ALPHA-CYPERMETHRIN and CYPERMETHRIN

First draft prepared by

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ADDENDUM

To the alpha-cypermethrin and cypermethrin monographs prepared by the 47th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/9, Rome 1997; the 54th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/13, Geneva 2000, for cypermethrin only; and the 58th meeting of the Committee and published in the FAO Food and Nutrition Paper 41/14, Rome 2002

IDENTITY

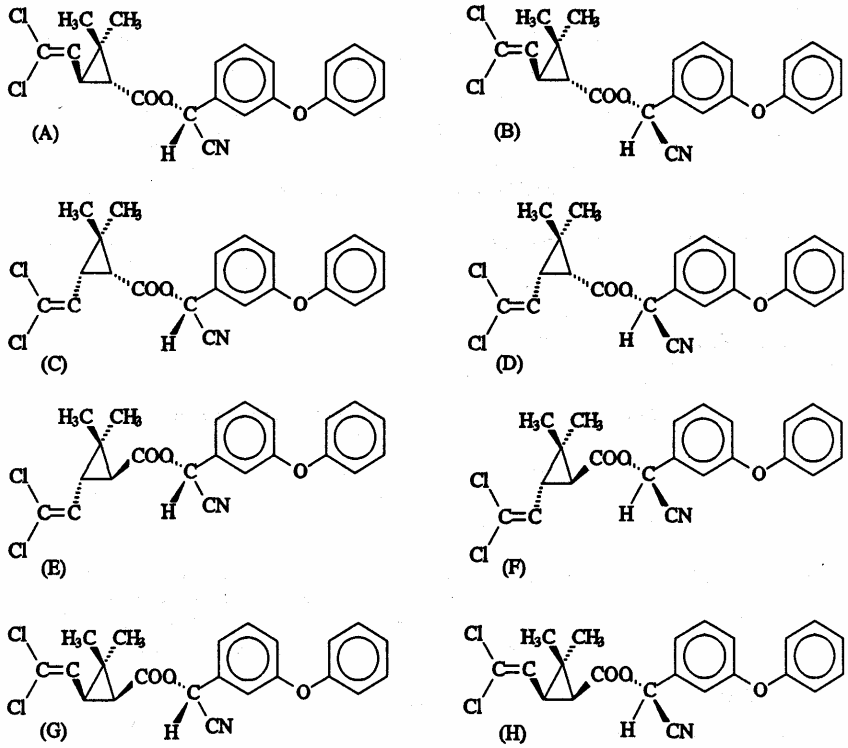
Chemical names:	Alphacypermethrin:
	A racemate of (S)-alpha-cyano-3-phenoxybenzyl (lR,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate and (R)-alpha-cyano-3-phenoxybenzy1 (IS,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate (IUPAC name); and a racemate of (S)-alpha-cyano-3-phenoxybenzyl (lR)-cis-3- (2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate and (R)-alpha-cyano-3-phenoxybenzyl (1S)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate. (CAS No. 67375-30-8)
	Cypermethrin:
	(RS)-alpha-cyano-3-phenoxybenzyl-(1RS, 3RS, 1RS, 3RS)-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 (CAS No. 52315-07-8)
Common names:	FASTAC, CONCORD, FENDONA, RENEGADE. (alpha-cypermethrin)
Structural formula:	See next page.
Molecular Formula:	$C_{22}H_{19}C_{l2}NO_3$
Molecular weight:	416.3
Appearance:	White-to-cream crystalline solid
Stability:	Highly stable to light and elevated temperatures. It is resistant to acidic hydrolysis but undergoes ester cleavage in environmental (basic) aquatic conditions. Its low water solubility indicates a low bioavailability in aquatic situations.
Melting point:	81.4-84.0°C
Boiling point:	200°C at 9.31 PA
Octanol-water partition coefficient:	p = 3.16x10s
Density:	1.330 g/ml (typical for pure material)

Solubility (g/l at 21°C)

n-Hexane	6.5
Propanol-2	9.6
Methanol	21.3
Ethyl acetate	584
Toluene	596
Fat	78
Water	2.06 μg/l at 20°C

Alphacypermethrin was determined to be miscible with acetone and dichloromethane at room temperature

Structural formula:



Chemical structure of eight cypermethrin stereoisomers. Alphacypermethrin comprises the (D) and (G) isomers.

(A) (1R,*trans*) (αR); (B) (1R,*trans*) (αS); (C) (1R,*cis*) (αR); (D) (1R,*cis*) (αS); (E) (1S,*trans*) (αR); (F) (1S,*trans*) (αS); (G) (1S,*cis*) (αR); and (H) (1S,*cis*) (αS)

Alphacypermethrin Cis 2: (D) and (G) isomers
Alphacypermethrin Cis 1: (C) and (H) isomer
Cypermethrin is a mixture of all isomers

INTRODUCTION

Alpha-Cypermethrin

Alpha-Cypermethrin was first reviewed by the Committee at its forty-seventh meeting in 1996 (FNP 41/9). Temporary MRLs for cattle, sheep and poultry were recommended at the 47th meeting: 500µg/kg in fat, 100µg/kg in muscle, liver and kidney, 25µg/kg for cattle whole milk and 50µg/kg for eggs, expressed as parent drug. The temporary MRLs accommodate the ADI and the recommended use of alpha-cypermethrin as a veterinary drug: The theoretical maximum intake of residues would be 406µg per day, compatible with the maximum 1200 µg based upon the ADI of 0-20 µg/kg body weight. In reaching its decision on MRLs for alpha-cypermethrin, the Committee took the following factors into consideration:

- An ADI of 0-20 µg/kg of body weight was established, equivalent to a maximum theoretical daily intake of 0-1200 µg for a 60 kg person.
- The parent drug was identified as the marker residue.
- Fat, milk and eggs were target tissues.
- The metabolism of the two isomers forming alpha-cypermethrin was similar to that of the other six isomers in cypermethrin. There was certain evidence, but not fully demonstrated, that no interconversion of the cis forms to the trans forms took place during metabolism.
- The metabolism and radio depletion studies were insufficient. Metabolite profiles were not determined in sheep or poultry. Limited studies were presented for cattle.

- The relationship between the concentration of alpha-cypermethrin and total residues was imprecise. A very conservative estimate of parent drug as a percent of total residues in all food species was proposed: muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80 and eggs, 30. These percentages were the same as proposed for cypermethrin by the Committee (the racemic mixture of eight isomers includes the two that correspond to alpha-cypermethrin).
- Adequate residue information from non-radiolabelled studies using the recommended formulations was provided.
- For cypermethrin, bound residues were lower than 20 % in liver and 10 % in other tissues (FNP 41/9)
- Analytical methods were available but validation was needed.

At its 47th meeting the Committee requested the following new information:

1. Radiodepletion studies in sheep and poultry which extended beyond the recommended withdrawal time using the drug in its topical formulation. The study must determine the depletion of the total residues and the parent drug;
2. The radio depletion studies submitted for cattle should be reassessed to determine the depletion of total residues and the parent drug;
3. Evidence of lack of interconversion of the cis isomers to the trans isomers during metabolism in the target species; and
4. Further information on the validation of the analytical methods, particularly data on the derivation of LOD and LOQ.

Since the information required at the 47th meeting of the Committee was not provided at the 54th meeting of the Committee, the temporary MRLs for cattle, sheep and chicken were not extended. The Committee requested similar data to be provided for evaluation at the 58th meeting of the Committee.

Two new radiolabel studies were submitted for evaluation at the 58th meeting of the Committee– one in sheep and one in cattle. As the market for poultry did not support conducting additional work, studies for poultry were not carried out. One additional report was provided on analytical methods. All the studies were carried out using appropriate and applicable good laboratory practices.

Based on the data provided, the 58th meeting of the Committee recommended the following MRLs of alpha-cypermethrin in cattle and sheep tissues and cattle milk: muscle, liver and kidney, 100µg/kg; fat, 1000µg/kg and cattle milk, 100µg/kg. Maximum residue limits in cattle and sheep liver and kidney are based on the LOQs of the GC-ECD method (50µg/kg for cattle and 20µg/kg for sheep) as residues were less than the LOQs at all sampling times. MRLs in fat, muscle and cattle milk were based on residue data of studies submitted for evaluation.

Cypermethrin

Cypermethrin was first reviewed by the .Joint Meeting on Pesticide Residues (JMPR) in 1979 and subsequently in 1981, 1986, 1988 and 1990. MRLs were recommended for a wide range of crops, meat and milk products and feed commodities. Whereas cypermethrin has been used on horses, deer, goats and sheep, it was evaluated for use only on cattle, sheep and pou1try by the 47th meeting of the Committee. The 47th meeting of the Committee recommended temporary MRLs for cattle, sheep and poultry of 200 µg/kg in muscle, liver and kidney, 1000µg/kg in fat, 50 µg/kg for cattle whole milk and 100µg/kg for eggs expressed as the parent drug. The JMPR exposure intake calculations use approximately 300 µg for pesticide use, leaving 2700 µg for veterinary use. The theoretical maximum daily intake was 810µg for use as a veterinary drug. In reaching its decision, the Committee took the following factors into consideration:

The ADI was 0-50 µg/kg body weight established by JMPR (1981), equivalent to 0-3000µg for a 60 kg person. The marker residue is the parent drug, cypermethrin. Fat, milk and eggs are marker tissues

The metabolism and radio depletion studies were not adequate and, therefore, very conservative estimated of the marker compound as a percent of total residues in all food species was applied. The percentages proposed for the estimation in individual tissues of total residues from the parent drug were: muscle, 30; liver, 10; kidney, 5; fat, 60; milk, 80; and eggs, 30.

There is adequate information from the non-radiolabel studies using the recommended formulations.

There are analytical methods available, however, evidence of adequate validation was needed.

The 47th Committee requested the following information to further elaborate MRLs at the 54th meeting of the Committee:

1. Radiodep1etion studies that 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 concerning no-interconversion of isomeric forms during metabolism in the target species.
3. Further information on the validation of the analytical methods, particularly data on how the LOD and LOQ were determined.

The 54th meeting of the Committee considered a new radiolabelled study in sheep treated orally with a 80:20 cis:trans isomer ratio, not topically treated as requested. The Committee considered that no information was submitted to the first two requests so the temporary MRLs recommended for all animal tissues and milk were not extended. The Committee also noted that no information was made available for the toxicological evaluation of the 80:20 cis:trans cypermethrin. In answer to request 3, a

suitable analytical method to measure the sum of isomers in mixtures of cypermethrin by GC was submitted. For re-evaluation at the 58th meeting, the Committee requested similar data to be provided (items 1, 2 and 3).

The 47th meeting of the Committee only considered the 45:55 cis-trans cypermethrin mixture and the recommended use of cypermethrin as an ectoparasiticide. No toxicological evaluation was performed for the 80:20 cypermethrin isomeric mixture. The 58th meeting of the Committee considered a conservative approach, using the toxicology assessment of alpha-cypermethrin (100% cis isomers). An ADI of 0-20µg/kg body weight was established at the 47th meeting of the Committee for alpha-cypermethrin and used for the 80:20 cis-trans isomer cypermethrin product.

Based upon the new information provided, the 58th meeting of the Committee recommended MRLs in sheep, measured as cypermethrin equivalents of: 20µg/kg muscle, liver and kidney and 200µg/kg for fat.The MRLs in muscle, liver and kidney were recommended using the limit of quantitation of the method (10µg/kg) as residues at 7 days post-treatment are above the LOQ only in fat tissue. MRLs for fat were based on the residue studies using a pour-on formulation reported at the 54th Committee.

The theoretical maximum daily intake accounts for approximately 6 percent of the alpha-cypermethrin ADI. As the sponsor did not indicate support for MRLs in species other than sheep, the temporary MRLs in cattle and poultry were not retained.

The Joint Meeting on Pesticide Residues plans to review cypermethrin in 2005.

The 14th Session of the Codex Committee on Residues of Veterinary Drugs in Food (CC/RVDF) considered the recommendations of the Committee (Codex Alimentarius Commission, Alinorm 03/31A, 2003) and decided to retain the recommended MRLs from the 58th meeting of the Committee at Step 4 in view of concerns expressed on the elaboration of separate MRLs for both compounds and requested that JECFA consider the establishment of one ADI and one set of MRLs for the entire cypermethrin group.

RESIDUES IN FOOD AND THEIR EVALUATION

General

Alpha-cypermethrin and cypermethrin are highly active synthetic pyrethroid insecticides which are effective against a wide range of pests relevant to public health and animal husbandry.

Alpha-cypermethrin consists of two of the four cis isomers present in cypermethrin. These isomers are the most biologically active enantiomeric pair. It is used in veterinary medicine for the control of ectoparasites such as ticks, fleas, lice and blowflies (EMEA, 1998).

Cypermethrin consists of a mixture of 4 cis- and 4 trans-isomers. The cis isomers are more acutely toxic than the trans isomers. It may be used, as a pesticide in a 45:55 cis:trans formulation or as a veterinary drug in two formulations, either 45:55 cis:trans or 80:20 cis:trans, named high cis cypermethrin (HCC). Cypermethrin commercial formulations for cattle and sheep are available as ear tags, sprays, dips and pour-on formulations (12.5mg /kg, 0.72-0.75g/animal)

Previous studies on alpha-cypermethrin

Dosage

Alpha-cypermethrin is applied as pour-on preparation for cattle (15 g/L, 0.15-0.75 g/animal) and sheep (2.5 g/L, 0.3-0.5 g/animal) and also as a dip for sheep and as a spray for poultry (10 mg/animal) (EMEA, 1998)

Radiolabelled drug studies in cattle

One lactating cow was dosed orally twice daily for 8 days, (0.25 g/day) with ^{14}C- alpha-cypermethrin. Of the administered dose, 58% was recovered (34% in faeces, 23% in urine, <1% milk) (Morrison and Richardson, 1994). Residue amounts were 390-480µg/kg for fat, 19-29µg/kg for muscle, 560µg/kg for liver, 220µg/kg for kidney and less or equal to 200µg/kg for milk. (FNP 41/9, pg.62 , table 1).

In another study four cows were dosed with 0.15 g pour-on treatment and sampled at 7, 14, 28 and 35 days post-dose (Redgrave et al, 1992). Total radiolabelled residues were mostly below the limit of quantitation (LOQ =10-30µg/kg) with the exception of some samples of fat (maximum 30µg/kg). Radiocounting estimates were not different from levels of alpha-cypermethrin measured by GC-ECD analysis (LOQ = 10ug/kg). In milk, maximum levels reached 7µg/kg by day 2-3 (71% determined as alpha-cypermethrin by GC), falling to the LOD for radiocounting (1µg/kg) by day 7. (FNP 41/9, pg 62, reference study 2, table 1)

In a third study (FNP 41/14, pg 23-36), ^{14}C-alpha-cypermethrin formulated at a nominal concentration of 15 g/L was topically administered to 8 steers (140-190 kg bw) and 8 lactating cows (510-560 kg) along the region of the back between shoulders and rump along the mid-dorsal line. Cows were treated following the morning milking. Mean doses achieved were 3 mg/kg body-weighth. Two steers and two cows were sampled at each time point. The total radiolabelled residues and extractability of different tissues were measured using a radiometric method for tissues and direct liquid scintillation counting for milk. Results are summarized in Table 1.

Table 1. Depletion of ^{14}C-alpha-cypermethrin in tissues and milk of cattle (mg/kg equivalents mean concentration ±std dev)

Tissue/Time	Post treatment time			
	3 day	7 day	14 day	21 day
Back fat	0.08 ±0.05	0.34 ±0.34	0.17 ± .03	0.65 ±0.07
Omental fat	0.05 ±0.04	0.20 ±0.14	0.28 ±0.12	0.31 ±0.12
Liver	0.08 ±0.05	0.18 ±0.08	0.17 ±0.06	0.10 ± .05
Kidney	0.03 ±0.03	0.04 ±0.02	0.06 ± .01	0.03 ± .02
Muscle*	<0.01(NA)	0.01 ±0.02	0.00 ± .00	0.01 ±0.00
Milk	0.04 ±0.03	0.02 ±0.01	0.00 ±0.00	0.00 (NA)

NA= not applicable
Data expressed as mean (n=4) ± SD in tissue, number of milk samples is variable
* Many results were calculated from data less than 30 dpm above background

^{14}C-total residues were detected at all times points post treatment in all tissues. The maximum ^{14}C alpha-cypermethrin residues as analyzed by HPLC radio-analysis were <35µg/kg in kidney and muscle, 647µg/kg in back fat and 421µg/kg in omental fat and 83µg/kg in milk. No ^{14}C-alpha-cypermethrin was detected in liver tissue. The percent parent drug to total residues at different time points were: 84 ±11% for back fat, 91±10% for omental fat, 90% in muscle (only one sample) and 16±13% for kidney and 96±23% in milk.

Using a GC-ECD method of analysis following the topical treatment noted above, alpha-cypermethrin residues followed the same tendencies with time as the radiolabel measures and also in milk at different milking times (60-126 h, n=13). The GC-ECD maximum results were: <50µg/kg for kidney, muscle and liver, 713µg/kg for back fat, and 337µg/kg for omental fat and 89µg/kg for milk. The percent of alpha-cypermethrin to TRR calculated from GC-ECD analyses of alpha-cypermethrin were lower than from studies using HPLC radio analysis: 76±44%, 70±16% and 76±13% in back fat, omental fat and milk, respectively. The results are summarized in Table 2.

Table 2. Maximum concentration of alpha-cypermethrin residues in cattle tissues following topical treatment.

	Post treatment (days)	Tissue	α-Cypermethrin µg/kg	Ratio (α-Cyper/total residues)
HPLC-radioanalysis study	21	Back fat	647	84
	21	Omental fat	421	91
	14	Kidney	22 (<35)	16
	7	Muscle	35	90
		Liver	ND.	
	60h	Milk	83	86
CG-ECD study	21	Back fat	713	76
	21	Omental fat	337	70
	All times post treatement	Kidney	< LOQ *	ND
		Muscle		
		Liver		
	60h	Milk	89	76

Note: ND=non detected . *LOQ tissues= 50µg/kg
References: Table 3 and Table 4, FNP 41/14, pg 23-36

The metabolite profiles in tissues and in cattle milk were defined. The ratio of parent drug to total ^{14}C- radiolabelled residues (TRR) in edible tissues was investigated at various withdrawal periods. In cattle, depletion rates of alpha-cypermethrin residues in edible tissues from steers and lactating cows were provided beyond the recommended 14 day withdrawal time for tissues and 0 day for milk following a single topical application.

The identified metabolites indicated that alpha-cypermethrin in steers and lactating cows, following topical application, underwent phase I oxidative hydroxylation at the phenyl ring and hydrolysis at the ester linkage to finally produce 3-phenoxybenzoic acid and its conjugates. The ester hydrolysis products were further oxidized to form 3-phenoxybenzoic acid (3- PBA) and 4-hydroxy-3-phenoxybenzoic acid (4'-OH-3-PBA). These compounds contained a free carboxylic and hydroxyl moiety, respectively, that underwent phase II metabolism to form glutamic acid and sulfate conjugates. Thus, the metabolism of alpha-cypermethrin was the same in orally treated cows (Morrison and Richardson 1994) and rat (Crawford and Hutson, 1977). Conversion from cis to trans configuration did not occur in the milk or steer tissues.

Identification and quantification of extracted radiolabelled residues showed that alpha-cypermethrin as the cis isomeric form was the main metabolite in both omental and back fat, milk and in the only sample of muscle analyzed. Extensive metabolism was also shown in liver and kidney, where the main metabolite identified was 3-PBA glutamate. Others metabolites tentatively identified were 3-PBA, 4-OH-3-PBA and 3-PBA-4-O-sulfate. A number of unknown extractable residues and bound residues were also detected in liver and kidney.

Two analytical methods for the determination of alpha-cypermethrin residues in cattle tissues (muscle, fat, kidney and liver) and milk were reported, bearing the titles SAMS 456-1 and SAMS 461-1, respectively. They were validated for determination of the LOQ values reported. They proved to be suitable for determination of alpha-cypermethrin in fat tissues of cattle and milk of cattle (LOQ = 50 µg/kg for cattle tissues, 10 µg/kg for cattle milk). However, because of the low residue concentrations in muscle, liver and kidney, the methods were of limited value for determination of residues in these tissues (most values were below the LOQ). The analysis of the chromatograms suggested that lower LOQs might be possible.

Radiolabelled drug studies in sheep

A sheep study extending beyond the recommended withdrawal time of 7 days for the pour-on formulation was reviewed by the 58th meeting of the Committee. Alpha-cypermethrin was formulated at a nominal concentration of 12.5 g/l and was topically administered on either side of the spine and around the rump to 6 male and 6 female sheep (28-39 kg BW) at a dose level of 15 mg/kg BW. This was the maximum recommended dose. (FNP 41/14, pg 23-36). The metabolite profile in tissues and in cattle milk was defined using only two samples (one ram and one ewe). The ratio of parent drug to total ^{14}C- radiolabelled residues (TRR) in edible tissues was investigated at various withdrawal periods. The total ^{14}C- radiolabelled residues (TRR) were determined using a radiometric method in tissues. For fat, residues were by direct liquid scintillation counting (LSC) and for other tissues, LSC following combustion. Results are summarized in Table 3.

Table 3. Depletion of ^{14}C-alpha-cypermethrin (mean concentration, mg/kg equivalents±std dev) in sheep tissues

Tissue/Time	Post treatment time			
	2 day	4 day	7 day	14 day
Back fat	0.62 ±0.74	0.66 ±0.70	0.17 ±0.06	0.08 ±0.03
Omental fat	0.11 ±0.06	0.23 ±0.01	0.19 ±0.12	0.14 ±0.06
Liver	0.04 ±0.02	0.08 ±0.05	0.04 ±0.02	0.02 ±0.00
Kidney	0.07 ± 0.03	0.14 ±0.06	0.06 ±0.02	0.02 ±0.01
Muscle	0.02 ±0.01	0.01 ±0.01	0.01 ±0.00	0.01 ±0.00

Data expressed as the mean (n=3 ±SD)

The maximum ^{14}C alpha-cypermethrin residues as analyzed by HPLC radio-assay were 1323µg/kg for back fat, 314µg/kg for omental fat, 22µg/kg for kidney and <20µg/kg for muscle and liver . The percentage of parent drug to total residues at different time points were 85 ±5% for back fat, 83±17% for omental fat and 62 ±23% in muscle, 9±6% in liver and 6 ±8% in kidney.

Using a GC-ECD method, alpha-cypermethrin residues followed the same tendencies with time as in the radiolabel study. The GC-ECD maximum levels were: 1360 µg /kg for back fat, 218 µg /kg for omental fat, and <20µg/kg for muscle, kidney and liver. The percentage of alpha-cypermethrin to TRR calculated from GC-ECD analyses of alpha-cypermethrin were lower than results from HPLC radioanalysis, 85±20% for back fat and 59±18% for omental fat. These results are summarized in Table 4.

Table 4. Maximum alpha-cypermethrin residues in sheep tissues from topical treatment.

	Post treatment (days)	Tissue	α-Cypermethrin µg/kg	Ratio (α-Cyper/total residues)
HPLC-radioanalysis study	2	Back fat	1323	85
	7	Omental fat	314	83
	2	Kidney	22	6
	2	Muscle	18	62
	2	Liver	10	9
CG-ECD study	4	Back fat	1360	85
	7	Omental fat	218	59
	All times post treatement	Kidney	< LOQ *	ND
		Muscle		
		Liver		

ND=non detected . *LOQ tissues= 20µg/kg

Reference: Table 8, FNP 41/14, pg 23-36

The metabolic fate of alpha-cypermethrin was the same as found in steers. Interconversion of the cis to trans isomeric form of alpha-cypermethrin was not observed. The cis isomeric form of alpha-cypermethrin was the main residue in fat tissues and

muscle. The main metabolite in liver was the 4-OH-parent and in kidney was 3-PBA-glutamate. A number of unknown extractable residues and bound residues were also detected.

Other residue depletion studies (with unlabelled drug) in cattle

Fifteen calves were dosed with 0.16g per animal as a pour-on treatment (Sherren, 1988b). Sampling time of residues were 3, 7 and 14 days post-dose. Maximum residues in both subcutaneous and perirenal fat occurred at day 7 (80-270 µg /kg). Residues were detected in kidney (<30 µg /kg), but were not detectable in muscle and liver (LOQ = 10µg /kg) (FNP 41/9, pg 64, reference study 2, table 2).

In a second study, two groups of 11 female calves were dosed at 0.15g pour-on treatment per animal (Cameron et al., 1993). Residue sampling times were 3, 7, 14, 21 and 28 days post-dose. Maximum residues in both subcutaneous and perirenal fat occurred at day 14 (20-100 µg /kg), then declined until day 28 (<40µg/kg) (FNP 41/9, pg 64, reference study 3, table 2). In both studies residues were higher in perirenal fat than in subcutaneous fat.

In another study 15 cows (five per treatment group) were treated at 0.1, 0.15 and 0.2g per cow (Sherren, 1988a). Tissues were sampled at 1, 2, 3, 4, 7, 14 and 21 days post-dose. Maximum residues (up to 5 µg /kg) were observed between days 2-5 after treatment and were all less than the LOQ (2 µg /kg) by day 21 for all treatments. The residue profile follows closely that seen with the radiolabelled study using the 0.15 g dose (FNP 41/9, pg 64, reference study 1, table 2).

Similar residue profiles to those obtained in the radiolabelled studies are found, showing residues principally in fat tissues (perirenal higher than subcutaneous), followed by kidney and minor quantities in muscle. Maximum residues in fat tissues occurred between 7 and 14 days at the same pour-on dose. Results were not corrected for recovery although they were determined. In milk, maximum residues were generally observed at short times after treatment (2-5 days) declining thereafter. Analytical data suggests that the residues were mainly alpha-cypermethrin. Most measurements were near the LOQ of methods employed.

Other residue depletion studies (with unlabelled drug) in sheep

Six sheep, three treated with a pour-on and three dip-treated, dosed at 0.2 g pour-on and 60 mg/l dip, were analyzed for residues in fat, skin and wool at 3, 7 and 14 days post-dose (Francis and Gill,1989). High residues were found in skin (up to 1400 ug/kg) for at least two weeks in both treatments. Subcutaneous fat residues were not detectable within 7 days of dosing in the pour-on treatment, but in dip treated sheep, residues were 40 ug/kg at 7 and 14 days of dosing (minimum concentration measured was 10 ug/kg) (FNP 41/9, pg 64, reference study 4, table 2).

Ten sheep treated with a pour-on formulation (five dosed at 0.01 g/kg bw and five at 0.02 g/kg bw), were sampled at 7 days post-dose (White, 1987). Residues after treatment in both perirenal and omental fat were 0.2-8 ug/kg and 3-11 ug/kg, respectively, at the 0.1 g/kg bw treatment and 5-18 ug/kg and 2-19 ug/kg, respectively, at the 0.2 g/kg bw treatment (FNP 41/9, pg 64, reference study 5, table 2).

In these studies, residues were measured only in fat and uncorrected for recovery. Others tissue residues were not measured. The majority of residues seemed remained unabsorbed (high concentrations in skin and wool) for the external treatments. Bound residues were less than 20% in liver and 10% in other tissues (FNP 41/9, pg 53).

Previous studies on cypermethrin

Radiolabelled drug studies in sheep

Two male sheep were topically treated (21.9 mg/kg BW) and a third was treated orally (3.9 mg/kg BW) with ^{14}C-cypermethrin cis:trans 45:55 isomer mixture (Crawford and Hutson, 1977b) (FNP 41/9, pg 42). Tissues were extracted and analyzed using gas chromatography for cypermethrin. In the oral treatment, maximum TRR concentrations were 390, 360 and 410 µg /kg in liver, kidney and renal fat, respectivley, at day 2 after treatment. The percent of total residues attributable to cypermethrin was 65%, 8%, <1% and 33% in fat, liver, kidney and muscle, respectively. In the pour-on treatment, TRR were higher in fat tissues: 170-300 µg/kg and up to 3300-100000 µg/kg in subcutaneous fat at the site of application. Residues in liver, kidney and muscle were 100-140 µg/kg, 140-120 µg/kg and 30-60 µg/kg respectivelly, between 1 and 6 days post treatment. Percent of total residues attributable to cypermethrin were between 80-92%, 13-17% and <4% in fat, liver and kidney, respectively. In muscle, cypermethrin was not quantifiable. Results of these previous studies are summarized in Tables 5 and 6.

Table 5. Percent cypermethrin of total residues following treatment with ^{14}C-cypermethrin in sheep.

Tissue	Topical (24 h)	Topical (6 d)	Oral (2 d)
Liver	13	17	8
Kidney	< 3	< 4	<1
Muscle	NQ	NQ	33
Renal fat	88	80	63
Subcutaneous fat	-	92	67

NQ = non quantifiable

Table 6. Total residues (ug/kg equivalents) of ^{14}C-cypermethrin in sheep

Treatment	Time Post Treatment	Muscle	Liver	Kidney	Renal fat	Subcutaneous fat
Topical	1 day	30-40	100	140	170	100000*
	6 day	30-60	140	120	300	3300*
Oral	2 day	30-40	390	360	410	260

A study investigating the radiodepletion of a mixture of 80:20 cis:trans ^{14}C-cypermethrin administered orally (1 mg/kg BW) to adult sheep was submitted (FNP 41/13 pg 19). Three groups of five sheep (two sexes) were slaughtered at 1, 3 and 5 days after dosing. Both radiolabelled cis and trans cypermethrin were measured by radio-TLC only at the 1-day time point due to small quantities in later post treatment times. No residues of the trans isomer were detected. Maximum concentrations of TRR reached 334, 408 and 50µg/kg in liver, kidney and fat, respectively, at day 1 after treatment. The percent of total residues attributable to cypermethrin was 86%, 4%, 1.2% and 22% in fat, liver, kidney and muscle. See Table 7.

Table 7. Concentration of total residues and marker residue (ug/kg) in sheep 1 day post treatment following oral treatment with 14C-cypermethrin (1 mg/kg BW) using an 80:20 isomer mixture.

Tissue	TRR	cis-Cyp	trans-Cyp	Ratio (%) cyp:TRR
Liver	334 ± 23	13 ± 5	0	4
Kidney	408 ± 105	5 ± 1	0	1.2
Muscle	13± 3	3 ± 2	0	22
Fat	50 ± 13	43 ± 17	0	86

Other Residue Depletion Studies (with unlabelled drug)

Residue information was provided using dip and pour-on preparations (FNP 41/9, pg 42). The main residue measured was the parent compound, cypermethrin, determined by GC-ECD with non-validated methods. In sheep following a dip treatment, residues were close or below the LOQ in most cases for all tisúes. Residues were only found in perirenal and omental fat. In one of the pour-on studies, 20 sheep were treated with 0.375 g of cypermethrin and 20 sheep with 0.75g. Residues of cypermethrin reached maximum values of 40 µg/kg at 3-7 days after treatment, descending to 20 µg /kg at 28 days after treatment in both perirenal and omental fat. In a second study, 10 sheep were treated with 0.375 g of cypermethrin in two different pour-on formulations. Residues at 7 days post treatment were 18-35 µg /kg in omental fat and 4-10 µg /kg in perirenal fat (very low recoveries). Residues in subcutaneous fat were not measured.

In another study, **t**wenty four wethers were dunked into a dip containing 0.01% cypermethrin (FNP 41/13, pg 23). Residues were detected in omental fat, perirenal fat and muscle from <10 µg /kg (0 day) up to 170 µg /kg in perirenal fat at day 14. Residues could not be detected in liver and kidney.

A study with Merino ewes treated using a 2.5% cypermethrin pour-on at 15 ml (normal maximun dosa rate) and 30 ml (FNP 41/13, pg 23) was reported. For the recommended maximum dose rate of 15 ml, residues in both omental and perirenal fats reached peak values of 40 µg/kg after 7 days. Values for a double dose rate of 30 ml also peaked after 7 days at 70µg/kg for omental fat and 80 µg/kg for perirenal fat. For muscle, liver and kidney samples, results were all less than 20 µg /kg.

Forty two female Suffolk cross sheep (approximately 50-60 kg body weight and 9 months old) were treated with cypermethrin at a rate of 1 ml/kg BW (12.5 mg/kg; mean dose level 0.72-0.75 g/animal). The drug was applied by pin-stream application to the backline, directly onto the skin. Groups of five sheep were sacrificed at 7, 14, 21, 28, 35 and 42 days. Duplicate samples of liver, muscle, kidney and subcutaneous fat were taken from each animal and analyzed for cis-cypermethrin. The remaining two slaughter groups were not required for analysis. Analysis for muscle and kidney samples were stopped at 14 days post-treatment because residues were below the limit of quantitation or not detected in all samples at 7 and 14 days post treatment. Similarly, liver sample analysis was stopped at 21 days post treatment. Analysis of subcutaneous fat samples was terminated at 28 days post treatment. The limit of quantitation (LOQ) was 10µg /kg and the limit of detection (LOD) was 4µg /kg. To estimate mean values, analytical results below the LOQ and LOD were allocated values of half the LOQ. Results are presented in Table 8 and 9.

Table 8. Cypermethrin residues in Suffolk sheep following topical treatment at 12.5 mg/kg body weight

Post treatment (days)	Liver	Kidney	Muscle	Fat (µg /kg)
7	5 <LOD	2 <LOQ, 3 <LOD	3 <LOQ, 2 <LOD	33.9, 17.2, 25.8, 20.2, 36.2
14	3 <LOD, 2 <LOD	5 <LOD	5 <LOD	17.1, 17.9, 1 <LOQ, 2 <LOD
21	1 <LOD, 4 <LOD	NA	NA	5 <LOD
28	NA	NA	NA	5 <LOD

NA= samples not analyzed, previous analysis showed levels of BLQ or ND for two consecutive timepoints.

Table 9. Estimated cis-cypermethrin residues (ug/kg) in sheep tissues after topical treatment (12.5 mg/kg body weight)

Days		Liver	Kidney	Muscle	Subcutaneous Fat
7	Max	2.0	5.0	5.0	36.2
	Mean	2.0	3.2	3.8	26.7
	S.D.	2.0	1.6	1.6	8.3
14	Max	5.0	2.0	2.0	17.9
	Mean	3.8	2.0	2.0	8.8
	S.D.	1.6	0.0	0.0	8.0
21	Max	5.0	NA	NA	2.0
	Mean	2.6	NA	NA	2.0
	S.D.	1.3	NA	NA	0.0
28	Max	NA	NA	NA	2.0
	Mean	NA	NA	NA	2.0
	S.D.	NA	NA	NA	0.0

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Three analytical methods for the determination of cypermethrin residues in cattle tissues (MCY/01/51), in cattle milk (MC/01/50) and in sheep tissues (MCY/99/31) were submitted. The submission also enclosed supplements to the method reference MCY/99/31 (the stability of HCC in sheep kidney and LOD determination in sheep liver and muscle). The methods have been properly validated.

Determination of high cis-cypermethrin (HCC) in cattle tissue using GC-ECD (Method Reference MCY/01/51)

This method describes the determination of the concentration of HCC cypermethrin present in cattle tissues using capillary gas chromatography with on column injection and electron capture detection, following solid phase extraction.

Principle: The analyte was extracted from fat, kidney and muscle with acetonitrile whereas from liver, chloroform is the indicated extraction solvent. HCC residues were extracted from kidney, fat and muscle (0.9-1.1g) by homogenisation with acetonitrile and anhydrous sodium sulphate twice and organic phases separated. This combined extract was partitioned with hexane twice, the hexane being discarded. After centrifugation, the supernatant was decanted and evaporated to dryness under nitrogen. Same procedure was employed for liver using chloroform rather than acetonitrile. The dry residues from all tissues were reconstituted in hexane and further cleaned up on a FL Isolute SPE cartridge. The eluate was evaporated to dryness and redissolved in hexane. The final analysis was carried out using capillary gas chromatography with electron capture detection. The analyte eluted with ethyl acetate: hexane 10:90

Standards: The differing MRL values (those established by EMEA) for cattle fat and others tissues dictated the level of dilution of the stock standard solutions for the particular tissue being analysed. The nominal concentration of working standard and the nominal concentration of analytical quality control (AQC) standard were 200 ng/ml and 2000 ng/ml respectively for fat and 20 ng/ml and 200 ng/ml respectively for muscle, liver and kidney. Tissue blank samples were prepared as the test tissue samples but using tissue free of HCC. AQC standards were prepared as for the test tissue samples but using blank tissue sample fortified with 100 µl of the appropriate spiking solution. Residues of HCC are determined by GC with ECD detection (2µl injection volume). GC was performed in a SGE BPI column (15m x 0.53 mm ID, 1.0 µm) with a temperature gradient.

Linearity: The linearity of detector response was determined over the range of approximately 10 ng/ml to 400 ng/ml, with a correlation coefficient of 0.9979.

Accuracy and Precision: To establish the accuracy and repeatability, three fortification concentrations levels (MRL, 1/2x MRL and 2x MRL) were used for recovery determinations on day 1 (n=6 at each fortification level). Three more fortified tissue samples were analysed, at each of the above concentration levels, on Day 2 and Day 3 and involving more than one operator in order to determine the within laboratory reproducibility part of precision.

For fat, recoveries and coefficient of variation (CV) at each respective level (100, 200 and 400 µg/kg) were: 91.1% (5.1), 82.8% (4.1), 76.8% (7.5), respectively. Interday variation of fat analysis had a mean CV of 8.62% (n=12). For liver, recoveries and coefficient of variation (CV) at each respective level (10, 20 and 40 µg/kg) were: 79.5% (9.9), 82.8% (6.1), 77.9% (3.5), respectively. Interday variation of liver analysis had a mean CV of 9.36 % (n=12). Similarly, for kidney recoveries at the same levels were 88.7% (7.9), 83.0% (3.2) and 77.4% (7.7), respectively. Interday variation of kidney analysis had a mean CV of 9.36 % (n=12). For muscle, recoveries at the same levels were 89.3 % (4.2), 81.1% (4.7) and 78.7% (8.1), respectively. Interday variation of muscle analysis had a mean CV of 10.6 % (n=12).

Specificity: A blank tissue extract was analysed together with three tissue extracts spiked with the five compounds most likely associated with typical cattle tissue samples (ivermectin, moxydectin, permethrin, 3-phenoxybenzaldehyde and DCVC acid). The tissue extracts were spiked with 2x MRL of the compounds together with HCC at MRL level. No significant interference was detected and the percentage recoveries obtained for HCC were within the acceptable limits.

Stability: The stability of HCC in the final tissue hexane extracts was assessed by spiking cattle tissue with HCC at MRL level and then following the extraction procedure. The extract was stored under ambient conditions of temperature and light and analysed initially to a maximum of 6 days in the four tissues. The final hexane extracts are stable at least 5-6 days under the mentioned conditions.

The stability of HCC in cattle tissue, when stored at -23ºC± 3ºC, was determined by spiking blank tissue samples with HCC at MRL level, samples then stored frozen and analysed at set intervals. The data indicated that analyte was stable in fat, kidney and muscle for at least 4 weeks and in liver for at least 2 weeks. The sponsor claims that stock analytical standard solutions and the subsequent dilutions in hexane were stable for up 29-30 days from a previous method.

Limit of quantitation (LOQ): This was defined as the lowest level at which precision and accuracy have been determined, nominally 10µg/kg in liver, muscle and kidney and 100µg/kg in fat. Limit of Detection (LOD): This was calculated as the mean background level at the retention time of HCC plus 3x SD of the mean from the analysis of 20 independently extracted blank samples of tissues. For fat, liver, kidney and muscle the LODs were 5µg/kg, 5µg/kg, 2µg/kg, 2µg/kg and 2µg/kg, respectively.

Practicability: The analyses were performed using commercially available reagents and equipments. Sponsors declared that the method was performed safely by a trained analyst and a large number of samples were analysed in a reasonable time period.

Note: raw data presented by sponsor included chromatograms for cattle tissues blanks, an HCC standard at the MRL corresponding to each tissue, all tissues spiked with HCC at the respective LOQs and cattle tissues (kidney, muscle and fat) with HCC standard at the MRL in the presence of possible interfering compounds.

Determination of high cis-cypermethrin (HCC) in cattle milk by GC-ECD (Method Reference MCY/01/50)

The principle of this method is the same as in the method described before, differing in two points: in this case,the solvent used in the first extraction was acetonitrile and in the solid phase extraction, florisil cartridges were used. Standard, milk blank samples and AQC standards were prepared in identical way to the previous described method.

Linearity: The linearity of detector response was determined over the range of approximately 10 ng/ml to 400 ng/ml, with a correlation coefficient of 0.9979.

Accuracy and precision: To establish the accuracy and repeatability, three fortification concentrations levels (MRL, 1/2x MRL and 2x MRL) were used for recovery determinations on day 1 (n=6 at each fortification level). Three more fortified milk samples were analysed, at each of the above concentration levels, on Day 2 and Day 3 and involving more than one operator in order to determine the within laboratory reproducibility part of precision. Mean recoveries and coefficient of variation (CV) at each respective level (10, 20 and 40 µg/l) were 79.9% (3.6), 79.3% (4.5), 71.2% (3.9), respectively. Interday variation of milk analysis had a mean CV of 5.6% (n=12).

Specificity: Blank milk extracts were spiked with solutions containing the five compounds most likely associated with typical cattle milk samples (ivermectin, moxydectin, permethrin, 3-phenoxybenzaldehyde and DCVC acid). The tissue extracts were spiked with 2x MRL (40µg/kg) of the compounds together with HCC at MRL level (20µg/kgl) No significant interference was detected and the percentage recoveries obtained for HCC were within the acceptable limits.

Stability: The stability of HCC in the final hexane extracts was demonstrated for up to 7 days at ambient temperature. The stability of HCC in cattle milk, when stored at -23ºC±3ºC, was demonstrated for up to 13 days. The sponsor declared that stock analytical standard solutions and the subsequent dilutions in hexane were stable for up 29-30 days from a previous method.

Limit of quantitation (LOQ): This was defined as the lowest level at which precision and accuracy have been determined, nominally 10µg/kg. Limit of determination (LOD): This was calculated as the mean background level at the retention time of

HCC plus 3x SD of the mean from the analysis of 21 independently extracted blank samples of milk. The rounded up calculated LOD was 6µg/l.

Practicability: The analysis was performed using commercially available reagents and equipments. Sponsors declared that the method was performed safely by a trained analyst and a large number of samples were analysed in a reasonable time period. The sponsor provided the following chromatograms: a typical cattle milk blank, a typical HCC standard spiked at the MRL level (20µg/kgl), cattle milk spiked with HCC at the LOQ (10µg/kg) and HCC standard in the presence of possible interfering compounds

Determination of High Cis Cypermethrin (HCC) in sheep tissue using GC-ECD (Method Reference MCY/99/31)

The principle of this method is the same as in the previous described methods, differing in solvents of the first extraction: chloroform was used for liver, kidney and muscle and acetonitrile for fat. Standard, milk blank samples and AQC standards were prepared in identical way to the previous described methods.

Linearity: The linearity of detector response was determined over the range of approximately 10 ng/ml to 400 ng/ml, with a correlation coefficient of 0.998.

Specificity: A blank tissue extract was analysed together with tissue extracts from fat and kidney spiked with the two compounds most likely associated with typical cattle tissue samples (propetamphos and abarmectin). The tissue extracts were spiked with 20ng/ml and 40 ng/ml, but no significant interference was detected.

Limit of quantitation (LOQ): This was defined as the lowest level at which precision and accuracy have been determined, nominally 10µg/kg in the four tissues.

Note: raw data presented by sponsor included chromatograms for kidney and fat tissue blanks, an HCC calibration standard, all tissues spiked with HCC at the LOQs and sheep kidney and fat with HCC standard in the presence of possible interfering compounds.

Addendum I to the method reference MCY/99/31

The results of a study about the stability of HCC in sheep kidney were provided (raw data were not submitted). The stability in sheep kidney tissue, when stored at -23ºC±3ºC, was determined by spiking blank tissue samples with HCC at MRL level (20µg/kg), samples then stored frozen and analysed at set intervals. The measured percentage recovery appeared to drop off rapidly after 11 days (under 65%) and hence 11 days was chosen as the maximum period of stability under the stated storage conditions.

Addendum II to the method reference MCY/99/31

The information submitted complement the original report of the method reference MCY/99/31. The results of a study to determine accuracy and precision, LODs and stability of HCC in sheep tissues were provided.

Accuracy and precision: For sheep liver and kidney, the sponsor noted that three fortification concentrations corresponding to MRL (20µg/kg), ½x MRL and 2x MRL were used for recovery determinations on day 1 (n=6 at each fortification level) in the original method. Three more fortified tissue samples were analysed, at each of the above concentration levels, on Day 2 and Day 3 and involving more than one operator in order to determine the within laboratory reproducibility part of precision. For sheep fat, the accuracy and precision were determined using the same procedure employed for liver and kidney, but fortification concentrations levels corresponded to the fat revised MRL (200µg/kg), 1/2x MRL and 2x the MRL. For muscle, the accuracy and precision were determined in a similar way but using the sheep fat extraction procedure to overcome the chromatographic problems due to the variable fat content of muscle. Spiking levels were the same as for liver and kidney (identical MRL). For fat, recoveries and coefficient of variation (CV) at each respective level (100, 200 and 400 µg/kg) were: 95.0% (8.9), 83.9 % (10.1), 90.4% (5.1), respectively. Interday variation of fat analysis had a mean CV of 11.0% (n=12). For liver, recoveries and coefficient of variation (CV) at each respective level (10, 20 and 40 µg/kg) were: 94.2% (5.6), 85.2% (6.3), 90.0% (8.0), respectively. Interday variation of liver analysis had a mean CV of 12.9% (n=12). Similarly, for kidney recoveries at the same levels were 81.6% (9.0), 95.2% (5.9) and 85.8% (8.7), respectively. Interday variation of kidney analysis had a mean CV of 13.4 % (n=12). For muscle, recoveries at the same levels were 85.3% (11.6), 90.5% (5.1) and 74.6% (7.5), respectively. Interday variation of muscle analysis had a mean CV of 11.3% (n=12).

Limit of detection (LOD): This was calculated as the mean background level at the retention time of HCC plus 3x SD of the mean from the analysis of 20-21 independently extracted tissue blank samples. Samples were processed as per original method MCY/99/31 for the liver, kidney and fat but using the fat extraction technique for muscle. From the calculations, an LOD of 5µg/kg was proposed for all tissues.

Stability: The stability of HCC in the final tissue hexane extracts was assessed by spiking tissues with a particular level and processing them as per original method MCY/99/31 as regards the liver, kidney and fat but using the fat extraction technique for muscle. The extract were kept under ambient conditions and analysed on three occasions. The results indicated that a stability period of 4-5 days for each tissue type was acceptable. The sponsor provided raw data presented as the following chromatograms: a typical sheep tissue blank, a typical HCC standard for each tissue spiked at the MRL level (20µg/l), fat and muscle spiked with HCC at the LOQ level.

APPRAISAL

Alpha-cypermethrin is a pyrethroid insecticide consisting of two of the four cis isomers present in cypermethrin (100% cis-isomers). These isomers comprise the most biologically- active enantiomeric pair. It is used in veterinary medicine. Cypermethrin consists of a mixture of 4 cis- and 4 trans-isomers (contains 20-40% alpha-cypermethrin). It may be used as a pesticide or as a veterinary drug in at least two formulations: either 45:55 cis:trans or 80:20 cis:trans, named high cis cypermethrin (HCC).

Studies on metabolism and residues of both compounds (FNP 41/9, 41/13, 41/14) indicates that there is no interconversion of cis to trans isomers and that the trans isomers deplete more rapidly from treated animals than cis isomers. Consequently, the residues found after veterinary treatment with cypermethrin and alpha-cypermethrin consist only of cis isomers and the source of the residue might be difficult to determine.

Under standard analysis conditions, the isomers of cypermethrin were not resolved and a single fused peak was obtained.

The 58th JECFA noted to national authorities the possible difficulty to determine whether residue concentrations comply with the recommended MRLs since the MRLs for cypermethrin and alpha-cypermethrin are different.

No new depletion studies were presented to the 62th meeting of the Committee. Results of studies provided to the 58th meeting of the Committee, indicate that for alpha-cypermethrin residues in cattle treated at a 3mg/kg dose using a 14C-alpha-cypermethrin formulation, the maximum concentration of residues as analyzed by either by HPLC analysis with a radio-label detector or by GC-ECD were 647µg/kg for back fat, 421µg/kg for omental fat, 22µg/kg for kidney, 35 µg/kg for muscle and <30µg/kg for liver. For alpha-cypermethrin in sheep treated with a topical dose of 15 mg/kg, maximum concentration of residues were 1323µg/kg for back fat, 314µg/kg for omental fat, 22µg/kg for kidney and <20µg/kg for muscle and liver. In milk, the highest concentration of residues found were 89µg/kg (60h). For cypermethrin, in a study on sheep treated with the recommended topical dose, the highest concentration of residues found in fat measured using a GC-ECD method was 34µg/kg while residues in liver, muscle and kidney were below the LOQ (10µg/kg).

Three analytical GC-ECD methods for the determination of cypermethrin residues in cattle tissues (MCY/01/51), in sheep tissues (MCY/99/31) and in cattle milk (MC/01/50) were submitted to the present Committee. They were properly validated. The submission also enclosed supplements to the method reference MCY/99/31 for determining cypermethrin in sheep tissues (the stability of HCC in sheep kidney and LOD determination in sheep liver and muscle).

The methods describe the determination of the concentration of HCC present in cattle and sheep tissues and milk using gas chromatography and electron capture detection, following extraction. The methods have almost identical extraction procedure differing in two points: the solvents used in the first extraction (acetonitrile is used with preference to chlroroform for safety reasons) and different cartridges in solid phase extraction. The instrumental GC-ECD conditions were identical. The methods have been validated in a similar way. The following criteria were evaluated: linearity, accuracy and precision, assay specificity, stability and practicability and were found to be adequate. Methods are suitable for determining the concentration of HCC in cattle tissues and milk over the range of 10µg/kg to 400µg/kg.

The limit of detection (LOD) and limit of quantitation (LOQ) were estimated for all methods. For cattle and sheep tissues LOQs were 100µg/kg for fat and 10µg/kg for liver, muscle and kidney respectively. For cattle tissues LODs were 5µg/kg for fat and 2µg/kg for liver, muscle and kidney. For sheep tissues, LODs were 5µg/kg for all tissues. For cattle milk: LOQ was 10µg/kg and LOD was 6µg/kg.

RECOMMENDED MAXIMUM RESIDUE LIMITS

The following factors can be considered in recommending a suitable marker residue and one set of maximum residue limits for the entire cypermethirn group:

- Alpha-cypermethrin (100% cis) consists of two of the four cis isomers presented in cypermethrin.
- A common ADI of 0-20µg mg/kg body weight, equivalent to 0-1200µg/kg was established for the most toxicologically active substance by the present Committee.
- The metabolism of cypermethrin and alpha-cypermethrin is similar in all species studied.
- The parent drugs cypermethrin and alpha-cypermethrin were the only recommended marker residues by the previous Committees.
- Residues of cypermethrin and alpha-cypermethrin found after treatment consists only of cis isomers.
- Using the common analytical methods for residue control, the eight isomers of cypermethrin are not resolved and a single fused chromatographic peak is obtained. Therefore, residues are reported as the sum of all isomers.
- MRLs of alpha-cypermethrin in cattle and sheep tissues and cattle milk recommended by the 58th Committee were: muscle, liver and kidney 100µg/kg; fat 1000µg/kg and cattle milk 100µg/kg. MRLs in liver and kidney were recommended on the basis of the limit of quantification of methods (LOD=20 µg/kg for sheep tissues, 50µg/kg for cattle tissues). MRLs in fat, muscle and cattle milk were based on residue data of studies submitted for evaluation.

- MRLs of cypermethrin in sheep tissues recommended by the 58th Committee were 20µg/kg muscle, liver and kidney and 200µg/kg in fat. The MRL in muscle, liver and kidney were recommended using the limit of quantitation of the method (10µg/kg) as residues at 7 days post-treatment are above the LOQ only in fat tissue. MRLs for fat were based on the residue studies using a pour-on formulation reported at the 54th Committee.
- New submitted methods are suitable to determine residues of both substances as the sum of isomers with the following LOQs: LOQ=100µg/kg for fat, 10µg/kg for liver, muscle and kidney respectively for cattle and sheep tissues. For cattle milk: LOQ=10µg/kg.

In considering a common set of recommendations for residues of cypermethrin and alpha-cypermethrin in cattle and sheep tissues and rounding, as appropriate, the following MRLs, expressed as of total cypermethrin residues, are 50µg/kg for muscle, liver and kidney; 1000µg/kg for fat, and 100µg/kg for milk.

The recommended MRLs in muscle, liver and kidney are based on the limits of quantitation of the new methods considering that residues are ≤ 35 µg/kg in both cattle and sheep tissues.

The recommended MRLs for fat and cattle milk are based on residue data from studies using the recommended treatments.

Residues in sheep tissues are lower than in cattle tissues, therefore, the same MRLs can apply to both species.

Using the daily food consumption figures for the theoretical diet, the residue equivalents of cypermethrin and alpha-cypermethrin are summarized in Table 10 (368 µg). The pesticide exposure for cypermethrin calculated by JMPR is 300 µg, therefore, total theoretical exposure for the cypermethrins would be approximately 650 µg.

The previously recommended MRLs for cypermethrin and alpha-cypermethrin are replaced by the following MRLs in cattle and sheep, as equivalents of total cypermethrin residues, 50µg/kg for muscle, liver and kidney; 1000µg/kg for fat, and 100µg/kg for cattle milk.

These MRLs are recommended for consideration by JMPR to harmonize MRLs of cypermethrin and alpha-cypermethrin.

Table 10. Theoretical maximum daily intake of residues of cypermethrin

Tissue	Recommended MRL (µg/kg)	Food Consumption Factor (kg)	Ratio MR/TR	Cypermethrins Equivalents (µg)
Muscle	50	0.3	0.3	50
Liver	50	0.1	0.1	50
Kidney	50	0.05	0.05	50
Fat	1000	0.05	0.8	60
Cattle Milk	100	1.5	0.95	158
Total				**368 µg**

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