

CYPERMETHRIN

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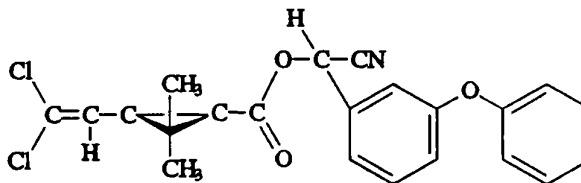
IDENTITY

Chemical name: (RS)-alpha-cyano-3-phenoxybenzyl-(1RS,3RS,1RS,3SR)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC name)
(RS)-cyano(3-phenoxyphenyl)methyl(1RS)-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate (Chemical Abstracts name)

C.A.S. number: 52315-07-8

Cypermethrin is a mixture of all eight possible chiral isomers (see alphacypermethrin monograph)

Structural formula:



Molecular formula: $C_{22}H_{19}Cl_2NO_3$

Molecular weight: 416.3

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Appearance: Yellow-brown viscous liquid to semi-solid crystalline mass

Purity: The commercial preparation contains 94.2% Cypermethrin

Melting point: 80.5°C

Vapour pressure: 1.9×10^{-7} pascals at 20°C

Solubility (g/l at 20°C):

Water	9.0×10^{-6}
Cyclohexane	> 600
Xylene	> 600
Ethanol	> 337
Hexane	103
Acetone	> 450

Density:	1.23 kg/l at 20°C								
Octanol-water partition coefficient (P):	2.0 x 10 ⁶								
Stability:	<table> <tr> <td>Hydrolytic:</td><td>Stable under acid or neutral conditions but not alkaline conditions</td></tr> <tr> <td>Photolytic:</td><td>Stable</td></tr> <tr> <td>Thermal:</td><td>Stable to 220°C</td></tr> <tr> <td>Oxidation:</td><td>Stable in air at ambient temperatures</td></tr> </table>	Hydrolytic:	Stable under acid or neutral conditions but not alkaline conditions	Photolytic:	Stable	Thermal:	Stable to 220°C	Oxidation:	Stable in air at ambient temperatures
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RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Cypermethrin is a synthetic pyrethroid used for the control of ectoparasites which infest cattle, sheep, poultry and some companion animals. There are in progress investigations into the use of the compound to control sea-lice infestations in farmed fish. However Cypermethrin is toxic to aquatic life and it is important to avoid contamination of surface waters. Cypermethrin may be applied orally or topically (ear tag, dipping, spraying, pour-on).

Dosage

The commercial formulations are in the form of ear tags, sprays, dips and pour-on formulations.

METABOLISM

Radiolabel and metabolite nomenclature

Studies on the metabolism of cypermethrin in animals have been conducted using ¹⁴C-cypermethrin labeled primarily in the rings of both the acid and alcohol portions in the molecule. These will be referred to as ¹⁴C-cyclopropyl and ¹⁴C-benzyl (or ¹⁴C-phenoxy), respectively. The following abbreviations are used throughout: 3PBA = 3-phenoxybenzoic acid; 4HO3PBA = 3-(4-hydroxyphenoxy)benzoic acid; DCVA = 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (present as the cis- and trans-isomers); cis- and trans-HO-DCVA = DCVA hydroxylated at the cis- and trans-methyl groups, respectively.

Pharmacokinetics - Excretion

Rat

When the cis and trans isomers of cypermethrin were dosed orally to rats, both were metabolised and eliminated rapidly. For example, 98-101% of the radioactivity derived from the ¹⁴C-benzyl-labeled compounds could be recovered in 3 days (Crawford and Hutson, 1977a). 53% (in males) and 66% (in females) of the dose was excreted in the urine and 27-29% in the faeces (Crawford, 1977). Tissue residues were low apart from those in the fat derived from the cis-isomer. This residue (about 1 mg/kg at a dose of 2 mg/kg) did not seem to be eliminated during the 8 day period of the experiment. In a follow up study the slow elimination of radioactivity in the later stages from liver and kidney was a consequence of the slow elimination from fat (Crawford and Hutson, 1978). The lipophilic character of the cis and trans-isomers are likely to be very similar and therefore it is not immediately obvious why the trans-isomer is released from fat more quickly than the cis-isomer (Crawford and Hutson, 1977a). It is possible that fat tissue contains an esterase (or lipase) that exhibits carboxylesterase activity towards the pyrethroids; trans-isomers generally are hydrolysed approximately 50 times faster than cis-isomers (Casida et al, 1976) thus the rate-limiting step in the release of cypermethrin from fat may be its metabolism in that tissue.

Cattle

Cypermethrin is used as a pesticide on crops (e.g. cotton) and the by-products may be fed to cattle. In three studies, lactating cows were fed daily for three weeks diet containing 0.2 mg of ^{14}C -Cypermethrin per kg diet (Hutson & Stoydin, 1979), or daily for one week 5 mg of ^{14}C -Cypermethrin (labels for both rings) per kg diet (Crawford, 1978) or daily for one week 10 mg of ^{14}C -Cypermethrin (label in benzyl ring) per kg diet (Hutson, 1980). Milk, faeces and urine were collected and the excretion of radioactivity monitored. An equilibrium between ingestion and excretion was reached after 3-4 days. The major routes of excretion were via the urine (49-54%) and faeces (43-44%) with <1% in the milk. Almost all (>97%) of the administered radioactivity was recovered.

Sheep

Two male sheep were topically treated (21.9 mg/kg BW) while a third was orally dosed (3.9 mg/kg BW) with labeled in the cyclopropyl and benzyl positions, see Table 3 (Crawford and Hutson, 1977b). Cypermethrin was slowly absorbed and eliminated when applied topically to sheep. Less than 0.5% of the dose was excreted in urine within 24 h and only 2% over a six day period. Faecal elimination was also slow, 0.5% of the dose being eliminated in six days. Approximately 30% of the applied dose was recovered from the application areas of both sheep. The elimination of radioactivity from the sheep, orally treated, was rapid, 61% of the administered dose being eliminated 48 h after dosing. Urinary elimination comprised 41% of the dose and faecal elimination 20.5%.

Poultry/Hens

A study in laying hens was conducted at a single dose level equivalent to 10 mg/kg in the diet, 0.7 mg/kg BW, administered twice a day for 14 consecutive days (Hutson & Stoydin, 1987). Eggs and excreta (combined urine and faeces) were collected once a day. The total output of radioactivity in the faeces (and urine) averaged 95% of the dose. The total radioactivity in whole eggs reached maximum values, 50-70 $\mu\text{g/kg}$ expressed as parent drug equivalents, between 5-8 days on treatment and remained at this level for the remainder of the dosing period (see also radiodepletion studies).

Metabolism

Cattle

Methods

In general samples were extracted with organic solvents and the radioactivity measured following radio-TLC or radio-HPLC. A cream/whey separation was carried out on milk. The content of cypermethrin in milk extracts was also measured by GC.

Urine and faecal metabolites

Three studies (see excretion section above) were carried out on lactating cows in which ^{14}C -Cypermethrin was fed in the diet. The lowest dose (0.2 mg/kg feed) did not permit a quantitatively accurate metabolite analysis; however, qualitative analysis of the urinary metabolites showed the presence of 3PBA-glutamic acid and 4HO3PBA in a ratio of 4:1. At the 5.0 mg/kg feed dose, the urinary metabolites identified were 3PBA-glutamate (68%) (confirmed by MS), 3PBA-glycine (16%), 3PBA (9%), and 4HO3PBA-sulphate (1%). Urinary metabolites at the 10.0 mg/kg dose were not analyzed. Faecal radioactivity was 88% extractable into organic solvent. TLC analysis showed that 85% of this was unchanged cypermethrin.

Tissue metabolites

Metabolite analysis on tissues was derived from the 10.0 mg/kg diet dose study (Hutson, 1980). The residue in milk was 90% extractable and shown to be cypermethrin. Both *cis* and *trans* isomers were present. Radioactive residue in fat that was removed by solvent extraction was 98%; in addition, 90% of the fat residue

was shown to be the parent Cypermethrin. The isomeric ratio was found to be 1 : 1 cis/trans in both animal matrices. The majority (80%) of the radioactive residue in muscle was extracted by methanol, but the extract was not further investigated since the residue level amounted to $< 10 \mu\text{g/kg}$. 80% radioactivity was extracted with hot methanol from liver. Hot methanol extraction was more effective compared to extraction at room temperature. The extract was further hydrolysed to liberate free metabolites, especially 3PBA and 4HO3PBA from their conjugates. The unextracted (bound) liver residue was hydrolysed in 5M HCl for about 4 hours to yield an additional 17% of the label. A level of 92% of the kidney residue could be extracted with methanol at room temperature. Bound residue in kidney was $< 10 \mu\text{g/kg}$ and was not further investigated. Metabolite data derived from analysis of liver and kidney is summarized in Table 1.

Table 1. Metabolites as mg/kg of ^{14}C -Cypermethrin in bovine liver and kidney

Metabolite	Liver Extract	Liver Extract (hydrolysed)	Liver Bound Residue (hydrolysed)	Kidney Extract
Polar	0.036	0.008	0.003	0.023
3PBA-Glutamate	0.067	0.011	0.015	0.070
3PBA-Glycine	0.003	-	-	0.004
Unknown	-	0.003	0.001	0.002
4HO3PBA	0.007	0.025	0.009	0.005
Unknown	0.007	-	0.002	0.004
3PBA	0.010	0.078	0.014	0.009
Cypermethrin	0.010	-	-	0.001
Unknown	-	0.014	-	-
Aqueous phase				0.012
Unextracted				0.011
Total	0.140	0.139	0.044	0.141

Original concentration of radioactivity was $0.216 \text{ mg } ^{14}\text{C}\text{-Cypermethrin/kg}$, thus about 85% of the residues were isolated and approximately 77% of the residue identified. There was very little parent Cypermethrin present in the residues in liver and kidney. A significant proportion ($> 90\%$) of the bound residues could be liberated to yield the same metabolites as in the free fraction (Croucher, Hutson, and Stoydin, 1985).

Sheep

Two male sheep were topically treated (21.9 mg/kg BW) while a third was orally dosed (3.9 mg/kg BW) with labeled in the cyclopropyl and benzyl positions (Crawford and Hutson, 1977b).

The metabolite profile was not determined in sheep. However the portion of the total radioactive residue attributable to cypermethrin was measured and varied in each tissue. A higher contribution was observed in fat as compared to the muscle and organ tissues; the results are summarized in Table 2.

Table 2. Percent of total radioactive residues in sheep tissues attributable to cypermethrin

Tissue/Route of application Withdrawal period	Topical 24 h	Topical 6 d	Oral 2 d
Liver	13	17	8
Kidney	< 3	< 4	< 1
Muscle (shoulder)	nq	nq	33
Fat (renal)	88	80	63
Fat (subcutaneous)	-	92	67

nq = not quantifiable

Hens/Poultry

About 60% of the residue in fat was present as cypermethrin in the original cis/trans isomer ratio. In contrast, the egg yolk residue consisted of 50 µg cypermethrin per kg and 100 µg other lipophilic compounds per kg. This component behaved as a lipid in that it was retained in hexane when cypermethrin was extracted from hexane into acetonitrile. It could not be separated from the natural yolk lipids during several chromatographic separations. It is relevant, in this context, that 3-phenoxybenzoic acid, when dosed to rats, appeared (in low concentration) in the skin as a mixture of triglycerides including the 3-phenoxybenzoyl dipalmitoyl glycerols (Crayford & Hutson, 1980).

Of the tissues analysed, the liver contained the highest residue (370 µg/kg) of which only 50 µg/kg was accounted for as cypermethrin. The common amino acid conjugates of 3-PBA were not substantial residual metabolites. The major excreted metabolite of 3-PBA in laying chickens, α -N-acetyl- δ -5-N-(3-phenoxybenzoyl)ornithine (Huckle et al., 1982) might be expected as a metabolite in liver, but this could not be confirmed. The hepatic metabolites appear to be very polar compounds, which cannot be converted into substantial quantities of 3-phenoxybenzoic acid or to its 4'-hydroxy derivative. Residues in breast and leg muscle (10-20 µg/kg) were too low for characterisation.

Laboratory animals

The majority of the total residues in the fat of rats after oral administration for 8 and 25 days was unchanged Cypermethrin (Crawford and Hutson, 1978). In another study rats were administered 1-2 mg/kg BW of either cis or trans ¹⁴C-Cypermethrin and the metabolites investigated. The major metabolites were in the cis form from the cis isomer, namely DCVA (36% of radioactivity) as the free or glucuronide conjugate or the parent compound (30%). Similarly for the trans isomer, 59% was trans-DCVA and parent compound (30%) (Crawford et al, 1981).

Summary

In all species there was hydrolysis of the ester bond and residues of each half of the molecule were found in different proportions in rats, cattle, poultry and humans.

In cattle the products were mostly identified as containing the phenoxy ring structure, whereas in humans and rats the cycloproponyl derivatives were mainly identified.

Cypermethrin, is the single most significant intact pyrethroid occurring in milk, eggs, and tissues following oral administration to food producing animals.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Radiodepletion studies were carried out using equal mixtures of the *cis* and *trans* forms of ^{14}C -Cypermethrin labeled in one or both rings. The compound was administered orally to cattle, sheep and hens and also topically to sheep as listed in Table 3. Oral administration of Cypermethrin is not the normal method of application of the compound to farm animals and only the one study in sheep was carried out using a topical route of administration. Thus there is limited information on the residues at the sites of application, in particular there is a lack of information on residues in the subcutaneous fat layers of the skin when spray, dip and pour-on preparations were used.

Table 3. ^{14}C -Cypermethrin radiodepletion studies in cattle, sheep and poultry

Animals	n	^{14}C -Label	Route	Dose (mg/kg)	Days dosed	Sampling times	Tissues	Ref
Cows	2	B	oral	0.2 (diet)	20/21	< 4 h daily	M,L,K,F,Mk	1
Cows	1	B	oral	5 (diet)	7	< 4 h daily	M,L,K,F,Mk	2
	2	C	oral	5 (diet)	7	< 4 h daily	M,L,K,F,Mk	
Cows	1	B	oral	10 (diet)	7	16 h daily	M,L,K,F,Mk	3
Sheep	1	BC	oral	3.9 (BW)	1	2d	M,L,K,F	4
	2	BC	topical	21.9 (BW)	1	1d, 6d	M,L,K,F,S	
Hens	4	B	oral	10 (diet) 0.7 (BW)	14	4.5 h daily	M,L,F Eggs	5

B and C are labels in benzyl ring or cyclopropyl ring respectively. Mk is milk sampled at each milking.

M = muscle; L = liver; K = kidney; F = fat; S = skin at application area.

References: 1. Hutson and Stoydin, (1979); 2. Crawford, (1978); 3. Hutson, (1980); 4. Crawford and Hutson, 1977b; 5. Hutson and Stoydin, (1987)

Cattle

The total residues of ^{14}C -Cypermethrin were measured in the edible tissues of dairy cows receiving labeled Cypermethrin in their feed as listed in Table 3. In the study where the cows received ^{14}C -Cypermethrin labeled in either of the rings, no differences in the total residues were observed for the respective tissues. The total residues of ^{14}C -Cypermethrin for the edible tissues for the three studies are shown in Table 4.

Table 4. Total residues ($\mu\text{g/kg}$) of ^{14}C -Cypermethrin in orally dosed cattle slaughtered on the last day of dosing

Reference Table 3	Dose (mg/kg feed)	Muscle	Liver	Kidney	Renal Fat	Subcut Fat
1	0.2	< 1	4, 8	3, 4	10, 12	8, 9
2	5	< 40	100 (3)	50-130	30-100	10-60
3	10	10	210	110	100	80

Milk

Milk was collected from the cows during the period of treatment and the radioactivity measured in the twice daily milkings. In each of the three studies the concentrations were lowest in the first day samples and then attained plateau levels throughout the period of dosing. The maximum concentrations in μg Cypermethrin equivalents/l were 1.2, 13.1 and 31 for the cows receiving 0.2, 5 and 10 mg ^{14}C -Cypermethrin/kg feed, respectively. No measurements were made after the last day of dosing. Residues in the whole milk ranged from 0.2-1.2 $\mu\text{g/kg}$ with 60-70% of the radioactivity in the cream phase for the lowest dose and 11-31 $\mu\text{g/kg}$ with ca 90% in the cream at the highest dose. The extracted residue (>90%) was shown to be Cypermethrin.

Sheep

Two male sheep were topically treated while a third was orally dosed with ^{14}C -labeled cypermethrin (see Table 3) (Crawford and Hutson, 1977b). Liver, kidney, fat (from three different areas), and muscle (shoulder and rear leg) samples were assayed for total radioactivity content. Tissues were also extracted using hexane/acetone (2:1), and analyses were performed by liquid scintillation counting (LSC) with product analysis conducted using gas chromatography (GC) and limited to cypermethrin.

Residues in tissues were comparable except for liver and kidney where higher values were observed in the tissues from the orally-dosed animals. A summary of the radioactive residue values is provided in Table 5.

Table 5. Total residues ($\mu\text{g/kg}$) of ^{14}C -Cypermethrin in sheep

Route	Time post dosing (days)	Muscle	Liver	Kidney	Renal Fat	Subcut Fat
Topical	1	30-40	100	140	170	100000*
Topical	6	30-60	140	120	300	3300*
Oral	2	30-40	390	360	410	260

* At site of application

Laying Hens

Four hens were administered ^{14}C -Cypermethrin in the feed (see Table 3) twice daily for 14 days. Eggs were collected throughout the study and the birds were sacrificed 4.5 hours after the last dose. Total radioactivity and residues of Cypermethrin and some metabolites were measured in the eggs, (white and yolk), muscle, liver and fat. The results are given in Table 6.

Table 6. Residues ($\mu\text{g/kg}$) of ^{14}C -Cypermethrin in four laying hens and their eggs

	Muscle leg	Muscle breast	Liver	Fat	Whole Egg	White	Yolk
Total ^{14}C	16-25	9-14	320-410	60-110	50-70	7-10	130-190
% Cypermethrin	nm	nm	14 %	56 %	nm	nm	33 %

The values for residues in eggs are for the maximum levels reached for each of the four hens. The residues in eggs reached plateau values about 6 days after the start of administration of radiolabel.

Rats

Male rats (430 g) and female rats (240 g) were given a single oral dose of 0.5 mg ^{14}C -Cypermethrin (cyclopropyl). The radioactivity in the tissues was determined at 3 days post-dosing. The mean values in $\mu\text{g/kg}$ were; muscle, 10 (M), 9 (F); liver, 370 (M), 120 (F); kidney, 100 (M), 60 (F); fat, 310 (M), 720 (F). Although the dose is much lower in females the residues in female fat were more than twice those in males (Crawford, 1977).

Summary

Cypermethrin is the only option for the choice of parent drug as the marker compound from the radiodepletion studies. Except in fat, there was not a good correlation between the concentration of Cypermethrin and the total residues in the various tissues. Fat, milk and eggs are obvious choices as marker tissues.

Other Residue Depletion Studies (with unlabeled drug)

Residue information was provided for the recommended topical uses of the insecticide in cattle, sheep and poultry. The main residue measured was the parent compound, Cypermethrin, determined by the method of gas chromatography with electron capture detection (GC-ECD). In nearly all studies the concentrations were reported as not corrected for recovery although the recoveries were determined by spiking experiments. Other than giving the recovery data, most of the results are reported without any validation data for the methods; e.g. the LOQ were given but no information on how these were derived. Also the two detailed procedures supplied by the sponsors were without validation information McKee et al., (1981) Baldwin et al., (1977b). In all studies residues were lower in muscle, liver and kidney than in fat tissues or the fat in milk (butterfat).

Cattle

A summary of the studies is shown in Table 8.

Results

The results for the maximum concentrations found in the studies are in table 9. Residues were less likely to be found in muscle and liver, occasionally residues were observed in kidneys, residues were mostly associated with body and milk fat. There were virtually no residues when ear tags were used, some residues were seen in fat and milk fat with both the spray and dip treatments, the highest and most persistent residues were found with the pour-on preparations.

Table 8. Residue studies with cattle

	n	Dose	Sampling period (d)	Samples	LOQ $\mu\text{g/kg}$ or l	Reference
Ear Tag	2 8	2 x 0.8 g 2 x 0.8 g	21 21	Mk-BF Mk-BF	4 4	1 1
Ear Tag	12	? x 0.8 g	1,3,8,15	M,L,K,F	5, 10(F)	2
Ear Tag	2	1 x ? g	77	M,L,K,F, Mk, Hair	10,2(Mk)	3
Spray	2	3 ^A x 1.13 g	21 ^D	M,L,K,F,Mk,	1, 10(F)	4
Spray	9 6	0.2 - 0.4 g 2 x 0.2-0.4 g	1,3,8 7 ^D	M,L,K,F M,L,K,F	5,10(F)	5
Spray	9	2.25 g	0.5,3,7	M,L,K,F	10	6
Spray	5	0.5 g	1-10	Mk-BF	10	7
Dip	4	2 ^B x 170 mg/l	4 ^D ,14 ^D	M,L,K,F	10	8
Dip	3 3	1 x 750 mg/l 2 ^C x 750 mg/l	0,1,3,7 7 ^D	M,L,K,F,Mk M,L,K,F,Mk	10,2(Mk)	9, 10
Pour-on	5 5	0.5 g 1.0 g	0 - 21 0 - 21	Mk Mk	2 (LOD?)	11
Pour-on	15	0.5 g	3,7,14	M,L,K,F	10	12

^A is at 2 week intervals; ^B dipped again after 10.5 weeks; ^C dipped again after 1 week; ^D is time after last treatment; References: 1. Braun et al. (1984); 2. Bosio (1979a); 3. Wallace (1982), 4. Baldwin et al (1977c); 5. Bosio (1979b); 6. McKee (1981); 7. Solly (1988); 8. Baldwin (1977a); 9. Sherren (1979); 10. McKee (1980); 11. Roberts et al. (1987a); and 12. Roberts et al. (1987b).

Table 9. Residues of Cypermethrin in bovine tissues, showing the maximum concentrations found in $\mu\text{g/kg}$ or l

Treatment	M, L, K	Fat	Whole Milk	Milk fat ^A
Ear Tags	None	None	None	9 ¹
Spray	None	100 ⁶	9 ⁴	180 ⁷
Dip	None 20(K) ⁸	130 ⁸	5 ⁹	n.m.
Pour-on	M 40 ¹² L <10 ¹² K 130 ¹²	610 ¹² (subcut) ^B 1400 ¹² (perit)	140 ¹¹	n.m.

The superscript numbers refer to the study number given in Table 8; ^A milk fat represents about 5 % of whole milk; ^B the subcutaneous fat was taken from under the area of application of Cypermethrin.

Ear tags - Studies 1-3

No residues were detected in all four edible tissues. In the first trial in Study 1, milk fat levels reached a mean level at day 3 of 5.1 µg/kg rising to a maximum mean level of 8.3 µg/kg at day 7 and declining thereafter. In the second trial, the only residues detected in milk fat were 4.2 µg/kg at day 3 in one of the eight cows and 9.2 and 8.9 µg/kg in two cows at day 21. In Study 3 no residues were detected in whole milk samples at any time.

Sprays - Studies 4-7

No residues of Cypermethrin were detected in muscle, liver and kidney at any sampling time. Residues were present in some of the fat and milk samples. The residues in fat were highest (mean 90 µg/kg) at 7 days post dosing. No measurements were made beyond this time point. In Study 6 residues of 3PBA were investigated, no evidence of this metabolite was found (LOD fat 50 µg/kg, other tissues 10 µg/kg). Residues persisted in the milk fat over the 10-day sampling period. The peak value (mean 110 µg/kg) in milk fat was at 4 days post dosing and had declined to a mean value of 30 µg/kg by day 10. In the study of residues in whole milk, no residues were detectable at 21 days post dosing.

Dips - Studies 8-10

Residues of Cypermethrin were either below or very close to the LOQ (10 µg/kg) for muscle, liver and kidney. Residues were present in renal, omental and subcutaneous fat. The concentration in the fat had not declined by 14 days post dosing, the last sampling time studied. However, the highest residue in fat was 180 µg/kg with most of the values < 100 µg/kg.

Pour-on - Studies 11-12

The residues in calf tissues are shown in Table 10. The concentrations in muscle and liver are low or not detectable. There were residues in the kidneys throughout the study period and much higher levels were found in both peritoneal and subcutaneous fat. The levels in fat were the highest recorded for any treatment. The residues although still present at 14 days were declining. The study was made using a dose which at 0.5 g per 125 kg calf was the same amount as that recommended for larger mature animals. Thus residues in larger animals may be lower.

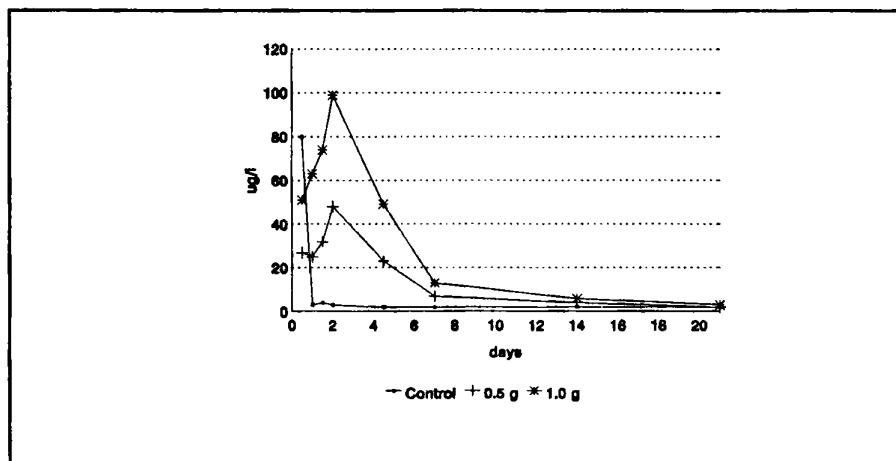
Table 10. Mean Residues of Cypermethrin in calves administered 0.5 g Cypermethrin in a pour-on preparation

Days post dosing	Muscle	Liver	Kidney	Peritoneal fat	Subcutaneous fat
3	20	< 10	50	840	470
7	< 10	< 10	70	670	260
14	< 10	< 10	40	330	140

Values in µg/kg are means of five calves per group.

Residues were measured in whole milk in Study 11 for up to 21 days post dosing. The results are plotted in Figure 1. There was an unexplained high value for the control animal at 12 hours post dosing, otherwise the residues follow a predictable pattern, with the highest values for the higher dose and all values declining to control values within 21 days.

Figure 1. Residues of Cypermethrin in whole milk after application of Cypermethrin in pour-on preparations



Sheep

A summary of the studies using dips and pour-on preparations are given in Table 11.

Table 10. Residue studies with sheep

	n	Dose and interval (days) between dips	Sampling period ^A (days)	Samples	LOQ µg/kg or l	Reference
Dip*	12	0.005%	1,3,7,14	M,L,K,F	10	1
	1	0.05%	7	M,L,K,F	10	1
Dip*	3	0.01%	0	M,L,K,F	10	2
	3	2 x 0.01% (4)	0	M,L,K,F	10	2
	5	3 x 0.01% (4)	0,1,3,6,10	M,L,K,F	10	2
Dip*	12	2 x 0.005% (7)	1,2,5,7	M,L,K,F	10	3
Dip*	6	0.015%	1,3,7,10,15	Mk	5	4
Pour-on*	20	0.375 g	1,3,7,14,28	M,L,K,FM,	0.2	5
	20	0.75 g	1,3,7,14,28	L,K,F	0.2	5
Pour-on*	5	0.375 g ^B	7	M,L,K,F	10	6
	5	0.375 g ^C	7	M,L,K,F	10	6

* Results were not corrected for recovery - recoveries were normally >75%;

^A Sampling done after last application; ^B ^C Two different solvents were used;

References: 1. Baldwin (1977b); 2. McKee and Wallace (1981); 3. Wallace (1980);

4. Bosio (1981a); 5. Perret (1982); and 6. White (1987).

Residues of Cypermethrin were close to or in most cases below the LOQ in all the studies of dipped sheep. Residues were found in both the perirenal fat and omental fat. Residues were measured in the subcutaneous fat in one study only, but they were higher than in either omental or perirenal fat. Surprisingly in view of the radiometric data, residues were not measured in the subcutaneous fat at the site of application in the pour-on studies. The mean values in fats are shown in Table 12.

Table 12. Residues of Cypermethrin in fat of sheep after dipping or pour-on preparations

WT (d)	Study - see reference number in Table 11									
	1 ^A			2		3	5		6	
	OF	PF	SF	OF	PF	Fat	OF	PF	OF	PF
0				70	80	50				
1	15	<10	20	110	140		20	30		
2						27				
3	10	<10	25	130	140		40	40		
5						60				
6				120	150					
7	<10	<10	<20			70	40	40	35, 18	4 ^{R1} , 10 ^{R2}
10				60	60					
14	15	10	<20 ^B				30	40		
28							20	20		

^A Values for the recommended dose; ^B Does not include one value of 70 thought to be an outlier; ^{R1} & ^{R2} Recoveries were low at 40% and 46% respectively; OF = omental fat; PF = perirenal fat; and SF = subcutaneous fat

Sheep Milk

Residues were measured in the whole milk of ewes after dipping once with a 0.015% formulation (Study reference 4, Table 11). Residues persisted throughout the 15 days post-dipping period. The mean values in $\mu\text{g/l}$ at 1, 3, 7, 10 and 15 days were 13, 10, 9, 7 and 7 respectively. The milk fat was about 5.8% of the milk and if all the residues are in the fat then the maximum concentration in the fat was 206 $\mu\text{g/kg}$ on day 1 and also a high value of 143 $\mu\text{g/kg}$ was seen on day 7. The results were not corrected for recoveries which were 70-85%.

Poultry - Laying Hens

Cypermethrin was sprayed on domestic hens, on a single occasion, diluted with water at dosage rates of 10 and 20 mg Cypermethrin/animal. In each group animals were sacrificed at various intervals after treatment, from 1 to 14 days, and samples of tissues were taken. In addition, eggs were collected from each group, from 3-day periods, between treatment and sacrifice. All the samples were analysed for residues of cypermethrin (Bosio, 1981b). The results are given in Table 13. Residues were at or below the LOQ in muscle, liver, kidney and eggs but were present in fat and skin throughout the 14 day post dosing period. The levels in skin were

higher than those in fat.

Table 12. Residues of Cypermethrin in laying hens after spraying with Cypermethrin

	Range of Cypermethrin ($\mu\text{g/kg}$) n=3	
	Dose 10 mg	Dose 20 mg
Muscle	10 - 20	< 10 - 30
Liver	< 10	< 10
Kidney	< 10	< 10 - 20
Fat	30 - 80	25 - 140
Skin	80 - 400	170 - 1300
Eggs	< 10	< 10

Bound Residues/ Bioavailability

There was evidence of bound residues particularly in liver and kidney, but these usually amounted to <20% in the liver and <10% in other tissues. The bound residues in the liver were treated with HCl and this liberated >90% of the radiolabel to yield metabolites similar to those in the free fraction (Croucher et al., 1985).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES, EGGS AND MILK

Tissues

A method for the determination of Cypermethrin and 3PBA in the edible tissues was submitted. Tissue (5 g) was extracted with acetone/petroleum ether. The residuum was separated and contained 3PBA residues. After drying the extract, the dried extract was partitioned between acetonitrile and petroleum ether. The phases were separated and processed as follows:

1. 3PBA: The acetonitrile extract was made to a 40% solution in water and partitioned with petroleum ether. The aqueous phase contained more 3PBA and was combined with the first residuum. The combined phases were hydrolysed with NaOH, acidified with HCl and the 3PBA back extracted into petroleum ether. 3PBA was assayed by reverse phase HPLC.

2. Cypermethrin: The petroleum ether phase was concentrated and cleaned up by liquid solid chromatography on a Florisil column. The eluate was analysed for Cypermethrin content using GC-EC (McKee et al., (1981).

The limits of determination (LOQ) for Cypermethrin were claimed (no data) as 10 $\mu\text{g/kg}$ and 50-100 $\mu\text{g/kg}$ for 3PBA. Recoveries were not appended but should be measured by spiking for a batch of analyses (Baldwin et al., 1977b).

Milk

Samples of milk are treated with potassium oxalate solution, ethanol, diethyl ether and hexane. The extract is evaporated to dryness to measure the fat content of the milk. The extract is purified further by partitioning between hexane and acetonitrile, followed by column chromatography on Florisil. The residues of

Cypermethrin were determined by GC-EC. The limit of detection was claimed (no raw data) as approximately 2 µg/l milk. Recoveries of spikes of 5 and 10 µg/l were 99 ± 8% (Baldwin et al., 1977d).

Eggs

A validated method was not supplied.

APPRAISAL

Cypermethrin is a synthetic pyrethroid insecticide applied topically to cattle, sheep and poultry. Cypermethrin is a mixture of all eight possible chiral isomers. The pharmacokinetic, metabolism and depletion studies using radiolabeled cypermethrin were carried out in cattle, sheep and poultry using oral administration (except for the topical application in two sheep) and not spray, dip or pour-on formulations.

Following oral administration of ¹⁴C-Cypermethrin to cattle, poultry or rats the radioactivity was excreted rapidly in both the urine and faeces. Less than 1% of the dose was found in milk or eggs. The topical application of ¹⁴C-Cypermethrin to sheep resulted in less than 3% of the dose being excreted over a six day period.

In all species after oral administration there was evidence of hydrolysis of the ester bond and residues of each part of the molecule were found in different proportions in rats, cattle, poultry and humans. There was extensive metabolism in bovine liver and kidney with the major metabolites consisting of either the conjugates or free forms of 3-phenoxybenzoic acid (3-PBA) or 4-hydroxy-3PBA. 97% of the radioactivity was extracted from liver. 92% of the residues in kidney could be extracted at room temperature. Bound residues in kidney were less than 10 µg/kg and were not further investigated. The residues in milk and fat were, respectively, 90% and 98% extractable and shown to be cypermethrin. In fat and milk the cis and trans isomers were present in an equal ration which suggests that no interconversion of the isomers occurs. No information on metabolism following absorption through the skin of cattle or poultry was provided and no residue profile data were available for sheep.

Radiolabeled depletion studies were carried out in cattle or poultry to only one day after the last oral dose and only at 1 and 6 days after topical dosing of two sheep. Thus it is not possible to determine the depletion of the total residues in these species.

The radiolabeled studies in farm animals show that the residues were higher in fat, liver and kidney (up to 410 µg/kg) than in muscle (up to 60 µg/kg). When sheep received the pour-on application the residues in the subcutaneous fat at the site of application were more than ten fold higher than following an oral dose.

Based on the limited data cypermethrin is the only residue possible for selection as a marker compound. This is suitable for fat, milk (in the milk fat) and eggs. However, the relationship between the concentration of cypermethrin and the total residues in muscle, liver and kidney was imprecise and not studied in the post-dosing period.

There were a large number of trials in which the residues of cypermethrin were measured in cattle, sheep and poultry following the recommended field uses. In all studies residues were lower in muscle, liver and kidney than in fat tissues or milk fat.

The residues following the use of ear tags impregnated with cypermethrin for cattle were mostly below the limit of quantification. The residues measured in cattle and hens after applying a spray formulation were at low levels (<10-30 µg/kg) in muscle liver, kidney and eggs. Higher residues were observed in fat (mean concentration was 90 µg/kg at 7 days post dosing) and in the milk fat (mean peak value was 110 µg/kg at 4 days post dosing) and had declined to a mean value of 30 µg/kg by day 10.

The residues of cypermethrin following the dipping of cattle in the commercial formulations were either below or very close to the LOQ (10 µg/kg) for muscle, liver and kidney. Residues were present in renal, omental and

subcutaneous fat. The concentration in the fat had not declined to the LOQ by 14 days post dosing, the last sampling time studied. However the highest residue in fat was 180 $\mu\text{g/kg}$ with most of the values less than 100 $\mu\text{g/kg}$.

The residues in calf tissues were measured following the use of a 0.5 g pour-on application. The concentrations in muscle and liver are low or not detectable. There were residues (up to 130 $\mu\text{g/kg}$) in the kidneys throughout the 14 days post dosing study period and much higher levels were found in both peritoneal and subcutaneous fat. The levels of up to 1400 $\mu\text{g/kg}$ in fat were the highest recorded for any treatment. The residues, although still present at 14 days, were declining. The study was carried out using a dose of 0.5 g per 125 kg calf which was the same amount as that recommended for larger, mature animals. Thus residues in larger animals may be lower.

The residues were measured in whole milk for 21 days after applying a dose of either 0.5 g or 1 g as a pour-on preparation to lactating cows. The residues followed a predictable pattern, with the highest values (up to 168 $\mu\text{g/l}$) being reached during day 2 for the higher dose and all values declining to control values (2 $\mu\text{g/l}$) within 21 days.

In sheep, the residues were measured following either the application of dip or pour-on preparations. Residues of cypermethrin were close to or in most cases below the LOQ in all the studies for muscle, liver and kidney. Residues were found in both the perirenal fat (<10 to 150 $\mu\text{g/kg}$) and omental fat (<10 - 130 $\mu\text{g/kg}$). Residues were only measured in the subcutaneous fat (<20 - 25 $\mu\text{g/kg}$) in one study, but in this study they were higher than in either omental or perirenal fat. Surprisingly in view of the radiometric data, residues were not measured in the subcutaneous fat at the site of application in the pour-on studies. Residues were measured in the whole milk of ewes after dipping once using a 0.015% formulation. Residues persisted throughout the 15 days post-dipping period. The mean values in $\mu\text{g/l}$ at 1, 3, 7, 10 and 15 days were 13, 10, 9, 7 and 7, respectively. The milk fat was about 5.8% of the milk and if all the residues are in the fat then the maximum concentration in the fat was 206 $\mu\text{g/kg}$ on day 1 and a high value of 143 $\mu\text{g/kg}$ was seen on day 7 also. The results were not corrected for recoveries which were 70-85%.

Residues were measured in hens over a 14 day period after applying either a dose of 10 or 20 mg per bird as a spray. Residues were at or below the LOQ in muscle, liver, kidney and eggs but were present in fat (25 - 140 $\mu\text{g/kg}$) and skin (80 - 1300 $\mu\text{g/kg}$).

There were low concentrations of bound residues and >90% of the bound material could be chemically released and shown to be metabolites.

Two detailed analytical methods were submitted, one for cypermethrin (LOQ was 10 $\mu\text{g/kg}$) and 3-phenoxy benzoic acid (LOQ was 50 $\mu\text{g/kg}$) in tissues and one for cypermethrin (LOD was 2 $\mu\text{g/l}$) in milk. Confirmatory methods using GC-MS are contained in the submitted papers. The analytical methods were submitted without adequate validation data. Evidence is required of the LOQ and LOD of the methods.

Maximum Residue Limits

The JMPR in 1981 set an ADI for Cypermethrin of 0.05 mg/kg/day which equates to a daily intake of 3 mg for a 60 kg person.

In recommending MRLs the Committee took account of the following factors:

- The ADI is 0-50 $\mu\text{g/kg}$ equivalent to 0-3000 μg for a 60 kg person. The ADI equates with that established by JMPR;
- The marker residue is parent drug, cypermethrin;
- Fat, milk and eggs are marker tissues but muscle, liver and kidney should be considered;

- The metabolism and radiodepletion studies are not adequate and, therefore, very conservative estimates of the marker compound as a percentage of total residues in all food species is proposed. The percentages proposed for the estimation in individual tissues of total residues from the parent drug are; muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80; eggs, 30;

- There is adequate residue information from the non-radiolabelled studies using the recommended formulations; and

- There are available analytical methods, however, evidence of adequate validation are needed;

The Committee recommends temporary MRLs for cattle, sheep and poultry of 200 µg/kg in muscle, liver and kidney, 1000 µg/kg in fat, 50 µg/kg for cattle whole milk and 100 µg/kg for eggs expressed as parent drug.

Estimates of residue intake are tabulated as follows:

Tissue	Food Basket (g)	MRL (µg/kg)	µg	Percent UD/TR	Intake (µg)
Muscle	300	200	60	30	200
Liver	100	200	20	10	200
Kidney	50	200	10	5	200
Fat	50	1000	50	60	83
Milk	1500	50	75	80	94
Eggs	100	100	10	30	33
				Total	810 µg

UD is unchanged drug; TR is total residues

The JMPR food basket takes approximately 300 µg leaving 2700 µg. The above MRLs accommodate the ADI and the recommended use of this compound as a veterinary drug and as a pesticide.

The Committee requires the following information:

1. Radiodepletion studies which extend beyond the recommended withdrawal times and using the drug in its topical formulation. The study should determine the depletion of the total residues and the parent drug;
2. Evidence to verify the limited information of no-interconversion of isomeric forms during metabolism in the target species; and
3. Further information on the validation of the analytical methods; particular data on the derivation of LOD and LOQs.

The committee will need to ascertain the contribution of ingested pesticide from non-food animal sources and subtract this from the ADI to calculate the allowed ADI for Cypermethrin from food animals.

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