LEVAMISOLE

First draft prepared by
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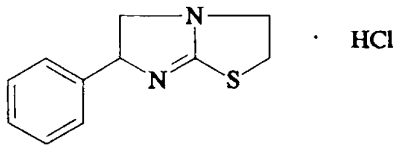
IDENTITY

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

CAS No. 16595-8-5; Merck Index 9055; Janssen Res. Code R12564.

Synonyms: Levamisole hydrochloride, Tetramisole, Ergamisol, Nemicide, Solaskil, Stimamizol, Tramisol, Worm-Chek; Also used as free base or phosphate

Structural formula:



Molecular formula: $C_{11}H_{13}ClN_2S$

Molecular weight: 240.75

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Levamisole: The levorotatory isomer of tetramisole

Appearance: White crystalline powder - stable

Melting Point: 227-229°C

Solubility: Very soluble in water (>500 g per litre).

Optical Rotation: $[\alpha]_D^{20} = -124° \pm 2°$ (c = 0.9 in water)

UV maxima: 213 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Levamisole is used as a broad-spectrum anthelmintic in large animals (cattle, sheep, pigs and goats) and poultry species (chickens and pigeons). It is used orally and parentally against many gastrointestinal nematodes and lungworms. A pour-on formulation for cattle is also available.

Dosages

Depending on the target species and the worm species, optimal doses vary from 5 to 40 mg per kg body weight (bw). The majority of the formulations recommend a dose of 5 to 8 mg per kg bw with a much higher dose for avian species.

METABOLISM

Pharmacokinetics

Levamisole is readily absorbed independent of the route of administration and is rapidly excreted, predominantly via the urine. An increasing fraction of the portion of the dose remaining in the animal is bound to larger molecules forming non-extractable residues.

In the target species the rate of absorption and the elimination half-lives of the drug differ with the route of administration (oral, parental or dermal). The drug is most rapidly absorbed (t_{max} 1-2 h) following intra-muscular or subcutaneous injection and the highest plasma levels, > 1 mg per litre are observed. Several oral formulations gave similar absorption rates (t_{max} ca. 3 h) and dermal applications were slower still (t_{max} ca. 4-6 h). Only about half the drug was bioavailable by the dermal route compared to the parental route.

Comparative Metabolism in Food Animals, Experimental Animals and Humans

Levamisole is rapidly metabolized to a large number of metabolites. Some of the major metabolites were identified and their structure and code names (re Janssen) are shown in Figure 2. The information for metabolism in *in vivo* studies is limited to studies in cattle, dogs, rats and monkeys. The sponsors have carried out a new definitive study (K54, V8386) using ^{14}C-levamisole administered to cattle, which provides new information on the metabolism of levamisole in urine, plasma and liver. The metabolism in other farm animal species, dogs and humans was investigated using *in vitro* studies of liver metabolism. The data for these studies are summarised in Table 1.

The sponsors claim that the results of all the studies based on both *in vivo* and *in vitro* in target species and laboratory animals indicate that qualitatively the routes of metabolism of levamisole are similar in most species.

Five of the seven major metabolites found in *in vitro* studies in cattle were found in *in vivo* studies using dogs, rats and monkeys (K16)(see Table 1). The other two major metabolites found in cattle, M1 and MS7, are the S-peptide and O-glucuronide derivatives of the two common major metabolites R 9280 and R 92535, respectively (Figure 3).

In the Japanese study of urinary metabolites in dogs, rats and monkeys (K16) there was very good similarity in the profiles of the metabolites for all three species. Unfortunately the study was not extended to farm animals.

The incubation of radiolabelled levamisole with either liver hepatocytes or liver microsome fractions of cattle, sheep, swine, dogs and humans produced qualitatively similar metabolic profiles between the species. However

the profiles for the human materials were not as well defined and although the same metabolites were probably present they were not fully identified.

Cattle

In vivo. A new study using ^{14}C-levamisole (V8386) investigated the metabolism of levamisole in urine, plasma and liver. There is ample evidence of extensive and rapid metabolism of levamisole. Unchanged drug (UD) accounted for only 0.4 - 2.3% of the dose in urine or faeces and less than 3% of the total residues (TR) in tissues.

The main metabolites are shown in Figure 3. There appears to be two main pathways:

1. Oxidation at the 2 position of the imidazothiazole ring followed by oxidation to a carbonyl and hydrolysis to a thiohydantoic acid to form metabolite R 92535. R 92535 is a major (26-47%) metabolite in urine. This metabolite forms a glucuronyl conjugate (MS7) and is a major metabolite in urine at 3 days after drug administration. R 92535 may also lose the acid side chain and form R 8418 and also the S group may be replaced by oxygen (R 9372).

2. Hydrolysis of the thiazolidine ring to yield a mercaptoethyl intermediate which forms the polar conjugate -S-cysteinyl-glycine (M1).

There was no evidence of para-hydroxylation of the phenyl ring. A major unidentified metabolite (M7) and M1 were found in liver.

Figure 1. General Metabolism of Levamisole

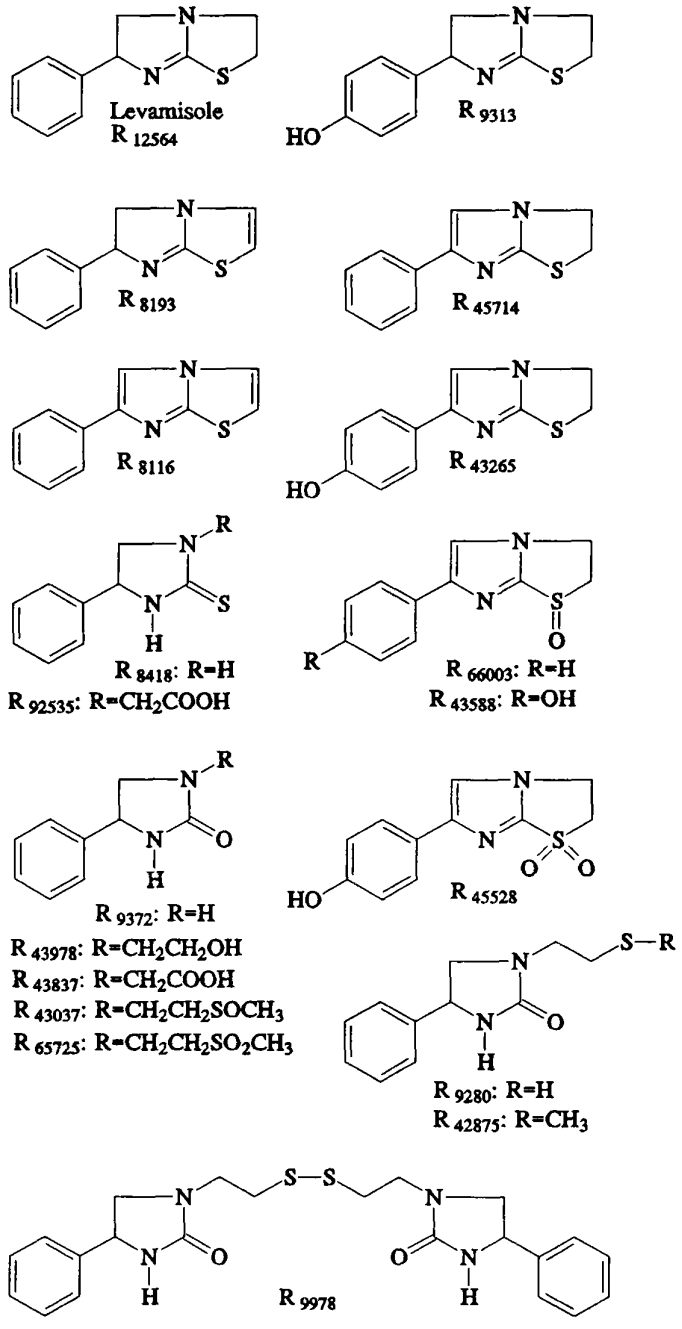


Figure 3. Metabolism in Cattle

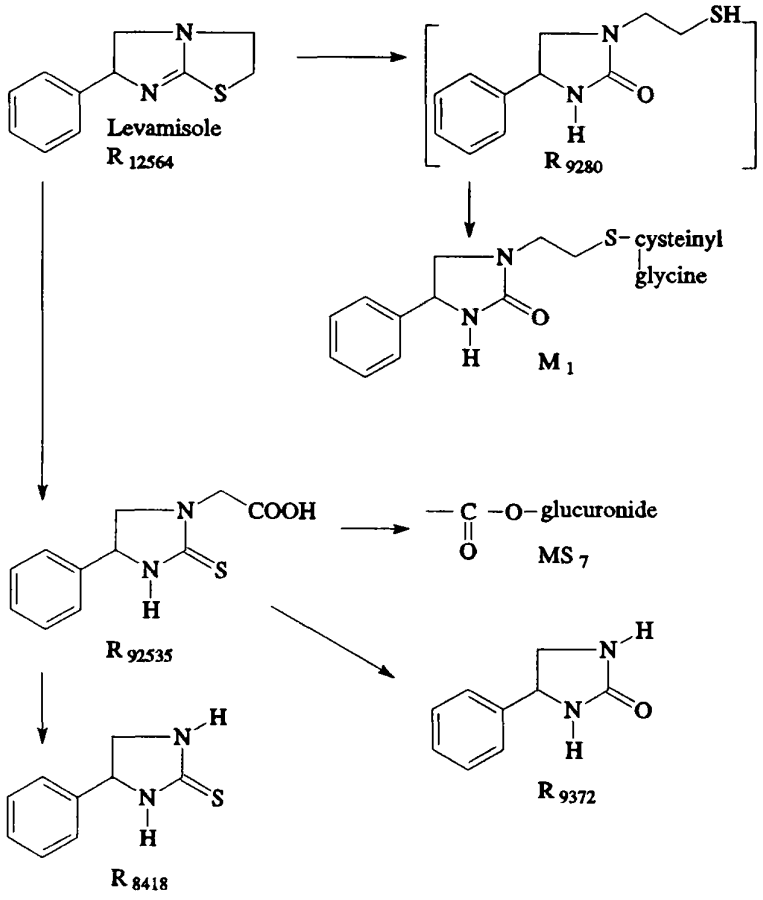


Table 1. Hepatic metabolite profiles of levamisole in both target and laboratory animals.

	IN VIVO				*IN VITRO*				
Metab-olite, R No	Cattle	Rat	Dog	Monkey	Cattle	Sheep	Swine	Dog	Human
12564	+	+++	+++	+++	+++	+++	+++	+++	+++
92535	+++	+++	+++	+++	++	+++	+	++	
MS7	++								
8418	+	+	+	+	0	0+	0	0	poss
9372	+	+	++	+	0	+	+	++	poss
9280		+	+	+	0	0+	0	0	poss
M1	++								
43837		+++	++	++	+	+	+	0+	
43037		+	++	+	+	+++	+++	+++	poss
65725		+							
43978					0+	0	0	0	poss
66003					+	++	+++	+	poss
45714		+	+	+	+	+	+	+	poss
8348		+							
8116		+							
43265		+	+	+					+
43588		++	+	+					
45528		++	+	+					
9978		+	+	+	++	++	++	++	++
9913		++	++	++					++

poss means possibly identified.

Metabolite: + minor, ++ medium, +++ major.
The metabolites are identified using Figures 2 and 3.
Data from V1357 (K4), V8386 (K54), V8414 (K14), V8424 (K15), V8426 (K16).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Cattle

The sponsors have carried out a study (V 8386, K54) in cattle in answer to the request by the 36th meeting. Six heifers and six steers weighing 189-279 kg were administered a single intra-muscular injection of ^{14}C-levamisole, labeled at the 7a position, at a dose rate of 7.5 mg per kg body weight. Urine and faeces were collected over a three day phase for three animals and over 21 days for another three cattle. The animals were slaughtered in groups of three of mixed sex at 3, 7, 14 and 21 days after dosing. Tissue samples and bile were collected at slaughter. The tissues were minced before storage at -20°C prior to analysis.

The pharmacokinetics of the excretion into bile, urine and faeces and the distribution in plasma are discussed in the earlier pharmacokinetics section. The metabolites found in urine are described above.

The total radioactivity in the tissues was determined and the data are presented in Table 2.

Table 2. Total residues as ^{14}C-levamisole equivalents (mg/kg) in bovine tissues after single i.m. injection at 7.5 mg per kg body weight

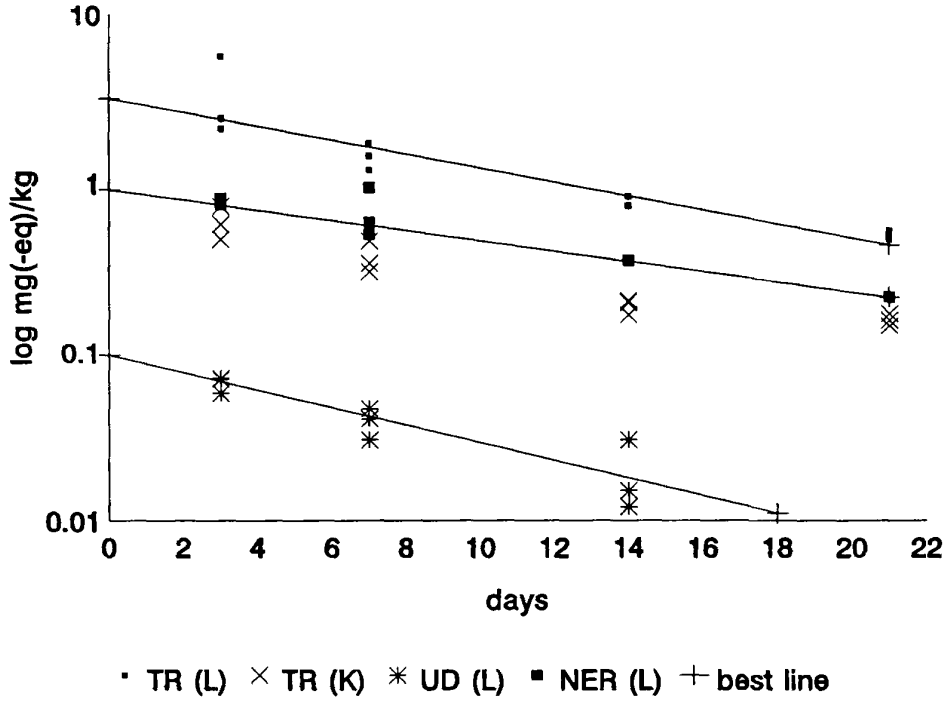
Days WT	Plasma	Liver	Kidney	Muscle	Fat	Inj. site
3	0.16	3.35 (0.76)	0.59 (0.20)	0.14	0.02	1.9-6.5
7	0.12	1.45 (0.60)	0.37 (0.15)	0.12	0.03	0.17-11.75
14	0.07	0.79 (0.35)	0.19 (0.09)	0.07	0.01	0.15-5.26
21	0.04	0.49 (0.21)	0.16 (0.07)	0.06	0.01	0.08-1.62

Data from V 8386, Table 12. Each value is the mean of three animals except for the injection site where the range of three values is given. The values in parentheses are the amount of total residue present as non-extractable residues.

The highest concentrations of residues are found in the liver and kidney. The nature of the residues was investigated further in the liver samples by determining the non-extractable residues (see Table 2) and identifying and quantifying the major metabolites in the free fraction.

The major metabolites in liver were M1 (R 9280) and M7 (unidentified) (see Figure 3) and it was not possible to measure radiometrically the concentration of parent drug. This was measured using the more sensitive GC assay used for the unlabelled drug. The results showing the relationship between the total residues and unchanged drug are shown in Figure 4. From this it can be concluded that over a 14 day period the unchanged drug forms a small, 1.3-3.6%, mean 2.4 $\pm$ 0.7%, but constant proportion of the total residues. M7 does not have a linear log ratio with total residues. M1 accounted for 7-8% of TR at 3 days.

Figure 4. **The depletion of radiolabeled residues after i.m. administration of 7.5 mg per kg ^{14}C-levamisole to cattle**



TR is Total Residues, UD is Unchanged Drug, NER is Non-Extractable Residues, L is liver, and K is kidney

The depletion of total residues from liver and kidney ($t_{½}$ 7 and 9 days) occurred at the same rate as in plasma ($t_{½}$ 9 days), the depletion in muscle and fat was much slower ($t_{½}$ 15 and 14 days).

The bound residues formed a significant and increasing percentage of the total residues in liver.

No other extensive radiolabeled depletion studies are reported for other species of farm animals.

Other Residue Depletion Studies (with unlabelled drug)

The sponsors have submitted new studies in which the limit of detection for levamisole is 5 or 10 μg per kg.

Cattle

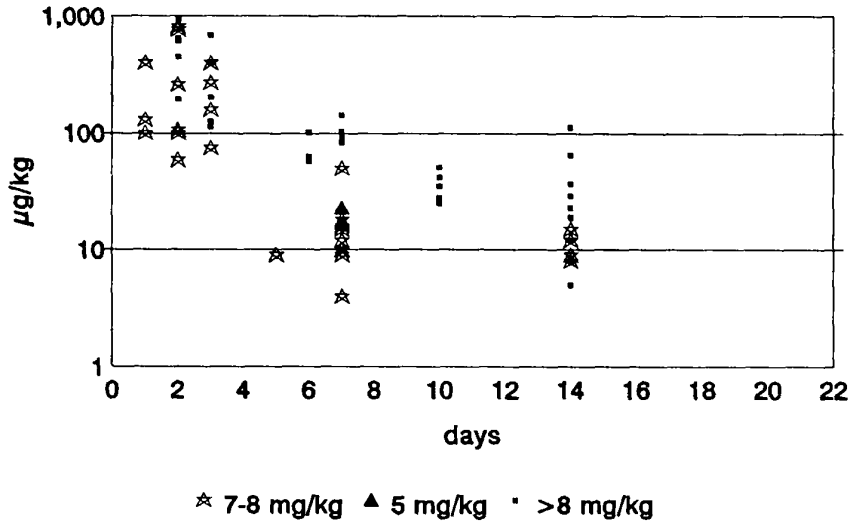
Several studies including three new ones (see Table 3) using a number of different formulations, dose rates and routes of administration are reported in which the concentration of levamisole in liver were determined. The results are shown graphically in Figure 5. When the dose is not greater than 7.5 mg per kg of body weight. the residues are < 100 μg per kg at 7 days post-dosing but can be > 10 μg per kg at 14 days, and < 10 μg per kg at 21 days. With doses > 8 mg per kg of body weight, most of the values are above 10 μg per kg for at least 14 days and can be above 100 μg per kg at 14 days post-dosing.

Table 3. Residues of levamisole in cattle

Study Nº	Dose mg/kg	Route	LOD µg/kg	Sampling times (days post dosing)
K 24	10	pour-on	5	3, 7, 14
K 24	15	pour-on	5	3, 7, 14
K 33	10	pour-on	10	2, 6, 10, 14
K 40	7.5	oral	10	7, 14, 21, 28
K 41	8	oral	100	2h, 1, 2, 4
K 44	8	i.m.	100	7, 8
K 53	6.5-9.5	paste	100	2h, 2, 3, 5, 7
K 54	7.5	i.m.	10	3, 7, 14, 21
K 56*	5	i.m.	10	7, 14, 21, 28
K 57*	7.5	oral	10	7, 14
K 59*	7.5	s.c.	10	7, 14

* New studies since 36th JECFA meeting in 1990

Figure 5. Residues above LOD in bovine liver



6/6 values at day 28 & 5/5 at day 21 are <10 µg/kg
2/4 <10 µg/kg at day 21 with triclabendazole
At 5 mg per kg dose all values <10µg/kg 14, 21 & 28 days

Swine

The studies reported for residues of levamisole in swine are listed in Table 4. Three studies (K92, K93, K94) were done with a method with an LOD of ≤10 µg per kg, the other methods having LODs of 100 µg per kg.

The results of those values > LOD are plotted for liver and kidney in Figure 6. Residues in liver are < 100 µg

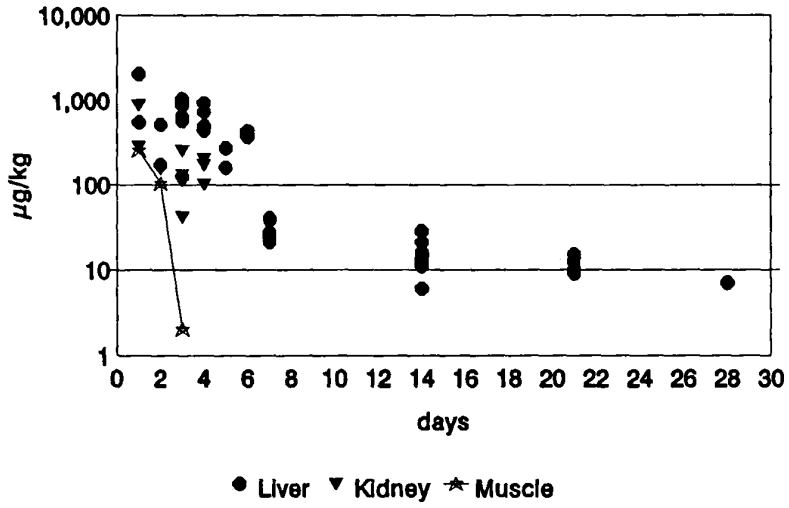
per kg at day 14 although they persist above 10 μg per kg at 14 days and in some pigs at 21 days. The residues are lower in kidney than liver and normally not detectable in kidney, muscle and fat at 7 days post-dosing.

Table 4. Residues of levamisole in swine

Study No	Dose mg/kg	Route	LOD μg/kg	Sampling times (days post dosing)
K 81	10	i.m.	100	1, 2, 3
K 83	8	oral	100	3, 4, 5, 6, 7
K 84	8	oral	100	4h, 1, 2, 3, 4
K 85	8	s.c.	100	4, 8, 12, 16, 20
K 86	8	oral	100	4h, 3, 4
K 87	8	s.c.	100	2h, 4, 7, 10
K 89	8	oral	100	3, 4, 5
K 90	8	oral	100	6, 7
K 92*	8	oral	10	7, 14, 21, 28
K 93*	5	i.m.	10	7, 14, 21, 28
K 94*	7.5	s.c.	3	14, 21, 28

* New studies since 36th JECFA meeting in 1990

Figure 6. Residues of levamisole above the LOD in swine tissues



Residues in kidney and muscle often below LOD at >7d
9/12 values in liver <LOD (3 or 10 μg/kg) at 21d

Sheep

The studies of residues of levamisole in sheep are listed in Table 5.

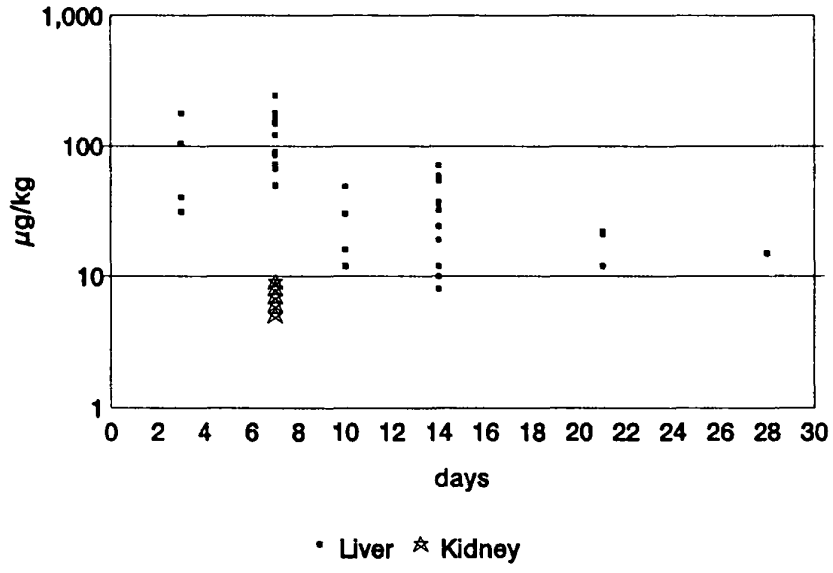
Table 5. Residues of levamisole in sheep

Study No	Dose mg/kg	Route	LOD µg/kg	Sampling times (days post dosing)
K 67	7.5	oral	10	7, 14, 21, 28
K 72	8	s.c.	50	7, 8, 9, 10
K 74*	8	oral	10	7, 14, 21, 28
K 76*	7.5	oral	10	7, 14
K 77*	7.5	s.c.	10	7, 14
K 78*	7.5	oral	5	3, 7, 10, 14, 21, 28
K 79*	7.5	s.c.	5	4, 21, 28

*New studies since 36th JECFA meeting in 1990

The residues in liver and kidney are plotted in Figure 7. Residues in liver are < 100 µg per kg at day 14 although they persist above 10 µg per kg at 10, 14, 21 and 28 (in 1 animal) days.
The residues are lower in kidney than liver and normally not detectable in muscle and fat after 7 days.

Figure 7. Residues of levamisole above LOD in sheep tissues



Most values <LOD from day 21 onwards

Poultry and Pigeons

Table 6. Residues of levamisole in poultry and pigeons

Study No	Dose mg/kg	Route	LOD µg/kg	Sampling times (days post dosing)
K 95[c]	40	oral	ca.100	7, 14, 28
K 96[p]	20 mg/bird	oral	10	1 or less
K 97[c]	36 and 72	oral	100	1, 2
K 98[c]	36 and 72	oral	25 (eggs)	1, 3, 5, 6, 7

[p] is pigeons, [c] are chickens

In poultry treated at the recommended dose and twice this dose the residues disappear rapidly from tissues within 1 day. The concentrations of parent drug in eggs of poultry administered the recommended dose were approximately 800 µg/kg of yolk and 200 µg/kg of egg white but were not detectable in eggs at 6-7 days drug withdrawal time. The nature and quantitation of the residues other than parent drug were not studied in detail.

Bound Residues/Bioavailability

A considerable fraction of the total residues in liver and kidney is non-extractable. The non-extractable fraction increased from about 23% in liver and 39% in kidney at 3 days to about 45% at 7 days and 50% at 14 and 21 days in both tissues. The non-extractable fraction in the liver from the 7 day sample was refed to bile-cannulated rats and the bioavailability calculated as approximately 15%.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Numerous methods are available for measuring residues of levamisole in biological samples. All the methods use a chromatographic separation step. The LOD for the best methods are better than 10 µg per kg and are likely to be suitable for monitoring MRLs at this level. Two methods are selected for routine monitoring and these can be supplemented by confirming with mass-spectrometry (MS).

Method 1. Gas chromatography-nitrogen-selective thermionic specific detection. (GC-TSD). 1 g samples may be analyzed at the 5-10 µg per kg level with acceptable accuracy and precision (CV 4% in liver and 20% for muscle). The recoveries are >78%. Throughput is about 20 samples per day.

Method 2. HPLC-UV. 20 g samples may be analyzed at the 10 µg per kg level with acceptable accuracy and precision. The recoveries are about 75% in bovine tissues.

APPRAISAL

At 36th meeting the sponsors were requested to provide further information in certain areas. The requests and the appraisal of their response are as follows:

1. A comparison of metabolism in humans, laboratory and target (farm) animals.

The sponsors have submitted information from both *in vitro* and *in vivo* studies. There is also an independent Japanese study identifying metabolites in dogs, rats and monkeys.

The incubation of radiolabelled levamisole with either liver hepatocytes or liver microsome fractions of cattle, sheep, swine, dogs and humans produced qualitatively similar metabolic profiles between the species. However the profiles for the human materials were not as well defined and although the same metabolites were probably present they were not fully identified.

A new study in cattle using ^{14}C-levamisole (V8386), administered intramuscularly at the proposed/recommended dose, investigated the metabolism of levamisole in urine, plasma and liver. There is ample evidence of extensive and rapid metabolism of levamisole. Unchanged drug (UD) accounted for only 0.4 - 2.3% of the dose in urine or faeces and less than 3% of the total residues (TR) in tissues when measured over a 14 day period.

The main metabolites are shown in Figure 3. There appears to be two main pathways:

- oxidation at the 2 position of the imidazothiazole ring followed by oxidation to a carbonyl and hydrolysis to a thiohydantoic acid to form metabolite R 92535. R 92535 is a major (26-47%) metabolite in urine. This metabolite forms a glucuronyl conjugate (MS7) and is a major metabolite in urine at 3 days after drug administration. R 92535 may also lose the acid side chain and form R 8418 and also the sulphur group may be replaced by oxygen (R 9372).

- hydrolysis of the thiazolidine ring to yield a mercaptoethyl intermediate which forms the polar conjugate -S-cysteinyl-glycine (M1).

There was no evidence of para-hydroxylation of the phenyl ring.

Only the metabolites M1 and MS7 were not found in the *in vitro* studies.

No comparison of metabolites formed *in vivo* in other farm species are reported although extrapolation from the liver incubation studies might suggest that major differences between cattle and other farm animals would be unexpected.

A summary of the metabolic profiles is given in Table 1.

2. A radiolabeled depletion study in the target animals (cattle).

A full radiometric study is reported and provides useful information for the evaluation of the total residues, the non-extractable residues, a marker compound and the setting of suitable MRLs.

The highest concentrations of residues are found in the liver and kidney. The nature of the residues was investigated further in the liver samples by determining the non-extractable residues and identifying and quantifying the major metabolites in the free fraction.

There is a typical log based decline of the total residues which is paralleled by the decline in the non-extractable residues and the unchanged drug. Neither of the major metabolites M1 or M7 were shown to have a linear log ratio with the total residues.

It was not possible to measure radiometrically the concentration of parent drug. This was measured using the more sensitive GC assay used for the unlabeled drug. It can be concluded that over a 14 day period the unchanged drug forms a small, 1.3-3.6%, mean 2.4 $\pm$ 0.7%, but constant proportion of the total residues; M7 does not have a linear log ratio with total residues.

The non-extractable residues form about 50% of the total residues and new information estimates about a 15% bioavailability.

Maximum Residue Limits

Based on the ADI of 0-6 μg/kg bw for parent drug established by the Committee, the permitted daily intake of parent drug and/or its equivalents is 360 μg for a 60 kg person.

The following factors were considered in estimating the MRLs:

1. The ADI.
2. The parent drug is a suitable residue marker and is 2.4% of the total residues.
3. All of the residues in muscle and fat are equivalent to parent drug.
4. 50% of the residues in liver are bound and 15% of these bound residues are bioavailable.
5. The residues in kidney are qualitatively similar to those in liver.
6. The residues are similar in cattle, sheep and pigs.

The Committee recommends MRLs of 10 μg/kg for muscle, kidney and fat and 100 μg/kg for liver of cattle, sheep, poultry and swine expressed as parent drug. Because residues in eggs at recommended dose level, at 1 day withdrawal, are approximately 1000 μg/kg, the Committee did not recommend a MRL for eggs. No new data was submitted by the sponsors to support the reevaluation of levamisole residues in milk. Therefore, the Committee withdrew the temporary MRL for milk allocated by the 36th meeting of JECFA in 1990. The sponsors recommend that levamisole should not be used in lactating cows.

The above assumptions can be used to calculate maximum theoretical daily intake of levamisole equivalents if a consumer ate the standard meat diet containing concentrations of levamisole at the proposed MRLs. The maximum ingested residue of parent drug and its equivalents is 397 μg per day which consists of 14 μg/day of parent drug and 383 μg/day of Levamisole equivalents. The calculation is shown in Table 7 below.

Table 7. Calculation of maximum ingested residue of parent drug and its equivalents

Tissue	Standard intake (kg)	MRL (μg/kg)	UD (μg)	EQ (μg) free	EQ (μg) bound	Total (μg)
Muscle	0.300	10	3	125	0	125
Liver	0.100	100	10	208	31	239
Kidney	0.050	10	0.5	10	2	12
Fat	0.050	10	0.5	21	0	21
Total	**0.500**	-	**14**	**364**	**33**	**397**

UD = unchanged drug, EQ = parent drug equivalents

Considering the inherent uncertainty of the total levamisole equivalents based upon levamisole as the marker residue and considering that only a small proportion of the total residues are used to estimate the total levamisole equivalents (397 μg) the Committee considered this value to be equivalent to the maximum ADI.

There are very good analytical methods for regulating the parent drug at the level of 10 μg/kg.

REFERENCES

All references were submitted as reports K1-K122 by the joint sponsors and included the reference:

Koyama, K, Oishi, T., Ishii, A. and Deguchi, T. (1983). Levamisole: Metabolic fate of levamisole in Rats, Dogs and Monkeys. Oyo Yakuri, 26, 869-876.