TRICLABENDAZOLE

First draft prepared by
Dr. Janenuj Wongtavatchai, Bangkok, Thailand
Dr. J. D. MacNeil, Saskatoon, Saskatchewan, Canada

ADDENDUM
to the monograph prepared by the 40th meeting of the Committee
and published in FAO Food & Nutrition Paper 41/5

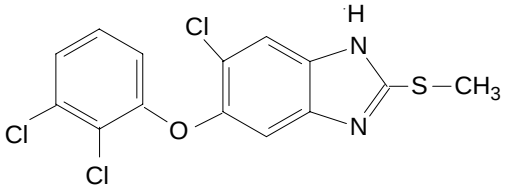
IDENTITY

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

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

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

Structural formula:



Molecular formula: $C_{14}H_9Cl_3N_2OS$

Molecular weight: 359.66

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: 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

The 40th meeting of the Committee established an ADI of 0 – 3 µg/kg body weight based on a long-term study in mice with a NOEL of 0.27 mg/kg body weight per day and a safety factor of 100. The Committee recommended MRLs in cattle and sheep. In cattle, the MRLs expressed as triclabendazole equivalents were: muscle and fat, 200µg/kg; liver and kidney, 300µg/kg. For sheep, MRLs for muscle, liver, kidney and fat were 100µg/kg. The marker residue was identified as 5-Chloro-6-(2', 3'-dichlorophenoxy)-benzimidazole-2-one. The 40th Committee concluded that more accurate estimates of total residues in edible tissues and the ratio of total residue concentrations to marker residue concentrations would be required before the MRLs for

triclabendazole in sheep could be reconsidered. The 15th Session of CCRVDF (Codex Committee on Residues of Veterinary Drugs in Foods) requested a new evaluation. Data were provided for cattle and sheep.

Conditions of Use

Triclabendazole is a benzimidazole anthelmintic used for the control of liver fluke, *Fasciola hepatica* and *Fasciola gigantica*, in sheep, goats and cattle. It is related both by chemical structure and pharmacological activity to other benzimidazole compounds, such as fenbendazole and thiabendazole.

Dosage

Triclabendazole is applied to cattle as a drench at 12 mg/kg body weight or as a pour-on at 30 mg/kg body weight. The recommended therapeutic oral dose for cattle is 12 mg/kg body weight and for sheep and goats is 10 mg/kg body weight. Veterinary advice differs from country to country regarding the interval for a possible repeat treatment, but typically at 8-10 week intervals during the fluke season, or at 5 to 6 week intervals when acute or sub-acute infection is present (FAO, 1993; EMEA, 1997).

PHARMACOKINETICS AND METABOLISM

The evaluation by the 40th Committee, that addressed studies using ^{14}C-labelled triclabendazole in rats, rabbits, dogs, sheep, goats, cattle and pigs, considered that the absorption, distribution, metabolism and excretion of triclabendazole were qualitatively similar in both laboratory animals and food-producing animals. The biotransformation and excretion of triclabendazole was very rapid, with two major metabolic pathways identified: oxidation of the methylthiol group initially to a sulfoxide and subsequently to a sulfone metabolite, and 4-hydroxylation of the dichlorophenoxy ring (Figure 1). Five identified metabolites - triclabendazole, sulfoxide, sulfone, ring-hydroxylated metabolites and keto-triclabendazole accounted for approximately 40-60% of the administered dose, with quantitative differences in relative proportions of metabolites being observed between species. Faecal excretion of triclabendazole and its metabolites accounted for a principal portion of the dose.

Laboratory Animals

Rats

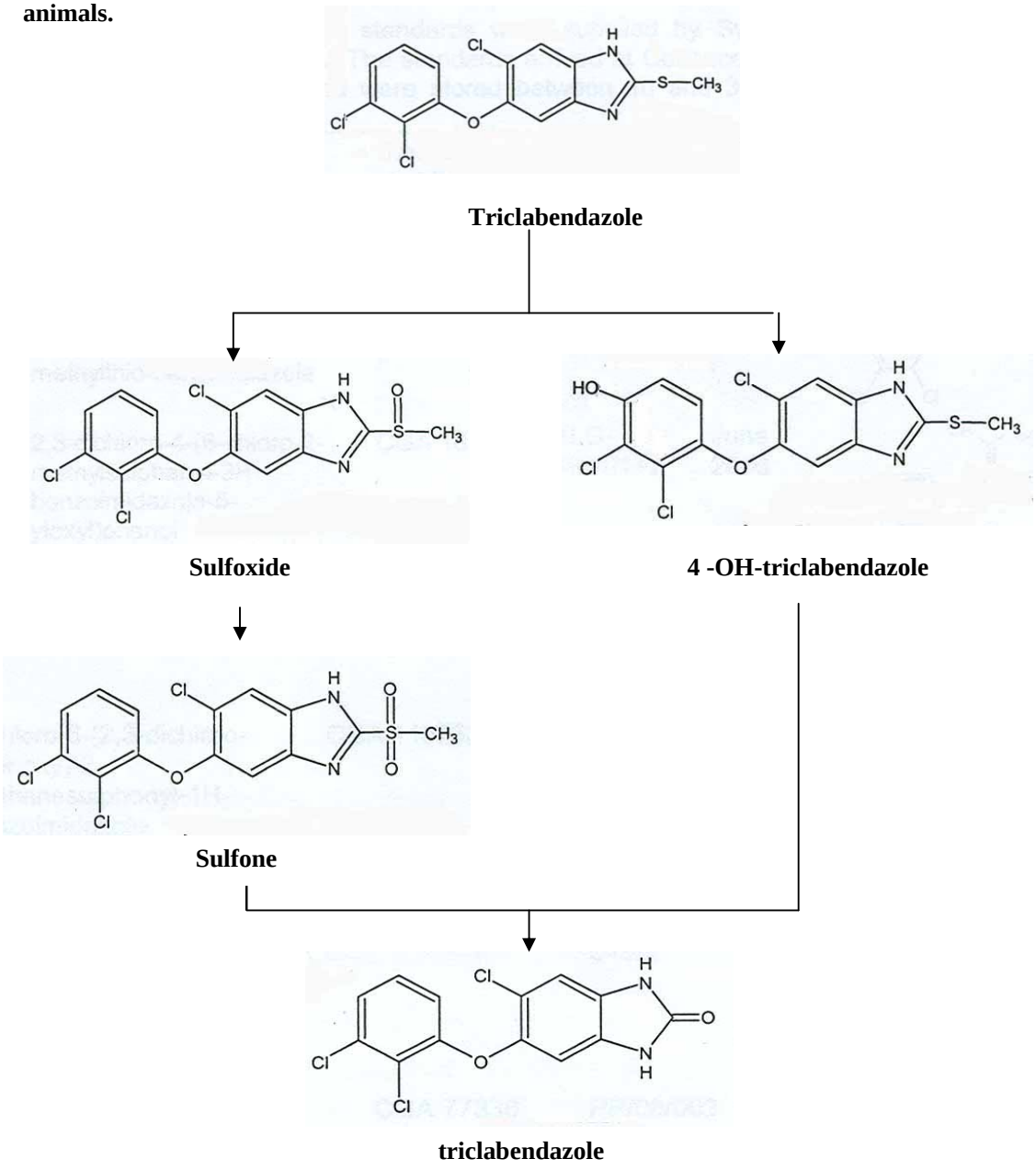
The absorption and disposition of ^{14}C-triclabendazole was investigated in male rats following a single intravenous administration of 1 mg/kg body weight and by oral administration at different doses: single doses of 1, 10 and 80 mg/kg body weight, respectively; 10 daily doses of 1 mg/kg body weight (Study DM23/1991, 1996). Peroral absorption of triclabendazole was almost complete at the lower doses (1 mg/kg body weight) and decreased to about 50% at higher doses (10 mg/kg body weight). Within 48 hours, 87-92% of a 1 mg/kg dose (oral or intravenous administration) was excreted in urine and faeces. The major route of excretion was via the faeces, accounting for 82-85% of the dose. Maximum concentration of radioactivity in plasma was observed 8 hours after oral administration, irrespective of the dose levels. Concentrations of radioactivity measured at different periods after the administration indicated wide distribution of triclabendazole metabolites in rat tissues following oral and intravenous treatment.

In a subsequent GLP study, twelve male rats were given a single oral gavage dose of 12 mg/kg body weight ^{14}C-triclabendazole (Study 1969/024, 2004). Recovery of radioactivity was quantified in the excreta and expired carbon dioxide collected daily from four rats for 10 days. Blood and tissues were collected at necropsy on days 10 (6 rats) and 28 (6 rats) for determination of radioactivity. The mean recovery in faeces from rats within 10 days after dosing was 96%, with a

mean recovery of 8% in urine. Less than 0.01% was detected in exhaled air. The major elimination of radioactivity in the faeces suggests that biliary excretion is significant in rats. Distribution of radioactivity in tissues (liver, kidney and muscle) showed the highest residue in kidney after 10 days and in kidney and muscle after 28 days. Chromatographic analysis of the radioactive extracts from excretions and tissues of rats killed on day 10 showed 4-12 metabolite fractions, none of which could be identified by comparison with the available reference standards.

In a GLP study of bioavailability of triclabendazole residues, male rats received a single dose of ^{14}C-triclabendazole either orally by gavage or by intravenous administration or by addition to powdered diet or by dietary dose of ^{14}C-triclabendazole derived residues from cattle tissues (Study 1969/026, 2004). Groups of rats which received ^{14}C-triclabendazole derived residues from cattle tissues were given lyophilized liver, muscle and kidney from cattle that had been killed 28 days after receiving a single oral dose of 12 mg/kg body weight (Study 1969/023, 2004).

Figure 1: Proposed metabolic pathway of triclabendazole in laboratory and food-producing animals.



The maximum concentration of radioactivity in blood from rats after dietary administration of ^{14}C-triclabendazole was found at 4 hours after gavage and 4.8 hours after dose in powdered diet, while the T_{max} was 12 and 6 hours in the groups administered lyophilised muscle and liver containing incurred residues of triclabendazole. Measurements of the area under the blood concentration time curve showed marked differences in the bioavailability of triclabendazole and triclabendazole-derived residues. The bioavailability of triclabendazole was 71.5% when given by gavage and 67.6% powdered diet, but reduced to 6.4% and 9.8% for triclabendazole- derived residues from lyophilized cattle muscle and liver, respectively.

The bioavailability of ^{14}C-triclabendazole-derived residues in tissues collected from cattle and sheep was reported in two additional GLP studies using bile duct-cannulated male rats (Study 017AM04, 1995; Study 1969/025, 2004). In the first study (Study 017AM04, 1995), tissues containing triclabendazole residues were obtained from cattle that received a single oral dose of 12 mg/kg body weight (Study 380/214-1011, 1994) and sheep that received a single oral dose of 10 mg/kg body weight (Study 380/215-1011, 1994) respectively; and were terminated 28 days post-dosing. The lyophilized tissues were orally administered to rats as diet mixture for 24 hours. The observed amount of radioactivity into systemic circulation after the administration, the sum of the % of dose in urine, bile, tissues and carcass, defined the bioavailability. The bioavailability was 14% and 7% in kidney, 9% and 8% in liver, 4% and 5% in muscle for cattle and sheep, respectively. Similar to the bioavailability study using the areas under plasma concentration time curve (AUC) (Study 1969/026, 2004), the bioavailability of triclabendazole-derived residues from the lyophilized tissues was very low compared to the concentrations of triclabendazole observed following oral dosing to rats. The administered dose was almost completely eliminated within 48 hours, predominantly in faeces. The rats were killed 48 hrs after the initial administration and residues in tissues were determined by radioactivity. The residues were considered negligible and were only detected in livers and kidneys at concentrations less than the limit of quantification, 0.002 mg/kg.

In the second new GLP study of residue bioavailability (Study 1969/025, 2004), rats were offered, over a 24 hour period, lyophilized liver, muscle or kidney containing ^{14}C triclabendazole residues from a cow dosed with triclabendazole at 12 mg/kg body weight and terminated at 28 days post administration (Study 1969/023, 2004). The bioavailability (defined as the % of dose in urine, bile, tissues and carcass) was 20%, 3.3% and 18% from liver, kidney and muscle, respectively. The mean recovery of radioactivity was > 90% with the majority eliminated in faeces (> 80%). Recovery in bile was 3-19% and in urine was < 1% of the administered dose. Absorbed residues did not depot in tissue of rats, as determined at 72 hours after dosing. Concentrations of radioactivity in tissues were very low or below the limit of quantification.

Dogs

A non-GLP study was reported in which 2 male dogs received doses of 0.5, 5 and 40 mg/kg body weight orally and 0.5 mg/kg body weight intravenously of ^{14}C-triclabendazole (Study DM23/1991, 1996). The concentrations of ^{14}C-triclabendazole-related residues in blood and plasma following oral administration showed that approximately 35-53% of a 0.5 mg/kg dose was absorbed at the lower doses, and the absorption decreased to about 25% at higher doses. In plasma, radioactivity attained the maximum concentration 8-24 hours after oral administration. Elimination of triclabendazole was almost exclusively *via* faeces. 79% and 54%, respectively, of the radioactivity associated with 0.5 mg/kg intravenous and oral dose of triclabendazole was recovered in faeces. Overall recovery in faeces and urine accounted for 80% of the intravenous dose and only 55% of the oral dose after 168 hours. Highest recovery of radioactivity in the excreta (90% of the dose) was found in the high dose of oral administration (40 mg/kg).

Food Producing Animals

Cattle

Studies considered by the 40th meeting of the Committee indicated rapid metabolism of triclabendazole following intravenous administration in cattle. The sulfoxide metabolites reached maximum concentrations in blood 4 hours after treatment, with terminal half-life of approximately 13 hours. The sulfone metabolites were produced more slowly with maximum plasma concentrations occurring 32 hours after dosing and terminal elimination half life of 40 hours (Study 86/12/1099, 1986; FAO, 1993).

In a new GLP study, two ruminating calves, one male and one female, were each given a single oral dose of ^{14}C triclabendazole 12 mg/kg body weight (Study 380/214-1011, 1994). Excretion of radioactivity was measured in urine and faecal samples collected at 24 hour intervals for 7 days following administration. A small portion of the administered radioactivity was found in urine (2.2%), with excretion primarily in faeces (76%). As 4% of the dose was found in the faeces at 7 days post-dosing, it was considered that faecal elimination may not have been complete. Distribution of radioactivity was extensive into tissues at 28 days post-dosing. Highest concentrations of radioactivity were detected in liver (0.461 mg equivalents/kg), followed by muscle (0.33-0.35 mg equivalents/kg) and kidney (0.195 mg equivalents/kg). Only very low residues were detected in fat (0.05 - 0.08 mg equivalents/kg).

Further investigation of the urinary and faecal metabolites was conducted in another GLP study (Study 017AM02, 1995). Four metabolites were detected in the urine but could not be identified with the reference standards. However, no sulfate or glucuronide conjugates were detected in the urine. Eight metabolite fractions were detected in the faecal extracts, with the most polar fractions displaying a similar chromatographic behavior to the fractions found in urine. Parent triclabendazole was the major fraction, accounting for 17% of the dose. The other metabolites identified in the faecal extracts were 5-chloro-6-(2,3-dichloro-phenoxy)-2-methanesulfinyl-1H-benzimidazole, 5-chloro-6-(2,3-dichloro-phenoxy)-2-methanesulfonyl-1H-benzimidazole, 2,3-dichloro-4-(6-chloro-2-methylsulfanyl-3H-benzimidazole-5-yloxy)-phenol and 2,3-dichloro-4-(6-chloro-2-methanesulfonyl-3H-benzimidazole-5-yloxy)-phenol, representing, respectively, 3%, 5%, 4% and 3% of the dose.

In a subsequent GLP study (Study 1969/023, 2004); the distribution, metabolism and excretion of ^{14}C triclabendazole was investigated in a ruminating male calf. Following a single oral dose of 12 mg/kg body weight, urine and faeces were collected for 10 days and the animal was killed 28 days after dosing. Similar to the previous studies (Study 380/214-1011, 1994; Study 017AM02, 1995), faecal elimination predominated, accounting for 78.2%, and urine contained only 3.4% of the dose. Triclabendazole was the major metabolite in faeces, with smaller amounts of sulfone, sulfoxide and hydroxylated metabolites. The majority of urinary metabolites were more polar than triclabendazole, including small amounts of 5-chloro-6-(2,3-dichloro-phenoxy)-2-methanesulfinyl-1H-benzimidazole, 5-chloro-6-(2,3-dichloro-phenoxy)-1,3-dihydro-benzimidazole-2-one, 2,3-dichloro-4-(6-chloro-2-methanesulfonyl-3H-benzoimidazol-5-yloxy)-phenol, 2,3-dichloro-4-(6-chloro-2-methylsulfanyl-3H-benzimidazole-5-yloxy)-phenol and 2,3-dichloro-4-(6-chloro-2-methanesulfinyl-3H-benzimidazole-5-yloxy)-phenol. At 28 days after dosing, levels of triclabendazole residues measured by concentrations of radioactivity were highest in liver (0.283 mg equivalents/kg) and muscle (0.209 mg equivalents/kg). The residues in kidney were lower (0.163 mg equivalents/kg) and much lower residue amounts were detected in fat (0.026 mg equivalents/kg). The systemic levels of radioactivity in the blood (0.070 mg equivalents/kg) and plasma (0.051 mg equivalents/kg) were generally lower than in tissues.

The studies demonstrated that triclabendazole was rapidly absorbed following administration of oral therapeutic dose to cattle (12 mg/kg body weight) and principally excreted *via* faeces. The major metabolites in faeces were similar to those of rats. Residue concentrations in tissues 28 day

after dosing were highest in liver followed by muscle and kidney. The residues in fat were negligible compared to the other tissues.

Sheep

The absorption, distribution and excretion studies of triclabendazole in sheep and goats reported in the previous evaluation by the Committee indicated a similar metabolism of triclabendazole in sheep and goats to other species tested (FAO, 1993). The orally administered dose was absorbed effectively and metabolized rapidly. The same metabolites were excreted by sheep and goats, and the elimination was nearly complete by 10 days post dosing. More than 90% of the administered dose was recovered in faeces, 2-3% in urine and 0.5% in milk. Disposition of triclabendazole residues in sheep and goat tissue indicated by the amount of radioactivity was similar to that observed in cattle. At 28 days after dosing, liver contained the highest concentration, followed by muscle and kidney, whereas radioactivity amounts in fat tissue were comparatively minor.

New GLP studies of triclabendazole metabolism in ruminating sheep demonstrated a corresponding metabolic pattern to the previous studies. Following a single oral administration of ^{14}C triclabendazole at nominal dose level (10 mg/kg body weight) to a male and a female sheep, the average recovery of radioactivity was 85% of dose at 7 days post-dosing (Study 380/215-1011, 1994). The primary route of excretion was in faeces, accounting for 77% of the administered dose. Absorption of triclabendazole, as indicated by radioactivity in plasma, was highest at 8 hours (19.59 mg equivalents/kg) and decreased to 10.05 mg equivalents/kg at 48 hours post-dosing. Distribution of radioactivity in tissues at 28 days post-dosing revealed highest levels in muscle (0.237-0.306 mg equivalents/kg), followed by liver (0.237 mg equivalents/kg) and kidney (0.198 mg equivalents/kg); while substantially lower levels were detected in fat tissue (0.02 mg equivalents/kg).

Chromatographic analysis of the excreta revealed 11 metabolite fractions in the faecal extracts and 5 metabolite fractions in urine (Study 017AM03, 1995). Only 5 components in faecal extracts co-chromatographed with the reference standards and corresponded to sulfoxide, sulfone, hydroxylated metabolites and unchanged triclabendazole. The major metabolic pathways in sheep were oxidation to the sulfoxide, with further oxidation to the sulfone, and hydroxylation of the dichlorophenyl moiety, essentially the same as found in cattle. As with the metabolite pattern observed for excretion in cattle (Study 380/214-1011, 1994; Study 017AM02, 1995; Study 1969/023, 2004), unchanged triclabendazole recovered from faecal samples was a dominant metabolite fraction, accounting for 16-17% of the dose. Metabolism and elimination in sheep was similar to that observed in the rat.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabelled Residue Depletion Studies

Cattle

In a pilot GLP study to investigate depletion of total residues of triclabendazole, two ruminating cows (7-month old heifers) each received a single oral dose of ^{14}C-triclabendazole at 12 mg/kg body weight (Study 400-05, 1991). The cows were sacrificed at 28 and 42 days, respectively, following drug administration and liver, kidney and composite muscle and fat samples were analyzed by combustion analysis to determine total residues in each tissue. Limits of determination were 0.002 mg/kg for liver, 0.003 mg/kg for kidney and muscle and 0.007 mg/kg for fat. The total residues found in tissues from each animal are given in Table 1.

Table 1: Total residues of ^{14}C-triclabendazole in tissues of cows after treatment with a single oral dose (12 mg/kg body weight) of ^{14}C-triclabendazole

Days post-treatment	Total ^{14}C-triclabendazole residues in tissues (Mean±SD, mg/kg)			
	muscle	liver	kidney	fat
28	0.131 ± 0.017	0.241 ± 0. 013	0.106 ±0.016	0.013 ± 0.003
42	0.097 ± 0.007	0.093 ± 0.009	0.069 ± 0.011	<0.008 ± 0.001

Tissues collected from the cow sacrificed at 28 days following drug administration were subsequently analyzed after approximately 5 months frozen storage at -20°C (Study 400-05A, 1992). Tissue samples (20 g) were extracted sequentially with 3 40-mL portions of methanol, followed by 3 portions of ethyl acetate. Recoveries of radiolabelled residues were low (liver, 13.8%; kidney, 4.7%; muscle, 4.7%; fat, 0.0%), so further characterization of the extracted residue was not attempted.

In a subsequent GLP study, liver, kidney and muscle tissues from a single male calf sacrificed 28 days following administration of ^{14}C-triclabendazole, 12 mg/kg body weight, were analyzed to determine the relationship between the residues measured as keto-triclabendazole and the total residues (Study 1969/023, 2004). Residues measured as keto-triclabendazole accounted for 24% of the total residue in liver, 27% of the total residue in kidney and 32% of the total residue in muscle at 28 days post-administration.

Tissues collected from two ruminating calves slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 12 mg/kg body weight (Study 380/214-1011, 1994), were analyzed to determine both keto-triclabendazole residues and total radiolabelled residues (Study 132/94, 1995). The results of the keto-triclabendazole analyses were not corrected for recovery in this study, conducted in compliance with GLP. Residues measured as keto-triclabendazole accounted for 13% of the total residue in liver, 21% of the total residue in kidney and 31% of the total residue in muscle at 28 days post-administration. After correcting the residues measured as keto-triclabendazole for recovery using the mean recoveries reported in the study for muscle, liver and kidney fortified at 0.1 mg/kg, the corrected percentages of marker-to-total residues were: muscle, 42%; liver, 19%; kidney, 24%.

Sheep

Muscle and liver tissues collected from two sheep slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 10 mg/kg body weight (Study 380/215-1011, 1994), were analyzed to determine both keto-triclabendazole residues and total radiolabelled residues (Study 132/94, 1995). Concentrations of keto-triclabendazole (not corrected for recovery) accounted for 29% of the total residues in muscle and 17% of the residues in liver. After correction for analytical recovery, residues measured as keto-triclabendazole account for 39% and 24%, respectively, of the total residues in muscle and liver.

Residue Depletion Studies with Unlabelled Drug

Cattle

Three residue studies were reported in the monograph prepared by the 40th Committee (FAO, 1993) in which cattle received a single oral dose of 12 mg/kg body weight triclabendazole. In these studies, residues were measured as 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one and converted to triclabendazole equivalents using a conversion factor of 1.0913. Two animals were slaughtered at each time point in the first two studies, while four cattle were slaughtered at each time point in the third study. The first and third studies included time points from 2 - 28 days,

while the second study was extended to 42 days. The studies demonstrated a consistent depletion profile, although the residue concentrations varied at the same or equivalent time points in the studies. In general, highest residues were observed in liver and kidney at times up to 7 days following treatment, while residues in fat were near or below the detection limits of the analytical methods used (0.03-0.06 mg/mg) at 14 days post-treatment. Residue concentrations in muscle tissue were lower than those in liver and kidney at 7-14 days post-treatment, but were similar at later time points. It was not stated whether these studies were conducted in compliance with GLP.

A new study was conducted in compliance with GLP in which 24 cattle (168 - 367 kg) were treated orally with triclabendazole at 18 mg/kg body weight, with a repeat treatment 28 days following the initial dose (Study Y03/49, 2004). Six animals were slaughtered at each of the 14, 28, 42 and 56 days sampling times following the second treatment. Samples of muscle (tenderloin), liver, kidney and renal fat were collected and analyzed for residues of keto-triclabendazole using a liquid chromatography method with a limit of quantification of 0.05 mg/kg. Results were corrected for analytical recovery. As in the earlier studies (FAO, 1993), highest residues were observed in kidney and liver at 14 days post-treatment, while mean residues in fat were <0.10 mg/kg. At 42 days post-treatment, residues in muscle and liver were similar, approximately 2.5 times higher than those in kidney, while residues were only detectable in muscle and liver at 56 days post-treatment (Table 2).

Table 2: Residues of triclabendazole measured as keto-triclabendazole in cattle tissues following two oral treatments (28 days between treatments) with triclabendazole (Fasinex®-10%) at 18 mg/kg body weight

Time following second treatment(days)	Residues of triclabendazole measured as keto-triclabendazole (Mean±SD, mg/kg)			
	Muscle	Liver	Kidney	Fat
14	0.238 ± 0.027	0.979 ± 0.235	0.656 ± 0.265	0.089 ± 0.029
28	0.14 ± 0.027	0.365 ± 0.083	0.129 ± 0.020	<0.050 - 0.062[a]
42	0.127 ± 0.021	0.228 ± 0.054	0.066 ± 0.015	<0.050
56	0.091 ± 0.015	0.102 ± 0.033	<0.050	N.A.[b]

[a] Only one of 6 fat samples contained residues at >0.050 mg/kg. [b] Not analyzed.

Sheep

Three residue depletion studies in which sheep were orally dosed with triclabendazole (single dose of 10 mg/kg body weight or 15 mg/kg body weight) were reported in the monograph prepared for the 40th meeting of the Committee (FAO, 1993). Residues depleted to below detection limits in fat (0.03 mg/kg) within 14-21 days after administration, but remained detectable in liver, kidney and muscle for 28 days. Highest residues were reported in liver at 21 days post-treatment, but results were similar in liver, kidney and muscle at 28 days post-treatment. It was not stated whether these studies were conducted in compliance with GLP.

A GLP study was provided to the current meeting of the Committee in which 24 lambs (29-42 kg body weight) received a single oral dose of 0.2 mL, equivalent to 10 - 13 mg/kg of triclabendazole body weight (Study Y04/22, 2004). Six animals were slaughtered at each of the time points 14, 28, 42 and 56 days post-treatment. Two control animals did not receive the treatment and were slaughtered prior to the first group of treated animals. Samples of liver, kidney, muscle and fat were collected from each animal and analyzed for residues of keto-triclabendazole by liquid chromatography, with results corrected for analytical recovery. Samples of some tissues were not analyzed from the later collection dates as all samples of these tissues collected at the previous date had been below the limit of quantification. Most persistent residues were found in muscle and were detectable in 3 of 6 animals at 56 days post-treatment. Residues were not detected in liver or kidney samples at 42 days post-treatment, or in fat samples at 14 days post-treatment.

Table 3: Residues of triclabendazole measured as keto-triclabendazole in sheep tissues following a single oral treatment with triclabendazole (Fasinex®-5 %) at 10 - 13 mg/kg b.w.

Days Post-Treatment	Triclabendazole residues measured as keto-triclabendazole (Mean±SD mg/kg)			
	Muscle	Liver	Kidney	Fat
Control	<0.05	<0.05	<0.05	<0.05
14	0.154 ± 0.030	0.429 ± 0.073	0.242 ± 0.031	<0.05
28	0.112 ± 0.030	0.158 ± 0.037	0.096 ± 0.022	N.A.
42	0.065 ± 0.014	<0.05	<0.05	N.A.
56	0.054 ± 0.003[a]	N.A[b]	N.A.	N.A.

[a] Average for muscle from 3 animals with detectable residues. [b] Not analyzed.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Analytical methods for triclabendazole residues reviewed in the monograph prepared for the 40th meeting of the Committee were based on reversed phase liquid chromatography with UV-detection (FAO, 1993). The method used in the depletion studies considered by the 40th Committee measures the hydrolyzable residues of triclabendazole after oxidation to keto-triclabendazole, or 5-chloro-6-(2',3'-dichlorophenoxy)-benzimidazole-2-one, designated as the marker residue. Analytical recoveries were reported for tissues from cattle and sheep. A factor of 1.0913 was applied to convert the marker residue to triclabendazole equivalents. Several methods from the open literature were also noted in the monograph. However, validation of the methods reported was not to contemporary standards.

A report 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 present Committee (Study V03/57, 2004). This is 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)-benzimidazole-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, 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. Quantification is by external standard curve. Performance characteristics determined for the method are summarized in Table 4. It should be noted that the limits of detection and quantification are based on estimates from calibration curves. The lowest concentration at which accuracy and recovery were tested and demonstrated to meet acceptable performance criteria was 0.050 mg/kg triclabendazole equivalents (0.046 mg/kg keto-triclabendazole).

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. The metabolites of triclabendazole, triclabendazole sulphoxide and triclabendazole sulphone, were detected, as was parent drug triclabendazole, but are fully separated by the chromatography conditions used in the method. It should be noted that 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.

Table 4: Summary of validation study results for analysis of triclabendazole residues by liquid chromatography in various edible tissues

Species	Edible Tissue	Limit of Detection[a] (mg/kg)	Limit of Quantification[b] (mg/kg)	Mean Recovery (%)	Repeatability[c] (%)
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	2.8-8.9
	Fat	0.007	0.020	78-90	10.1-6.1
Sheep	Muscle	0.014	0.041	80-102	3.1-5.2
	Liver	0.008	0.024	90-102	2.3-4.3
	Kidney	0.012	0.034	89-93	5.0-6.5
	Fat	0.015	0.042	79-102	10.0-6.8

[a] Based on mean response of blank, plus 3 standard deviations
[b] Based on mean response of blank, plus 10 standard deviations
[c] Within run, measured at 0.050, 0.100 and 0.200 mg/kg

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

The method has also been extended to the analysis of tissues from goats (study Y04/51, 2004). Results, given in Table 5, are based on analysis of three replicates at each of three concentrations for the three tissues tested (muscle, liver, kidney).

Table 5: Recovery and precision for determination of keto-triclabendazole residues in goat tissues

Tissue	Concentration keto-triclabendazole(mg/kg)					
	50		100		100[a]	
	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

[a] Fortified samples, analyzed after storage at room temperature for 16-24 hours.

The stability of residues of triclabendazole in cattle tissues, measured as keto-triclabendazole, was determined using incurred tissues from two animals collected in Study Y03/49 (Study V03/57, 2004a). Three replicates of each tissue were analyzed 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, given in Table 6, demonstrate that the residues remain essentially stable during this time period, with some decrease (maximum 33 %) being seen at the final time point.

Table 6: Stability of incurred triclabendazole residues in cattle tissues under typical conditions of frozen storage

	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 ± 0.006
Kidney 1	0.25 ± 0.013	0.27 ± 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

A similar study of residue stability in sheep tissues under typical conditions of frozen storage was undertaken using incurred muscle, liver and kidney samples from two animals from Study Y04/22 (Study Y04/22, 2004a). Three replicates of each tissue were analyzed prior to storage and after 2 and 4 months of frozen storage. The storage temperature varied from -5°C to -21°C during the period of storage. As with the cattle tissues, there was minimal change in the residue concentrations during the period of storage (Table 7).

Table 7: Stability of incurred triclabendazole residues in sheep tissues under typical frozen storage conditions

	Residues measured as keto-triclabendazole, corrected 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

APPRAISAL

The previous evaluation by the 40th meeting of the Committee addressed studies using ^{14}C-labelled triclabendazole in rats, rabbits, dogs, sheep, goats, cattle and pigs. The absorption, distribution, metabolism and excretion of triclabendazole were qualitatively similar in both laboratory animals and food producing animals. Following an oral dose of ^{14}C-labelled triclabendazole, the biotransformation and excretion of triclabendazole was very rapid. Absorbed triclabendazole which entered the circulation was rapidly metabolized *via* two major pathways: oxidation of the methyl thiol group initially to a sulfoxide and later to a sulfone metabolite, and 4-hydroxylation of the dichlorophenoxy ring. Five identified metabolites, triclabendazole, sulfoxide, sulfone, ring-hydroxylated metabolites and keto-triclabendazole accounted for approximately 40-60% of the administered dose. Quantitative differences in relative proportions of metabolites were observed between species. Elimination of triclabendazole was nearly complete within 10 days after administration. Faecal excretion of triclabendazole and its metabolites accounted for the principal portion of the dose, with only minor elimination *via* urinary excretion.

New studies confirmed the findings in the report of the 40th Committee that absorption is rapid and is not dose-dependent in rats after oral dosing, reaching a maximum plasma concentration 8 hrs post-dose. The studies also confirmed that elimination is rapid, with approximately 90% of the dose eliminated through urine and faeces within 48 hrs, primarily through faeces (>80%). Studies in which rats were fed lyophilized tissues containing incurred residues of triclabendazole demonstrated that the bioavailability of these residues was low (<20%)

The biotransformation of triclabendazole is also rapid in dogs, with the sulfoxide and sulfone metabolites accounting for approximately all of an administered oral dose in plasma. Following oral administration, approximately 35-53% of a 0.5 mg/kg dose was absorbed, with a decrease to about 25% at higher doses. Maximum concentration in plasma occurred 8-24 hours after oral administration and elimination of triclabendazole was almost exclusively *via* faeces.

Previous studies have indicated rapid metabolism of triclabendazole following intravenous administration in cattle, with the sulfoxide metabolite achieving maximum concentrations in blood 4 hours after treatment, with terminal half-life of approximately 13 hours. Maximum plasma concentrations of the sulfone metabolite were observed 32 hours after dosing, with terminal elimination half-life of 40 hours.

The new studies confirmed that absorption and elimination were rapid in cattle and that, as in rats and dogs, elimination is primarily in faeces. The major metabolites in cattle faeces were similar to those of rats. Residue concentrations in tissues 28 day after dosing were highest in liver followed by muscle and kidney. The residue in fat was negligible compared to other edible tissues.

The studies of absorption, distribution and excretion of triclabendazole reviewed by the 40th Committee indicated that the metabolism of triclabendazole in sheep and goats was similar to that seen in other species tested. At 28 days after dosing with ^{14}C- triclabendazole, liver contained the highest residue concentrations, followed by muscle and kidney, whereas residues were relatively negligible in fat tissue. The results of new studies of triclabendazole metabolism in ruminating sheep were consistent with the metabolic pattern seen in the previous studies. Faecal elimination accounted for 77% of the administered dose. Distribution of radioactivity in tissues at 28 days post-dosing revealed highest total residues in muscle, followed by liver and kidney.

Three residue depletion studies in cattle considered by the 40th Committee demonstrated that at 28 days following a single oral dose of 12 mg/kg body weight, average residue concentrations were 0.12 mg/kg in liver, 0.07 mg/kg in kidney, 0.11 mg/kg in muscle and 0.05 mg/kg in fat, representing, respectively, 50%, 66%, 84% and >100% of the total residues in these tissues.

Two new GLP studies using ^{14}C-triclabendazole by oral administration at 12 mg/kg body weight provided additional information on the relationship between the marker and the total residues in cattle. In the first study, two ruminating calves were slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 12 mg/kg body weight. Residues measured as keto-triclabendazole, corrected for analytical recovery, accounted for 24% of the total residue in liver, 19% of the total residue in kidney and 42% of the total residue in muscle at 28 days post-administration. Subsequently, residues were measured as keto-triclabendazole in tissues from a single calf killed 28 days after a single oral dose of ^{14}C-triclabendazole, 12 mg/kg body weight. Residues measured as keto-triclabendazole accounted for the following percentages of the total residue in each tissue: liver 24%, kidney 27% and muscle 32%. These studies demonstrated that the relationships of marker to total residue calculated from information available to the 40th meeting of the Committee over-estimated the proportion of total residue present as marker residue.

In muscle and liver tissues collected from two sheep slaughtered 28 days after administration of a single oral dose of ^{14}C-triclabendazole, 10 mg/kg body weight, residues measured as keto-triclabendazole, corrected for analytical recovery, accounted for 39% of the total residues in

muscle and 24% of the residues in liver. These results are consistent with those found in incurred tissues from cattle and with the relationships reported for sheep by the 40th Committee.

Based on the results of the new studies in cattle and sheep, factors for conversion of recovery-corrected residues measured as keto-triclabendazole to total residues are 4 for liver, 3.7 for kidney and 2.5 for muscle. No conversion factor was considered necessary for fat, given the limited distribution of residues in that tissue.

The new GLP studies in cattle and sheep gave results consistent with the distribution and depletion studies reviewed by the 40th Committee. Unlike the earlier studies, however, the new GLP study in cattle was conducted under conditions of repeat treatment, 28 days subsequent to the initial treatment, using a dose 1.5 times the recommended treatment. Highest residues measured as keto-triclabendazole were found in liver at all time points (14, 28, 42, 56 days), but residues in muscle were similar to those found in liver at 56 days after the second treatment. Residues were below the detection limit of 0.05 mg/kg in kidney at 56 days and were only detectable in fat at 14 days post-treatment. There were no detectable residues in fat at the later timepoints when residues in other tissues were approaching the MRLs. This eliminated residues in fat from inclusion in the calculation of theoretical maximum daily intake.

The three residue depletions studies with sheep orally dosed with triclabendazole (single dose of 10 mg/kg body weight or 15 mg/kg body weight) considered by the 40th Committee demonstrated that residues depleted to below detection limits in fat (0.03 mg/kg) within 14-21 days after administration, but remained detectable in liver, kidney and muscle for 28 days. Highest residues were reported in liver at 21 days post-treatment, but results were similar in liver, kidney and muscle at 28 days post-treatment. In the new GLP study considered by the present Committee, sheep received a single oral dose of 10-13 mg/kg body weight. Most persistent residues were in muscle, detectable in muscle from 50% of the animals at 56 days post-treatment. Residues were not detected in liver or kidney samples at 42 days post-treatment or in fat samples at 14 days post-treatment.

The results of the studies demonstrate that while liver is the tissue in which highest residues are initially found, particularly for cattle within approximately 4 weeks of treatment, muscle is also a suitable target tissue and is the preferred target tissue at longer times following treatment.

A suitable validated liquid chromatographic method was available for regulatory use to detect and quantify residues as keto-triclabendazole. However, a confirmatory method which meets contemporary criteria in many countries, such as one based on LC/MS, was not provided for review.

The median residues calculated for the residues represented by an MRL established at the 95/95 limit, reflecting the normal distribution in a group of animals in which the residues are below the MRL in 95% of the treatment group, were also considered as an alternative approach to estimation of a dietary intake. The median residue concentrations calculated from the data were: muscle, 88.5 μg/kg; liver, 99.5 μg/kg; kidney, 25 μg/kg; fat, 12.5 μg/kg. The value for fat is a default value intermediate between the LODs of the proposed regulatory method for fat of cattle and sheep and was selected to reflect that there were no detectable residues in fat when residues in liver and muscle are approaching the MRLs. Using the median residues approach, an estimate of dietary intake of residues from fat is included in the estimated daily intake calculation. Based on the median values, the corresponding daily intake estimate is 121.7 μg.

MAXIMUM RESIDUE LIMITS

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

- The marker residue is keto-triclabendazole.
- The appropriate target tissue is muscle.
- A validated analytical method is available for analysis of triclabendazole residues in edible tissues of cattle, sheep and goats.
- The bioavailability of incurred triclabendazole residues in tissues fed to rats did not exceed 20%.

- The factors calculated for conversion of marker-to-total residue in cattle tissues by the 40th meeting of the Committee, based on the then available studies, have been demonstrated to be incorrect by data from the more recent GLP studies which provided both total residue and marker residue concentrations from the same tissues.

- The factors to convert from marker to total residue , derived from mean results of the new GLP studies in cattle and sheep, are 4.3 for liver, 4.2 for kidney and 2.7 for muscle of cattle and sheep, calculated at 28 days after a single administration at the recommended dose. When multiple doses are used, these factors are sufficiently conservative to apply to the various timepoints where residues are at the MRLs. The same factors are applicable to goats, based on the available information. A factor was required for kidney as detectable residues were present below the LOQ at the timepoint used for calculation of the MRLs for liver and muscle, requiring their inclusion in the intake estimate. Due to the rapid depletion of residues in fat, a factor to convert marker to total residues was not required as the intake estimate based on one-half the LOQ of the analytical method provides a conservative estimate.

- Maximum residue limits for liver and muscle of cattle, sheep and goats were based on the mean residue concentrations plus 3 standard deviations from the new GLP studies in cattle and sheep. The time point for which the MRLs for cattle were calculated is 56 days following the second treatment at 1.5 times the recommended dose. Residues deplete more rapidly in cattle following a single treatment at the recommended dose. These MRLs will be achieved in sheep at a time point intermediate between 28 and 42 days after a single treatment at the recommended dose.
- Maximum Residue Limits for kidney and fat were based on twice the limit of quantification.
- An ADI of 0-3 μg per kg of body weight was established by the 40th meeting of the Committee, equivalent to 0-180 μg for a 60 kg person (WHO, 1993).

On the basis of the above considerations, the Committee recommended the following MRLs for edible tissues of cattle, sheep and goats, expressed as the marker residue, keto-triclabendazole.

Muscle	150 μg/kg
Liver	200 μg/kg
Kidney	100 μg/kg
Fat	100 μg/kg

The MRLs recommended above would result in a theoretical daily maximum intake of 230 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, or 128% of the upper bound of the acceptable daily intake. An estimate of residue intake calculated as the TMDI is tabulated as follows:

Table 8: Theoretical Maximum Daily Intake (TMDI) of Triclabendazole Residues

Food Item	MRL (μg/kg)	Standard Food Basket (kg)	MR/TR[1]	TMDI (μg)
Muscle	150	0.300	2.7	121.5
Liver	200	0.100	4.5	90
Kidney	100	0.050	3.7	18.5
Fat	100	0.050	1	-
Total:				230.0

[1] MR = marker residue (parent drug); TR = total residue

The Committee also calculated long term (chronic) exposure an estimated median daily intake, using the median values from the residue data used in recommending the MRLs. The median value of the distribution of residue concentrations from which the MRL is derived is used as new point estimate instead of the MRL. The Committee considers that this represents a more realistic estimate of intake, consistent with the approach to intake calculations used by the Joint FAO/WHO meeting on Pesticide Residues (JMPR). The median residue concentrations calculated from the data are: muscle, 88.5 μg/kg; liver, 99.5 μg/kg. kidney, 25 μg/kg; fat, 25 μg/kg. In the absence of quantifiable residues in kidney and fat at the time point where the MRLs were recommended, a value equal to one-half the LOQ was assigned as a conservative estimate of intake from kidney and fat. The median residue concentrations above would result in an estimated daily intake of 121 μg, based on a daily food intake of 300 g of muscle, 100 g of liver, 50 g each of kidney and fat, or 67% of the upper bound of the acceptable daily intake. Estimates of the residue intake calculated as the EDI are tabulated in Table 9.

Table 9: Estimated Daily Intake of triclabendazole residues

Tissue	Median residue concentration (μg/kg)	Standard Food Basket (kg)	MR/TR[1]	EDI (μg)
Muscle	88.5	0.300	2.7	71.7
Liver	99.5	0.100	4.3	42.8
Kidney	25	0.050	4.2	5.2
Fat	25	0.050	1	1.3
Total:				121.0

[1] MR = marker residue (parent drug); TR = total residue

REFERENCES

EMEA (1997). Committee for Veterinary Medicinal Products. Triclabendazole. Summary report (3), EMEA/MRL/196/97-FINAL.

FAO (1993). Triclabendazole. Residues of some veterinary drugs in animals and food (Monographs prepared by the fortieth meeting of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Paper 41/5, 63-86 Rome.

Study 86/12/1099 (1986). The Bioavailability of Triclabendazole and Its Sulphoxide Metabolite in Cattle. Ciba-Geigy Australia Ltd.

Study 400-05 (1991). Report No. 1375: Depletion of Total Drug-Related residues in Tissues of Beef Cattle treated with [14C]Triclabendazole (Pilot Study). Ciba-Geigy Corporation, Greensboro, North Carolina, U.S.A.

Study 400-05A (1992). Report No. 1375: The Characterization of [^{14}C]Triclabendazole-Related Residues in Tissues from a Cow Treated with a Single Dose of [^{14}C]Triclabendazole. Ciba-Geigy Corporation, Greensboro, North Carolina, U.S.A.

Study 380/214-1011 (1994). [^{14}C]-CGA 89317: Absorption, Distribution and Excretion Following a Single Oral Administration of Fasinex®-10% to Ruminating Cattle. Hazleton Europe, Department of Metabolism and Environmental Chemistry, Harrogate, England.

Study 380/215-1011 (1994). [^{14}C]-CGA 89317: Absorption, Distribution and Excretion Following a Single Oral administration of Fasinex®-5% to Ruminating Sheep. Hazleton Europe, Department of Metabolism and Environmental Chemistry, Harrogate, England.

Study 017AM02 (1995)**.** Report No. 2/95. The Metabolite Profiles in Urine and Faeces Extract of Ruminating Cattle after Administration of [U-^{14}C]Benz-Imidazole CGA 89317. Ciba-Geigy.

Study 017AM03 (1995). Report No. 3/95. The Metabolite Profiles in Urine and Faeces Extract of Ruminating Sheep after Administration of [U-^{14}C]Benz-Imidazole CGA 89317. Ciba-Geigy.

Study 017AM04 (1995). Report No. 6/95. Bioavailability Study in Rats Fed with CGA 89317 Related Tissue Residues from Cattle and Sheep. Ciba-Geigy.

Study 132/94 (1995). Validation of Method REM 15/83: Determination of Common Moiety CGA 110754 in Muscle, Liver, Kidney and Fat of Cattle as well as in Muscle and Liver of Sheep After Administration of ^{14}C-CGA 89317 by High Performance Liquid Chromatography (HPLC). Ciba-Geigy Limited, Basel, Switzerland.

Study DM23/1991 (1996). [^{14}C]-CGP 23030: Absorption and Distribution Studies in Rats and Dogs. Ciba-Geigy Limited, Basel, Switzerland.

Study 1969/023 (2004). Report No. 1969/023-D1145. [14C]Triclabendazole: Distribution and Excretion Following Oral Administration to Cattle. Covance Laboratories Ltd, Harrogate, England.

Study 1969/024 (2004). Report No.1969/024-D1145. [^{14}C]Triclabendazole: Absorption, Distribution, Excretion and Metabolism in the Rat. Covance Laboratories Ltd, Harrogate, England.
S**tudy 1969/025** (2004). [^{14}C]-Triclabendazole: Bioavailability of Total Residues in Bovine Tissue to Rats. Covance Laboratories Ltd.

Study 1969/026 (2004). Report No. 1969/026-D1145. [^{14}C]-Triclabendazole: Pharmacokinetics in the Rat Following Oral and Intravenous Administration. Covance Laboratories Ltd, Harrogate, England.

Study V03/57 (2004). Report No. TR 04/05/1886. Validation of Analytical procedure No. 193F.00 - Determination of Residues of Triclabendazole in Animal Tissues by HPLC. Novartis Animal Health Australasia Pty. Ltd.

Study V03/57 (2004a). Report No. TR 04/07/1895. Addendum 1. Validation of Analytical procedure No. 193F.00 - Stability of Incurred Residues of Triclabendazole in Cattle Tissues Under Freezer Storage Conditions. Novartis Animal Health Australasia Pty. Ltd.

Study Y03/49 (2004). Tissue Residues Measured as CGA 110 754 in Cattle Following Repeated Oral Dosing with Fasinex 10%. Novartis Animal Health Australasia Pty. Ltd.

Study Y04/22 (2004). Tissue Residues of Triclabendazole Measured as CGA 110 754 in sheep following oral dosing with Fasinex 5%. Novartis Animal Health Australasia Pty. Ltd.

Study Y04/22 (2004a). Addendum 1. Tissue Residues of Triclabendazole Measured as CGA 110 754 in sheep following oral dosing with Fasinex 5% - Stability of Incurred Residues of Triclabendazole in Sheep Tissues Under Freezer Storage Conditions. Novartis Animal Health Australasia Pty. Ltd.

Study Y04/51. (2004). Report No. TR 04/09/1901. Validation of Analytical Procedure No. 193F.00 for Goat Tissues. Novartis Animal Health Australasia Pty. Ltd.

Study V05/24. (2005). Report No. TR 05/06/1945. Extended Validation of Analytical Procedure 193F.00 for Sheep and Cattle Tissues. Novartis Animal Health Australasia Pty. Ltd.

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.