LEVAMISOLE

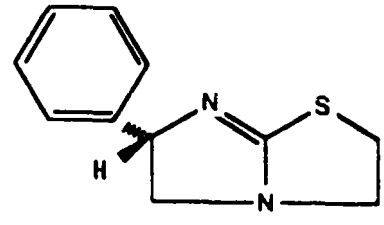
IDENTITY

Chemical name: (-)-2,3,5,6-Tetrahydro-6-phenylimidazo-[2,1-b]thiazole

(-)-6-Phenyl-2,3,5,6-tetrahydroimidazo-[2,1-b]thiazole

Synonyms: Decaris-Vet; Levasole; Narpenol; Nemicide; Nilverm; Ripercol; Solaskil; Tramisol; Vermisol; Worm-Chek

Structural formula:



Molecular formula: $C_{11}H_{12}N_2S$ (levamisole)

$C_{11}H_{13}ClN_2S$ (levamisole hydrochloride)

Molecular weight: 204.29 (levamisole)
240.75 (levamisole hydrochloride)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

	Levamisole	Levamisole hydrochloride
Appearance:	white to slightly yellowish powder	white to pale cream crystalline powder
Melting point:	58-63°	229-230.1°
Optical rotation:	$[a]_D^{20}$ = -144° (c 5%, 1N HCl)	$[a]_D^{20}$ = -128.6° (c 5%, H_2O)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Levamisole, the levorotatory enantiomer of tetramisole, is used as a nematocidal anthelmintic in cattle, sheep, goats, pigs and poultry. It is effective against lungworms and gastrointestinal nematodes, while ineffective against cestodes, trematodes and arthropods.

Depending upon the formulation, levamisole is administered as levamisole base, levamisole hydrochloride or levamisole phosphate. The base is applied via pour-on formulations, while the hydrochloride and phosphate are used in injectables, oral drenches and feed premixes. Of the two salts, the hydrochloride is more widely used; the phosphate is used mainly in North America.

Dosages

Therapeutic dose levels of levamisole generally vary between 5 and 40 mg/kg body weight, depending on the worm species and host animal. The usual maximum dose rates, given in terms of levamisole hydrochloride, are summarized as follows: cattle, 8 mg/kg; sheep, 8 mg/kg; swine, 8 mg/kg; goats, 8 mg/kg; and chickens, 36 mg/kg.

METABOLISM STUDIES

Rats

Levamisole hydrochloride labeled with 14C in the 8-position (see Figure 1 for the structure) was administered orally in a single dose of 15 mg/kg to male Sprague-Dawley rats. Urine and feces were collected from rats for up to 8 days after dosing. Groups of 3 rats were sacrificed at withdrawal periods of 12, 24, 48, 96 and 192 hours. At the withdrawal times, samples of tissues, including muscle, liver, kidney and fat, were collected for combustion radioanalysis. The amount of $14CO_2$ in expired air was determined. Thin-layer chromatography was used to study the nature of the urine radioactivity.

The concentrations of radioactivity in tissues of rats treated with levamisole are given in Table I. Levels of radioactivity were highest in liver and kidney. Only 0.2% of the dose was found in the respiratory gases, indicating that complete metabolism of levamisole to $14C0_2$ is not a significant pathway.

Table I. Total Residues of Levamisole Hydrochloride in Tissues of Rats Treated at 15 mg/kg Orally (ppm)

Withdrawal (hours)	Muscle	Liver	Kidney	Fat
12	0.38	3.38	2.50	0.11
24	0.18	1.35	0.94	0.07
48	0.13	0.80	0.57	0.06
96	0.08	0.34	0.28	0.05
192	0.07	0.20	0.13	0.02

The excretion of levamisole was found to be rapid, with 40% of the dose eliminated in the urine and 34% in the feces within 12 hours of treatment. Urine and fecal excretion was virtually complete at 24 hours (46% of the dose) and 48 hours (40% of the dose), respectively.

Thin-layer chromatography of urine samples collected at various times after treatment indicates that levamisole undergoes extensive metabolism. Thus, 13 metabolites were observed at 2 hours and 21 metabolites at 8 hours after treatment. In some samples of urine, it was possible to distinguish ~50 spots on thin-layer chromatography. Examination of extracts of tissues with thin-layer chromatography showed that the metabolites appear to be the same as those in urine, and that the predominant metabolite is levamisole.

Several of the metabolites from this study were isolated and examined by a number of physico-chemical procedures. From the results of this work, structures were postulated for some metabolites and confirmation achieved by comparison with standards.

Four major metabolic pathways were proposed for levamisole based on the metabolites that were identified in this radiolabeled study:

1. Oxidative introduction of a double bond in the imidazoline ring - Quantitatively the most important pathway, the double bond formation is followed by para-hydroxylation of the phenyl ring and oxidation of the sulfur to sulfoxide and sulfone. Some of the metabolites can become conjugated.

2. Hydroxylation of the thiazolidine ring - This is followed by formation of the thiolactone, and hydrolysis to the thiohydantoic acid metabolite or, by O replacement of the S, the corresponding hydantoic acid metabolite. N-Dealkylation can also lead to the oxo-imidazolidine metabolite.

3. Formation of para-hydroxylevamisole - This is likely to be followed by the formation of conjugates.

4. Hydrolysis of the thiazole ring - Quantitatively the least important pathway, opening of the ring is followed by oxidation of sulfur or methylation of the sulfur.

The metabolism of levamisole in rats is detailed in Figure 1. (Boyd, et al., 1969) (Gatterdam, et al., 1966) (Graziani and De Martin, 1977)

Male Sprague-Dawley rats (250 g) were administered 7.5 mg/kg levamisole hydrochloride, labeled with tritium in the 2-position of the phenyl ring, orally or by intramuscular injection. Groups of treated rats were sacrificed at 1, 4, 8 and 24 hours after dosing. A group of rats was used to determine urinary and fecal excretion. Radioactivity was measured with liquid scintillation counting.

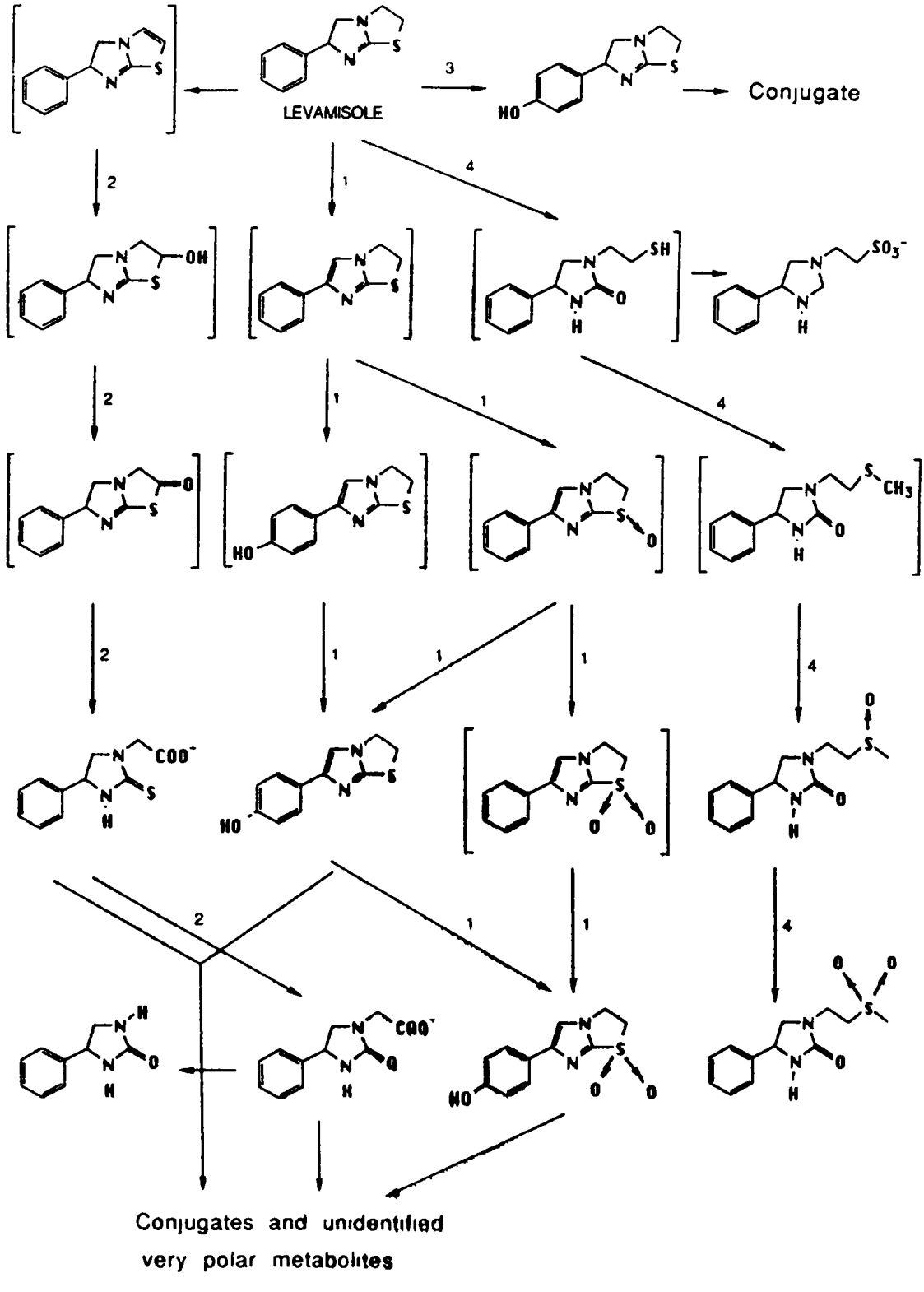
The depletion of total residues from tissues of treated rats is given in Table II. The values in Table I (administration of 14C-levamisole) generally show comparability with those in Table II (administration of 3H-levamisole).

Table II. Total Residues of Levamisole Hydrochloride in Tissues of Rats Treated at 7.5 mg/kg Orally or IM (ppm)

	Oral				IM			
Tissue	**1 hr**	**4 hr**	**8 hr**	**24 hr**	**1 hr**	**4 hr**	**8 hr**	**24 hr**
Liver	1.46	2.29	1.40	0.26	9.09	3.97	1.20	0.29
Kidney	4.44	5.27	3.03	0.14	15.36	10.47	1.27	0.22

For the two routes of administration, tritium was excreted mainly in the urine (68-78%) rather than in the feces (33-17%). Levamisole was found to be 6.3-8.5% of the urinary radioactivity, 4-hydroxylevamisole 5.8-8%, chloroform-soluble compounds 36-47% and water-soluble compounds 37-52%. In the feces, levamisole was observed to be <1% of the radioactivity. Overall, therefore, levamisole was extensively (91-94%) metabolized in the excreta. (Galtier, et al., 1983a)

Figure 1 - Metabolic Pathways for Levamisole in Rats



Pigs

Tritium labeled levamisole was administered intravenously to 5 pigs at 5 mg/kg. Urine and feces were collected through the course of the study. Radioactivity was determined by liquid scintillation counting. The excretion of levamisole was fast. The total excretion of radioactivity in the urine was 80-85%, most of which was recovered in the first 12 hours after dosing. Only 5-10% of the drug was recovered with the feces. Many metabolites were observed, with levamisole comprising 5-10% of the urinary excretion. (Nielsen and Rasmussen, 1982)

A single oral (10 mg/kg) or intramuscular (7.5 mg/kg) dose of 3H-levamisole (phenyl 2-position) was given to pigs. In this study, ~60-70%, depending upon the route of administration, of the dose was eliminated via the urine within 72 hours after dosing. However, in contrast to the study above levamisole comprised 18-26% of the urine excretion. Also, only 4% of the dose was eliminated via the bile in the same time period, with <1% representing levamisole. (Galtier, et al., 1983b)

Goats

Tritium labeled levamisole was administered intravenously to 3 goats at 5 mg/kg. Urine and feces were collected through the course of the study. Radioactivity was determined by liquid scintillation counting. The excretion of levamisole was fast. The total excretion of radioactivity in the urine was 50%, most of which was recovered in the first 12 hours after dosing. About 30% of the drug was recovered with the feces. Many metabolites were observed, with levamisole comprising 5-10% of the urinary excretion. (Nielsen and Rasmussen, 1982)

RADIOLABELED RESIDUE DEPLETION STUDIES

Total residue depletion data in food-producing animals are not available. Radiolabeled levamisole has been administered to rats, pigs and goats to study pharmacokinetics and metabolism. These studies will be summarized later in this monograph.

RESIDUE DEPLETION STUDIES

Chickens

Drinking water providing 36 mg/kg levamisole hydrochloride was offered in a half-day's supply of water to 8-week old broilers. Edible tissues were collected from the birds the morning after treatment (0 withdrawal) and for the next 5 days. Levamisole was determined with a cathode ray polarographic procedure with a detection limit of 0.1 ppm. At 0 withdrawal levamisole hydrochloride averaged (6 samples) <0.1 ppm in muscle, <0.1 ppm in skin, <0.1-0.13 in fat, <0.1-0.12 in kidney and 0.59 ppm in liver. At 1-day and 2-day withdrawals all samples contained <0.1 ppm levamisole. (Berger, et al., 1973a)

Laying hens weighing ~4 lb were treated with drinking water providing 36 mg/kg levamisole hydrochloride. Egg samples were collected daily up to ten days post medication. Egg yolks and white were analyzed (6 samples of each) for levamisole

hydrochloride with a cathode ray polarographic method validated to 0.025 ppm. At 1 day withdrawal, egg yolks contained an average of 0.79 ppm levamisole hydrochloride and egg whites, 0.19 ppm. At 3 days withdrawal, egg yolks averaged 0.12 ppm levamisole hydrochloride and egg whites, <0.025-0.035 ppm. At 5 days withdrawal, egg yolks contained <0.025-0.097 ppm and egg whites, <0.025 of levamisole hydrochloride. (Berger, 1973)

Sheep

Twelve wether sheep (average weight, ~20.3 kg) were treated with a single dose of levamisole at 8 mg/kg via oblet. Samples of muscle, liver, kidney and fat were collected from treated animals sacrificed at 3, 24, 72 and 120 hours after treatment. Residues of levamisole were measured with a cathode ray differential polarographic method with a limit of detection of 0.10 ppm. The results of the assays are given in Table III. (Berger, et al., 1969)

Table III. Average Levamisole Residues in Sheep Treated at 8 mg/kg via Oblet (ppm)

Withdrawal (hours)	Muscle	Liver	Kidney	Fat
3	1.30	20.00	7.49	<0.12
24	<0.12	1.57	1.20	<0.10
72	<0.10	<0.10	<0.10	<0.10
120	<0.10	<0.10	<0.10	<0.10

Fifteen lambs (average weight, ~29.5 kg) were injected subcutaneously in the mid-neck area with levamisole phosphate to provide a dose equivalent to 8 mg/kg levamisole hydrochloride. Samples of edible tissues were taken at 0 (2 hr), 7, 8, 9 and 10 days of withdrawal. Levamisole hydrochloride was measured with the cathode ray polarographic procedure that was validated to 0.05 ppm in sheep tissues. At 0-withdrawal, the following concentrations of levamisole hydrochloride were observed: injection site, 106 ppm; muscle, 8.8 ppm, liver, 50.2 ppm; kidney, 84.1 ppm, and fat, 2.9 ppm. At all other times, samples contained <0.05 ppm with the exception of injection site from one animal sacrificed at 8 days of withdrawal whose average concentration was 5.6 ppm. (Berger and Colavita, 1974)

Eight ewes weighing ~50 kg were treated with 7.5 mg/kg levamisole hydrochloride via drench. Samples of muscle, liver, kidney and fat were taken from two animals sacrificed after withdrawal times of 7, 14, 21 and 28 days. Levamisole was measured using a gas chromatographic procedure with nitrogen/phosphorous detection that had a 10 ppb detection limit. Concentrations of levamisole in liver averaged 87 ppb at 7 days, 57 ppb at 14 days and <39 ppb at 21 days. All other tissue samples contained levamisole in amounts <10 ppb. (Van Leemput, et al., 1989a)

Swine

Three male pigs weighing ~63 kg were injected intramuscularly with 10 mg/kg levamisole hydrochloride. The animals were sacrificed at 24, 48 and 72 hours post dosing. Samples of injection site, muscle, liver, kidney and fat were taken for analysis of levamisole with a polarographic procedure validated to 0.1 ppm. The results of the assays are given in Table IV. The data show a t1/2 of ~12 hours for residues of levamisole in liver and kidney. (Heykants, et al., 1974a)

Table IV. Concentration of Levamisole in Tissues of Swine Treated with Levamisole Injection at 10 mg/kg (ppm)

Withdrawal (hours)	Injection Site	Muscle	Liver	Kidney	Fat
24	0.26	0.10	2.05	0.87	0.16
48	0.10	ND	0.51	0.15	0.15
72	ND	ND	0.12	0.04	ND

ND = not significantly different from control tissue

Seventeen sows weighing 144 to 342 kg were treated orally with levamisole hydrochloride gel to provide 8 mg/kg. Three animals were sacrificed on days 3, 4 and 5 post treatment and four animals on days 6 and 7. Samples of edible tissues were analyzed for levamisole with a gas chromatographic method validated to 0.1 ppm. Levamisole residues were <0.1 ppm in muscle and fat at 3 days of withdrawal. In kidney, levamisole residues were 0.16 ppm and 0.19 ppm on withdrawal days 3 and 4, respectively, and <0.1 thereafter. In liver, levamisole residues averaged 0.78 ppm (3 days), 0.65 ppm (4 days), 0.31 ppm (5 days), 0.14 ppm (6 days) and 0.12 ppm (7 days). (Berger, et al., 1987)

Fifteen pigs averaging 55 lbs were given 8 mg/kg levamisole hydrochloride via the drinking water. Samples of edible tissues were collected at withdrawal times of 4, 24, 48, 72 and 96 hours. Concentrations of levamisole in the samples were determined with the cathode ray polarographic method having a limit of detection of 0.1 ppm. The results of this study are summarized in Table V. (Colavita, et al., 1970)

Table V. Average Concentrations of Levamisole in Tissues of Pigs Treated with 8 mg/kg Levamisole Hydrochloride in the Drinking Water (ppm)

Withdrawal (hours)	Muscle	Liver	Kidney	Fat
4	1.31	13.77	13.28	1.68
24	<0.1	<0.1-0.55	<0.1	<0.1
72	<0.1	<0.1-0.11	<0.1	<0.1

Fifteen pigs, average weight ~40 kg, were injected subcutaneously with levamisole phosphate to deliver 8 mg/kg on a levamisole hydrochloride basis. Three animals were sacrificed at each of the following withdrawal periods: 0 (2 hours), 4, 8, 12, 16 and 20 days. Samples of edible tissues were analyzed for levamisole hydrochloride with the cathode ray polarographic method having a detection limit of 0.1 ppm. At 0 withdrawal, residues of levamisole hydrochloride averaged: 34.9 ppm, injection site; 3.2 ppm, muscle; 13.4 ppm, liver; 24.5 ppm, kidney; and 2.6 ppm, fat. Because no residues were detected in any tissue at 4 and 8 days of withdrawal, the remaining samples were not analyzed. (Berger, et al., 1973b)

Nine pigs, weight range 16 to 22.3 kg, were administered feed containing sufficient levamisole hydrochloride to provide a dose of 8 mg/kg. Three animals were sacrificed at withdrawal times of 4, 72 and 96 hours. Samples of edible tissues were analyzed with the cathode ray polarographic procedure having a detection limit of 0.1 ppm. At 4 hours withdrawal, residues of levamisole hydrochloride averaged 2.19 ppm in muscle, 9.8 ppm in liver, 14.2 ppm in kidney, and 1.08 ppm in fat. Levamisole hydrochloride was <0.1 ppm in the samples taken at 72 and 96 hours of withdrawal. (Berger, et al., 1975)

Twelve pigs (7 M, 5 F, average weight ~20 kg) were injected with levamisole base in benzyl benzoate to provide a dose equivalent to 8 mg/kg levamisole hydrochloride. Groups of three animals were sacrificed at 0 (2 hours), 4, 7 and 10 days of withdrawal. Samples of tissue were assayed with the cathode ray polarographic procedure (detection limit, 0.1 ppm). At 2 hours withdrawal, residues of levamisole hydrochloride averaged 2775 ppm at the injection site, 1.4 ppm in muscle, 12.1 ppm in liver, 21.2 ppm in kidney and 2.1 ppm in fat. Because residues of levamisole hydrochloride were <0.1 ppm in all samples taken on 4 and 7 days of withdrawal, analyses were not done on samples taken at 10 days of withdrawal. (Colavita and Garces, 1977)

Twelve sows, average weight ~263 kg, were orally dosed with 8 mg/kg levamisole hydrochloride gel. Groups of four animals were sacrificed on 3, 4, and 5 days of withdrawal. Samples of edible tissues were collected for analysis of levamisole hydrochloride using a gas chromatographic method with a detection limit of 0.1 ppm. Residues of levamisole hydrochloride in muscle and fat were <0.1 ppm at all withdrawal times. Residues of levamisole hydrochloride averaged 0.78 ppm at 3 days of withdrawal, 0.65 ppm at 4 days and 0.31 at 5 days. In kidney, the residues of levamisole hydrochloride were 0.16 ppm, 0.19 ppm and <0.1 ppm at withdrawal times of 3, 4 and 5 days, respectively. (Garces, et al., 1984)

Eight sows weighing an average of 249 kg were dosed orally with 8 mg/kg levamisole hydrochloride gel. Groups of four animals were sacrificed on 6 and 7 days of withdrawal. Samples of liver were collected from each animal for analysis of levamisole hydrochloride using a gas chromatographic procedure validated to 0.05 ppm. Livers from the 6-day withdrawal sows contained 0.06 ppm, 0.10 ppm, 0.08 ppm and 0.31 ppm levamisole hydrochloride. Livers from the 7-day withdrawal sows contained <0.05 ppm, <0.05 ppm, 0.18 ppm and 0.19 ppm levamisole hydrochloride. (Fisher, et al., 1985)

Cattle

Residues in Tissues

Three bulls weighing 111-211 kg were injected intramuscularly with 8 mg/kg levamisole hydrochloride. The animals were sacrificed at 24, 48 and 72 hours post medication. Samples of muscle, liver, kidney and fat were collected for analysis of levamisole with a polarographic procedure. At 24 hours withdrawal, residues of levamisole base were 0.016 ppm in muscle, 0.402 ppm in liver, 0.043 ppm in kidney and <0.002 ppm in fat. Only liver contained detectable residues at 24 (0.059 ppm) and 48 hours (0.075 ppm). (Heykants, et al., 1974b)

Nine calves having an average weight of 163 kg were injected with levamisole phosphate to provide 8 mg/kg levamisole hydrochloride. Three animals were sacrificed at each of the following times after injection: 0 (2 hours), 7 days, and 8 days. Samples of edible tissues were analyzed for levamisole with the cathode ray polarographic method (detection limit 0.1 ppm). At 0 withdrawal, residues of levamisole hydrochloride were reported to average: 460 ppm in injection site; 2.4 ppm in muscle; 16 ppm in liver; 15 ppm in kidney; and 1.5 ppm in fat. At 7 and 8 days of withdrawal, residues in all tissue samples were <0.1 ppm. (Bohn and Moyer, 1971a)

Eight cattle, ~200-250 kg, were administered 7.5 mg/kg levamisole hydrochloride as a drench. Two animals were sacrificed at 7, 14, 21 and 28 days post medication. Samples of edible tissues were analyzed for levamisole using gas chromatography with nitrogen/phosphorus detection (limit of detection 0.01 ppm). At 7 days of withdrawal, levamisole in liver averaged 50 ppb. All other tissue samples at all times averaged <10 ppb. (Van Leemput, et al., 1989b)

Twelve female calves, average weight ~187 kg, were treated with 8 mg/kg levamisole hydrochloride via bolus. The animals were sacrificed in groups of three at 2, 24, 48 and 96 hours after treatment. Levamisole hydrochloride was determined in the edible tissues using the cathode ray polarographic procedure with a detection limit of 0.1 ppm. The results of the study are shown in Table VI. (Colavita, et al., 1968)

Table VI. Average Concentrations of Levamisole Hydrochloride in Tissues of Cattle Treated with 8 mg/kg via Bolus (ppm)

Withdrawal Time (hours)	Muscle	Liver	Kidney	Fat
2	1.41	3.33	5.07	0.61
24	<0.1	0.11	<0.3	<0.1
48	<0.1	<0.1	<0.1	<0.1
96	<0.1	<0.1	<0.1	<0.1

Six heifer calves weighing an average of 137 kg received 8 mg/kg levamisole hydrochloride through pellets in the feed.Three animals were sacrificed at 0 (2 hours) and 2 days withdrawal. Samples of edible tissues were analyzed for levamisole

hydrochloride with the cathode ray polarographic method. The results of this study are shown in Table VII. (Berger, et al., 1970)

Table VII. Average Concentrations of Levamisole Hydrochloride in Tissues of Heifers Dosed at 8 mg/kg Via Pellets (ppm)

Withdrawal Time (hours)	Muscle	Liver	Kidney	Fat
2	0.41	13.05	7.88	1.18
48	<0.1	~0.1	<0.1	<0.1

A study was conducted in two phases. In Phase I, nine cattle, mixed as to sex and weighing ~232 kg, were administered 8 mg/kg levamisole hydrochloride paste. The animals were sacrificed at 2, 48 and 72 hours after treatment. Samples of edible tissues were analyzed for levamisole hydrochloride using a gas chromatographic method validated to 0.1 ppm. The results for Phase I are presented in Table VIII.

Table VIII. Average Concentrations of Levamisole Hydrochloride in Tissues of Cattle (ppm)-Phase I

Withdrawal Time (hours)	Muscle	Liver	Kidney	Fat
2	1.87	14.3	9.12	1.20
48	<0.1	0.61	<0.1	<0.1
72	<0.1	0.28	<0.1	<0.1

Phase II was performed to determine when residues of levamisole fall to 0.1 ppm in liver. Six cattle, treated as in Phase I, were sacrificed in groups of three at 120 and 168 hours. Levamisole hydrochloride was found to be <0.1 in all liver samples. (Garces, et al., 1980)

Eighteen cattle, average weight 273 kg, were assigned to six groups of three. Three groups received levamisole pour-on at 10 mg/kg and three at 15 mg/kg. For each dosage level, groups were sacrificed with withdrawal times of 3, 7, and 14 days. Samples of edible tissues were analyzed for levamisole with a gas chromatographic method having a detection limit of 0.005 ppm. The results of the study are given in Table IX. (Marsboom, et al., 1981)

Table IX. Average Concentrations of Levamisole in Tissues of Cattle Treated with the Pour-on Formulation (ppm)

Withdrawal Time (days)	Dose mg/kg	Muscle	Liver	Kidney	Fat
3	10	0.011	0.334	0.025	0.009
3	15	0.006	0.219	0.013	0.038
7	10	<0.005	0.108	0.014	<0.005
7	15	<0.005	0.094	0.027	0.007
14	10	<0.005	0.007	<0.005	<0.005
14	15	0.010	0.071	<0.005	0.006

Four groups of five cattle each were treated topically with levamisole pour-on at 10 mg/kg. At 2, 6, 10 and 14 days after treatment, one group was sacrificed and samples of edible tissues, including application site, were collected. Levamisole was determined using a gas chromatographic procedure having a detection limit of 0.01 ppm. The results of this study are shown in Table X. (Woestenborghs, et al., 1985)

Table X. Average Concentrations of Levamisole in Cattle Treated with the Pour-on Formulation at 8 mg/kg (ppm)

Withdrawal Time (days)	Muscle (shoulder)	Muscle (appl. site)	Liver	Kidney	Fat
2	0.039	0.347	0.566	0.100	0.063
6	<0.01	0.095	0.068	0.010	<0.01
10	<0.01	0.033	0.036	<0.01	<0.01
14	<0.01	<0.01	0.017	<0.01	<0.01

Residues in Milk

Three dairy cows weighing an average of 490 kg were injected intramuscularly with 8 mg/kg levamisole hydrochloride. Samples of milk were collected at various times up to 168 hours post treatment. Levamisole was determined in the samples using a polarographic method with a 2 ppb detection limit. The results of this study are given in Table XI (beyond 64 hours all samples of milk were <2 ppb). (Heykants, et al., 1974c)

Table XI. Levamisole in Milk of Dairy Cows Injected Intramuscularly with 8mg/kg Levamisole HCl (ppb)

Time After Dosing	Average Concentration of Levamisole
16 hours	763
24 hours	306
40 hours	50
48 hours	<15
64 hours	<2

In four separate trials, five cows (weight range 400-600 kg) each were treated with 8 mg/kg levamisole hydrochloride through drench, bolus, feed or injection. Samples of milk were taken at 1"-hour intervals up to 72 hours after dosing. Levamisole hydrochloride was determined using a gas chromatographic method validated to 10 ppb. The results of this study are shown in Table XII (nearly all samples at 48 hourc were <10 ppb.) (Simkins, et al., 1976) (Colavita, et al., 1974)

Table XII. Levamisole in Milk of Dairy Cows Treated with 8 mg/kg Levamisole HCl fia Different Formulations (ppb)

	Average Concentration at Time After Dosing		
Formulation	12 hr.	24 hr.	36 hr.
Drench	500	50	<15
Pellets	550	60	<16
Bolus	580	100	<26
Injectable	320	<100	<20

Two cows weighing 460 and 539 kg were injected subcutaneously with 7.1 mg/kg levamisole hydrochloride. Samples of milk were taken at various times post dosing for determination of levamisole using high-performance liquid chromatography (HPLC) with a detection limit of 20 ppb. The average concentration of levamisole was 845 ppb at 8 hours, 210 ppb at 16 hours and 45 ppb at 24 hours. All samples were <20 ppb at 48 hours and beyond. (Osterdahl, et al., 1985)

Forty-two cows weighing 500-650 kg were injected intramuscularly with 7 mg/kg levamisole hydrochloride. Samples of milk were taken at various times for determination of levamisole with the HPLC method described above. The data are summarized in Table XIII. Beyond 25 hours levamisole in milk samples was mostly not detectable. (Osterdahl, et al., 1986)

Table XIII. Levamisole in Milk of Dairy Cows Injected Intramuscularly with 7 mg/kg Levamisole HCl (ppb)

Time After Dosing	Average Concentration of Levamisole
1 hour	2220
3 hours	1425
5 hours	1000
7 hours	773
14.5 hours	228
16.5 hours	131
18.5 hours	100
20.5 hours	123
25 hours	<100

Five dairy cows were injected subcutaneously with 8 mg/kg levamisole hydrochloride. Milk samples were collected at 12, 48 and 60 hours post-injection and residues of

levamisole hydrochloride were measured using the cathode ray polarographic procedure (validated to 10 ppb). Detectable residues were observed only at 12 hours, at which time levamisole hydrochloride averaged 560 ppb. (Bohn and Moyer, 1971b)

Five cows were treated with a single oral drench of levamisole hydrochloride at 8 mg/kg. Residues of levamisole hydrochloride in milk collected at 12-hour intervals up to 72 hours were measured with the polarographic method noted above. Levamisole hydrochloride averaged 1020 ppb at 12 hours, 40 ppb at 24 hours and 10 ppb at 36 hours. Levamisole was not detected in milk after 36 hours. (Alford, et al., 1970)

Seven dairy cows weighing ~550 kg were treated topically with 10 mg/kg levamisole pour-on. Residues of levamisole in milk collected at 12-hour intervals up to 72 hours were measured using a gas chromatographic method with a 10 ppb detection limit. The results of the study are presented in Table XIV. (Michiels, et al., 1988)

Table XIV. Residues in Milk of Dairy Cows Treated with the Pour-on Formulation of Levamisole at 10 mg/kg (ppb)

Time After Dosing	Average Concentration of Levamisole
12 hours	722
24 hours	122
36 hours	34
48 hours	19
60 hours	<10
72 hours	<10

METHODS OF RESIDUE ANALYSIS

Differential polarographic, gas liquid chromatographic and high-performance liquid chromatographic procedures have been developed for the detection and measurement of residues of parent levamisole in plasma, tissues and milk.

Most of the data from the early residue depletion work (i.e., through ~1975) was collected using the differential polarographic method. The method generally involves extraction, purification and analysis of the sample for levamisole using differential pulse polarography. Limits of detection for the method are reported to range from 5 to 100 ppb. (Holbrook and Scales, 1967) (Dominicus, et al., 1974)

The gas liquid chromatographic procedure is currently used for the determination of levamisole in biological samples. The United States Food and Drug Administration has accepted a gas liquid chromatographic procedure with alkali-flame ionization detection as the official method (validated to 0.1 ppm) for the analysis of levamisole in milk and tissues. This method is reported to have a limit of detection of 10 ppb in milk and 10-20 ppb in tissues. (Wynants, et al., 1975) (Smith, et al., 1976)

The use of thermionic specific detection with gas liquid chromatography has also been reported. This method can detect levamisole in tissues and plasma in the 5-10 ppb range. (Woestenborghs, et al., 1981, 1984)

Gas liquid chromatography has also been coupled with electron impact mass spectrometry to provide enhanced sensitivity for levamisole. The quantification limit for this procedure is reported to be 5 ppb for levamisole in tissues. (Timmerman, et al., 1985)

High-performance liquid chromatography with UV-detection has been investigated for the determination of levamisole in biological fluids. The detection limit of the method was found to be 20-50 ppb for levamisole in milk. (Marriner, et al., 1980) (Osterdahl, et al., 1985)

APPRAISAL

The depletion of residues of levamisole from the edible tissues of cattle, sheep, swine, and chickens has been studied using unlabeled drug only. In those studies, it could be seen that residues of parent levamisole, following administration via a number of different formulations including bolus, soluble powder, pour-on and injection, fall well below the 100 ppb level in tissues of food-producing animals at 2 to 5 days of withdrawal. Residues of parent levamisole dropped below 10 ppb at 60 hours in milk of cattle treated orally, parenterally or dermally. The analyses of tissue samples for levamisole in these depletion studies were conducted with gas chromatography, polarography or high-performance liquid chromatography.

The metabolism of levamisole has been studied in the rat using 14C-levamisole. The work has evidenced extensive metabolism of levamisole, with at least 50 metabolites identified in some samples of urine from treated rats. Four major metabolic pathways have been proposed to account for many of the metabolites.

Very limited metabolism work has been done in pigs and goats. However, the results are generally consistent with those observed for rats: excretion of the drug is rapid; a large portion of the administered dose is excreted in the urine; and metabolism apparently is extensive. It could be expected that the metabolism of levamisole in food-producing animals would be qualitatively similar to that in rats, although this would need to be demonstrated in suitably conducted studies.

Based on the temporary ADI of 0-3 μg/kg established by JECFA, the permitted daily intake of levamisole would be 180 μg of total drug-related residue contributed by 500 g of food animal meat and 1.5 L of milk in the diet of a 60-kg person. On the basis of data in rats, the sponsor estimated that levamisole parent would represent approximately 10% of the total residues in food animals. Consequently, the daily allowable intake, on a parent levamisole basis, would be about 18 μg. Since this amount would be contributed through 2 kg of diet, the necessary quantitation limit of an analytical method would be 18 μg/2 kg or 9 μg/kg. The sponsor indicated that its gas chromatographic method has a detection limit of 10 μg/kg. Consequently, JECFA recommended a temporary MRL of 10 μg/kg for parent levamisole for tissues of all species and milk.

REFERENCES

Alford, B.T., Drain, J.J., and Colavita, J. (1970). Tetramisole: Disappearance rate of TRAMISOLR levamisole hydrochloride in milk. Unpublished report on Experiment B-70-18-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H. (1973). Levamisole: Egg residues and safety of TRAMISOLR levamisole hydrochloride administered in the drinking water of laying hens. Unpublished report on Experiment A-72-58-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H. and Colavita, J.H. (1974). Levamisole: Tissue residues of levamisole in sheep following subcutaneous injection of 18.2% TRAMISOLR levamisole phosphate solution. Unpublished report on Experiment O-73-9-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H., Colavita, J.H., and Wang, G.T. (1973a). Levamisole: Safety and tissue residue study with TRAMISOLR levamisole hydrochloride in broilers. Unpublished report on Experiment A-72-19-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H., Colavita, J.H., and Kemmerer, R.W. (1973b). Levamisole: Tissue residues of TRAMISOLR levamisole in swine following injection of levamisole dihydrogen phosphate solution, 9.1%, at the rate of 8 mg levamisole HCl equivalent/kg body weight. Unpublished report on Experiment P-72-17-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H., Colavita, J., and Drain, J.J. (1969). Tetramisole: Tissue residues of l-tetramisole in sheep following oral administration of l-tetramisole oblets at the rate of 8 mg l-tetramisole/kg body weight. Unpublished report on Experiment O-67-13-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H., Garces, T.R., Fisher, R.K., DeLay, R.L., Gale, G.O., Boyd, J.E., and Simkins, K.L. (1987). Efficacy, safety, and residue evaluation of levamisole gel formulation in sows. Am. J. Vet. Res., 48, 852.

Berger, H., Colavita, J.H., and Miller, R.F. (1975). Levamisole: Tissue residues of levamisole in swine given feed containing TRAMISOLR levamisole resinate, 10%. Unpublished report on Experiment P-74-23-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Berger, H., Colavita, J., and Drain, J.J. (1970). Tetramisole: Tissue residues of l-tetramisole in cattle following feeding of 0.8% l-tetramisole alfalfa pellets at the rate of 8 mg l-tetramisole/kg body weight. Unpublished report on Experiment B-69-29-FT from

American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Bohn, W.R. and Moyer, M. (1971a). Levamisole injectable 18.2%: Levamisole residue in bovine tissues, blood and urine. Unpublished report on Experiment B-71-17-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Bohn, W.R. and Moyer, M. (1971b). Levamisole injectable 18.2%: Residue of levamisole in milk. Unpublished report on Experiment B-71-18-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Boyd, J.E., Bullock, M.W., Champagne, D.A., Gatterdam, P.E., Morici, I.J., Plaisted, P.H., Spicer, L.D., Wayne, R.S. and Zulalian, J. (1969). Metabolism of l-tetramisole in rats. Lecture given at 8th National Meeting of the American Chemical Society, New York.

Colavita, J.H. and Garces, T.R. (1977). Levamisole: Tissue residues of levamisole in swine following subcutaneous injections of levamisole base in benzyl benzoate, 9.1% levamisole hydrochloride equivalent. Unpublished report on Experiment P-76-2-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Colavita, J., Eggert, E.G., Phillips, A., and Wang, G.T. (1970). Tetramisole: Tissue residues of levamisole in swine following medication of 8 mg levamisole/kg body weight in drinking water. Unpublished report on Experiment P-69-14-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Colavita, J., Drain, J.J., and Schumacher, W.E. (1968). Tetramisole: Tissue residues of l-tetramisole in cattle following oral administration of l-tetramisole bolus or soluble powder drench at the rate of 8 mg l-tetramisole/kg body weight. Unpublished report on Experiment BB-67-42-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Colavita, J.H., Miller, R.F., and Simkins, K.L. (1974). Levamisole: Milk residue studies in cows treated with TRAMISOLR levamisole drench, feed, oblet and injectable formulations. Unpublished report on Experiment B-74-9-FT from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Dominicus, J., Hofkens, R., and Heykants, J. (1974). Polarographic determination of levamisole in animal tissues. Unpublished report N 8590. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Fisher, R.K., Colavita, J.H., and Samuel, J.L. (1985). Levamisole: A tissue residue study using TRAMISOLR gel in sows. Unpublished report on Experiment P-85-1 from

American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Galtier, P., Coche, Y., and Alvinerie, M. (1983a). Tissue distribution and elimination of [3H]levamisole in the rat after oral and intramuscular administration. Xenobiotica, 13, 407-413.

Galtier, P., Escoula, L., and Alvinerie, M. (1983b). Pharmacokinetics of [3H]levamisole in pigs after oral and intramuscular administration. Am. J. Vet. Res., 44, 583-587.

Garces, T.R., Colavita, J.H., and Drews, K.D. (1984). Levamisole: Tissue residues of levamisole in sows following a single oral dose of TRAMISOLR 11.5% gel. Unpublished report on Experiment P-83-15 from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Garces, T.R., Blagdan, B.B., and Colavita, J.H. (1980). Levamisole: Tissue residues of levamisole in cattle following a single oral dose of TRAMISOL 11.5% paste. Unpublished report on Experiment B-80-12 from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Gatterdam, P.E., Champagne, D.A., Boyd, J.E., and Hall, R. (1966). Tetramisole: Preliminary studies of the absorption, metabolism, and excretion in rats of CL 105,276 (Janssen R-8299), a potent anthelmintic agent. Unpublished report on Project No. (35)53-9-32-96 from American Cyanamid Company, Princeton, NJ, USA. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Graziani, G. and De Martin, G.L. (1977). Pharmacokinetic studies on levamisole. Absorption, distribution, excretion and metabolism of levamisole in animals-a review. Drugs Exptl. Clin. Res., 2, 221-233.

Heykants, J., Dominicus, J., and Marsboom, R. (1974a). Plasma and tissue levels of levamisole (R 12564) in the pig after a single intramuscular dose of 10 mg/kg. Unpublished report N 8719. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Heykants, J., Dominicus, J., and Marsboom, R. (1974b). Plasma and tissue levels of levamisole in cattle after intramuscular injection. Unpublished report N 8588. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Heykants, J., Dominicus, J., and Marsboom, R. (1974c). The excretion of levamisole in cow's milk. Unpublished report N 8589. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Holbrook, A. and Scales, B. (1967). Polarographic determination of tetramisole hydrochloride in extracts of animal tissue. Anal. Biochem., 18, 46-53.

Marriner, S., Galbraith, E.A., and Bogan, J.A. (1980). Determination of the anthelmintic levamisole in plasma and gastro-intestinal fluids by high-performance liquid chromatography. Analyst, 105, 993-996.

Marsboom, R., Woestenborghs, R., and Heykants, J. (1981). Levamisole tissue residues in cattle following topical treatment with the RYPA/C/2 pour-on formulation. Unpublished report V 4083. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Michiels, M., Monballu, J., Woestenborghs, R., Heykants, J., and Veys, P. (1988). Residual levels of levamisole and of butyldioxitol in the milk of cattle after a single topical treatment with levamisole pour-on at 10 mg/kg. Unpublished report V 6573. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Nielsen, P. and Rasmussen, F. (1982). The pharmacokinetics of levamisole in goats and pigs. Pharmacol. Toxicol. Veter., les Colloques de l'INRA, No. 8, 431-432.

Osterdahl, B., Johnsson, H., and Nordlander, I. (1985). Rapid extrelut column method for determination of levamisole in milk using high-performance liquid chromatography. J. Chromatogr., 337, 151-155.

Osterdahl, B., Nordlander, I., and Johnsson, H. (1986). Levamisole residues in milk from a herd suffering from lungworms. Food Additives and Contaminants, 3, 161-165.

Simkins, K.L., Smith, J.E., and Eggert, R.G. (1976). Excretion of levamisole in milk from cows treated with various formulations. J. Dairy Sci., 59, 1440-1443.

Smith, J.E., Pasarela, N.R., and Wyckoff, J.C. (1976). Gas-liquid chromatographic determination of levamisole residues in bovine milk. J. Ass. Offic. Anal. Chem., 59, 954-958.

Timmerman, P., Woestenborghs, R., Pauwels, C., and Heykants, J. (1985). A confirmatory GC/MS assay method for levamisole in bovine plasma and tissues. Unpublished report V 5911. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Van Leemput, L., Monballu, J., Woestenborghs, R., Pauwels, C., Goris, I., and Heykants, J. (1989a). August 22, 1989, Interim Report: Plasma kinetics and tissue residues of levamisole in sheep after oral drench treatment with levamisole (7.5 mg levamisole HCl/kg sheep) or levamisole/triclabendazole (7.5 mg levamisole HCl/kg sheep/10 mg triclabendazole/kg sheep). Interim report of project 139642 from Inveresk Research International Limited, Musselburgh, Scotland. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Van Leemput, L., Monballu, J., Woestenborghs, R., Pauwels, C., Goris, I., and Heykants, J. (1989b). August 24, 1989, Interim Report: Plasma kinetics and tissue residues of levamisole in cattle after oral drench treatment with levamisole (7.5 mg levamisole HCl/kg cattle) or levamisole/triclabendazole (7.5 mg levamisole HCl/kg

cattle/12 mg triclabendazole/kg cattle). Interim report of project 139794 from Inveresk Research International Limited, Musselburgh, Scotland. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Woestenborghs, R., Pauwels, C., Heykants, J., and Desplenter, L. (1985). Levamisole tissue residues in cattle following a single topical dose of levamisole pour-on at 10 mg/kg. Unpublished report V 5646. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Woestenborghs, R., Michielsen, L., and Heykants, J. (1981). Determination of levamisole in plasma and animal tissues by gas chromatography with thermionic specific detection. J. Chromatogr., 224, 25-32.

Woestenborghs, R., Michielsen, L., Pauwels, C., Timmerman, P., and Heykants, J. (1984). Determination of levamisole residues in bovine tissues by gas chromatography with thermionic specific detection. Unpublished report V 5389. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Wynants, J., Woestenborghs, R., and Heykants, J. (1975). The gas chromatographic determination of levamisole in body fluids and tissues. Unpublished report V 2274. Submitted to FAO by Janssen Pharmaceutica, B-2340 Beerse, Belgium.