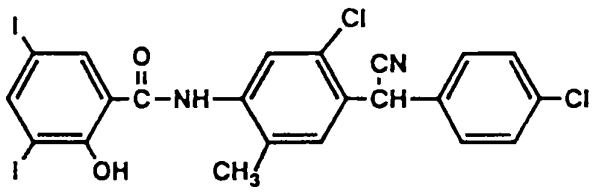
# CLOSANTEL

## IDENTITY

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

**Synonyms:** Flukiver
Seponver

**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°C 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 sheep and cattle orally or parenterally via drench, bolus or injectable formulations. The common use of the injectable formulation, delivered subcutaneously or intramuscularly, is 5.0 mg/kg in sheep and 2.5 mg/kg in cattle. The dose range for the oral route is primarily 5 to 10 mg/kg in sheep and cattle; however, the use of 15 mg/kg is recommended for treatment of *Fascioloides*, which is a minor indication. A single application of the drug is recommended.

### METABOLISM

**Pharmacokinetics**

The pharmacokinetic studies on absorption, distribution, metabolism, excretion, and tissue depletion that are summarized were conducted using closantel labelled with carbon-14 in the carbonyl carbon of the benzamide ring.

Rat

Five male Wistar rats were given a single oral dose of closantel at 20 mg/kg, provided as a 0.5% suspension. Maximum concentrations of closantel in serum ($C_{max}$) of 73.1 ± 10.3 μg/ml (X ± 1 SD) occurred at 1 day after administration ($t_{max}$). Closantel depletion in serum from $t_{max}$ was monoexponential with a $t_{1/2}$ of 2.8 days. The area under the curve ($AUC_{0-\infty}$) was 408 μg·day/ml. (Van Beijsterveldt et al., 1989)

Sheep

Several pharmacokinetic studies have been conducted in sheep but only one with $^{14}C$-labelled closantel and it will be summarized.

$^{14}C$-labelled closantel was administered orally or intramuscularly to two groups of five sheep at doses of 10 and 5 mg/kg, respectively. Peak radioactive plasma concentrations, occurring at 8-24 hours after drug administration, were similar by both routes, amounting to 47.0 ± 11.1 and 47.9 ± 4.4 μg-eq/ml for the oral and intramuscular route respectively. Plasma radioactivity was 86 to 91% unmetabolized drug. Closantel was eliminated from plasma with a $t_{1/2}$ of 26.7 and 22.7 days after oral and intramuscular administration respectively. The $AUC_{0-\infty}$ values for the respective routes were similar, 1303 and 1027 μg·day/ml, indicating that the systemic bioavailability of orally administered closantel is half that of an intramuscular dose.

Within 8 weeks after oral or intramuscular dosing, about 80 % of the administered dose was excreted with the feces, and only 0.5 % with the urine. (Meuldermans, W. et al., 1982)

Cattle

As with sheep, several pharmacokinetic studies have been conducted in cattle but only one with $^{14}C$-labelled closantel and it will be summarized.

$^{14}C$-labelled closantel was administered orally to five Friesian heifers and four Friesian steers at a single dose of 10 mg/kg. The average $C_{max}$ values for total radioactivity (TRA) in blood and plasma were 26.8 and 35.7 $\mu$g-eq/ml, occurring after 48 hours ($t_{max}$). The $AUC_{0-\infty}$ for TRA in plasma was 593 $\mu$g-eq·day/ml and a half-life of 11 days. All TRA in the plasma was unmetabolized drug. Within 42 days, 90 % of the dose was excreted with the feces and less than 0.25% in the urine. (Van Leemput et al., 1991)

**Metablolism in Food and Laboratory Animals**

Rat

Five male Wistar rats (~242 g) were dosed orally with $^{14}C$-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-h periods up to 10 days post dosing. Plasma was collected at sacrifice after the 10-day excreta collection period. Samples were analyzed for total radioactivity by combustion and counting or by direct counting. Unchanged drug and metabolites were determined using HPLC 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 h) and monoiodoclosantel (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

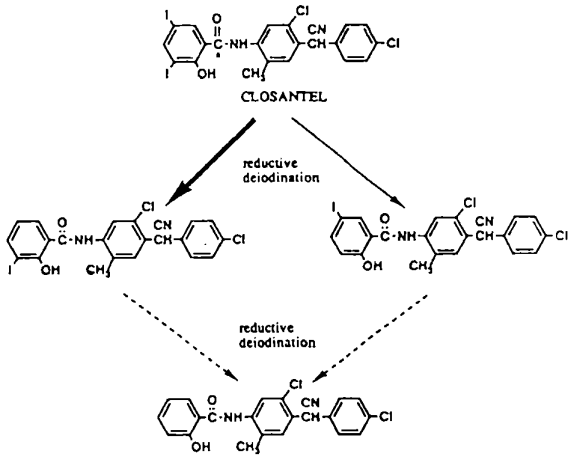
possibility of amide hydrolysis occurring after the initial deiodination step. (Mannens et al., 1989)

Sheep

Following the administration of $^{14}$C-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 $^{14}$C 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)

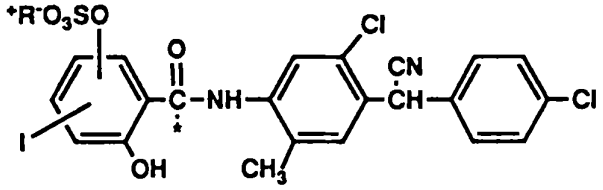
Cattle

$^{14}$C-labelled closantel was administered orally to five Friesian heifers and four Friesian steers at a single dose of 10 mg/kg. Parent closantel was found to be the major constituent in feces (82 % of the total residue) and in kidney, muscle and

fat (70-80, 80-100 and 60-100 % of the total residue, respectively). However, unmetabolized closantel only accounted for 6-15 % of the total residue in liver. Characterization of the closantel-derived radioactivity in the liver showed that 40-77 % of the radioactivity could be accounted for by monoiodoclosantel. Further analysis of the monoiodoclosantel revealed that it was the 3-monoiodoclosantel metabolite present, resulting from the deiodination at the 5-position of the benzamide moiety.

Besides the unmetabolized drug, only one notable metabolite was detected in the methanol extracts of the feces. This metabolite accounted for approximately 6% of the administered dose in the feces extracts of the samples collected during the first two weeks. This metabolite also occurred in the bile. Elucidation of this metabolite was performed by mass spectrometry and UV analysis and determined to be a sulfate conjugate of a closantel-derivative where one iodine had been removed and an hydroxyl substituent attached on the benzamide moiety (see Figure 2 below). (Van Leemput et al., 1991)

**Figure 2.** **Metabolite of Closantel in Cattle Feces and Bile. The Position of $^{14}C$ is Indicated by the Asterisk.**




R = H, Na or K

## TISSUE RESIDUE DEPLETION STUDIES

### Radiolabelled Residue Depletion Studies

Sheep

Five Texel sheep (3MA, 2F) were dosed intramuscularly with 5 mg/kg $^{14}C$-closantel while another five (3MA, 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 $^{14}C$-closantel had a specific activity of 23.5 $\mu$Ci/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 $\mu$Ci/mg.

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 h and were similar for both routes of administration (~32 ppm for blood, ~47 ppm for plasma). Closantel was eliminated from plasma with a $t_{1/2}$ 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 1 and 2 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 ($t_{1/2}$ ~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 1. Concentration of Total Residue (TR) and Unchanged Closantel (C) in Tissues of Sheep Treated with 5 mg/kg $^{14}$C-Closantel Intramuscularly (ppm)**

| Withdrawal | Muscle | | Liver | | Kidney | | Fat | |
|---|---|---|---|---|---|---|---|---|
| Time (days) | 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 2. Concentration of Total Residue (TR) and Unchanged Closantel (C) in Tissues of Sheep Treated Orally with 10 mg/kg $^{14}$C-Closantel (ppm)**

| Withdrawal | Muscle | | Liver | | Kidney | | Fat | |
|---|---|---|---|---|---|---|---|---|
| Time (days) | 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 3, 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 3. Tissue to Plasma Ratios of Radioactivity in Sheep After Oral 10 mg/kg (PO) or Intramuscular (IM) 5 mg/kg Dose of $^{14}$C-Closantel**

| Withdrawal | Muscle | | Liver | | Kidney | | Fat | |
|---|---|---|---|---|---|---|---|---|
| Time (days) | 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 |

Cattle

Five Friesian heifers and four Friesian steers, weighing 203-252 kg, were administered a single oral dose (via stomach tube) of 10 mg/kg of $^{14}$C-labelled closantel. For the 6-week phase, the $^{14}$C-closantel had a specific activity of 2.39 $\mu$Ci/mg and a radiochemical purity of 98.1 % as determined by radio-HPLC. The labeled drug was diluted with cold drug to give material with a specific activity of 1.62 $\mu$Ci/mg. For the 2- and 4-week phases, the $^{14}$C-closantel had a specific activity of 2.12 $\mu$Ci/mg and a radiochemical purity of 100 % as determined by radio-HPLC. The labeled drug was diluted with cold drug to give material with a specific activity of 2.08 $\mu$Ci/mg.

For the 6-week phase, there were two heifers and one steer included in the study of the mass balance of the $^{14}$C-closantel-derived radioactivity. Blood samples were collected on heparin from a jugular vein before and at 4, 8, 24, 48, 72 and 96 hours after drug administration, and weekly up to 42 days. Urine and feces were collected daily from dosing up to the time of sacrifice. At 42 days after treatment,

the three animals were sacrificed. From each animal, samples of liver, muscle, mesenteral fat and kidneys were taken.

For the 2- and 4-week phase, there were three heifers and three steers included in the study of tissue depletion of the $^{14}$C-closantel-derived radioactivity. Blood samples were collected on heparin from a jugular vein before and at 4, 8, 24, 48, 72 and 96 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. Group 1 was comprised of two heifers and one steer and they were sacrificed at 14 days after treatment. Group 2 was comprised on one heifer and two steers and they were sacrificed at 28 days after treatment. 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 liquid scintillation counting. Feces samples were 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.

The results of the pharmacokinetics and metabolism from this study have been described previously in their respective sections.

The results of assays on tissues for total radioactivity and unchanged closantel are given in Table 4. Of the edible tissues, liver contained the highest levels of total residue and kidney contained the highest concentrations of unmetabolized closantel. Thus, after oral dosing at 10 mg/kg, liver contained ~3.7 ppm total residue at 14 days and 1.1 ppm at 42 days. An examination of the residue in the tissues using HPLC shows that in fat, muscle and kidney a large portion of the total residue can be attributed to closantel. Parent closantel accounts for approximately 100, 80 and 70 % of the total residues in muscle, kidney and fat, respectively. In liver, closantel by HPLC represents ~10 % of the total residue. The main metabolite in liver was shown to be the 3-monoiodoclosantel derivative by radio-HPLC. (Van Leemput et al., 1991)

**Table 4. Mean Concentration (n=3/group) of Total Residue (TR) and Unchanged Closantel (C) in Tissues of Cattle Treated with 10 mg/kg $^{14}$C-Closantel Orally (ppm)**

| Withdrawal | Muscle | | Liver | | Kidney | | Fat | |
|---|---|---|---|---|---|---|---|---|
| Time (days) | TR | C | TR | C | TR | C | TR | C |
| 14 | 0.71 | 0.57 | 3.71 | 0.54* | 2.50 | 1.87 | 1.09 | 0.78 |
| 28 | 0.34 | 0.37 | 2.41 | 0.17* | 1.28 | 1.05 | 0.83 | 0.52 |
| 42 | 0.13 | 0.16* | 1.13 | ≤0.1 | 0.47 | 0.38 | 0.20 | 0.18 |

* - This mean does not include one value which was ≤ 0.1 ppm.

In Table 5, the tissue to plasma ratios for radioactivity are given. As with sheep, the data suggest that the ratios are independent of time and, therefore, that the plasma elimination reflects the depletion of residues from the tissues.

**Table 5. Tissue to Plasma Ratios of Radioactivity in Cattle After Oral 10 mg/kg (PO) Dose of $^{14}$C-Closantel**

| Withdrawal Time (days) | Muscle | Liver | Kidney | Fat |
|---|---|---|---|---|
| 14 | 0.044 | 0.227 | 0.153 | 0.067 |
| 28 | 0.044 | 0.312 | 0.169 | 0.108 |
| 42 | 0.050 | 0.423 | 0.192 | 0.077 |
| Mean | 0.046 | 0.321 | 0.171 | 0.084 |

## OTHER RESIDUE DEPLETION STUDIES

Sheep

Two groups of 4 sheep (2MA, 2F) weighing 35.7-51.4 kg 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 h. The drug was eliminated from plasma with a $t_{1/2}$ of ~16 days. The concentrations of closantel in sheep tissues are given in Table 6. 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 6. 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 | NA | 0.4 | <0.5 | 1.2 | 0.8 | 0.9 | <0.5 |

Ten sheep (7MA, 3F) weighing 26.9 ± 2.1 kg were treated orally with closantel at 5 mg/kg. Groups of three animals (2MA, 1F) 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 7. 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 7. 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 8. 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 8. Concentration of Closantel in Tissues of Sheep After a Single Oral Dose at 5 or 10 mg/kg (ppm)**

| Withdrawal | Muscle | | Liver | | Kidney | | Fat | |
|---|---|---|---|---|---|---|---|---|
| Time (days) | 5 | 10 | 5 | 10 | 5 | 10 | 5 | 10 |
| 14 | 2.0 | 2.3 | 1.7 | 0.9 | 2.7 | 2.6 | 2.6 | 1.7 |
| 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

Twelve Friesian cattle (6MA, 6F), mean body weight 270 ± 28 kg, were administered a single oral dose of closantel at 10 mg/kg using a 5% suspension formulation (Seponver®). A group of four animals were sacrificed at 14, 28 and 42 days after treatment. Samples of liver, kidney, muscle and fat were taken for analysis of closantel using the validated HPLC method having a detection limit of 0.1 ppm (Woestenborghs et al., 1979; Woestenborghs et al., 1985). Blood samples were collected from all animals before dosing and at 1, 2, 3, 4, 7 and 14 days after administration and from all animals at 21, 28, 35 and 42 days after administration for those animals remaining in the experiment.

The maximum concentrations of closantel in plasma occurred at ~2 days after treatment and were 30.7 μg/ml on average. The $AUC_{0-\infty}$ averaged 517 μg day/ml and the mean $t_{1/2}$ for the elimination of closantel from plasma was ~11 days.

The highest concentrations of closantel in tissues were found in kidney: from 3.29 ppm at day 14 to 0.11 ppm at day 42. Muscle concentrations ranged from 0.58 ppm at day 14 to ≤ 0.10 ppm at day 42. Concentrations in fat varied from 0.93 ppm to ≤ 0.10 ppm at day 42. Liver concentrations decreased from a maximum of 1.55 ppm after 14 days to ≤ 0.10 ppm at day 42. The mean results are described in Table 9 below. (Van Beijsterveldt et al., 1991)

**Table 9. Concentration of Closantel in Tissues of Cattle (n=4/group) Treated with a Single Oral Dose of 10 mg/kg (ppm)**

| Withdrawal Time (days) | Muscle | Liver | Kidney | Fat |
|---|---|---|---|---|
| 14 | 0.41±0.10 | 0.68±0.52 | 2.14±1.00 | 0.65±0.20 |
| 28 | 0.19±0.05[1] | 0.16±0.02[1] | 0.83±0.31 | 0.18±0.03[2] |
| 42 | 0.19±0.10[2] | 0.49[3] | 0.33±0.26 | ND |

1 - This mean does not include two values which are ≤ 0.10 ppm.
2 - This mean does not include one value which is ≤ 0.10 ppm.
3 - This mean does not include three values which are ≤ 0.10 ppm.
ND - All values are ≤ 0.10 ppm.

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 $t_{1/2}$ 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 Day | Dose (mg/kg) | Sampling 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 10. Highest levels of closantel were observed in the kidneys of treated steers. (Michiels et al., 1980b)

**Table 10. 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 MA) were sacrificed at 14, 28 and 42 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 11. 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 11. 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 | NA | NA | NA | NA | 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 MA, 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 (detection limit, 0.1 ppm).

The results of this experiment are presented in Table 12. The data for this study, in contrast those in Table 11, show depletion of closantel in fat. (Michiels et al., 1979)

**Table 12. 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 | NA |
| 84 | NA | NA | NA | NA | 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 $t_{1/2}$ of ~12 days. The depletion of closantel from milk is shown

in Table 13. Mean concentrations of closantel in milk peaked at 4 to 7 days post dose. (Michiels et al., 1977b)

**Table 13. 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 ANALYSIS FOR RESIDUES IN TISSUES

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. (Woestenborghs et al., 1985)

## APPRAISAL

The depletion of residues of closantel from the edible tissues of sheep and cattle has been studied using radiolabelled and unlabeled drug.

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 parent 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 $t_{1/2}$ normally in the 2-3 week range; in sheep, 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).

From the studies in which sheep were treated either orally or parenterally with radiolabelled 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 60-70% 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. In cattle treated orally, parent closantel accounts for approximately 100, 80 and 70 % of the total residues in muscle, kidney and fat, respectively. However, in cattle liver approximately 10% of the radioactivity was parent closantel, and 40-77% of the radioactivity was accounted for by 3-monoiodoclosantel. In addition, a metabolite accounting for 6% of the radioactivity was identified as a sulfate conjugate of a closantel-derivative. No radiolabelled studies have been conducted with closantel administered parenterally in cattle.

The metabolism work in rats demonstrates a similarity with that in sheep and cattle. It appears that metabolites present in the edible tissues of sheep and cattle are produced as well in rats. The only exception is the small amount of the sulfate conjugate metabolite in cattle. 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 within species which is independent of time (see Tables 3 and 5). 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.

JECFA used the residue chemistry data in the monograph developed for the 36th Session of JECFA to recommend MRLs in sheep. However, since the last meeting it has been recognized that the MRL calculation of 1.5 ppm for kidney at 28 days may be exceeded at that time due to the concentrations of parent closantel in the oral radiolabelled study in sheep at 35 and 42 days (Meuldermans et al., 1982). Therefore, the MRL has been reevaluated and determined that an increase to 5 ppm in kidney will not impact the drug's human food safety. This change has been incorporated in Table 14.

Sheep - Based on the ADI of 0-0.03 mg/kg established by the 36th Meeting of 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. The doses studied were 10 mg/kg orally or 5 mg/kg intramuscularly. At 28 days of withdrawal, the intake of residues of closantel 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 5000 μg/kg for parent closantel in kidney and 1500 μg/kg in muscle and liver and 2000 μg/kg in fat of sheep. See Table 14.

**Table 14. Recommended MRLs μg/kg for Closantel in Sheep**

| | Observed Concentration at Day 28 Withdrawal, mg/kg | | mg Closantel Consumed | | | |
|---|---|---|---|---|---|---|
| Tissue | 10 mg/kg Oral | 5 mg/kg IM | Oral | IM | Rec. MRL (parent) | mg(a) Consumed (Theory) |
| Muscle | <0.4 | 1.1 | 0.12 | 0.33 | 1500 | 0.45 |
| Liver | 0.8(1.14)b | 0.7(1.17)c | 0.11 | 0.12 | 1500 (2500)c | 0.25 |
| Kidney | 0.7 | 1.2 | 0.04 | 0.06 | 5000 | 0.25 |
| Fat | 0.7 | 0.4 | 0.04 | 0.02 | 2000 | 0.10 |
| Total | | | 0.31 | 0.53 | | 1.05 |

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 - Temporary MRLs in cattle were proposed at the 36th Session of JECFA, but based on the development of a more complete residue package for cattle having been submitted, the Committee recommends the new MRLs. At 42 days of withdrawal, the intake of residues of closantel is below the ADI of 1.8 mg. Based on the data from the studies using closantel at 10 mg/kg orally and 2.5 mg/kg intramuscularly, JECFA recommended an MRL of 1000 μg/kg for parent closantel in muscle and liver, and 3000 μg/kg in kidney and fat (see Table 15).

**Table 15. Recommended MRLs μg/kg for Closantel in Cattle**

| Tissue | Observed Concentration at Day 28* or 42* Withdrawal, mg/kg | | mg Closantel Consumed | | Rec. MRL (parent) | mg(a) Consumed (Theory) |
|---|---|---|---|---|---|---|
| | 10 mg/kg Oral | 2.5 mg/kg IM | Oral | IM | | |
| Muscle | 0.19 | 0.29 | 0.06 | 0.09 | 1000 | 0.30 |
| Liver | 0.16(1.6)b | 0.56(5.6)b | 0.16 | 0.56 | 1000(10000)b | 1.00 |
| Kidney | 0.83(1.0)c | 1.39(1.7)c | 0.05 | 0.09 | 3000(3750)c | 0.19 |
| Fat | 0.7(1.0)d | 2.36(3.4)d | 0.05 | 0.17 | 3000(4290)d | 0.21 |
| Total | | | 0.32 | 0.91 | | 1.70 |

* - The oral data is summarized from Day 28 withdrawal and the parenteral data is summarized from Day 42 withdrawal.
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 10% to estimate total residues.
c) Adjusted observed value by 80% to estimate total residues.
d) Adjusted observed value by 70% to estimate total residues.

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