TRICLABENDAZOLE

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Addendum to the monographs prepared by the 40th and 66th meetings of the Committee and published in FAO Food & Nutrition Paper 41/5 and FAO JECFA Monographs 2, respectively.

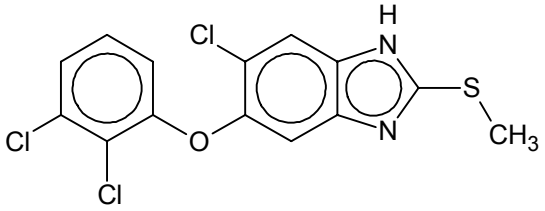
IDENTITY

Chemical name: 5-Chloro-6-(2,3-dichlorophenoxy)-2-methylthio-1H-benzimidazole {International Union of Pure and Applied Chemistry name}

Chemical Abstracts Service (CAS) number: 68786-66-3

Synonyms: Triclabendazole (common name); CGA 89317, CGP 23030; proprietary names Fasinex®, Soforen®, Endex®, Combinex®, Parsifal®, Fasimec®, Genesis®, Genesis™ Ultra.

Structural formula:



Molecular formula: $C_{14}H_9Cl_3N_2OS$

Molecular weight: 359.66

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredients: Triclabendazole

Appearance: White crystalline solid

Melting point: 175-176°C (Merck), α-modification; 162°C, β-modification

Solubility: Soluble in tetrahydrofuran, cyclohexanone, acetone, iso-propanol, n-octanol, methanol; slightly soluble in dichloromethane, chloroform, toluene, xylene, ethyl acetate; insoluble in water, hexane.

RESIDUES IN FOOD AND THEIR EVALUATION

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed triclabendazole at its 40th and 66th meetings (FAO/WHO, 1993, 2006). At the 40th meeting the Committee established an ADI of 0-3 µg/kg of bodyweight (0-180 µg per day for a person of 60 kg bodyweight) and recommended the following Maximum Residue Limits (µg/kg):

Species	MRLs recommended by the 40th JECFA (µg/kg)			
	Muscle	Liver	Kidney	Fat
Sheep	100	100	100	100
Cattle	200	300	300	200

The FAO Food Nutrition Paper residue monograph prepared at the fortieth meeting (FAO, 1993) states: "The marker residue for triclabendazole is 5-chloro-6-(2', 3'-dichlorophenoxy)-benzimidazole-2-one and is produced when common fragments of triclabendazole-related residues are hydrolysed under alkaline conditions at 90-100°C… …Marker residue levels can be converted into triclabendazole equivalents by multiplying by a conversion factor of 1.09." In the report from the fortieth meeting of the Committee (FAO/WHO, 1993), it is noted in Annex 2 that the MRLs are expressed as 5-chloro-6-(2', 3'-dichlorophenoxy)-benzimidazole-2-one.

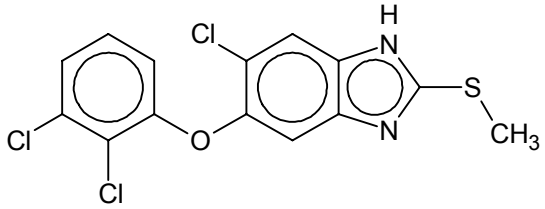
The 66th meeting defined the marker residue as "keto-triclabendazole" and recommended the following Maximum Residue Limits (µg/kg):

Species	MRLs in Tissues (µg/kg)			
	Muscle	Liver	Kidney	Fat
Cattle	150	200	100	100
Sheep	150	200	100	100
Goat	150	200	100	100

The sponsor (correctly) defined the marker residue as "sum of the extractable residues that may be oxidised to keto-triclabendazole" and proposed MRLs below as consistent with withdrawal periods of 35 days after oral administration to cattle and 27 days after oral administration to sheep and goats.:

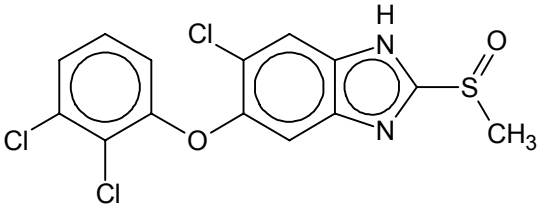
Species	MRLs in Tissues (µg/kg)			
	Muscle	Liver	Kidney	Fat
Cattle	275	600	375	200
Sheep	275	600	375	200
Goat	275	600	375	200

Triclabendazole is 6-chloro-5-(2', 3'-dichlorophenoxy)-2-methylthio-1-*H*-benzoimidazole (CAS number 68786-66-3). Its structure and the structure of some compounds related to it (e.g., metabolites and conversion products) are given in the scheme below:



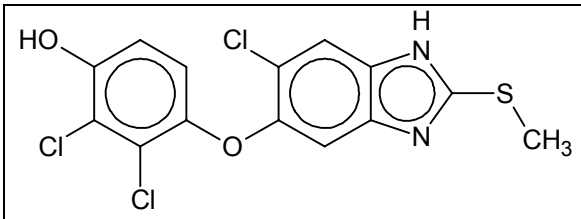
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylthio)-1*H*-benzimidazole
Molecular Formula = $C_{14}H_9Cl_3N_2OS$
Formula Weight = 359.65
Synonyms and abbreviations are:
CGA 89 317 and CGP 23 030.

Triclabendazole



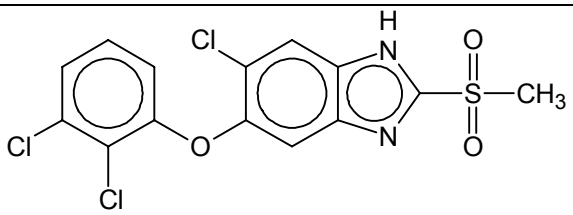
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylsulfinyl)-1*H*-benzimidazole
Molecular Formula = $C_{14}H_9Cl_3N_2O_2S$
Formula Weight = 375.65
Synonyms and abbreviations are:
CGA 110 752

Triclabendazole sulphoxide



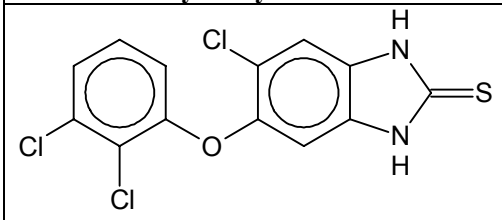
2,3-dichloro-4-{[6-chloro-2-(methylthio)-1*H*-benzimidazol-5-yl]oxy}phenol
Molecular Formula = $C_{14}H_9Cl_3N_2O_2S$
Formula Weight = 375.65
Synonyms and abbreviations are:
CGA 161 944

4-Hydroxytriclabendazole

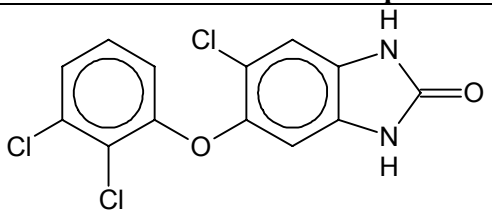


6-chloro-5-(2,3-dichlorophenoxy)-2-(methylsulfonyl)-1*H*-benzimidazole
Molecular Formula = $C_{14}H_9Cl_3N_2O_3S$
Formula Weight = 391.65
Synonyms and abbreviations are:
CGA 110 753

Triclabendazole sulphone



5-chloro-6-(2,3-dichlorophenoxy)-1,3-dihydro-2*H*-benzimidazole-2-thione
Molecular Formula = $C_{13}H_7Cl_3N_2OS$
Formula Weight = 345.63
Synomyms and abbreviations are:
CGA 77 336



5-chloro-6-(2,3-dichlorophenoxy)-1,3-dihydro-2*H*-benzimidazol-2-one
Molecular Formula = $C_{13}H_7Cl_3N_2O_2$
Formula Weight = 329.56
Synonyms and abbreviations are:
CGA 110 754

Keto-triclabendazole

Conditions of use

Triclabendazole is an anthelmintic used for the control of liver fluke, *Fasciola hepatica* and *F. gigantica*, in cattle, sheep and goats. Triclabendazole is contained in oral suspensions for cattle, sheep and, in some countries, goats as well as in pour-on formulations for cattle. Triclabendazole is also used for the treatment of fascioliasis in humans.

Dosage

Triclabendazole is administered to cattle as a drench at a nominal dose rate of 12 mg/kg of bw and as a pour-on application at a nominal dose rate of 30 mg/kg of bw. It is administered orally to sheep and goats at a nominal dose rate of 10 mg/kg of bw. Veterinary advice regarding the interval for repeat treatments differs from country to country; however, the recommended interval for routine treatment during the Fasciola season is reported to be 10 weeks.

PHARMACOKINETICS AND METABOLISM

Laboratory Animals

Rats

In a study conducted by Muecke (1981), two female and two male rats were each given a single oral dose of either 0.5 or 25 mg [^{14}C]-triclabendazole/kg of bw. The radioactive label was at the carbon atom in position 2 of the benzimidazole ring system. Radioactivity was determined by liquid scintillation counting. Urine was directly added to scintillation fluid for counting whereas tissues were directly combusted before counting and faeces were lyophilised, homogenized and combusted prior to

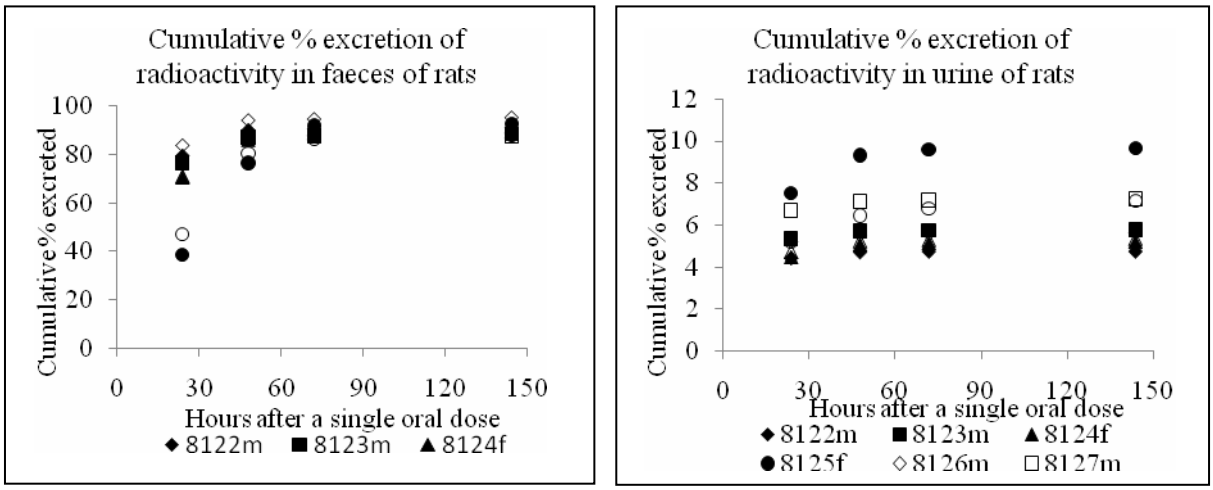
counting. Samples of faeces were extracted with methanol/water 80:20 and subjected to co-chromatography on TLC plates with reference standards.

Amounts of expired $^{14}CO_2$ were minimal (<0.05% of the administered dose). Radioactivity was primarily excreted in faeces and to a lesser and more variable extent in urine. Table 1 shows the cumulative percentage excretion of radioactivity of total dose administered in faeces and urine calculated over a time period of 144 hours (6 days). The results suggest that recovery was approximately 97% after 144 hours in this study. Individual data points are given in Figure 1.

Table 1: Cumulative percentage excretion of radioactivity in rats after a single oral dose of either 0.5 or 25 mg [^{14}C]-triclabendazole/kg of bw, relative to dose administered.

	Results obtained with the low dose			Results obtained with the high dose			Results of both dose levels combined		
	Faeces	Urine	Faeces plus urine	Faeces	Urine	Faeces plus urine	Faeces	Urine	Faeces plus urine
Parameter estimate	Percent of radioactivity recovered in 144 hours after a single oral dose								
Mean	90.9	6.1	97.0	90.1	6.3	96.5	90.5	6.2	96.7
St Dev	1.8	2.4	3.5	3.5	1.0	2.9	2.6	1.7	3.0
Min	88.4	4.2	94.2	87.8	5.3	95.0	87.8	4.2	94.2
Max	92.6	9.6	102.2	95.2	7.3	100.8	95.2	9.6	102.2

Figure 1: Cumulative percentage excretion of radioactivity in rats after a single oral dose of either 0.5 or 25 mg [^{14}C]-triclabendazole/kg of bw, relative to dose administered.

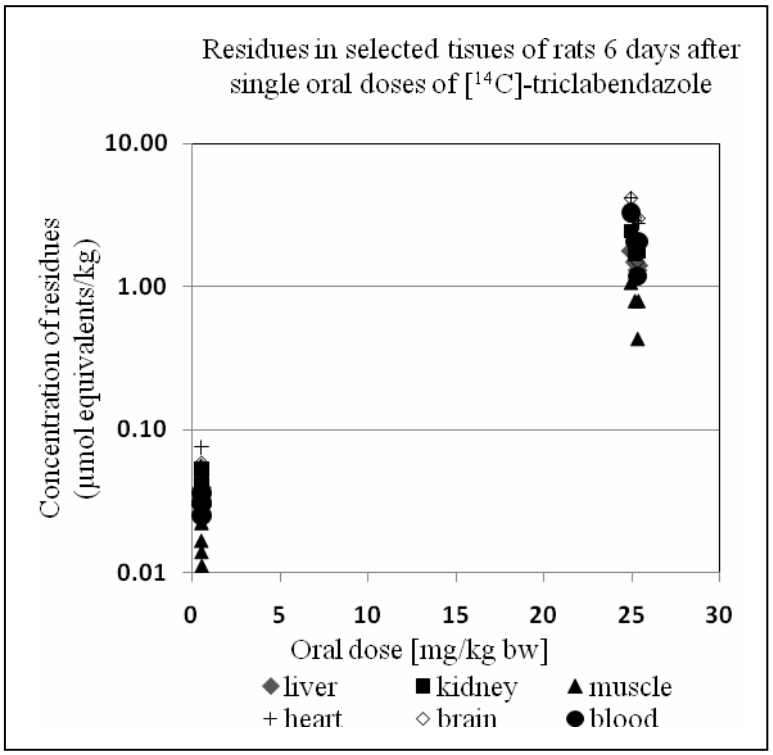


The code numbers in the legend refer to animal ID; m = male; f = female. The filled symbols indicate results obtained with the lower dose (approximately 0.5 mg/kg of bw); the open symbols indicate results obtained with the higher dose (approximately 25 mg/kg of bw).

The extracts of faeces contained some unchanged drug (7% of dose), but mainly the corresponding sulphoxide (24% of dose) and small amounts of the sulphone (2% of dose) metabolites. Approximately 27% of the radioactivity in faeces was not extracted with three sequential extractions with the methanol/water solvent. The dose had no significant influence on the qualitative metabolite pattern. The structure of the more polar metabolites in urine could not be determined in this study.
Residues in selected tissues were determined six days after dose administration. Residue concentrations found were highest in heart, brain and blood. The individual results for some selected tissues (liver, kidney, muscle, heart, brain and blood) are given in Figure 2. Residues in fat were below

the limit of detection (0.06 mg/kg), except in one sample obtained from a rat that had received the higher dose. The administered high and low doses differed by a factor of 47.4. The ratio of radioactivity found in the tissues (geometric mean) represented in Figure 2 was 40.7, 42.9, 47.8, 49.4, 57.9 and 67.2 for liver, kidney, muscle, heart, brain, and blood, respectively. An increase in dose had an over-proportional effect on residue distribution into certain tissues.

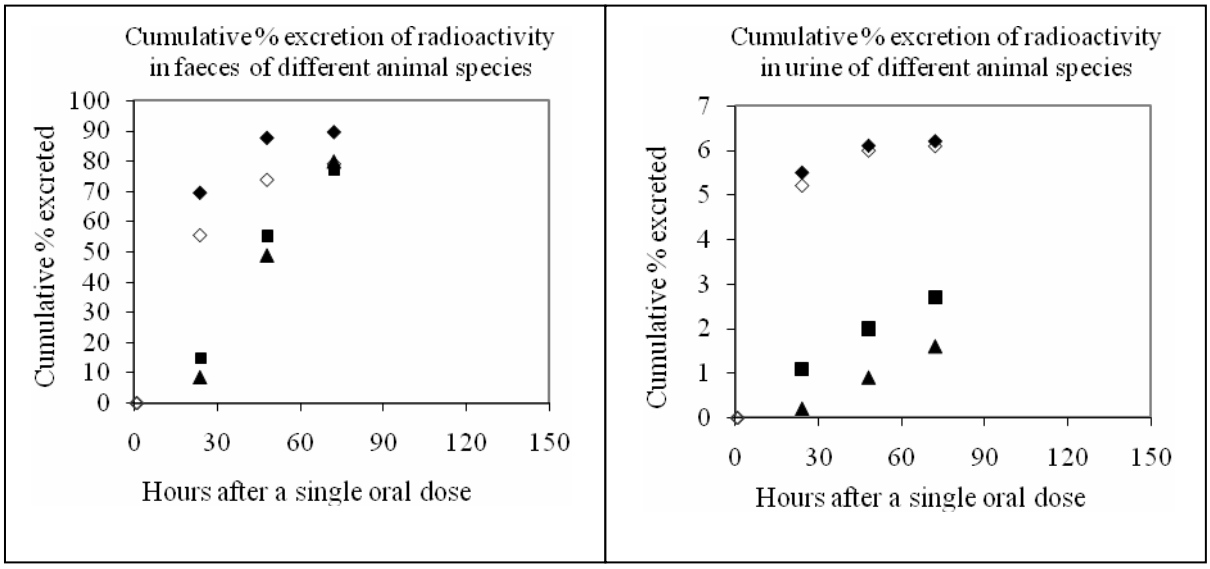
Figure 2: ^{14}C residues in tissues of rats six days after a single oral dose of approximately 25 mg [^{14}C]-triclabendazole/kg of bw.



Rats, sheep and goats

After a single oral dose of 10 mg/kg of bw to one sheep and to one goat and 0.5 or 25 mg/kg of bw in two rats (one male and one female), excretion of radioactivity was monitored for 72 hours in faeces and urine (Hamböck, 1983). The rates of excretion in faeces and urine of rats relative to the total dose administered were similar to those in the study of Muecke, (1981); however, they were lower for both routes in the female sheep and in the female goat at early time intervals. Excretion was slowest in the goat. The results obtained with the individual animals are shown in Figure 3.

Figure 3: Excretion data obtained in the study of Hamböck (1983).



Legend: Solid square = female sheep; solid triangle = female goat; open diamond = low dose male rat; solid diamond = high dose female rat

Metabolites were determined in samples of pooled faeces (0-72 hours in a sheep, a goat and a male rat; 0-48 hours in a female rat); the radioactivity in these samples corresponded to 76.7, 79.8, 90.0, and 87.6 % of the total dose in the sheep, goat, male rat and female rat, respectively. Some 50-72% of the radioactivity was extractable with methanol. Metabolites were identified by co-chromatography with reference standards on TLC plates. Structures were further confirmed by specific transformations using chemical reduction/oxidation reactions, mass spectrometry and nuclear magnetic resonance. Similarly, pooled urine samples were analysed. Metabolites in urine were generally more polar than metabolites in faeces. The least polar metabolite in urine was keto-triclabendazole.

Four major metabolites in addition to the parent drug were identified in faeces of all three species. In the sheep and goat, most of the excreted metabolites were unchanged parent drug, however, in rats, the sulphoxide was the major excreted metabolite (Table 2). The difference between the two ruminant species and rats was assumed to reflect differences in intestinal flora rather than differences in biotransformation pathways.

Table 2: Characterisation of radioactive substances extracted from pooled faeces.

Species	Rat		Sheep	Goat
Sex	male	female	female	female
Dose (mg/kg bw)	0.5	25	10	10
Identification of the radioactive zone on TLC plates	% of administered dose			
6-chloro-5-(2', 3'-dichlorophenoxy)-2-methylthio-1-*H*-benzoimidazole (parent drug) (CGA 89 317) $C_{14}H_9Cl_3N_2OS$; MW: 359.66	6	9	19	25
6-chloro-5-(2',3'-dichlorophenoxy)-2-methylsulfinyl-1-*H*-benzimidazole (sulphoxide) (CGA 110 752) $C_{14}H_9Cl_3N_2O_2S$; MW: 375.66	20	27	7	6
6-chloro-5-(2',3'-dichlorophenoxy)-2-methylsulfonyl-1-*H*-benzimidazole (sulphone) *plus minor unknowns* CGA 110 753 $C_{14}H_9Cl_3N_2O_3S$; MW: 391.66	3	3	2	2
5-chloro-6-(2',3'-dichlorophenoxy)-1,3-dihydro-2*H*-benzimidazol-2-one (keto-triclabendazole) (CGA 110 754) $C_{13}H_7Cl_3N_2O_2$; MW: 329.57	8	10	2	3
Minor unknowns plus 6-chloro-5-(2',3'-dichloro-4-hydroxyphenoxy)-2-methylthio-1-*H*-benzimidazole (hydroxy-triclabendazole) (CGA 161 944) $C_{14}H_9Cl_3N_2O_2S$; MW: 375.66	11	12	13	9
Unknowns	9	13	6	6
Non-extractable	32	16	27	29

The elimination of triclabendazole and its metabolites was also investigated in a bile duct-cannulated male rat receiving 4.55 mg/kg as a single oral dose. In this study, 34% of the dose was excreted with the bile. Comparison of the results obtained with bile duct-cannulated and non-cannulated rats found that a significant proportion of the absorbed dose was eliminated in bile and only a small proportion of the radioactivity in faeces is unabsorbed triclabendazole. The biliary metabolites were not further characterised; however, the investigators noted that they were not acid-labile.

Rats

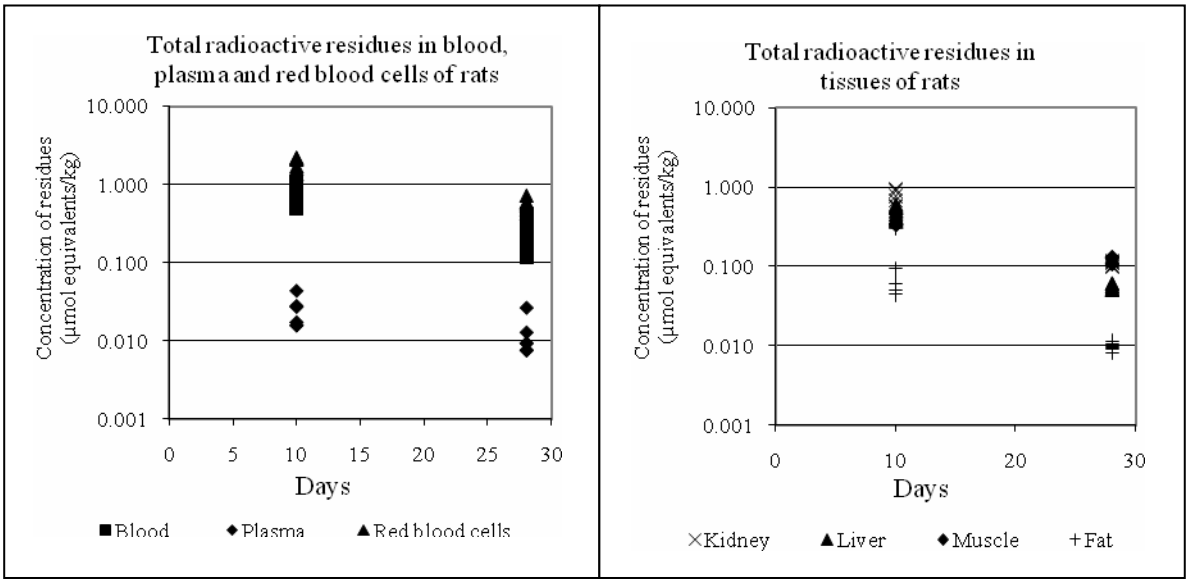
Excretion balance and tissue distribution studies (Hardwick, 2004a) were conducted in twelve Sprague Dawley rats dosed orally by gavage at a nominal dose rate of 12 mg (range 10.32-12.14 mg) triclabendazole per kg of bw. Triclabendazole was labelled in the benzene ring of the benzimidazole moiety (specific activity 13.9 MBq/mg). Urine and faeces and expired air were collected from six rats for up to 10 days. At 10 days after dose administration these rats were sacrificed and samples of blood, liver, kidney, muscle and fat were collected. At 28 days after dosing, the remaining six rats were sacrificed and the same types of samples obtained. Radioactivity was determined in blood, plasma, red blood cells, urine, faeces, expired air, cage washes, liver, kidney, muscle and fat. The recovery after 10 days from faeces and urine was variable (Table 3) ranging from 88.2 to 127.7 % of the administered dose per animal, suggesting methodological uncertainties. None of the radio-labelled residues showed similar chromatographic properties to the supplied reference standards; however, co-chromatography showed that all the residues present in cow tissues were also present in rat tissues.

Table 3: Total recovery of radioactivity 0-10 days following a single oral dose of 12 mg [^{14}C]-triclabendazole/kg of bw to male rats.

Animal ID:	101M	102M	103M	104M	105M	106M
Dose (mg/kg)	10.6	10.3	12.1	10.4	11.1	10.3
Matrix	Recovery (% of dose)					
Urine	7.7	10.0	6.8	10.1	7.8	3.9
Faeces	86.5	78.2	98.1	88.9	119.8	106.6
Cage Wash	6.2	3.3	2.0	2.3	1.4	0.3
Cage Debris	<LOQ	0.009	0.010	0.003	0.025	<LOQ
Expired Air	0.007	0.003	0.006	0.008	0.005	<LOQ
Tissues	0.16	0.19	0.19	0.28	0.17	0.17

Residues in tissues after 10 and 28 days, respectively, are shown in Figure 4. Concentrations of residues were highest in erythrocytes and lowest in fat. The rate of depletion between the two time points was highest in fat, followed by liver and kidney and the lowest in muscle and the constituents of blood. Approximately 80% of the residues in liver were non-extractable. The extractable residues showed a wide range of polarities. Alkaline hydrolysis of the tissues followed by acidification increased the extraction efficiency. The reference standards were unaffected by alkaline hydrolysis, with the exception of triclabendazole which hydrolysed to a less polar compound. The authors reported that it is probable that the triclabendazole moiety in the residues extracted after alkaline hydrolysis was intact, although covalently bound (via the sulphur atom) to a cellular component that had been cleaved by hydrolysis. At least seven bound residues were present in alkaline tissue extracts.

Figure 4: Residue depletion in selected tissues of rats dosed orally at a nominal dose of 12 mg [^{14}C]-triclabendazole/kg of bw.



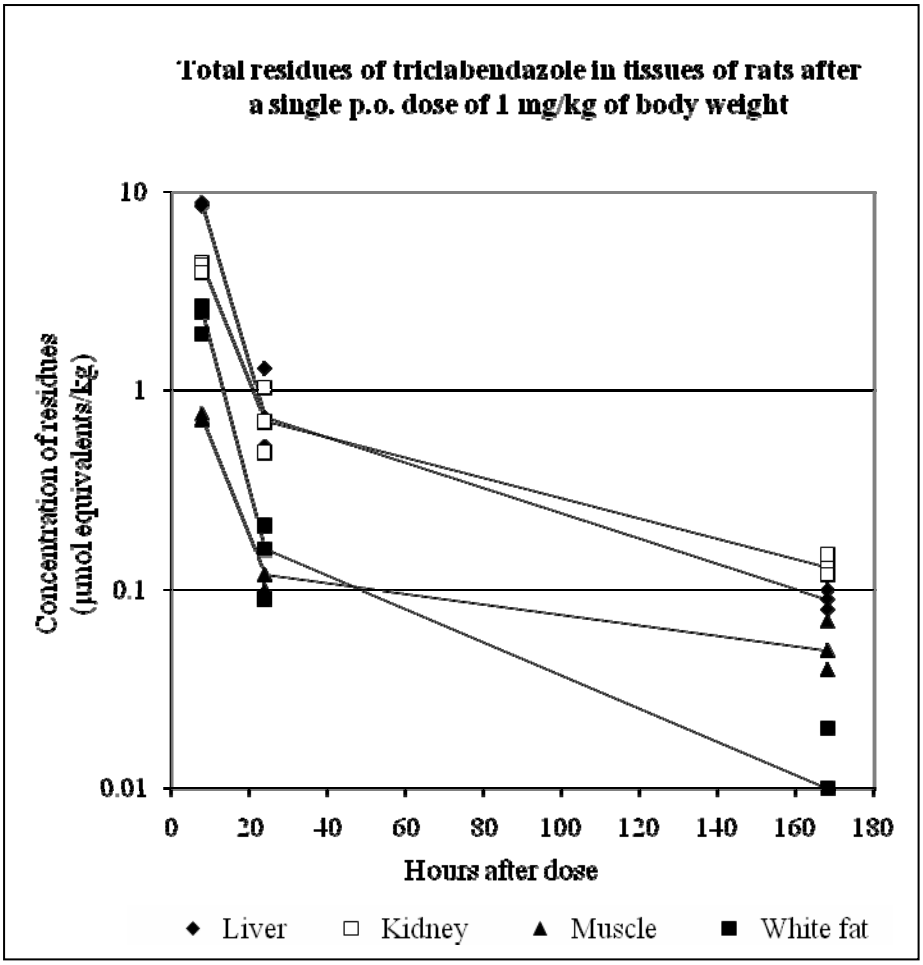
The results of studies into the extractability following NaOH hydrolysis are shown in Table 4 and indicate that 2M NaOH was equally efficient in solubilising parts of the residues in red blood cells, liver and kidney.

Table 4: Partitioning of radioactive residues between dichloromethane and water following treatment with sodium hydroxide (NaOH).

Tissue	Treatment	Dichloromethane Extractable (%)	Remaining in aqueous phase (%)
Red blood cells	2M NaOH	80	12
Liver	0.2 M NaOH	49	39
Liver	2M NaOH	78	25
Muscle	0.2 M NaOH	65	26
Kidney	2M NaOH	75	14

The distribution of radioactivity in blood, plasma and 22 organs and tissues of rats was determined after single i.v. and p.o. administrations and multiple p.o. dosing of 1 mg [^{14}C]-triclabendazole/kg of bw. At 8 hours after an oral dose, residue concentrations were highest in liver, followed by kidney, heart, white fat and lung, brain and muscle. The kinetics of depletion were biphasic with overall rates decreasing in the order of white fat, liver, lung and kidney, muscle, heart and brain. Concentrations in most tissues at 168 hours after dosing were still slightly lower after p.o. dosing compared to i.v. administration. Figure 5 shows some examples of depletion kinetics (the lines connect the median values of three data points of the same tissue type). Once daily dosing with 1 mg [^{14}C]-triclabendazole/kg of bw for 10 days resulted in significant accumulation of residues in all tissues except plasma. The accumulation was most significant in brain and heart.

Figure 5: Depletion of radioactive residues after a single oral dose of 1 mg [^{14}C]-triclabendazole/kg of bw to rats.



Excretion of total radioactivity in urine and faeces of some rats and dogs was determined at some of the same dose levels used for establishing the kinetics in blood and plasma. Excretion was not complete in rats and even less complete in dogs after 168 hours (see Table 5). The fraction of the dose that was excreted in urine was smaller in dogs than in rats and decreased further with increasing oral doses in both species.

Table 5: Cumulative excretion of total radioactivity in urine and faeces of rats and dogs

Dose (mg/kg bw)	Route	Rats						Dogs	
		RA16	RA17	RA 18	RA4	RA5	RA6	1014	1016
		Cumulative excretion (0-168 hrs) in urine and faeces (%)							
0.5	i.v.							83.3	77.2
0.5	p.o.							58.9	51.8
1	i.v.	89.7	88.3	90.4					
1	p.o.				92.7	95.1	94.3		
5	p.o.							68.8	
40	p.o.								89.7

The pharmacokinetics of [^{14}C]-triclabendazole (specific radioactivity of 13.9 MBq/mg and radiochemical purity of 99.7%) was studied following p.o. and i.v. administration to 6-12 weeks old male Sprague Dawley rats weighing 0.27 - 0.37 kg (Needham, 2004a). Lyophilised tissue test material in the study was obtained from cattle treated with [^{14}C]-triclabendazole of specific radioactivity of 6.585 MBq/mg (Needham, 2004b). The design of the study is shown in Table 6.

Table 6: Design of the Needham (2004a) Sprague Dawley rat study

Group	Route and method of administration	Test material	Dose (mg/kg bw)	Number of animals
A	Oral gavage	[^{14}C]-triclabendazole	0.25 $\pm$ 0.001	6
B	Intravenous	[^{14}C]-triclabendazole	0.30 $\pm$ 0.006	6
C	Dietary	[^{14}C]-triclabendazole	0.24 mg $\pm$ 0.034	6
D		lyophilised muscle [1]	0.0013 – 0.0059	6
E		lyophilised liver	0.25 - 3.46 µg	6
F		lyophilised kidney	0.00022 – 0.0023	3
G		lyophilised muscle	0.0035 – 0.0079	5
H		lyophilised liver [2]		5
I	Oral gavage	lyophilised liver	0.0015	3

[1] [^{14}C]-triclabendazole equivalents

[2] Rats did not eat the dose and were removed from the study and allowed to recover for one week. Three of the rats were then dosed orally by gavage with an aqueous suspension of lyophilised liver (Group I).

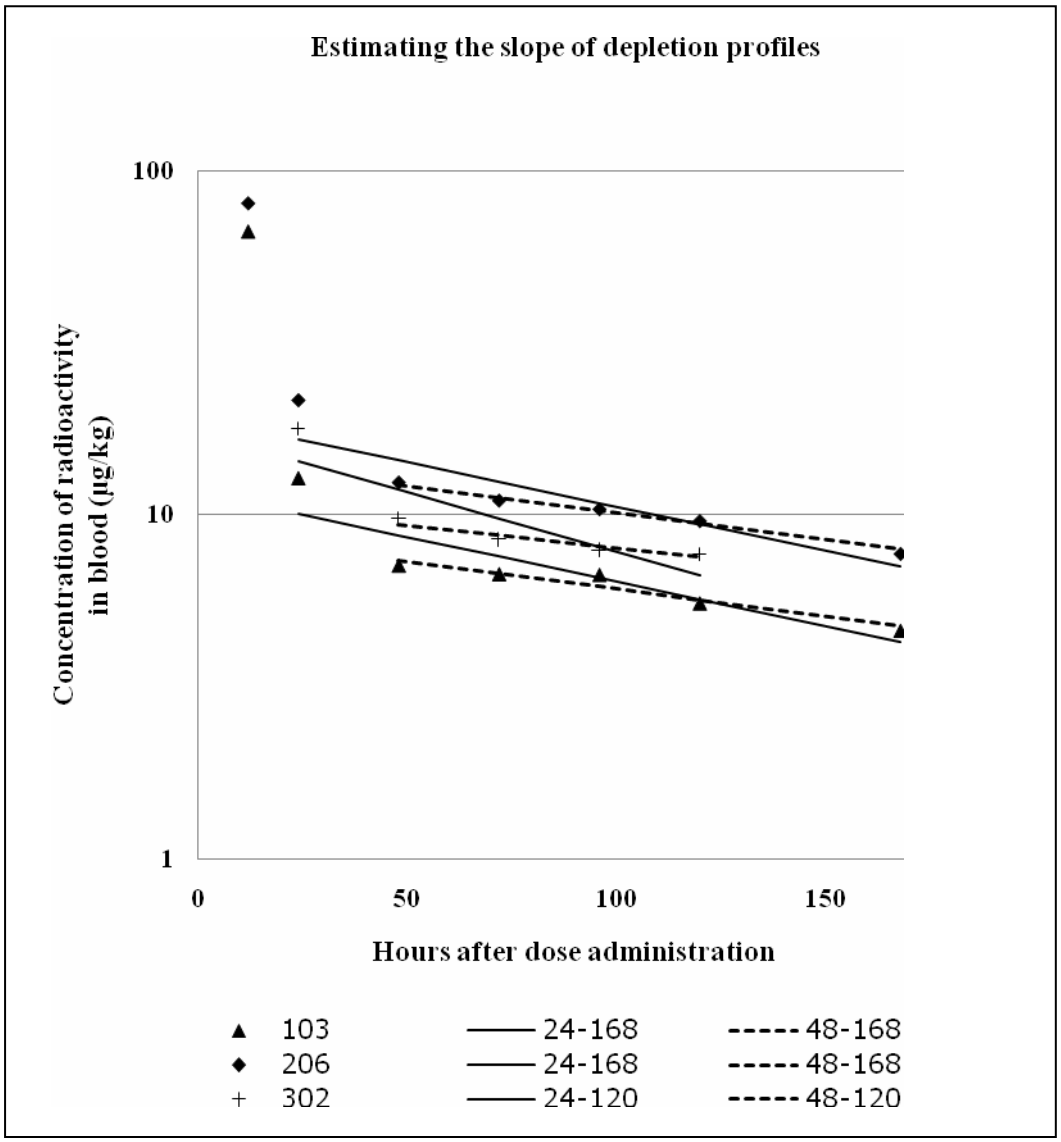
Rats receiving lyophilised tissues were allowed to eat the diet for 4 h before it was removed, weighed, and replaced with normal diet. Blood samples (150 µL) were taken 1, 2, 3, 4, 6, 9, 12, 24, 48, 72, 96 and 120 hours after initial exposure to the diet containing [^{14}C]-triclabendazole (Group C) or lyophilised tissues with incurred residues (Groups D-H). Blood samples were also collected 30 minutes (Group A) and 20 minutes (Group B) after dosing with [^{14}C]-triclabendazole, and 168 hours (Groups C-F at necropsy) after dietary exposure to lyophilised tissues. Liver, kidney and muscle were taken from the rats in Groups A and C-F at necropsy. With Group G, blood samples were taken only from the three animals that consumed the largest quantity of tissue.

The kinetics of the concentration of radioactivity were studied for 168 hours in animals of Groups A and B and for 120 hours in Groups C, G, and I. Detectable concentrations of radioactivity in blood were measured by liquid scintillation counting for all animals following oral, intravenous or dietary

dosing with [^{14}C]-triclabendazole (Group A-C). Data received from Groups D-F were insufficient to determine the pharmacokinetic parameters of the absorbed radioactivity in these animals. Using accelerated mass spectrometry (AMS), it was also possible to determine the levels of radioactivity in the groups that had received lyophilised tissues.

The estimation of bioavailability of the radioactive marker is based on calculations of the $AUC_{0\text{-infinity}}$. These calculations showed that the terminal elimination was not yet complete at 120-168 hours after dosing, the last time point at which blood samples were taken. Figure 6 highlights a problem when estimating the slope of the depletion profiles. The study authors consistently used the results obtained 24 hours after dosing for calculating terminal half-life; however, it is evident from the three examples given in Figure 6 that the concentrations measured at 24 h are dependent on earlier phases of the disposition kinetics.

Figure 6: Estimation of the slope of depletion profiles for calculating terminal half-life and $AUC_{t\text{-infinity}}$ in the Needham (2004a) study.



The graph shows (solid triangle symbols) the last 6 data points of the kinetics obtained with animal 103 (dosed by gavage with 0.25 mg/kg of bw of labelled triclabendazole). The solid line shows the basis for the calculation of the terminal half-life by the authors of the study. The dotted line shows the difference if the calculation is based on the last five data points only. The difference is significant. The

same is true regarding the results obtained with animal 206 (dosed i.v. with 0.30 mg/kg of bw) (shown as solid diamond symbols). The influence on the calculated $AUC_{t\text{-infinity}}$ is significant due to the steeper slopes, the terminal half-lives calculated by the authors are typically shorter and the values of AUC smaller compared with the results of a more adequate calculation. However, since each pair of lines run in parallel the influence on the ratios of the AUCs is minimal and "correct" estimates of the blood bioavailability of doses given by gavage are obtained. The situation is different if one looks at the evaluation of the results obtained with animal 302 (exposed to 0.26 mg/kg of bw in the diet). In this case (cross symbols), the "incorrectly" calculated lines no longer run in parallel, however, the "correctly" calculated lines still do.

The results of the whole experiment were re-calculated in this way. Graphs of all depletion curves were prepared and the data points primarily influenced by the terminal elimination were selected. Using these points the terminal half-lives and the $AUC_{t\text{-infinity}}$ were recalculated and the following results were obtained. All terminal half-lives calculated in this way were longer than those reported by the authors and all values for the $AUC_{t\text{-infinity}}$ were higher. This had no significant influence on the estimated bioavailability when the animals were dosed by gavage; however, in the case of dietary exposure to incurred residues, the calculated bioavailability was increased. Table 7 compares the results of the re-calculation with those obtained by the authors.

Table 7: Results of recalculation of selected results of the Needham (2004) study.

Treatment group	Mean Bioavailability		Mean Terminal Half-life (hrs)	
	Calculated by the authors	Re-calculated	Calculated by the authors	Re-calculated
A	0.715	0.694	147.7	197.4
C	0.676	0.913	91.4	289.4
G	0.064	0.086	90.7	203.7
I	0.098	0.094	135.9	164.9

The re-calculated terminal half-lives are significantly longer than those reported by the authors. The effects on calculated bioavailability are negligible for the experiments with gavage administration (Groups A and I), however they are significant for the dietary exposure (Groups C and G). In general, terminal half-lives are longer than estimated by the authors. In terms of dietary exposure, a weakness of the study design was the absence of sampling points later than 120 hours after exposure. This was also problematic for the re-calculation insofar as frequently too few data points were available for a fully adequate estimation. The main finding of the authors remains unchallenged, namely the bioavailability of residues from incurred tissues (animals sacrificed 28 days after treatment) is low.

These data demonstrated that the absolute bioavailability of [^{14}C]-triclabendazole was approximately 70% when given by gavage to rats. By comparison, the absolute bioavailability of incurred residues administered by gavage to rats was 9.2% for liver, which was higher than for other tissues. Therefore the calculated bioavailability of incurred liver residues in cattle was 13% (9.2/70 x 100) relative to the oral gavage treatment.

Rabbits

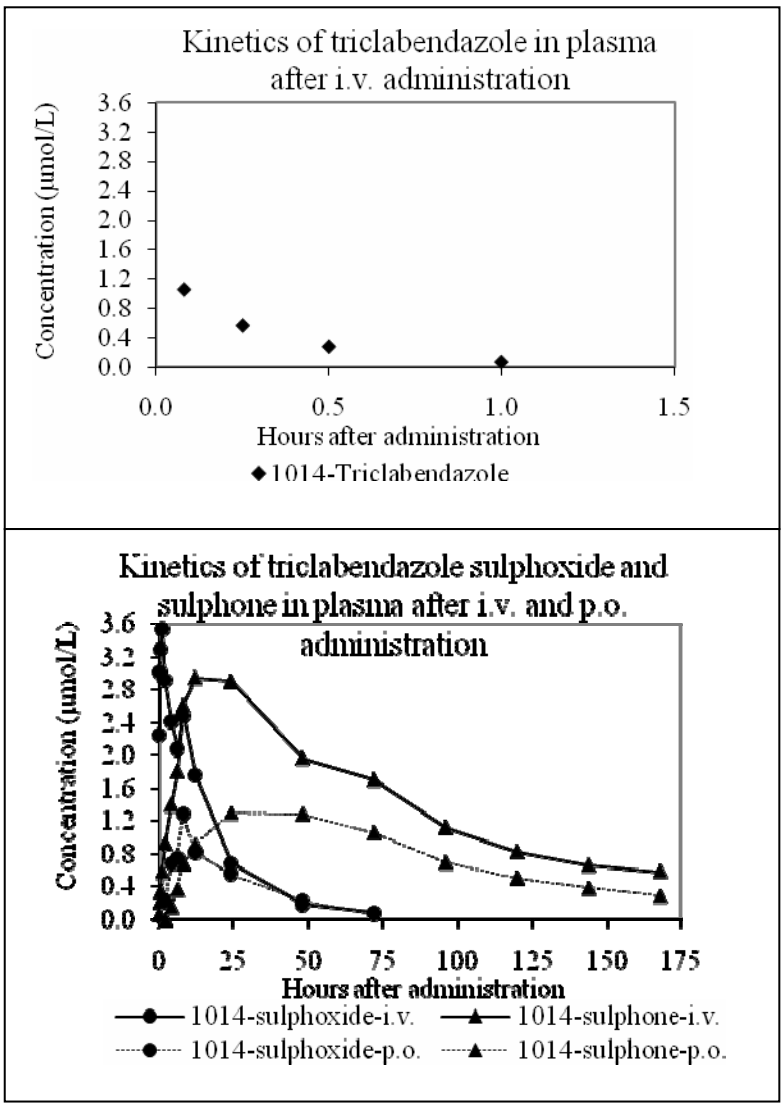
[^{14}C]-labelled triclabendazole was administered i.v. and p.o. to two female Chinchilla rabbits (Wiegand, et al., 1991a). The animals ranged from 2.7 to 4.3 kg over the duration of the study. The doses were administered at intervals of at least 4 weeks, first with an i.v. dose of 3 mg/kg of bw, then with oral doses of 3 mg/kg and 26 mg/kg of bw. The concentration of total radioactivity in blood and plasma, and excretion with urine and faeces, were measured. The absorption of triclabendazole from the gastrointestinal tract was complete irrespective of the dose rate. Radioactive substances in blood demonstrated a biphasic decay in plasma. Most of the radioactivity was cleared from the circulation within 72 hours, predominantly in bile. However, approximately 17-20% of the radioactivity had not

been excreted 7 days after dosing. In addition, plasma concentrations of unchanged triclabendazole, and of its sulphoxide and sulphone metabolites, were determined (Wiegand, et al., 1991b). At 5 minutes after i.v. injection, the concentration of triclabendazole sulphoxide was higher than that of triclabendazole. Following oral dosing, no triclabendazole was detected in plasma. The formation of the sulphone was slower than for the sulphoxide. These two metabolites represented the total radioactivity measured in plasma for the first 8 hours after dosing.

Dogs and Rats

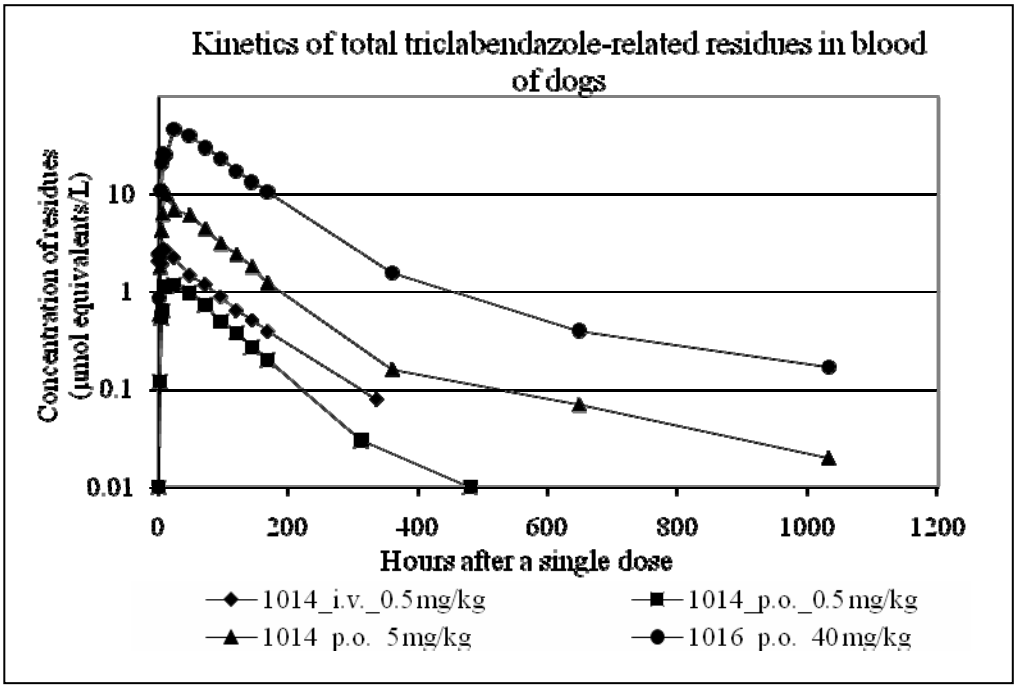
A large study in dogs and rats was conducted that investigated the absorption, distribution and excretion of [^{14}C]-triclabendazole (Schütz, et al., 1991). The concentrations of triclabendazole and its sulphoxide and sulphone metabolites in plasma and urine of dogs and rats, following i.v. and p.o. administration of [^{14}C]-labelled triclabendazole, were reported. The plasma kinetics of the parent drug after i.v. administration of 0.5 mg/kg of bw to one of two beagle dogs, and of the sulphoxide and sulphone metabolites after p.o. administration of the same dose to the same dog, are shown in Figure 7. Triclabendazole was rapidly converted to its sulphoxide and sulphone metabolites. After i.v. administration, the parent drug rapidly disappeared and the concentration of triclabendazole sulphoxide immediately increased. No unchanged drug could be detected beyond 1 hour after injection. After oral administration of 0.5 and 5 mg/kg doses, no triclabendazole was detected in plasma; the sulphone was slowly formed and eliminated. The renal elimination of triclabendazole was negligible in dogs.

Figure 7: Plasma kinetics of triclabendazole and its major metabolites after a single i.v. or oral dose of 0.5 mg/kg of body weight to a beagle dog (animal 1014).



The kinetics of total radioactivity in plasma and blood were also determined after i.v. and p.o. administration of 0.5 and 5 mg/kg of bw and 40 mg/kg of bw p.o. in dogs, and after 1 mg/kg of bw i.v. and p.o. and 10 mg/kg and 80 mg/kg of bw p.o. in rats (Schütz, et al., 1996). Figure 8 shows selected results of blood analyses for total radioactivity obtained with two dogs (animals 1014 and 1016).

Figure 8: Kinetics of total radioactive residue in blood of dogs dosed with [^{14}C]-triclabendazole



The concentrations of total radioactivity in plasma were initially higher than those determined in whole blood. The ratio of the concentrations in blood to plasma was approximately 0.59 at the lowest dose level and was the same for the i.v. and p.o. routes of administration. The ratio decreased with increasing oral doses, but was constant over several days for a given dose. The sum of the concentrations of the sulphoxide and the sulphone in plasma (given in Figure 7) correlates well with the concentrations of total radioactive residue.

The ratio of the concentrations in blood to plasma was in the order of >0.7 after an i.v. dose of 1 mg/kg of bw and >0.6 after an oral dose of 1mg/kg of bw in rats. However, the ratio increased over time and was >1 after 24 hours. After two days, the ratio was 3.2-5.5 (n=3) for the i.v. treatment and 7-8 (n=3) after p.o. treatment.

The kinetic profiles of the radioactive residues were also used to determine the area under the concentration-time curves. The results are summarised in Table 8. Calculated plasma bioavailability was practically 100% in rats at an oral dose of 1 mg/kg bw (n=3). It decreased slightly up to a 10-fold dose level and decreased significantly further up to an 80-fold dose level. The calculated plasma bioavailability in dogs was approximately 37.7 - 55.6% on the basis of the $AUC_{0\text{-infinity}}$ (n=2), 43.8 % at a dose of 0.5 mg/kg bw (n=1) and 26.8% (n=1) at a dose of 40 mg/kg of bw. Each rat was tested only at one dose level in this experiment; however, each of the two dogs of the experiment was tested at three dose rates.

Table 8: Specific AUC (dose corrected; time from 0 to 168 hours after treatment) of total radioactive residues in blood and plasma of dogs and rats.

Dose (mg/ kg bw)	Route	Matrix	Dogs		Rats									
			1014	1016	RA13	RA14	RA15	RA1	RA2	RA3	RA101	RA102	RA105	RA106
			Specific AUC [µmoles x h/(L x mg/kg)]											
0.5	i.v.	Blood	405	503										
		Plasma	686	881										
	p.o.	Blood	215	174										
		Plasma	353	303										
1	i.v.	Blood			49	61	55							
		Plasma			55	66	61							
	p.o.	Blood						53	61	55				
		Plasma						58	66	57				
5	p.o.	Blood	141											
		Plasma	252											
10	p.o.	Blood									46	37		
		Plasma												
40	p.o.	Blood		106										
		Plasma		213										
80	p.o.	Blood											26	27
		Plasma												

Food Producing Animals

Cattle

A dose of 12 mg of [^{14}C]-triclabendazole (specific activity of 82.6 µCi/mg and radiochemical purity of 95.7%)/kg of bw was administered by oral capsule to one Angus heifer (animal 159) and one Hereford heifer (animal 156), both approximately 7 months of age and weighing 177 kg and 160 kg, respectively (Downs, et al., 1991). Animal 159 was sacrificed at 28 days and animal 156 was sacrificed at 42 days after dosing and tissue samples were collected for combustion analysis to determine [^{14}C]. The results are summarised in Table 9.

Table 9: Total [^{14}C] residues in tissues from treated beef heifers.

Tissue	Beef Heifers	
	Animal 159 (sacrificed 28 days after dosing)	Animal 156 (sacrificed 42 days after dosing)
	Residues (mg/kg equivalents*	
Liver	0.24 ± 0.013	0.09 ± 0.009
Kidney	0.11 ±0.016	0.07 ± 0.011
Muscle (composite)	0.13 ± 0.017	0.10 ± 0.007
Fat (composite)	0.01 ± 0.003	<0.01 ± 0.001

* Data are mean ± standard deviation

Tissue samples derived from the Angus heifer (animal 159) sacrificed 28 days after drug administration in the study by Downs, et al. (1991) were sequentially extracted on three occasions each with methanol and ethyl acetate, and the extracts were radioassayed (Krautter, 1992). Low extraction efficiencies did not allow for the incurred tissue residues to be characterised by chromatography.

[^{14}C]-triclabendazole (specific radioactivity of 5.96 MBq/mg and radiochemical purity of 95.7%) was administered by gavage as a single dose of 12 mg/kg of bw to one female Aberdeen Angus and one male Friesian/Limousin cross ruminating calf weighing 63 kg and 96 kg, respectively, at the time of dosing (Ferguson, 1994a). Faecal and urinary excretion for 0-168 hours post-dosing accounted for 76% and 2.2% of the administered radioactivity, respectively. Plasma protein binding exceeded 99% in all samples. Both animals were sacrificed at 28 days after dosing. Radioactivity was determined in liver, kidney, tenderloin muscle, hindquarter muscle, forequarter muscle, perirenal fat, subcutaneous fat, plasma and red cells. Radioactivity was present in all tissues sampled with highest levels in liver, followed by muscle and kidney with the lowest levels present in fat.

In a separate study by Dieterle and Kissling (1995), the tissue samples from the above study (Ferguson, 1994a) were analysed in the context of a validation study for method REM 15/83. Extractability with dichloromethane was 102% (muscle), 64% (liver), 82% (kidney), and 97% (perirenal fat) for cattle. The accountability (not corrected for procedural recoveries) of method REM 15/83 with UV detection was 34% (muscle), 14% (liver), and 22% (kidney) of total residues for cattle. These results are discussed later in relation to dietary intake and are summarised in Table 21.

A study by Thanei (1995a) was a continuation of the Ferguson (1994a) study. Specifically, the metabolite pattern in extracts of urine and faeces derived from cattle was quantitatively determined and the individual metabolites were characterised. Approximately 2% of the administered dose was eliminated in urine collected up to 168 hours after dosing. Four metabolites of triclabendazole but no parent *per se* were detected in urine. By comparison, approximately 76% of the administered dose was eliminated in faeces collected up to 168 hours after dose administration. The major metabolites in faeces were triclabendazole, its sulphoxide and sulphone, and 2,3-dichloro-4-(6-chloro-2-methylsulfanyl-3H-benzoimidazol-5-yloxy)-phenol and its sulphone.

A male ruminating Holstein Friesian calf aged 9 weeks and weighing 91 kg was administered a single oral dose by capsule of 12 mg [^{14}C]-triclabendazole (specific radioactivity of 13.9 MBq/mg and radiochemical purity of 99.5%)/kg of bw (Needham, 2004b). Urine and faeces collected at 24 h intervals until 10 days after dosing accounted for 78.2% and 3.4% of the administered radioactivity, respectively. The calf was sacrificed at 28 days after dosing and the tissue distribution of radioactivity was determined (Table 10).

Table 10: Concentration of radioactive residue in the tissues of a calf at 28 days after administering a single oral dose of 12 mg [^{14}C]-triclabendazole/kg of bw.

Tissue	Concentration of radioactivity as µg equivalents of triclabendazole/kg of tissue
Liver	283.3
Kidney	163.3
Muscle	209.1
Fat	25.8
Blood	70.4
Red blood cells	63.1
Plasma	51.1

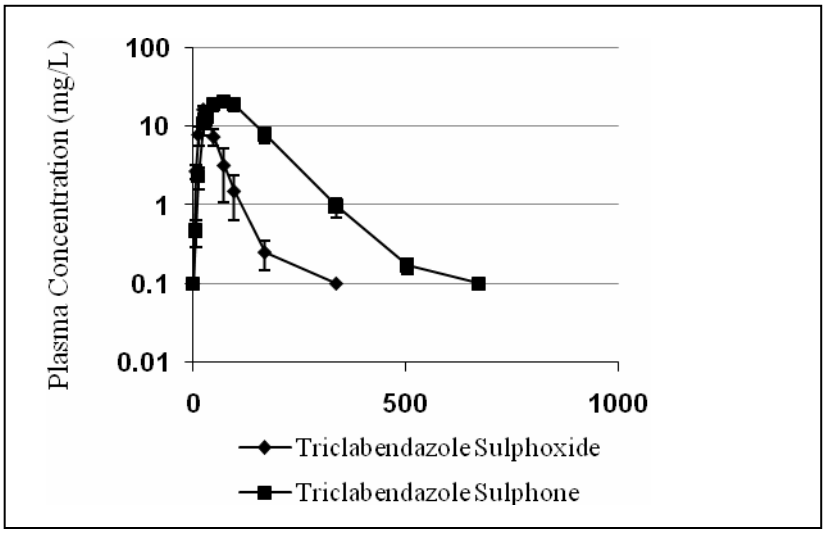
A fractionation study (Needham, 2004b) showed that 92-98.6% of the radioactivity in liver and muscle was associated with either lipid or protein. Changing the pH of the extracting solvent from acidic to basic did not release the tissue residues, suggesting that they are covalently bound to macromolecules in cells. The triclabendazole-derived moiety of the residue was not released as a free metabolite with either alkaline hydrolysis or protease digestion whereas oxidation cleaved the ketone CGA-110 754 (keto-triclabendazole). Other metabolites, derived from phenolic metabolites of triclabendazole (eg, CGA-161 944 and CGA-183 196), could be released from the extract but were not carried through the clean-up process of the residue analysis method. The extraction of radiolabelled incurred residues was

determined for a range of solvents (Needham, 2004b). The most efficient extraction involved alkaline hydrolysis of the tissue with 2M NaOH. Under these conditions, 70–85% of the total radioactive residues was extractable with dichloromethane; however, the resultant extracts were difficult to analyse by HPLC, and no data were obtained from HPLC/MS.

A validated residue method with a limit of quantification of 0.03 mg/kg (expressed as triclabendazole equivalents for each bovine tissue) accounted for 26.5% (liver), 29.4% (kidney) and 34.9% (muscle) of the total radioactivity present in these tissues (Needham, 2004b). In plasma, the presence of triclabendazole-protein conjugates resulted in 90% of the radioactivity precipitating with the protein fraction. Storage stability of incurred residues in samples of cattle tissues stored frozen for 184 days (muscle) and 194 days (liver and kidney) was investigated (Needham, 2004b). Residues in muscle and kidney were stable during storage whereas with bovine liver, the mean concentration of triclabendazole after 6 months frozen storage declined to 72% of the initial concentration.

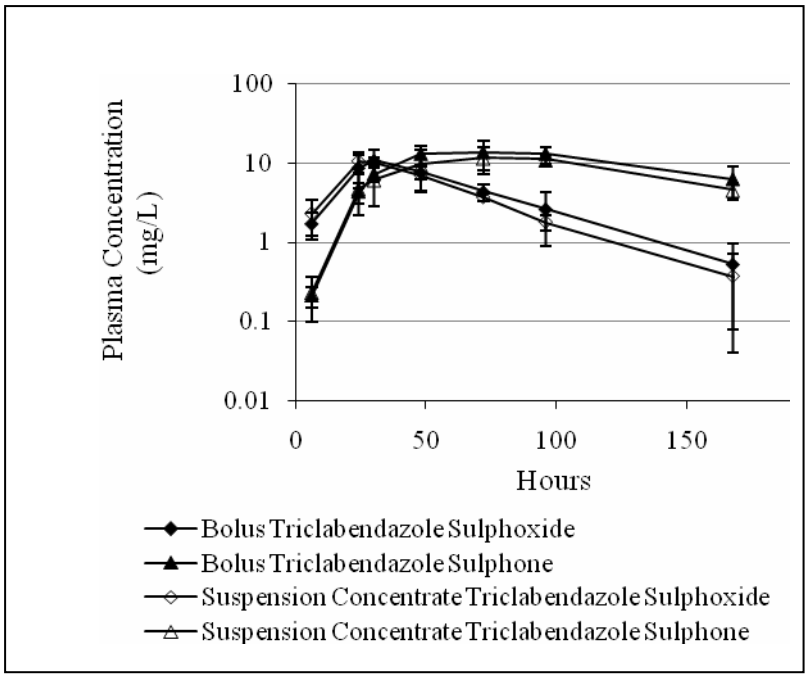
Four calves weighing 165-196 kg were administered an oral dose of 12 mg of triclabendazole (as a 10% w/v suspension)/kg of bw (Bull, et al., 1986a). Plasma samples were taken and analysed by HPLC for triclabendazole sulphoxide and triclabendazole sulphone. The semi-logarithmic plots of metabolite plasma concentration *versus* time are shown in Figure 9. The maximum plasma concentrations of the sulphoxide and the sulphone occurred at 24 h and 72 h, respectively.

Figure 9: Plasma concentration *versus* time profiles for triclabendazole sulphoxide and triclabendazole sulphone in cattle following an oral dose of 12 mg triclabendazole/kg of bw.



Ten 9 month-old Hereford-crossed calves weighing 192-238 kg were dosed with 12 mg triclabendazole/kg of bw as either a bolus (n=5) or 10% w/v suspension (n=5)(Bull et al, 1990). Animals receiving boluses were dosed to the nearest half bolus and the precise treatment rate was then calculated. Plasma samples were collected and analysed for triclabendazole sulphoxide and triclabendazole sulphone. The semi-logarithmic plots of metabolite plasma concentration *versus* time are shown in Figure 10. The bioavailability of triclabendazole was similar when administered to cattle by bolus and liquid suspension.

Figure 10: Plasma concentration *versus* time profiles for triclabendazole sulphoxide and triclabendazole sulphone in calves following an oral dose of 12 mg triclabendazole/kg of bw by bolus or 10% w/v suspension concentrate.



A study was conducted in cattle (Bull, et al., 1986b) which was similar to the sheep study conducted by Strong, et al.(1983). Six Friesian bulls, 10 months of age and weighing 186-236 kg, were assigned to one of two groups. Group 1 (n=3) were dosed i.v. with 12 mg triclabendazole (as a 10% w/v suspension)/kg of bw. Group 2 was dosed i.v. with 12 mg triclabendazole sulphoxide (as a 10% w/v suspension)/kg of bw. All animals in Groups 1 and 2 displayed adverse clinical signs after i.v. administration and one animal in Group 2 died. Plasma samples were collected for analysis by HPLC.

Semi-logarithmic plots of plasma metabolite concentrations *versus* time for the two groups are shown in Figures 11 and 12. With Group 1, the average maximum concentration of triclabendazole sulphoxide of 30.1 mg/l was observed approximately 4 hours after dosing and the maximum concentration of triclabendazole sulphone of 23.9 mg/l was observed approximately 32 hours after dosing. Plasma concentrations of triclabendazole were <0.1mg/l in 2 of the 3 animals by 12 hours after dosing. With Group 2, the average maximum concentration of triclabendazole sulphoxide of 159 mg/l was observed in the first blood sample taken at 2 minutes after dosing and the average maximum concentration of triclabendazole sulphone of 41.3 mg/l was observed at 32 hours after dosing.

Figure 11: Plasma concentration of triclabendazole sulphoxide and triclabendazole sulphone *versus* time after i.v. administration of 12 mg triclabendazole/kg of bw to cattle.

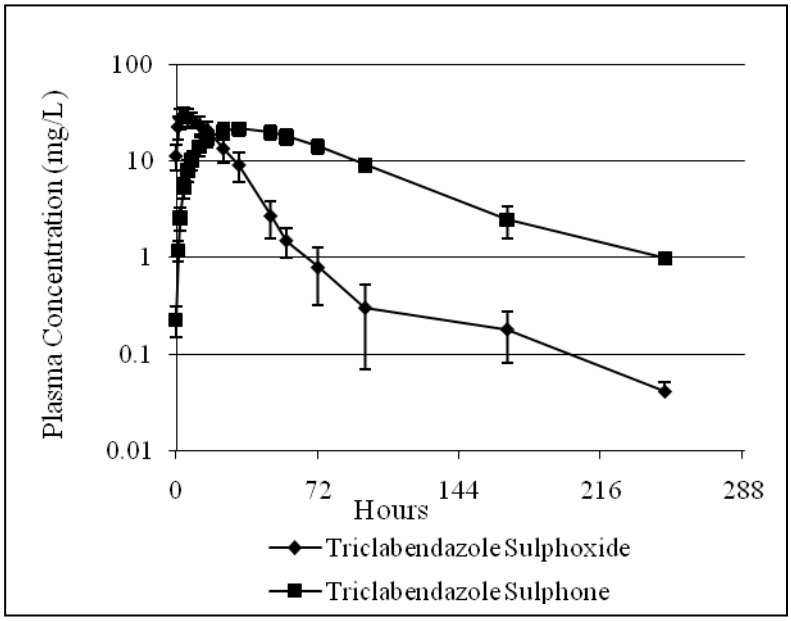
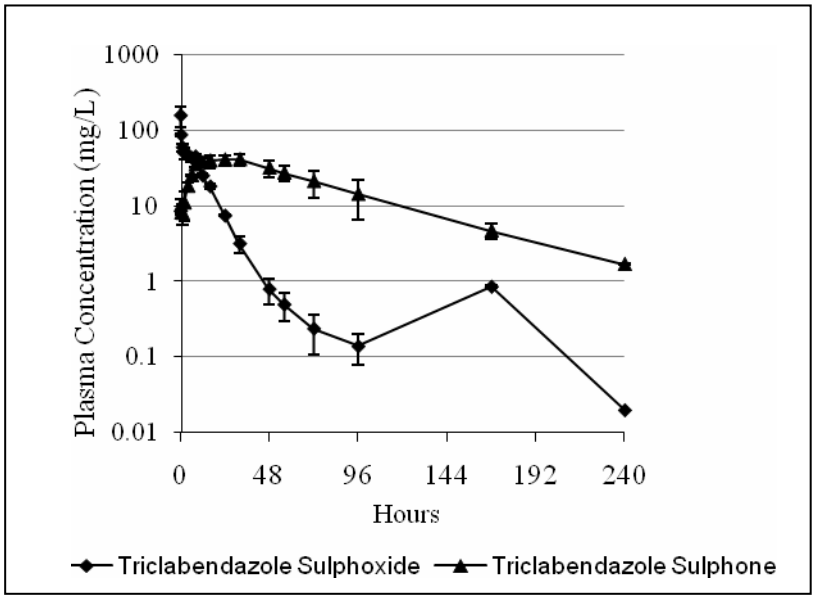


Figure 12: Plasma concentration of triclabendazole sulphoxide and triclabendazole sulphone *versus* time after i.v. administration of 12 mg triclabendazole sulphoxide/kg of bw to cattle.



In studies to determine whether the co-administration of triclabendazole and levamisole altered the pharmacokinetic behaviour of either compound, 21 calves were assigned to three groups (each n=7) (Strong, et al., 1987). Group 1 was dosed with 12 mg triclabendazole/kg of bw; Group 2 was dosed with 7.5 mg levamisole hydrochloride/kg of bw and 12 mg triclabendazole/kg of bw; and Group 3 was dosed with 7.5 mg levamisole hydrochloride/kg of bw. Plasma samples were collected and analysed for triclabendazole sulphoxide and triclabendazole sulphone and/or levamisole hydrochloride. Pharmacokinetic parameters for triclabendazole sulphoxide, triclabendazole sulphone and levamisole were calculated. The data demonstrated that co-administration of triclabendazole and levamisole did not significantly alter the pharmacokinetics of either compound in cattle.

Sheep

A comparison of the kinetic parameters of triclabendazole sulphoxide (CGA-110 752) and triclabendazole sulphone (CGA-110 753) in plasma following i.v. administration of triclabendazole (CGA-89 317) to sheep and cattle is shown in Table 11.

Table 11: Kinetic parameters of triclabendazole sulphoxide and triclabendazole sulphone in plasma following i.v. administration of triclabendazole to sheep and cattle.

Animal	Dose of CGA-89 317	Plasma profile	AUC (mg/L.h)	C_{max} (mg/L)	T_{max} (h)	$t_{1/2}$ (h)
Sheep	10 mg /kg of bw	CGA-110 752 CGA-110 753	651 596	42 13	42 13	14 27
Cattle	12 mg/kg of bw	CGA-110 752 CGA-110 753	795 2043	34 24	34 24	13 40

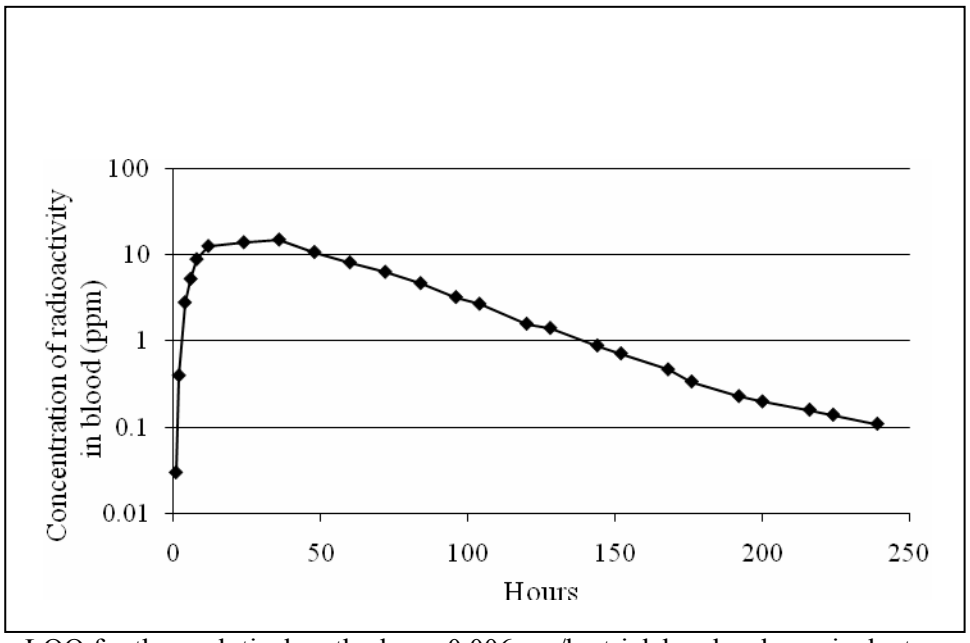
[^{14}C]–CGA-89 317 (specific radioactivity of 1.129 MBq/mg and radiochemical purity of 99%) was administered orally in gelatine capsules at a rate of 10.5 mg/kg to a 4 months old female sheep (Swiss White Alp X Ile de France breed), that weighed 28.5 kg (Hamböck and Strittmatter, 1982). Blood samples were collected and radioactivity in the samples was determined. The semi-logarithmic plot of radioactivity concentration in blood *versus* time is shown in Figure 13. The excretion of radioactivity in urine and faeces was measured every 24 hours to 10 days post-dosing. Faecal and urinary excretion accounted for 100.9% and 3.5% of the administered dose, respectively. The faecal extract (0-72 hours) contained unchanged triclabendazole (19.3% of the administered dose), triclabendazole sulphoxide (6.6%), triclabendazole sulphone (2.2%) and some unknown metabolites. The urine contained only polar fractions. The sheep was sacrificed 10 days after dosing and the tissue distribution of radioactivity investigated. The results are shown in Table 12.

Table 12: Radioactivity in selected tissues and organs of a sheep 10 days after oral administration of 10.5mg [^{14}C]-triclabendazole/kg of bw.

Tissue	mg/kg triclabendazole equivalents	Tissue	mg/kg triclabendazole equivalents
Blood	0.11	Rumen - wall	0.21
Liver	1.84	content	0.02
Gall bladder	0.40	Intestine- wall	0.17
Kidney	1.11	- content	0.09
Lung	1.35	Bone marrow–yellow	~LOQ
Spleen	0.24	- red	~LOQ
Heart	0.92	Spinal cord	1.13
Brain	0.95	Lymph node(s)	0.22
Muscle rump	0.58	Eye	0.29
- round steak	0.58	Ovary	0.14
- tenderloin	0.53	Adrenal gland	1.07
Fat perirenal	0.09	Thyroid gland	1.67
- subcutaneous	0.08	Pancreas	0.41
		Thymus	0.11

LOQ values: blood = 0.006 ppm; tissues 100 mg = 0.023 ppm; tissues 150-250 mg = 0.012 ppm; tissues 300-400 mg = 0.008 ppm.

Figure 13: Radioactivity in sheep blood after the oral administration of 10.5 mg [^{14}C]-triclabendazole/kg of bw.



The LOQ for the analytical method was 0.006 mg/kg triclabendazole equivalents**.**

Two Texel-cross sheep, one male and one female weighing 33 kg and 27 kg at dosing, respectively, were administered [^{14}C]-triclabendazole (specific radioactivity of 5.96 MBq/mg and radiochemical purity of 97.0%; - specific radioactivity of 6.13 MBq/mg and radiochemical purity of 98.5%) by oral gavage at a nominal dose rate of 10 mg/kg of bw (Ferguson, 1994b). Up to 168 hours after dosing, 77% and 4.7% of the administered dose was excreted in faeces and urine, respectively. Plasma protein binding determined *in vitro* in fortified samples and *ex vivo* in plasma collected from animals at 8 and 48 hours post-dosing was 99%. The animals were sacrificed 28 days after dosing, and the tissue distribution of radioactivity was determined. The concentration of radioactivity (in units of mg/kg equivalents of [^{14}C]-triclabendazole/kg) were 0.24 mg in forequarter muscle; 0.24 in tenderloin muscle; 0.24 in liver; 0.20 in kidney; 0.02 in subcutaneous fat; and 0.02 in renal fat.

Tissues from sheep orally dosed with [^{14}C]-triclabendazole and sacrificed at 28 days after dosing in the above study by Ferguson (1994b) were analysed in the context of a validation study for method REM 15/83 (Dieterle and Kissling, 1995). Extractability with dichloromethane was 91% (muscle) and 78% (liver), and the accountability (not corrected for procedural recoveries) of method REM 15/83 with UV detection was 32% (muscle) and 19% (liver) of the total residues for sheep.

[^{14}C]-Triclabendazole was administered by oral capsule at a dose of 10 mg/kg of bw to a goat and a sheep weighing 42.5 kg and 28.5 kg, respectively (Hamböck, 1982). The animals were sacrificed 10 days after dosing. The extractability of residues with various organic solvents and with 0.01M aqueous phosphate buffer solution was low for both animals. By comparison, the percentage of [^{14}C]-residues extracted with dichloromethane from tissues that had been solubilized using 2N aqueous NaOH and then acidified to pH<3 were 85% (liver), 89% (kidney) and 82% (muscle) for the goat and 78% (liver), 79% (kidney) and 89% (muscle) for the sheep. Oxidation using hydrogen peroxide transformed 40% and 42% of [^{14}C]-tissue residues to the common moiety keto-triclabendazole (CGA-110 754) in muscle of the goat and sheep, respectively.

The sheep study of Ferguson (1994b) was continued by determining the metabolic patterns in urine and faeces collected up to 168 hours after dosing (Thanei, 1995b). Urine and faeces accounted for 4.7% and 77% of the administered radioactivity, respectively, and contained five and eleven metabolic fractions, respectively. Unchanged triclabendazole was not detected in urine but accounted for 16% of

the dose in faeces. The major metabolic pathways of triclabendazole in sheep were oxidation to the sulphoxide and ultimately to the sulphone, and hydroxylation in position 4 of the dichloro-phenyl-ring. Therefore, the metabolic pathways of triclabendazole in sheep and cattle are essentially the same.

Triclabendazole was administered intraruminally at a dose rate of 10 mg/kg of bw to sheep surgically fitted with a bile duct cannula (Hennessy, et al., 1987). The profiles of triclabendazole metabolites in plasma and bile were determined. In plasma, only triclabendazole sulphoxide and triclabendazole sulphone were present and were bound to plasma albumin. In bile, the major triclabendazole metabolites were hydroxylated in the 4 position and excreted predominantly as sulphate esters with lesser proportions as glucuronide conjugates. Of the administered triclabendazole dose, 9.7% was excreted as free metabolites in bile, 35.8% was excreted as conjugated metabolites, and 6.5% was excreted in urine.

The absorption of triclabendazole and triclabendazole sulphoxide at different dose rates (5 and 10 mg/kg of bw); in different formulations (aqueous suspensions and aqueous solution); and administered via different routes (oral, intraruminal and i.v.) was studied in sheep (Strong, et al., 1982). Preliminary information only was reported and this study was not considered further.

Plasma levels of triclabendazole and triclabendazole sulphoxide in sheep were measured following the administration of triclabendazole on two occasions and in different formulations (Strong, et al., 1983). The authors reported that intra-animal variability in the pharmacokinetic behaviour of triclabendazole was small whereas inter-animal variability was large. In addition, the absorption of triclabendazole from an aqueous suspension and from a peanut oil formulation was reported to be similar. Administering triclabendazole on two occasions 8 weeks apart resulted in unchanged plasma levels of triclabendazole sulphoxide and triclabendazole sulphone in individual sheep. Partial stimulation of the oesophageal groove reflex was reported to occur in one of 15 sheep with no closure of the oesophageal groove occurring in the remaining 14 animals. It is concluded that when doses of triclabendazole are administered orally to ruminating sheep, they will generally enter the rumen.

A rapid and simple HPLC method for estimating triclabendazole and its metabolites in plasma was reported (Sanyal, 1994). The method was used to determine the pharmacokinetics of intraruminally administered triclabendazole in five sheep and five goats. The values of C_{max}, T_{max}, AUC and $t_{1/2}$ were similar for the two species.

Twenty-four sheep were assigned to three groups (each n=8)(Strong et al, 1988). Group 1 was dosed orally with 10 mg triclabendazole/kg of bw; Group 2 was dosed orally with 7.5 mg levamisole hydrochloride/kg of bw plus 10 mg triclabendazole/kg of bw; and Group 3 with 7.5 mg levamisole hydrochloride/kg of bw. The plasma kinetics of neither triclabendazole nor levamisole were affected by the other compound.

Six sheep of different breed and sex, aged 1 to 5 years and weighing 36-61 kg were assigned to 3 groups (each n=2) (Mohammed Ali, et al., 1986). Group 1 was drenched orally with 10 mg triclabendazole/kg of bw; Group 2 with 10 mg triclabendazole and 10 mg fenbendazole per kg of bw; and Group 3 with 10 mg fenbendazole/kg of bw. Each treatment was subsequently administered to the other two groups at 4-weekly intervals. The pharmacokinetics of triclabendazole were not altered when administered with fenbendazole.

Goats

A three year-old lactating goat weighing 42.5 kg bw was dosed orally with 10.1 mg [^{14}C]-triclabendazole (specific activity 1.129 MBq/mg and radiochemical purity 99%) labelled at the carbon atom in position 2 of the benzimidazole ring system (Hamböck and Strittmatter, 1981). Blood, milk, faeces and urine were collected until 10 days after dosing when the goat was sacrificed. Radioactivity in all samples including tissues collected at slaughter was determined by liquid scintillation counting. In blood, the peak level of radioactivity of 13.7 mg/kg triclabendazole equivalents was observed at 36

hours. The maximum concentration of radioactivity in milk was 1.8 mg/kg triclabendazole equivalents in the 8-24 hour sample post-dosing. The overall recovery of radioactivity was 103.9% with excretion in urine and faeces accounting for 2% and 98% of the administered dose, respectively. Faeces but not urine contained triclabendazole and its sulphoxide and sulphone. The distribution of radioactivity at 10 days after dosing was highest in liver (1.0 mg/kg triclabendazole equivalents) and thyroid gland (1.3 mg/kg triclabendazole equivalents); lower levels were observed for fat and blood (each 0.08 mg/kg triclabendazole equivalents), red bone marrow (0.06 mg/kg triclabendazole equivalents) and yellow bone marrow (<0.02 mg/kg triclabendazole equivalents).

TISSUE RESIDUE DEPLETION STUDIES

Residue Depletion Studies with Unlabeled Drug

Cattle

A group of Hereford cattle comprising 12 males and 12 females aged 7-10 months and weighing 168-367 kg was treated orally at a dose of 18 mg triclabendazole/kg of bw and retreated 28 days later (Adams, 2004a). This treatment regimen corresponded to the minimum re-treatment interval in the directions for use on the product label. Six animals were sacrificed at each of 14, 28, 42 and 56 days following the second treatment. Samples of muscle (tenderloin), kidney, liver and renal fat were collected and analysed by HPLC for triclabendazole residues, measured as keto-triclabendazole. The limit of quantitation of the analytical method was 0.05 mg/kg. The results, corrected for recovery, are shown in Table 13.

Table 13: Residues of triclabendazole determined as keto-triclabendazole, following oral treatment of cattlewith *Fasinex 100* at 18 mg triclabendazole/kg of bw.

Sampling time (DALT[1])	Concentration of residues of triclabendazole measured as keto-triclabendazole (μg/kg)[2]			
	Muscle	Liver	Kidney	Renal fat
14	194, 221, 237, 248, 254, 271	797, 845, 862, 871, 1084, 1413	476, 487, 514, 586, 706, 1169	<50, <50, 72, 74, 78, 132
28	104, 118, 128, 155, 159, 175	263, 300, 339, 377, 424, 489	109, 118, 118, 129, 133, 165	<50, <50, <50, <50, <50, 62
42	103, 109, 124, 129, 132, 162	149, 183, 219, 262, 269, 288	49, 53, 62, 69, 75, 89	<50, <50, <50, <50, <50, <50
56	70, 85, 87, 90, 104, 111	48, 91, 96, 103, 131, 142	<50, <50, <50, <50, <50, <50	na, na, na, na, na, na

[1]. DALT = days after last treatment. [2]. Corrected for recovery. na = not analysed

Residue data corrected for recoveries from the cattle study by Adams (2004a) were analysed by linear regression (Strehlau, 2004a) in accordance with the EMEA/CVMP guideline (EMEA, 1996). One-sided, upper 95% tolerance limits with 95% confidence were calculated for muscle, liver and kidney. Model assumptions were checked using diagnostic tests. The linear regression assumptions regarding homogeneity of variances and homogeneity of normal distribution of errors were valid for muscle, liver and kidney; the assumption of linearity was valid for liver only.

Another GLP-compliant residue depletion study (Study No. AA031, 2001) involving a pour-on application to beef cattle was reviewed by the Committee. The animal phase of this study was conducted at Armidale, NSW, Australia. Beef cattle (n=25; Hereford/Hereford × Angus; 15 females and 10 male castrates; 126-192 kg bw) were treated with a single pour-on application of 0.75 mg abamectin/kg of bw and 45 mg triclabendazole/kg of bw. Groups of 5 animals were sacrificed on days 14, 21, 28, 35 and 42 after application, and samples of fat (back and perirenal), liver, kidney and muscle were collected. All tissue samples were stored frozen until analysed for residues.

A further 5 animals (3 females and 2 male castrates; 114 to 166 kg bw) were treated with a single pour-on application of 1.5 mg abamectin/kg of bw and 90 mg triclabendazole/kg of bw.. The cattle were held in covered pens overnight after their treatment. On the following day, the cattle were returned to open grazing paddocks with animals from the different treatment groups being held in separate paddocks. Animals were sacrificed at 35 days post-treatment, and samples of fat (back and perirenal), liver, kidney and muscle were collected for residues analysis. The concentrations of triclabendazole residues in tissue samples were determined using a validated method and analyses were completed within 14 months of sample collection. The results are shown in Table 14.

Table 14: Residues of triclabendazole following a single pour-on application of *Genesis Ultra Pour-on Roundworm, Liver Fluke & External Parasiticide for Cattle* to beef cattle at a dose rate of 45 mg triclabendazole/kg of bw.

Treatment Regimen	Sampling time (DALT[1])	Concentration of triclabendazole residues (mg/kg)[2,3]				
		Muscle	Liver	Kidney	Back fat	Perirenal fat
Single pour-on application of 0.75 mg abamectin/kg b.w. and 45 mg triclabendazole/kg b.w.	14	0.08, 0.34, 0.57, 0.59, 0.82	1.32, 2.76, 2.99, 3.59, 4.18	0.75, 1.36, 1.85, 2.08, 2.52	0.35, 2.08, 2.59, 2.92, 4.07	0.29, 1.12, 1.49, 1.52, 10.83
	21	0.13, 0.17, 0.21, 0.44, 0.53	0.75, 1.26, 1.63, 1.74, 1.88	0.40, 0.85, 1.01, 1.08, 1.17	0.29, 0.73, 0.82, 0.91, 0.93	0.16, 0.41, 0.42, 0.46, 0.76
	28	0.12, 0.15, 0.26, 0.28, 0.35	1.33, 1.57, 1.80, 1.85, 2.72	0.56, 0.91, 0.97, 1.00, 1.20	0.33, 0.43, 1.06, 1.06, 1.25	0.23, 0.35, 0.59, 0.76, 0.78
	35	0.09, 0.14, 0.14, 0.15, 0.26	0.39, 0.86, 0.95, 0.97, 2.29	0.15, 0.41, 0.45, 0.48, 0.89	0.15, 0.21, 0.33, 0.39, 0.63	0.09, 0.11, 0.20, 0.26, 0.62
	42	0.15, 0.22, 0.27, 0.27, 0.42	0.72, 0.74, 0.90, 0.93, 2.23	0.28, 0.28, 0.36, 0.42, 0.96	<0.03, 0.10, 0.18, 0.27, 1.37	0.04, 0.05, 0.11, 0.16, 0.57
Single pour-on application of 1.5 mg abamectin/kg bw and 90 mg triclabendazole/kg b.w.	35	0.16, 0.16, 0.20, 0.25, 0.30	1.40, 1.54, 1.63, 1.86, 1.91	0.62, 0.67, 0.82, 0.98, 1.22	0.30, 0.42, 0.45, 0.51, 1.31	0.20, 0.26, 0.26,0.33, 0.85

$LOQ_{triclabendazole}$ (all tissues) = 0.03 mg/kg; [1].DALT = days after last treatment; [2]. Residue results have not been corrected for method recoveries; [3].The residue results are expressed as triclabendazole equivalents, which can be converted to keto-triclabendazole equivalents by multiplying by a factor of 0.916.

Another GLP-compliant residue depletion study (Study No. ANT 1274, 2002) using the same pour-on product was applied to beef cattle. The animal phase of this study was conducted at Armidale/Dangersleigh, NSW, Australia. Twenty cattle (Hereford or Angus cross breed; 10 females and 10 male castrates; 208-290 kg bw) were treated with a single pour-on application of 0.75 mg abamectin/kg of bw and 45 mg triclabendazole/kg of bw. The cattle were treated and held in covered pens for 48 hours post-treatment. Thereafter, the cattle were returned to open grazing paddocks, and were observed on a weekly basis. Groups of 5 animals were sacrificed on days 49, 56, 63 and 70 after application, and samples of fat (back and perirenal), liver, kidney and muscle were collected. All tissue samples were stored frozen until analysed for residues.The concentration of triclabendazole residues in tissue samples were determined using a validated HPLC method and analyses were completed within 6 months of sample collection. The results are shown in Table 15.

Table 15: Residues of triclabendazole following a single pour-on application of *Genesis Ultra Pour-on Roundworm, Liver Fluke & External Parasiticide for Cattle* to beef cattle at a dose rate of 45 mg triclabendazole/kg of bw.

Treatment Regimen	Sampling time (DALT[1])	Concentration of triclabendazole residues (mg/kg)[2,3]				
		Muscle	Liver	Kidney	Back fat	Perirenal fat
Single pour-on application of 0.75 mg abamectin/kg bw and 45 mg triclabendazole/kg bw	49	ND, 0.03, 0.04, 0.11, 0.15	0.51, 0.55, 0.99, 1.15, 1.27	0.26, 0.28, 0.57, 0.73, 0.82	ND, 0.10, 0.14, 0.2, 0.33	ND, 0.12, 0.16, 0.31, 0.68
	56	ND, 0.07, 0.08, 0.10, 0.12	0.45, 0.72, 0.73, 0.76, 1.01	0.26, 0.37, 0.40, 0.46, 0.49	0.09, 0.19, 0.24, 0.30, 0.36	0.04, 0.05, 0.11, 0.32, 0.39
	63	ND, ND, 0.01, 0.03, 0.05	0.31, 0.35, 0.39, 0.66, 0.84	ND, 0.18, 0.20, 0.36, 0.43	ND, ND, ND, 0.16, 0.19	ND, ND, ND, ND, 0.04
	70	ND, ND, ND, ND, 0.01	0.35, 0.37, 0.41, 0.45, 0.63	ND, 0.19, 0.20, 0.31, 0.39	ND, ND, ND, ND, 0.15	ND, ND, ND, ND, ND

[1]. DALT = days after last treatment; [2]. Residue results have been corrected for method recoveries; [3]. The residue results are expressed as triclabendazole equivalents, which can be converted to keto-triclabendazole equivalents by multiplying by a factor of 0.916.

A GLP-compliant residue trial (Study EL-55021, 2004) was conducted in beef cattle in New Zealand, using 21 females, weighing between 106-148 kg. One animal was included as an untreated negative control while 4 groups, each of 5 animals, were treated with a single pour-on application of 0.68 mg of abamectin/kg of bw and 38 mg of triclabendazole/kg of bw. Samples of fat (perirenal), muscle, liver and kidney were taken from groups of animals sacrificed at 77, 91, 105 and 119 days after treatment and analysed by HPLC for triclabendazole residues. The results are presented in Table 16.

Table 16: Residues of triclabendazole following a single pour-on application of *Genesis Ultra Pour-on Roundworm, Liver Fluke & External Parasiticide for Cattle* to beef cattle at a dose rate of 38 mg triclabendazole/kg of bw.

Treatment Regimen	Sampling time (DALT[1])	Concentration of triclabendazole residues measured as keto-triclabendazole (mg/kg)			
		Muscle	Liver	Kidney	Perirenal fat
Single pour-on application of 0.68 mg abamectin/kg b.w. and 38 mg triclabendazole/ kg b.w.	77	<LOQ, <LOQ, <LOQ, 0.10, 0.13	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, 0.10, 0.13
	91	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ
	105	<LOQ, <LOQ, <LOQ, <LOQ, 0.10	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ
	119	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, <LOQ	<LOQ, <LOQ, <LOQ, <LOQ, 0.10

LOD = 0.03 mg/kg; LOQ = 0.1 mg/kg; [1]DALT = days after last treatment

Sheep

Twenty-four sheep comprising 12 males and 12 females, 7 months old and weighing 29-42 kg bw, were treated orally at a dose of 10–13 mg triclabendazole/kg of bw and assigned to four groups (each 3 males and 3 females) One group was sacrificed at each of 14, 28, 42 and 56 days following treatment. Samples of muscle (tenderloin), kidney, liver and renal fat were collected and analysed by HPLC for triclabendazole residues, measured as keto-triclabendazole. The limit of quantitation of the analytical method was 0.05 mg/kg. Results, corrected for recovery, are shown in Table 17.

Table 17: Residues of triclabendazole determined as keto-triclabendazole, following oral treatment of sheep with *Fasinex 50* at 10–13 mg triclabendazole/kg of bw.

Sampling time (DALT[1])	Concentration of residues of triclabendazole measured as keto-triclabendazole (μg/kg)[2]			
	Muscle	Liver	Kidney	Renal fat
14	111, 143, 148, 152, 171, 200	327, 353, 428, 473, 487, 503	200, 219, 228, 258, 265, 279	<50, <50, <50, <50, <50, <50
28	70, 99, 101, 117, 140, 144	106, 128, 148, 181, 183, 201	68, 73, 93, 99, 118, 122	na, na, na, na, na, na
42	50, 51, 60, 64, 80, 83	<50, <50, <50, <50, <50, <50	<50, <50, <50, <50, <50, <50	na, na, na, na, na, na
56	<50, <50, <50, 51, 54, 57	na, na, na, na, na, na	na, na, na, na, na, na	na, na, na, na, na, na

[1]. DALT = days after last treatment; [2]. Corrected for recovery; na = not analysed

Residue data corrected for recovery for muscle, liver and kidney from the sheep study by Adams (2004a) were analysed in accordance with the EMEA/CVMP guideline (EMEA, 1996) by Strehlau (2004b). Depletion curves were estimated and one-sided, 95% tolerance limits with 95% confidence limits calculated after a single dose and extrapolated to a repeated administration 28 days after the first dose. Model assumptions were checked using diagnostic tests with the exception of linearity for liver and kidney residues. The latter could not be checked because there were only two time points. The model assumptions tested were shown to be satisfied. The residue depletion curves and corresponding tolerance limits predicted on the basis of repeated administration 28 days after the first dose were presented.

Bound residues and bioavailability

Tissues originating from cattle (Ferguson, 1994a) and sheep (Ferguson, 1994b) which were sacrificed 28 days after oral dosing with 12 mg (cattle) or 10 mg (sheep) [^{14}C]-triclabendazole/kg of bw were lyophilised, homogenised and mixed with powdered standard rat chow in a ratio of 80/20 w/w and fed to bile duct-cannulated rats (Hassler, 1995). In addition, diet mixtures containing cattle kidney, and sheep kidney and liver, were homogenised in water and orally administered by syringe during the first 8 hours of access to the fortified rat chow. This assured uptake of the diet mixture by the bile duct-cannulated rats. Urine, faeces and bile were collected from the rats until they were sacrificed 2 days after commencing the diet. Tissues including abdominal fat, kidney, liver and skeletal muscle were sampled at necropsy. The faeces of rats accounted for 85% (liver), 68% (kidney) and 91% (muscle) of the radioactivity present in the respective cattle tissue ingested, and for 88% (liver), 88% (kidney) and 88% (muscle) of the radioactivity present in the respective sheep tissue ingested. The bioavailability of the radioactivity from cattle and sheep tissues is shown in Table 18.

Table 18: Recovery of radioactivity (% of administered dose) following administration of cattle and sheep tissues containing [^{14}C]-triclabendazole derived residues to male bile duct-cannulated rats.

Tissue	Cattle			Sheep		
	Liver[1]	Kidney[2]	Muscle[1]	Liver[2]	Kidney[2]	Muscle[1]
Number of male rats	5	4	5	4	6	3
Urine 0–48 h	0.9	0.4	1.7	1.5	0.5	1.0
Bile 0–48 h	6.3	9.3	1.4	4.9	5.6	2.8
Residues						
Tissue & carcass	1.6	4.0	0.7	1.8	1.0	1.7
Bioavailability (%)	8.8	13.7	3.7	8.2	7.0	5.5

[1]. Tissue specimens were lyophilised, homogenised and mixed with powdered standard rat chow in a ratio of 80/20 w/w in a blender; [2]. The diet mixture was suspended in water.

The bioavailability of radioactive residues of [^{14}C]-triclabendazole-derived compounds was investigated in Sprague Dawley rats (Hardwick, 2004b) using tissues collected in an earlier cattle study (Needham, 2004b). Muscle, liver and kidney tissues were freeze-dried, powdered and prepared as a thick paste; additionally, a suspension of lyophilised muscle was prepared in water. Bile duct-cannulated rats were allocated to three groups. One group (n=6) was administered bovine muscle as an oral paste for 24 h followed by an additional gavage dose of 0.5 g lyophilised muscle suspended in water. A second group (n=6) was administered bovine liver as an oral paste for 24 hours. A third group (n=3) was administered bovine kidney as an oral paste for 24 h. Urine, faeces and bile were collected for 24 h on three occasions up to 72 h when the rats were sacrificed. Muscle, liver, kidney and the gastrointestinal tract plus contents were collected at necropsy. The mean recovery of radioactivity (expressed as mean ± sd) following dietary exposure was 91.4 ± 17.3% of the administered dose for muscle; 116.7 ± 6.8% for liver; and 90.6 ± 1.7% for kidney. The recovery of radioactivity in urine, faeces and bile is shown in Table 19.

Table 19: Recovery of radioactivity (% of administered dose) following administration of cattle tissues containing [^{14}C]-triclabendazole-derived residues fed to bile duct-cannulated rats.

	Muscle administration	Liver administration	Kidney administration
Number of rats	(n=6)	(n=6)	(n=3)
Sample			
Urine	0.78 ± 0.79	0.53 ± 0.45	<LOQ
Faeces	72.5 ± 19.3	93.6 ± 9.4	87.3 ± 1.3
Bile	17.2 ± 7.7	19.2 ± 5.0	3.3 ± 0.4

Data are rounded values of mean ± sd

In the same study, radioactivity in bile was detectable 48 h following the cessation of dietary exposure to bovine muscle, liver and kidney (Table 20). The percent of the administered dose of liver recovered as radioactivity in bile was highest in the 48-72h sample, suggesting that the duration of collection was inadequate and the bioavailability of incurred residues in bovine liver was under-estimated.

Table 20: Recovery of radioactivity (% of administered dose) in the bile of bile duct-cannulated rats following the administration of cattle tissues containing [^{14}C]-triclabendazole derived residues.

	Muscle administration	Liver administration	Kidney administration
Number of rats	(n=6)	(n=6)	(n=3)
Time (h)			
24	4.2 ± 1.8	5.6 ± 2.0	1.8 ± 0.8
48	11.7 ± 6. 5	4. 7 ± 5.4	0.7 ± 0.6
72	1.2 ± 0.6	8.9 ± 5.3	<LOQ
Total	17.2 ± 7.7	19.2 ± 5.0	2.5 ± 1.5

Data are rounded mean ± sd

ESTIMATION OF DAILY INTAKE

Calculation of the Estimated Daily Intake (EDI) of triclabendazole residues requires data on the median concentrations of marker residues and the ratios of marker to total residues, the quantities of the food commodities consumed (as defined by the standard food basket) and the bioavailability of residues. The latter discounts unreleased and undissolved residues, thereby providing a more realistic estimate of dietary intake. The median concentration of the marker residue in a specified tissue is derived from the predicted value of the regression line at the same time point used for establishing the MRL. In the case of triclabendazole, the choice of time points is limited because the ratio of marker to total residue concentrations in cattle tissues is known only at day 28. The corresponding information in sheep is even more limited.

Data from the following studies in cattle and sheep were used to prepare the summary of the available information on the ratio of marker to total residue concentrations (Table 21).

Two ruminating calves, one female (Aberdeen Angus) 63 kg bw and one male (Friesian/Limousin cross) 96 kg bw at the time of dosing, received a nominal dose of 12 mg [^{14}C]-triclabendazole/kg of bw by gavage (Ferguson, 1994a). Both animals were sacrificed 28 days after dosing. Radioactivity was determined in liver, kidney, muscle (tenderloin, hindquarter and forequarter), perirenal fat, subcutaneous fat, plasma, and red cells. The tissues obtained in this study were later analysed in the context of a method validation study (Dieterle and Kissling, 1995). Concentrations of the marker residue in liver, kidney and muscle were determined for the male animal only.

A male ruminating calf of 91 kg bw at the time of dose administration was studied. A dose of 12.55 mg [^{14}C]-triclabendazole/kg of bw was administered by oral capsule and the animal was sacrificed on day 28 after treatment. Radioactivity was determined in liver, kidney, muscle, fat, blood, red blood cells, and plasma. Concentration of the marker residue was determined in liver, kidney and muscle (Needham, 2004b).

The above evaluation demonstrates that the ratio of marker to total residue concentrations is only known for liver, kidney and muscle of two young male animals of a small subpopulation with regard to age and bw.

A study similar in design to the study in cattle (Ferguson, 1994a) mentioned above was conducted in sheep (Ferguson, 1994b). Two sheep of 27 kg (female) and 33 kg (male) pre-dose bw were given a nominal dose of 10 mg [^{14}C]-triclabendazole/kg of bw orally by gavage. Animals were sacrificed 28 days after dosing. Radioactivity was determined in liver, kidney, muscle (hindquarter, forequarter, and tenderloin) and perirenal and subcutaneous fat. The concentration of marker residue was determined in muscle and liver of the male sheep in the context of the method validation study conducted by Dieterle and Kissling (1995). There were significant inconsistencies in the use of the specific radioactivities for

the calculation of total residue and also a major discrepancy in the total residue concentration given for liver in the two studies. The final results shown below were obtained following independent re-calculations of the data taking into account error propagation:

Ratio	Mean	Standard error
Muscle	0.400	0.104
Liver	0.248	0.011

A report providing very few details provides some limited information on the ratio of marker to total residue concentrations in muscle of goat and sheep sacrificed at an earlier time point after treatment. A goat of 42.5 kg bw and a sheep of 28.5 kg bw received a single oral dose of 10.1 and 10.5 mg/kg bw, respectively. The animals were sacrificed ten days following dosing. The reported ratio of marker to total residue concentrations in muscle was 0.4 in the goat and 0.42 in the sheep (Hamböck, 1982).

Table 21: Summary of available information on the ratio of marker to total residue concentrations.

Species	Bw (kg)	Dose (mg/kg of bw)	Days after dose administration	Ratio of marker to total residue concentrations		
				Liver	Kidney	Muscle
Bovine	96	12	28	0.19	0.24	0.41
	91	12.55	28	0.24	0.27	0.32
Ovine	33	10.45	28	0.25		0.4
	28.5	10.5	10			0.42
Caprine	42.5	10.1	10			0.4

The median concentrations of the marker residue in cattle tissues were based on data collected on day 28, the only day when the ratio of marker to total residue concentrations is known. Accordingly, two residue depletion studies with unlabeled drug in cattle were considered. One study involved the oral treatment of twenty-four Hereford cattle which was repeated 28 days later (Adams, 2004b). The second study involved the application of a pour-on to beef cattle (Study No. AA031, 2001). The samples collected in this study were stored frozen for up to 14 months prior to analysis; however, no stability data were provided to support the validity of these storage conditions. An earlier study (Needham, 2004b) demonstrated that the mean concentration of triclabendazole after 6 months frozen storage declined to 72% of the initial concentration. The data from the pour-on study were therefore considered to be unsuitable for the purpose of deriving median residues, or for recommending MRLs.

Data from the residue depletion study in cattle dosed orally (Adams, 2004b) were evaluated using the procedure adopted by the 66th meeting of the Committee (WHO Technical Report Series, No. 939, 2006). Accordingly, the points on the curve describing the upper one-sided 95% confidence limit over the 95th percentile and the linear regression line at day 28 were derived for muscle, liver and kidney. The results are shown in Table 22 as “Tol28” and “Median28”, respectively. Also shown in Table 22 is “f”, the inverse of the marker to total residue concentration ratio, for muscle, liver and kidney. The corresponding values for fat are, of necessity, conservative estimates because observed values are not available. The “true” median of the marker residue concentrations in fat may be about 50 µg/kg (compared with the conservative value of 100 µg/kg used in Table 22). The extrapolation shows that fat probably contributes <<5 % to the total intake; the conservative estimate of the median residue concentration in fat would therefore appear to be acceptable.

Table 22: Estimates of intakes based on the residue concentrations found in tissues of cattle on day 28 after treatment.

Estimates of dietary intakes							
Cattle	"Tol28"	"Median28"	Marker (µg/ person*day)	Total/ marker	Intake total (µg/person* day	Bioavailability	EDI (µg/person* day)
	µg/kg			f			
Muscle	246	161	48.2	3.1	149.4	0.13	19.4
Liver	827	423	42.3	5.4	228.5	0.13	29.7
Kidney	390	173	8.6	4.2	36.2	0.13	4.7
Fat		100	5.0	2.5	12.5	0.13	1.6
Sum					426.6		55.5

The results show that MRLs established on the basis of the tolerance limits of the marker residue concentrations found on day 28 after the last treatment would result in the EDI significantly exceeding the ADI (0-180 µg/person per day) when bioavailability is not taken into account. However, when bioavailability is factored in, which results in a more realistic estimate of consumer intake, the EDI of 55.5 µg per 60 kg person represents 30.8% of the ADI.

Similar considerations were applied to the evaluation of the residue data for sheep. The available database for sheep is even smaller than in cattle since measurable quantities of the marker residue were only found on days 14 and 28 in kidney and liver. In fat, all concentrations were < 50 µg/kg.

Table 23: Estimates of intakes on the basis of the residue concentrations found in tissues of sheep on day 28 after treatment.

Estimates of dietary intakes							
Sheep	"Tol28"	"Median28"	Intake marker (µg/person *day)	Total/ marker f	Intake total (µg/person *day)	Bioavailability	EDI (µg/person *day)
	µg/kg						
Muscle	174	103	31.0	2.50	77.6	0.13	10.1
Liver	288	154	15.4	4.00	61.6	0.13	8.0
Kidney	164	93	4.7	4.20	19.6	0.13	2.6
Fat		50	2.5	2.50	6.3	0.13	0.8
Sum			51.1		165.1		21.5

In Table 23, the median residue for fat is an hypothetical value, which is intentionally conservative and with kidney, the conversion factor for cattle kidney is used. The combined contribution of the total intake of fat and kidney is about 15%. In sheep, the EDI accounts for approximately 92% of the ADI when the bioavailability of the residues is not considered, and less than 12% of the ADI when the bioavailability of residues is taken into account. The tolerance limits of the marker residue concentrations found on day 28 after treatment are therefore an acceptable starting point for the recommendation of MRLs.

METHODS OF ANALYSIS

A report by Adams (2004c) on the validation of an analytical method for the determination of triclabendazole residues in cattle and sheep tissues (liver, kidney, muscle, fat) was reviewed by the 66th meeting of the Committee. This was an up-dated version of a method considered by the 40th Committee. Tissues are initially digested with hot alkali solution to release bound residues, then acidified, cooled and extracted with dichloromethane. For fatty tissues, an additional step to remove lipids by hexane-acetonitrile partitioning is included. The extract is evaporated to dryness, then taken up in ethanol:glacial acetic acid (1:1) and heated following addition of hydrogen peroxide to oxidize

the residues to keto-triclabendazole (the marker residue, identified as 5-chloro-6-(2, 3-dichlorophenoxy)-benzimidozole-2-one in the report of the 40th Committee). After a further partitioning step and evaporation to remove acetic acid, the residues are dissolved in dichloromethane and loaded on an anion exchange solid phase extraction cartridge and eluted with isopropyl alcohol/dichloromethane (12% v/v). The dried eluate is dissolved in acetonitrile and injected into the liquid chromatograph, with separation on a reversed phase (C-18) column and UV-detection at 296 nm. Quantitation is by external standard curve. Performance characteristics determined for the method are summarised in Table 24. The limits of detection and quantification for the method are based on estimates from calibration curves. The lowest concentration to meet acceptable performance criteria was 0.05 mg triclabendazole equivalents/kg (corresponding to 0.046 mg keto-triclabendazole/kg).

Table 24: Summary of validation study results for analysis of triclabendazole residues by liquid chromatography in various edible tissue.

Species	Edible Tissue	Limit of Detection[1] (mg/kg)	Limit of Quantification[2] (mg/kg)	Mean Recovery (%)	Repeatability[3] (%)
Cattle	Muscle	0.012	0.036	81-100	2.1-8.5
	Liver	0.024	0.074	84-87	1.7-9.6
	Kidney	0.020	0.058	89-97	3.1-9.4
	Fat	0.007	0.020	78-90	5.8-12
Sheep	Muscle	0.014	0.041	80-102	3.9-5.8
	Liver	0.008	0.024	90-102	2.4-4.8
	Kidney	0.012	0.034	89-93	5.6-7.0
	Fat	0.015	0.042	79-102	1.0-7.4

[1]. Based on mean response of blank, plus 3 standard deviations; [2]. Based on mean response of blank, plus 10 standard deviations; [3]. Within run, measured at 0.050, 0.100 and 0.200 mg/kg

No endogenous substances present in extracts produced a response in excess of the limit of quantification for keto-triclabendazole in any tissue. Other benzimidazole drugs, such as fenbendazole, thiabendazole and albendazole were not detected.

It was noted that the detection wavelength of 296 nm limits potential interferences. Triclabendazole sulphoxide and triclabendazole sulphone were detected, as was the parent drug triclabendazole; however, all three compounds were fully separated by the chromatography conditions used in the method. These compounds would normally be oxidized to keto-triclabendazole during the analysis. A confirmatory method was proposed which uses a phenyl liquid chromatography column as an alternative liquid chromatography system. Limits of quantification were higher than for the original method and the information obtained does not provide sufficient evidence for structural confirmation.

The stability of residues of triclabendazole in cattle tissues, measured as keto-triclabendazole, was determined (Adams, 2004d) using incurred residues in the tissues from two animals collected in Study Y03/49 (Adams, 2004b). Three replicates of each tissue were analysed prior to storage and then at 1.5, 3 and 6.5 months after storage in a freezer room that was maintained at a temperature ranging from a maximum average of –8°C to a minimum average of –22°C over the time period of the study. The average results, corrected for recovery, shown in Table 25, demonstrate that the residues remain essentially stable during this time period, with some decrease (maximum 33%) being seen at the final time point.

Table 25: Stability of incurred triclabendazole residues in cattle tissues under typical frozen storage conditions.

	Residues measured as keto-triclabendazole, for analytical recovery (mg/kg)			
	0 months (pre-storage)	1.5 months	3 months	6.5 months
Muscle 1	0.23 ± 0.01	0.24 ± 0.00	0.21 ± 0.00	0.19 ± 0.02
Muscle 2	0.25 ± 0.01	0.24 ± 0.02	0.20 ± 0.01	0.17 ± 0.02
Kidney 1	0.48 ± 0.03	0.42 ± 0.01	0.43 ± 0.05	0.36 ± 0.02
Kidney 2	0.47 ± 0.03	0.47 ± 0.06	0.44 ± 0.04	0.41 ± 0.02
Liver 1	0.85 ± 0.04	0.80 ± 0.02	0.78 ± 0.15	0.70 ± 0.04
Liver 2	0.75 ± 0.04	0.76 ± 0.04	0.81 ± 0.05	0.62 ± 0.01

A study reported the steps in Method REM 3/38 for determining residues that are hydrolysable and oxidisable to keto-triclabendazole (CGA-110 754) (Giannone, 1983). The sample is hydrolysed under alkaline conditions at 99-100^{o}C and the entire hydrolysate extracted with dichloromethane under acidic conditions. The dichloromethane is evaporated to dryness and the residue dissolved in a mixture of acetic acid/ethanol and oxidised overnight with hydrogen peroxide at 90^{o}C. The mixture is acidified and keto-triclabendazole is partitioned into dichloromethane. Further cleanup of the residue is carried out on a Silica Gel column followed by a C_{18} Sep-Pak column prior to the final determination by HPLC on a LiChrospher Si 100 column. The limit of quantification of the method is 0.027mg/kg keto-triclabendazole, which corresponds to 0.03 mg/kg triclabendazole.

Residues hydrolysable and oxidisable to keto-triclabendazole were quantified by method REM 3/38 and compared with total radioactivity in muscle from a goat (Adams, 2004e). A goat weighing 42.5 kg bw was dosed orally with [^{14}C]-labelled triclabendazole at a rate of 10.12 mg/kg of bw and sacrificed at 10 days after dosing. Tissue samples were collected and stored at –20^{o}C. Incurred residues in muscle were investigated. Total radioactivity was measured by scintillation counting after combustion; keto-triclabendazole was determined by HPLC according to method REM 3/38 except the residue was not cleaned up using a Sep-Pak column. Residues determined by method REM 3/38 accounted for 32-39% of the total radioactivity present with goat muscle (Table 26).

Table 26: Comparison of total radioactivity and total residues determined by HPLC in muscle of a goat dosed with [^{14}C]-labelled triclabendazole and sacrificed 10 days later.

Sample	Total radioactivity calculated as triclabendazole equivalent mg/kg	Total residues[1] determined by HPLC with method REM 3/83	
		mg/kg	% of total radioactivity
1	0.44	0.17	39
2		0.15	34
3		0.14	32
4		0.14	32

[1]. Residues determined as keto-triclabendazole and converted to triclabendazole with the conversion factor 1.09.

A study of Method REM 15/83, which is a replacement for method REM 3/83, was reported (Giannone and Formica, 1983). The two methods are identical through all steps up to and including the cleanup of residues on a C_{18} Sep-Pak column. Following C_{18} Sep-Pak column cleanup, final determination of keto-triclabendazole with method REM 3/38 is carried out on a LiChrospher 100 column. With the replacement method REM 15/38, keto-triclabendazole is determined by HPLC on a LiChrospher Si 100 column (as for method REM 3/83) or by a column switching technique involving two LiChrospher Si 100 columns. Recovery data for the determination of keto-triclabendazole with

and without column switching are comparable (Table 27). The limit of quantitation for method REM 15/83 is 0.027 mg keto-triclabendazole or 0.03 mg triclabendazole per kg.

Table 27: Recovery of keto-triclabendazole in muscle, liver, kidney and fat of sheep and cattle

Tissue	Fortification (mg/kg)	Recoveries after Sep-Pak cleanup (%)		Recovery after column switching (%)
		Cattle	Sheep	Sheep
Muscle	0.1	109	85, 69, 95	97, 95
	0.5	76	87, 67, 70, 74	72, 79
Liver	0.1	71	82, 76, 68, 68	67, 75
	0.5	77	85, 70, 60, 66	76, 79
Kidney	0.1	80	80, 73, 83, 74	89, 98
	0.5	70	77, 72, 75, 67, 75	75, 76
Fat	0.1	69	53	71, 73, 68
	0.5	55	69	54, 60, 61

Further validation of the method for analysis of sheep and cattle tissues was provided in Study V05/24 (Adams, 2005). The study demonstrated no background interferences; confirmed that precision was ≤15% at concentrations >0.10 mg/kg; and demonstrated the stability of the residues under freeze/thaw conditions.

In Study Y04/51, the method was extended to the analysis of tissues from goats (Adams, 2004e). Results, shown in Table 28, are based on analysis of three replicates at each of three concentrations for the three tissues tested (muscle, liver, kidney).

Table 28: Recovery and precision for determination of keto-triclabendazole residues in goat tissues.

Tissue	Concentration of keto-triclabendazole (µg/kg)					
	50		100		100[1]	
	Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)
Muscle	99	2.2	102	2.2	95	4.1
Liver	110	13	97	3.4	91	11
Kidney	98	1.7	85	2.7	85	5.4

[1]. Fortified samples analysed after storage at room temperature for 16-24 hours.

The stability of residues of triclabendazole in sheep tissues, measured as keto-triclabendazole, was determined (Adams, 2004f) using incurred residues in tissues obtained from two animals in Study Y04/22 (Adams, 2004a). Three replicates of each tissue were analysed prior to storage and after 2 and 4 months of frozen storage. The storage temperature varied from –5°C to 21°C during the study. There was minimal change in the residue concentration during the period of storage (Table 29).

Table 29: Stability of incurred triclabendazole residues in sheep tissues under typical conditions of frozen storage.

Tissue	Residues measured as keto-triclabendazole corrected for analytical recovery (mg/kg)		
	0 months (pre-storage)	2 months	4 months
Muscle 1	0.17 ± 0.01	0.15 ± 0.00	0.16 ± 0.01
Muscle 2	0.13 ± 0.01	0.12 ± 0.00	0.11 ± 0.01
Kidney 1	0.25 ± 0.01	0.23 ± 0.01	0.24 ± 0.00

Kidney 2	0.17 ± 0.01	0.15 ± 0.02	0.15 ± 0.00
Liver 1	0.47 ± 0.02	0.41 ± 0.04	0.41 ± 0.05
Liver 2	0.34 ± 0.01	0.27 ± 0.02	0.28 ± 0.02

Two new methods (Study No. AA031, 2001; Study No. ANT1274, 2002) for determining triclabendazole residues in animal tissues (referred to below as method 1 and method 2, respectively) were submitted for review by the 70th Committee. These methods are similar to the method reported by Adams (2004c) discussed above. Briefly, both methods involved alkaline hydrolysis of tissue homogenates at 90-100^{o}C, followed by extraction with dichloromethane under acidic conditions. In method 1, the solvent extracts were cleaned up using liquid/liquid partitioning. No clean up step was included in method 2. In both methods, the extracts were then oxidised overnight with hydrogen peroxide at 85-90^{o}C. Subsequently, the keto-triclabendazole analyte was partitioned into dichloromethane before clean up on an SPE column (method 2 only), and quantitation by HPLC with UV detection at 295-297 nm. Residue levels (expressed in keto-triclabendazole equivalents) were determined using an external standard calibration curve. Validation data for the analytical methods were provided to demonstrate the linearity of detector response, recoveries from fortified samples, method precision, and the limits of quantitation and detection.The following validation parameters were investigated in Study No. AA031 (2001) for method 1: linearity, precision, accuracy, specificity, limit of quantitation and limit of detection. The validation results are presented in Table 30.

Table 30: Summary of validation study results for analysis of triclabendazole residues by liquid chromatography (method 1) in bovine tissues.

Validation parameter	Details of test	Tissue matrix	Fortification level (mg/kg)	Linearity (r^2)	Accuracy (% recovery)	Precision (% RSD)
Linearity	Calibration standards extracted from tissues	Muscle	0.1-2.5	0.9995	--	--
		Kidney	0.05-2.5	0.9998	--	--
		Liver	0.05-2.5	0.9997	--	--
		Fat	0.05-2.5	0.9994	--	--
Recovery	% Recovery from fortified tissue samples (n=3)	Muscle	0.007-0.46	--	74-112	--
		Kidney	0.02-1.0	--	85-121	--
		Liver	1.2-1.6	--	94-100	--
		Fat	0.015-2.3	--	95-158	--
Precision	Replicate analyses of fortified samples	Muscle	0.007-0.46	--	--	10.9
		Kidney	0.02-1.0	--	--	10.3
		Liver	1.2-1.6	--	--	2.3
		Fat	0.015-2.3	--	--	24.0
Specificity	Determine whether there are method interferences associated with tissue components or related compounds			No known interferences detected and chromatographic runs showed specificity for all tissue types. No interference due to fenbendazole or oxfendazole.		
LOQ	Limit of quantitation (mg/kg)			LOQ (all tissues) = 0.045 mg/kg		
LOD	Limit of detection (mg/kg)			LOD (all tissues) = 0.03 mg/kg		

The validation parameters investigated in Study No. ANT1274 (2002) for method 2 were linearity, recovery from fortified samples, precision and limit of quantitation. The validation results are presented in Table 31.

Table 31: Summary of validation study results for analysis of triclabendazole residues by liquid chromatography (method 2) in bovine tissues.

Validation parameter	Details of test	Tissue matrix	Fortification level (µg/tube)	Linearity (r^2)	Accuracy (% Recovery)	Precision (% RSD)
Linearity	Calibration standards extracted from tissues	Muscle	--	0.9999	--	--
		Kidney	--	0.9870	--	--
		Liver	--	0.9983	--	--
		Fat	0.19-25	0.9992-0.9998	--	--
Recovery	% Recovery from fortified tissue samples (n=4-6)	Liver	0.39-25.0	--	78-120	--
		Fat	0.39-6.25	--	62-121	--
Precision	Replicate analyses of fortified samples	Liver	0.4 3.1 25.0	--	--	20.5 6.6 5.1
		Fat	0.4 1.6 6.3	--	--	20.4 10.0 15.2
LOQ	Limit of quantitation (mg/kg)			Muscle Kidney Liver Fat		0.13 1.25 0.39 0.19

It is noted that the LOQ for kidney of 1.25 mg/kg is anomalously high. The validation data for both methods are acceptable.

APPRAISAL

The 17th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) requested that the MRLs for triclabendazole in cattle and sheep be re-evaluated, including reconsideration of the data on bioavailability. No new studies on pharmacokinetics or metabolism were provided for evaluation; however, three new residue studies in cattle using a pour-on formulation were submitted. In its re-evaluation of the MRLs for triclabendazole in cattle and sheep, the Committee therefore re-evaluated the pharmacokinetic and metabolism data considered at the 40th and 66th meetings; evaluated the three new studies in cattle and re-evaluated the residue studies considered by the previous meetings; and reconsidered the studies which investigated the bioavailability of incurred residues of triclabendazole. This monograph reports the Committee's considerations and MRL recommendations for triclabendazole in cattle and sheep. The recommended MRLs were derived using the procedure adopted by the 66th meeting of the Committee (WHO Technical Series Report, No. 939, 2006).

Re-evaluation of the pharmacokinetic and metabolism data considered at the 40th and 66th meetings of the Committee confirmed the earlier findings. The ratio of marker residue concentration to total residue concentration in cattle tissues (muscle, liver and kidney) and sheep tissues (muscle and liver) on day 28 were derived from the metabolism studies and are shown in Table 21.

The modelling of dietary intake of residues present in cattle tissues was conducted at day 28, the only day when the ratio of the marker residue concentration to total residue concentration is known. The results of modelling show that the bioavailability of residues must be taken into account when establishing cattle MRLs; otherwise the EDI exceeds the ADI. Three studies (Hassler, 1995; Hardwick, 2004b; Needham, 2004a) on bioavailability were evaluated by the 66th meeting of the

Committee and were reconsidered by the present Committee. Only the study by Needham (2004) was suitable for determining the absolute bioavailabilities of [^{14}C]-triclabendazole administered by gavage, and of [^{14}C]-triclabendazole residues derived from lyophilised cattle tissues in the diet, and in turn, the bioavailability of [^{14}C]-triclabendazole-derived residues in lyophilised cattle tissues relative to the bioavailability of [^{14}C]-triclabendazole administered by gavage. Measurements of areas under the radioactivity-time curve indicated that the absolute bioavailability of [^{14}C]-triclabendazole approximated 70% when administered by oral gavage to rats. It is important to note that this value was used when establishing the ADI for triclabendazole. The absolute bioavailability of incurred residues in cattle tissues was the highest for liver at 9.2%. Based on these values, the bioavailability of incurred liver residues in cattle was calculated to be 13% (9.2/70 x 100) relative to gavage administration. Studies by Hassler (1995) and Hardwick (2004b) using the bile duct-cannulated rat model confirmed that the bioavailability of incurred residues from liver was higher than for muscle or kidney. However, the relative bioavailability could not be calculated based on the data from these studies. Therefore, the relative bioavailability for liver of 13% was used in the calculation of the EDI, as it represents the worse-case scenario.

Three new residue depletion studies involving pour-on applications to cattle were provided (Study No. AA031, 2001; Study No. ANT1274; Study EL-55021, 2004). In these studies, animals were sacrificed at days 14, 21, 28, 35 and 42; days 49, 56, 63 and 70; and days 77, 91, 105, and 119, respectively. For the purpose of recommending MRLs using the procedure adopted at the 66th meeting of the Committee, the ratio of marker to total residue concentrations must be known at the time point under consideration. In the case of triclabendazole, such information is available for day 28 only and in this regard, only the first of the three pour-on studies analysed tissues sampled on day 28 after the last treatment. In this study, however, samples were stored frozen for up to 14 months prior to being analysed for residues, and no data were provided from studies which investigated the stability of residues stored for this duration. Earlier studies by Needham (2004b) and Adams (2004d) found that triclabendazole residues in liver declined to 72% and 83% of the initial concentration, respectively, after 6 months of storage. On account of the uncertainty surrounding the stability of residues when stored frozen for 14 months, the data from Study No. AA031 were not considered suitable for the purpose of recommending MRLs.

A residue depletion study (Adams, 2004b) evaluated at the 66th meeting of the Committee was reconsidered. Bioavailability of incurred residues was taken into account and MRLs were recommended using the procedure adopted at the 66th meeting of the Committee (FAO/WHO, 2006). The sponsor's statistician noted: "The linear regression assumptions regarding homogeneity of variances and of normal distribution of errors are met for the muscle, liver and kidney. The assumption of linearity is solely met for liver." Nevertheless, this approach was used with minimal numerical differences (0.6-1.7%) in the calculated tolerance limits, compared to the present evaluation. The results of the study are summarised in Figure 14.

Figure 14: Depletion kinetics of residues convertible to keto-triclabendazole in tissues of cattle treated orally with a nominal dose of 18 mg triclabendazole/kg of bw on two occasions at an interval of 28 days.

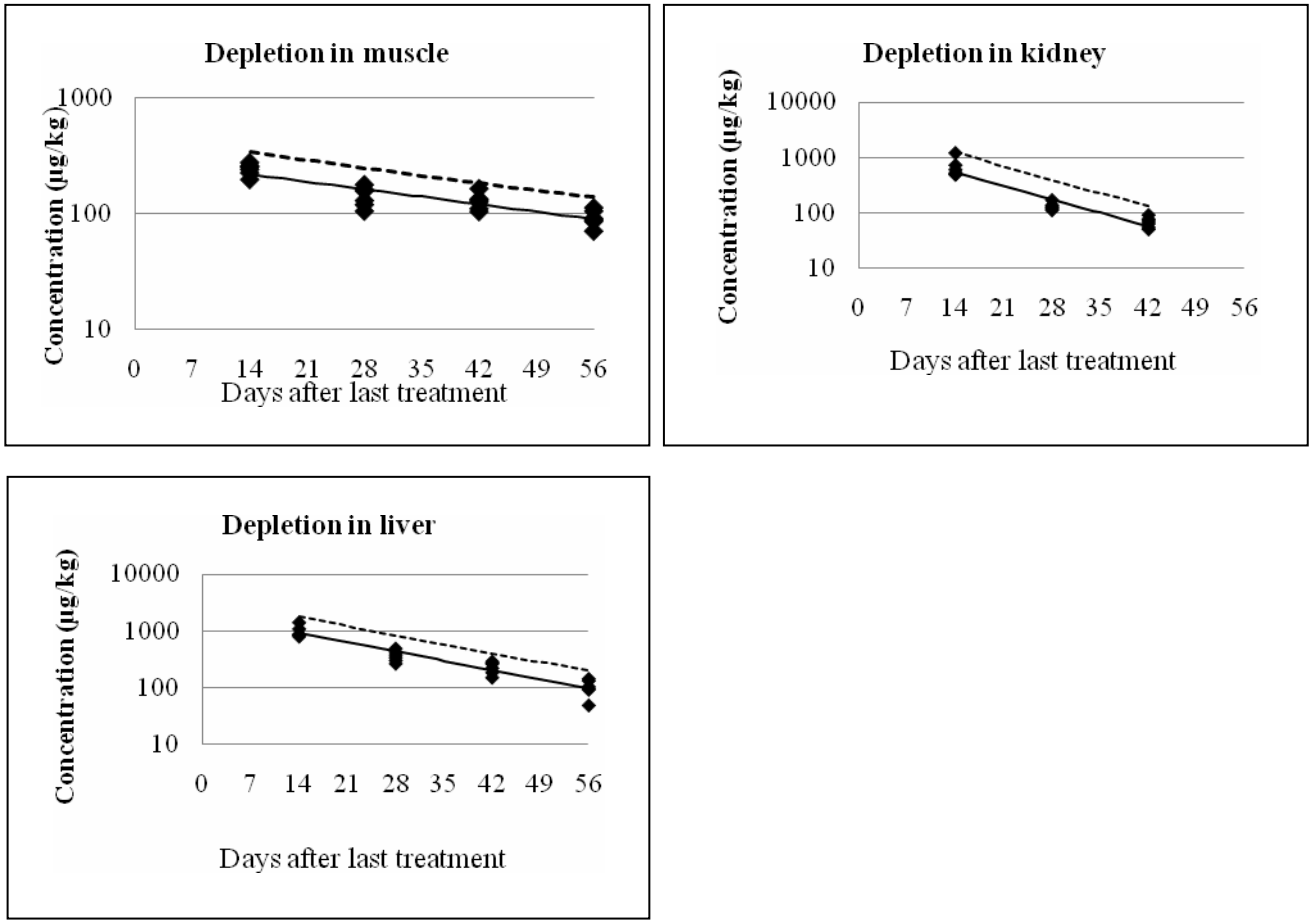
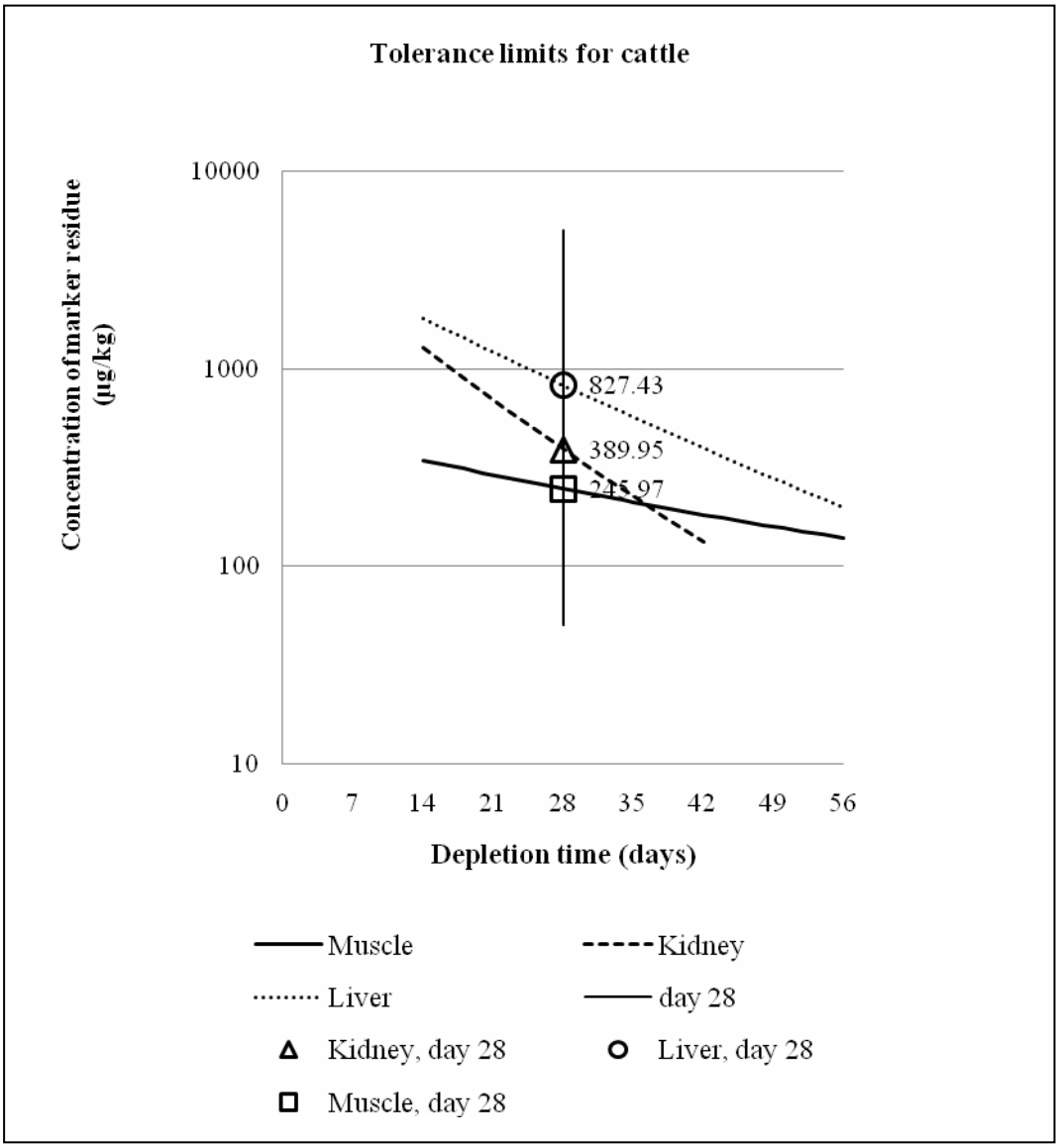


Figure 15 shows only the tolerance limit curves for the concentration of marker residue in liver, kidney and muscle of cattle. It also highlights the corresponding values for day 28, the only day when the ratios of the concentrations of marker and total residue are known. This is the only day for which dietary intake estimates are possible.

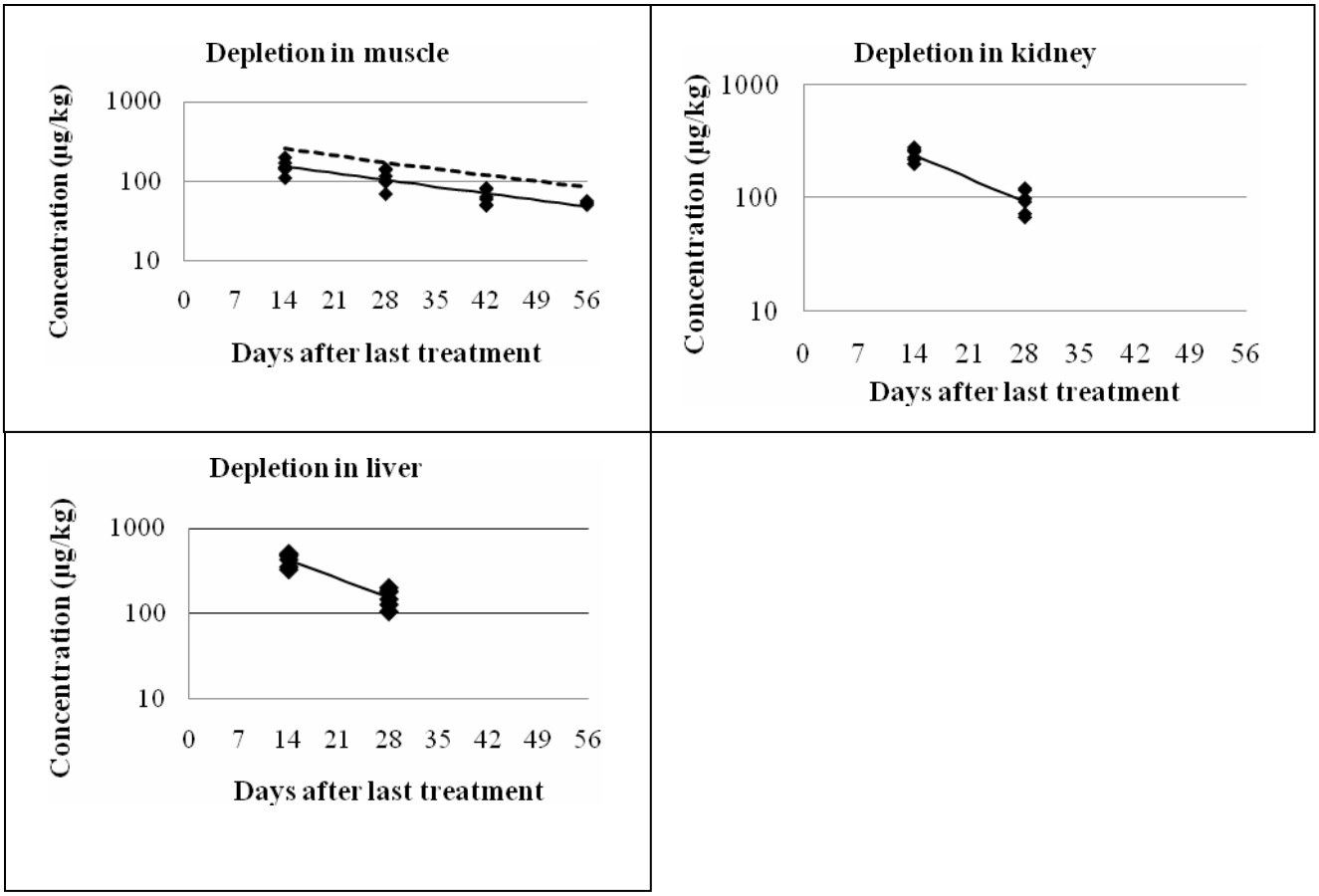
Figure 15: Tolerance limit curves for the concentration of marker residue in tissues of cattle and the concentration of marker residue in these tissues at day 28.



The proportions of the tolerance limits calculated for day 28 are 827:390:246 for liver:kidney:muscle. Table 22 shows some model calculations from the perspective of MRLs in cattle.

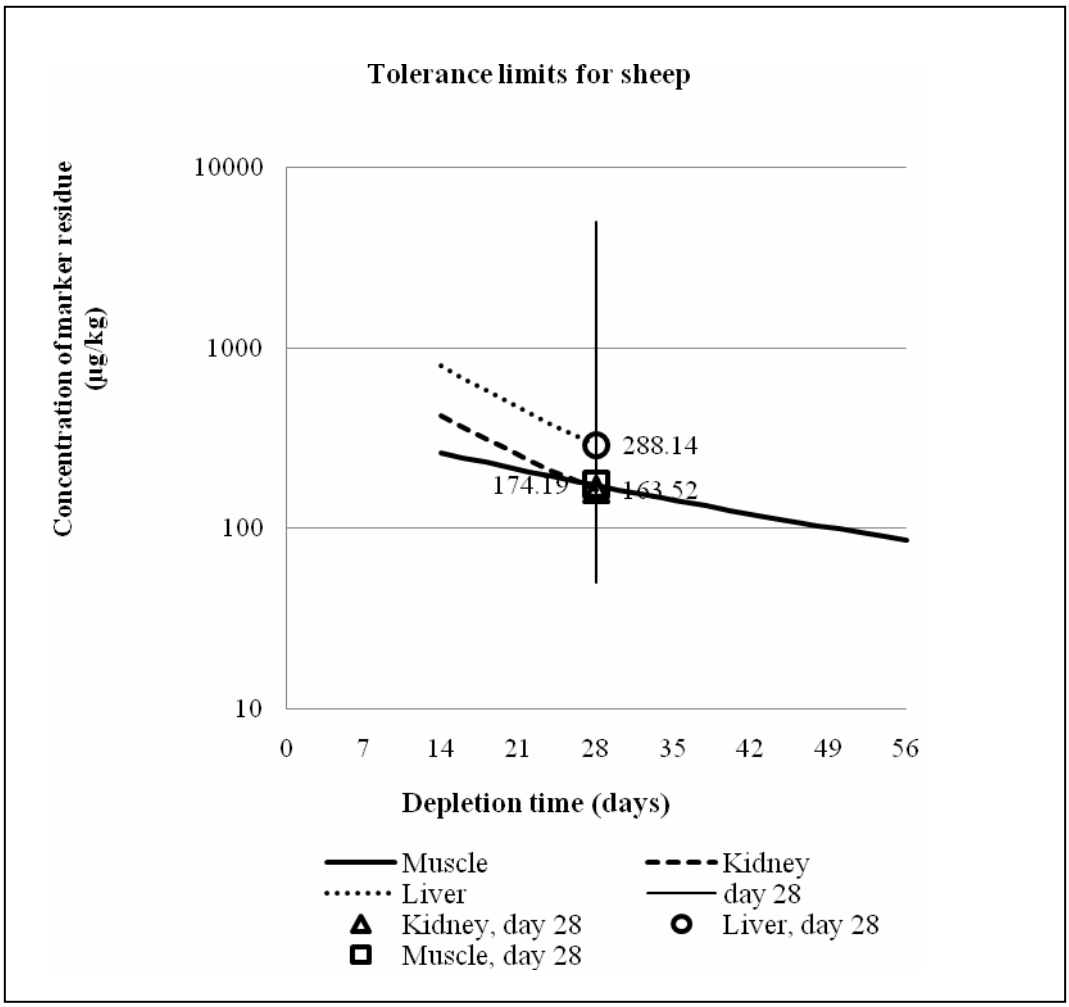
Similar considerations to those described above for cattle were applied to the evaluation of the sheep residue data. Figure 16 shows the depletion of marker residue in tissues of sheep.

Figure 16: Depletion kinetics of residues convertible to keto-triclabendazole in sheep tissues treated with a single oral dose of 10.5-13 mg triclabendazole/kg of bw.



The available database for sheep is smaller than that of cattle as measurable quantities of the marker residue were only found on days 14 and 28 in kidney and liver. In fat, all concentrations were < 50 µg/kg. Figure 17 summarises the results of statistical data analysis which is analogous to Figure 15.

Figure 17: Tolerance limit curves for the concentration of marker residue in tissues of sheep and the concentration of marker residue in these tissues on day 28.

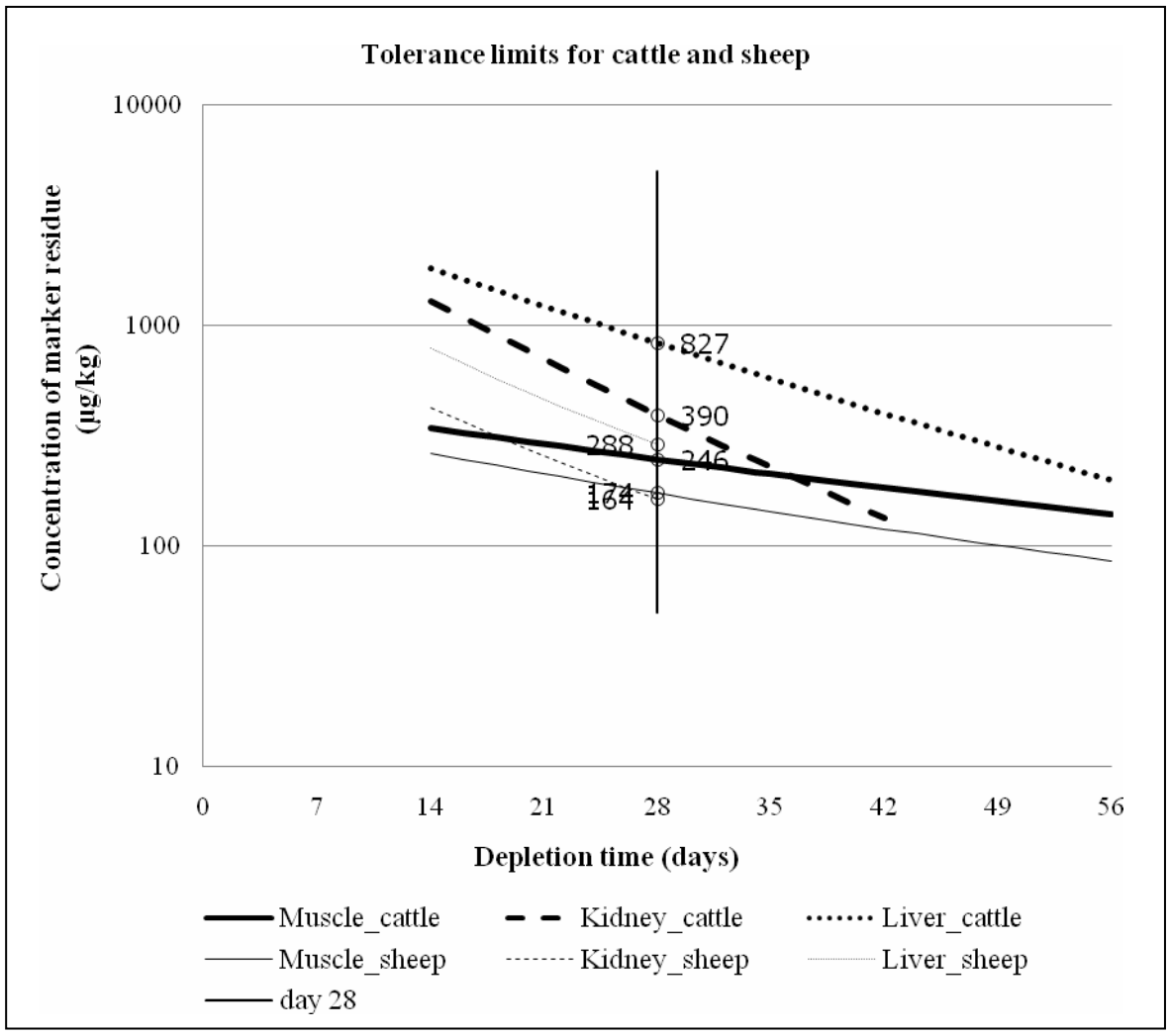


The proportions of the tolerance limits calculated for day 28 are 288:164:174 for liver:kidney:muscle. Table 23 shows calculations from the modelling of MRLs in sheep.

The dietary intake estimate shows with sheep, the tolerance limits of the marker residue concentrations found on day 28 after treatment are suitable for the establishment of MRLs.

Figure 18 combines the results of the depletion studies in cattle and sheep. The graph shows that the kinetic behaviour of triclabendazole is distinctly different in cattle and sheep and that there is no basis for establishing MRLs of identical numerical values for the two species.

Figure 18: Tolerance limit curves for the concentration of marker residue in tissues of cattle and sheep.



MAXIMUM RESIDUE LIMITS

In recommending the MRLs, the Committee took into account the following factors:

- An ADI of 0-3 µg/kg of bw was established by the fortieth meeting of the Committee, equivalent to 0-180 µg for a 60 kg-person.
- The marker residue is the sum of all residues extracted and converted to keto-triclabendazole.
- Liver and muscle are suitable target tissues.
- A validated analytical method is available for analysis of triclabendazole residues in edible tissues of cattle and sheep.
- The bioavailabilities of [^{14}C]-triclabendazole and [^{14}C]-triclabendazole-derived incurred residues administered to rats by oral gavage was 70% and 9.2%, respectively. Based on these data, the relative oral bioavailability of incurred residues was 13%.
- In cattle, the ratios of marker residue concentration to total residue concentration were 0.32 for muscle, 0.19 for liver, 0.24 for kidney and 0.4 for fat on day 28. In sheep, the ratios were 0.4 for muscle, 0.25 for liver, 0.24 for kidney and 0.4 for fat (a conservative value based on that for fat from cattle).

- The kinetic behaviour of triclabendazole is distinctly different in cattle and sheep and there is no basis for establishing MRLs with the same numerical values for the two species.
- MRLs for liver, kidney and muscle from cattle and sheep were derived from the curve describing the upper one-sided 95% confidence limit over the 95th percentile of the residues of the marker residue keto-triclabendazole on day 28 after the last treatment and are thus higher than those recommended by the 66th meeting of the Committee, which were based on the time point of 56 days.
- MRLs for fat were based on twice the LOQ of the analytical method.

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, expressed as the marker residue, keto-triclabendazole: muscle, 250 μg/kg; liver, 850 μg/kg; kidney, 400 μg/kg; and fat, 100 μg/kg. These values were derived from the curve describing the upper one-sided 95% confidence limit over the 95th percentile of the residues on day 28 after the last treatment. The latter are depicted as "Tol28" in Table 22.

The Committee also recommended MRLs for triclabendazole for edible tissues of sheep, expressed as the marker residue, keto-triclabendazole, as follows: muscle, 200 μg/kg; liver, 300 μg/kg; kidney, 200 μg/kg; and fat, 100 μg/kg. These values were derived from the curve describing the upper one-sided 95% confidence limit over the 95th percentile of the residues on day 28 after the last treatment. The latter are depicted as "Tol28" in Table 23.

The Committee calculated the EDI using the median concentrations of marker residues in cattle tissues at day 28. The data for cattle (shown in Table 22) and not sheep (shown in Table 23) were chosen for the EDI calculation because the concentration of median residues in all tissues was higher for cattle than for sheep. Accordingly, the EDI represents 47.4 % of the ADI.

Tissue	Median residue	Standard Food Basket	Total residue concentration/Marker residue concentration	Bioavailability	EDI
Muscle	160.6 μg/kg	0.3 kg	3.1	0.13	19.4 μg
Liver	423.1 μg/kg	0.1 kg	5.4	0.13	29.7 μg
Kidney	172.5 μg/kg	0.05 kg	4.2	0.13	4.7 μg
Fat	100 μg/kg	0.05 kg	2.5	0.13	1.6 μg
EDI					55.4 μg

The MRLs previously recommended by the sixty-sixth meeting of the Committee for triclabendazole for cattle and sheep were withdrawn. As the Committee recommended significantly different MRLs for cattle and sheep and upon reviewing the limited data base for residues in goats, the Committee concluded that there was insufficient data to extend the recommended MRLs for goats. Therefore, the MRL for goats recommended at the sixty-sixth meeting of the Committee were withdrawn.

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