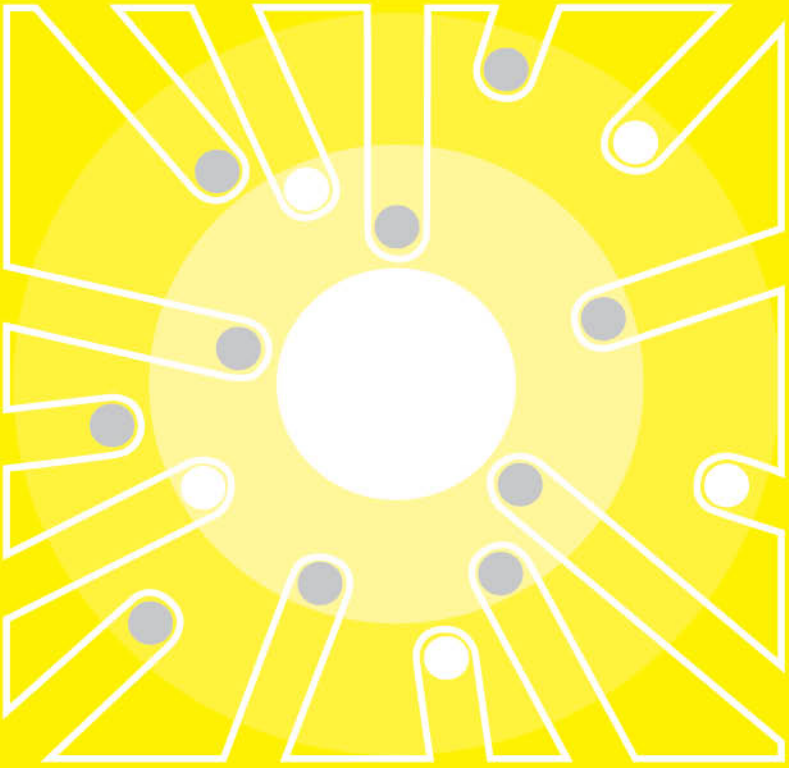
WHO FOOD ADDITIVES SERIES: 66

# Toxicological evaluation of certain veterinary drug residues in food

Prepared by the
Seventy-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Food and Agriculture Organization of the United Nations

World Health Organization



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## CONTENTS

## PREFACE

The monographs contained in this volume were prepared at the seventy-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, on 8–17 November 2011. These monographs summarize the data on the safety of residues in food of selected veterinary drugs reviewed by the Committee.

The seventy-fifth report of JECFA has been published by WHO as WHO Technical Report No. 969. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication; a summary of the conclusions of the Committee is given in Annex 4. Some of the substances listed in Annex 4 were considered at the meeting for residue evaluation only.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The toxicological monographs contained in this volume are based on working papers that were prepared by temporary advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers. The monographs were edited by M. Sheffer, Ottawa, Canada.

Many unpublished proprietary reports are unreferenced. These were voluntarily submitted to the Committee by various producers of the veterinary drugs under review and in many cases represent the only data available on those substances. The temporary advisers based the working papers they wrote on all the data that were submitted, and all these reports were available to the Committee when it made its evaluations.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of the compounds evaluated in this publication should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

# RESIDUES OF VETERINARY DRUGS

# *AMOXICILLIN*

***First draft prepared by***

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## 1. EXPLANATION

Amoxicillin (Chemical Abstracts Service [CAS] No. 26787-78-0) is a moderate-spectrum, semi-synthetic β-lactam antimicrobial agent used for treatment or prevention of bacterial infections in animals and humans. It exerts bactericidal effects by inhibiting the transpeptidase that catalyses the cross-linking of bacterial cell wall peptidoglycan. It is susceptible to degradation by β-lactamase and is often combined with clavulanic acid (a β-lactamase inhibitor) to improve its effectiveness against β-lactamase-producing bacteria.

In animals, amoxicillin is approved for use in cats, dogs, pigs, pre-ruminating calves, including veal calves, cattle, sheep, horses, chickens, ducks, pigeons and turkeys to treat a variety of Gram-positive (e.g. α- and β-haemolytic streptococci, *Streptococcus pneumoniae*, non-penicillinase-producing staphylococci and *Streptococcus faecalis*) and Gram-negative (e.g. many strains of *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Escherichia coli* and *Proteus mirabilis*) bacterial infections (all strains of *Pseudomonas* and most strains of staphylococci, *Klebsiella* and *Enterobacter* are resistant). Not all jurisdictions, however, have approved the drug in all species of animals listed above (USCFR, 2010; APVMA PUBCRIS, 2011; DPD, 2011; PID, 2011). Amoxicillin is stable in the presence of gastric acid and is a drug of choice within the class because of better oral bioavailability.

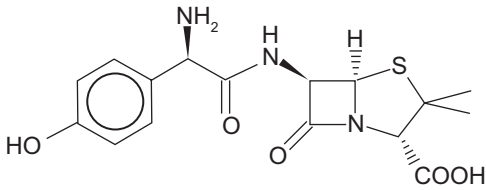
Amoxicillin, either as amoxicillin trihydrate (CAS No. 61336-70-7) or as sodium salt, has been used extensively in humans to treat a variety of infections (e.g. otitis media, pharyngitis and tonsillitis, lower respiratory tract infections, skin and skin structure infections, urinary tract infections, chlamydial infections, *Helicobacter pylori* infections and lyme disease). The World Health Organization (WHO) has categorized it as a critically important antimicrobial agent in human medicine.

In food-producing animals, amoxicillin is approved for use as amoxicillin trihydrate in oral suspensions equivalent to 40 mg of amoxicillin for baby pigs under 4.5 kg; as a soluble powder of amoxicillin trihydrate at 400 mg/45.5 kg body weight (bw) for pre-ruminating calves, including veal calves, administered by drench or by mixing in milk replacer; as amoxicillin trihydrate boluses containing 400 mg of amoxicillin for pre-ruminating calves; as a sterile amoxicillin trihydrate powder for use as a suspension, administered by intramuscular or subcutaneous injection in cattle; as a sterile intramuscular injection suspension containing amoxicillin at 50 mg/ml at a dose rate of 7 mg/kg bw for sheep; as a 150 mg/ml long-acting amoxicillin trihydrate oily intramuscular injection suspension for sheep; and as a 200 mg/ml intramuscular injection of amoxicillin for sheep, cattle and pigs.

Amoxicillin has not previously been reviewed by the Committee. The Committee carried out the present evaluation of amoxicillin to establish an acceptable daily intake (ADI) and to recommend maximum residue limits (MRLs) in cattle, sheep and pig tissues and cattle and sheep milk at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011).

Data supporting the evaluation of amoxicillin were provided by the United States Food and Drug Administration (USFDA) with the permission of the pharmaceutical sponsor. In addition, a literature search was conducted, which

***Figure 1. Structure of amoxicillin***




identified a number of additional pharmacokinetic and epidemiological studies that were used in this evaluation.

Amoxicillin, which has the International Union of Pure and Applied Chemistry name (2*S*,5*R*,6*R*)-6-[[(2*R*)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, has a hydroxyl group in the *para* position of the phenylglycine side-chain of ampicillin (Figure 1).

## *2. BIOLOGICAL DATA*

### *2.1 Biochemical aspects*

Studies conducted by the sponsor were performed prior to the implementation of good laboratory practice (GLP) guidelines, but consistent with the standards existing at the time of the study. Published journal articles reviewed often did not declare the GLP compliance status of their studies.

A few studies conducted by the sponsor on pharmacokinetics or toxicology identified the test article as BRL 2333 (the sponsor's code for amoxicillin) free acid or BRL 2333 sodium, but most identified it only as BRL 2333. The compound referred to as BRL 2333 is likely to be the amoxicillin trihydrate, but this could not be verified. This is, however, unlikely to have a significant impact on the interpretation of the studies, as the mass difference between amoxicillin and amoxicillin trihydrate is only 15%.

#### *2.1.1 Absorption, distribution and excretion*

##### *(a) Laboratory animals*

Amoxicillin administered orally to mice, rats and dogs was quickly absorbed. Peak plasma concentrations ($C_{max}$) above 5 µg/ml were observed within 1–2 hours post-administration (Table 1). Plasma concentrations were not proportional to the dose administered, suggesting a saturable absorption mechanism for amoxicillin. Amoxicillin distributed well into tissues and extravascular fluids. Protein binding in plasma, peripheral lymph and thoracic lymph in dogs was 15%, 9% and 7%, respectively (Acred et al., 1970). The drug was quickly eliminated from plasma, with an elimination half-life of 0.22 hour in mice (Woodnutt & Berry, 1999) and 1.5 hours in dogs (Küng & Wanner, 1994), with no accumulation.

***Table 1. Pharmacokinetics of oral amoxicillin when administered to laboratory animals***

| Formulation | Species (strain, sex) | Dose (mg/kg bw) | $C_{max}$ (μg/ml) | $T_{max}$ | Reference |
|---|---|---|---|---|---|
| Free acid | Mouse (CS1, M) | 50 | 11.2 | 10 min | Acred et al. (1970) |
| Trihydrate | Mouse (CS1/ OLAC, M/F) | 25 | 9 | 10 min | Comber, Osborne & Sutherland (1975) |
| Trihydrate | Mouse (CS1, F?) | 20 | 8.2 | 15 min | Woodnutt & Berry (1999) |
| Free acid | Rat (Sprague-Dawley, M) | 100 | 33 | 30 min | Acred et al. (1970) |
| Free acid | Dog (Beagle, M/F) | 100 | 15.1 | 2 h | Acred et al. (1970) |
| Free acid | Dog (Beagle, M/F) | 100 | 35.9 | 1 h | Beecham Research Laboratories (1971a) |
| | | 1000 | 53.7 | 1 h | |
| | | 2000 | 50.0 | 1 h | |
| Not specified | Dog (Beagle, M/F) | 11 | 5.5 | 1 h | Beecham Research Laboratories (1976) |
| Trihydrate | Dog (Beagle, M/F) | 20 | 18.1–20.7[a] | 1.4–2.0 h[a] | Küng & Wanner (1994) |

$C_{max}$, peak plasma concentration; F, female; M, male; $T_{max}$, time to $C_{max}$
[a] Three different commercial formulations, suspension, tablet and drops, were investigated.

Studies using [$^{14}$C]amoxicillin at 100 mg/kg bw suggested that about 35.5% of the total radioactivity administered orally to rats was excreted in urine (Fairclough, Filer & Smith, 1971; Filer, 1971) and more than half (50.2%) was excreted in faeces (Fairclough, Filer & Smith, 1971). Radiolabelled amoxicillin administered to dogs at an oral dose of 85 mg/kg bw resulted in a higher amount (54%) of total radioactivity in urine compared with rats. Only 40.2% of radioactivity was detected in faeces of dogs by day 7 post-administration (Filer & Smith, 1971). Urinary excretion values for non-radiolabelled drug reported in many studies were lower than those reported in the radiolabelled studies (Acred et al., 1970), most likely because the microbiological assay would not detect metabolites that are microbiologically inactive.

The reported bioavailability of amoxicillin in rats was 44% when administered orally (Filer, 1971) and 51% when administered into the duodenal lumen (Chesa-Jiménez et al., 1994). In dogs, oral bioavailability ranged from 54% (Filer & Smith, 1971) to 77% (Küng & Wanner, 1994). Studies conducted in rats suggested the lack of hepatic first-pass metabolism for amoxicillin and that the low bioavailability of the drug in this species is related to presystemic degradation of amoxicillin in the intestine (Beecham Research Laboratories, 1971b; Chesa-Jiménez et al., 1994).

*(b) Humans*

As in laboratory animals, the pharmacokinetics of amoxicillin after oral administration to humans was characterized by rapid absorption, distribution and

***Table 2. Pharmacokinetics of oral amoxicillin in humans***

| Formulation | Number of subjects | Dose (mg/person) | $C_{max}$ (µg/ml) | $T_{max}$ (h) | Plasma elimination half-life (h) | Reference |
|---|---|---|---|---|---|---|
| Salt not specified | 8 | 500 | 7.6 | 1.5 | 1.0 | Gordon, Regamey & Kirby (1972) |
| Sodium salt | 30 | 250 | 14.8 | 1.1 | NR | Spyker et al. (1977) |
| | | 500 | 11.8 | 1.2 | NR | |
| | | 1000 | 9.5 | 1.6 | NR | |
| Trihydrate | 9 | 500 | 9.5 | 1.5 | 1.0 | Arancibia et al. (1980) |
| Trihydrate | 16 | 500 (fasted) | 8.9 | 1.9 | 8.9 | Eshelman & Spyker (1978) |
| | | 500 (non-fasted) | 8.8 | 2.4 | 8.8 | |
| Trihydrate | 12 | 375 | 8.1 | 1.0 | 1.7 | Sjövall, Alván & Westerlund (1985) |
| | | 750 | 13.0 | 1.5 | 1.5 | |
| | | 1500 | 17.9 | 1.75 | 1.6 | |
| | | 3000 | 28.7 | 2.0 | 1.7 | |
| Amoxicillin trihydrate–potassium clavulanate | 144 | 250/125 | 4.3 | 1.1 | 1.4 | Vree, Dammers & Exler (2003) |
| | | 500/125 tablet | 6.8 | 1.5 | 1.5 | |
| | | 500/125 suspension | 7.6 | 1.1 | 1.5 | |
| | | 875/125 | 9.9 | 2.0 | 1.8 | |

$C_{max}$, peak plasma concentration; NR, not reported; $T_{max}$, time to $C_{max}$

elimination, with no accumulation. The $C_{max}$ for a single 500 mg/person dose of amoxicillin was between 5 and 10 µg/ml, with a time to $C_{max}$ ($T_{max}$) of 1–2 hours (Table 2). The oral bioavailability was more than 80% (Spyker et al., 1977) and was not affected by the presence of food in the stomach (Eshelman & Spyker, 1978). Similar to laboratory animals, amoxicillin absorption in humans has a saturable mechanism, probably by a carrier-mediated transport (Spyker et al., 1977; Sjövall, Alván & Westerlund, 1985). The body clearance ranged from 0.2 l/kg bw per hour (Arancibia et al., 1980) to 0.34 l/kg bw per hour (Spyker et al., 1977). Within the normal dose range, amoxicillin pharmacokinetics was not affected by the presence of clavulanic acid (Vree, Dammers & Exler, 2003).

The urinary recovery of amoxicillin in humans as determined by a microbiological assay ranged from 43.4% to greater than 60% of the total dose (Beecham Research Laboratories, 1969b; Gordon, Regamey & Kirby, 1972; Spyker

***Table 3. Amoxicillin recovery in 0- to 6-hour urine after oral administration to humans, rats and dogs***

| Subject (route) | % of dose recovered as amoxicillin | % of dose recovered as AMA | % of dose recovered as amoxicillin + AMA | % of amoxicilloic acid in total amoxicillin + AMA recovered in urine |
|---|---|---|---|---|
| Human (oral) | 60 | 14 | 74 | 19 |
| Rat (oral) | 8 | 2 | 10 | 20 |
| Rat (intramuscular) | 62 | 10 | 72 | 14 |
| Dog (oral) | 11 | 7 | 18 | 39 |
| Dog (intramuscular) | 61 | 5 | 66 | 8 |

From Beecham Research Laboratories (1969b, 1970b)
AMA, amoxicilloic acid

et al., 1977; Arancibia et al., 1980). It is, however, realized that the microbiological methods would not detect biologically inactive metabolites. Studies conducted by the sponsor have demonstrated that metabolites constitute almost 20% of the total drug excreted in urine of rats, dogs and humans (Table 3). Renal excretion of amoxicillin occurs through both glomerular filtration and active tubular secretion (Sjövall, Westerlund & Alván, 1985).

### *2.1.2 Biotransformation*

Most absorbed amoxicillin is excreted unchanged in the urine (Table 3). An in vitro incubation (with shaking) of amoxicillin at room temperature in hydrochloric acid solution (0.1 mol/l) for 1 hour identified amoxicilloic acid (AMA), amoxilloic acid, 4-hydroxyphenylglycyl amoxicillin and amoxicillin diketopiperazine-2′,5′-dione (DIKETO) as the degradation products (Nägele & Moritz, 2005). However, whether all of these metabolites are formed in vivo is not known. High-performance liquid chromatographic analysis has identified three metabolites: (5*R*,6*R*)-AMA, the (5*S*,6*R*) epimer and amoxicillin-(2*R*)-piperazine-2′,5′-dione in humans and pigs (Haginaka & Wakai, 1987; Reyns et al., 2008).

The pharmacokinetics of the major metabolites of amoxicillin (i.e. AMA and DIKETO) were compared in portal and jugular venous plasma after oral and intravenous administration to pigs (Reyns et al., 2009). Almost immediately after intravenous administration, high AMA and DIKETO concentrations were measured in plasma, whereas following oral dosing, the metabolites appeared in plasma after almost complete absorption of amoxicillin. No significant differences in pharmacokinetic parameters of amoxicillin, AMA and DIKETO, derived from the concentration–time profiles in portal and jugular venous plasma, were identified after intravenous or oral administration ($P > 0.05$). After oral administration, the half-life of elimination was 0.75 hour for amoxicillin and 2.7 hours for AMA, indicating a slower clearance of the metabolite. It is hypothesized that, compared with amoxicillin, which is excreted by both glomerular filtration and active secretion, AMA is eliminated only by glomerular filtration, as its open β-lactam structure might not be recognized by the transport carrier in the proximal tubule of the kidney.

***Table 4. Results of studies of the acute toxicity of amoxicillin***

| Species | Sex | Route | $LD_{50}$ (mg/kg bw) | Reference |
|---|---|---|---|---|
| Mouse | Male | Oral | >5000 | Beecham Research Laboratories (1969a) |
| Rat | Male | Oral | >5000 | Beecham Research Laboratories (1969a) |
| Rat | Male/female | Oral | >5500/8200 | Hardy, Palmer & Cozens (1971) |
| Dog | Female | Oral | >20 000 | Noel et al. (1970b) |

$LD_{50}$, median lethal dose

Various studies suggest that only the parent compound has microbiological activity (Beecham Research Laboratories, 1969b, 1970b). However, both parent and some metabolites (e.g. AMA) could be potential allergens.

## *2.2 Toxicological studies*

### *2.2.1 Acute toxicity*

Results of acute toxicity studies with amoxicillin are summarized in Table 4.

#### *(a) Mice*

Groups of non-fasted male mice (10 per group) of the Ash CS1 strain, weighing 20–25 g, were administered amoxicillin by gavage at doses up to 5000 mg/kg bw. No deaths occurred and no clinical signs of toxicity were observed during the 5-day observation period (Beecham Research Laboratories, 1969a). The acute oral median lethal dose ($LD_{50}$) in mice was greater than 5000 mg/kg bw.

#### *(b) Rats*

Groups of non-fasted male Sprague-Dawley rats (six per group), weighing 140–160 g, were administered amoxicillin by gavage at doses up to 5000 mg/kg bw. No deaths occurred and no clinical signs of toxicity were observed during the 5-day observation period (Beecham Research Laboratories, 1969a). The oral $LD_{50}$ in rats was greater than 5000 mg/kg bw.

Groups of CFY strain rats of both sexes, aged 3, 22 and 36 days, were administered a single dose of amoxicillin by gavage. The 3- and 22-day-old rats were treated at doses up to 5.5 g/kg bw. Among the 3-day-old male rats, five deaths occurred, one in each dose group, which were not treatment related. The 36-day-old male rats were treated at doses up to 5.5 g/kg bw, and females at doses up to 8.2 g/kg bw. No clinical signs of toxicity were observed during the 14-day observation period or at necropsy at the end of the observation period (Hardy, Palmer & Cozens, 1971). The oral $LD_{50}$ was in excess of 5.5 g/kg bw.

#### *(c) Dogs*

Three female Beagle dogs, weighing 8.9–10.3 kg, were administered through a stomach tube single doses of amoxicillin at 10, 15 and 20 g/kg bw, with

***Table 5. Results of short-term studies of the toxicity of amoxicillin***

| Species (sex) | Route | Doses | Treatment | NO(A)EL | Reference |
|---|---|---|---|---|---|
| Rat (M/F) | Oral | 500 mg/kg bw per day | 21 days | 500 mg/kg bw per day (NOAEL) | Wheldon et al. (1968) |
| Rat (M/F) | Oral | 500 mg/kg bw per day | 21 days | 500 mg/kg bw per day (NOAEL) | Beecham Research Laboratories (1970a) |
| Rat (M/F) | Oral | 200, 500, 2000 mg/kg bw per day | 26 weeks | 2000 mg/kg bw per day (NOAEL) | Wheldon et al. (1970) |
| Cat (M/F) | Oral | 100, 300, 500 mg/day | 28 days | 500 mg/day (NOAEL) | Ridglan Farms (1975) |
| Dog (M/F) | Oral | 250 mg/kg bw per day | 14 days | 250 mg/kg bw per day (NOEL) | Noel et al. (1967) |
| Dog (M/F) | Oral | 200, 500, 2000 mg/kg bw per day | 6 months | 2000 mg/kg bw per day (NOAEL) | Noel et al. (1970a) |

F, female; M, male; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level

a 7-day gap between each dose. Some vomiting occurred 1–3 hours after dosing. No other clinical signs or adverse effects were seen during the 7-day observation period or at necropsy at the end of the study (Noel et al., 1970b). The $LD_{50}$ in Beagle dogs was greater than 20 g/kg bw.

### *2.2.2 Short-term studies of toxicity*

Results of short-term studies of the toxicity of amoxicillin are summarized in Table 5.

#### *(a) Rats*

Amoxicillin was administered by gavage to Sprague-Dawley rats (10 of each sex) at a dose of 500 mg/kg bw per day for 21 days and compared with vehicle-only controls. At the end of the study, all rats were necropsied. There were no mortalities and no marked effects on feed consumption, body weight or body weight gain. There were isolated intergroup differences in haematology and urine analysis parameters, which were considered not treatment related. The mean serum calcium level in treated females, but not males, was significantly lower, and albumin concentration was marginally elevated in treated females. The absolute and relative liver weights were lower in treated males ($P < 0.001$) and females ($P < 0.05$). No histological abnormalities were observed. There was no evidence of any changes in morphology that could be related to treatment (Wheldon et al., 1968). The no-observed-adverse-effect level (NOAEL) for this study was 500 mg/kg bw per day, the only dose tested.

In another 21-day study, groups of 10 male (132–139 g) and 10 female (123–126 g) Sprague-Dawley rats were administered amoxicillin in a 1% methylcellulose vehicle by gavage at a dose of 500 mg/kg bw per day. Control groups received the vehicle only. With the exception of three deaths (group not specified) due to errors of dosing (no information available on type of error), all other animals remained in good condition throughout the study. No treatment-related changes were observed in body weight gain, feed intake, haematology, blood chemistry or urine analysis. Histopathology revealed a minimal degree of statistically non-significant fatty change in the liver of treated females (Beecham Research Laboratories, 1970a). The NOAEL for this study was 500 mg/kg bw per day, the only dose tested.

Groups of Sprague-Dawley rats (25 animals of each sex per group) with mean body weights of 136 g (males) and 124 g (females) were administered amoxicillin by gavage at a dose of 0, 200, 500 or 2000 mg/kg bw per day, 6 days/week, for 26 weeks. At 13 weeks, 10 males and 10 females from each group were killed for interim study. On completion of treatment at 26 weeks, all remaining rats were necropsied. Moderate enlargement of caecum in male and female rats in the 2000 mg/kg bw per day group was the only treatment-related change observed at the interim necropsy. Absolute and relative liver weights were significantly higher in treated females of all groups, but relative liver weights in males were decreased in the high- and intermediate-dose groups. The relative spleen weight was depressed in females in the 2000 mg/kg bw per day treatment group. There were no dose-related changes in histology or in glycogen distribution. Therefore, changes in liver and kidney weights were considered not treatment related. At the terminal necropsy, there were no apparent changes in the absolute organ weights. Relative liver weight was marginally elevated in males, but not females, of the high-dose group. No treatment-associated changes were observed in hepatic structure or in glycogen distribution. No treatment-related changes were observed in blood biochemistry, haematology or urine analysis (Wheldon et al., 1970). The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

*(b) Cats*

Groups of cats (breeds not specified, two of each sex per group) were treated with amoxicillin trihydrate film–coated tablets at 0, 100, 300 or 500 mg/day, 5 days/week, for 4 weeks. The only lesion observed in the highest-dose males, but not females, was an increase in the level of renal tubular lipid. In the absence of related lesions in females, this effect was not considered of toxicological significance. No other treatment-related changes were identified (Ridglan Farms, 1975). The NOAEL for this study was the highest dose of 500 mg/animal, equal to 149 mg/kg bw per day, based on the group mean animal body weight of 3.36 kg at the initiation of the study.

In a second study, young cats (six of each sex per group) were treated with amoxicillin trihydrate at 11, 33 or 55 mg/kg bw per day by either the intramuscular or the subcutaneous route for 15 days. There were no effects on clinical signs, survival or blood parameters (Wren, 1977).

*(c) Dogs*

One male and one female Beagle dog received amoxicillin orally in gelatine capsules at a dose of 250 mg/kg bw per day for 14 days. Gross pathological and histopathological examination of major organs at the end of the study did not reveal any significant changes. There were no abnormalities that could be considered related to drug treatment (Noel et al., 1967). The no-observed-effect level (NOEL) for this study was 250 mg/kg bw per day, the only dose tested.

Amoxicillin was administered orally to groups of Beagle dogs (six dogs of each sex per group) 7 days/week for 6 months. Animals were dosed at 200, 500 or 2000 mg/kg bw per day in gelatine capsules. A control group was left untreated (no placebo administration). At the end of 3 months of dosing, three males and three females from each group were killed for interim study. On completion of 6 months of dosing, the remaining three females and three males from each group were necropsied. There were no deaths during the dosing period. Some vomiting did occur either immediately after dosing or 1–4 hours after dosing in the highest-dose group. Grey-coloured faeces were observed mainly in the first 3 weeks in a dose-related manner in dogs receiving 500 or 2000 mg/kg bw per day. Weight gain in the highest-dose group was decreased compared with control dogs ($P < 0.01$ between 0 and 13 weeks and $P < 0.05$ between 13 and 26 weeks). At the interim necropsy, relative kidney weights were increased in the high- and intermediate-dose groups, but no treatment-related microscopic abnormalities were found. At the completion of the study after 6 months, relative liver weights were increased in the highest-dose group. Microscopic examination revealed no changes in morphology associated with drug administration (Noel et al., 1970a). The decreased weight gain observed in the highest-dose group was likely associated with the disturbance in the gastrointestinal flora from the microbiological effect. The microbiological effect of amoxicillin is evaluated separately in section 2.2.6. The NOAEL for this study was the highest treatment level of 2000 mg/kg bw per day.

### *2.2.3 Long-term studies of toxicity and carcinogenicity*

Long-term animal studies to evaluate the carcinogenic potential of amoxicillin were not performed. Multiple searches of the published literature and the web sites of public and environmental health organizations (e.g. International Agency for Research on Cancer, United States National Institute of Environmental Health Sciences) did not indicate any studies or reports on the carcinogenic effects of amoxicillin.

### *2.2.4 Genotoxicity*

In an in vitro comet assay, amoxicillin induced deoxyribonucleic acid (DNA) damage at a 5 mmol/l concentration in human peripheral blood lymphocytes as well as in *Helicobacter pylori*–infected and non-infected human gastric mucosal cells. The damage was more pronounced in *H. pylori* non-infected gastric mucosal cells than in the infected gastric mucosal cells or peripheral lymphocytes. Amoxicillin-induced DNA damage was repaired within 60 minutes. The drug did not induce DNA strand breaks in isolated pUC19 plasmid DNA. There is some indication

that amoxicillin-induced DNA damage is an indirect effect due to the formation of oxidative free radicals (Arabski et al., 2005).

In a modified comet assay, amoxicillin at 5 mmol/l induced DNA lesions in vitro in human AGS cells. Some DNA damage was also noted at doses as low as 1 mmol/l. Repair of the amoxicillin-induced DNA lesions was essentially completed within 4 hours. Amoxicillin induced intracellular reactive oxygen species at a pace similar to that of induction of DNA lesions, suggesting that DNA damage caused by amoxicillin is likely an indirect effect due to the formation of reactive oxygen species (Li et al., 2007). Earlier studies have indicated that other β-lactams can also cause DNA damage in vitro at high concentrations, again possibly through induction of reactive oxygen species (Quinlan & Gutteridge, 1988).

Another in vitro study investigated the potential genotoxic effects of amoxicillin at concentrations of 400, 600, 800 and 1000 µg/ml, using sister chromatid exchange, chromosomal aberration and micronucleus tests in human peripheral blood lymphocytes. Amoxicillin did not induce sister chromatid exchanges or chromosomal aberrations in human peripheral blood lymphocytes in both the presence and the absence of a metabolic activator. Amoxicillin did not induce the formation of micronuclei or decrease the nuclear division index in human peripheral blood lymphocytes in both the presence and the absence of a metabolic activator (Istifli & Topaktas, 2010).

The Center for Drug Evaluation and Research of the USFDA reviewed additional genotoxicity data while assessing the safety of a human drug consisting of a combination of amoxicillin and potassium clavulanate. In the studies reviewed by the USFDA, but not available to the Committee for review, the combination of amoxicillin–clavulanic acid gave negative results for genotoxicity in vitro in an Ames test, a human lymphocyte cytogenicity assay and a yeast recombination assay. Amoxicillin–clavulanic acid was weakly positive in a mouse lymphoma assay only at cytotoxic concentrations. It was negative in vivo in a mouse micronucleus test and a dominant lethal test (USFDA, 2006).

Results of genotoxicity tests with amoxicillin are summarized in Table 6.

### *2.2.5 Reproductive and developmental toxicity*

Results of reproductive and developmental toxicity studies are summarized in Table 7.

#### *(a) Reproductive toxicity*

##### *(i) Rats*

Groups of Specific Pathogen Free rats of the CD strain (11 males and 22 females per group) were treated with amoxicillin at a dose of 0, 200 or 500 mg/kg bw per day by gavage. Drug administration commenced when the males were 42 days of age and the females were 91 days of age and continued throughout the remainder of the investigation (the length of the treatment period was not specified). The animals were 105 days of age at cohabitation. On day 13 of gestation,

***Table 6. Results of genotoxicity tests with amoxicillin***

| Test system/end-point | Test object | Concentration/dose | Result | Reference |
|---|---|---|---|---|
| Comet nuclear extraction assay for DNA strand breaks | Human AGS cell line | 1–10 mmol/l | Positive (maximum effect observed at 5 mmol/l) | Li et al. (2007) |
| | Human NB4 cell lines | 5 mmol/l | | |
| | Chinese hamster CHP-K1 and UV24 cell lines | 5 mmol/l | | |
| Comet nuclear extraction assay for DNA strand breaks | Human peripheral lymphocytes | 1–5 mmol/l | Positive (at 5 mmol/l) | Arabski et al. (2005) |
| | *H. pylori*–infected human gastric mucosa | 1–5 mmol/l | Positive (at 5 mmol/l) | |
| | *H. pylori* non-infected human gastric mucosa | 1–5 mmol/l | Positive (at 5 mmol/l) | |
| | Isolated pUC19 plasmid DNA | 1–5 mmol/l | Negative | |
| Sister chromatid exchange | Human peripheral blood lymphocytes with/without S9 mix | 400–1000 µg/ml | Negative | Istifli & Topaktas (2010) |
| Chromosomal aberration | | | Negative | |
| Micronucleus test | | | Negative | |

DNA, deoxyribonucleic acid; S9, 9000 × *g* rat liver supernatant

***Table 7. Results of reproductive and developmental toxicity studies of amoxicillin in laboratory animals***

| Species (sex) | Dose (mg/kg bw per day) | Treatment schedule and study description | Outcome | Reference |
|---|---|---|---|---|
| Mouse (F) | 0, 200, 500, 2000 | Treated on days 6–15 and killed on day 17 of gestation | Equivocal results on fetal loss, NOAEL could not be identified | Palmer & Cozens (1969b) |
| Rat (F) | 0, 200, 500, 2000 | Treated on days 6–15 and killed on day 20 of gestation | NOEL for developmental toxicity 2000 mg/kg bw per day | Palmer & Cozens (1969a) |
| Rat (M/F) | 0, 200, 500 | Males treated from 42 days of age, females from 91 days of age, cohabited on day 105 and killed on day 13 of gestation (first mating) or allowed to rear pups, remated and killed on day 20 of gestation (second mating) | NOAEL for reproductive toxicity 500 mg/kg bw per day | Palmer & Cozens (1970) |
| Rat (F) | 0, 200, 500 | Treated from day 15 of gestation through lactation day 21 | NOAEL for perinatal and postnatal developmental toxicity 500 mg/kg bw per day | Palmer & Cozens (1969c) |

F, female; M, male; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level

10 females from each group were necropsied for examination of the reproductive tract and the fetuses. The remaining rats were allowed to rear their pups. They were mated again for a second time and killed on day 20 of gestation.

Parent males showed a treatment-related retardation of body weight gain. A similar trend was not apparent among females. Among rats necropsied at the first gestation, there was a reduction in the corpora lutea count in the 500 mg/kg bw per day group. This was not considered to be of toxicological significance, as a similar effect was not observed in the second mating, and the implantation rate and the number of viable pups in treated animals were not statistically different from those of controls. No abnormal pups were observed and no other differences were observed between the treated and control animals or their pups after both the first and second matings (Palmer & Cozens, 1970).

The NOAEL for reproductive toxicity was 500 mg/kg bw per day, the highest dose tested.

*(b) Developmental toxicity*

*(i) Mice*

Amoxicillin was administered to groups (20 per group) of Specific Pathogen Free CD-1 mice. Animals were dosed at 0, 200, 500 or 2000 mg/kg bw daily by gavage on days 6 through 15 of pregnancy. On day 17, animals were killed and the ovaries and uterine contents were examined for the number of resorption sites, number of viable pups and developmental abnormalities in pups.

Among the parent animals, body weight change and pregnancy rate were unaffected by treatment, and no deaths or other abnormalities were identified. Embryonic and fetal development as assessed by the incidence of major malformations, minor visceral anomalies and the distribution of skeletal variants was unaffected by treatment. Fetal losses of 4.8%, 13.9%, 15.2% and 13.0% were observed in the 0, 200, 500 and 2000 mg/kg bw per day dose groups, respectively. Although the difference in fetal loss between the control and treated groups was significant, these values were within the historical control range of 3.8–14.3% (mean 7.9%) for the laboratory. Additionally, there was no dose–response effect on fetal loss (Palmer & Cozens, 1969b).

The results of this study were considered equivocal, and a NOAEL for developmental toxicity in mice could not be identified.

*(ii) Rats*

Amoxicillin was administered to groups of Specific Pathogen Free rats (20 per group) of the CD strain at a dose of 0, 200, 500 or 2000 mg/kg bw daily by gavage on days 6 through 15 of pregnancy. On day 20, the animals were killed and the ovaries and uterine contents were examined for the numbers of corpora lutea, resorption sites, viable pups and abnormalities. There were no alterations in clinical signs, survival, pregnancy rate, preimplantation loss, litter size, litter weight, major malformations, minor visceral anomalies or the distribution of skeletal variants

in any treatment group. In the medium- and high-dose groups, there was a dose-related increase in mean pup weight (Palmer & Cozens, 1969a). The NOEL for developmental toxicity was the highest tested dose of 2000 mg/kg bw dose per day.

Amoxicillin was administered to groups of Specific Pathogen Free CD strain rats (20 per group) at a dose of 0, 200 or 500 mg/kg bw per day by gavage from day 15 of gestation through to day 21 of lactation. After parturition, all pups were counted, weighed and examined. There were no effects on clinical signs, body weight gain, pregnancy rate, duration of gestation, and pup weights or abnormalities at birth. Pups from treated groups had a better weight gain postpartum. The total litter size at birth was 11.5, 10.3 and 9.6 for the 0, 200 and 500 mg/kg bw treatment groups, respectively, which was dose dependent. However, there was no difference in the number of dead pups at birth (Palmer & Cozens, 1969c). The difference in the litter size was likely due to differences in the implantation rate and was not considered treatment related, as drug administration started post-implantation (i.e. day 15 of gestation). The NOAEL for perinatal and postnatal toxicity was 500 mg/kg bw per day, the highest dose tested.

The effects of amoxicillin on Sprague-Dawley rat kidney development were studied by in vitro metanephros organ cultures and in vivo maternal treatment. For the in vitro experiments, metanephroi were removed from 14-day-old fetuses and grown in cultures containing amoxicillin at a concentration of 0, 10, 100 or 1000 µg/ml for 2 days. For the in vivo experiments, pregnant rats were treated with amoxicillin from day 11 to day 15 of gestation at 100 mg/kg bw per day through a subcutaneous implant. Amoxicillin altered renal development in vitro in a dose-dependent manner at concentrations at or above 100 µg/ml. In pups exposed to amoxicillin in utero, no reduction in the number of nephrons per gram of body weight was detected, but kidney histology showed mild cystic tubule dilatation (Nathanson et al., 2000). The toxicological relevance of this study is not clear.

### *2.2.6 Special studies on microbiological effects*

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for amoxicillin (VICH, 2004). The decision-tree approach initially seeks to determine if there may be microbiologically active amoxicillin residues entering the human colon. If the answer is “no” to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria. At Step 4 of the decision-tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both of these end-points. At Step 5, a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used.

The Committee evaluated in vitro minimum inhibitory concentration (MIC) susceptibility testing data and in vivo human volunteer, biological activity of

***Table 8. MIC of amoxicillin against* Lactobacillus *and* Bifidobacterium *species***

| Strains | Total no. of strains | No. of strains with indicated MIC values | | | |
|---|---|---|---|---|---|
| | | ≤0.25 µg/ml | 0.5 µg/ml | 1 µg/ml | 2 µg/ml |
| *Bifidobacterium longum* | 47 | 35 | 10 | 1 | 1 |
| *B. bifidum* | 16 | 16 | — | — | — |
| *B. pseudocatenulatum* | 11 | 7 | 4 | — | — |
| *B. catenulatum* | 2 | 2 | — | — | — |
| *Lactobacillus gasseri* | 20 | 9 | 11 | — | — |
| *L. delbrueckii* | 8 | 8 | — | — | — |
| *L. casei/L. paracasei* | 7 | — | 5 | 2 | — |
| *L. rhamnosus* | 5 | — | 3 | 2 | — |
| *L. acidophilus* | 2 | — | 2 | — | — |
| *L. plantarum* | 1 | — | 1 | — | — |
| *L. parabuchneri* | 1 | — | 1 | — | — |
| *L. brevis* | 1 | 1 | — | — | — |
| *L. vaginalis* | 1 | — | — | 1 | — |

From Delgado, Flórez & Mayo (2005)

amoxicillin residue and antimicrobial resistance studies for use in the decision-tree to answer the following questions in the assessment of amoxicillin.

*Step 1: Are residues of the drug, and/or its metabolites, microbiologically active against representatives of the human intestinal microflora?*

Yes. Amoxicillin is microbiologically active against some bacterial genera and species representative of the human intestinal microflora. The MIC of amoxicillin was determined for 122 strains of *Bifidobacterium* and *Lactobacillus* species isolated from the faeces of eight healthy adult volunteers. Most of the bifidobacteria and lactobacilli strains assayed were susceptible to amoxicillin, with MIC values ranging from ≤0.25 to 0.5 µg/ml. Results are tabulated in Table 8 (Delgado, Flórez & Mayo, 2005). In an investigation of the antimicrobial susceptibility of 50 strains of bifidobacteria belonging to eight species, isolated from humans, animals or probiotic products, all 50 strains were sensitive to amoxicillin, with a minimum inhibitory concentration required to inhibit the growth of 50% of organisms ($MIC_{50}$) range of ≤0.06–0.25 µg/ml (Moubareck et al., 2005). In another study, the susceptibility of 492 anaerobic bacteria to 23 antimicrobial agents, including amoxicillin, was presented as per cent susceptible to indicate an amoxicillin concentration for each species. Amoxicillin was active against many of the strains tested at 8 µg/ml or less. The antibacterial spectrum is shown in Table 9 (Sutter & Finegold, 1976). The $MIC_{50}$ values for the predominant and relevant intestinal microbiota were determined from

***Table 9. Activity of amoxicillin against anaerobic bacteria***

| Bacteria | No. of strains | Cumulative % susceptible to indicated concentration (μg/ml) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ≤0.1 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | ≥256 |
| *Bacteroides fragilis*[a] | 42 | 2 | 5 | 10 | 17 | 26 | 31 | 52 | 83 | 91 | 93 | 100 |
| *Bacteroides melaninogenicus* | 59 | 71 | 83 | 86 | 90 | 95 | 100 | — | — | — | — | — |
| Other *Bacteroides* and *Selenomonas* species | 21 | 43 | 57 | 62 | 67 | 76 | — | 86 | — | 95 | 100 | — |
| *Fusobacterium nucleatum* | 8 | 63 | 88 | — | 100 | — | — | — | — | — | — | — |
| Other *Fusobacterium* species | 12 | 42 | 67 | — | — | — | — | 75 | | 83 | 92 | 100 |
| *Peptococcus* and *Gaffkya* species | 19 | 42 | 90 | 95 | 100 | — | — | — | — | — | — | — |
| *Peptostreptococcus* species | 15 | 53 | 87 | 93 | — | — | — | 100 | — | — | — | — |
| Anaerobic and microaerophilic streptococci | 6 | 67 | 83 | — | — | — | — | 100 | — | — | — | — |
| Gram-negative cocci | 7 | 57 | 86 | — | — | — | 100 | — | — | — | — | — |
| *Eubacterium* species | 7 | 57 | 100 | — | — | — | — | — | — | — | — | — |
| *Arachnia propionica* | 2 | — | 100 | — | — | — | — | — | — | — | — | — |
| *Propionibacterium* species | 4 | 25 | 75 | 100 | — | — | — | — | — | — | — | — |
| *Actinomyces* species | 16 | 63 | 100 | — | — | — | — | — | — | — | — | — |
| *Lactobacillus* species | 10 | 60 | 90 | — | 100 | — | — | — | — | — | — | — |
| *Clostridium perfringens* | 8 | 75 | 88 | — | — | — | — | — | 100 | — | — | — |
| Other *Clostridium* species | 26 | 8 | 77 | 92 | 96 | — | 100 | — | — | — | — | — |

From Sutter & Finegold (1976)

[a] Includes all strains identified as subspecies of *B. fragilis.*

the antimicrobial susceptibility studies described for amoxicillin—i.e. *Escherichia coli* ($MIC_{50}$ = 5 µg/ml), *Bifidobacterium* ($MIC_{50}$ = 0.06 µg/ml), *Clostridium* ($MIC_{50}$ = 0.1 µg/ml), *Bacteroides* ($MIC_{50}$ = 0.5 µg/ml), *Lactobacillus* ($MIC_{50}$ = 0.25 µg/ml), *Fusobacterium* ($MIC_{50}$ = 0.1 µg/ml), *Eubacterium* ($MIC_{50}$ = 0.1 µg/ml) and *Peptostreptococcus* ($MIC_{50}$ = 0.1 µg/ml).

Several studies were available on the effects of orally administered amoxicillin (with and without clavulanic acid) on the intestinal microflora of humans. Ten healthy volunteers received 500 mg amoxicillin tablets every 8 hours for 7 days, and the impact on the oral and intestinal microflora was studied. Amoxicillin administration induced small alterations in the faecal microflora, although all subjects had overgrowth of new colonizing amoxicillin-resistant microorganisms, mainly *Escherichia coli*, *Klebsiella* and *Enterobacter*. β-Lactamase activity was detected in the flora of six volunteers (Brismar, Edlund & Nord, 1993). In another study, 12 healthy subjects (6 females, 6 males; ages 18–40 years) were given amoxicillin–clavulanic acid as 1000 mg tablets once daily for 7 days. Faecal samples were collected prior to, during and after administration (days –2 to 35) for microbiological analyses. The samples were diluted in pre-reduced media and inoculated aerobically and anaerobically on non-selective and selective media. Different colony types were identified to genus level by morphological, biochemical and molecular analyses. Amoxicillin–clavulanic acid administration caused increased numbers of enterococci and *Escherichia coli* in the aerobic intestinal microflora, whereas numbers of bifidobacteria, lactobacilli and clostridia decreased significantly. *Clostridium difficile* strains were recovered from three of the volunteers. The intestinal microflora of the volunteers returned to normal levels 35 days after the cessation of treatment (Lode et al., 2001).

*Step 2: Do residues enter the human colon?*

Yes. A number of residue studies using [$^{14}$C]amoxicillin were conducted to detect amoxicillin in food animal species (pigs, cattle, poultry, goats and sheep) and laboratory animals (rats, mice, monkeys, pigs, dogs and rabbits) by analytical chemistry (high-performance liquid chromatography–mass spectrometry) and microbiological assay methods. Muscle, liver, kidney, fat and skin from cattle and pig tissue and milk contain relatively low or no amoxicillin-derived residues, regardless of the period between withdrawal of medication and slaughter. Under the most conservative assumptions, amoxicillin residues may be present at low levels in meat products consumed by humans. Therefore, amoxicillin-related residues could enter the colon of a person ingesting edible tissues from treated animals.

*Step 3: Do the residues entering the human colon remain microbiologically active?*

Yes. As the submitted evaluation did not contain measurements of the amount of drug residue in the intestinal tract, the Committee used the pharmacokinetic and bioavailability studies to determine the fraction of oral dose available to the human intestinal microbiota. Pharmacokinetic and bioavailability studies indicated that amoxicillin is rapidly absorbed after oral administration. Human studies in healthy volunteers and patients gave a range of bioavailability figures for recovery of amoxicillin in urine. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. A considerable amount of the administered amoxicillin was

detected in faecal samples. Mastrandrea et al. (1984) reported that 50–60% of intact amoxicillin was detected in the urine 24 hours after dosing. Arancibia et al. (1980) showed that 43.4% and 57.4% of amoxicillin were recovered after oral and intravenous doses, respectively. Eshelman & Spyker (1978) showed 47% and 44% amoxicillin recovery in the urine of fasting and non-fasting children, respectively, after oral dosage. Beecham Research Laboratories (1969b) recovered 60% amoxicillin in the urine after an oral dose. Amoxicillin is metabolized to two major metabolites, AMA and DIKETO. Numerous studies indicate that only the parent drug amoxicillin has biological activity. However, no studies were available to evaluate whether metabolites detected in human urine are biologically active against bacteria. Therefore, amoxicillin residues entering the human colon will remain microbiologically active.

The lowest amoxicillin urinary recovery value from the human studies was 43.4%. Therefore, the fraction of oral dose available would be conservatively estimated as 56.6%.

*Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern, i.e. disruption of the colonization barrier or resistance development?*

Yes. The Committee noted that there is scientific justification to eliminate resistance development as an end-point of concern. However, there is the potential for an adverse effect on the disruption of the colonization barrier in the human intestinal microbiota, as amoxicillin residues do occur in food animals in small amounts. Therefore, if edible tissues are ingested, they could have an impact on the intestinal microbiota. Furthermore, the susceptibility of bifidobacteria, a predominant microorganism in the gastrointestinal tract, to amoxicillin may affect the barrier effect, which prevents colonization of pathogenic bacteria in the gut (Moubareck et al., 2005). In terms of antimicrobial resistance, amoxicillin is a broad-spectrum β-lactam antibiotic used in both human and veterinary medicine. The widespread use of this antibiotic may give rise to resistant bacterial strains and reduce its efficacy in the treatment of disease. In a national and university hospital survey, high use of amoxicillin–clavulanic acid contributed to high rates of resistance in Enterobacteriaceae, including *E. coli*, up to 50%. Nationally, 8% of *E. coli* strains were resistant or intermediately susceptible to amoxicillin–clavulanic acid. Results of the influence of restriction on resistance from those antibiotics accounting for 90% of the university hospital antibiotic use were analysed for microbiological susceptibility of *E. coli* during two 9-month periods, before and after restricting the use of amoxicillin–clavulanic acid as well as other antibiotics. Using defined daily doses per 100 bed-days as the hospital measurement base, use of antibiotics including amoxicillin–clavulanic acid was 30% before intervention and declined to 4% after the intervention, and resistance of *E. coli* declined significantly from 37% to 11% (Matanovic et al., 2010). Although increased prevalence of resistance may be linked to amoxicillin used after therapeutic administration, amoxicillin residue levels in food-producing animals were either not detected or present at such low levels (below the $MIC_{50}$ values of the representative human intestinal microbiota evaluated in this report) that it is unlikely that the development of resistance to amoxicillin would occur.

*Step 5: Derivation of a microbiological ADI using the VICH GL36 approach*

The formula for calculating the microbiological ADI from in vitro (MIC) data is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

*$MIC_{calc}$*: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC) ($MIC_{calc}$) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean $MIC_{50}$ for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the $MIC_{calc}$ were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the $MIC_{50}$ values for *Escherichia coli* ($MIC_{50}$ = 5 µg/ml), *Bifidobacterium* ($MIC_{50}$ = 0.06 µg/ml), *Clostridium* ($MIC_{50}$ = 0.1 µg/ml), *Bacteroides* ($MIC_{50}$ = 0.5 µg/ml), *Lactobacillus* ($MIC_{50}$ = 0.25 µg/ml), *Fusobacterium* ($MIC_{50}$ = 0.1 µg/ml), *Eubacterium* ($MIC_{50}$ = 0.1 µg/ml) and *Peptostreptococcus* ($MIC_{50}$ = 0.1 µg/ml), the $MIC_{calc}$ is 0.10 µg/ml.

*Mass of colon content*: A value of 220 g is based on the colon content measured from humans.

*Fraction of oral dose available to microorganisms*: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. The lowest amoxicillin urinary recovery data from the human studies was 43.4%. Therefore, the fraction of oral dose available would be 1 – 0.434 = 0.566.

*Body weight*: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for amoxicillin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{0.10 \text{ µg/ml} \times 220 \text{ g}}{0.566 \times 60 \text{ kg bw}}$$

$$= 0.65 \text{ µg/kg bw}$$

Therefore, a microbiological ADI of 0–0.7 µg/kg bw (rounded to one significant figure), or 42 µg for a 60 kg adult, was derived from in vitro MIC susceptibility testing.

## 2.3 Observations in humans

### 2.3.1 Gastrointestinal effects

Diarrhoea and vomiting are the most common adverse gastrointestinal effects reported in people treated orally with amoxicillin either alone or in combination with clavulanic acid. In a randomized trial in 354 children for treatment of otitis media, 164 children completed the study in the amoxicillin–clavulanic acid (90/6.4 mg/kg bw) treatment group. Among them, 17.9% had diarrhoea, 5.8% had vomiting, 6.4% had diaper rash and 1% had severe abdominal pain (Sher et al., 2005). Two systematic reviews of treatment of acute otitis media in children with amoxicillin and other antimicrobials (Takata et al., 2001; Coker et al., 2010) identified diarrhoea, rash and vomiting as reportable adverse effects related to treatment. Children receiving amoxicillin or ampicillin had a pooled diarrhoeal rate difference of 5% more than those receiving placebo, with rates as high as 14% in some studies (Coker et al., 2010). In another study, patients treated with amoxicillin–clavulanic acid had 18% more diarrhoea than did those treated with azithromycin (Takata et al., 2001). Other reviews of randomized clinical trials involving children (Marmor & Newman, 2011) reported an incidence of 20–33% diarrhoea in patients treated with amoxicillin–clavulanic acid. Diarrhoea occurred in 6% and 5% of breastfeeding infants when their mothers were treated with amoxicillin–clavulanic acid or amoxicillin, respectively (Benyamini et al., 2005).

### 2.3.2 Allergen and immune response

The cross-reactivity of amoxicillin to sera from benzylpenicillin-hypersensitive patients was demonstrated in vitro using a radioallergosorbent test or histamine release test (Anfosso, Leyris & Charpin, 1979). Several recent investigations in penicillin-sensitive humans have identified that amoxicillin is often the most frequently reactive penicillin tested (Blanca et al., 2001; Torres et al., 2003; Lin, Saxon & Riedl, 2010). Furthermore, about 80% of the patients that test positive to amoxicillin in the skin prick or intradermal tests are also positive to benzylpenicillin, and vice versa. There is also an indication that subjects who are positive to amoxicillin determinants become negative sooner than those positive to benzylpenicillin. In a prospective study, 60% of patients who were positive to benzylpenicilloyl or a minor determinant mixture (benzylpenicillin and benzylpenicilloic acid) remained skin test reactive after 5 years. However, all subjects who were positive to amoxicillin alone became skin test negative within the same period (Blanca et al., 1999).

Despite the widespread use of amoxicillin in food-producing animals for many years, hypersensitivity reactions associated with the consumption of amoxicillin residues in food were not identified in the literature search. However, oral exposure to a small quantity of amoxicillin in sensitized individuals was shown to elicit allergic reactions. For example, hypersensitivity was recorded in a mother who kissed her baby 3 hours after oral administration of amoxicillin to the baby

***Table 10. Concentrations recommended for both prick and intradermal testing with β-lactams***

| Hapten | Dose |
|---|---|
| Benzylpenicilloyl | $5 \times 10^{-5}$ mmol/l |
| Minor determinant mixture | $2 \times 10^{-2}$ mmol/l |
| Amoxicillin | 20–25 mg/ml (51.7 mmol/l) |
| Ampicillin | 20–25 mg/ml (54 mmol/l) |
| Cephalosporin | 1–2 mg/ml |

Adapted from Torres et al. (2003)

(Mancuso & Berdondini, 2006) and in a woman who kissed her boyfriend a few minutes after he had taken oral amoxicillin (Pétavy-Catala, Machet & Vaillant, 2001). Hypersensitivity to amoxicillin has also been recorded in sensitized individuals from licking by a dog treated with amoxicillin (Tosoni et al., 2011), from various types of oral or contact exposure (Blanca et al., 1996; Kounis, Giannopoulos & Goudevenos, 2011), through sexual intercourse with a partner receiving amoxicillin or by drinking from a glass previously used for administering amoxicillin (Blanca et al., 1996).

Published literature comparing the allergenic potential of amoxicillin with that of other penicillins, including benzylpenicillin, could not be found. However, there is limited evidence that, compared with benzylpenicillin, amoxicillin is chemically less reactive with proteins to form haptens, both in vitro and in vivo. The concentrations used in routine β-lactam allergy testing to elicit hypersensitivity reactions may also be an indication of the relative allergenic potency of these compounds. Routine clinical allergic skin testing concentrations (expressed as millimoles per litre) of amoxicillin are typically 2500 times greater than those of benzylpenicillin (part of the minor determinant mixture) (Table 10).

The Committee previously assessed the toxicological information on benzylpenicillin (Annex 1, reference *91*) and procaine benzylpenicillin (Annex 1, reference *134*) and concluded that allergy was the determining factor in the safety evaluation of residues of benzylpenicillin. The thirty-sixth meeting of the Committee (Annex 1, reference *91*) recommended that the daily intake of benzylpenicillin from food be kept as low as practicable, and in any case below 30 µg of the parent drug. The risk associated with the occurrence of mild hypersensitivity reactions to benzylpenicillin at levels lower than this was considered to be insignificant. Given the cross-reactivity of amoxicillin and benzylpenicillin in a high proportion of penicillin-sensitive patients and the occurrence of hypersensitivity in sensitized individuals through oral exposure to a small amount of amoxicillin, the Committee considered the applicability of the previously established maximum allowable exposure limit of 30 µg of benzylpenicillin per person to amoxicillin.

### *2.3.3 Hepatotoxicity*

Use of amoxicillin alone or in combination with clavulanic acid has been associated with liver injury in humans. Hepatotoxicity associated with the use of

amoxicillin alone is reported less frequently (e.g. Bolzan et al., 2000; Schwarze et al., 2002) than the hepatotoxicity associated with the use of amoxicillin in combination with clavulanic acid (Chang & Schiano, 2007; Robles et al., 2010; Andrade & Tulkens, 2011). Liver toxicity associated with amoxicillin–clavulanic acid is usually mild. It is generally observed 2 weeks after the initiation of treatment, and symptoms resolve within a few weeks (Andrade & Tulkens, 2011). There are, however, occasional reports of a protracted, more serious course of liver toxicity, including liver failure and death (Lucena et al., 2006). Prolonged or repeated course of treatment, heightened sensitivity in older (>65 years) males (Andrade & Tulkens, 2011) and certain genetic predisposition (O'Donohue et al., 2000) are considered risk factors for hepatotoxicity associated with amoxicillin–clavulanic acid.

The reported incidence of hepatotoxicity for amoxicillin in different studies ranges from less than 0.02 (Thiim & Friedman, 2003) to 3 (Garcia Rodriguez, Stricker & Zimmerman, 1997) per 100 000 prescriptions. In contrast, hepatotoxicity associated with amoxicillin–clavulanic acid has a much higher estimated incidence of 1–17 per 100 000 prescriptions (Larrey et al., 1992; Garcia Rodriguez, Stricker & Zimmerman, 1997; Hussaini et al., 2007). Hepatotoxicity is most frequently linked to the clavulanic acid moiety, as the use of the combination product increases the risk by about 9-fold compared with amoxicillin alone (Andrade & Tulkens, 2011). In epidemiological studies, the use of the amoxicillin–clavulanic acid combination was much more frequently associated with hepatotoxicity than the use of amoxicillin alone. Other studies have shown that in patients with a history of liver injury induced by amoxicillin–clavulanic acid, subsequent treatment with amoxicillin caused no hepatotoxicity, but treatment with amoxicillin and clavulanic acid led to a second episode of hepatitis (Stricker et al., 1989; Berg & Hahn, 2001). These observations indicate the predominant role of clavulanic acid in liver toxicity induced by the amoxicillin–clavulanic acid combination.

### *2.3.4 Reproductive toxicity*

Several epidemiological studies evaluated the developmental toxicity or teratogenic potential of amoxicillin or amoxicillin–clavulanic acid used in pregnant women. In general, penicillins, including amoxicillin, are considered to have minimal reproductive toxicity. The potential reproductive toxicity of amoxicillin in humans was reviewed by Nahum, Uhl & Kennedy (2006). The results, summarized in Table 11, did not identify an increased risk of major congenital anomalies, malformations or teratogenic potential in children when their mothers were treated with amoxicillin during various stages of gestation. Based on these data, the increased risk of teratogenicity in humans is considered "unlikely".

An in vitro study investigated the effects of amoxicillin on sperm movement characteristics, viability and the ability of spermatozoa to undergo the acrosome reaction. Spermatozoa were cultured for 24 hours with increasing concentrations of antimicrobials. Amoxicillin had no effect on sperm movement characteristics over the dose ranges tested (2.5–500 µg/ml), although it inhibited viability at 1 mg/ml (44.7% in controls versus 38.4% in amoxicillin-treated groups). No effects were seen on the ability of spermatozoa to undergo the acrosome reaction (Hargreaves et al., 1998).

***Table 11. Summary of the teratogenic and toxic potential of amoxicillin based on human and animal data***

| Human data: Teratogenic and toxic effects | Animal data: Teratogenic and toxic fetal effects |
|---|---|
| 1. OR (adjusted) for congenital anomalies = 1.16 (95% CI 0.54–2.50) in a Danish study (1991–2000) of 401 primiparous women who filled prescriptions for amoxicillin during pregnancy (rate = 4.0%) compared with 10 237 controls who did not redeem any prescription drug (rate = 4.1%) (Jepsen et al., 2003).<br>2. No increased rate of congenital malformations among 147 women who received prescriptions for amoxicillin during the first trimester (Jepsen et al., 2003).<br>3. No increased rate of congenital anomalies among 284 infants whose mothers were administered amoxicillin or ampicillin during the first trimester or in 1060 infants whose mothers were treated at any time during pregnancy (Colley, Kay & Gibson, 1983).<br>4. No adverse effects in offspring exposed to amoxicillin during the second and third trimesters in three controlled clinical trials of antibiotic treatment for premature preterm rupture of membranes (Almeida, Schmauch & Bergstrom, 1996; Lovett et al., 1997; Mercer et al., 1997).<br>5. No significantly increased rate of major or minor anomalies in the children of 14 women treated with amoxicillin and probenecid during the first 14 weeks of gestation or among 57 women treated after the 14th week in a controlled clinical trial on the treatment of gonorrhoea during pregnancy (Cavenee et al., 1993).<br>6. OR for major congenital anomalies = 1.4 (95% CI 0.9–2.0) for women using amoxicillin–clavulanic acid during pregnancy in a case–control study of 6935 malformed infants (no increased risk) (Czeizel et al., 2001).<br>7. An association of necrotizing enterocolitis in newborns and maternal amoxicillin and clavulanic acid treatment during the third trimester was observed in a randomized controlled trial including 4826 pregnant patients (Kenyon et al., 2001; Kenyon, Taylor & Tarnow-Mordi, 2002). | 1. No increased congenital malformations in mice treated with 3–7 times the maximum human therapeutic dose of amoxicillin (Abou-Tarboush, 1994).<br>2. Increased frequency of embryonic death in mice treated with amoxicillin at 6–7 times the maximum therapeutic human dose (Abou-Tarboush, 1994).<br>3. No adverse reproductive effects in rats given amoxicillin–clavulanic acid at doses of 400 and 1200 mg/day prior to fertilization and during the first 7 days of gestation (Hirakawa et al., 1983).<br>4. No adverse fetal effects in pigs given amoxicillin with clavulanic acid at a dose of 600 mg/kg bw on days 12–42 (James, Hardy & Koshima, 1983). |

Modified from Nahum, Uhl & Kennedy (2006)
CI, confidence interval; OR, odds ratio

## 3. COMMENTS

Amoxicillin is an old drug with a long history of use. Studies were performed prior to the implementation of GLP guidelines, but consistent with standards existing at the time of study. Published journal articles reviewed often did not declare the GLP compliance status of their studies.

Data supporting the evaluation of amoxicillin were provided by the USFDA with the permission of the pharmaceutical sponsor. In addition, a literature search was conducted, which identified a number of additional pharmacokinetic and epidemiological studies that were used in this evaluation.

### 3.1 Biochemical data

Amoxicillin is rapidly absorbed through oral and parenteral routes of administration and distributed in various tissues. Peak plasma concentrations of amoxicillin greater than 10 µg/ml are observed generally within an hour after oral administration in mice (50 mg/kg bw), rats (100 mg/kg bw), dogs (100 mg/kg bw) and humans (500 mg/person). Oral bioavailability is approximately 50% in rats, about 65% in dogs and greater than 80% in humans. There is a dose-dependent saturability of amoxicillin absorption. Partial degradation of amoxicillin may occur in the intestine, thereby reducing its bioavailability. Plasma protein binding is not high (<15%). Amoxicillin has a short plasma elimination half-life (~1 hour), and the absorbed drug is quickly eliminated unchanged from the body, principally via urinary excretion through both glomerular filtration and active secretion. Hepatic first-pass metabolism is not observed. Within the normal dose range, amoxicillin pharmacokinetics is not influenced by the presence of clavulanic acid. Only the parent compound has microbiological activity.

### 3.2 Toxicological data

Acute toxicity results in mice, rats and dogs suggest that the drug is well tolerated at high doses following oral exposure ($LD_{50}$ > 5000 mg/kg bw).

Short-term toxicity studies were conducted in rats, cats and dogs. In one study, rats of both sexes were administered amoxicillin by gavage at 500 mg/kg bw per day for 21 days. The absolute and relative liver weights in treated groups were significantly lower than those in the controls, but there were no histological changes that were treatment related. The NOAEL from this study was 500 mg/kg bw per day, the only dose tested.

In another study, male and female rats were treated with amoxicillin by gavage at 500 mg/kg bw per day for 21 days. Histopathological examination revealed minimal, statistically non-significant fatty changes in the livers of treated females. The NOAEL for this study was 500 mg/kg bw per day, the only dose tested.

Groups of male and female cats were treated orally with amoxicillin at 100, 300 or 500 mg/animal per day, 5 days/week, for 28 days. In the highest-dose males, there was an increase in the level of renal tubular lipid, but it was not considered to be treatment related. No other treatment-related lesions were identified. The NOAEL for this study was the highest dose tested, 500 mg/day per animal, equal

to 149 mg/kg bw per day based on the group mean animal body weight of 3.36 kg at the initiation of the study.

One male and one female dog were orally administered amoxicillin at a dose of 250 mg/kg bw per day for 14 days. Gross examination during necropsy and histopathological examination of major organs did not reveal any significant changes. The NOEL for this study was 250 mg/kg bw per day, the only dose tested.

In a 6-month study, male and female rats were dosed with amoxicillin by gavage at 0, 200, 500 or 2000 mg/kg bw per day, 6 days/week, for 26 weeks. At 13 weeks, some males and females from each group were necropsied; the remaining animals were necropsied at the end of the study. At the interim necropsy, moderate enlargement of the caecum in the highest-dose group was the only treatment-related change. The only change noticed at termination was elevated relative liver weights in the highest-dose males, but it was considered not to be of toxicological relevance. The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

In a 6-month study, male and female dogs were treated orally with amoxicillin at a dose of 200, 500 or 2000 mg/kg bw per day for 6 months. Control dogs were left untreated (no placebo administration). At the end of 3 months, half of the males and females from each group were necropsied; the remaining animals were necropsied at the end of 6 months. Some vomiting occurred either immediately or within 1–4 hours of dosing in some animals in the highest-dose group. Grey-coloured faeces were observed mainly in the first 3 weeks in a dose-related manner in dogs receiving 500 or 2000 mg/kg bw per day. The weight gain in the highest-dose group was decreased compared with controls. At termination of the study, relative liver weights were increased for the highest-dose dogs, but no associated histology was observed. The NOAEL for this study was 2000 mg/kg bw per day, the highest dose tested.

Amoxicillin was negative in vitro for sister chromatid exchange, chromosomal aberration and the induction of micronuclei in human peripheral lymphocytes with and without exogenous metabolic activation. In studies reviewed by the USFDA[1], but not available to the Committee, the combination of amoxicillin–clavulanic acid gave negative results for genotoxicity in vitro in an Ames test, a human lymphocyte cytogenicity assay and a yeast recombination assay. Amoxicillin–clavulanic acid was weakly positive in a mouse lymphoma assay only at cytotoxic concentrations. It was negative in vivo in a mouse micronucleus test and a dominant lethal test. As with other β-lactam antimicrobial agents, amoxicillin caused DNA damage in vitro in cultured cells at high concentrations. There is some evidence that this is an indirect effect due to the formation of reactive oxygen species.

The Committee concluded that it was unlikely that amoxicillin would be genotoxic in vivo following exposure to residues arising from its use as a veterinary drug.

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[1] Based on the USFDA's label on the approved human drug amoxicillin–clavulanate potassium.

Data from studies of long-term toxicity and carcinogenicity were not available for the Committee to review. However, the long history of use of amoxicillin in a wide range of species, including humans, has not identified an association between the use of amoxicillin and carcinogenicity. Similarly, there were no indications of potential carcinogenicity effects (e.g. increased incidence of tumours or preneoplastic lesions) in the 6-month rat study. These findings, together with the genotoxicity data reviewed, led the Committee to conclude that amoxicillin is unlikely to be carcinogenic to humans at levels of exposure to residues in food-producing animals.

In a reproductive toxicity study, rats were treated orally with amoxicillin at 200 or 500 mg/kg bw per day for several weeks before mating, during cohabitation and thereafter for the study duration, and animals were followed through first and second matings. No significant differences were noted between treated and control animals or their pups in most parameters measured after both the first and second matings, except reduced weight gain in treated males. The NOAEL from this reproductive study was 500 mg/kg bw per day, the highest dose tested.

Groups of pregnant mice were treated with amoxicillin at 0, 200, 500 or 2000 mg/kg bw per day by gavage between days 6 and 15 of pregnancy and killed on day 17. Fetal losses of 4.8%, 13.9%, 15.2% and 13.0%, respectively, were observed. No other treatment-related abnormalities were noted in either the dams or the pups. Although the difference in fetal losses between the control and treated groups was significant, these values were within the historical control range of 3.8–14.3% (mean 7.9%) for the laboratory, and there was no dose–response relationship. These results were considered equivocal, and a NOAEL for developmental toxicity in mice could not be identified.

Groups of pregnant rats were treated with amoxicillin at doses up to 2000 mg/kg bw per day by gavage between days 6 and 15 of pregnancy and killed on day 20. No significant effects were observed in either the dams or the pups from treated animals. The NOEL from this developmental toxicity study was 2000 mg/kg bw per day, the highest dose tested.

In another rat study, animals were treated with amoxicillin at 200 or 500 mg/kg bw per day by gavage from day 15 of gestation through to lactation day 21. There was no effect on dams, pup mortality or growth, and no abnormal pups were observed. The NOAEL for perinatal and postnatal toxicity was 500 mg/kg bw per day, the highest dose tested.

Epidemiological studies that investigated the risk of teratogenicity of amoxicillin in humans have not identified any increased risk. Based on the overall data reviewed, the Committee considered that amoxicillin is unlikely to cause reproductive or developmental toxicity in humans.

In humans, the use of amoxicillin–clavulanic acid has been reported to be associated with hepatotoxicity. However, hepatotoxicity is rarely associated with the use of amoxicillin alone. Information in the published literature suggests that clavulanic acid is likely to be responsible for this toxicity.

Hypersensitivity is considered to be the most relevant toxicological end-point for the safety assessment of amoxicillin. Individuals who are hypersensitive

to amoxicillin are also sensitive to other β-lactams, including benzylpenicillin. In recent studies, amoxicillin was often the most frequent positive allergen among several β-lactams tested in human patients, and a high proportion of patients who are reactive to amoxicillin (~80%) were also allergic to other penicillins. Although published literature indicates that hypersensitivity in sensitized individuals could be evoked by oral exposure to a small quantity of amoxicillin, no reports were found on hypersensitivity reactions occurring in humans from exposure to the residues of amoxicillin in foods of animal origin. The Committee previously evaluated benzylpenicillin and procaine benzylpenicillin, which dissociates to procaine and benzylpenicillin in the body (Annex 1, references *91* and *134*), and concluded that allergy was the determining factor in the safety evaluation of these residues, recommending that daily intake of benzylpenicillin from food be kept as low as practicable, and in any event below 30 µg of the parent drug.

Limited information available suggests that, compared with benzylpenicillin, amoxicillin is chemically less reactive with proteins to form haptens. Additionally, the recommended starting dilution of amoxicillin used in the diagnosis of hypersensitivity in sensitized individuals by the skin prick test is more than 2500 times higher than that of benzylpenicillin.

### *3.3 Microbiological data*

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with VICH GL36 was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for amoxicillin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated. Amoxicillin was active against *Escherichia coli* ($MIC_{50}$ = 5 µg/ml), *Bifidobacterium* ($MIC_{50}$ = 0.06 µg/ml), *Clostridium* ($MIC_{50}$ = 0.1 µg/ml), *Bacteroides* ($MIC_{50}$ = 0.5 µg/ml), *Lactobacillus* ($MIC_{50}$ = 0.25 µg/ml), *Fusobacterium* ($MIC_{50}$ = 0.1 µg/ml), *Eubacterium* ($MIC_{50}$ = 0.1 µg/ml) and *Peptostreptococcus* ($MIC_{50}$ = 0.1 µg/ml).

Amoxicillin residues may be present at low levels in meat products consumed by humans; therefore, amoxicillin-related residues could enter the colon of a person ingesting edible tissues from treated animals. Although amoxicillin was rapidly absorbed after oral administration in animals, a considerable amount of the administered amoxicillin was excreted through faeces. In the absence of data to support faecal inactivation, it is considered that amoxicillin residues entering the human colon will remain microbiologically active.

There is potential for disruption of the colonization barrier in the human gastrointestinal tract, as MIC values for the most relevant and predominant bacteria in the gastrointestinal tract indicated that they were susceptible to amoxicillin. Because the majority of amoxicillin residue levels detected in target tissue were below the lowest $MIC_{50}$ of any of the representative human intestinal microbiota tested, it is unlikely that the development of resistance to amoxicillin residues would occur.

The formula for calculating the microbiological ADI is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

$MIC_{calc}$: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC ($MIC_{calc}$) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean $MIC_{50}$ for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the $MIC_{calc}$ were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the $MIC_{50}$ values for *Escherichia coli* ($MIC_{50}$ = 5 µg/ml), *Bifidobacterium* ($MIC_{50}$ = 0.06 µg/ml), *Clostridium* ($MIC_{50}$ = 0.1 µg/ml), *Bacteroides* ($MIC_{50}$ = 0.5 µg/ml), *Lactobacillus* ($MIC_{50}$ = 0.25 µg/ml), *Fusobacterium* ($MIC_{50}$ = 0.1 µg/ml), *Eubacterium* ($MIC_{50}$ = 0.1 µg/ml) and *Peptostreptococcus* ($MIC_{50}$ = 0.1 µg/ml), the $MIC_{calc}$ is 0.10 µg/ml.

*Mass of colon content*: A value of 220 g is based on the colon content measured from humans.

*Fraction of oral dose available to microorganisms*: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Amoxicillin is rapidly absorbed and is excreted in urine primarily in unchanged form. The lowest amoxicillin urinary recovery data from the human studies was 43.4%. Therefore, the fraction of oral dose available would be 1 – 0.434 = 0.566.

*Body weight*: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for amoxicillin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{0.10\ \mu g/ml \times 220\ g}{0.566 \times 60\ kg\ bw}$$

$$= 0.65\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–0.7 µg/kg bw (rounded to one significant figure), or 42 µg for a 60 kg adult, was derived from in vitro MIC susceptibility testing.

## *4. EVALUATION*

As hypersensitivity is frequently encountered in the therapeutic use of penicillins, including amoxicillin, in human medicine, this was considered to be the toxicological effect of concern.

The thirty-sixth meeting of the Committee based its toxicological guidance value of 30 µg/person for benzylpenicillin on only four case-studies of allergy to oral exposure to residues of penicillins, in the absence of information related specifically to benzylpenicillin.

Available limited evidence indicates that, compared with benzylpenicillin and possibly other penicillins, amoxicillin is less chemically reactive with proteins to form haptens. While the Committee recognizes that adverse reactions are generally underreported, it was unable, through a search of the published literature, to identify any cases of allergy following oral exposure to residues of amoxicillin in food. The Committee therefore concluded that the value of 30 µg/person previously established by the thirty-sixth meeting to protect against allergic reactions from residues of penicillins would be unnecessarily conservative in protecting against residues of amoxicillin.

A microbiological ADI of 0–42 µg/person could be established on the basis of disruption of the colonization barrier of the gastrointestinal tract. The Committee therefore concluded that this ADI is sufficient to ensure that allergic reactions would be very unlikely to occur following oral exposure to residues of amoxicillin in food.

The Committee therefore established an ADI of 0–0.7 µg/kg bw on the basis of microbiological effects.

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## *APRAMYCIN*

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## 1. EXPLANATION

Apramycin (Chemical Abstracts Service No. 65710-07-8) is a broad-spectrum aminocyclitol antibiotic produced by a strain of *Streptomyces tenebrarius*. It is effective against both Gram-positive and Gram-negative bacteria, including isolates of *Escherichia coli* and *Salmonella* species, and some strains of mycoplasma from farm animals and human origin. Apramycin is extracted from the fermentation medium as apramycin sulfate at a purity of at least 85%, and activity is expressed as equivalents of apramycin base using a microbiological assay.

Apramycin exerts its antibacterial effect by inhibiting protein synthesis at the level of peptidyl translocation. It is used for the treatment of a variety of enteropathogenic infectious diseases in target species such as cattle, swine, poultry and rabbits (e.g. colibacillosis, salmonellosis and other bacterial infections in calves and pigs, *Escherichia coli* septicaemia, colibacillosis, salmonellosis and other bacterial infections in poultry and bacterial enteritis including colibacillosis in rabbits). Apramycin is marketed as the sulfate in soluble powder (to be incorporated in drinking-water) and premix formulations (to be incorporated in feed). Apramycin has also been used as an intramuscular injection in calves and in an oral dosage form in neonatal lambs, calves and pigs. Apramycin is not used in human medicine.

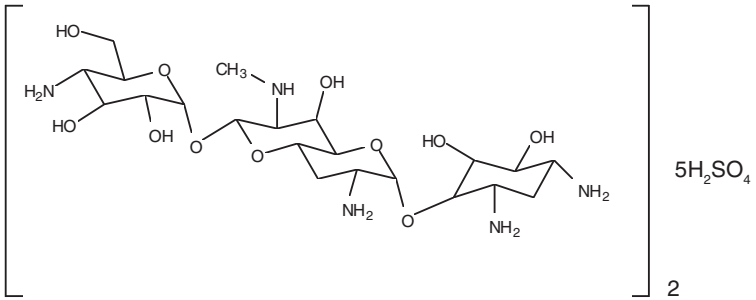
The recommended doses are 20–40 mg/kg body weight (bw) per day of soluble power incorporated in drinking-water or milk replacer or of premix incorporated in feed for calves for 5 days; 7.5–12.5 mg/kg bw per day of soluble power incorporated in drinking-water for 7 days or 4–8 mg/kg bw per day of premix incorporated in feed for up to 28 days in pigs; 20–80 mg/kg bw per day of soluble power incorporated in drinking-water for 5–7 days or 2–5 mg/kg bw per day of premix incorporated in feed for 5 days in poultry; and 10–15 mg/kg bw per day of soluble powder incorporated in drinking-water for 5–8 days or 5–10 mg/kg bw per day of premix incorporated in feed for up to 21 days in rabbits.

Apramycin has not been previously evaluated by the Committee. The Committee evaluated apramycin to establish an acceptable daily intake (ADI) and recommend maximum residue limits (MRLs) in cattle, pig and chicken tissues at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011).

In addition to a submission from the sponsor, a literature search was conducted using Toxnet. Only one further relevant study report was identified in the published literature.

The structure of apramycin ((2*R*,3*R*,4*R*,5*S*,6*R*)-5-amino-2-[((1*R*,2*R*,3*R*,4*R*,6*R*,8*R*)-8-amino-9-[(1*R*,2*S*,3*R*,4*R*,6*R*)-4,6-diamino-2,3-dihydroxy-cyclohexyl]-oxy-2-hydroxy-3-methylamino-5,10-dioxabicyclo[4.4.0]dec-4-yl)oxy]-6-(hydroxy-methyl)oxane-3,4-diol) sulfate is shown in Figure 1.

***Figure 1. Structure of apramycin sulfate***




## 2. BIOLOGICAL DATA

### 2.1 Biochemical aspects

#### 2.1.1 Absorption, distribution and excretion

*(a) Rats*

In a preliminary study, which did not comply with good laboratory practice (GLP), rats (number and sex not specified) were given a single dose of 4 mg [$^{14}$C]-apramycin (form not specified), either orally or subcutaneously. Radioactivity was determined in excreta and, at sacrifice 4 days after dosing, in the kidneys. The subcutaneous dose was excreted predominantly in urine (93%), with the remainder in faeces. Following an oral dose, only 0.5% was found in urine, with 99.5% in faeces, indicating very low absorption from the gastrointestinal tract. The levels of radioactivity in kidneys were 12 µg/g and 0.2 µg/g after the subcutaneous and oral doses, respectively. The primary route of excretion in rats following oral administration was in the faeces (Donoho et al., 1976).

*(b) Dogs*

In a 6-month non-GLP-compliant toxicity study, Beagle dogs (six animals of each sex per group) received an oral dose of 0, 25, 50 or 100 mg of apramycin activity per kilogram body weight per day in capsules (see section 2.2.2(c)). Venous blood was collected 1, 2, 3, 4, 6 and 24 hours after apramycin administration on days 1, 64, 119 and 182. Serum concentrations of apramycin peaked at 2 hours and were proportional to the dose, with means of 2.2, 5.5 and 11.0 µg/ml at doses of 25, 50 and 100 mg/kg bw per day, respectively. Essentially no apramycin was detected in plasma 24 hours after administration, and there was no evidence of accumulation or altered plasma levels following repeated administration. Urine collected for a 24-hour period after dosing on days 1, 64, 119 and 182 contained between 0.3% and 10.5% of the administered dose. The mean concentrations of apramycin in kidneys at the end of the treatment period were proportional to the dose: 45.2, 113.2 and 170 µg/g tissue at doses of 25, 50 and 100 mg/kg bw per day, respectively. Lower mean levels of apramycin were detected in kidneys of dogs

allowed a 3-month treatment-free period after 6 months of dosing: 10.2, 25.8 and 29.4 µg/g tissue at 25, 50 and 100 mg/kg bw per day, respectively (Howard et al., 1977c).

In another study that did not comply with GLP, Beagle dogs (4–5 animals of each sex per group) were given an oral dose of 0, 25, 50 or 100 mg of apramycin activity per kilogram body weight per day in capsules for 1 year (see section 2.2.2(c)). Venous blood was collected 1, 2, 3, 4 and 6 hours after apramycin administration during weeks 15, 28 and 51. Serum concentrations of apramycin peaked at 1–2 hours and were proportional to the dose; mean concentrations were between 1.12 and 3.63 µg/ml, 4.86 and 6.3 µg/ml and 5.85 and 11.9 µg/ml at doses of 25, 50 and 100 mg/kg bw per day, respectively. There was no evidence of apramycin accumulation or altered plasma levels following repeated administration. The mean concentrations of apramycin in kidneys at the end of the treatment period were proportional to the dose: 20.7, 41.9 and 82.2 µg/g tissue at 25, 50 and 100 mg/kg bw per day. The results from the studies in dogs suggested low absorption after oral dosing. The primary route of excretion in dogs was in the faeces (Howard, Johnson & Bridge, 1980).

### *2.1.2 Biotransformation*

In the Donoho et al. (1976) study in rats described in section 2.1.1, the radioactivity was identified using thin-layer bioautography as primarily apramycin, suggesting that no metabolism had occurred.

## *2.2 Toxicological studies*

### *2.2.1 Acute toxicity*

#### *(a) Single-dose toxicity*

The results of non-GLP-compliant studies of the acute toxicity of different forms of apramycin are summarized in Table 1.

Following oral dosing, there were no deaths in mice, rats, rabbits or dogs. Clinical signs of toxicity were diarrhoea for 4–6 hours after dosing and cachectic appearance for 4–5 days in mice, thinness, poor grooming and diarrhoea in rats on the first day, anorexia, weight loss and hypoactivity in rabbits and mild diarrhoea and vomiting in dogs. Guinea-pigs exhibited anorexia, alopecia, distended stomach, reduced weight gain, renal injury and delayed death in 2 of 10 animals. Apramycin demonstrated low acute toxicity when administered by the oral route.

Mortality occurred within 1 hour of intravenous dosing in rodents and appeared to be due to central nervous system toxicity. The higher lethality of the sulfate forms was shown to be due to the lower pH of the dosing solutions. Mice and rats that survived high doses revealed varying degrees of renal damage.

Rats were exposed to an atmosphere containing particulate apramycin for 1 hour. There were no deaths or toxic signs at the single dose used. The median lethal concentration ($LC_{50}$) was greater than 211 mg apramycin activity per cubic metre (Arthur et al., 1977).

***Table 1. Summary of acute toxicity (lethal dose) studies with apramycin***

| Apramycin form | Species, sex | Route of administration | $LD_{50}$ (mg activity/kg bw) | References |
|---|---|---|---|---|
| Sulfate | Mouse, M + F | Oral | >5200 | Howard & Van Duyn (1976); Worth et al. (1976c) |
| Sulfate | Rat, M + F | Oral | >4160 | Howard & Van Duyn (1976); Worth et al. (1976c) |
| Sulfate | Guinea-pig, M + F | Oral | >1250 | Howard & Van Duyn (1976); Worth et al. (1976c) |
| Sulfate | Rabbit, M + F | Oral | >832 | Howard & Van Duyn (1976); Worth et al. (1976c) |
| Sulfate | Dog, M + F | Oral | >520 | Howard & Van Duyn (1976); Worth et al. (1976c) |
| Base | Mouse, M + F | Intravenous | 570–573 | Howard & Van Duyn (1976); Worth et al. (1976b) |
| Base | Rat, M + F | Intravenous | 1596–1640 | Howard & Van Duyn (1976); Worth et al. (1976b) |

F, female; $LD_{50}$, median lethal dose; M, male

*(b) Irritation and sensitization*

*(i) Rabbits*

Dermal application of 2000 mg apramycin sulfate (52% activity) per kilogram body weight to the intact and abraded skin of six rabbits caused slight erythema at the application site for a period of 5 days. There were no deaths or signs of systemic effects. This study was not GLP compliant (Worth et al., 1976a).

The instillation of 36 mg apramycin sulfate (52% activity) into the left eye of six rabbits caused slight conjunctival redness in two animals, which cleared within 48–96 hours. There were no deaths or signs of systemic effects. This study was not GLP compliant (Worth et al., 1976a).

*(ii) Guinea-pigs*

Guinea-pigs received dermal applications of 40 mg apramycin activity 3 times a week for 3 weeks for a total of 10 doses, followed by a 2-week rest period. Body weight gains were unaltered. When guinea-pigs were challenged in the fifth week, there was no evidence of skin sensitization. This study was not GLP compliant (Worth et al., 1976a).

### 2.2.2 Short-term studies of toxicity

#### (a) Mice

In two separate non-GLP-compliant studies, apramycin base was admixed in the diet and fed to B6C3F1 mice for 3 months. In the first study, doses of 0, 5000, 10 000 and 20 000 mg/kg of feed were administered to groups of 10 males and 10 females. In the second study, doses of 0 and 50 000 mg/kg of feed were given to groups of 15 males and 15 females. Dietary assays generally revealed apramycin concentrations well below the target level. Virtually no apramycin was present in the 10 000 mg/kg feed; therefore, this group was terminated early and not reported. There were no treatment-related deaths, and the sole clinical sign of toxicity was alopecia in males given 20 000 and 50 000 mg/kg feed. Body weight gains were reduced in both sexes given 50 000 mg/kg feed and in males given 20 000 mg/kg feed. Slightly decreased lymphocyte counts and increased neutrophil counts were observed at the highest dose. Blood chemistry was not meaningfully affected. Absolute and relative liver and kidney weights were lower in males and females given 50 000 mg/kg feed, but pathology was unaffected. Because the concentrations of apramycin in feed were considerably different from the expected levels, there was uncertainty as to the administered doses. Therefore, a no-observed-adverse-effect level (NOAEL) could not be determined (Howard et al., 1986).

#### (b) Rats

In a non-GLP-compliant study, groups of 10 male and 10 female Fischer 344 rats were fed diets containing 0, 10 000, 25 000 or 50 000 mg apramycin activity per kilogram feed for 1 month. No deaths occurred in any group. During the last week of the study, rats from the 25 000 and 50 000 mg/kg feed groups excreted dark to black faeces. Body weight gain was depressed and serum glucose and blood urea nitrogen were increased in males and females given 50 000 mg/kg feed. Also at this dose level, there were slight decreases in serum levels of creatinine in females and in alkaline phosphatase activity in males. Haematological parameters and organ weights were not examined, and only limited pathology was undertaken. No lesions were seen in the kidneys, whereas the caeca of all high-dose rats were 2–3 times normal size. Because an inadequate range of parameters was investigated, a NOAEL was not determined (Howard & Johnson, 1981).

In a non-GLP-compliant study, groups of 10 male and 10 female weanling Harlan rats were fed diets containing 0, 200, 400 or 1000 mg apramycin sulfate per kilogram feed. Another group received apramycin sulfate in the drinking-water at a concentration of 10 mg/ml. The treatments were given for a period of 3 months. The time-weighted average doses of apramycin sulfate were 0, 11, 21 and 50 mg/kg bw per day in males and 0, 13, 26 and 63 mg/kg bw per day in females treated via the diet and 1040 mg/kg bw per day in males and 1359 mg/kg bw per day in females treated via the drinking-water. All animals survived, and mild diarrhoea in the drinking-water group was the only clinical sign. There were no meaningful findings in body weight gain, ophthalmology, haematology, blood chemistry, organ weights or pathology. The no-observed-effect level (NOEL) was the highest dietary dose of 1000 mg apramycin sulfate per kilogram feed, equal to 26 and 33 mg of apramycin

activity per kilogram body weight per day in males and females, respectively (Worth et al., 1976d).

In another non-GLP-compliant 3-month study, groups of 15 male and 15 female Fischer 344 rats were fed diets containing 0, 1800, 2750, 6200 or 10 000 mg apramycin activity per kilogram feed. The time-weighted average doses were 0, 129, 198, 460 and 738 mg/kg bw per day in males and 0, 153, 228, 556 and 896 mg/kg bw per day in females. There was no mortality, and there were no effects on clinical signs, haematology, blood chemistry or organ weights. Feed consumption in all treated groups and body weight gains in females given 6200 and 10 000 mg/kg feed were slightly elevated, but not in a dose-related manner. Mild nephrosis was observed in three male rats at the highest dose. Based on kidney toxicity, the NOAEL was 6200 mg/kg feed, equal to 460 mg/kg bw per day in males (Howard & Owen, 1981).

In a non-GLP-compliant study, Wistar rats were fed diets containing 0, 1000, 2500 or 5000 mg apramycin activity per kilogram feed for up to 6 months. Groups of 4–6 rats of each sex were necropsied after 4 weeks, and groups of 10 rats of each sex were necropsied after 29–30 weeks. Drug intake during the first month was 0, 96, 236 and 461 mg/kg bw per day in males and 0, 94, 241 and 475 mg/kg bw per day in females. Over the full 6 months, the time-weighted average doses were 0, 69, 170 and 343 mg/kg bw per day in males and 0, 77, 191 and 388 mg/kg bw per day in females. There was no mortality, and there were no effects on feed consumption, body weight gain or urine analysis. Darker, softer faeces with increased moisture were found in all treated groups in a dose-related manner and were associated with a concomitant increase in water intake. Erythrocyte counts, haemoglobin and haematocrit were lower in males given 5000 mg/kg feed for 1 month, but not at the end of the study. Neutrophil counts were lower in high-dose females at the end of treatment. Serum glutamate dehydrogenase activity was increased in animals given 5000 mg/kg feed for 6 months. Absolute and relative kidney weights were lower in high-dose males, but kidney pathology was unchanged. Based on haematological and biochemical findings, the NOAEL was 2500 mg/kg feed, equal to 170 mg/kg bw per day in males and 191 mg/kg bw per day in females (Tarrant et al., 1976).

*(c) Dogs*

In a non-GLP-compliant study, groups of three male and three female Beagle dogs were administered 0, 5, 10 or 25 mg apramycin sulfate per kilogram body weight per day in capsules for a period of 3 months. Survival, clinical signs, body weight gain, haematology, blood chemistry, urine analysis, myeloid/erythroid ratios and pathology were unaffected. The weights of kidney, heart and testes were elevated in dogs at the highest dose when compared with concurrent controls. These apparent changes were not significantly different from historical control values. The NOEL was the highest dose of 25 mg apramycin sulfate per kilogram body weight per day, equal to 13 mg apramycin activity per kilogram body weight per day (Worth et al., 1976e).

In a non-GLP-compliant study, Beagle dogs received oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Groups of four males and four females were terminated after 6 months of treatment, and

further groups of two dogs of each sex per dose were treated for 6 months and retained for a 3-month drug-free period. Body weight gain was lower in both sexes given 100 mg/kg bw per day, but no dog died during treatment. One high-dose dog had decreased appetite during the treatment phase and developed anorexia in the recovery period. As a result of weight loss and weakness, this animal was killed on day 236. During months 3–5, one dog given 50 mg/kg bw per day and four dogs given 100 mg/kg bw per day did not respond to noise stimuli, but all responded at 6 months. Soft stools occurred in nearly all treated dogs, which is likely to be a consequence of an effect on the bacterial flora rather than a toxic effect in the dog. Erythrocyte counts were consistently lower in both sexes during treatment with 50 and 100 mg/kg bw per day, but not in a dose-related manner, and statistical significance was achieved on only three of the eight occasions on which haematology was examined. Blood chemistry, urine analysis, organ weights and pathology (including electron microscopy of renal proximal convoluted tubules) were unremarkable. Based on reduced body weight gain, the NOAEL was 50 mg/kg bw per day (Howard et al., 1977c).

In a non-GLP-compliant study, Beagle dogs were given oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules for 1 year. There were five males and four females in the control group and four dogs of each sex per dose in the treated groups. There were no effects on survival, clinical signs, ophthalmology, auditory response, body weight gain, haematology, blood chemistry, urine analysis or pathology. Adrenal weights were increased in males at the highest dose but were not associated with pathological alterations. The NOAEL was the highest dose of 100 mg/kg bw per day (Howard, Johnson & Bridge, 1980).

*(d) Chickens*

In a GLP-compliant study, groups of 60 male and 60 female Cobb broiler chicks received drinking-water containing 0, 500, 1500 or 2500 mg of apramycin sulfate per litre when they were 22–36 days of age. The birds were sacrificed when they were 37–44 days of age. Survival, clinical signs, body weight gain, feed consumption, haematology, blood chemistry, organ weights and pathology were unaffected (Roberts et al., 1985).

*(e) Pigs*

In a non-GLP-compliant study, groups of five male and five female pigs (strain not specified) were fed diets containing 0, 110, 330 or 550 mg apramycin activity per kilogram feed for 28 days. There were no effects on survival, clinical signs, body weight gain, haematology, blood chemistry, urine analysis, organ weights or pathology (Van Duyn & Zimmerman, 1978).

In another non-GLP-compliant study, groups of four male and four female weanling pigs (strain not specified) were administered drinking-water containing 0, 0.2, 0.6 or 0.9 g apramycin activity per litre for 28 days. There were no treatment-related effects on survival, body weight gain, haematology, blood chemistry, urine analysis, organ weights or pathology (Van Duyn et al., undated).

*(f) Cattle*

In a non-GLP-compliant study, groups of five male and five female Holstein dairy calves received 0, 20, 40, 80 or 120 mg apramycin activity per kilogram body weight per day, as apramycin sulfate, in reconstituted milk replacer for 5 days. All treated animals remained in good health throughout the study. There were no treatment-related effects on survival, body weight gain, haematology, blood chemistry, urine analysis, organ weights or pathology (Van Duyn & Johnson, 1978).

### *2.2.3 Long-term studies of toxicity and carcinogenicity*

*(a) Mice*

In a GLP-compliant study, B6C3F1 mice were fed diets containing 0, 1500, 5000, 15 000 or 45 000 mg apramycin activity per kilogram feed for 2 years. Each group consisted of 60 mice of each sex. The time-weighted average doses were 0, 189, 623, 1928 and 7183 mg/kg bw per day in males and 0, 213, 668, 2043 and 7570 mg/kg bw per day in females. There were no effects on survival or clinical signs of toxicity. During the majority of the study, body weight gains were lower in males and females given 5000 mg/kg feed and above. At a dose of 45 000 mg/kg feed, haemoglobin and haematocrit were increased in both sexes, erythrocyte counts were increased in females and slightly decreased lymphocyte counts and increased neutrophil counts were observed in males. Serum alkaline phosphatase activity was increased in females given 5000–45 000 mg/kg feed. Serum glucose level was decreased and blood urea nitrogen was increased in both sexes at the highest dose. Organ weights and gross pathology were unaffected. Histopathology revealed a dose-related cytoplasmic basophilia involving the renal cortical tubular epithelium in both sexes at doses of 5000–45 000 mg/kg feed. The incidences of tumours were not increased. Based on reduced body weight gain and kidney pathology, the NOAEL was 1500 mg/kg feed, equal to 189 mg/kg bw per day in males and 213 mg/kg bw per day in females (Williams & Fisher, 1987).

*(b) Rats*

In a GLP-compliant study, Fischer 344 rats were maintained on diets containing 0, 2500, 5000, 10 000 or 50 000 mg apramycin activity per kilogram feed for 2 years. There were 59 males and 61 females in the control group and 50 rats of each sex per treatment group. The animals were sourced from the offspring of rats given similar diets for 3 months. The time-weighted average doses were 0, 124, 245, 488 and 2772 mg/kg bw per day for males and 0, 154, 301, 610 and 3451 mg/kg bw per day for females. Mortality, auditory response, feed consumption, haematology, blood chemistry and pathology were unaffected. Body weight gains and liver and kidney weights were reduced in both sexes at the highest dose. The incidences of tumours were not influenced by treatment. Based on reduced body weight gain and reduced organ weights, the NOAEL was 10 000 mg/kg feed, equal to 488 mg/kg bw per day in males and 610 mg/kg bw per day in females (Howard, Johnson & Owen, 1981).

***Table 2. Results of tests for genotoxicity with apramycin***

| End-point | Test object | Concentration (form of apramycin) | Result | Reference |
|---|---|---|---|---|
| Reverse mutation in vitro[a] | *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 | 3–300 µg/plate ±S9 (apramycin sulfate) | Negative | Haworth (1979) |
| Reverse mutation in vitro[b] | *S. typhimurium* strains TA98, TA100, TA1535, TA1537 *Escherichia coli* strain WP2*uvrA*$^-$ | 0.1–1000 µg/ml ±S9 (form of apramycin not specified) | Negative | Rexroat et al. (1995) |
| Forward mutation in vitro[c] | Mouse lymphoma L5178Y cells | 62.5–1000 µg/ml ±S9 (apramycin sulfate) | Negative | Rinzel & McMahon (1979) |
| DNA repair in vitro[d] | Rat hepatocytes | 0.5–500 nmol/ml (apramycin sulfate) | Negative | Howard, Probst & Neal (1980) |

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant of rat liver used for metabolic activation

[a] Positive controls were 2-nitrofluorene, 1,3-propane sultone and 9-aminoacridine in the absence of S9, 2-aminoanthracene in the presence of S9.

[b] Positive controls were 2-nitrofluorene and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine in the absence of S9, 2-aminoanthracene and streptozotocin in the presence of S9.

[c] Positive controls were 2-aminofluorene and streptozotocin.

[d] Positive controls were *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine and 2-acetylaminofluorene.

### *2.2.4 Genotoxicity*

Apramycin was evaluated for potential genotoxicity in a battery of in vitro tests. The results are summarized in Table 2. The tests included 1) in vitro assays for the induction of gene mutations in strains of *Salmonella typhimurium* and *Escherichia coli*; 2) an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells; and 3) an in vitro assay for the induction of deoxyribonucleic acid (DNA) repair in rat hepatocytes. The results were negative in all cases.

### *2.2.5 Reproductive and developmental toxicity*

#### *(a) Multigeneration studies in rats*

In a GLP-compliant study, Fischer 344 rats were fed diets containing 0, 2500, 5000 or 10 000 mg apramycin activity per kilogram feed continuously over four generations. The initial groups of rats were bred on three occasions; the $F_{1a}$ offspring were killed due to poor survival in all groups, the $F_{1b}$ offspring were assigned to the chronic toxicity and carcinogenicity study and the $F_{1c}$ offspring were bred to give the $F_2$ generation. The $F_2$ animals were mated twice to give the $F_{3a}$ offspring, which were reared to weaning, and the $F_{3b}$ pregnancy, which was terminated on gestation day 20 for examination of fetuses. Each mating group consisted of 25 males and

25 females, and the time-weighted average doses were 0, 194, 388 and 785 mg/kg bw per day. In adult rats, there were no effects on mortality, clinical signs, body weight gain, pregnancy rate or gestation length. Litter size, birth weight and sex ratio of offspring, body weight gain and survival during lactation, and gross, visceral and skeletal fetal examinations were unaffected by treatment. The NOEL was the highest dose of 10 000 mg/kg feed, equal to 785 mg/kg bw per day (Howard, Owen & Adams, 1981).

*(b) Reproductive toxicity in pigs*

In a non-GLP-compliant study, a single Yorkshire boar and groups of 17 sows (Yorkshire or Hampshire × Yorkshire) were given drinking-water containing 0 or 0.53 g apramycin activity per litre, as apramycin sulfate. Apramycin intake was 4 g/day for the boar and varied between 23.4 and 35.5 mg/kg bw per day for the sows. The boar was treated from 5 days prior to collection of sperm for artificial insemination. The sows were treated for 7 days at the time of insemination, on days 21–28 of gestation and on lactation days 1–7. The number of pregnancies, litter size, body weight at birth, and survival and weight gain of offspring to lactation day 14 were similar between groups (Melliere, Waitt & Paxton, undated).

*(c) Developmental toxicity*

*(i) Rats*

In a non-GLP-compliant study, groups of 25 pregnant female Wistar rats were given a gavage dose of 0, 250, 500 or 1000 mg apramycin activity per kilogram body weight per day on gestation days 6–15. The animals were killed on gestation day 20. All maternal animals survived and showed no effects on feed consumption or body weight gain. There were no intergroup differences in the numbers of implants, resorptions or dead fetuses. Fetal sex ratio, body weight and the incidences of gross, visceral and skeletal abnormalities were unaffected by treatment. There was no evidence of maternal toxicity, fetotoxicity or teratogenicity at any dose level tested. The NOEL was the highest dose tested, 1000 mg/kg bw per day (Howard et al., 1977a).

*(ii) Rabbits*

In a non-GLP-compliant study, groups of 15 pregnant female Dutch Belted rabbits were given a gavage dose of 0, 2, 8 or 32 mg apramycin activity per kilogram body weight per day on gestation days 6–18. The animals were killed on gestation day 28. In all treated groups, maternal feed consumption and body weight gains were markedly reduced, and the numbers of animals aborting were increased in a dose-related manner. Most of the dams aborting showed an empty gastrointestinal tract. In the remaining females, the numbers of implants and dead fetuses were similar between groups. The number of resorptions was increased at 32 mg/kg bw per day. Fetal body weight was depressed in a dose-related manner, and the incidence of bilateral 13th ribs was increased at the highest dose. The incidences of fetal malformations and external and visceral abnormalities were unaffected. The maternal toxicity was likely related to the particular sensitivity of the gut flora in

rabbits given certain antimicrobial agents (FAO/WHO, 2009); therefore, a NOEL could not be identified. The fetal toxicity was probably secondary to the severe toxicity in dams (Howard et al., 1977b).

### *2.2.6 Special studies*

#### *(a) Gastrointestinal irritation*

In a non-GLP-compliant study, groups of four male and six female pigs (strain not specified) were fed diets containing 0 or 110 mg apramycin sulfate per kilogram for 5 weeks. There were no effects on survival, body weight gain or gross examination of the gastrointestinal tract (Van Duyn & Rathmacher, undated).

#### *(b) Kidney toxicity*

In a non-GLP-compliant study, groups of 24 female Sprague-Dawley rats were hydrated with 0.9% saline, by gavage, at 25 ml/kg bw, and urine was collected for 5 hours. Apramycin sulfate was included in the saline at a dose of 0, 0.2, 1, 2, 5, 10 or 50 mg/kg bw. The highest dose resulted in a 38% decrease in urine volume, which was associated with higher concentrations of sodium, potassium, chloride and creatinine and increased osmolality. However, the absolute amounts of each electrolyte and creatinine excreted were not significantly altered (Tust & Garrity, 1981).

#### *(c) Ototoxicity*

##### *(i) Cats*

In a non-GLP-compliant study, mongrel cats (two males and two females) received daily subcutaneous injections of 100 mg apramycin activity per kilogram body weight, approximately 1.5 hours after feed, for up to 30 days. The animals displayed anorexia and body weight loss, leading to early death or sacrifice of three cats on days 12, 16 and 25. Post-rotatory nystagmus gradually declined, from 23 to 9 seconds, in one cat, whereas the righting reflex was not affected in any cat. Pathological examination of the deceased cats revealed severe nephrosis in the kidneys (Worth et al., 1976f).

##### *(ii) Pigs*

In a non-GLP-compliant study, groups of five male and five female pigs (strain not specified) were fed diets containing 0 or 110 mg apramycin activity per kilogram for 8 weeks. Prior to commencement of dosing, the animals were conditioned to respond to an auditory stimulus to avoid an electrical shock. The response was unchanged in the treatment group (Anon, undated).

#### *(d) Pharmacology*

##### *(i) Isolated tissues*

The pharmacological activity of apramycin ($10^{-8}$ to $10^{-5}$ mol/l) was investigated in isolated preparations of guinea-pig ileum, trachea and atria and rat aorta, vas

deferens and estrogen-primed uterus. The smooth and cardiac muscle tissues were suspended in an organ bath with Kreb's bicarbonate solution at 37 °C (ileum, trachea, aorta, atria, vas deferens) or Jalon's solution at room temperature (uterus) and aerated with 95% oxygen and 5% carbon dioxide. The rate of spontaneously beating guinea-pig atria was slightly increased (4%) at an apramycin concentration of $10^{-5}$ mol/l (5.4 µg apramycin activity per millilitre), but agonist activity was not observed in any other tissue. At a concentration of $10^{-5}$ mol/l, apramycin slightly enhanced the response to carbamylcholine (cholinergic agonist) in guinea-pig ileum and slightly inhibited the response to phenylephrine (sympathomimetic $\alpha_1$ agonist) in rat aorta. The responses to histamine in the guinea-pig ileum, norepinephrine in rat vas deferens, oxytocin in rat uterus and isoproterenol in guinea-pig trachea were not changed, indicating that apramycin did not block the muscarinic, histamine $H_1$ or β-adrenergic receptors. These experiments were not GLP compliant (Ruffolo & Beckhelm, 1981).

*(ii) Rats*

In a non-GLP-compliant study, anaesthetized rats were given single doses of apramycin at 25 mg/kg bw intravenously or at 250 and 400 mg/kg bw by gavage. The twitch response of the anterior tibialis muscle, resulting from stimulation of the sciatic nerve, was unaffected at each dose, indicating no activity at the neuromuscular junction. The positive control was (+)-tubocurarine (Ruffolo, 1980).

*(iii) Dogs*

In a GLP-compliant study, anaesthetized dogs received intravenous infusions of apramycin at cumulative doses of 0, 4, 8, 12, 16 and 20 mg apramycin activity per kilogram body weight. Mean arterial blood pressure was increased at 8–20 mg/kg bw, but not at 4 mg/kg bw. Heart rate declined in saline controls but was maintained at doses of 8–20 mg/kg bw. These changes were abolished when animals were pretreated with either phenoxybenzamine (α-receptor antagonist) or propranolol (β-receptor antagonist), suggesting a minor sympathomimetic action for apramycin. There were no alterations in electrocardiogram, cardiac output, stroke work, vascular resistance, respiratory parameters or blood gases at any dose (Holland et al., 1981).

*(e) Microbiological effects*

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for apramycin (VICH, 2004). The decision-tree approach initially seeks to determine if there may be microbiologically active apramycin residues entering the human colon. If the answer is "no" to any of the first three steps, then no microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: 1) disruption of the colonization barrier and 2) increase of the population(s) of resistant bacteria. At Step 4 of the

decision-tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points. At Step 5, a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological or pharmacological ADI would be used. The Committee evaluated a GLP-compliant study of minimum inhibitory concentration (MIC) susceptibility testing and studies on the biological activity of apramycin residue and used the decision-tree to answer the following questions in the assessment of apramycin.

*Step 1: Are residues of the drug, and/or its metabolites, microbiologically active against representatives of the human intestinal microflora?*

Yes. Apramycin is microbiologically active against some bacterial genera and species representative of the human intestinal microflora. In radiolabel studies, no apramycin metabolites were identified.

The MIC of apramycin was determined against 100 bacterial strains, comprising 10 isolates from each of 10 groups of genera representing the normal intestinal microbiota: *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Bacteroides fragilis*, other *Bacteroides* species ("non-*fragilis* group"), *Fusobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Enterococcus* and *Escherichia coli* (Pridmore & Cheetham, 2011). All strains were sourced from faecal samples of healthy, unmedicated humans. Each volunteer experienced no symptoms of diarrhoea within the 4-week period preceding sample collection and no antimicrobial therapy during the 3-month period prior to sample collection. The test system was standardized agar dilution MIC methodology using quality control strains as described in the Clinical and Laboratory Standards Institute guidelines (CLSI, 2004).

Apramycin activity against each bacterial group is summarized in Table 3. $MIC_{50}$, $MIC_{90}$, geometric mean MIC and MIC range were calculated for each bacterial group. Apramycin activity was clearly shown against *Escherichia coli* ($MIC_{50}$ = 4 µg/ml). Apramycin exerted no measurable antibacterial activity (MIC values > 128 µg/ml) against *Bifidobacterium*, *Clostridium*, *Bacteroides fragilis* and "non-*fragilis*" *Bacteroides* strains. Apramycin exerted relatively poor activity against *Lactobacillus* ($MIC_{50}$ = 64 µg/ml), *Enterococcus* ($MIC_{50}$ = 32 µg/ml), *Fusobacterium* ($MIC_{50}$ = 16 µg/ml), *Eubacterium* ($MIC_{50}$ = 16 µg/ml) and *Peptostreptococcus* ($MIC_{50}$ = 16 µg/ml).

*Step 2: Do residues enter the human colon?*

Yes. A number of residue and bioavailability studies using [$^{14}$C]apramycin and analytical (high-performance liquid chromatography) and microbiological assay methods were conducted to detect apramycin in pigs, cattle and poultry. Muscle, kidney, fat and skin contain little or no apramycin-derived residues, regardless of the period between withdrawal of medication and slaughter, for the various routes of administration. However, apramycin residues were present at low levels in the liver. Therefore, apramycin residues may be present at low levels in meat products consumed by humans. Apramycin is poorly absorbed from the gastrointestinal tract; therefore, residues could enter the colon of a person ingesting edible tissues from treated animals.

***Table 3. Susceptibility of representative human intestinal bacteria to apramycin***

| Bacterial group | Summary of apramycin MIC parameters (μg/ml) | | | |
|---|---|---|---|---|
| | MIC range | $MIC_{50}$ | $MIC_{90}$ | Geometric mean MIC |
| *Bifidobacterium* spp. | All >128 | >128 | >128 | >128 |
| *Eubacterium* and related species | 4 to >128 | 16 | >128 | 26 |
| *Clostridium* spp. | All >128 | >128 | >128 | >128 |
| *Bacteroides fragilis* group | All >128 | >128 | >128 | >128 |
| *Bacteroides* (non-*fragilis* group) | 128 to >128 | >128 | >128 | >128 |
| *Fusobacterium* spp. | 2 to >128 | 16 | >128 | 34 |
| *Peptostreptococcus* spp. | 2 to >128 | 16 | 128 | 13 |
| *Lactobacillus* spp. | 32 to 128 | 64 | 64 | 60 |
| *Enterococcus* spp. | 32 to 128 | 32 | 64 | 37 |
| *Escherichia coli* | 4 to 8 | 4 | 4 | 4.3 |
| All strains (*n* = 100) | 2 to >128 | 128 | >128 | 44 |

*Step 3: Do the residues entering the human colon remain microbiologically active?*

Yes. As the studies submitted for evaluation did not contain measurements of the amounts of drug residue in the intestinal tract, the Committee used the pharmacokinetic and bioavailability studies to determine the fraction of oral dose available to the human intestinal microflora. Pharmacokinetic and bioavailability studies indicated that apramycin is poorly absorbed after oral administration. A considerable amount of the administered apramycin was detected as unmetabolized parent compound in faecal samples. No metabolites were identified in any of the food animals dosed with apramycin. Therefore, apramycin residues entering the human colon will remain microbiologically active.

*Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern, i.e. disruption of the colonization barrier or resistance development?*

Yes. The Committee noted that there is scientific justification to eliminate resistance development as an end-point of concern. However, there is the potential for an adverse effect on disruption of the colonization barrier of the human gastrointestinal tract, as apramycin residues do occur in chicken, pigs, sheep, calves and rabbits, and, if edible tissues are ingested, they could have an impact on the intestinal microbiota. Furthermore, diarrhoea occurred in toxicity studies in mice, rats, guinea-pigs, rabbits and dogs after oral administration of apramycin, suggesting adverse effects of apramycin on the intestinal microbiota.

In terms of antimicrobial resistance, results from microbiological studies indicate that resistance was shown to occur in various bacteria isolated from animals treated with apramycin (Mortensen et al., 1996). Although apramycin is solely used in veterinary medicine, cross-resistance to other aminoglycosides has been documented (Pohl et al., 1993; Johnson et al., 1994; Yates et al., 2004; Jensen et al., 2006). Apramycin is recommended for the treatment of intestinal bacterial infections in animals, including colibacillosis. Apramycin-resistant *E. coli* have been isolated from farm animals and farm workers (Zhang, Ding & Fan, 2009).

Therefore, apramycin could modify the intestinal microbiota by creating a selective pressure favouring the development of apramycin-resistant bacteria. However, as the majority of apramycin residue levels detected in target tissues were below the lowest $MIC_{50}$ value of any of the representative human intestinal microbiota listed in Table 3 and the clinical breakpoint for *E. coli* is 32 µg/ml, it is unlikely that the development of resistance to apramycin residues and cross-resistance to commonly used aminoglycosides in veterinary and human medicine would occur.

*Step 5: Derivation of a microbiological ADI using the VICH GL36 approach*

The formula for calculating the microbiological ADI from in vitro (MIC) data is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

*$MIC_{calc}$*: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC) ($MIC_{calc}$) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean $MIC_{50}$ for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the $MIC_{calc}$ were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a $MIC_{50}$ of 32 µg/ml or less (i.e. *Escherichia coli*, *Enterococcus*, *Fusobacterium*, *Peptostreptococcus* and *Eubacterium* species), the $MIC_{calc}$ is 8.3 µg/ml.

*Mass of colon content*: A value of 220 g is based on the colon content measured from humans.

*Fraction of oral dose available to microorganisms*: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be

lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Apramycin is poorly absorbed and is excreted in faeces primarily in unchanged form; therefore, the value is 1.

*Body weight*: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for apramycin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{8.3\ \mu g/ml \times 220\ g}{1 \times 60\ kg\ bw}$$

$$= 30.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–30 µg/kg bw (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing.

### *2.3 Observations in humans*

Apramycin has not been used in the treatment of humans. No data could be identified relating to effects in humans.

## *3. COMMENTS*

In addition to a submission from the sponsor, a literature search was conducted, which identified only one further study report. The Committee considered the results of studies on pharmacokinetics, acute, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, pharmacology, ototoxicity, kidney toxicity and microbiological safety. A number of critical studies, namely the 2-year studies in mice and rats, the multigeneration reproductive toxicity study in rats, two of the four genotoxicity studies and the microbiology studies, were carried out according to appropriate standards. The majority of the other studies were performed prior to the establishment of standards for study protocol and conduct.

### *3.1 Biochemical data*

In a preliminary study in rats, an oral dose of apramycin was poorly absorbed from the gastrointestinal tract, and approximately 99.5% was excreted unchanged in the faeces. Primary data were not supplied for this study.

In 6-month and 12-month toxicity studies in dogs given daily oral doses, absorption was low and was proportional to the dose. Essentially no apramycin was detected in serum at 24 hours, and there was no evidence of accumulation following repeated administration. Between 0.3% and 10.5% of the administered dose was excreted in urine, which represents the absorbed fraction. The primary route of excretion was in the faeces.

### *3.2 Toxicological data*

Apramycin sulfate exhibited low acute toxicity by the oral route, which was probably a reflection of low absorption. The $LD_{50}$ values (as apramycin activity)

were greater than 4000 mg/kg bw in mice and rats, greater than 1250 mg/kg bw in guinea-pigs, greater than 832 mg/kg bw in rabbits and greater than 520 mg/kg bw in dogs. High doses in mice, rats, guinea-pigs, rabbits and dogs resulted in gastrointestinal effects that were presumably related to antimicrobial activity on the intestinal microflora. Renal injury was also observed in guinea-pigs. In mice and rats, single intravenous doses of apramycin base were more toxic than oral doses, and animals showed signs of central nervous system toxicity and renal damage.

In a 3-month study in which mice were fed diets containing 0, 5000, 10 000, 20 000 or 50 000 mg of apramycin activity per kilogram, dietary assays generally revealed apramycin concentrations well below the target level. Virtually no apramycin was present in the feed containing a nominal concentration of 10 000 mg/kg. Alopecia was observed in males given 20 000 and 50 000 mg/kg feed. Body weight gain was reduced in both sexes given 50 000 mg/kg feed and in males given 20 000 mg/kg feed. Slightly decreased lymphocyte counts and increased neutrophil counts were observed at the highest dose. Absolute and relative liver and kidney weights were lower in males and females given 50 000 mg/kg feed. Because of the uncertainty in the administered doses, a NOAEL could not be determined.

In a 1-month study, rats were fed diets containing 0, 10 000, 25 000 or 50 000 mg apramycin activity per kilogram. During the last week of the study, rats from the 25 000 and 50 000 mg/kg feed groups excreted dark to black faeces. Body weight gain was depressed and blood glucose and blood urea nitrogen were increased in males and females given 50 000 mg/kg feed. Also at this dose level, there were slight decreases in serum levels of creatinine in females and in alkaline phosphatase activity in males. Haematological parameters were not examined, and only limited pathology was undertaken. The caeca of all high-dose rats were 2–3 times normal size. Because only a limited range of parameters was investigated, a NOAEL was not determined.

In a 3-month study, rats were fed diets containing 0, 200, 400 or 1000 mg apramycin sulfate per kilogram. Another group received apramycin sulfate in the drinking-water at a concentration of 10 mg/ml. Mild diarrhoea in the drinking-water group was the only finding. The NOEL was the highest dietary dose of 1000 mg apramycin sulfate per kilogram feed, equal to 26 mg of apramycin activity per kilogram body weight per day.

In another 3-month study, rats were fed diets containing 0, 1800, 2750, 6200 or 10 000 mg apramycin activity per kilogram. Feed consumption in all treated groups and body weight gains in females given 6200 and 10 000 mg/kg feed were slightly elevated, but not in a dose-related manner. Mild nephrosis was observed in some male rats at the highest dose. Based on kidney toxicity, the NOAEL was 6200 mg/kg feed, equal to 460 mg of apramycin activity per kilogram body weight per day.

In a 6-month study, rats were fed diets containing 0, 1000, 2500 or 5000 mg apramycin activity per kilogram. Darker, softer faeces with increased moisture were found in all treated groups in a dose-related manner and were associated with a concomitant increase in water intake. Erythrocyte counts, haemoglobin and haematocrit were lower in males given 5000 mg/kg feed for 1 month, but not at

the end of the study. Serum glutamate dehydrogenase activity was increased in animals given 5000 mg/kg feed for 6 months. Absolute and relative kidney weights were lower in high-dose males. Based on haematological and biochemical findings, the NOAEL was 2500 mg/kg feed, equal to 170 mg apramycin activity per kilogram body weight per day.

In a 3-month study, dogs were administered 0, 5, 10 or 25 mg apramycin sulfate per kilogram body weight per day in capsules. There were no treatment-related effects. The NOEL was the highest dose of 25 mg apramycin sulfate per kilogram body weight per day, equal to 13 mg apramycin activity per kilogram body weight per day.

In a 6-month study, dogs received oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Body weight gain was lower in both sexes given 100 mg/kg bw per day. One dog out of eight at the high dose had decreased appetite and developed anorexia; as a result of weight loss and weakness, this animal was killed. During months 3–5, one dog of eight given 50 mg/kg bw per day and four dogs of eight given 100 mg/kg bw per day did not respond to noise stimuli, but all responded at 6 months. Soft stools occurred in all treated dogs, which is likely to be a consequence of an effect on the bacterial flora rather than a toxic effect in the dog. Erythrocyte counts were consistently lower in both sexes during treatment with 50 and 100 mg/kg bw per day, but not always in a dose-related manner and rarely statistically significantly; consequently, they were considered unrelated to treatment. Based on reduced body weight gain, the NOAEL was 50 mg apramycin activity per kilogram body weight per day.

In a 1-year study, dogs were given oral doses of 0, 25, 50 or 100 mg apramycin activity per kilogram body weight per day in capsules. Adrenal weights were increased in males at the highest dose, but were not associated with pathological alterations. The NOAEL was the highest dose of 100 mg apramycin activity per kilogram body weight per day.

In a long-term study in which mice were fed diets containing 0, 1500, 5000, 15 000 or 45 000 mg apramycin activity per kilogram for 2 years, body weight gains were lower in males and females given 5000 mg/kg feed and above. At a dose of 45 000 mg/kg feed, haemoglobin and haematocrit were increased in both sexes, erythrocyte counts were increased in females and slightly decreased lymphocyte counts and increased neutrophil counts were observed in males. Serum alkaline phosphatase activity was increased in females given 5000 mg/kg feed and higher. Serum glucose level was decreased and blood urea nitrogen was increased in both sexes at the highest dose. Cytoplasmic basophilia involving the renal cortical tubular epithelium was observed in both sexes at doses of 5000 mg/kg feed and higher. The incidences of tumours were not increased. Based on reduced body weight gain and kidney pathology, the NOAEL was 1500 mg/kg feed, equal to 189 mg apramycin activity per kilogram body weight per day. There was no evidence for carcinogenicity in mice.

In a study in which rats were given diets containing 0, 2500, 5000, 10 000 or 50 000 mg apramycin activity per kilogram for 2 years, body weight gains and liver and kidney weights were reduced in both sexes at the highest dose. The incidences

of tumours were not influenced by treatment. Based on reduced body weight gain and reduced organ weights, the NOAEL was 10 000 mg/kg feed, equal to 488 mg apramycin activity per kilogram body weight per day. There was no evidence for carcinogenicity in rats.

Apramycin was evaluated for potential genotoxicity in two in vitro assays for the induction of gene mutations in strains of *Salmonella typhimurium* and a strain of *Escherichia coli*, an in vitro assay for the induction of gene mutations in L5178Y mouse lymphoma cells and an in vitro assay for the induction of DNA repair in primary cultures of rat hepatocytes. The results were negative in all cases.

Because the carcinogenicity and genotoxicity studies were negative, the Committee concluded that apramycin is unlikely to pose a carcinogenic risk to humans.

In a multigeneration reproductive toxicity study, rats were fed diets containing 0, 2500, 5000 or 10 000 mg apramycin activity per kilogram continuously over four generations. No adverse effects on fertility and reproductive indices or on growth and survival of offspring were found. The NOEL was the highest dose of 10 000 mg/kg feed, equal to 785 mg apramycin activity per kilogram body weight per day.

In a developmental toxicity study, pregnant female rats were given a gavage dose of 0, 250, 500 or 1000 mg apramycin activity per kilogram body weight per day on gestation days 6–15. There were no effects on maternal toxicity, fetal growth and development or the incidences of fetal abnormalities. The NOEL was the highest dose of 1000 mg apramycin activity per kilogram body weight per day.

In another developmental toxicity study, pregnant female rabbits were given a gavage dose of 0, 2, 8 or 32 mg apramycin activity per kilogram body weight per day on gestation days 6–18. Maternal feed consumption and body weight gains were markedly reduced, and the numbers of animals aborting were increased, in a dose-related manner. Most of the dams aborting showed an empty gastrointestinal tract. The number of resorptions was increased at 32 mg/kg bw per day. Fetal body weight was depressed in a dose-related manner, and the incidence of bilateral 13th ribs was increased at the highest dose. The incidences of fetal malformations and external and visceral abnormalities were unaffected. The maternal toxicity was likely related to the particular sensitivity of the gut flora in rabbits given certain antimicrobial agents (FAO/WHO, 2009), and therefore it was not possible to identify a NOEL. The fetal toxicity was probably secondary to the severe toxicity in the dams.

In a special study to assess renal toxicity in rats, urine volume was decreased (38%) by a single oral apramycin sulfate dose of 50 mg/kg bw. However, the absolute amounts of urinary electrolytes and creatinine excreted were unchanged. Other special studies indicated that ototoxicity and intrinsic pharmacological activity would not be expected.

No data could be identified relating to effects in humans.

The most relevant study for determining a toxicological ADI was the 6-month study in dogs. The NOAEL was 50 mg/kg bw per day, based on reduced body

weight gain. A safety factor of 100 was considered appropriate. Therefore, an ADI of 0–0.5 mg/kg bw could be established on the basis of the toxicological data.

### *3.3 Microbiological data*

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with VICH GL36 was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for apramycin. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated. Apramycin was active against *Escherichia coli* ($MIC_{50}$ = 4 µg/ml). Apramycin exerted no measurable antibacterial activity (MIC values > 128 µg/ml) against *Bifidobacterium*, *Clostridium*, *Bacteroides fragilis* and "non-*fragilis*" *Bacteroides* strains. Apramycin exerted relatively poor activity against *Lactobacillus* ($MIC_{50}$ = 64 µg/ml), *Enterococcus* ($MIC_{50}$ = 32 µg/ml), *Fusobacterium* ($MIC_{50}$ = 16 µg/ml), *Eubacterium* ($MIC_{50}$ = 16 µg/ml) and *Peptostreptococcus* ($MIC_{50}$ = 16 µg/ml).

Apramycin residues may be present at low levels in meat products consumed by humans; therefore, apramycin-related residues could enter the colon of a person ingesting edible tissues from treated animals. As the data submitted for evaluation did not contain measurements of the amount of drug residue in the intestinal tract, the Committee used the pharmacokinetic studies to determine the fraction of the oral dose available to the human intestinal microbiota. Apramycin was poorly absorbed after oral administration in animals, and a considerable amount of the administered apramycin was detected as unmetabolized parent compound in faecal samples. Therefore, apramycin residues entering the human colon will remain microbiologically active.

There is potential for disruption of the colonization barrier of the human gastrointestinal tract. Diarrhoea occurred in some toxicity studies in animals after oral administration of apramycin, suggesting adverse effects of apramycin on the intestinal microbiota. Because the majority of apramycin residue levels detected in target tissue were below the lowest $MIC_{50}$ of any of the representative human intestinal microbiota tested and the clinical breakpoint for *E. coli* is 32 µg/ml, it is unlikely that the development of resistance to apramycin residues would occur.

The formula for calculating the microbiological ADI is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

*$MIC_{calc}$*: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC ($MIC_{calc}$) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean $MIC_{50}$ for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the $MIC_{calc}$ were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a $MIC_{50}$ of 32 µg/ml or

less (i.e. *Escherichia coli*, *Enterococcus*, *Fusobacterium*, *Peptostreptococcus* and *Eubacterium* species), the $MIC_{calc}$ is 8.3 µg/ml.

*Mass of colon content*: A value of 220 g is based on the colon content measured from humans.

*Fraction of oral dose available to microorganisms*: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Apramycin is poorly absorbed and is excreted in faeces primarily in unchanged form; therefore, the value is 1.

*Body weight*: The body weight of an adult human is assumed to be 60 kg.

Therefore, the upper bound of the microbiological ADI for apramycin is calculated as indicated below:

$$\text{Upper bound of ADI} = \frac{8.3\ \mu g/ml \times 220\ g}{1 \times 60\ kg\ bw}$$

$$= 30.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–30 µg/kg bw (rounded to one significant figure) could be derived from in vitro MIC susceptibility testing.

## 4. EVALUATION

The Committee considered that microbiological effects were more appropriate than toxicological effects for the establishment of an ADI for apramycin. Therefore, the Committee established an ADI of 0–30 µg/kg bw on the basis of the data for disruption of the colonization barrier.

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# *DERQUANTEL*

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## 1. EXPLANATION

Derquantel (Chemical Abstracts Service No. 187865-22-1), also known as 2-desoxoparaherquamide, 2-deoxyparaherquamide, 2-DOPH, PF-520904 and PNU-141962, is a semi-synthetic spiroindole characterized by an indole or oxindole moiety fused with a cyclopentyl ring at position C-3 of the indole (Figure 1) (Lee, Clothier & Johnson, 2001; Lee et al., 2002; Little et al., 2011). Derquantel is a broad-spectrum anthelminthic agent with activity against the adult and larval stages of gastrointestinal nematodes in sheep.

Derquantel acts as a nicotinic cholinergic receptor antagonist. Its mode of action is blockage of cation channels in nematode muscle cell membranes.

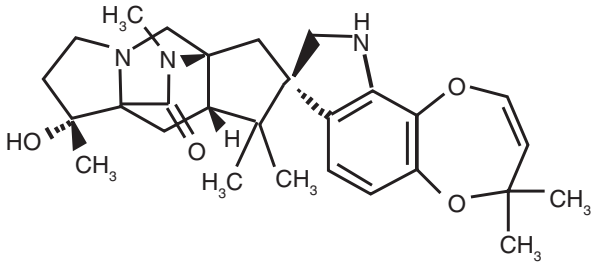
When used as an anthelminthic agent for sheep, derquantel is combined with abamectin to control infections by nematodes that are resistant to macrocyclic lactone-, levamisole-, benzimidazole- and closantel-based drenches (and combinations of these). The combination product is composed of derquantel at a concentration of 10 mg/ml and abamectin at a concentration of 1 mg/ml. It is administered to sheep and lambs 3–4 times per year as a single-dose oral drench at a dose rate of 2 mg/kg body weight (bw) for derquantel and 0.2 mg/kg bw for abamectin.

Abamectin was previously evaluated by the Committee at its forty-seventh meeting (Annex 1, reference *125*), at which maximum residue limits (MRLs) for cattle fat, liver and kidney were proposed. An acceptable daily intake (ADI) of 0–1 µg/kg bw was established by the 1995 Joint FAO/WHO Meeting on Pesticide Residues for abamectin used as a veterinary drug (FAO/WHO, 1996).

Derquantel has not previously been evaluated by the Committee. Derquantel was placed on the agenda of the current meeting at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011). The Committee was asked to establish an ADI and recommend MRLs for derquantel in sheep tissues.

The Committee considered the results of studies on receptor pharmacology, pharmacokinetics, acute and short-term toxicity, in vitro, in vivo and in silico genotoxicity, reproductive toxicity and microbiological safety in humans. The majority of the studies were performed in accordance with good laboratory practice (GLP) standards. In addition, a literature search in Embase database was performed using the keywords derquantel, abamectin, Startect, toxicity, tolerance, mechanism, human, animal, mice, mouse, rat, dog, rabbit, monkey, guinea pig, sheep, in vivo, in

***Figure 1. Chemical structure of derquantel, a spiroindole anthelminthic***




vitro, toxic metabolites and paraherquamide. No additional information relevant to this evaluation was identified.

## *2. BIOLOGICAL DATA*

### *2.1 Biochemical aspects*

#### *2.1.1 Absorption, distribution and excretion*

*(a) Rats*

In a study that was conducted in compliance with GLP, male and female Sprague-Dawley rats (three of each sex per group) were dosed by oral gavage once daily with vehicle control (1% carboxymethylcellulose) or derquantel at 200 mg/kg bw per day for 4 days. Blood samples were collected from each animal at 0.5, 1, 2 and 24 hours post-dosing on day 4. The peak plasma concentrations ($C_{max}$), 15.7 and 22.3 µg/ml, occurred at 2 hours post-dosing ($T_{max}$) for both male and female rats, respectively (Coffing, 2008).

Male and female Sprague-Dawley rats (one of each sex per group) were given a single oral administration of [$^{14}$C]derquantel at a dose rate of 100 mg/kg bw (14.8 MBq/kg bw) in a non-GLP-compliant study. Urine and faeces were collected for 7 days post-dosing, and blood and tissues were sampled at 7 days post-dosing and analysed for total radioactivity content. Ninety-five per cent of the radioactive dose was recovered in urine and faeces. Approximately 87–90% of the dose was excreted in the faeces during the 7-day post-dosing period. Faecal excretion of radiolabel in the male rat was 37.5% of the administered dose at day 1 and 47.2% at day 2. The female rat had 0.005% and 67.0% of the administered dose in the day 1 and day 2 faecal samples, respectively. Urinary excretion of radiolabel was low, with total recoveries of 5.5% and 7.6% in the male and female rats, respectively. Negligible amounts of the dose were detected in the carcass and terminal blood samples. Overall, derquantel was excreted mainly in the faeces in 2 days after oral administration and may not be distributed in tissues (Marsh, 2006).

In a GLP-compliant study, Sprague-Dawley rats were dosed orally with [$^{14}$C]-derquantel at 50 mg/kg bw per day to identify metabolite profiles in urine, faeces,

bile and tissues. Four male and four female rats were dosed daily for 7 days with a single oral administration of [$^{14}$C]derquantel. Urine and faeces were collected 24 hours after the first dose and until 6 hours after the seventh dose, and liver, kidney, muscle and fat were sampled at 6 hours after the seventh dose. In a second group, bile duct–cannulated rats (one of each sex) were given a single oral administration of [$^{14}$C]derquantel at 50 mg/kg bw, and then bile, urine and faeces were collected up to 48 hours post-dosing, and liver, kidney, muscle and fat were obtained at 48 hours post-dosing. The majority of the radioactive dose (69–75%) was recovered in faeces, with small amounts, 3–4% and 1%, recovered in urine and tissues, respectively. In bile duct–cannulated rats, the majority of the radioactive dose was recovered in the bile (64–70%). This supports a high oral bioavailability of derquantel in rats and the high percentage of dose excreted in the faeces via biliary elimination (Wu & Liu, 2008).

In a 90-day dosing study performed under GLP conditions, derquantel was administered to Sprague-Dawley rats by oral gavage once daily at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day for 3 months. Blood samples were collected from two males and two females 1 hour after dosing on days 0, 7, 14, 21, 29, 61 and 90. The mean concentrations of derquantel in plasma across the entire treatment period showed a dose-dependent increase: 118.3, 317.5, 2127 and 5272 ng/ml at 1, 5, 50 and 150 mg/kg bw per day, respectively. There was no accumulation of derquantel with time (Goldenthal, 2006).

Male and female Sprague-Dawley rats were given derquantel orally by gavage once daily at 0, 1, 5 or 50 mg/kg bw per day for 1 year under GLP conditions. Plasma samples were collected from male and female rats (three of each sex per time point per dose group) at 0.5, 1, 2, 4, 8 and 12 hours post-dosing on days 1, 178 and 365 and 2 hours post-dosing on days 90 and 270. The area under the plasma concentration–time curve ($AUC_{0–t(last)}$) for both sexes ranged from 179 to 182 ng·h/ml at 1 mg/kg bw per day and increased to 2150–4000 ng·h/ml at 5 mg/kg bw per day and 33 300–50 000 ng·h/ml at 50 mg/kg bw per day. Mean peak plasma concentrations ($C_{max}$) ranged from 82.0 to 109 ng/ml at 1 mg/kg bw per day and increased dose dependently. The time to $C_{max}$ ($T_{max}$) occurred at 0.5 hour at 1 and 5 mg/kg bw per day and ranged from 0.5 to 2 hours at 50 mg/kg bw per day. Females showed a tendency towards higher plasma concentrations of derquantel throughout the exposure period. There was no accumulation of derquantel with time (Yancey & Grover, 2008).

Derquantel was administered to male and female Sprague-Dawley rats (nine of each sex per group) by oral gavage at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day for 1 year in a GLP-compliant study. Plasma samples were collected on days 1, 84 and 365 at 0, 1, 2 and 4 hours post-dosing from three animals of each sex per time point. $AUC_{0–t(last)}$ and $C_{max}$ increased with dose dependency, and derquantel appeared to accumulate at all doses, with a concentration on day 365 that was 2–3 times higher than on days 1 and 84 at the highest dose. The plasma $T_{max}$ occurred at 1–2 hours post-dosing for all dose groups. Exposure as measured by AUC and $C_{max}$ was consistently higher in females than in males (Bonnette, 2009b).

In a two-generation reproduction study performed in compliance with GLP, male and female Sprague-Dawley rats were given derquantel by oral gavage once

daily at 0, 1, 5 or 25 mg/kg bw per day through two generations. Mean plasma derquantel concentrations on day 50, 2 hours post-dosing, in the $P_1$ generation (adult females) were 44.1, 525 and 2240 ng/ml at 1, 5 and 25 mg/kg bw per day, respectively. Mean plasma derquantel concentrations on day 76, 2 hours post-dosing, in the $F_1$ generation of adult females were 59.1, 414 and 2230 ng/ml at 1, 5 and 25 mg/kg bw per day, respectively. Mean plasma derquantel concentrations in the $F_1$ and $F_2$ generation pups ranged from 0.01 to 2.69 ng/ml on lactation day 4 and from less than 0.050 to 0.622 ng/ml on lactation day 21 across the dose ranges. In general, mean plasma derquantel concentrations were similar in the $P_1$ and $F_1$ generation adult female rats and in the $F_1$ and $F_2$ generation lactating pups (Barnett, 2009).

Ten male and 10 female Sprague-Dawley rats were dosed once daily by oral gavage for 7 days with the combination product containing a 10:1 ratio of derquantel and abamectin, at doses of 0/0, 1/0.1, 2/0.2, 10/1 and 20/2 mg/kg bw per day. Plasma samples were collected from four animals of each sex per time point per dose group at 0, 2, 6 and 24 hours post-dosing on days 1 and 7. Plasma concentrations of derquantel and abamectin increased with increasing doses of the combination product. Derquantel appeared not to accumulate, based on no difference in $AUC_{0-t(last)}$ and $C_{max}$ values on day 7 compared with day 1. However, abamectin showed accumulation, with increased $AUC_{0-t(last)}$ and $C_{max}$ values on day 7 compared with day 1. Greater derquantel concentrations in female rats were noted at all time points for all doses on both days 1 and 7 when compared with male rats. This trend was not noted for abamectin. $T_{max}$ occurred mainly at 2 hours post-dosing for derquantel and 6 hours post-dosing for abamectin across the dose range (May, 2009d).

*(b) Rabbits*

In a GLP-compliant study, three female New Zealand White rabbits per treatment group were given a dose of derquantel by stomach tube once daily on days 7 through 19 of presumed gestation at 0.1, 1 and 10 mg/kg bw per day. Plasma samples were collected on days 7 and 19 of gestation at 0 hour (prior to dosing) and at 0.5, 2, 4, 8 and 24 hours post-dosing. $AUC_{0-t(last)}$ and $C_{max}$ increased with dose. $T_{max}$ was observed at 0.5 hour, with the exception of the high-dose group on day 7, for which $T_{max}$ occurred at 0.5–2 hours post-dosing (Lewis, 2007).

*(c) Dogs*

In a non-GLP-compliant study, derquantel was administered daily as a single oral dose via capsule to two male and two female Beagle dogs at 1, 5 or 10 mg/kg bw per day for 28 days. Plasma samples were collected at 0, 0.5, 1, 2, 4, 12 and 24 hours post-dosing on days 0 and 27, but only at 2 hours post-dosing on day 14. Plasma derquantel concentrations from the male and female dogs were not different. $AUC_{0-24}$ and $C_{max}$ increased at a rate greater than proportional with dose and indicated accumulation on day 27, only in the highest-dose group. $T_{max}$ ranged from 0.5 to 2.25 hours, and the measured half-life was from 2.24 to 5.88 hours (Savides, 2006).

Derquantel was administered orally to Beagle dogs for 1 month in a GLP-compliant study. Four dogs of each sex per dose received derquantel once daily by oral gavage for 28 days at 0.01, 0.03 or 0.1 mg/kg bw per day. An additional group of two males and two females was dosed 3 times at 0.01 mg/kg bw for a total daily dose of 0.3 mg/kg bw per day. Plasma samples were collected at 0, 0.5, 1, 3, 8 and 24 hours post-dosing on days 3 and 26. There were no observable differences in plasma derquantel $AUC_{0-t(last)}$ or $C_{max}$ values between male and female dogs. The mean plasma derquantel $AUC_{0-t(last)}$ increased from 0.0935 to 1.60 ng·h/ml on day 3 and from 0.0494 to 1.37 ng·h/ml on day 26 with doses ranging from 0.01 to 0.1 mg/kg bw per day. The mean plasma derquantel $C_{max}$ showed a similar trend across dose groups between day 3 and day 26. There was no accumulation noted in any dose group for either $AUC_{0-24}$ or $C_{max}$. $T_{max}$ occurred primarily at 0.5 hour post-dosing in the majority of subjects (May, 2009a).

In a GLP-compliant study, derquantel was administered daily as a single oral dose via capsule to each of four male and four female Beagle dogs at a dose of 0.1, 0.5, 1 or 10 mg/kg bw per day for 3 months. Plasma samples were collected at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post-dosing on days 0 and 90, but only at 2 hours post-dosing on days 15, 30, 45, 60 and 75. The $C_{max}$ values for derquantel indicated no accumulation over the duration for doses of 0.1, 0.5 and 1 mg/kg bw per day; at 10 mg/kg bw per day, however, the $C_{max}$ on day 90 was approximately 10–80 times greater than on day 0. The $AUC_{0-24}$ showed modest increases between days 0 and 90 in the three lowest-dose groups (1.4- to 1.8-fold increases), with an increase of 15-fold for the highest-dose group. $T_{max}$ was generally between 0.5 and 2 hours in the three lowest-dose groups on days 0 and 90, but ranged from 2.5 to 8.9 hours in the highest-dose group. Half-lives generally increased with dose; however, all were relatively short, less than 7 hours (Savides, 2008).

Derquantel was administered daily as a single oral dose via oral gavage to male and female Beagle dogs (four of each sex per group) at a dose of 0.1, 0.5, 1 or 5 mg/kg bw per day for 3 months in a GLP-compliant study. Plasma samples were collected at 0, 0.5, 1, 3, 8 and 24 hours post-dosing on days 3, 30, 61 and 91. Mean plasma derquantel $AUC_{0-t(last)}$ and $C_{max}$ tended to be higher in males than in females in all dose groups and on all study days. Mean plasma derquantel $AUC_{0-t(last)}$ on study day 3 in males ranged from 1.77 to 1750 ng·h/ml as the dose increased from 0.1 to 5 mg/kg bw per day. A similar trend was found in the $AUC_{0-t(last)}$ for females. On study day 3, the mean plasma derquantel $C_{max}$ in males and females indicated a 194-fold and 269-fold increase over the dose range from 0.1 to 5 mg/kg bw per day. There was no trend in accumulation for the duration of the study. Plasma derquantel $T_{max}$ was 0.5 hour most frequently at doses up to 1 mg/kg bw per day and 1–3 hours at 5 mg/kg bw per day (May, 2009b).

*(d) Horses*

One female and one castrated male horse were administered a single oral dose of derquantel at 2 mg/kg bw via nasogastric intubation as a 0.5% hydroxymethylcellulose suspension. Plasma samples were collected at 0, 1, 2, 4, 8, 24 and 48 hours post-dosing. Maximum concentrations of derquantel in plasma ($C_{max}$) of 0.157 and 0.075 µg/ml for the male and female horse, respectively, were

seen at 1 hour ($T_{max}$). This corresponded with the onset of severe adverse reactions in both horses at 1–2 hours post-dosing. AUC values of 0.48 µg·h/ml and 0.27 µg·h/ml were calculated for the male and female horse, respectively. A mono-hydroxylated metabolite of derquantel, previously not seen in other species, was identified at higher apparent plasma concentrations than for the parent derquantel (Seaman, 2000b).

### *2.1.2 Biotransformation*

One male and one female Sprague-Dawley rat were given a single oral administration of [$^{14}$C]derquantel at a dose of 100 mg/kg bw (14.8 MBq/kg bw) in a non-GLP-compliant study. Urine and faeces were collected for 7 days post-dosing and analysed for metabolism/degradation by high-performance liquid chromatography–mass spectrometry. Only 13% of the parent drug was found in urine and 3% in faecal samples at 24 hours post-dosing. The formation of approximately 11 metabolites or degradation products was observed during this time interval. Some of the molecular weights of the metabolites were indicative of normal metabolism products of glucuronidation and hydroxylation (Marsh, 2006).

In a GLP-compliant study, Sprague-Dawley rats were dosed orally with [$^{14}$C]derquantel at 50 mg/kg bw to identify metabolite profiles in urine, faeces, bile and tissues. Metabolic profiles found in urine, faeces and bile indicated a high degree of biotransformation to a large number of metabolites. Metabolite profiles in males and females were similar, although the metabolic rate was higher in males than in females. Small amounts of parent derquantel along with a large number of metabolites were found in faeces at the 144- to 150-hour collection interval, whereas parent drug was found mainly at the 0- to 24-hour collection interval. The metabolite profiles became increasingly complex between the 0- to 6-hour sample and the 24- to 48-hour samples. Parent derquantel and metabolites M1, M2, M4 and M5 were the most common components found in the tissues. In rat liver, derquantel comprised 6–8% of the total radioactivity at 6 hours following the final dose. The metabolite profiles were similar between males and females, except that metabolite M2 comprised 21% of the total profile in male liver and only 2% in female liver. Compared with liver, rat kidney contained higher percentages of derquantel, 21% in males and 40% in females. Metabolite M2 comprised a higher percentage of the profile in the kidney in males (11%) than in females (3%). In rat muscle and fat, derquantel comprised higher percentages of the total residues in tissues of females (21% and 53%, respectively) relative to the males (5% and 31%, respectively) (Wu & Liu, 2008).

In sheep, [$^{14}$C]derquantel was extensively metabolized. Parent derquantel and metabolites M1, M2, M4 and M5 were the most common components found in the tissues. The majority of metabolites found in tissues were less polar (longer retention times) than the parent (Wu & Liu, 2008).

A GLP-compliant in vitro metabolism study was conducted to determine the degree of metabolism and profiles of metabolites of derquantel in liver microsomes of rat, sheep, dog and human. [$^{14}$C]Derquantel (1 µmol/l) was incubated in triplicate with each of rat, sheep, dog and human liver microsomes at 37 °C for 1 hour in the presence of reduced nicotinamide adenine dinucleotide phosphate (1 mmol/l)

and magnesium dichloride (2 mmol/l). 7-Ethoxycoumarin (100 µmol/l) was also incubated with each of the liver microsomes as a positive control. Microsomal incubation samples were analysed by high-performance liquid chromatography to determine the $^{14}C$ metabolite profiles. All microsomal preparations used were determined to be enzymatically active, as 55.0%, 67.6%, 100.0% and 75.0% of 7-ethoxycoumarin were metabolized to mainly 7-hydroxycoumarin and other metabolites by rat, sheep, dog and human liver microsomes, respectively, after 1 hour. Derquantel was metabolized extensively in dog liver microsomes, as only 2.8% of the radioactive derquantel was detected after a 1-hour incubation. Derquantel was metabolized moderately by rat, sheep and human liver microsomes, as 45.2%, 39.8% and 55.0% of the radioactive derquantel were detected, respectively, after 1 hour. Unchanged [$^{14}C$]derquantel and 18 radioactive components were detected in rat, sheep, dog and/or human liver microsomal incubations. M3, M4, M5a and M5b were the predominant metabolites in dog liver microsomes, comprising 40.1%, 8.3%, 8.7% and 7.6% of the radioactivity. Rat, sheep and human liver microsomes presented a similar pattern of metabolites; M1, M9 and M10 were the major metabolites, accounting for 3.6–12.9% of the radioactivity, but M8 was also a major metabolite in rats, comprising 14.0% of the radioactivity (Ma, 2006a).

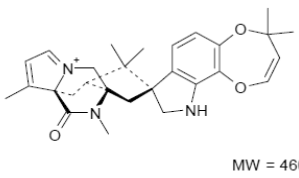
In another GLP-compliant study, the metabolism of derquantel was evaluated in hepatocytes of rat, sheep, dog and humans. [$^{14}C$]Derquantel (1 µmol/l) was incubated with cryopreserved hepatocytes from rats, dogs and humans and freshly prepared hepatocytes from sheep for 4 hours. Non-radiolabelled 7-ethoxycoumarin and 7-hydroxycoumarin were included as positive controls. The cellular viability was 36.4% for the rat hepatocytes and 70.0% for the sheep, dog and human hepatocytes at the 4-hour incubation. The prepared hepatocytes were determined to be enzymatically active, as 35.4%, 94.6%, 45.8% and 29.5% of 7-ethoxycoumarin and 18.5%, 100%, 96.3% and 90.4% of 7-hydroxycoumarin were metabolized by rat, sheep, dog and human hepatocytes, respectively. After a 4-hour incubation, unchanged derquantel comprised approximately 18.7%, 25.1%, 14.3% and 51.0% of the radioactivity in rat, sheep, dog and human hepatocytes, respectively. Unchanged [$^{14}C$]derquantel and 26 radioactive metabolites were detected in rat, sheep, dog and/or human hepatic incubations. M1, M8, M10, M12 and M19/20 were detected commonly as the major metabolites in rat, sheep, dog and/or human hepatic incubations, with varying compositions. M18 was one of the major metabolites, accounting for 7.6% and 7.7% of the radioactivity in rat and human hepatocytes, respectively. M16/17 was another major metabolite, detected at 7.8% in rat hepatocytes. M15 was a predominant metabolite, comprising 10.9% of the radioactivity only in dog hepatocytes, and M2 was a major metabolite in sheep hepatocytes, comprising 8.7% of the radioactivity (Ma, 2006b).

The chemical structures of some of the derquantel metabolites are shown in Figure 2.

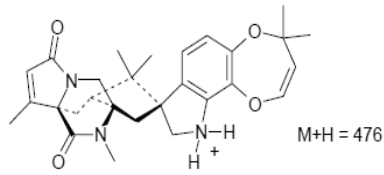
### *2.1.3 Effects on enzymes and other biochemical parameters*

The antagonistic potency of derquantel on both α7 and α3 nicotinic acetylcholine receptors (nAChR) endogenously expressed in rats and canine dorsal root ganglion neuronal cells was evaluated using whole-cell patch clamp assay in

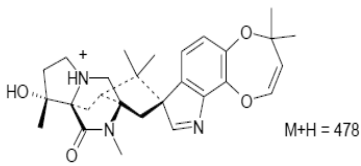
***Figure 2. Chemical structures of selected derquantel metabolites***



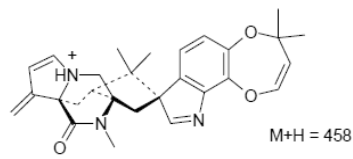

Structure of M1



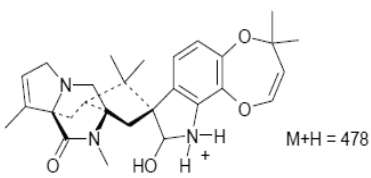

Structure of M2



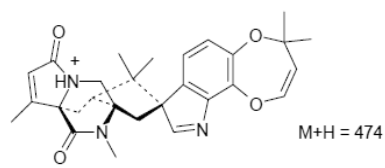

Structure of M3



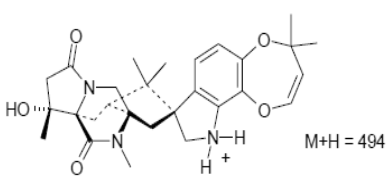

Structure of M4




Structure of M5




Structure of M6




Structure of M7

a GLP-compliant study. Mecamylamine, a known nAChR antagonist and a human antihypertensive agent, was used as a positive control. The antagonist properties of mecamylamine against acetylcholine at 1 mmol/l were similar in both rat and canine α3 nAChR, with median inhibitory concentrations ($IC_{50}$ values) of 0.14 and 0.09 μmol/l, respectively, and approximately 17–27 times greater in relative potency when compared with antagonist activity at rat α7 nAChR ($IC_{50}$ value of 2.41

μmol/l). Derquantel displayed considerably more potent antagonist activity against acetylcholine at 1 mmol/l at canine α3 nAChR ($IC_{50}$ value of 1.16 μmol/l) than at rat α3 nAChR ($IC_{50}$ value of 31.51 μmol/l). The antagonistic potency of derquantel on α7 nAChR could not be assayed for the non-expression of the type of receptor in dog ganglion cells. Derquantel displayed approximately 2- to 3-fold more potent antagonist activity against acetylcholine at 1 mmol/l at rat α3 nAChR than at rat α7 nAChR ($IC_{50}$ value of 70.14 μmol/l). Derquantel was approximately one log unit less potent than mecamylamine. These results suggested that derquantel blocks nAChR, acting more potently in dogs than in rats (May, 2009c).

Derquantel was evaluated for its binding affinity to subtypes of human nAChR using SH-Sy5Y cells (a human neuroblastoma cell line) that endogenously express α3 nAChR, α7/5-HT3-SHEP cells (a human neuroblastoma cell line) that stably express a human α7 nAChR and TE-671 cells (a human medullablastoma cell line) that endogenously express muscle-type nAChR α subunit. The cells were analysed on a fluorescent imaging plate reader to detect the change in calcium flux after the addition of derquantel at 30–100 μmol/l (agonistic activity) and a second addition of nicotine at 100 μmol/l (antagonistic activity). Derquantel had no agonist activity in any of the human nAChR models tested at concentrations of 30–100 μmol/l. Derquantel blocked nicotinic stimulation of cells expressing human α3 ganglionic ($IC_{50}$ value of approximately 9 μmol/l) and muscle-type ($IC_{50}$ value of approximately 10 μmol/l) nicotinic cholinergic receptors, but was inactive ($IC_{50}$ > 100 μmol/l) on the α7 central nervous system subtype (Zinser et al., 2002).

## *2.2 Toxicological studies*

### *2.2.1 Acute toxicity*

#### *(a) Single-dose toxicity studies*

The results of single-dose toxicity studies with derquantel are summarized in Table 1. Following oral exposures, abnormal clinical signs in mice were observed at and above 12.5 mg/kg bw and included ptosis, decreased activity, loss of righting reflex, rapid or laboured breathing, jerky movements or ataxia. There were no deaths, and clinical signs returned to normal 4 hours post-dosing.

In rats, a single oral derquantel dose of 500 mg/kg bw in the 60:40 propylene glycol:glycerol formal vehicle produced severe toxicity in both test article–treated and control rats; therefore, the toxicity was attributed to the vehicle. The vehicle was changed to 1% carboxymethylcellulose, and rats (one of each sex per dose) were administered single oral doses of 0 (carboxymethylcellulose vehicle), 200, 350, 500, 1000, 1500 or 2000 mg/kg bw and observed for 7 days. One rat at the highest dose of 2000 mg/kg bw died. Doses of 350 mg/kg bw and above produced jerky movements within 15 minutes of dose administration. Other clinical signs at greater than 1000 mg/kg bw included subdued behaviour, piloerection, tilted posture and reduced somatomotor activity. Clinical signs subsided at 6 hours in all animals and recurred on day 7 in the 1500 mg/kg bw group and the surviving 2000 mg/kg bw animal. Body weight loss occurred at and above 500 mg/kg bw (Brook, 2004a).

***Table 1. Single-dose toxicity studies with derquantel***

| Species | No. of each sex per group | Route | Dose (mg/kg bw) | $LD_{50}$ (mg/kg bw) | Reference |
|---|---|---|---|---|---|
| Mouse | 3M, 3F | Oral[a] | 0, 12.5, 25, 50 | >50 | Frank, Kechele & Palmer (1996a) |
| Rat | 1M, 1F | Oral[a] | 0[b], 0[c], 200[c], 350[c], 500[b], 500[c], 1000[c], 1500[c], 2000[c] | ≥ 2000 | Brook (2004a) |
| Rat | 5M, 5F | Oral[a] | 0, 200, 500, 1200 | >1200 | Brook (2005e) |
| Rat | 1M, 1F | Dermal[d] | 500, 2000 | >2000 | Brook (2004c) |
| Rat | 5M, 5F | Dermal[d] | 2000 | >2000 | Brook (2005f) |
| Rat | 5M, 5F | Inhalation | 2.4 mg/l for 4 h | >2.4 mg/l for 4 h[e] | Kirkpatrick (2005) |
| Dog | 1M, 1F | Oral[f] | 5, 10, 20 | >20 | Frank, Kechele & Palmer (1996b) |
| Dog | 2M, 2F | Oral[a,c] | 1, 5 | >5 | May (2008) |
| Horse | 1M, 1F | Oral[a,b] | 0, 20 | 20 | Seaman (2000a) |
| Horse | 1M, 1F | Oral[a,c] | 2 | 2 | Seaman (2000b) |

F, female; $LD_{50}$, median lethal dose; M, male
[a] Via gavage.
[b] 60:40 propylene glycol:glycerol formal vehicle.
[c] 1% carboxymethylcellulose vehicle.
[d] Neat test article applied.
[e] $LC_{50}$.
[f] Via capsule.

Single dermal applications of derquantel to rats at up to 2000 mg/kg bw resulted in no mortality and no signs of toxicity (Brook, 2004c, 2005f). Inhalation exposure of rats at 2.4 mg/l for 4 hours resulted in prostration, hypothermia, hypoactivity, rough haircoat and reduced faecal output, but no mortality. All animals were normal by study day 2 (Kirkpatrick, 2005).

Derquantel administered to young Beagle dogs at a dose level of 5, 10 or 20 mg/kg bw for 1 day was well tolerated. Clinical signs included scleral injection (bilateral) and mydriasis (bilateral) at and above 5 mg/kg bw, ptosis (bilateral), relaxed nictitating membranes and dry mouth at and above 10 mg/kg bw and dry conjunctiva at 20 mg/kg bw. All dogs appeared clinically normal after 24 hours post-dosing (Frank, Kechele & Palmer, 1996b). In an acute neurobehavioural study in dogs, oral administration of derquantel at 1 or 5 mg/kg bw for 1 day was without effect on mortality, did not produce qualitative or quantitative electrocardiogram abnormalities and was without effect on the functional observational battery measurements of activity/arousal, neuromuscular function and sensorimotor function. Doses of 1 and 5 mg/kg bw were associated with clinical signs of scleral

injection, partial/slow constriction of pupils and slightly drooping eyelids, as well as a dose-related increase in heart rate. Administration of derquantel at 5 mg/kg bw was associated with decreased direct and indirect pupillary light reflex on both eyes, mild to moderate photosensitivity, a prolapsed nictitating membrane, QTc prolongation, slight decreases in body temperature and single incidences of coughing, stereotypic chewing and an exaggerated patellar reflex response on both hindlimbs (May, 2008).

Abnormal clinical signs in horses included ataxia, falling, marked sweating, distended subcutaneous blood vessels, hypothermia, marked and unresponsive mydriasis, marked ptosis, paraphimosis, slow, laboured breathing, tremors, muscle fasiculations and body weight loss. Mortality by death or euthanasia occurred at 46 (2 mg/kg bw) to 58 (20 mg/kg bw) hours post-dosing. Postmortem findings included gaseous distension of the lower gastrointestinal tract and microscopic vacuolations and mild degeneration of centrilobular hepatocytes (Seaman, 2000a,b).

Clinical signs observed in these studies were consistent with effects associated with derquantel antagonism of nAChR. The greater sensitivity in dogs compared with rats or mice was attributed to a greater sensitivity of dog α3 nicotinic receptors in the dorsal root ganglion in in vitro studies. The antagonistic potency of derquantel in these studies was measured as $IC_{50}$ values, which were 1.16 µmol/l for the dog and 31.51 µmol/l for the rat (Lowney, 2009a; May, 2009c). The severe toxicity observed in horses may have been related to a difference in drug metabolism, as evidenced by the presence of a hydroxylated metabolite of derquantel seen in plasma at a higher concentration than for the parent derquantel and not seen in other species tested, or due to an enhanced pharmacological sensitivity of this species to the mode of action of this class of compound (Seaman, 2000b).

*(b) Dermal sensitization*

The skin sensitization potential of derquantel (lot no. 5789-MAM-9901) in corn oil vehicle was tested using the Magnusson & Kligman guinea-pig maximization test in accordance with Organisation for Economic Co-operation and Development (OECD) guideline 406 and OECD principles of GLP. Thirty-two guinea-pigs (16 of each sex) were used for the test (2 pigs for the preliminary test and 30 pigs for the main test). Skin sensitization of the derquantel/abamectin combination was tested in a modified Buehler technique in guinea-pigs in accordance with United States Environmental Protection Agency (USEPA) GLP standards and OECD guideline 406. Seventy-four guinea-pigs (37 of each sex) were used for the test (2 pigs of each sex for the range-finding phase, 15 of each sex for the induction phase and 20 of each sex for the challenge phase). Derquantel and the derquantel/abamectin combination were non-sensitizing to the skin of guinea-pigs in these studies (Brook, 2005d; Lloyd, 2008a).

*(c) Dermal irritation*

The skin irritation potential of derquantel (lot no. 5789-MAM-9901) was tested in three adult female rabbits using a topical dose of 0.5 g of neat test article, which was applied to a shaved area of skin on each rabbit. The study was performed

in accordance with OECD guideline 404 and OECD principles of GLP. The skin irritation potential of the derquantel/abamectin combination was tested in three adult male rabbits in accordance with OECD guideline 404 and USEPA principles of GLP. A topical dose of 0.5 ml of a 10 mg/ml / 1 mg/ml derquantel/abamectin combination was applied to an area of shaved skin on each rabbit. Derquantel and the derquantel/abamectin combination were non-irritating to the skin of rabbits in these studies (Brook, 2004b; Lloyd, 2008b).

*(d) Ocular irritation*

In a GLP-compliant study, the ocular irritation potential of derquantel (lot no. E010002720) was tested in adult rabbits (one of each sex) by application of a single 100 mg dose of neat derquantel into the cupped lower conjunctival sac of the right eye of each animal. Positive conjunctival irritation was noted for both animals. All irritation subsided by study termination on day 4. The maximum average score for derquantel was 8 out a total maximum score of 110 at 1 hour post-instillation. The ocular irritation potential of the derquantel/abamectin combination was tested in a GLP-compliant study in adult rabbits (three males). A single 0.1 ml dose of the 10 mg/ml / 1 mg/ml derquantel/abamectin combination was instilled into the cupped lower conjunctival sac of the right eye of each animal. Chemosis or discharge scores of 1 (minimal) were obtained in one animal each at the 1 hour post-dosing time point. The effects had cleared in less than 24 hours. The derquantel/abamectin combination was non-irritating to rabbit eyes in this study (Hurley, 2005; Lloyd, 2008c).

### *2.2.2 Short-term studies of toxicity*

*(a) Rats*

In a non-GLP-compliant, 3-day escalating oral dose range–finding study, 3-month-old rats (two of each sex per group) received derquantel (PNU-141962, batch no. 31082-MAM-149A) via gastric intubation at a dose of 0, 25, 50, 100, 200, 300, 400 or 600 mg/kg bw per day. Dose volumes increased with dose up to a maximum volume of 12 ml/kg bw for the 600 mg/kg bw per day group. Owing to clinical signs of excessive toxicity, dosing of the 400 mg/kg bw per day group was interrupted, and the animals were allowed a 2-week washout period. Dosing then resumed at a dose level of 300 mg/kg bw per day for 2 days. The 600 mg/kg bw per day dose was given only once due to excessive toxicity. Control animals received the formulation vehicle (60% propylene glycol and 40% glycerol formal, volume per volume) at a dose volume of 12 ml/kg bw, the maximum dose volume used during the study. A 2-week observation period followed the dosing phase. Feed and water were provided ad libitum. The rats were inspected at least daily, and clinical observations were made at least 4 times per day during the dosing phase. Body weight was determined daily. All rats that died during the study or survived for 2 weeks post-dosing were necropsied, followed by a gross examination of the brain and thoracic and abdominal cavities.

There was one test article–related death. A female dosed once at 600 mg/kg bw per day died 2 days after showing severe toxicity soon after dosing. Clinical

signs prior to this animal's death included ataxia and hypoactivity for the first 3 hours post-dosing. The condition worsened at approximately 4 hours post-dosing, when the animal became prostrate with no righting reflex and laboured respiration. Opisthotonos (abnormal body position) was observed at approximately 5.5 hours post-dosing, and the animal never recovered.

For animals that survived the initial 600 mg/kg bw per day dose, the female became prostrate soon after dosing and exhibited loss of the righting reflex, jerky body movements and tremors when handled, but appeared normal approximately 5.5 hours post-dosing. Males had decreased activity and ataxia within 20 minutes post-dosing, lasting until approximately 4 hours post-dosing.

At 400 mg/kg bw per day, males showed no signs of toxicity until day 2 of dosing, when one male was ataxic and hypoactive within 10 minutes of dosing until approximately 3 hours post-dosing. The second male showed prostration, ataxia, loss of the righting reflex and opisthotonos within 11 minutes of dosing, but returned to normal by 6 hours post-dosing. Females showed signs of toxicity after the first dose. Both exhibited ataxia and hypoactivity within 20 minutes of dosing, and one lost its righting reflex. On day 2, within 20 minutes of dosing, both females exhibited ataxia, prostration, loss of the righting reflex and jerky movements, and one also had ptosis. They did not return to normal until the next day.

There were no clinical signs of toxicity at doses of 25, 50, 100, 200 and 300 mg/kg bw per day.

Decreases in mean body weight were observed during the dosing phase in all treated groups. Losses in males ranged from 2% to 8%, and losses in females ranged from 0% to 9%. Control males and females had 13% and 9% losses in mean body weight, respectively, during the dosing phase, which suggested a vehicle effect on body weight. All groups regained weight lost during the dosing phase by 4 days after the last dose administered, and mean values exceeded pre-dosing means by 10 days after the last dose administered.

There were no treatment-related findings in the postmortem data (Seaman, 1999). Based on the absence of test article–related clinical signs, the no-observed-adverse-effect level (NOAEL) for this study was 300 mg/kg bw per day.

In a GLP-compliant, 12-day oral dose range–finding study, 6- to 8-week-old rats (two of each sex per group) received derquantel (PF-520904, batch no. 5789-MAM-9901) via gastric intubation at a dose of 0, 30, 50, 100, 150, 200, 250, 400, 600 or 800 mg/kg bw per day. Controls were treated with the vehicle (1% carboxymethylcellulose). The dose volume given to all groups was approximately 10 ml/kg bw. Feed and water were provided ad libitum. Animals were observed at least once daily. Body weights were determined prior to the first dose, on day 8 and at study termination. At study termination, animals were euthanized and subjected to a gross necropsy. Liver, kidneys, adrenals, gonads and spleen from all groups were collected and weighed, and livers from animals in the 30 and 150 mg/kg bw per day groups were processed for histopathological examinations.

All animals in the 400, 600 and 800 mg/kg bw per day groups were either found dead 24 hours after dose administration on day 2 (one 400 mg/kg bw per

day female) or euthanized on day 2 because of clinical signs of severe toxicity. At 250 mg/kg bw per day, all animals were euthanized on day 3 as a result of severe clinical signs. At 600 and 800 mg/kg bw per day, clinical signs prior to death or euthanasia included hypoactivity, piloerection and jerky movements 15 minutes after dose administration on day 1. After dosing on day 2, the signs appeared with increased severity. At 400 mg/kg bw per day, clinical signs included hypoactivity and jerky movements after day 1 dose administration. After dosing on day 2, all animals were prostrate and had convulsions. Clinical signs in the 250 mg/kg bw per day group were seen after dosing on day 2 and included prostration and paralysis, with recovery 4 hours later. Following dosing on day 3, convulsions and excessive salivation were seen in addition to prostration and paralysis.

At 200 mg/kg bw per day, test article–related clinical signs were observed beginning on day 3 and included wobbly gait, porphyrin staining around the nose, piloerection, hunched or tilted posture and jerky movements. These clinical signs appeared 10–15 minutes after dose administration and resolved within 4 hours. The appearance of animals was assessed as normal prior to dosing on the following day. A significant reduction in body weight gain compared with controls was observed for both males and females in this group after 7 days of treatment. No difference was observed for the remainder of the dosing days.

No test article–related clinical signs or effects on body weight were seen at 30, 50, 100 or 150 mg/kg bw per day, and no abnormal clinical signs were seen in the control group.

Treatment-related gross pathology findings were limited to a dose-related increase in mean liver weight of males and females in all surviving test article–treated groups compared with controls. The greatest increases were seen in the 150 and 200 mg/kg bw per day groups. No organ weights were collected for animals in the 250 mg/kg bw per day dose groups and higher. Histopathological examinations of the liver were performed on animals in the 30 and 150 mg/kg bw per day groups only. At 150 mg/kg bw per day, two of four animals (one of each sex) had mild, periacinar hepatocyte swelling and cytoplasmic vacuolation with a few scattered necrotic hepatocytes. There were no findings at 30 mg/kg bw per day.

Based on a slight increase in mean liver weight with no associated histopathological findings, the NOAEL for this study was 30 mg/kg bw per day (Brook, 2005a).

In a GLP-compliant, 28-day oral toxicity study, 6- to 8-week-old rats (seven of each sex per group) received derquantel (PF-520904, batch no. 5789-MAM-9901) via gastric intubation at a dose of 0, 20, 100 or 200 mg/kg bw per day. Controls were treated with the vehicle (1% carboxymethylcellulose). The dose volume given to all groups was approximately 10 ml/kg bw. Feed and water were provided ad libitum. Feed consumption was determined weekly. The animals were observed daily approximately 1 and 3 hours after dosing. Detailed clinical examinations were performed weekly, and functional observations were performed in the last week of the experimental period. Body weights were determined prior to the first dose, weekly thereafter, at death or 1 day before study termination. Blood samples for haematology and biochemistry were collected on day 29 prior to euthanasia. Urine

was collected for urine analysis on day 29 by manual expression or cystocentesis at necropsy. Following gross necropsy examination, liver, kidneys, adrenals, brain, gonads and spleen were collected, weighed and preserved. Sections of these organs as well as sections from 27 additional tissues or organs were prepared for histological examination. Histological examinations were performed in the vehicle control and 200 mg/kg bw per day groups, and livers of 20 and 100 mg/kg bw per day animals were also examined.

Test article–related mortality was seen in females at 200 mg/kg bw per day. One female died on day 7. Abnormal clinical signs were first seen in this animal on day 5, 1–2 hours after dosing, and included wobbly gait, prostration and convulsions, all of which had resolved within 4 hours. On day 6, wobbly gait, prostration and convulsions were seen again 1 hour post-dosing, resolving 3 hours later. The animal was found dead on day 7. A second female died on day 10. Abnormal clinical signs were first seen in this animal on day 8, 30 minutes after dosing, and consisted of subdued behaviour lasting 3 hours. On day 9, the animal became prostrate immediately after dosing, had convulsions for 15 minutes and had no or subdued somatomotor activity for 4 hours. On day 10, the animal became prostrate again after dosing. Piloerection, laboured breathing, subdued behaviour and convulsions were also seen. The animal was found dead 2.5 hours after dosing.

Clinical signs of toxicity were seen in surviving 200 mg/kg bw per day males and females throughout the dosing phase, beginning early in week 1 for males and the middle of week 1 for females. The predominant clinical signs included subdued behaviour, prostration, convulsions, paralysis and tilted posture. Other clinical signs included wobbly gait, jerky movements, piloerection, laboured breathing, tremors and splayed hindlimbs. No signs of toxicity were seen in males during week 4. Females had no clinical signs of toxicity in week 3, but signs of toxicity were observed in week 4. Clinical signs of toxicity in the 100 mg/kg bw per day group include subdued behaviour seen in three males and three females during week 3 lasting approximately 2 hours, and porphyrin staining around the nose was seen in one male during week 2. Porphyrin staining was also seen transiently during weeks 2 and 3 in one control female and three males and one female in the 20 mg/kg bw per day group.

Treatment-related decreases in body weight gain were seen in males and females at 200 mg/kg bw per day. These decreases corresponded with decreases in feed consumption.

Results of functional observations performed on all animals in the last week of dosing, prior to test article administration, showed an increase in forelimb grip strength in both males and females in each group compared with controls. This finding is of uncertain toxicological relevance.

Effects on haematology parameters included a slight decrease in mean red blood cells, haemoglobin and haematocrit values relative to controls for 20, 100 and 200 mg/kg bw per day males and 100 and 200 mg/kg bw per day females. A dose-related decrease was observed in females, but not males. A slight, dose-related increase in mean platelet values relative to controls was seen in males and females at all dose levels. None of the differences were statistically significant. These findings are of uncertain toxicological relevance.

Effects on blood biochemistry analyses included slight decreases in mean glucose levels in 200 mg/kg bw per day males and 100 and 200 mg/kg bw per day females; slight increases in urea levels in 200 mg/kg bw per day males and females; lower albumin levels in 200 mg/kg bw per day females; lower albumin to globulin ratios (as a result of increased globulin levels) in females in all treatment groups and males at 100 and 200 mg/kg bw per day; elevated cholesterol levels in males and females at 200 mg/kg bw per day; and increased gamma-glutamyltransferase (GGT) levels at 200 mg/kg bw per day. An elevated serum GGT level is consistent with cholestasis. The other changes are of uncertain toxicological significance. None of the changes were statistically significant.

Relative mean liver weight was significantly increased compared with controls in males and females in the 100 and 200 mg/kg bw per day groups.

Treatment-related histopathology findings were limited to bile duct hyperplasia, which was of minimal severity in two males and three females at 20 mg/kg bw per day; minimal to mild severity in six males and three females at 100 mg/kg bw per day; and minimal to severe severity in five males and five females at 200 mg/kg bw per day.

Based on the significant increases in mean liver weights at 100 and 200 mg/kg bw with an associated dose-related increase in the incidence and severity of bile duct hyperplasia and elevated serum GGT levels and mortality at 200 mg/kg bw per day, the NOAEL for this study was 20 mg/kg bw per day (Rozinova, 2005).

In a GLP-compliant, 28-day oral toxicity study, 7- to 8-week-old rats (15 of each sex per group) received derquantel (batch no. 115883-tis-111) enriched with 4% *N*-oxide (batch no. 115883-tis-7) by gastric intubation at a dose level of 0, 0.5, 5, 25 or 175 mg/kg bw per day, at a dose volume of 5 ml/kg bw. Owing to excessive toxicity and deaths in females, the 175 mg/kg bw dose level was lowered to 150 mg/kg bw per day for females only beginning on day 9, by lowering the dose volume to 4.3 ml/kg bw. Controls were dosed with 1% (weight per volume [w/v]) carboxymethylcellulose. *N*-Oxide is a degradation product of derquantel seen during accelerated stability testing of the derquantel/abamectin drug product. Feed and water were provided ad libitum. Animals were observed for morbidity, mortality, injury and the availability of feed twice daily. Detailed clinical examinations were performed twice daily (pre-dosing and 1 and 4 hours post-dosing). Functional observational battery evaluations were performed once pretest, 1 hour post-dosing on day 3, pre-dosing on day 4, 2 hours post-dosing on day 11 and pre-dosing on day 28. Locomotor activity evaluations were performed once pretest, immediately post-dosing on day 3 and pre-dosing on day 28. Body weights were measured pretest and weekly thereafter. Feed consumption was measured weekly. Ophthalmoscopic examinations were performed pretest and prior to terminal necropsy. Clinical pathology evaluations were conducted on fasted animals prior to the terminal necropsy. At study termination, animals were humanely killed and a gross necropsy was performed. A standard assortment of organs was collected and weighed. Tissues from weighed organs along with a comprehensive assortment of other organs and tissues collected at necropsy were processed for microscopic examination. All tissues from the 0 and 175/150 mg/kg bw per day groups were examined, whereas only the epididymis, kidney, liver, testis and thyroid gland (with

parathyroid) were examined from animals at 0.5, 5 and 25 mg/kg bw per day. A peer review of the histopathological findings was performed.

Test article–related toxicity was the cause of death for four females given 175 mg/kg bw per day through day 8. The dose was lowered to 150 mg/kg bw per day on day 9, and two more females died, one each on days 10 and 14. Adverse clinical signs associated with mortality included decreased activity, ataxia, prostration, an impairment or loss of righting reflex, head tilt, salivation, stereotypy, thin appearance, coldness to the touch and laboured respiration. After the dose was lowered on day 9, these signs persisted through day 14. There were no test article–related clinical signs in males at this dose level or in males or females at the lower dose levels.

Test article–related effects in the functional observational battery observations included a significantly increased incidence, compared with controls, of rearing activity in 175 mg/kg bw per day males. This finding was considered incidental, as it was not seen in females, and there were no adverse test article–related effects on the test battery indices for neuromuscular function or sensorimotor measurements.

Test article–related effects on autonomic measurements included increased palpebral closure in 4 of 10, 6 of 10 and 4 of 6 females at 5, 25 and 175/150 mg/kg bw per day, respectively, on day 11. The incidence was significant at and above 25 mg/kg bw per day. These effects were not seen on day 28 and therefore were not considered adverse because of their transient nature. Similarly, decreased pupillary response was observed in three of nine 175/150 mg/kg bw per day females on day 3 and only in one of nine on day 4. Pupillary response was normal 2 hours post-dosing on day 11 and prior to administration on day 28. This effect was not considered adverse because of its transient nature. A decrease in mean body temperature in 175/150 mg/kg bw per day females was also observed on days 3, 4 and 11, but not on day 28. Decreased body temperature was associated with mortality in four animals.

Test article–related effects on locomotor activity included lower rearing counts (0–240 minutes) in males and females at and above 5 mg/kg bw per day on day 28. For females, the decrease was dose related, with significant differences ($P < 0.01$) at 5, 25 and 175/150 mg/kg bw per day. For males, the dose-related decrease was apparent at 5 mg/kg bw per day, and differences were significant ($P < 0.05$) at 25 and 175 mg/kg bw per day. This finding is considered adverse where the differences were statistically significant.

Mean body weights for 175 mg/kg bw per day males during weeks 2 through 4 and for 175/150 mg/kg bw per day females during week 2 were decreased by 5–8% compared with controls. Corresponding test article–related decreases in mean feed consumption were noted for males and females at this dose level during week 2. These test article–related effects on body weight and feed consumption were assessed as minor and not adverse.

The haematology data for 175 mg/kg bw per day males and 175/150 mg/kg bw per day females reflected a mild anaemia of inflammation characterized by mild decreases in red blood cell mass (erythrocytes, haemoglobin and haematocrit),

which was slightly more severe in females. This diagnosis was based on evidence of an inflammatory response in the clinical chemistry data (increases in globulin and fibrinogen; decreases in albumin), which can suppress erythroid function and cause the observed changes. A test article–related decrease in lymphocyte and eosinophil counts was also observed in males and females at this dose level.

The coagulation data showed a treatment-related but non-adverse increase in platelets, activated partial thromboplastin times and fibrinogen in males and females and non-adverse decreases in prothrombin times in females at the 175/150 mg/kg bw per day dose level. In 25 mg/kg bw per day females, a treatment-related but non-adverse decrease in prothrombin time and an increase in fibrinogen level were also noted.

Treatment-related effects in the clinical chemistry data included increases in GGT, alanine aminotransferase (ALT) and sorbitol dehydrogenase activities in males and females at 175/150 mg/kg bw per day. Urea nitrogen levels were also increased in both sexes in this group; the increases were attributable to mild dehydration resulting from reduced feed intake. Decreases in albumin levels with increases in globulin levels were observed in both sexes at 175/150 mg/kg bw per day. Minimal increases in globulin levels were seen in 25 mg/kg bw per day females. These changes resulted in decreased albumin to globulin ratios for 175/150 mg/kg bw per day males and females and 25 mg/kg bw per day females. Non-adverse treatment-related increases in cholesterol levels were observed in 25 mg/kg bw per day females and in both sexes at 175/150 mg/kg bw per day, and a mild decrease in triglyceride was seen in 175 mg/kg bw per day males.

There were no treatment-related macroscopic findings at necropsy. Significantly ($P < 0.05$ or $P \leq 0.01$) increased mean absolute and relative liver weights in 25 and 175/150 mg/kg bw per day males and females corresponded microscopically with panlobular hepatocellular hypertrophy at 175/150 mg/kg bw per day and was therefore considered adverse at this dose level. There was an increased presence of hepatocellular mitotic figures in two of six early-death females from the 175/150 mg/kg bw per day group, but no evidence of hepatocellular hypertrophy in early-death animals. Similarly, significantly ($P < 0.01$) increased absolute and relative mean thyroid weights in 175/150 mg/kg bw per day females were an adverse effect corresponding microscopically with follicular cell hypertrophy. Minimal to mild follicular cell hypertrophy was seen in the thyroid glands of males and females at 175/150 mg/kg bw per day and in females at 5 and 25 mg/kg bw per day.

Based on significantly lower rearing counts on day 28 in females at 5, 25 and 175/150 mg/kg bw per day, adverse treatment-related effects seen at the 175/150 mg/kg bw per day dose level, which included mortality and evidence of thyroid toxicity in females, and evidence of hepatotoxicity in males and females at 25 and 175/150 mg/kg bw per day, the NOAEL for this study was 0.5 mg/kg bw for females and 5 mg/kg bw per day for males (May, 2009f).

In a GLP-compliant, 90-day oral toxicity study, rats (10 of each sex per group) less than 9 weeks of age received derquantel (PF-520904, batch no. E010002418) by gastric intubation at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day. Controls

were dosed with the vehicle, carboxymethylcellulose. The dose volume given to all groups was 3 ml/dose, except in the latter part of the study, when some animals heavier than 600 g required slightly more than 3 ml. Feed and water were provided ad libitum. Animals were observed twice daily for signs of morbidity, mortality or change in behaviour or appearance. Clinical examinations were performed weekly, approximately 1 hour after dose administration. Body weights were measured weekly. Ophthalmoscopic examinations were performed prior to dose initiation and on days 56/57 and 84/85. Neurological examinations consisting of a standard functional observational battery were performed on days 29 and 90. Blood samples for haematology and clinical chemistry evaluations were collected prior to necropsy on day 90. At study termination, animals were humanely killed with an overdose of anaesthetic agent followed by cardiac puncture. Gross necropsies were performed, and heart, kidneys, thymus, brain, liver, adrenals, testes and ovaries were collected and weighed and processed for histopathological evaluation, along with a comprehensive assortment of other tissues and organs. All tissues from controls and 150 mg/kg bw per day animals and livers from 1, 5 and 50 mg/kg bw per day animals were examined microscopically.

There were no test article–related deaths during the study. Treatment-related clinical signs included a dose-related increased incidence of palpebral closure and decreased alertness at and above 1 mg/kg bw per day, increased incidence of ataxia at and above 5 mg/kg bw per day and piloerection at and above 50 mg/kg bw per day. Test article–related decreases in mean body weight were seen in 150 mg/kg bw per day males and females. In males, the difference was significant ($P < 0.0001$) compared with all male groups and was associated with a lower mean weekly feed consumption compared with controls. Treatment-related neurological effects were observed at 50 and 150 mg/kg bw per day and included changes in behavioural response, piloerection at both dose levels and reduced response to stimuli, tremors and abnormal posture at 150 mg/kg bw per day.

Test article–related changes in the clinical chemistry data included significant increases in serum protein in females at and above 5 mg/kg bw per day ($P = 0.036$, $P = 0.003$ and $P < 0.0001$, respectively) and in males at and above 1 mg/kg bw per day ($P = 0.028$, $P = 0.039$, $P = 0.002$ and $P = 0.001$, respectively); significant ($P = 0.0009$) decreases in albumin in 150 mg/kg bw per day females; significant ($P < 0.0001$) increases in globulin levels in females at and above 5 mg/kg bw per day and in males at and above 50 mg/kg bw per day; significant decreases in the albumin to globulin ratio in females at and above 50 mg/kg bw per day ($P = 0.029$ and $P < 0.001$) and in 150 mg/kg bw per day males ($P < 0.0001$); significantly ($P \leq 0.0001$) increased urea values for females at and above 50 mg/kg bw per day ($P = 0.028$ and $P < 0.0001$) and males at 5 and 150 mg/kg bw per day ($P = 0.032$ and $P = 0.009$); and significant increases in triglycerides in males and females at 150 mg/kg bw ($P = 0.013$ and $P = 0.036$, respectively). ALT and aspartate aminotransferase (AST) levels were increased in 150 mg/kg bw per day males. There were no changes in the haematology data that were attributable to test article toxicity.

Gross necropsy findings associated with treatment included abnormal appearance of the liver, described as enlarged, pale or lobular, which occurred with a dose-related incidence at and above 5 mg/kg bw per day. There was a treatment-

related increase in mean liver weight in 50 and 150 mg/kg bw per day males and females. The differences compared with controls were significant ($P = 0.0003$ and $P < 0.0001$ for females and $P = 0.027$ and $P < 0.0001$ for males). Mean heart weight was significantly increased in 50 and 150 mg/kg bw per day males ($P = 0.049$ and $P = 0.013$, respectively). Microscopically, test article–related changes were characterized as increased incidence and severity of biliary cell degeneration and multifocal biliary cell proliferation. Focal, minimal biliary cell degeneration was seen in one male and one female control animal. Minimal to slight biliary degeneration was seen in three 1 mg/kg bw per day males, but no females. At 5 mg/kg bw per day, minimal to slight biliary cell degeneration was seen in six males. In five females with this finding, the severity was minimal to moderate. At 50 mg/kg bw per day, multifocal biliary cell degeneration was seen in six males (minimal to slight severity) and five females (minimal to moderate severity). At 150 mg/kg bw per day, biliary proliferation was seen in nine males (minimal to marked severity) and seven females (multifocal, minimal to slight severity). An additional two males had minimal to moderate biliary cell degeneration with portal inflammatory cell aggregates. An additional two females had moderate biliary cell degeneration. One female had no remarkable liver findings (Goldenthal, 2006).

A cover memo accompanying this study report reviewed reasons why the results of this study should be considered unreliable and not suitable for an assessment of the toxicity of derquantel. In particular, it was found that the liver slides evaluated by the study pathologist were of poor quality; as a result, some of the biliary degeneration reported by the study pathologist could have been due to tissue shrinkage and artefact. Other deficiencies noted included the limited histopathological evaluation of the 1, 5 and 50 mg/kg bw per day groups and post-dosing monitoring that was insufficient to adequately capture clinical signs (Lowney, 2009b).

Owing to the questionable quality or reliability of the clinical observations and histopathology data, a NOAEL could not be established in this study.

In a GLP-compliant, 1-year oral toxicity study, 8-week-old rats (27 of each sex per group) received derquantel (PF-520904, batch no. E010002940) by gastric intubation at a dose of 0, 1, 5 or 50 mg/kg bw per day. Controls were treated with the vehicle (1% carboxymethylcellulose). The dose volume given to all groups was 5 ml/kg bw. Feed and water were provided ad libitum. In addition to twice daily assessments for mortality or moribundity, detailed clinical observations were performed once prior to treatment and weekly prior to dosing. Cage-side observations were performed daily within 3 hours following dose administration. Body weights were determined prior to dose initiation and weekly thereafter. Feed consumption was measured weekly. Ophthalmological examinations were performed prior to dose initiation and during the last week of the treatment period. Blood samples for haematology, biochemistry and coagulation evaluations were collected at study termination (day 366). Samples for urine analysis were collected by cage pan drainage overnight at study termination. At necropsy, 17 organs were collected for organ weight determinations. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. All tissues from control and 50 mg/kg bw per day animals

were examined microscopically. The liver, eyes, testes, epididymis and kidneys were identified as target organs and were therefore processed and examined from the 1 and 5 mg/kg bw per day groups. The histopathology evaluation was peer reviewed.

There were no treatment-related mortalities. A treatment-related significant ($P < 0.05$) decrease in mean body weight occurred in 50 mg/kg bw per day males during the last 3 weeks of the study, although feed consumption was not affected.

There were no clear test article–related changes in haematology, coagulation or red blood cell morphology data.

Test article–related changes in the clinical chemistry data included elevated mean levels of GGT for 50 mg/kg bw per day males and females. Mean bilirubin levels were significantly ($P < 0.05$) elevated, as were mean cholesterol levels ($P < 0.01$), and mean triglyceride levels were increased in 50 mg/kg bw per day females. In 50 mg/kg bw per day males, a significant ($P < 0.01$) increase in mean globulin levels resulted in a significant ($P < 0.01$) increase in mean total protein levels and a lower albumin to globulin ratio compared with controls. In females, mean globulin levels were increased at all dose levels compared with controls. The difference was significant ($P < 0.01$) at 50 mg/kg bw per day, resulting in a significant ($P < 0.01$) decrease in the albumin to globulin ratio and significantly ($P < 0.01$) increased total protein levels at this dose level. In addition, mean total protein levels were significantly increased in all test article–treated groups ($P < 0.05$, $P < 0.05$ and $P < 0.01$, respectively).

Ophthalmoscopic findings were limited to an increased incidence of cataracts in males at 50 mg/kg bw per day, although only 2 of 27 cataracts seen on ophthalmic examination were confirmed histologically. Cataracts in males at 50 mg/kg bw per day are likely a treatment-related effect in this study, given the relatively high incidence of cataracts observed by the board-certified ophthalmologist who performed the in-life examinations and the absence of this finding at lower dose levels.

Test article–related necropsy findings were limited to enlarged livers observed in 9 of 27 males and 8 of 27 females at 50 mg/kg bw per day. Significant changes in organ weights relative to controls were seen at 50 mg/kg bw per day and included increases in mean absolute and relative liver weights in males and females, decreased mean absolute and relative thyroid gland and thymus weights in males and increased mean absolute and relative thyroid weights in females.

Test article–related histopathology findings were observed in the liver, kidney, testis and epididymis. Liver findings were seen in males and females at all dose levels, including controls, and were characterized as spontaneous, non-adverse, age-related biliary hyperplasia. In many cases, bile duct proliferation was accompanied by periportal (and periductal) fibrosis or infiltration of mononuclear cells. The incidence or severity of the liver findings increased with increasing dose, starting at 1 mg/kg bw per day, indicating that the test article had an accelerating effect on the development of this lesion. Biliary hyperplasia and associated elevations in ALT and bilirubin levels and increased mean liver weight at 50 mg/kg bw per day were considered adverse effects. Kidney findings were seen in males

and females at all dose levels, including controls, and were characterized as age-related, spontaneous, chronic progressive nephropathy with a treatment-related increase in severity at 50 mg/kg bw per day. In the absence of clinical pathology changes, these kidney findings were not considered adverse. Adverse test article–related effects were noted in 50 mg/kg bw per day males and consisted of mild to marked degeneration of seminiferous tubules in 3 of 27 animals and mild to marked hypospermia in 4 of 27 animals in this dose group. Minimal degeneration of seminiferous tubules without corresponding hypospermia was considered a non-adverse finding seen in 2 of 25 males at 5 mg/kg bw per day. Marked degeneration of seminiferous tubules was also seen in one male in the 1 mg/kg bw per day group. This finding was not test article related because the lesion had moderate mononuclear cell infiltration and was therefore qualitatively different from the degeneration seen at 50 mg/kg bw per day. Marked hypospermia also seen in this animal was an incidental finding associated with degeneration of the testes. There were no abnormal findings in the testis or epididymis of control animals.

Based on adverse testicular effects in males and evidence of hepatotoxicity in males and females at 50 mg/kg bw per day, the NOAEL was 5 mg/kg bw per day (Bonnette, 2009a).

A second GLP-compliant, 1-year oral toxicity study in the rat was conducted with lower dose levels, which allowed for a better evaluation of the hepatobiliary effects of derquantel seen in the previous study. In this study, 8-week-old rats (15 of each sex per group) received derquantel (PF-520904, batch nos E010002940 and NO70041) by gastric intubation at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day. Controls were treated with the vehicle (1% [w/v] carboxymethylcellulose). An additional 15 rats of each sex per group were assigned to the control and 0.01 mg/kg bw per day groups for interim termination on day 91. The dose volume given to all groups was 5 ml/kg bw. Feed and water were provided ad libitum. In addition to twice daily assessments for mortality or moribundity, detailed clinical observations were performed once prior to treatment and weekly prior to dosing. Cage-side observations were performed daily within 3 hours following dose administration. Body weights were determined prior to dose initiation and weekly thereafter. Feed consumption was measured weekly. Ophthalmological examinations were performed prior to dose initiation, for interim-phase animals on day 84 and during the last week of the treatment period. Blood samples for haematology, biochemistry and coagulation evaluations were collected from control and 0.01 mg/kg bw per day animals at interim sacrifice (day 91) and from all groups at study termination (day 366/367). Samples for urine analysis were collected by cage pan drainage overnight at both intervals. At necropsy, 17 organs were collected for organ weight determinations. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. All tissues and organs collected at necropsy from control and 0.3 mg/kg bw per day main-phase animals, the liver from control and 0.01 mg/kg bw per day interim-phase animals and all tissues from early-death animals in 0.01 mg/kg bw per day interim-phase and 0.01 and 0.03 mg/kg bw per day main-phase groups were processed and examined microscopically. Subsequently, the livers from 0.01, 0.03 and 0.1 mg/kg bw per day animals were processed to slides and examined microscopically.

There were no test article–related deaths in the study. Test article–related effects in this study were limited to histopathological findings of an increased incidence and severity of biliary hyperplasia and fibrosis in the liver in male main-study animals at and above 0.03 mg/kg bw per day, as compared with females or controls. These findings were assessed as non-adverse age-related spontaneous findings, the incidence or severity of which was accentuated by the test article, and there were no associated changes in the serum chemistry data.

The only changes of toxicological relevance in this 1-year study were the increased incidence and severity of biliary hyperplasia and fibrosis of the liver seen in males compared with controls or females at 0.03, 0.1 and 0.3 mg/kg bw per day. In the absence of corresponding elevations in hepatic enzymes indicative of hepatobiliary injury or increased liver weights, this finding was not considered to be adverse. Therefore, the NOAEL in this study was 0.3 mg/kg bw per day, the highest dose tested (Bonnette, 2009b).

Biliary hyperplasia occurs spontaneously in rats and has not been correlated with liver carcinogenicity or neoplasia. A statistical analysis of the biliary hyperplasia response in the two chronic rat studies with derquantel comparing any positive response in each dose group with the control response showed that the incidence of biliary hyperplasia was significantly elevated relative to controls only at the 50 mg/kg bw per day dose level. In addition, this dose level was associated with significant increases in liver weight and changes in GGT levels consistent with hepatotoxicity and is therefore a clear adverse effect level. There was no statistically detectable difference in the incidence of biliary hyperplasia seen at lower dose levels, nor were there significant increases in liver weights or changes in serum chemistry parameters indicative of hepatocellular injury.

Thus, 5 mg/kg bw per day would be considered the NOAEL on the basis of this analysis. This NOAEL is further supported by benchmark dose (BMD) modelling of the pooled data from both 1-year toxicity studies in the rat using various models. This modelling was performed by the sponsor and reviewed by the Committee. The lower bound of the one-sided 95% confidence interval for a benchmark dose representative of 10% extra risk compared with placebo ($BMDL_{10}$) derived from these models ranged from 2.0 to 7.6 mg/kg bw per day, which was in close agreement with the previously statistically verified NOAEL of 5 mg/kg bw per day (Lowney, 2011).

*(b) Dogs*

In a non-GLP-compliant, 28-day preliminary oral toxicity study, Beagle dogs 6–7 months of age (two of each sex per group) received derquantel (PF-520904, batch no. E01000242) as neat test article in gelatine capsules at a dose of 0, 1, 5 or 10 mg/kg bw per day. Control animals were dosed with an empty gelatine capsule. Feed was offered daily in the morning for approximately 2 hours, and water was provided ad libitum. Dose administration occurred after the feeding period. In addition to morning and afternoon checks for signs of morbidity or mortality, clinical observations were performed daily at 1, 2.5 and 4 hours following dosing. Detailed clinical evaluations were performed weekly. Body weights were measured prior to

treatment initiation, weekly thereafter and at study termination. Feed consumption was measured daily. Blood samples for haematology, biochemistry and coagulation evaluations were collected from fasted animals prior to dose initiation and on days 14 and 28. At study termination, animals were euthanized and necropsied, and adrenals, kidneys, heart and thyroids with parathyroids were collected, weighed, preserved and processed for microscopic evaluation.

All animals survived to study termination. Test article–related clinical signs were seen within 4 hours of dosing in all dogs during the first week of dosing, with a generally dose-related increase in incidence, and included dry conjunctiva, dry eyes, relaxed nictitating membrane, ptosis, red eyes, dilated pupils and dry mouth. The incidence of dry conjunctiva and dry eyes was lower in weeks 3 and week 4 (compared with the first 2 weeks of the study). These clinical signs were absent in 1 mg/kg bw per day males and females and 5 mg/kg bw per day females in week 4. Red eyes, dilated pupils and relaxed nictitating membrane were sporadically seen in males or females as a pre-dose observation weekly at all dose levels. Ptosis was seen sporadically as a pre-dose observation in 5 and 10 mg/kg bw per day females beginning in week 2 and in 10 mg/kg bw per day males during week 4. No other test article–related effects were observed.

The lowest-observed-adverse-effect level (LOAEL) for this study was 1 mg/kg bw per day, based on test article–related clinical observations seen at all dose levels (Savides, 2006).

In a GLP-compliant, 28-day oral toxicity study, 7.5- to 9-month-old Beagle dogs (four of each sex per group) received derquantel (PF-520904, batch no. 07VMDA010201A) by gavage at a dose of 0, 0.01, 0.03 (0.01 mg/kg bw per dose, 3 times per day), 0.03 or 0.1 mg/kg bw per day. The dose volume was 1 ml/kg bw. Control animals received the vehicle, 0.1% (w/v) carboxymethylcellulose. Owing to the absence of clinical signs at 0.03 mg/kg bw per day once per day and 3 times per day, dosing and data collection were suspended in the 3 times per day group after 7 days of dosing. Feed was provided ad libitum except during designated periods. Water was provided ad libitum. All animals were observed twice daily for signs of morbidity or mortality, and detailed clinical examinations were performed twice daily and following each functional observational battery evaluation. Functional observational battery evaluations were conducted prior to dose initiation, 1 hour post-dosing on day 1 and prior to dosing on days 2 and 28. Neurological examinations were scheduled at the same intervals, following the functional observational battery evaluation and 1 hour post-dosing on day 28. Body weights and feed consumption were measured prior to dose initiation and weekly thereafter. Ophthalmoscopic examinations were conducted at study termination. Electrocardiographic examinations were performed prior to dose initiation and pre-dosing and 1 hour post-dosing on days 2 and 27. Clinical pathology evaluations were performed on all animals pretest and on fasted animals at study termination. At study termination, animals were returned to stock to be used in a subsequent study with this test article.

All animals survived to study termination. There was no evidence of toxicity in any of the parameters evaluated.

Based on the absence of evidence of toxicity at all dose levels, the NOAEL was 0.1 mg/kg bw per day, the highest dose tested (May, 2009a).

In a GLP-compliant, 90-day oral toxicity study, Beagle dogs (four of each sex per group) that were approximately 6 months of age received neat derquantel (PF-520904, batch no. E010002421) by capsule at a dose of 0, 0.1, 0.5, 1 or 10 mg/kg bw per day. Control animals received an empty gelatine capsule. Feed was offered daily for approximately 1 hour in the morning. Dose administration occurred after the feeding period. Water was provided ad libitum. All animals were observed twice daily for signs of morbidity or mortality. Once dosing began, animals were observed for clinical signs daily pre-dosing, between 1 and 2 hours after dosing every day and 5 hours after dosing on Tuesdays and Fridays. Body weights were measured prior to dose initiation and weekly thereafter and at study termination. Feed consumption was measured daily. Ophthalmoscopic examinations were conducted prior to dose initiation, midway through the study and during the last week of dosing. Blood samples for evaluation of haematology, biochemistry and coagulation were collected from feed-fasted animals prior to dose initiation and on days 45 and 90. Samples for urine analysis were collected onto clean cage pans at the same intervals. At termination (day 92), animals were euthanized and necropsied, and adrenals, kidneys, liver, testes, thymus, heart, thyroid with parathyroids, brain, spleen and ovaries were collected and weighed. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. Microscopic examinations were performed on all animals and all tissues.

All animals survived to study termination. Test article–related clinical observations included dry mouth, dilated pupils, ptosis, dry conjunctiva, dry eyes, red eyes and relaxed nictitating membrane. Less commonly seen were vomit in the cage, salivation and abnormalities of faeces, and rarely seen were tremors and decreased activity. In general, these findings were first seen during the first week of dosing and occurred with a dose-related increase in numbers of animals affected, persistence of the finding post-dosing and number of days affected.

Dry mouth was observed in all test article–treated groups, with a dose-related increase in incidence. The incidence was highest 2 hours post-dosing; by 24 hours post-dosing, dry mouth was seen in only a few cases at 0.5 mg/kg bw per day and higher.

Dilated pupils were seen in all treated animals at both the 2- and 5-hour observations at one point or another in the study. The incidence increased with dose: at 0, 0.1, 0.5, 1 and 10 mg/kg bw per day, the average respective incidence was approximately 0%, 5%, 57%, 90% and 97% at 2 hours post-dosing and 8%, 10%, 37%, 65% and 98% at 5 hours post-dosing (on two occasions for each animal, mid-point and end of study, eyes were dilated with Mydriacil® for an ophthalmic examination; this accounts for some observations of dilated pupils). At 24 hours post-dosing, the pupils were still dilated, with incidences of 4%, 7% and 47% at 0.5, 1 and 10 mg/kg bw per day, respectively.

Ptosis was noted with a dose-related increase in incidence in all test article–treated groups at the 2- and 5-hour post-dosing observations. Ptosis was seen as

a single incident in one male and one female control animal. By 24 hours post-dosing, ptosis was present in all test article–treated groups (including the control group); however, the incidence in the 10 mg/kg bw per day group remained high, averaging about 20%.

Dry conjunctiva was seen occasionally 2 hours post-dosing in half of the 1 mg/kg bw per day animals and in all 10 mg/kg bw per day animals. The incidence of this finding decreased over time, with only one 10 mg/kg bw per day female seen with this finding 24 hours post-dosing.

Dry eyes were seen with increasing incidence at 0.5, 1 and 10 mg/kg bw per day. The highest incidence occurred at 10 mg/kg bw per day 2 and 5 hours post-dosing, then decreased over time, with one 0.5 mg/kg bw per day female and one male and one female in the 10 mg/kg bw per day group seen with this finding 24 hours post-dosing.

Red eyes were seen in one 0.1 mg/kg bw per day male, half of the 0.5 mg/kg bw per day animals and all 1 and 10 mg/kg bw per day animals 2 hours post-dosing. By 24 hours post-dosing, red eyes were seen in only two males and two females at 10 mg/kg bw per day.

Relaxed nictitating membrane was seen in all groups, including two males and one female in the control group. In test article–treated groups, this finding was seen at all dose levels at 2 and 5 hours post-dosing. Twenty-four hours post-dosing, the incidence of this finding at 1 and 10 mg/kg bw was still rather substantial (9% and 23%, respectively).

Tremors were noted sporadically in three of four 10 mg/kg bw per day females. Tremors were seen at 2 and 5 hours post-dosing and were not seen in any of the animals after week 8.

Test article–related decreases in mean body weight relative to controls occurred in males and females at 10 mg/kg bw per day. The decrease was associated with significantly ($P = 0.0023$) lower mean daily feed consumption.

Possible test article–related effects included significant decreases in kidney weight ($P = 0.0015$) and absolute and relative heart weights ($P < 0.002$) relative to controls at 10 mg/kg bw per day. There were no corresponding histopathological findings in these tissues.

On the basis of observed test article–related clinical signs at all dose levels, the LOAEL for this study is 0.1 mg/kg bw per day (Savides, 2008).

In a GLP-compliant, 90-day oral toxicity study, non-naive Beagle dogs (four of each sex per group) that were 10.5–12 months old received derquantel (PF-520904, batch no. 07VMDA010201A) by gavage at a dose of 0, 0.1, 0.5, 1 or 5 mg/kg bw per day. Control animals were dosed with the vehicle, 1.0% (w/v) carboxymethylcellulose. The dose volume for all groups was 2 ml/kg bw. The animals used in this study were transferred from the May (2009a) study previously reported. The washout period was at least 6 weeks. Feed was available ad libitum except during designated periods, and water was available ad libitum. Animals were checked twice daily for signs of morbidity or mortality. Detailed clinical examinations

were performed twice daily during the dosing phase and at the end of each functional observational battery evaluation. Functional observational battery evaluations were performed on all animals once prior to dose initiation, 2 hours post-dosing on day 1, pre-dosing on days 2 and 28, again 2 hours post-dosing on day 59 and pre-dosing on day 90. A neurological examination was conducted on each animal following each functional observational battery evaluation. Ophthalmoscopic examinations were performed on all animals prior to dose initiation and prior to the terminal necropsy. Electrocardiographic examinations were performed prior to dose initiation and twice on days 2, 27, 58 and 89 (pre-dosing and 2 hours post-dosing). Indirect blood pressures were measured following each electrocardiographic examination. Blood samples for haematology, biochemistry and coagulation evaluations were collected from feed-fasted animals prior to dose initiation and immediately prior to terminal necropsy. Samples for urine analysis were collected via cystocentesis at terminal necropsy. Bone marrow smears were collected at necropsy and examined microscopically. At study termination, animals were euthanized and necropsied. Sections of organs that were collected and weighed as well as sections from a comprehensive assortment of other tissues and organs were prepared for histological examination. A complete list of tissues from control and 5 mg/kg bw per day animals was examined microscopically. Only liver and gross lesions collected from animals in the 0.1, 0.5 and 1 mg bw per day groups were examined.

All animals survived to scheduled necropsy. Treatment-related clinical signs included protruding nictitating membrane, dilated pupils and red eyes.

Protruding nictitating membrane and dilated pupils were seen in all test article–treated groups, with a general dose-related increase in incidence and number of animals affected. Protruding nictitating membrane was seen in males and females (0/0, 1/1, 2/4, 4/3 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day), which resolved prior to the next dose in all 0.1, 0.5 and 1 mg/kg bw per day males and females. Dilated pupils were seen in males and females (0/0, 1/1, 3/1, 4/4 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day), which resolved prior to the next dose in all 0.1, 0.5 and 1 mg/kg bw per day females and in 0.1 and 1 mg/kg bw per day males.

Eye redness was seen in control animals, but the incidence and number of animals affected increased to levels suggesting a test article–related effect in males and females at 1 and 5 mg/kg bw per day. Eye redness resolved prior to the next dose in 5 mg/kg bw per day females.

In functional observational battery tests 2 hours post-dosing on days 1 and 59, decreased pupil response and increased pupil size were noted in males and females at 5 mg/kg bw per day, occasionally reaching statistical significance. Decreases in these same parameters were also noted in males at 1 mg/kg bw per day on day 1. Also at 2 hours post-dosing on days 1 and 59, ptosis scores were noted in one male and three or four females at 5 mg/kg bw per day. Scores for females were occasionally statistically significant. Ptosis was also noted in one female during the pre-dose functional observational battery on day 2.

Veterinary neurological examinations performed 2 hours post-dosing on days 1 and 59 found all males and two females at 5 mg/kg bw per day and one

male at 1 mg/kg bw per day with decreased or absent direct and indirect pupillary light reflex. Decreased indirect pupillary light reflex was also noted in one male at 0.1 mg/kg bw per day on day 59.

There were no test article–related findings in any of the other parameters evaluated. None of the test article–related findings were considered adverse, because there were no associated pathological changes observed.

Based on observations of protruding nictitating membranes, dilated pupils, eye redness and decreased indirect pupillary light response at all dose levels, the LOAEL for derquantel was 0.1 mg/kg bw per day (May, 2009b).

In an attempt to better define a point of departure for nictitating membrane protrusion in the dog, which was among the most sensitive effects seen in the dog, BMD modelling was performed. The $BMDL_{10}$ was calculated for a benchmark response of 10% incidence above the modelled background incidence. The USEPA's BMDS version 2.2 was used for modelling the dose–response relationship for this sensitive end-point in derquantel-treated male and female dogs using data from the two 90-day oral toxicity studies reviewed above.

The dose–response models were fitted to the dose–incidence data: gamma, logistic, logistic with background, log-logistic, multistage, multistage with background, probit, log-probit, Weibull, Weibull with background and quantal-linear models. The BMD and BMDL values for an extra 10% risk compared with the modelled background incidence ($BMD_{10}$ and $BMDL_{10}$) were estimated by performing 250 iterations.

The $BMD_{10}$ values from the accepted models ranged from 0.0112 to 0.0695 mg/kg bw per day, and the $BMDL_{10}$ values from 0.000 15 to 0.0682 mg/kg bw per day. Most models presented $BMD_{10}$ and $BMDL_{10}$ values as 0.03 and 0.02 mg/kg bw per day, respectively, with good fitness (Akaike information criterion 62.543–62.787; *P*-value: 0.6026–0.6068).

However, considering the limited data available with which to model a dose–response relationship for elicitation of protrusion of the nictitating membrane in the dogs, the high degree of uncertainty at the low end of the dose–response curve and the fact that the two studies from which the data were derived differed in the age of dogs on study (juvenile versus adult) and mode of test article administration (capsule versus gavage), the Committee concluded that the $BMDL_{10}$ approach was not appropriate for the purpose of this evaluation.

### *2.2.3 Long-term studies of toxicity and carcinogenicity*

No long-term studies of toxicity and carcinogenicity were performed.

### *2.2.4 Genotoxicity*

Derquantel was tested for the potential to induce microbial gene mutations in six Ames *Salmonella typhimurium* tester strains and in *Escherichia coli* strain WP2*uvrA*. Derquantel was also tested in a chromosomal aberration assay in human lymphocytes. The in vitro microbial and mammalian cell gene assays were

***Table 2. Results of genotoxicity assays with derquantel***

| Test system | Test object | Concentration | Results | References |
|---|---|---|---|---|
| **In vitro** | | | | |
| Bacterial mutagen screen[a] | *Salmonella typhimurium*[b] TA97A, TA98, TA100, TA102 | 0–2500 µg/plate | Not mutagenic | Mayo (1998) |
| Ames reverse mutation assay[a,c] | *S. typhimurium* TA98, TA100, TA102, TA1535, TA1537 | 10–1000[d] µg/plate | Not mutagenic | Brook (2005b) |
| Ames reverse mutation assay[a] | *S. typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA* | 1.6–5000 µg/plate | Not mutagenic | Farabaugh (2009) |
| Chromosomal aberration assay in human peripheral lymphocytes[a,e] | Adult human peripheral blood lymphocyte primary cell cultures | Fifteen concentrations from 25 to 1000 µg/ml[f] | Positive; chromosomal aberrations were induced without metabolic activation[g] | Mauthe (2005) |
| **In vivo** | | | | |
| Micronuclei induction[h] | Mouse bone marrow | 50, 100 and 200 mg/kg bw | Negative; not clastogenic | Brook (2005c) |
| Micronuclei induction[i] | Juvenile rat hepatocytes | 25, 50, 100 and 200 mg/kg bw | Negative; no micronuclei formation | Coffing (2008) |

[a] With and without Aroclor 1254–induced rat liver S9 fraction.
[b] Non-GLP Ames assay.
[c] A positive control without metabolic activation was not included in the assay, and 2-aminoanthracene was used as the sole indicator of the efficacy of the S9 mix.
[d] Maximum soluble dose.
[e] Cyclophosphamide and mitomycin C were used as positive controls.
[f] For 3 hours with or without metabolic activation and for 24 hours without metabolic activation.
[g] Only seen at concentrations that produced at least 50% toxicity.
[h] 9,10-Dimethylbenzanthracene was used as the positive control.
[i] Diethylnitrosamine was used as the positive control.

completed in the presence and absence of metabolic activation using S9 prepared from the livers of Aroclor 1254–induced rats to compare the direct genotoxic potential of derquantel and the genotoxic potential of any major derquantel metabolites. In vivo testing included screening for micronuclei induction in mouse bone marrow and juvenile rat hepatocytes.

The results of these genotoxicity assays are shown in Table 2.

### *2.2.5 Reproductive and developmental toxicity*

#### *(a) Multigeneration studies*

##### *(i) Rats*

In a USEPA and OECD GLP-compliant, two-generation (one litter per generation) reproduction study, male and female Sprague-Dawley rats (30 of each sex per group) were dosed with derquantel (lot no. E010002940) by oral gavage at a dose of 0, 1, 5 or 25 mg/kg bw per day. Control animals received the vehicle, 1% aqueous carboxymethylcellulose. The dose volume was 10 ml/kg bw. Males were treated once daily beginning 70 days prior to cohabitation and continuing through the last day of cohabitation and then euthanized and necropsied. Sperm parameters were evaluated. Females were treated once daily beginning 70 days prior to cohabitation and continuing throughout gestation and 21 days postpartum and then euthanized and necropsied. Reproductive organs, including the pituitary gland, and gross lesions were also retained from all $P_1$ generation males and females for possible histological evaluation. Prior to the initiation of dose administration, 10 male and 10 female $F_1$ generation rats per dose group received a motor activity evaluation. The same rats received a functional observational battery and an additional motor evaluation on postpartum days 63–65. Feed and water were provided ad libitum. Rats were observed for signs of morbidity or mortality at least twice daily, and observations for treatment-related clinical signs were performed daily before dosing and approximately 2 hours post-dosing. Body weights were recorded at least weekly during gestation and on lactation days 1, 4, 7, 10, 14 and 21. Feed consumption was recorded weekly prior to cohabitation, on gestation days 0, 6, 10, 15, 18, 20 and 25 (non-pregnant rats) and on lactation days 1, 4, 7, 14 and 21. Clinical observations of pups were performed daily, and body weights were recorded on lactation days 1, 4, 7, 14 and 21.

Test article–related findings in $P_1$ males included significantly increased ($P \leq 0.05$ or $P \leq 0.01$) absolute and relative liver weights and thyroid follicular epithelial hypertrophy and hyperplasia at 25 mg/kg bw per day. Findings in $P_1$ females included a test article–related significant ($P \leq 0.05$) increase in the number of $F_1$ pups in the 25 mg/kg bw per day group observed with discoloration (purple, dark purple, green, grey and/or black) of the head, nose, whole body, both hindlimbs, both hindpaws, lower midline, tail or tip of tail. There were no other findings to verify that discoloration was treatment related or adverse. Postmortem findings in $P_1$ females included a significant ($P \leq 0.01$) increase in absolute and relative thyroid weights in all test article–treated groups compared with controls. This finding was considered adverse at 25 mg/kg bw per day, because it corresponded with microscopically observed thyroid follicular epithelial hypertrophy and hyperplasia. Terminal body weight of $P_1$ females was significantly ($P \leq 0.01$) increased at 25 mg/kg bw per day, as were absolute and relative mean liver weights and paired adrenals weight ($P \leq 0.05$ or $P \leq 0.01$). These findings were test article related. There were no test article–related macroscopic or microscopic changes observed in any of the reproductive tissues from $P_1$ males or females.

Test article–related findings in $F_1$ generation males included a significant increase in absolute and relative liver weights at 25 mg/kg bw per day. For $F_1$ females, test article–related findings were observed at 25 mg/kg bw per day and limited to significantly ($P \leq 0.01$) increased absolute and relative liver weights, significantly ($P \leq 0.05$ or $P \leq 0.01$) increased absolute terminal body weight with associated decrease in brain to body weight ratio, and increased absolute spleen weight. An increased incidence of thyroid follicular epithelial hyperplasia was observed in $F_1$ males and females at 25 mg/kg bw per day. Thyroid follicular hyperplasia was also observed in the 0, 1 and 5 mg/kg bw per day groups but was not test article related, based on lower incidence or severity compared with 25 mg/kg bw per day.

There were no test article–related findings in reproductive or developmental parameters.

A NOAEL of 5 mg/kg bw per day was established for males, based on histopathological findings of thyroid follicular epithelial hyperplasia at 25 mg/kg bw per day. For females, a NOAEL of 1 mg/kg bw per day was established, based on significant increases in thyroid weights of $P_1$ female rats. The NOAEL for reproductive effects was 25 mg/kg bw per day, the highest dose tested (Barnett, 2009).

*(b) Developmental toxicity*

*(i) Rats*

A GLP-compliant dose range–finding study for developmental toxicity was conducted to determine doses for the subsequent main study in rats. Derquantel (lot no. 5789-MAM-9901) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant Sprague-Dawley rats (seven per group) at a dose of 0 (vehicle), 2, 10, 50 or 100 mg/kg bw per day on gestation days 5–20, at a dose volume of approximately 10 ml/kg bw. Feed and water were provided ad libitum. Body weights and feed consumption were determined every 3 days throughout the study. The animals were observed daily at approximately the same time each day between 1 and 4 hours post-dosing. On day 21, rats were euthanized and caesarean sectioned. A gross necropsy of the thoracic and abdominal viscera was performed. The uteri were removed to ascertain the pregnancy status. The number of corpora lutea on the ovaries was recorded. The fetuses were removed, weighed and examined for sex, live or dead status, and early or late resorptions.

No mortalities occurred during the study. Clinical signs observed only at 100 mg/kg bw per day included subdued behaviour, tilted posture and laboured breathing. Porphyrin staining around the nose was seen in all groups, including controls (with an incidence of 2/7, 1/7, 3/7, 2/7 and 7/7 animals per group, respectively). Decreased body weight gain and lower average total feed intake were observed at 100 mg/kg bw per day when compared with the vehicle control group. A lower mean number of corpora lutea in the 100 mg/kg bw per day group compared with the control group (11.4 versus 15.0) corresponded with the mean number of live fetuses found in the uteri of these two groups (10.7 versus 14.2). The mean number of female fetuses in the 100 mg/kg bw per day group was about half that of the vehicle control group. This difference between the 100 mg/kg bw per

day and the control groups was reflected in the mean litter weights. Based on the findings in this preliminary study, doses selected for the main study were 20, 70 and 120 mg/kg bw per day (Henderson, 2006).

In the definitive, GLP-compliant study, derquantel (lot no. 5789-MAM-9901) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant Sprague-Dawley rats (23 per group) at a dose of 0 (vehicle), 20, 70 or 120 mg/kg bw per day on gestation days 5 through 20, at a dose volume of approximately 10 ml/kg bw. Feed and water were provided ad libitum. Body weights and feed consumption were determined every 3 days throughout the study. The animals were observed daily at approximately the same time each day between 1 and 4 hours post-dosing. On day 21, rats were euthanized and caesarean sectioned. A gross necropsy of the thoracic and abdominal viscera was performed. The uteri were removed to ascertain the pregnancy status. The number of corpora lutea on each ovary was recorded. The fetuses were removed, weighed and examined for sex, live or dead status, early or late resorptions, gross external alterations and fetus allocation. Soft tissue and skeletal examinations were performed.

Two mortalities occurred during the experimental period, but neither was attributable to treatment with the test article.

No signs of maternal toxicity were observed at the lowest dose of 20 mg/kg bw per day. Test article–related increased incidences of porphyrin discharge and piloerection were seen at 70 and 120 mg/kg bw per day. At the highest dose of 120 mg/kg bw per day, test article–related signs of toxicity included increased porphyria, subdued behaviour, laboured breathing, thin appearance, piloerection and a significant ($P < 0.05$) reduction in body weight gain correlating with significantly ($P < 0.05$) reduced feed intake. There were no biological differences in the number of corpora lutea, number of implantation sites, number of live fetuses and percentage of resorptions between treated groups and the vehicle control group. A lower trend in uterus weight was observed in dams in the 120 mg/kg bw per day group. The number and distribution of fetuses were not affected by treatment. Differences in sex ratio were considered to be of no biological significance. Male fetus weight in the 120 mg/kg bw per day group was found to be significantly ($P < 0.05$) lower than that of vehicle control group animals. Ossification retardation was observed in fetuses in the 120 mg/kg bw per day group, which may be due to nonspecific inhibition or retardation of physiological growth due to the effects of maternal toxicity at this dose. Skeletal development was related to the reduced body weight of the fetuses. No soft tissue abnormalities were identified in any treatment group.

Based on the results of this study, derquantel was not teratogenic. The NOAEL for maternal toxicity was 20 mg/kg bw per day, and the NOAEL for developmental toxicity was 70 mg/kg bw per day (Rozinova, 2006).

*(ii) Rabbits*

A GLP-compliant dose range–finding study for developmental toxicity was conducted to determine doses for the subsequent main study in rabbits. Derquantel (lot no. E010002720) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant New Zealand White rabbits (five per

group) at a dose of 0 (vehicle), 0.1, 1, 10 or 100 mg/kg bw per day on gestation days 7 through 19, at a dose volume of 5 ml/kg bw. Owing to severe signs of test article–related toxicity and mortality at 100 mg/kg bw per day, this group was terminated on gestation day 10. Approximately 150 g feed per rabbit per day was offered until the first day of dosing, at which time the daily feed allotment was increased to 180–185 g feed per rabbit per day. Water was available ad libitum. Animals were observed for clinical signs daily at approximately 1-hour intervals post-dosing for 4 consecutive hours and once at the end of the day. Daily observations were performed on non-dosing days. Body weight and feed consumption were measured daily. On gestation day 29, surviving rabbits were euthanized and caesarean sectioned. Examinations of the number and distribution of corpora lutea, implantation sites and uterine contents and a gross necropsy of the thoracic, abdominal and pelvic viscera were performed. Fetuses were weighed and examined for gross external alterations and sex.

There were no test article–related deaths at doses below 100 mg/kg bw per day.

A test article–related reduction in body weight gain was observed in the 1 mg/kg bw per day group during the post-dosing phase (gestation days 20 through 29), which corresponded with lower feed consumption compared with controls. At 10 mg/kg bw per day, body weight gains and feed consumption relative to the control group were reduced during the dosing period. During the post-dosing phase, body weight gains and feed consumption rebounded, but body weight gain remained reduced for the entire gestation period.

There were no abnormal findings in the caesarean section or fetal examination data. Based on the results of this study, doses of 0 (vehicle), 0.1, 1 and 10 mg/kg bw per day were recommended for the definitive study of developmental toxicity in rabbits (Lewis, 2006).

In the definitive GLP-compliant study, derquantel (lot no. E010002720) suspended in 1% aqueous carboxymethylcellulose was administered orally by gavage to pregnant New Zealand White rabbits (20 per group) at a dose of 0 (vehicle), 0.1, 1 or 10 mg/kg bw per day on gestation days 7 through 19 at a dose volume of 5 ml/kg bw. Approximately 150 g feed per rabbit per day was offered until the first day of dosing, at which time the daily feed allotment was increased to 180–185 g feed per rabbit per day. Water was available ad libitum. Animals were observed for clinical signs daily at approximately 1-hour intervals post-dosing for 4 consecutive hours and once at the end of the day. Daily observations were performed on non-dosing days. Body weight and feed consumption were measured daily. On gestation day 29, surviving rabbits were euthanized and caesarean sectioned. The number and distribution of corpora lutea were recorded. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses and early and late resorptions. Placentae were examined for size, colour and shape. Fetuses were weighed and examined for gross external alterations and sex. Live fetuses were euthanized, and soft tissue and skeletal examinations were performed.

There were no abortions or premature deliveries. One rabbit in the 10 mg/kg bw per day group was found dead on day 10 of gestation. Although the cause of this death was not determined, it was presumed that it was test article related.

Test article–related clinical signs in does were limited to scant faeces at 10 mg/kg bw per day, the incidence of which was significantly ($P \leq 0.01$) increased relative to controls and correlated with low feed consumption in this dose group.

Test article–related effects on body weight of does were seen at 10 mg/kg bw per day and consisted of significant ($P \leq 0.01$) body weight loss relative to controls during the dosing phase and reduced to significantly reduced ($P \leq 0.05$) body weight gains between gestation days 13 and 20. Reduced body weight gain and body weight losses in the 10 mg/kg bw per day group were associated with decreases in absolute and relative feed consumption.

Increased post-implantation loss due to an increase in total resorptions was a test article–related effect seen at 10 mg/kg bw per day. One doe in this group had a litter that consisted of all resorbed conceptuses. Fetal body weights in this group were also reduced to significantly reduced ($P \leq 0.05$) relative to controls. These findings were secondary effects resulting from maternal toxicity.

Developmental toxicity was seen at 10 mg/kg bw per day and consisted of significant increases ($P \leq 0.01$) in the incidence of supernumerary thoracic ribs with associated significant increases and decreases ($P \leq 0.01$) in the numbers of thoracic and lumbar vertebrae, respectively. This variation is commonly seen at maternally toxic doses. Decreases in fetal ossification sites are indicative of a delay in development, which corresponds with the decreased fetal weights observed in this group.

Based on these findings, the NOAEL for developmental toxicity was 1 mg/kg bw per day (Lewis, 2007).

### *2.2.6 Special studies*

#### *(a) Structural toxicity alerts for derquantel and related impurities*

An assessment of the chemical structure of derquantel and related impurities paraherquamide, *N*-methyl-2-desoxyparaherquamide, PF-03334456, PF-03198957, 2-desoxyparaherquamide E and *N*-oxide for potential structural alerts for genotoxicity or carcinogenicity was conducted using the computer program DEREK (Deductive Estimation of Risk from Existing Knowledge) (Vogel, 2005; Kenyon, 2009). No structural alerts were identified for derquantel or any of the related compounds tested (Kenyon, 2009).

#### *(b) Impurities*

2-Desoxyparaherquamide E is an impurity that can occur during the synthetic conversion of the paraherquamide E fermentation impurity in paraherquamide to the derquantel moiety equivalent. It has been present at variable concentrations up to 1.4% in development batches used in toxicity studies. An acceptance criterion of not more than 1% is proposed for the active pharmaceutical ingredient. 2-Desoxyparaherquamide E was tested for the potential to induce microbial gene

***Table 3. Results of a genotoxicity assay with 2-desoxyparaherquamide E***

| Test system | Test object | Concentration | Results | Reference |
|---|---|---|---|---|
| **In vitro** | | | | |
| Ames reverse mutation assay[a] | *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvrA* | 6.67–5000 µg/plate | Not mutagenic | Mecchi (2009) |

[a] With and without Aroclor 1254–induced rat liver S9 fraction.

mutations in four Ames tester strains and in *Escherichia coli* strain WP2*uvrA*. The results are shown in Table 3.

*(c) Microbiological effects*

A JECFA decision-tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for derquantel. The decision-tree approach initially seeks to determine if there may be microbiologically active derquantel residues entering the human colon.

Derquantel is not classified as an antimicrobial agent and is not structurally related to antimicrobial agents used in animal or human medicine. The Committee considered an in vitro evaluation of derquantel against 62 strains representing a wide range of Gram-positive and Gram-negative bacteria of veterinary importance. Derquantel showed limited strain-dependent activity against the staphylococci (minimum inhibitory concentrations [MICs] ranging from 64 to 128 µg/ml for all strains). Against the streptococci, derquantel showed very limited activity (MICs ≥128 µg/ml). Against *Pasteurella* spp., derquantel showed limited activity (MICs ranging from 32 to >128 µg/ml). Derquantel was not active against the enteric organisms tested (Salmon, Case & Watts, 1998).

The Committee concluded that a microbiological ADI for derquantel was not necessary.

### *2.3 Toxicological studies with derquantel/abamectin (Startect)*

A summary of these studies is included to assess the toxicity of derquantel when used in combination with abamectin as an anthelminthic drug combination product for veterinary use. The formulation consists of derquantel at 10 mg/ml and abamectin at 1 mg/ml.

#### *2.3.1 Acute toxicity*

Single-dose toxicity studies with the derquantel/abamectin combination are summarized in Table 4.

***Table 4. Acute toxicity studies with derquantel/abamectin***

| Species | No. of each sex per group | Route | Dose | $LD_{50}$ | Reference |
|---|---|---|---|---|---|
| Rat | 7F | Oral | 55/5.5, 100/10, 175/17.5, 200/20 mg/kg bw | 88.72/8.872 mg/kg bw | Lloyd (2008d) |
| Rat | 5M, 6F | Dermal | 30/3 mg/kg bw | >30/3[a] mg/kg bw | Lloyd (2008e) |
| Rat | 5M, 5F | Inhalation | 5.04 mg/l[b] for 4 h | >5.04 mg/l[c] | Rodabaugh (2008) |

F, female; $LD_{50}$, median lethal dose; M, male

[a] No dermal toxicity observed.

[b] Calculated dose from this exposure was 0.05 mg/l derquantel and 0.005 mg/l abamectin.

[c] $LC_{50}$. No clinical signs of toxicity were observed.

### *2.3.2 Short-term studies of toxicity*

#### *(a) Rats*

In a non-GLP-compliant, 7-day oral dose range–finding study, rats approximately 6 weeks of age (four of each sex per group) were given derquantel/abamectin (batch no. 071027-1) by gavage at a dose of 0/0, 1/0.1, 2/0.2, 10/1 or 20/2 mg/kg bw per day. Control animals were dosed with the vehicle, 0.05% (w/v) butylated hydroxytoluene, 40% (w/v) triacetin and Miglyol 840. The dose volume given to all groups was 5 ml/kg bw. Feed and water were provided ad libitum. Animals were checked twice daily for signs of morbidity or mortality. Detailed clinical examinations were performed twice daily pre-dosing and 2–3 hours post-dosing. Body weights were measured prior to dose initiation and at termination. Feed consumption was measured once (weekly). At study termination, animals were euthanized and necropsied. Fourteen organs were collected, weighed and preserved, and a comprehensive assortment of other tissues and organs was collected and preserved.

All animals survived to scheduled necropsy. Clinical signs of salivation were observed at 20/2 mg derquantel/abamectin per kilogram body weight per day in a single male and three of four females on days 6 and 7. This observation was treatment related but not considered adverse. No other clinical signs were attributable to treatment with the test article, and there were no effects on body weight or feed consumption.

Mean absolute and relative liver weights were increased in 20/2 mg derquantel/abamectin per kilogram body weight per day females relative to controls. The difference for the mean relative value was statistically significant. There were no associated histopathological findings. Cytoplasmic clearing of hepatocytes without vacuolation was noted in males and females in all groups, except control females. This finding is consistent with glycogen accumulation and was likely a vehicle effect.

On the basis of an increased incidence of salivation seen in males and females and increased liver weight without corresponding increases in biochemical markers associated with hepatocellular injury or microscopic evidence of toxicity in females, the NOAEL was 20/2 mg derquantel/abamectin per kilogram body weight per day, the highest dose tested (May, 2009d).

In a GLP-compliant, 28-day oral toxicity study, rats approximately 8 weeks of age (15 of each sex per group) were given derquantel/abamectin (batch no. 080523-1) by gavage at a dose of 0/0, 3/0.3, 20/2 or 30/3 mg/kg bw per day, at dose volumes of 3, 1, 2 and 3 ml/kg bw, respectively. Control animals received placebo 16957A, which consisted of 0.05% (w/v) butylated hydroxytoluene, 5% (w/v) glycerol formal, 40% (w/v) triacetin and approximately 54.95% (w/v) Miglyol 840. The 3/0.3 mg derquantel/abamectin per kilogram body weight per day dose was prepared by diluting a 10/1 stock solution with placebo 16957A. Undiluted test article was used for the higher dose levels. Owing to early deaths, the 20/2 mg/kg bw per day dose level was lowered to 10/1 mg/kg bw per day between days 1 and 3, and the 30/3 mg/kg bw per day dose was lowered to 20/2 mg/kg bw per day on day 4. Dose volumes were respectively lowered to 1 and 2 ml/kg bw. Feed was available ad libitum, except during designated periods. Water was available ad libitum. All animals were checked twice daily for signs of morbidity or mortality. Detailed clinical examinations were performed during the acclimation period and 4 times daily (pre-dosing and 1, 4 and 6 hours post-dosing). The 6-hour post-dosing observation was added on day 4. Functional observational battery evaluations were conducted prior to dose initiation, 2 hours post-dosing on day 1, prior to dosing on day 2, 4 hours post-dosing on day 21 and prior to dosing on day 28. Locomotor activity evaluations were conducted on five designated main-study animals of each sex post-dosing on day 1 and pre-dosing on day 28. Body weights were measured prior to dose initiation and weekly during the study. Feed consumption was recorded weekly. Ophthalmic examinations were conducted on all animals prior to dose initiation and just prior to terminal necropsy. Samples for clinical pathology evaluations were collected just prior to terminal necropsy. Blood was collected from feed-fasted animals, and urine was collected while animals were housed in metabolism cages. Bone marrow smears were collected at necropsy and examined microscopically. At study termination, animals were euthanized and necropsied. Fourteen organs were collected and weighed. Sections of these as well as sections from a comprehensive assortment of other tissues and organs were prepared for microscopic examination. All tissues from the control and 20/2 mg/kg bw per day groups and gross lesions or tissue masses from all groups were examined microscopically. In addition, the adrenals, liver, pituitary, thyroid gland and vagina were identified as target organs and were examined from the 3/0.3 and 10/1 mg/kg bw per day groups. A peer review of the histopathological findings was also conducted.

There were three deaths during the study. On day 3, a 30/3 mg/kg bw per day female was euthanized owing to severe signs of test article–related toxicity. On day 5, a 20/2 mg/kg bw per day female was euthanized owing to severe signs of test article–related toxicity after having received three 30/3 mg/kg bw and two 20/2 mg/kg bw dose administrations. On day 7, a 10/1 mg/kg bw per day female was euthanized as a result of a malocclusion of the incisors and associated loss of ability to eat. The malocclusion was assessed as being caused by the animal

biting the wire of the cage bottom during post-dosing tremors observed before the dose level was lowered from 20/2 to 10/1 mg/kg bw per day. Therefore, this animal's death was not directly related to treatment with the test article.

Test article–related clinical signs in animals given 30/3 and 20/2 mg/kg bw per day were decreased activity, ataxia, difficult and shallow breathing, head tilt, prostration, tremors, vocalization, hunched posture, partially/completely closed eyes, piloerection, salivation, and thin and unkempt appearance. The majority of these signs were associated with 30/3 mg/kg bw per day dose administrations. In animals given 10/1 mg/kg bw per day, clinical signs were tremors, piloerection, abnormal respiration, salivation, and thin and unkempt appearance, all of which were noted in a limited number of animals. Salivation was the only abnormal clinical sign at 3/0.3 mg/kg bw per day; however, the incidence was comparable to that in controls and therefore was not a test article–related effect. Salivation was noted as a test article–related effect in males and females at 20/2 mg/kg bw per day based on the markedly increased numbers of animals affected compared with lower-dose groups and controls.

Increases in mean forelimb and hindlimb grip strength were noted in 20/2 and 10/1 mg/kg bw per day males and females on days 21 and 28. The effect was test article related but not considered adverse, because the differences were of low magnitude and only sometimes statistically significant.

Tail pinch response was significantly decreased in 20/2 mg/kg bw per day females on day 28. This finding was test article related.

On day 28, rearing counts were decreased compared with controls for males and females at all dose levels. Differences (0–240 minutes) were significant ($P < 0.01$) for males in all test article–treated groups and females at 20/2 and 10/1 mg/kg bw per day. The majority of statistically significant decreases were noted at measurement intervals during the first 90 minutes of monitoring and were not associated with decreases in any other dimension of locomotor activity evaluated. Mean rearing counts decreased with a dose-related response in males, but not females. Based on the magnitude of the decreases noted, this effect was test article related at all dose levels, but not considered adverse, as the finding was not associated with adverse effects on the overall health or well-being of the animals.

Test article–related effects on body weight included significant decreases relative to controls in mean body weight for 20/2 mg/kg bw per day males at weeks 2 ($P < 0.01$), 3 ($P < 0.05$) and 5 ($P < 0.05$). Mean body weight values for females were slightly lower (≤5%) compared with controls at these intervals. The lower value for week 2 was associated with significantly lower mean feed consumption for males and females in this group during week 1. Mean feed consumption during subsequent weekly intervals was comparable to that of controls, indicating a direct, adverse test article–related effect on body weight gain in males and females at 20/2 mg/kg bw per day.

There were no test article–related effects in the clinical pathology data.

Test article–related changes in organ weights included increased mean liver weights (absolute and relative) in males and females at 10/1 and 20/2 mg/kg bw per

day; increased adrenal weights (absolute and relative) in males at 10/1 and 20/2 mg/kg bw per day; and increased thyroid weights (absolute and relative) in females at 10/1 and 20/2 mg/kg bw per day. In addition, there was a significant increase in relative pituitary weight in males at 20/2 mg/kg bw per day.

Test article–related histopathology findings included minimal centrilobular hepatocellular hypertrophy and minimal follicular cell hypertrophy in the thyroid gland in females at 20/2 mg/kg bw per day. Both of these findings were adverse because they correlated with increases in respective organ weights. Minimal diffuse cortical hypertrophy was observed in adrenal glands of males and females at 10/1 and 20/2 mg/kg bw per day and corresponded with significant adrenal weight changes in males. Based on the absence of a dose–response relationship for adrenal weight changes in males and females relative to controls, the adrenal gland cortical hypertrophy observed is of uncertain toxicological relevance. Minimal to mild cellular hypertrophy of cells in the pars distalis of the pituitary gland in 10/1 and 20/2 mg/kg bw per day males was an adverse finding that correlated with increased relative pituitary weights.

The NOAEL for this study was 3/0.3 mg derquantel/abamectin per kilogram body weight per day. This is based on signs of neurotoxicity and adverse effects on body weight of males and females and hepatotoxicity, thyroid toxicity and mortality observed in females at the higher dose levels (May, 2009e).

### *2.4 Observations in humans*

No data were available, as derquantel is not a drug used in human medicine, and there were no reports of accidental human exposures submitted or identified in the literature search.

## *3. COMMENTS*

The Committee considered results of studies on receptor pharmacology, pharmacokinetics, acute and short-term toxicity, in vitro, in vivo and in silico genotoxicity, reproductive toxicity and microbiological safety. The majority of the studies were performed in accordance with GLP standards. In addition, a literature search in Embase database was performed. No additional information relevant to this evaluation was identified.

### *3.1 Biochemical data*

In rats dosed orally with derquantel at 200 mg/kg bw per day for 4 days, peak plasma concentrations ($C_{max}$) of approximately 20 µg/ml occurred at 2 hours post-dosing ($T_{max}$). When rats were administered daily oral doses of derquantel ranging from 0.01 to 150 mg/kg bw per day for a longer duration (90 days, 1 year or multigeneration), the mean concentration of derquantel in plasma showed a dose-dependent increase, with no accumulation. $T_{max}$ was mainly 2 hours (ranging from 0.5 to 2 hours), according to the dose administered. Exposure, as measured by AUC and $C_{max}$, was consistently higher in females than in males.

In rats dosed once daily by oral gavage for 7 days with the combination product containing a 10:1 ratio of derquantel and abamectin at doses up to

20/2 mg/kg bw per day, the plasma concentration of derquantel and abamectin increased with increasing doses of the combination product. Derquantel appeared not to accumulate, whereas abamectin showed accumulation. $T_{max}$ was 2 hours post-dosing for derquantel and 6 hours post-dosing for abamectin.

When pregnant rabbits were given derquantel orally by stomach tube once daily on days 7 through 19 of gestation at doses up to 10 mg/kg bw per day, the $AUC_{0-t(last)}$ and $C_{max}$ values increased with increasing dose, and $T_{max}$ was consistently 0.5 hour post-dosing.

When dogs were given daily oral doses of derquantel of up to 10 mg/kg bw per day for 28 days, the $AUC_{0-24}$ and $C_{max}$ values increased at a rate greater than proportional to increasing dose. Accumulation was found on day 27 only at 10 mg/kg bw per day. $T_{max}$ ranged from 0.5 to 2 hours, and the elimination half-life was 2–6 hours.

In two studies, derquantel was given to dogs as a single oral dose via capsule at dose levels up to 10 mg/kg bw per day or by oral gavage at dose levels up to 5 mg/kg bw per day for 3 months. The $C_{max}$ values for derquantel indicated no accumulation over time for doses of 0.1, 0.5, 1 and 5 mg/kg bw per day. Accumulation was seen at 10 mg/kg bw per day. $T_{max}$ was generally between 0.5 and 2 hours in the three lowest dose groups, but ranged from 1 to 9 hours at 5 and 10 mg/kg bw per day. Half-lives generally increased with dose; however, all were relatively short, less than 7 hours.

One male and one female horse were given a single oral dose of derquantel at 2 mg/kg bw. Maximum plasma concentrations occurred at 1 hour post-dosing, with $C_{max}$ values of 0.157 µg/ml and 0.075 µg/ml and AUC values of 0.48 µg·h/ml and 0.27 µg·h/ml for the male and female, respectively. Additionally, a mono-hydroxylated metabolite of derquantel, previously not seen in other species, was identified at higher apparent plasma concentrations than for the parent derquantel.

Derquantel (1 µmol/l) was metabolized extensively in dog liver microsomes, with only 2.8% of parent detected after a 1-hour incubation. In rat, sheep and human liver microsomes, derquantel was metabolized moderately, with approximately half of the derquantel unchanged. Among 18 metabolites occurring in microsomal cultures, M3, M4, M5a and M5b were predominant in dog liver microsomes, whereas M1, M9 and M10 were found as predominant metabolites in rat, sheep and human liver microsomes. The M8 metabolite was another predominant metabolite in rats.

Hepatocytes from rat, sheep, dog and human metabolized 49–81% of derquantel (1 µmol/l) over a 4-hour incubation. Unchanged derquantel and 26 radioactive metabolites were detected in rat, sheep, dog and/or human hepatic incubations. Hepatocytes from rats, dogs, sheep and humans exhibited similar patterns of metabolites, as M1, M8, M10, M12 and M19/20 commonly occurred as predominant metabolites, with variations in the relative levels of each. Also, M18 was a prominent metabolite, accounting for 8% of the total in rat and human hepatocytes. M15 was found as a predominant metabolite only in dogs, comprising 11% of the total.

Rats (one of each sex) were given a single oral dose of [$^{14}$C]derquantel at 100 mg/kg bw (14.8 MBq/kg bw). Most of the radiolabel (85% and 67% for the male and

female, respectively) was recovered in the faeces in the first 2 days. Approximately 95% of the radioactive dose was recovered in urine and faeces during the 7-day post-dosing period, with 87–90% of the dose in the faeces and 5.5–7.6% in the urine. Negligible amounts of the dose were detected in the carcass and terminal blood samples. The formation of approximately 11 metabolites was observed during 7 days post-dosing. The molecular weights of some of the metabolites indicated metabolism by glucuronidation and hydroxylation.

When cannulated rats were dosed orally with [$^{14}$C]derquantel at 50 mg/kg bw to identify metabolite profiles in urine, faeces, bile and tissues, the majority (69–75%) of the radioactive dose was recovered in faeces, and small amounts (3–4% and 1%, respectively) were recovered in urine and tissues. The majority of the radioactive dose (64–70%) was excreted in bile. The results showed that derquantel has high oral bioavailability. Large numbers of metabolites were found in urine, faeces and bile, indicating a high degree of biotransformation in the rat. Metabolic profiles in males and females were similar, although the rate of metabolism was more rapid in males than in females. Faeces contained mainly the parent drug at the 0- to 24-hour collection interval, whereas only small amounts of parent derquantel along with a large number of metabolites were found at the 144- to 150-hour collection interval. Parent derquantel and metabolites M1, M2, M4 and M5 are the most common components found in the tissues. In rat liver, derquantel comprised 6–8% of the total radioactivity. In rat muscle and fat, derquantel comprised higher percentages of the total residues in tissues of females (21% and 53%, respectively) relative to the males (5% and 31%, respectively).

### *3.2 Toxicological data*

The acute oral toxicity of derquantel varies between species. Derquantel was extremely toxic to horses, but showed low toxicity in rats ($LD_{50}$ >2000 mg/kg bw). Clinical signs of acute toxicity in mice, rats and dogs were attributable to the pharmacological activity of derquantel as an antagonist of nAChR. Whether higher sensitivity in horses was due to a toxic metabolite or greater sensitivity to the pharmacological activity of derquantel could not be determined from the data. The primary clinical signs of acute toxicity included various effects on the nervous system, such as prostration, seizures, hypoactivity and ataxia, and various ocular effects, including mydriasis, ptosis and relaxation of the nictitating membrane. Derquantel was not a skin sensitizer in guinea-pigs or a skin or ocular irritant in rabbits.

In a non-GLP-compliant, 3-day escalating oral dose range–finding study, 3-month-old rats (two of each sex per group) received derquantel via gastric intubation at a dose of 0, 25, 50, 100, 200, 300, 400 or 600 mg/kg bw per day. Dose volumes increased with dose up to a maximum volume of 12 ml/kg bw for the 600 mg/kg bw per day group. Because of clinical signs of excessive toxicity, dosing of the 400 mg/kg bw per day group was interrupted, and the animals were allowed a 2-week washout period. Dosing then resumed at a dose level of 300 mg/kg bw per day for 2 days. The 600 mg/kg bw per day dose was given only once due to excessive toxicity. Clinical signs of toxicity included death, hypoactivity, loss of righting reflex, ataxia, prostration, jerky movements, abnormal posture, laboured respiration, tremors and ptosis. There were no clinical signs of toxicity at doses up

to and including 300 mg/kg bw per day. There was no microscopic examination of tissues. The NOAEL for this study was 300 mg/kg bw per day.

In a 12-day oral dose range–finding study, 6- to 8-week-old rats (two of each sex per group) received derquantel via gastric intubation at a dose of 0, 30, 50, 100, 150, 200, 250, 400, 600 or 800 mg/kg bw per day. Clinical signs of toxicity included death (≥250 mg/kg bw per day), prostration, paralysis, convulsions, jerky movements, ataxia, hypoactivity, hunched or tilted posture, piloerection, porphyrin staining around the nose and excessive salivation. No test article–related clinical signs or effects on body weight were seen up to and including 150 mg/kg bw per day. Test article–related macroscopic findings included a dose-related increase in mean liver weight of males and females in all test article–treated groups. Microscopic examinations were limited to livers of animals in the 30 and 150 mg/kg bw per day groups. Test article–related microscopic findings included periacinar hepatocyte swelling and hepatic cytoplasmic vacuolation with a few scattered necrotic hepatocytes at 150 mg/kg bw per day. There were no microscopic findings in the liver to correspond with increased liver weight at 30 mg/kg bw per day. Based on a slight increase in mean liver weight with no associated histopathological findings, the NOAEL for this study was 30 mg/kg bw per day.

In a 28-day oral toxicity study, 6- to 8-week-old rats received derquantel via gastric intubation at a dose of 0, 20, 100 or 200 mg/kg bw per day. Clinical signs of toxicity included death (200 mg/kg bw per day), ataxia, prostration, convulsions, hypoactivity, piloerection, laboured breathing, paralysis, tilted posture, jerky movements, tremors, splayed hindlimbs and porphyrin staining around the nose. Decreases in body weight gain and feed consumption occurred in males and females at 200 mg/kg bw per day. Mean relative liver weights were significantly increased in males and females in the 100 and 200 mg/kg bw per day groups. Elevated serum GGT levels were seen in males and females at 200 mg/kg bw per day. Microscopically, bile duct hyperplasia of minimal severity was seen in males and females at 20 mg/kg bw per day. The severity of this finding increased to minimal to mild in males and females at 100 mg/kg bw per day and to minimal to severe in males and females at 200 mg/kg bw per day. Based on the significant increases in mean liver weights at 100 and 200 mg/kg bw per day with an associated dose-related increase in the incidence and severity of bile duct hyperplasia and elevated serum GGT levels and mortality at 200 mg/kg bw, the NOAEL for this study was 20 mg/kg bw per day.

In a 28-day oral toxicity study, 7- to 8-week-old rats received derquantel enriched with 4% *N*-oxide, a degradation product of derquantel seen during accelerated stability testing of the derquantel/abamectin drug product. Treatment was given by gastric intubation at dose levels of 0, 0.5, 5, 25 and 175 mg/kg bw per day. As a result of excessive toxicity and deaths in females, the 175 mg/kg bw per day dose level was lowered to 150 mg/kg bw per day in females only beginning on day 9. Significantly lower rearing counts were seen in females at 5 mg/kg bw per day and in males and females at 25 and 175/150 mg/kg bw per day. Additional treatment-related effects included significantly increased liver weight and hepatocellular hypertrophy at 25 mg/kg bw per day and elevations of serum GGT, ALT and sorbitol dehydrogenase enzymes in males and females and significantly

increased thyroid weight and follicular cell hypertrophy in females at 175/150 mg/kg bw per day. Based on lower rearing counts in females at 5 mg/kg bw per day and higher, the NOAEL for this study was 0.5 mg/kg bw per day.

In a 90-day oral toxicity study, rats less than 9 weeks of age received derquantel by gastric intubation at a dose of 0, 1, 5, 50 or 150 mg/kg bw per day. The quality and reliability of the histopathology data were limited due to the poor quality of the slides. There were also concerns about whether the timing of the clinical observations was adequate to capture the potential neurobehavioural effects of derquantel. As a result, the Committee could not determine a NOAEL for this study.

In a 1-year oral toxicity study, 8-week-old rats received derquantel by gastric intubation at a dose of 0, 1, 5 or 50 mg/kg bw per day. There were no test article–related deaths and no clinical signs of toxicity. Evidence of toxicity in the serum chemistry data included elevated mean levels of GGT at 50 mg/kg bw per day in both males and females. In 50 mg/kg bw per day females, mean bilirubin level was significantly elevated, and mean cholesterol and triglyceride levels were increased. In males at 50 mg/kg bw per day, a significant increase in mean globulin level resulted in a significant increase in mean total protein level and a lower albumin to globulin ratio compared with controls. In females at 50 mg/kg bw per day, mean globulin levels were increased at all dose levels compared with controls. The difference was significant, resulting in a significant ($P < 0.01$) decrease in the albumin to globulin ratio and significantly increased total protein at this dose level. In addition, mean total protein levels were significantly increased in all test article–treated groups. Cataracts seen in males at 50 mg/kg bw per day were treatment related. Treatment-related effects on the liver included enlarged livers and increased mean liver weight. Increased incidence and severity of age-related biliary hyperplasia were also seen starting at 1 mg/kg bw per day. Macroscopic and microscopic liver effects were considered adverse at 50 mg/kg bw per day in males and females due to associated elevations in serum chemistry parameters indicative of hepatocellular injury. The severity of spontaneous, chronic progressive nephropathy was increased in males and females at 50 mg/kg bw per day. Mild to marked hypospermia and degeneration of the seminiferous tubules were adverse effects seen in males at 50 mg/kg bw per day. Minimal degeneration of the seminiferous tubules was a non-adverse test article–related finding in males at 5 mg/kg bw per day.

A second 1-year oral toxicity study in the rat was conducted with lower dose levels, which allowed for a better evaluation of the hepatobiliary effects of derquantel seen in the previous study. In this study, 8-week-old rats received derquantel by gastric intubation at a dose of 0, 0.01, 0.03, 0.1 or 0.3 mg/kg bw per day. There were no test article–related deaths or clinical signs of toxicity. Test article–related effects in this study were limited to histopathological findings of an increased incidence and severity of biliary hyperplasia and fibrosis in the liver in males at and above 0.03 mg/kg bw per day. These were considered to be non-adverse and age-related spontaneous findings, the incidence or severity of which was accentuated by the test article. There were no associated changes in liver weights or in the serum chemistry data.

Biliary hyperplasia occurs spontaneously in rats and has not been correlated with liver carcinogenicity or neoplasia (Allen et al., 2004). A statistical analysis of the biliary hyperplasia response in the two chronic rat studies with derquantel comparing any positive response in each dose group with the control response showed that the incidence of biliary hyperplasia was significantly elevated relative to controls only at 50 mg/kg bw per day. In addition, this dose level was associated with significant increases in liver weight and changes in GGT levels consistent with hepatotoxicity and is therefore a clear adverse effect level. There was no statistically detectable difference in the incidence of biliary hyperplasia seen at lower dose levels, nor were there significant increases in liver weights or changes in serum chemistry parameters indicative of hepatocellular injury. Thus, 5 mg/kg bw per day would be considered the NOAEL on the basis of this analysis. This NOAEL is further supported by BMD modelling of the pooled data from both 1-year toxicity studies in the rat using various models. This modelling was performed by the sponsor and reviewed by the Committee. The $BMDL_{10}$ derived from these models ranged from 2.0 to 7.6 mg/kg bw per day, which was in close agreement with the previously statistically verified NOAEL of 5 mg/kg bw per day. On the basis of these two analyses, the Committee concluded that the NOAEL for biliary hyperplasia in the rat was 5 mg/kg bw per day. The Committee further noted that this NOAEL is consistent with the overall NOAEL that the Committee determined for both 1-year studies, which was also 5 mg/kg bw per day based on testicular effects.

In a non-GLP-compliant 28-day preliminary oral toxicity study, dogs received derquantel as neat test article in gelatine capsules at a dose of 0, 1, 5 or 10 mg/kg bw per day. Control animals were dosed with an empty gelatine capsule. Clinical signs of dry conjunctiva, dry eyes, relaxed nictitating membrane, ptosis, red eyes or mydriasis were seen in every dog dosed with derquantel during the first week of dosing, but the incidence of dry conjunctiva and dry eyes was lower in weeks 3 and 4 (compared with the first 2 weeks of the study). There was no associated pathology. The LOAEL for this study was 1 mg/kg bw per day, based on test article–related clinical observations seen at all dose levels.

In a 28-day oral toxicity study, dogs received derquantel by gavage at a dose of 0, 0.01, 0.03 (0.01 mg/kg bw per dose, 3 times per day), 0.03 or 0.1 mg/kg bw per day. Owing to the absence of clinical signs at 0.03 mg/kg bw per day given once daily or at 0.01 mg/kg bw given 3 times daily, dosing and data collection were suspended in the 3 times daily group after 7 days of dosing. There were no test article–related effects at any dose tested. Therefore, the NOAEL for this study was 0.1 mg/kg bw per day, the highest dose tested.

In a 90-day oral toxicity study, adolescent dogs received neat derquantel by capsule at a dose of 0, 0.1, 0.5, 1 or 10 mg/kg bw per day. Clinical signs seen in all animals in all treatment groups included dry mouth, mydriasis and relaxed nictitating membranes. Dilated pupils were seen in all treated animals at both 2 and 5 hours post-dosing at one point or another in the study. The incidence increased with dose. At 24 hours post-dosing, the pupils were still dilated, with incidences of 4%, 7% and 47% at 0.5, 1 and 10 mg/kg bw per day, respectively. Ptosis was also seen in all treatment groups, with a dose-related increase in the number of animals affected and the incidence of observations in individual animals. Dry conjunctiva,

dry eyes, red eyes and tremors were also treatment related in some groups based on increased numbers of animals affected and increased incidence of observations in individual animals relative to controls. Test article–related decreases in mean body weight and feed consumption of dogs at 10 mg/kg bw per day were also noted. On the basis of observed test article–related clinical signs at all dose levels, the LOAEL for this study was 0.1 mg/kg bw per day.

In a second 90-day oral toxicity study, mature dogs received derquantel by gavage at a dose of 0, 0.1, 0.5, 1 or 5 mg/kg bw per day. Test article–related clinical signs included relaxed nictitating membrane (in 0/0, 1/1, 2/4, 4/3 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day) and mydriasis (in 0/0, 1/1, 3/1, 4/4 and 4/4 males/females at 0, 0.1, 0.5, 1 and 5 mg/kg bw per day). In affected animals, the incidence of observations in individual animals increased with increasing dose. An increased incidence of eye redness was seen at 1 and 5 mg/kg bw per day for males and females. There was no pathology associated with the clinical signs observed. Based on treatment-related observations consistent with the known pharmacological activity of the test article at all dose levels, the LOAEL for derquantel was 0.1 mg/kg bw per day.

The Committee performed and considered the results of BMD modelling to better define a point of departure for nictitating membrane protrusion, which was among the most sensitive effects seen in the dog. The Committee concluded that the $BMDL_{10}$ approach was not appropriate for the purpose of this evaluation. This conclusion was based on the high degree of uncertainty at the low end of the dose–response curve, the limited data available with which to model a dose–response relationship for elicitation of protrusion of the nictitating membrane in the dog and the fact that the two studies from which the data were derived differed in the age of dogs on study (juvenile versus adult) and mode of test article administration (capsule versus gavage).

In an adequate range of in vitro and in vivo genotoxicity studies, derquantel produced positive results in an in vitro chromosomal aberration assay in human peripheral lymphocytes, without metabolic activation, at dose levels that produced at least 50% cytotoxicity. Derquantel was not genotoxic in in vitro tests for bacterial mutagenicity or in in vivo studies in mouse bone marrow or rat hepatocytes. The Committee concluded that derquantel was weakly clastogenic in vitro, but was unlikely to be genotoxic in vivo. Derquantel had no structural alerts for mutagenicity, carcinogenicity or clastogenicity as determined by in silico analysis using the computer program DEREK.

No carcinogenicity studies were performed. Based on the absence of genotoxicity in vivo, the absence of structural alerts for carcinogenic, mutagenic or clastogenic potential by in silico analysis and lack of evidence of carcinogenicity or preneoplastic changes in rats dosed up to 1 year, the Committee concluded that derquantel is unlikely to pose a carcinogenic risk to humans at exposure levels likely to occur in food.

In a two-generation (one litter per generation) reproduction study, male and female rats were dosed with derquantel by oral gavage at dose levels of 0, 1, 5 or 25 mg/kg bw per day. Derquantel was well tolerated, with no evidence of reproductive

impairment or adverse effects on progeny at 0, 1, 5 or 25 mg/kg bw per day. At 25 mg/kg bw per day, an increase in liver weight was seen in $P_1$ males, and $P_1$ females had increased thyroid weight and increased incidence of thyroid follicular epithelial cell hypertrophy and hyperplasia. Terminal body weight, liver weight and adrenal weight were also increased in 25 mg/kg bw per day $P_1$ females. At 25 mg/kg bw per day, increased liver weights were also seen in $F_1$ males and females, and terminal body weight was increased in $F_1$ females. Increased incidence and severity of thyroid follicular epithelial hyperplasia were seen in $F_1$ males and females at 25 mg/kg bw per day. The NOAEL for reproductive effects was 25 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study, derquantel was administered orally by gavage to pregnant rats at doses of 0 (vehicle), 20, 70 or 120 mg/kg bw per day on gestation days 5 through 20. The dose levels were established in a previously conducted pilot study in pregnant rats using dose levels of 0, 2, 10, 50 and 100 mg/kg bw per day. No signs of maternal toxicity were observed at the lowest dose of 20 mg/kg bw per day. Test article–related increased incidences of porphyrin discharge and piloerection were seen at 70 and 120 mg/kg bw per day. At the highest dose of 120 mg/kg bw per day, test article–related signs of toxicity included increased porphyria, subdued behaviour, laboured breathing, thin appearance, piloerection and a significant reduction in body weight gain correlating with significantly reduced feed intake. There were no biological differences in the number of corpora lutea, number of implantation sites, number of live fetuses and percentage of resorptions between treated groups and the vehicle control group. A lower trend in uterus weight was observed in dams in the 120 mg/kg bw per day group. Male fetus weight was significantly lower in the 120 mg/kg bw per day group than in vehicle control–treated animals. Ossification retardation was observed in fetuses in the 120 mg/kg bw per day group and was attributed to nonspecific inhibition or retardation of physiological growth due to the effects of maternal toxicity at this dose. Skeletal development was related to the reduced body weight of the fetuses. No soft tissue abnormalities were identified in any treatment group. Based on the results of this study, derquantel was not teratogenic. The NOAEL for maternal toxicity was 20 mg/kg bw per day, and the NOAEL for developmental toxicity was 70 mg/kg bw per day.

In another developmental toxicity study, derquantel was administered orally by gavage to pregnant rabbits at doses of 0 (vehicle), 0.1, 1 or 10 mg/kg bw per day on gestation days 7 through 19. Dose levels were determined by a previously conducted pilot study in pregnant rabbits given derquantel at dose levels of 0, 0.1, 1, 10 or 100 mg/kg bw per day. There was one death attributable to test article toxicity at 10 mg/kg bw per day. Other clinical findings at 10 mg/kg bw per day included low feed consumption, resulting in scant faeces, significant body weight loss and reduced body weight gains. At 10 mg/kg bw per day, an increase in total resorptions was seen, and fetal body weights were significantly reduced. Developmental toxicity characterized by an increased incidence of supernumerary thoracic ribs with associated increases and decreases in the numbers of thoracic and lumbar vertebrae, respectively, at 10 mg/kg bw per day was attributable to maternal toxicity and the decreased fetal weights observed in this group. Based on the findings at 10 mg/kg bw per day, the NOAEL for developmental toxicity was 1 mg/kg bw per day.

***Table 5. Toxicity comparison of derquantel/abamectin combination product***

| Study type | NOAEL | | |
|---|---|---|---|
| | Derquantel/abamectin | Derquantel | Abamectin[a] |
| Acute oral, rat | 88.72/8.872 mg/kg bw[b] | >2000 mg/kg bw[b] | 8–2.8 mg/kg bw[b] |
| Acute dermal, rat | No dermal toxicity | No dermal toxicity | >330 mg/kg bw[b] |
| Acute (4 h) inhalation, rat | >5.04 mg/l[c] | >2.4 mg/l[c] | ND |
| Short-term oral, rat | 3/0.3 mg/kg bw per day[d]<br>20/2 mg/kg bw per day[e] | 0.5 mg/kg bw per day[d]<br>30 mg/kg bw per day[f] | 1.5 mg/kg bw per day[g] |

ND, no data
[a] WHO (1992).
[b] Estimated $LD_{50}$.
[c] $LC_{50}$.
[d] Twenty-eight-day study.
[e] Seven-day study.
[f] Twelve-day study.
[g] Eight-week study.

The Committee observed that adverse effects on developmental parameters occurred only at dose levels that were maternally toxic. Therefore, the Committee concluded that derquantel had no direct reproductive or developmental toxicity potential.

For comparative toxicity purposes, results of toxicity studies with the 10 mg/ml derquantel and 1 mg/ml abamectin combination product, derquantel and abamectin are shown in Table 5.

The Committee concluded that there was no indication that the derquantel/abamectin combination showed any greater toxicity than the more toxic component, abamectin. It was further noted that the pharmacokinetic profile for derquantel when given in the combination product was similar to the pharmacokinetic profile of derquantel given alone in the laboratory species tested.

When the functional antagonistic potency of derquantel on α3 nAChR endogenously expressed in rat and canine dorsal root ganglion neuron cell cultures was evaluated using whole-cell patch clamp assays, derquantel displayed more potent antagonist action against acetylcholine at canine α3 nAChR ($IC_{50}$ of 1 µmol/l) than at rat α3 nicotinic acetylcholine receptors ($IC_{50}$ of 30 µmol/l).

In an in vitro study of functional agonistic and antagonistic potency using a fluorescent imaging plate reader assay to measure calcium flux, derquantel showed no agonist activity in human α3, muscle-type or α7 nAChR models. Antagonist activity of derquantel was demonstrated by inhibiting the activity of nicotine at α3 nAChR of humans with an $IC_{50}$ of 9 µmol/l. In addition, in vitro antagonistic potency of derquantel at human muscle-type nAChRs was reported with an $IC_{50}$ value of 10 µmol/l, but derquantel was inactive (with an $IC_{50}$ value of >100 µmol/l) at the central

nervous system α7 nAChR subtype. Direct comparison of the results of this study with those from studies in dogs and rats was not possible because of differences in study design.

### *3.3 Microbiological data*

A JECFA decision-tree approach that was adopted at the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with VICH GL36 was used by the Committee to determine the need to establish a microbiological ADI for derquantel. The decision-tree approach initially seeks to determine if there may be microbiologically active derquantel residues entering the human colon.

Derquantel is not classified as an antimicrobial agent and is not structurally related to antimicrobial agents used in animal or human medicine.

The Committee considered an in vitro evaluation of derquantel against 62 strains representing a wide range of Gram-positive and Gram-negative bacteria of veterinary importance. Derquantel showed limited strain-dependent activity against the staphylococci (MIC values ranging from 64 to 128 µg/ml for all strains). Against the streptococci, derquantel showed very limited activity (MIC values ≥128 µg/ml). Against *Pasteurella* species, derquantel showed limited activity (MIC values ranging from 32 to >128 µg/ml). Derquantel was not active against the enteric organisms tested.

The Committee concluded that a microbiological ADI for derquantel is not necessary.

## *4. EVALUATION*

The Committee considered the acute clinical observations in dogs, which were consistent with the antagonistic activity of derquantel on nAChR, as the most relevant toxicological effect for the establishment of an ADI for derquantel. The LOAEL for this effect was 0.1 mg/kg bw per day. The Committee established an ADI of 0–0.3 µg/kg bw by applying an uncertainty factor of 300, using the default uncertainty factor of 100 and an additional uncertainty factor of 3 to account for setting the ADI on the basis of a LOAEL instead of a NOAEL. The Committee noted that the dog is appreciably more sensitive than the rat to the anti-nicotinergic effects of derquantel, but had no information to allow a relative comparison with humans. The Committee further noted that it may be possible to refine the ADI with additional studies, in particular on the comparative sensitivity of the nicotinic receptors to derquantel.

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## *MONEPANTEL*

***First draft prepared by***

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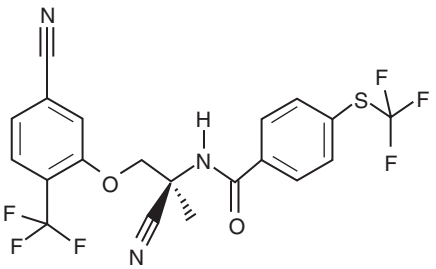
***Figure 1. Structure of monepantel***




## *1. EXPLANATION*

Monepantel (Chemical Abstracts Service No. 887148-69-8; *N*-[(1*S*)-1-cyano-2-(5-cyano-2-trifluoromethyl-phenoxy)-1-methyl-ethyl]-4-trifluoromethylsulfanyl-benzamide), also known as AHC 2102225, is an anthelminthic of the amino-acetonitrile derivative class, indicated for the treatment of nematodes in sheep. The recommended dose is 2.5 mg/kg body weight (bw), to be administered as an oral drench, and the maximum dose used is 3.75 mg/kg bw. Monepantel is also being developed for use in goats (proposed therapeutic dose 3.75 mg/kg bw, maximum dose 5.625 mg/kg bw). Monepantel is the *S*-enantiomer of an optically active molecule. Its chemical structure is shown in Figure 1.

Monepantel exerts its nematocidic action through activation of a nematode-specific subfamily of nicotinic acetylcholine receptors. Genetic screening of resistant *Caenorhabditis elegans* mutants suggests that the ACR-23 protein, which belongs to a nematode-specific subfamily of nicotinic acetylcholine receptors, is key to the activity of amino-acetonitrile derivative anthelminthics (Kaminsky et al., 2008). Mutations resulting in a non-functional ACR-23 protein produced resistance. Although one explanation for this would be that amino-acetonitrile derivative anthelminthics are direct agonists of ACR-23-containing ion channels, there is some evidence to indicate that they may in fact act as positive allosteric modulators of the receptor (Rufener et al., 2010).

Monepantel has not previously been evaluated by the Committee. It was included on the agenda of the current meeting of the Committee at the request of the Nineteenth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2011).

The present Committee considered data on pharmacodynamics, pharmacokinetics, short-term and long-term toxicity, reproductive toxicity, genotoxicity and carcinogenicity. All pivotal studies reported were conducted in line with good laboratory practice (GLP) and other relevant standards. In addition to data provided by the sponsor, a literature search was undertaken using the Embase database and the keywords monepantel, AHC 2102225 and toxicity. The literature search identified only one study of interest that was not available in the package provided by the sponsor.

## *2. BIOLOGICAL DATA*

### *2.1 Biochemical aspects*

#### *2.1.1 Absorption, distribution and excretion*

*(a) Rats*

In a non-GLP-compliant study, [$^{14}$C]monepantel was administered to male Ico:WI(AF/Han) rats as a single intravenous dose of 0.5 mg/kg bw (three animals per dose) or as a single oral dose of 2.5 or 50 mg/kg bw suspended in polyethylene glycol 300:tap water (3:7) by gavage (three animals per dose). Blood was taken for analysis at time points up to 96 hours after administration, and urine and faeces were collected for up to 168 hours. A fourth group of animals was administered seven daily doses of [$^{14}$C]monepantel at 2.5 mg/kg bw, and blood was collected for up to 96 hours after administration. In the same study, excretion via the bile was monitored in bile duct–cannulated rats administered a single oral dose of 2.5 mg/kg bw (three animals), with urine, bile and faeces collected and monitored for up to 72 hours post-dosing. Radioactivity was determined in blood, plasma and excreta by liquid scintillation counting, plasma concentrations of monepantel and its sulfone metabolite (AHC 2144670, also referred to M2) were determined by liquid chromatography–mass spectrometry and metabolite patterns were monitored by high-performance liquid chromatography with radioactivity detection. Finally, whole-body luminography was performed on three groups of rats: one group of three pigmented rats (crt:LE strain) administered a single oral dose of 2.5 mg/kg bw, one group of three pigmented rats (crt:LE strain) administered multiple oral doses of 2.5 mg/kg bw and one group made up of a single albino rat (Ico:WI(AF/Han) strain) orally administered a single oral dose of 2.5 mg/kg bw.

Total absorption of radioactivity (ratio of areas under the plasma concentration–time curves for oral and intravenous administration: $AUC_{oral}/AUC_{iv}$) following oral administration was 30%, 27% and 25% following doses of 2.5, 50 and 7 × 2.5 mg/kg bw, respectively. Bioavailability of unchanged monepantel was estimated to be 9.4% after a single 2.5 mg/kg bw dose and 8.3% after a single 50 mg/kg bw dose, indicating significant first-pass metabolism.

Radioactivity was poorly distributed to tissues. Twenty-four hours after the last of seven daily oral doses, the highest levels of radioactivity were seen in liver and hair follicles, with lower levels present in glandular stomach, white fat, skin, subcutis, brown fat and salivary gland. Levels in all other tissues were below the limit of detection (0.015 nmol/g).

There was little evidence of accumulation of radioactivity in tissues after seven daily doses, with levels in liver being approximately twice those seen after a single dose.

Following oral administration, maximal radioactivity concentrations in blood ($C_{max}$) were seen between 4 and 8 hours post-dosing ($T_{max}$) and then declined to approximately 10% of the peak concentration between 48 and 72 hours. Terminal

elimination of radioactivity from blood occurred with a half-life of approximately 55–60 hours.

Most (92–98%) of the administered radioactivity was excreted by 168 hours after both intravenous and oral dosing. The bulk of the administered radioactivity was excreted in the faeces (approximately 91% following intravenous administration, 90% following an oral dose of 2.5 mg/kg bw and 97% following an oral dose of 50 mg/kg bw). Urinary excretion accounted for 4% of the dose following intravenous administration and between 1% and 2% following oral administration of single doses. Excretion was monitored in bile duct–cannulated animals for up to 72 hours post-dosing. In these animals, urinary excretion accounted for approximately 2% of the administered dose, biliary excretion for 31% of the dose and faecal excretion for 30% of the dose (indicating that at least 32% of the dose was absorbed) (Wirz, Schweitzer & Smal, 2006).

In a study using Hanover-Wistar (HanBrl:WIST) rats (for which a signed quality assurance statement indicating GLP compliance was available), animals were administered, by oral gavage, [$^{14}$C]monepantel suspended in polyethylene glycol 300 and labelled at one of two positions: the "cyano" label was present at the site of the cyano group on the trifluoromethyl-phenyl ring, whereas the "amide" label was present at the site of the amide bond.

Groups 1 (four males) and 2 (four females) were administered seven daily oral (gavage) doses of monepantel labelled at the cyano position at 10 mg/kg bw, whereas groups 3 (four males) and 4 (four females) were administered seven daily doses of monepantel labelled at the amide position at 10 mg/kg bw. These rats were killed 6 hours after the last dose. A fifth group (nine males) received a single dose of monepantel labelled at the amide position at 10 mg/kg bw. Blood was sampled for 10 time points up to 96 hours after dosing.

After oral administration of a single 10 mg/kg bw dose, radioactivity reached peak levels in blood and plasma at 2 hours post-dosing. After seven daily doses of 10 mg/kg bw, levels of radioactivity were generally highest in liver and fat, followed by adrenals, pancreas and ovaries. Radioactivity was present in kidney at an intermediate level and in blood and muscle at comparably low levels. Residue levels in organs and tissues were generally higher in females than in males (e.g. radioactivity levels in liver were approximately 3 times higher in females than in males).

After seven single daily doses, the bulk of radioactivity was excreted in faeces (83%, 72%, 78% and 63% in groups 1, 2, 3 and 4, respectively) by 6 hours after the last dose. Urinary excretion accounted for 3%, 5%, 3% and 5% in groups 1–4, respectively. The major part of the residual radioactivity was recovered in the gastrointestinal tract (12%, 10%, 12% and 9% in groups 1–4, respectively). Systemic exposure was seen particularly in liver (0.5–2% of radioactivity) and fat (0.29–2.91% of radioactivity), with residue levels in other organs and tissues generally accounting for less than 0.1% of the mass balance (Gassen, 2007).

*(b) Dogs*

In a preliminary, non-GLP-compliant study, three groups of two dogs were administered a 2.5 mg/kg bw intravenous dose of NG-96 (the racemic mixture of

monepantel and its *R*-enantiomer) formulated at 2% (weight per volume [w/v]) (administered in three parts: at 8 a.m., 10 a.m. and 10:30 a.m.), a single oral 12.5 mg/kg bw dose as a capsule containing a liquid formulation of NG-96 formulated at 10% (w/v) or a single dermal (pour-on) application of NG-96 formulated at 10% (w/v) at a dose of 12.5 mg/kg bw. Blood samples were taken several times on day 1 and up to 83 days after dosing, with the concentrations of parent NG-96 and the sulfone metabolite (NG-236) measured.

Oral absorption of NG-96 was rapid, with a time to $C_{max}$ ($T_{max}$) of 1–2 hours and a $C_{max}$ of 238 ng/ml. The half-life of elimination of NG-96 was 44 hours. After dermal administration of NG-96, absorption was slow and peak concentrations were low, with a $T_{max}$ of 2–8 days and a $C_{max}$ of 4 ng/ml. The half-life of elimination was 185 hours. The oral bioavailability of NG-96 and NG-236 combined was 24%, and dermal bioavailability was 15% (although the dermal application was applied to the shoulders, but without occlusion, so the possibility of lick-off cannot be ruled out). The sulfone metabolite was still measurable in blood at 83 days after oral, dermal and intravenous applications (levels of 2.7–4.6 ng/ml after oral dosing, 6.2–16.2 ng/ml after dermal application and 3.6–3.7 ng/ml after intravenous application) (Chardonnens & Jung, 2005).

Blood levels of monepantel and its sulfone metabolite (ACH-2144670) seen in the 1-year toxicity study in dogs were measured in samples taken on days 115 and 365 and are detailed in two separate reports. Animals received monepantel in the diet at a level of 100, 300 or 3000 parts per million (ppm) (equal to approximately 3, 10 or 100 mg/kg bw per day, respectively). Levels of monepantel were far lower than those of the sulfone metabolite and were not quantifiable in the 100 ppm group on day 365. Mean blood levels of monepantel and the sulfone metabolite increased in a roughly dose-proportional manner in samples from dogs from the 100 and 300 ppm dose groups. The increases in blood levels of monepantel and of its sulfone metabolite between the dose levels of 300 and 3000 ppm were less than dose proportional (mean monepantel levels at day 115 were 58.2 and 157 ng/ml in the 300 and 3000 ppm groups, respectively; mean levels of the sulfone metabolite at day 115 were 2917 and 7921 ng/ml, respectively). This could be indicative of saturation of metabolic pathways at these doses (Browning, 2007 [a statement of GLP compliance was available]; Chardonnens & Jung, 2007 [GLP status for this analytical report is not indicated]).

*(c) Sheep*

For studies on absorption, distribution and excretion in sheep, the reader is referred to the residues monograph (Annex 1, reference *210*).

*(d) Protein binding in vitro*

Protein binding was investigated in an in vitro study (for which a signed quality assurance statement indicating GLP compliance was available) using rat, dog, sheep and bovine plasma. [$^{14}$C]Monepantel was incubated at a concentration of 30, 100 or 1000 ng/ml with plasma at 37 °C for 30 hours. Following equilibrium dialysis against deproteinized plasma, free test item in the dialysate was quantified

by liquid scintillation counting. Results showed that 96.2–99.5% of the test item was bound to plasma protein and that protein binding was independent of test item concentration and was similar in rat, dog, sheep and bovine plasma. It is noteworthy that protein binding of the sulfone metabolite (AHC 2144670) was not evaluated (Sagelsdorff, 2008).

### *2.1.2 Biotransformation*

#### *(a) Rats*

In a non-GLP-compliant study in rats (see section 2.1.1 for study outline), the main residues present in plasma and excreta were analysed. Metabolite patterns in plasma were comparable following intravenous and oral administration. Following oral administration of [$^{14}$C]monepantel, parent compound accounted for between 10% and 12% of the total $^{14}$C plasma $AUC_{0–24}$, whereas the sulfone metabolite (AHC 2144670) accounted for approximately 27–34% of the $^{14}$C plasma $AUC_{0–24}$. A further seven minor metabolites were also present. No parent compound was detected in urine, where the predominant metabolite was the sulfate conjugate of 3-hydroxy-4-trifluoromethyl-benzonitrile (M5). After intravenous administration, faecal extract contained almost no parent compound, whereas after oral administration, parent compound accounted for 52–75% of administered radioactivity, presumably due to non-absorbed material. M3, an oxidized metabolite of the sulfone, was the main metabolite in all faecal extracts. No parent compound was observed in the bile, the main component being M6 (the *O*-glucuronide of M3), with other components being minor unknown metabolites (Wirz, Schweitzer & Smal, 2006).

Metabolite patterns were assessed in blood, excreta and tissues of male and female rats after single and multiple oral doses of [$^{14}$C]monepantel, labelled at two different positions, at 10 mg/kg bw (Gassen, 2007; see section 2.1.1 for study outline). In addition, tissue and blood samples collected from the metabolism study in sheep (Jung et al., 2007; see below) were analysed in parallel in order to allow comparison of metabolites seen in these two species.

Parent compound was consistently detected in faeces and was present at highest levels at early time points (accounting for up to approximately 70% of activity in this matrix over the first 24 hours following oral dosing). Additionally, up to 13 different metabolite fractions were detected in faeces. In urine, the metabolite profile was complex, with a total of 16 metabolite fractions detected and no parent compound. In blood, parent compound accounted for up to 39% of the radioactivity in this matrix, with 11 metabolite fractions also detected. The predominant metabolite in blood was the sulfone derivative (AHC 2144670), which accounted for up to 52% of the activity.

In general, exposure of tissues to total residues was higher in females than in males. For example, exposure of liver after administration of the cyano label was 9.4 µg equivalents (eq) per gram in males versus 26.4 µg eq/g in females, and exposure of fat was 7.8 µg eq/g in males versus 53.3 µg eq/g in females.

In muscle, parent compound accounted for approximately 20% of the radioactivity in males and approximately 30% of the radioactivity in females. In addition, 13 metabolite fractions were detected, with the predominant metabolite being the sulfone (AHC 2144670), which accounted for 54–71% of the radioactivity. M1 (the sulfoxide derivative) accounted for approximately 5% of the radioactivity, and a further metabolite designated as M13 accounted for 1.7–19.8% of the activity. All other fractions contributed less than 2% of the radioactivity.

In liver, parent compound accounted for approximately 10% of the radioactivity in males and 25% of the radioactivity in females. The predominant metabolite was the sulfone metabolite (AHC 2144670), which accounted for approximately 30% of the radioactivity in males and 50% in females. M3 and M6 were found predominantly in males, whereas M1 was found predominantly in females.

In fat, parent compound accounted for 37–48% of the radioactivity. The predominant metabolite was the sulfone (AHC 2144670), which accounted for 50–61% of the radioactivity. M1 was a minor metabolite (2.5–3%).

In kidney, parent compound accounted for approximately 15% of the radioactivity in males and approximately 24% in females. Twelve metabolite fractions were detected, with the predominant metabolite being the sulfone (AHC 2144670), which accounted for 42.5–51.3% of the radioactivity. Other significant metabolites were K4 (6.1–8.2% in males and 11.5–11.9% in females), M1 (5.4–8.4%) and K1 (2.1–6.2%). K3 was found in males only.

Overall, 25% of the daily dose was excreted unchanged through faeces, with a further 52% accounted for by the hydroxylated sulfone metabolite. After seven daily doses, tissue residues were characterized as mainly parent and sulfone, accounting for 1.6% and 2.2% of the total dose, respectively. These were detected in all investigated tissues. Three more metabolites were detected in tissues at amounts corresponding to between 0.1% and 1% of the daily dose, and a further five metabolites were detected at an amount corresponding to less than 0.1% of the dose.

The proposed main biotransformation pathways in the rat are shown in Figure 2.

Table 1 provides an overview of the main metabolites found in rat and sheep tissues (see below). With one exception, all main metabolites seen in sheep tissues were also seen in rat tissues. Mu1 in muscle, which is equivalent to Fa1 in fat, was seen in sheep tissues, but not in rat tissues. However, in a subsequent analysis of rat fat samples from the 2-year carcinogenicity study (Kress, 2008 [signed quality assurance statement indicating GLP compliance included]), this metabolite, referred to as AHC 2197876 or G32, was confirmed to be present in both renal and peritoneal fat of rats.

*(b) Sheep*

In a preliminary (non-GLP-compliant) study in sheep, two animals received a single oral dose of “cyano” radiolabelled monepantel, prepared in a combination of Cremophor RH 40 (macrogolglycerol hydroxystearate), Transcutol (diethylene

***Figure 2. Proposed main biotransformation pathways in rats***

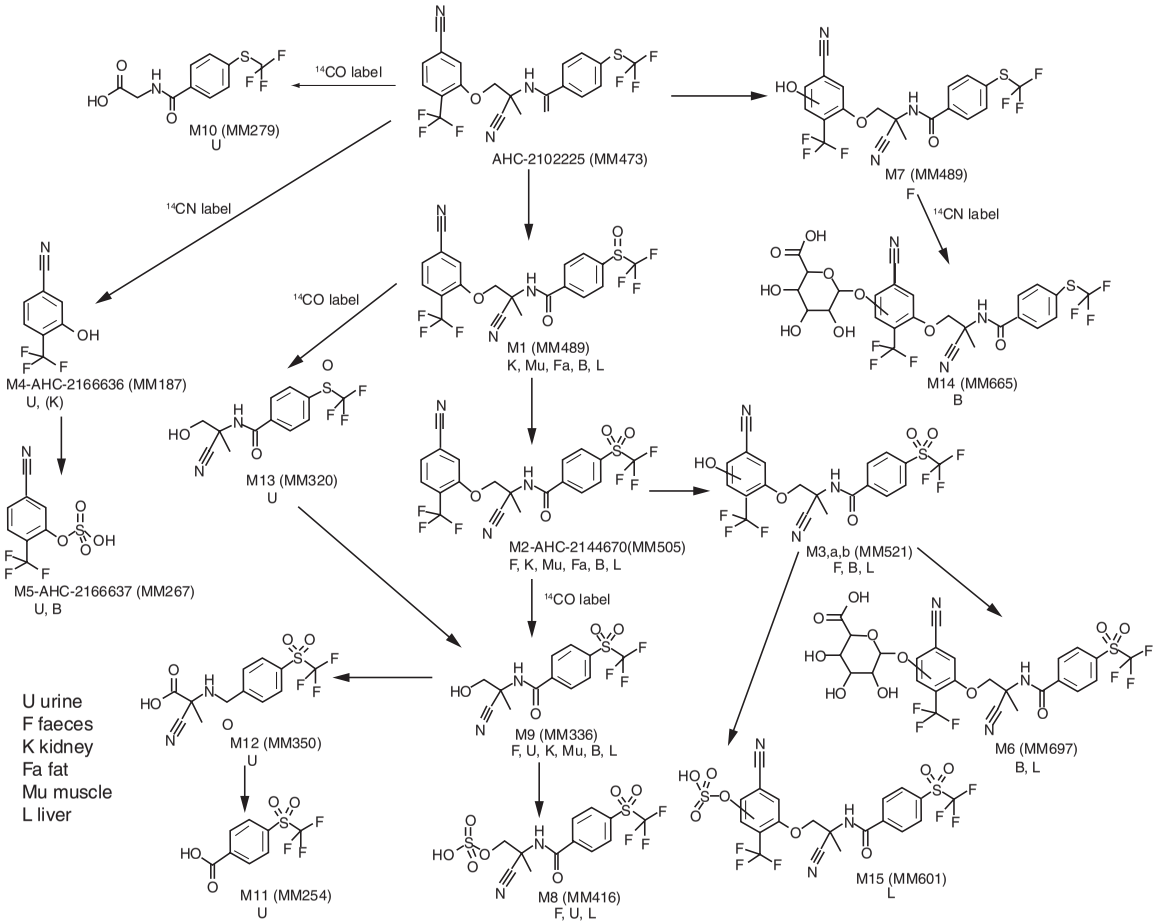


***Table 1. Comparison of residues in rats and sheep***

| Tissue | Rats (% activity in matrix) | Sheep (% activity in matrix) |
|---|---|---|
| Blood | Parent (23%), AHC 2144670 (45%) | AHC 2144670 (100%) |
| Muscle | Parent (24%), AHC 2144670 (62%) | Parent (5.2%), AHC 2144670 (93%), Mu1 (2%) |
| Liver | Parent (19%), AHC 2144670 (41%), M3 (14%), M9 (6.8%), L21 (4.2%), M6 (1.1%) | Parent (1.1%), AHC 2144670 (92%), M9 (4.5%), L21 (1.4%) |
| Fat | Parent (44%), AHC 2144670 (54%) | Parent (14%), AHC 2144670 (77%), Fa1 (7.4%) |
| Kidney | Parent (20%), AHC 2144670 (48%), K3 (2%) | Parent (5.8%), AHC 2144670 (73%), K3 (22%) |

glycol monoethyl ether), Capryol 90 (1,2-propanediol monocaprylate) and Miglyol 840 (propylene glycol diester of caprylic and capric acids), into the oesophagus and were kept in metabolism cages for 12 days. Excreta were collected, as were blood and tissues, and analysed for total radioactivity as well as for metabolites (liquid chromatography–mass spectrometry). Highest residue levels in edible tissues were seen in fat, followed by liver, then muscle and kidney at similar levels (Jung, 2006).

Metabolite profiling was performed in a study in 34 Suffolk cross-bred sheep (for which a signed quality assurance statement indicating GLP compliance was available). Groups of four animals were administered a single oral dose of “cyano” radiolabelled monepantel at a target dose of 5 mg/kg bw, formulated at 2.5% (w/v) in the dosing vehicle (TG 1778/30), which contained Cremophor, Polysorbate, Capryol and Miglyol, with residue levels monitored in fat, muscle, kidney and liver as well as in bile and wool at 2, 7, 14, 21, 28 and 35 days post-dosing. In addition, urine and faeces were collected daily, and terminal blood samples were taken. Another group of four sheep was administered single oral doses of “amide” radiolabelled monepantel and killed 14 days after administration, whereas a further group of four sheep received a 50:50 mix of “cyano” and “amide” raiolabelled monepantel and was killed 21 days after administration. Finally, two untreated sheep served as controls.

In muscle, fat, kidney and liver, the sulfone (AHC 2144670) was the most prominent residue, whereas the parent compound was present as a minor constituent. A third component, referred to as G32 (AHC 2197876), was also present in muscle and fat. Other metabolites were present at very low levels. In blood, the sulfone (AHC 2144670) was also the major component, with parent compound being measurable only at early time points. Two minor metabolites were also seen in blood. Monepantel and the main metabolite were present in blood as the pure enantiomers, indicating that the molecule does not undergo racemization after administration as the pure enantiomer (Jung et al., 2007).

*(c) In vitro*

A non-GLP-compliant in vitro biotransformation study of [$^{14}$C]monepantel was performed using commercially available mouse, rat, dog and human pooled hepatic microsomal fractions and a substrate concentration of 1 µmol/l. The dog and mouse samples yielded a more modest intrinsic clearance rate than the rat and human samples (45, 90, 147 and 162 µl/min per milligram of microsomal protein in dog, mouse, human and rat, respectively). It is notable that there were no replicates in the study and that clearance of the sulfone metabolite was not assessed.

Biotransformation with and without cofactors was investigated at a 10 µmol/l substrate concentration. Without cofactors, the parent compound was stable, whereas in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), two main metabolite peaks (the sulfone and the intermediate sulfoxide metabolites) were seen, as well as the parent compound and a further unidentified minor peak. The presence of uridine diphosphate–glucuronic acid had only a marginal further impact, and the presence of glutathione had practically no effect. Comparable patterns of metabolites were seen in liver microsomes from all four species. Besides the sulfone and sulfoxide metabolites, three trace metabolites were detected in rat and human samples, but not in mouse and dog (Wirz, 2005).

A further in vitro study (for which a signed quality assurance statement indicating GLP compliance was available) using liver microsomes was performed specifically to compare the metabolism of [$^{14}$C]monepantel in dogs and humans. Metabolism was investigated using hepatic microsomal preparations (with an NADPH regenerating system) from young, middle-aged and old male and female human donors (one sample each) and pooled samples from male and female Beagle dogs. Incubations were performed in duplicate with the human samples and twice in triplicate (except for the second incubation for dogs, which was in duplicate). Clearance rates were calculated based on the half-life of the parent compound in the incubations. Radioactivity was monitored using high-performance liquid chromatography and online radioactivity detection.

Monepantel was metabolized exclusively to its sulfoxide and sulfone metabolites, with no other metabolites seen during the 3-hour incubation period. Mean clearance rates were 217, 213 and 138 µl/min per nanomole for young, middle-aged and old male human donors and 166, 164 and 121 µl/min per nanomole for young, middle-aged and old female human donors, respectively. Clearance rates seen in the dog samples were similar for males and females (89 and 84 µl/min per nanomole, respectively). The data suggest that monepantel is metabolized more rapidly by human liver microsomes than by dog liver microsomes (Sagelsdorff, 2007).

### *2.1.3 Effects on enzymes and other biochemical parameters*

Possible liver enzyme induction of the cytochrome P450 (CYP) system was investigated in a non-GLP-compliant study (Sagelsdorff, 2005) using liver samples collected from all female animals in the control and top-dose groups in the 4-week repeated-dose toxicity study in rats (Bachmann, Flade & Waldvogel, 2004; see

section 2.2.2 below). The study also investigated the effects of monepantel on drug metabolizing enzymes and plasma levels of thyroid hormones. The study was performed following observed increases in liver and thyroid weights in the 4-week rat toxicity study.

The protein content of hepatic microsomal fractions was determined, as well as the microsomal cytochrome P450 content and the activities of probe substrates for CYP1A1, CYP1A2, CYP2B1 and CYP4A1. The activity of hepatic microsomal uridine diphosphate glucuronosyltransferase, responsible for the conjugation of the thyroid hormone thyroxine ($T_4$), and plasma levels of the thyroid hormones thyroid stimulating hormone (TSH), $T_4$ and triiodothyronine ($T_3$) were determined.

No effect was seen on the protein content of microsomal fractions. Total cytochrome P450 content was increased to 167% of control values. There was no increase in 7-methoxyresorufin *O*-demethylase (CYP1A2) or 7-pentoxyresorufin *O*-dependentylase (CYP2B1) activities, but there was a slight increase in 7-ethoxyresorufin *O*-deethylase (CYP1A1) activity (to 157% of control, $P < 0.05$) as well as in microsomal lauric acid 11- and 12-hydroxylase (CYP4A1) activities, to 178% and 144% of control, respectively ($P < 0.05$).

Treatment increased microsomal glucuronidation of 3-methyl-2-nitrophenol to 245% of control (not statistically significant, results in treated group were quite variable), whereas plasma concentrations of TSH, $T_4$ and $T_3$ were not affected.

It is concluded that monepantel induced liver metabolizing enzymes to a slight degree. Although there was some evidence that microsomal uridine diphosphate glucuronosyltransferase activity might have been induced, there was no effect on plasma levels of thyroid hormones.

## *2.2 Toxicological studies*

### *2.2.1 Acute toxicity*

Following oral and dermal administration, the median lethal dose ($LD_{50}$) of monepantel was greater than 2000 mg/kg bw in rats (European Medicines Agency, 2011).

Monepantel was not irritating to the skin of rabbits and was slightly irritating to the rabbit eye (European Medicines Agency, 2011).

In a non-GLP-compliant target species toxicity study using a dose escalation design, two groups of two Suffolk ewes were orally administered monepantel at doses of 50 mg/kg bw followed by 100 mg/kg bw or 75 mg/kg bw followed by 125 mg/kg bw. All doses were well tolerated, with no clear signs of toxicity (Hosking, 2006).

### *2.2.2 Short-term studies of toxicity*

#### *(a) Mice*

In a 13-week oral toxicity study in CD-1 mice (Bachmann, Flade & Krinke, 2005 [signed quality assurance statement indicating GLP compliance included]),

groups of 10 male and 10 female mice were administered monepantel in the diet at a concentration of 0, 30, 120, 600 or 6000 ppm (equal to 0, 4.53, 17.97, 97.51 and 959.10 mg/kg bw per day in males and 0, 5.27, 22.04, 114.88 and 1212.51 mg/kg bw per day in females). Animals were monitored for clinical signs, feed consumption and body weights. Laboratory observations (urine analysis, haematology, clinical biochemistry, organ weights) were performed at the end of treatment, and histopathology evaluations were undertaken.

All animals survived to the scheduled termination date, with no clinical signs or effects on feed consumption or body weight. No relevant haematological effects or effects on urine parameters were reported. Clinical biochemistry revealed increased mean plasma aspartate aminotransferase (AST) levels in males at all test doses, but with no dose–response relationship (+69%, +91%, +42% and +20% at 30, 120, 600 and 6000 ppm, respectively). The effect was statistically significant at 30 and 120 ppm, but not at the higher doses. In females, there was no effect on mean AST level at 30 ppm, but a dose-related increase was seen at higher doses (+60%, 85% and 121% at 120, 600 and 6000 ppm, respectively), although the increases were not statistically significant. Mean alanine aminotransferase (ALT) levels were increased in females only at 120, 600 and 6000 ppm (+11%, +113% and +113%, respectively), with only the effect at 600 ppm reaching statistical significance. Mean alkaline phosphatase level was increased in males at 600 ppm (+20%) and 6000 ppm (+56%), although the increases were not statistically significant. In females, mean alkaline phosphatase level was increased, but not statistically significantly, at 120 ppm (+17%), 600 ppm (+29%) and 6000 ppm (+27%). Mean total bilirubin levels were increased in males and females at 600 ppm (+56% in both sexes) and 6000 ppm (+102% in males and +109% in females), with the increases being statistically significant at both doses in males and only at 6000 ppm in females. In females, mean cholesterol level was increased at all dose levels (+32%, +34%, +50% and +61% at 30, 120, 600 and 6000 ppm, respectively), whereas in males, it was increased only at 6000 ppm (+10%). Only the cholesterol level increases in females at 600 and 6000 ppm reached statistical significance.

Absolute and relative mean liver weights were statistically significantly increased at 600 ppm (males) and 6000 ppm (males and females). Higher absolute and relative weights of adrenals and spleen were seen at 6000 ppm (females), although without any associated histological findings.

No treatment-related gross lesions were seen at necropsy.

Histological findings were confined to the liver. Centrilobular fatty change was seen in males and females at all doses, with the incidence increasing with dose. Increased focal necrosis and lymphoid cell infiltration were seen in females at 600 and 6000 ppm. In relation to the centrilobular fatty change, the incidence in females at 30 and 120 ppm (6/10 at both doses) was greater than in concurrent controls (3/10) and historical controls (4/10), whereas for males, the incidence at these doses (2/10 at both doses) was greater than in concurrent controls (0/10), but below that in historical controls (3/10). The grading seen in females at 30 and 120 ppm was below that seen in the concurrent controls.

Liver was concluded to be the target organ, with increased total bilirubin seen at 600 and 6000 ppm. A possible effect on lipid metabolism was demonstrated

by slightly increased cholesterol levels at 600 and 6000 ppm, corroborated by increased incidences of fatty liver change at all doses. In addition, females at 600 and 6000 ppm had increased levels of focal necrosis and lymphoid cell infiltrates in liver. It is notable that histological effects of the test item were seen predominantly in females. Liver enzyme effects were seen at a range of doses, but no consistent pattern was revealed.

The lowest-observed-adverse-effect level (LOAEL) was 30 ppm (equal to 5.27 mg/kg bw per day), the lowest dose tested, based on an increased incidence of fatty change in the liver of females.

*(b) Rats*

In a 4-week non-GLP-compliant range-finding study in Wistar rats, monepantel was administered to groups of five males and five females in the diet at a concentration of 0, 1000, 4000 or 12 000 ppm (equal to 0, 86, 346 and 1044 mg/kg bw per day in males and 0, 90, 362 and 1017 mg/kg bw per day in females). Animals were monitored for clinical signs, feed and water consumption and body weights, and laboratory observations (haematology, clinical biochemistry, urine analysis, organ weights and histopathology) were performed at the end of the treatment period.

All animals survived to the scheduled termination date, with no clinical signs or effects on feed and water consumption or body weight. No relevant haematological effects were reported. The only urinary parameter findings consisted of minimally higher glucose levels and a marked increase in leukocytes at 12 000 ppm in females.

Clinical biochemistry revealed slightly reduced glucose levels at 4000 and 12 000 ppm (males only), increased cholesterol levels at 1000 (females only), 4000 and 12 000 ppm (males and females), increased triglyceride levels at all doses (females only), increased phospholipid levels at 1000 (females only), 4000 and 12 000 ppm (males and females) and increased globulin levels at 12 000 ppm (females).

Increased absolute and relative liver weights were seen in females at all doses and in males at 4000 and 12 000 ppm. Histopathology revealed centrilobular hepatocellular hypertrophy at all doses in both sexes and diffuse follicular hypertrophy in the thyroid in all dose groups, with the exception of low-dose females. The incidence and severity of these effects were seen to increase in a dose-dependent manner.

The LOAEL was 1000 ppm (equal to 86 mg/kg bw per day), the lowest dose tested (Bachmann, Flade & Waldvogel, 2004).

In a 3-month study in Wistar rats (for which a signed quality assurance statement indicating GLP compliance was available), groups of 10 animals of each sex per dose were administered monepantel in the diet at a concentration of 0, 50, 200, 1000 or 12 000 ppm (equal to 0, 3.61, 14.64, 73.56 and 899.81 mg/kg bw per day in males and 0, 3.98, 15.20, 81.45 and 946.81 mg/kg bw per day in females).

A further five rats of each sex were administered the high dose level for 90 days, followed by a 4-week treatment-free recovery period.

Animals were regularly monitored for clinical signs, feed and water consumption, body weights and effects on a functional observational battery. Ophthalmoscopic observations were made during acclimatization, at treatment end and at the end of the recovery period. Haematology, plasma biochemistry and urine analysis parameters were measured at the end of the dosing and recovery periods. Necropsies were performed, with macroscopic observations on organs and tissues. Histology was carried out on organs and tissues from the control and high-dose groups, with additional histology on organs and tissues from low-dose, mid-dose and recovery groups if effects were noted at the high dose or if gross lesions were noted.

Treatment was well tolerated. All animals survived to scheduled termination, and no clinical signs or effects on feed consumption or body weight were seen. No treatment-related ophthalmoscopic changes were noted. Haematology findings consisted of a decreased neutrophilic granulocyte count and slightly increased platelet count in females at 12 000 ppm, slightly increased thromboplastin time at 1000 and 12 000 ppm (males and females) and slightly decreased activated partial thromboplastin time in females at 12 000 ppm.

Clinical biochemistry findings consisted of minimal hypoglycaemia at 12 000 ppm (males and females), minimally decreased bilirubin concentrations in females at 1000 ppm and in both sexes at 12 000 ppm, elevated cholesterol and phospholipid levels at 1000 and 12 000 ppm (females), elevated triglyceride levels in males and females at 12 000 ppm (not statistically significant), increased total protein level in males at 12 000 ppm, increased albumin concentrations in males at 1000 and 12 000 ppm, increased sodium levels in males at 200, 1000 and 12 000 ppm and decreased sodium levels in females at 1000 and 12 000 ppm. Given the inconsistency of the effects on sodium, these were not considered to be test item related.

In urine analysis, the only effect seen was reduced urine volume at 12 000 ppm in both sexes.

Absolute and relative liver weights were increased in females at 1000 and 12 000 ppm, and absolute and relative adrenal and spleen weights were increased in females at 12 000 ppm. Histology findings included minimal hypospermatogenesis in 8 of 10 males at 12 000 ppm, which was still present in 1 of 5 males at recovery; moderately increased intratubular debris in epididymides in 9 of 10 males at 12 000 ppm (versus 3/10 controls), still present at recovery in 2 of 5 males (versus 1/5 controls); centrilobular hepatocellular hypertrophy in 3 of 10 females at 1000 ppm and in all females at 12 000 ppm, but not after recovery; and minimal sex cord stromal hypertrophy or hyperplasia in the ovary in 3 of 10 females at 12 000 ppm, but not at the end of recovery.

The main target organ was the liver, reflected by increased absolute and relative liver weights and centrilobular hepatocellular hypertrophy in females at 1000 ppm. Biochemical findings indicative of liver effects included indications of effects on lipid metabolism (elevated cholesterol: females at 1000 and 12 000 ppm;

phospholipids: females at 1000 and 12 000 ppm; and triglycerides: males and females at 12 000 ppm) and increased total protein (males at 12 000 ppm) and albumin (males at 1000 and 12 000 ppm) levels. In addition, absolute and relative weights of adrenals and spleen were increased at the highest dose level in females, although without associated histological findings. High-dose findings also included hypospermatogenesis in males and sex cord stromal hypertrophy or hyperplasia in females. Histological findings were largely reversible, although some evidence of hypospermatogenesis was still present at the end of the 4-week recovery period.

The no-observed-adverse-effect level (NOAEL) was 200 ppm (equal to 15.20 mg/kg bw per day), based on effects on the liver (increased absolute and relative liver weights, centrilobular hypertrophy and clinical chemistry changes, i.e. increased cholesterol and phospholipids) in females at 1000 ppm (equal to 81.45 mg/kg bw per day) (Bachmann, Flade & Heider, 2005).

In a 52-week study in Wistar rats (for which a signed quality assurance statement indicating GLP compliance was available), groups of 20 male and 20 female animals per dose received monepantel in the diet at a concentration of 0, 50, 200, 1000 or 12 000 ppm (equal to 0, 2.69, 10.67, 54.45 and 656.08 mg/kg bw per day in males and 0, 3.36, 13.92, 67.36 and 778.29 mg/kg bw per day in females). Animals were regularly monitored for clinical signs, feed consumption and body weight. Ophthalmoscopic observations were made before treatment and at week 51. Haematology, blood biochemistry and urine parameters were analysed in samples taken at weeks 13, 26 and 52. Necropsies were performed, with macroscopic observations on organs and tissues. Histology was carried out on an extensive range of organs and tissues from the control and high-dose groups and on one animal from the 1000 ppm group killed in extremis.

One control male and one 1000 ppm female were killed in extremis in weeks 48 and 49, respectively. No clinical signs or test item–related effects on feed consumption were noted.

Mean body weights were consistently reduced throughout the study at 1000 and 12 000 ppm in males, although the effect was less marked at 12 000 ppm (not statistically significant reduction at most time points) than at 1000 ppm. Body weights were consistently reduced to a statistically significant degree at 200 ppm in females (over most of the study), but to a lesser degree at 1000 and 12 000 ppm. Similarly, body weight gains in males were consistently lower in treatment groups than in controls, but the effect was not clearly dose related, often reaching statistical significance at 1000 ppm but not at 12 000 ppm. In females, the effect on body weight gain was most marked at 200 ppm. In the absence of a clear dose–response relationship, the effect on body weight was not considered test item related.

No effects were seen in the ophthalmoscopic examinations. Haematological findings were minor, inconsistent and reported to be within historical control range. Effects were considered incidental.

Statistically significant effects seen in clinical biochemistry parameters at all three time points (weeks 13, 26 and 52) consisted of decreased glucose levels in males at 12 000 ppm (–14%, –22% and –15%), increased cholesterol and

phospholipid levels in males and females at 12 000 ppm and increased triglyceride levels in females at 12 000 ppm. Effects on protein variables were also noted (increased total protein level in 12 000 ppm males and 1000 and 12 000 ppm females, increased albumin level in 12 000 ppm males and increased globulin level in 12 000 ppm females), but no clear dose–response relationship was seen, and values were outside the historical control range only at 12 000 ppm and only at week 26 (increased total protein level in females, increased albumin level in males and increased globulin level in females). Increased sodium levels were seen in males at all doses and in females at 12 000 ppm, but as the only group in which values were outside the historical control range was the 12 000 ppm females (and only at week 26), and as there were no relevant changes in urine parameters or histopathology, the effect on sodium was not considered toxicologically relevant.

No significant findings were noted in the urine analysis.

Liver weights and liver weight ratios were statistically significantly increased in females at 1000 and 12 000 ppm. Liver weight ratios were also statistically significantly increased in males at 12 000 ppm. An increased kidney to body weight ratio was seen in 12 000 ppm females.

All histology results are reported to have been within the normal background range.

Overall, it can be concluded that an effect on lipid metabolism was evidenced by increased cholesterol, phospholipid and triglyceride levels at 12 000 ppm. Evidence of an effect on carbohydrate metabolism is suggested by decreased glucose levels in males at 12 000 ppm. Effects on liver weights and kidney to body weight ratios were also seen at this dose.

At 1000 ppm, increased protein levels were noted in females, as well as increased liver weights in females. In the absence of a clear dose–response relationship, the effect on protein at this dose was not considered adverse, and in the absence of histopathological findings in the liver, the effect on liver weight was not considered adverse.

The NOAEL was 1000 ppm (equal to 54.45 mg/kg bw per day), based on increased absolute and relative liver weights and increased cholesterol, triglyceride and phospholipid levels, indicative of effects on the liver, at 12 000 ppm (equal to 656.08 mg/kg bw per day) (Broich, Flade & Weber, 2007).

*(c) Dogs*

In a 4-week dose range–finding study (for which a signed quality assurance statement indicating GLP compliance was available), groups of four dogs (two males and two females each) received monepantel in the diet at a concentration of 0, 5000, 15 000 or 40 000 ppm (equal to 0, 161.2, 566.1 and 1216.6 mg/kg bw per day in males and 0, 183.6, 561.0 and 1472.4 mg/kg bw per day in females). Animals were regularly monitored for clinical signs, feed consumption and body weight. Haematology and blood biochemistry parameters were analysed in samples taken before the start of treatment and at the end of treatment. Urine parameters were analysed at the end of treatment. Necropsies were performed, with macroscopic

observations on organs and tissues. Histology was carried out on selected tissues and organs from control and high-dose animals.

No unscheduled deaths or treatment-related clinical signs were noted. A reduced mean body weight gain associated with slightly reduced feed consumption was seen in males at 40 000 ppm. No test item–related effects were noted in haematology parameters. The only notable finding in the clinical biochemistry analyses was an increased alkaline phosphatase activity at all test doses. Individual values were 2- to 3-fold higher in males and 3- to 9-fold higher in females than pre-dosing levels, without a clear dose–response relationship.

There were no treatment-related findings in urine parameters.

Absolute and relative thymus weights were reduced at all doses, and absolute and relative adrenal gland weights were increased at all doses. Relative liver weights were increased at all doses, although without a clear dose–response relationship. Absolute and relative thyroid weights were increased in females at all test doses.

The only finding noted during histology was an increased severity of thymus involution in the 40 000 ppm group. It is noted that the incidence of thymus involution was not increased.

The study identified the liver, thymus, adrenal gland and thyroid as targets of toxicity (Haag, 2006a).

In a 13-week study in Beagle dogs (for which a signed quality assurance statement indicating GLP compliance was available), animals received monepantel in the diet at concentrations of 0 ppm (six males and six females), 300 ppm (four males and four females), 3000 ppm (four males and four females) and 30 000 ppm (six males and six females) (equal to actual doses of 0, 9.9, 106.8 and 963.0 mg/kg bw per day in males and 0, 10.7, 96.8 and 1176.1 mg/kg bw per day in females). At the end of the treatment period, two males and two females from the 300 and 30 000 ppm groups were kept for a further 4 weeks to investigate the reversibility of effects. Animals were regularly monitored for clinical signs, feed consumption and body weight. Ophthalmoscopic observations were made before treatment and at the end of treatment. Haematology, blood biochemistry and urine parameters were monitored before treatment, at week 7, at treatment end and at the end of the recovery period. Necropsies were performed, with macroscopic observations on organs and tissues. Histology was carried out on selected tissues and organs from control and high-dose animals, on macroscopic lesions, adrenals, kidneys, liver, thymus, thyroid with parathyroids, pancreas, duodenum, jejunum and ileum on all animals and on liver, pancreas, duodenum, jejunum and ileum of animals after the recovery period.

There were no unscheduled deaths or treatment-related clinical signs reported. A reduced body weight gain was noted in females at 30 000 ppm and considered to be treatment related. The effect was not associated with decreased mean feed consumption. Body weight gain was also reduced in males at 30 000 ppm, although the effect was less marked.

No ophthalmological findings were noted at the end of the treatment period.

Haematology results revealed decreased leukocyte counts due to decreased neutrophil counts in males at 30 000 ppm (noted at weeks 7 and 12). Recovery was not apparent by week 17. The other notable haematology finding was a reduced activated partial thromboplastin time in males and females at 3000 and 30 000 ppm. The effect was reversible.

Blood biochemistry revealed increased alkaline phosphatase activity in both sexes. At 300 ppm, alkaline phosphatase activity was approximately double the level seen in controls, but the increase was not statistically significant. The effect did reach statistical significance at 3000 ppm, at which the level of activity in the test group was at least 3 times that seen in the control group. The effect remained statistically significant at 30 000 ppm, although in males, the level of activity was slightly lower than that seen at 3000 ppm. In females, activity was slightly increased at 30 000 ppm compared with 3000 ppm. At week 17, alkaline phosphatase activity was reduced compared with levels seen at week 13, but had not returned to control levels. Blood biochemistry also revealed decreased total protein, albumin and albumin to globulin ratios at all doses in weeks 7 and 12, although none of the changes were statistically significant at 300 ppm, and the severity of the effects did not clearly increase between 3000 and 30 000 ppm or between 7 and 12 weeks. Evidence of reversibility was mixed. Statistically significant decreases in calcium levels were also noted in males at 3000 and 30 000 ppm and in females at 30 000 ppm, but were minor and not considered to be of toxicological significance.

No relevant findings were seen in the urine analysis.

Increased liver weights (absolute and relative) were seen at all doses in both sexes. The effect was dose related in males but did not reach statistical significance at any dose, whereas in females, the effect was statistically significant at all doses but did not demonstrate a clear dose–response relationship. Higher absolute and relative ovary weights were reported in females at 300 and 30 000 ppm, but were considered to have been related to the estrous cycle and not to the test item.

Macroscopic examination of organs and tissues did not reveal relevant findings.

Histology findings in the liver consisted of minimal to slight hepatocellular hypertrophy in males at 30 000 ppm and in females at all doses, biliary proliferation in males and females at 3000 and 30 000 ppm, and brown pigment in Kupffer cells and hepatocytes at 30 000 ppm in males and at 3000 and 30 000 ppm in females. All histology effects in the liver were reversible.

Other notable histology findings consisted of dilated intestinal glands in the duodenum, jejunum and ileum, containing cellular debris and inflammatory cells, noted in all treated groups, and minimal to moderately increased apoptosis in the pancreas at 3000 and 30 000 ppm in males and at all doses in females. These effects were fully reversible. The effects on the intestine were minor and showed no increase in either severity or incidence with dose and were not considered to be toxicologically relevant. The effect on the pancreas represented an exaggeration of a normal process and was also considered not to be toxicologically relevant.

The main target organ of toxicity was the liver, with increased alkaline phosphatase activity seen at all doses, increased liver weights seen at all doses and histology findings also seen in the liver, particularly biliary proliferation at 3000 and 30 000 ppm. Liver effects seen at 300 ppm were considered to be non-adverse, as they consisted of biochemical changes that did not reach statistical significance and the severity of which did not increase between weeks 7 and 12, hepatocellular hypertrophy in a single female, and increased relative liver weights that were not associated with consistent histological effects and were not statistically significantly different from control values. The NOAEL was 300 ppm (equal to 9.9 mg/kg bw per day), based on hepatocellular hypertrophy, biliary hyperplasia, increased alkaline phosphatase activity and reduced activated partial thromboplastin time at 3000 ppm (equal to 96.8 mg/kg bw per day) (Haag, 2006b).

In a 52-week study in Beagle dogs (for which a signed quality assurance statement indicating GLP compliance was available), groups of four males and four females received monepantel in the diet at a concentration of 0, 100, 300 or 3000 ppm (equal to 0, 2.96, 8.21 and 91.03 mg/kg bw per day in males and 0, 3.16, 10.18 and 99.39 mg/kg bw per day in females). Animals were regularly monitored for clinical signs, feed consumption and body weight. Ophthalmoscopic observations were made before treatment, at week 25/26 and at the end of treatment. Haematology, blood biochemistry and urine parameters were monitored before treatment and at weeks 12, 25 and 51. Necropsies were performed, with macroscopic observations on organs and tissues. Histology was carried out on a comprehensive range of tissues and organs from all doses. In addition, liver and jejunum tissues from male control and high-dose animals were examined by electron microscopy (it is reported that only tissues of males were examined, as light microscopy indicated that relevant lesions were similar in both sexes and that effects were more marked in males). Blood samples for the determination of blood levels of the test item and its metabolite were taken in week 17 and just before study termination.

There were no unscheduled deaths or treatment-related clinical signs reported. Body weights and body weight gains were reduced at 3000 ppm, although the effects did not reach statistical significance. There was no clear effect of the test item on feed consumption.

Ophthalmoscopy did not reveal findings of note.

The only haematology finding of note was a reduced activated partial thromboplastin time in males at 300 and 3000 ppm and in females at 3000 ppm. No clinical signs or other pathological findings suggestive of a coagulation disorder were observed.

Blood biochemistry revealed increased (2- to 9-fold) alkaline phosphatase activity in both sexes at all test doses throughout the test period. The effect was statistically significant in males at 3000 ppm and in females at 300 and 3000 ppm. Lower total protein levels were seen in males (ranging from –9% to –16%) and females (–13%) at 3000 ppm, and lower plasma albumin levels (from –16% to –24%) and albumin to globulin ratios (from –12% to –25%) were seen in both sexes at 3000 ppm and in females at 300 ppm. Increased ALT activity was seen

in males and females (from 1.4- to 4-fold) at 3000 ppm, and increased gamma-glutamyltransferase activity was seen in males (4-fold) at 3000 ppm. Finally, lower calcium levels were seen in males and females (from –7% to –8%) at 3000 ppm.

Macroscopic examination of organs revealed enlarged adrenal glands in one male at each of 300 and 3000 ppm. Increased absolute and relative liver weights were seen in all test groups, although statistical significance was reached only in males at 3000 ppm and in females at 300 ppm (but not at 3000 ppm). Increased absolute and relative adrenal gland weights were also seen in all test groups, although the effect was not statistically significant at any dose. Increased absolute and relative thyroid gland weights were also noted in all test groups, although the effect was statistically significant only in males at 3000 ppm and in females at 300 and 3000 ppm.

Histology findings in the liver consisted of bile duct hyperplasia in males and females at 3000 ppm, hepatocellular hypertrophy in all test groups (both sexes) and brown pigment corresponding to lipofuscin in hepatocytes, Kupffer cells and macrophages in males and females at 300 and 3000 ppm. Histology in the intestine revealed dilated Lieberkühn glands in the duodenum and/or jejunum with or without intraluminal cellular debris at all test levels. These effects were also seen in control animals, although the frequency and severity of the effect were less. In the adrenal glands, cortical cell hypertrophy was noted at all test levels and correlated with enlarged adrenal glands seen macroscopically. The effect may have been associated with the stress of treatment. In the kidney, slightly increased incidence and severity of brown pigment corresponding to lipofuscin were noted in tubular cells at 300 and 3000 ppm in males and at 3000 ppm in females. The effect represents a slight exaggeration of a background finding and was not considered toxicologically relevant. In the pancreas, increased acinar cell apoptosis was noted in males at 3000 ppm and also represents a slight exaggeration of a background effect.

Electron microscopic examination of the liver revealed a moderate proliferation of smooth endoplasmic reticulum membranes in hepatocytes at 3000 ppm and a reduced amount of glycogen in the hepatocytes of these animals. All other cellular organelles of the hepatocytes had the same morphology and distribution as those of control animals. Proliferation of smooth endoplasmic reticulum could be interpreted as a morphological expression of induction of drug metabolizing enzymes. Electron microscopic examination of the jejunum indicated that the dilated Lieberkühn glands were associated with slightly enlarged goblet cells, and it is hypothesized that both may have been the result of increased mucous production and secretion, possibly due to local irritation.

The study report includes information on blood levels of monepantel and its sulfone metabolite (AHC 2144670) prior to termination. Monepantel levels were below the limit of quantification (8 ng/ml) in the 100 ppm group, 17.4 ng/ml in the 300 ppm group and 60.5 ng/ml in the 3000 ppm group. The corresponding levels of the sulfone metabolite were 1057, 2565 and 6030 ng/ml, respectively. Whereas blood levels increased in a roughly dose-proportional manner between 100 and 300 ppm, the increases were not dose proportional between 300 and 3000 ppm.

Overall, the study demonstrated that the primary target organ of toxicity was the liver, as demonstrated by blood biochemistry findings at all doses, increased liver weights at all doses and histopathology findings at all doses. At doses of 100 and 300 ppm, relevant findings in the liver were limited to increased alkaline phosphatase activity, hepatocellular hypertrophy and increased liver weights. At 300 ppm, the increased alkaline phosphatase activity was statistically significant in females.

The NOAEL was 100 ppm (equal to 2.96 mg/kg bw per day), based on increased alkaline phosphatase activity, decreased albumin to globulin ratio, increased thyroid weights and increased pigmentation in liver at 300 ppm (equal to 8.21 mg/kg bw per day) (Haag, 2007).

*(d) Target species: sheep and lambs*

In a target species tolerance study (for which a quality assurance statement indicating GLP compliance was available), monepantel was administered as a single oral dose of 37.5 mg/kg bw (i.e. 10 times the recommended dose) to 2- to 4-week-old lambs. Three deaths occurred, but were attributed to inadvertent administration of the test item into the respiratory tract. With the exception of these animals, no test item–related clinical signs were noted, and no effects on growth weight or body weight were seen (lambs were monitored for 21 days after administration). Haematology and blood biochemistry analyses did not reveal any consistent and clearly test item–related adverse effects. No test item–related effects were seen in macroscopic or histological examinations (Malikides, 2007).

In a target species tolerance/margin of safety study (for which a signed quality assurance statement indicating GLP compliance was available), four groups of Merino lambs (seven of each sex per group) were administered saline or monepantel at an oral dose of 3.75 mg/kg bw (i.e. recommended dose), 11.25 mg/kg bw (i.e. 3 times the recommended dose) and 18.75 mg/kg bw (i.e. 5 times the recommended dose) every 3 weeks, on a total of eight occasions. No findings considered to be toxicologically relevant were seen (Malikides et al., 2008a).

*2.2.3 Long-term studies of toxicity and carcinogenicity*

*(a) Mice*

The carcinogenic potential of monepantel was investigated in a 78-week mouse (CD-1, SPF) study (for which a signed quality assurance statement indicating GLP compliance was available). Groups of 50 male and 50 female mice were administered monepantel in the diet at a concentration of 0, 10, 30, 120 or 500 ppm (equal to 0, 1.3, 4.2, 16.2 and 69.1 mg/kg bw per day in males and 0, 1.8, 5.5, 22.8 and 91.7 mg/kg bw per day in females). Clinical signs, feed consumption and body weights were monitored regularly. At week 52 and at the end of the treatment period, blood was taken for haematology analyses. At the end of treatment, all animals were killed, necropsied and subjected to a postmortem examination. Histology was performed on organs and tissues from all control and high-dose animals and on all gross lesions seen in other groups. Owing to test

item–related findings noted in high-dose groups, the liver was examined in all dose groups.

The mortality rate in females at 500 ppm was slightly increased compared with controls. No effect on survival was seen at the other doses. Clinical signs were considered to be similar in the treatment and control groups and not test item related. No effect on feed consumption, mean body weight or mean body weight gain was noted.

No test item–related haematology findings were noted at 10, 30 or 120 ppm. At 500 ppm, there was a test item–related statistically significant decrease in red blood cell count in males (–4.2%) and females (–3.5%) at week 52. Red blood cell counts were also decreased at week 79, but the effect was not statistically significant. At week 79, statistically significant increases in white blood cell counts (+60%) and absolute large unstained cell counts (+267%) were seen in males at 500 ppm. In females at 500 ppm at 79 weeks, the absolute eosinophil cell count was statistically significantly decreased (–28.6%).

No effects on organ weights were noted at 10 or 30 ppm. At 120 and 500 ppm, statistically significant increases in absolute and relative liver weights were seen in females. Absolute and relative spleen weights were statistically significantly increased in males at 500 ppm.

The macroscopic examination at necropsy revealed nothing to distinguish between treated and untreated animals.

The only test item–related effects noted in the histological examinations occurred in the liver and were non-neoplastic in nature. An increased incidence of fatty change was recorded at all doses. The effect demonstrated a dose–response relationship in females and was statistically significant at 120 and 500 ppm in males and females. Although the effect did not reach statistical significance at 10 and 30 ppm, the effect in females at 30 ppm was considered toxicologically relevant. In addition, a statistically significant increase in the incidence of hepatocellular hypertrophy was noted in males at all test item doses and in females at all test item doses with the exception of the top dose. Although the hypertrophy was clearly increased at all dose levels, there was no dose–response relationship.

There was no indication of carcinogenic potential of monepantel in this study. The NOAEL was 10 ppm (equal to 1.8 mg/kg bw per day), based on increased incidence of fatty change in liver in females at 30 ppm (equal to 5.5 mg/kg bw per day) and supported by increased hepatocellular hypertrophy (Fischer, 2008).

*(b) Rats*

Monepantel was also investigated for carcinogenicity in a 2-year study in (HanRcc:WISt, SPF) rats (for which a signed quality assurance statement indicating GLP compliance was available). Groups of 50 male and 50 female rats were administered monepantel in the diet at a concentration of 0, 100, 1000 or 12 000 ppm (equal to 0, 4.63, 47.40 and 578.17 mg/kg bw per day in males and 0, 5.61, 56.66 and 706.65 mg/kg bw per day in females). Clinical signs, feed consumption and body weights were monitored regularly. At weeks 53, 78 and 104 (after the end

of treatment), blood was taken for haematology analyses. At the end of treatment, all animals were killed, necropsied and given a postmortem examination. Histology was performed on organs and tissues from all control and high-dose animals and on all gross lesions seen in other groups, as well as in all animals that died spontaneously or were killed in extremis.

Survival rate was not influenced by the test item, and no test item–related clinical signs were noted. At 12 000 ppm, body weights in females were consistently slightly lower than in controls (except in week 18). The effect was statistically significant for most of the fourth quarter of the study. By the end of treatment, the mean body weight of females in the 12 000 ppm group was 6.6% lower than that of controls. Body weights in females in other test groups were in line with those in the control group. In males, body weights were increased in the 100 ppm group, although the effect was not statistically significant at most time points. Nevertheless, by the end of treatment, the mean body weight in males in the 100 ppm group was 7.1% greater than in controls. Body weights in males in other test groups were in line with those seen in the control group. The pattern seen in mean body weight gains was similar to that seen for body weights. No clear effect of the test item on feed consumption was noted.

Haematology results did not reveal consistent or dose-related effects on any parameters over the course of the study.

Increases in absolute and relative liver, heart and kidney weights were noted in females at 1000 and 12 000 ppm. Absolute and relative mean thymus weights were increased at all doses in males, but the effect did not reach statistical significance. At 1000 and 12 000 ppm, highly increased thymus weights were seen, but these were caused by markedly increased thymus weights in two single males per group.

Macroscopic examination revealed increased foci in the adrenal glands in females (present in 2% of controls, 4% at 100 ppm, 6% at 1000 ppm and 16% at 12 000 ppm; statistically significant at 12 000 ppm).

Histology did not reveal any clearly test item–related changes. The NOAEL was 100 ppm (equal to 4.63 mg/kg bw per day), based on organ weight changes and macroscopic observations in the adrenal glands at 1000 ppm (equal to 47.40 mg/kg bw per day) (Broich, 2008).

### *2.2.4 Genotoxicity*

Monepantel was tested for its ability to induce mutations in *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535, both with and without S9 metabolic activation. Four experiments were conducted, the first of which was a dose range–finding experiment (using the plate incorporation method) in one strain only (TA100) using monepantel concentrations of 5, 50, 500 and 5000 µg/plate. A subsequent experiment (using the plate incorporation method) was performed in all five strains identified above, using concentrations of 8, 40, 200, 1000 and 5000 µg/plate. Two further experiments were performed (using the preincubation method) in all five strains, the first of which used monepantel concentrations of 312.5, 625, 1250, 2500 and 5000 µg/plate and the second of which used concentrations of

25, 50, 100, 200 and 400 µg/plate. Monepantel was seen to precipitate at 5000 µg/ml using the plate incorporation method and at greater than 200 µg/plate using the preincubation method. No evidence of bacteriotoxicity was seen at any concentration. No increase in revertant numbers was seen in any strain at any concentration. It was concluded that no evidence of mutagenicity was seen (Martus, 2005 [signed quality assurance statement indicating GLP compliance included]).

Monepantel was tested in a chromosomal aberration test with cultured human peripheral blood lymphocytes both with and without S9 metabolic activation. Monepantel decreased the mitotic index in a concentration-dependent manner, and concentrations for analysis in the chromosomal aberration test were selected based on these data. In experiments without S9 activation, concentrations were 25.8, 33.4 and 43.1 (20 hours of continuous treatment) and 57.2, 81.8 and 97.8 µg/ml (3-hour treatment, 17-hour recovery). In experiments with S9 activation, concentrations were 77.5, 100.1 and 129.3 µg/ml (3-hour treatment, 17-hour recovery) and 81.8, 117 and 139.9 µg/ml (3-hour treatment, 17-hour recovery) in a repeat study. No evidence of clastogenic potential was seen at any concentration (Elhajouji, 2005 [signed quality assurance statement indicating GLP compliance included]).

Monepantel was tested in an in vivo mouse bone marrow micronucleus test. Groups of five male and five female mice received vehicle, monepantel or cyclophosphamide (positive control) by the oral route. Monepantel was administered at a dose of 2000 mg/kg bw per day for 2 days. Treated and control animals were killed 24 hours after the last administration, and bone marrow smears were prepared. A preliminary test was carried out with a dose of 2000 mg/kg bw per day for 2 days, and no observable toxic effects were seen. This dose was used as a limit dose in the final assay. No clinical signs or mortalities were seen. In both male and female test animals, the number of micronucleated polychromatic erythrocytes as well as the ratio of polychromatic erythrocytes to normochromatic erythrocytes were not influenced by the test item. It was concluded that monepantel did not induce damage to the chromosomes or the mitotic apparatus of mouse bone marrow cells after two oral administrations of 2000 mg/kg bw, 24 hours apart.

No proof of exposure was provided for this study, as no toxicity was seen, and no blood measurements were performed. It is accepted, however, that systemic exposure to monepantel has been seen in rats, dogs and sheep following oral administration. In the rat absorption, distribution, metabolism and excretion study (Gassen, 2007), repeated dosing at 10 mg/kg bw for 7 days was seen to lead to radioactivity in the bone: mean levels of up to 0.53 and 2.34 µg eq/g tissue in male and female rats, respectively. It was concluded that the lack of acute toxicity seen in mice in the micronucleus study is not evidence that the test item did not reach the target tissue (Haddouk, 2006 [signed quality assurance statement indicating GLP compliance included]).

A brief report was provided for a non-GLP-compliant study investigating the mutagenic potential of the racemic sulfone metabolite AHC 2092404 in a screening version of the Ames test that used six well plates in place of petri dishes (Glowienke, 2004). AHC 2092404 was incubated at a concentration of 30, 100,

***Table 2. Results of genotoxicity assays on monepantel***

| Test system | Test object | Concentration | Results | Reference |
|---|---|---|---|---|
| **In vitro** | | | | |
| Ames test[a] | *Salmonella typhimurium* TA97a, TA98, TA100, TA102, TA1535 | 8–5000 µg/plate | Negative | Martus (2005) |
| Chromosomal aberration test[a] | Human peripheral lymphocytes | 25.8–139.9 µg/ml | Negative | Elhajouji (2005) |
| **In vivo** | | | | |
| Cytogenetics test (micronucleus test) | Mouse bone marrow | 2000 mg/kg bw per day for 2 days | Negative | Haddouk (2006) |

[a] Both with and without rat liver S9 fraction.

300 or 1000 µg/well with *Salmonella typhimurium* strains TA97a, TA98, TA100, TA102 and TA1535, with and without addition of S9 metabolic activation. The report indicates that the top concentration of 1000 µg/well in this system is approximately equivalent to a concentration of 5000 µg/plate in the standard Ames test. The test item did not precipitate and did not induce signs of bacteriotoxicity. No signs of mutagenicity were seen.

A brief report was also provided for a non-GLP-compliant study investigating the genetic toxicity of the racemic sulfone metabolite AHC 2092404 in an in vitro micronucleus test using TK6 cells (Frieauff, 2004). Cells were incubated with AHC 2092404 at a concentration of 64.6, 107.8 or 179.8 µg/ml with and without an S9 metabolic activation system. Precipitation was seen at the top dose. The test item did not induce micronucleus formation.

Results of genotoxicity assays on monepantel are summarized in Table 2.

### *2.2.5 Reproductive and developmental toxicity*

#### *(a) Multigeneration studies*

A two-generation reproductive toxicity study (for which a signed quality assurance statement indicating GLP compliance was available) was performed using Han Wistar rats with the aim of assessing gonadal function, estrous cycles, mating behaviour, conception, parturition, gestation, lactation, weaning, and growth and development of offspring. Information was also provided on neonatal morbidity, mortality and behaviour, and limited data were provided on teratogenesis. Monepantel was administered in the diet at a concentration of 0, 200, 1500 or 12 000 ppm. P generation animals were administered monepantel for a 70-day pre-pairing period and during pairing, gestation and lactation. $F_1$ generation animals were either terminated following weaning on day 21 postpartum or selected for breeding the $F_2$ generation. Those selected for breeding were maintained on the

monepantel diet from weaning through growth to adulthood (approximately 90 days) and during pairing, gestation and subsequent lactation.

Mean achieved doses in parental generation animals were 0, 13.3, 99.8 and 798 mg/kg bw per day in males and 0, 15.8, 119 and 950 mg/kg bw per day in females during the pre-pairing period; 0, 10.5, 79.3 and 647 mg/kg bw per day in males and 0, 13.5, 103 and 863 mg/kg bw per day in females during the after pairing (males) or gestation period; and 0, 32.3, 245 and 2055 mg/kg bw per day during lactation. In the $F_1$ generation, the mean achieved doses were 0, 16.8, 125 and 1014 mg/kg bw per day in males and 0, 18.6, 141 and 1109 mg/kg bw per day in females during the pre-pairing period; 0, 11.2, 81.7 and 694 mg/kg bw per day in males and 0, 15.1, 114 and 918 mg/kg bw per day in females during the after pairing (males) or gestation period; and 0, 30.8, 241 and 2028 mg/kg bw per day during lactation.

In parent animals (P and $F_1$ generations), all animals survived until scheduled necropsy, and no clinical signs were noted. At necropsy performed at the end of treatment, enlarged livers were observed in P generation females in the 1500 and 12 000 ppm groups, which correlated with histopathology findings. No other macroscopic findings were reported. Statistically significantly increased mean liver weights were seen in P generation females at 1500 and 12 000 ppm. Liver to body weight ratios were also statistically significantly increased in P generation females at 1500 and 12 000 ppm. The liver to brain weight ratio was slightly, but statistically significantly, increased in 200 ppm females. At 12 000 ppm, statistically significantly increased absolute and relative adrenal weights were seen in P generation females.

In $F_1$ generation parental animals, statistically significantly increased absolute and relative liver and adrenal gland weights were seen in females at 200, 1500 and 12 000 ppm, although the increase in relative weights was not statistically significant at 200 ppm. There was no histopathological correlate in either organ at 200 ppm.

No treatment-related effects were seen on sperm motility, sperm morphology or epididymal or testicular sperm count.

Histology findings were noted in female parental animals from the P and $F_1$ generations in the liver, adrenal glands and ovaries ($F_1$ generation only). In the P and $F_1$ generations, centrilobular hepatocellular hypertrophy was seen at higher incidences in females at 1500 and 12 000 ppm. The effect was associated with increased mean liver weights and with liver enlargement seen in several animals at necropsy. In the adrenal glands of the P and $F_1$ generation parental animals, cortical cell hypertrophy of the zona glomerulosa was seen at higher incidences in females at 1500 and 12 000 ppm. In the ovaries of the $F_1$ generation parental animals, a higher incidence of hyperplasia of the sex cord stromal cells (interstitial cell hyperplasia) was seen at 12 000 ppm (incidence was 6/24, 6/24, 7/24 and 12/24 at 0, 200, 1500 and 12 000 ppm, respectively).

In parental animals ($F_1$ females), no test item–related effect was noted on counts of ovarian follicles or corpora lutea.

In P and $F_1$ generation animals, no treatment-related effects were seen on estrous cycles, mean precoital time, fertility indices, mean duration of gestation, number of implantation sites, incidences of post-implantation loss, number of pups and postnatal pup loss.

No test item–related effects were noted on mean pup weights on day 1 postpartum or during the lactation period or on sex ratios or age of sexual maturation.

Increased mean absolute and relative liver weights were seen in female $F_1$ pups at 200, 1500 and 12 000 ppm and in male $F_1$ pups at 12 000 ppm. There was little or no change in females between 200 and 1500 ppm. In $F_2$ pups, relative liver weights were increased at 12 000 ppm. No associated histology findings were seen in livers of $F_2$ pups (livers of $F_1$ pups were not examined histologically).

Overall, it can be concluded that monepantel did not induce reproductive toxicity in this study; the NOAEL for reproductive toxicity was therefore 12 000 ppm (equal to 647 mg/kg bw per day), the highest dose tested. The NOAEL for general effects in the P and $F_1$ generation parental animals was 200 ppm (equal to 13.5 mg/kg bw per day), based on macroscopically observed enlarged livers (P generation females), increased absolute and relative liver weights (P and $F_1$ generation females) associated with centrilobular hepatocellular hypertrophy (P and $F_1$ generation females), increased absolute and relative adrenal gland weights ($F_1$ generation females) and cortical cell hypertrophy (P and $F_1$ generation females) seen at 1500 ppm (equal to 103 mg/kg bw per day). The NOAEL for offspring toxicity was 1500 ppm (equal to 103 mg/kg bw per day), based on increased relative liver weights seen at 12 000 ppm (equal to 863 mg/kg bw per day) in $F_1$ and $F_2$ generation pups (Gerspach, Schaetti & Flade, 2006).

*(b) Developmental toxicity*

*(i) Rats*

In a prenatal developmental toxicity study in the Han Wistar rat, four groups of 22 mated female rats were administered 0, 100, 300 or 1000 mg/kg bw per day by oral gavage from day 6 (implantation) to day 20 post-coitum (a signed quality assurance statement indicating GLP compliance was included in the study report).

In maternal animals, no test item–related mortality occurred, mean feed consumption and body weight development were similar in all groups, no effects on reproduction variables were noted and no test item–related necropsy findings were seen.

Mean fetal weights, calculated on a litter basis, were not significantly different between test and control animals. When mean fetal weights were calculated on an individual basis, they were statistically significantly higher in all test groups than in controls. However, the difference was minimal (the mean fetal weight in the control group was 4.8 g, whereas it was 4.9 g in all test groups). Given the size of the effect and the absence of a clear dose–response relationship, the effect is considered

to have occurred by chance. The only other effect of note was a statistically significantly decreased incidence of non-ossified proximal phalanges of toes 2, 3, 4 and 5 at 300 and 1000 mg/kg bw per day, possibly reflecting advanced ossification corresponding to the minimally increased fetal body weights and not considered to be test item related. With the exception of these effects on fetal weights and ossification of proximal phalanges, fetuses were macroscopically unaffected, with no findings on visceral, skeletal or cartilaginous structures noted. There were no effects noted on fetal sex ratio. The NOAEL for maternal and for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Gerspach & Flade, 2006b).

*(ii) Rabbits*

In a prenatal developmental toxicity study in Himalayan rabbits, groups of 20 female rabbits were administered 0, 100, 300 or 1000 mg/kg bw per day by oral gavage from day 6 to day 27 post-coitum (a signed quality assurance statement indicating GLP compliance was included in the study report).

In maternal animals, no test item–related mortality occurred. Mean feed consumption was slightly reduced at 100, 300 and 1000 mg/kg bw per day (–3.2%, –8.4% and –5.3%, respectively). Owing to the absence of a clear dose–response relationship, the effect was not considered to be test compound related. Corrected body weight gain (i.e. difference between body weight at termination minus the uterus weight and body weight at day 6 post-coitum) was slightly reduced at 100, 300 and 1000 mg/kg bw per day compared with controls (–2.7%, –2.8% and –2.5% compared with –1.2%, respectively). Owing to the absence of a clear dose–response relationship, the effect was not considered to be test compound related. There were no effects seen on reproduction end-points (post-implantation loss and number of fetuses per dam) and no test item–related effects noted at necropsy.

In the fetuses, no effects were seen on fetal body weight or sex ratio. In the external and visceral examination, there was one finding of note: two fetuses (from the same litter) in the 1000 mg/kg bw per day group were observed to have masses within the nasopharyngeal region and resultant interruption of the nasopharyngeal tract and protrusion of the soft palate into the buccal cavity. As the findings were confined to one litter, they were considered incidental. There were no abnormal findings noted during the skeletal or cartilage examination.

The NOAEL for both maternal and fetal effects was 1000 mg/kg bw per day, the highest dose tested (Gerspach & Flade, 2006a).

*(iii) Sheep*

In a target species repeated-dose reproduction study (for which a signed quality assurance statement indicating GLP compliance was available), a group of 14 Merino breeding rams and 56 Merino breeding ewes were administered monepantel at an oral dose of 11.25 mg/kg bw (i.e. 3 times the recommended dose). Control rams and ewes were administered saline. Rams were administered the test material every 5 days over one spermatogenic cycle (approximately 63 days), through mating and ending approximately 4 weeks after mating was complete (the

total treatment period was 100 days). Ewes were administered the test material every 5 days during estrus and mating, during gestation (apart from a treatment-free period of approximately 2 weeks up to lambing) and up to weaning (treatment started when lambs were approximately 3 weeks old). Variables monitored included clinical effects, body weight, faeces, haematology, coagulation, clinical chemistry parameters, reproductive indices (i.e. ram scrotal circumference, ram semen quality, ram and ewe serving capacity and breeding ability, pregnancy percentage, gestation length, lambing percentage and twinning percentage) and macroscopic findings at necropsy of parental animals and offspring of treated animals (variables monitored included failed embryos, abortions, teratogenic defects, stillbirths, ewe reproductive problems, perinatal mortality, lamb growth). No test item–related toxicologically significant effects were reported (Malikides, 2008b).

### *2.2.6 Special studies*

#### *(a) Cardiovascular and respiratory end-points*

In a study of effects on cardiovascular and respiratory end-points (a signed quality assurance statement indicating GLP compliance was available), two groups of four anaesthetized male rats received a single intraduodenal dose of either vehicle or monepantel at a dose of 2000 mg/kg bw. Systolic, diastolic and mean blood pressure, heart rate, electrocardiogram, respiratory rate, tidal volume and minute volume were monitored for up to 4.5 hours after test item administration. No evidence of an effect of monepantel on cardiovascular end-points was seen. Small decreases in mean absolute values for respiratory rate and minute volume were seen, but, because of the modest size of these effects, they are not considered to be biologically significant (Earl, Boehlmann & Gisin, 2007).

#### *(b) Behavioural end-points*

In a modified Irwin screen test in rats (a signed quality assurance statement indicating GLP compliance was available), two groups of six male rats received single doses of either vehicle or monepantel at a dose of 2000 mg/kg bw by oral gavage. Rats were assessed for behavioural changes before dosing and at 1, 2, 4 and 6 hours after dosing. No evidence of an effect of monepantel on general behaviour was observed (Earl & Gisin, 2007b).

#### *(c) Gastrointestinal end-points*

In a charcoal propulsion test (a signed quality assurance statement indicating GLP compliance was available), two groups of seven male rats received single doses of either vehicle or monepantel at a dose of 2000 mg/kg bw by oral gavage. Four and a half hours later, animals were administered a charcoal suspension by oral gavage. Thirty minutes later, animals were killed, the gastrointestinal tract was removed and the distance travelled by the charcoal was measured. No evidence of an effect of monepantel on intestinal motility was observed (Earl & Gisin, 2007a).

*(d) Immune responses*

Monepantel was tested for its potential to induce delayed contact hypersensitivity in the mouse local lymph node assay (a signed quality assurance statement indicating GLP compliance was available in the study report). Groups of four female CBA/J mice were administered monepantel at a concentration of 1%, 2.5%, 5%, 10% or 25%. Two further groups of four animals received vehicle or positive control. The test item was applied over the ears (25 µl/ear) for 3 consecutive days during the induction phase. After 2 days of resting, proliferation of lymphocytes in the lymph node draining the application site was measured by incorporation of tritiated methyl thymidine. The obtained values were used to calculate stimulation indices. In addition, the irritant potential of the substance was assessed in parallel by measurement of ear thickness on days 1, 2, 3 and 6. No clinical signs were seen, no cutaneous reactions or increases in ear thickness were noted and no noteworthy lymphoproliferation was seen at any test concentration. It is concluded that monepantel did not induce delayed contact hypersensitivity in the mouse local lymph node assay (Sire, 2006).

*(e) Microbiological effects*

No data on microbiological effects of monepantel were available for evaluation. In the absence of any evidence to suggest that the substance has a microbiological effect, such data are not required.

### *2.3 Observations in humans*

No data were identified on the effects of monepantel in humans.

## *3. COMMENTS*

The Committee considered data from studies on pharmacodynamics, pharmacokinetics, including metabolism, short-term toxicity, genotoxicity, carcinogenicity, reproductive toxicity and developmental toxicity submitted by the sponsor. A supplementary literature search was performed that revealed only one additional study considered to be of relevance. All pivotal studies reported were conducted in line with GLP and other relevant standards.

### *3.1 Biochemical data*

Pharmacokinetic studies were performed in rats, dogs and sheep, as well as in vitro.

Following oral administration of monepantel to rats, uptake and elimination were rapid. Absorption was approximately 30%, whereas bioavailability of unchanged monepantel was slightly less than 10%, indicating significant first-pass metabolism. The peak concentration of radiolabelled material in blood was reached between 2 and 8 hours after oral administration. Radioactivity was poorly distributed to tissues, with highest levels in liver and fat, followed by adrenals, pancreas and ovaries. Levels in kidney were intermediate, and those in muscle were lowest.

Residue levels in organs and tissues were generally higher in females than in males. There was little evidence of accumulation of radioactivity in tissues after seven daily doses. The terminal elimination half-life of radioactivity from blood was 55–60 hours. More than 90% of orally administered monepantel was eliminated in the faeces by 168 hours after dosing, with less than 5% excreted in urine.

In a non-GLP-compliant study in the dog using the racemic mixture of monepantel and the corresponding *R*-enantiomer, absorption after oral administration was rapid, with maximal blood concentrations occurring 1–2 hours after administration. The half-life of elimination was 44 hours. The oral bioavailability of combined parent substance and sulfone metabolite was 24%. Blood levels of the sulfone metabolite far exceeded those of the parent compound.

Protein binding of radiolabelled monepantel was investigated in vitro using rat, dog, sheep and bovine plasma. The results showed a high level of protein binding (96.2–99.5%), independent of monepantel concentration and the species from which the plasma was obtained.

Following oral administration of radiolabelled monepantel in the rat, the predominant compounds seen in blood were the sulfone derivative and the parent compound. The main components in faeces were M3 (an oxidized metabolite of the sulfone), the parent compound and the sulfone derivative. Parent compound was not present in bile or urine. The predominant compound seen in muscle, fat, liver and kidney was the sulfone metabolite, although parent compound was also seen in significant amounts. All significant residues seen in edible sheep tissues were also detected in rat tissues.

In in vitro studies, intrinsic clearance rates for monepantel were lower in mouse and dog liver microsomes than in rat and human liver microsomes.

In liver microsomal fractions from rats administered daily doses of monepantel for 4 weeks, there was an increase in total cytochrome P450 content, a slight increase in 7-ethoxyresorufin *O*-deethylase (CYP1A1) activity, as well as a slight increase in lauric acid 11- and 12-hydroxylase (CYP4A1) activities. CYP1A2- and CYP2B1-dependent activities were not increased. Microsomal glucuronidation of 3-methyl-2-nitrophenol was increased. Plasma concentrations of TSH, $T_4$ and $T_3$ were not affected. It is concluded that there is some evidence that monepantel is a weak inducer of xenobiotic metabolizing enzymes, but there is little evidence that it resembles phenobarbital in its induction profile.

### *3.2 Toxicological data*

The $LD_{50}$ of monepantel in rats following oral or dermal administration has been reported to be greater than 2000 mg/kg bw. Monepantel has been reported to be non-irritating to the skin of rabbits and slightly irritating to the rabbit eye.

In a 13-week study in mice with dietary concentrations of 0, 30, 120, 600 and 6000 ppm, liver was the target organ. Increased total bilirubin was seen at 600 and 6000 ppm, and a possible effect on lipid metabolism was demonstrated by slightly increased cholesterol levels at 600 and 6000 ppm, corroborated by increased incidences of fatty liver change at all doses. At 600 and 6000 ppm,

females had increased levels of focal necrosis and lymphoid cell infiltrates. It is notable that histological effects of the test substance were seen predominantly in females. Liver enzyme effects (increased mean plasma AST, ALT and alkaline phosphatase activities) were seen at a range of doses, but often without a clear dose–response relationship. The LOAEL was 30 ppm (equal to 5.27 mg/kg bw per day), the lowest dose tested, based on an increased incidence of fatty change in the liver of females.

In a 4-week study in rats with dietary concentrations of 0, 1000, 4000 and 12 000 ppm, disturbed fat metabolism was suggested by increased cholesterol, triglyceride and phospholipid levels in females at all doses and increased cholesterol and phospholipid levels in males at 4000 and 12 000 ppm. Increased absolute and relative liver weights were seen in females at all doses and in males at 4000 and 12 000 ppm. Centrilobular hepatocellular hypertrophy (males and females) and diffuse follicular hypertrophy of the thyroid (males only) were seen at all doses. The LOAEL was 1000 ppm (equal to 86 mg/kg bw per day), the lowest dose tested.

In a 13-week study in rats with dietary concentrations of 0, 50, 200, 1000 and 12 000 ppm, the main target organ was the liver, reflected by increased absolute and relative liver weights and centrilobular hepatocellular hypertrophy at 1000 and 12 000 ppm in females. Biochemical findings indicative of liver effects included elevated levels of cholesterol and phospholipids (females at 1000 and 12 000 ppm) and triglycerides (males and females at 12 000 ppm), indicative of effects on lipid metabolism, and increased total protein (males at 12 000 ppm) and albumin levels (males at 1000 and 12 000 ppm) in blood. In addition, absolute and relative weights of adrenals and spleen were increased at the highest dose level in females, although without associated histological findings. High-dose findings also included hypospermatogenesis in males and sex cord stromal hypertrophy or hyperplasia in females. Histological findings were largely reversible, although some evidence of hypospermatogenesis was still present at the end of the 4-week recovery period. The NOAEL was 200 ppm (equal to 15.20 mg/kg bw per day), based on effects on the liver (centrilobular hypertrophy and increased cholesterol and phospholipid levels) in females at 1000 ppm (equal to 81.45 mg/kg bw per day).

In a 52-week study in rats with dietary concentrations of 0, 50, 200, 1000 and 12 000 ppm, an effect on lipid metabolism was evident from increased cholesterol, phospholipid and triglyceride levels at 12 000 ppm. Increases in absolute and relative liver and relative kidney weights were also seen at this dose. The NOAEL was 1000 ppm (equal to 54.45 mg/kg bw per day), based on increased absolute and relative liver weights and increased cholesterol, triglyceride and phospholipid levels, indicative of effects on the liver, at 12 000 ppm (equal to 656.08 mg/kg bw per day).

A 4-week dose range–finding study in dogs with dietary concentrations of 0, 5000, 15 000 and 40 000 ppm (equal to 0, 161.2, 566.1 and 1216.6 mg/kg bw per day in males and 0, 183.6, 561.0 and 1472.4 mg/kg bw per day in females) identified the liver, thymus, adrenal gland and thyroid as targets of toxicity. Alkaline phosphatase activity was increased at all test doses, although not in a clearly dose-dependent manner. Absolute and relative thymus weights were reduced at all doses, and increased severity of thymus involution was seen at 40 000 ppm.

Absolute and relative adrenal gland, liver and thyroid weights (females only) were also altered, but with no associated histological effects. Body weight gain and feed consumption were reduced in males at 40 000 ppm.

In a 13-week study in dogs with dietary concentrations of 0, 300, 3000 and 30 000 ppm, the main target of toxicity was the liver, with increased alkaline phosphatase activity seen at all doses, increased absolute and relative liver weights seen at all doses, and histological findings, which were reversible, also seen in the liver (hepatocellular hypertrophy in males at 30 000 ppm and in females at all doses, biliary proliferation at 3000 and 30 000 ppm and brown pigment in Kupffer cells and hepatocytes at 30 000 ppm in males and at 3000 and 30 000 ppm in females). Other effects of note included reduced activated partial thromboplastin time in males and females at 3000 and 30 000 ppm. Liver effects seen at 300 ppm were considered to be non-adverse, as these consisted of biochemical changes that did not reach statistical significance and the severity of which did not increase over time, hepatocellular hypertrophy in a single female animal and increased relative liver weights that were not associated with consistent histological effects and were not statistically significantly different from control values. The NOAEL was 300 ppm (equal to 9.9 mg/kg bw per day), based on hepatocellular hypertrophy, biliary hyperplasia, increased alkaline phosphatase activity and reduced activated partial thromboplastin time at 3000 ppm (equal to 96.8 mg/kg bw per day).

In a 52-week study in dogs with dietary concentrations of 0, 100, 300 and 3000 ppm, alkaline phosphatase activity was statistically significantly increased at 300 ppm (females only) and 3000 ppm (both sexes). Other effects on the liver included effects on blood protein (3000 ppm, both sexes), decreased plasma albumin to globulin ratios (females only at 300 ppm, both sexes at 3000 ppm), increased ALT activity (3000 ppm, both sexes), increased gamma-glutamyltransferase activity (3000 ppm, males only), increased absolute and relative liver weights at all doses (statistically significant in females at 300 ppm and in males at 3000 ppm), hepatocellular hypertrophy (all doses, both sexes), brown pigment corresponding to lipofuscin in hepatocytes, Kupffer cells and macrophages (300 and 3000 ppm, both sexes) and bile duct hyperplasia (3000 ppm, both sexes). Increased absolute and relative thyroid weights (with no histological correlate) were seen at 300 ppm (females only) and 3000 ppm (both sexes). Electron microscopy of the liver revealed smooth endoplasmic reticulum proliferation, consistent with induction of xenobiotic metabolizing enzymes. The NOAEL was 100 ppm (equal to 2.96 mg/kg bw per day), based on increased alkaline phosphatase activity, decreased albumin to globulin ratio, increased thyroid weights and increased pigmentation in liver at 300 ppm (equal to 8.21 mg/kg bw per day).

Single-dose and repeated-dose target species tolerance studies were performed in sheep using oral administration. The single-dose study used a dose of 37.5 mg/kg bw (10 times the recommended dose), whereas the repeated-dose study used doses of 3.75, 11.25 and 18.75 mg/kg bw administered every 3 weeks, on a total of eight occasions. No findings considered to be toxicologically significant were seen in either study.

In an 18-month carcinogenicity study in mice, animals received monepantel in the diet at a concentration of 0, 10, 30, 120 or 500 ppm. At the top dose,

slightly increased mortality in females was noted, as well as some changes in haematological parameters (decreased red blood cell count, increased white blood cell count, decreased eosinophil count). Increased absolute and relative liver weights were seen in females at 120 and 500 ppm. The only test item–related effects noted in the histological examinations consisted of an increased incidence of fatty change in liver at all doses. The effect demonstrated a dose–response relationship in females and was statistically significant at 120 and 500 ppm in males and females. Although the effect did not reach statistical significance at 10 and 30 ppm, the Committee considered that the effect in females at 30 ppm could not be dismissed. A statistically significant increase in the incidence of hepatocellular hypertrophy was noted at all test substance doses except for the top dose in females. Although the hypertrophy was clearly increased in all treated groups, there was no dose–response relationship. There was no indication of carcinogenic potential of monepantel in this study. The NOAEL was 10 ppm (equal to 1.8 mg/kg bw per day), based on increased incidence of fatty change in liver of females seen at 30 ppm (equal to 5.5 mg/kg bw per day) and supported by increased hepatocellular hypertrophy.

In a 2-year carcinogenicity study in rats, animals received monepantel in the diet at a concentration of 0, 100, 1000 or 12 000 ppm. Decreased body weights in females relative to controls were seen at 12 000 ppm. Increased absolute and relative liver, heart and kidney weights were seen in females at 1000 and 12 000 ppm, and absolute and relative mean thymus weights were increased at all doses in males, although the effect did not reach statistical significance. The other finding of note was an increase in macroscopic foci in adrenal glands in females (present in 2% of controls, 4% at 100 ppm, 6% at 1000 ppm and 16% at 12 000 ppm) that reached statistical significance at 12 000 ppm. No evidence of carcinogenicity was seen in the rat. The NOAEL was 100 ppm (equal to 4.63 mg/kg bw per day), based on organ weight changes and macroscopic observations in the adrenal glands at 1000 ppm (equal to 47.40 mg/kg bw per day).

The genotoxicity of monepantel was investigated in vitro in mutation studies in *Salmonella typhimurium* (Ames test) and a chromosomal aberration test in cultured human peripheral blood lymphocytes and in vivo in a mouse bone marrow micronucleus test. No evidence of genotoxicity was seen.

The racemic sulfone metabolite AHC 2092404 was also tested in a screening version of the Ames test, with no evidence of mutagenicity.

In light of the negative results in genotoxicity studies and the lack of carcinogenicity in mice and rats, monepantel is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats, animals received monepantel in the diet at a concentration of 0, 200, 1500 or 12 000 ppm. The main effects seen on parent animals were consistent with those seen in the repeated-dose studies (increased liver weights associated with hepatocellular hypertrophy, increased adrenal gland weights associated with cortical cell hypertrophy). Increased relative liver weights were also noted in $F_1$ and $F_2$ pups, but no associated histopathology was seen in $F_2$ pups ($F_1$ pups were not examined histologically).

The other notable effect was an increased incidence of hyperplasia of the sex cord stromal cells in the ovary at 12 000 ppm in $F_1$ parent animals. No evidence of reproductive toxicity was seen in this study, and the NOAEL for reproductive toxicity was therefore 12 000 ppm (equal to 647 mg/kg bw per day), the highest dose tested. The NOAEL for general effects in the P and $F_1$ generation parental animals was 200 ppm (equal to 13.5 mg/kg bw per day), based on macroscopically observed enlarged livers in P generation females, increased absolute and relative liver weights associated with centrilobular hepatocellular hypertrophy in P and $F_1$ generation females, increased absolute and relative adrenal gland weights in $F_1$ generation females and cortical cell hypertrophy in P and $F_1$ generation females seen at 1500 ppm (equal to 103 mg/kg bw per day). The NOAEL for offspring toxicity was 1500 ppm (equal to 103 mg/kg bw per day), based on increased relative liver weights seen at 12 000 ppm (equal to 863 mg/kg bw per day) in $F_1$ and $F_2$ generation pups.

Developmental toxicity studies were performed in rats and rabbits with doses of up to 1000 mg/kg bw per day from day 6 post-coitum to day 20 (in rats) or 27 (in rabbits). Monepantel was well tolerated in maternal animals, and no findings considered to be test substance related were seen in maternal or fetal animals. The NOAEL for maternal toxicity and for embryo and fetal toxicity was 1000 mg/kg bw per day, the highest dose tested, in both rats and rabbits.

The Committee concluded that monepantel was not teratogenic or otherwise developmentally toxic in rats or rabbits.

Special studies were carried out in rats to investigate the effects of monepantel on cardiovascular and respiratory end-points, behavioural end-points (modified Irwin screen test), intestinal motility and delayed contact hypersensitivity. Monepantel did not induce notable effects on any of these end-points.

No data were identified on the effects of monepantel in humans.

On the basis of the toxicological findings, the Committee considered liver to be the primary target of toxicity for monepantel, as demonstrated by effects on relevant biochemical variables, absolute and relative liver weights and histological findings. The Committee observed that, although, in some cases, effects on the relevant end-points failed to demonstrate a clear dose–response relationship, there was a consistent effect of monepantel on the liver across studies and species.

The Committee considered the most appropriate point of departure for the derivation of the toxicological ADI to be the NOAEL of 10 ppm (equal to 1.8 mg/kg bw per day) in a 78-week oral dosing study in mice, based on an increased incidence of fatty change in the liver of females seen at 30 ppm (equal to 5.5 mg/kg bw per day) and supported by increased hepatocellular hypertrophy.

### *3.3 Microbiological data*

No data on microbiological effects of monepantel were available for evaluation. In the absence of any evidence to suggest that the substance has a microbiological effect, such data are not required.

## 4. EVALUATION

The Committee established an ADI of 0–20 µg/kg bw, based on a NOAEL of 1.8 mg/kg bw per day in a 78-week oral dosing study in mice, based on an increased incidence of fatty change in the liver of females seen at 5.5 mg/kg bw per day, application of a safety factor of 100 and rounding to one significant figure.

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# ANNEXES

## *ANNEX 1*

## *REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES*

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. I. *Antimicrobial preservatives and antioxidants*, Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives*, Vol. II. *Food colours*, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours*. FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
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21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.

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26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
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28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
31. *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate.* FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
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35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.

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45. *Summary of toxicological data of certain food additives.* WHO Food Additives Series, No. 12, 1977.
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58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives).* FAO Food and Nutrition Paper, No. 19, 1981.
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62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
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91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
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## *ANNEX 2*

## *ABBREVIATIONS USED IN THE MONOGRAPHS*

| | |
|---|---|
| ADI | acceptable daily intake |
| ALT | alanine aminotransferase |
| AMA | amoxicilloic acid |
| AST | aspartate aminotransferase |
| AUC | area under the plasma concentration–time curve |
| BMD | benchmark dose |
| $BMD_{10}$ | benchmark dose for an extra 10% risk compared with the modelled background incidence |
| $BMDL_{10}$ | lower bound of the one-sided 95% confidence interval for a benchmark dose representative of 10% extra risk compared with modelled background incidence |
| bw | body weight |
| CAS | Chemical Abstracts Service |
| CI | confidence interval |
| $C_{max}$ | peak plasma concentration |
| CYP | cytochrome P450 |
| DEREK | Deductive Estimation of Risk from Existing Knowledge |
| DIKETO | amoxicillin diketopiperazine-2′,5′-dione |
| DNA | deoxyribonucleic acid |
| eq | equivalent |
| F | female |
| FAO | Food and Agriculture Organization of the United Nations |
| GGT | gamma-glutamyltransferase |
| GL36 | Guideline 36 |
| GLP | good laboratory practice |
| $IC_{50}$ | median inhibitory concentration |
| iv | intravenous |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| $LC_{50}$ | median lethal concentration |
| $LD_{50}$ | median lethal dose |
| LOAEL | lowest-observed-adverse-effect level |
| M | male |
| MIC | minimum inhibitory concentration |
| $MIC_{50}$ | minimum inhibitory concentration required to inhibit the growth of 50% of organisms |
| MRL | maximum residue limit |
| nAChR | nicotinic acetylcholine receptor |
| NADPH | reduced nicotinamide adenine dinucleotide phosphate |
| ND | no data |
| NOAEC | no-observed-adverse-effect concentration |
| NOAEL | no-observed-adverse-effect level |
| NOEL | no-observed-effect level |
| NR | not reported |
| OECD | Organisation for Economic Co-operation and Development |

| | |
|---|---|
| OR | odds ratio |
| S9 | 9000 × *g* rat liver supernatant |
| $T_3$ | triiodothyronine |
| $T_4$ | thyroxine |
| $T_{max}$ | time to $C_{max}$ |
| TSH | thyroid stimulating hormone |
| USA | United States of America |
| USEPA | United States Environmental Protection Agency |
| USFDA | United States Food and Drug Administration |
| VICH | International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products |
| WHO | World Health Organization |
| w/v | weight per volume |

## *ANNEX 3*

## *JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES*

### *Rome, 8–17 November 2011*

### *MEMBERS*

Professor A. Anadón, Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense de Madrid, Madrid, Spain

Dr D. Arnold, Consultant, Berlin, Germany *(Vice-Chairman)*

Professor A.R. Boobis, Centre for Pharmacology & Therapeutics, Department of Experimental Medicine, Division of Medicine, Faculty of Medicine, Imperial College London, London, England *(Chairman)*

Dr R. Ellis, Consultant, Myrtle Beach, SC, United States of America (USA) *(Joint Rapporteur)*

Dr A. Fernández Suárez, Ciencias Veterinarias, Universidad del Salvador, Buenos Aires, Argentina

Dr L.G. Friedlander, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA

Dr K.J. Greenlees, Office of New Animal Drug Evaluation, Center for Veterinary Medicine, Food and Drug Administration, Department of Health and Human Services, Rockville, MD, USA *(Joint Rapporteur)*

Professor J. Palermo-Neto, Department of Pathology, Faculty of Veterinary Medicine, University of São Paulo, São Paulo, Brazil

Dr L. Ritter, Canadian Network of Toxicology Centres, Professor Emeritus, School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada

Dr P. Sanders, National Reference Laboratory for Veterinary Drug Residues and Antimicrobial Resistance, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Fougères, France

Professor G.E. Swan, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa[1]

[1] Dr Swan was invited but unable to attend the meeting.

***SECRETARIAT***

Dr J. Boison, Centre for Veterinary Drug Residues, Canadian Food Inspection Agency, Saskatoon, Saskatchewan, Canada *(FAO Expert)*

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Codex Secretariat)*

Dr C.E. Cerniglia, Division of Microbiology, National Center for Toxicological Research, Food and Drug Administration, Department of Health and Human Services, Jefferson, AR, USA *(WHO Temporary Adviser)*

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Professor B. Le Bizec, Laboratoire d'Étude des Résidus et des contaminants dans les aliments (LABERCA), École Nationale Vétérinaire, Agroalimentaire et de l'Alimentation Nantes Atlantique (ONIRIS), Nantes, France *(FAO Expert)*

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Dr G. Roberts, Consultant, Preston, Victoria, Australia *(WHO Temporary Adviser)*

Ms M. Sheffer, Orleans, Ontario, Canada *(FAO/WHO Editor)*

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland *(WHO Joint Secretary)*

Dr P. Verger, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland *(WHO Joint Secretary to Joint FAO/WHO Meeting on Pesticide Residues)*

Dr A. Wennberg, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy *(FAO Joint Secretary)*

## *ANNEX 4*

## *RECOMMENDATIONS ON COMPOUNDS ON THE AGENDA AND FURTHER INFORMATION REQUIRED*

### **Amoxicillin** (antimicrobial agent)

Acceptable daily intake: The Committee established an acceptable daily intake (ADI) of 0–0.7 µg/kg body weight (bw) on the basis of microbiological effects.

Estimated dietary exposure: The Committee did not calculate an estimated daily intake (EDI) for amoxicillin owing to the small number of quantifiable residue data points. Using the model diet of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 litres of milk with the maximum residue limits (MRLs) recommended, the theoretical maximum daily intake (TMDI) is 31 µg/person, which represents 74% of the upper bound of the ADI.

Residue definition: Amoxicillin

***Recommended maximum residue limits (MRLs)***

| Species | Fat[a] (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) | Milk (µg/kg) |
|---|---|---|---|---|---|
| Cattle | 50 | 50 | 50 | 50 | 4 |
| Sheep | 50 | 50 | 50 | 50 | 4 |
| Pigs | 50 | 50 | 50 | 50 | |

[a] Includes skin plus fat in pigs.

### **Apramycin** (antimicrobial agent)

Acceptable daily intake: The Committee established an ADI of 0–30 µg/kg bw on the basis of microbiological effects.

Estimated dietary exposure: Using the limits of quantification (LOQs) of the analytical methods as calculated by the Committee as residue levels for muscle, fat and liver, together with the proposed MRL for kidney, the theoretical intake in the worst-case scenario would be around 1400 µg/day and would not exceed the upper bound of the ADI.

Residue definition: Apramycin

***Recommended maximum residue limits (MRLs)***

| Species | Kidney (µg/kg) |
|---|---|
| Cattle | 5000[a] |
| Chickens | 5000[a] |

[a] The MRLs are temporary. Because of data limitations, the Committee was unable to recommend MRLs in tissues and species other than cattle kidney and chicken kidney. The sponsor is requested to provide improved analytical methods with better performance and lower LOQs and residue depletion studies with appropriate sampling points close to the zero withdrawal periods for all tissues and species. The validated analytical methods and residue depletion studies are requested by the end of 2014.

## **Derquantel** (anthelminthic)

Acceptable daily intake: The Committee established an ADI of 0–0.3 µg/kg bw on the basis of a lowest-observed-adverse-effect level (LOAEL) of 0.1 mg/kg bw per day for acute clinical observations in dogs, consistent with antagonistic activity on the nicotinic acetylcholine receptors. A safety factor of 300 was applied to the LOAEL.

Estimated dietary exposure: As the ADI was based on an acute effect, the Committee did not calculate an EDI. Using the model diet of 300 g muscle, 100 g liver, 50 g kidney, 50 g fat and 1.5 litres of milk with the MRLs recommended, the TMDI is 8 µg/person, which represents 45% of the upper bound of the ADI.

Residue definition: Derquantel

***Recommended maximum residue limits (MRLs)***

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Sheep | 0.7 | 0.2 | 2.0 | 0.2 |

The Committee was not able to recommend an MRL for sheep milk, as no residue data were provided.

## **Ivermectin** (anthelminthic)

No data on ivermectin were submitted following a public request for data.

The Committee previously established an ADI of 0–1 µg/kg bw at its fortieth meeting (WHO TRS No. 832, 1993). The Committee concluded at the current meeting that there was a need to evaluate the toxicological information on ivermectin with a view

to identifying a critical effect other than in the CF-1 mouse for the establishment of an ADI. Information that would be of value in future toxicological evaluation of ivermectin includes reports on the effects of ivermectin when used as a therapeutic agent in humans and information from in vitro and/or in vivo studies evaluating the critical effects upon which recent ADIs for other avermectins have been established.

MRLs were proposed at the fifty-eighth meeting (WHO TRS No. 911, 2002). Before it could re-evaluate the residue depletion of ivermectin and propose updated MRLs, the Committee would need a submission indicating the animals and products for which MRLs are requested, marker residue depletion studies in support of proposed withdrawal times and/or in support of applications for MRLs, and pharmacokinetics and metabolism studies in food-producing animals that might enable interspecies extrapolations. A complete up-to-date list of approved products on the market together with documentation of approved uses, including withdrawal times and all relevant parts of proprietary studies directly or indirectly supporting the approved uses, and an expert report summarizing the above content of the submission and additional relevant published data are also requested. Suitably validated analytical methods should be provided for regulatory control based on contemporary analytical techniques for any future re-evaluation of ivermectin.

## **Monensin** (antimicrobial agent and production aid)

| | |
|---|---|
| Acceptable daily intake: | The Committee established an ADI of 0–10 µg/kg bw at its seventieth meeting (WHO TRS No. 954, 2009). |
| Estimated dietary exposure: | Using the revised MRL, the exposure estimate (TMDI) from the seventieth meeting of the Committee was recalculated, resulting in a value of 481 µg/person, which represents 80% of the upper bound of the ADI. |
| Residue definition: | Monensin A |

***Recommended maximum residue limits (MRLs)***

| Species | Liver (µg/kg) |
|---|---|
| Cattle | 100 |

The Committee was unable to revise the current MRLs for goats and sheep, as no additional residue data were provided.

## **Monepantel** (anthelminthic)

| | |
|---|---|
| Acceptable daily intake: | The Committee established an ADI of 0–20 µg/kg bw on the basis of a no-observed-adverse-effect level (NOAEL) of 1.8 mg/kg bw per day, |

considering liver effects in mice, and a safety factor of 100, with rounding to one significant figure.

Estimated dietary exposure: Using the model diet and a ratio of marker residue to total residue of 100% for muscle and 66% for fat, liver and kidney, and applying a correction factor of 0.94 to account for the mass difference between the marker residue and monepantel, the EDI is 201 µg/person, which represents 17% of the upper bound of the ADI.

Residue definition: Monepantel sulfone

***Recommended maximum residue limits (MRLs)***

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Sheep | 5500 | 700 | 3000 | 300 |

The Committee was unable to propose an MRL for sheep milk, as no data were provided.

## **Narasin** (antimicrobial agent and production aid)

Acceptable daily intake: The Committee established an ADI of 0–5 µg/kg bw on the basis of a NOAEL of 0.5 mg/kg bw per day and a safety factor of 100 at its seventieth meeting (WHO TRS No. 954, 2009).

Residue definition: Narasin A

***Recommended maximum residue limits (MRLs)***

The Committee recommended full MRLs, as a validated analytical method for residue control purposes is available and was evaluated as satisfactory for the purpose.

| Species | Fat (µg/kg) | Kidney (µg/kg) | Liver (µg/kg) | Muscle (µg/kg) |
|---|---|---|---|---|
| Cattle | 50 | 15 | 50 | 15 |

## **Triclabendazole** (anthelminthic)

The Committee concluded that the available database on the residues of triclabendazole in goat was too limited to allow a scientifically justifiable extrapolation of MRLs for cattle and sheep tissues to this species of animal.

This volume contains monographs prepared at the seventy-fifth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 8 to 17 November 2011.

The toxicological monographs in this volume summarize data on the veterinary drug residues that were evaluated toxicologically by the Committee: the antimicrobial agents amoxicillin and apramycin and the anthelminthics derquantel and monepantel.

This volume and others in the WHO Food Additives Series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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