CLOSANTEL

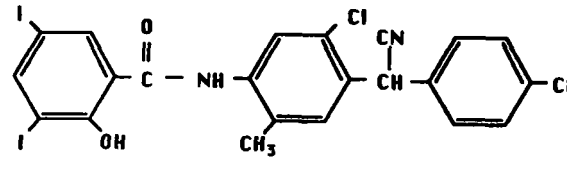
IDENTITY

Chemical name: N-[5-chloro-4-[(4-chlorophenyl)cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide

5'-Chloro-a4-(p-chlorophenyl)-a4-cyano-3,5-diiodo-2',4'-salicyloxylidide

Synonyms: Flukiver

Structural formula:



Molecular formula: $C_{22}H_{14}Cl_2I_2N_2O_2$

Molecular weight: 663.08

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: almost white to beige powder

Melting point: 215-235° with decomposition

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Closantel is used primarily in cattle and sheep for the treatment and control of adult and immature liver flukes, haematophagous nematodes and larval stages of some arthropods.

Dosages

Closantel may be administered to cattle and sheep orally or parenterally via drench, bolus or injectable formulations. The usual use range of the injectable formulation, delivered subcutaneously or intramuscularly, is 2.5 to 7.5 mg/kg. The dose range for the oral route is 5 to 15 mg/kg. A single application of the drug is recommended.

METABOLISM STUDIES

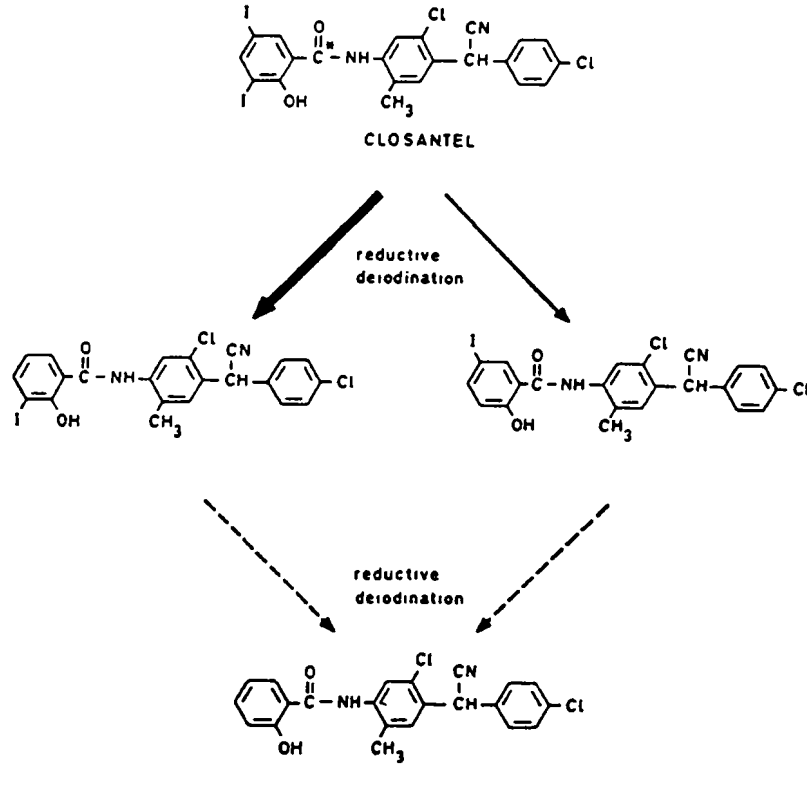
Sheep

Following the administration of 14C-closantel to sheep via the intramuscular (5 mg/kg) or oral (10 mg/kg) route, parent closantel was found to be the major constituent of the residue in feces (80-90% of the total residue) and in liver (~60-70% of the total residue). In muscle, kidney and fat, the total residue was very nearly attributable to closantel exclusively.

In feces, two metabolites were identified, by reference to standards, to be 3-monoiodoclosantel and 5-monoiodoclosantel. The 3-monoiodoclosantel isomer was present in a larger amount than the 5-isomer. In feces, no evidence of completely deiodinated closantel was found. In liver, closantel was 61% of the total residue after intramuscular treatment and 71% following oral dosing. Monoiodoclosantel was the main metabolite in liver.

The metabolic pathways for closantel in sheep are presented in Figure 1.

Figure 1. Metabolic Pathways of Closantel in Sheep. The Position of 14C is Indicated by the Asterisk.



The primary route of metabolism of closantel, therefore, is reductive deiodination leading to the monoiodoclosantel isomers. Although complete deiodination is possible, no evidence for deiodinated closantel has been observed. While amide hydrolysis would appear to be a possible alternate pathway, no metabolites which would result from the pathway (e.g., 3,5-diiodosalicylic acid) have been identified. It may well be that steric hindrance around the amide bonds prevents the hydrolysis. (Meuldermans, et al., 1982; Michiels, et al., 1987)

Rats

Five male Wistar rats (~242 g) were dosed orally with 14C-closantel at 10 mg/kg. The drug, labeled in the carbonyl carbon, had a radiochemical purity of ~97%. Urine and feces were collected at 24-hr periods up to 10 days post dosing. Plasma was collected at sacrifice after the 10-day collection period. Samples were analyzed for total radioactivity by combustion and counting or by direct counting. Unchanged drug and metabolites were determined using HPLC and co-chromatography with reference standards.

Radioactivity was found to be excreted primarily through the feces. After 10 days, fecal excretion amounted to 88.4% of the dose. Over that same time period, only 0.4% of the dose was in the urine. Approximately half the dose was excreted within 2 days after dosing.

An examination of the feces for metabolites evidenced unchanged closantel (90% of the radioactivity in the feces at 0-24 hr collection period, 76% at 192-240 hr) and monoiodo-closantel (3.4% of the sample radioactivity at the first collection, 19% at the last). The monoiodoclosantel is stated to be predominantly the 3-iodo isomer. Also present in feces were deiodinated closantel (trace amounts) and an unidentified metabolite (~3-6% of the fecal radioactivity).

In the urine, closantel and a metabolite that co-eluted with monoiodosalicylic acid were observed. This latter compound would result from reductive deiodination and amide hydrolysis of closantel. No sulfate or glucuronide conjugates were detected.

Total residues of closantel in plasma amounted to 3.54 ppm 10 days after dosing. Even after that 10-day period, closantel was 93.4% of the total radioactivity. Monoiodoclosantel was 4.7% of the plasma radioactivity.

These data demonstrate the similarity of the metabolism of closantel in rats and sheep. The scheme shown in Figure 1 would, therefore, apply to rats, with the possibility of amide hydrolysis occurring after the initial deiodination step. (Mannens, et al., 1989)

RADIOLABELED RESIDUE DEPLETION STUDIES

Sheep

Five Texel sheep (3M, 2F) were dosed intramuscularly with 5 mg/kg 14C-closantel while another five (3M, 2F) were treated orally (stomach tube) with 10 mg/kg labeled drug. The body weights of the sheep ranged from 27 to 35 kg. The 14C-closantel had a

specific activity of 23.5 μCi/mg and a radiochemical purity of 97% as determined by radio-HPLC. The labeled drug was diluted with cold drug to give material with a specific activity of 4.2 μCi/mg. The 14C label was in the carbonyl carbon of closantel.

Blood samples were collected on heparin from a jugular vein before and at 4, 8, 24, 48, 96 and 168 hours after drug administration, and weekly up to the time of sacrifice. Urine and feces were collected daily from dosing up to the time of sacrifice. At 14, 21, 35, 42 and 56 days after treatment, one animal from each group was sacrificed. From each animal, samples of liver, muscle, mesenteral fat and kidneys were taken.

Whole blood was analyzed by combustion and liquid scintillation counting, while plasma was measured for radioactivity by direct counting. Urine samples were analyzed by air drying of the sample followed by combustion and counting. Feces samples were also analyzed by combustion and counting. Tissue samples were homogenized in water and counted directly. The metabolite pattern in some urine, feces, liver or plasma samples was investigated by radio-HPLC.

Peak concentrations of closantel in blood and plasma occurred at 24 hr and were similar for both routes of administration (~32 ppm for blood, ~47 ppm for plasma). Closantel was eliminated from plasma with a half-life (t1/2) of ~27 and ~23 days after oral or intramuscular administration, respectively. By extraction and HPLC analysis of the plasma, it was found that 90% of the radioactivity represented unchanged closantel.

Analysis of the excreta showed that within 8 weeks after oral or intramuscular dosing ~80% of the administered radioactivity was excreted with the feces and only ~0.5% with the urine. The larger fecal excretion of the radioactivity during the first two days after oral dosing (43.3%) as opposed to intramuscular dosing (10.4%) reflects the smaller systemic availability by the oral route. HPLC analysis of excreta showed that unchanged closantel accounted for 80 to 90% of the eliminated radioactivity. In feces, two other radioactive peaks were detected. These peaks were determined, by reference to standard compounds, to be the 3- and 5-monoiodoclosantel derivatives, indicating reductive deiodination to be a main metabolic pathway. These monoiodoclosantel derivatives were present in urine in only small amounts. Other metabolites in urine, comprising only <0.5% of the total dose, did not correspond to available reference compounds.

The results of assays on tissues for total radioactivity and unchanged closantel are given in Tables I and II below. Of the edible tissues, kidney contained the highest levels of total residue and closantel. Thus, after intramuscular dosing at 5 mg/kg or oral dosing at 10 mg/kg, kidney contained ~3.3 ppm total residue at 14 days and 1.4-1.7 ppm at 56 days (t1/2 ~25 days). An examination of the residue in the tissues using HPLC shows that in fat, muscle and kidney virtually all the total residue can be attributed to closantel. In liver, closantel by HPLC represents ~60-70% of the total residue. The main metabolites in liver were shown to be the 3- and 5-monoiodoclosantel derivatives by radio-HPLC. (Meuldermans, et al., 1982)

Table I. Concentration of Total Residue (TR) and Unchanged Closantel (C) in Tissues of Sheep Treated with 5 mg/kg Closantel Intramuscularly (ppm)

Withdrawal Time (days)	Muscle		Liver		Kidney		Fat	
	TR	C	TR	C	TR	C	TR	C
14	0.59	0.58	2.11	1.59	3.44	3.53	0.25	0.23
21	0.41	0.32	1.95	0.59	2.54	2.45	0.42	0.40
35	0.39	0.35	1.05	0.79	1.83	1.90	0.07	<0.1
42	0.20	0.20	1.00	0.70	1.48	1.42	0.07	<0.1
56	0.19	0.10	0.67	0.36	1.66	1.36	0.09	<0.1

Table II. Concentration of Total Residue (TR) and Unchanged Closantel (C) in Tissues of Sheep Treated Orally with 10 mg/kg Closantel (ppm)

Withdrawal Time (days)	Muscle		Liver		Kidney		Fat	
	TR	C	TR	C	TR	C	TR	C
14	0.75	0.78	1.54	1.24	3.27	3.20	0.17	0.17
21	0.75	0.75	1.58	1.15	2.96	2.87	0.08	<0.1
35	0.44	0.39	0.99	0.67	1.97	1.91	0.09	<0.1
42	0.31	0.30	1.92	1.18	2.15	1.95	0.06	<0.1
56	0.24	0.15	0.67	0.49	1.40	0.88	0.11	<0.1

In Table III, the tissue to plasma ratios for radioactivity are given. It is suggested that the ratios are independent of time and, therefore, that the plasma elimination reflects the depletion of residues from the tissues. (Meuldermans, et al., 1982)

Table III. Tissue to Plasma Ratios of Radioactivity in Sheep After Oral 10 mg/kg (PO) or Intramuscular (IM) 5 mg/kg Dose of 14C-Closantel

Withdrawal Time (days)	Muscle		Liver		Kidney		Fat	
	PO	IM	PO	IM	PO	IM	PO	IM
14	0.039	0.023	0.081	0.083	0.172	0.135	0.009	0.010
21	0.040	0.025	0.084	0.119	0.157	0.155	0.004	0.026
35	0.034	0.033	0.077	0.090	0.153	0.156	0.007	0.006
42	0.016	0.021	0.099	0.107	0.111	0.158	0.003	0.007
56	0.028	0.027	0.078	0.095	0.163	0.235	0.013	0.013
Mean	0.031	0.026	0.084	0.099	0.151	0.168	0.007	0.012

RESIDUE DEPLETION STUDIES

Sheep

Two groups of 4 sheep (weight range 35.7-51.4 kg, 2M, 2F) were treated with closantel either at 5 mg/kg intramuscularly or 10 mg/kg orally. Blood samples were taken from each animal before and at various times after dosing. At 2, 4, 6 and 8 weeks after

dosing, one male and one female animal of either group were sacrificed and samples of edible tissues were taken. Samples were analyzed for closantel using a GLC-electron capture method (stated limit of detection, 0.1 ppm) that used an internal standard.

Peak plasma levels of ~50 ppm were reached at 24 hr. The drug was eliminated from plasma with a t1/2 of ~16 days. The concentrations of closantel in sheep tissues are given in Table IV. Tissue levels were generally comparable for the oral and intramuscular routes. Of the edible tissues, kidney contained the highest concentration of closantel (~2.7 ppm at 14 days). (Michiels, et al., 1977a)

Table IV. Concentrations of Closantel (ppm) in Sheep After a Single Oral (PO, 10 mg/kg) or Intramuscular (IM, 5 mg/kg) Dose

Withdrawal	Muscle		Liver		Kidney		Fat	
Time (days)	PO	IM	PO	IM	PO	IM	PO	IM
14	2.0	2.3	1.7	0.9	2.7	2.6	2.6	1.7
28	<0.4	1.1	0.8	0.7	0.7	1.2	0.7	0.4
42	<0.4	<0.5	0.8	0.3	0.6	1.2	0.5	<0.4
56	<0.3	-	0.4	<0.5	1.2	0.8	0.9	<0.5

Ten sheep (weight 26.9 ± 2.1 kg, 3 F, 7 M) were treated orally with closantel at 5 mg/kg. Groups of three animals (1 F, 2 M) were sacrificed at 14, 18 and 42 days and samples of edible tissues were collected for analysis. A serum sample was taken from the tenth animal at day 56. Closantel was determined with an HPLC method (limit of detection, 0.1 ppm). The results of this study are given in Table V. Levels of closantel at 14 days were highest in kidney (2.43 ppm) and fat (2.17 ppm). By day 42, concentrations of closantel in kidney dropped to 0.62 ppm and in fat to 0.80 ppm. The concentration of closantel in serum was 1.6 ppm. (Michiels, et al., 1979)

Table V. Concentration of Closantel in Tissues of Sheep Treated with a Single Oral Dose of 5 mg/kg (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat
14	1.13 ± 0.11	1.00 ± 0.50	2.43 ± 0.71	2.17 ± 0.75
28	0.20 ± 0.07	0.48 ± 0.33	0.78 ± 0.62	0.45 ± 0.26
42	0.22 ± 0.04	0.23 ± 0.09	0.62 ± 0.35	0.80 ± 0.33

Two groups of six sheep weighing 47 ± 11 kg were treated orally with 5 or 10 mg/kg closantel. Three animals of either group were sacrificed 56 days after treatment and the remaining ones after an 84-day withdrawal period. Samples of liver, kidney, muscle and fat were taken for analysis of closantel. Blood samples were collected from all animals every two weeks up to the time of sacrifice. Closantel was measured with an HPLC procedure, limit of detection 0.1 ppm.

In this study, maximal concentrations of closantel in plasma of sheep were 14.5 ppm and 33.9 ppm for the 5 and 10 mg/kg doses at 14 days. The levels for the respective doses

decreased with a half-life of ~24 days to 1.7 ppm and 3.8 ppm after 84 days of withdrawal. Residues of closantel in tissues are summarized in Table VI. After the 5 mg/kg dose, residue levels at 56 days were 0.06-0.09 ppm for fat and muscle and up to 0.47 ppm for kidney and liver. At 84 days, residues were not detected in fat and muscle, while they had decreased to 0.06-0.17 ppm for liver and kidney. After the 10 mg/kg dose, residue levels at 56 days were 0.65-0.81 ppm for kidney and liver and 0.19-0.24 ppm for fat and muscle. At 84 days, the residue levels were ~0.1 ppm for liver, fat and muscle and 0.25 ppm for kidney. (Michiels, et al., 1980a)

Table VI. Concentration of Closantel in Tissues of Sheep After a Single Oral Dose at 5 or 10 mg/kg (ppm)(ND = not detected)

Withdrawal	Muscle		Liver		Kidney		Fat	
Time (days)	5	10	5	10	5	10	5	10
56	0.09	0.24	0.43	0.81	0.47	0.65	0.06	0.19
84	ND	0.13	0.06	0.10	0.17	0.25	ND	0.10

Cattle

Two groups of six calves, mean body weight 118 ± 22 kg, were injected intramuscularly with closantel at 2.5 mg/kg. A group of three animals was sacrificed at 56 and 84 days after treatment. Samples of liver, kidney, muscle and fat were taken for analysis of closantel. Blood samples were collected from all animals every two weeks up to the time of sacrifice. Closantel was determined with an HPLC procedure having a 0.1 ppm detection limit.

The concentration of closantel in plasma averaged 10 ppm at 14 days. The t1/2 for the elimination of closantel from plasma was ~12 days. By 70 days after treatment, closantel was mostly undetectable in plasma. Residues of closantel were not detected in any tissue at 56 days. (Michiels, et al., 1980a)

Five groups of three steers, body weight ~200 kg, were treated subcutaneously with closantel according to the schedule below:

Treatment		**Sampling**	
day	dose: mg/kg	day	sample
0	15		
21	15	21	plasma
		35	plasma + tissue
50	10		
		65	plasma + tissue
80	10		
		95	tissue
120	10		
		135	plasma + tissue
		150	tissue

Blood samples were obtained on EDTA from the three animals of the last surviving group at the time of the second injection and further at 2 weeks after the second, third and fifth dose. Groups of three animals were sacrificed at 2 weeks after each injection beginning from the second dose and at four weeks after the last dose. Samples of edible tissues were collected for analysis of closantel with the HPLC method. The sampling scheme is summarized in the chart above.

Plasma concentrations of closantel ranged between 88 and 150 ppm for the period of drug administration. The results of the tissue residue analyses are summarized in Table VII. Highest levels of closantel were observed in the kidneys of treated steers. (Michiels, et al., 1980b)

Table VII. Mean Tissue Concentrations of Closantel in Steers After Multiple Administration (ppm)

	Closantel Concentration on Day of Experiment				
Tissue	35	65	95	135	150
muscle-psoas	8.47	6.69	4.99	5.25	2.94
muscle-semitendinosus	4.36	4.15	3.84	2.79	2.82
liver	15.6	14.6	14.1	15.7	10.3
kidney	20.5	19.7	20.1	19.4	12.7
fat-subcutaneous	7.65	7.51	9.04	10.1	2.48
fat-perirenal	3.55	6.29	5.77	11.2	4.56

Four female and six male calves averaging 166 kg were injected intramuscularly with closantel at 2.5 mg/kg. Groups of three animals (1 F, 2 M) were sacrificed at 14, 28 and 56 days of withdrawal. Samples of muscle, liver, kidney and perirenal fat were taken for analysis. A serum sample was taken from one animal of each group and from one surviving female at 56 days of withdrawal. Closantel was determined using an HPLC method having a detection limit of 0.1 ppm.

The results of this study are shown in Table VIII. Highest concentrations of closantel were seen initially in kidney. Very little, if any, depletion of closantel occurred in the edible tissues of calves over the first 28 days of withdrawal. In fact, over the time period studied the concentration of closantel in fat seemed to increase slightly. (Michiels, et al., 1979)

Table VIII. Concentration of Closantel in Tissues of Calves Treated with a Single Intramuscular Dose of 2.5 mg/kg (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat	Serum
14	0.67	1.54	2.84	2.08	21
28	0.70	1.43	2.93	1.97	13
42	0.29	0.56	1.39	2.36	9
56	*	*	*	*	6.8

Five male and two female calves averaging 203 kg were injected intramuscularly with closantel at 5 mg/kg. A group of three animals (2 M, 1 F) were sacrificed at 28 and 56 days of withdrawal. Samples of edible tissues were taken for analysis. A serum sample was taken from one female at 28 days and one male at 84 days of withdrawal. Analyses for closantel were done with an HPLC procedure (limit, 0.1 ppm).

The results of this experiment are presented in Table IX. The data for this study, in contrast those in Table VIII, show depletion of closantel in fat. (Michiels, et al., 1979)

Table IX. Concentration of Closantel in Tissues of Calves Treated with a Single Intramuscular Dose of 5 mg/kg (ppm)

Withdrawal Time (days)	Muscle	Liver	Kidney	Fat	Serum
28	0.94	1.71	4.95	6.03	20
56	0.39	0.58	1.58	1.31	*
84	*	*	*	*	2.3

Three dairy cows, average weight 350 kg, were treated with a single intramuscular dose of closantel at 5 mg/kg. Milk and blood were taken at various times post dose. Samples were examined for closantel using HPLC with UV detection (limit of detection, ~0.5 ppm). Plasma concentrations of closantel were highest 1 to 5 days post dose, ranging ~44-45 ppm. After 5 days, closantel depleted from plasma with a t1/2 of ~12 days. The depletion of closantel from milk is shown in Table X. Mean concentrations of closantel in milk peaked at 4 to 7 days post dose. (Michiels, et al., 1977b)

Table X. Mean Concentrations of Closantel in Milk of Three Dairy Cows After a Single Intramuscular Dose at 5 mg/kg (ppm)

Time (days)	Concentration
1	0.47
2	0.80
3	0.66
4	1.01
5	0.88
6	0.92
7	1.07
14	0.48
21	0.52
28	0.08
35	0.22

METHODS OF RESIDUE ANALYSIS

Gas liquid chromatographic (GLC) and high pressure liquid chromatographic (HPLC) methods have been investigated for the determination of closantel in plasma and tissues of treated animals.

A GLC procedure for closantel in plasma and tissues of sheep has been developed. The method involves extraction of the sample, silylation of closantel and added internal standard, and analysis using electron capture detection. The limit of detection is 0.1 ppm. Continuous use of this method in analyzing tissue samples yielded a number of difficulties, including the appearance of interference peaks and a severe decrease in the sensitivity of the electron capture detector. (Woestenborghs, et al., 1977; Woestenborghs, et al., 1979)

The earliest investigations with HPLC for the determination of closantel led to a procedure for plasma of sheep. In this method, samples, to which an internal standard has been added, are extracted and then analyzed using HPLC with UV detection. The detection limit is ~0.5 ppm. (Hendrickx, et al., 1976)

An HPLC procedure was developed for closantel in animal tissues as well as plasma using improved extraction techniques. In this method samples to which internal standard has been added are extracted with a SEP-PAK™ C18 cartridge. Following separation with HPLC, the samples are quantified using UV detection. The limit of detection is 0.1 ppm. (Woestenborghs, et al., 1979)

The HPLC method above was modified to make the determination of closantel in plasma at levels exceeding 1 ppm more convenient. In the new procedure, sample containing internal standard is deproteinated and then analyzed using HPLC with UV detection. No separate purification step is necessary. (Van Rompaey, et al., 1985)

APPRAISAL

The depletion of residues of closantel from the edible tissues of sheep has been studied using radiolabeled and unlabeled drug; in cattle, the depletion of closantel has been examined using unlabeled drug only.

The characteristics of residue depletion in the edible tissues of cattle and sheep that have been treated with closantel are similar. In particular, residues of the drug are highest in kidney over the entire range of the withdrawal periods studied (usually to 56 days); the residues deplete from tissues with a t1/2 normally in the 2-3 week range; within a species, the parenteral and the oral routes of administration yield comparable residue concentrations when the oral dose is twice the parenteral dose (an oral dose is roughly 50% as bioavailable as the parenteral dose); dose linearity is observed for residue concentrations in the tissues (doubling the dose for a particular route of administration, doubles the residue).

From the study in which sheep were treated with radiolabeled closantel, it was found that parent closantel accounted for nearly all the total radioactivity in muscle, fat and kidney; i.e., virtually no metabolism occurs in those tissues. In sheep liver, approximately

61-71% of the radioactivity was parent closantel, with the remaining residue being comprised of 3- and 5-monoiodoclosantel. No evidence for alternate metabolic pathways (e.g., amide hydrolysis or complete deiodination) was reported.

The metabolism work in rats demonstrates a similarity with that in sheep. It appears that metabolites present in the edible tissues of sheep are produced as well in rats. In addition, the presence of deiodinated closantel and monoiodosalicylic acid is reported in rats.

Of particular interest from the standpoint of residue monitoring, the depletion of residues of closantel from plasma parallels that from the edible tissues. That is,there is a fairly constant tissue:plasma ratio which is independent of time (see Table III). The extrapolation of concentrations of closantel residues from plasma to edible tissues therefore seems feasible. By extension, the monitoring of plasma may then be of potential use in determining when treated animals may be marketed.

The residue chemistry data in this monograph was used to recommend MRLs in sheep and temporary MRLs in cattle as follows:

Sheep - Based on the ADI of 0-0.03 mg/kg established by JECFA, the permitted daily intake of closantel would be 1.8 mg of total drug-related residue contributed by 500 g of food animal meat in the diet of a 60-kg person. For all dose levels studied in sheep, the ADI is not exceeded at 14 days. However, the doses studied were 10 mg/kg orally or 5 mg/kg intramuscularly, whereas the maximum use levels are 15 mg/kg orally or 7.5 mg/kg intramuscularly. If extrapolation of the amount of closantel residues ingested is increased by 50 percent to estimate intake at the maximum use limits, then the ADI of 1.8 mg would be met at approximately 14 days. At 28 days of withdrawal, the intake of residues of closantel, even with this adjustment of 50 percent, is well below the ADI. Based on the data from the studies using closantel at 10 mg/kg orally and 5 mg/kg intramuscularly, JECFA recommended an MRL of 1.5 mg/kg for parent closantel for all edible tissues of sheep. See Table XI.

Table XI. Recommended MRLs for Closantel in Sheep

	Observed Concentration at Day 28 Withdrawal, mg/kg		mg Closantel Consumed		mg/kg	mg(a)
Tissue	10 mg/kg Oral	5 mg/kg I.M.	Oral	I.M.	Recommended MRL (parent)	Consumed (Theory)
Muscle	<0.4	1.1	0.12	0.33	1.5	0.45
Liver	0.8(1.14)(b)	0.7(1.17)(c)	0.11	0.12	1.5(2.5)(c)	0.25
Kidney	0.7	1.2	0.04	0.06	1.5	0.075
Fat	0.7	0.4	0.04	0.02	1.5	0.075
		Total	0.31	0.53		0.85

(a) Based on a daily intake of 0.3 kg muscle, 0.1 kg liver, 0.05 kg kidney and fat.
(b) Adjusted observed value by 70% to estimate total residues.
(c) Adjusted observed value by 60% to estimate total residues.

Cattle - Total residue depletion data were not presented for cattle. However, in view of the metabolism data in sheep and rat, JECFA believed it reasonable to estimate that in muscle, kidney and fat parent closantel was not less than 50 percent of the total residue, and in liver not less than 30 percent of the total residue. Using those estimates to adjust observed concentrations to a total residue basis, the ADI would not be exceeded at 42 days. This conclusion was derived from studies in which animals were dosed at 2.5 mg/kg intramuscularly. At 42 days, the concentrations of parent closantel were 0.29 mg/kg in muscle, 1.39 mg/kg in kidney and 0.56 mg/kg in liver. Accordingly, JECFA recommended temporary MRLs of 0.5 mg/kg in muscle, 2 mg/kg in kidney and 1 mg/kg in liver. An MRL was not set for fat because of unacceptable variability in the data provided. JECFA did not extend these temporary MRLs to use more of the ADI in cattle because (1) the residue studies were done at 2.5 mg/kg whereas the maximum possible dose is 7.5 mg/kg via the injectable, and (2) the lack of total residue depletion and metabolism studies in cattle represents a major deficiency in the residue chemistry data package. See Table XII.

Table XII. Recommended Temporary MRLs for Closantel in Cattle

Tissue	Observed Concentration (Total Residue) at Day 42 Withdrawal, mg/kg 2.5 mg/kg I.M.	mg Closantel Consumed	Recommended Temporary MRL (parent) mg/kg	mg Consumed (Theory)(a)
Muscle	0.29 (0.58)(b)	0.17	0.5	0.30
Liver	0.56 (1.87)(c)	0.19	1.0	0.20
Kidney	1.39 (2.78)(b)	0.14	2.0	0.20
Fat	-	-	(d)	-
	Total	0.50		0.70

(a) Based on a daily intake of 0.3 kg muscle, 0.1 kg liver and 0.05 kg kidney.
(b) Adjusted by factor of 2 (closantel estimated to be 50% of the total residue).
(c) Adjusted by factor of 3.3 (closantel estimated to be 30% of the total residue).
(d) MRL not assigned for fat because of variability in the data; based on available data, fat is not expected to contribute more than about 0.25 mg to the daily intake of total residues of closantel at day 42 of withdrawal.

REFERENCES

Hendrickx, J., Wynants, J. and Heykants, J. (1976). A "HPLC" assay method for closantel (R 31520) in sheep plasma. Unpublished report V 2710. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Mannens, G., Mostmans, E., Verboven, P., Hendrickx, J., Hurkmans, R., Van Leemput, L., Meuldermans, W. and Heykants, J. (1989). The excretion and metabolism of 14C-closantel in male Wistar rats after a single oral dose of 10 mg/kg. Unpublished report V 7201. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Meuldermans, W., Michiels, M., Woestenborghs, R., Van Houdt, J., Lorreyne, W., Hendrickx, J., Heykants, J. and Desplenter, L. (1982). Absorption, tissue distribution, excretion and metabolism of closantel-14C after intramuscular and oral administration in sheep. Unpublished report V 4523. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Woestenborghs, R., Heykants, J. and Marsboom, R. (1977a). On the absorption and distribution of closantel (R 31520) in sheep after oral and intramuscular administration. Unpublished report V 2709. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Hendrickx, J., Heykants, J. and Marsboom, R. (1977b). Plasma and milk concentrations of closantel in cattle after a single intramuscular administration. Unpublished report V 2706. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Woestenborghs, R., Michielsen, L., Hendrickx, J., Heykants, J. and Marsboom, R. (1979). Residual plasma and tissue concentrations of closantel (R 31520) in cattle and sheep. Unpublished report V 3173. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Woestenborghs, R., Embrechts, L., Heykants, J. and Marsboom, R. (1980a). Plasma levels and residual tissue concentrations of closantel (R 31520) in sheep and cattle. Unpublished report V 3413. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Woestenborghs, R., Embrechts, L., Heykants, J. and Marsboom, R. (1980b). Residual plasma and tissue concentrations of closantel (R 31520) in cattle. Unpublished report V 3716. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Meuldermans, W. and Heykants, J. (1987). The metabolism and fate of closantel (Flukiver) in sheep and cattle. Drug Metab. Rev., 18, 235-251.

Van Rompaey, F., Woestenborghs, R., Prinsen, P. and Heykants, J. (1985). A modified HPLC-method for the rapid determination of closantel in animal plasma. Unpublished report V 5907. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Woestenborghs, R., Michiels, M. and Heykants, J. (1977). Electron capture GLC determination of closantel (R 31520) in plasma and animal tissues. Unpublished report V 2708. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Woestenborghs, R., Hendrickx, J., Michiels, M., Michielsen, L. and Heykants, J. (1979). A new HPLC-method for the determination of closantel (R 31520) in plasma and animal tissues. Unpublished report V 3190. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.