CYHALOTHRIN

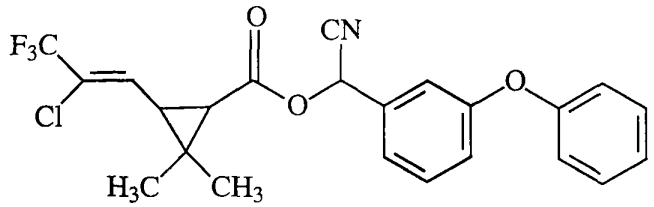
First draft prepared by
James MacNeil, Saskatchewan, Canada
Michael Morgan, Leeds, United Kingdom

IDENTITY

Chemical Name: (RS)-α-cyano-3-phenoxybenzyl (Z)-(1RS,3RS)-2-(chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate; CAS No. 68085-85-8

Synonyms: PP-563, Coopertix™, Grenade®

Structural formula:



Cyhalothrin is composed of two pairs of enantiomers, designated **A** and **B**, in an approximately 60:40 ratio (**A**:**B**);
A pair: *Z*(1*R*) *cis* (*R*) α-CN and *Z*(1*S*) *cis* (*S*) α-CN;
B pair: *Z*(1*R*) *cis* (*S*) α-CN and *Z*(1*S*) *cis* (*R*) α-CN.

Molecular formula: $C_{23}H_{19}ClF_3NO_3$

Molecular weight: 449.9

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Purity: The commercial product is a mixture consisting of 95% *cis*-isomers.

Appearance: Yellow to dark greenish-brown viscous liquid with no characteristic odour.

Melting point: Approximately 10°C

Boiling point: Decomposes >275 °C (atmos.); 187-190 °C (0.2 mm Hg)

Solubility: water, 0.003; acetone, >500; dichloromethane, >500; diethyl ether, >500, ethyl acetate, 500; hexane, >500; methanol, >500; toluene, >500 (all g/L)

Partition coefficient: *n*-octanol/water ($logP_{ow}$) at 20 °C = 6.9

Relative density: 1.25 (25 °C)

Refractive Index: $n_d^{24} = 1.534$

Ultraviolet maxima: not reported

RESIDUES IN FOOD AND THEIR EVALUATION

Conditions of use

Cyhalothrin is a synthetic pyrethroid insecticide used in many countries on various food-producing animals for the control of a broad range of ectoparasites, including flies, lice and ticks. Major use is on cattle and sheep, with lesser use on pigs and goats.

Typical product formulations include a 20 g/L liquid intended for direct dermal application as a "pour-on" or "spot-on", and emulsifiable concentrate formulations containing from 16 g/L to 200 g/L cyhalothrin, intended for use as a spray or dip after dilution with water. Cyhalothrin is applied topically as a pour-on at up to 60 ml (1.2 g) for ticks and at 10 ml (0.2 g) for lice or flies on cattle and at 5 ml (0.1g) on sheep and pigs for all applications. Cyhalothrin is also available as a 20% (w/v) emulsifiable concentrate for use as a spray or a dip prepared by dilution to 0.002-0.2% and applied at a dose of 0.1-4 L per animal, depending on the size of animal and the pest for which it is applied. The recommended repeat treatment intervals are 2-4 weeks for the spray or dip, and 4-8 weeks for the pour-on formulations. More frequent applications may be required for the control of ticks.

PHARMACOKINETICS AND METABOLISM

Laboratory Animals

Rat

Excretion

Groups each consisting of 6 male and 6 female rats (200-250 g BW) received single oral doses of ^{14}C-labelled cyhalothrin at 1 mg/kg and 25 mg/kg, labelled at two different positions, the 1-position of the cyclopropane ring (cyclopropyl-label) or the α-carbon of the benzyl group, or benzyl-label (Harrison, 1981a,b The radiolabelled cyhalothrin was dissolved in corn oil for administration to animals that had fasted overnight. Excreta were collected from each rat at 24-hour intervals for 6 to 7 days after exposure, following which the animals were euthanized and selected tissues were collected for measurement of residual radioactivity.

Radiolabelled residues were rapidly excreted in urine and faeces following all treatments, with most of the benzyl-labelled cyhalothrin dose was excreted within 24 hrs post treatment (Harrison, 1981a). Monitoring of expired air from rats (2 males, 2 females) dosed orally at 1 mg/kg did not reveal any elimination via this route. Different patterns of urinary metabolites were observed following treatment with cyhalothrin labelled at each of the two positions, with extensive metabolism and excretion of polar metabolites in the urine was determined by thin-layer chromatographic analysis. No significant difference in elimination rate or route was observed between males and females at either dose, or with either label. Urine contained 30.0-41.5% of the excreted radiolabel in all groups treated with the benzyl-label, while the balance of each dose was in the faeces. Following treatment with the cyclopropyl-label cyhalothrin, excretion was slower at the high dose than with the benzyl-label, with only 20-30% of the total radioactivity eliminated in the first 24 hrs. Overall, less radiolabel product was excreted in the urine from the rats treated with the cyclopropyl-label material (18.6–39.3%). Much of the radiolabel in the faeces was unchanged parent compound, indicating incomplete absorption. Only 1-3% of the dose remained in animal tissues at 7 days after exposure, predominantly in the fat.

In the same study, other rats received a single subcutaneous injection of 1 mg/kg benzyl-labelled cyhaothrin. Excretion was slower than from oral dosing, with 58% of the dose remaining in the tissues after 7 days. This was attributed to retention of the oil formulation in the subcutaneous fat. This study also included a treatment of bile duct canulated rats in which the benzyl-labelled cyhalothrin was administered orally at 1 mg/kg. Biliary excretion accounted for only 4.8–8.9% of the radiolabel residues, while urinary excretion was 7.2–8.3%, much less than observed in intact animals, but this increased to 16.8% in urine and 11.2% in bile when bile duct canulated rats were co-administered bile with the oral cyhalothrin dosing. The results suggested that bile strongly influenced the digestion process. Peak concentrations of cyhalothrin were observed in blood at 4-7 h after oral dosing for the various treatments and it depleted exponentially, with a half-life of approximately 11 h. This study report did not include a statement of GLP compliance.

Three studies contained GLP compliance statements. In one report on residues in tissues from rats included in the oral dosing study described above, residues were over 10-fold higher, as expected, in tissues from animals that received the 25 mg/kg dose and residues were detectable in all tissues, including brain, bone, fat, heart, liver, lungs, kidney, muscle and spleen from all rats at both doses (Harrison, 1981b). Residues were also detected in blood and bone. Residues in white fat varied from 0.17 to 0.34 mg/kg for the four treatment groups (males and females, 2 label positions) that received the 1 mg/kg dose and from 6.41-11.84 mg/kg for those which received the 25 mg/kg dosing. There was no significant difference in residue distributions between males and females, or between the residues in fat resulting from treatment with cyhalothrin labelled at the different positions. Residues in other tissues were much lower (>100-fold difference at 25 mg/kg treatment).

Rats (divided into two groups, each consisting of 6 males and 6 females) received 0.5 mL of a corn oil solution containing 0.5 mg/mL of either benzyl-labelled or cyclopropyl-labelled ^{14}C-cyhalothrin, administered orally by gavage tube, once daily for 14 days (Harrison, 1984a). The rats were kept in metabolism cages and urine and faeces were collected for each 24-hour period until the animals were killed at intervals up to 7 days after the last dose. Two males and two females from each group were killed at each of 48 hrs, 120 hrs and 7 days after the last dose and tissue samples were collected for analysis. Over 90% of the dose was eliminated in urine and faeces within 7 days after the final administration, with <5% remaining in the carcasses. Overall recovery of the total radioactive dose was 96% for both males and females and for both labelled forms of cyhalothrin. While elimination was rapid for both labelled forms, there were significant differences in the distribution of radioactive residues in urine and faeces, depending on the site of labelling. Residues in the tissues tested were primarily in fat, as parent compound. The estimated half-life for the residues in fat was 23 days. The distribution and elimination observed for cyhalothrin in this multiple dosing study was similar to that observed in the previous single dose study.

Metabolism

In one study, rats were administered ^{14}C-cyhalothrin (labelled at the benzyl-position) orally for 8 days, so that each animal received a total dose of 25 mg. Approximately 64% was recovered in the urine (Harrison, 1983). Urine was also collected and pooled from 14 rats that received 14 consecutive daily doses (1 mg/kg/day) of cyclopropyl-labelled ^{14}C-cyhalothrin. ^{14}C-components in urine from the two dosing experiments were separated by thin-layer chromatography (TLC) and analysed by high performance liquid chromatography (HPLC) using a radiochemical detector in combination with a uv-detector. Spectral identification techniques used included electron impact mass spectrometry, fast atom bombardment mass spectrometry (FAB-MS) in both positive and negative ion modes and ^{13}C-nuclear magnetic resonance (NMR). TLC analysis of urine from animals dosed with the benzyl-labelled ^{14}C-cyhalothrin indicated the presence of one major metabolite, designated M1, that accounted for about 75% of the radioactivity in urine. Three minor metabolites, designated M2, M3 and M4 were also observed, of which the least polar, M2, co-chromatographed with a standard of 3-phenoxybenzoic acid in the two solvent systems used for separation. No parent compound were observed. Further analysis by TLC again indicated that the least polar component from each urine sample co-chromatographed with M2. The fractions containing M2 were pooled and concentrated for mass spectral analysis using both direct inlet mass spectrometry and GC/MS after derivatization, with both techniques confirming the identification of M2 as 3-phenoxybenzoic acid. Following further separation of the remaining fractions by HPLC, one major peak, identified as M1 by TLC, and two minor peaks (<5%) were separated. The M1 was hyrolyzed by incubation with aryl sulphatase enzyme to a single component that co-chromatographed with M3. Treatment of M1 with β-glucuronidase resulted in only about 10% hydrolysis. Structural examination by NMR of purified M1 and its hydrolysis product indicated that the latter is phenol 3-(4'-hydroxyphenoxy) benzoic acid, while M1 is the sulphate conjugate. Urine from rats treated with the cyclopropyl-labelled ^{14}C-cyhalothrin was shown by TLC to contain one major ^{14}C-component (about 50%) and four minor components. The major component was completely hydrolyzed using β-glucuronidase, resulting in a corresponding increase in MII, one of the minor metabolites. This material co-chromatographed with the acid moiety of cyhalothrin, (1*RS*)cis-3-(*Z*-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid (CPA). Using HPLC and TLC, a main fraction was separated that contained the major ^{14}C-cyclopropyl metabolites. The main metabolite, MIII, accounting for 55% of the residual radioactivity, was purified by additional HPLC and examined by electron impact mass spectrometry and by FAB-MS, confirming that it was a glucuronide conjugate of MII.

Dog

Excretion

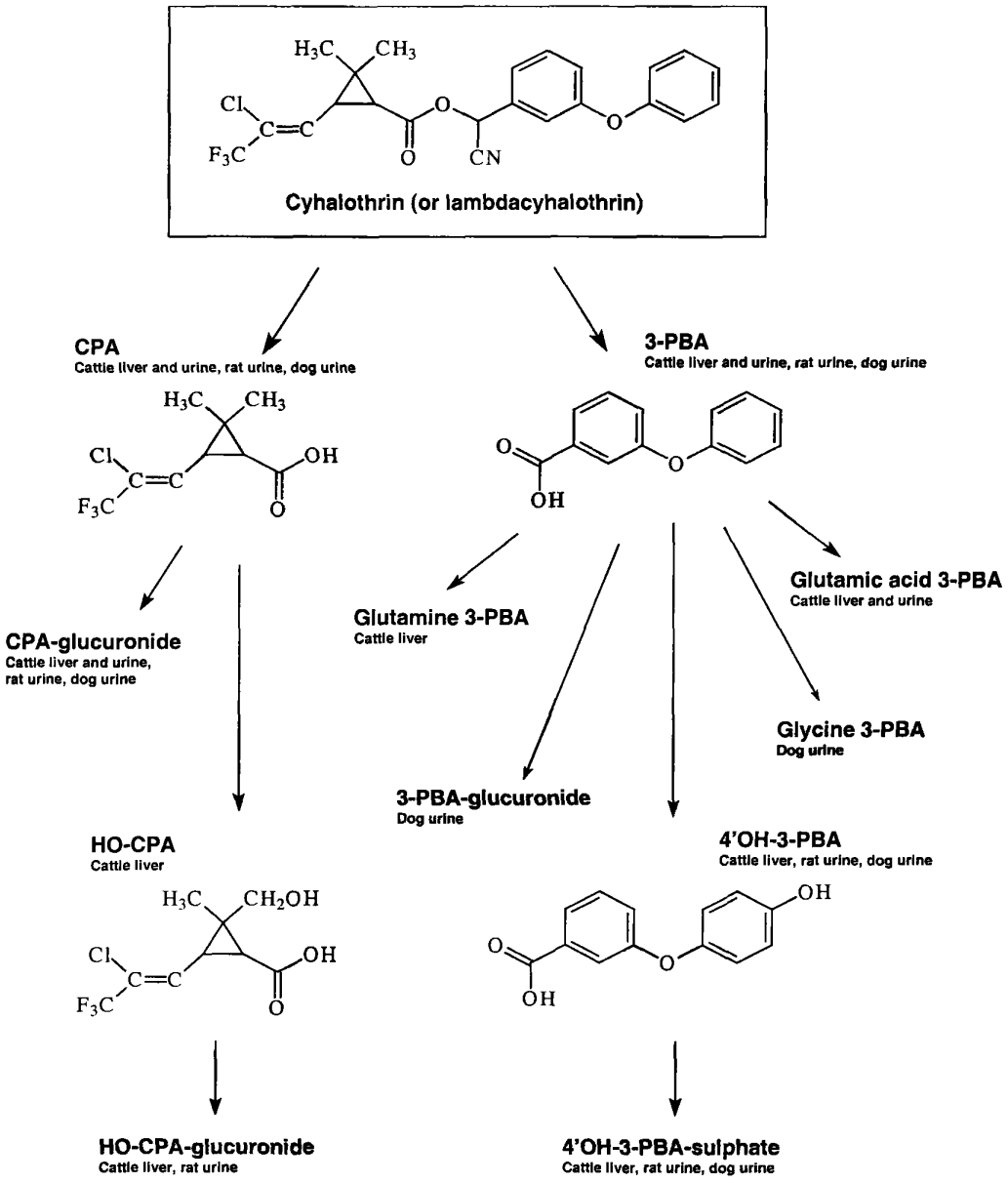
A series of studies were conducted in beagle dogs (3 males, 3 females, each approx. 15 kg BW), dosed orally (1 and 10 mg/kg BW) and intravenously (0.1 mg/kg) with benzyl-labelled and cyclopropyl-labelled ^{14}C-cyhalothrin (Harrison, 1984b). In each dosing regimen, each animal was dosed twice, once with each labelled form of cyhalothrin, at the chosen dose, in the following order: 1 mg/kg oral, 10 mg/kg oral, 0.1 mg/kg intravenous; benzyl-label first, cyclopropyl-label second in the oral studies; the reverse in the intravenous study. At least three weeks elapsed between dose treatments. The dogs were individually housed in metabolism cages for sample collection. Urinary metabolites were separated by TLC and detected by autoradiography. Spectral identification was by electron impact mass spectrometry or FAB-MS. After oral administration of the benzyl-label drug, most of the radiolabel was excreted within 48 hrs, with 65-70% in the faeces and 16-20% in the urine. Oral administration of the cyclopropyl-label drug gave a similar overall result, but with 46-55% of the radioactive residue in the faeces and 25-40% in the urine. After administration of the cyclopropyl label drug, peak concentrations occurred in the blood at 4 and 12 hrs, respectively, for the 1 and 10 mg/kg doses. With the benzyl-label drug, peak concentrations in blood were at 2-12 hrs for 1 mg/kg and at 12 hrs for 10 mg/kg doses, with half-lives of 28 and 32 h, respectively. No significant differences in absorption or elimination were observed between the sexes. Overall, from 79-96% of the dose was accounted for in the urine, faeces and cage washings. Excretion was slower after intravenous dosing, but was largely complete within three days, with similar distribution of residues in urine (36-44%) and faeces (34-43%), irrespective of label position.

Metabolism

Twelve urinary metabolites were detected following oral or intravenous administration, with the major components being CPA and its glucuronide. Other urinary metabolites identified are shown in Figure 1. Parent compound was the main residue in the faeces following oral administration, but little parent compound was found in faeces after intravenous administration. This study included a statement of GLP compliance.

Results of these metabolism studies are shown in Figure 1.

Figure 1 **Metabolic pathway for cyhalothrin (or lambda-cyhalothrin) in rats, dogs and cattle. (Some identifications are tentative. Metabolism by goats is similar)[a]**



[a] Figure provided courtesy of Sponsor.

Food Producing Animals

Cattle

Metabolism

Two lactating cows were given ^{14}C-labelled cyhalothrin orally to determine the metabolism and distribution resulting from a treatment corresponding to the total dose received in a typical dermal application (Harrison, 1984c). Each cow was dosed orally twice daily at 1 mg/kg bw/day for 7 days, one with benzyl-labelled cyhalothrin, the second with cyclopropyl-labelled cyhalothrin. Concentrations of cyhalothrin in blood rose rapidly for the first 50 hrs of the study, then slowly for the remainder of the study. Maximum concentrations of 0.16 and 0.27 mg eq./L, respectively, for the benzyl-label and cyclopropyl-label drug. An equilibrium was observed between ingestion and excretion within 3 days of the start of dosing. Overall, 76-77% of both the benzyl-label and the cyclopropyl-label cyhalothrin residues were accounted for- 49% in faeces, 27% in urine; 0.6% in pen wash; and 0.8%in milk, when the animals were slaughtered 16 hours after the final dose. Parent compound was the major residue in faeces, while CPA glucuronide was the major urinary metabolite from the cyclopropyl-labelled cyhalothrin. The major urinary metabolite from the benzyl-label cyhalothrin was tentatively identified by TLC as the glutamic acid derivative of 3-phenoxybenzoic acid (3-PBA). Parent compound was 11-28% of the residue in kidney, but only 3% of the total residue in liver. CPA and its glucuronide were major metabolites identified from the cyclopropyl-label ^{14}C-cyhalothrin in both liver and kidney. These and other metabolites (some only tentatively) are shown in Figure 1. Residues in milk were determined daily during the study and residues in fat, liver, kidney and muscle were determined at slaughter. Highest total residues of cyhalothrin found in the tissues, resulting from the treatment with the cyclopropyl label, were: perirenal fat, 2.69 mg/kg; subcutaneous fat, 1.61 mg/kg; liver, 1.28 mg/kg; kidney, 0.60 mg/kg; muscle, 0.19 mg/kg. In milk, the highest residue observed was 0.59 mg/kg, from the afternoon milking on the final day of dosing with the cyclopropyl label. All the radiolabel in milk partitioned into the cream, with parent compound accounting for 96% and 88% of the total residues from treatment with the cyclopropyl- and benzyl-label ^{14}C-cyhalothrin, respectively. Parent compound accounted for 85% and 91% of the total residues in fat associated, respectively, with the cyclopropyl- and benzyl-label. drug. The usual 60:40 ratio of A:B isomers was essentially reversed in the perirenal fat and in milk, suggesting that the B isomers are more persistent than the A isomers. The study was GLP compliant.

In another study, two lactating cows were given 15 mL of a pour-on formulation of ^{14}C- lambda-cyhalothrin {*RS*-α-cyano-3-phenoxybenzyl (1*RS*) –*cis*-3-(*Z*-2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropane carboxylate} (containing a 1% benzyl- label) in maize oil and volatile silicone, once daily for 3 days (Dow, et al, 1989). Application was as a streak down the midline of the back and followed the morning milking. The animals were killed after the morning milking, 24 hours after the third application of the formulation. As in the oral dosing study with ^{14}C-cyhalothrin discussed above, the residues in milk were predominantly parent drug (87-113%). The maximum residue found in milk was 0.16 mg/kg, in the final milking. In fat, 80-113% of the total residue was parent compound, while residues in muscle were 80-88% parent compound. Seven components were separated from liver, with parent compound comprising less than 5% of the total residue and the remainder attributed to metabolites or unassigned. At least 6 components were separated in kidney, with parent compound accounting for up to 20% of the total radioactive residue. The main metabolite identified was 3-PBA, accounting for 7% of the total residue in liver and 8% in kidney. Maximum total residues found in tissues were: perirenal fat, 0.15 mg/kg; subcutaneous fat, 0.21 mg/kg; omental fat, 0.12 mg/kg; liver, 0.31 mg/kg; kidney, 0.13 mg/kg; and in muscle, 0.03 mg/kg. This study was also GLP compliant.

Further work was done to identify three unidentified metabolites, designated L1, L2 and L3, separated in the previous study (Dow & Parker, 1989). Following fractionation by HPLC, the metabolites were methylated and studied using mass spectrometry (electron impact direct probe, FAB-MS and GC/MS), in comparison with reference compounds. L3, which accounted for 25% of the total residue in liver, was identified as a glutamic acid conjugate of 3-PBA. Mass spectral data also suggested that L1 (2% of the total residue) was a 3-PBA conjugate. No identification of L2 (11% of the total residue) could be made from the mass spectral data, but the chromatographic behavior suggested a similar structure to L1 and L2. Metabolites L1 and L3 could be analyzed as 3-PBA following acid hydrolysis of samples.

In a related study, two lactating cattle were treated according to the same protocol as described above with 1% cyclopropyl-labelled ^{14}C-lambda-cyhalothrin in maize oil and volatile silicone (Knight et al, 1989). Excreta were not collected in this study. The maximum residue found in milk was 0.15 mg/kg, in the final milking. Maximum total residues found in tissues were: perirenal fat, 0.13 mg/kg; subcutaneous fat, 0.07 mg/kg; omental fat, 0.08 mg/kg; liver, 0.23 mg/kg; kidney, 0.14 mg/kg; muscle, 0.03 mg/kg. Parent compound was the major residue in milk, accounting for 92-100% of the total residue. Parent compound was 85-92% of the total residue in muscle, 10% in liver, 37% in kidney and 54-77% in muscle. The major metabolites in liver and kidney were CPA, hydroxy-CPA and their conjugates.

Goats

A single lactating goat (50 kg bw) was dosed orally at a concentration equivalent to 10.8 mg/kg in the total diet, twice daily at milking for 7 days, with cyclopropyl-labelled ^{14}C-lambda-cyhalothrin formulated in gelatin capsules (Leahey et al, 1985). Milk, urine and faeces were collected throughout the dosing period and until the goat was killed at 16 hrs

after the final dose was administered. No blood was tested in this study. Distribution of total residues was similar to that observed in cattle, with highest residues in perirenal fat (0.44 mg/kg), followed by liver (0.34 mg/kg), omental fat (0.33 mg/kg), kidney (0.20 mg/kg), subcutaneous fat (0.13 mg/kg) and muscle (0.024–0.028 mg/kg). Parent compound accounted for 89% of the total residue in fat, 94% of the total residue in muscle and 6% of the total residue in liver. CPA was the major metabolite in liver (21%, mainly unconjugated) and in kidney (58%, mainly conjugated). Total residues in milk, mainly as parent drug, were from 0.005 mg/kg after the first dose to a maximum of 0.27 mg/kg on day 5, then declining to 0.17 mg/kg in the final sample collected. This report included a statement of GLP compliance.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

No depletion studies using radiolabeled cyhalothrin were reported.

Residue Depletion Studies with Unlabelled Drug

The studies reported in this section were all conducted according to GLP requirements.

Cattle

Twenty-two calves (105-165 kg) were randomly divided into 4 treatment groups (5 animals per group), two control animals (Bicknell et al, 1986) . Each of the animals in the treatment groups received a cyhalothrin ear tag-tape on day 0. In addition, the calves in the treatment groups were sprayed on day 0 with a solution containing 500 mg/L cyhalothrin, prepared from a 20% emulsifiable concentrate with water, corresponding to a dose of about 2 mg cyhalothrin/kg body weight per treatment. Spray treatments were repeated at 14 day intervals until each animal had received a total of 7 treatments (day 84). Treatment groups were then slaughtered at 0, 3, 7 and 14 days following final treatment. The two control animals were maintained and slaughtered separately from the treated animals. Samples of perirenal and subcutaneous fat, liver, kidney and muscle were collected from all carcasses, but only analyses of fat were reported in this study. The analytical method used gas chromatography with electron capture detection, with recoveries of 83% and a limit of detection (LOD) of 0.003 mg/kg. Residues, corrected for recovery, are given in Table 1. Residues of cyhalothrin from the animals in the day 0 withdrawal group, not corrected for recovery, were: 0.005 ± 0.003 mg/kg in liver; 0.010 ± 0.003 mg/kg in kidney; and 0.005± 0.001 mg/kg in muscle(Bicknell et al, 1989a).

Table 1. Residues in cattle fat (mg/kg) using eartags containing cyhalothrin and 7 weekly spray applications (approximate dose of 2 mg/kg body weight per treatment).

	Cyhalothrin residues (mg/kg), n=4	
Withdrawal time (days)	Subcutaneous fat	Perirenal fat
Control	<0.003	<0.003
0	0.26 ± 0.12	0.32 ± 0.13
3	0.15 ± 0.04	0.17 ± 0.07
7	0.19 ± 0.05	0.21 ± 0.05
14	0.12 ± 0.02	0.16 ± 0.03

Twenty-four heifers (250-350 kg BW) were randomly divided into four groups of five animals and two groups of two animals (Bicknell et al, 1987). The four 5-animal groups were each treated with 30 mL of a formulation containing 2% cyhalthrin in maize oil and volatile silicone, applied by syringe along the back, on days 1, 7, 14 and 21. This represents a dose of about 2 mg cyhalothrin/kg BW. In the remaining groups, two animals were treated with 60 mL of the cyhalothrin formulation (about 4 mg cyhalothrin/kg BW) as described, while the second group of two animals was segregated on a separate farm as controls. The control animals were slaughtered separately on day 21. One group of 5 heifers, plus the 2 animals that received the doubled dose of cyhalothrin, were slaughtered at 5-7 h. after the final treatment. The remaining groups of 5 heifers were slaughtered at 3, 7 and 14 days after the final treatment. Samples of subcutaneous and perirenal fat, kidney, liver and quadriceps muscle were collected at slaughter, but only fat and liver were analyzed by gas chromatography with electron capture detection. Mean recoveries on fortified fat samples were 78% at 0.1 and 0.6 mg/kg using the analytical procedure described for fat. The assay for liver gave a mean recovery of 82% at 10-95 mg/kg. The limit of quantitation (LOQ) was 0.01 mg/kg for both fat and liver. The results reported in Table 2 were corrected for recovery.

Table 2. Residues in fat and liver of heifers treated with 4 weekly applications of a 2% cyhalothrin formulation.

Withdrawal period	Treatment	Cyhalothrin Residue Concentration (mg/kg), n=4		
		Subcutaneous fat	Perirenal fat	Liver
Control	None	<0.01	<0.01	<0.01
7 hrs.	30 mL	0.67 ± 0.30	0.91 ± 0.37	<0.01
7 hrs.[1]	60 mL	0.57, 0.68	0.94, 1.14	<0.01, 0.01
3 days	30 mL	0.61 ± 0.14	0.87 ± 0.24	<0.01
7 days	30 mL	0.48 ± 0.09	0.64 ± 0.14	*
14 days	30 mL	0.35 ± 0.17	0.91 ± 0.55	*

Note. [1] only 2 animals in this treatment group;* = not analyzed.

Five dairy cattle (320-392 kg BW) each received two tag-tapes containing cyhalothrin, attached to an ear-tag, while an additional five cattle (260-422 kg BW) were each sprayed 7 times, at 14-day intervals, with a spray solution at about 2 mg cyhalothrin/kg BW (Knight et al, 1985). Milk samples were collected from both groups of animals throughout the study and analyzed by a gas chromatographic method using electron capture detection (LOQ of 0.005 mg/kg, LOD of 0.001 mg/kg and recoveries of 89%). Control milk samples were collected from the animals prior to treatment and from untreated animals. Control samples from the animals selected for treatment with the tag-tapes were estimated to contain approximately 0.002 mg/kg cyhalothrin prior to treatment. 105 milk samples were collected and analyzed following application of the tag-tapes with only 9 of these samples having residues in excess of the LOQ (0.005 mg/kg). The highest residue observed in a milk sample from this treatment group was 0.012 mg/kg. The majority of the 150 milk samples collected and analyzed from the group treated by spray application contained cyhalothrin residues in excess of 0.005 mg/kg, but only 15 samples exceeded 0.01 mg/kg, with the highest concentration found being 0.018 mg/kg. Results were not corrected for recovery in this study.

Five dairy cattle (260-435 kg BW) were each sprayed with 4 liters of an aqueous solution corresponding to a dose of about 0.4 mg cyhalothrin/kg body weight (Bicknell et al, 1989a). These animals were those used in the previous study that received 7 spray treatments, (Knight et al, 1985). There was a 7-day interval between the two studies. The 0.4 mg/kg BW treatment was repeated at 7-day intervals for four spray applications. The animals were slaughtered at zero withdrawal. Milk was collected twice daily during this study and stored in a freezer (-15 to -20 °C) until analyzed. Milk and tissue samples were analyzed by gas chromatography with electron capture detection. (recoveries were 89% for residues in milk, with an LOD of 0.001 mg/kg; in tissues, recoveries ranged from 82% in fat and kidney to 84% in liver and 100% in muscle, with corresponding LODs of 0.005-0.01 mg/kg for the various tissues). Residues in tissues were: 0.10 ± 0.03 mg/kg in perirenal fat; 0.16 ± 0.22 mg/kg in subcutaneous fat; <0.01 mg/kg liver, kidney and muscle: Overall, mean concentrations of cyhalothrin in milk ranged from 0.005 ± 0.001 to 0.008 ± 0.003 mg/kg at the first milking following a spray application and from 0.004 ± 0.001 to 0.008 ± 0.002 mg/kg at the 14th milking . Residues in milk typically peaked at 3-4 days following a spray application, with a maximum mean value of 0.013 ± 0.004 mg/kg observed at the 6th milking, 3 days after the third spray application.

In another study, four dairy cattle (480-535 kg BW) received 4 treatments, at 7-day intervals, of a pour-on formulation containing 2% cyhalothrin in maize oil and volatile silicone, or a dose of about 1.2 mg cyhalothrin/kg BW (Knight et al, 1987a). Two other dairy cattle (415 and 495 kg BW) received the same series of treatments, but with 3.6 mg cyhalothrin/kg BW (treatment 1) followed by three weekly treatments of 2.4 mg cyhalothrin/kg BW (treatments 2-4), of the pour-on product. Control milk was collected from all the animals in the two treatment groups for four days prior to the first administration of pour-on, with additional control milk obtained from untreated animals. Samples were collected at each milking for 35 days following the initial application of cyhalothrin and stored frozen until analyzed. Analysis was by gas chromatography with electron capture detection, similar to that reported in the previous study (Knight et al, 1985). Validation work for this study indicated recoveries of 97 ± 21%, with an LOD of 0.001 mg/kg and an LOQ of 0.009 mg/kg. The Highest residue concentrations were observed at the 3-5 milkings following application of the 1.2 mg/kg BW treatment, with a maximum observed value of 0.31 mg/kg. At this treatment rate, residues were consistently <0.005 mg/kg by the 7th milking following final application. In milk from the cows that received the over-dose treatments, the highest residue observed in milk was 0.47 mg/kg on day 3 following the application of 3.6 mg/kg BW of the product, while the highest residue observed with the 2.4 mg/kg BW formulation was 0.30 mg/kg at the third milking following the first application. Highest residues were observed in each case from 3-7 milkings post-treatment and did not remain below 0.005 mg/kg until 16 milkings after final treatment.

In a subsequent study, five dairy cattle (470-590 kg BW) were treated with a single application of a 2% pour-on solution of cyhalothrin in maize oil and volatile silicone (1.2 mg cyhalothrin/kg BW), applied directly along the mid-line of the back (Bicknell et al, 1989b). Two other cattle served as controls. Samples were collected for analysis at each of the twice-daily milkings for 7 days following treatment and analyzed as in previous studies. Highest residue concentrations of cyhalothrin were found in milkings 3-5 post-treatment, at concentrations ranging from 0.02 – 0.07 mg/kg, falling to <0.01 mg/kg by day 7.

Pig

Twelve healthy pigs (39-50 kg BW) each received a single application of a formulation containing 2% cyhalothrin in maize oil and volatile silicone, administered by syringe as a single streak about 5 cm long on the back of the neck (Bicknell et al, 1988). This corresponds to a dose of about 3 mg cyhalothrin/kg BW. The pigs were randomly divided into 3 groups of 4, then slaughtered at 3, 7 and 14 days after treatment, respectively. Four other pigs served as controls and were slaughtered prior to application of the cyhalothrin. Tissue samples were collected at slaughter and analyzed by gas chromatography with electron capture detection (LOD, 0.001 mg/kg; LOQ, 0.010 mg/kg; recovery 75-97%, depending on tissue). Analytical results were corrected for recovery and are summarized in Table 3.

Table 3. Residues of cyhalothrin in pigs treated with a single dermal application of 2% cyhalothrin in maize oil and volatile silicone to the neck.

Withdrawal Time (days)	Mean concentration of cyhalothrin (mg/kg), n=4						
	Liver	Kidney	Muscle	Subcutaneous fat	Abdominal fat	Skin (flank)	Skin (Application site)
Controls	<0.01	<0.01	<0.01	<0.01	na	<0.01	na
3	<0.01	<0.01	<0.01	0.08 ±0.05	0.08 ± 0.10	0.23 ± 0.04	0.82 ± 0.44
7	-na	na	na	0.04 ±0.02	0.05 ± 0.04	0.15 ± 0.03	0.35 ± 0.20
14	na	na	na	<0.01	0.04 ± 0.01	0.09 ± 0.08	0.33 ± 0.13

Note. na, means not analyzed.

Sheep

Fifteen sheep (28-36 kg BW) were randomly divided into 5 groups of 3 each. One group served as controls and sheep in the remaining 4 groups each received 3 applications of cyhalothrin in maize oil and volatile silicone, at 14 day intervals, administered by syringe directly onto the skin down the midline of the back (Knight et al, 1987b). This corresponds to a dose of about 2.25 mg cyhalothrin/kg BW. The treated animals were killed in groups at 16 h, 3, 7 and 14 days after the third application and tissue samples were collected for analysis. The animals in the control group were killed prior to the commencement of the treatments. Analysis was by gas chromatography with electron capture detection ((LOD, 0.001 mg/kg; LOQ, 0.01 mg/kg for fat, kidney, muscle, and 0.05 mg/kg for liver; recovery 78-84%, depending on tissue). Residues were measured in tissues and are reported in Table 4.

Table 4. Residues in sheep administered 3 treatments of a pour-on formulation of 2% cyhalothrin in maize oil and volatile silicone.

Withdrawal time	Cyhalothrin residues found in tissues (mg/kg), n = 3				
	Liver	Kidney	Muscle	Subcutaneous fat	Perirenal fat
Controls	<0.05	<0.01	<0.01	<0.01	<0.01
16 hrs	<0.05	<0.01	<0.01	0.04 – 0.13	0.03 – 0.08
3 days	<0.05	<0.01	<0.01	0.03 – 0.05	0.03 – 0.05
7 days	<0.05	<0.01	<0.01	0.03 – 0.08	0.06 – 0.08
14 days	<0.05	<0.01	<0.01	<0.01	0.04 – 0.10

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

The sponsor has provided an analytical method originally developed for the analysis of fat, with extensions to other tissues, based on gas chromatography with electron capture detection (Knight and Parker, 1988). A 20 g fat sample is mixed with 5 g anhydrous sodium sulphate and acetone-hexane (1:1) and homogenized, warmed in a water bath at 60°C to dissolve the fat, then partitioned with water. Following solvent partition the hexane extract is transferred to a cyano-bonded SPE cartridge, and cyhalothrin is eluted with 10% THF in hexane. Analyis is by gas chromatography using a 1.5 m x 4 mm id column packed with 5% OV-101 on 60-80 mesh Gas Chrom Q, with nitrogen carrier gas at 20 mL/min flow. The recommended injection volume is 5 µL. A typical retention time for cyhalothrin under these conditions is 2.8 min. Muscle samples are also analyzed using this procedure.

For liver, the method is modified by adding 5 g celite and 20 g anhydrous sodium sulphate to the initial 20 g sample of liver. Following homogenization with acetonitrile, the sample is further purified on a florisil column and analyzed by

gas chromatography, as above. For kidney samples, the procedure for fat is followed to the column clean-up step, then the clean-up using the florisil column for liver is used.

A confirmatory procedure has also been described for residues of cyhalothrin in fat. Following the purification for gas chromatographic analysis, an additional clean-up step is added using a florisil column, prepared as described in the procedure for liver analysis. The final extract is added to the florisil column and eluted. The GC/MS analysis is conducted using a 25 m x 0.32 mm id fused silica column coated with SE54, or equivalent, with a helium flow rate of 1 mL/min. Sample injection is at near-ambient temperature, using an oven temperature . The ion fragments detected are m/z 197, 199 and 208.

Similar methodology has been reported for the analysis of milk samples (Bicknell et al, 1989a). Whole milk (100 mL) is freeze-dried and extracted with hexane. Cyhalothrin is then partitioned into DMF and 1% aqueous sodium sulphate is added to the DMF, after which the mixture is again extracted with hexane. Following solvent partition, this residue eluate is transferred to a cyano-bonded SPE cartridge as described above in the procedure for fat. Conditions for gas chromatographic analysis are as described for fat samples.

Variations on the methodology have been used in the various depletion studies reported by the sponsor, with method performance tests included in each report. The performance of the GC methodology used for various matrices in these studies has been summarized in Table 5.

Table 5. Method performance characteristics of the gas chromatographic methods for various sample matrices as reported in depletion trials.

Species	Matrix (Tissue or Milk)	Analytical Range (mg/kg)	Recovery (%)	LOD* (mg/kg)	LOQ** (mg/kg)	Precision over Analytical Range (%)
Bovine	Milk	0.01–0.05 to 0.01–0.47	74–97	0.001	0.01	8–25
	Liver	0.01–0.11	82–84	0.003–0.005	0.01	7–21
	Kidney	0.01–0.05	82	0.005	0.01	12
	Muscle	0.01–0.05	100	0.005	0.01	9
	Fat	0.01–0.62	78–83	0.001–0.003	0.01	11–13
Porcine	Liver	0.01–0.60	97	0.001	0.01	6
	Kidney	0.01–0.60	86	0.001	0.01	10
	Muscle	0.01–0.60	80	0.001	0.01	10
	Fat	0.01–0.60	89	0.001	0.01	11
	Skin	0.01–0.60	75	0.001	0.01	10
Ovine	Liver	0.05–0.09	84	0.001	0.05	5
	Kidney	0.01–0.09	79	0.001	0.01	9
	Muscle	0.01–0.09	84	0.001	0.01	12
	Fat	0.01–0.40	78	0.001	0.01	32

*Determined statistically from linear regression approach using control and fortified samples.
** The lowest concentration at which recovery and precision were investigated.

More recently, a method for the analysis of cyhalothrin residues in milk using negative ion chemical ionization gas chromatography-mass spectrometry (GC/MS-NICI) has been proposed (McCormack, 1999a). A 1 g sub-sample of milk is mixed with 20 mL acetonitrile using ultrasonication for 10 min, then left to stand in a water bath at 50°C for 15 min. The homogenate is centrifuged and the supernatant liquid is decanted. The extract is washed with 20 mL hexane. The acetonitrile solution is reduced to <10 mL at 40 °C and cleaned up on a C-18 solid phase extraction cartridge. The eluate from the cartridge containing cyhalothrin is further cleaned up using a florisil cartridge. GC/MS analysis is conducted using a 30m x 0.32 mm id CP-SIL 8 column (0.25 µm film thickness. A flow rate of 2 mL/min helium (20:1 v/v split) is used, with methane as reagent gas. The sample injection volume is 3 µL. Cyhalothrin is detected as two pairs of enantiomers, at 11.6 and 11.8 min, monitored as the fragment ion m/z 205. The method also has been applied to bovine muscle, liver, kidney and fat, with variations in the clean-up procedure according to the tissue analyzed. In a second report, the method was applied to ovine muscle, liver, kidney and fat (McCormack, 1999b). Performance characteristics of the GC/MS-NICI method for various matrices are given in Table 6.

Table 6. Performance characteristics reported for GC/MS-NICI determination of cyhalothrin residues in bovine milk and tissues and ovine tissues.

Species	Matrix (Tissue or Milk)	Analytical Range (mg/kg)	Recovery (%)	LOD* (mg/kg)	LOQ** (mg/kg)	Precision at LOQ (%)
Bovine	Milk	0.01–0.04	96	0.004	0.01	18
	Liver	0.02–0.10	95	0.02	0.02	24
	Kidney	0.02–0.10	88	0.02	0.02	20
	Muscle	0.02–0.10	80	0.02	0.02	24
	Fat	0.25–1.0	75	0.05	0.25	8
Ovine	Liver	0.02–0.10	85	0.02	0.02	28
	Kidney	0.02–0.10	97	0.01	0.02	20
	Muscle	0.02–0.10	97	0.02	0.02	22
	Fat	0.25–1.0	100	0.17	0.25	32

*Determined statistically from linear regression approach using control and fortified samples.
** The lowest concentration at which recovery and precision were investigated.

APPRAISAL

Cyhalothrin is a synthetic pyrethroid insecticide comprised of of two pairs of enantiomers, designated **A** and **B**, in an approximately 60:40 ratio (**A**:**B**). This ratio is usually reversed in residues found in fatty tissues. The product is intended for dermal application as a pour-on, dip or spray, with some potential oral intake from treated animals through grooming. Cyhalothrin may also be registered for horticultural uses and some oral ingestion may occur through feeds, but no specific information was provided on such exposure. Any MRLs assigned by JMPR, and their contribution to the TMDI, need to be considered in assigning MRLs for veterinary use. Cyhalothrin is also a compound with a long history of use that could be considered under JECFA provisions for such compounds.

Information was provided on metabolism and distribution in both laboratory animals (rats, dogs) and food animals (cattle, sheep, goat), with generally consistent results across species . Cyhalothrin is excreted via urine and faeces, with <5% of the total dose remaining as a residue in treated animals. The highest residues of parent compound are found in fat, with concentrations generally being higher in renal fat than in other fatty deposits. Residues are much lower in kidney and muscle and are also primarily parent compound. Cyhalothrin is excreted in milk as parent compound and is associated with the fatty constituents of the milk. Metabolism occurs primarily in the liver, where radiolabel studies indicate the highest concentrations of total residue, but very little parent compound. Metabolism and distrtibution are similar to that observed for other synthetic pyrethroids Some of the studies on metabolism and distribution were conducted with lambda-cyhalothrin, a product with different toxicity but similar metabolism to cyhalothrin. It appears appropriate to use these studies to support the information gained in studies with cyhalothrin.

Adequate data were provided to recommend maximum residue limits (MRLs) for cattle, swine and sheep, where GLP studies with unlabelled compound were provided. The marker residue in all species is parent compound and fat (preferably renal) is recommended as the target tissue for testing, both in national residue control programs and for tissues in trade.

Performance data were provided for both GC and GC/MS determinative methods and for GC/MS confirmation of residues. While the methods require a relatively involved clean-up, they are well within the capabilities of a normally well-equippped residue laboratory.

Based on the data provided, MRLs can be assigned for edible tissues of cattle, swine and sheep, and for milk from cattle. The single metabolic study with radiolabelled lambda-cyhalothrin is not sufficient information on which to extend MRLs to goats.

MAXIMUM RESIDUE LIMITS

In considering the recommendation of Maximum Residue Limits, the Committee took into account the following:

- A temporary ADI of 0-2 μg/kg of body weight was recommended by the Committee, based on a toxicological endpoint, that allows for a maximum daily intake of 120 μg for a 60 kg person.
- The appropriate marker residue of cyhalothrin is the sum of the isomers, as previously established by JMPR.
- Cyhalothrin isomers account for less than 5% of the total residue found in liver of cattle and about 10 % or more of the total residue found in kidney. The metabolites are considered two-fold less toxic than the parent compound and

were accounted for using a factor of 16 for liver and 5 for kidney to adjust the marker residue to total residues in calculating the theoretical daily intake.

- Residues found in muscle, fat and milk are parent compound.
- The weekly application of the pour-on formulation to cattle and the volumes of this product used in excess of 10 mL leads to much higher residue concentrations in fat and milk than those found in the other residue depletion studies. While these uses may fall within the range of recommended applications for cyhalothrin, they were not considered as a suitable basis for the establishment of MRLs as they represent extreme uses. Use of the pour-on product can result in residue concentrations in excess of the MRL in milk.
- The maximum intakes assigned for horticultural use by JMPR account for 10% or less of the temporary ADI established by the Committee.
- A suitable analytical method is available for analysis of cyhalothrin residues in edible tissues and milk.
- MRLs for liver, kidney and muscle can be harmonized at twice the LOQ of the analytical method as validated for tissues from cattle and pigs.
- MRL recommended for fat are based on the highest mean residues, plus 3 standard deviations, as determined in depletion studies using treatments consistent with good practice in the use of veterinary drugs.
- The MRL recommended for milk is based on the highest mean residues, plus 3 standard deviations, as determined in depletion studies which used treatments with the spray formulation consistent with good practice in the use of veterinary drugs.

On the basis of the above considerations, the Committee recommended the following temporary MRLs for edible tissues of cattle, pigs and sheep, expressed as parent drug; muscle, 20 μg/kg; liver, 20 μg/kg; kidney 20 μg/kg; and fat, 400 μg/kg. The Committee also recommended a temporary MRL of 30 μg/kg for cyhalothrin in milk from cattle.

Based on consumption of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat and 1.5 kg of milk and using the factors for liver and kidney as given above, the theoretical maximum daily intake of cyhalothrin residues from veterinary use is 108 μg. The remainder of the ADI has been allocated to other uses by the JMPR.

Table 7. Theoretical maximum daily intake (TMDI) of cyhalothrin residues

Food Item	MRL (μg/kg)	Food Basket (g)	μg	MR/TR[1]	TMDI (μg)
Muscle	20	300	6	1	6
Liver	20	100	2	1/16	32
Kidney	20	50	1	1/5	5
Fat	400	50	20	1	20
Milk	30	1500	45	1	45
Total:					108

Note: [1] MR = marker residue (parent drug); TR = total residues

REFERENCES

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1986). Residues of cyhalothrin in bodyfat of calves following multiple sprayings of 500 ppm cyhalothrin ex 20%E.C. and season long application of tap-tapes. Coopers Animal Health, Report CIBH 85-C4, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1987). Residues of cyhalothrin in bodyfat and liver of cattle following multiple treatment with a 2% cyhalothrin in maize oil and volatile silicone pour-on. Coopers Animal Health, Report CIBH 87-C4, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1988). Residues of cyhalothrin in the skin and tissues of pigs following treatment with a 2% cyhalothrin in maize oil and volatile silicone pour-on. Coopers Animal Health, Report CIBH 87-C5, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1989a). Residues of cyhalothrin in bovine milk and tissues following multiple sprayings of 250 ppm cyhalothrin ex 20%E.C. Coopers Animal Health, Report CIBH 85-C6, Sponsor Submitted.

Bicknell, C.E., Knight, P.J., Parker, R.C., and Woollon, R.M. (1989b). Milk residues of cyhalothrin following a single application of a 2% pour-on. Coopers Animal Health, Report CIBH 89-1, Sponsor Submitted.

Dow, P.R., Knight, P.J., Parker, R.C., and Woollon, R.M. (1989). Metabolism of C-14 alcohol labelled lambda-cyhalothrin in lactating cows following dermal application. Coopers Animal Health, Report CIBH 88-4, Sponsor Submitted.

Dow, P.R., and Parker, R.C. (1989). Attempted identification of 3 major liver metabolites following dermal application of C-14 alcohol lambda-cyhalothrin to lactating cows. Coopers Animal Health, Report CIBH 89-2, Sponsor Submitted.

Harrison, M.P. (1981a). Cyhalothrin: the metabolism and disposition of carbon-14 labelled material in rats; Part I. Imperial Chemical Industries Report YIBH 85-C6, Sponsor Submitted.

Harrison, M.P. (1981b). Cyhalothrin (ICI 146,814): the metabolism and disposition of carbon-14 labelled material in rats; Part II. Tissue residues derived from carbon-14 benzyl or carbon-14 cyclopropyl labelled cyhalothrin after a single oral dose of 1 or 25 mg/kg. Imperial Chemical Industries Report YIBH 85-C7, Sponsor Submitted.

Harrison, M.P. (1983). Cyhalothrin:. the metabolism and disposition of carbon-14 labelled material in rats; Part IV. Isolation and identification of the major urinary metabolites derived from carbon-14 labelled benzyl or carbon-14 labelled cyclopropyl-cyhalothrin following oral administration. Imperial Chemical Industries Report YIBH 85-C9, Sponsor Submitted.

Harrison, M.P. (1984a). Cyhalothrin: the metabolism and disposition of carbon-14 labelled material in rats; Part III. Studies to determine radioactive residues in the rat following 14 days repeated oral administration. Imperial Chemical Industries Report YIBH 85-C8, Sponsor Submitted.

Harrison, M.P. (1984b). Cyhalothrin (ICI 146,814): the disposition and metabolism of carbon-14 labelled material in the dog. Imperial Chemical Industries Report YIBH 85-C10, Sponsor Submitted.

Harrison, M.P. (1984c). Cyhalothrin (ICI 146,814): the metabolism, excretion and residues in the cow after 7 days oral administration with two carbon-14 labelled forms of cyhalothrin at 1 mg/kg/day. Imperial Chemical Industries Report YIBH 85-C11, Sponsor Submitted.

Knight, P.J., Parker, R.C., Winter, R.L, and Woollon, R.M. (1985) Residues of cyhalothrin in bovine milk following multiple sprayings of 500 ppm cyhalothrin ex 20% E.C. or application of ear tag-tapes. Coopers Animal Health, Report CIBH 85-C3, Sponsor Submitted.

Knight, P.J., Mallard, K.L., Parker, R.C. and Woollon, R.M. (1987a) Residues of cyhalothrin in bovine milk following weekly application of a 2% cyhalothrin pour-on. Coopers Animal Health, Report CIBH 87-C3, Sponsor Submitted.

Knight, P.J., Parker, R.C., and Woollon, R.M. (1987b). Residues of cyhalothrin in sheep tissue following multiple treatment with a 2% cyhalothrin in maize oil and volatile silicone pour-on. Coopers Animal Health, Report CIBH 87-C2, Sponsor Submitted.

Knight, P.J, and Parker, R.C. (1988). A method for the analysis of cyhalothrin in animal tissues. Coopers Animal Health, Report CDBT 87-1, Sponsor Submitted.

Knight, P.J., Parker, R.C., and Woollon, R.M. (1989). Metabolism of C-14 acid labelled lambda-cyhalothrin in lactating cows following dermal application. Coopers Animal Health, Report CIBH 88-4, Sponsor Submitted.

Leahey, J.P., French, D.A., and Heath, J. (1985). PP321: Metabolism in a goat. ICI Plant Protection Division Report YIBH 85-C12, Sponsor Submitted.

McCormack, A. (1999a). Cyhalothrin: validation of an analytical method for determination in bovine edible tissues and milk. Report 808/115-D2140, Sponsor Submitted.

McCormack, A. (1999b). Cyhalothrin: validation of an analytical method for determination in ovine edible tissues. Report 808/116-D2140, Sponsor Submitted.