicles used. The signs of intoxication were salivation, tremors, ataxia, increased startle response, sinuous writhing of the whole body (choreoathetosis), and clonic seizures (IPCS, 1996). Myelin and axon degeneration were noted in the sciatic nerve at near lethal doses. The onset of symptoms was rapid and disappeared within several days in animals that survived (IPCS, 1996).

Acute toxicity following dermal exposure is low for cypermethrin and α-cypermethrin, with LD_{50} values >2000 mg/kg bw irrespective of the vehicle used (IPCS, 1996).

Cypermethrin was moderately to severely irritating (depending on the vehicle), when applied to the skin or the eye of the rabbit (see also below). In guinea-pigs, a mild skin sensitising potential was found using the maximization test (Combs *et al.*, 1976)**.**

3.2 Repeat dose toxicity

Many data are available to determine the potential for cypermethrin to cause toxicity following repeated exposures and to compare cypermethrin with α-cypermethrin.

Cypermethrin

A study on occupational exposure to cypermethrin in humans reported short term paraesthesia effects on the face and other body areas. The paraesthesia was considered to be due to cypermethrin reducing the spontaneous repetitive firing of the local sensory nerve endings. Details of the route, level and duration of exposure were not described in the EMEA Summary Report (EMEA, 2004).

Wistar rats (12/sex/dose) were fed diets containing cypermethrin at concentrations of 0 (24 animals), 25, 100, 400 or 1600 mg/kg for 91–95 days. In the highest dose group reduced body weight gain was observed; however, reduced food consumption was only noted in the first week, and week 13 in females. Males in the highest dose group had decreased haemoglobin, mean corpuscle volume and eosinophil counts, as well as an increased prothrombin time. At 400 mg/kg, there was a decrease in eosinophil numbers. Mean plasma urea concentration was

increased in the 1600 mg/kg group. Relative liver weights were increased in both sexes at the highest dose, but only in males at 400 mg/kg. Males also had increased kidney weights at 1600 mg/kg. The NOAEL for this study was 100 mg/kg feed, which is equivalent to 5 mg/kg bw/day (Pickering, 1981).

Beagle dogs were administered doses of cypermethrin (0, 1, 5, 15 mg/kg/day) in corn oil for 52 weeks. Males and the females in the high dose group showed body tremors, abnormalities (specifics were not available) and in-coordination, disorientation, and hypersensitivity to noise. For all dose levels an increase in vomiting occurred during the first week. The passing of liquid faeces occurred throughout the study, the incidence was 10-fold for groups dosed with 5 mg/kg/day and 30 fold for groups dosed with 15 mg/kg/day. No further details concerning the control group was available. A NOEL for systemic effects of 1 mg/kg/day was derived based on the increased incidence of liquid faeces (Sax, 1987).

In the only available dermal toxicity study, New Zealand White rabbits (10/sex/dose) were exposed via occluded patches to abraded and intact skin, to doses of 0, 2, 20 or 200 mg cypermethrin/kg bw in PEG 300. The applications were for 6 hours/day, 5 days/week for three weeks. Slight to severe skin irritation was observed at the highest dose, and slight to moderate irritation at the other two doses. Food consumption and body weight gain were reduced in both sexes at the highest dose, as were absolute and relative gonad weights in males. No effects on haematology, clinical chemistry or following macroscopic or microscopic examination were observed (Henderson & Parkinson, 1981).

In vitro studies indicate cypermethrin may possess an immunomodulatory effect and antibody production effects in rats and rabbits *in vivo* were also shown. However, no effects were observed for immunological, haematology and pathological tests using α-cypermethrin following oral doses of 0, 4, 8 or 12 mg/kg bw/day to rats for 28 days (EMEA, 2004).

α-Cypermethrin

In a 13-week feeding study, CD-1 mice (12/sex/dose) received diets containing 0, 50, 250 or 1000 mg α-cypermethrin/kg feed. Four males that received the highest dose died during week 12, which the authors suggested was probably due to the stress caused by the treatment and consequent refusal to eat or drink. The relative kidney weights of these animals were slightly higher than those of the controls (statistical significance not given). One animal died at 250 mg/kg feed. Clinical signs observed at 1000 mg/kg feed included thin build, ungroomed coat, hair loss and encrustations of the dorsal body surface. At the middle dose ungroomed hair was noted in both sexes, and two males also had hair loss and encrustations. Two males had hair loss at the lowest dose. It is not known whether these effects were observed in the control group. Body weight gain was significantly decreased at the highest dose. The food conversion efficiency of animals at 1000 mg/kg feed was lower than that of the controls. In addition, males at the highest dose had decreases in haematocrit, haemoglobin, red blood cells, total white blood cells and leucocyte counts. There was a dose-dependent increase in serum aspartate aminotransferase in males at 250 and 1000 mg/kg feed, and a decrease in glucose at 1000 mg/kg feed. Serum alkaline phosphatase was increased in females at the highest dose. Urinary specific gravity was increased in both sexes at the highest dose. Numerous organ weight changes were observed, mainly at the highest dose, but also at 250 mg/kg feed. In males, there were increases in relative brain, adrenal, heart, kidney, liver, spleen, lung and testes weight at the highest dose. In females, increases in relative brain and spleen weight were noted at 1000 mg/kg feed, and increases in relative liver weight at 250 and 1000 mg/kg feed. However, no histological changes were observed. Two male and 11 female mice that had received the highest dose were considered to be emaciated at necropsy. The authors concluded that a NOAEL could not be determined from this study, as hair loss was observed at all doses (Amyes *et al.*, 1994). Similar effects were noted in a study that dosed CD-1 mice with 0, 200, 400, 800, 1200 or 1600 mg α-cypermethrin/kg feed for 29

days. In addition to the effects on general appearance, organ weights and blood parameters observed in the above study, some neurological disturbances (not specified) were also observed in animals at the two highest doses (Green, 1993).

In a 13-week study, Wistar rats (30/sex/dose; control group 60/sex) were fed 0, 20, 60, 180 or 540 mg α-cypermethrin/kg diet (equivalent to 0, 1, 3, 9 or 27 mg/kg bw). After six weeks of exposure, 10 rats/sex (controls 20/sex) were killed for interim haematological, clinical chemical and gross post-mortem examinations. The remaining animals were killed after 13 weeks. Signs of intoxication, such as abnormal gait characterised by splayed hind limbs, were found in three males fed 540 mg/kg diet (duration of exposure not given). No axonopathy was observed in these animals. Several instances of transient skin sores and fur loss were observed in all males, particularly at the highest dose, and in females of the control and 540 mg/kg dose group. There was decreased growth, which correlated with decreased food intake, in both sexes fed 540 mg/kg from the first week onwards. During the second part of the study, body weight gain was also reduced in males at 60 and 180 mg/kg, but not in a dose-dependent manner. No clear effects on the haematological and clinical chemical parameters were found. There were increases in the relative kidney, liver weights at the highest dose, as well as increased relative liver weights at 180 mg/kg, in both sexes. In females, increases in relative spleen, heart and brain weights, were also noted at the highest dose only. An increase in testes weight was observed in males at 540 mg/kg. The statistical significance of these organ weight changes is not known. No histopathological abnormalities were found except sparse axonal degeneration in the sciatic nerve, without clinical signs of toxicity, in two males fed 540 mg/kg. No effects were found in the 60 mg/kg group, which is equivalent to 3 mg/kg bw/day (Clark, 1982).

Two additional sub-chronic studies in rats are available. Although these are not ideal studies for predicting the long-term effects of α-cypermethrin in humans, they do support the findings of the above study in rats.

Groups of Wistar rats (10/sex/dose) received diets that contained 0, 20, 100, 200, 400 or 800 mg α-cypermethrin/kg for five weeks. At the highest dose, two males had to be killed owing to development of severe neurological disturbances. Other animals also showed signs of neurotoxicity, such as abnormal gait and increased sensitivity to noise. Abnormal gait was observed in only one animal at 400 mg/kg. At the two highest doses, mean body weights and food intake were significantly lower than those of the controls. There were increased brain kidney and liver weights at the highest dose, but also at 400 mg/kg to a lesser extent. One male that was removed from the study showed scanty axonal lesions of the sciatic nerves. The NOAEL for this study was 200 mg/kg feed, which is equivalent to 10 mg/kg bw/day (Thorpe, 1982).

In a range-finding study, rats (5/sex/dose) were given diets that contained α-cypermethrin at concentrations of 0, 50, 200, 800 or 1200 mg/kg feed for six weeks. No effects on food consumption, haematology, clinical chemistry and microscopy were found. However, all animals at 1200 mg/kg and all males at 800 mg/kg were killed in weeks 2–4 due to severe clinical signs, which included high stepping, splayed gait, abasia and hypersensitivity. At 800 mg/kg female rats had a lower mean body weight, food intake and leucocyte count than the control animals. Lymphocytolysis and lymphocyte depletion of the cortical region of the thymus in males at 800 mg/kg and both sexes at the highest dose, were recorded following a microscopic examination (Fokkema, 1994). The NOAEL was 200 mg/kg, which is equivalent to 10 mg/kg bw/day.

The following study using beagle dogs was conducted in two parts. In the first part of the study, the dogs (one/sex/dose) were fed α-cypermethrin in the diet at the following concentrations: 200 mg/kg diet for 7 days, 300 mg/kg diet for 7 days and 400 mg/kg diet for 2 days (dosing discontinued due to severe intoxication). No signs of toxicity were observed at

the lowest dose. However, dosing with 300 or 400 mg/kg caused weight loss, ataxia, subdued behaviour, head nodding, food regurgitation, inflammation of gums and tongue, body tremors and diminished response to stimuli. Haematological, clinical chemical and gross pathological examination showed no effects (Greenough & Goburdhun, 1984). In the second part of the study, beagle dogs (one/sex) received 300 mg/kg diet for 3 (male dog) or 4 days (female dog) and 250 mg/kg diet for 7 days. Both animals showed the above-mentioned signs of intoxication, the only difference being that when it was being fed the lower concentration, the female dog showed these signs more frequently than the male dog. There were no effects on haematology, clinical chemical parameters, urinalysis, or gross pathology (Greenough & Goburdhun, 1984). A NOAEL could not be obtained from this study.

Beagle dogs (4/sex/dose) received a diet containing α-cypermethrin at 0, 30 or 90 mg/kg diet for 13 weeks, an additional dose group of six dogs per sex was given α-cypermethrin in the diet at a concentration of 270 mg/kg for 13 weeks. All animals at 270 mg/kg diet exhibited whole body tremors, head nodding, 'lip-licking', subduedness, ataxia, agitation and a high-stepping gait, which increased in both intensity and duration as the study progressed, and one female was killed because the effects were severe. Food consumption, body weight gain, organ weights, ophthalmoscopy, haematological and clinical chemical parameters, urinalysis, gross pathology and microscopy of 18 organs and tissues of all test groups showed no dose-related effects. In this study, the NOEL was considered to be 90 mg/kg diet, which is equivalent to 2.25 mg/kg bw/day (Greenough *et al.*, 1984).

In a more recent study, groups of beagle dogs (4/sex/dose) received α-cypermethrin via the diet at concentrations of 0, 60, 120 or 240 mg/kg for 52 weeks. Two males at the highest dose developed skin reddening on their tails, which in one of these animals led to ulceration and necrosis that meant that part of the tail had to be amputated. Abdominal skin reddening and alopecia were observed in one male at 240 mg/kg and one female at 120 mg/kg. Treatment did not affect body weights, food consumption, ophthalmology, haematology, clinical chemistry, or urinary parameters, or organ weights. In addition, no changes were observed in macroscopic and microscopic examinations. The NOAEL for this study was 60 mg/kg diet, which is equivalent to 1.5 mg/kg bw/day (Dean & Jackson, 1995).

No long-term/life-time repeat dose studies were available for α-cypermethrin (IPCS, 1996).

3.3 Carcinogenicity and mutagenicity

Cypermethrin

SPF-Swiss-derived mice (70/sex/dose) were fed diets containing cypermethrin at concentrations of 0 (140/sex), 100, 400, or 1600 mg/kg feed for up to 101 weeks. Ten mice per sex were killed after 52 weeks of treatment for interim examinations. Several haematological changes consistent with mild anaemia were noted at the highest concentration at 52, but not 101 weeks. Other effects consisted of increased liver weights at 400 and 1600 mg/kg diet, and decreased body weight and thrombocytosis at 1600 mg/kg diet. There were no compound-related changes in non-neoplastic histopathology or increases in tumours of types that are not commonly associated with the mouse strain used. The incidence of tumours was similar in all groups, with the exception of a slightly increased incidence of benign alveolar lung tumours in the females in the 1600 mg/kg group. However, the magnitude of this increase was within the historical control incidence. There was no evidence for a decreased latency of benign alveolar lung tumours in these female mice, and this tumour type was not accompanied by any increase in malignancy. Furthermore, benign alveolar lung tumours are known to occur in both sexes at a high and variable incidence in this strain of mouse. Therefore, it is considered that the occurrence of benign alveolar lung tumours in the female mice receiving the highest dose was not related to treatment with cypermethrin. Feeding cypermethrin at concentrations up to 1600 mg/kg diet to mice for a life-time did not

produce any evidence of carcinogenicity. The NOAEL for this study was 400 mg/kg feed, equivalent to 57 mg/kg bw/day (Lindsay *et al.*, 1982)**.**

In a 2-year combined long-term/carcinogenicity study, Wistar rats (groups of 48 males and 48 females; 96 of each sex were used as controls) were fed dietary concentrations of 0, 1, 10, 100, or 1000 mg cypermethrin/kg feed. The effects observed were reduced body weight and food consumption in both sexes at the highest concentration. No evidence for carcinogenicity was found. The NOEL was 100 mg/kg feed, or 5 mg/kg bw/day (McAusland *et al.*, 1978).

In a 2-year dietary study no increase in tumour incidence was observed when Wistar rats were given diets containing 0, 20, 150, or 1500 mg cypermethrin/kg diet (equivalent to 0, 1, 7.5, or 75 mg/kg bw). The NOAEL was 7.5 mg/kg bw, based on a decrease in the normal body weight gain at 1500 mg/kg bw/day (Milburn *et al.*, 1982).

Groups of beagle dogs (4/sex/dose) were fed diets that contained cypermethrin at concentrations of 0, 3, 30, 300 or 1000 mg/kg feed for two years. Satellite groups that consisted of four dogs per sex received diets that contained 0, 300 or 1000 mg cypermethrin/kg feed. Owing to severe adverse effects the highest concentration had to be reduced to 750 mg/kg feed during week four. However, signs of toxicity persisted into weeks 6–8, so animals were fed the control diet for 10 days, then treatment was continued at a dietary concentration of 600 mg/kg feed. The signs of intoxication, which appeared within 24 hours of initiation of treatment, consisted of licking and chewing of the paws, stiff high stepping gait, whole body tremors, head shaking, incoordination, ataxia and in some cases, convulsions. These effects occurred at 1000 mg/kg and to a lesser extent at 750 mg/kg, but not at 600 mg/kg bw. Significantly reduced body weight gain was observed in male dogs at the highest dose in the main study, but not in the satellite group. No evidence for carcinogenicity was noted. The NOAEL for this study was 300 mg/kg feed, which is equivalent to 7.5 mg/kg bw/day (Buckwell, 1981).

When concentrations of up to 2 mg cypermethrin/plate were tested in bacterial assays, no increases in the reversion rates where observed in *E. coli* or *S. typhimurium* TA1535, TA1537, TA1538, TA98, and TA100, in the presence or absence of a rat liver microsomal activation system. In addition, cypermethrin did not cause an increase in the rate of mitotic gene conversion when *S. cerevisiae* (in liquid culture) was exposed to cypermethrin at concentrations of up to 5 mg/ml, both in the presence and absence of metabolic activation (Brooks, 1980, Dean, 1981). Cypermethrin was tested for mutagenicity in V79 Chinese hamster cells at concentrations of up to 20 µg/ml, in the presence and absence of a metabolic activation system. No cytotoxicity was observed and cypermethrin was not mutagenic with or without metabolic activation (Dean, 1977). Cypermethrin was shown not to be mutagenic in the following *in-vivo* test systems; in Chinese hamsters a chromosomal aberration test, in mice the dominant lethal test and host-mediated assay. Studies conducted using bacteria *in vitro* tests found negative results when using bacteria, yeast and Chinese hamster cells (Dean, 1977). However, genotoxicity was reported in cypermethrin in mouse spleen (including cultured mouse spleen), bone marrow and chromosomal aberrations and sister chromatid exchange (Amer *et al.*, 1993).

Following *in vivo* administration of cypermethrin via intraperitoneal, oral and dermal routes, the number of micronuclei was studied in mouse bone marrow. Cypermethrin showed mutagenic potential after oral administration of 900 mg/kg diet for 7 and 14 consecutive days (no additional details given). No effects were observed following intraperitoneal administration of a single injection of 60 or 180 mg/kg bw or double and triple injections of 60 mg/kg bw (Amer and Aboul-Ela, 1985). Up to four dermal treatments with 360 mg cypermethrin/kg bw resulted in a significant increase in the frequency of polychromatic erythrocytes with micronuclei (no additional details given (Amer & Aboul-ela, 1985).

Chinese hamsters (12/sex/dose) were orally dosed with 20 or 40 mg cypermethrin/kg bw (in DMSO) for two successive days. The incidence of chromosome abnormalities in bone marrow cells, 8 and 24 hours after dosing, did not differ from that in the DMSO control animals. The positive control group (100 mg/kg cyclophosphamide) responded appropriately (Dean, 1977**).**

The effects of cypermethrin on sister chromatid exchange were studied in the bone marrow cells of 3-month-old mice. Cypermethrin was injected subcutaneously at doses of 0.75, 1.5, or 3 mg/kg bw, or 2.5, 5.0, or 10 mg Ripcord/kg bw (a formulation that included cypermethrin). Both the technical and the formulated products showed a dose-related increase in sister chromatid exchanges in the dividing cells at all dose levels. The highest doses of both cypermethrin and Ripcord completely inhibited mitotic division (Seehy *et al.*, 1983).

α-Cypermethrin

α-Cypermethrin (in DMSO) at concentrations of 31.25, 62.5, 125, 250, 500, 1000, 2000 or 4000 µg/ml did not increase reverse gene mutation in *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538, or in *E. coli* or *S. cerevisiae*, either in the presence or absence of a metabolic activation system. Positive controls gave appropriate results (WHO, 1989). In addition, cypermethrin did not cause an increase in the rate of mitotic gene conversion when *S. cerevisiae* (in liquid culture) was exposed at concentrations of 10, 100, 500, 1000 or 5000 µg/ml, both in the presence and absence of metabolic activation. This study was conducted with four positive control compounds (IPCS, 1992).

In a study reported by IPCS (1998), groups of five male and five female Wistar rats were administered a single oral dose of 2, 4 or 8 mg α-cypermethrin/kg bw (in corn oil) and killed 24 hours after dosing. The control group received corn oil alone, and cyclophosphamide was used as a positive control. α-Cypermethrin caused no increase in the incidence of chromatid or chromosome aberrations, or polyploidy in bone marrow cells. In addition, α-cypermethrin (in aqueous carboxymethylcellulose) at concentrations of up to 40 µg/ml did not increase the frequency of chromatid gaps, chromatid breaks, or total chromatid aberrations in rat liver cell cultures (no further details available). Also, α-cypermethrin (in DMSO; 20%) was administered to Wistar rats as a single oral dose of 40 mg/kg bw, and after six hours liver DNA was analysed. α-Cypermethrin did not produce any detectable DNA single-strand damage using alkaline elution profiles of liver DNA. Methylmethane sulfonate was used as a positive control and DMSO as the solvent control (IPCS, 1992).

Cypermethrin and α-Cypermethrin

The reviews cited and the EMEA (2001) are all in agreement that there is no evidence that cypermethrin and α-cypermethrin are genotoxic. The evidence also suggests that cypermethrin is not a carcinogen. There are no available carcinogenicity studies for α-cypermethrin.

3.4 Reproductive and developmental toxicity

Cypermethrin

Wistar rats (30/sex/dose) were mated following treatment with cypermethrin at dietary concentrations of 0, 10, 100, or 500 mg/kg for five weeks. Two successive litters were produced from each pair (F_{1a} and F_{1b}), of which the first was discarded at weaning. Randomly-selected pups from the second litters were mated to produce the next generation (F_{2a} and F_{2b}). The study was continued until two litters from each of three successive generations had been bred. Treatment was continued throughout pregnancy and lactation for all three generations. It was noted that the adult animals in the highest dose group of all three generations had reduced food consumption compared with the controls, and this was accompanied by a reduction in

body weight gain. No other effects on the parent animals were observed. Cypermethrin did not cause any adverse effects on the reproductive performance of the animals, or on the pup survival. No consistent changes were observed in mean litter weight between birth and weaning in any treatment group. However, there was a reduction in total litter weights in the 500 mg/kg F_{1a} litters at birth and on days 7, 14, and 21 of lactation. There was also a statistically significant decrease in total litter weights and size in the F_{1b} litters at this dose. These effects on pup weights and size are probably secondary to the reduced maternal body weights observed at the highest dose. No effects were observed in rats administered 100 mg/kg diet. Hence, the NOEL for this study (based on maternal and reproduction toxicity) was 5 mg/kg bw/day (Hend *et al.*, 1978, Fish, 1979, Thorpe, 1985).

Groups of pregnant Sprague–Dawley CD rats (25/dose) were administered cypermethrin by gavage (1% solution in corn oil) at doses of 0, 17.5, 35, or 70 mg/kg bw/day, on days 6 to 15 of gestation. One animal died, and one animal had to be killed due to severe convulsions, after receiving the highest dose. At 35 and 70 mg/kg bw/day, respectively, a dose-related reduction of maternal body weight gain was recorded. In addition, at 70 mg/kg per day, slight to severe neurological disturbances (slight splaying of the hind legs while walking ranging to severe splaying of all limbs, involuntary movements of the jaws, convulsive spasms, and hypersensitivity to noise) were observed in 11 of the females. The NOEL for maternal toxicity was 17.5 mg/kg bw/day. Despite this maternal toxicity, there were no indications of any embryotoxic or teratogenic effects of cypermethrin. The NOEL for developmental toxicity was ≥70 mg/kg bw/day (Tesh *et al.*, 1978).

Groups of pregnant Banded Dutch rabbits (30 controls and 20/dose) were dosed orally with 0, 3, 10, or 30 mg/kg bw/day (in corn oil by gelatin capsule) on days 6 to 18 of gestation. Treatment did not affect growth, number of pre-implantation losses, resorptions, or fetal deaths, or the number and sizes of foetuses. The incidence of fetal visceral and skeletal abnormalities was comparable to that in the vehicle control group. No developmental effects were found in this study. Therefore the NOAEL for this study was 30 mg/kg bw/day (IPCS, 1996).

In a further study, groups of pregnant New Zealand White rabbits were dosed with 0, 20, 50 or 120 mg/kg bw/day cypermethrin (in corn oil) by gavage on days 6 to 18 of gestation. The number of implantations, live young and resorptions, pre- and post-implantation losses and fetal and placental weights were unaffected by the treatment. There was no indication of developmental toxicity. The NOAEL for this study was 120 mg/kg bw/day (Tesh *et al.*, 1984 & 1988).

α-Cypermethrin

α-Cypermethrin has not been tested for reproductive effects. However, from the available reproductive toxicity and teratogenicity studies with cypermethrin it is clear that no influence on reproductive performance occurs at a level of 100 mg/kg diet (IPCS, 1989). Furthermore, the NOEL of cypermethrin for reproduction is comparable with the NOELs from other toxicity studies. Therefore, there is no evidence to suggest that α-cypermethrin, which consists of two *cis*-isomers, also present in cypermethrin, would behave differently (IPCS, 1992).

In an unpublished study, groups of pregnant Sprague–Dawley rats (24/dose) were dosed with α-cypermethrin (in corn oil) by gavage at doses of 0, 3, 9, or 18 mg/kg bw/day on days 6 to 15 of gestation. The highest dose had to be reduced to 15 mg/kg bw/day on day 10 of gestation. On gestation day 20 the dams were killed and necropsied, and the fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. Signs of toxicity at 18 mg/kg bw/day, which were reduced at 15 mg/kg bw/day, were unsteady gait, piloerection, limb splay and hypersensitivity to noise. At the highest dose, body weight gain and food

consumption were reduced. This effect was only slight. Mean fetal weights were only slightly reduced at the highest doses. No abnormalities were observed. The NOEL for this study was 9 mg/kg bw/day (Irvine, 1994b).

In a further unpublished study, groups of pregnant New Zealand White rabbits (16/dose) were dosed with α-cypermethrin (in corn oil) by gavage at doses of 0, 3, 15 or 30 mg/kg bw/day on gestation days 7 to 19. The dams were killed on gestation day 28 and examined along with the fetuses. In all groups there was a similar mean body weight loss following the onset of treatment, which continued until day 11. At the highest dose there was also decreased body weight gain towards the end of the treatment period. In all cases food consumption reflected the reduced mean body weight gains. There was no indication of developmental toxicity. The NOELs for maternal and fetal toxicity were 3 and 30 mg/kg bw/day, respectively (Irvine, 1994a).

4 Guidelines and standards

JMPR has not yet evaluated α-cypermethrin.

In 2004, IPCS (2004) concluded that as α-cypermethrin and cypermethrin are qualitatively similar in their toxicity and metabolism, and cypermethrin includes a substantial proportion of α-cypermethrin, the ADI already established for α-cypermethrin could apply to both substances. An ADI of 0–15 µg/kg bw was established for α-cypermethrin and cypermethrin based on the NOAEL of 1.5 mg/kg bw/day in a 52-week study in dogs and the application of the standard uncertainty factor of 100.

At the same meeting, JECFA also recommended MRLs, which are expressed as total cypermethrin residues. The MRLs are 50 µg/kg for muscle, liver and kidney, 1000 µg/kg for fat, and 100 µg/kg for milk (IPCS, 2004). JECFA recommended that the JMPR should also consider the above approach.

Using daily food consumption factors, recommended MRLs would result in a theoretical maximum daily intake of 368 µg of residues of cypermethrin equivalents or 30% of the upper bound of the ADI for a 60 kg person (IPCS, 2004). The exposure to cypermethrin from use of pesticides, as estimated by JMPR, is approximately 300 µg; the total theoretical exposure for the cypermethrins would therefore be approximately 650 µg.

According to the EMEA (2003; 2004) an ADI of 50 µg/kg bw (3000 µg/person) was established for cypermethrin, a safety factor of 100 was applied to the NOEL of 5 mg/kg bw/day. The NOEL was established in both the 3-month and 2-year studies in rats, and the 3-generation reproduction study in rats. This ADI was found to equal the ADI adopted by the Joint WHO/FAO Expert Committee on Food Additives (JECFA). A lower ADI of 15 µg/kg bw (900 µg/person) has been established for α-cypermethrin, from a one year study in dogs (EMEA, 2003 & 2004). This value has been used in the current assessment.

Table 4.1 MRLs for cypermethrin and α-cypermethrin

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Comments
Cypermethrin	Cypermethrin (sum of all isomers)	Bovine	20 µg/kg	Muscle	
			200 µg/kg	Fat	
			20 µg/kg	Liver	
			20 µg/kg	Kidney	

			20 µg/kg	Milk	Further provisions in Council Directive 98/82/EC are to be observed
		Ovine	20 µg/kg	Muscle	Not for use in animals from which milk are produced for human consumption
			200 µg/kg	Fat	
			20 µg/kg	Liver	
			20 µg/kg	Kidney	
		Salmonidae	50 µg/kg	Muscle and skin in natural proportions	
α-cypermethrin		Bovine, Ovine	20µg/kg	Muscle	
			200µg/kg	Fat	
			20µg/kg	Liver	
			20µg/kg	Kidney	
			20µg/kg	Milk	Further provisions in Council Directive 98/82/EC are to be observed

(Source EMEA 2003; 2004)

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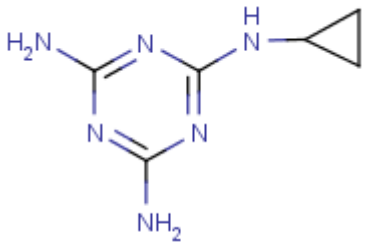
Cyromazine

1 Introduction

Cyromazine (N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine; molecular formula $C_6H_{10}N_6$; CAS No. 66215-27-8; Figure 1.1; Box 1) is a triazine derivative (ChemID Plus).

Figure 1.1 Molecular structure of cyromazine



From ChemID Plus

Box 1 Synonyms and trade names for cyromazine

2,4-Diamino-6-(cyclopropylamino)-s-triazine (8CI); 2-Cyclopropylamino-4,6-diamino-s-triazine; AI3-52713; Armor; Azimethiphos; BRN 0882879; CGA 72662; Caswell No. 167B; Ciromazina; Ciromazina [INN-Spanish]; Citation; Cyclopropylmelamine; Cypromazine; Cyromazine; Cyromazinum; Cyromazinum [INN-Latin]; EINECS 266-257-8; EPA Pesticide Chemical Code 121301; HSDB 6602; Larvadex; N-Cyclopropyl-1,3,5-triazine-2,4,6-triamine; Neporex; OMS-2014; Trigard; UNII-CA49Y29RA9; Vetrazin; Vetrazin (pesticide); Vetrazine

From ChemID Plus

Cyromazine is used in veterinary medicine as a topical insect growth regulator to protect animals against insects. It is used topically in a pour-on formulation (0.9 - 3.6 g/sheep, every 8-10 weeks) for the prevention of blowfly strike (*Lucilla sericata*) on sheep and lamb. Cyromazine is also used to protect plants. Cyromazine is not used on sheep producing milk for human consumption or in human medicine (EMEA, 2001).

The mode of action of cyromazine differs from conventional insecticides. Cyromazine interferes with the first dipteran larval moult and possibly with metamorphosis, resulting in morphological transformations of larvae and pupae before death (EMEA, 2001).

2 Toxicokinetics

No human toxicokinetic data has been identified. In Charles River albino rats given a single oral dose of ^{14}C-cyromazine at 0.5 mg/kg, in the first 24 hours after dosing most of the administered dose was excreted in the urine with some via the faeces. Hence the gastrointestinal absorption of cyromazine is considered rapid and extensive. Tissue residues were below the limit of detection except for the liver where levels were still too low to quantify (Simoneaux & Cassidy, 1978). In a study where one sheep was given ^{14}C-cyromazine at 0.15 mg/kg/day orally for nine days, rapid absorption was again noted with total excretion being achieved within 24 hours of the final dose. Blood levels plateaued at approximately 0.1% by day 5. Concentrations were highest in the liver, followed by decreasing amounts in the gastrointestinal tract, kidney, muscle, brain and heart . Levels were at or below the limit of quantification in fat (Simoneaux & Cassidy, 1981).

In the above study in which rats were given a single oral dose of ^{14}C-cyromazine (0.5 mg/kg), approximately 80% of the administered dose was recovered unchanged in the urine (Simoneaux & Cassidy, 1978). The three metabolites identified were melamine (most abundant) and, to lesser extent, hydroxyl-cyromazine and methyl-cyromazine (EMEA, 2001).

In the repeat dose sheep study 84% of that recovered in urine, 44% in faeces and 11.6% in liver was the parent compound. Four metabolites were identified, amounting to under 7% of total radioactivity; of these melamine accounted for 43% (Simoneaux & Cassidy, 1981). Melamine is not discussed in the current study, for further details please see screening information data sheet for melamine (OECD SIDS, 2002).

These studies showed that excretion in the rat occurred mostly within the first 24 hours and by 72 hours, 95% of dose was excreted in the urine. Approximately 3% in total was excreted in the faeces within the first 24 hour. In sheep, excretion was again mostly complete by 24 hours and approximately 90% was via the urine and only approximately 3.7% via the faeces (Simoneaux & Cassidy, 1981).

In another study, groups of sheep treated dermally with a pour-on formulation (therapeutic route of administration) of 82 mg ring-labelled ^{14}C-cyromazine/kg, were killed 2, 6 and 10 days after treatment. Run-off of administered dose accounted for approximately 28%. Peak plasma levels of radioactivity were observed 24 hours after treatment and declined biphasically. Levels of radioactivity up to 33000 mg cyromazine equivalents/kg occurred in the wool of the treatment area with up to 2500 mg/kg cyromazine in ventral body surface wool. The highest level of cyromazine equivalents (1.15 mg/kg) was found in the fat underlying the treatment area at six days after treatment. Levels here declined to 1 mg/kg by 10 days. The level of radioactivity in muscle was highest six days after treatment. Levels in liver and kidney were highest two days post treatment. Levels in all tissues declined with time; the lowest levels were detected 10 days post treatment. The parent compound was the most abundant component of the residue in muscle, fat, kidney, urine and faeces; N-methyl cyromazine and melamine were present but at much lower levels. Within ten days after treatment, approximately 3 - 4% had been excreted in the urine (1%) and faeces (2.5%) (EMEA, 2001).

3 Toxicity profile

3.1 Acute toxicity

No studies on the acute toxicity of cyromazine have been identified.

Cyromazine has low acute oral toxicity; oral LD_{50} values are 2029 mg/kg for mice, 3387 mg/kg for rats (HSD:SD) and 1467 mg/kg for rabbits (Bathe & Sachsse, 1978a, Bathe & Sachsse, 1978b, Ulrich & Blair, 1979). In Tif:RAIf rats, dermal LD_{50} is > 3170 mg/kg and inhalation LC_{50} (four hour exposure) is > 3.6 mg/L air. Signs of toxicity reported include sedation, dyspnoea, hunched position and ruffled fur after oral or dermal dosing (Sachsse & Bathe, 1978b, Sachsse & Bathe, 1978a) and, after inhalation, decreased activity, piloerection and nasal discharge which resolved by two days after exposure (Holbert, 1994).

3.2 Repeat dose toxicity

No repeat dose toxicity studies with cyromazine have been identified.

When male and female weanling Sprague-Dawley rats were fed 0, 30, 300, 1000 or 3000 ppm cyromazine in the diet for 90 days, decreased body weight at 1000 and 3000 ppm and food consumption at 3000 ppm were noted. A decrease in absolute and relative liver weights was

reported though no associated histopathology was seen. A NOAEL of 300 ppm (equivalent to 23.5-26.9 mg/kg body weight /day, was established (Goldenthal & Hughes, 1979).

In a 12 month study in Beagle dogs, animals received cyromazine in the diet at 0, 50, 200, 800 or 3500 mg/kg feed (equivalent to 0, 1.4, 5.7, 23 or 94 mg/kg bw/day in males and 0, 1.5, 6.0, 25 or 110 mg/kg bw/day in females). Changes in haematological parameters (comprising decreased haemoglobin, haematocrit and red blood cell count), and increased total protein were noted at 800 mg/kg feed/day in males and at 3500 mg/kg feed/day in both sexes. At the high dose, low mean corpuscular cell volume, mean corpuscular haemoglobin, triglyceride, basophil counts and plasma creatine kinase activities, were observed. Absolute and relative heart and liver weights were increased at 800 mg/kg feed/day in females and in both sexes at 3500 mg/kg feed/day as were kidney weights in females at this dose. High dose animals also showed myocarditis and one male had foci of cartilaginous metaplasia in the heart muscle. Also in high dose animals, hypercellularity of bone marrow and tubular lesions in the kidney were noted. At 50 mg/kg food/day, no effects were observed. At 200 mg/kg food/day, some variation in total plasma protein in males and globulin concentration (calculated from protein and albumin data) were not attributed toxicological significance. Hence, the NOAEL was considered to be 200 mg/kg feed/day, (equivalent to 5.74 mg/kg bw/day) based on changes in haematology and histopathology (Altmann, 1997).

In an inhalation study in Tif:RAIf (SPF) rats, animals were exposed to cyromazine aerosol at 0, 0.058, 0.206 or 0.706 mg/L, for 4 hours/day, for 4 consecutive weeks. A recovery period of three weeks followed; median aerodynamic diameter of particles was 1.6 – 6 µm, with 52 - 57% of particles <3 µm. Dose-related piloerection, dyspnoea and hunched posture were observed in treated groups and reduced spontaneous activity in the mid and high doses. Symptoms reversed during the recovery period except for piloerection, which remained moderate. Reduced body weight gain and food consumption were noted in males of all treated groups though there was no dose-relationship and females were unaffected. In high dose males, slight increases in erythrocyte count, haemoglobin and haematocrit were noted; these reversed by the end of the recovery period. No treatment-related changes in clinical chemistry were observed. Liver weights (absolute or relative not stated) were increased in mid and high dose females while increased pituitary weights (absolute or relative not stated) were noted in males. By the end of the treatment phase, all mid and high dose females showed hepatocytic cytoplasmic vacuolation. However, by at the end of the recovery period, both control and treated animals showed this change. Changes seen at 0.058 mg/L were considered inconsequential and this level was considered to be the no-observed-adverse-effect-concentration (NOAEC) (Hartmann, 1988).

In briefly reported unpublished studies, cyromazine was not a skin sensitiser in the Pirbright white Guinea pig but was irritant to the cornea, iris or conjunctiva of Himalayan rabbits and was a mild dermal irritant to scarified skin of Himalayan rabbits (Ullmann & Sachsse, 1978a,b&c).

3.3 Reproductive and developmental toxicity

In a two-generation study, weanling Sprague-Dawley rats were fed diets containing 0, 30, 1000 or 4000/3000 ppm cyromazine; the high dose of 4000 ppm caused toxicity and was therefore reduced to 3000 ppm from week 4. F_0 generation animals were fed cyromazine for 100 days prior to mating. Body weight and food consumption were reduced in F_0 and F_1 animals of the mid and high doses. There was no treatment-related effect on pregnancy or gestation. However, reduced fertility was apparent for F_0 generation high dose males compared with other treatment groups. This effect was not seen in F_1 generation males. Average litter sizes were slightly reduced in high dose F1 animals and, in both generations, increased perinatal mortality was observed at the high dose. Pup weight gain from birth to lactation was reduced in high doses of both generations. At the mid dose, only F_1 male pups at

21 days age had reduced body weight gains; relative organ weight changes were also noted and attributed to this change in growth performance. There were no treatment-related effects on pup behaviour, appearance or at histopathological evaluation of adults or pups. The NOAEL was 1000 ppm, equivalent to 64 mg/kg/day in males and 51 mg/kg/day in females; this was based on changes noted at this dose not being attributed biologically significance (Blair, 1981)

In an embryo-/feto-toxicity study, pregnant Sprague Dawley rats were orally dosed with cyromazine at 0, 100, 300 or 600 mg/kg/day on days 6 – 19 of gestation. Instances of a clear oral discharge were observed in all high dose, some mid and a few low dose animals on several days during dosing. There were no mortalities during the study. Dams in the mid and high dose groups showed body weight loss during the first few days of dosing and reduced body weight gain for the rest of the dosing period. However, there was no effect on number of dams with litters, number of viable fetuses per litter or number of resorptions per litter, and no dead fetuses were noted in any group. The mean body weight of fetuses in the high dose group was low compared with controls. The ratio of male to female pups was similar in all groups and there was no evidence of treatment-related teratogenic effects although the incidence of unossified sternebrae was higher than controls in the high dose group and slightly higher at the low and mid doses. Cyromazine was considered not to be teratogenic at up to 600 mg/kg/day by oral gavage. Signs of maternal toxicity were seen at 300 mg/kg/day or above and fetal toxicity was noted at the high dose. Therefore, the maternal NOAEL was 100 mg/kg/day and developmental NOAEL was 300 mg/kg/day (Rodwell, 1979).

Several teratology studies have been conducted in the rabbit. However only the most significant is discussed here (WHO. JMPR, 2008). Artificially inseminated New Zealand White rabbits were orally dosed at 0, 5, 10 or 30 mg/kg/day cyromazine on gestation days 7 - 19. The number of mortalities and abortions that occurred did not show dose-relationship. However the high dose group showed the greatest incidence of decreased urination, defecation and a reduction of body weight and food consumption was noted compared with other groups. No treatment-related effects were apparent in numbers of viable fetuses although total number of implantations was slightly reduced at the high dose. Fetal body weight and sex ratio were unaffected by treatment. The incidence of external and skeletal malformations (including omphalocele, umbilical hernia, gastroschisis, spina bifida, microphthalmia, macroglossia, agnathia) and visceral and skeletal variations was also unaffected. In females left to deliver their litters and rear the offspring to weaning, no difference in mean gestation length or in number of dams with live litters, number of kits per litter, total number of dead kits per group, kit survival to day 4, number of dams failing to raise their litter to weaning, sex ratio of kits, or body weight of kits were noted. On post partum day 4, some litters were culled and examined. No malformations were observed except for one kit in the low dose that had a cataract. A slightly higher number of kit deaths occurred during postnatal days 4 - 28 for the high dose only. Of those dying during postnatal days 0 - 4, one control kit had omphalocele and one high dose kit had cyclopia, omphalocele and cleft palate; malformations and variations in cyromazine treated groups were considered not to be biologically meaningful or statistically different from controls. There were no malformations in kits dying during postnatal days 4 - 28. At necropsy, there were no treatment-related gross pathology in dams. The maternal NOAEL was 10 mg/kg/day based on changes in body weight and food consumption, and a fetal NOAEL of 30 mg/kg/day was stated (Nemec & Rodwell, 1986).

3.4 Carcinogenicity and mutagenicity

In a 104 week carcinogenicity study in CD-1 mice fed with diets containing 0, 50, 1000 or 3000 ppm cyromazine, no treatment-related signs were noted and mortality to week 93 was unaffected. By the end of the study, survival was, however, slightly lower in mid and high dose males and low and high dose females than their controls. The differences in survival

were considered chance events because the mid-dose females had better survival than their controls. A dose-related decrease in body weight was observed at mid and high doses whilst weights of low dose males and all treated females showed no dose relationship. Food consumption was slightly reduced in all treated groups. There were no treatment-related changes in haematological parameters or organ weights. A variety of neoplasms were found in males and females of all groups but the incidence and distribution of neoplasms were similar in control and treated groups; the most frequent tumours were alveolar and bronchiolar adenoma and carcinoma of the lung, adenoma and carcinoma of the Harderian glands and malignant lymphoma (involving primarily the thymus, spleen and lymph nodes). The NOAEL was 1000 ppm (equivalent to 126 mg/kg bodyweight/day based on body weight (Blair & Hardisty, 1982b).

In a 104 weeks carcinogenicity study in Sprague-Dawley rats fed diet containing 0, 30, 300 or 3000 ppm cyromazine, no treatment-related clinical signs or deaths were noted. Body weight gain was reduced at the high dose; this became more pronounced as the study progressed. Body weight was also lower than controls in the mid dose females but to a lesser extent than at the high dose. Food consumption was reduced at the high dose only. No treatment-related changes in haematology or clinical chemistry parameters were noted. Changes in organ weights were attributed to the body weight effect rather than a specific effect on the organs. There were some increases in incidence of bronchiectasis and renal pelvic epithelial hyperplasia. However, these were not dose-related and were not accompanied by other related changes, and so were considered a consequence of ageing and not related to treatment. A higher incidence of testicular interstitial cell tumour in high dose males and mammary gland adenocarcinoma in high dose females were noted but were within historical control ranges. The NOAEL for this study was 300 ppm (equivalent to 15 mg/kg bw/day in males and 19 mg/kg bw/day in females) based on body weight and food consumption effects (Blair & Hardisty, 1982a).

Cyromazine was negative in a series of *in vitro* and *in vivo* mutagenicity studies (Table 3.1) except for one giving an inconclusive result.

Table 3.1 Summary of mutagenicity studies with cyromazine

	In vitro		
Test system	**Test organism/cell type & strain**	**Concentrations**	**Results**
Ames test, reverse mutation	*Salmonella typhimurium*; TA98, TA100, TA1535, TA1537, TA1538 & *Escherichia coli* WP2*uvrA*	20 – 5000 µg/0.1ml	Negative
Chromosome aberration	Human peripheral blood lymphocytes	0 - 1000 µg/ml ± S93 hour treatment, harvesting 46 hours after treatment end	Negative
Unscheduled DNA synthesis	Rat (F344) primary hepatocytes	- 1000 µg/ml, 18 hour exposure	Negative
DNA repair assay	Mouse (male CD-1) primary hepatocytes	0.5 - 1000 µg/ml, 18 hour exposure	Negative
Gene mutation	Mouse lymphoma cells L5178YTK+/-	50 - 500 µg/ml; 4 hour exposure	Negative
Point mutation test	V79 Chinese hamster cells *Hprt* locus	25-1000 µg/ml, -S9 21 hour exposure; 100-4000 µg/ml +S9 5 hour exposure	Negative

Gene mutation, mitotic gene conversion & mitotic recombination	*Saccharomyces cerevisiae* D7	375 - 3000 µg/ml	Negative

In vivo

Test system	Test organism	Dose	Results
Dominant lethal test	Mouse (Tif:MAGf SPF, NMRI derived)	0, 226, 678 mg/kg	Negative
Spot test	Mouse (males: T-stock; females:C57Bl/6)	0, 150, 300, 600 mg/kg	Inconclusive
Micronucleus (bone marrow) test	Mouse (Tif:MAGf SPF, NMRI derived)	0, 360, 1080 mg/kg sampling at 24, 48 and 72 hours after treatment	Negative
Nucleus anomaly test	Chinese hamster	2000, 4000, 8000 mg/kg	Negative

Cited from WHO. JMPR, 2008

4 Guidelines and standards

An updated toxicologically-based ADI of 0.06 mg/kg was established by JMPR by applying a safety factor of 100 to a NOAEL of 5.7 mg/kg/day from a one-year repeat dose toxicity study in dogs (WHO. JMPR, 2008).

The CVMP recommended inclusion of cyromazine in Annex I of Council Regulation (EEC) No 2377/90 using an MRL of 300 µg/kg in ovine muscle, fat, liver and kidney, with the proviso that cyromazine was not used in animals from which milk is being produced for human consumption (EMEA, 2001).

It is estimated by the EMEA that consumer intake from food sources would be 53% of the ADI (EMEA, 2001).

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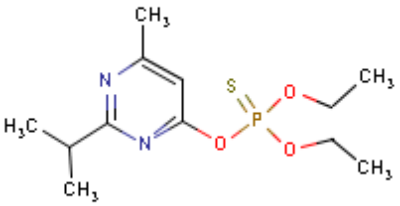
Diazinon

1 Introduction

Diazinon (O,O-Diethyl 2-isopropyl-6-methyl-4-pyrimidinylphosphorothioate, molecular formula $C_{12}H_{21}N_2O_3PS$, CAS No. 333-41-5; Figure 1.1; Box 1) an organophosphorous insecticide, is a clear colourless liquid with a faint ester-like odour and is soluble in most organic solvents (HSDB, 2005, ChemID Plus). It is more stable at alkaline pH than at neutral or acidic pH (O'Neil *et al.*, 2001). There is evidence that, as well as being a neurotoxicant, diazinon has adverse effects on reproduction. The levels at which these effects occur are discussed below.

Figure 1.1 Molecular structure of diazinon



From ChemID Plus

Box 1 Synonyms and trade names of diazinon

Alfa-tox; Antigal; BRN 0273790; Bassadinon; Basudin; Basudin 10 G; Basudin S; Bazuden; CCRIS 204; Caswell No. 342; Ciazinon; Compass; Compass (insecticide); Cooper's Flystrike Powder; Dacutox; Dassitox; Dazzel; Delzinon; Diazajet; Diazide; Diazinon ag 500; Diazinone; Diazitol; Diazol; Dicid; Diethyl 2-isopropyl-4-methyl-6-pyrimidinyl phosphorothionate; Diethyl 4-(2-isopropyl-6-methylpyrimidinyl)phosphorothionate; Dimpilato [INN-Spanish]; Dimpylat; Dimpylate; Disonex; Dizictol; Dizinil; Dizinon; Drawizon; Dyzol; EINECS 206-373-8; ENT 19,507; EPA Pesticide Chemical Code 057801; Ektoband; Exodin; Flytrol; Galesan; Garden Tox; Geigy 24480; HSDB 303; Isopropylmethylpyrimidyl diethyl thiophosphate; KFM Blowfly Dressing; Kayazinon; Kayazol; Kleen-Dok; Knox Out 2FM; Knox Out Yellow Jacket Contorl; Knox-out; Meodinon; NCI-C08673; NSC 8938; Nedcidol; Neocidol; Neocidol (oil); Neodinon; Nipsan; Nucidol; O,O-Diethyl 2-isopropyl-4-methylpyrimidyl-6-thiophosphate; O,O-Diethyl O-(2-isopropyl-4-methyl-6-pyrimidyl); thionophosphate; O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl); phosphorothioate; O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate; O,O-Diethyl O-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl)phosphorothioate; O,O-Diethyl O-6-methyl-2-isopropyl-4-pyrimidinyl phosphorothioate; O,O-Diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl)-phosphorothioate; O,O-Diethyl-O-(2-isopropyl-4-methyl-6-pyrimidyl)phosphorothioate; O-2-Isopropyl-4-methylpyrimidyl-O,O-diethyl phosphorothioate; OMS 469; Oleodiazinon; Optimizer; PT 265; Phosphorothioate, O,O-diethyl O-6-(2-isopropyl-4-methylpyrimidyl); Phosphorothioic acid, O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) ester; Phosphorothioic acid, O,O-diethyl O-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl) ester; Phosphorothioic acid, O,O-diethyl O-(isopropylmethylpyrimidinyl) ester; Sarolex; Spectracide; Srolex; Terminator; Thiophosphoric acid 2-isopropyl-4-methyl-6-pyrimidyl; diethyl ester

From ChemID Plus

Diazinon is an organophosphorous insecticide used as both a veterinary medicinal product and for plant protection purposes (EMEA, 1995). Environmental levels are generally low, and the routes of human exposure are inhalational and dietary (HSDB, 2005).

2 Toxicokinetics

A validated gas chromatography method for the determination of diazinon in animal tissues with a limit of quantitation (LOQ) of 0.1 mg/kg is available. In addition, a gas–liquid chromatography protocol is available for the detection of diazinon in milk (LOQ = 0.008 mg/kg).

A number of experimental studies of the metabolic fate of diazinon have been conducted in the rat (as well as other species where appropriate) and have been reported in detail (IPCS, 1994). A summary of these findings is presented here. Total urinary excretion has been shown to amount to 96% of the orally administered dose, with 3% excreted in the faeces. Complete absorption takes place after intragastric administration, and the small amount excreted in the faeces may be of biliary origin. No accumulation of diazinon or its metabolites has been shown to occur (Capps *et al.*, 1990, Craine, 1989). Studies in both rats and sheep have shown that diazinon is readily absorbed through the skin (Capps *et al.*, 1990, Ballantine *et al.,* 1984, Pickles & Seim, 1990). *In vitro* studies of the biotransformation of diazinon have shown that it is rapidly converted to water-soluble metabolites (among which are hydroxydiazinon, diazoxon and hydroxydiazoxon; (Dahm, 1970)). Study of the *in vivo* metabolism of diazinon in mammals has shown that it is readily degraded and the metabolites formed are mainly eliminated via the kidneys (Robbins *et al.*, 1957). The main degradative pathway for diazinon in mammals is the oxidase/hydrolase-mediated cleavage of the ester bond (IPCS, 1994). The metabolites with intact pyrimidinyl phosphorous ester bond detected in *in vitro* studies are ultimately cleaved *in vivo* to their corresponding pyrimidine analogues (Hagenbuch & Mücke, 1985).

3 Toxicity profile

3.1 Acute toxicity

Diazinon exposure causes clinical signs consistent with the neurotoxic effects of organophosphate insecticides, including decrease in spontaneous activity, sedation, dyspnea, ataxia, tremors, convulsions, lacrimation and diarrhoea (IPCS, 1994). Diazinon is not considered to be either an irritant or to have skin-sensitising potential (Kuhn 1989b,c,e). A review of 60 cases of diazinon poisoning demonstrated the need for early treatment with atropine in such cases (Gupta and Patel, 1968). The interaction between diazinon with cholinesterase occurs in two phases — an early reversible phase, and a late irreversible phase (Grob, 1956).

Diazinon exhibits moderate acute oral, inhalation and dermal toxicity and is classified by the WHO as moderately hazardous (WHO, 2005), where an LD_{50} value of 1000 mg/kg is reported. A wide range of reported LD_{50} values are presented in a number of documents (IPCS, 1994; HSDB, 2005), and the disparity in these values has been ascribed to the presence of an impurity (TEPP) in the technical grade material used in studies conducted prior to 1979. The only effect of concern is the dose-related inhibition of acetyl cholinesterase activity (HSDB, 2005).

3.2 Repeat dose toxicity

The clinical effects of repeated oral exposure of humans to diazinon were investigated in a study of four human male volunteers, each receiving 0.025 mg/kg bw/day for 34–36 days.

There was no treatment-related effect on plasma or erythrocyte cholinesterase activity, blood chemistry or urinalysis, therefore a NOAEL of 0.025 mg/kg bw/day was established.

A greater than 20% inhibition of the erythrocyte cholinesterase activity was regarded as significant adverse effect of anticholinesterase pesticides. Data from a number of toxicity tests have been presented (IPCS, 1994). The lowest reported NOAELs in tests of this format are 0.4 mg/kg bw/day (90-day repeat dose toxicity test in rats) and 0.02 mg/kg bw/day (52-week repeat dose toxicity test in dogs (Singh *et al.,* 1988).

3.3 Carcinogenicity and mutagenicity

At a recent conference in the USA, diazinon was also not classifiable as a human carcinogen (ACGIH, 1986). In addition no evidence for carcinogenicity has been observed in long-term toxicity tests with diazinon in rats or mice (NCI, 1979, NIEHS, 1991, Kirchner *et al.,* 1991) and in a 99-week, long-term oral toxicity study of diazinon in rats, a NOAEL of 0.07 mg/kg bw/day has been reported..

Diazinon has been subjected to extensive testing for genotoxicity (IPCS, 1994). In summary, diazinon has tested negative for genotoxicity in *in vitro* tests such as the Ames test (Geleick & Arni, 1990, Marshall *et al.*, 1976), mouse lymphoma assay (Dollenmeier & Müller, 1986), sister chromatid exchange assay (using hamster, human lymphoid and whole blood lymphocyte cells) (Chen *et al.*, 1981, Sobti *et al.*, 1982, Strasser & Arni, 1988, Murli & Haworth S.R., 1990) and the autoradiographic DNA repair test (Hertner & Arni, 1990). Positive results in two *in vitro* genotoxicity (mouse lymphoma and chromosomal aberration) assays were noted (Matsuoka *et al.*, 1979). *In vivo* tests of genotoxicity, including sister chromatid exchange, nucleus anomaly test (Hool & Müller, 1981d, Hool & Müller, 1981c), mouse micronucleus test (Ceresa, 1988), dominant lethal study (Fritz, 1975) and chromosome studies in male germinal epithelium (Hool & Müller, 1981a, Hool & Müller, 1981b), all returned negative results for diazinon. A review of these data led to the conclusion that diazinon does not represent a genotoxic hazard (IPCS, 1994).

3.4 Reproductive and developmental toxicity

A two-generation study of the potential reproductive and developmental effects of diazinon has been conducted in rats (Giknis, 1989). No treatment-related malformations were found in the pups (no evidence for teratogenicity). The NOAEL in this study was reported to be 0.5 mg/kg bw/day based on reduced parental body-weight gain, weights and reduced viability of pups above this concentration. No evidence for teratogenicity has been observed in a number of special studies on teratogenicity in mammalian species. In contrast, experimental evidence exists for a teratogenic effect of diazinon on chicken embryos. However, the chicken embryo is anatomically and physiologically distinct from the mammalian embryo, therefore diazinon is considered not to be a teratogen of relevance to mammals (EMEA, 1995).

In a study of Sprague-Dawley rat pups neurotoxicity effects were observed and significant behavioral abnormalities in adolescence and adulthood. However, pups administered diazinon once daily (0, 0.5, or 2mg/kg) via subcutaneous injections on PND 1-4 produced no systemic toxicity determined by assessment of impaired gait or weight gain, weakened motor activity or loss of viability) (Slotkin *et al.*, 2008).

4 Guidelines and standards

Based on the NOAEL of 0.025 mg/kg bw/day established in a study of diazinon in human male volunteers, and incorporating a safety factor of 10, JMPR has established an ADI of 0-0.002 mg/kg bw/day (IPCS, 1994).

The major (pyrimidinyl) metabolites in liver and kidney show no acetylcholinesterase inhibition activity and have LD_{50} values more than 10 times lower than diazinon. Therefore, the parent compound (diazinon) has been identified as the marker residue.

Annex I of Council Regulation (EEC) No 2377/90 lists MRLs for diazinon (Table 4.1). These MRLs have been established in view of the fact that diazinon, in addition to its use as a veterinary medicinal product, is used in vegetable crop protection. Therefore the MRLs set for tissues of animal origin have an inherent allowance for a contribution from vegetable origin within the overall ADI (EMEA, 1995).

It is estimated by the EMEA that based on these MRLs, consumer intake from food sources would be approximately 100% of the ADI where 62% would be from animal sources and 38% would be compatible with the Estimated Maximum Daily Intake of residues of diazinon from vegetable sources (EMEA, 1995).

Table 4.1 Maximum residue limits – diazinon

Pharmacologically active substance	Marker residue	Animal species	Tissue	MRL, µg/kg
Diazinon	Diazinon	Bovine, Ovine, Porcine and Caprine	Fat	700
			Muscle	20
			Liver	20
			Kidney	20
		Bovine, Ovine and Caprine	Milk	20

From EMEA, 1995

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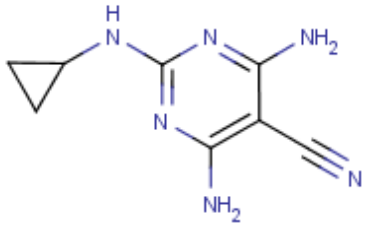
Dicyclanil

1 Introduction

Dicyclanil (5-Pyrimidinecarbonitrile, 4,6-diamino-2-(cyclopropylamino)-; molecular formula $C_{18}H_{10}N_6$; CAS No. 112636-83-6; see figure 1.1 and Box 1) is a pyrimidine derived insect growth regulator used for the prevention of myiasis or fly-strike, predominantly in sheep (EMEA, 2000a).

Figure 1.1 Molecular structure of dicyclanil



From ChemID Plus

Box 1 Synonyms and trade names

2-(Cyclopropylamino)-4,6-diamino-5-cyanopyrimidine; 4,6-Diamino-2-(cyclopropylamino)-5-pyrimidinecarbonitrile; Dicyclanil

From ChemID Plus

The recommended dose for dycyclanil for sheep is 30 – 100 mg/kg once per season, and it is administered topically in a suspo-emulsion as a pour-on formulation with dicyclanil at a concentration of 50 mg/ml. Dicyclanil has a long lasting action and is known to interfere with moulting and pupation in dipteran species. However, its exact mode of action is not known (EMEA, 2000b). More recently, dicyclanil has been successfully used in a preventative manner against Wohlfahrtiosis (infestation with the fleshfly *Wholfahrtia magnifica* or *Diptera: Sarcophagidae*) to reduce overall incidence to 4.5 - 5.5% in treated flocks, compared with 13% in untreated control flocks); Wohlfahrtiosis is a major cause of traumatic myiasis in sheep in the Mediterranean basin and continental Europe (Sotiraki *et al.*, 2005). Dicyclanil is not used in human medicine.

2 Toxicokinetics

No human toxicokinetics studies have been identified

After oral gavage dosing of [2-^{14}C] pyrimidyl-labelled dicyclanil at 0.5 or 20 mg/kg/day, for seven consecutive days, absorption from the gastrointestinal tract was found to have occurred for 80 - 85% of the administered dose. Twenty-four hours after a final dose of 0.5 mg/kg/day, the concentration of radiolabel in the liver, blood and kidneys was 270 µg/kg, 170 µg/kg and 37 µg/kg respectively; concentrations in other tissues were less than 4 µg/kg and the level in the residual carcass was 23 µg/kg. By 72 hours after dosing had finished, concentrations had declined to 40 - 80% of the 24 hour levels except for blood where the decline was slower; the radiolabel in blood was found to associate with erythrocytes (Hassler, 1994). A similar but dose-related pattern was seen in tissue levels, with levels at 20 mg/kg/day being

approximately 10 - 60 times higher than those at 0.5 mg/kg/day. Within 72 hours of the last dose, residues were approximately 30 – 80% of those at 24-hours. At the highest dose, levels identified in blood remained nearly constant for the whole study duration (EMEA, 2000b).

In rats, metabolism of dicyclanil involves the oxidative opening of the cyclopropyl ring at various positions, followed by oxidation and cleavage of the cyclopropyl-N-bond (i.e. dealkylation). Most metabolites result from more than one transformation; the most abundant (48 - 54% of l administered dose) urinary metabolite is N-(4,6-diamino-5-cyano-pyrimidin-2-yl)proprionamide. Other urinary metabolites include 2,4,6-triaminopyrimidin-5-carabonitrile (9-10%), 3-(4,6-diamino-5-cyanopyrimidin-2-ylamino) proprionic acid (4-10%) and 2-(4,6-diamino-5-cyanopyrimidin-2-ylamino) -3-hydroxypropionic acid (1-3%). All were also present in faeces but at markedly lower concentrations (each metabolite represented < 3% of total faecal radioactivity). The parent compound accounted for 2 - 7% of that present in urine and approximately 1% of that in faeces. The major metabolite in the liver and kidneys is 2,4,6-triaminopyrimidin-5-carbonitrile though smaller amounts of the parent and N-4,6-diamino-5-cyanopyrimidin-2-yl) proprionamide were present. Muscle and fat also contained the same metabolites as in liver and kidneys though levels were higher in fat (Hassler, 1994, Thanei, 1996). Within 24 hours of final dose, 93 - 96% of total dose had been excreted via urine (79 - 83%) or faeces (6 - 12%). Over the subsequent 48 hours, an additional 2 - 3% was excreted, thus confirming the rapid total elimination of absorbed material (Hassler, 1994).

In another study, dicyclanil was applied as a single topical application (jetting technique) to Oxford- Down sheep at 35 mg/kg. Approximately 37 - 59% of total dose remained on the animals with the rest collected as run off. Dermal absorption accounted for approximately 2% of retained radioactivity and peak whole blood levels occurred four to six hours post dose. There was no evidence for prolonged continued absorption from the application site. Seven days after dosing, 0.83% and 1.05% of the retained dose was excreted in the urine and faeces respectively; radiolabel was also detected in bile. Detected in the urine were 2,4,6-triamino-pyrimidine-5-carbonitrile and unchanged parent as well as three other separate compounds . In contrast, the faecal radiolabel was mainly attributable to unchanged dicyclanil. High levels of radioactivity were detected in the wool; these did not decrease with time.

In a separate study in Greyface sheep dosed with ^{14}C-dicyclanil at 35 mg/kg, maximum blood levels were reached 12 - 48 hours after dosing; approximately 4% of applied dose had been absorbed by seven days. In contrast to the study in Oxford Down sheep, this study suggested there was continued low level dermal absorption. Absorbed radioactivity was widely distributed and, overall, depletion of residues was slow although considerable inter-animal variation was noted. The metabolites were similar to the previous study though metabolite ratios differed (quantitative differences not presented in the EMEA summary report). However, radioactivity was again eliminated via urine and faeces (EMEA, 2000b).

In a series of radiolabel residue depletion studies, sheep given a single topical pour-on suspo-emulsion of dicyclanil at up to 100 mg/kg showed the highest residue levels in the liver, fat, kidney and muscle. Levels had depleted significantly by days 14 - 21 after dosing. The most significant residues present were the unchanged parent and the 2,4,6-triamino-pyrimidine-5-carbonitrile metabolite; the sum of these was established as the appropriate marker with muscle, fat, liver and kidneys established as the marker tissues (EMEA, 2000b).

3 Toxicity profile

3.1 Acute toxicity

No acute toxicity data in humans have been identified.

In a OECD test guideline 401 and GLP compliant study, Tif:RAIf rats had an oral LD_{50} of 560 mg/kg in males and 500 mg/kg in females; common signs were piloerection, hunched posture and dyspnoea. All animals had reduced locomotor activity and some males showed ataxia. At necropsy, involuted testes were seen in two males given 200 mg/kg (Hartmann, 1992b). The acute dermal LD_{50} was > 2000 mg/kg in this species (in a OECD test guideline 402 and GLP compliant study); piloerection and hunched posture were observed (Hartmann, 1992a). Acute exposure to dicyclanil aerosol for four hours to Tif:RAIf rats (nose-only) gave LC_{50} values of 3400 mg/m^3 in males and 3000 mg/m^3 for females (using an OECD test guideline 403 and GLP compliant design); effects noted included piloerection, hunched posture, dyspnoea and reduced locomotor activity. Spotting of the lungs was seen in high dose animals, as was abdominal distension in high dose males surviving the observation period. For all these studies, irrespective of route of exposure, animals surviving the immediate post-dosing period recovered within two to 12 days (Hartmann, 1993).

Single oral doses of 0, 1, 10, 50 or 100 mg/kg dicyclanil to male NMRI mice caused changes in general behaviour, locomotor activity and motor co-ordination at the high dose; locomotor activity was slightly affected at 10 mg/kg and very slightly at 1 mg/kg (Pfister & Hussherr, 1996). There were no treatment-related effects on gastrointestinal motility (Pfister & Gisin, 1996a).

In male Han Wistar rats, a single oral dose of 0, 1, 10, 50 or 100 mg/kg dicyclanil led to changed heart rate and tidal and minute lung volume and body temperature at the high dose; there were no treatment-related effects on blood pressure, electrocardiogram or respiration rate(Pfister & Nordmann, 1996). Treatment at 50 mg/kg had no effect on sperm motility, concentration or morphology while minor non-significant increases in abnormal sperm morphology were observed six weeks after dosing. These had completely reversed by 12 weeks after dosing (Pfister & Gisin, 1996b).

Acute dermal toxicity was evaluated in three New Zealand white rabbits which received 0.5 g dicyclanil as a semi-occlusive topical application to the shaved flank (in accordance with OECD test guideline 404; GLP compliant study). Very slight erythema was observed in all animals one hour after, and in one animal 24 hours after, removal of the patch (Hagemann, 1992a).

Acute ocular toxicity was evaluated in three New Zealand white rabbits which received an instillation of 0.1ml (84 mg) dicyclanil to the conjunctival sac of one eye; the other eye was used as control (in accordance with OECD test guideline 405; GLP compliant study). One animal showed an affected iris one hour after instillation but recovered by 24 hours. Slight chemosis of the conjunctiva was seen in two animals one hour after instillation; this also recovered within 24 hours. All animals had conjunctival redness of differing severities but recovered within one to seven days (Hagemann, 1992b).

No significant skin sensitisation (1 out of 20 had a positive reaction) was observed with 20% dicyclanil in an evaluation on 20 Pirbright white Tif:DHP Guinea pigs using an optimization test design conducted according to OECD test guideline 406 in a GLP compliant study. However, when dicyclanil was administered intradermally (at 0.1%), 13 out of 20 animals were positive, compared with three out of 20 given vehicle control (10% propylene glycol) only (Hagemann, 1993).

3.2 Repeat dose toxicity

In a three month toxicity study according to OECD test guideline 408 that was GLP compliant, Tif:RAIf rats were fed diet containing 0, 5, 25, 125 or 500 mg of dicyclanil/kg feed (equivalent to average intakes of 0, 0.31, 1.6, 8.0 or 33 mg/kg body weight/day in males and 0, 0.31, 1.7, 8.4 or 34 mg/kg bodyweight/day in females). Ten additional animals were

assigned to the control and high dose groups and were retained for a four week recovery period at the end of the treatment phase. No treatment-related deaths or clinical signs were noted. Slight reductions in body weight gain and food consumption were noted in all animals of the high dose and in males at 125 mg/kg diet; these showed reversal by the end of the recovery period. Increases in relative kidney, brain and testes weights were noted in male high dose rays and liver and brain weights in females of this dose. These changes reversed during the recovery period. Increased relative epididymidal weight was noted in males at ≥ 25 mg/kg but was considered not to be of toxicological significance as the absolute weights were unaltered and there was no associated histopathology. There were no treatment-related ophthalmoscopic, haematology or gross or microscopic changes. A single high dose recovery phase female developed a mammary tumour; the tumour was considered spontaneous. The NOEL was considered to be 25 mg/kg diet (equivalent to 1.6 mg/kg body weight/day) based on the changes noted in body weight gain (Bachmann, 1993).

In a dermal application study on Tif:RAIf rats were given 0, 5, 30, 300 or 1000 mg/kg/day dicyclanil under an occlusive dressing to a shaved area of dorsal skin for 6 hours/day, 5 days per week for four weeks, at the end of each application period the treated area was washed with lukewarm water. No treatment-related deaths or clinical signs occurred and, with the exception of a few incidental findings, no skin irritation was detected. At 300 or 1000 mg/kg/day, a dose-related decrease in body weight, body weight gain and food consumption was observed; in these animals, plasma sodium and calcium concentrations were also slightly reduced. High dose females had raised absolute and relative liver weights; a similar but not statistically significant effect was seen at 300 mg/kg/day. Absolute, but not relative brain, weights were increased in females given ≥ 30 mg/kg/day but no associated histopathology was noted. No gross effects were noted though histopathology examination showed hepatocyte hypertrophy in high dose animals and females only at 300 mg/kg/day. The NOEL was considered to be 30 mg/kg/day based on the reduction in body weight gain and the liver effects (Marty, 1995).

In a one year toxicity study in Beagle dogs (in accordance with OECD test guideline 452 and GLP compliant), dicyclanil was given in the diet at concentrations of 0, 5, 25, 150 or 750 mg/kg feed (equivalent to average intakes of 0, 0.16, 0.71, 4.4 or 23 mg/kg body weight/day in males and 0, 0.15, 0.77, 5.1 or 23 mg/kg body weight/day in females). Two additional animals per sex were included in the control and high dose groups which were kept for a further four week recovery period. One high dose female was found dead on day 13 with no associated clinical signs and one high dose male was killed on day 32 after signs of vomiting, marked apathy and lying in a lateral position; this animal also showed body weight loss and reduced food intake. High does females surviving until the end of the study showed vomiting, reduced body weight gain and food consumption. No treatment-related ocular, neurological, haematology or urinary effects were noted. Plasma cholesterol levels were increased in high dose animals and males given 150 mg/kg feed/day; effects in males did not reverse during the recovery period. Slightly reduced plasma calcium level in high dose males and reduced plasma bilirubin and urea concentrations and reduced alkaline phosphatase activity were noted in high dose males and females; these changes had partially reversed by the end of the recovery period. Absolute and relative liver weights were increased in high dose animals but absolute and relative heart weights were decreased in high dose females; changes in organ weights reversed during the recovery period. Macroscopic and microscopic examinations were conducted on animals found dead or killed on humane grounds only. Macroscopically, haemorrhagic abdominal cavity contents in the female and a scar in the liver and pale kidneys in the male were found. Microscopy revealed marked diffuse liver necrosis and kidneys lesions; these were more severe in the male which also showed testicular and prostatic atrophy. The female had a thrombus in a peritoneal blood vessel. The two animals had acute, severe liver failure resulting in cardiocirculatory disturbance, and weight loss induced stress in the male. Because of the marked difference in response of these two animals compared with findings in shorter term studies, it was concluded that these lesions were incidental.

Therefore the NOEL was considered to be 25 mg/kg of diet (equivalent to 0.71 mg/kg body weight/day in males and 0.77 mg/kg body weight/day in females (Altmann, 1995).

In an 18-month study, Tif:MAGf mice were given diets containing dicyclanil at 0, 10, 100, 500 or 1500 mg/kg diet (equivalent to average intakes of 0, 1.1, 12, 59 or 210 mg/kg body weight/day in males and 0, 1.1, 12, 65 or 200 mg/kg body weight/day in females). The study was conducted according to OECD test guideline 451 (except that histopathology was limited to high dose liver and lungs only) and was GLP compliant. At the highest dose self-inflicted injury and deaths were noted at higher than expected incidences. The poor condition of surviving high dose animals led to a decision to terminate this group during weeks 58 - 59. The maximum tolerated dose was therefore considered to have been exceeded at 1500 mg/kg diet/day in males and at 500 and 1500 mg/kg diet/day for females. Doses of ≤500 mg/kg diet did not affect survival. At 1500 mg/kg diet, reduced body weights and body weight gain were noted while females at 500 mg/kg diet had decreased body weight gain but not food intake. Higher food consumption ratios were noted at 1500 mg/kg diet and females at 500 mg/kg diet. There were no treatment related changes in haematological parameters. Absolute and relative liver weights were increased at 500 mg/kg diet. In females at 500 mg/kg diet, increases were also noted in relative (but not absolute) kidney, brain and adrenal weights. Pigmentation in the olfactory epithelium was observed at increased incidence and severity at 100 and 500 mg/kg diet; males with this finding also showed increased incidence of inflammatory cell infiltration in the underlying Bowman's glands, though the cell type was not specified. At 500 mg/kg diet, increased incidences of pigmentation of the adrenal glands, recorded as ceroid (i.e. a partly oxidised form of lipofuscin) deposition and of hypercellularity of the bone marrow. This study is further considered below in respect of carcinogenic effects observed (Bachmann, 1996a).

In a two year study, Tif:RAIf rats were given dicyclanil at 0, 5, 25, 125 or 500 mg/kg diet (equivalent to average intakes of 0, 0.19, 0.97, 4.8 or 22 ng/kg body weight/day in males or 0, 0.23, 1.2, 6.0 or 26 mg/kg body weight/day in females). The study was in accordance with OECD test guideline 453 and GLP compliant. Decreased food consumption in the high dose and reduced body weight gain in the mid- and high-dose animals was seen. Following evaluation of blood chemistry, levels of inorganic phosphate were increased in the high dose group and males at 125 mg/kg diet. Triglyceride levels were also reduced in high dose males. Due to the effect on body weight, almost all relative organ weights were increased at the high dose while absolute epididymal weights were also increased at this level. Non-neoplastic findings included a dose-related increase in incidence of pigmentation of the olfactory epithelium in males given 25 mg/kg diet or above and in females given 125 mg/kg diet or above. Macroscopically an increase in the incidence of liver cysts characterised as unilocular or multilocular biliary cysts in high dose females was noted. In high dose males, an increased incidence of masses and nodules in the exocrine pancreas was seen, characterised as foci or areas of hyperplasia. The NOEL for the 2 year rat carcinogenicity study was considered to be 125 mg/kg diet, equivalent to 22 mg/kg body weight/day due to the changes in body weight and histopathology observed in the liver and pancreas (Bachmann, 1996b). The pigmentation of the olfactory epithelium was examined in a separate study using tissue samples from the two year and three month rat studies described above. It was concluded that this was the consequence of enhanced natural age-related changes. In the absence of other morphological changes or effects on survival, behaviour or general wellbeing, these were not attributed significance (Weber, 1998) .

3.3 Carcinogenicity and mutagenicity

In the 18 month carcinogenicity study in Tif:MAGf mice (discussed above) macroscopic evaluation showed liver masses and/or nodules in females given 500 or 1500 mg/kg diet. Histopathologically, the liver macropathology was found to associate with a spectrum of non-neoplastic and neoplastic changes. These included Kupffer cell pigmentation (mainly

haemosiderin) and hepatocellular necrosis in males at 100 mg/kg diet and higher, and increased hepatocellular mitotic figures and multinucleated hepatocytes in high dose males. At the highest dose, frequency of foci of cellular changes was increased. The incidence of hepatocellular adenoma was increased in females at 500 and 1500 mg/kg diet compared with control while hepatocellular carcinoma incidence was higher in females given the highest dose. Dicyclanil did not affect the numbers of animals with malignant lymphoma but females given 500 mg/kg diet were observed to have more sites of infiltration of malignant lymphoma cells that their controls or other treated groups. The NOEL for this study was considered to be 10 mg/kg diet (1.1 mg/kg body weight/day) based on the liver effects noted (Bachmann, 1996a). More recent mechanistic studies have identified oxidative stress as a result of free radical generation is involved in liver tumour development following dicyclanil administration (Moto *et al.*, 2006).

In the two year carcinogenicity study in Tif:RAIf rats (described above), no treatment related effect of neoplasia was identified (Weber, 1998).

The available genetic toxicology studies (summarised in Table 3.1) have all proved negative, further suggesting that the neoplastic effect noted in the livers of mice (see above) may operate via a non-mutagenic mechanism (Ogorek & Arni, 1987, Hertner, 1992, Hertner, 1993b, Hertner, 1993a, Hertner, 1993c, Geleick, 1992).

Table 3.1 Summary of mutagenicity studies

In vitro			
Test type	**Test organism, cell and strain**	**Concentration**	**Result**
Reverse mutation	*Salmonella typhimurium* TA1527, TA98, TA 100	20 – 5000 µg/plate	Negative[a]
Reverse mutation	*S typhimurium* TA1535, TA1537, TA98, TA100 *Escherichia coli* WP2 uvrA	313 – 5000 µg/plate	Negative[b]
Gene mutation	V79 Chinese hamster lung cells, hprt locus	12.4 – 400 µg/ml – S9[c] 24.7 – 667 µg/ml +S9[d,e]	Negative
Chromosomal aberration	Chinese hamster ovary cells	20.8 – 83.4 µg/ml – S9[c] 166.75 – 667 µg/ml + S9[d,e]	Negative
Unscheduled DNA synthesis	Primary rat hepatocytes	6.2 – 670 µg/ml[e]	Negative
In vivo			
Test system	**Test organism**	**Dose**	**Result**
Micronucleus formation	Mouse bone marrow	47 – 188 mg/kg, single dose by oral gavage	Negative[f]

From IPCS, 2000

[a] With and without rat liver S9 fraction; results of cytoxicity test not disclosed and precipitation not reported
[b] With and without rat liver S9 fraction; precipitation at 5000µg/plate
[c] Cytotoxic at the highest concentration
[d] Slightly cytotoxic at the highest concentration
[e] The highest concentration represents the limit of solubility in dimethyl sulfoxide
[f] At all doses and all time points, the ratio of polychromatic to normochromatic erythrocytes did not deviate from that in controls; clinical signs of toxicity (including reduced locmotor activity, unkempt fur, ataxia, piloerection and diarrhoea) were observed at the highest dose tested; doses ≥312.5 mg/kg body weight resulted in death

Overall, dicyclanil is considered not to possess mutagenic activity (EMEA, 2000b) and that in the 18 month mouse study, the hepatic tumours observed (common in test species, of uncertain relevance to humans) were at doses that was above the maximum tolerated dose (MTD) (IPCS, 2000).

3.4 Reproductive and developmental toxicity

In a two generation reproduction study, Tif:RAIf rats were given dicyclanil at 0, 5, 30, 200 or 500 mg/kg diet from 10 weeks before the first mating until necropsy at the end of lactation. Dams were allowed to litter and suckle their pups naturally. The F_{1a} litters were culled to four pups per sex per litter at day 4 post partum. After weaning, some of the F_{1a} pups were necropsied. The F_0 parents were mated for a second time; F_{1b} litters were again culled to four pups per sex per litter and, after weaning, F_{1b} pups and F_0 parents were necropsied. The same protocol was followed for the F_1 parents and F_{2a} and F_{2b} generations. The study was conducted in accordance with OECD test guideline 416 and GLP.

There were no effects of dicyclanil on F_0 or F_1 generation parents with respect to mortality, clinical signs, male or female mating, fertility indices, maternal gestation, parturition indices or duration of gestation while parental animals from these generations showed no macro- or histo-pathological effects of treatment. In the F_0 generation, decreased food intake and body weight gain were noted in males and females of the high dose group and to a lesser extent at 200 mg/kg diet. As a consequence of the body weight effect, relative weights of most organs were increased in the high dose except for heart and liver where the absolute values were

decreased. In the F_1 generation during gestation periods for F_{2a} and F_{2b}, body weight gain was slightly lower than the controls in females although, in both lactation periods, the overall body weight gain was increased in the high dose and also slightly increased at 200 mg/kg diet. The absolute weight of most organs in the high dose males were decreased, except for the testis and brain. In the high dose females, only the heart, liver and kidney absolute weights were decreased. At 200 mg/kg diet, absolute weight of the liver was decreased in some animals of each sex, whilst most of the relative organ weights were increased, due to the body weight changes.

In the F_1 and F_2 generation pups, no treatment-related effects were seen in sex ratio, clinical signs, litter size, development of physical landmarks (surface righting and eye opening) or macroscopic evaluation. At the high dose, F_{1a} pup weights were reduced at birth; effects were also noted, due to reduced weight gain from day 4 post partum, at day 14 and 21 post partum. In contrast, weights of high dose F_{1b} pups were similar to controls from birth to day 14 post partum. However, thereafter, lower pup weights were noted on day 21. Reduced pup weights were also noted on days 14 and 21 post partum in the F_2 generation high dose group.

The parental NOEL was 30 mg/kg diet, equivalent to 2 mg/kg body weight/day, based on body weight effects. The reproductive NOEL was 500 mg/kg diet (equivalent to 24 mg/kg body weight/day), the highest dose tested. Offspring NOEL was 200 mg/kg diet (equivalent to 21 mg/kg body weight/day) because of the reductions in body weight gain (Khalil, 1995).

In a developmental toxicity study, pregnant Tif:RAIf rats were orally dosed at 0, 1, 5, 25 or 75 mg/kg bw/day dicyclanil on days 6-15 of gestation. On gestation day 21, dams were killed and necropsied and fetuses examined. There were no deaths or treatment-related clinical signs. At the high dose, reductions in maternal body weight, body weight gain, food consumption were noted; less marked changes in these parameters were noted at 25 mg/kg/day. One high dose animal had total early resorptions and haemorrhagic fluid in the uterus. For other high dose animals, observations comprised: gravid uterine weight reduction, increase early post implantation loss and lower numbers of fetuses per litter. High dose fetal effects included reduced weight, increased frequency of renal pelvic dilatation, a number of mainly sternebral defects and variations due to poor or absent ossification. There were no fetal effects at other doses and no evidence of teratogenicity. The NOEL for maternal toxicity was 5 mg/kg/day due to body weight reductions and the NOEL for developmental toxicity was 25 mg/kg/day based on the presence of fetal anomalies and growth impairment (FitzGerald, 1993b).

In a developmental toxicity study, pregnant Chbb:HM rabbits were dosed with 0, 1, 3, 10 or 30 mg/kg/day dicyclanil by oral gavage on days 7-19 of gestation. On gestation day 29, dams were necropsied and fetuses examined. There no deaths and no effect of treatment on clinical signs, macropathology or reproductive parameters. At the high dose, maternal body weight, body weight gain, food consumption and carcass weight were reduced and a reduction in maternal body weight gain was also noted at 10 mg/kg/day. At the high dose, fetuses showed body weight reduction and skeletal variation indicative of slightly delayed ossification, but no evidence of teratogenicity. The NOEL for maternal toxicity was 3 mg/kg/day due to reduced body weight gain, and the NOEL for developmental toxicity was 10 mg/kg/day based on reduced fetal weight and delayed ossification (FitzGerald, 1993a).

4 Guidelines and standards

A toxicological ADI of 0.007 mg/kg, equivalent to 0.42 mg per person, was derived by applying a safety factor of 100 to the NOEL of 0.7 mg/kg/day identified in a one year toxicity study in the dog.

The sum of dicyclanil and metabolite 2,4,6-triamino-pyrimidine-5-carbonitrile was identified as marker residue; this is considered to represent approximately 100%, 100%, 15% and 25% in muscle, fat, liver and kidney respectively at 21 days after treatment.

The CVMP recommended the inclusion of dicyclanil in Annex I of Council Regulation (EEC) No 2377/90 in accordance with MRL as shown in Table 4.1.

Table 4.1 MRLs for dicyclanil

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissue
Dicyclanil	Sum of dicyclanil and 2,4,6-triamino-pyrimidine-5-carbonitrile*	Ovine	200 µg/kg 150 µg/kg 400 µg/kg 400 µg/kg	Muscle Fat Liver Kidney

* With the only provision that dicyclanil should not be used in animals from which milk is produced for human consumption (EMEA, 2000a)

Based on these MRL the daily intake from food sources would represent approximately 98.5% of the ADI as estimated by the EMEA (EMEA, 2000a).

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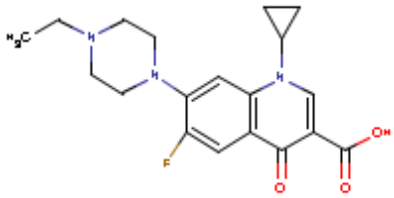
Enrofloxacin

1 Introduction

Enrofloxacin (1,4-dihydro-1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-4-oxo-3-quinoline-carboxylic acid, molecular formula $C_{19}H_{22}FN_3O_3$, CAS No. 93106-60-6; Figure 1.1; Box 1) is a fluoroquinolone antibiotic (ChemID Plus); it acts by inhibition of bacterial DNA gyrase in gram-negative bacteria. It is administered by subcutaneous (s.c.) injection to cattle, by intramuscular (i.m.) injection to pigs and orally to cattle, pigs, turkeys and chickens for treatment of infections of the respiratory and alimentary tract (EMEA, 1998c). In some countries, enrofloxacin is authorised for use in sheep, goats and rabbits (EMEA, 1999).

Figure 1.1 Molecular structure of enrofloxacin



From ChemID Plus

Box 1 Synonyms and trade names of enrofloxacin

1,4-Dihydro-1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-4-oxo-3-quinolinecarboxylic acid; 1-Cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid; BAY VP 2674; BRN 5307824; Baytril; CCRIS 8214; CFPQ; Enrofloxacin; HSDB 6952; UNII-3DX3XEK1BN

From ChemID Plus

Ciprofloxacin, a major metabolite of enrofloxacin, is used in human medicine by oral administration for the treatment of a wide variety of infectious diseases (EMEA, 1999).

In veterinary medicine in the UK, enrofloxacin is used for treating enrofloxacin-sensitive infections in cattle, pigs, chickens, turkeys, dogs, cats, and exotic species such as small mammals, reptiles and birds, for which a range of licensed products are available. There are concerns over the use of fluoroquinolones in food-producing animals due to the emergence of strains of Campylobacter and Salmonella with reduced susceptibilities to this group of compounds and the potential impact on human health (Bishop, 2004).

Cattle and pigs are treated by s.c. or i.m. injection, respectively, at a dose rate of 2.5 mg/kg daily for 3 days with either Baytril 5%™or 10%™ (50 or 100 mg/ml enrofloxacin w/v). The dose rate may be increased to 5 mg/kg for 5 days for salmonellosis or treatment of complicated respiratory disease. A single, depot injection containing 100 mg/mℓ enrofloxacin is available for use in cattle (Baytril Max™). An oral solution containing 25 mg/mℓ is available for the treatment of calves at 2.5 mg/kg (Baytril 2.5% Oral Solution™). Piglets up to 10 kg bw, may be treated orally with 1–2 mℓ of an oral solution containing 5 mg/mℓ

enrofloxacin at a dose rate of approximately 1.5 mg/kg (Baytril Piglet Doser™). Withdrawal periods for cattle are 14 days for meat and 84 hours for milk and for pigs 10 days for meat (NOAH, 2004).

Chickens and turkeys are medicated by addition to the drinking water at a dose rate of 10 mg/kg daily for 3–10 days (Baytril Oral 10% solution — 100 mg/ml enrofloxacin w/v). Treated birds should not be slaughtered within 8 days and the drug should not be given to birds within 14 days of commencement of laying (Bishop, 2004).

Dogs and cats are treated at 5 mg/kg with either Baytril 2.5%™or 5%™ (25 or 50 mg/ml enrofloxacin w/v) once daily or twice daily by subcutaneous injection, or by oral tablets (Baytril Tablets™) containing either 15, 50 or 150 mg enrofloxacin, according to weight and size (Bishop, 2004).

Potentially, humans might be exposed via ingestion of animal products containing drug residues.

1.1 Exposure concentrations in meat

Enrofloxacin is well absorbed following oral administration in all target species and is widely distributed to all tissues with the highest concentrations in liver and kidney and rapid excretion via faeces and urine. The main metabolite, ciprofloxacin, is also readily absorbed. Enrofloxacin binds strongly to cattle faeces, but less so to poultry excreta.

Experimentally-derived data are available on concentrations of enrofloxacin in meat products. Cattle were treated with enrofloxacin at 2.5 mg/kg bw/d for five days. Chickens were treated following typical treatment regimes (10 mg/kg bw/d for 10 d). Following treatment, the cattle were slaughtered and concentrations of enrofloxacin and the major metabolite, ciprofloxacin, were then measured in meat material. Enrofloxacin concentrations ranged from 0.7–5.75 mg/kg whereas ciprofloxacin concentrations ranged from 0.07–3.55 mg/kg (Table 1.1). Maximum concentrations of both enrofloxacin and ciprofloxacin were detected in the liver of both species.

Data are also available on the concentrations of enrofloxacin in eggs (Heitzman, 1997). Laying hens were administered enrofloxacin and concentrations of both enrofloxacin and ciprofloxacin were measured in the egg white and the egg yolk. The results are shown in Table 1.1.

Table 1.1 Concentrations of enrofloxacin and its metabolite, ciprofloxacin, in meat from cattle and chickens

	Concentration of enrofloxacin (mg/kg)	Concentration of ciprofloxacin (mg/kg)
Beef – muscle	0.7	0.86
Beef – kidney	1.3	3.25
Beef – liver	1.31	3.55
Chicken – muscle	2.05	0.07
Chicken – liver	5.75	2.1
Laying hen – egg white (1 d withdrawal)	3.63	0.200
Laying hen – egg yolk (1 d withdrawal)	3.14	0.21

2 Toxicokinetics

An analytical method is available for the determination of the combined residues of enrofloxacin and ciprofloxacin (the marker residue) in sheep tissues. The method involves extraction of the residues followed by column clean-up and analysis by HPLC with fluorescence detection. The limits of quantification were 25 µg/kg for enrofloxacin and for ciprofloxacin for ovine muscle and fat and 50 µg/kg for ovine liver and kidney (EMEA, 1999).

Enrofloxacin was well absorbed after oral administration to rats and the target species. In rats, the bioavailability of enrofloxacin was estimated to be 75% after a single oral dose of 5 mg/kg bw of radiolabelled enrofloxacin. The compound was widely distributed to all tissues with the highest concentrations in the liver and kidney. Elimination was rapid via urine and faeces, with most of the administered radioactivity excreted during the first 24 h after administration. Rat urine, after dosing with enrofloxacin (5 × 5 mg/kg bw/day), contained the following compounds: enrofloxacin 17%, ciprofloxacin 31%, oxociprofloxacin 5%, enrofloxacin amide 23%, dioxociprofloxacin 9%, desethylene ciprofloxacin 3%, desethylene enrofloxacin 2%, N-formyl ciprofloxacin <2%, oxoenrofloxacin <2% and hydroxy oxoenrofloxacin 3% (EMEA, 1998b).

The main metabolite of enrofloxacin, ciprofloxacin, is well absorbed. In humans, the bioavailability of orally administered ciprofloxacin was 63–69%. The main sites of absorption in humans were the duodenum and jejenum. Ciprofloxacin was eliminated by both renal and non-renal routes. It was calculated that about 14% of orally administered ciprofloxacin was excreted in human faeces. In humans, oxociprofloxacin was the main metabolite of ciprofloxacin found in urine (EMEA, 1998b).

Enrofloxacin was well absorbed in rabbits after oral, i.m. and s.c. administration of 5 mg/kg bw. C_{max} values of 0.45, 3.04 and 2.07 µg/mℓ, respectively, were attained 1–2 hours after administration. The respective bioavailabilities were estimated to be 61%, 92% and 72%. In an *in vitro* study using fractions of rabbit liver, the metabolism of enrofloxacin was shown to be similar to that of the rat, with ciprofloxacin as the main metabolite (12–15% of radioactivity). Rabbits were dosed orally, in drinking water, with 13 mg enrofloxacin/kg bw/day for 7 consecutive days. Residues of enrofloxacin in liver and muscle were detectable only in samples taken one day after treatment (<10–20 µg/kg). No residues were detected in fat. In kidney, residues of enrofloxacin declined from 13–100 µg/kg, one day after treatment, to <10–30 µg/kg, 4 days after the end of treatment (EMEA, 1998a). Rabbits were given a single oral dose of 5 mg/kg enrofloxacin and killed at intervals up to 12 h later.

The mean tissue levels (enrofloxacin + ciprofloxacin) at 1 h were: liver 1411 µg/kg; kidney 1683 µg/kg; muscle 543 µg/kg; and lung 151 µg/kg. These tissue levels declined rapidly: at 12 hours the respective values were 179 and 225 µg/kg for liver and kidney and non-detectable for muscle and lung (Cagnardi *et al.*, 2003).

The pharmacokinetics of enrofloxacin and ciprofloxacin were studied in dogs after an oral tablet of 5 mg/kg enrofloxacin and intravenous (i.v.) injections of 5 mg/kg enrofloxacin and ciprofloxacin. A model was used to examine enrofloxacin metabolism and fitted parameters were used to calculate plasma clearance: 0.729 ℓ/h/kg for enrofloxacin, 0.468 ℓ/h/kg for ciprofloxacin; distribution volume: 2.45 ℓ/kg for enrofloxacin, 1.92 ℓ/kg for ciprofloxacin; and mean residence time: 3.47 hours for enrofloxacin and 4.2 hours for ciprofloxacin. Enrofloxacin was mainly metabolised to ciprofloxacin. The fraction of metabolised drug was similar after i.v. and oral doses, the hepatic first pass effect being low at 7.5% (Cester & Toutain, 1997).

Enrofloxacin was administered to goats by a single i.m. injection of 2.5 mg/kg bw. The peak plasma concentrations of enrofloxacin (1.13 µg/ml) and ciprofloxacin (0.24 µg/ml) were found at 0.8 and 1.2 h, respectively. The elimination half-life, volume of distribution, total body clearance and mean residence time of enrofloxacin were 0.74 hours, 1.42 ℓ/kg, 1329 mℓ/h/kg and 1.54 hours, respectively. The metabolic conversion of enrofloxacin to ciprofloxacin was appreciable (36%) and the sum of the plasma concentrations of enrofloxacin and ciprofloxacin was maintained at or above 0.1 µg/ml for up to 4 hours. Enrofloxacin was given by single i.v. and i.m. administrations of 5 mg/kg bw to 5 adult Angora goats. Pharmacokinetics were best described by a two-compartment open model. The elimination half-life and volume of distribution after i.v. and i.m administrations were similar at 4.0–4.7 h and 1.2–1.5 ℓ/kg, respectively. Enrofloxacin was rapidly ($t_{½}$, 0.25 h) and almost completely absorbed (90%) after i.m. administration (Rao *et al.*, 2001).

Serum concentrations and pharmacokinetics of enrofloxacin were studied in 6 mares after i.v. and (intragastric) i.g. administration at a single dose of 7.5 mg/kg bw. At 5 minutes after the i.v. injection, the mean serum concentration was 9.04 µg/mℓ, decreasing to 0.09 µg/mℓ by 24 hours. The elimination half-life was 5.33 ± 1.05 h and the area under the serum concentration *versus* time curve (AUC) was 21.03 ± 5.19 mg.h/ℓ. After the i.g. dose the mean peak serum concentration was 0.94 ± 0.97 µg/mℓ at 4 hours, declining to 0.29 ± 0.12 µg/mℓ by 24 hours (Haines *et al.*, 2001).

Four Jersey cows, 3–4 years old, 370 ± 20 kg weight were injected with enrofloxacin i.v. at a single dose of 5 mg/kg. Blood samples were collected at intervals up to 30 hours post-injection. The plasma concentration of enrofloxacin was 2.7 µg/mℓ at 2 minutes after dosing. The therapeutic concentration (0.1 µg/ml) of enrofloxacin and ciprofloxacin remained in the plasma for more than 12 and 8 hours, respectively. The plasma concentration time profile following the single i.v. dose was adequately fitted to a two-compartment open model. The elimination rate constant was 0.282/h with an elimination half-life of 2.61 hours. Enrofloxacin was widely distributed in the extravascular compartments. The clearance of enrofloxacin in this study was comparable to that seen in dairy cows of 21 mℓ/kg/min. The AUC of enrofloxacin in plasma was 4.4 µg.h/mℓ. Ciprofloxacin, the active metabolite, appeared within 2 minutes of the enrofloxacin injection at a concentration of 0.26 µg/mℓ with a peak plasma level of 1 µg/mℓ at 30 minutes. Its elimination half-life was 3.1 h and the AUC was 3.7 µg.h/mℓ (Varma *et al.*, 2003).

The pharmacokinetics of i.v. and orally administered enrofloxacin were investigated in 6 adult sheep in single dose and multiple dose regimes. Single dose studies included an i.v. bolus of 5 mg/kg enrofloxacin and an oral suspension of 10 mg/kg crushed 68 mg enrofloxacin tablets. The multiple dose regimen consisted of a mixture of 10 mg/kg enrofloxacin solution and grain per 24 hours for 7 days. The results for the single i.v. dose were: $t_{1/2}$ = 4.76 ± 2.71 h, clearance

= 210 ± 0.25 mℓ/kg/h and volume of distribution = 0.40 ± 0.06 ℓ/kg. The mean AUC was 23.23 ± 12.60 µg.h/mℓ. For the oral dose , the parameters were: $t_{1/2}$ = 14.99 ± 1.14 h, the mean AUC was 29.92 ± 11.75 µg.h/mℓ and C_{max} = 1.24 ± 0.98 µg/mℓ. For the multiple dosing, the mean C_{max} was 3.11 ± 1.82 µg/mℓ. The amount of enrofloxacin metabolised to ciprofloxacin was <20% in all the studies. Enrofloxacin administered orally to sheep has a prolonged $t_{1/2}$ and acts as a sustained release product (Bermingham & Papich, 2000). The pharmacokinetics of enrofloxacin was also examined after its i.v. and i.m. administration in 6 lactating sheep. After i.v. injection, as a bolus, the elimination half-life, the volume of distribution and the AUC were 3.30 ± 0.36 h, 2.91 ± 0.17 ℓ/kg and 4.19 ± 0.18 µg.h/mℓ, respectively. The maximum milk concentrations of enrofloxacin (C_{max}) and the AUC were 2.38 ± 0.14 µg/mℓ and 23.76 ± 2.21 µg.h/mℓ, respectively. After i.m. administration, the elimination half-life, C_{max}, time of C_{max} and bioavailability were 3.87 ± 0.10 h, 0.74 ± 0.07 µg/mℓ, 0.83 ± 0.12 h and 75.35%, respectively. The C_{max} and AUC for milk were 1.94 ± 0.13 µg/mℓ and 24.81 ± 2.25 µg.h/mℓ, respectively (Haritova *et al.*, 2003).

Eight male pigs were used to study the pharmacokinetics of enrofloxacin after single i.v. and i.m. administrations at 2.5 mg/kg bw. Twelve pigs were used to study tissue residue distribution; they were given daily doses of enrofloxacin at 2.5 mg/kg, i.m. for 3 days. The mean elimination half-life and mean residence time (MRT) of enrofloxacin in plasma were 9.64 ± 1.49 and 12.77 ± 2.15 hours, respectively, after i.v. administration and 12.06 ± 0.68 and 17.15 ± 1.04 hours, respectively, after i.m. administration. The bioavailability of enrofloxacin after i.m. administration was 74.53 ± 5.20%; the maximal plasma concentration of 1.17 ± 0.23 µg/mℓ was detected 1.81 ± 0.23h after i.m. administration. After i.m. dosing, the metabolite, ciprofloxacin, was 51.5% of the parent drug plasma concentrations. The peak ciprofloxacin plasma level of 0.71 ± 0.14 µg/mℓ occurred at 1.75 ± 0.63 h after enrofloxacin administration. Mean concentrations of enrofloxacin and ciprofloxacin ranging between 0.029 and 0.079 µg/mℓ were detected 5 days after the last injection in muscle, liver , kidney and adipose tissue. After 10 days, ciprofloxacin was not detected in any tissues and enrofloxacin was detected only in liver (0.02 µg/g) and kidney (0.01 µg/g; (Anadon *et al.*, 1999)).

Plasma enrofloxacin concentrations after single i.v. dosing to healthy and *E. coli*-infected broilers were best described by a two-compartment model. The disposition kinetics in the healthy and infected birds were as follows: elimination half-life 4.75 *vs* 3.63 hours; apparent volume of the central compartment 1.11 *vs* 1.57 ℓ/kg; rate constant for transfer from peripheral to central compartment 1.15 *vs* 1.4 ℓ/h; and total body clearance 0.35 *vs* 0.53 ℓ/h/kg. After oral administration, the absorption half-life in infected birds was significantly longer than in healthy birds, while the elimination half-life and MRT were significantly shorter. Bioavailability was higher in infected birds at 72.5% compared with healthy birds at 69.8% (Soliman *et al.*, 2000).

Several studies have been undertaken to quantify and characterise tissue residues in target species. When pigs were injected s.c. with 5 mg ^{14}C-enrofloxacin/kg bw/day for 5 consecutive days, mean total residue levels (3 animals) in kidney, liver, muscle and fat fell from 2080, 1790, 862 and 148 µg equivalents/kg, respectively, at one day after the last dose, to 141, 277, 28 and 10 µg equivalents/kg, respectively, at 5 days. The residues, from 1 gilt and 1 barrow killed 12 hours after dosing, were characterised as approximately 80, 80, 90 and 99% as enrofloxacin in liver, muscle, kidney and fat; some ciprofloxacin was also present. When cattle were injected s.c. with 5 mg ^{14}C-enrofloxacin/kg bw/day for 5 consecutive days, total residue levels in liver, kidney, muscle and fat fell from 10 050, 7470, 1540 and 625 µg/kg, respectively, at 8 hours after the last dose to 639, 66, 4 and 6 µg/kg, respectively, at 14 days. In the residues of tissues from 1 steer and 1 heifer killed 8 hours after the last dose, enrofloxacin accounted for 21–26%, 27–31%, 55–56% and 35–59% in the liver, kidney, muscle and fat, respectively. Ciprofloxacin accounted for 38–44%, 50–55%, 33–34% and 5–7% of the residues in these same tissues. Oxoenrofloxacin was the major component of the residues in fat. When 3 lactating cows were dosed i.v. with 5 mg ^{14}C-enrofloxacin/kg bw/day

for 5 consecutive days, the total residue levels in milk samples fell from approximately 3267 µg/ℓ at 6 hours after dosing to <17–140 µg/kg at 57 hours. About 80% of the residues in milk samples taken 6–24 hours after dosing were ciprofloxacin and 8% were enrofloxacin. In neonatal sheep given a single oral dose of 7.5 mg enrofloxacin/kg bw, the mean residue levels of enrofloxacin, 2 days after dosing, in liver, kidney, muscle and fat were 520, 1370, 993 and 1370 µg/kg, respectively, and these declined to 48, 13, 13 and 13 µg/kg 4 days after dosing. Over the same time period, mean residues of ciprofloxacin in these tissues declined from 483, 175, 265 and 175 µg/kg in liver, kidney, muscle and fat to 53, 13, 13 and 13 µg/kg. When poultry were dosed orally with 10 mg ^{14}C enrofloxacin/kg bw for 7 days, the total residue levels in liver samples taken over 6–24 hours after the last dose declined from 4890 µg equivalents/kg to 89 µg/kg in chickens and from 8240 µg equivalents/kg to 2662 µg/kg in turkeys. In chickens, 61–66%, 51%, 53–62% and 85% of the total residues present in kidney, liver, fat and muscle, 6 hours after dosing, were composed of enrofloxacin. The comparative values for turkeys were 58–60%, 94–97%, and 99% for liver, kidney and muscle, respectively (EMEA, 1998a; 1998b).

In an *in vitro* experiment, the inhibition of P-450 reductase by enrofloxacin was assessed by measuring the NADPH-cytochrome c reductase activity and the inhibition of P-450 1A1, 1A2 and 11B in rat liver microsomes. NADPH-cytochrome c reductase was not affected. Enrofloxacin induced a strong concentration-dependent inhibition of P-450 1A1 and 1A2. In an *in vivo* study, the effects of five administrations of 5, 25, or 100 mg enrofloxacin/kg bw/day were assessed in rats. A slight induction of P-450 11B1 and 11B2 expression and activity (140% of controls) was present only at the 5 mg/kg bw/day dose level (Vancutsem & Babish, 1996). Enrofloxacin was administered orally to 25-day old commercial broiler chickens at a dosage of 10 mg/kg bw daily for a 3-day period. Enrofloxacin was found significantly to inhibit microsomal cytochrome P-450 monooxygenases up to 9 days post treatment (Shlosberg *et al.*, 1997).

3 Toxicity profile

3.1 Acute toxicity

Although there appear to be no conventional acute toxicity tests for enrofloxacin, the compound is considered to be of low acute toxicity on the basis of its reactions in other types of toxicity testing. For example, acute signs were not seen at high dose levels (up to about 1000 mg/kg bw) of enrofloxacin in a 13-week dietary study in rats (EMEA, 1998c).

The main metabolite of enrofloxacin, ciprofloxacin, has been widely used in human medicine for several years; side effects reported include gastrointestinal disturbances, some hypersensitivity reactions and crystalluria (EMEA, 1998c).

3.2 Repeat dose toxicity

Frequencies of 0.5–5% of nervous manifestations have been described after a dose of 250 mg ciprofloxacin in humans (= 3.6 mg/kg bw). The intensity of these manifestations is diverse and includes headaches, dizziness, tremors, hallucinations, psychosis and seizures (Vancutsem *et al.*, 1990).

In a 13-week study in rats, enrofloxacin was administered in the feed at doses up to 7500 mg/kg. Reduced body weight gain was observed in both sexes receiving the top dose. Caecal distension occurred with no corresponding microscopic alterations, a common finding in rats given large doses of antibiotics. Histopathological changes were observed in the knee joints in 3 out of 30 animals receiving the top dose and, in male rats at this dose level, round or oval cells in the epididymides (12/15 rats) and in the seminiferous tubules (5/15 rats). These cells probably represented a degenerating or necrotic ‘cap phase’ spermatid. The no-

effect level for the study was considered to be 300 mg/kg enrofloxacin in diet (equal to 40 mg/kg bw). In order to examine the testicular effects further, a study incorporating recovery periods was undertaken. In rats at the lowest dietary dose administered (125 mg/kg = 10 mg/kg bw/day), no abnormal spermatozoa were found. Neither were abnormal spermatozoa found in the seminiferous tubules of any rat at the end of a 90-day recovery phase and recovery of the epididymides was also apparent. The effects on rat testes were therefore reversible. Degenerative changes, typical of those induced by floroquinolone antibiotics, were found in the articular cartilage after administration of enrofloxacin to immature animals such as calves, piglets and puppies. Dogs were the most sensitive to this effect and a NOEL of 3 mg/kg bw/day was established for arthropathy in a 13-week repeat dose study in which the dogs were 3 months old at the start (EMEA, 1998c). Following a re-evaluation of the results of the 13-week repeat dose toxicity studies in rats and dogs, it was concluded that effects seen in the testes of dogs were inconsistent, not dose related and were of a different type from those seen in rats. The toxicological ADI for enrofloxacin should therefore be based on the NOEL of 3 mg/kg bw/day for arthropathy in juvenile dogs (EMEA, 1998b).

Ten Turkish Shepherd dogs, 24–30 months old, were given enrofloxacin i.m. at 5 mg/kg bw daily for 14 days and blood samples were taken regularly during the study period for investigation of biochemical, haematological and blood gas parameters. Acidosis and temporary increases in indirect bilirubin, sodium, partial pressure of CO_2 and mean corpuscular volume and decreased levels of inorganic phosphorus, ionised calcium, potassium, partial pressure of O_2 and standard bicarbonate were observed (Tras *et al.*, 2001).

The possible relationship between the administration of parenteral enrofloxacin and the onset of acute retinal degeneration in cats was evaluated in a retrospective clinical study. The cats identified were all of the domestic shorthair breed; 7 were females and 10 were males. Ages ranged from 3 to 16 years. The daily and total dosage of enrofloxacin and number of days of administration were highly variable. The clinical signs were most often mydriasis and acute blindness. All cats had diffuse retinal degeneration as evidenced by increased tapetal reflectivity and retinal vascular attenuation. Vision returned in a few cats, but retinal degeneration persisted or even progressed. Histopathology of 2 eyes revealed primarily outer retinal degeneration with diffuse loss of outer nuclear and photoreceptor layers and hypertrophy and proliferation of the retinal pigment epithelium (Gelatt *et al.*, 2001).

No evidence of leg weakness was observed in poultry treated at the recommended rate of enrofloxacin. No evidence of arthropathy was observed in calves and pigs treated according to the recommended dosage regime. However, oral administration of 30 mg/kg bw/day for 14 days to calves and 50 mg/kg bw/day to pigs produced histopathological evidence of arthropathy. No adverse effects were observed in neonatal lambs treated orally with 5–7 mg/kg bw enrofloxacin and the lambs grew normally over the first 3 months of life (EMEA, 1998a). Toxicity problems associated with cartilage and bone development have occurred with enrofloxacin use in young horses and it is therefore not recommended for use in this species (Vancutsem *et al.*, 1990).

3.3 Carcinogenicity and mutagenicity

In a rat carcinogenicity study on enrofloxacin, the incidence of endocardial tumours was elevated in females at the top dose (level not stated). When the incidence of endocardial tumours was combined with the incidence of proliferative lesions, the difference from the controls was significant in both sexes at the top dose. Also, the incidence of bile duct hyperplasia was increased in a dose-related manner. However, an independent review of the histopathology indicated that both the endocardial neoplasms and the Schwann cell-like hyperplasia were not associated with the administration of enrofloxacin. In a second carcinogenicity study in the same strain of rat, no neoplastic change attributable to

enrofloxacin treatment was observed. However, because evidence of bile duct hyperplasia was apparent at the lowest dose level, a third study was carried out. Histopathological examinations after 12 months showed no evidence of a dose-related increase in bile duct hyperplasia at dose levels of up to 50 mg/kg enrofloxacin in the diet. Although the incidence of malignant lymphoma was increased in treated groups in a carcinogenicity study in the mouse with enrofloxacin, the incidence was within the historical control range. It was concluded from these studies that enrofloxacin was not carcinogenic (EMEA, 1998c).

Several *in vitro* mutagenicity assays have been reported for enrofloxacin. The compound produced no increase in revertants in a bacterial assay for gene mutation though only very low drug concentrations could be used because of the high toxicity shown towards the tester strains of bacteria. Sporadic increases in mutant frequency were observed in a CHO HGPRT forward mutation assay but with no evidence of a dose–response and an *in vitro* UDS assay gave negative results (EMEA, 1998c). Other reported studies included a *S. typhimurium* assay with the TA102 tester strain with enrofloxacin at 0.015 µg/plate (- S9) which gave a positive result and a mouse lymphoma L5178Y (TK+/TK-) test (- S9) with enrofloxacin at 500 µg/mℓ which also gave a positive result. Another mouse lymphoma L5178Y (HPRT) 6-thioguanine test gave a negative result. A Chinese hamster V-79 assay (- S9) with enrofloxacin at 600 µg/mℓ was also negative (CCRIS, 2000). Chromosomal aberrations were evaluated in cultures of human peripheral lymphocytes exposed to enrofloxacin or ciprofloxacin. The control analysis resulted in 3.6 ± 0.6 chromosomal aberrations/100 cells while the treated cultures resulted in 8.3 ± 0.8 and 9.6 ± 1.2 aberrations/100 cells at 5 and 50 µg/mℓ enrofloxacin and 5.6 ± 1.3 and 7.7 ± 3.5 aberrations/100 cells at 5 and 25 µg/mℓ ciprofloxacin. Some cytotoxic effect was noted with enrofloxacin at 50 µg/ml. These results indicate a genotoxic effect for these 2 compounds in this system (Gorla *et al.*, 1999).

There was a small but dose-related increase in sister chromatid exchanges per metaphase in an *in vivo* assay in hamster bone marrow but the increase was not statistically significant and no evidence of mutagenicity was found in either an *in vivo* mouse micronucleus test nor in an *in vivo* cytogenetics assay in rat bone marrow (EMEA, 1998c).

3.4 Reproductive and developmental toxicity

Effects on the male rat epididymides and seminiferous tubules have been described in a 13-week study in Section 3.2. Similar effects on the male reproductive tract due to enrofloxacin were observed in several other studies in rats including a multigeneration study in which reproductive performance was impaired at the top dose level of 7500 mg/kg. The reported NOEL was 300 mg/kg (equivqlent to 40 mg/kg bw) (EMEA, 1998c). A separate reproductive study administered enrofloxacin via subcutanenous injection to 20 male mice. The dose administered was 150 mg/kg for 15 days. Effects observed included deterioration of sperm content, motility and morphological abnormalities due to disruption of spermatogenesis (Aral *et al.*, 2008). These effects on spermatogenesis were not observed in repeat dose studies in other species. Enrofloxacin was not teratogenic in either the rat or the chinchilla and NOELs for fetotoxicity of 50 and 25 mg/kg bw/day, respectively, were established (EMEA, 1998c). Treatment of rabbits according to the recommended dosage regime had no adverse effects on general health, reproductive performance or development of the offspring (EMEA, 1998a).

The placental transfer of enrofloxacin and ciprofloxacin was evaluated in a two-step infusion programme to establish steady state maternal plasma concentrations for these drugs. For each compound, the placenta in 5 rabbits was perfused for 200 minutes with Earle's enriched bicarbonate buffer at a flow rate of 1.5 mℓ/min. Plasma protein binding estimation indicated no difference between the drugs. Placental clearance of the drugs was significantly different: 0.88 ± 0.13 mℓ/min for enrofloxacin and 0.06 ± 0.02 mℓ/min for ciprofloxacin. These values accounted for 81 and 5%, respectively, of the placental clearance found for antipyrene, a commonly used indicator of placental exchange (Aramayona *et al.*, 1994).

4 Guidelines and standards

The EMEA CVMP has based a toxicological ADI for enrofloxacin on the NOEL of 3 mg/kg bw/day for arthropathy in juvenile dogs (see Section 3.2). By applying a safety factor of 100 the toxicological ADI for enrofloxacin was determined to be 30 µg/kg bw (EMEA, 1998b)

A microbiological ADI for enrofloxacin has also been calculated, using a formula recommended by the CVMP involving a daily faecal bolus of 150 g. The calculated microbiological ADI was shown to be 6.2 µg/kg bw, that is 372 µg/person (EMEA, 1998b).

JECFA obtained a microbiological ADI of 620 µg/60 kg equivalent bw/day using the results from a simulated human gut model (Cerniglia & Kotarski, 1999).

The CVMP recommended the inclusion of enrofloxacin in Annex 1 of Council Regulation (EEC) No. 2377/90 in accordance with the MRLs shown in Table 4.1. In coming to these recommendations, the Committee took into account that a microbiological ADI of 372 µg/person had been established for enrofloxacin (EMEA, 2002).

Table 4.1 MRLs for enrofloxacin

Marker residue	Animal species	MRL (µg/kg)	Target tissue
Sum of enrofloxacin & ciprofloxacin	Bovine,ovine,caprine	100	Muscle
		100	Fat
		300	Liver
		200	Kidney
		100	Milk
	Porcine, rabbits	100	Muscle
		100	Fat
		200	Liver
		300	Kidney
	Poultry[a]	100	Muscle
		100	Skin+fat
		200	Liver
		300	Kidney
	All food-producing species except the above	100	Muscle
		100	Fat
		200	Liver
		200	Kidney

[a] Not for use in animals from which eggs are produced for human consumption
From EMEA 2002

It was estimated by the EMEA that extending the MRLs to all food-producing species, as proposed above, would result in a consumer intake from food sources not exceeding 74% of the ADI (EMEA, 2002).

The US FDA has set a tolerance of 0.3 ppm for residues of enrofloxacin (marker residue) in muscle (target tissue) of chickens and turkeys. For cattle, a tolerance of 0.1 ppm for desethylene ciprofloxacin (marker residue) has been established in liver, the target tissue (CFR).

In October 2000 the FDA approved the withdrawal of a new animal drug application for enrofloxacin in poultry. The CVMP is addressing this withdrawal and is currently reviewing the use of fluoroquinolones in food-producing animals; the final publication is to follow. The reason for withdrawal in poultry has not been disclosed (EMEA, 2006).

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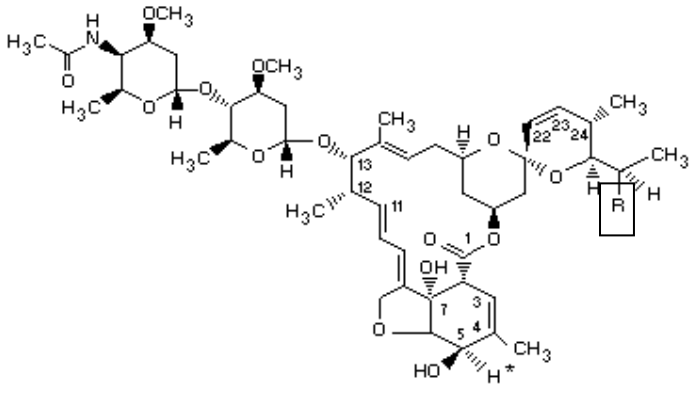
Eprinomectin

1 Introduction

Eprinomectin (4”-(Acetylamino)-4”-deoxy-avermectin B1, (4”R)-; CAS No. 123997-26-2;, molecular formula is unspecified; Figure 1.1; Box 1 (ChemID Plus) is a semi-synthetic compound of the avermectin family. Eprinomectin is not intended for use in human medicine.

Figure 1.1 Molecular structure of eprinomectin[1]



[1]B1a component: R = C_2H_5

[1]B1b component: R = CH_3

From ChemID Plus

Box 1 Synonyms and trade names of eprinomectin

A mixture of two components having a ratio of 90% or more of Eprinomectin Component B1a and 10% or less of Eprinomectin Component B1b; A mixture of two components having a ratio of 90% or more of epinometin component B1a and 10% or less of eprinometin component B1b; Eprinex; Eprinomectin; MK 397; MK-397; UNII-75KP30FD8O

From ChemID Plus

Eprinomectin is intended for the treatment of internal and external parasites in cattle and lactating cows; the recommended treatment regimen is a single topical dose of 0.5 mg/kg. Eprinomectin is a mixture of two homologues eprinomectin B1a (90%) and eprinomectin B1b (10%) which differ by a methylene group in the C25 position (highlighted in figure 1.1) (EMEA, 1996). The precise mode of action of eprinomectin is uncertain but avermectins generally affect specific high affinity receptors present in target organisms, resulting in paralysis and death (Turner & Schaeffer, 1989). Physiologically, avermectin binding lead to increased membrane permeability to chloride ions independent of gamma-aminobutyric acid (GABA) mediated chloride channels, except at high levels where avermectins can interact with GABA-gated chloride channels. Neurotoxicity is elicited only at higher concentrations in mammals than nematodes, possibly because of differences in numbers of specific high

[1] [1]where the R group, highlighted in the box, represents the point of substitution to generate eprinomectin B1a or B1b

affinity sites or the limited transport through the blood-brain barrier (Lankas & Gordon, 1989).

2 Toxicokinetics

There are no data available on the toxicokinetics of eprinomectin in humans.

The most relevant experimental information relates to an oral toxicokinetic study in the rats. In this, Sprague-Dawley rats were given [5-^{3}H]-eprinomectin at 6 mg/kg/day for one week. Although information on the rate and percentage of absorption was not stated, it was noted that at seven hours after the last dose tissue levels were highest (in abundance order) in the gastrointestinal tract, liver, fat, kidney and muscle; levels were lower in plasma and erythrocytes. The parent compound was the major residue (75 - 94%) in all tissues including the plasma by seven hours and accounted for 82-87% of that remaining after one day. A number of metabolites were present, including N-deacetylated B1a which was the major metabolite at these time points. Other minor metabolites, each representing <7% of the total radioactivity, were also found including the 24α-mydroxymethyl, 24α-hydroxy and 26α - hydroxymethyl metabolites of eprinomectinB1a. Thus, the primary route of metabolism involves N-deacetylation. Overall excretion was 90% via the faeces and < 1% in urine and by five days of treatment, total levels in all tissues were < 0.1mg/kg (Halley *et al.*, 1995).

Although of limited relevance, two studies have also reported on the toxicokinetics of dermal administration to Angus and Hereford beef cattle and Holstein dairy cattle. These demonstrated that single topical application led to a slow and prolonged dermal absorption over a two week period, although approximately half the administered dose was not absorbed after 28 days. Excretory patterns were, however, similar to those noted in orally dosed rats, with only 0.35% of administered dose excreted via the urine and 14% in the faeces after 28 days (Green-Erwin *et al.*, 1994, Faidley, 1995).

3 Toxicity profile

3.1 Acute toxicity

There is no information on the acute toxicity of eprinomectin to humans.

The oral LD_{50} was, in mice, 70 mg/kg and, in rats, 55 mg/kg. The intraperitoneal LD_{50} was somewhat similar at 35 mg/kg in both species. Signs of toxicity included ataxia, tremor, loss of righting reflex, ptosis and bradypnoea. Surviving animals recovered in four to five days (Bagdon & McAfee, 1990).

3.2 Repeat dose toxicity

There is no information on the repeat dose toxicity of eprinomectin to humans.

Sprague Dawley rats were fed diet containing eprinomectin at levels intended to achieve 0, 1, 5 or 30 mg/kg body weight/day, for 90 days. However, because of low food consumption, the high dose group received only 25 mg/kg body weight/ day. However, profound decreases in body weight and body tremors were noted and the dosage was reduced to 20 mg/kg body weight/day at week 4 in females and week 5 in males. No other clinical signs or ophthalmic effects were noted in this group. However, they showed an increase in blood urea nitrogen. In males only, haematocrit and erythrocyte count were increased slightly while females showed increases in serum protein and albumin and decreased mean lymphocyte count. Urinalysis for this group showed a slight increase in urine specific gravity and slight decrease in urine volume. Organ weight effects for this group included changes in liver, uterus, pituitary,

adrenal, ovary, spleen, thymus and prostate weight. Arrest of normal ovarian follicular maturation was apparent in 15/20 high dose females and the uteri of four animals showed endometrial squamous metaplasia. These changes were attributed to an oestrogen:progesterone imbalance which was also apparent as a decrease in remodelling of the femora (primary spongiosa) in 12/20 high dose females. Slight sciatic degeneration was also reported in three males and three females in the high dose. The NOEL was 5 mg/kg day (Kloss *et al.*, 1990).

In a one year Beagle dog study, oral administered of eprinomectin at 0, 0.5, 1 or 2 mg/kg/day resulted in mydriasis in high dose animals. One high dose dog was also less active and showed salivation and ataxia progressing to lateral recumbency; decreased body weight and food consumption was also noted; it was killed on humane grounds and necropsied at week 13. No treatment related changes in ophthalmoscopy, electrocardiography, haematology, clinical chemistry, urinalysis or organ weight were noted in the other animals although macroscopic evaluation revealed pin-point dark brown/black foci in the mucosa of the neck of the gall bladder (identified as thickened/dry bile) in animals of all groups including the controls. Very slight focal degeneration of one to three neurons per dog were however noted in the pons area and/or cerebellar nuclei of high dose animals. This degeneration was characterised by neuronal enlargement resulting from increased eosinophilic, vacuolated cytoplasm with nuclear displacement; no such effect was noted at other doses or in controls. The NOEL based on mydriasis and neuronal changes, was 1 mg/kg/day (Kloss *et al.*, 1994).

3.3 Carcinogenicity and mutagenicity

No carcinogenicity studies have been conducted on eprinomectin.

Table 3.1 shows the mutagenic studies that have been conducted with eprinomectin; all were negative.

Table 3.1 Summary of mutagenicity studies

End point	Test system	Concentration	Result
	In vitro		
Reverse mutation	*S typhymurium* TA97a, TA98, TA100, TA1535; *E coli* WP2, WP2uvrA, WP2 urApKM101	100 – 10,000 µg/plate, ± S9	Negative[a]
Gene mutation	V-79 Chinese hamster lung cells, *hprt* locus	1 – 40 µmol/plate, -S9; 10 – 4-µmol/plate, +S9	Negative[b]
Cytogenetic alterations	CHO cells	8 – 12 µmol/plate, -S9; 5 – 7 µmol/plate, +S9	Negative[b]
DNA damage	Primary rat hepatocytes	10 – 51 µmol/plate	Negative
End point	**Test system**	**Dose**	**Result**
	In vivo		
Micronucleus formation	Mouse bone marrow	10 – 40 mg/kg, single oral dose	Negative[c]

From IPCS, 1998

[a] precipitation at 10,000 µg/plate
[b] dose related cytotoxicity in absence and presence of S9
[c] at all doses and all time points, ratio of polychromatic to normochromatic erythrocytes did not deviate from controls, however clinical signs of toxicity including decreased activity, ataxia and tremors were observed at the highest dose. The absence of apparent mutagenic activity or structural similarity to known carcinogens, together with the absence of carcinogenic potential among other avermectins, suggests that eprinomectin is unlikely to be carcinogenic. Therefore, the CVMP and JECFA considered eprinomectin to be unlikely to possess carcinogenic activity (EMEA, 1996, IPCS, 1998).

3.4 Reproductive and developmental toxicity

In a two-generation study in Sprague Dawley rats were given eprinomectin at 0, 6, 18 or 54 mg/kg diet and two litters were produced for each of the F_1 and F_2 generations, Treated animals of each generation tended to demonstrate reductions in food intake, particularly during the first few weeks of exposure. Particularly in high dose pups of each generation this associated with reduced bodyweight gains during the period leading up to weaning. Signs of toxicity included body tremors in treated pups of F_1 and F_2 pups during the first two weeks of post natal life. For F_{2b} generation pups doses were reduced to 0, 3, 9 or 27 mg/kg diet and no body tremors were noted in treated animals. However, in those animals given 27 mg/kg diet, litter size and mean pup weight was decreased although to a lesser extent than noted in the F_1 and F_{2a} generations. Other reproductive effects included a delay in sexual maturity in F_1 animals that associated with their delayed growth performance. At the first mating of the F_1 generation, pregnancy rates were slightly reduced. Impairment was, however, more severe at the second mating where there was also a 50% reduction in pregnancy rate, with consequent reductions in numbers producing live litters (although litter size was not affected). The maternal toxicity NOEL was considered to be 18 mg/kg diet (equivalent to 2.5 mg/kg body weight/day) based on the decrease in food intake in F_0 and F_1 dams. The reproductive NOEL is 18 mg/kg diet, equivalent to 1.6 mg/kg body weight/day. The fetal NOEL was 9 mg/kg diet (equivalent to 1.3 mg/kg body weight/day) based on the body tremors and growth effects noted in the F_{2a} and F_{2b} generations, respectively (Brooker *et al.*, 1992).

To determine levels of eprinomectin in maternal plasma and milk Sprague Dawley rats were fed treated diets designed to achieve intakes of 0, 0.4, 4.0 or 4.1 mg/kg body weight/day from gestation day 15 and 0, 1.2, 4.5 or 6.6 mg/kg body weight/day through lactation to day 21.

Dams showed no treatment-related deaths, abortions, clinical signs or effects on gestation length or number of live pups per female. Food consumption was reduced at the mid and high doses during lactation days 8 – 21. Eprinomectin was present in maternal plasma and milk (ratio of 1:3) during lactation days 7 – 21. In pups of the mid and high dose, dose-related effects were noted on or after day 5 of lactation. Effects included a decrease in body weight gain, body tremors and, at the high dose, increased mortality (Mattson, 1992). These changes are common in this family of chemicals, for example, a multi-generation reproductive study with ivermectin, a related substance, in the rat also showed higher ratio of ivermectin in milk vs plasma, and similarly related toxic effects (Lankas & Gordon, 1989, Lankas *et al.*, 1989).

In pregnant Sprague Dawley rats dosed at 0, 0.5, 1, 3 or 12 mg/kg bodyweight/day on days 6 – 17 of gestation, there were no treatment-related signs, deaths, abortions or gross lesions. At 3 or 12 mg/kg/day, an initial increase in body weight gain and food consumption was reported followed by a decrease on days 18 – 20. There was no evidence of developmental toxicity or teratogenicity based on post-implantation survival, fetal weight, and external, visceral and skeletal examinations. The maternal NOEL was 1 mg/kg day based on body weight and food consumption effects while the developmental NOEL was 12 mg/kg day (Cukierski, 1991)

Two other developmental toxicity studies on eprinomectin are available, conducted in the New Zealand White rabbit. In the first, rabbits were given 0, 0.5, 2 or 8 mg/kg/day on gestation days 6 – 18 and dams were killed on gestation day 28. There were no treatment-related deaths, abortions or gross lesions. Dams showed a slow pupillary reflex at the mid and high dose and mydriasis and decreased body weight gain at the high dose. The numbers of implants and live fetuses per litter were lower at the mid and high doses, though values were still within historical controls. No effect on weight of live fetuses or evidence of teratogenicity was observed. The maternal NOEL was 0.5 mg/kg/day based upon pupillary reflex while the developmental NOEL was 8 mg/kg/day (Wise, 1991). In the second study, animals were given 0, 1.2, 2 or 8 mg/kg day orally on days 6 – 18 of gestation, and dams were killed on day 28. There were no treatment-related deaths or abortions. At the high dose, a slowed pupillary reflex and/or mydriasis and decreased body weight gain were again noted, but embryonic and fetal survival was unaffected. The maternal NOEL was therefore 2 mg/kg/day while the developmental NOEL was 8 mg/kg/day (Cukierski, 1994).

4 Guidelines and standards

An ADI of 10 µg/kg has been established by JECFA for eprinomectin based on a NOEL of 1 mg/kg day in a one year study in dogs; a safety factor of 100 was applied (IPCS, 1998). However CVMP has established a lower ADI of 5 µg/kg based on the same study but applying a safety factor of 200 based on the effect in mydriasis. This higher safety factor was considered necessary to account for the uncertain sensitivity of this finding as a marker of possible neurotoxicity (EMEA, 1998). JECFA MRL values are as µg/kg, muscle 100, liver 2000, kidney 300, fat 250 and milk 20 (IPCS, 1998).

Adopting a precautionary approach, the lower of the ADI values, 5 µg/kg, will be adopted for the current assessment.

The MRL values are currently included in Annex I of Council Regulation (EEC) No 2377/90, as shown below in Table 4.1:

Table 4.1 MRLs for eprinomectin

Pharmacologically active substance	Marker residue	Animal species	MRL (µg/kg)	Target tissue
Eprinomectin	Eprinomectin	Bovine	50	Muscle

B1a	250	Fat
	1500	Liver
	300	Kidney
	20	Milk

From EMEA, 1998

Assuming a maximum daily intake by humans of 276.23 µg per day, based on the MRLs in table 4.1, this would equate to an intake of approximately 92% of ADI from food sources as estimated by the EMEA (EMEA, 1998).

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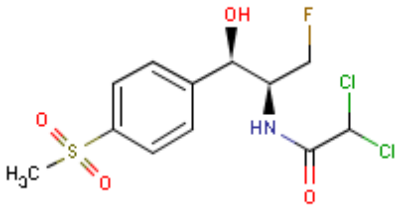
Florfenicol

1 Introduction

Florfenicol (Acetamide, 2,2-dichloro-N-((1S,2R)-1-(fluoromethyl)-2-hydroxy-2-(4-(methylsulfonyl) phenyl)ethyl)-, molecular formula $C_{12}H_{14}Cl_2FNO_4S$, CAS No. 73231-34-2; Figure 1.1; Box 1) is a broad-spectrum, synthetic antibacterial drug, used in the treatment of a number of species including cattle, pigs, chickens and fish (ChemID Plus). Florfenicol is structurally related to *D*(-)threo chloramphenicol (CAP), differing by the presence of a *p*-methyl sulfonyl group and a fluorine atom in place of the *p*-nitro and hydroxyl groups of CAP, respectively. Florfenicol acts by inhibition of peptidyl transferase activity on 70S ribosomes. Bacterial strains highly resistant to other chloramphenicol drugs are sensitive to the fluorinated florfenicol alternative (EMEA).

Figure 1.1 Molecular structure of florfenicol



From ChemID Plus

Box 1 Synonyms and trade names of florfenicol

2,2-Dichloro-N-((alphaS,betaR)-alpha-(fluoromethyl)-beta-hydroxy-p-(methylsulfonyl)phenethyl)acetamide ; Acetamide, 2,2-dichloro-N-(1-(fluoromethyl)-2-hydroxy-2-(4-(methylsulfonyl)phenyl)ethyl)-, (R-(R*,S*)); D-threo-2,2-Dichloro-N-(alpha-(fluoromethyl)-beta-hydroxy-p- (methylsulfonyl)phenethyl)acetamide (-)-Florfenicol; 2,2-Dichloro-N-(1-(fluoromethyl)-2-hydroxy-2-(4-(methylsulfonyl)phenyl)ethyl)acetamide; Nuflor; Sch 25298; Sch-25298; UNII-9J97307Y1H

From ChemID Plus

The route of administration of florfenicol differs among species, with cattle treated intramuscularly, pigs treated either orally or intramuscularly and fish and chickens treated by oral administration. Florfenicol is not used in human medicine (EMEA).

2 Toxicokinetics

An analytical method based on the conversion of florfenicol and its metabolites to florfenicol amine has been developed and validated. The limits of quantification and detection for florfenicol amine in target tissues of species treated with florfenicol are presented in Table 2.1. Therefore, the marker residue for the sum of florfenicol and its metabolites is measured as florfenicol amine. Validated analytical methods are available for the determination of

florfenicol marker residue in fish muscle and skin in natural proportions, and edible tissues of chicken.

Table 2.1 LOQ and LOD[a] of analytical methods for florfenicol amine

Marker residue	Species	Tissue	LOQ, µg/kg	LOD, µg/kg
Florfenicol amine	Bovine	Muscle	0.1	0.058
		Kidney	0.1	0.07
		Liver	0.1	0.045
	Fish	Muscle & skin in natural proportion	500	152
	Chicken	Muscle	100	5
		Skin & fat	200	39
		Kidney	150	5
		Liver	1500	250
	Porcine	Muscle	150	7
		Skin & fat	50	15
		Kidney	50	16
		Liver	1000	64

[a]LOQ = limit of quantification; LOD = limit of detection

Metabolism studies of florfenicol in cattle have identified five metabolites — florfenicol, florfenicol-amine, florfenicol alcohol, florfenicol oxamic acid and monochloride florfenicol. The primary route for excretion of florfenicol is the urine (63–71%; (EMEA). A total residues depletion study has demonstrated that the highest concentrations were found in the liver and the injection site at 30 days post treatment (EMEA). The metabolite composition of various tissues has been studied and the percentage of metabolites present as microbiologically active florfenicol is 5, 35 and 100% for liver, kidney and muscle, respectively. In fish, 4% of the residues assayed at 15 days after the end of treatment are microbiologically active (EMEA, 1997, EMEA, 2000). In a metabolism study carried out in broiler chickens, the microbiologically active compound could not be detected in liver and muscle, and was detected in skin and fat and kidney at low levels (4 and 1%, respectively) 24 hours after the end of oral treatment for three days (EMEA, 1999a). In a metabolism study in pigs using radioactively labelled florfenicol, florfenicol (the only microbiologically active metabolite) represented a small fraction of the total radioactivity extractable 12 days after the end of treatment (1.2, 11, 0.1 and 0.5% in muscle, skin and fat, liver and kidney, respectively; (EMEA, 1999b).

3 Toxicity profile

3.1 Acute toxicity

Orally administered florfenicol was not acutely toxic to either mice or rats, and an LD_{50} value could not be established (> 2000 mg/kg bw). After intraperitoneal administration to rats, the LD_{50} value was approximately 2000 mg/kg bw (EMEA).

The sensitivity of the bacterial strains which constitute normal human gut microflora to florfenicol and its metabolites has also been tested (EMEA). The only microbiologically active compound is florfenicol, with its metabolites significantly less active or inactive. The minimal inhibitory concentration (MIC) is 0.36 µg/mℓ for the most sensitive microorganism (*Fusobacterium* sp).

3.2 Repeat dose toxicity

Tests of repeated dose toxicity have been carried out in a number of species including mice, rats and dogs, and a number of NOAEL values have been established (see Table 3.1). The lowest observed NOAEL of 1 mg/kg bw/day is used to establish the ADI (see Section 4).

Table 3.1 NOAEL values for florfenicol

Study	Toxic effect	NOAEL, mg/kg bw/day
13-week, rat	Testicular atrophy at 100 mg/kg bw/day	30
13-week, dog (with recovery period)	Increased liver weight at 12 mg/kg bw/day	3
2-generation reproductive toxicity, rats	Low viability index in F2b pups at > 3 mg/kg bw/day	1
Teratogenicity, rats	Embryotoxicity (↓ fetal weight, retarded ossification) in the presence of maternotoxicity >12 mg/kg bw/day	4
104-week, rat (52-week interim kill)	↓ body weight gain and ↓ testes weights > 12 mg/kg bw/day	3
	Testicular tubular degeneration/atrophy >12 mg/kg bw/day	
52-week, dog	↑ Hepatocyte rarefaction at 12 mg/kg bw/day	3

Adapted from FOI summary, FDA (1996)

3.3 Carcinogenicity and mutagenicity

No evidence for carcinogenicity was observed in 104-week oral carcinogenicity studies of florfenicol in rats or mice (FDA, 2009).

Extensive testing for genotoxicity of florfenicol has been carried out both *in vitro* and *in vivo,* presented in detail elsewhere (FDA, 2009) and summarised here. On balance, it may be concluded that florfenicol does not represent a genotoxic hazard.

No evidence for induction of DNA damage in rat hepatocyte assay for unscheduled DNA synthesis was observed. Equivocal results in a mouse lymphoma reverse mutation assay were not supported in a subsequent repeat of the assay, where florfenicol was evaluated as negative for inducing forward mutations at the TK locus of mouse lymphoma cells in the presence of S9 metabolic activation. Florfenicol was found to be clastogenic at toxic concentration and to induce endoreduplication at non-toxic and toxic doses in an *in vitro* test for chromosome aberration. However, florfenicol tested negative in an *in vivo* mouse bone marrow cytogenetics assay, alleviating previous concerns for the clastogenic potential *in vitro* of the drug. In addition, florfenicol was devoid of clastogenic effects in a further *in vivo* (bone marrow micronucleus) test.

3.4 Reproductive and developmental toxicity

A two-generation study has been conducted in rats to test for potential adverse effects of florfenicol on reproduction. Treatment-related effects observed included low viability index, low lactation index and low overall survival index in F2b pups. The NOAEL, based on low viability index, was 1 mg/kg bw/day. Biologically and statistically significant reduced epididymal weights was noted in F0 and F1 generation males (FDA, 2009).

No evidence for teratogenicity was observed in a study in rats. Embryotoxicity, in the form of reduced fetal weight and retarded ossification was observed, but only in the presence of maternal toxicity (FDA, 2009).

4 Guidelines and standards

Based on the lowest observed NOAEL of 1 mg/kg bw/day from a two generation reproduction study in rats, EMEA CVMP (EMEA, 2001) derived a toxicological ADI of 10 µg/kg by applying a safety factor of 100. A microbiological ADI (important where a significant proportion of the residues detected after a period of treatment with the drug possess microbiological activity) of 3 µg/kg bw was established using the MIC for *Fusobacterium* sp and is used in the current assessment. The microbiological ADI (lower than the toxicological ADI) was used to establish the MRLs for cattle and fish. For chicken and pigs, it was also considered relevant to compare the amount of residues likely to be consumed to the toxicological ADI because of the presence in small amounts only of microbiologically active residues in the target tissues. Since data were provided for broiler chickens only (EMEA, 1999a), the established MRLs may not be applied to animals producing eggs for human consumption.

The MRLS for cattle, pigs and chickens are not identical, and therefore it was not possible to recommend entry in Annex I of Council Regulation (EEC) No. 2377/90 in such a way that the same MRL values would apply to all food producing species (EMEA, 2002). It was not considered necessary to reduce the existing MRLs to the lowest values in order to guarantee consumer safety. It was considered appropriate to recommend the extension of the existing MRLs for bovine species to ovine and caprine species and those for chickens to poultry. Finally, it was considered appropriate to extend the lowest MRL values to all food-producing species except bovine, ovine, caprine, porcine, poultry and fish.

Since the analytical method for florfenicol is based on conversion to florfenicol amine, the MRLs have been established on the basis of florfenicol amine. In order to achieve this, the relative content of florfenicol in the target tissues and a conversion factor (ratio of molecular masses) have been used to derive the MRLs listed in Table 4.1.

Table 4.1 MRLs for florfenicol

Pharmacologically active substance	Marker residue	Animal species	Target tissue	MRL, µg/kg	Other provisions	%ADI
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol amine	Bovine ovine & caprine	Muscle Liver Kidney	200 3000 300	n/a	
		Fin fish	Muscle and skin in natural proportions	1000	n/a	
		Poultry	Muscle Skin & fat Liver Kidney	100 200 2500 750	Not for use in animals from which eggs are produced for human consumption	99.95% toxADI <1% micADI
		Porcine	Muscle Skin & fat Liver Kidney	300 500 2000 500	n/a	99.74% toxADI <5% micADI
		All food-producing species except those listed above	Muscle Fat Liver Kidney	100 200 2000 300	n/a	

From EMEA, 2002

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Halofuginone

1 Introduction

Halofuginone (7-Bromo-6-chloro-3-(3-((2R,3S)-3-hydroxy-2-piperidinyl)-2-oxopropyl-4(3H-quinazolinone; molecular formula $C_{16}H_{17}BrClN_3O_3$; CAS No. 55837-20-2; Figure 1.1; Box 1 (ChemID Plus)) belongs to the quinazolone family of chemicals.

Figure 1.1 Molecular structure of halofuginone

From ChemID Plus

Box 1 Synonyms and trade names for halofuginone

(-)-trans-7-Brom-6-chlor-3-(3-(3-hydroxy-2-piperidyl)acetonyl-4(3H)-chinazolinon; (-)-trans-7-Bromo-6-chloro-3-(3-(3-hydroxy-2-piperidyl)acetonyl-4(3H)-quinazolinone; (+/-)-trans-7-bromo-6-chloro-3-(3-(3-hydroxy-2-piperidyl)-acetonyl)-4(3H)-quinazolinone ; 4(3H4(3H)-Quinazolinone, 7-bromo-6-chloro-3-(3-(3-hydroxy-2-piperidinyl)-2-oxopropyl)-, trans-(+/-)- ; Halofuginona; Halofuginona [INN-Spanish]; Halofuginone ; Halofuginonum; Halofuginonum [INN-Latin] ; UNII-L31MM1385E; trans-7-Bromo-6-chloro-3-(3-(3-hydroxy-2-piperidinyl)-2-oxopropyl)-4(3H)-quinazolinone

From ChemID Plus

Halofuginone is a synthetic analogue of febrifugine, which is the active moiety isolated from a naturally-occurring traditional Chinese medical herb used as an anti-malarial agent (Jiang *et al.*, 2005). The *trans*-isomer of halofuginone is the active ingredient and the *cis*-isomer is regarded as an impurity.

In addition to its potential as an anti-malarial agent (Jiang *et al.*, 2005), halofuginone has a number of other medical applications. These relate to the action of halofuginone in avian species and humans as a specific, but transient, inhibitor of collagen α1 gene expression, which affects collagen synthesis in skin fibroblasts. This effect has been reported in both healthy human and scleroderma patients at concentrations in the nanomolar range or below (EMEA, 2000). More recently, it has been reported that inhibition of collagen synthesis by halofuginone involves inhibition of transforming growth factor (TGF)-β-dependent Smad3 phosporylation. The Smad family of proteins and their signaling pathway are involved in regulation of matrix proteins and collagen type I gene expression, particularly by TGF-β (Pines *et al.*, 2003). TGF-β is known to be involved in the overproduction of extracellular matrix proteins in normal wound healing and in various fibrotic conditions (Verrecchia *et al.*, 2006). Furthermore, increased presence of nuclear Smad3 has been detected in fibroblasts of systemic sclerosis patients (Pines *et al.*, 2003).

In a recent EMEA publication (June, 2009), the Committee for Orphan Medicinal Products summarised the orphan designation status (first given in 2001) of halofuginone hydrobromide for treatment of systemic sclerosis in humans associated with build up of collagen (type I) (EMEA, 2009). Topical halofuginone has also been used in the successful treatment of skin

and internal organ fibrosis occurring in chronic graft versus host disease and scleroderma (Pines *et al.*, 2003).

Two salts of halofuginone, lactate and hydrobromide, are used in veterinary medicine. Oral halofuginone lactate at a dose of 0.1 mg halofuginone/kg/day for seven days is indicated for prevention of diarrhea caused by *Cryptosporidium parvum* in non-ruminating calves from 4 – 15 days of age. Halofuginone hydrobromide is used as an anticoccidial feed additive in broilers and turkeys; there is a withdrawal period of five days (EMEA, 2000).

2 Toxicokinetics

In patients with advanced solid tumours, dosing with halofuginone at up to 3.5 mg/day gave highly variable results but suggested that maximum plasma concentrations were achieved by approximately 3.5 hours. Within the first 24 hours of administration, 4-12% of dose was excreted as unchanged parent, mainly in urine (de Jonge *et al.*, 2006).

In CD_2F_1 mice given halofuginone at 1.5 mg/kg orally, no halofuginone was detected in plasma at any time point (Stecklair *et al.*, 2001). However, in an oral dose study in mice with 0.25 mg/kg ^{14}C-halofuginone hydrobromide 82.7% of the administered dose was excreted within 48 hours. High levels of radioactivity (220 µg) were detected in the liver at 24 hours post dose, declining to 130 µg by 48 hours (EMEA, 2000).

Oral dosing of Sprague Dawley rats with halofuginone at 3 mg/kg showed peak plasma concentrations by 90 minutes in this species, though levels, at approximately 30 ng/ml, were at the limit of quantitation. In bile duct cannulated rats given ^{14}C-halofuginone in aqueous solution or as liver homogenates from treated chickens, oral bioavailability (as percentage of radioactivity excreted in urine and bile) was 44 and 36% respectively; only 18% was excreted in bile only (EMEA, 2000).

Halofuginone given to mice (i.v., 1.5 mg/kg) distributed to tissues rapidly except for brain, where none was detected. The highest concentrations were in the kidney and lung, with lower concentrations in heart, liver, spleen, skeletal muscle, fat and red blood cells. All concentrations declined with time however, detectable levels of halofuginone were still found in all tissues for at least 90 minutes after administration and were still detectable in the lung and liver 72 hours after dosing. After oral dosing, halofuginone was detected in kidney (1648 ng/g), liver (102 ng/g) and lung (474 ng/g) of mice two and three hours after dosing, persisting for up to 48 hours after dosing. In rats, levels were 1848 ng/g in liver, 1960 ng/g in kidney and 2934 ng/g in lungs at 15, 120 and 240 minutes respectively after dosing, with the peak levels being much greater in the tissues than in the plasma; halofuginone was still present in tissues at above 30 ng/g by seven hours after dosing (Stecklair *et al.*, 2001).

No metabolites of halofuginone were detected in plasma, urine or organs of CD_2F_1 mice or Fischer 344 rats. Seven, 24 and 48 hours after oral dosing urinary excretion of halofuginone accounted for 3.2 – 7.1%, 9.1 – 11.4% and 15.9% respectively (Stecklair *et al.*, 2001). However, after oral administration of 0.25 mg/kg ^{14}C-halofuginone hydrobromide to mice, the main route of excretion was the faeces, accounting for approximately 65% of administered dose of which approximately 90% was present as the parent (EMEA, 2000). Another oral dose study in rats found approximately 78% of an administered dose of 5 mg/kg ^{14}C-halofuginone hydrobromide was recovered within 24 hours, of which about 60% was in the faeces (EMEA, 2000).

In three week old calves treated orally with 0.1 mg/kg/day halofuginone lactate (therapeutic dose) for seven days, peak plasma levels (9 µg/L) were achieved 6 hours after the last dose falling to below the limit of quantification (1 µg/L) by seven days after treatment. In another

study, 22 - 32 day old calves were orally given 0.1 mg/kg/day halofuginone lactate for seven days and peak plasma levels of 6.66 µg/L were found eight hours after the last dose; by36 hours after the final dose, plasma level was 2.3 µg/L. Levels subsequently fell below the limit of quantification (1 µg/L). A mean terminal half life of 32.8 hours was found and no accumulation was observed. In a further study calves given halofuginone lactate at 0.1 mg/kg/day orally on two separate occasions, peak plasma levels were 4.12 µg/L by 11 hours post dose. The oral half-life of elimination was 30.84 hours which was noted to be three-fold higher than that following i.v. administration demonstrating that oral absorption is the rate limiting step of the kinetics of halofuginone. An oral bioavailability of 81.1% was established (EMEA, 2000).

Other studies have reported on the toxicokinetics of halofuginone by parenteral routes but are not considered of relevance to the current study, so are not discussed here.

3 Toxicity profile

Most toxicity studies of halofuginone have been conducted on either the lactate or hydrobromide forms. To aid comparison, wherever possible, doses are expressed as halofuginone base equivalents (EMEA, 2000).

3.1 Acute toxicity

In a Phase I clinical trial, 26 healthy male volunteers were given single oral doses of halofuginone in diet controlled meals. Single doses of 0.07 - 0.5 mg/day were well tolerated. At 1.5 – 2.5 mg/person moderate dose-related signs were noted including nausea and vomiting. A daily dose of 1.5 mg was deemed the maximum tolerated dose (MTD). In eight healthy male volunteers given three dose regimens (0.25 mg x 8 doses; 0.5 mg x 4 doses or 2 mg x 1 dose) a reduced slope increment in plasma levels was achieved resulting higher overall exposure without gastrointestinal side effects (Pines *et al.*, 2003).

Experimentally, oral LD_{50} values of approximately 30 mg/kg in rats and 5 mg/kg in mice were noted for halofuginone lactate and hydrobromide. The inhalation LC_{50} in rats was 53 µg/L and the dermal LD_{50} in rabbits was 16 mg/kg. An oral LD_{50} of approximately 430 mg/kg was noted for the *cis*- isomer in the mouse (EMEA, 2000).

In a bioequivalence study, mice were given a single oral dose of halofuginone hydrobromide or lactate at 2 mg/kg. The $AUC_{0\text{-}8\ h}$ was 103.37 and 82.65 µg.hr/L for halofuginone lactate and hydrobromide respectively. Large inter-individual variation resulted in a lack of definitive conclusion regarding pharmacokinetics, however with respect to toxicity, both compounds were tolerated at this dose (EMEA, 2000).

Halofuginone and its two salts have been shown to cause delayed systemic toxicity when applied to shaved skin and are irritant to the eye mucosa of rabbits (EMEA, 2000). Halofuginone caused a slight cutaneous reaction (delayed contact hypersensitivity) in 35% of Guinea pigs in a Magnusson and Kligman Maximization test but this was not noted in a modified Buehler assay (EMEA, 2000).

3.2 Repeat dose toxicity

Repeated topical application of 0.1% halofuginone to 14 healthy volunteers was tolerated , with no reports of skin irritation or systemic absorption (Pines *et al.*, 2003).

In a Phase II clinical trial in patients with diffuse systemic sclerosis, a once daily topical application of 0.01% halofuginone for six months was well tolerated. There were no clinically-relevant changes in blood pressure, heart rate, body temperature, body weight,

haematological or clinical chemistry parameters. The most common effect was dermatitis of varying degree and severity though this did not cause any patients to cease treatment (Pines *et al.*, 2003).

Four weeks treatment of mice with given (route of administration not stated) halofuginone hydrobromide or lactate at 0.07, 0.16 or 0.35 mg halofuginone base/kg/day showed changes in haematological parameters (mean cell volume and mean cell haemoglobin) at the mid and high doses. At the high dose, changes in urea and cholesterol were also noted. The toxicological profiles of these salts were similar, with a NOEL established for both of 0.07 mg/kg/day (EMEA, 2000).

In a thirteen week study, rats of an unspecified strain received halofuginone hydrobromide in the diet at 0, 2, 5 or 10 mg/kg feed (equivalent to 0, 0.13, 0.33 or 0.7 mg/kg body weight/day in males and 0, 0.16, 0.41 or 0.88 mg/kg body weight/day in females). At the high dose, approximately 80% of females had hepatic fat depositions and vacuolation and a slight decrease in glycogen in periportal hepatocytes. No other changes in haematology or blood chemistry parameters were reported. The NOEL was 0.13 mg/kg/day in males and 0.16 mg/kg/day in females (EMEA, 2000).

In a 26 week study in dogs fed diet containing halofuginone hydrobromide at 0, 1.25, 2.5 or 5 mg/kg diet (equivalent to 0, 0.045, 0.086, 0.16 mg/kg body weight/day in males and 0, 0.039, 0.075 or 0.17 mg/kg body weight /day in females), significantly decreased mean cell volume, mean cell haemoglobin concentration and/or haemoglobin levels were reported at the high dose. Haematological changes were also noted at lower doses although these were within the range of historical controls. The NOEL was 2.5 mg/kg feed (EMEA, 2000).

Halofuginone given to calves of 4 – 10 days of age at the recommended therapeutic regimen (0.1 mg/kg/day for seven days) was well tolerated. At doses 15-fold to 25-fold the therapeutic level, deaths occurred and at one, two or three times the therapeutic, reversible gastro-intestinal inflammatory/necrotic lesions was seen (EMEA, 2000).

3.3 Carcinogenicity and mutagenicity

Due to the anti-fibrotic and anti-angiogenic activity of halofuginone, its clinical value as an anti-tumour agent has been investigated in a number of animal and human cell models (Bruck *et al.*, 2001, Elkin *et al.*, 1999, Pines *et al.*, 2000, Abramovitch *et al.*, 2004). However in bile duct obstructed rats, halofuginone caused a worsening of liver fibrosis (Van de Casteele *et al.*, 2004).

In a 26-month study in Sprague Dawley rats, halofuginone bromide at dietary levels of 0, 0.25, 5 or 10 mg/kg feed /day (equivalent to 0, 0.09, 0.18, 0.36 mg/kg body weight/day in males and 0, 0.11, 0.23 or 0.47 mg/kg body weight/day in females) resulted in no increase in tumour incidence. However, changes in haematology and histopathology (details not reported), resulted in the establishment of a NOEL of 2.5 mg/kg feed for non-neoplastic endpoints (EMEA, 2000).

In a carcinogenicity study in Swiss mice given halofuginone hydrobromide in diet at levels designed to achieve doses of 0, 0.03, 0.07 or 0.24 mg/kg body weight/day, no carcinogenic potential was identified (EMEA, 2000).

Halofuginone lactate was positive in an Ames *S. typhimurium* (strain TA98 at 1000 µg/plate) assay with or without metabolic activation. However, no dose-relationship was apparent and a mouse lymphoma assay was negative. In other studies for which only limited details are given in the EMEA summary report, halofuginone (salt not stated) was negative in an *in vitro* mouse lymphoma assay, chromosomal aberration assay using cultured human lymphocytes

and in a DNA repair assay on human epitheloid cells. It was also negative in a *in vivo* bone marrow mouse micronucleus assay, a metaphase analysis in rats and a host-mediated assay in mice. Overall halofuginone is considered unlikely to be genotoxic (EMEA, 2000).

3.4 Reproductive and developmental toxicity

In dogs (strain not stated) administered 0, 2.5 or 5 mg halofuginone/kg feed (equivalent to 0, 0.67 or 0.134 mg/kg body weight) for 68 weeks, decreased testicular length and width and fertility index were noted in treated animals. Dose-relationship was apparent and no NOEL was identified (EMEA, 2000).

In a poorly reported, three generation study in mice, halofuginone in the diet at 0, 0.25, 0.5 or 1 mg/kg feed (equivalent to 0, 0.034, 0.063 or 0.126 mg/kg body weight/day) resulted in a reduction in body weight for males in F_0 and F_1 generation parents at the mid and high dose and in F_2 generation parents at the high dose only. F_3 generation pups also showed decreased mean body weight at the high dose and a transient decrease at the mid dose. The NOEL was 0.25 mg/kg feed, equivalent to 0.034 mg/kg body weight/day (EMEA, 2000).

In a teratogenicity study in rats (strain not stated), halofuginone hydrobromide was given by oral gavage at 0, 10.17, 0.34 or 0.67 mg/kg day from day 6 – 17 of gestation. Maternal toxicity included deaths, clinical signs and abortion at the high dose. No adverse fetal effects were noted. Therefore, the maternal NOEL was 0.34 mg/kg/day and the fetal NOEL was 0.67 mg/kg/day (EMEA, 2000). In another study in rabbits (strain not stated) given halofuginone hydrobromide orally at 0, 0.0084, 0.025 or 0.076 mg/kg day on gestation days 6 – 18, maternal toxicity comprised mortality, lower body weight and increased abortion rate in the high dose only. There were no adverse effects in the fetuses. The maternal NOEL was 0.025 mg/kg/day and the fetal NOEL was 0.076 mg/kg/day (EMEA, 2000).

4 Guidelines and standards

A toxicological-based ADI of 0.3 µg/kg (equivalent to 18 µg/person) was derived by applying a safety factor of 100 to the maternal NOEL of 0.03 mg/kg day identified in the reproduction study in the mouse (NOEL = 0.034 mg/kg/day) and teratogenicity studies in the rabbit (NOEL = 0.025 mg/kg/day) respectively (EMEA, 2000).

The CVMP recommended the inclusion of halofuginone in Annex I of Council Regulation (EEC) No. 2377/90 in accordance with MRLs as shown in Table 4.1.

Table 4.1 MRLs for halofuginone

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues
Halofuginone	Halofuginone	Bovine	10 µg/kg	Muscle
			25 µg/kg	Fat
			30 µg/kg	Liver
			30 µg/kg	Kidney

From EMEA, 2000

Based on these MRLs, EMEA estimate that the daily intake from bovine species will represent approximately 85% of the ADI (EMEA, 2000).

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Lasalocid

1 Introduction

Lasalocid ((2R-2alpha(2S*(3R*,4S,5S*,7R*)3S*,5S*),5alpha,6beta))-6-(7-(5-ethyl-5-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)tetrahydro-3-methyl-2-furyl)-4-hydroxy-3,5-dimethyl-6-oxonyl)-3-methylsalicylic acid; molecular formula $C_{34}H_{54}O_8$; CAS No. 25999-31-9; Figure 1.1; Box 1) is an antibiotic from the group of carboxylic ionophores and is used as the sodium salt (ChemID Plus).

Figure1.1 Molecular structure of lasalocid

From ChemID Plus

Box 1 Synonyms and trade names for lasalocid

6-(7(R)-(5(S)-Ethyl-5-(5(R)-ethyltetrahydro-5-hydroxy-6(S)-methyl-2H-pyran-2(R)-yl)tetrahydro-3(S)-methyl-2(S)-furyl)-4(S)-hydroxy-3(R),5(S)-dimethyl-6-oxononyl)-2,3-cresotic acid; 6-(7(R)-(5(S)-Ethyl-5-(R)-ethyltetrahydro-5-hydroxy-6(S)-methyl-2H-pyran-2(R)-yl)tetrahydro-3(S)-methyl-2(S)-furyl)-4(S)-hydroxy-3(R),5(S)-dimethyl-6-oxononyl)-2,3-cresotic acid; 6-(7-(5-Ethyl-5-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-2-yl)tetrahydro-3-methyl-2-furanyl)-4-hydroxy-3,5-dimethyl-6-oxononyl)-2-hydroxy-3-methylbenzoic acid (2R-(2alpha(2S*(3R*,4S*,5S*,7R*),3S*,5S*),5alpha,6beta))-; Antibiotic X 537A; Benzoic acid, 6-(7(R)-(5(S)-Ethyl-5-(5(R)-ethyltetrahydro-5-hydroxy-6(S)-methyl-2H-pyran-2(R)-yl)tetrahydro-3(S) methyl-2(S)-furanyl)-4(S)-hydroxy-3(R),5(S)-dimethyl-6-oxononyl)-2-hydroxy-3-methyl-; EINECS 247-401-9; Ionophore X 537A; Lasalocid A; Lasalocide; Lasalocide A; Lasalocide [INN-French]; Lasalocido; Lasalocido [INN-Spanish]; Lasalocidum; Lasalocidum [INN-Latin]; NSC 243048; UNII-W7V2ZZ2FWB; X 537A

From ChemID Plus

Lasalocid sodium (the formulation that lasalocid is used as) is a carboxylic acid ionophore that binds divalent and monovalent cations (EMEA, 2004). Lasalocid is produced by fermentation of *Streptomyces lasaliensis* subsp. *lasaliensis*, and recovered from fermentation media by acidification before extraction. Lasalocid is a mixture of several closely-related homologues, A, B, C, D and E, although lasalocid sodium A accounts for 90% of the active ingredient in authorised products while the other lasalocid homologues account for 10% of the total lasalocid (EFSA, 2004).

Lasalocid is active against Gram positive bacteria and is used as an additive in feed and as a veterinary medicine for the prevention of coccidiosis caused by *Eimeria spp* in chicken and turkeys. Intended doses are, in the feed, 75 – 125 ppm for broiler chickens (EMEA, 2004, Galitzer & Oehme, 1984); for fattening chickens from birth to up to 16 weeks of age; 90 – 120 mg/kg for turkeys to up to 12 weeks of age and 90 – 120 mg/kg for pheasants, partridges and quails. Use of lasalocid requires a withdrawal period of five days before use of animals for human consumption (a withdrawal time for eggs is not stated) (EMEA, 2004). In countries outside of the EU, lasalocid is also used as a growth promoting agent for fattening ruminants

(lamb and cattle) and pigs at maximum feed concentrations of 33 mg/kg feed, and also for hepatic coccidiosis in rabbits at 68 – 113 mg/kg feed (EFSA, 2007).

The mode of action of lasalocid sodium involves formation of lipid soluble and dynamically reversible complexes with cations. Lasalocid sodium can bind divalent cations (e.g. Ca^{2+} and Mg^{2+}) and monovalent ions (such as Na^{+} and K^{+}) increasing their passage across biological membranes. This leads to disruption of normal physiological processes in pro-and eukaryotic cells.

Lasalocid is not used in human medicine.

2 Toxicokinetics

No toxicokinetic data in humans has been identified

In mice and rats (strains not specified) given single oral doses of ^{14}C-lasalocid (1 mg/kg), peak blood concentrations of 0.7 µg/ml and 0.05 µg/ml were noted after 0.25 hours and 3 hours in mice and rats respectively. The blood elimination half-life was 3 hours in mice and 4.8 hours in rats (EMEA, 2004). Studies in chickens orally dosed with unlabelled lasalocid (75 mg/kg feed) for sixteen days followed by ^{14}C-labelled lasalocid for 5 mg/kg for three days, showed peak plasma concentrations of 5.62 µg/ml within two hours of the last dose (EMEA, 2004). In another study, one day old chickens dosed with 125 mg/kg ^{14}C- lasalocid for seven days showed the mean plasma radioactivity of 0.56 µg/ml immediately after completion of treatment. By seven days after treatment, plasma radioactivity had fallen to approximately 0.003 µg/ml (EMEA, 2004).

Lasalocid is widely distributed in mice and rats in many tissues including muscle, skin, fat, heart, thymus, lung and spleen; the highest concentration was in the liver. Chickens given unlabelled lasalocid followed by ^{14}C-labelled lasalocid for three days showed peak levels of 10.3 mg/kg in liver, 0.76 mg/kg in muscle, 1.4 mg/kg in fat and 3 mg/kg in kidney (EMEA, 2004). Details of the metabolism of lasalocid in mice and rats have not been reported but in chickens lasalocid sodium is extensively metabolised although no single metabolite accounts for more than 10% of the total radioactivity excreted (EFSA, 2004).

Excretion in rats and mice was between 90 and 95% in the faeces within 48 hours of dosing with urinary excretion accounting for approximately 1% in both species (EMEA, 2004). On the last day of treatment (125 mg/kg ^{14}C- lasalocid for seven days) in one day old chickens, 88% of the administered dose was excreted; by seven days after the end of treatment, the dose fraction excreted had risen slightly to 91%. Analysis of the composition of the excreted metabolites showed 74 – 77% to be lasalocid A, 0.8 – 4% lasalocid A homologues and 0.3 – 4% unidentified components (EMEA, 2004).

In biliary duct cannulated rats given 1 mg/kg ^{14}C-lasalocid by oral gavage, 60% of the administered dose was absorbed through the gastrointestinal tract into the blood, and 58% was recovered in bile showing that virtually all of the administered dose must be excreted via the biliary duct in the normal animal; approximately 1.1% was recovered in the urine (EMEA, 2004).

3 Toxicity profile

3.1 Acute toxicity

Neurological toxicity is a main sign of intoxication consistent with the mode of action of ionophoric polyether coccidiostats (EFSA, 2007).Toxicity is moderate in mice and acute in

rats with the oral LD_{50} in mice and rats was 146 and 122 mg/kg respectively. Oral lasalocid is highly toxic to neonatal rats and rabbits, with LD_{50} values of 33 and 40 mg/kg respectively. Lasalocid showed low acute toxicity with a dermal LD_{50} of approximately 1400 mg/kg in the rabbit. After a single i.p. dose LD_{50} values were 68 and 26.5 mg/kg in mice and rats respectively; signs of toxicity included tremors in mice and cyanosis, decreased motor activity and respiratory depression in rats. In mice, s.c. administration gave an LD_{50} of 140 mg/kg (EMEA, 2004).

In Broiler chickens given a single oral dose of lasalocid sodium (capsules) at 39 – 317 mg/kg, signs included lethargy, dropped wings and reluctance to move; death occurred generally within 24 hours of dosing. Surviving birds were emaciated and dehydrated. At necropsy, nephromegaly, splenomegaly and hepatomegaly with scattered foci of necrosis were noted; LD_{50} values were between 59 and 84 mg/kg . In another study, where broiler chickens given oral lasalocid sodium gave LD_{50} values of 112 and 84 mg/kg respectively, depending on vehicle used; a dose-related decrease in body weight was also noted (EFSA, 2007).

Acute inhalation of lasalocid sodium in CD-1 mice (12.5 mg/L) and Sprague Dawley rats (up to 8.05 mg/L) for four hours was poorly tolerated in both species with signs of toxicity including corneal opacity, excessive lacrimation and ocular and nasal discharge in the mouse and similar discharge in the rat with laboured respiration. Both species had discoloured lungs. Inhalation exposure to Hartley guinea-pigs (11.5 mg/L for one hour) led to similar signs of toxicity as in the rats and mice (EFSA, 2004).

Lasalocid sodium was evaluated for dermal toxicity in New Zealand white rabbits) up to 2000 mg/kg on intact or abraded skin. Mid and high dose animals died though a dose-relationship was not stated. A separate study in rabbits showed that lasalocid sodium did not possess dermal toxicity at a dose of 500 mg. Lasalocid sodium (challenge dose of 25%) did not induce skin sensitisation in Hartley guinea pigs (EFSA, 2004).

Ocular toxicity has also been assessed by the instillation of 0.036 g (0.1 ml) lasalocid sodium dry powder into the conjunctival sac of one eye of each of three rabbits. Eyes were rinsed with water either 5 minutes or 24 hours after instillation. Conjunctival redness was observed in all treated animals (EFSA, 2004).

3.2 Repeat dose toxicity

A series of 13 week studies on Sprague Dawley rats of various ages, that had also been exposed pre-natally, showed, independent of maturity, decreased haematocrit and haemoglobin levels, leukocytosis, small numbers of target cells, a resistance of erythrocytes to lyse by osmotic stress, increased haemosiderin levels in the liver and kidneys, elevated alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase and vacuolation of cardiac muscle, were noted. Overall, females were more sensitive than males. From these studies, a NOEL of 1 mg/kg/day was identified (EMEA, 2004).

Lasalocid sodium was given to Fisher 344 rats at 0, 10, 35 or 120 mg/kg feed for one week, then the animals were mated and treatment continued until weaning. At weaning, the offspring were treated for a further 130 weeks (see also section 3.3). Overall survival was low (21.8 – 43.6%) but was greater than 50% at 104 weeks. In all groups treated with lasalocid sodium, a decrease in serum blood urea nitrogen level was noted at weeks 26 and 78, though there was no associated histopathology in the kidneys. A dose-related increase in slow grasping or righting reflexes in mid and high dose females and in high dose males were noted (EMEA, 2004). An increase in liver weight in mid and high dose animals and in adrenal weight in mid and high dose females was reported (absolute or relative not stated) (EFSA, 2004). There were no treatment-related macro or non-neoplastic microscopic findings. A

NOEL of 10 mg/kg feed, equivalent to approximately 0.5 and 0.6 mg/kg body weight/day, was established in males and females respectively (EMEA, 2004).

In a two year mouse dietary study (see also carcinogenicity and mutagenicity section), mice were given 0, 10, 35 or 120 mg lasalocid sodium/kg feed, except for the first five weeks when low and mid dose concentrations were 20 and 60 mg/kg feed. There were no treatment-related effects on mortality incidence, clinical signs, ophthalmoscopy or consistent changes in body weight or food consumption. At the end of the study, no treatment related pathology was reported (EMEA, 2004). A NOEL for this study was not stated in the EMEA summary report.

In Beagle dogs treated with 10, 35 or 180 mg lasalocid/kg feed for two years, food consumption was slightly decreased at the high dose in the first three months of the study only. Clinical chemistry evaluations revealed increased alkaline phosphatase levels from month six until the end of the study and intermittent paralysis of the limbs was seen in high dose animals. However, no associated histopathological changes in the central nervous system and sciatic nerves were noted. Reductions in prostate weight (not stated whether this was absolute or relative weight) at the high dose were reported without associated histopathological change. A NOEL of 35 mg lasalocid/kg feed was identified, equivalent to approximately 1 mg/kg body weight/day in males and females, based on changes in food consumption, clinical chemistry and organ weights (EMEA, 2004).

In one day old chicks fed lasalocid sodium at up to 375 mg/kg feed for 13 weeks, results showed increased mortality, reduced body weight gain and reduced feed efficiency at 225 and 375 mg/kg feed. No haematology effects were reported. There were no adverse effects at 75 and 150 mg lasalocid/kg feed (150 mg/kg feed is equivalent to approximately 8.8 mg/kg body weight/day (EMEA, 2004).

3.3 Carcinogenicity and mutagenicity

In the two year study in mice given 0, 10, 35 or 120 mg lasalocid sodium/kg feed reported above, the incidence of neoplasms was similar in all groups. In females euthanized or found dead, an increase in lymphosarcoma was noted at low and high doses but not found in the mid dose, in treated males or in animals killed at study termination. A NOEL for this study was not stated in the EMEA summary report (EMEA, 2004), however a dose-relationship was not reported in the findings.

In the rat carcinogenicity study reported above in which animals were given t 0, 10, 35 or 120 mg/kg feed the incidence of neoplasms were similar in all groups. A NOEL of 10 mg/kg feed, equivalent to approximately 0.5 and 0.6 mg/kg body weight/day in males and females respectively, was reported (EMEA, 2004).

In vitro studies conducted to assess the mutagenicity of lasalocid were negative (see table 3.1 below).

Table 3.1. Summary of mutagenicity studies

End point	Test system	Concentration	Result
DNA repair (rec assay)	*Bacillus subtilis* strain M45 and H17	0, 1, 10, 100 µg/disc	Negative
Reverse mutation	*Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strains trp¯B/rWP2 and trp¯WP2hcr	0, 100, 200, 500, 1000, 2000 µg/plate, ± S9 metabolic activation	Negative

Reverse mutation and mitotic cross over	*Saccharomyces cerevisiae* D7	0, 0.05, 0.17, 0.5, 1.67 and 5 mg/ml ± S9 metabolic activation	Negative
Forward mutation	Chinese hamster V79 cells	0, 0.5, 1.0, 10, 15, 20 µg/ml –S9; 0, 1, 10, 20, 40, 60 µg/ml +S9	Negative at up to 15 µg/ml ± S9
Unscheduled DNA synthesis	Rat hepatocytes	0, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 12.5 µg/ml repeated at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 µg/ml	Negative up to 4 µg/ml
Chromosome aberration	Peripheral human blood lymphocytes	0, 4.0, 6.0, 7.0 µg/ml repeated at 0, 5.0, 6.0, 7.0 µg/ml – S9; 0, 2.0, 4.0, 6.0, 8.0, 10 µg/ml + S9	Negative up to 8 µg/ml ± S9

From (EFSA, 2004)

3.4 Reproductive and developmental toxicity

In a three generation study, rats (strain not stated) were given 0, 10, 35 or 120 mg lasalocid sodium/kg diet continuously from nine weeks prior to mating of the F_0 animals. This study is believed to be the same study as that discussed in the section 3.3. Only offspring from the F_1 and F_3 generations were evaluated for teratological changes. Reduced body weights, and transient reductions in food consumption, were noted in high dose females of each. Pregnancy and fertility rates at the high dose were decreased but this was only significantly in the third generation. Weaning and lactation survival indices were also reduced in the third generation offspring at the high dose. When assessed at postnatal day 4, pup body weights were reduced in the F_2 and F_3 generation. A reduced number of corpora lutea and implantations were noted in the mid and high dose groups in the F_1 and F_3 generations. An increase in delayed ossification was reported in the high dose group in the third generation though no effect on incidence of visceral or skeletal abnormalities was noted. A NOEL of 10 mg/kg feed, equivalent to approximately 0.5 – 0.8 mg/kg body weight/day, was identified (EMEA, 2004).

In New Zealand White rabbits orally dosed with 0, 0.5, 1 or 2 mg/kg/day lasalocid sodium from days 6 – 28 of gestation and necropsied on day 29, a dose-related decrease in food consumption in the mid and high dose groups was observed; there was an associated reduction in faecal output, body weight and body weight gain at the high dose. At 2 mg/kg/day, an increased incidence in fetuses of corneal opacity and at mid and high doses increased pale spleens were noted. At the highest dose only, greater numbers of fetuses with zygomatic bone malformations connected to the maxilla, complete 13^{th} supernumerary ribs and displaced pelvic girdle were noted and animals at this dose also showed an increased incidence of fetuses with incomplete ossification. The NOEL for fetal and maternal toxicity was 0.5 mg/kg/day (EMEA, 2004).

4 Guidelines and standards

A toxicological ADI of 2.5 µg/kg/day has been established for lasalocid based on a NOEL of 0.5 mg/kg/day (in the two year study in rats and reproduction studies in rabbits); a safety factor of 200 was applied due to the limited neurotoxicity data available (EMEA, 2004). Microbiological studies have also been conducted that gave an ADI of 5 µg/kg/day. As the toxicological ADI is lower, this will be used for the safety assessment (EMEA, 2004).

MRLs have been evaluated for edible tissues and eggs, and CVMP recommended the inclusion of lasalocid in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the levels as shown in Table 4.1.

Table 4.1 MRLs for lasalocid

Pharmacologically active substance	Marker residue	Animal species	MRLs (µg/kg)	Target tissues
Lasalocid	Lasalocid A	Poultry	20	Muscle
			100	Skin + fat
			100	Liver
			50	Kidney
			150	Eggs

From EMEA, 2007

Based on the MRL values for eggs and tissues, the theoretical adult daily intake from poultry represents approximately 78.4% of the ADI as estimated by EMEA (EMEA, 2004).

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Lincomycin

1 Introduction

Lincomycin (D-erythro-alpha-D-galacto-Octopyranoside, methyl 6,8-dideoxy-6-((((2S,4R)-1-methyl-4-propyl-2-pyrrolidinyl)carbonyl)amino-1-thio; molecular formula $C_{18}H_{34}N_2O_6S$; CAS No. 154-21-2; Figure 1.1; Box 1) is an antibiotic derived from *Streptomyces lincolnensis* (ChemID Plus, EMEA, 2000).

Figure 1.1 Molecular structure of lincomycin

From ChemID Plus

Box 1 Synonyms and trade names for lincomycin

BRN 0707677; Cillimycin; D-erythro-D-galacto-Octopyranoside, methyl 6,8-dideoxy-6-(1-methyl-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-, trans-alpha- ; D-erythro-alpha-D-galacto-Octopyranoside, methyl 6,8-dideoxy-6-(((1-methyl-4-propyl-2-pyrrolidinyl)carbonyl)amino)-1-thio-, (2S-trans)-; EINECS 205-824-6; HSDB 3109; Jiemycin; Lincolcina; Lincolnensin; Lincomicina; Lincomicina [INN-Spanish]; Lincomycin; Lincomycin A; Lincomycine; Lincomycine [French]; Lincomycine [INN-French]; Lincomycinum; Lincomycinum [INN-Latin]; Methyl 6,8-dideoxy-6-(1-methyl-4-propyl-2-pyrrolidinecarboxamido)-1-thio-D-erythro-D-galacto-octopyranoside (alpha-form); Methyl 6,8-dideoxy-6-trans-(1-methyl-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-erythro-alpha-D-galacto-octopyranoside; NSC-70731; U 10,149A; UNII-BOD072YW0F

From ChemID Plus

Lincomycin is part of the lincosamide group which also includes pirlimycin and clindamycin. In human medicine, lincomycin is given orally at 500 – 2000 mg per day for between 7 and 10 days. It can also be given as an i.m. injection at 600 – 1800 mg/person/day. Lincomycin is an antibiotic active mainly against Gram negative bacteria, though no specific conditions are targeted by lincomycin. It has largely been replaced by clindamycin (a structurally similar compound with very similar properties) (EMEA, 2000).

In veterinary medicine, lincomycin is used alone or in combination with other antibiotics, such as spectinomycin, sulfadimidine and gentamicin. In poultry, it is given in feed or drinking water at up to 10 mg/kg body weight/day for up to 21 days or as an i.m. injection at 15 mg/kg/day for up to 4 days; in swine at oral doses of up to 10 mg/kg/day for up to 21 days or i.m. at 15 mg/kg/day for up to seven days; in calves and sheep by i.m. injection at up to 15 mg/kg/day for up to four days. Lincomycin can also be given in a mixed preparation with neomycin and methylprednisolone for intramammary administration to dairy cattle at one tube (containing 200 mg lincomycin) per teat (EMEA, 2000). In an antibiotic associated colitis model in the Syrian hamster a NOEL of 0.1 mg/kg/day was identified (IPCS, 2000).

The mode of action of lincomycin is mainly against Gram positive bacteria, exerting antibacterial action by inhibition of RNA-dependent protein synthesis by acting on the 50S ribosome subunit (EMEA, 2000). Compared to the parent, no metabolites have been found to have significant antimicrobial activity; the main metabolites N-desmethyl lincomycin and

lincomycin sulfoxide are 15 and 100 times, respectively, less antimicrobiolgical activity than the parent (EMEA, 1998).

Lincomycin has been used in safety studies as either US Pharmacopoeia (USP) grade, or "premix"grade. The grades differed by up to 5% and 10% in USP and "premix" grade respectively. This difference is considered not to affect toxicity study outcomes (EMEA, 1998).

2 Toxicokinetics

The kinetic profile of lincomycin has been evaluated following i.m., i.v. or oral administration in humans. The presence of food in the stomach has been shown to impair the absorption of lincomycin (oral bioavailability approximately 5% with a meal compared with approximately 25 – 50% after fasting) (Hornish *et al.*, 1987). After oral administration (1000 mg/person; fasting state of individuals not stated in this study), peak serum concentrations occurred approximately 4 hours after dosing, with levels reaching 2.5 – 6.7 mg/ml. This compared with peak serum levels of 17 µg/ml approximately 1.5 hours after i.m. at the same dose, while i.v. injection of 600 mg/person have mean concentrations of 16 – 21 mg/ml (C_{max} and t_{max} exact values not given) (Fass, 1981, Smith *et al.*, 1981). In pregnant women given i.m. lincomycin at 600 mg/person, it was shown that lincomycin crossed the placenta; peak levels of 0.2 – 3.8 mg/ml were detected in amniotic fluid for up to 52 hours after dosing (Fass, 1981). After dosing, approximately 72% is bound to serum proteins and lincomycin is widely distributed throughout the body (e.g. bile, peritoneal fluid, pleural fluid, eye, brain, bone, bone marrow, joint capsules, synovial fluid and, in cases of CNS inflammation only, cerebrospinal fluid) irrespective of the route of administration, (Fass, 1981). In humans following oral or i.m. dosing lincomycin is excreted as unchanged parent (EMEA, 1998) generally via the faeces although some biliary excretion also occurs (Fass, 1981).

Experimentally, pigs orally dosed with 4.4, 11 (therapeutic dose) or 22 mg/kg ^{14}C-lincomycin showed therapeutic serum concentrations of 1.8, 3.9 and 5.1 mg/ml respectively, approximately 0.5 hours after dosing (EMEA, 1998). As for humans, dogs excrete lincomycin as unchanged parent following oral or i.m. dosing (EMEA, 1998). However in other species - rats, cows, pigs and chickens - lincomycin is extensively metabolised in all tissues, with the exception of cow's milk. Metabolism is primarily by S-oxidation to the sulphoxide or de-methylation to the N-desmethyl derivative (EMEA, 1998). Approximately 16 metabolites have been identified in most cases though 26 metabolites were identified for the pig liver. The main residues are unchanged parent and the N-desmethyl lincomycin and lincomycin sulfoxide (Hornish *et al.*, 1987, Nappier, 1998).

In oral dosed rats, approximately 5% was excreted in the urine (of which 97% was lincomycin and lincomcyn sulfone). The remaining 95% of administered dose was present in the gastrointestinal tract. This is somewhat different from the profile that occurs when given I.V. when 43% was excreted in the urine (73% as unchanged parent) while faeces contained 40% lincomycin and 60% unidentified metabolites (Hornish *et al.*, 1987). In pigs, urine contained 11 – 21 % of administered dose, half of which was unchanged parent. Trace amounts of N-desmethyllincomycin were also reported. Most of the excreted residues were in the gastrointestinal tract (79 – 86%) with the faeces containing 17% parent, the remainder was uncharacterized metabolites(Hornish *et al.*, 1987). In lactating cattle dosed with 11 mg/kg lincomycin, approximately 32% is excreted in the urine irrespective of route of administration. After i.v. injection, 1.5% appears in the urine while intramammary dosing leads to over 85% being eliminated in the milk (EMEA, 1998).

Studies using radiolabelled lincomycin have been performed in chickens (including on eggs), cattle (including milk) and pigs to determine the residue profile of this compound. In the

chicken, doses of ^{14}C-lincomycin at up to 11 mg/kg in drinking water, feed or oral gavage for up to 35 days led to residues being found in liver, kidney, muscle, skin and fat. Radiolabel was detectable for up to seven 7 days after dosing. In laying hens, 12 consecutive days treatment led to residues in liver, kidney, muscle, skin and fat and levels in egg yolk of approximately three times that in egg white by three days after the last dose (EMEA, 1998). In cattle dosed i.m. at up to 15 mg/kg lincomycin for up to six administration cycles (two doses on day 1, then once per day for the next four days), residues were again detected in liver, kidney, muscle and fat for up to 21 days. In lactating cattle, intramammary doses after three consecutive milkings resulted in lincomycin being detected in milk for up to 48 hours after the last dose, although levels were 320 times lower than at 12 hours after last dose (EMEA, 1998). In pigs, residues were present in a range of organs for up to twelve days after dosing (EMEA, 1998).

3 Toxicity profile

3.1 Acute toxicity

Acute toxicity reports in humans have not been identified.

In mice, oral LD_{50} was 20,000 mg/kg and i.v. LD_{50} was 210 mg/kg; signs in surviving animals included severe sedation for one – two minutes (Gray & Purmalis, 1962a). The sub-cutaneous LD_{50} was 780 mg/kg in new born rats and 10,000 mg/kg in adult rats, while the i.v LD_{50} was 340 mg/kg (Gray & Purmalis, 1963).

In rabbits a single i.v. injection of 0.5 mg/kg led to 50% of animals dying within two weeks and a further 2 dying within six weeks of dosing (Gray *et al.*, 1965a).

In contrast, single intrathecal injections of lincomycin at 15 mg or 50 mg to dogs did not induce clinical, macroscopic or microscopic changes. The only change was increased cell counts (mainly lymphocytes) in the cerebrospinal fluid (Gray *et al.*, 1965b).

In a rat model of *Escherichia coli* (gram negative) induced sepsis, a single i.v. dose of lincomycin (5 mg/kg) led to increased levels of endotoxin, compared with untreated rats. This may be clinically important since increases in plasma endotoxin levels have been noted in deteriorating septic patients receiving empirical lincomycin therapy (Horii *et al.*, 2000).

3.2 Repeat dose toxicity

In patients, gastrointestinal effects are the most commonly reported adverse effect; signs include nausea, vomiting, abdominal cramps and diarrhoea. Pseudomembranous colitis following lincomycin (or clindamycin) therapy may occur in up to 20% of patients, two to 25 days after the start of treatment (Goodman & Gilman, 1975, Kucers & Bennet, 1979). Rarely, hypersensitivity has been reported (Kucers & Bennet, 1979).

Rabbits are intolerant of lincomcyin, suffering gastrointestinal stasis and haemorrhagic suffusion of serosal surface of the caecum leading to death; this is attributed to gastrointestinal Gram positive floral imbalance (IPCS, 2000).

In a 90 day study, B6C3F$_1$ mice given lincomycin in the diet at 0, 70, 200, 700, 2000 or 20,000 mg/kg feed (equivalent to 0, 10, 30, 100, 300 and 3000 mg/kg body weight/day), at the high dose, reduced body weight, increased food consumption and intestinal weight (with pancreas), decreased serum glucose concentration and, in females, increased serum corticosterone concentrations, decreased serum globulin and decreased mean absolute thymus weight, were noted. At this dose, all animals showed decreased absolute organ weights for the heart, liver and spleen, and, in males, kidney. The lumina of the large and small intestine were

distended and dilated at the high dose. At the next highest level (300 mg/kg body weight/day), intestinal weight (with pancreas) and luminal distension and dilatation of the small and large intestines was increased and serum glucose levels reduced. The NOEL was 100 mg/kg body weight/day (Platte & Seaman, 1981).

In rats (strain not stated) given lincomycin at 0, 30, 100 or 300 mg/kg/day by oral gavage for one year, no treatment related effects were noted at body weight, organ weight, haematological or histological analysis. A difference in absolute, but not relative, liver weights was noted for high dose animals. The NOEL was 300 mg/kg/day (Gray & Purmalis, 1963).

Pregnant Sprague Dawley rats were given oral doses at 0, 0.38, 0.75 or 1.5 mg/kg/day premix grade lincomycin or 1.5 or 100 mg/kg/day USP-grade lincomycin (treatment duration of pregnant rats not stated). Treatment of offspring continued for a further 26 months. The percentage survival, clinical and ophthalmoscopy evaluations, food consumption, organ weights, haematological, serum chemistry and urinary parameters were not affected by treatment. Increases in incidence of non-neoplastic lesions of the prostate and seminal vesicles was noted at ≥0.75 mg/kg/day in both grades of lincomycin, however the increases in incidence or severity were not dose related and therefore considered not to be treatment related. Details of this study with respect to carcinogenicity can be in section 3.3 (IPCS, 2000).

Lincomycin in oral capsules to dogs (strain not stated) at 0, 0.38, 0.75 or 1.5 mg/kg/day for one year caused no treatment related findings in clinical or ophthalmic parameters, food consumption, body weights, clinical pathology, urinary parameters, organ weights, macroscopic or microscopic assessments. The NOEL was 1.5 mg/kg/day (Goyings *et al.*, 1979a).

3.3 Carcinogenicity and mutagenicity

Pregnant Sprague Dawley rats were given oral doses at 0, 0.38, 0.75 or 1.5 mg/kg/day premix grade lincomycin or 1.5 or 100 mg/kg/day USP-grade lincomycin for 26 months (as described in section above). The numbers of benign and malignant tumours were similar to controls in all treated groups. A range of specific malignancies including subcutaneous fibroma, lymphosarcoma, mammary adenocarcinoma and carcinoma, pituitary adenoma and mammary fibroadenoma, were noted but were considered common at this age and strain, and as incidence was not dose-related and/or within historical control values these tumours were not considered to be attributable to lincomycin. However, the low maximum dose used and poor survival limited a definitive conclusion. Nonetheless, a NOEL of 100 mg/kg/day was established (IPCS, 2000).

In a series of mutagenicity studies (Table 3.1 below), lincomycin was negative in all but one unscheduled DNA synthesis assay. This positive result was repeated in a similar assay but, after use of an improved microscope slide preparation procedure, findings were negative for the same lot of lincomycin. It was considered, based on weight of evidence, that lincomycin is not genotoxic (Aaron, 1988, Seaman, 1982, Harbarch & Aaron, 1987).

Table 3.1. Summary table of genotoxicity studies conducted with lincomycin

In vitro			
End point	**Test system**	**Concentration**	**Result**
Reverse mutation	*S. typhimurium*, TA98, TA100, TA1535, TA1537, TA1538	120 – 1000 µg/plate ± S9	Negative
Reverse mutation	*S. typhimurium*, TA98, TA100, TA102, TA1535, TA1537	620 – 5000 µg/plate ± S9	Negative

Forward mutation	Chinese hamster V79 lung fibroblasts, hprt locus	30 – 3000 µg/ml ± S9	Negative
DNA damage (alkaline elution)	Chinese hamster V79 lung fibroblasts	13 – 1300 µg/ml ± S9	Negative
Unscheduled DNA synthesis	Primary rat hepatocytes	10 – 2500 µg/ml	Negative[a]
Unscheduled DNA synthesis	Primary rat hepatocytes	0.17 – 17 µg/ml	Positive[b]
DNA repair	Human peripheral lymphocytes	2800 – 5000 µg/ml ± S9	Negative

In vivo

End point	Test system	Dose	Result
Cytogenicity	Rat bone marrow	1500 – 3000 mg/kg[c]	Negative
Cytogenicity	Mouse bone marrow	150 – 600 mg/kg	Negative
Sex-linked recessive lethal mutation	*Drosophila melanogaster*	25,000 and 50,000 µg/ml	Negative

From IPCS, 2000
[a] toxicity observed at concentrations of 50 µg/ml
[b] concentrations of above 16.7 µg/ml were lethal to cell cultures
[c] one half of the dose given at 0 and 24 hours, 3000 mg/kg as single dose was lethal

3.4 Reproductive and developmental toxicity

In humans, oral administration of lincomycin (2 g/person for seven days) to treat cervicitis or vaginitis of pregnant women in different trimesters of pregnancy, found no evidence of adverse effect on offspring when compared with babies of untreated mothers delivered at the same time when monitored for seven years (EMEA, 1998).

In a three-generation study in Sprague Dawley rats, dietary lincomycin at 0, 0.38, 0.75 or 1.5 mg/kg body weight /day (given as a premix grade) or 1.5 or 100 mg/kg body weight/day (USP grade) from weaning of F_0 rats through to weaning of F_{3a} litters, resulted in no treatment-related effects on clinical signs, fertility or maintenance of pregnancy. Litter parameters (including pup viability, growth rate, sex ratio, survival rates, clinical status and macroscopic or histopathological evaluation) were unaffected. The NOEL was considered to be the highest dose tested for both premix and USP grade lincomycin, i.e. 1.5 mg/kg body weight/day and 100 mg/kg body weight/day, respectively (Goyings *et al.*, 1979b).

In another study on SPF rats of two generations duration, treatment with lincomycin at 0, 100, 300 or 1000 mg/kg/day by oral gavage (males treated for sixty days before mating until delivery of F_1 generation and females for 14 days before mating until 21 days post partum), a transient decrease in body weight was noted in the first 14 days of treatment but weights were similar to controls by day 21. There were no treatment-related changes in reproductive or developmental toxicity. The maternal and fetal NOEL was 1000 mg/kg/day (Black *et al.*, 1988).

In a developmental study, pregnant rats (strain not stated) were given 0, 10, 30, 100 mg/kg/day by oral gavage on gestation days 6 – 15 and the dams killed on gestation day 20. There was no maternal toxicity but an increase in fetal resorptions was noted at the high dose with a corresponding decrease in number of live fetuses. However there was no evidence of teratogenicity. The maternal NOEL was 100 mg/kg/day and the fetal NOEL was 30 mg/kg/day (Morris *et al.*, 1980).

A range of studies with limited experimental details was performed in the 1960's, lincomycin administered s.c. to the rat during gestation (up to 300 mg/kg/day), prior to and through two reproduction cycles (75 mg/kg/day) and from birth to adulthood (30 mg/kg) did not give rise

to any treatment related maternal or fetal abnormalities (Gray & Purmalis, 1962a, Gray & Purmalis, 1963, Gray & Purmalis, 1962b, Mulvihill & Gray, 1965).

4 Guidelines and standards

A toxicologically-based ADI of 300 µg/kg was derived by applying a safety factor of 100 to the NOEL of 30 mg/kg/day for fetal toxicity in the rat.

However, because of the activity of lincomycin in the human gastrointestinal flora and given the excretion of unchanged parent into the gut, the most sensitive endpoint is considered to be based on the microbiological activity of the compound.

The microbiological ADI of 10 µg/kg was based on a NOEL of 0.1 mg/kg/day from an antibiotic associated colitis model in the Syrian hamster and a safety factor of 10 applied. A factor of 10 was applied for inter-animal variation. No correction factor was applied for extrapolation from animals to humans due to the sensitivity of the hamster model (IPCS, 2000). This ADI has been used for the current assessment.

Lincomycin was included in Annex I of Council Regulation (EEC) No. 2377/90 with MRL quoted for specific species in 1998 (EMEA, 1998). However, the CVMP conducted a risk assessment review and adopted a Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin in 2000 (EMEA, 2000) that allowed for extrapolation of MRLs to all food producing species. Therefore the CVMP currently recommends inclusion of lincomycin in Annex I of Council Regulation (EEC) No. 2377/90 based on the MRLs quoted in Table 4.1 (EMEA, 2002).

Following assessment by JECFA, temporary MRLs were established for cattle tissues of 100 µg/kg in muscle, 500 µg/kg in liver, 1500 µg/kg in kidney, 100 µg/kg in fat and 150 µg/kg in milk. Studies in pigs and chickens have significant differences between some animal species with levels in pig's kidneys being three times higher than in liver. In chickens, liver and kidney levels are similar. Hence extrapolation of kinetics of lincomycin residues between species was considered not possible (IPCS, 2000).

Table 4.1 MRLs for lincomycin

Pharmacologically active substance	Marker residue	Animal species	MRLs (µg/kg)	Target tissues
Lincomycin	Lincomycin	All food producing species	100 50 500 1500 150 50	Muscle* Fat** Liver Kidney Milk Eggs

*For fin fish, this MRL relates to muscle and skin in natural proportions
** For porcine and poultry species, this MRL relates to skin and fat in natural proportions
From (EMEA, 2002)

EMEA estimated that based on these MRLs for all food producing species, consumer intake from food sources would not exceed 64% of the microbiological ADI (EMEA, 2002).

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Luprostiol

1 Introduction

Luprostiol ((+-)-(Z)-7-((1R*, 2S*, 3S*, 5R*)-2-(((2R*)-3-(m-chlorophenoxy)-2-hydroxypropyl)thio)-3,5-dihydroxycyclopentyl)-5-heptenoic acid; molecular formula $C_{21}H_{29}ClO_6S$; CAS No. 67110-79-6; Figure 1.1; Box 1) is a synthetic prostaglandin F2α analogue (ChemID Plus).

Figure 1.1 Molecular structure of luprostiol

From ChemID Plus

Box 1 Synonyms and trade names

(+-)-(Z)-7-((1R*,2S*,3S*,5R*)-2-(((2R*)-3-(m-Chlorophenoxy)-2-hydroxypropyl)thio)-3,5-dihydroxycyclopentyl)-5-heptenoic acid; EMD 34946; Equestrolin; Luprostiol; Luprostiolum; Luprostiolum [INN-Latin]; Reprodin; UNII-HWR60H5GZB

From ChemID Plus

Luprostiol is generally used for the control of oestrus or induction of parturition in cattle, sheep, goat, horse and pig. It is used therapeutically in single doses of between 3 and 15 mg per animal as an i.m. injection. In cattle, luprostiol is also used in the control of suboestrus, induction of abortion, chronic endometritis, pyometra and mummified or macerated fetuses. In horses it can be used to induce "second heat" twenty days post partum, anoestrus, treatment following early fetal death and resorption, pymetra and endometritis. In sheep and goats, luprostiol can be used for the luteolytic effect in planned breeding (EMEA, 1997).

Luprostiol is not used in human medicine.

The EMEA report and FDA new animal drug application (NADA) document are the primary sources for this assessment; no other authoritative literature reviews are available.

2 Toxicokinetics

Studies in rats, cows, mini-pigs, sheep and goats showed that luprostiol is absorbed rapidly (within 15 – 30 minutes) following i.m. or oral (rat only) administration with ^{35}S-luprostiol at doses ranging from 0.02 – 0.5 mg/kg. Distribution studies revealed that luprostiol is not retained in any specific organ although in the rat, radioactivity was eliminated from liver, kidney and intestinal tract within 24 hours (EMEA, 1997).

The degree of metabolism varied widely between species and metabolites were only characterized in terms of polarity relative to the parent. Chromatographic analysis revealed the metabolites to be closely related to luprostiol and the major pathway of degradation was found to be the β-oxidation of the carboxylic acid side chain. In the rat, luprostiol is largely metabolized with only 2 – 3% of administered dose excreted as unchanged parent (ratio of metabolites to parent not stated). In the cow, several metabolites have been identified in the urine, liver and kidney. In sheep within 24 hours of administration plasma levels were between 70 – 80% unchanged parent; urinary excretion 24 hours post dose comprised 73% unchanged drug. In sheep milk, by 24 hours post dose approximately 10% was unchanged parent and the rest was a less polar metabolite considered to be the same metabolite as that present in plasma. In mini-pigs, four hours after dosing 20 – 30% was present as parent in the plasma while 50 – 60% was present as a metabolite that was less polar than the parent. After 8 hours, approximately 40% unchanged parent was excreted in urine and the remainder was a less polar metabolite. However in faeces no unchanged parent, only the less polar metabolite, was detected. In goats in the first 24 hours after dosing, 76% of the administered dose was excreted, 70% of which was a less polar metabolite and 20% unidentified metabolites. Urinary excretion accounted for 69% and faecal for 18%, and the ratio of unchanged parent and metabolite was 3:2 after 15 minutes and 2:3 after 2 hours in the plasma. In Shetland ponies, 8 hours after dosing 50 – 60% of the administered dose was excreted as unchanged parent. However, in the plasma 24 hours after dosing 70 – 80% was present as unchanged drug. A less polar metabolite was also observed in both plasma and urine however the levels were not quantified (EMEA, 1997).

Residue depletion studies in cows demonstrated that luprostiol was present three days after i.m. administration (15 – 30 mg/animal) at < 1 µg/kg in the liver and kidney; no residues were noted in muscle and fat, while levels of 0.04 µg/kg occurred in milk 48 hours after dosing. These studies showed the rapid elimination of luprostiol from milk and many organs; luprostiol when detected, was mainly in the excretory organs. Studies in horses, pigs, sheep and goats gave similar results (EMEA, 1997).

3 Toxicity profile

3.1 Acute toxicity

No specific incidences of luprostiol exposure of humans have been identified although there is concern since luprostiol is readily absorbed through the skin and can cause abortion and/or bronchospasms due to its pharmacological activity. Hence, it is suggested by FDA that women of childbearing age, asthmatics and individuals with respiratory conditions should be extremely careful when handling this product (FDA, 2009).

Mice and rats (strain not stated) were given single doses of luprostiol orally or by s.c., i.m., i.p. or i.v. (dose ranged used not stated). No histopathological abnormalities were found; i.v. LD_{50} values were 116 mg/kg in male rats and 130 mg/kg in female rats. Overall, the parenteral NOEL was 1 mg/kg in the rat and 3 mg/kg in the mouse, and the oral NOEL was 75 mg/kg in the rat and 150 mg/kg in the mouse (EMEA, 1997).

3.2 Repeat dose toxicity

In rats subject to subcutaneous injection of 100 mg/kg/day luprostiol for two weeks led to reductions in body weight and food consumption (FDA, 2009).

In pigs orally dosed with luprostiol (exact dose range not stated) for 30 days, doses of 1.5 or 30 mg/kg/day led to reduced growth, food intake and polydipsia (excessive thirst), polyuria (passage of large volumes of urine) and a trend towards higher urea-nitrogen levels; changes

in renal morphology were also observed. These changes were reversed during a four week period of withdrawal of treatment. A NOEL of 0.1 mg/kg/day was established (EMEA, 1997).

Oral administration of luprostiol at 0, 0.1, 3 or 100 mg/kg/day to Sprague-Dawley rats for 13 weeks resulted, at the high dose, in retained corpora lutea, haematopoiesis of spleen and liver, nephrosis and interstitial nephritis, degeneration of the Harderian gland, excessive formation of bone trabeculae and osteoid tissue in the femur, excessive formation of osteoid tissue and/or callus in the tibia/fibula and fractures in the tibia/fibula. At the mid dose, only retention of corpora lutea was noted in some animals. The NOEL for this study was 0.1 mg/kg/day (FDA, 2009).

In a 14 week study, Beagle dogs were given luprostiol at 0, 0.025, 0.25 or 2.5 mg/kg/day (route of administration not stated). The high dose led to multifocal serositis (inflammation of the serous layer) of the liver, transient reductions in body weight gain and food consumption. At the mid dose, increased adrenal weight (absolute or relative not stated) was noted in males with associated vasculitis in 50% of males and 17% of females. There were no effects of toxicological significance at 0.025 mg/kg/day (FDA, 2009).

3.3 Carcinogenicity and mutagenicity

No carcinogenicity studies have been identified.

Luprostiol has been examined for its mutagenic potential.

In an Ames test and an *in vivo* rat micronucleus study (where rats were dosed orally with 0, 0.1, 3 or 100 mg/kg/day) luprostiol did not show mutagenic potential. Furthermore, luprostiol belongs to a class of prostaglandins that are not generally considered to have carcinogenic activity (EMEA, 1997).

3.4 Reproductive and developmental toxicity

Male rats (strain not stated) were orally dosed with luprostiol at 0, 0.1, 0.3 or 1.0 mg/kg/day for nine weeks prior to and throughout mating. Females were also dosed for two weeks prior to mating, throughout gestation and lactation. No treatment-related changes in condition, behaviour, body weight, mating performance or fertility were noted and no teratogenic effects were observed. The only effect of treatment was, at the mid and high dose, premature delivery (EMEA, 1997).

In a two generation study, Sprague Dawley rats dosed with 0, 0.1, 1, 10 or 2500 µg/kg/day by oral gavage showed signs of toxicity in the high dose first parental generation; signs of toxicity including death, clinical signs, gross lesions and reduced body weight gain. Effects in the high dose were decreased fertility and abortion, as well as a decrease in viable offspring. Other groups showed no treatment-related effects and the NOEL was established at 10 µg/kg/day (FDA, 2009).

In a developmental study in pregnant rats (strain not stated) given 0, 0.12, 1.2, 12 or 2500 µg/kg/day luprostiol by oral gavage on days 6 – 15 of gestation, embryo/fetal effects (not specified) were noted at the high dose. Maternal effects included increased resorption, fetal death and decreased litter size at the high dose only. The developmental NOEL was 12 mg/kg/day (FDA, 2009).

In rabbits orally dosed with luprostiol at 0, 0.01, 0.02, 0.03 or 0.3 mg/kg/day on days 6 – 18 or 10 – 18 of gestation, no signs of maternal toxicity were noted at any dose. Doses up to 0.02 mg/kg/day did not show any embryotoxicity. However, at 0.03 mg/kg/day a clear disruption

of pregnancy was noted with total fetal resorption; no mention of any effects at 0.3 mg/kg/day is made. The oral NOEL was 0.02 mg/kg/day (EMEA, 1997),

A study in hamsters further evaluated the effects of oral luprostiol dosing on day 6 of gestation. Reflecting the established pharmacological activity of luprostiol as a prostaglandin F2α analogue, doses of 0.8 mg/kg induced abortion while doses of 0.025 mg/kg did not (EMEA, 1997).

4 Guidelines and standards

An ADI of only 0.2 µg/kg has been established for luprostiol based on a NOEL of 0.02 mg/kg/day in a developmental toxicity study in rabbits, with a safety factor of 100 applied (EMEA, 1997).

Due to the therapeutic use of luprostiol, for oestrus control and parturition, it is not intended to be used in animals slaughtered immediately after treatment. Given its established rapid clearance and lack of accumulation potential, the CVMP considered there was no need to establish MRL and it was recommended for inclusion in Annex II of Council Regulation (EEC) No 2377/90 as applicable to all mammalian species (EMEA, 1997).

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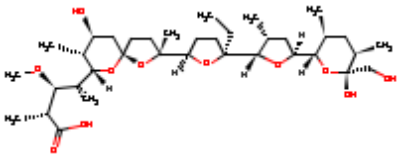
Monensin

1 Introduction

Monensin (1,6-Dioxaspiro(4.5)decane-7-butyric acid, 2-(5-ethyltetrahydro-5-(tetrahydro-3-methyl-5-(tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl)-2-furyl)-2-furyl)-9-hydroxy-beta-methoxy-alpha,gamma,2,8-tetramethyl-, molecular formula $C_{36}H_{62}O_{11}$, CAS No. 17090-79-8; SIS NLM, 2004, Figure 1.1; Box 1), is a growth-promoting agent and a coccidiostat, used in beef cattle, sheep, chickens and turkeys (de Souza Spinosa *et al.*, 1999; SIS NLM, 2004).

Figure 1.1 Molecular structure of monensin



From SIS NLM (2004)

Box 1 Synonyms and trade names for monensin

A 3823A; ATCC 15413; Coban (as sodium salt); EINECS 241-154-0; Elancoban; HSDB 7031; Lilly 673140; Monelan; Monensic acid; Monensin A; Rumensin (as sodium salt); Stereoisomer of 2-(2-ethyloctahydro-3'-methyl-5'-(tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl)(2,2'-bifuran-5-yl))-9-hydroxy-beta-methoxy-alpha,gamma,2,8-tetramethyl-1,6-dioxaspiro(4.5)decan-7-butanoic acid

From SIS NLM (2004)

Monensin is also a carboxylic ionophore antibiotic that offers a broad range of uses in research and agriculture (Langston *et al.*, 1985). Monensin is intended to be used in lactating dairy cattle as an oral device (controlled release capsule). The device will release monensin in the rumen (maximum rate of 400 mg/day) over approximately 100 days to control ketosis (EMEA, 2007). It is mainly composed of analogue A (equivalent to 98%); analogues B, C and D are also components (equivalent to 98%) (EMEA, 2007). Monensin interferes with the transport of ions through membranes, causing an influx of sodium ions that indirectly increases intracellular calcium ion concentration, with perturbation of calcium ion homeostasis and concentration. This increased intracellular concentration of calcium ions is believed to be important in the pathogenesis of monensin toxicity in animals (de Souza Spinosa *et al.*, 1999). Antimicrobial and anticoccidial activity is exhibited by monensin. Its mode of action is via complexation of monovalent cations. The lipophilic structure of monensin can help facilitate the transport of cations through biological membranes. This can therefore alter the normal concentration gradients. Generally, monensin is active against Gram-positive bacteria (EMEA, 2007).

The therapeutic effects of monensin administered to cattle are due to the shift in ruminal fermentation characteristics. This leads to higher propionate concentrations at the expense of other volatile fatty acids (e.g. acetate, butyrate) and effects on energy metabolism including reduced blood ketones and increased serum glucose. Growth efficiency may be enhanced due to these changes and because of improvements in energy capture from feed and protein utilization (EMEA, 2007).

The majority of the data presented in this assessment has been taken from articles by the European Food Safety Authority (EFSA, 2004b) and Todd *et al*. (1984; the primary source of many of the key studies reported in the EFSA review).

2 Toxicokinetics

Monensin is well absorbed from the gastrointestinal tract of most mammals. However, extensive hepatic metabolism coupled with the relatively low dose used for therapeutic purposes results in only trace amounts of the substances being absorbed into the general circulation. Consequently, there is only limited distribution to tissues (Langston *et al.*, 1985). The pharmacokinetics of monensin following oral administration has been evaluated in cattle and rats using ^{14}C-monensin. Monensin was shown to be rapidly absorbed and extensively metabolised, mainly in liver in both species (EMEA, 2007). *In vitro* studies comparing interspecies metabolic clearance (using horse, pooled dog and pooled human microsomal preparations) indicate that monensin in humans and dogs is rapidly metabolised when compared to the horse. Unfortunately experimental limits mean that conclusions cannot be drawn regarding the variability within the human population to metabolise monensin. The EMEA report found that the *in vitro* studies concluded the average clearance of monensin in humans and dogs may be comparable. Therefore the dog is an appropriate species in which to assess the pharmacological NOEL (EMEA 2007).

Many metabolites of monensin have been isolated from the excreta and tissues, of which eight major metabolites have been identified, each representing less than 10% of the total residues. Metabolites are produced by either single or combined demethylation, decarboxylation and hydroxylation reactions (EFSA, 2004c). Monensin and its metabolites have been found to be mainly excreted in the bile, excretion rates in cattle and rats reportedly corresponded with approximately 35% and 40% of the oral dose, respectively. Oral doses of radioactivity was largely recovered in faeces, in comparison, urinary excretion rates were negligible in cattle and rats.

The production of metabolites occurred by either single or combined demethylation, decarboxylation and hydroxylation along the ionophore backbone at various locations. This resulted in a number of unknown polar metabolites occurring at low concentrations. Parent monensin and five metabolites have been identified in liver, bile and/or faeces from cattle treated with monensin, as a result of O-demethylation and oxidation reactions. The compounds represented about 30% of total radioactivity in liver and 67% in faeces. Only a limited fraction of the monensin-related compounds was represented as unchanged in liver and faeces (less than 10 %) and 2 % in milk (EMEA, 2007).

The pattern of metabolites in cattle and rats was found to be qualitatively comparable and similar routes of metabolism were also reported for other species such as poultry. *In vitro* studies, have found the cytochrome P450 (and also probably the cytochrome P4503A subfamily) in rats, cattle, pigs, chickens, and horses to be involved in the oxidative metabolism of monensin.

An estimate established on available information suggests that the major monensin metabolites have less than 50% pharmacological and microbiological activity than the parent

compound (EMEA 2007). It is also known that the use of monensin in combination with other medicinal products (e.g. macrolides) can hinder its metabolism. This can result in accumulation of the ionophore and lead to increased toxicity (EMEA, 2007).

In a residue depletion study 5 cattle animals were administered twice daily with oral doses of 0.9 mg ^{14}C-monensin/kg bw in gelatine capsules, via a rumen fistula over a period of 9.5 days (918 to 1125 mg ^{14}C-monensin/day). Cows were milked at 12 hour intervals (twice daily). 6 hours following the last dose, animals were slaughtered and milk, liver, kidney and fat tissues were analysed. Levels of radioactivity detected in the kidney, muscle and fat were too low to identify metabolites. The total residues in milk reached a steady-state 5 days following administration. Mass spectrometry of milk identified monensin and the metabolite M-6 (demethylated ketoderivative, decarboxylated). In milk, approximately 2% of the total radioactivity was monensin and approximately 26.5% was incorporated into endogenous fatty acids. The liver had the highest concentrations of total mean residues (1280 µg/kg). The kidney was found to contain 70 µg/kg, fat had 20 µg/kg and muscle content was below the limit of detection. The parent monensin concentrations in liver and kidney were below the limit of quantification (25 µg/kg) of the HPLC method. Mass spectrometry of LC fractions of liver extract identified monensin and metabolites in the liver to be M-1 (demethylated and hydroxylated derivative), M-2 (demethylated and hydroxylated derivative with additional hydroxylation ring E) and M-6 (demethylated keto derivative, decarboxylated) and were thought to account for approximately 6.8%, 4.5%, 4.5%, and 18% of the total radioactivity, which was approximately 75% of extractable radioactivity (EMEA, 2007)

In a residue study, 16 lactating cows were treated orally with monensin (0.9 mg/kg bw/day) for 7 consecutive days. Monensin was administered twice daily in gelatine capsules and doses of 0.45 mg/kg bw were given at approximately 12 hour intervals. Tissue samples of the liver, kidney, muscle, fat were taken from 4 animals. The measured time points were 6, 12 and 24 hours following treatment. Milk samples were taken from 8 cows at the milkings prior to treatment and at 3 milkings, at the same time points. Only low concentrations of Monensin A were detected in tissues and milk. The highest concentrations were found in the liver with maximum values of 10.46 µg/kg (6 hours), 6.70 µg/kg (12 hours) and 5.43 µg/kg (24 hours). Concentrations of fat were found to be.24 µg/kg and 1.41 µg/kg at 6 and 12 hours, respectively and in the kidney were 1.03 µg/kg at 6 hours (one sample). Monensin A concentrations were below the limit of quantification in the liver and fat in all samples at 12 hours and 24 hours, respectively. No monensin was detected in muscle for any of the time points. The parent monensin was only detected in milk up to 12 hours, concentrations were very low (0.54 µg/kg (max) in the 1st milking and 0.32 µ/kg (max) in the 2nd milkings).

A routine analytical method for the determining monensin A in bovine tissues and milk has been derived. This is based on HPLC coupled with ESI tandem mass spectrometry (HPLC/MS/MS) using narasin A as the internal standard. The limits of quantification for all tissues were 1 µg/kg and 0.25 µg/kg in milk. The reported recovery ranged between 97 and 108 % in tissues and milk. Validation of the method was according to Volume 8 of the Rules Governing Medicinal Products in the European Union (EMEA, 2007).

3 Toxicity profile

3.1 Acute toxicity

Monensin is not used in human medicine and limited data exists on the effect of direct exposure of humans to monensin. One international report is available describing a 17 year old male who died from acute rhabdomyolysis with renal failure after ingesting an unknown amount of monensin premix. In another case, 2 deaths among 6 people occurred as a result of consuming baked goods made with monensin premix. The monensin exposure intake was in

excess of a 10 times daily dose fed to cattle (EMEA, 2007). The exact quantity of monensin in the "premix" is not known.

Allergic symptoms of monensin include urticaria, swelling of the face or tongue, pruritus, nasal congestion, contact dermatitis and local respiratory irritation; these symptoms have all been reported in workers handling monensin (EMEA, 2007)

Acute toxicity following therapeutic administration of monensin varies considerably between species. The LD_{50} ranges between 2–3 mg/kg bw in horses to 200 mg/kg bw in chickens. Rats and mice have an LD_{50} of 35 and 125 mg/kg bw, respectively. In all species, clinical signs of toxicity follow changes to feeding habits, and include anorexia, ataxia, mild diarrhoea, depression, dyspnoea, stiffness, weakness, recumbency and death. Some effects are more pronounced in some species. Clinical pathology findings at necropsy and histopathological findings all depend on species, and which of their organs are most severely affected. However, severe cardiac and skeletal lesions usually occur, from which recovery can be protracted (Langston *et al.*, 1985).

Todd *et al*. (1984) administered a single oral dose of monensin to several experimental animal species (rats, mice, rabbits, monkeys, chicken, cattle, sheep, goats, swine, horses, dogs and trout) and observed for signs of toxicity for 14 days (no further details reported). Consistent signs of toxicity were observed including anorexia, hypoactivity, skeletal weakness, ataxia, diarrhoea, decreased weight gain and death. Of the animals that survived, all showed improvements in condition during the observation period. LD_{50} values were similar to those reported by Langston *et al*. (1985), with an additional value for monkeys of >160 mg/kg bw, which showed that these animals were amongst the least sensitive species.

Due to the ionophoretic activity of monensin,secondary pharmacological activity, such as cardiovascular effects have also been noted. The susceptibility for these secondary effects was seen to vary widely between species and the therapeutic margin was found to be narrow. For example following oral dosing of monensin to dogs, transient increases in coronary blood flow were observed though heart rate and mean blood pressure did not significantly change at the doses tested (0.138, 0.345, 0.69 and 1.38 mg/kg bw) with a NOEL of 0.345 mg/kg bw. However after intravenous administration of monensin, to dogs (conscious and anaesthetised) and pigs (anaesthetised) (0.00069 – 1.4 mg/kg bw), increases in coronary flow, blood pressure and heart rate were noted with NOELs of 0.0345 mg/kg bw in conscious dogs and 0.0035 mg/kg bw in anaesthetised dogs. A NOEL was not established in pigs (EMEA, 2007).

In contrast to observations of other animal species, in cats no cardiovascular effects were observed at oral doses of 30 mg/kg bw. Furthermore, no related effects of oral doses of 10 mg/kg or higher were noted on the central, peripheral, and autonomic nervous systems or respiratory and digestive systems in cats or in other laboratory species such as mice (EMEA, 2007).

According to HSDB (2002) monensin is a skin and eye irritant. EFSA concluded that monensin and Elancoban, a product that contains monensin as the active ingredient, are very irritating to the eyes, but not the skin. EFSA also regarded monensin and Elancoban to be weak skin sensitisers and potential respiratory sensitisers (EFSA, 2004b).

The oral NOEL (from the species studied) established for cardiovascular effects observed in dogs appears to be similar to oral doses that have potentially lethal effects in horses, with a 4-fold margin. The available scientific literature indicates a difference between species for the potential of monensin to induce acute effects. It is possible a correlation may exist between the rate and capacity of monensin turnover by microsomal liver enzymes (cytochrome P450) (EMEA, 2007).

3.2 Repeat dose toxicity

Wistar rats (15/sex/dose) were fed diets containing 50, 150 or 500 ppm monensin (equivalent to 3-5, 5-15 and 39-47 mg/kg bw/day; EFSA, 2004a) for three months. The control group was given untreated feed. Daily examinations were made to check for physical signs of toxicity. In addition, food consumption and body weight were measured weekly. A blood sample was collected from each animal before termination for evaluation of haematology and blood chemistry parameters. Following necropsy, pathological examinations of all major organs and tissues were conducted. The highest dose group was the only group to show significant treatment-related toxicity. These effects were severe depression of body weight gain, 17 deaths during the first three weeks, moderate skeletal muscle degeneration and necrosis, and slight cardiac degeneration. Blood and haematological parameters were not affected. The NOAEL was 150 ppm, although there was a slight reduction in body weight gain at this dose (Todd *et al.*, 1984).

In an unusual 2-year chronic toxicity/carcinogenicity study, three groups of 100 male and 100 female Wistar rats derived from parents given diets containing monensin (see Section 3.4), were given 33, 50 or 80 ppm (equivalent to 1.4, 2.18, and 3.6 mg/kg bw/day in males, and 1.72, 2.86 and 5.02 mg/kg bw/day in females; EFSA, 2004a) monensin in the diet for two years. Control animals were kept on an untreated diet. The animals' physical appearance was noted daily. Body weights, and haematological and blood chemistry parameters were determined at intervals before and during the test period. All animals were necropsied and special attention paid to chronic and neoplastic lesions during the pathological evaluation. There were no treatment-related effects, except for a slight decrease in body weight at the highest dose for both sexes and at 5 ppm in females. The test compound did not increase the incidence or severity of chronic lesions including those in skeletal and cardiac muscles. The NOAEL for this study was >80 ppm (Todd *et al.*, 1984).

B6C3F1 mice (15/sex/dose) were fed diets containing 37.5, 75, 150 or 300 ppm monensin for 3 months. The control group was given untreated feed. Daily examinations were made to check for physical signs of toxicity. In addition, food consumption and body weight were measured weekly. A blood sample was collected from each animal before termination for evaluation of haematology and blood chemistry parameters. Following necropsy, pathological examinations of all major organs and tissues were conducted. A dose-related decrease in body weight gain occurred in all dosed groups compared with the controls. Serum creatine phosphokinase (CPK) levels were slightly increased in several animals that received the highest dose, and it was thought that this probably came from skeletal or heart muscle. On microscopic examination, minimal heart lesions were observed in the highest dose group. No other toxic effects were observed. A NOAEL could not be determined from this study (Todd *et al.*, 1984).

In a 2-year chronic toxicity/carcinogenicity assay reported in EFSA (2004a), B6C3F1 mice (60/sex) were maintained on diets containing mycelial monensin at levels of 0, 10, 25, 75 and 150 mg/kg (equivalent to doses of monensin sodium of 0, 1.2, 3.1, 10.2 and 22.6 mg/kg bw/day for males and 0, 1.4, 3.5, 11.7 and 25.6 mg/kg bw/day for females) for two years. At all but the lowest dose, body weight was decreased in both sexes, and leukocyte counts decreased in males. No adverse effects on mortality, physical appearance, behaviour, haematology (except leucocytes), clinical chemistry, gross pathology, organ weights, or histopathology were observed. The NOAELs for this study were 1.2 and 1.4 mg/kg bw/day for males and females, respectively (EFSA, 2004a).

Beagle dogs (2/sex/dose) were given daily oral doses of 5, 15 or 50 mg/kg bw monensin for 3 months by gelatine capsules. A control group remained untreated. The physical condition of each animal was assessed on a daily basis and body weights were measured weekly. Haematological and blood biochemistry values were obtained on all animals before, and five

times during the test period. Following necropsy, pathological examinations of all major organs and tissues were conducted. No adverse effects were observed in the low dose group, and blood parameters were normal for all groups. However, during weeks one to four, anorexia, weakness, ataxia, laboured breathing and body weight loss were observed in the mid and high dose groups. Significant elevation of aspartate transaminase (SGOT) levels, a sign of muscle injury, occurred in six of the eight mid and high dose groups. After week four the levels returned to normal. Two animals died and one was killed in a moribund condition during the first two weeks of the study. These animals had severe skeletal muscle lesions and minimal cardiac lesions. At the termination of the study, only minimal skeletal muscle damage was found in the two top dose groups. However, there were some signs of repair to skeletal muscle in some animals. The NOAEL for this study was 5mg/kg bw/day (Todd *et al.*, 1984).

Two further studies in dogs were reported by EFSA (2004a). In one of these studies, cross breed dogs (2/sex/dose) were fed monensin by capsule for 90 days. There were no signs of toxicity for doses of 2.5 or 5 mg/kg bw/day. At higher doses of 11 and 25 mg/kg bw/day ataxia, loss of motor coordination, relaxation of the nictitating membrane, salivation, vomiting and tremors were observed. Both males that received 25 mg/kg bw/day, and one female that was dosed with 11 mg/kg bw/day died after three doses. Treatment of females in the 25 mg/kg bw/day group was stopped after the fourth dose due to increasing morbidity. Histopathology showed evidence of hepatic toxicity at the two highest doses. A NOAEL of 5 mg/kg bw/day was identified for this study. In the second study, Beagle dogs (4/sex/dose) were given doses of monensin in mycelium for one year. At 5 and 7.5 mg/kg bw/day transient anorexia, hypoactivity and weakness were observed. Elevated serum alanine transaminase and creatine phosphokinase were found in some dogs at these doses during the first four weeks of treatment. Body weight gain was decreased at 7.5 mg/kg bw/day. Based on these findings the NOAEL was 2.5 mg/kg bw/day (EFSA, 2004a).

Todd *et al*. (1984) conducted a further one-year study in Beagle dogs. Animals (4/sex/dose) were given daily oral doses of 1.25, 2.5, 5 or 7.5 mg/kg bw monensin. An additional, untreated group served as the control. The animals' physical appearance was noted daily. Body weights, and haematological and blood chemistry parameters were determined at intervals before and during the test period. Ophthalmoscopic and electrocardiographic evaluations were performed before, during and at the end of treatment. The dogs were necropsied and all of the major organs and tissues were examined histopathologically. Since the heart, diaphragm and skeletal muscles were all known to be affected by monensin, specific evaluations of these tissues were undertaken. There were no deaths in any dose group, and no evidence of toxicity in the two lowest dose groups. Animals that received the two highest doses had signs of transient anorexia, hypoactivity and weakness. In six of these dogs, alanine transaminase levels were elevated during the first two weeks of dosing, and CPK levels were elevated in 13 animals during weeks one to four. This pattern of enzyme alteration is indicative of muscle damage during the first four weeks, with recovery thereafter. There was also a decrease in body weight in the highest dose group. No other signs of toxicity or any pathological lesions were seen in any dose group. The NOAEL for this study was 2.5 mg/kg bw/day (Todd *et al.*, 1984).

Todd *et al*. (1984) concluded that the target organs damaged by toxic doses of monensin were the skeletal and cardiac muscles, and that the damage and recovery from it can be monitored by measuring serum muscle enzymes. The muscles that were most severely damaged were those that have the highest level of activity, such as the diaphragm, abdominal and heart muscles.

3.3 Carcinogenicity and mutagenicity

The EFSA (2004a) concluded that monensin is not carcinogenic, based on the lack of genotoxicity (see below) and studies into the carcinogenic potential of monensin in rats and mice. In a 2-year chronic toxicity/carcinogenicity study in Wistar rats (described in Section 3.2) the type, latency and prevalence of benign and malignant neoplasms were similar in control and treated animals. The NOAEL for carcinogenicity in this study was >1.4 and >1.72 mg/kg bw/day for males and females, respectively (Todd *et al.*, 1984). In a 2-year chronic toxicity/carcinogenicity study in B6C3F1 mice (reported in Section 3.2) there was no increase in benign or malignant tumour incidence at any dose tested. The NOAEL for carcinogenicity was >22.6 and >25.6 mg/kg bw/day for males and females, respectively.

The EFSA (2004c) also concluded that monensin is not genotoxic, based on three pre-GLP bacterial reverse mutation assays, an *in vitro* cytogenetics assay and an *in vivo* micronucleus study. Monensin showed no mutagenic activity or growth inhibition with and without metabolic activation in *S. typhimurium* G46, TA1535, TA100, TA1537, TA1538, TA98, D3052 and C3076, and *E. coli* WP2 and WP2 uvrA. In the cytogenetics assay, monensin sodium was dissolved in dimethyl sulphoxide and tested for potential to cause chromosomal aberrations in CHO cells, with (50, 80 or 100 µg/ml) and without (25, 50 or 100 µg/ml) metabolic activation. The EFSA concluded that monensin did not cause chromosomal damage. In the *in vivo* micronucleus test, monensin (0, 181, 363 and 725 mg/kg bw/day) was given by oral gavage to groups of five male and five female ICR mice on two consecutive days. There was no indication of mutagenic activity; however, the value of this result is compromised by the small group sizes resulting from high mortality in the mid- and high dose groups.

3.4 Reproductive and developmental toxicity

Todd *et al*. (1984) conducted a multigeneration reproductive study in Wistar rats. Three groups of 25 to 30 male and female rats were continuously maintained on diets containing 33, 50 or 80 ppm (equivalent to 3.3, 5 or 8 mg/kg bw/day) monensin over four generations. A similar group was given an untreated diet and served as a control. Reproductive parameters and the health status of the offspring were evaluated. The offspring from the F_2 generation were evaluated for teratogenic defects by macroscopic and microscopic examinations for visceral and skeletal abnormalities. The F_{1a} offspring were assigned to the two-year chronic/oncogenic study described above. There was no apparent treatment-related effect on fertility, litter size, length of gestation, parent and progeny survival and sex distribution. There was a slight decrease in body weight gain in each generation, but the statistical significance of this was not reported. There was no evidence of teratogenicity in any dose group or generation. The NOAEL for developmental and reproductive toxicity in this study was ≥80 ppm (Todd *et al.*, 1984).

De Souza Spinosa *et al*. (1999) conducted a study into the development of female Wistar rats and their offspring following administration of monensin in their diet. Young female rats (14/dose) received 100 or 300 ppm monensin in their diet until adulthood, when they were mated and their offspring examined for physical and neurobehavioural development. Reproductive parameters, including gestation length, maternal body weight gain, litter size, sex ratio and pup weight, were evaluated. The only significant treatment-related effect was on body weight at the top dose. Dams showed a significantly reduced body weight gain, as did weaning offspring that had begun to eat the treated food. Weight gain during pregnancy was similar in treated and untreated animals. None of the reproductive parameters were affected by treatment with monensin, nor were there any developmental effects, including teratogenicity. The NOAEL for this study was 100 ppm (de Souza Spinosa *et al.*, 1999).

Two multigeneration reproduction studies were undertaken. One crystalline (non-GLP compliant study) and one mycelial (GLP-study). Monensin was administered to three generations of rats, via their diets. In the first study, crystalline monensin was administered at levels of 2.5 mg/kg (equivalent to 0.14 to 0.2 mg/kg bw/day), 12.5 mg/kg (equivalent to 0.74 to 0.97 mg/kg bw/day), and 25 mg/kg (equivalent to 1.43 to 2.3 mg/kg bw/day) to 30 parent rats and 20 generation 1 and 40 generation 2 animals/per sex/per dose. There were no treatment related changes reported for the groups receiving crystalline monensin up to the highest dose tested. The established NOEL was 25 mg/kg, which corresponded to 1.43 to 2.3 mg/kg bw/day. In the second study, 30 parent rats and 25 rats from generation 1 and 2 per sex and dose group were administered with either 33 mg/kg (equivalent to 1.6 to 2.2 mg/kg bw/day), 50 mg/kg and 80 mg/kg (equivalent to 4 to 8 mg/kg bw/day). Effects were noted relating to reduced weight gain. The weight gain was depressed during the growth phase in; F_0 males for all monensin-treated groups, F_2 males of middle and high dose groups, in F_2 females of the low and middle doses and F_0, F_1, and F_2 females of the high dose groups. There were also reductions in the weight of pregnant and lactating females of the middle and high dose groups. In relation to maternal toxicity, a NOEL could not be identified. This was because slight body weight effects occurred at the 33 mg/kg dose (equivalent to 1.6 to 2.2 mg/kg bw/day). The reproductive performance (fertility, litter size, gestation length, parent and offspring survival and sex distribution) was not affected. No evidence of teratogenicity in the offspring from parents in both studies was observed. Doses up to 80 mg/kg (equivalent to 4 to 8 mg/kg bw/day) of monensin showed reproductive processes were not adversely affected (EMEA, 2007).

In a teratology study (non GLP), 15 pregnant rabbits were given oral gavage doses of 0.076, 0.38, or 0.76 mg monensin/kg daily, on gestation days 6-18. At day 28 of gestation, females reproductive performance and the fetuses were examined for any abnormalities. |No effects were observed on the reproductive parameters and indices (not specified), and fetal viability, sex distribution, and weight were all normal. In a small number of offspring, developmental deviations were observed, however no evidence of a treatment effect existed. Conclusions indicated that oral administration of monensin to pregnant rabbits at doses up to 0.76 mg/kg affected neither the reproductive performance nor fetal development. A treatment-related reduction in mean daily food consumption was observed in the highest dose group. However, no body weight changes occurred. No embryo/foetotoxicity was reported up to the highest dose tested. The reported NOEL for maternal and embryo/foetotoxicity was 0.76 mg/kg bw (higher doses were not tested; EMEA 2007).

4 Guidelines and standards

The EFSA identified the lowest NOEL from toxicological studies as 1.1 mg/kg bw/day in a two-year chronic toxicity/carcinogenicity assay in rats (EFSA, 2004c). A NOEL of 0.345 mg/kg bw/day for acute pharmacological effects on the cardiovascular system was identified from a dog study (EMEA, 2007). This was the lowest NOEL from toxicological and pharmacological studies. No data were available for humans, so an uncertainty factor of 100 was applied to the NOEL of 0.345 mg/kg bw/day to derive an ADI of 3 µg/kg bw (rounded figure; EFSA, 2004b). In the EMEA report (2007) a pharmacological ADI was established as 3.45 µg/kg bw i.e. 207 µg/person was derived, using an uncertainty factor of 100 using the same NOEL and study. This ADI has been used in the current assessment.

Established MRLs for monensin have been derived using monensin A as the marker residue and bovine as the target species. MRLs for muscle, fat, liver, kidney and milk are respectively; 2, 10, 30, 2, 2µg/kg. Based on these MRLs, the theoretical maximum daily intake from tissue and milk of cattle represents 57% of the ADI. Taking into account the MRLs established by the FEEDAP Panel of the EFSA for chicken tissues and the CVMP MRLs for milk and the consideration of 50% activity of monensin metabolites, EMEA

estimated the theoretical maximum daily intake from chicken tissue and milk to represent 82% of the ADI (EMEA, 2007)

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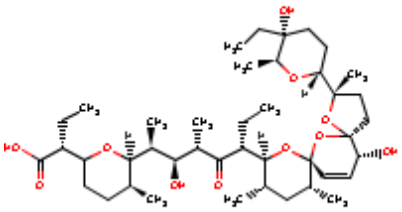
Salinomycin

1 Introduction

Salinomycin (molecular formula $C_{42}H_{70}O_{11}$, CAS No. 53003-10-4; Figure 1.1, Box 1 (ChemID Plus) is a polyether ionophoric antibiotic with coccidiostatic activity, which is used for fattening chickens, turkeys, rabbits and cattle, and as a growth promoter for piglets and lambs.

Figure 1.1 Molecular structure of salinomycin



From ChemID Plus

Box 1 Synonyms and trade names of salinomycin

AHR 3096; Bio-cox; Coxistac; EINECS 258-290-1; HSDB 7032; K 364; UNII-62UXS86T64

From ChemID Plus

The majority of the toxicological information included in this assessment is taken from the published reports of the EU Scientific Committee for Animal Nutrition (SCAN, 1992), which considered the submissions for the use of salinomycin as a food additive to fatten or induce growth of specific animals. However, the data reviewed by SCAN are not publicly available, so could not be retrieved for the purposes of this assessment. We have also included more recent information on the mutagenicity and environmental safety of the chemical from the Scientific Panel on Additives and Products or Substances used in Animal Feed (EFSA, 2005)

2 Toxicokinetics

The kinetics of ^{14}C-salinomycin have been investigated in rabbits. It is rapidly and extensively absorbed from the gut following oral administration. Maximum tissue concentrations of labelled salinomycin in a 15-day oral dosing study were achieved by 24 hours and remained constant. The main target for residues was the liver, with residues also detected in the kidney, fat, muscle and gut wall. Salinomycin is metabolised to numerous metabolites, mainly mono-, di- and trihydroxylated derivatives, which are reported to be similar to those produced in the rat and mouse. Although the parent compound was not detected in bile, metabolites rapidly appeared in the bile; it is suspected that they undergo enterohepatic recirculation. In the 15-day feeding study in rabbits, the four main metabolites of salinomycin were undetectable after 24 hours of withdrawal of treatment. Elimination occurred mainly via the faeces (56–80%

within 3–8 days), in addition to 8–15% recovery in urine. No label was present in expired air (SCAN, 1992).

3 Toxicity profile

3.1 Acute toxicity

Accidental inhalation exposure to approximately 1 mg/kg salinomycin in a single adult male led to immediate nausea, shortness of breath and dizziness. 48 hours after exposure limb weakness occurred. The side effects increased and severe pain in both legs spreading to arms and chest occurred 14 days following exposure to salinomycin. Rhabdomyolysis (rapid breakdown of skeletal muscle tissue) was diagnosed, with pain and disability. Six weeks following exposure to salinomycin, the patient had made a slow recovery, and was discharged. However, the patient had a limited exercise tolerance. The symptoms observed were similar to that observed in the dog (Story & Doube, 2004).

Acute oral toxicity studies have been performed in a number of species (mouse, rat, rabbit, chicken, dog, pig, bull and horse). The oral LD_{50} values were all in the range of 21–60 mg/kg bw (SCAN, 1992). For mice, rats, chickens and rabbits the signs of toxicity were mainly neurological, while for the other species the toxic effects occurred in the liver and myocardium (SCAN, 1992).

Acute dermal toxicity testing has shown that salinomycin is a moderate irritant (SCAN, 1992).

3.2 Repeat dose toxicity

In subchronic toxicity studies in mice, rats, dogs and pigs, SCAN (1992) indicated that the main toxicity observed in mice and rats was liver and spleen toxicity (NOEL of 3.75 and 2.5 mg/kg bw/day, respectively).

Chronic toxicity was investigated in mice over 24 months and in rats over 30 months. In these studies most of the observed toxicity involved organ weight changes, and changes in clinical biochemistry parameters. The NOEL was <1.4 and 2.5 mg/kg bw/day for mice and rats, respectively (SCAN, 1992).

3.3 Carcinogenicity and mutagenicity

There were no signs of carcinogenicity in the chronic toxicity studies in rats and mice briefly described in Section 3.2 (SCAN, 1992).

Salinomycin was negative for genotoxic effects in Ames tests, a host-mediated assay in mice, recessive-lethal mutation tests in *D. melanogaster*, hepatocyte unscheduled DNA synthesis and a mouse lymphoma forward mutation assay (SCAN, 1992). The FEEDAP panel noted a positive *in vitro* cytogenetic assay (metaphase analysis of CHO cells) for clastogenicity (EFSA, 2004), however, there are also now two *in vivo* studies demonstrating negative results in two mammalian somatic tissues; these are a mouse bone marrow micronucleus assay and a UDS test in hepatocytes from male Fisher rats (EFSA, 2005).

3.4 Reproductive and developmental toxicity

SCAN (1992) reported that a two-generation reproductive toxicity study in rats gave a NOEL of 5 mg/kg bw/day. Maternal and fetal toxicity were observed in mice and rabbits in an embryotoxicity study, in which the NOEL was 4 mg/kg bw/day. Rabbits showed increased

resorptions, likely to have been due to maternal toxicity. The NOEL for this effect was 0.25 mg/kg bw/day.

No developmental effects were observed in rats in a two-generation study or in teratogenicity studies in mice and rabbits (SCAN, 1992).

4 Guidelines and standards

SCAN (1992) derived an ADI for salinomycin of 2.5 µg/kg bw based on the NOEL from the rabbit reproductive toxicity study, and an uncertainty factor of 100.

The Australian Government's Department of Health and Ageing has recommended an ADI of 10 µg/kg bw. The background to this recommendation is not described, but it is known to be based on a NOEL of 1 mg/kg bw/day (Department of Health and Ageing. Australian Government, 2005).

The new *in vivo* data on genotoxicity confirmed the lack of genotoxic potential of salinomycin, and allowed the FEEDAP to propose an ADI of 5 µg/kg bw (EFSA, 2004), and it is this value that will be adopted for the current assessment. An MRL for all target tissues (liver, muscle, kidney, skin and fat) has now been recommended for salinomycin of 5 µg kg^{-1} wet tissues, with a one day withdrawal time (EFSA, 2005).

5. Environmental Risks

It has been observed in liver that only about 20 % of all salinomycin residue still posses the ionophore properties of binding cations (Dimenna *et al.*, 1989), as cited by EFSA, 2005), and the FEEDAP panel concluded that only 20 % of the total salinomycin-related excretory products are environmentaly relevant to environmental risk assessments.

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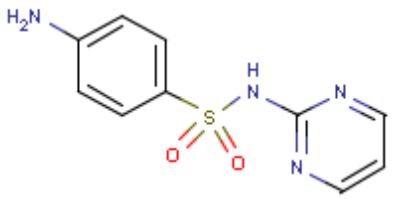
Sulfadiazine

1 Introduction

Sulfadiazine (molecular formula, $C_{10}H_{10}N_4O_2S$, CAS No. 68-35-9) belongs to the sulphonamide group of antimicrobial drugs. When potentiated by co-administration with other drugs, the short-acting sulphonamide has a broad spectrum of activity that is useful in human and veterinary medicine. Sulfadiazine is known by a range of synonyms (Box 1) and its structure is shown in Figure 1.1 (ChemID Plus).

Figure 1.1 Molecular structure of sulfadiazine



From ChemID Plus

Box 1 Synonyms and trade names for sulfadiazine

2-Sulfanilamidopyrimidin(e); A 306; A-306 (VAN); Al3-01047; Adiazin(e); BRN 0235192; Coco-Diazine; Cremodiazine; Cremotres; Debenal; Deltazina, Di-Azo-Mul; Diazin; Diazolone; Diazovit; Diazyl; EINECS 200-685-8; Eskadiazine; Honey diazine; Lipo-Diazine; Lipo-Levazine; Liquadiazine; Metha-Meridiazine; Microsulfon; N(sup 1)-2-Pyrimidinylsulfanilamide; NSC 35600; Neazine; Neotrizine; Palatrize; Pecta-diazine, suspension; Piridisir; Pirimal; Pyrimal; Pyrimidine, 2-sulfanilamido-; Quadetts; Quadramoid; RP 2616; S.N. 112; Sanodiazine; Solfadiazina [DCIT]; Spofadrizine; Sterazine; Sulfadiazene; Sulfadiazin; Sulfanilamide, N1-2(1H)-pyrimidinylidene-; Sulfanilamidopyrimidine; Sulfapyrimidin(e); Sulfatryl; Sulfazine; Sulfolex; Sulfonsol; Sulfose; Sulphadiazine; Sulphadiazine E; Terfonyl; Theradiazine; Thi-Di-Mer; Tri-Sulfameth; Trifonamide; Triple Sulfas; Trisem; Truozine; Benzenesulfonamide, 4-amino-N-2-pyrimidinyl-; Sulfanilamide, N(sup 1)-2-pyrimidinyl-; Sulfanilamide, N1-2-pyrimidinyl- (8Cl)

From ChemID Plus

Sulfadiazine belongs to a class of compounds called sulphonamides, which are antibiotics used against agents like *Pneumonocystis carinii* and ampicillin-resistant *Haemophilus influenzae*. When sulfadiazine is potentiated by combination with other drugs such as trimethoprim or ormetoprim, it has a broad spectrum of activity and may be used to treat urinary, prostatic, skin and respiratory tract bacterial infections, and opportunistic mycobacterial infections and protozoal infections (Trepanier, 2004).

Sulfadiazine is also associated with adverse hypersensitivity reactions in some individuals, which may be fatal depending on the severity of reaction. This has led to a reduction in use of sulfadiazine in human medicine but it has recently re-emerged as an agent of clinical importance because, when combined with pyrimethamine, it is effective against

toxoplasmosis in patients with acquired immunodeficiency syndrome (AIDS) and in newborns with congenital infections (Catalano-Pons *et al.*, 2004).

Sulfadiazine is also used in veterinary medicine to treat microbiological pathogens, and normal therapeutic doses in dogs range from 30–60 mg/kg bw/day (Trepanier, 2004).

2 Toxicokinetics

No information on the analytical methods for the detection of sulfadiazine in human or animal tissues was found and data on the absorption, distribution and elimination of sulfadiazine are unavailable for review.

3 Toxicity profile

Dose-dependent adverse reactions to sulfadiazine include non-regenerative anaemia, haematuria and inhibition of thyroid hormone synthesis. In humans, prolonged high exposure to potentiated sulphonamides is reported to result in megaloblastic anaemia. In dogs, prolonged doses above therapeutic levels are reported to lead to normocytic, rather than megaloblastic, anaemia. Sulfadiazine may also form insoluble crystals in acidic urine, which can lead to obstruction and haematuria in humans (Catalano-Pons *et al.*, 2004; Trepanier, 2004).

The most common signs of hypersensitivity reactions associated with sulphonamides in humans occur 1–2 weeks after treatment and initially present as skin rash or fever with possible progression to multi-organ toxicity involving the liver, kidney, bone marrow, lung, heart or central nervous system (Rieder *et al.*, 1988).

The bioactivation of sulfadiazine to more reactive metabolites has been suggested as the cause of sensitisation in humans. The increased susceptibility of some individuals is considered to reflect human genetic polymorphism of *N*-acetyl transferase, with slow and fast acetylation phenotypes existing within the general population. People who demonstrate hypersensitivity to sulfadiazine (potentiated by the co-administration of another drug) are often slow acetylators. Consequently, a slow acetylating stage in the metabolism of sulfadiazine may give rise to larger amounts of the reactive intermediates of sulfadiazine, because more of the drug is available for oxidative metabolism by cytochrome P-450 (Rieder *et al.*, 1988).

All of the potentiated sulphonamides used in the USA have been associated with hypersensitivity reactions but it is unclear that the sulphonamides themselves are wholly responsible. For example, trimethoprim can cause skin eruption and hepatopathy in humans when administered alone although at a much lower frequency than when sulphonamides are administered alone (Trepanier, 2004).

3.1 Acute toxicity

A study on dogs (breed unspecified) given a combination of trimethoprim–sulfadiazine at a dose of 60 mg sulfadiazine/kg bw/day for six weeks resulted in lower serum thyroxine levels in 57% of dogs with pyoderma. Hypothyroidism was also reported in a clinical study on a dog given two 10-week courses of trimethoprim–sulfadiazine at 48 mg/kg bw/day. However, another study in which dogs were given trimethoprim–sulfadiazine at 30 mg/kg bw/day for four weeks, found no effect on serum total or free thyroxine or on the response to exogenous thyroid-stimulating hormone (Trepanier, 2004).

Studies have shown that the idiosyncratic, hypersensitivity reactions seen in some humans also occur in dogs given therapeutic doses of trimethoprim-sulfadiazine, 5–36 days after

therapy. The syndrome in dogs is characterised by severe multisystemic toxicity consisting of fever (in 50% of cases), polyarthropathy, blood dyscrasias (neutropenia, thrombocytopenia or haemolytic anaemia), hepatic toxicity (cholestasis or necrosis), skin eruptions, uveitis or keratoconjunctivitis sicca. Although not fully understood, the effect may be due to a T-cell mediated response to proteins haptenated by oxidative sulphonamide metabolites. Hypersensitivity reactions are also seen in cats although less commonly than dogs. Such idiosyncratic reactions are relatively uncommon. In a retrospective study on cats and dogs, an overall incidence of 0.25% (2.5 cases per 1000 prescriptions) was estimated. A small survey in the UK reported that 10% of adverse reactions to drugs in animals are due to sulphonamide treatments, and that of all antimicrobial drugs, sulphonamides are responsible for 82% of adverse reactions (Trepanier, 2004).

Two surveys were conducted in Florida, USA in 1987 (pilot study) and 1989–1990. A total of 1552 owners of domestic dogs and cats were given questionnaires (60% response rate) on the adverse reactions observed in their pets following treatment with 12 different oral antimicrobial, veterinary drugs. Trimethoprim–sulfadiazine was the most commonly used drug in dogs and associated with the widest variety of adverse reactions (in 18%). It was ranked intermediately among the 12 antimicrobial drugs that were tested in terms of probability of causing any adverse reaction. Only four of the 12 drugs surveyed caused an adverse reaction in cats; trimethoprim–sulfadiazine caused an adverse reaction in 25% and effects noted were non-specific — vomiting, diarrhoea, personality change, depression, polyuria/polydipsia and salivation (Trepanier, 2004).

3.2 Repeat dose toxicity

No data are available.

3.3 Carcinogenicity and mutagenicity

No data are available.

3.4 Reproductive and developmental toxicity

One paper reported findings from three *in vitro* test systems: whole chick and whole rat embryo cultures (to determine the teratogenic potential of sulfadiazine) and the aggregating embryonic brain cell culture (to assess toxicity). Sulfadiazine at 40 µg/mℓ in dimethyl sulphoxide (DMSO) induced no adverse response in the rat embryo culture but brachial limb buds were reported in the chick embryos. However, the effects in chick embryos were not reproducible and no concentration–response relationship could be established. The effects observed may be attributable to the vehicle DMSO, a substance previously associated with teratogenic effects *in vitro*. The results are therefore considered equivocal. The embryonic brain cell culture assay indicated that sulfadiazine was moderately toxic, predominantly to oligodendrocytes (Kucera *et al.*, 1993).

In vivo data for reproductive or developmental end-points sulfadiazine have not been identified but classified it as an ‘improbable human teratogen’ (Kucera *et al.*, 1993).

4 Guidelines and standards

An ADI of 20 µg/kg bw has been recommended, based on a NOEL of 37.5 mg/kg/day in a rat reproductive study and applying a safety factor of 2000 (NRA, 2000).

The CVMP considered that a tissue MRL of 100 µg/kg of the original drug substance should be applied for all compounds of the sulphonamide group. It was also stated that this value should also be applied to milk from cattle, sheep and goats (EMEA, 1995).

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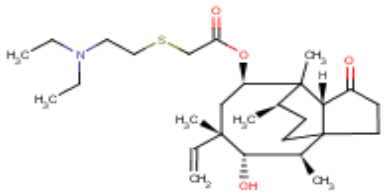
Tiamulin

1 Introduction

Tiamulin (Acetic acid, ((2-(diethylamino)ethyl)thio)-,(3aS,4R,5S,6S,8R,9R,9aR,10R)-6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3a,9-propano-3aH-yclopentacycloocten-8-ylester; Acetic acid, ((2-(diethylamino)ethyl)thio)-, 6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3a,9-propano-3aH-cyclopentacycloocten-8-yl ester (3aS-(3aalpha,4beta,5alpha,6alpha,8beta,9alpha,9abeta,10S*))-; molecular formula C_{28}-H_{47}-N-O_4-S; CAS No 55297-95-5; Figure 1.1; Box 1) is a diterpene antimicrobial with a pleuromutilin chemical structure. It is similar to valnemulin (EMEA, 2008, EMEA, 2000). The activity of tiamulin is largely confined to gram-positive micro-organisms and *mycoplasma* (EMEA, 2008) and it is used to treat or prevent dysentery, pneumonia and mycoplasmal infections in animals (EMEA, 2008). The mode of action involves inhibition of protein synthesis at the ribosomal level. The EMEA CVMP has evaluated the use of tiamulin in pigs, chickens and turkeys and an application has been received for the use of tiamulin in rabbits (EMEA, 2000). No information could be found to identify if this application has been supported. Tiamulin is not used in human medicine (EMEA, 2008)

Figure 1.1 Molecular structure of tiamulin



From ChemID Plus

Box 1 Synonyms and trade names of tiamulin

((2-(Diethylamino)ethyl)thio)-, 6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3a,9-propano-3aH-cyclopentacycloocten-8-yl ester (3aS-(3aalpha,4beta,5alpha,6alpha,8beta,9alpha,9abeta,10S*)); ((2-(Diethylamino)ethyl)thio)acetic acid 8-ester with (3aS,4R,5S,6S,8R,9R,9aR,10R)-octahydro-5,8-dihydroxy-4,6,9,10)-tetramethyl-6-vinyl-3a,9-propano-3aH-cyclopentacycloocten-1(4H)-one ((2-(Diethylamino)ethyl)thio)acetic acid 8-ester with (3aS,4R,5S,6S,8R,9R,9aR,10R)-octahydro-5,8-dihydroxy-4,6,9,10-tetramethyl-6-vinyl-3a,9-propano-3aH-cyclopentacycloocten-1(4H)-one; 14-Deoxy-14-((2-diethylaminoethyl-thio)-acetoxy)mutiline; Acetic acid, ((2-(diethylamino)ethyl)thio)-, 6-ethenyldecahydro-5-hydroxy-4,6,9,10-tetramethyl-1-oxo-3a,9-propano-3aH-cyclopentacycloocten-8-yl ester (3aS-(3aalpha,4beta,5alpha,6alpha,8beta,9alpha,9abeta,10S*)); BRN 2229396; Denagard; EINECS 259-580-0; HSDB 7026; SQ 14055; Thiamutilin; Tiamulin; Tiamuline; Tiamulinum; Tiavet P; UNII-E38WZ4U54R

From ChemID Plus

Tiamulin can be administered in a variety of forms including as a solution or water soluble powder (for use with drinking water) and, for pigs only, as an injection (EMEA, 2008). Doses depend on species and administration route, varying from 10 – 320 mg/kg bw (EMEA, 2008).

2 Toxicokinetics

In a residue depletion study in turkeys given tiamulin via drinking water at 0.025% for five consecutive days residues (based on hydrolysis to 8-α-hydroxymutilin) were less than 50 μg/kg in muscle while those in skin and fat were 72, 90 and 71 μg/kg at 0 hours, 8 hours and after one day, Two days following dosing all skin and fat samples contained less than 50 μg/kg. In the liver, the average concentrations were 905, 518, 527, 253 and 228 μg/kg after 0 hours, 8 hours, 1 day, 2 days or 3 days, respectively (EMEA, 2008). In a second study, turkeys were given 50mg radiolabelled tiamulin (^{3}H-tiamulin/kg bw/day) for five consecutive days. Residue levels two hours following dosing in the liver, muscle, skin and fat were 87,000, 3150 and 4600 μg equivalents/kg, respectively. The appropriate marker residue in turkeys is 8-α-hydroxymutilin which represents about 10% in the liver, 4% in the muscle and 3% in the skin and fat of all residues (EMEA, 2000).

Using the same marker residue (sum of all metabolites hydrolysed to 8-α-hydroxymutilin), rabbits were administered tiamulin in their diet for 21 days. The target dose was 11 mg/kg bw/day and the achieved mean dose was 13 mg/kg bw/day. Animals were killed at 0 and 8 hours, 1, 2 and 3 days following treatment. This resulted in mean renal residues of 35 μg/kg immediately after treatment falling to below the level of quantification for the remaining time points. In the liver, the mean residue was 529 μg/kg immediately after treatment, 283 μg/kg at 8 hours, 127 μg/kg at 1 day, 63 μg/kg at 2 days and 37 μg/kg at 3 days. Residues in the muscle and fat were below the level of quantification at all times (EMEA, 2000).

3 Toxicity profile

3.1 Acute toxicity

In patch tests in humans, tiamulin (0.05%) did not result in skin irritation or sensitisation. In another study three volunteers were given tiamulin orally as five doses of 0.125 – 7.2 mg/kg bw with 72 hours between, while a further three were given a single dose of 8.2 – 10.7 mg/kg bw. No changes in blood pressure, serum chemistry or electrocardiogram were observed (EMEA, 2000).

3.2 Repeat dose toxicity

In a chronic dietary study in CFE Carworth rats, diet containing tiamulin at 0, 5 or 30 mg/kg bw/day was fed for 26 weeks. A further group of rats received 180 mg/kg bw/day for 10 weeks, followed by 270 mg/kg bw/day for 16 weeks. Some rats were killed following treatment while others were maintained on untreated control diets for a four to eight week recovery period. Increases in serum chloesterol and water intake were observed at 180 mg/kg bw/day, it was not stated if these changes were during treatment or recovery, while at 270 mg/kg bw serum alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase activities and urine specific gravity were increased. Abdominal distention and dense faeces were also noted at the 270 mg/kg bw dose group. Relative and absolute liver weights were increased in both the males and the females and histopathological examination showed hepatic fatty infiltration, again in the 270 mg/kg bw dose group. A degree of recovery in the liver was noted after withdrawal of treatment, although it was not specified how extensive this recovery was. The NOEL was considered to be 5 mg/kg bw/day (EMEA, 1999).

In healthy male turkeys, not receiving monensin in their feed, treatment with tiamulin was assessed using a range of treatment regimens for different ages of turkey Intramuscular injection of 12.5 mg/kg tiamulin for up to 145 days was found to cause no toxicity and did not impair growth. Similarly, up to 25 mg/kg given via drinking-water did not cause effects in turkeys up to 160 days (the age for market; (Weisman *et al.*, 1983).

In a chronic study Beagle dogs were given tiamulin in gelatin capsules at 0, 3 or 10 mg/kg bw/day for 26 weeks. Additional groups of beagle dogs were given 30 mg/kg bw/day for 13 weeks, the dose was increased to 45 mg/kg bw/day. Two hours following the administration of tiamulin at the 45 mg/kg bw/day dose two male dogs died. The dose was subsequently reduced to 30 mg/kg bw/day for the remaining dogs. The NOEL was 3 mg/kg bw/day. Emesis was observed in all dogs, including controls, and at 10 mg/kg bw/day serum alanine aminotransferase increased significantly. Electrocardiography showed a prolongation of QT interval at the dose 10mg/kg bw/day and an increase in liver weights and fatty deposits were noticed in dogs receiving the highest dose (30 mg/kg bw/day; EMEA, 2000).

In a chronic study in Beagle dogs given tiamulin in gelatin capsules at 0, 3, 10 or 30 mg/kg bw/day for 54 weeks, vomiting was noted at 30 mg/kg bw/day. In addition, in the 10 mg/kg bw/day and 30 mg/kg bw/day group, serum potassium level was decreased while electrocardiography showed a prolongation of QT interval. Serum lactate dehydrogenase (LDH) activity was increased at some time-points for some groups (details not specified) but dose-relationship was not consistently demonstrated. However, no increase in LDH1 isoenzyme activity was apparent. The NOEL was 3 mg/kg bw/day (EMEA, 1999).

3.3 Carcinogenicity and mutagenicity

In a dietary study in which Charles River CD rats were given diet containing tiamulin at levels designed to achieve doses of 0, 2, 8 or 32 mg/kg bw/day for 30 months, a NOEL for neoplastic effects of 8 mg/kg bw/day was established based on a statistically significant increase in cholangiofibrosis, bile duct proliferation and hepatocellular vacuolation in males and females given 32 mg/kg bw. In females, a significant reduction in bodyweight was also noted at this dose The findings as presented relate to a reread of tissue slides approximately 13 years after the original study was completed (EMEA, 2000).

In a study on Charles River CD1 COBS mice were given a diet containing tiamulin at 0, 1, 6 or 48 mg/kg bw/day for up to 123 weeks. At 26, 53 or 78 weeks 5 mice per dose group and sex were sacrificed, no effects of treatment on tumour incidence were noted; a NOEL was not stated (EMEA, 2000). Again, the findings as presented relate to a reread of tissue slides approximately 13 years after the original study was completed (EMEA, 2000).

3.4 Reproductive and developmental toxicity

Effects of tiamulin on male fertility were studied in a non-GLP study in male OFA rats. Groups of 15 male rats were orally dosed with tiamulin at 0, 30, 55 or 100 mg/kg bw/day prior to mating with untreated females. Each male was allowed to mate with two females and thirteen days following mating half of the females were killed and the remainder were allowed to litter and wean their offspring. No treatment effects on fertility or the survival and growth of the offspring were identified (EMEA, 2000). A NOEL was not reported.

In Yellow-silver rabbits orally dosed with tiamulin at 0, 30, 55 or 100 mg/kg bw/day from day 6 of gestation through to day 18, deaths of some dams and reduced maternal body weight gain occurred at 55 mg/kg bw/day. At this dose, litter size and fetal weight were also reduced. However, there was no evidence of teratogenicity at any dose. A NOEL of 30 mg/kg bw/day was established for foetoxicity and maternal toxicity (EMEA, 2000). The study was undertaken before GLP was established, however it is well documented.

4 Guidelines and standards

An ADI of 30 µg/kg bw (equivalent to 1800 µg per 60 kg person) was established based on a NOEL of 3 mg/kg bw/day from 26 and 54 week studies in dogs. A safety factor of 100 was applied (EMEA, 2000).

According to the EMEA (2008) the expected intake from food sources will represent approximately about 90% of the ADI. This is based on the MRLs values and those for eggs in Table 4.1.

Table 4.1 MRLs for tiamulin

Pharmacologically active substances	Marker residue	Animal species	MRLs (µg/kg)	Target tissues	Other provisions
Tiamulin	Sum of metabolites that may be hydrolysed to 8-α-hydroxymutilin	Porcine	100	Muscle	
			500	Liver	
		Chicken	100	Muscle	
			100	Skin & Fat	
			1000	Liver	
	Tiamulin	Chicken	1000	Eggs	
	Sum of metabolites that may be hydrolysed to 8-α-hydroxymutilin	Turkey	100	Muscle	
			100	Skin & Fat	
			300	Liver	

From EMEA, 2008

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Tilmicosin

1 Introduction

Tilmicosin (4(supA)-O-de(2,6-dideoxy-3-C-methyl-alpha-L-ribo-hexopyranosyl)-20-deoxo-20-(3,5-dimethyl-1-piperidinyl)-, 20(cis)tylosin; molecular formula C46H80N2O13, CAS no. 108050-54-0; (ChemID Plus); Figure 1.1; Box 1) is a macrolide antibiotic synthesised from tylosin (EMEA, 2002).

Figure 1.1 Molecular structure of tilmicosin

From ChemID Plus

Box 1 Synonyms and trade names of tilmicosin

4(sup A)-O-de(2,6-Dideoxy-3-C-methyl-alpha-L-ribo-hexopyranosyl)-20-deoxo-20-(cis-3,5-dimethylpiperidino)tylosin; HSDB 7446; Micotil; Micotil 300; Tilmicosin; Tilmicosina; Tilmicosina [INN-Spanish]; Tilmicosine; Tilmicosine [INN-French]; Tilmicosinum; Tilmicosinum [INN-Latin]

From ChemID Plus

Tilmicosin is composed of one cis- and two trans-isomers in a ratio of approximately 85:15. It has anti-bacterial activity with a similar spectrum to that of tylosin with enhanced activity against *Pasteurella multocida* and *P. haemolytica*. It is used in the treatment of respiratory diseases in cattle and sheep at doses of 10 mg/kg in an injectable form or in pigs as a premix to be added to feed at 200 – 400 mg tilmicosin/kg feed (IPCS, 1996).

Tilmicosin is not used in human medicine.

2 Toxicokinetics

Specific oral absorption details have not been identified from the kinetic studies that have been conducted.

In Beulah cross lambs given a single s.c. dose of 20 mg/kg ^{14}C-tilmicosin, radioactivity was mostly detected in the liver, kidneys and urine as unchanged parent; low levels of desmethyl derivative (T1) and an impurity with two macrolide rings and one piperidine (T2) were noted (Hawkins *et al.*, 1993). The T2 form is considered to be an impurity because it has the same HPLC characteristics as the dimer of tilmicosin (EMEA).

In chickens, rats, cattle, pigs and sheep, as well as formation of T1 and T2, studies have identified other metabolites including a hydroxylated form of tilmicosin, referred to as T3, and reduced or sulphated forms of T4. Also, in chickens, a reductive glycosidic cleavage of

the mycinose sugar moiety has been observed to yield metabolites T9 and T10 (EMEA, 1997).

In pigs given ^{14}C-tilmicosin in the feed at 400 mg for five days, 60 - 70% of radioactivity was detected in the liver and kidney, mainly as the parent with low levels of T1. In the urine and faeces the parent was at higher levels than T1 but another metabolite (T4) accounting for 14% of the faecal and 25% of the urinary radioactivity was also present (Donoho *et al.*, 1993). Pigs given ^{14}C-tilmicosin in the feed at 110 mg for one day or up to 400 mg for five days showed excretion of between 4 and 15% of the radioactivity in the urine and 62 - 85% in the faeces (Donoho *et al.*, 1993, Giera & Thomson, 1993, Donoho & Thomson, 1988).

In Fischer 344 rats given oral ^{14}C-tilmicosin (labelled at both the desmycosin macrolide ring and piperidine ring) at 20 mg/kg/day for three days, radioactivity was detected in the liver for unchanged parent and as T1. In the urine, only the unchanged parent was detected. However, as well as the parent, faeces contained T1 and T2 (Donoho, 1988). In Fischer 344 rats given ^{14}C-tilmicosin at 50 mg/kg for five days, faecal analysis showed a sulphate metabolite (T4), as was also found in pig studies (Donoho & Kennington, 1993).

Studies have been conducted in cattle given ^{14}C-tilmicosin (details of dose, route and duration of administration not given) have established a similar profile in the faeces as was observed in the liver of treated rats. Radioactivity in the kidney was mainly in the form of unchanged parent (Donoho, 1988).

Lactating dairy cattle were given tilmicosin at 1200 mg (in 4 ml) administered by intramammary infusion followed, 12 hours later, by a repeat treatment. Tilmicosin was found to be present in milk samples collected 40 days after the last dose and also was detected in milk from untreated mammary gland teats of these cattle for up to 30 – 35 days (Smith *et al.*, 2009).

3 Toxicity profile

3.1 Acute toxicity

Over twelve months, a total of 241 cases of accidental human exposure to tilmicosin were reported to the Rocky Mountain Poison Center, USA. Needle stick / scrapes and accidental injections accounted for 112 and 43 reports respectively. Some cases reported no effects. However, others reported local reactions such as soreness, numbness, stinging, swelling, redness, burning and stiffness. Some injected patients reported anxiety, sweating, headache and light-headedness. Dermal exposures (50 cases) led to redness and tingling of the skin and eye exposures (11 cases) resulted in stinging and swelling. Thirty-nine cases of oral ingestion were reported; effects in some instances included bitter taste, nausea, numbness of lips and tongue, vomiting, thirst and headache (Montanio & Dart, 1993). In one case, accidental injection of approximately 1 ml of tilmicosin in the arm resulted in chest pains, electrocardiographic abnormalities and intraventricular conduction delays. However, ECG changes were not detected in other patients injected with tilmicosin (Crown & Smith, 1999, Von Essen *et al.*, 2003). A death occurred following accidental i.v. injection where the blood level of tilmicosin was 15 µg/ml and gastric content was 3.1 µg/g. Another fatality was noted following self-injection with a blood level of 30 µg/ml. Peak blood levels in cattle after a therapeutic dose of 10 mg/kg are <1 µg/ml, and toxicity in pigs and cows is associated with blood levels of 3 – 7 µg/ml (Mueller & Bottei, 2003).

Oral LD_{50} values in the ICR mouse are 97 mg/kg in males and 109 mg/kg in females. In Sprague-Dawley rats, the oral LD_{50} is 850 and 800 mg/kg in males and females respectively. However in Fischer rats, the oral LD_{50} is >2000 mg/kg and the s.c. LD_{50} 185 and 440 mg/kg

in males and females respectively (Jordan *et al.*, 1986c, Jordan *et al.*, 1986a, Jordan *et al.*, 1986b).

In New Zealand white rabbits topically dosed with 5000 mg/kg tilmicosin on to shaved skin under a non-occlusive dressing for 24 hours, no signs of irritation occurred; the dermal LD_{50} was >5000 mg/kg (Jordan *et al.*, 1987).

A dose of 17 mg of tilmicosin in 0.1 ml (vehicle not stated) instilled in to the eye of New Zealand white rabbits led to conjunctival hyperaemia and chemosis for several days; these signs had reversed by one week after exposure (Jordan *et al.*, 1987).

3.2 Repeat dose toxicity

In a three month study in Sprague-Dawley rats, tilmicosin given by oral gavage at 0, 50, 250 or 1000 mg/kg/day resulted in increased deaths in high dose rats and mid dose females. Signs at the high dose included thinness, ventral soiling, pigmented nasal secretions, bloody secretions from the tear ducts, alopecia and poor grooming. This dose group also showed decreased food consumption in males and reduced body weight gain in males and females (which was also seen in mid dose females). Serum alanine aminotransferase activity in males and blood urea nitrogen level in males and females were increased. Urinary pH was slightly lower in high dose females and there was an increased observation of occult blood in the high dose group. Increased kidney, liver and heart weights (absolute or relative) in high dose females and adrenal weights (absolute and relative) in mid dose females and high dose animals were reported. Enlarged or distended caecums were noted in the mid and high doses and smaller spleens were noted in a few high dose rats. Slight nephrosis was seen in two males at the mid and two males at the high dose. Other findings in the high dose were hypertrophy of the zona fasciculata of the adrenal cortex in most animals and increased myocardial degeneration and lymphoid depletion in the spleen and thymus in some rats. The NOEL was 50 mg/kg/day (Jordan, 1988, FDA, 2009).

Beagle dogs were exposed to tilmicosin in aerosols at 0, 12, 47 or 251 mg/m^3 for four hours per day on 12 days out of a 16 day period (i.e. n=2 /sex/group). One high dose male died on the last day of exposure without showing signs of toxicity. High dose dogs had increased heart rates but food intake, body weight, haematology and clinical chemistry parameters were unaffected. At necropsy, increased lung weight (absolute or relative weight not stated) in high dose females and inflammation of the respiratory tract in mid dose females and high dose animals were observed (Jordan *et al.*, 1991). A NOEL was not stated for this study; however no effects were reported for groups in the low dose group.

In a year study, Beagle dogs (n = 4 /sex/group) were given 0, 4, 12 or 36 mg/kg/day in capsules administered using a twice daily dosing regimen with doses given six hours apart. There were no treatment related deaths. Body weight gain was reduced at the mid and high dose. There were no treatment-related effects on ophthalmoscopy, haematology, blood chemistry or urinalysis parameters. Heart rates were increased in the high dose and a sporadic depression of the ST segment was noted in some dogs with increased heart rate. Heart weight was increased in all four high dose males (absolute and relative) and in one high dose female (relative weight only). Mild chronic dermatitis was noted in the external ears of dogs from all treated groups; effects included minimal thickening of the epidermis, foci of acantholysis (breakdown of epidermal cell layer) and inflammatory cell infiltration in the dermis. These changes were slight and not dose-related. Macroscopic observations of the heart showed slight dilatation in the high dose but it was histologically normal. The NOEL was 4 mg/kg/day (FDA, 2009, Jordan & Bernhard, 1989).

In a sensitisation study, Hartley guinea pigs were given ten intracutaneous doses of tilmicosin (0.05 – 0.1 ml of a 50 mg/ml solution, vehicle and dosing regimen not stated) in an induction

phase. Fourteen days after induction phase, each animal received an intracutaneous injection of 0.05 ml tilmicosin. A skin sensitisation response was not induced (Jordan *et al.*, 1989).

In a *in vivo* microbiological study, germ-free female rats were orally dosed once with 1 ml of 10% (w/v) pooled human faecal suspension. After three weeks, groups of two females and two males were dosed by oral gavage with 0, 0.12 or 0.4 mg/kg/day tilmicosin for five days. Body weight gain was unaffected during the study. Animals received faecal suspensions from different donors which may account for the variation in results. However, the total anaerobes were not reduced by tilmicosin and the total enterobacterial count and proportion of enterobacteria with respect to total anaerobe count was transiently increased at the high dose only. Tilmicosin resistant enterobacteria were not significantly increased at any dose and the NOEL for tilmicosin in this study was 0.4 mg/kg/day (Rumney, 1993).

3.3 Carcinogenicity and mutagenicity

The longest duration study that tilmicosin has been evaluated for is one year and hence the carcinogenic potential has not been fully investigated. However, the chronic repeat dose toxicity studies have not identified any evidence of potential carcinogenicity (IPCS, 1996). In particular, no treatment-related proliferative lesions have been identified and tilmicosin was negative in genotoxicity studies (see below) (FDA, 2009). Tilmicosin is a macrolide antibiotic, a chemical class which has been used for many years in humans with no association to carcinogenicity (IPCS, 1996).

Tilmicosin was negative in a series of *in vitro* and *in vivo* mutagenicity studies, including reverse mutation assays in *S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538) and *E. coli* (WP2uvrA) with and without S9 metabolic activation as well as in forward mutation assays in L5178 mouse lymphoma cells and hgprt locus of CHOcells. It was also negative in an unscheduled-DNA synthesis assay in primary rat hepatocytes. *In vivo*, tilmicosin was negative in a sister chromatid exchange assay in Chinese hamster bone marrow and in a chromosome aberration assay in rat bone marrow (IPCS, 1996)

3.4 Reproductive and developmental toxicity

In a two-generation study, Sprague-Dawley rats were given 0, 10, 45 or 200 mg/kg/day tilmicosin by oral gavage. Males were treated for seventy days prior to mating and females for fourteen days prior to mating and throughout two consecutive litter generations. In F_1 generation adult animals, salivation was noted at the high dose and body weight gain and food consumption were reduced in mid and high dose females in the premating period. There were no treatment-related effects on mating performance, pregnancy rates, duration of gestation, litter size and weight and weight gain of offspring in any generation. The F_1 generation high dose had slightly increased pup mortality up to day 4 post partum, but this was not seen in the F_2 generation. The NOEL was 10 mg/kg/day in adult rats, a fetal NOEL was not stated (IPCS, 1996) although the conclusion was drawn that there was no treatment-related effect on perinatal mortality or the reproductive process in the rat (Jordan *et al.*, 1993) indicating that the NOEL would be 200 mg/kg/day or above.

Pregnant Sprague-Dawley rats were given 0, 10, 70 or 500 mg/kg/day tilmicosin by oral gavage on gestation days 6 – 15 and killed on gestation day 20. There were no intercurrent deaths or abortions. Increased salivation was noted at the mid- and high- doses and alopecia occurred in the high dose only. Body weight was reduced at mid and high doses and food consumption was reduced at the high dose. There were no treatment related effects on number of resorption or live fetuses, fetal weight, sex ratio or incidence of fetal malformations. An increased incidence of total skeletal and visceral anomalies was noted in treated groups but did not show dose dependency and incidences fell within historical control values. The NOEL

for maternal toxicity was 10 mg/kg/day and for teratogenicity was 500 mg/kg/day (Jordan & Higdon, 1988, Jordan *et al.*, 1993).

Oral gavage dosing of tilmicosin at 0, 8, 19 or 48 mg/kg/day to pregnant Japanese White-NIBS rabbits (n = 15 / group) on gestation days 6 – 18 and necropsy examination on gestation day 28 found that one high dose female aborted on gestation day 26 prior to death. Reduced amounts of faeces were noted in the mid and high doses; a transient change was also noted at the low dose. Food consumption was reduced in a dose-dependent manner and body weight decreases were noted at the mid and high dose. Fetal and placental weights were decreased at the mid and high dose but no treatment-related effect on incidence of resorptions, fetal deaths or sex ratio was noted. Eyelids were noted to be open in fetuses from the mid and high doses and some had cleft palate or club foot. The affected fetuses had low body weight and were from dams that had decreased actual body weights during treatment. Skeletal retardations in fetal development were noted in mid and high dose animals. In another study on the effect of diet restriction in rabbits, similar fetal effects were noted suggesting that these effects may be secondary to maternal malnutrition (Noda, 1993). A NOEL for this study was not stated.

4 Guidelines and standards

A microbiological ADI of 4 µg/kg was derived by applying a safety factor of 100 to the NOEL of 0.4 mg/kg/day identified in an *in vivo* microbiological study in the rat (EMEA, 2000).

According to JECFA (2009) and FOI (2009), a toxicologically-based ADI of 40 µg/kg is acceptable (WHO. JECFA, 2009) based on applying a safety factor of 100 to the NOEL of 4 mg/kg/day identified in a 12 month dog study (FDA, 2009).

Adopting a precautionary approach, the lower of the ADI value of 4 µg/kg will be adopted for the current assessment.

The CVMP recommended the inclusion of tilmicosin in Annex I of council regulation (EEC) No 2377/90 in accordance with MRLs as shown in Table 4.1.

Table 4.1 MRLs for tilmicosin

Pharmacologically active substance	Marker residue	Animal species	MRL (µg/kg)	Target tissues	Other comments
Tilmicosin	Tilmicosin	All food producing species except poultry	50	Muscle*	
			50	Fat**	
			1000	Liver	
			1000	Kidney	
			50	Milk	
		Poultry	75	Muscle	Not for use in animals from which eggs are produced for human consumption
			75	Skin + fat	
			1000	Liver	
			250	Kidney	

*For fin fish, this MRL related to "muscle and skin in natural proportions"
** For porcine species, this MRL relates to "skin and fat in natural proportions".
From EMEA, 2002

EMEA estimated that based on these MRLs consumer intake from food sources would not exceed 103% of the ADI (based on EMEA established ADI of 4 µg/kg). It should also be noted that a fraction of the total residue would be microbiologically inactive compounds and/or compounds less microbiologically active than the parent (EMEA, 2002).

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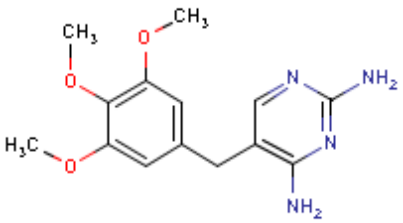
Trimethoprim

1 Introduction

Trimethoprim (2,4-Pyrimidinediamine, 5-((3,4,5-trimethoxyphenyl)methyl)-, molecular formula $C_{14}H_{18}N_4O_3$, CAS No. javascript:;738-70-5; Figure 1.1; Box 1 (ChemID Plus) is a diaminopyrimidine antimicrobial agent, which is active against a wide range of Gram-positive and Gram-negative organisms (EMEA, 1997), including *E. coli* and some *Klebsiella*, *Proteus* and *Staphylococcus* species (EMEA). Trimethoprim is a structural analogue of the pteridine moiety of dihydrofolic acid and is an active competitive inhibitor of dihydrofolate reductase, which allows conversion of folic acid into folinic acid. The ultimate effect of inhibition of the target enzyme is inhibition of DNA synthesis, which results in bacterial cell elongation without division, and eventually cell death. The bacterial/protozoal dihydrofolate reductase is inhibited at concentrations many times lower than that required to inhibit the mammalian enzyme (EMEA).

Figure 1.1 Molecular structure of trimethoprim



From ChemID Plus

Box 1 Synonyms and trade names of trimethoprim

2,4-Diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine; 2,4-Pyrimidinediamine, 5-((3,4,5-trimethoxyphenyl)-methyl)-; 5-((3,4,5-Trimethoxyphenyl)methyl)-2,4-pyrimidinediamine; 5-(3,4,5-Trimethoxybenzyl)-2,4-diaminopyrimidine; AI3-52594; Abaprim; Acuco; Alcorim-F; Anitrim; Antrima; Antrimox; Apo-Sulfatrim; BRN 0625127; BW 56-72; Bacdan; Bacidal; Bacide; Bacin; Bacta; Bacterial; Bacticel; Bactifor; Bactoprim; Bactramin; Bactrim DS; Bencole; Bethaprim; Biosulten; Briscotrim; CCRIS 2410; Centrim; Chemotrin; Cidal; Co-Trimoxizole; Colizole; Colizole DS; Conprim; Cotrimel; DRG-0030; Deprim; Diseptyl; Dosulfin; Duocide; EINECS 212-006-2; Esbesul; Espectrin; Euctrim; Exbesul; Fermagex; Fortrim; Futin; HSDB 6781; Ikaprim; Kombinax; Lagatrim; Lagatrim Forte; Lastrim; Lescot; Metoprim; Monoprim; Monotrim; Monotrimin; NIH 204; NIH 204 (VAN); NSC-106568; Novotrimel; Omstat; Pancidim; Proloprim; Protrin; Purbal; Pyrimidine, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)-; Resprim; Resprim Forte; Roubac; Roubal; Salvatrim; Septrin DS; Septrin Forte; Septrin S; Setprin; Sinotrim; Smz-Tmp; Stopan; Streptoplus; Sugaprim; Sulfamar; Sulfamethoprim; Sulfamethoxazole Trimethoprim; Sulfoxaprim; Sulmeprim; Sulthrim; Sultrex; Syraprim; Tmp Smx; Toprim; Trimanyl; Trimeth/Sulfa; Trimethoprim; Trimexol; Trimez-IFSA; Trimezol; Trimono; Trimopan; Trimpex; Triprim; Trisul; Trisulcom; Trisulfam; Trisural; U-Prin; Uretrim; Uro-D S; Urobactrim; Utetrin; Velaten; Veltrim; WR 5949; Wellcoprim; Wellcoprin; Xeroprim; Zamboprim

From ChemID Plus

The data presented in this assessment are taken from the summary report of the Committee for Veterinary Medicinal Products from the European Agency for the Evaluation of Medicinal Products (EMEA, 1997; EMEA). A literature search of Medline, Toxfile, CancerLit, Embase and Biosis previews revealed a small number of published additional supporting toxicological studies, which have been included in this assessment where appropriate.

In veterinary medicines, trimethoprim is usually used in combination with a sulphonamide (concentration ration of 1:5), and is administered orally as a bolus, paste or in drinking water or feed of calves, pigs, horses, poultry and fish. There are also injectable formulations for pigs, cattle, goats and horses (EMEA, 1997).

2 Toxicokinetics

The toxicokinetics of trimethoprim have been studied in humans and the results reported by EMEA, (1997). It is not clear from the EMEA review, whether the humans were volunteers or patients, or indeed how well the study was conducted, and when. It was reported that trimethoprim (dose not given) was rapidly and almost completely absorbed (>95%) following oral administration. After a single oral dose of 100 mg, the peak plasma concentrations were 1 μg/mℓ at 1–4 hours. The mean plasma half-life was 10 hours. Trimethoprim was widely distributed throughout the body and crossed the placenta. Trimethoprim concentrations measured during pregnancy showed that 75% of the maternal plasma concentration was present in amniotic fluid, 57% in the cord blood and 50% in fetal tissue. Trimethoprim also bound to plasma proteins (42–46%), but this was reduced in the presence of sulphonamides. Around 80% of an administered dose was excreted unchanged, with the remaining 20% metabolised to one of five metabolites — trimethoprim-1-oxide, trimethoprim-3-oxide, 4-hydroxytrimethoprim, 3-hydroxytrimethoprim and α-hydroxy-trimethoprim. The majority of an orally administered dose was excreted in urine, with 70–90% of the dose recovered from urine within 24 hours and 92–102% within 3 days. Only a small amount was excreted into bile (EMEA, 1997).

In rats and dogs, most of a dose of radiolabelled trimethoprim was excreted in urine and the metabolic pattern was similar to that observed in humans. The main difference between humans and rats was that only 21% of the excreted radioactivity in urine was unchanged trimethoprim, and only 6% of the radioactivity in plasma was trimethoprim (EMEA, 1997).

3 Toxicity profile

3.1 Acute toxicity

In humans, trimethoprim has been associated with gastrointestinal disturbances, pruritis, rashes and depression of haematopoiesis (EMEA, not dated).

According to EMEA (1997) trimethoprim has oral LD_{50} values in the range 1500 (rat) –5400 (female mouse) mg/kg bw; therefore it is of low acute toxicity to mammals. EMEA also noted that there is no evidence of a change in the acute toxicity of trimethoprim when administered with a sulphonamide.

There are no reliable data regarding the sensitising potential of trimethoprim.

3.2 Repeat dose toxicity

There are several studies relating to repeat dose toxicity in rats, dogs and primates. However, the EMEA (1997) stated that these were badly designed and poorly reported; this is probably

because they were conducted in the 1960s, and were therefore not conducted to current test guidelines and standards. The main effects observed were on the haematopoietic system.

In a 13-week study, Sprague–Dawley rats were given oral doses of up to 300 mg/kg bw/day trimethoprim. The effects observed included changes in the bone marrow and significant effects on some organ weights (no further details given). The NOEL was 0.3 mg/kg bw/day based on the organ weight changes at higher doses (EMEA, 1997).

Other studies in rats investigated the effect of administering trimethoprim in combination with sulphamethoxazole. Wistar rats were given oral doses of 0, 100 or 300 mg/kg bw/day, 0, 400 or 1200 mg/kg bw/day sulphamethoxazole, or the same doses of each combined. Dosing took place for either one or six months. In the one-month studies, body weight gain and food consumption were reduced in the groups that received the highest sulphamethoxazole and combination doses. From blood samples taken at termination, the combination of sulphamethoxazole and trimethoprim caused dose-related reductions in leucocyte and neutrophil counts. After six months of treatment, reductions in erythrocyte counts were also observed in all treated groups, except the lowest trimethoprim dose group. Bone marrow hyperplasia was reported after one month, and hypoplasia after six months of treatment (dose levels not specified). Fatty changes in the liver were observed, but the treatment period and doses at which this effect occurred were not reported by EMEA. Histopathological changes, which were considered to be typical of those induced by large doses of sulphonamides in rats, were found in the thyroid and pituitary. A NOAEL could not be established from this study (EMEA, 1997).

Oral doses of 300 mg/kg bw/day trimethoprim given to Patas monkeys for 14 days were associated with marked changes in the bone marrow. However, following a four-day period during which treatment stopped (days 15–18), there was significant recovery from the effects on bone marrow in all animals. There were no histopathological changes in the thyroid or pituitary. Leucopenia was observed in only one animal (out of 4) when trimethoprim was given with leucoverin, which indicated that the effects were due to folate metabolic inhibition (EMEA, 1997).

In other studies, monkeys were orally dosed with trimethoprim alone (100 or 300 mg/kg bw/day) or in combination with a sulphonamide (at a ratio of 1:5; trimethoprim dose was 33 or 100 mg/kg bw/day) for between 14 days and six months. The effects on haematology parameters and bone marrow were similar to those observed in rats. Fatty changes in the liver were also reported (EMEA, 1997).

As with the rat and primate studies, Beagle dogs have been administered with oral doses of trimethoprim, or trimethoprim with sulphadiazine for up to 90 days. Following oral gavage doses of 135 mg/kg bw/day of trimethoprim, effects including reduced white blood cell counts, increased serum cholesterol concentrations and changes in thyroid weights were observed. Only minor changes in haematological parameters were observed following doses of 45 mg/kg bw/day for 90 days. The NOEL was 2.5 mg/kg bw/day (EMEA, 1997).

3.3 Carcinogenicity and mutagenicity

EMEA (1997) noted that no carcinogenicity studies have been conducted for trimethoprim. However, the use of trimethoprim in human medicine has not been associated with any carcinogenic effect.

Trimethoprim tested negative for gene mutation activity in *S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538. One positive result was obtained in strains TA98 and TA1538, but the EMEA CVMP (EMEA, 1997) concluded that no reliance could be placed upon this study, for which the test substance purity was not given and the doses tested were

too high. Ono *et al*. (1997) tested *S. typhimurium* TA97, TA98, TA100, TA102 and *E. coli* WP2 with concentrations of trimethoprim up to 5 µg/plate, with and without metabolic activation. Toxicity was observed at concentrations of 5 µg/plate, but there was no evidence of mutagenicity in any of the tester strains, with or without metabolic activation.

Negative results were obtained in *in vitro* mammalian cell gene mutation assays (at the HPRT locus in Chinese hamster ovary cells (CHOs), and the TK locus in mouse lymphoma cells). Trimethoprim was also negative in a study for unscheduled DNA synthesis in cultured A549 cells (EMEA, 1997).

The potential for trimethoprim to cause chromosomal damage has been investigated in numerous studies. The results of these studies were mixed. Three *in vitro* chromosomal aberration studies that used human lymphocytes were negative, although there were limitations as there was no independent repeat of the assay in one study, and the details available for the other two studies were limited. A positive chromosomal aberration study (in CHOs in the presence of S9), and a sister chromatid exchange (SCE) study (in CHOs) had important limitations. The first study was not confirmed when the study was repeated, and the second study had no clear dose–response and the two participating laboratories produced conflicting results (EMEA, 1997). Ono *et al*. (1997) conducted an *in vitro* chromosomal aberration study using Chinese hamster CHL cells, with and without metabolic activation, up to cytotoxic doses of 800 µg/mℓ. Trimethoprim did not exhibit clastogenic activity at any of the doses tested. Abou-Eisha *et al*. (1999) investigated the potential for trimethoprim to induce SCEs and micronuclei in cultured human lymphocytes from two healthy volunteers. In the SCE test, trimethoprim was added to the lymphocyte cultures up to cytotoxic concentrations of 100 µg/mℓ. There was a statistically significant increase in the number of SCEs at concentrations of 25 and 100 µg/mℓ, but this was not the two-fold increase over the controls that is required for the results to be considered a clear indication of genotoxic effects. In the micronuclei test, trimethoprim was added to whole blood up to a cytotoxic concentration of 100 µg/mℓ. There was a statistically significant increase in the number of micronuclei at some doses, but there was no dose relationship and the response was different between the two donors. Therefore a clear association between the compound and genotoxicity was not established.

All *in vivo* studies reported by EMEA (1997) were negative. NMRI mice were given a single oral dose of 2000 mg/kg bw trimethoprim and killed either 12, 24 or 48 hours later. There was no increase in the micronucleus frequency in any of the groups (cell not given). In a second micronucleus study, CD-1 mice were given three daily oral doses of 100, 250, 500, 700 or 1000 mg/kg bw trimethoprim, but there was no increase in the frequency of micronuclei in bone marrow cells. Trimethoprim was also negative in a dominant lethal assay in rats. There are also four published studies in which the effects of trimethoprim therapy, in adults and children, on chromosomal aberrations in peripheral blood lymphocytes was negative (EMEA, 1997).

Overall, trimethoprim was negative for gene mutation and chromosomal aberration. Despite some evidence for positive results for chromosomal aberration, this effect was not evident in any of the *in vivo* studies, and studies that used human cells. EMEA (1997) concluded that trimethoprim is not genotoxic.

3.4 Reproductive and developmental toxicity

In humans, trimethoprim may predispose to folate deficiency through its action as a competitive inhibitor of dihydrofolate reductase, so it is contraindicated in pregnancy and neonates (EMEA, 1997; (Shepard *et al.*, 2002). Shepard *et al*. (2002) reported that there have been associations between maternal treatment with trimethoprim early in pregnancy and various birth defects (neural tube defects, congenital heart defects and cleft palate), but that

these observations have been discounted because the findings were not reproduced in other studies.

Female Sprague–Dawley rats (20/dose) were treated with oral doses of 0, 20, 180 or 420 mg/kg bw/day of a mixture of trimethoprim and sulfamoxole for two weeks prior to mating with untreated male rats. The actual dose of trimethoprim was therefore 0, 3.3, 30 or 70 mg/kg bw/day. Half of the females had a laparoscopic examination on day 13 of gestation. The remaining animals were allowed to litter. In addition, male rats (20/dose) were given the same doses of trimethoprim and sulfamoxole mixture for ten weeks prior to mating with untreated female rats. The uterine contents were examined on gestation day 19. None of the groups showed treatment-related effects on male or female fertility, mating activity, the number of *corpora lutea* or the implantation rate. Pup weights at birth and at four weeks of age were significantly reduced in comparison with other groups. It is not clear which of the two substances caused this effect, but the NOEL for the mixture was 180 mg/kg bw/day, or 30 mg/kg bw/day of trimethoprim EMEA (1997).

In a study conducted by de Pascale *et al*. (1979) Sprague–Dawley rats and New Zealand White rabbits were given trimethoprim-sulfamethoxypyrazine at doses from 2–8 times higher, and 25 times higher than the maximum therapeutic dose, respectively. No reproductive or developmental effects were observed in either species (only the abstract was available in English).

There are a number of developmental toxicity studies, but like in the reproductive study described above, a mixture of trimethoprim and a sulphonamide has been tested in a ratio of 1:5, probably because this is how trimethoprim is usually administered. No further details regarding the methodology or results of the studies, beyond those described below, are available in the EMEA (1997) report.

Groups of Sprague–Dawley rats (20/dose) were orally dosed with 0, 10, 30, 100, 300 or 600 mg/kg bw/day of trimethoprim and sulfamoxole from day 16 of gestation until the end of lactation. At the highest dose the mothers showed signs of toxicity including sedation and reduced body weight gain. At this dose the pup weights at birth, and pup weight gain during lactation were significantly reduced. The NOEL was 300 mg/kg bw/day for the mixture, which is equivalent to 50 mg/kg bw/day of trimethoprim (EMEA, 1997).

On gestation days 8–15, Sprague–Dawley rats (20/dose) were given oral doses of trimethoprim and sulfamoxole at doses of 0, 180, 420 or 600 mg/kg bw/day. The dams were sacrificed on day 19 of gestation and the uterine contents examined. At the two highest doses the dams showed signs of toxicity, including sedation, and reduced body weight gain and food consumption. Malformations in pups, including micrognathia, cleft palate and phocomelia, as well as fetotoxicity in the form of increased post-implantation losses, reduced fetal weight, increased incidence of skeletal variations and retarded ossification, were also observed at these doses. Therefore the NOEL for maternal and developmental toxicity was 180 mg/kg bw/day of the mixture, or 30 mg/kg bw/day of trimethoprim (EMEA, 1997).

In a third study, Sprague–Dawley rats (15–18/dose) were given oral doses of 0, 50, 200 or 350 mg/kg bw/day of trimethoprim and sulfamethoxypyrazine on gestation days 6-15. On day 20 of gestation the animals were sacrificed and the uterine contents examined. There was a low incidence of fetal malformations, including two fetuses with microphthalmia/anophthalmia at the highest dose. In addition, fetal weights were significantly reduced at 200 and 350 mg/kg bw/day. No information on whether the dams showed signs of toxicity was provided. The NOELs for developmental toxicity and fetotoxicity were 200 and 50 mg/kg bw/day of the mixture, respectively. This corresponds to 33.3 and 8.3 mg/kg bw/day of trimethoprim, respectively (EMEA, 1997).

Groups of rabbits (15/dose) were given oral doses of 0, 60, 180 or 600 mg/kg bw/day of trimethoprim and sulfamoxole on gestation days 8–14. On day 16/17 of gestation, five animals per dose were euthanased and their uterine contents examined. The remaining animals were allowed to deliver. The highest dose caused reduced maternal body weight gain and food consumption, and there was an increase in resorptions at this dose level. There were no developmental effects. The NOEL was 180 mg/kg bw/day of the mixture (equivalent to 30 mg/kg bw/day of trimethoprim) for maternal and fetal toxicity (EMEA, 1997).

In another study in rabbits, smaller groups of 5–8 animals were given oral doses of 0, 150, 300 or 600 mg/kg bw/day of a combination of trimethoprim and sulfamethoxypyrazine on days 6–8 of gestation. The number of dead fetuses increased at the top dose, but there were no malformations in any group. Effects on the dams were not discussed by EMEA (1997). The NOEL was 300 mg/kg bw/day, equivalent to 50 mg/kg bw/day of trimethoprim, based on fetotoxicity at the highest dose (EMEA, 1997).

Finally, when oral doses of 0, 10, 16 or 32 mg/kg bw/day of a combination of trimethoprim and sulfamethoxazole were administered to hamsters at various stages of organogenesis, there was no evidence of developmental effects. However, fetal weight and size were significantly reduced in all treated groups in comparison with the controls. A NOEL could not be determined from this study (EMEA, 1997).

4 Guidelines and standards

The EMEA CVMP (EMEA, 1997) derived a toxicological ADI of 12.5 µg/kg bw by applying an uncertainty factor of 200 to the NOEL of 2.5 mg/kg bw/day determined from the 90-day study in Beagle dogs. The uncertainty factor of 200 was chosen due to the poor quality of the 90-day study. The quality and therefore reliability of the repeated dose studies is unknown from the level of reporting by EMEA (1997), and there is one 13-week study in rats that gave a NOEL of 0.3 mg/kg bw/day. It is not clear why this, the lowest NOEL available was not chosen to derive the ADI.

The MRLs for trimethoprim are summarised in Table 4.1. They were based on a microbiological ADI of 4.2 µg/kg bw/day (based on microbiological activity in most sensitive gut bacteria). The desmethyl and α-hydroxy metabolites of trimethoprim retain the pteridine structure and therefore contribute to the microbiological activity. However, the majority of residues are trimethoprim, which is the marker residue (EMEA, 2002). In considering the potential effects of this substance, the microbiological ADI of 4.2 µg/kg bw was used. The intake from food sources as described below is estimated by the EMEA not to exceed 46% of the ADI (EMEA, 2002).

Table 4.1 MRLs for trimethoprim

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Comments
Trimethoprim	Trimethoprim	All food producing species except equidae	50 µg/kg	Muscle (for fin fish this MRL relates to muscle and skin in natural proportions)	Not for use in animals from which eggs are produced for human consumption

		50 µg/kg	Fat (for porcine and poultry species this MRL relates to skin and fat in natural proportions)
		50 µg/kg	Liver
		50 µg/kg	Kidney
		50 µg/kg	Milk
	Equidae	100 µg/kg	Muscle
		100 µg/kg	Fat
		100 µg/kg	Liver
		100 µg/kg	Kidney

From EMEA, 2002

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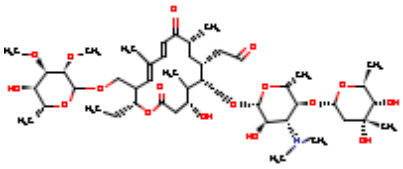
Tylosin

1 Introduction

Tylosin (tylosin, molecular formula $C_{46}H_{77}NO_{17}$, CAS No. 1401-69-0; Figure 1.1, Box 1) is a macrolide antibiotic produced by a strain of *Streptomyces fradiae* and is active against most Gram-positive bacteria, mycoplasma and some Gram-negative bacteria. The commercial product predominantly contains tylosin (factor A), with varying amounts of desmycosin (factor B), macrocin (factor C) and relomycin (factor D). It is not a recognised human medicine but is employed in many countries (including Europe, North America, Australia and New Zealand) as a veterinary medicine including prophylactic applications (IPCS, 1991, EMEA(a), HSDB, 2002, ChemID Plus).

Figure 1.1 Molecular formula of tylosin



From ChemID Plus

Box 1 Synonyms and tradenames for tylosin

Tylan; Tylon; (10E,12E)-(3R,4S,5S,6R,8R,14S,15R)-14-((6-deoxy-2,3-di-O-methyl-beta-D-allopyranosyl)oxymethyl)-5-((3,6-dideoxy-4-O-(2,6-dideoxy-3-C-methyl-alpha-l-ribo-hexopyranosyl)-3-dimethylamino-beta-d-glucopyranosyl)oxy)-6-formylmethyl-3-hydroxy-4,8,12-trimet; AI3-29799; EINECS 215-754-8; Fradizine; HSDB 7022; Tilosina; Tilosina [INN-Spanish]; Tylan; Tylocine; Tylosin; Tylosin A; Tylosine; Tylosine [INN-French]; Tylosinum; Tylosinum [INN-Latin]; UNII-YEF4JXN031; Vubityl 200

From ChemID Plus

Human exposure may occur through inhalation or dermal contact in workplaces where tylosin is produced or used (HSDB, 2002).

In veterinary medicine, tylosin is used in pigs, cattle and poultry for the treatment of infections. In calves, tylosin can be given in the milk replacer at 40 mg/kg and to calves as an i.m. injection at 4 – 10 mg/kg. In pigs, tylosin can be given in the drinking water (25 mg/kg) in the feed (3 – 7 mg/kg) or as an i.m. injection (2 – 10 mg/kg) for the prevention and control of diseases such as swine dysentery and enzootic pneumonia. In poultry, tylosin is given in the drinking water at 75 mg/kg mainly for treating chronic respiratory disease complex in chickens and infectious sinusitis in turkeys. Tylosin has also been used for emergency control of American foulbrood (bacterial disease) in honeybees (WHO. JECFA, 2009).

Previously, tylosin was also used as a feed additive in pigs at 5 – 20 mg/kg feed at up to 6 months of age and 10 – 40 mg/kg feed in animals up to 4 months of age. However, tylosin (along with some other antibiotics) was withdrawn as a growth promoter in the European Union, in 1998 on the basis of the Precautionary Principle. There was concern that bacterial resistance that could occur in animal tissue could be transmitted to humans and be harmful to

human health (Casewell *et al.*, 2003). However the use of tylosin as an anti-biotic for the prevention and treatment of diseases remains (NOAH, 2009).

2 Toxicokinetics

An HPLC method using UV detection, with a limit of detection of 0.02 mg/kg and a limit of quantitation of 0.05 mg/kg, is available for the determination of tylosin in tissue, eggs and milk. A bioassay using *Sarcina lutea* with a detection limit of 0.05 mg/ℓ, is also available to determine residue levels in milk (EMEA (a&b)).

Bioavailability following oral administration is approximately 22.5% in pigs but is lower in rats, dogs and cattle. In most species investigated, peak plasma levels are attained 1–2 hours after administration (Shionogo & Co. Ltd, 1981, EMEA(b)). Tylosin is reported to be extensively metabolised, and no individual metabolite has been shown to occur at concentrations greater than that of the parent. Metabolism appears to be quite similar across a range of species, although quantitative differences in the amounts of individual metabolites have been reported (EMEA(a), EMEA, 1997).

There is little evidence of accumulation, and clearance has been shown to occur relatively rapidly following withdrawal of treatment. For example, after completion of treatment, levels fell to below the limit of detection within 7 hours in rats and 24 hours in rabbits (Anderson *et al.*, 1966).

The principal routes of elimination are believed to be the faeces (involving biliary excretion) and, to a lesser extent, the urine. However, the route of administration is known to influence the rate at which tylosin is cleared from body tissues. If given orally, tylosin is depleted most slowly in the kidney whilst hepatic clearance is slowest if given parenterally (EMEA (a)).

Studies in rats and dogs have shown significant biliary secretion following parenteral administration, while studies in which radiolabelled-tylosin was given after 3 days of oral administration of unlabelled material showed excretion of 99% of the radioactivity in the faeces and 1% in the urine; excretory products were predominantly factor A, factor D and dihydrodesmycosin (EMEA, 1997).

In a radiotracer study in pigs in which tylosin was administered in the food, 12.3% and 7.6% of the total residue at 4 hours after the last dose, were found to be tylosin A in the liver and kidney, respectively. Smaller amounts of tylosin D, dihydrodesmycosin and cysteinyl-tylosin A were also detected (EMEA(a)). In studies in which pigs were given food containing radiolabelled tylosin at 220 mg/kg food/day (equivalent to approx. 3.2 mg/kg bw/day) for 5 days, or drinking water containing 250 mg/ℓ for 10 days, residues were no longer detectable by 3 days after completion of the treatment period; in the study in which pigs were given tylosin in the food, 94% of radioactivity was excreted in the faeces and 6% in the urine. When analysed for 2 of the animals, 43% of this was in the form of tylosin D and 44% as dihydrodesmycosin while, in the third pig, 56% was the seco acid of factor D and 6% tylosin D (EMEA, 1997).

In a study in cattle, the majority of radioactivity was also excreted in the faeces, with approximately 30% as tylosin A (EMEA(b)). When calves were killed 4 hours after the third daily intramuscular injection of 17.6 mg/kg bw of radiolabelled tylosin, residue levels corresponded to 10.5%, 14.5%, 24.6% and 61.8% of the total in the liver kidney, muscle and fat, respectively. Of the residue, tylosin A represented 36.7 %, 31% and 70% (as assessed microbiologically) in the kidney, liver and muscle respectively (EMEA, 1997).

In chickens given tylosin in drinking water at 500 mg/ℓ for 8 days and then killed 6 hours, 1, 5 or 10 days after completion of the treatment phase, residues were found only in occasional livers and kidneys sampled within a few hours of treatment completion. Only one liver sample showed a value above the level of quantitation by the 6 hour sacrifice point. In a similar study on turkeys, tissue levels had also fallen to below the limit of detection by 48 hours after completion of a 7 day exposure period to tylosin (EMEA, 1997).

3 Toxicity profile

In humans, clinically-relevant drug-drug interactions have been reported between some macrolide antibiotics. These involve the formation of stable complexes with enzymes of the CYP3A subfamily, and may result in decreased biotransformation rates of co-administered pharmaceuticals. Similar interactions with CYPP450s, mainly CYP3A, have been noted for tylosin in *in vitro* studies on microsomes and, for related antibiotics, in pigs, rats, goats and cattle (HSDB, 2002).

3.1 Acute toxicity

No information is available on the effects of acute exposure of humans to tylosin. However, accidental exposure to the closest structural analogue, tilmicosin.

Experimentally, tylosin has been shown to have low acute toxicity. In rats, the oral LD_{50} is in excess of 5 g/kg, while those for the i.v., i.p. and s.c. routes are 695 mg/kg, >1 g/kg and >3 g/kg, respectively; an LC_{50} of >1.05 mg/ℓ has also been reported following inhalation exposure. However, in a comparative study in female rats, iv LD_{50} values of 321, 193 and 189 mg/kg were noted for tylosin, desmycosin and macrocin, respectively; effects include tremor, colonic convulsion and limb weakness for all compounds, with poor grooming apparent in those given tylosin and hypoactivity and ataxia in those given desmycosin. In mice, LD_{50} values are >5 g/kg oral, >784 mg/kg s.c., >493 mg/kg i.p., and >386 mg/kg i.v. In rabbit, the dermal LD_{50} is >2 g/kg. The oral LD_{50} value in dogs is in excess of 800 mg/kg; at this dosage, dogs survived but showed salivation, emesis and defaecation (IPCS, 1991; EMEA (a); 1997; HSDB, 2002).

3.2 Repeat dose toxicity

In a human volunteer study of 6 months duration, a dose of 20 mg/day is reported to have had only a marginal effect on a number of resistant streptococci; no other changes were reported. Also, in a study on 2 female volunteers given tylosin orally at 2 or 5 mg/day for 3 months, no effects were noted (EMEA(a); 1997).

In a Swedish study, sensitization to tylosin was reported in 6 out of 9 veterinary surgeons with pre-existing contact dermatitis, and there have been case reports of sensitisation of animal feed workers (EMEA, 1997). A case of occupational asthma and rhinitis has also been reported following repeated exposure to tylosin tartrate dust. The individual had a history of vasomotor rhinitis but not of asthma or eczema (Lee *et al.*, 1989). Experimentally, tylosin has been noted to posses a mild sensitisation potential in a study in which guinea-pigs were exposed at a concentration of 50 mg/mℓ (EMEA, 1997). Further more, quantitative structure–activity relationship (QSAR) modelling using a Bayesian approach to combine findings from a computer automated structural evaluation programme (CASE/Multicase system) suggested that tylosin may have limited respiratory sensitisation potential (Rodford *et al.*, 2003)

With regard to general toxic properties following repeated treatment, no effects were noted following s.c. dosing of female Harlan rats with tylosin at 10–100 mg/kg/day for 1 month. In a further study in which Harlan rats were given tylosin at 100–1000 mg/kg/day s.c. for 1 month, diarrhoea was noted from the first week at dosages of 250 mg/kg/day or above.

Thereafter, the stools of animals given this dosage remained soft, a finding that was also occasionally noted at 100 mg/kg/day. Scarring at the injection sites was also noted on occasion at dosages of 100 mg/kg/day or above. Growth performance, clinical pathology, organ weights and pathology were unaffected in either study (EMEA, 1997, Anderson *et al.*, 1966).

Dietary administration of male Wistar rats at 0.1 or 5 mg/kg/day for 65 days resulted in statistically significant increases in some hormone levels and pituitary weights. However, these changes did not show any temporal or dosage-related pattern; furthermore this study was considered to be methodologically flawed (EMEA, 1997).

In a study on Harlan rats (that had been exposed to tylosin *in utero* during a multigenerational study), tylosin base was fed at 0.1 to 1% tylosin base (equivalent to approx. 50–1000 mg/kg bw/day) for 1 year. Effects were restricted to increased irritability and hyperactivity, and a dosage-related increase in blood lymphocytes and concomitant decrease in blood neutrophil counts, together with minor changes in serum glucose and urinary alkalinity. The NOEL for this study was considered to be 0.1% (50 mg/kg bw/day; (EMEA, 1997, Broddle *et al.*, 1978a). Similar findings were seen in another study in rats where tylosin was given in the feed at doses of up to 5000 mg/kg, and a NOAEL of 1000 mg/kg feed, equivalent to 39 mg/kg bw/day (WHO. JECFA, 2009).

A number of studies have been conducted on dogs. One of 30 dogs given tylosin at 40 mg/kg/day for 3 weeks for superficial pyoderma was noted to develop a transient gastritis that required treatment with a different antibacterial agent (HSDB, 2002). Vomiting, diarrhoea and, in some dogs, nephrosis, pyelonephritis and cystitis were also noted in a 2-year oral study in dogs at dosages of 200 mg/kg/day or above; a NOAEL of 100 mg/kg/day was established (EMEA (a); WHO. JECFA, 2009).

In a study in cats to investigate neurotoxicity, effects were limited to a slight reduction in post-rotatary nystagmus in those given tylosin tartrate at 200 mg/kg/day s.c. for 90 days (Anderson *et al.*, 1966).

In a study in which tylosin was fed to pigs at 30 µg/g (not stated whether per gram of food or body weight, or if feeding restricted) for 20 days, a significant and immediate decrease in the levels of erthryomycin-resistant enterococci and *Staphylococcus hyicus* was noted in the intestinal and dermal bacterial compositions, when compared with those for pigs fed an antibiotic-free diet. However, no information on toxicologically significant end points were reported (HSDB, 2002).

3.3 Carcinogenicity and mutagenicity

A number of studies with tylosin of 17- to 24-months duration, at dietary inclusion levels of up to 20%, have reported no treatment-related effect, but are considered inadequate for the assessment of carcinogenic risk. However, a replicated 2-year feeding study on Wistar rats (whose parents had been exposed to tylosin in the diet prior to mating) using dietary treatment levels of 0.1–1% (approx. 50–1000 mg/kg/day) has also been reported. Treated males showed some increase in food intake, elevated body weight and prolonged survival compared with the controls and, in males given tylosin at 150 or 300 mg/kg/day, an increased incidence of benign pituitary adenomas was observed. However, this latter finding was considered not to represent evidence of carcinogenicity since the incidence of this tumour type in this species is considered to be influenced by body weight, and the incidence in the treated rats was similar to the upper limit of a set of 13 historical control studies (EMEA, 1997, IPCS, 1996). The NOAEL in rats was 5000 mg/kg feed, equivalent to 192 mg/kg bw/day (WHO. JECFA, 2009).

In a mouse L5178Y TH lymphoma assay using levels of 1–100 mg/mℓ, a weak positive response was reported at cytotoxic levels in the absence of metabolic activation. However, tylosin was non-mutagenic in an *in vitro* chromosome aberration assay using CHOat levels of 50–100 mg/mℓ, and in a CHO HGPRT gene mutation assay at 10–150 mg/mℓ. A *in vivo* mouse bone marrow micronucleus test in which ICR mice were dosed twice daily at 1250–50 000 mg base/kg, was also negative. Furthermore, a series of *in vitro* and *in vivo* studies on the closest structural analogue tilmicosin were all negative. Overall, EMEA CVMP has concluded that tylosin is unlikely to present a risk of mutagenicity (IPCS, 1991, EMEA (a&b), EMEA, 1997, IPCS, 1996)

3.4 Reproductive and developmental toxicity

Tylosin has been reported to cause an increase in pituitary luteinising hormone (LH) stores and decreased gonadotrophin releasing hormone (GnRH)-induced LH secretion in rats fed 0.1, but not 5.0, mg/kg feed. No effect on these parameters were, however, noted in rams given tylosin at 10 mg/kg bw i.m. (Yildiz *et al.*, 2004) and, although noted to be poorly reported and subject to a number of methodological limitations, no evidence of any adverse effects on reproduction were noted in a two-generation study in ICR mice (with 2 litters per generation) and a three-generation study in rats in which tylosin was administered via the diet at 0.1 or 1%(EMEA, 1997, Anderson *et al.*, 1966). In a further study, weanling Wistar rats were given tylosin in the diet at 0.1, 0.5 or 1% tylosin base (equivalent to approximately 50, 500 or 1000 mg/kg bw/day) for 10 weeks before mating and for 6 months thereafter; no effects on reproductive performance and offspring development were found (EMEA, 1997, Broddle *et al.*, 1978b).

No treatment-related effects on reproductive or fetal parameters were noted in a study in which mice of the CBA or A/Jax strains were orally dosed at 100, 500 or 1000 mg/kg on days 7–12 of gestation. Similarly, no effects on developmental parameters were noted in a study in which the offspring of female A/Jax and male CBA given tylosin at dosages of up to 1000 mg/kg/day were allowed to develop to weaning (Tsuchikawa & Akabori). A slight reduction in pup weight and a delay in ossification were noted with tylosin at a dietary level of 10% (equivalent to 4800 mg/kg bw) when Wistar rats were fed treated diet on days 1–20 of gestation; a NOAEL of 1.0% in the diet (725 mg/kg/day) was established. In a second study at the same treatment levels in which offspring were followed to weaning, the only effect reported was a slight growth impairment at the high dosage (EMEA, 1997, Terashima).

4 Guidelines and standards

A toxicological ADI of 1 mg/kg was derived by applying a safety factor of 100 to the NOAEL of 100 mg/kg/day identified in a two year study in dogs. However, a lower microbiological ADI of 30 µg/kg based on *in vitro* MIC susceptibility testing and faecal binding data was identified. In addition, JECFA have recommended MRLs, using tylosin A as the marker residue, with levels of 100 µg/kg in liver, kidney, muscle and fat in cattle and pigs; 100 µg/kg in chicken skin and fat; 100 µg/kg for mild and 300 µg/kg for eggs. It was estimated by JECFA that these MRLs would result in the consumer intake from food sources not exceeding 13% of the ADI (WHO. JECFA, 2009).

However, EMEA CVMP has derived a toxicologically-based ADI of 500 µg/kg bw for tylosin. For this, a safety factor of 100 was applied to the NOEL of 50 mg/kg bw/day established in a 1-year dietary study in rats. However, a lower ADI, based on a microbiological end-point in the most sensitive human gut bacteria, of 6.06 µg/kg (i.e. 363.3 µg for a 60 kg person) was also derived by this committee. Based on the lower microbiological ADI, MRLs were also established at 50 µg/kg in milk, 100 µg/kg in the tissues of food-producing species and 200 µg/kg in eggs. It was estimated by the EMEA that

these MRLs would result in the consumer intake from food sources not exceeding 80% of the ADI (EMEA, 1997, EMEA, 2002, EMEA, 2000).

Both JECFA and EMEA derived levels have been discussed here due to the difference in microbiological ADI; 30 µg/kg by JECFA and 6 µg/kg by EMEA. Adopting a precautionary approach, the lower of the ADI values, 6 µg/kg, will be adopted for the current assessment (WHO. JECFA, 2009, EMEA, 2000).

5 References

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