Monepantel

First draft prepared by
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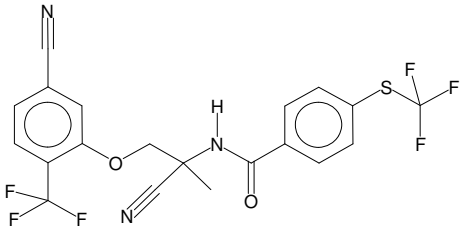
Identity

IUPAC Name: N-[(1S)-1-Cyano-2-(5-cyano-2-trifluoromethyl-phenoxy)-1-methyl-ethyl]-4--trifluoromethylsulfanyl-benzamide

Synonyms: N-[2-(5-cyano-2-trifluormethyl-phenyloxy)-1-(S)-1-cyano-1-methyl-ethyl]-4-trifluoromethylthio-benzoic amide

Chemical Abstracts Service Number: 887148-69-8

Structural formula:



Molecular formula: $C_{20}H_{13}F_6N_3O_2S$

Molecular weight: 473.4

Other information on identity and properties

Pure active ingredient: AHC-2102225 (N-[2-(5-cyano-2-trifluormethyl-phenyloxy)-1-(S)-1-cyano-1-methyl-ethyl]-4-trifluoromethylthio-benzoic amide), the active S-enantiomer

Appearance: White powder

Melting point: 125°C (polymorphic form A;, 142–149°C (polymorphic form B)

Solubility in water: 0.1 mg/L at 20°C

Solubility in organic solvents: dichloromethane: 175 g/L; ethanol: 60.7 g/L; n-octanol: 7.3 g/L; propylene glycol: 6.9 g/L; polyethylene glycol: 156.1 g/L

pH: 6.2–6.3 (suspension in water)

Partition coefficient: Octanol/water partition coefficient: log P_{ow} = 3.0 (shake flask method, pH 7, at 20°C)

Storage: At room temperature, protect from light

Chirality: Monepantel has one chiral centre

Optical Density: Optical rotation $[\alpha]_{580nm}$ -32° (methanol)

Purity: AHC-2155367 = N-[2-(5-cyano-2-trifluormethyl-phenyloxy)-1-(S)-1-cyano-1-methylethyl]-4-chloro-benzoic amide, residual solvents, etc., each specified at <0.5%

Residues in food and their evaluation

Conditions of use

Monepantel is an anthelmintic of the amino-acetonitrile derivative class, indicated for the treatment and control of gastrointestinal roundworms (nematodes) in sheep. It is marketed under the trade name Zolvix® and licensed for use in Australia, New Zealand, Switzerland, South Africa, Uruguay and Argentina.

Dosage

The recommended dose is as a single oral drench of 2.5 mg/kg bw and the maximum dose used is 3.75 mg/kg bw. The label has a warning that the product should not be administered to female sheep which are producing or may in the future produce milk or milk products for human consumption.

Pharmacokinetics and metabolism

Absorption, distribution, metabolism and excretion

Sheep

A non-GLP-compliant pilot study investigating the absorption, distribution, metabolism and excretion (ADME) of ^{14}C-monepantel, following oral dosing, in two sheep was conducted (Jung, 2006). ^{14}C-monepantel diluted with unlabelled drug was dissolved in a formulation similar to the proposed final formulation. Blood, urine and faeces were collected for 12 days post-treatment, at which time the animals were sacrificed for the collection of tissues. Total radioactive residue (TRR) in collected samples was measured and the metabolic profiles determined by LC-MS and HPLC/LSC (liquid scintillation counter) in selected samples, comparing with available authentic reference compounds. Due to the non-homogeneity of the test formulation, the doses administered were less than anticipated, and results could only be estimated. Consequently, accurate figures for excretion and balance were not possible. A large fraction (about 90%) of the dose was excreted via the urine and faeces, although the latter contained a significant proportion of unabsorbed drug over the first 1–2 days. Between animals there was variability in the ratio of faecal versus urinary excretion, ranging from 2:1 to 3:1 during the last few days before sacrifice. Figure 5.1 shows the cumulative excretion profile of radioactivity after a single oral administration of ^{14}C-monepantel to one of the sheep. The remainder of the total radioactivity (17–27%) was distributed in fat (11–21%) and muscle (4%). The distribution of radioactivity in edible tissues is summarized in Table 5.1.

Radioactivity in blood peaked between 8 and 24 h after administration, and declined slowly thereafter. Radioactivity was neither exclusively bound in the plasma fraction, nor in the red cell fraction. Liver and fat were the tissues with the highest radioactivity. Muscle and kidney were relatively low in residues, and the variability observed for muscle residues may reflect the variability in fat content, as drug residues are lipophilic in nature. Various extraction experiments were conducted to determine the recovery of incurred radioactivity from samples. In general, simple extraction with organic solvents yielded 90% or better recovery, except for liver, where only about 70% was recovered following high-speed homogenization with acetonitrile (or other tested solvents).

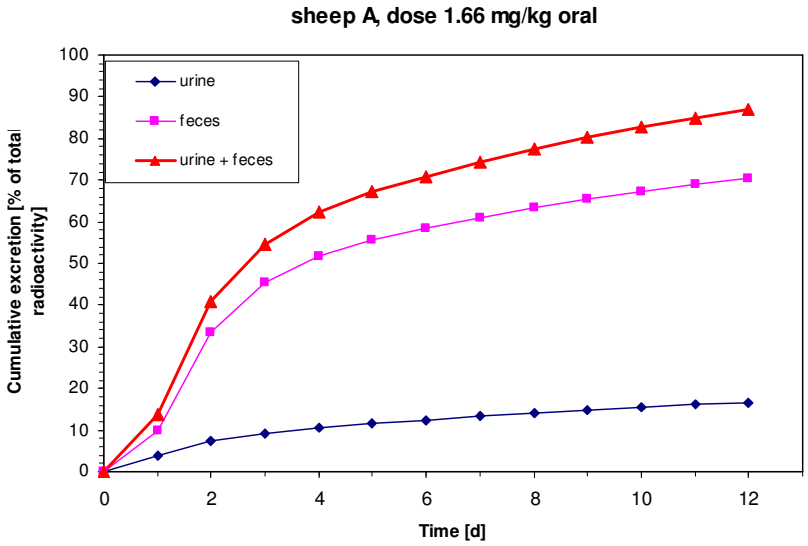


Figure 5.1. Cumulative excretion of radioactivity after oral administration of 14C-monepantel at 1.66 mg/kg bw to sheep

Table 5.1. Distribution of radioactivity in edible tissues of sheep (pilot study)

Tissue	Monepantel equivalents (µg/kg) 12 days after treatment	
	Sheep A (dose ~1.7 mg/kg bw)	Sheep B (dose ~4.6 mg/kg bw)
Fat (subcutaneous)	1887	2665
Fat (peritoneal)	2250	3402
Liver	884	1932
Kidney	228	392
Muscle (shoulder)	330	979
Muscle (thigh)	169	342
Skin	204	324
Blood	11	28
Plasma	10	31
Wool (back)	23	63

Metabolite profiling indicated that in tissue, blood, plasma and faeces, the sulphone metabolite is predominant, together with minor amounts of parent drug. For blood and faeces, the initial samples had a proportionately higher amount of the parent drug, especially in faeces collected within two days of treatment. Trace amounts of sulphoxide metabolite were observed in blood at 8 h post treatment, and also in faeces up to two days post-treatment.

Faeces contained two additional metabolites: the phenol M4, and the hydroxylated sulphone M3. Urine contained no parent compound or oxidized (unconjugated) metabolites of the parent. Two metabolites were observed; the minor one was M4 and the major one was its sulphate conjugate M5. The latter metabolite was confirmed by hydrolysis with sulphatase to M4. The structures of monepantel and identified metabolites are shown in Figure 5.2.

A GLP-compliant study investigating the ADME and residue depletion of ^{14}C-monepantel, using test material labelled at either of two rings (on the cyano group, referred to as label 2, or on the amide group, referred to as label 3) of the parent molecule, was

reviewed (Jung *et al.*, 2007). The radioactive substances were dissolved in formulation TG 1778/30, and 17 male and 17 female Suffolk sheep were orally dosed at 5.0 mg/kg bw with either label 2, label 3 or an equimolar mixture of each labelled substance (labels 2+3). Blood, excreta, wool and edible tissues (fat, muscle, kidney and liver) were collected 2, 7, 14, 21, 28 and 35 days post-dose and analysed for TRR. Tissue residues were extracted and the analytes quantified using the validated HPLC/UV (LOQ = 0.05 µg/kg) method (Karadzovska, 2007a). Blood and plasma were extracted and analysed by the validated method for blood for monepantel and monepantel sulphone (Karadzovska, 2007b).

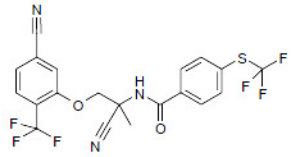
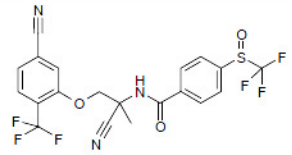
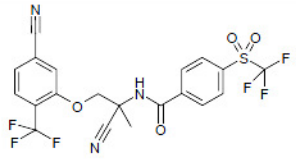
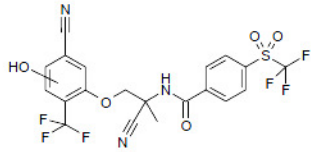
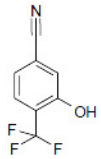
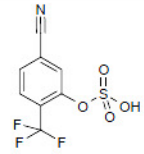
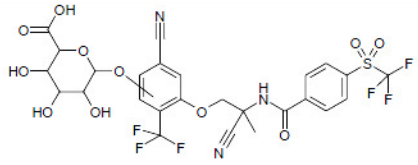
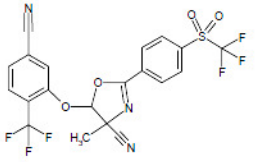
Metabolite ID	Code ID	Description/other names	Structure
Parent (monepantel)	AHC-2102225	NG-96 is the racemate	
M1		Sulfoxide of parent	
M2	AHC-2144670	Monepantel sulfone NG-236 is the racemate	
M3		Hydroxylated M2	
M4	AHC-2166636	Phenol	
M5	AHC-2166637	Sulfate of phenol	
M6		Glucuronide of M3	
G32	AHC-2197876	M32	

Figure 5.2. Identified metabolites of monepantel

The cumulative excretion is summarized in Table 5.2. The data show that radioactivity is predominantly excreted through the faeces, with a significant contribution from urinary elimination. Faecal excretion is high in the first 3 days (30%), but subsequently the rate declines, with about 2–3 weeks required for 90% elimination. Blood and plasma profiles, obtained from 4 sheep dosed with the label 2 substance, are shown in Table 5.3. Data show slow elimination from the systemic circulation, and radioactivity is distributed approximately equally between the cellular and plasma compartments.

Table 5.2. Mean cumulative excretion of radioactivity from sheep as % of dose

Label	No. of sheep	Days	Urine (%)	Faeces (%)	Cage Wash (%)	Total (%)
2	2	0 – 14	30.8 ± 1.3	52.9 ± 5.0	1.8 ± 0.3	85.4 ± 6.0
3	2	0 – 14	28.8 ± 0.8	60.6 ± 0.7	2.2 ± 1.1	93.3 ± 1.5
		0 – 14	29.2 ± 5.8	52.8 ± 4.1		
2 + 3	4	0 – 21	30.7 ± 6.0	55.3 ± 3.4	4.2 ± 2.5	90.2 ± 5.4

Table 5.3. Mean sheep blood and plasma radioactivity profiles expressed as monepantel equivalents

Day	Blood (mean ± SD) (µg/kg equivalents)	Plasma (mean ± SD) (µg/kg equivalents)
1	254 ± 27	300 26
2	145 ± 26	176 ± 21
4	77 ± 21	91 ± 23
7	53 ± 21	63 ± 23
14	31 ± 17	36 ± 20
21	19 ± 14	21 ± 15
28	9 ± 9	12 ± 9

Residues in tissues and other matrices are shown in Table 5.4. The position of the label did not affect the excretion or distribution of residues. The highest residues in edible tissues were found in the fat, with slightly higher residues in rendered pure fat compared with composite fat tissue, followed by liver, kidney and then muscle. The approximate TRR proportions were 100 (fat):50 (liver):20 (kidney):10 (muscle):1 (blood):1 (plasma), respectively.

Table 5.4. Depletion of total radioactive residues (TRR) from sheep tissues

	Mean TRR ± SD in mg equivalents/kg							
Label position	2	2	2	3	2	2+3	2	2
Days post-dose	2	7	14	14	21	21	28	35
Bile	4.6 ±1.6	1.1 ±0.52	0.34 ±0.24	0.33 ±0.17	0.13 ±0.10	0.08 ±0.10	0.19 ±0.23	0.08 ±0.09
Blood	0.14 ±0.20	0.04 ±0.10	0.02 ±0.10	0.02 ±0.10	0.01 ±0.01	0.01 ±0.00	0.01 ±0.01	0.01 ±0.00
Plasma	0.16 ±0.04	0.05 ±0.01	0.03 ±0.01	0.02 ±0.01	0.01 ±0.01	0.01 ±0.00	0.01 ±0.01	0.01 ±0.00
Fat tissue	15.5 ±4.0	5.8 ±2.9	2.2 ±1.2	1.7 ±0.83	1.1 ±0.50	0.74 ±0.52	1.1 ±0.62	0.46 ±0.26
Pure fat	19.3 ±5.2	7.3 ±2.3	2.9 ±1.4	2.1 ±0.90	1.3 ±0.57	0.99 ±0.52	1.3 ±0.67	0.55 ±0.28
Liver	6.7 ±0.23	2.7 ±0.75	1.5 ±0.71	1.1 ±0.47	0.77 ±0.37	0.50 ±0.33	0.71 ±0.55	0.33 ±0.25
Kidney	2.4 ±0.15	0.81 ±0.26	0.38 ±0.24	0.32 ±0.16	0.16 ±0.10	0.12 ±0.08	0.18 ±0.1	0.06 ±. 06
Muscle	1.50 ±0.34	0.45 ±0.23	0.22 ±0.15	0.14 ±0.08	0.11 ±0.06	0.06 ±0.05	0.09 ±0.06	0.03 ±0.03

The distribution of non-radiolabelled monepantel and its metabolite, monepantel sulphone in tissue, is summarized in Table 5.5. The approximate proportions of monepantel sulphone in the tissues were 10 (fat):5 (liver):2 (kidney):1 (muscle).

Table 5.5. Depletion of monepantel and its metabolite monepantel sulphone from sheep tissues

Day	Label position	Fat Tissue	Pure Fat	Liver	Kidney	Muscle
Monepantel [mean concentration ± SD (mg/kg)]						
2	2	3.5 ± 1.4	5.1 ± 2.0	0.39 ± 0.12	0.14 ± 0.05	0.28 ± 0.08
7	2	0.61 ± 0.41	0.92 ± 0.61	All <0.05	All <0.05	0.81 + 3 at <0.05
14	2, 3	0.09 ± 0.02 + 3 at <0.05	0.13 ± 0.04 + 3 at<0.05	All <0.05	All <0.05	All <0.05
21	2, 2 + 3	All <0.05	All <0.05	All <0.05	All <0.05	All <0.05
Monepantel sulphone [mean concentration ± SD (mg/kg)]						
2	2	10.2 ± 2.1	13.4 ± 3.1	5.2 ± 0.13	1.5 ± 0.23	1.4 ± 0.32
7	2	4.2 ± 2.0	5.7 ± 2.5	1.9 ± 0.57	0.60 ± 0.26	0.47 ± 0.26
14	2, 3	1.6 ± 1.0	2.2 ± 1.2	0.87 ± 0.49	0.30 ± 0.08 + 2 at <0.05	0.20 ± 0.10 + 2 at <0.05
21	2, 2 + 3	0.60 ± 0.56	0.76 ± 0.67	0.34 ± 0.30	0.13 ± 0.07 + 3 at <0.05	0.15 ± 0.06 + 5 at <0.05
28	2	1.1 ± 0.47 + 1 at <0.05	1.5 ± 0.73 + 1 at <0.05	0.550 ± 0.32 + 1 at <0.05	0.18 ± 0.10 + 1 at <0.05	0.15 ± 0.07 + 2 at <0.05
35	2	0.60 ± 0.02 + 2 at <0.05	0.70 ± 0.09 + 2 at <0.05	0.26 ± 0.04 + 2 at <0.05	0.10 ± 0.01 + 2 at <0.05	0.06 ± 0.01 + 2 at <0.05

Metabolite identification and profiling of residues in sheep samples

Selected samples of edible tissues (except pure fat), blood, wool and excreta were extracted and profiled by HPLC with LSC detection. If a simple extraction failed to recover a large proportion of TRR, then further extractions with harsher conditions were attempted.

Some samples were extracted and profiled after prolonged storage in the freezer and compared with the initially obtained results; there were no significant changes in the profiles, indicating acceptable stability (except for urine). HPLC profiling of selected blood samples on a chiral column indicated that there was no racemization of parent or sulphone. Attempts were made to profile bile and wool, but no conclusions could be drawn because of poor extractability, low levels or complex profiles. Monepantel was not detected, but the sulphone and the M6 may have been present in bile. The proposed metabolic pathway for monepantel in sheep is shown in Figure 5.3.

The chromatographic and spectral data support the proposed metabolites and pathways. The information obtained formed the basis for selection of the marker residue and the regulatory analytical method. The distribution of metabolites in edible sheep tissues was remarkably comparable to those obtained during non-radiolabelled drug analysis and metabolite profiling studies. Little or no bound residues were observed in edible tissues. There are two main metabolic pathways:

- One route involves oxidation of the parent to the transient sulphoxide M1, with rapid oxidation to the sulphone M2. There is a further slow oxidation to M3; the site of the hydroxylation was not elucidated but was thought to be on the phenolic ring.
- The other route involves cleavage to yield the phenol M4, together with its sulphate conjugate M5. From the corresponding benzamide portion, an alcohol and a peptide hydrolysis product, an acid that is formed and eliminated via the urine.

Elimination is predominantly via faeces, with significant contribution from urine. Bile excretion contributes to total faecal elimination. A tiny fraction of the drug is excreted with wool.

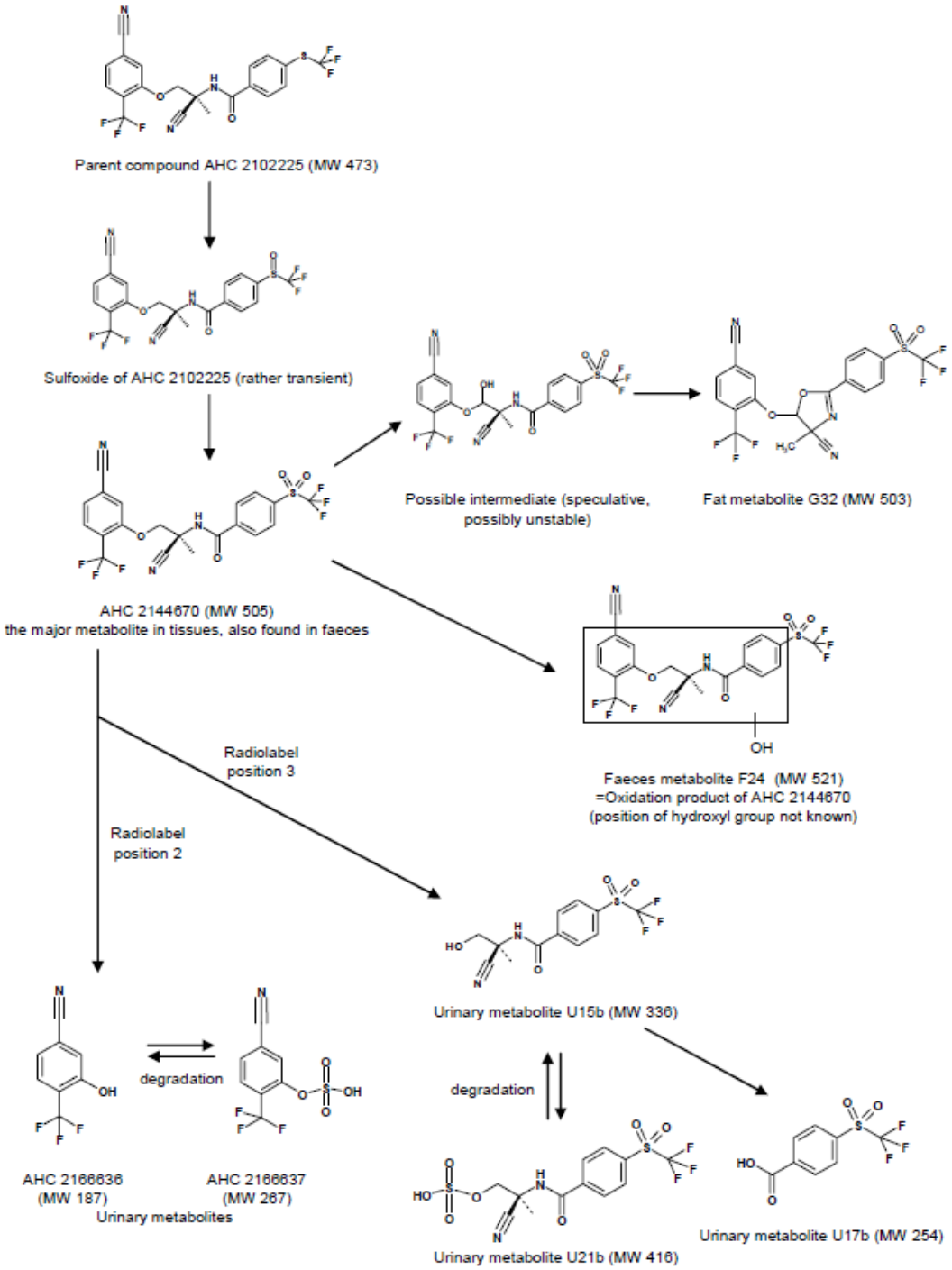


Figure 3. Proposed metabolic pathway of monepantel in sheep

Radioactive residues in fat tissue (most samples) were readily extractable with hexane (>90%) and the metabolites were identified. The sulphone was a dominant metabolite, with monepantel being a minor contributor at early times. A cyclized metabolite (G32), appeared at day 7, and was still observed at 35 days. Its distribution, as a percent of TRR, was variable between animals and increased with time to about one-third (mean value) of TRR at day 35. However, the highest individual residues (400 µg/kg) occurred at 7 to 21 days post-treatment.

Radioactive residues in liver (selected representative samples only) were readily extractable with acetonitrile at room temperature (70%) and more vigorous conditions (polar solvents, reflux, extreme pH) could extract another 16%, suggesting a very small amount of bound residues. Monepantel sulphone was the major metabolite, together with minor amounts of parent at day 2, plus two or three minor metabolites that were less than 10% of TRR. The metabolite G32 was not detected.

Kidney samples (selected representative samples only) also were readily extractable with acetonitrile at room temperature (80%). The pattern of metabolites was very similar to that of liver, with some additional very minor metabolites, one of which is tentatively the sulphoxide. Again, G32 was not detected.

Radioactivity in muscle samples (selected representative samples only) was readily extractable with acetonitrile at room temperature (90%). The sulphone was the major metabolite, with minor amounts of parent only at day 2. Minor amounts of G32 (<14 µg/kg) were observed in some samples at day 14 and 21 (only these samples were profiled), contributing between 0 and 27% to individual TRR.

The higher percentages of G32 were found in those animals with high contributions of G32 to fat TRR. It is reasonable to assume that G32 in muscle tissues arises from intramuscular fat, which is naturally present. G32 was not detected at day 2. Faeces metabolites were readily extractable with acetonitrile/water, and were dominated by (unabsorbed) parent during the first few days, and then by the sulphone and M3. Some minor polar metabolites, such as the phenol, were also observed.

A complex pattern was observed in urine, which contained numerous polar metabolites resulting from the cleavage of the ether linkage in monepantel, and, in some cases, followed by conjugation. The major label-2 urinary metabolites were identified as the phenol and its sulphate, the latter being unstable and degrading to the former. The major label-3 urinary metabolites were the alcohol formed after cleavage of the phenylether bond (U15b) plus its corresponding sulphate (U21b) and the carboxylic acid formed after cleavage of the peptide bond (U17b). G32 was not detected in excreta or in blood.

Pharmacokinetics

A pharmacokinetic study of monepantel and its main metabolite, monepantel sulphone, was performed in 36 sheep, 18 castrated males and 18 females aged 6–8 months (Karadzovska, 2007c). The animals were allocated to five treatment groups on the basis of sex and body weight. In the first group of 6 animals, monepantel was administered intravenously at a dose of 1 mg/kg bw. In the second group of 6 animals, monepantel sulphone was administered intravenously at a dose of 1 mg/kg bw. The three remaining groups of 8 animals received monepantel via oral route at a dose of 1, 3 or 10 mg/kg bw. Blood specimens were collected from the animals in the groups treated intravenously at pre-defined time points: pre-treatment, 2, 5, 10, 30 minutes, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 h and 7, 10, 14, 21, 28 days post-treatment. Blood specimens were collected from the animals in the groups treated orally at pre-defined time points: pre-treatment, 30 minutes, 1, 2, 4, 8, 12, 24, 36, 48, 72, 96 h, and 7, 10, 14, 21, 28, 35 days post-treatment. Specimens were analysed for monepantel and its metabolite, monepantel sulphone (LOQ = 3 ng/ml). Faecal specimens were collected from groups 1, 2 and 4 over two 8-hour periods to estimate faecal clearance. The calculated

pharmacokinetic parameters after i.v. or oral administration of monepantel are summarized in Table 5.6.

Table 5.6. Pharmacokinetic parameters of monepantel after i.v. and oral administration

Route	Intravenous	Oral	Oral	Oral
Dose (mg/kg bw)	1	1	3	10
Parameter (Unit)				
$AUC_{0\text{-}7d}$ (ng-h/ml)	676.1 ± 89.3	220.4 ± 78	703.1 ± 234.4	1967.2 ± 472.4
T_{max} (h)		2-8	4-24	4-6
C_{max} (ng/ml)		7 ± 1.9	18.9 ± 6.9	130.4 ± 110.2
MRT (h)	5.3 ± 2	24.0 ± 5.7	30.0 ± 3.2	22.3 ± 4.9
CL (L/(kg-h))	1.5 ± 0.2			
Vss (L/kg)	7.68 ± 2.34			

After i.v. administration of 1 mg/kg bw of monepantel, the blood monepantel concentration fell rapidly and the last quantifiable blood level was detected at 48 h. Total blood clearance was high (1.49 L/kg/h) and it was not possible to determine the half-life of the parent drug. Peak blood concentration of monepantel sulphone was achieved approximately 2 h after i.v. administration of monepantel, and monepantel sulphone concentrations remained above the LOQ for more than 7 days, depending on the animal. The calculated pharmacokinetic parameters after i.v. and oral administration of monepantel and i.v. administration of monepantel sulphone are summarized in Table 5.7. After i.v. administration of 1 mg/kg bw of monepantel sulphone, monepantel sulphone concentration remained above the LOQ for more than 4 days. Total blood clearance was smaller than for the parent compound (0.28 L/kg/h) and the terminal half-life was approximately 4.5 h. The volume of distribution at steady state of monepantel sulphone was 31.2 L/kg.

Bio-availability of monepantel after oral administration of 1 mg/kg bw was approximately 31% ($Cl_{90\%}$ = 23–44%). Calculated bio-availability of monepantel sulphone obtained after oral administration of 1 mg/kg bw of monepantel was 94% ($Cl_{90\%}$ = 71–124%). It is not a true bio-availability because the animals are different, but it shows that approximately the same amount of monepantel sulphone is formed whether monepantel is given orally or intravenously, hence demonstrating that the oral route has an excellent bio-availability of amounts of monepantel sulphone generated after administration of monepantel. The difference in oral bio-availability of monepantel and monepantel sulphone can be explained by a complete first-pass effect and a total absorption of the oral dose of monepantel.

Dose linearity of the blood concentration of monepantel after oral administration of monepantel appears to hold for oral administration of 1–10 mg/kg bw but is only seen for the dose of 3 mg/kg bw for blood concentration of monepantel sulphone (Table 5.7). Dose corrected AUC, terminal half-life and MRT are significantly lower at 10 mg/kg bw than at 1 mg/kg bw.

A small fraction of monepantel (4%) is excreted unchanged in faeces and the great majority is converted to monepantel sulphone (94%). This confirms that the conversion of monepantel into monepantel sulphone is the most important metabolic pathway. When monepantel sulphone was administered, 27% was excreted in faeces.

Table 5.7. Pharmacokinetic parameters of monepantel sulphone after i.v. and oral administration of monepantel and i.v. administration of monepantel sulphone

Route	Intravenous	Intravenous	Oral	Oral	Oral
Compound	Monepantel	Monepantel sulphone	Monepantel	Monepantel	Monepantel
Dose	1 mg/kg bw	1 mg/kg bw	1 mg/kg bw	3 mg/kg bw	10 mg/kg bw
Parameter (Unit)					
$AUC_{0\text{-}7d}$ (ng-h/ml)	2344 ± 290	2705 ± 631	2 442 ± 433	7 207 ± 1 230	1519 ± 1 251
$AUC_{0\text{-}\infty}$ (ng-h/ml)	3651 ± 721	3701 ± 1056	3 564 ± 1 419	11 571 ± 3 600	19 200 ± 1 937
T_{max} (h)			24	24	24
C_{max} (ng/ml)			30.3 ± 4.6	31.8 ± 5.4	29.4 ± 12.2
MRT (h)	127.47 ± 43.14	119.79 ± 46.58	139.15 ± 43.58	170.3 ± 47.15	104.47 ± 32.18
CL (L/(kg-h))		0.292 ± 0.096			
Vss (L/kg)		32.13 ± 8.27			

Tissue residue depletion studies

Residue studies with radiolabelled monepantel

A pivotal ADME study was conducted in sheep, with radiolabelled monepantel to demonstrate the depletion of residues in edible tissues with a non-final formulation, at a dose rate (5 mg/kg bw) higher than the maximum recommended rate of 3.75 mg/kg bw.

The collected tissue and blood samples were analysed by HPLC with UV detection for monepantel and monepantel sulphone (Karadzovska, 2007b). Tissues were extracted once with acetonitrile and the analytes quantified using the validated analytical method; results were summarized earlier, in Table 5.5. Blood and plasma were analysed by the method validated for blood. Blood levels were consistent with data observed in pharmacokinetic studies and plasma results confirmed the blood:plasma distribution ratio of about 1, for both parent drug and monepantel sulphone. The ratios of sulphone were 200 (fat):100 (liver):30 (kidney):20 (muscle):1 (blood):1 (plasma).

The ratio of the concentration of monepantel sulphone (marker residue) to total radioactive residue (expressed as equivalent monepantel concentration) for each tissue was calculated using all valid data provided. Mean ratios of marker residue:total residues of 1 for muscle and of 0.66 for liver, kidney and fat were determined.

Residue depletion studies with unlabelled monepantel

Three GLP-compliant residue depletion studies using unlabelled monepantel were reviewed. The final commercial formulation at the proposed maximum dose rate of 3.75 mg/kg bw was used, with a single administration individually adjusted to the individual animal body weight. In all 3 studies, equal numbers of females and castrated males were used, and sheep were maintained on green pasture. Renal fat, subcutaneous back fat (if present), liver, kidney and muscle were collected and analysed for monepantel sulphone only, using a validated analytical method (Karadzovska, 2007a, b). The additional SPE clean-up was used to reduce the LOQ to 10 µg/kg, and consequently increase the number of quantifiable values for statistical purposes.

Residue depletion in Suffolk lambs after single dose administration

Thirty two Suffolk lambs, 3–4 months old were used (Karadzovska, 2007d). The mean dose received was 3.8 mg/kg bw. A group of four animals served as controls. Groups of eight were sacrificed at 7, 18, 29 and 40 days after treatment. Mean residues are shown in Table 5.8.

Table 5.8. Monepantel sulphone residues in edible tissues of Suffolk lambs after a single oral administration of monepantel

Day	Mean residue concentration ± SD (µg/kg)				
	Renal fat	Subcutaneous fat	Liver	Kidney	Muscle
7	3256 ± 1106	2 417 ± 1 153	1 757 ± 516	591 ± 201	222 ± 114
18	490 ± 326	538 ± 281	212 ± 141	71 ± 52	43 ± 17 + 3 <10 µg/kg
29	115 ± 67 + 1 <10 µg/kg	114 ± 38	91 ± 55 + 1 <10 µg/kg	21 ± 9 + 2 <10 µg/kg	15 ± 3 + 5 <10 µg/kg
40	109 ± 73 + 2 <10 µg/kg	114 ± 78	59 ± 43 + 2 <10 µg/kg	18 ± 9 + 4 <10 µg/kg	12 + 7 <10 µg/kg

Residue depletion in Suffolk lambs after repeated administration

A GLP-compliant repeat dosing study (Smal, 2007) with 5-month-old Suffolk lambs, 30–39 kg, was conducted with equal numbers of females and castrated males, maintained on green pasture. The animals were dosed every 21 days at a rate of 3.75 mg/kg bw, with the dose adjusted each time to the new body weight. Up to 4 doses were administered and the mean dose rate received was 3.7 to 3.8 mg/kg bw. Between the 1st and 2nd doses the animals gained about 25% in weight, but then lost weight (about 3%) between the 2nd and 3rd treatments due to poor pasture growth. Afterwards they gained about 12% in weight. Groups of 6 animals were sacrificed at 21 days after the 2nd and 3rd doses, and at 14 and 21 days after the 4th dose. Samples were analysed for monepantel and monepantel sulphone only to the LOQs of 50 µg/kg, using the validated analytical procedure. There were no quantifiable residues of monepantel, and mean residues of monepantel sulphone were compared with the single-dose Suffolk lamb study. The results are shown in Table 5.9.

Table 5.9. Comparison of residues of monepantel sulphone after repeat dosing of monepantel

Study	Doses/sacrifice	Renal fat	Liver	Kidney	Muscle
Y06/93	2/21 days	133	108	<50	<50
	3/21 days	<50	<50	<50	<50
	4/14 days	395	305	80	<50
	4/21 days	328	227	66	<50
Y07/21	1/18 days	490	212	71	<50

NOTES: Values the mean (n = 6) of residues of monepantel sulphone (µg/kg). For calculation of means, residues <LOQ were replaced by half LOQ.

Residue depletion in cross-bred lambs

In another study, 47 second-cross-bred lambs (Merino × Dorset), 3–4 months old, were used (Karadzovska, 2007e; Strehlau, 2007). The mean dose received was 3.9 mg/kg bw. Another group of four served as controls. Groups of eight animals were sacrificed at 7, 19, 29, 40, 70 and 77 days after treatment. Mean residues are shown in Table 5.10.

Table 5.10. Monepantel sulphone residues in edible tissues of Merino × Dorset cross-bred lambs after a single oral administration of monepantel

Day	Tissue [mean (n = 8) residues in µg/kg (±SD)]				
	Renal fat	Subcutaneous fat	Liver	Kidney	Muscle
7	3 068 ± 1050	3 667 ± 1316	2 056 ± 733	460 ± 170	155 ± 76
19	681 ± 298	751 ± 364	354 ± 169	99 ± 47	32 ± 11 + 1 at <10 µg/kg
29	83 ± 45	114 ± 91	51 ± 40	18 ± 5 + 5 at <10 µg/kg	All <10 µg/kg
40	22 ± 13 + 2 at <10 µg/kg	21 ± 14 + 2 at <10 µg/kg	18 ± 7 + 1 at <10 µg/kg	All <10 µg/kg	All <10 µg/kg
70	All <10 µg/kg	All <10 µg/kg	11 + 7 at <10 µg/kg	All <10 µg/kg	Not analysed
77	15 + 7 at <10 µg/kg	All <10 µg/kg	12 ± 2 + 2 at <10 µg/kg	Not analysed	Not analysed

Residue depletion in Merino sheep

A residue depletion study with 2–3 year old Merino sheep was reported (Karadzovska, 2007f). The mean oral dose received was 3.8 mg/kg bw. A group of 4 animals served as controls. Animals were sacrificed at 7, 18, 29, 35, 70, 120 and 127 days after treatment; mean tissue residues are shown in Table 5.11.

Table 5.11. Monepantel sulphone residues in edible tissues of Merino sheep after a single oral administration of monepantel

Day	Tissue [mean residues in µg/kg (±SD)]				
	Renal Fat	Subcutaneous Fat	Liver	Kidney	Muscle
7	3 109 ± 834	3 010 ± 1028	1 376 ± 258	366 ± 90	199 ± 110
18	474 ± 380	638 ± 497	325 ± 259	82 ± 62	40 ± 39
29	202 ± 132	265 ± 197	138 ± 84	35 ± 12 + 2 at <10 µg/kg	23 ± 6 + 2 at <10 µg/kg
35	67 ± 52	89 ± 46	54 ± 47 +1 at <10 µg/kg	22 ± 6 +6 at <10 µg/kg	15 ± 4 +6 at <10 µg/kg
70	22 ± 10 + 1 at <10 µg/kg	27 ± 15 + 2 at <10 µg/kg	15 ± 6 + 3 at <10 µg/kg	All <10 µg/kg	All <10 µg/kg
120	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg
127	All <10 µg/kg	All <10 µg/kg	All <10 µg/kg	Not analysed	Not analysed

Comparison of residue data from all residue studies

Statistical analysis of variance (significance level of 5%) of the data from the three single-dose administration studies indicated that there was no difference in observed residues amongst the three studies. Therefore, data from the above single-dose administration studies were pooled **(**Karadzovska, 2007f), as shown in Table 5.12.

Table 5.12. Pooled monepantel sulphone residues in edible tissues of sheep

Day	Tissue [mean residues in µg/kg (±SD)]				
	Renal fat	**Subcutaneous fat**	**Liver**	**Kidney**	**Muscle**
7	3 145 ± 962	3 031 ± 1235	1 730 ± 588	472 ± 180	192 ± 101
18/19	548 ± 335	652 ± 391	297 ± 198	84 ± 53	38 ± 26 + 4 at<10 µg/kg
29	134 ± 101 + 1 at <10 µg/kg	169 ± 147	93 ± 70 + 1 at <10 µg/kg	26 ± 12 + 9 at <10 µg/kg	20 ± 6 +15 at <10 µg/kg
35	67 ± 52	89 ± 46	54 ± 47 + 1 at <10 µg/kg	22 ± 6 + 6 at <10 µg/kg	15 ± 4 +6 at <10 µg/kg
40	65 ± 67 + 4 at <10 µg/kg	63 ± 70 + 2 at <10 µg/kg	37 ± 35 + 3 at <10 µg/kg	18 ± 9 + 12 at <10 µg/kg	12 + 15 at <10 µg/kg

The residues were highest in fat, with renal and subcutaneous fat having similar levels, as would be expected for a lipid-soluble molecule, followed by liver, kidney and muscle. The relative magnitude of residues in tissues is about 20:10:2:1, respectively, during early phase depletion (≤2 weeks). Muscle residues presumably arise, in part, from the interstitial fat in muscle tissue. Beyond day 35, the magnitude of liver residues approaches those of fat.

Methods of analysis for residues in tissues

Analytical method for monepantel sulphone residues in sheep tissues

A validated analytical procedure (Karadzovska, 2007b) was used for the quantification of monepantel sulphone residues in edible sheep tissues from the ADME (Karadzovska, 2007g) and residue depletion studies. A ground tissue sample (1 g) was extracted at room temperature with acetonitrile (9 ml), either by mechanical homogenization for 2 minutes or by mechanical shaking for 10 minutes. The mixture was centrifuged briefly, and the supernatant was clarified by syringe filtration prior to dilution. The diluted extract was injected onto HPLC for quantification to an LOQ of 50 µg/kg. When an increased sensitivity was required, the stored extract (prior to filtration) would be cleaned up on a SPE cartridge (either silica based C_{18} for liver, or polymeric for the other tissues). Following evaporation and re-dissolution, an aliquot was injected into the same HPLC system for quantification to an LOQ of 10 µg/kg. The HPLC system was an isocratic 2-column switching system, with a narrow band of eluate from the first column (Luna 3 µm C_{18} (2) silica) being directed to the second column (NovaPak Phenyl 4 µm silica). The same mobile phase (acetonitrile/methanol/water) was used for both columns. The eluate from column 2 was monitored with a UV detector set at 230 nm. The combined retention time was approximately18 minutes. The HPLC was calibrated by injection of pure standards from 2 to 1000 ng/ml.

The method is simple, uses commonly available reagents and without any hazardous steps. The extraction, centrifugation, filtration and dilution steps can be performed by any experienced analyst. Switching HPLC systems, while not common, are easily set-up using readily available HPLC modules plus a switching valve, which can be controlled by instrument software. Up to 18 samples can be processed in a working day and automated HPLC analysis can be performed overnight. The SPE option, however, is more labour-intensive. The method was used extensively in the analysis of samples from several depletion studies and it was determined to be robust and there were no critical control points identified in the procedure.

Confirmation of monepantel sulphone residues was conducted on a separate LC-MS/MS system. The LC-MS/MS system was a gradient system with increasing concentrations of acetonitrile in water, on an Atlantis T3 (C_{18} silica, 3 µm) column. In the enhanced product ion mode, the specific *m/z* ions at 487 and 186 were monitored. Analytical Procedure 272B.00 was validated under GLP conditions.

The performance characteristics of the method including selectivity, accuracy and precision, stability of analyte in solution and under frozen storage conditions, LOD and LOQ were validated as follows.

Selectivity

Numerous drug-free control samples of each tissue type were tested and no interferences were observed at the specified retention time. Similarly, a range of commonly used drugs approved for use in sheep (or their metabolites) were tested, and no interferences were observed. Of the metabolites derived from monepantel, only the parent itself was tested and did not co-elute. The only other major metabolites known to occur in sheep tissues (G32 and sulphoxide M1) will not interfere as their retention differs sufficiently on a C_{18} column, as demonstrated in the pivotal sheep ADME study. The method is not stereo-specific, so the enantiomer of monepantel sulphone would be detected, but the ADME study demonstrated that racemization does not occur.

Accuracy and precision

Precision and accuracy of the method was tested by analysing batches of 6 replicates at different levels encompassing 0.5 × MRL and 2 × MRL. Mean accuracies were acceptable, within -30 ± 10%. Precision, measured as %CV, were 12% or less for fortifications at 50 µg/kg or higher. Within-laboratory reproducibility was assessed by comparing results obtained by two analysts, each completing a batch of 6 replicates at 10 µg/kg (with SPE) and 50 µg/kg (without SPE) for each tissue type. Mean recoveries were within 9% of each other at 50 µg/kg and within 25% at 10 µg/kg.

Limit of detection (LOD) and limit of quantitation (LOQ)

The calculated LODs and LOQs are based on analyses of drug-free samples. The LODs for the regulatory method option (without SPE cleanup) were 23, 22, 6 and 8 µg/kg for fat, liver, kidney and muscle tissue, respectively. The corresponding LOQs were 51, 56, 13 and 15 µg/kg. For the more sensitive method option that uses an additional SPE cleanup, the LODs were 6, 4, 5 and 5 µg/kg in fat, liver, kidney, and muscle tissues, respectively. The corresponding LOQs were 15, 7, 14 and 10 µg/kg, respectively.

Stability

The stock solution of monepantel sulphone in acetonitrile was stable for 12 months (Karadzovska, 2007g). The corresponding fortification and calibration solutions were also stable for 6 months. The primary extract of tissue samples was stable for 7 days when stored in a refrigerator. Prepared HPLC extracts were stable after 24 h storage at room temperature. A GLP-compliant study investigating the stability of incurred samples under freezer storage conditions, room temperature storage and freeze-thaw (3 cycles) was reviewed. Triplicates of each sample were stored under each of the conditions, and then analysed by the above method (without SPE). After 3 cycles of freeze-thaw, incurred residues of monepantel sulphone were within 94–100% of initial values. After 4 h storage at room temperature, results were within 97–107% of initial values. Samples under freezer storage (-20°C) were analysed after 1.5, 3 and 6 months storage. For liver and fat, there was no change in residue values, but for kidney there was a small decrease (17%) after 6 months storage. Muscle was stable under long-term freezer storage conditions for 1 year.

Analytical method for monepantel and its sulphone in blood

As numerous studies involved analysis of blood samples, the sponsor developed and validated an analytical method for sheep blood (Karadzovska, 2007h; Browning and Karadzovska, 2008). A 0.5 ml aliquot of blood, with anti-coagulant, was extracted with acetonitrile (1.3 ml) and water (0.5 ml) and, following centrifugation, the extract was diluted with water and cleaned-up on a polymeric SPE cartridge. The eluate was evaporated and re-dissolved in mobile phase for HPLC quantification, using the same system as the tissue method, except that a separate switch for monepantel was performed within the same run. The estimated LODs were 0.5 and 0.7 ng/ml for monepantel and its sulphone respectively. The LOQs of 3 ng/ml for each analyte were validated with at least 6 replicate samples showing acceptable accuracy and precision. The method was validated in the range 3 to 1000 ng/ml for both analytes with fortified samples. Accuracy and precision (both repeatability and within-laboratory reproducibility) were excellent. Solutions of the analytes in solvent or mobile phase were stable for 6 months or longer. Prepared blood samples for HPLC injection were stable after storage overnight at room temperature. Blood samples, both fortified and incurred, were stable under 3 cycles of freeze-thaw, after storage at room temperature for 4 h, and after 4 months storage in the freezer (-20°C).

The version of the validated analytical method without SPE, described above, with a LOQ of 50 µg/kg (Browning, 2010), was determined to be a suitable regulatory control method. In addition, a validated LC-MS/MS method is available to confirm the presence of monepantel sulphone residues at the LOQ of 50 µg/kg.

Appraisal

Monepantel has not previously been evaluated by the Committee. Monepantel was included in the agenda for the current meeting of the Committee at the request of the 19th Session of the CCRVDF. Monepantel 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 bw and the maximum dose used is 3.75 mg/kg bw.

Data from two pharmacokinetic GLP-compliant studies were available, one with i.v. administration and another as an oral drench. In both studies, blood samples were collected up to 28 days post-dose. Monepantel and monepantel sulphone concentrations in blood were quantified by a validated HPLC method. In blood and plasma, monepantel concentration decreased rapidly after i.v. administration and was detected until 48 h. Monepantel sulphone concentrations declined in blood and were quantified during 4 days. The oral bio-availability of monepantel was 31%. The area under the curve of monepantel sulphone concentrations obtained after oral administration of monepantel at a dose of 1 mg/kg bw was similar to the value obtained from those given i.v. administration of monepantel sulphone, demonstrating a first-pass effect and complete absorption of the oral dose of monepantel.

In an ADME and residue depletion GLP-compliant study with a single oral dose of ^{14}C-monepantel as a 2.5% (w/v) solution was administered to sheep. Approximately 50% of the administered radioactivity was recovered in faeces and 30% in urine after 14 days. Two different positions of the [^{14}C] labelling in monepantel were used to assess other possible modes of metabolism for the compound. The position of the radiolabel on either ring did not influence the interpretation of the total radioactivity or the metabolic profiling in tissue and excreta. The metabolite profile was analysed and metabolites identified. Monepantel was metabolized to a sulphoxide and a sulphone, identified as the predominant metabolites. A second metabolite pathway involved cleavage to yield the phenol metabolite together with its sulphate conjugate. Monepantel sulphone was the major metabolite found in blood and tissue and represented 100% of radioactivity in blood. Fat was the tissue with the highest

concentration of radioactivity followed by liver, kidney and muscle. The approximate total radioactive residue proportions were 10 (fat): 5 (liver): 2 (kidney): 1 (muscle).

In the ADME and residue depletion GLP-compliant study, monepantel and monepantel sulphone were quantified in tissue using a validated HPLC/UV method with a chiral column and a LOQ of 50 µg/kg, and the results were compared. The concentrations of radio-labelled monepantel in the tissue matrices decreased in the order: fat > liver > kidney > muscle. The corresponding concentrations of monepantel sulphone residues decreased in the same order. Total radioactive residue (TRR) expressed as monepantel-equivalent were compared with monepantel and monepantel sulphone tissue concentrations at different time points from 2 to 35 days to calculate the ratio of marker residue and TRR. The ratio of the mean concentration of the marker residue and that of the total residue was calculated as 1 for muscle and 0.66 for fat, liver and kidney.

Three depletion studies were evaluated: one using Suffolk lambs; the second using cross-bred lambs (Merino × Dorset); and the third using Merino sheep. In each study, sheep were administered monepantel at 3.8–3.9 mg/kg bw using the same general study design. Animals were sacrificed at 7, 18, 29, 35, 70, 120 and 127 days after treatment. Residue concentration data from the three single-dose administration studies were statistically compared. An analysis of variance study of the data indicated that there was no significant difference in observed residues amongst the 3 studies at the significance level of 5%. Therefore, the 3 data sets were pooled and used for the estimated daily intake analysis. The median concentration of monepantel sulphone measured in the animal tissues seven days post-dose were 2 620 µg/kg in fat, 1 295 µg/kg in liver, 406 µg/kg in kidney and 152 µg/kg in muscle.

Using the combined data from the three single oral dose monepantel administration studies, the upper one-sided 95% confidence limit over the 95th percentile of residue concentrations was calculated for each edible tissue. The ratio of the mean concentration of the marker residue to that of the TRR was calculated as 1.0 for muscle and 0.66 for fat, liver and kidney. The estimated daily intake (EDI) for monepantel was calculated after applying a correction factor of 0.94 to account for the mass difference between monepantel sulphone (the marker residue) and monepantel (See Figure 5.4). The time point at which the MRLs were recommended was based on an EDI < ADI approach described in the report of the 66th meeting of the Committee (FAO/WHO, 2006).

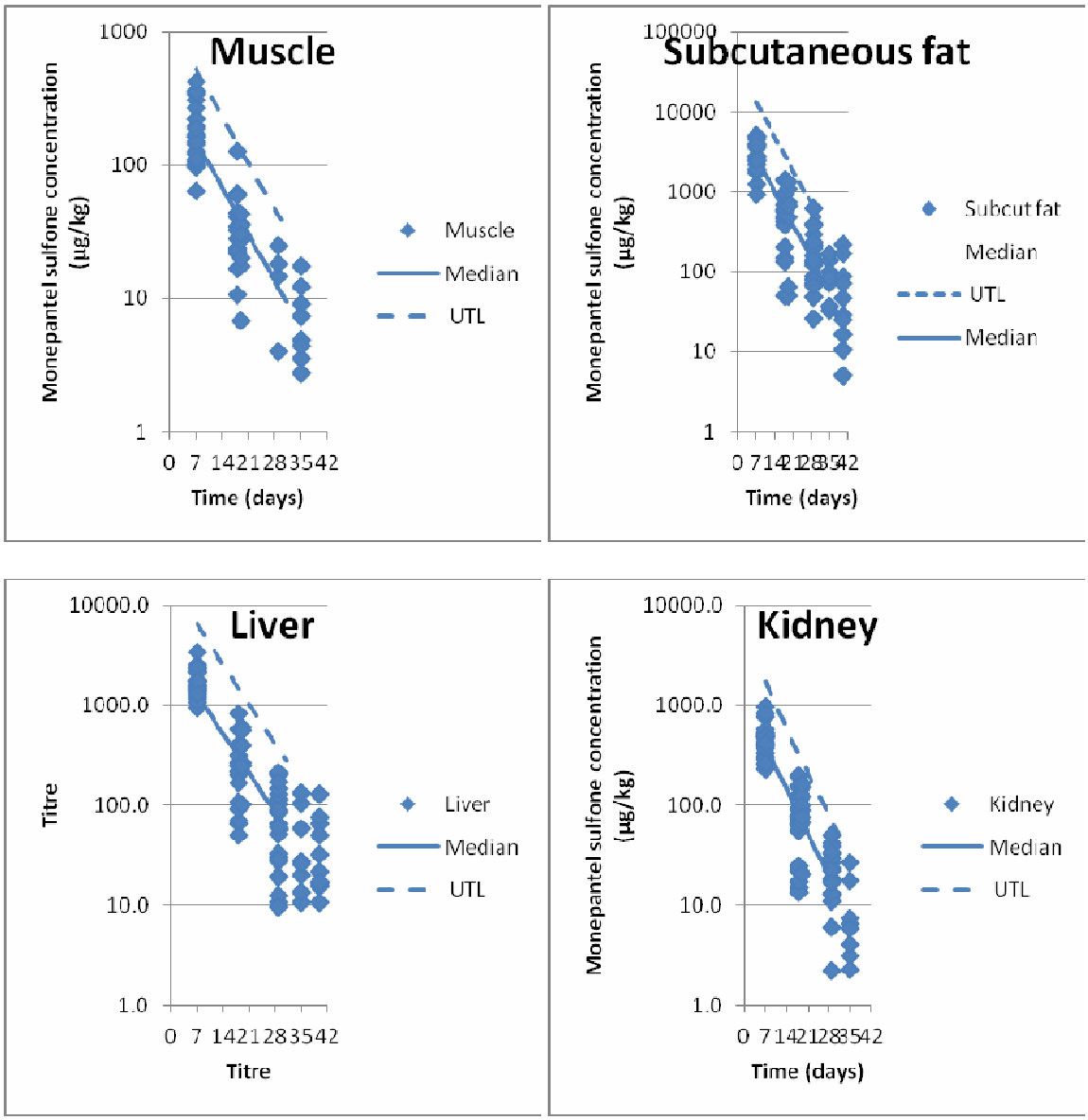


Figure 5.4. Monepantel sulphone residue depletion curves after oral administration of monepantel to sheep (combined data). Median and Upper 95/95 Tolerance Limit (UTL).
NOTE: In the liver figure, the Y-axis is monepantel sulphone (µg/kg) and X- axis is time (days).

A validated HPLC/UV method was available and was used for the analysis of incurred residues of monepantel as its sulphone metabolite in edible sheep tissues. The method provided fit for purpose performances for muscle, kidney, liver and fat samples. Concentrations in tissue samples were determined by reference to non-matrix-matched, external standard calibration curves and can be used for the regulatory monitoring of residues of monepantel and monepantel sulphone in edible tissues. Additionally, a validated LC-MS/MS method suitable for confirming the analytes at 50 µg/kg was available.

Maximum residue limits

In recommending MRLs for monepantel in sheep, the Committee considered the following factors:

- Monepantel is registered for use in sheep at a maximum recommended single oral dose of 3.75 mg/kg bw.
- An ADI of monepantel 0–20 µg/kg bw was established by the Committee, corresponding to an upper bound of acceptable intakes of 1200 µg/day for a 60 kg person.
- Monepantel is extensively metabolized.
- Monepantel sulphone is the marker residue in tissues.
- Fat contains the highest concentration of monepantel sulphone at all sampling times, followed by liver, then kidney and muscle. Liver and fat can serve as the target tissues.
- The ratios of the concentration of marker residue to total residues are 1.0 in muscle and 0.66 in fat, liver and kidney.
- Residue data evaluated were determined with a validated analytical method to quantify monepantel sulphone in tissue.
- A validated analytical method for the determination of monepantel sulphone in edible sheep tissues (liver, kidney, muscle and fat) is available and may be used for monitoring purposes.
- MRLs were calculated on the basis of the upper limit of the one-sided 95% confidence interval over the 95th percentile of residue concentrations.
- The time point at which the MRLs were set was based on an EDI < ADI approach described in the 66th meeting of the Committee.
- No data are provided for sheep milk.

The Committee recommended MRLs determined as monepantel sulphone in sheep tissue at 300 µg/kg in muscle, 700 µg/kg in kidney, 3000 µg/kg in liver and 5500 µg/kg in fat. Using the model diet and marker to total residue of 1 for muscle and 0.66 for fat, liver and kidney, and after applying a correction factor of 0.94 to account for the mass difference between monepantel sulphone (the marker residue) and monepantel, the EDI is 201 µg/person per day, which represents 17% of the upper bound of the ADI.

References

Browning, A. 2010. Amendment A to Analytical Procedure No 272B.01 Regulatory method for the determination of AHC-2144670 in sheep tissues by HPLC - extension to goat tissues. Unpublished report from Novartis Animal Health Australasia Pty Ltd. vol. 07, p. 191.

Browning, A. & Karadzovska, D. 2008. Regulatory method for determination of AHC-2144670 in sheep tissue by HPLC. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Analytical procedure No. 272B.01.

FAO/WHO. 2006. Evaluation of certain veterinary drug residues in animals and foods. Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series,* No. 939.

Jung, M. 2006. Pilot ADME study of [^{14}C]AHC 2102225 in sheep. Unpublished report from Novartis. Novartis Centre de Recherché Sante Animale, Switzerland. Study Report No. CRA 05/051, vol. 03, p. 3.

Jung, M., McLellan, G., Karadzovska, D. & Strehlau**, G.** 2007. ADME and residue depletion study of [^{14}C]AHC 2102225 in sheep. Study Report No. CRA 05/15, vol. 03, p. 99.

Karadzovska, D. 2007a. Validation of the analytical procedure 272A.00 for the determination of AHC-2102225 and AHC-2144670 in sheep blood. Unpublished report from Novartis Animal Health Australasia Pty Ltd. Study Y06/53, Report Y06/53/2075, vol. 07, p. 83.

Karadzovska, D. 2007b. Validation of a HPLC-UV Analytical Method (Procedure 272B.00) for the determination of AHC-2144670 in sheep tissues. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y07/38, Report Y07/38/2166, vol. 06, p. 230.

Karadzovska, D. 2007c. Determination of pharmacokinetic parameters of AHC-2102225 and AHC-2144670 in sheep following i.v. and oral administration of AHC-2102225 and i.v. administration of AHC-2144670. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y06/72, Report Y06/72/2159, vol. 05, p. 3. Later published as Karadzovska, D., Seewald, W., Browning, A., Smal, M., Bouvier, J. & Giraudel, J.M. 2009. Pharmacokinetics of monepantel and its sulfone metabolite, monepantel sulfone, after intravenous and oral administration in sheep. *Journal of Veterinary Pharmacology and Therapeutics,* 32(4): 359–367.

Karadzovska, D. 2007d. Residues of AHC-2102225 in edible tissues of Suffolk sheep following a single oral dose of AHC-2102225 at 3.75 mg/kg. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y07/21, Report Y07/21/2154, vol. 06, p. 3.

Karadzovska, D. 2007e. Residues of AHC-2102225 in edible tissues of cross-bred fattening lambs following a single oral dose of AHC-2102225 at 3.75 mg/kg. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y07/17, Report Y07/17/2157, vol 06, p. 59.

Karadzovska, D. 2007f. Residues of AHC-2102225 in edible tissues of Merino sheep following a single oral dose of AHC-2102225 at 3.75 mg/kg. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y07/16, Report Y07/16/2158, vol.06, p. 120.

Karadzovska, D. 2007g. Stability of incurred AHC-2102225 residues in sheep tissues. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y06/78, Report Y06/78/2138, vol. 07, p. 59.

Karadzovska, D. 2007h. Validation of the analytical procedure 272A.00 for the determination of AHC-2102225 and AHC-2144670 in sheep blood. Unpublished report from Novartis Animal Health Australasia Pty Ltd. Study Y06/53, Report Y06/53/2075.

Strehlau, G. 2007. Residues of AHC-2102225 in edible tissues of sheep of different breeds following a single oral dose of AHC-2102225 at 3.75 mg/kg. Unpublished report from Novartis Animal Health Inc, Switzerland. Statistical report of studies Y07/16, Y07/17 & Y07/21, vol. 06, p. 182.

Smal, M. 2007. Tissue residues following repeated oral administration of AHC 2102225. Unpublished report from Novartis Animal Health Australasia Pty. Ltd. Study Y06/93, Report Y06/93/2141, vol. 06, p. 204.