Triclabendazole

First draft prepared by
Dieter Arnold, Berlin, Germany

Addendum to the monographs prepared by the 40th, 66th and 70th Meetings of the Committee and published in *FAO Food & Nutrition Paper* 41/5 and *FAO JECFA Monographs* 2 and 6, respectively

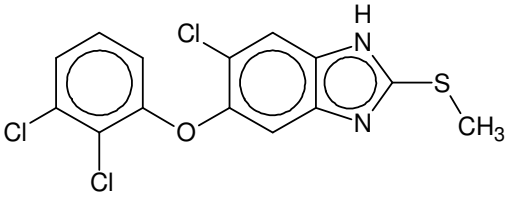
Identity

IUPAC name: 6-chloro-5-(2,3-dichlorophenoxy)-2-methylsulfanyl-1H-benzimidazole

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

Structural formula:

Benzimidazoles normally undergo an inter-molecular proton transfer between N-1 and N-3 in the imidazole ring which at room temperature is very rapid and yields to tautomers (Iddon *et al.*, 1992).



Molecular formula: $C_{14}H_9C_{l3}N_2OS$

Molecular weight: 359.66 g/mol

Other information on identity and properties

Pure active ingredient: Triclabendazole

Appearance: White crystalline solid

Melting point: The melting point of commercial products is frequently given as 175–176°C. This is also the value given in the Merck Index. Another source: (http://www.wolframalpha.com/entities/chemicals/triclabendazole/b0/ve/bh/) reports 177°C. Another form with a melting point of 85–90°C has also been described in the literature (Iddon *et al.*, 1992). Tothadi *et al.* (2012) describe two anhydrous forms, one consisting of one tautomer only and exhibiting the higher melting point of 177°C and the other consisting of an equimolar mixture of the two tautomers and exhibiting a melting point of 166°C.

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

Octanol water partition coefficient (P_{OW}): 1.75×10^6

UV_{max}: Approximately 305 nm, depending on solvent (Shrivastava, Kumar and Jain, 2011).

Residues in food and their evaluation

The Committee has reviewed triclabendazole at its 40th, 66th and 70th meetings (FAO/WHO, 1993, 2006, 2009). At the 40th meeting the Committee established an ADI of 0–3 μg/kg bw (0–180 μg/day for a 60 kg person) and recommended Maximum Residue Limits expressed as 5-chloro-6-(2′,3′-dichlorophenoxy)-benzimidazole-2-one for muscle, liver, kidney and fat of cattle and sheep. The marker residue on which the MRLs proposed by the 40th meeting were based is produced when common fragments of triclabendazole-related residues are hydrolysed under alkaline conditions at 90–100°C. Its concentrations can be converted into triclabendazole equivalents by multiplying with a conversion factor of 1.09. The 66th meeting defined the marker residue as "keto-triclabendazole" and recommended MRLs for muscle, liver, kidney and fat in cattle, sheep and goat. The sponsor correctly defined the marker residue as "sum of the extractable residues that may be oxidised to keto-triclabendazole". This definition was also used by the 70th meeting of the Committee: "The marker residue is the sum of all residues extracted and converted to keto-triclabendazole." On this basis the Committee recommended the MRLs listed in Table 7.1.

Table 7.1 MRLs for triclabendazole recommended by the 70th meeting of the Committee

Species	MRL (μg/kg)			
	Muscle	Liver	Kidney	Fat
Cattle	250	850	400	100
Sheep	200	300	200	100

The MRLs in muscle, liver and kidney of both species 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. MRLs for fat were based on twice the LOQ of the analytical method. 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 database for residues in goats, the Committee concluded that there was insufficient data to extend the recommended MRLs for goats. The MRLs for goats recommended at the sixty-sixth meeting of the Committee were withdrawn.

The Committee was requested to review triclabendazole by the 19th session of the CCRVDF, that had raised the specific question: "Can MRLs for goat (tissues) be established by extrapolation considering data used for recommending MRLs for cattle and sheep (tissues)." On the question of data , the CCRVDF had stated: "JECFA has established MRLs for sheep and cattle and extrapolation would be based on the data packages available to the 70th JECFA and literature review to be provided by the United States of America."

Since the dossier provided for evaluation by the 70th meeting has been extensively reviewed by the Committee, the present addendum focuses mainly on studies possibly suitable to answer the question raised by the CCRVDF. These are primarily studies that could be useful for extrapolation from sheep to goat. Some other available studies performed in cattle and sheep are not reviewed again in the present addendum.

Conditions of use

Table 7.2 summarizes recently received information on approved products that are regulated in different countries and which contain triclabendazole and include goat as target species.

Table 7.2. A selection of commercially available triclabendazole products for use in goat

Country	Product	TCBZ	Target animal	Dose	Withdrawal time
Australia	Fasinex 50 Flukicide	5%	cattle		
			sheep		
			goat	1 ml/5 kg	21 days
	Fasinex 100 Oral Flukicide	5%	cattle		
			sheep	1 ml/10 kg	21 days
			goat		
	Young's Tricla 50 Flukicide	5%	cattle		
			sheep	1 ml/5 kg	21 days
			goat		
France	Fascinex 5%	5%	sheep	1 ml/5 kg	28 days
			goat		
Mexico	Fasinex 10%	10%	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
	Fasimec	12% plus 2% ivermectin	cattle		
			sheep	0.5 ml/5 kg	21 days
			goat		
New Zealand	Fasinex 10	10%	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
South Africa	Endex 19.5%	12% plus 7.5% levamisole	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
	Fasinex 10%	10%	cattle		
			sheep	1 ml/10 kg	28 days
			goat		
Switzerland	Fasinex 5%	5%	sheep		
			goat	1 ml/5 kg	28 days

NOTES: TCBZ = triclabendazole; percentages are given on a weight/volume basis.

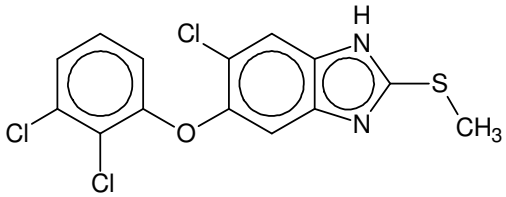
Dosage

Triclabendazole is typically administered orally to sheep and goat. The recommended dose is usually 10 mg/kg bw, occasionally 12 mg/kg bw in both sheep and goat. The dose is typically administered in liquid formulations (tablets are also available). Body weights are typically rounded up in steps of 5 or 10 kg when calculating the volume of the formulation to be administered. Overdosing may therefore occur systematically, and would be more significant in animals with comparatively low body weights.

Pharmacokinetics and metabolism

The following scheme shows some structures related to triclabendazole (metabolites and conversion products) that will be discussed in subsequent paragraphs.

TCBZ



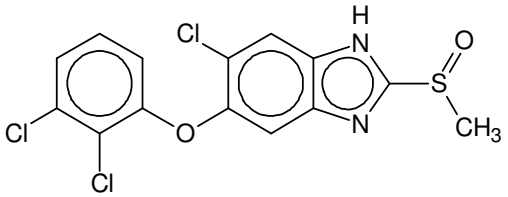
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylthio)-1*H*-benzimidazole

Molecular Formula = $C_{14}H_9Cl_3N_2OS$

Formula Weight = 359.66

Synonyms and abbreviations: CGA 89317 and CGP 23030.

TCBZ-sulphoxide



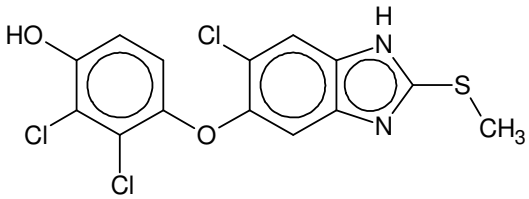
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylsulfinyl)-1*H*-benzimidazole

Molecular Formula = $C_{14}H_9Cl_3N_2O_2S$

Formula Weight = 375.66

Synonyms and abbreviations: CGA 110752

TCBZ-OH



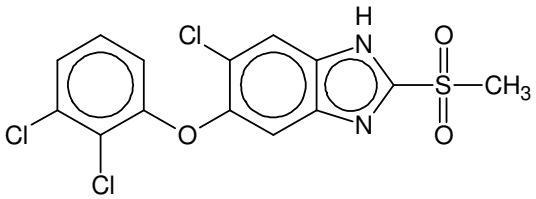
2,3-dichloro-4-{[6-chloro-2-(methylthio)-1*H*-benzimidazol-5-yl]oxy}phenol

Molecular Formula = $C_{14}H_9Cl_3N_2O_2S$

Formula Weight = 375.66

Synonyms and abbreviations: CGA 161944

TCBZ-sulphone



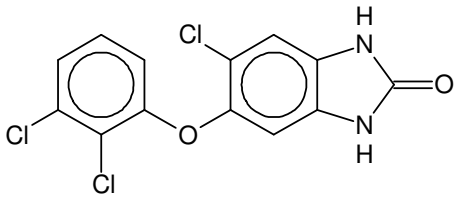
6-chloro-5-(2,3-dichlorophenoxy)-2-(methylsulfonyl)-1*H*-benzimidazole

Molecular Formula = $C_{14}H_9Cl_3N_2O_3S$

Formula Weight = 391.66

Synonyms and abbreviations: CGA 110753

Keto-TCBZ



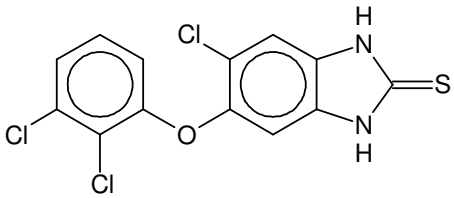
5-chloro-6-(2,3-dichlorophenoxy)-1,3-dihydro-2*H*-benzimidazol-2-one

Molecular Formula = $C_{13}H_7Cl_3N_2O_2$

Formula Weight = 329.56

Synonyms and abbreviations: CGA 110754

TCBZ-thione



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

Synonyms and abbreviations: CGA 77336

Food Producing Animals

The absorption, distribution, metabolism and excretion are very similar in the laboratory animals and food producing animals studied. A detailed review of the available information was performed by the 70th meeting of the Committee (FAO/WHO, 2009). Some studies are summarized here because the data could be helpful in considerations of between-species extrapolations (sheep and goat).

Sheep and goat

The distribution, degradation and excretion of ^{14}C-labelled triclabendazole were studied in a single female sheep (Hamböck and Strittmatter, 1982) and in a single lactating goat (Hamböck and Strittmatter, 1981). The same radiolabel preparation was used in both animals. The kinetic changes of the concentrations of radioactivity in blood, blood cells (only a few samples) and in plasma were measured during a period of 10 days. During the same period excretion in urine and in faeces (and in milk for goat) was determined. Animals were killed 10 days after the treatment and radioactivity was measured in a great number of tissues including, liver, kidney, muscle and fat. The tissues of both animals were also used to characterize the radioactive residues in these tissues (Hamböck, 1982). The metabolic fate of triclabendazole was finally summarized (including results of studies in the rat and analyses of metabolites in urine and faeces of sheep, goat, and rat) and a metabolic pathway was proposed (Hamböck, 1983). Table 7.3 summarizes the conditions of the experiments performed in the sheep and goat.

Table 7.3. Summary of the studies performed in a single sheep and a single goat

Species and breed	Animal	Age	Body weight	Product used	Formulation	Route	Dosage
Sheep Swiss White Alp × Ile de France	1 female	4 months	28.5 kg	^{14}C-CGA 89317 Specific activity 7.7 µCi/mg	2 gelatin capsules rinsed with ca. 0.5 L water	oral	10.5 mg/kg bw
Goat Chamoises des Alpes	1 female, lactating	Approx. 3 years	42.5 kg		3 gelatin capsules rinsed with ca. 0.5 L water	oral	10.1 mg/kg bw

Figure 7.1 shows the kinetics of the radioactivity in blood and plasma of the two animals and in milk obtained from the goat. The highest concentrations in plasma were measured in the samples taken 24 h after treatment. Concentrations in sheep plasma were higher than in goat plasma and the rates of distribution and elimination were apparently lower in the sheep.

More than 100% of the administered radioactivity was recovered during the observation period of 240 h. Most of the administered radioactivity was excreted in the faeces. The time course of cumulative recovery from urine and faces is shown in Figure 7.2. The curves were very similar in both animals. Cumulative excretion of the radioactivity is given in Table 7.4.

Table 7.4. Recovery of radioactivity in excreta of a sheep and a goat

Animal	Recovery of radioactivity (% of dose)			
	Urine	Faeces	Milk	Total recovery
Sheep	3.54	100.85		104.39
Goat	2.12	98.8	0.56	101.48

Faeces and urine pooled from both animals and over the period 0–72 h were analysed for radioactive metabolites of the parent drug. Using a variety of extraction procedures, chromatographic separations, chemical transformations and physical-chemical identification methods, several metabolite fractions could be separated and some of them could be identified. The pattern of metabolites was qualitatively and quantitatively similar in the two animals.

It was concluded that the predominant pathway of biotransformation was the oxidation of the 2-thiomethyl group producing the sulphoxide and the sulphone, and to a limited

extent the 2-benzimidazolone. A separate minor oxidative pathway leads to the 4'-hydroxy derivative (Hamböck, 1983). Several groups of authors have studied the metabolic pathways of triclabendazole. Virkel *et al.* (2006), for example, have shown that both flavin-containing mono-oxygenases (FMO) and cytochromes P450 are involved in the oxidation of triclabendazole in sheep liver. The FMO system is mainly involved in the sulphoxidation. Both enzyme systems participate in similar proportion in the formation of the sulphone.

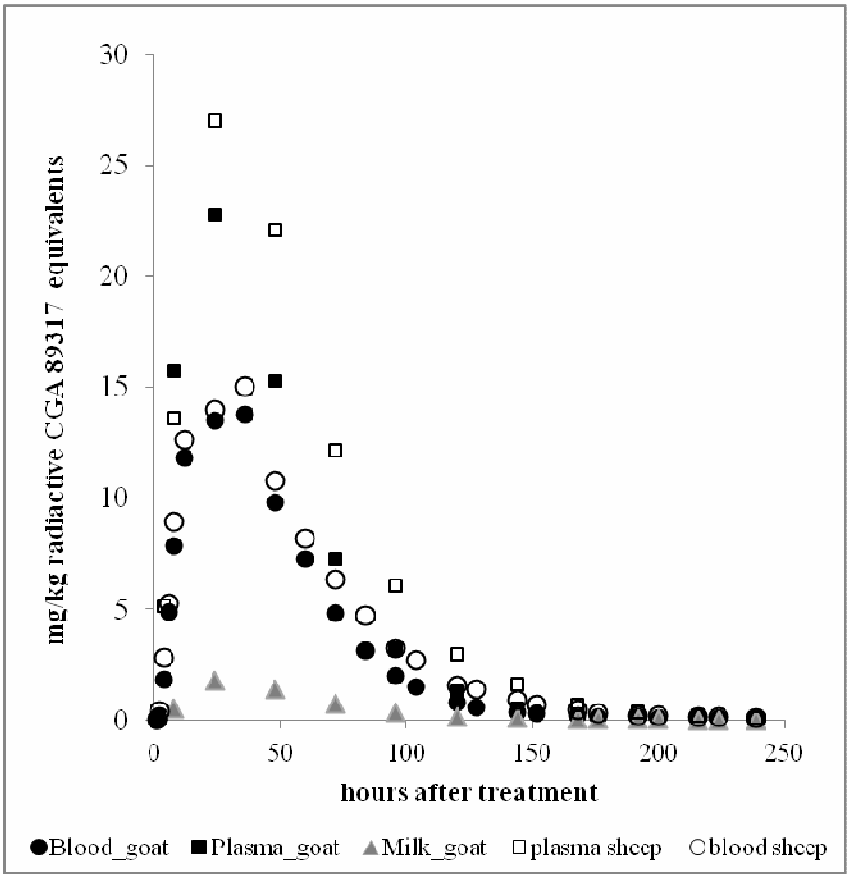


Figure 7.1. Time course of the changes of concentrations of the radioactivity in some body fluids of a sheep and a goat

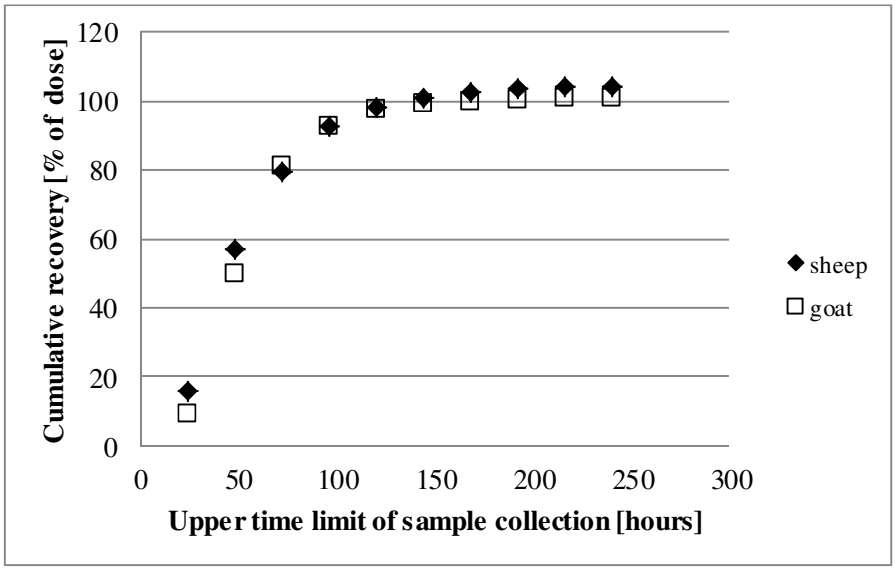


Figure 7.2. Cumulative recovery of the administered radioactivity in urine and faeces of a sheep and of a goat

The total residue equivalents calculated from the radioactivity found in tissues was higher in the tissues of the sheep than in those of the goat; the difference was particularly significant in liver and kidney (see Table 7.5), although the proportions of the concentrations of residues in liver, kidney, muscle and fat were similar in the two animals.

Table 7.5. Total radioactivity in selected tissues of a sheep and of a goat

Tissue		Sheep	Goat
		Total residue (mg/kg)	
Liver		1.84	1.00
Brain		0.95	0.79
Heart		0.92	0.73
Kidney		1.11	0.69
Muscle	rump	0.58	0.44
	round steak	0.58	0.59
	tenderloin	0.53	0.45
Lung		0.35	0.34
Rumen	wall	0.21	0.23
Intestine	wall	0.17	0.15
Thymus		0.11	0.11
Fat	perirenal	0.09	0.08
	subcutaneous	0.08	0.07

The radioactivity in sheep and goat tissues was not readily extractable into solvent systems. Alkaline solubilization of the tissues rendered 72–95% of the radioactivity into a form that could be partitioned into methylene chloride at pH <3 (Hamböck, 1982). Oxidation using hydrogen peroxide transformed 31–45% of the total radioactive tissue residues into the benzimidazole-2-one derivative CGA 110754 ("keto-triclabendazole"). The percentage CGA 110754 found using this procedure was 42% and 40% in muscle of the sheep and the goat, respectively; 45% and 36% in brain of sheep and goat, respectively; and 31% in the lungs of both animals.

Kinabo and Bogan (1988) studied the pharmacokinetics and efficacy of triclabendazole in normal goats (breed, age and body weight not given) and in goats with induced fascioliasis. The drug was administered orally (12 mg/kg bw). Plasma was collected over a period of seven days. Six weeks later the animals were infected and another six weeks later the kinetic experiment was repeated. Samples were analysed for the parent drug, the sulphoxide and the sulphone. The authors state that the observed differences in kinetic parameters (e.g. lower C_{max}, longer t_{max}, longer terminal half-life in infected animals) were not statistically significant. Although there are some discrepancies between the numerical and the graphical presentation of the data in this publication, it is evident that t_{max} of the concentration of the sulphone was almost twice as long as t_{max} of the concentration of the sulphoxide. This finding is consistent with the proposed metabolic sequence. The parent drug was not detected in any sample (LOD = 0.02 µg/ml). Qualitatively similar kinetic patterns of the same two metabolites were observed in a study of Sanyal (1994) after intraruminal administration of 10 mg/kg bw of triclabendazole to five goats (breed, age and body weight not given). In this study, a group of five sheep (breed, age and body weight not given) was also treated in the same way. The differences observed in the kinetic parameters of goat and sheep were considered statistically insignificant. A graph of the data shows that the observation period was probably too short to reliably estimate the half-life of the terminal elimination. The best-fitting curves of the computer modelling were not shown. The parent drug was not detected at any time (LOD = 0.02 µg/ml).

Gokbulut *et al.* (2007) found that the type of diet could have statistically significant effects on the kinetic parameters of the sulphoxide and of the sulphone in plasma of goats. The authors used 5–6-month-old goats (breed not given) weighing 15–18 kg. Two groups of six randomly allocated animals (similar mean body weight in each group) were formed. One group was kept indoors and fed concentrate plus hay rations; the other group was grazed outside. Following three weeks during which the groups were kept on their respective diets, all animals were treated orally with 10 mg/kg bw of triclabendazole. Plasma was obtained from blood samples taken up to 192 h after treatment. Parent drug and the sulphoxide and sulphone metabolites were determined, and the concentration vs time curve of each animal was analysed with the WinNonlin (4.1) software. Pharmacokinetic parameters for the two diets were compared by one-way ANOVA and a value of $P<0.05$ was considered significant. Triclabendazole was found at very low concentrations (t_{max} 12 h) during the first 20 h after treatment. All estimated parameters of the kinetics of the metabolites were significantly different between the two groups. The sulphoxide reached higher concentrations and was less rapidly metabolized to the sulphone in "indoor" animals. Also, fasting of goats for 24 h before and 6 h after treatment resulted in significantly higher absorption and systemic bio-availability of the drug and its metabolites (Gokbulut *et al.*, 2010).

Pharmacokinetic parameters that were determined in all the above studies are summarized in Table 7.6. The only investigation that compared the pharmacokinetics in sheep and goat (Sanyal, 1994) used the intraruminal route for administration (triclabendazole is more typically administered by mouth to sheep and goat). The number of animals used in the study (n=5) was very small for a comparative study. The observation period was too short to adequately cover the terminal elimination (e.g. in the case of the sulphone in sheep plasma, there were only two measurements made after the calculated t_{max}). The author does not indicate for which time period the AUC was determined. The author used a program "PHARMKIT". Such a program could not be found in the literature. The model parameters of the non-linear curve fitting are not given. Best fitting curves are not shown in the graph. Individual animal data are not provided, so the results cannot be independently verified. The author stated: "There were no differences in C_{max}, t_{max}, AUC and $t_{1/2}$ for each metabolite between the groups." This statement is not plausible in some cases, particularly when variability within a group was very low and differences between groups

were rather large (e.g. some AUC values seem to be significantly different). The author also stated: "Comparison between groups were carried out using t-test". It is not clear how these tests were calculated (e.g. in cases of unequal variances). There are also some discrepancies between the graphical presentation of the results and the calculated parameters. The results of the other studies performed with goat showed great variability and dependencies on several factors such as dietary conditions of the investigated animals.

Pharmacokinetic interactions between triclabendazole and other drugs were studied by some groups. Lifschitz *et al.* (2009) administered triclabendazole and ivermectin intravenously to Corriedale sheep with body weights of 20–30 kg, either alone or in combination. A two-compartment model and the software PK Solution® 2.0 was used for curve fitting. Higher C_{max} of the sulphoxide and the sulphone metabolites were observed in the presence of ivermectin.

Triclabendazole was administered intra-ruminally to male Corriedale sheep (14–16 months old, body weight 53.8 ± 2.6 kg, artificially infected with triclabendazole-resistant *F. hepatica*). No statistically significant changes in the pharmacokinetic behaviour of the metabolites of triclabendazole were seen between a group receiving triclabendazole alone and another group which received ivermectin in addition to triclabendazole, applied by s.c. injection, and methimazole given by i.m. injection (Ceballos *et al.,* 2010). It is difficult to interpret the discrepancies between the results of the two studies because too many potentially influential factors were different in the experimental design. It cannot generally be concluded from the results reported by Ceballos *et al.* (2010) that ivermectin has no influence on the pharmacokinetics of triclabendazole.

Inhibition of cytochrome P450 enhances the systemic availability of triclabendazole metabolites in sheep (Virkel *et al.*, 2009). The authors carried out pharmacokinetic studies in Corriedale × Merino weaned female lambs (18.6 ± 4.2 kg). Four treatment groups of five animals each were formed and treated intravenously with either triclabendazole alone or in combination with the FMO inhibitor methimazole (MTZ), or the P450 inhibitors piperonyl butoxide (PB) or ketoconazole (KTZ; this substance was administered orally). Pharmacokinetic data analysis was carried out with PK Solution® 2.0. Methamizole, which inhibits the formation of both the sulphoxide and the sulphone *in vitro,* had no influence under the experimental conditions of the study. Co-administration of PB drastically enhanced the AUC of both the sulphoxide and the sulphone. KTZ is known to inhibit several P450 subspecies. It also enhanced C_{max} and AUC of the metabolites in this study.

The variability of the array of parameters listed in Table 7.6 underlines the difficulty of comparing the results of such studies performed in different laboratories under different experimental conditions. The interesting results of these studies could not be used for between-species extrapolations. At the present time it cannot be excluded that the kinetic behaviour of triclabendazole is different in sheep and goat, and that the drug and/or its metabolites exhibit different kinetic behaviour in certain commercially available combination products.

Table 7.6. Summary of pharmacokinetic parameters of metabolites of triclabendazole in sheep and goats

Treatment group	Dose	Additional conditions	C_{max} (µg/ml)	t_{max} (h)	AUC (µg/h/ml)	Elimination $t_{½}$ (h)	Source
			Triclabendazole sulphoxide (Mean ± s.d.)				
5 sheep	10	Stall-fed, intraruminal dose	8.59 ±0.50	32.89 ±0.21	682.75 ±2.42	32.37 ±1.72	Sanyal, 1994
5 goats			10.34 ±0.60	27.82 ±0.38	760.97 ±3.91	32.18 ±1.32	
5 goats	12	Not infected	14.88 ±2.0	12.80 ±1.29	606 ±79	22.38 ±0.66	Kinabo and Bogan, 1988
		Artificially infected	12.99 ±1.2	17.60 ±2.99	490 ±55	23.53 ±3.23	
6 goats	10	Kept Indoors	13.22 ±2.8	18.40 ±2.19	613 ±137	24.77 ±1.92	Gokbulut *et al.,* 2007
6 goats		Grazing	10.17 ±1.5	14.00 ±2.19	406 ±98	16.16 ±1.17	
4 goats	10	Fed	6.49 ±1.7	34.00 ±15.14	376.34 ±51.65	26.96 ±14.98	Gokbulut *et al.,* 2010
4 goats		Fasted	12.98 ±5.4	29.00 ±6.00	654.14 ±171.32	20.93 ±3.67	
5 sheep	5	TCBZ only, i.v.	12.6 ±4.6	2.80 ±1.05	297 ±74.3	16.7 ±4.71	Lifschitz *et al.,* 2009
5 sheep		TCBZ + IVM, i.v.	23.2 ±7.7	1.50 ±0.68	319 ±70.2	10.8 ±1.03	
5 sheep	5	TCBZ only, i.v.	12.9 ±4.4	2.80 ±1.05	296.6 ±76[(1)]	15.6 ±1.77	Virkel *et al.,* 2009
5 sheep		TCBZ, KTZ i.v..	12.0 ±1.1	2.60 ±1.34	253.5 ±96.1	12.2 ±4.27	
5 sheep		TCBZ, PB, i.v.	20.9 ±2.7	5.00 ±2.00	592.5 ±145.2	17.8 ±2.00	
5 sheep		TCBZ i.v., KTZ oral	17.7 ±3.6	4.00 ±3.67	418.7 ±66.4	17.4 ±6.81	
			Triclabendazole sulphone (Mean ± s.d.)				
5 sheep	10	Stall-fed, intraruminal dose	7.95 ±0.4	78.1 ±0.22	1449.6 ±3.38	71.7 ±2.13	Sanyal, 1994
5 goats			10.81 ±0.4	59.08 ±0.24	1356.0 ±6.59	54.18 ±2.89	
5-goats	12	Not infected	12.37 ±1.2	25.60 ±1.94	730 ±99	19.36 ±1.11	Kinabo and Bogan, 1988
		Artificially infected	12.11 ±2.1	34.80 ±5.49	699 ±114	21.80 ±2.29	
6 goats	10	Kept Indoors	11.66 ±2.5	44.80 ±7.16	890 ±214	29.75 ±1.91	Gokbulut *et al.*, 2007
6 goats		Grazing	15.05 ±4.9	40.00 ±8.76	1108 ±445	21.43 ±2.00	
4 goats	10	Fed	6.45 ±1.2	56.00 ±11.31	533.93 ±114.05	34.15 ±12.96	Gokbulut *et al.,* 2010
4 goats		Fasted	12.07 ±5.7	52.00 ±4.62	882.93 ±370.26	27.04 ±5.83	
5 sheep	5	TCBZ only, i.v.	7.00 ±1.8	21.6 ±5.26	438 ±85.8	29.6 ±11.4	Lifschitz *et al.,* 2009
5 sheep		TCBZ + IVM, i.v.	10.4 ±2.1	16.0 ±7.35	489 ±116	16.3 ±2.31	
5 sheep	5	TCBZ only, i.v.	7.02 ±1.9	21.6 ±5.37	420.1 ±103.6[(2)]	24.3 ±5.22	Virkel *et al.,* 2009
5 sheep		TCBZ, KTZ i.v.	5.05 ±0.6	21.6 ±10.0	309.1 ±107.3	19.1 ±5.56	
5 sheep		TCBZ, PB, i.v.	11.4 ±1.9	30.0 ±6.93	643.0 ±151.2	23.5 ±6.76	
5 sheep		TCBZ i.v., KTZ oral	8.32 ±1.8	26.4 ±13.2	517.6 ±166.4	24.7 ±10.1	

NOTES; All doses are in mg/kg bw; TCBZ = triclabendazole; IVM = ivermectin; MTZ = methimazole; PB = piperonyl butoxide; KTZ = ketoconazole. (1) All AUC values in this study are for 0–120 h. (2) All AUC values in this study are for 0–144 h.

Tissue residue depletion studies

Radiolabelled residue depletion studies

Sheep and goat

No kinetic residue depletion studies with radiolabelled triclabendazole have been reported. The ratio of marker (keto-triclabendazole) to total residue concentrations can only be estimated from some experiments performed with single animals, including those of the Hamböck studies, and of another very limited study (Ferguson, 1994). In the latter study, two sheep, a 27 kg female and a 33 kg male received a dose of 10 mg/kg bw, orally by syringe and gavage tube. They were terminated 28 days later. The composition of the

residues in muscle and liver of the male animal was determined in a separate study. The ratio of marker to total residue concentrations re-calculated from the data was 0.40 for muscle and 0.25 for liver. Table 7.7 summarizes the ratios of marker to total residue concentrations for sheep and goat. Data for bovine animals are added for comparison from the corresponding table of the triclabendazole residue monograph of the 70th meeting of the Committee (FAO, 2009).

Table 7.7. Summary of available information on the ratio of marker to total residue concentrations in ruminants

Animal (sex)	Body weight	Dose (mg/kg bw)	Days after dosing	Ratio of marker to total residue concentrations			
				Liver	Kidney	Muscle	Fat
1 Calf (m)	96 kg	12	28	0.19	0.24	0.41	No data
1 Calf (m)	91 kg	12.55	28	0.24	0.27	0.32	No data
1 Sheep (m)	33 kg	10.45	28	0.25	No data	0.4	No data
1 Sheep (f)	28.5 kg	10.5	10	No data	No data	0.42	No data
1 Goat (f)	42.5 kb	10.1	10	No data	No data	0.4	No data

Residue depletion studies with unlabelled drug

Sheep

The kinetic residue depletion studies carried out with sheep and with unlabelled drug have been described in detail in the residue monograph of the 70th meeting of the Committee (FAO, 2009). The most relevant study for the calculation of MRLs by the 70th meeting of the Committee was the study by Adams (2004). These studies were conducted using a commercial formulation. Using these data, MRLs for liver, kidney and muscle were derived from the curves describing the upper one-sided 95% confidence limits over the 95th percentiles of the concentrations of the marker residue on day 28 after the last treatment. Figure 7.3 shows a modification of a graph published in the residue monograph of the 70th meeting of the Committee. It shows, as an example, the abovementioned tolerance limit curves for liver, kidney and muscle of sheep, as well as the data points for muscle on which the tolerance limit curve was based and, in addition, two single data points available for the marker residue concentration in muscle of a single goat and of a single sheep used in the studies of Hamböck and Strittmatter (1981, 1982) and Hamböck (1982, 1983). These two additional points are within the range of expected concentrations on the basis of the data of the Adams (2004) study.

Goat

No residue depletion study was carried out with the unlabelled drug in goat.

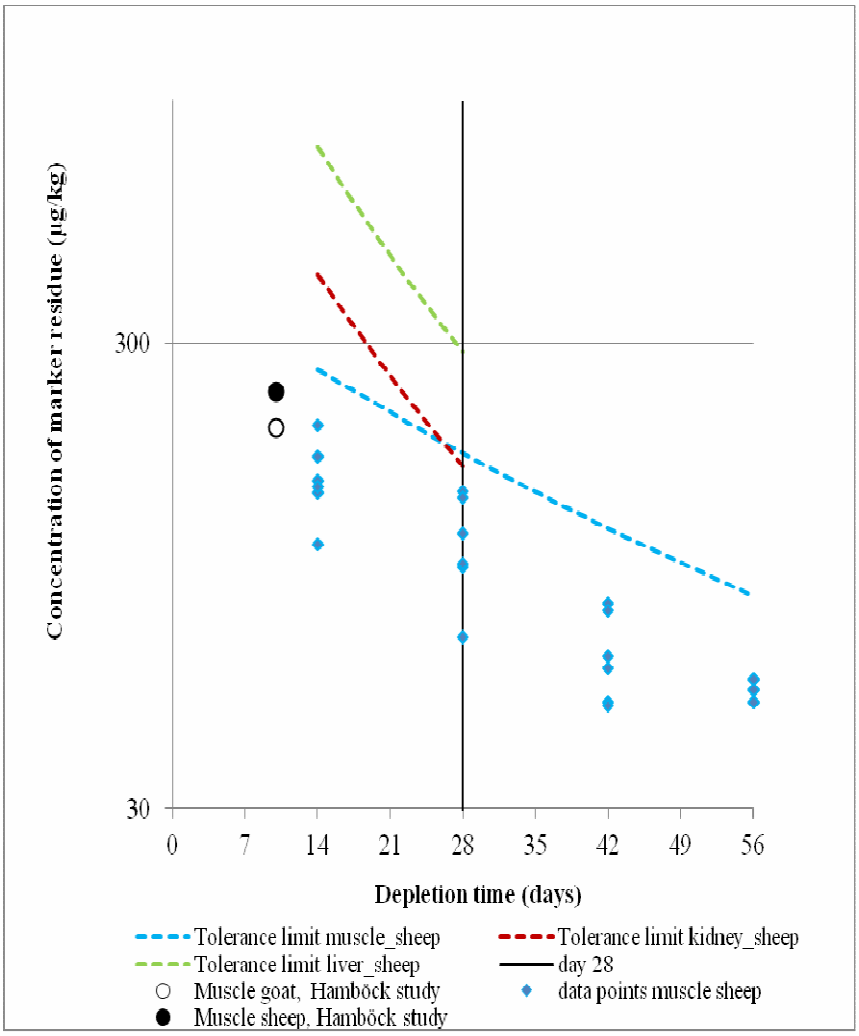


Figure 7.3. Selected data and calculated results of the study in comparison with data points on marker residue determination in muscle of a single goat and of a single sheep

Estimation of daily intake

Sheep

The 70th meeting of the Committee estimated the daily intake on the basis of the residue concentrations found in tissues of sheep on day 28 after withdrawal of treatment. The median concentration of the marker residue used for the calculation was based on a statistical evaluation of 12 to 21 data points per tissue. The calculated daily total intake of total residue equivalents of triclabendazole was 165 µg per person. This corresponds to approximately 92% of the ADI. This would be reduced to 21.5 µg per person (less than 12% of the ADI) when the bio-availability of the residues (approximately 13%) is taken into account.

Goat

A theoretical estimate of consumption of a standard food basket calculated from the data of Table 7.5 would result in intakes of 316 µg/day (176% of the ADI) on day 10 after treatment; however, taking the limited bio-availability of the residues into account and using the factor

of 0.13 developed by the 70th meeting of the Committee for tissues of cattle on day 28 after treatment (see FAO, 2009), this would be reduced to 41 µg/day (equivalent to 23% of the upper bound of the ADI). This estimate is based on one data point per tissue obtained from a study with a radiolabelled, non-commercial product in one single animal. The animal was slaughtered 10 days after treatment.

Methods of analysis for residues in tissues

The 70th meeting of the Committee reviewed available analytical methods according to their performance characteristics and results obtained in validation studies. Three methods were discussed for which the validation data were acceptable (FAO, 2009).

Several additional methods have been published in the open literature since, of which two are relevant. Cai *et al.* (2010) reported a method for the simultaneous determination of triclabendazole and its metabolites (the sulphoxide, sulphone and keto-triclabendazole) in bovine and goat tissues. The determinative step is based on HPLC-MS/MS with a deuterated triclabendazole internal standard. Validation data are provided. Cheng *et al.* (2011) published a multi-analyte method for several benzimidazoles, including triclabendazole and its sulphoxide and sulphone metabolites in edible tissues. The determinative step is also based on HPLC-MS/MS with deuterated fenbendazole as the internal standard. Validation data for several tissues were provided.

Appraisal

The 19th Session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) asked the question: "Can MRLs for goat (tissues) be established by extrapolation considering data used for recommending MRLs for cattle and sheep (tissues)". CCRVDF also noted that "JECFA has established MRLs for sheep and cattle and extrapolation would be based on the data packages available to the 70th JECFA and a literature review to be provided by the United States of America."

The sponsor of the dossier for the 70th JECFA re-submitted that information, together with an addendum to the expert report of 17 August 2005. The author of the addendum carried out a literature search with the keyword "triclabendazole" covering the period from January 2004 to 27 October 2010. No other literature review was received. The Committee extended the literature search to cover the period until 25 October 2011.

The Committee specifically re-evaluated pharmacokinetic, metabolism and residue data from the 40th, 66th, and 70th meetings that were considered relevant for possibly providing an answer to the question raised by CCRVDF, provided that linking data were found enabling between-species extrapolations. Some kinetic studies performed in cattle were not re-evaluated because it was unlikely to extrapolate from the kinetic residue data obtained with this species to the goat. The 70th meeting of the Committee had already concluded "that the kinetic behaviour of triclabendazole was distinctly different in cattle and sheep and that there was no basis for establishing MRLs of identical numerical values for the two species". However, many products used in sheep are also recommended for use in goat and the recommended doses are typically the same (with few exceptions, more or less uniformly, oral doses of 10 mg/kg bw). Therefore, it was important to examine the possibility to extrapolate from kinetic residue data obtained in sheep to goat.

No state-of-the-art comparative pharmacokinetic study conducted in the same laboratory and using a commercial product or equivalent formulation in a sufficient number of animals of both species of animal was available.

The complex comparative study carried out with radiolabelled triclabendazole and one single animal of both species (approximately 30 years ago) was of limited value. The cumulative excretion pattern of the radioactivity was very similar in both animals in that study and the metabolites identified were the same. However, these are insufficient criteria to conclude that residue kinetics would also be the same or quantitatively similar. Only about 2.4% of the administered radioactivity was calculated to be present in blood and tissues of the goat. Kinetics in plasma of the radioactivity was qualitatively similar in the treated goat and the treated sheep; however, they were quantitatively different. Radioactivity in most tissues was significantly different in the two animals, with higher concentrations found in the sheep. Whether this is a representative finding cannot be judged on the basis of a single treated animal. In the goat, the ratio of marker residue concentration to total residue concentration is only known for muscle of one animal.

Taking together all available data from all studies, two tissues of sheep and three tissues of goat are not covered by such a ratio, and therefore a full comparison between the two species cannot be made. All known figures are based on observations in one or two animals and the time points for which they are known are partly different for sheep and goat (28 and 10 days, respectively); variability and time trends are not known, except that for muscle the numerical values obtained for calves and sheep on day 28 in another study were similar to those obtained in sheep and goat on day 10 after treatment.

As the report of the 70th meeting has explained, the modelling of dietary intake of residues present in sheep tissues could only be conducted at day 28 after treatment, that being the only day when the ratio of the marker residue concentration to total residue concentration was known for two tissues. In the case of the goat, this ratio is only known for muscle and at day 10 after treatment.

The results of modelling performed by the 70th meeting have shown that the bioavailability of residues must be taken into account. The factor developed by the 70th meeting for incurred residues in liver of cattle (13%) would need to be re-applied for all tissues of the three ruminants in the absence of complete data for the other tissues and species.

For recommending MRLs for goat, the procedure adopted at the 66th meeting of the Committee could not be used, because of the absence of all necessary data except the ratio of marker to total residue concentrations in muscle of a single goat slaughtered on day 10 after treatment.

Maximum residue limits

The procedure for deriving MRLs adopted at the 66th meeting of the Committee could not be used, because the necessary data were not available. The Committee concluded that the available database on the residues of triclabendazole in goat did not allow a scientifically justifiable extrapolation of MRLs to this species of animal. The Committee recommended that the criteria described should be met and the corresponding data provided before triclabendazole is proposed for re-evaluation with the aim of obtaining MRLs based on extrapolations between species

References

Adams, S. 2004. Tissue residues of triclabendazole, measured as CGA 110754, in sheep following oral dosing with Fasinex 5%. Novartis Animal Health Australasia Pty Ltd, Report No 04/07/1894, Study Y04/22.

Cai, C., Zhang, L., Xue, F., Qiu, M. & Zheng, W. 2010. Simultaneous determination of triclabendazole and its metabolites in bovines and goat tissues by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B*, 878: 3106–3112.

Ceballos, L., Moreno, L., Alvarez, L., Shaw, L., Fairweather, I. & Lanusse, C. 2010. Unchanged triclabendazole kinetics after co-administration with ivermectin and methimazole: failure of its therapeutic activity against triclabendazole-resistant liver flukes. *BMC Veterinary Research,* 3 February 2010: 6–8.

Cheng, D., Tao, Y., Zhang, H., Pan, Y., Liu, Z., Huang, L., Wang, Y., Peng, D., Wang, X., Dai, M. & Yan, Z. 2011. Development of a liquid chromatography-tandem mass spectrometry with pressurized liquid extraction method for the determination of benzimidazole residues in edible tissues. *Journal of Chromatography B,* 879: 1659–1667.

FAO. 1993. Residues of some veterinary drugs in animals and foods. *FAO Food and Nutrition Paper*, 41/5:63–86.

FAO. 2006. Residue evaluation of certain veterinary drugs. *FAO JECFA Monographs*, 2: 71–88.

FAO. 2009. Residue evaluation of certain veterinary drugs. *FAO JECFA Monographs,* 6: 197–242.

FAO/WHO. 1993. Evaluation of certain veterinary drug residues in food. Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series,* No. 832.

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

FAO/WHO. 2009. Evaluation of certain veterinary drug residues in food. Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series,* No. 954.

Ferguson, E.G.W. 1994. [14C]-CGA 89317: Absorption, distribution and excretion following a single oral administration of Fasinex-5% to ruminating sheep. Hazleton Europe. Report No. 380/215-1011.

Gokbulut, C., Karademir, U., Boyacioglu, M. & Akar, F. 2007. The effect of diet type on the plasma disposition of triclabendazoloe in goats. *Research in Veterinary Science*, 82: 388–391.

Gokbulut, C., Boyacioglu, M., Karademir, U. & Aksit, D. 2010. The effect of fasting on the plasma disposition of triclabendazole following oral administration in goats. *Research in Veterinary Science*, 89: 415–417.

Hamböck, H. 1982. Characterization of tissue residues of CGA 89317 in sheep and goat. Ciba-Geigy Ltd, Project Report 50/82.

Hamböck, H. 1983. The metabolic fate of CGA 89317 in sheep, rat and the lactating goat. Ciba-Geigy Limited, Project Report No. 41/83.

Hamböck, H. & Strittmatter, J. 1981. Distribution, degradation and excretion of CGA 89317 in the lactating goat. Ciba-Geigy Limited, Project Report p. 34-81.

Hamböck. H. & Strittmatter, J. 1982. Distribution, degradation and excretion of CGA 89317 in sheep. Ciba-Geigy Limited, Project Report 10/82.

Iddon, B., Kutschy, P., Robinson, A.G., Suschitzky, H., Kramer, W. & Neugebauer, F.A. 1992. 2H-benzimidazoles (isobenzimidazoles). Part 7. A new route to Triclabendazole [5-chloro-6-(2,3-dichlorophenoxy)-2-methylthio-1H-benzimidazole] and congeneric benzimidazoles. *Journal of the Chemical Society, Perkins Transactions,* 1: 3129–3134.

Kinabo, L.D.B. & Bogan, J.A. 1988. Pharmacokinetics and efficacy of triclabendazole in goats with induced fascioliasis. *Journal of Veterinary Pharmacology and Therapeutics,* 11: 254–259.

Lifschitz, A., Virkel, G., Ballent, M., Sallovitz, J. & Lanuss, C. 2009. Combined use of ivermectin and triclabendazole in sheep: *In vitro* and *in vivo* characterization of their pharmacological interaction. *The Veterinary Journal,* 182: 261–268.

Sanyal, P.K. 1994. Pharmacokinetic study of triclabendazole in sheep and goat using a High Performance Liquid Chromatography method. *Indian Journal of Pharmacology,* 26: 200–203.

Shrivastava, A., Kumar, S.M. & Jain, A. 2011. Spectrophotometric method for quantitative determination of triclabendazole in bulk and pharmaceutical. *Chronicles of Young Scientists,* 2: 90–92.

Tothadi, S., Bhogala, B.R., Gorantla, A.R., Thakur, T.S., Jetti, R.K.R. & Desiraju, G.R. 2012. Triclabendazole: An intriguing case of co-existence of conformational and tautomeric polymorphism. *Chemistry - An Asian Journal,* 7(2): 330–342.

Virkel, G., Lifschitz, A., Sallovitz, J., Pis, A. & Lanusse, C. 2006. Assessment of the main metabolism pathways for the flukicidal compound triclabendazole in sheep. *Journal of Veterinary Pharmacology and Therapeutics,* 29: 213–223.

Virkel, G., Lifschitz, A., Sallovitz, J., Ballent, M., Scarcella, S. & Lanusse, C. 2009. Inhibition of cytochrome P450 activity enhances the systemic availability of triclabendazole metabolites in sheep. *Journal of Veterinary Pharmacology and Therapeutics*, 32(1): 79–86. (Article first published online 6 August 2008)