

Toxicological evaluation of certain veterinary drug residues in food

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MONEPANTEL

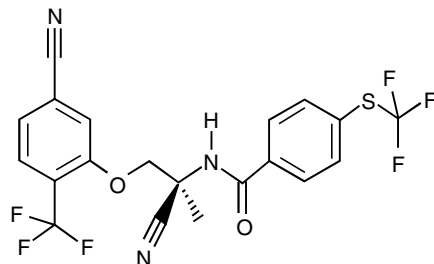
First draft prepared by

Dr Nicholas Jarrett¹ and Professor Alan R. Boobis²

¹ European Medicines Agency, London, England

² Faculty of Medicine, Imperial College London, London, England

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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-trifluoromethylsulfanylbenzamide), also known as AHC 2102225, is an anthelmintic 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 nematocidal 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 anthelmintics (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 anthelmintics 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: $\text{AUC}_{\text{oral}}/\text{AUC}_{\text{iv}}$) 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) (Chardonens & 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]; Chardonens & 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 (macroglycerol hydroxystearate), Transcutol (diethylene

Figure 2. Proposed main biotransformation pathways in rats

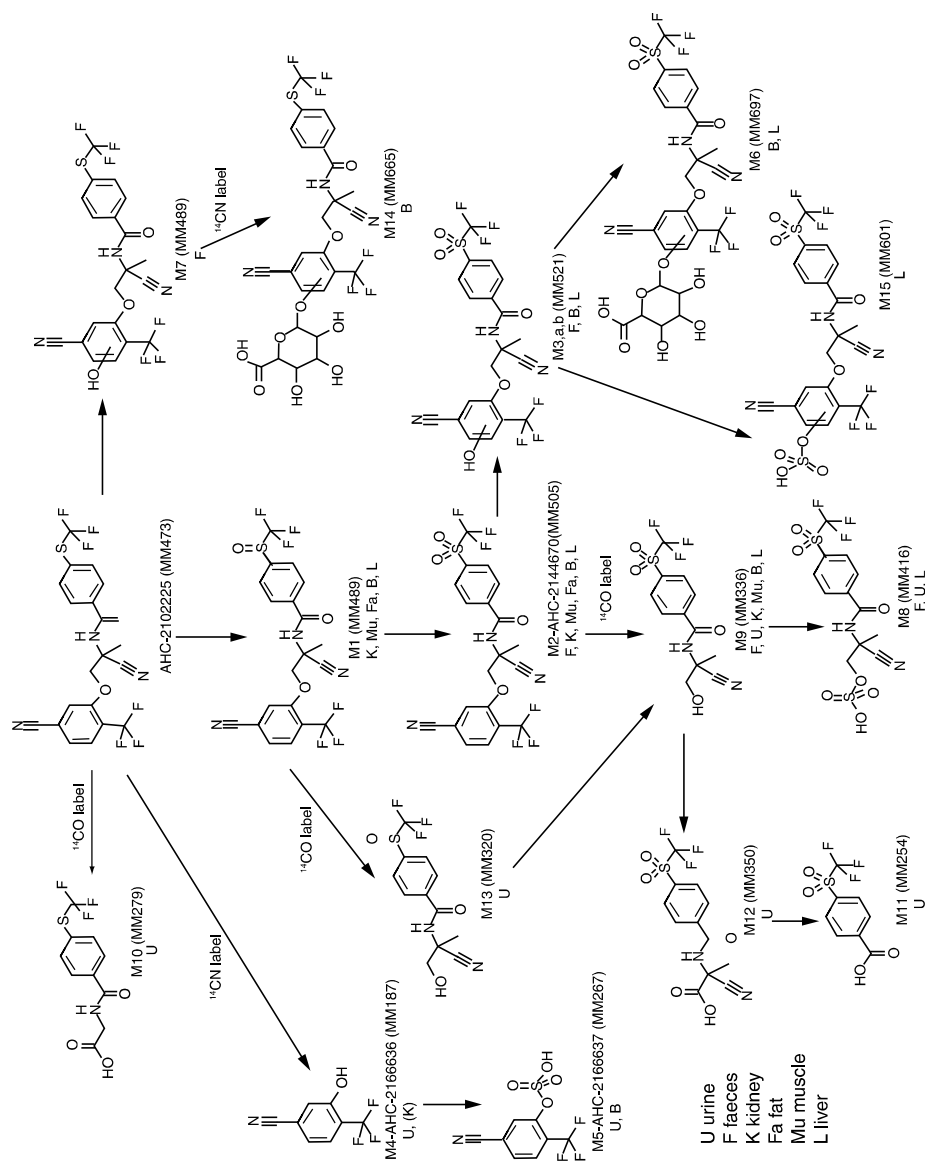


Table 1. Comparison of residues in rats and sheep

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” radiolabelled 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 [¹⁴C]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 [¹⁴C]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-pentoxymethylresorufin *O*-deethylase (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:W1St, 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	<i>Salmonella typhimurium</i> 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. F₁ generation animals were either terminated following weaning on day 21 postpartum or selected for breeding the F₂ 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 F₁ 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 F₁ pups at 200, 1500 and 12 000 ppm and in male F₁ pups at 12 000 ppm. There was little or no change in females between 200 and 1500 ppm. In F₂ pups, relative liver weights were increased at 12 000 ppm. No associated histology findings were seen in livers of F₂ pups (livers of F₁ 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 F₁ 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 F₁ generation females) associated with centrilobular hepatocellular hypertrophy (P and F₁ generation females), increased absolute and relative adrenal gland weights (F₁ generation females) and cortical cell hypertrophy (P and F₁ 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 F₁ and F₂ 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 LD₅₀ 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 F₁ and F₂ pups, but no associated histopathology was seen in F₂ pups (F₁ 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 F₁ 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 F₁ 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 F₁ generation females, increased absolute and relative adrenal gland weights in F₁ generation females and cortical cell hypertrophy in P and F₁ 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 F₁ and F₂ 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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