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Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 85th Meeting 2017

Monepantel

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Monepantel

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Addendum to the monographs prepared by the 75th and 78th meetings of the Committee and published in the FAO JECFA Monographs 12 and 15.

Identity

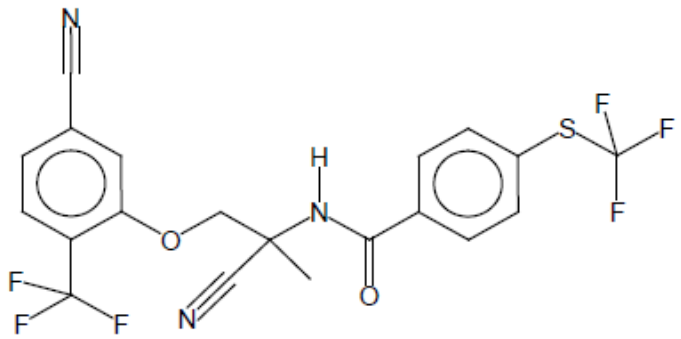
International Non-proprietary Names (INN): Monepantel

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

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

Chemical abstract Service No.: 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 , the active S-enantiomer of NG-96 (the racemic mixture of S- and R-enantiomers)

Appearance: White powder

Impurities: 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 %

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

Solubility: water: 0.1 mg/L at 20 °C

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

Log Ko/w or Partition Coefficient: Octanol/water partition coefficient: log Pow = 3.0 (shake flask method, pH 7, at 20 °C)

pH: 6.2-6.3 (suspension in water)

Optical rotation: [α] 580nm -32° (methanol)

UV_{max}: Note provided

Stability: Store at room temperature; protect from light

Background

Monepantel, an amino-acetonitrile derivative anthelminthic, was reviewed previously by the Committee at its seventy-fifth meeting (FAO, 2011). The Committee established an ADI of 0-20 μg/kg bw, corresponding to an upper bound of acceptable intake of 1200 μg/day for a 60 kg person. Used as an oral drench to control gastrointestinal nematodes (roundworms) in sheep, the Committee recommended MRLs, determined as monepantel sulphone, in sheep tissue of 300 μg/kg in muscle, 700 μg/kg in kidney, 3000 μg/kg in liver and 5500 μg/kg in fat. Because sufficient data were available to calculate median residue values, the EDI approach was used. Using the model diet and marker to total residue ratio 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 calculated was 201 μg/person per day, which represents 17 % of the upper bound of the ADI.

At the Twentieth of the Codex Committee on Residue of Veterinary Drugs in Food (CCRVDF), concerns were raised that the recommended MRLs were significantly lower than those already established in some countries and could create trade problems (FAO/WHO, 2012). It also was noted that the recommended MRLs were not consistent with the withdrawal times in some countries. The CCRVDF discussed higher MRLs, recognising that it was within the purview of the Codex Committee, as risk managers, to modify the MRLs recommended by JECFA. Some Delegations did not consider advancing higher MRLs appropriate without an evaluation of their safety by JECFA, in recognition of JECFA's role as risk assessor for Codex. The

CCRVDF agreed to request that JECFA evaluate the safety of the proposed higher MRLs in light of the information provided by the Committee. Specifically, JECFA was asked to consider if higher MRLs (Muscle, 700 ug/kg; Liver, 5000 ug/kg; Kidney, 2000 ug/kg; Fat, 7000 ug/kg) are compatible with the ADI and consistent with the JECFA process for the derivation of MRLs.

Monepantel was subsequently reviewed by the Committee at its seventy-eight meeting (FAO, 2013), which confirmed the ADI of 0–20 μg/kg bw. The Committee recommended revised MRLs in sheep tissue of 500 μg/kg in muscle, 1700 μg/kg in kidney, 7000 μg/kg in liver and 13000 μg/kg in fat. Again, using the model diet and marker to total residue ratio 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 revised EDI calculated was 443 μg/person per day, which represents 37 % of the upper bound of the ADI.

Monepantel was added to the priority list at the 23rd meeting of the CCRVDF (Houston, TX, 2016) with the request to recommend MRLs for cattle tissues.

Residues in food and their evaluation

Conditions of use

Monepantel is indicated for the treatment of roundworms in cattle. The product, formulated as a solution containing 25 mg monepantel per mL, has recently been approved for use in cattle in New Zealand and is marketed for use in sheep in many countries. For cattle, the EU has established an ADI of 0.03 mg/kg bw. The EU recommended MRLs are 7000, 2000, 1000 and 300 μg/kg for fat, liver, kidney and muscle, respectively (EMA, 2016). The same MRLs have been established in Australia. The assigned withdrawal period in New Zealand for cattle is 5 days. A milk discard time is assigned as part of the registration; however, a milk MRL is not requested from the JECFA and no milk residue data are provided for evaluation.

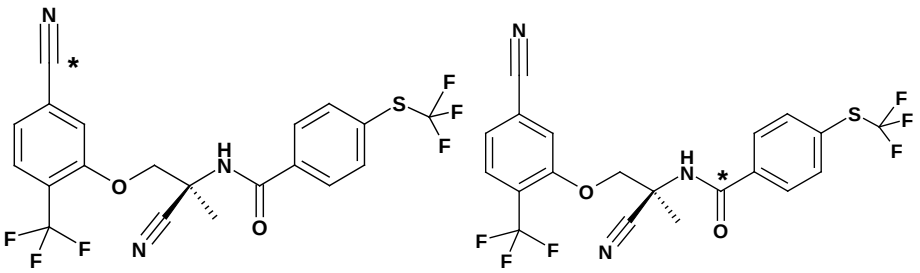
Dosage

Monepantel is administered to cattle orally, as a drench, at a minimum dose rate of 2.5 mg/kg bw and a maximum dose of 3.7 mg/kg bw. Up to three doses per season can be applied with a minimum retreatment interval of 21 days.

Pharmacokinetics and metabolism

For the pharmacokinetic and metabolism studies, monepantel was radiolabelled at either “label 2” or “cyano” or “label 3” or “amide” (Figure 1).

Figure 1. Positions of labelled [^{14}C]-monepantel



"Label 2" or "cyano" **"Label 3" or "amide"**

Pharmacokinetics in laboratory animals

The pharmacokinetics of monepantel in laboratory animals were evaluated at the 75th meeting of the Committee (WHO, 2012).

Pharmacokinetics in Food-producing Animals

Cattle

A GLP-compliant ADME study was conducted with [^{14}C]-monepantel (3.75 mg/kg bw) in a placebo base (Vance, 2014). The test material was determined to have a radiopurity of more than 99 % using two distinct chromatographic systems. Monepantel was administered orally once to twelve male and female Aberdeen/Angus cross beef cattle (Table 1). The monepantel was labelled at the amide carbon adjacent to the phenyl ring ("label 3" in Figure 1). Animal 11 was overdosed by 25 % due to complications with gavage dosing, and was slaughtered early at day 3; no correction for overdosing is made below.

Table 1. Study Group Assignments

Study Group	Animal Number	Sacrifice Time Point
1	11M*, 2F, 3F	Study Day 3
2	4M, 5M, 6F	Study Day 7
3	7M, 8F, 9F	Study Day 14
4	10M, 1M*, 12F	Study Day 21

M = Male, F = Female

Animal 1M originally was assigned to Group 1 but was swapped with Animal 11 on Study Day 0 to become a Group 4 animal due to mistaken dosing of Animal 11.

Blood, edible tissues, faeces, bile and urine were collected for up 21 days post-treatment. Three animals were held in metabolism crates for the first 3 days.

Blood samples (*ca.* 20 mL) were collected from Animal 11M pre-dose and at the following time points post dose: 4 h, 8 h, 12 h, 24 h, 36 h, 48 h and 72 h. Blood samples were collected from Animals 10M and 12F pre-dose and at the following time points post dose: 4 h, 8 h, 12 h, 24 h, 36 h, 48 h, 72 h, 96 h, 168 h, 240 h, 312 h, 408 h and 504 h. Blood samples were collected from Animal 1M at the following time points post dose: 24 h, 36 h, 48 h, 72 h, 96 h, 168h, 240

h, 312 h, 408 h and 504 h. Blood samples were collected within ± 10 min of the target time at 4 h, 8 h, 12 h and 24 h time point. The remaining blood samples were collected within ± 30 min of the target time. All blood was sampled from the jugular vein. Immediately following collection, the blood was transferred into 2 x 10 mL tubes containing lithium heparin as anti-coagulant and the samples thoroughly mixed.

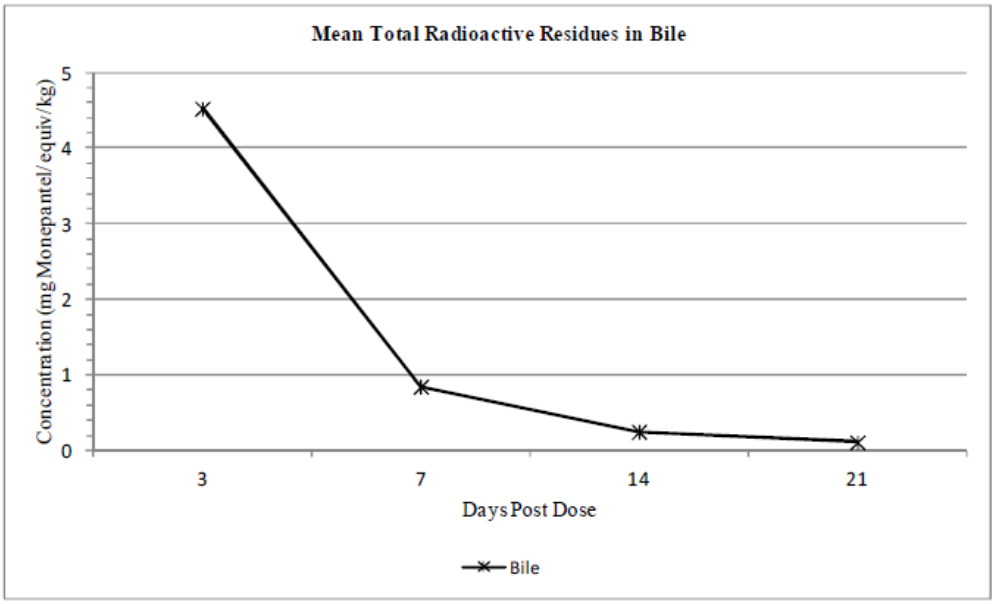
Systemic absorption was relatively high, with TRR reaching 216 µg equivalents/kg in blood and 268 µg equivalents/kg plasma at 24 h after dosing (Table 2). Absolute bioavailability was not determined. Initial elimination was rapid with a half-life of about 36 h, but this slowed progressively at later times. Residues were still detectable at the final sampling time point at *ca.* 5 µg equivalents/kg in blood and plasma.

Table 2. Mean TRR concentrations in blood and plasma (mg monepantel equiv/kg)

Time Point (Hours Post Dose)	Concentration (mg monepantel equiv/kg)	
	Blood	Plasma
4	0.073	0.102
8	0.135	0.172
12	0.181	0.230
24	0.216	0.268
36	0.177	0.204
48	0.159	0.175
72	0.103	0.109
96	0.070	0.076
168	0.041	0.045
240	0.027	0.028
312	0.016	0.021
408	0.008	0.009
504	0.005	0.005

Bile TRR was highest at day 3 (~4500 µg/kg) but declined progressively to ~100 µg/kg at day 21 (Figure 2).

Figure 2. Mean TRR in Bile



About 21 % of the dose was eliminated in the urine over 3 days. Mean total radioactive residues in urine were similar at one and 2 days post dose (16-18 mg equiv/kg). Subsequently, residues depleted to *ca.* 11 mg equiv/kg by 3 days post dose and to *ca.* 1 mg equiv/kg by 7 days post dose. By 14 and 21 days post dose, concentrations had declined to *ca* 500 and 200 µg equivalents/kg, respectively (Table 3).

Approximately 36 % of the dose was eliminated in the faeces. In faecal samples, concentrations at one day post dose were approximately half those observed in the urine (*ca* 9 mg equiv/kg). Concentrations subsequently doubled to *ca* 17 mg equiv/kg during the following 24-hour period (2 days post dose). By 3 days post dose, concentrations had decreased to similar levels observed at one day post dose (*ca* 7 mg equiv/kg), before depleting rapidly to *ca* 600 µg equivalents/kg at 7 days post dose and <100 µg equivalents/kg by 21 days post dose.

Table 3. Mean TRR in Urine and Faeces*

Time Point (Days Post Dose)	Concentration (mg equiv/kg)	
	Urine	Faeces
1	17.725	8.840
2	15.984	16.539
3	11.422	7.081
7	1.327	0.576
14	0.545	0.200
21	0.157	0.084

*Day 1-3 means of animals in metabolism cages; Day 7, 14, and 21 means are from animals at time of slaughter.

Cage rinse was collected at the end of 3 days. Less than 2 % of the administered radioactivity was recovered in the crate rinse.

About 60 % of the dose was recovered in excreta over 3 days, with the remaining material distributed in the tissues (Table 4).

Table 4. Mean Recovery of Total Radioactivity in Urine, Faeces and Cage Rinse*

Time Point (Hours Post Dose)	Total Radioactive Recovery (% of Dose Administered)			
	Urine	Faeces	Cage Rinse	Total
24	9.133	8.575	Not Required	17.708
48	5.894	17.568	Not Required	23.462
72	6.316	10.014	1.771	18.101
Total	21.343	36.157	1.771	59.271

*Means of animals in metabolism cages only

In a second GLP compliant study, the pharmacokinetic profiles of monepantel and monepantel sulphone in blood were investigated in beef cattle dosed three times 21 days apart at 3.75 mg/kg bw monepantel as part of the pivotal cold residue depletion study (Adams and Le, 2014).

Twenty male and female Angus cross cattle, *ca.* 250 kg, were enrolled in the study and allocated to 4 slaughter groups. Animals were maintained on pasture for the duration of the study. Blood was collected from one group at regular intervals after each dose, starting at 4 h. Samples were analysed using a validated LC-MS/MS method (Browning, 2014b, 2014c). Animals were slaughtered at 4, 7, 10 and 13 days following the third (final) dose.

Monepantel sulphone (AHC-2144670) was the dominant residue in the blood. Concentrations peaked at 24 h after each dosing and declined thereafter, with a terminal half-life of *ca.* 3 days. Residues at were 4.09-7.22 ng/mL by day 13. Monepantel concentrations peaked at *ca.* 24 h after the first and second dosing with the maximum concentration of monepantel occurring 12 h after the 3rd dosing, and depleted rapidly. Monepantel residues were less than the LOQ (0.25 ng/mL) at day 13 (Tables 5-7; Figures 3 and 4).

The blood profiles of the animals were compared and no accumulation of monepantel and monepantel sulphone in blood was evident following repeated administration of the test item. Overall, the blood profile (measuring individual metabolites) was similar to that observed in the beef ADME study where only TRRs were measured.

Table 5. Concentrations of monepantel and monepantel sulphone in cattle blood (group 4)- 1st Treatment.

	Time post 1st treatment (day)									
	[d]0.00	0.17	0.33	0.50	1	2	3	4	7	13
Animal no.	**monepantel (ng/mL)**									
229	<LOQ	9.17	12.2	13.4	19.9	7.93	4.60	1.34	0.279	<LOQ
239	<LOQ	27.5	19.9	20.0	15.6	5.40	2.02	0.810	<LOQ	<LOQ
242	<LOQ	17.2	20.0	21.1	31.0	11.1	3.26	1.75	0.501	<LOQ
243	<LOQ	6.91	9.78	12.2	14.1	5.11	1.72	0.742	<LOQ	<LOQ
245	<LOQ	15.9	18.1	18.0	18.5	8.14	2.62	1.23	<LOQ	<LOQ
Mean	**<LOQ**	**15.3**	**16.0**	**16.9**	**19.8**	**7.54**	**2.84**	**1.17**	**<LOQ**	**<LOQ**
sd	**NA**	**8.07**	**4.71**	**3.96**	**6.66**	**2.43**	**1.15**	**0.413**	**NA**	**NA**
	monepantel sulfone (ng/mL)									
229	<LOQ	28.1	49.1	62.5	110	95.4	56.8	40.0	16.4	8.69
239	<LOQ	97.7	128	133	156	91.3	59.6	39.2	18.5	4.26
242	<LOQ	59.4	87.0	91.5	146	132	77.3	55.6	29.8	5.50
243	<LOQ	25.1	47.0	67.3	99.5	70.2	44.1	30.9	12.2	3.01
245	<LOQ	55.6	80.1	84.7	116	103	68.5	53.5	18.8	3.34
Mean	**<LOQ**	**53.2**	**78.2**	**87.8**	**126**	**98.4**	**61.3**	**43.8**	**19.1**	**4.96**
sd	**NA**	**29.3**	**33.1**	**28.0**	**24.3**	**22.4**	**12.5**	**10.4**	**6.52**	**2.30**

[d]day -4 pre-treatment

NA = Not available

Figure 3. Mean blood concentrations of monepantel following oral administration of monepantel three times 21 days apart to beef cattle

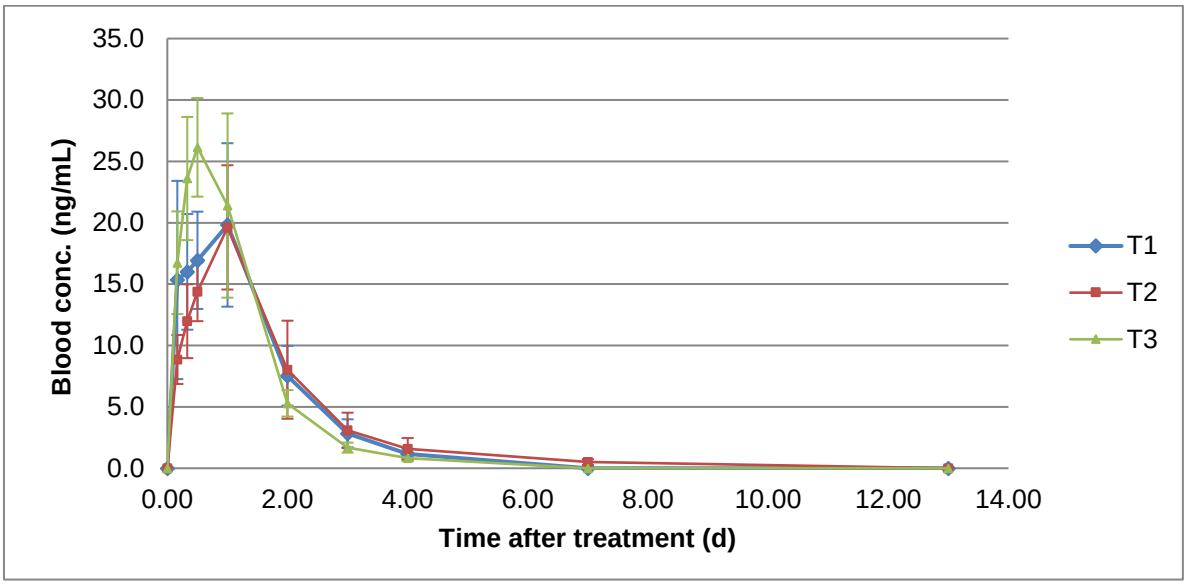
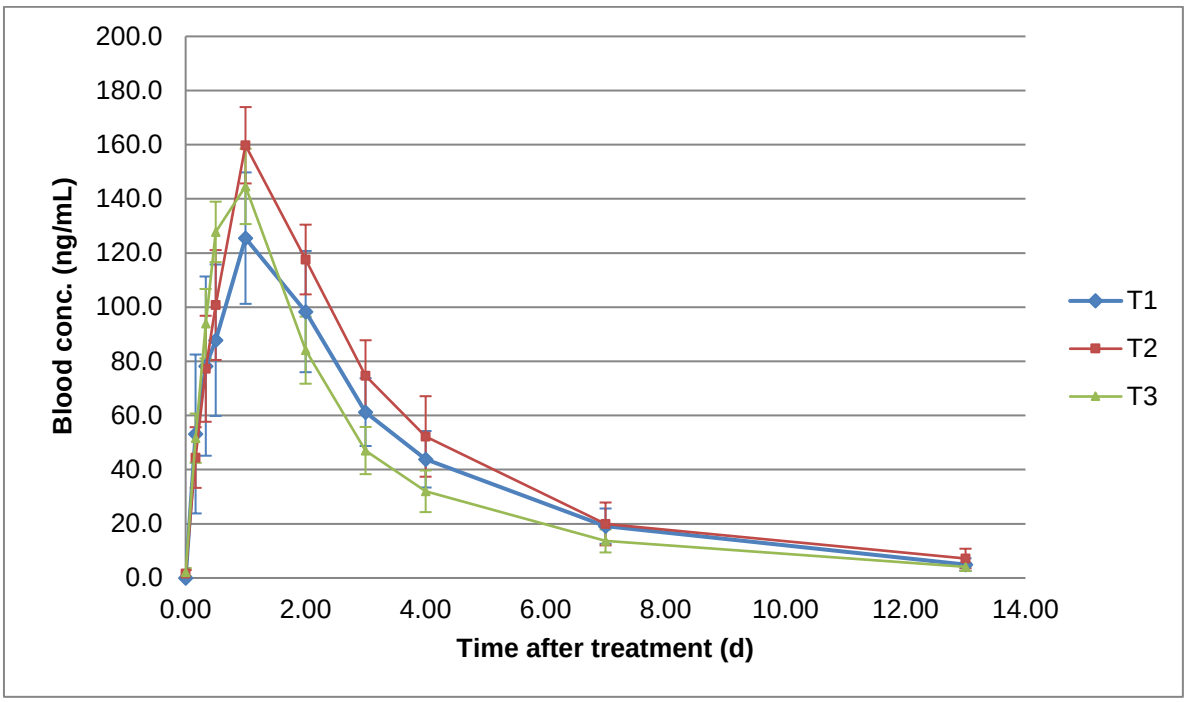


Figure 4. Mean blood concentrations of monepantel sulphone following oral administration of monepantel three times 21 days apart to beef cattle



Sheep

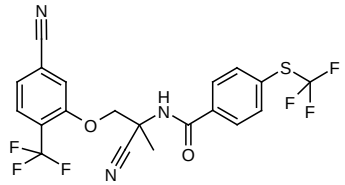
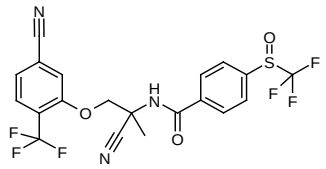
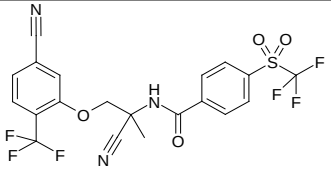
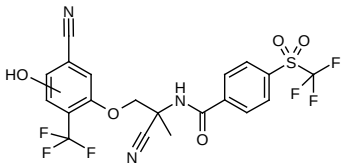
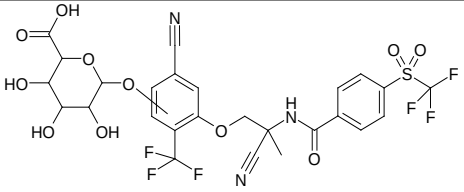
The pharmacokinetic behaviour of monepantel and monepantel sulphone in the blood and plasma of sheep was evaluated at the 75th meeting of the Committee (FAO, 2011; WHO, 2012).

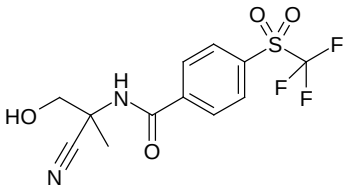
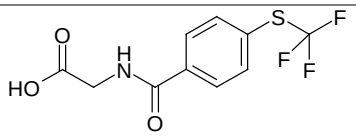
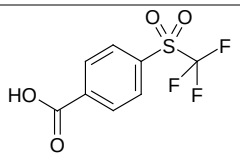
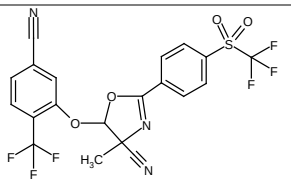
Metabolism in Laboratory Animals

Rats

The ADME of monepantel was evaluated by the 75th meeting of the Committee (FAO, 2011; WHO, 2012). Metabolism of monepantel proceeds by rapid oxidation of the sulphur to the sulphone with slower hydroxylation of the phenoxyl ring. The resulting metabolites are nonpolar. There are multiple cleavages of the amino-acetonitrile bridge to polar metabolites. Elimination is via the urine and faeces of the sulphation or glucuronidation of these metabolites. A minor metabolite, AHC-2197876, is found in fat and, to a lesser extent, muscle. The metabolic pathways in the rat are summarized in (Figure 2 in WHO, 2012).

Table 8. Metabolite nomenclature

Metabolite	Code	Description/other names	Structure
Monepantel	AHC-2102225	Parent	
M1		Sulfoxide of parent	
M2	AHC-2144670	Sulphone Monepantel sulphone	
M3		Hydroxylated M2 F24	
M6		Glucuronide of M3	

M9		Cleaved metabolite U15b	
M10		Glycine conjugate of trifluoromethylthiobenzoic acid	
M11		Cleaved metabolite U17b	
G32	AHC-2197876	Cyclized metabolite	

Metabolism in Food Producing Animals

Cattle

Samples of edible tissues, blood, bile and excreta from the GLP compliant ADME study (Vance, 2014) were investigated for extractability of residues, and metabolite profiles. Due to low TRR, kidney at day 21 and muscle at days 7 to 21 were not investigated (Table 9).

Radioactivity was extracted readily from fat, kidney and muscle, with simple solvent extraction. Less than 5 % of TRR was unextractable.

Liver residues proved more difficult to extract, especially with increasing time after dose administration. Unextracted residues increased from 37 % on Day 3 to 75 % on Day 14. Simple solvent extraction could remove about half of the TRR at day 3 and this dropped to *ca.* 13 % on day 21. Further extractions with more polar solvents, water and ammonia did not improve extraction significantly. Extracted day 21 TRR was too low to proceed with metabolite profiling.

Table 9. Metabolites in edible tissues of cattle

		%TRR (mg/kg)			
Tissue	Peak	Day 3	Day 7	Day 14	Day 21
Liver	Parent	0.5 (0.012)	ND	ND	-
	AHC 2144670	36.5 (0.855)	4.5 (0.074)	6.4 (0.042)	-
	Unknowns (by decreasing polarity)	13.7 (0.320) 5.8 (0.136)	1.1 (0.018) 2.1 (0.034) 1.1 (0.018)	1.8 (0.011) 0.7 (0.005) 0.6 (0.004)	-
Kidney	Parent	5.2 (0.068)	7.5 (0.040)	ND	-
	AHC 2144670	67.9 (0.888)	65.8 (0.350)	94.9 (0.214)	-
	Unknowns (by decreasing polarity)	4.8 (0.063) 11.6 (0.152) 7.2 (0.094)	12.0 (0.064)	ND	-
Muscle	Parent	ND	-	-	-
	AHC 2144670	76.3 (0.152)	-	-	-
	Unknown	10.4 (0.021)	-	-	-
Fat	Parent	9.5 (0.627)	4.8 (0.115)	10.0 (0.089)	ND
	AHC 2144670	88.2 (5.834)	73.5 (1.762)	82.0 (0.730)	77.7 (0.252)
	AHC 2197876	0.8 (0.051)	1.4 (0.033)	5.8 (0.051)	9.3 (0.030)

ND = Not Detected - = Not Analyzed

The major component present in all tissues cochromatographed with AHC 2144670, monepantel sulphone. The parent molecule, monepantel, was a minor constituent, present only at the early timepoints in fat, liver and kidney, and was not detected in the muscle. Minor unknown components of high polarity were detected in the liver (n=3), kidney (n =3) and muscle (n=1).

In liver, two minor polar metabolites (referred to as L1 and L2, Figures 5 and 6) were observed at days 3–14 (Strathdee, 2016). High resolution MS (accurate mass determination and fragmentation patterns via MS/MS and MS3) and radio-HPLC detection were used to identify metabolite L1 as the cleaved metabolite M9 and metabolite L2 as a further cleaved M11 (Table 8 above).

Figure 6. Postulated structure of Metabolite L1

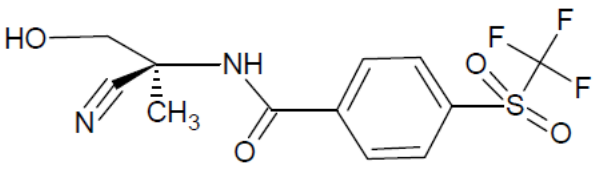
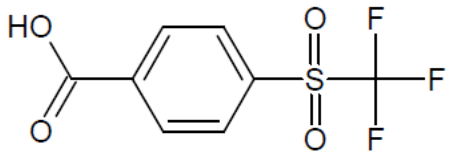


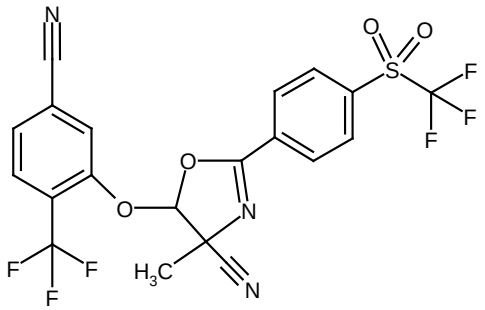
Figure 6. Postulated structure of Metabolite L2



Kidney profiles were similar to those in liver, including the presence of small amounts of L1 and L2. A third polar metabolite was observed only at day 3 and it was <5 % of TRR.

A component, detected only in the fat, cochromatographed with AHC 2197876 and was determined to be a cyclised metabolite (Figure 7).

Figure 7. Postulated structure of cyclised metabolite in fat



The small amount of TRR in muscle limited profiling to the Day 3 samples. Only a trace of L1 was detected.

The major metabolite in blood was monepantel sulphone, AHC 2144670. There was a small amount of parent monepantel and several minor metabolites (Table 10).

Table 10. Metabolic profile in Day 3 cattle blood

Metabolite	Retention Time (min)	%TRR	mg/kg
Combined and Concentrated			
-	6.63	3.9	0.004
-	7.88	2.9	0.003
-	10.63	10.3	0.011
-	13.13	2.6	0.003
AHC 2144670	24.88	13.7	0.014
Monepantel	25.63	1.8	0.002
Total characterised/ Identified		35.2	0.037

The metabolic profile in bile was complex (Table 11). Non-polar metabolites were readily identified by co-chromatography with authentic standards. In addition to parent monepantel, there was a trace of monepantel sulphone, which was confirmed by LC-MS/MS, as part of the analytical method validation used to establish the MR/TRR ratio (Browning, 2014a). Additionally, there was a large peak of intermediate polarity, a large polar peak with retention time corresponding to L1 and a cluster of more polar peaks.

Table 11. Metabolic profile in Day 3 cattle bile

Component	Retention Time (min)	%TRR (%)
Unknowns	0.63	0.7
	1.88	0.6
	3.63	2.2
	5.63	26.4
	9.88	18.1
	10.88	2.1
	12.38	1.6
	13.38	0.6
	14.13	0.6
	14.88	1.3
	15.88	29.8
	17.38	7.8
	20.38	0.6
	22.88	0.5
AHC 2144670	24.38	4.3
Monepantel	26.88	0.6
Unknowns	32.13	0.3
	33.88	1.0
Total Characterised by HPLC		99.1
Total Extracted		99.3
Unextracted		0.7
Total		100

In urine, there was one polar metabolite and four minor polar peaks (Table 12). No parent monepantel, monepantel sulphone (AHC-2144670) or hydroxylated monepantel sulphone was detected.

Table 12. Metabolic profile in Day 2 cattle urine

Component	Retention Time (min)	%TRR (%)
Unknowns	8.45	8.5
	10.63	9.7
	11.72	5.5
	13.25	66.3
	17.77	6.6
Total Characterised by HPLC		96.6
Total Extracted		96.7
Unextracted		3.3
Total		100

Faecal residues were easily extracted with simple solvent extraction (>80 %) (Table 13).

Table 13. Metabolites in Faeces

	%TRR in Faeces				
Extract/Peak	Day 1	Day 2	Day 3	Day 7	Day 14
Parent	43.9	25.9	16.3	3.8	ND
AHC 2144670	10.4	9.4	7.0	28.6	28.5
Unknowns	37.5	53.1	55.6	41.8	36.9

ND = Not Detected

Sheep

The metabolism of monepantel in tissues of sheep was evaluated at the 75th meeting of the Committee (FAO, 2011).

Comparative metabolism

A non-GLP in vitro metabolism study was conducted to investigate the metabolism of monepantel in intact rat, cattle and sheep hepatocytes (Anderson *et.al.*, 2016). Both ^{14}C-labeled analogues of monepantel (Figure 1) were used in the study. The activity of hepatocytes was evaluated using verapamil. The activity of rat and bovine hepatocytes was consistent with historical controls. Sheep hepatocytes were about 30 % less active than hepatocytes from cattle and rats but there were no historical data for comparison.

Following MeOH quenching at 0, 1, 2, and 4 h, incubations were profiled by HPLC with radio-detection. Metabolites in these extracts were identified by LC/MS techniques. The protein pellet was extracted further with various solvents to determine the extent of covalent binding.

Covalent binding was minimal, but occurred in all three species and for both label types. Binding was highest in bovine hepatocytes and lowest in ovine hepatocytes (bovine > rat >

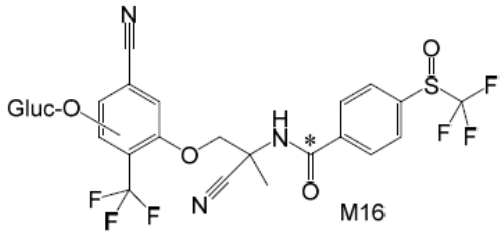
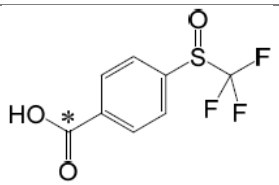
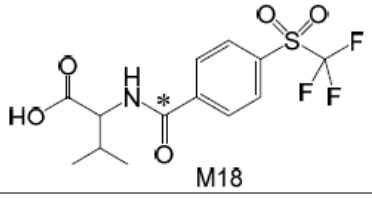
sheep). The lower binding in sheep hepatocytes was attributed to the lower metabolic turnover. The radioactivity recovered in the methanol extracts further indirectly supports the conclusion of minimal binding for monepantel and its metabolites, with between 96 to 105b% recovered at 1, 2, and 4 h.

Metabolism was greatest with rat hepatocytes. Less than 1 % monepantel remained after 2 h incubation. With bovine and sheep hepatocytes, 13 % and 66 % of monepantel remained after 4 h.

In incubations of rat hepatocytes, the predominant metabolites were M2 and M6. M2 peaked at 1 hour, accounting for 45 % of the extracted radioactivity. M6 peaked at 2 h, accounting for 49-52 % of the extracted radioactivity. In incubations of bovine hepatocytes, the major metabolites were M1, M2 and M10, accounting for 16 %, 27 % and 16 % respectively of extracted radioactivity, in the 4 hour sample. In incubations of sheep hepatocytes, the major metabolites were M1, M2 and M10, accounting for 10 %, 4 %, and 9 % respectively of extracted radioactivity, in the 4 hour sample.

Three new metabolites were observed in the in vitro study (Table 14). In rat hepatocytes, a new metabolite (M16) was observed at <2 % of applied radioactivity. In bovine hepatocytes, two new minor metabolites (M17 and M18) were observed at <5 % or less of applied radioactivity. Fewer metabolites were observed in incubations with sheep hepatocytes and these were limited to the sulphur oxidation products and only one cleavage product. No new metabolites were observed.

Table 14. New metabolites identified in the in vitro hepatocyte study

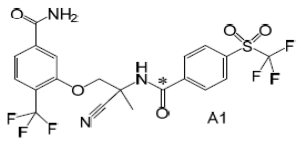
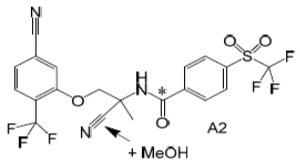
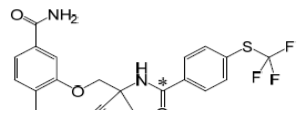
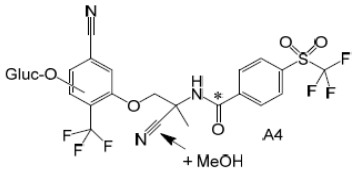
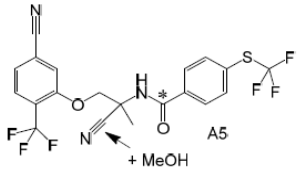
Metabolite	Description/other names	Structure*
M16	Glucuronide of hydroxylated M1	
M17	Trifluoromethylsulfinylbenzoic acid	
M18	Valine conjugate of M11	

* indicates position of ^{14}C label

Incubations with bovine hepatocytes metabolized monepantel in a similar manner to incubations with rat hepatocytes (Figures 8-10). Although two new metabolites were observed for cattle but not for rats, these were at very low concentrations. Additionally, because the valine conjugate (M18) potentially can be hydrolysed back to M11 by stomach juice and the M1 cleavage product (M17) could be oxidized to M11, these are likely of no toxicological significance (Anderson *et.al.*, 2016).

In addition to the reported metabolites, five chemical adducts (A1 to A5) also were observed in this study (Table 15). Adducts observed in the 0-hour samples were the result of addition at the aromatic or aliphatic cyano moieties of monepantel. Adducts observed in the incubated samples were the result of addition at the cyano moiety of either M2 or M6. Adducts were observed only for the predominant peaks in the metabolic profiles. Because these adducts did not appear to grow over the time course of the incubations, they were determined to be chemical artefacts formed during the workup procedure (Anderson *et.al.*, 2016).

Table 15. Water and MeOH adducts identified in the in vitro hepatocyte study

Peak [1]	[M-H]⁻	Proposed Metabolite Identification	Structure [2]	Rat	Bovine	Sheep
A1 [1]	524	M2 + H_2O to form amide on aromatic cyano group		X	X	MS
A2 [1]	538	M2 + MeOH to form N-methylamide on aliphatic cyano group		X	X	X
A3 [1,5]	492	P + H_2O to form amide on aromatic cyano group		X	X	X
A4 [1]	730	M6 + MeOH to form N-methylamide on aliphatic cyano group		X		
A5 [1,5]	506	P + MeOH to form N-methylamide on aliphatic cyano group		X	X	X

"X" indicates the metabolite was quantified in the radioprofile of at least one sample. "MS" indicates the metabolite was not quantified in a radioprofile but was detected by mass spectrometry, hence present only at low concentrations.

[1] Metabolites were labelled following the nomenclature from the in vivo rat metabolism study, RCC study number A89493 (Gassen 2007 in WHO, 2012), where metabolites M1 through M15 were previously reported. Metabolites M16 through M18 and chemical adducts A1 through A5 were observed for the first time in this study.

[2] The asterisk denotes the site of the ^{14}C label position 3 and indicates that the monitored M-H anion included the ^{14}C isotope. Due to lower specific activity, for compounds with radiolabel position 2, the ^{12}C isotope was used.

[3] Metabolites M1, M2, M5, M9, and M11 were previously reported in the in vivo sheep metabolism study (Jung *et.al*., 2007 in WHO, 2012).

[4] The identity of metabolite M5 was previously matched to standard AHC2166637 (Gassen, 2007, and Jung *et.al*., 2007, in WHO, 2012).

[5] Chemical adducts A3 and A5 were observed in the 0-hour rat, bovine, and/or sheep hepatocyte samples.

Figure 8. Metabolic Pathways and Metabolites of Monepantel in Rat (R), Bovine (B) and Sheep (S) hepatocytes

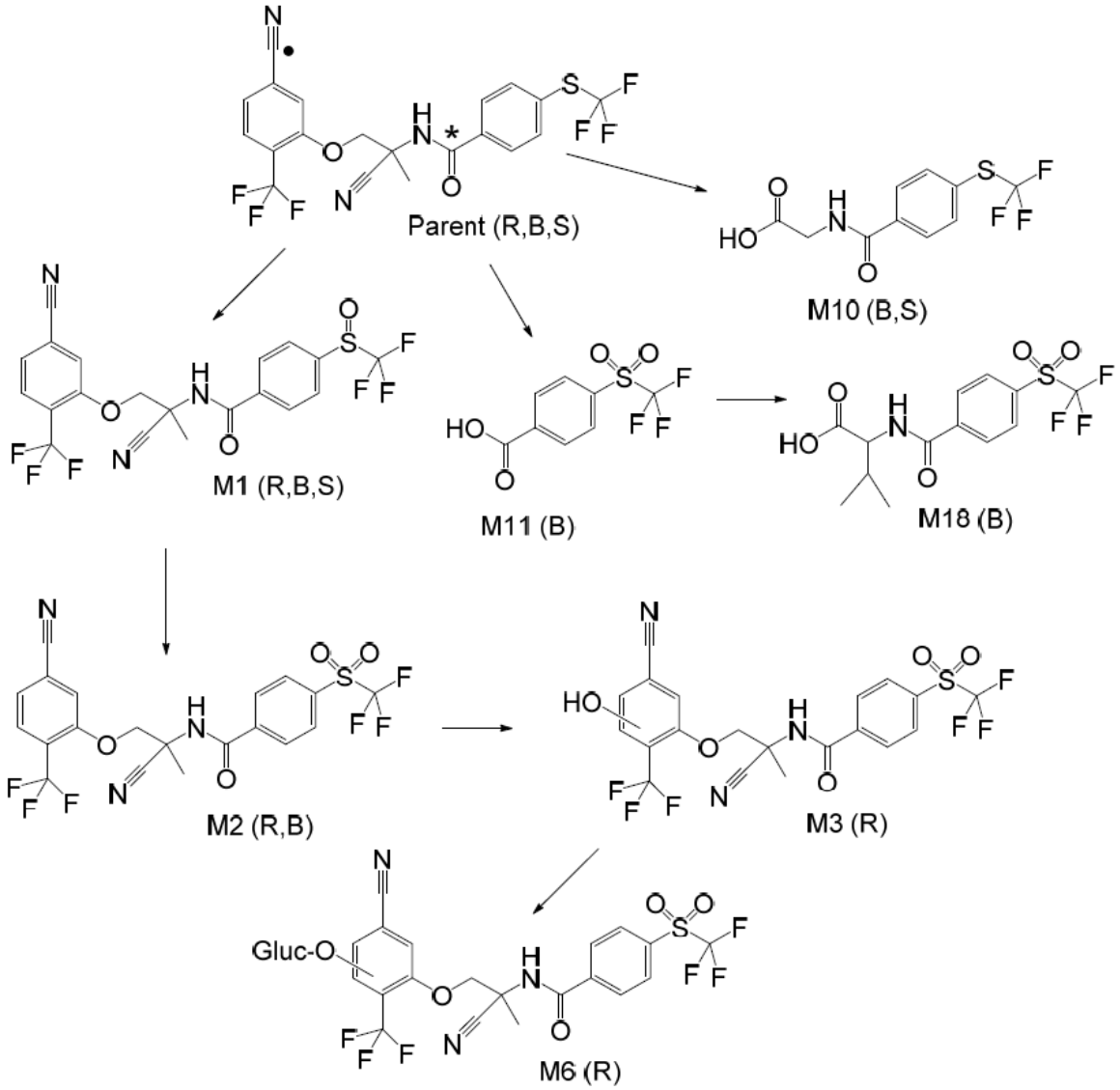
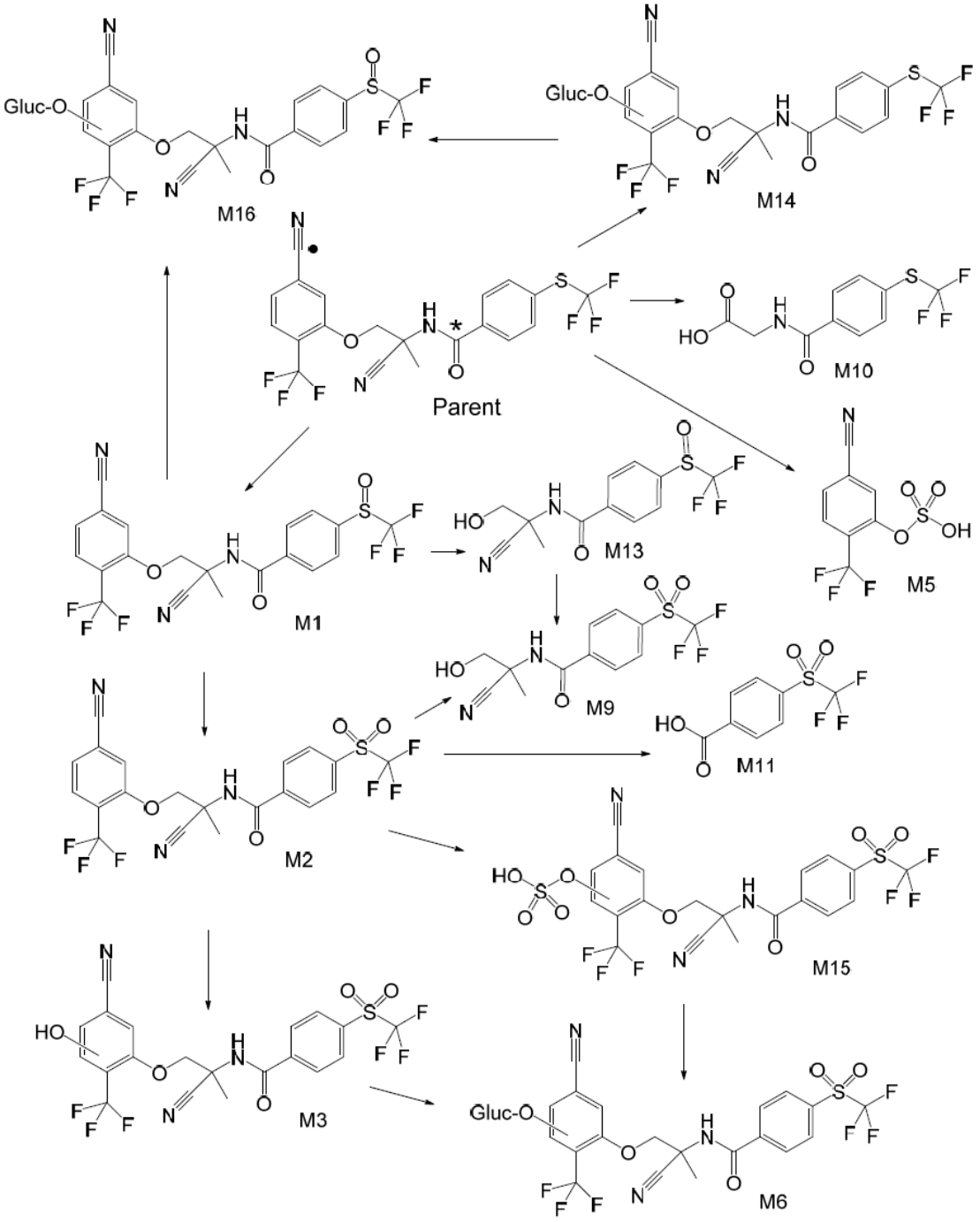


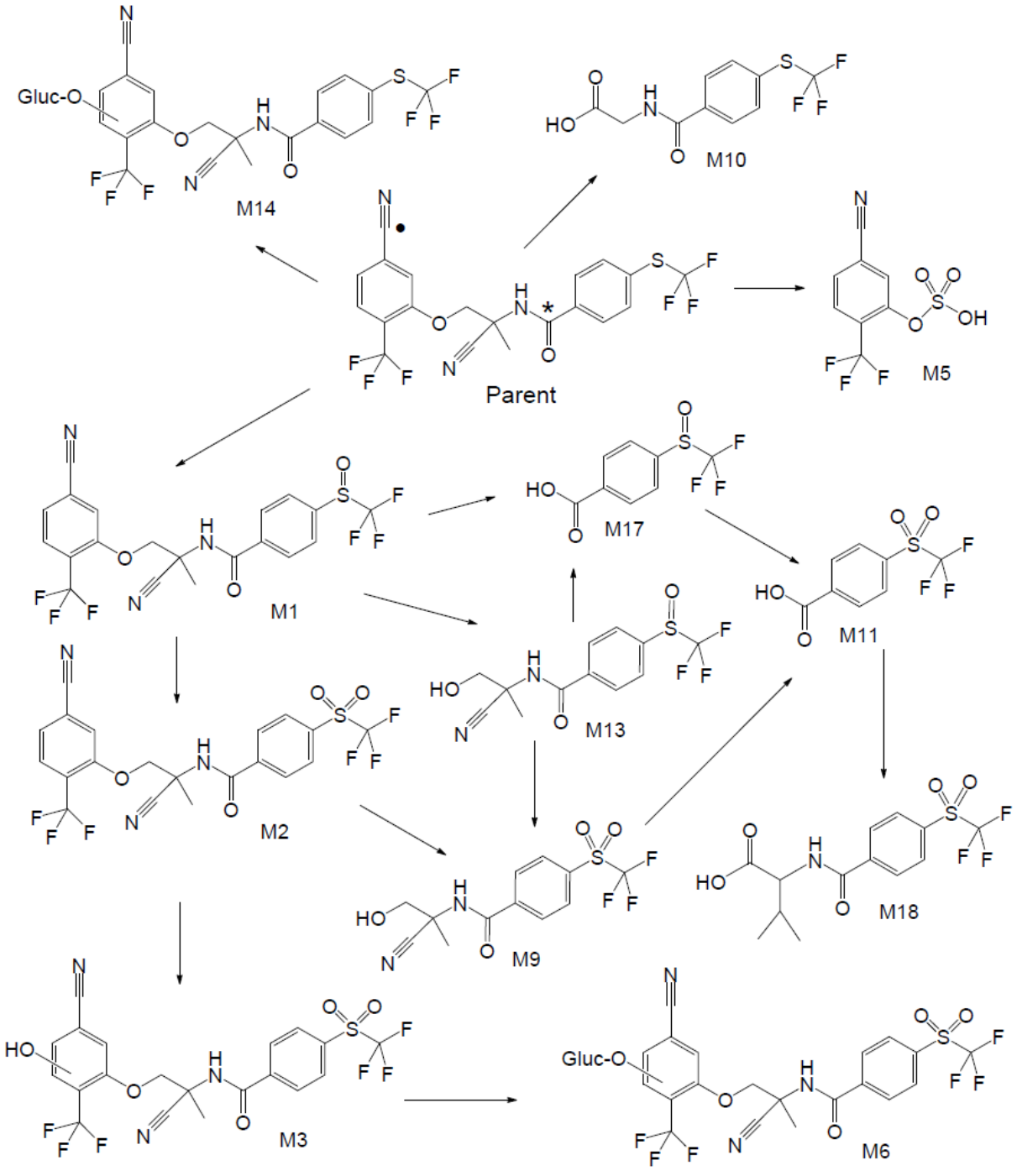
Figure 9. Proposed metabolic scheme for radiolabelled monepantel in rat hepatocytes



• Dot indicates ^{14}C-radiolabel position 2

* Asterisk indicates ^{14}C-radiolabel position 3

Figure 10. Proposed metabolic scheme for radiolabelled monepantel in cattle hepatocytes



• Dot indicates ^{14}C-radiolabel position 2

* Asterisk indicates ^{14}C-radiolabel position 3

The results of the in vitro study (Anderson *et.al*., 2016) are consistent with those in the in vivo study (Vance, 2014). The observed metabolites have previously been detected in in vivo studies in rat, and sheep (FAO, 2011; WHO, 2012; Table 8, above, and Table 16).

Table 16. Comparative metabolism in rats and sheep from WHO, 2012

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%)

Bovine liver residues are less extractable than sheep or rat liver residues. Bound residues are found in all three species. The higher bound residues in cattle liver are consistent with the shorter terminal half-life of monepantel sulphone in the blood of cattle, *ca.* 3 days following 3 oral doses of monepantel, compared to the terminal half-life of monepantel sulphone in the blood of sheep, nearly 6 days following a single IV dose of monepantel or more than 4 days following a single IV dose of monepantel sulphone (Karadzovska *et.al*., 2008).

The in vivo metabolism study demonstrated that the cleavage pathway is more prominent in cattle than in other species. The hydrolysis of the amide bond of intact monepantel metabolites forms free amines and carboxylic acids, both of which are candidates for peptide bond formation with free carboxylic acid and free amine groups of endogenous proteins. Indeed, a valine conjugate of M11 was observed demonstrating conjugation to available amino acids, and a glycine conjugate (M10) of trifluoromethylthiobenzoic acid was an in-vitro bovine metabolite.

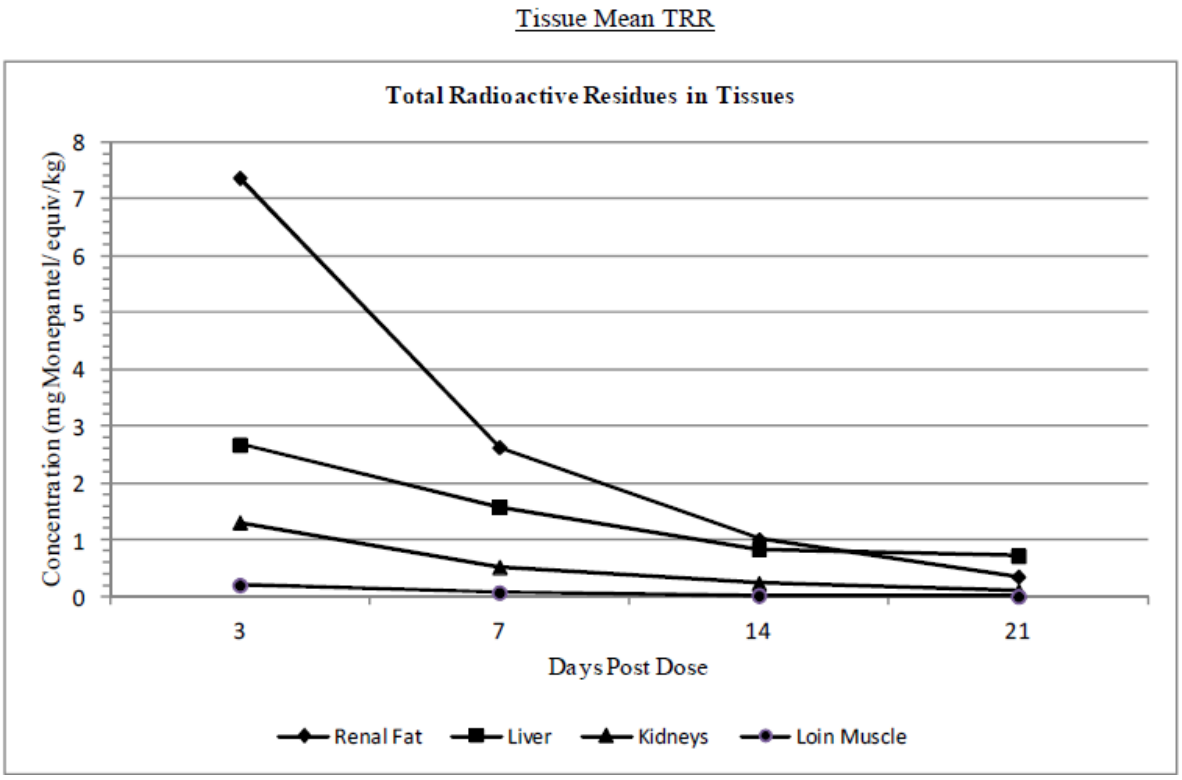
Tissue residue depletion studies

Radiolabelled residue depletion studies

Cattle

Selected samples of edible tissues from the GLP compliant ADME study (Vance, 2014) were investigated for TRR. Monepantel was labelled at the amide carbon adjacent to the phenyl ring ("label 3" in Figure 1). TRRs were determined after a single oral dose at 3.75 mg/kg using combustion and liquid scintillation counting (LSC). Residue data were subjected to log-linear regression analyses (Strehlau, 2014), and log-linearity was observed for fat, kidney and muscle, but not for liver. The calculated half-lives of TRR were 4-5 days for fat, kidney and muscle but about 10 days for liver, consistent with presence of bound residues. Of the edible tissues, kidney samples at day 21 and muscle samples at days 7 to 21 were not investigated due to low TRR.

Figure 11. Depletion profiles of radiolabelled residues in cattle tissues



Edible tissue TRRs are summarized in Table 17-20 and Figure 11. The order of residues in tissues is fat > liver > kidney > muscle, indicative of lipophilic substances, and is the same as seen for sheep. Total residues were more persistent in cattle liver than in the other tissues and more than in sheep liver (FAO, 2011).

Table 17. TRR (µg equiv/kg; mean ±SD) in edible tissue of beef cattle

Day	Fat	Liver	Kidney	Muscle
3	7373±1164	2677±256	1315±328	201±26
7	2636±466	1587±136	531±86	80±39
14	1019±107	843±42	246±25	28±10
21	362±182	725±177	111±51	11±4

Table 18. TRR as % of administered dose

Group	Euthanasia Days Post Dose	Total Radioactive Recovery (% of Dose Administered)	
		Liver	Kidney
1	3	0.982	0.075
2	7	0.613	0.037
3	14	0.321	0.017
4	21	0.281	0.007

Table 19. Metabolites as % TRR

		%TRR (mg/kg)			
Tissue	Peak	Day 3	Day 7	Day 14	Day 21
Liver	Parent	0.5 (0.012)	ND	ND	-
	AHC 2144670	36.5 (0.855)	4.5 (0.074)	6.4 (0.042)	-
	Unknowns (by decreasing polarity)	13.7 (0.320) 5.8 (0.136)	1.1 (0.018) 2.1 (0.034) 1.1 (0.018)	1.8 (0.011) 0.7 (0.005) 0.6 (0.004)	-
Kidney	Parent	5.2 (0.068)	7.5 (0.040)	ND	-
	AHC 2144670	67.9 (0.888)	65.8 (0.350)	94.9 (0.214)	-
	Unknowns (by decreasing polarity)	4.8 (0.063) 11.6 (0.152) 7.2 (0.094)	12.0 (0.064)	ND	-
Muscle	Parent	ND	-	-	-
	AHC 2144670	76.3 (0.152)	-	-	-
	Unknown	10.4 (0.021)	-	-	-
Fat	Parent	9.5 (0.627)	4.8 (0.115)	10.0 (0.089)	ND
	AHC 2144670	88.2 (5.834)	73.5 (1.762)	82.0 (0.730)	77.7 (0.252)
	AHC 2197876	0.8 (0.051)	1.4 (0.033)	5.8 (0.051)	9.3 (0.030)

ND = Not Detected - = Not Analyzed

Table 20. Ratio of MR to TRR in bovine tissues

Time	Animal	Fat			Liver			Kidney			Muscle		
(day)		TRR	Sulphone[1]	%	TRR	Sulphone[1]	%	TRR	Sulphone[1]	%	TRR	Sulphone[1]	%
		(µg/kg)	(µg/kg)	MR/TRR	(µg/kg)	(µg/kg)	MR/TRR	(µg/kg)	(µg/kg)	MR/TRR	(µg/kg)	(µg/kg)	MR/TRR
3	11M	8472	7393	87	2935	860	29	1520	812	53	226	127	56
	2F	7494	5862	78	2673	823	31	1488	753	51	202	132	65
	3F	6153	5410	88	2423	732	30	937	561	60	175	140	80
Mean		7373	6222	84	2677	805	30	1315	709	55	201	133	67
7	4M	2519	2262	90	1576	404	26	623	327	53			
	5M	2240	1916	86	1729	305	18	518	301	58			
	6F	3149	2801	89	1457	226	16	452	340	75			
Mean		2636	2326	88	1587	312	20	531	323	62			
14	7M	992	769	78	892	67	8						
	8F	928	769	83	817	104	13						
	9F	1136	855	75	820	154	19						
Mean		1019	798	79	843	108	13						
21	10M	573	364	64	921	89	10						
	1F	254	179	70	677	22	3						
	12F	260	199	77	578	22	4						
Mean		362	247	70	725	44	6						

[1] Raw sulphone result adjusted for MW (473/505) and for unmeasured [^{14}C] monepantel sulphone (2.7 % of TRR: ^{14}C monepantel (12.54 g) in 467.976 g placebo formulation) as only [^{12}C]-monepantel sulphone was measured by LC-MS/MS

Blank entries indicate no analysis for monepantel sulphone

As the major residue is monepantel sulphone (AHC-2144670), it is the marker residue identified for monepantel in cattle tissues. It also is the marker identified previously for sheep tissues (FAO; 2011).

Concentrations of the monepantel sulphone were determined as part of the method validation study (Browning 2014a) and used to determine the MR to TRR ratio cattle tissues (Table 21).

Table 21. MR to TRR ratios

Day	Fat	Liver	Kidney	Muscle
3	84	30	55	67
7	88	20	62	
14	79	13		
21	70	6		

Lactating dairy cows

No radiolabelled residue data were provided for the use of monepantel in lactating dairy cattle.

Sheep

Radiolabelled residue data for the use of monepantel in sheep were evaluated by the 75th meeting of the Committee (FAO, 2011).

Residue depletion studies with unlabelled drug

Cattle

Two GLP-compliant residue depletion studies were conducted in cattle using unlabelled monepantel.

The first study (Adams and Le, 2014) was conducted using 20, 9-month old crossbred beef cattle. Cattle were treated orally three times at a nominal dose of 3.75 mg monepantel/kg body weight. Doses were administered 21 days apart. Animals were slaughtered 4, 7, 10 and 13 days after the final treatment. Samples of muscle, kidney, liver, renal fat and subcutaneous fat were collected and analysed for monepantel sulphone.

The maximum monepantel sulphone residues were observed in the Day 4 samples: 4110, 4680, 1090, 478 and 231 µg/kg for subcutaneous fat, renal fat, liver, kidney and muscle, respectively. At the final sampling time, maximum residues were 1720, 770,146, 61.8 and 22.8 µg/kg for subcutaneous fat, renal fat, liver, kidney and muscle, respectively (Tables 22 - 24). While initial residues in renal fat were highest, by the later sampling times residues were highest in subcutaneous fat. The calculated half-life estimates for muscle, kidney, liver, renal and subcutaneous fat were 2.8, 2.7, 2.7, 2.9 and 5.1 days, respectively.

Table 22. Residues of monepantel sulphone in subcutaneous and renal fats of cattle treated three times, 21 days apart, with a nominal dose of 3.75 mg monepantel/kg body weight

			Subcutaneous fat(µg/kg)		Renal fat(µg/kg)	
Group	**Day post 3rd treatment**	**Animal no.**	**Uncorrected**	**Recovery corrected**	**Uncorrected**	**Recovery corrected**
1	4	250	3480	3497	4600	4623
		237	4110	4131	4630	4653
		238	3660	3678	4680	4704
		228	2690	2704	3920	3940
		246	3030	3045	4270	4291
2	7	240	2020	2030	1820	1829
		247	2730	2744	2940	2955
		232	2850	2864	3090	3106
		241	2080	2090	2440	2452
		249	2140	2151	2410	2422
3	10	233	1270	1276	1130	1136
		231	1340	1347	958	963
		236	1120	1126	1460	1467
		227	977	982	970	975
		230	1320	1327	1140	1146
4	13	245	527	530	346	348
		239	859	863	352	354
		243	1460	1467	759	763
		243	*1430*	1428	not reanalyzed	not reanalyzed
		243	*1310*	1308	not reanalyzed	not reanalyzed
		242	1220	1226	770	774
		242	*1420*	1418	not reanalyzed	not reanalyzed
		242	*1550*	1547	not reanalyzed	not reanalyzed
		229	1720	1729	538	541
		229	*1610*	1607	not reanalyzed	not reanalyzed
		229	*1720*	1717	not reanalyzed	not reanalyzed

Underlined italic values are results of repeated analysis

Table 23. Residues of monepantel sulphone in liver, kidney, and muscle of cattle treated three times, 21 days apart, with a nominal dose of 3.75 mg monepantel/kg body weight

			Liver(µg/kg)		Kidney(µg/kg)		Muscle(µg/kg)	
Group	Day post 3rd treatment	Animal no.	Uncorrected	Recovery corrected	Uncorrected	Recovery corrected	Uncorrected	Recovery corrected
1	4	250	1070	1119	439	468	96.3	96.8
		237	1010	1056	478	510	93.8	94.3
		238	1090	1140	386	412	113	114
		228	870	910	358	382	231	232
		246	978	1023	380	405	156	157
2	7	240	416	435	193	206	38.6	38.8
		247	666	696	337	359	82.8	83.3
		232	764	799	310	330	92.7	93.2
		241	477	499	237	253	42.5	42.7
		249	462	483	237	253	85.3	85.8
3	10	233	255	267	132	141	66.3	66.7
		231	218	228	100	107	29.9	30.1
		236	222	232	150	160	43.0	43.2
		227	284	297	94.3	101	20.2	20.3
		230	226	236	85.2	90.8	46.4	46.7
4	13	245	64.4	67.3	27.9	29.7	13.0	13.1
		239	87.7	91.7	34.0	36.2	11.0	11.1
		243	88.9	93.0	35.7	38.1	7.99	8.03
		242	146	153	61.8	65.9	22.8	22.9
		229	127	133	55.6	59.3	15.7	15.8

Table 24. Mean predicted residues, uncorrected for recovery, with corresponding 95 %/95 % tolerance limits:

Day	Muscle µg(kg		Kidney µg(kg		Liver µg(kg		Renal fat µg(kg		Subcutaneous fat µg(kg	
	Predicted residue	95%/95%	Predicted residue	95%/95%	Predicted residue	95%/95%	Predicted residue	95%/95%	Predicted residue	95%/95%
0	364.47	1231.74	1315.61	2945.49	3038.27	5866.36	12284.1	24752.2	5653.93	13199.6
1	285.14	926.78	1016.84	2218.54	2345.23	4433.78	9658.84	19030.4	4936.99	11217.0
2	223.08	698.57	785.92	1672.99	1810.27	3354.27	7594.62	14646.3	4310.95	9544.01
3	174.52	527.78	607.44	1263.53	1397.34	2540.79	5971.55	11287.3	3764.30	8133.76
4	136.54	399.97	469.49	956.22	1078.60	1927.78	4695.36	8714.00	3286.97	6946.66
5	106.82	304.32	362.87	725.58	832.57	1465.84	3691.90	6742.87	2870.17	5949.39
6	83.57	232.75	280.46	552.46	642.66	1117.71	2902.89	5233.20	2506.22	5113.71
7	65.38	179.18	216.77	422.45	496.06	855.26	2282.51	4076.73	2188.42	4415.32
8	51.15	139.00	167.54	324.69	382.91	657.16	1794.71	3189.93	1910.91	3832.81
9	40.02	108.74	129.49	250.95	295.57	507.26	1411.16	2508.20	1668.60	3346.79
10	31.31	85.80	100.08	195.05	228.15	393.35	1109.57	1981.78	1457.01	2939.65
11	24.49	68.22	77.36	152.38	176.11	306.28	872.44	1572.80	1272.26	2595.93
12	19.16	54.59	59.79	119.55	135.94	239.33	685.99	1252.89	1110.93	2302.78
13	14.99	43.91	46.21	94.12	104.93	187.54	539.39	1001.03	970.06	2050.12
14	11.73	35.47	35.72	74.29	80.99	147.27	424.11	801.65	847.05	1830.27
15	9.18	28.73	27.60	58.76	62.52	115.84	333.47	643.11	739.64	1637.49
16	7.18	23.33	21.34	46.55	48.26	91.23	262.21	516.62	645.85	1467.39

The second study (Le, 2015) was conducted using 20, 9-month old crossbred beef cattle. Cattle were treated orally three times at a nominal dose of 3.75 mg monepantel/kg body weight and again doses were administered 21 days apart. Animals were slaughtered 21, 42, 56 and 85 days after the final treatment. Samples of muscle, kidney, liver, renal fat and subcutaneous fat were collected and analysed for monepantel sulphone residues.

The maximum monepantel sulphone residues were observed in the Day 21 samples: 1010, 583, 143, 63.4 and 14.5 µg/kg for subcutaneous fat, renal fat, liver, kidney and muscle, respectively. At the final sampling time, all residues were below the method limit of quantification (LOQ=5 µg/kg) (Table 25).

Table 25. Residues of monepantel sulphone in tissues of cattle treated three times, 21 days apart, with a nominal dose of 3.75 mg monepantel/kg body weight.

Day post third treatment	Animal no.	Liver	Kidney	Muscle	Subcutaneous fat	Renal fat
21	301	98.6	40.2	10.8	604	409
	302	78.1	32.7	7.01	340	398
	304	143	63.4	14.5	1010	583
	323	54.8	27.5	6.90	375	348
	324	68.0	28.3	10.7	457	344
42	305	6.58	<LOQ (4.02, 3.94*)	<LOQ (1.58)	29.7	17.4
	307	6.68	<LOQ (3.71, 3.62*)	<LOQ (1.76)	28.0	22.7
	308	6.14	<LOQ (3.62, 3.06*)	<LOQ (1.75)	22.9	29.1
	313	<LOQ (4.37)	<LOQ (2.94, 2.83*)	<LOQ (1.50)	15.9	16.2
	316	<LOQ (1.84)	<LOQ (1.64, 1.47*)	<LOQ (1.21)	9.08	6.28
56	310	5.01	<LOQ (2.94)	<LOQ (2.20)	23.8	18.0
	312	<LOQ (2.60)	<LOQ (1.86)	<LOQ (1.32)	8.11	7.83
	315	<LOQ (1.08)	<LOQ (1.64)	<LOQ (1.01)	<LOQ (4.36)	<LOQ (4.53)
	319	<LOQ (0.705)	<LOQ (1.09)	<LOQ (1.04)	<LOQ (2.01)	<LOQ (2.14)
	322	<LOQ (2.81)	<LOQ (1.17)	<LOQ (1.10)	<LOQ (4.07)	<LOQ (3.49)
85	306	<LOQ (0.555)	Not analyzed	Not analyzed	<LOQ (0.669)	<LOQ (0.696)
	309	<LOQ (0.492)			<LOQ (0.901)	<LOQ (0.599)
	314	<LOQ (0.623)			<LOQ (0.778)	<LOQ (1.28)
	317	<LOQ (0.531)			<LOQ (0.715)	<LOQ (0.553)
	321	<LOQ (0.929)			<LOQ (0.950)	<LOQ (0.556)

*reanalyzed for confirmation

The incurred residues at the first sampling time in the second study (21 days post-last dose, Le, 2015) were generally consistent with the incurred residues at the last sampling time in the first study (13 days post-last dose, Adams and Le, 2014) and continued the depletion trend (Table 26).

Table 26. Comparison of the incurred residues at the final sampling time of the first study (Adams and Le, 2014) with those at the first sampling time of the second study (Le, 2015).

	Kidney	Liver	Perirenal fat	Subcutaneous Fat	Muscle
Day 13 (Adams and Le, 2014)	43.00	102.80	553.00	1157.20	14.10
Day 21 (Le, 2015)	38.42	88.50	416.40	557.20	9.98

Lactating dairy cows

No unlabelled residue data were provided for the use of monepantel in lactating dairy cattle.

Sheep

The depletion of unlabelled monepantel in tissues of sheep was evaluated at the 75th meeting of the Committee (FAO, 2011)

Methods of analysis for residues in tissues

An analytical procedure, validated under GLP conditions (Browning, 2014a), with a LOQ of 5 µg/kg and demonstrated to be suitable for routine analysis of monepantel in bovine tissue matrices, was available that met the requirements of the validation criteria established by the CCRVDF, as contained in CAC/GL 71-2009.

Briefly, the validated method for the extraction and analysis of monepantel residues in bovine tissue matrices is as follows: The ground tissue sample (0.5 g) is extracted with ACN (5 mL), either by mechanical homogenization for 2 min (for non-fat tissues) or by mechanical shaking for 10 min followed by ultra-sonication for 10 min (for fat). The mixture is centrifuged briefly, and the supernatant is diluted to 50 mL with ACN/MeOH/water. For samples with residues above 750 µg/kg, a further dilution is conducted (1:40 in the same solvent). Once diluted, the extract is injected onto the LC-MS/MS, which is calibrated with injections of varying concentrations of pure standards (0.05 to 7.5 ng/mL, equivalent to 5 to 750 µg/kg, for extracts not requiring further dilution).

Reversed-phase HPLC was conducted using a Waters Atlantis® T3 (3 µm, 2.1 X 50 mm; part number 186003717) column with a Waters Atlantis® T3 (3 µm, 2.1x10 mm; part number 186003756) guard column. The mobile phase A: 20 mM ammonium bicarbonate in water with 5 % acetonitrile, mobile phase B: acetonitrile/methanol (50/50, v/v), flow rate 0.3 mL with gradient elution were used to chromatographically separate monepantel sulphone from any other extracted components with detection by tandem MS with electrospray ionization in the negative mode. Monepantel sulphone was quantified with the transition ion (m/z 503.8 $\rightarrow$ 186) and its identity confirmed with the two qualifying transitions at m/z 504 $\rightarrow$ 166 and m/z 504 $\rightarrow$ 146 at the appropriate chromatographic retention time. Quantification in unknown samples was done by comparing the analyte area responses to those of the calibration curve generated by linear regression with 1/x weighting (origin excluded).

Selectivity

Control tissues from 20 different sources for each tissue type were analysed for interference by co-extractives and compared to the lowest STD. Mean interferences at the retention time were less than 20 % of the lowest standard at 5µg/kg.

Solutions of fenbendazole, thiabendazole, triclabendazole, triclabendazole sulphone, triclabendazole sulfoxide, levamisole, fluazuron, ivermectin, abamectin, doramectin, moxidectin, amoxicillin, oxytetracycline, ceftiofur, florfenicol, cypermethrin and monepantel each at a concentration equivalent to approximately 1000 µg/kg were injected and analysed for a response at the retention time and transition of the marker residue. Minor interference of 22 % of the lowest standard at 5µg/kg was observed for levamisole when it was injected as a single, neat solution at a 200-fold higher concentration of the target analyte.

Matrix samples containing the selected veterinary drugs including monepantel each at a concentration of 1000 µg/kg were extracted and fortified thereafter with monepantel sulphone at 350µg/kg. The responses from these samples were compared to matrix-matched and matrix-fortified extracts at the same concentrations. The detector response for monepantel sulphone did not show any suppression or enhancement in presence of the other drugs.

These experiments confirmed that the method was selective and that the method will accurately detect negative control samples as negative and that the presence of other drugs such as fenbendazole, thiabendazole, triclabendazole, triclabendazole sulphone, triclabendazole sulfoxide, levamisole, fluazuron, ivermectin, abamectin, doramectin, moxidectin, amoxicillin, oxytetracycline, ceftiofur, florfenicol, cypermethrin or their metabolites and the parent compound, monepantel, used in food animal production will not interfere with the quantification of monepantel sulphone. Other metabolites, as seen in the ADME cattle study (Vance, 2014), are well separated from the analyte on reversed-phase HPLC and should not interfere. The method, however, is not stereo-specific, so the other enantiomer of monepantel sulphone also would be detected but, because it was determined in the sheep ADME study (FAO, 2011) that this isomer is not formed, it is likely that this will also be true for cattle.

Recovery

The total recovery was obtained by comparison of the analytical response of blank matrix spiked with the analyte and extracted compared to the response of a STD solution analysed directly.

The extraction loss was obtained by comparison of the analytical response of a blank matrix sample spiked with the analyte and extracted compared to the response of blank matrix of the same origin first extracted and then spiked post-extraction with the analyte.

The matrix effect was obtained by comparison of the analytical response of blank matrix first extracted and then spiked ‘post extraction’ with the analyte compared to the response of a STD solution analysed directly (Table 27).

Table 27. Matrix effects and recovery of extraction from bovine tissue matrices taken from six different matrix sources for each tissue type and fortified at the LOQ of the method at a concentration of 5 µg/kg

Matrix	Fat	Liver	Kidney	Muscle
Qualitative Matrix Effect	Suppression	Suppression	Suppression	Enhancement
Mean Matrix Effect (%)	-7.42	-3.56	-0.391	-5.27
Mean Extraction loss (%)	-1.64	-7.49	-3.28	-12.0
Mean Total Recovery (%)	91.1	89.2	96.3	92.6

Accuracy

The accuracy data presented in Tables 28-31 for fat, liver, kidney and muscle tissues respectively, demonstrated that the method was accurate and met the acceptance criteria for accuracy: 60–120 % for concentrations ≥ 1 µg/kg < 10 µg/kg; 70–110 % for concentrations ≥ 10 µg/kg < 100 µg/kg; and 80–110 % for concentrations ≥ 100 µg/kg.

The precision data shown in Tables 28-31 for fat, liver, kidney and muscle tissues, respectively, showed that the method met the acceptance criteria for intra-day variability (CV): intra-day CV were all ≤ 25 % for all concentrations ≥ 1 µg/kg < 10 µg/kg, ≤ 15 % for concentrations ≥ 10 µg/kg < 100 µg/kg, and ≤ 10 % for concentrations ≥ 100 µg/kg.

The inter-day variability CV were all ≤ 32 % for concentrations ≥ 1 µg/kg < 10 µg/kg, ≤ 23 % for concentrations ≥ 10 µg/kg < 100 µg/kg, and ≤ 16 % for concentrations ≥ 100 µg/kg.

Table 28. Accuracy & Precision Data for Fat

Fat batch	Result (µg/kg)	Accuracy (%)	Result (µg/kg)	Accuracy (%)	Result (µg/kg)	Accuracy (%)
QC (µg/kg)	5.00		350.0		750.0	
Intra-day 1 mean	5.36	106	328	91.7	709	94.9
CV (%)	0.56	10.3	15.8	4.82	23.3	3.31
Intra-day 2 mean	4.72	93.4	348	97.2	744	99.5
CV (%)	0.27	5.70	19.4	5.45	35.3	4.69
Intra-day 3 mean	5.04	99.9	335	93.7	718	96.1
CV (%)	0.59	11.7	12.8	3.82	244	3.45
Inter-day mean	5.04	100	337	94.2	724	96.8
CV (%)	0.53	10.6	17.4	5.10	30.6	4.22

Table 29. Accuracy & Precision data for Liver

Liver batch	Result (µg/kg)	Accuracy (%)	Result (µg/kg)	Accuracy (%)	Result (µg/kg)	Accuracy (%)

QC (µg/kg)	5.00		350.0		750.0	
Intra-day mean	5.25	104	292	95.7	707	94.7
CV (%)	0.26	5.01	12.4	4.51	24	3.47
Intra-day mean	4.53	89.8	356	99.7	752	101
CV (%)	0.23	4.96	7.2	2.11	26	3.36
Intra-day mean	4.83	95.5	339	94.8	689	92.3
CV (%)	0.58	12.0	8.2	2.33	17.7	2.57
Inter-day mean	4.87	96.4	346	96.7	716	95.9
CV (%)	0.48	9.78	12.9	3.74	34.5	4.83

Table 30. Accuracy & Precision Data for Kidney

Kidney batch	**Result (µg/kg)**	**Accuracy (%)**	**Result (µg/kg)**	**Accuracy (%)**	**Result (µg/kg)**	**Accuracy (%)**
QC (µg/kg)	5.00		350.0		750.0	
Intra-day mean	5.33	106	356	100	745	100
CV (%)	0.31	5.93	10.4	3.00	21.9	2.95
Intra-day mean	4.80	95.0	351	98.3	720	96.4
CV (%)	0.17	3.44	6.9	2.04	8.2	1.15
Intra-day mean	5.36	106	331	92.5	701	93.8
CV (%)	0.14	2.70	8.0	2.37	16.6	2.37
Inter-day mean	4.89	102	347	96.9	722	96.7
CV (%)	1.04	6.59	14.2	4.17	24.4	3.40

Table 31. Accuracy & Precision Data for Muscle

Muscle batch	**Result (µg/kg)**	**Accuracy (%)**	**Result (µg/kg)**	**Accuracy (%)**	**Result (µg/kg)**	**Accuracy (%)**
QC (µg/kg)	5.00		350.0		750.0	
Intra-day mean	4.65	92.0	342	95.5	731	97.9
CV (%)	0.44	9.41	3.8	1.07	15.8	2.20
Intra-day mean	4.71	93.3	348	97.4	699	93.6
CV (%)	0.24	5.23	16	4.52	15.4	2.19
Intra-day mean	4.49	88.9	295	82.4	644	86.2
CV (%)	0.399	8.91	17.6	6.02	16.7	2.58

Inter-day mean	4.62	91.4	328	91.8	691	92.6
CV (%)	0.36	7.82	27.7	8.44	40	5.80

Stability

Monepantel sulphone was demonstrated to be stable when stored in the auto sampler chamber at room temperature for 3 days and at approximately 15 °C for 4 days and in the cool room at approximately 6 °C for 6 days.

To evaluate the stability in matrix extract, triplicate incurred samples of fat, liver, kidney and muscle were homogenized with extraction solvent and analysed following storage of extracts in capped polypropylene centrifuge tubes stored at approximately 6 °C for 2 weeks.

To evaluate stability, five subsamples of incurred residue samples for each tissue was analysed within 1 week of receipt; other samples were stored for the evaluation of short term bench top (4 h at room temperature prior to addition of solvent), over 3 freeze/thaw cycles and long-term storage stability (up to 7.5 months continually frozen at approximately -10 to -22 °C).

Monepantel sulphone was demonstrated to be stable when stored under all the above-specified conditions using incurred material in the studies (Table 32).

Table 32. Results of Stability Studies

	Matrix	**Fat**	**Liver**	**Kidney**	**Muscle**
Batch	**Time point**	**Residue (µg/kg)**	**Residue (µg/kg)**	**Residue (µg/kg)**	**Residue (µg/kg)**
Initial Concentration (1 week)	Mean	608	222	80.7	22.7
	CV (%)	2.67	1.22	2.14	5.78
Reference value T_0	**Accuracy (%)**	100	100	100	100
Extracts 14 days at ~6 ºC	Mean	608	212	67.5	19.0
	CV (%)	1.24	0.817	2.96	8.97
	**Accuracy (%)	106	99.5	98.8	91.8
4 h ambient prior to extraction	Mean	594	208	70.3	22.0
	CV (%)	1.22	2.17	3.56	6.46
	*Accuracy (%)	97.7	93.7	87.2	96.8
Freeze/thaw 3 cycles	Mean	595	214	65.7	21.1
	CV (%)	1.85	0.540	2.04	1.42
	*Accuracy (%)	97.9	96.4	81.5	93.0
	**Accuracy (%)	104	100	96.2	102
Continually frozen 3 months	Mean	575	213	68.3	20.7
	CV (%)	3.95	3.39	2.93	3.70
	*Accuracy (%)	94.5	96.1	84.7	91.0
	Mean	589	212	66.7	21.0
	CV (%)	1.79	1.25	6.32	5.24

Continually frozen 4 months	*Accuracy (%)	96.8	95.7	82.7	92.5
Continually frozen 7.5 months	Mean	578	197	65.5	21.6
	CV%	2.77	2.33	14.6	4.99
	*Accuracy (%)	95.1	88.9	81.2	95.3
* Accuracy compared to initial analysis (T_0) ** Accuracy compared to the 3 month frozen cycle results					

Appraisal

Monepantel was previously evaluated by the Committee for use in sheep. Monepantel for use in cattle was included in the agenda for the current meeting of the Committee at the request of the 23rd Session of the CCRVDF. Monepantel is an anthelmintic of the amino-acetonitrile derivative class indicated for the treatment of roundworms in cattle. The recommended dose is 2.5 mg/kg bw and the maximum dose used is 3.7 mg/kg bw. Up to three doses per season can be applied with a minimum retreatment interval of 21 days.

Two GLP-compliant ADME studies were evaluated. In the first study, cattle were treated orally with [^{14}C]-monepantel (3.75 mg/kg bw). Systemic absorption was relatively high, with TRR reaching peak concentrations at 24 h after dosing. Initial elimination was rapid with a half-life of about 36 h, but this slowed progressively at later times. Residues were still detectable at the final sampling time point at *ca.* 5 µg equivalents/kg in blood and plasma. About 21 % of the dose was eliminated in the urine over 3 days. Approximately 36 % of the dose was eliminated in the faeces. About 60 % of the dose was recovered in excreta over 3 days, with the remaining material distributed in the tissues.

In the second GLP compliant study, conducted as part of the pivotal cold residue depletion study, the pharmacokinetic profiles of monepantel and monepantel sulphone in blood were investigated in cattle orally dosed three times 21 days apart at 3.75 mg/kg bw monepantel. The blood samples were analysed for monepantel and monepantel sulphone using a validated LC-MS/MS method. Monepantel sulphone was the dominant residue in the blood. The blood profiles of the animals were compared and no accumulation of monepantel and monepantel sulphone in blood was evident. Additionally, the blood profile (measuring individual metabolites) was similar to that observed in the cattle ADME study where only TRRs were measured.

Edible tissues, blood, bile and excreta from the GLP-compliant radiolabelled ADME study in cattle were investigated for extractability of residues, and metabolite profiles. Radioactivity was extracted readily from fat, kidney and muscle, with simple solvent extraction. Liver residues proved more difficult to extract, especially with increasing time after dose administration.

The major component present in all tissues cochromatographed with monepantel sulphone. The parent molecule, monepantel, was a minor constituent. Minor unknown components of high polarity were detected in the liver, kidney and muscle.

A non-GLP in vitro metabolism study was conducted to investigate the metabolism of monepantel in intact rat, cattle and sheep hepatocytes. Incubations were profiled by HPLC and profiles were similar across the tested species. The results of the in vitro study were consistent with those in the in vivo ADME study.

Selected samples of edible tissues from the radiolabelled GLP-compliant ADME study in cattle were investigated for TRR. The calculated half-lives of TRR were 4-5 days for fat, kidney and muscle but *ca.* 10 days for liver, consistent with the presence of bound residues. The order of residues in tissues is fat > liver > kidney > muscle, indicative of lipophilic substances, and is the same as that seen for sheep. Because the major residue is monepantel sulphone, it is the marker residue identified for monepantel in cattle tissues. It also is the marker identified previously for sheep tissues. Concentrations of the monepantel sulphone residues in cattle tissue were determined as part of the method validation study and used to determine the MR to TRR ratio cattle tissues.

Two GLP-compliant residue depletion studies were conducted in cattle using unlabelled monepantel.

The first study was conducted using crossbred beef cattle. Cattle were treated orally three times at a nominal dose of 3.75 mg monepantel/kg body weight. Doses were administered 21 days apart. Animals were slaughtered 4, 7, 10 and 13 days after the final treatment. Samples of muscle, kidney, liver, renal fat and subcutaneous fat were collected and analysed for monepantel sulphone.

The maximum monepantel sulphone residues were observed in the Day 4 samples. While initial residues in renal fat were highest, by the later sampling times residues were highest in subcutaneous fat. The calculated half-life estimates for muscle, kidney, liver, renal and subcutaneous fat were 2.8, 2.7, 2.7, 2.9 and 5.1 days, respectively.

The second study also was conducted using crossbred beef cattle. Again, cattle were treated orally three times at a nominal dose of 3.75 mg monepantel/kg body weight and again doses were administered 21 days apart. In this second study, animals were slaughtered 21, 42, 56 and 85 days after the final treatment. Samples of muscle, kidney, liver, renal fat and subcutaneous fat were collected and analysed for monepantel sulphone residues.

The maximum monepantel sulphone residues were observed in the Day 21 samples. At the final sampling time, all residues were below the method limit of quantification (LOQ=5 μg/kg).

Using the data from the first GLP-compliant residue depletion study, the upper one-sided 95 % confidence interval over the 95th percentile of the 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 0.88 in fat, 0.2 in liver and 0.62 in kidney. The ratio of the concentration of marker residue to total residues in muscle is available at only the first sampling time and is 0.67. Additionally, a correction factor of 0.94 to account for the mass difference between monepantel sulphone (the marker residue) and monepantel was applied.

A validated LC-MS/MS, demonstrated to be suitable for routine analysis of monepantel in bovine tissue matrices, was available that met the requirements of the validation criteria established by the CCRVDF, as contained in CAC/GL 71-2009.

Maximum Residue Limits

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

- An ADI of 0-0.02 mg/kg bw was previously established by the Committee.
- An Acute Reference Dose was considered unnecessary.
- The metabolite, monepantel sulphone, is the marker residue in cattle tissues.
- Fat contains the highest concentration of monepantel sulphone at all sampling times, followed by liver, kidney and muscle. Liver and fat can serve as the target tissues.
- The ratios of the concentration of marker residue to total residues are 0.88 in fat, 0.20 in liver and 0.62 in kidney at 7 days post-treatment. The ratio of the concentration of marker residue to total residues in muscle is available only at the first sampling time, 3 days post-treatment, and is 0.67.
- A correction factor of 0.94 is applied to account for the mass difference between monepantel sulphone (the marker residue) and monepantel.
- A validated analytical method for the determination of monepantel sulphone in edible cattle 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 95^{th} percentile of the residue concentrations (UTL 95/95)

Consistent with the conditions of good veterinary practice in the Member State with an approved used of monepantel in cattle, the Committee recommended MRLs determined as monepantel sulphone, expressed as monepantel, in cattle tissue of 300 µg/kg in muscle, 1000 µg/kg in kidney, 2000 µg/kg in liver and 7000 µg/kg in fat.

Exposure Assessment

Exposure to monepantel residues is considered to occur only through its use as a veterinary drug in the muscle, liver, kidney and fat of sheep and other ovines and cattle and other bovines.

In the JECFA dietary exposure assessment, sheep and other ovine muscle, liver, kidney and fat and cattle and other bovine muscle, liver, kidney and fat, were contributors to dietary exposure. Where appropriate consumption data for a specific category (e.g., ovine kidney) were not available, the more generic category (e.g., mammalian kidney) was used in conjunction with the associated highest residue to estimate exposure.

The GECDE for monepantel was calculated based on median residues (STMR) 7 days or 5 days after administration of the drug in sheep and cattle respectively. The STMR in the specific tissues of both species were included in the exposure estimate wherever possible. In all other cases, the highest STMR from either species was used together with the most appropriate food consumption available for the tissue of interest.

The GECDE for the general population is 13.7 µg per kg bodyweight per day, which represents 68 % of the upper bound of the ADI of 20 µg per kg bw. The GECDE for children is 5.0 µg per kg bodyweight per day, which represents 25 % of the upper bound of the ADI of 20 µg per kg bw. The GECDE for infants is 4.4 µg per kg bodyweight per day, which represents 22 % of the upper bound of the ADI of 20 µg per kg.

Table 33. Exposure Assessment

Category	Type	Median concentration[1] (µg/kg)	Mean consumption[2] (whole population, g/kg bw)	Highest reliable percentile consumption[3] (consumers only, g/kg bw) /[percentile used]	MR:TR ratio	Correction	Exposure (µg/kg bw/day)		GECDE[4]	
							mean	Highest Reliable Percentile	µg/kg bw/day	% ADI
General Population										
Mammalian muscle	Sheep and other ovines	152	0.163	4.320 [97.5]	1.00	0.94	*0.023*	0.617		
Mammalian offal	Cattle liver	831	0.028	3.322 [97.5]	0.2	0.94	0.110	*12.976*		
Mammalian offal	Cattle Kidney	362	0.039	3.976 [97.5]	0.62	0.94	*0.021*	2.182		
Mammalian t fat[6]	Sheep fat	2660	0.002	0.105 [97.5]	0.66	0.94	*0.006*	0.399		
Mammalian muscle	Beef and other Bovines	107	0.959	4.442 [97.5]	0.67	0.94	*0.144*	0.667		
Mammalian offal	Sheep liver	1259	0.009	2.103 [97.5]	0.66	0.94	*0.017*	3.879		
Mammalian offal	Sheep Kidney	406	0.160	0.105 [97.5]	0.66	0.94	*0.001*	0.062		
Mammalian fat[6]	Cattle fat	3257	0.143	0.610 [97.5]	0.88	0.94	*0.497*	2.124		
TOTAL							0.709	12.976	13.684	68.4
Children										
Mammalian muscle	Sheep and other ovines	152	0.353	9.425 [97.5]	1.00	0.94	*0.050*	1.347		
Mammalian offal	Cattle offal[7]	831	0.049	0.504 [97.5]	0.20	0.94	*0.193*	1.969		
Mammalian fat[6]	Sheep fat	2660	0.001	0.146 [97.5]	0.66	0.94	*0.005*	0.554		
Mammalian muscle	Beef and other Bovines	107	1.940	8.394 [97.5]	0.67	0.94	*0.291*	1.260		

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