Bull. Environ. Contam. Toxicol. (1994) 53:907–912

Environmental Contamination and Toxicology

# Nonextractable (Bound) Residues of Cypermethrin in Soils

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Received: 5 December 1993/Accepted: 30 April 1994

Cypermethrin [(S,R)-∝-cyano-3-phenoxybenzyl (IR, IS, cis, trans) -2,2- dimethyl-3-(2,2-dichlorovinyl) cyclopropane carboxylate] is a highly potent synthetic pyrethroid insecticide widely used in agriculture. The major degradation route of cypermethrin in soils is by hydrolysis leading to the formation of 3-(2,2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylic acid ($Cl_2CA$) and 3-phenoxybenzoic acid (PB Acid) which under aerobic conditions may undergo further extensive degradation with the formation of $CO_2$ (Roberts and Standen, 1977). Furthermore, the metabolism of $^{14}C$-cypermethrin in soils has been shown to result in the formation of $^{14}C$-residues that are not extracted with solvent systems and methods devised for routine residue analysis of this insecticide from soils (Robert and Standen, 1977; 1981; Sakata et al., 1986). These nonextractable residues referred as "bound residues" were shown to be released in the form of $^{14}CO_2$ from soil (Robert and Standen, 1980). Musumeci et al. (1992) also observed the formation of bound $^{14}C$ residues in two different Brazilian soils treated with $^{14}C$-cypermethrin which were released and metabolized by microorganism. However, the identity or nature of the bound $^{14}C$-residues formed was not known. The present work is an extension of this investigation and describes our attempt to determine the nature of bound $^{14}C$-residues in the two Brazilian soils. Our approach to this problem consisted of extracting the soils containing bound $^{14}C$-residues by using two techniques, namely supercritical fluid extraction (SFE) and high temperature distillation (HTD). The extracted material was characterized by chromatographic techniques.

## MATERIALS AND METHODS

Chemicals: Cypermethrin (uniformly $^{14}C$ labeled in the benzyl ring) was supplied by Shell Bioscience Laboratory, England. The radiochemical purity and specific activity of cypermethrin were 94% and 22.5 $\mu$Ci/mg, respectively.

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All solvents used were of pesticide grade and were used as received.

Soils: Two Brazilian soils, a Red Yellow Latosol (RYL) (pH 5.1; organic matter 1.3%; clay 75%, silt 15%, sand 10%) and a Humic Gley (HG) (pH 6.5; organic matter 8.8%; clay 47%; silt 33%, sand 20%) were collected (0-15 cm) from a non-cultivated area in the Sao Paulo State. The soils were air dried and passed through a 2 mm sieve before use.

Soil Incubation and Extraction: RYL and HG soil samples (20.0 g, oven dry basis) at 70% field moisture capacity were treated with $^{14}$C-cypermethrin and unlabeled cypermethrin to give an insecticide concentration of 10$\mu$g/g. The solvent was evaporated with a gentle air stream and the soil was throroughly mixed. The samples were then incubated aerobically for a period of 120 days. After the incubation period the soils were extracted two times with a mixture of hexane: acetone (7:3) and then subjected to soxhlet methanol extraction to remove all the extractable $^{14}$C residues. The extracted soil material was air-dried to remove methanol and analyzed for bound $^{14}$C residues described below.

Determination of Radioactivity: Combustion of dried soil was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. The latter was absorbed in and admixed with appropriate volume of Carbo-Sorb and Permafluor-V. Aliquots of these solutions or of various extracts obtained by SFE or HTD described below were analyzed by liquid scintillation counting (LSC) using Beckman LS 3801 Liquid Scintillator Spectrometer.

Supercritical Fluid Extraction (SFE): A Supercritical Fluid Extractor Model SFE-50 (Suprex Corp., Pittsburg, PA) equipped with a 5ml stainless steel vessel containing sample was used to extract the bound $^{14}$C residues from soils. After equilibrating the oven to an appropriate temperature and the $CO_2$ syringe pump at 100 atm, the samples were extracted dynamically through 30$\mu$ fused silica tubing vented into the graduated tubes containing methanol. The extraction conditions for the two soils were: (i) RYL: 2 1/2 hours of extraction; mobile phase - $CO_2$ = 1.1 ml/min, methanol: water (85:15) = 0.8 ml/min; (ii) HG: 6 hours of extraction; mobile phase: $CO_2$ = 1.8 ml/min, methanol: water (85:15) = 0.7 ml/min. Aliquot of the extracts were radioassayed by LSC, concentrated to a small volume and chromatographically analyzed.

High Temperature Distillation (HTD): A Lindberg Tube furnace (Sola Basic S/B) was used for high-temperature Distillation (HTD) as described earlier (Khan and Hamilton, 1980). The released $^{14}$C material was collected in 4 different traps. The first three traps contained hexane, acetone and methanol, respectively. The last trap contained Carbosorb to capture released $^{14}CO_2$. At the end of distillation the quartz tube was washed with methanol, and an aliquot from each trap and washings was analyzed by LSC. The solvent traps were combined, concentrated to a small volume and subjected to chromatographic analysis.

Chromatographic Analysis: High-Performance Liquid Chromatography (HPLC) - A varian Model Vista 5500 equipped with a variable-wavelength UV detector (λ-243 mm) and a radioactivity monitor and data station (Berthold LB504 and LB512) was used. The operating conditions were as follow: injector volume - 20 $\mu$l; solid phase - Ultrasphere ODS, 5 $\mu$m, 25 cm x 4.6 mm i.d.; guard column - adsosbosphere C18 5 $\mu$m; flow rate - 1.1 ml/min. Two mobile phases were used: (a) for cypermethrin - methanol: water (85:15); (b) for metabolites - methanol: water (75:25).

Thin-Layer chromatography (TLC) - The extracted $^{14}C$ material was applied to 20 x 20 cm PLK5F linear-K silica gel TLC plate (Whatman), and developed in a solvent system consisting of toluene: ethyl ether: acetic acid (75:25:1). The developed TLC plates were photographed by a Berthold Beta camera LB 292 to detect radioactivity regions. These regions were scrapped off the plate, extracted with (i) hexane: acetone (1:1), (ii) acetone, and (iii) acetone: methanol (1:1). The extracted material was analyzed by HPLC as described above.

Gas Chromatography (GC) - The Gas Chromatograph was a Varian Model 3400 with $^{63}Ni$ detector equipped with an SE-30 coating thickness 0.25 $\mu$m) fused silica capillary column (30 m x 0.26 mm). The oven temperature was programmed at 5°C/min from 80°C to 290°C. The detector and the injector temperatures were 320°C and 180°C, respectively. The carrier gas was nitrogen at a flow rate of 20 ml/min.

## RESULTS AND DISCUSSION

Table 1 shows the recovery by SFE of $^{14}C$-cypermethrin spiked onto the two Brazilian control soils. Under the experimental conditions used the recoveries of $^{14}C$ ranged from 93.1 to 97.9%. It was observed that at high pressure (400 atm) and temperature (190°C) almost 50% of the parent compound in RYL soil was decomposed to $Cl_2CA$ (Table 1). However, no such breakdown of cypermethrin in this soil was observed when SFE was carried out at 365 atm and 145°C. The insecticide in HG soil was very stable when subjected to SFE and suffered no breakdown under the experimental conditions used (Table 1). Therefore, in subsequent studies the incubated soil samples containing

Table 1. Recovery of $^{14}C$-residues from control soils fortified with $^{14}C$-cypermethrin and extracted with supercritical fluid extraction (SFE).

| Soil | $^{14}C$-cypermethrin Added (dpm/g) x10$^{3}$ | % $^{14}C$ Recovery | Compounds Identified |
|---|---|---|---|
| RYL[a] | 218 | 93.1 | Cypermethrin |
| RYL[b] | 42.4 | 97.9 | Cypermethrin<br>$Cl_2CA$ |
| RYL[b] | 84.8 | 94.0 | Cypermethrin<br>$Cl_2CA$ |
| HG[a] | 218 | 93.6 | Cypermethrin |
| HG[b] | 118 | 96.4 | Cypermethrin |

[a] 365 atm., 145°C
[b] 400 atm., 190°C

bound $^{14}C$ residues were subjected to SFE under the operating conditions at which no breakdown of the compound occurred.

Table 2 shows the release of bound $^{14}C$ by SFE from the two soils. SFE did not result in quantitative release of bound $^{14}C$ from the soils. It was observed that the recovery of $^{14}C$ from the RYL soil was rather very low. Treatment of soil containing bound $^{14}C$ residues with water or 0.1N NaOH prior to SFE or change in pressure and/or temperature during SFE did not result in any further release of $^{14}C$. The reason for the low recoveries of bound $^{14}C$ residues from the two soils by SFE is not known. The released $^{14}C$ material was identified as 3PB acid in RYL soil; and 3PB acid and 3-Phenoxybenzaldehyde (PB ald) in HG soil. In addition, the SFE extracts from both soils contained an unknown compound (10-15%) which could not be identified by the experimental techniques used in this study.

Extraction of bound $^{14}C$ residues from the two soils by HTD was not successful. During the HTD of RYL soil, almost all the bound $^{14}C$ residues were thermally decomposed to $^{14}CO_2$. The amount of $^{14}C$ recovered in the trapping solutions was too small to be identified by chromatographic techniques. More than 50% of the bound $^{14}C$ residues was not released by HTD from HG soil. Furthermore, about 16% was thermally decomposed to $^{14}CO_2$

Table 2. Bound $^{14}$C-residues in $^{14}$C-cypermethrin treated soils extracted with supercritical fluid extraction (SFE).

| Soil | $^{14}$C-Residues[a] (dpm/g) x10$^3$ | % $^{14}$C Release by SFE | Compounds Identified |
|---|---|---|---|
| HG | 10 | 30[b] | 3PB acid<br>UR[c] |
| RYL | 18 | 69[b] | 3PB acid<br>3PB ald<br>UR[c] |

[a] Total bound $^{14}$C-residues determined by combustion.

[b] Average of 10 samples extracted under different SFE conditions (RYL range 20-40%; HG range 60-78%).

[c] Unidentified $^{14}$C residues.

during HTD and about 20% collected in the trapping solutions which could not be identified by the techniques used in this study.

The HG and RYL soils from Brazil contained 30% and 69% bound $^{14}$C residues of the initially applied $^{14}$C-cypermethrin. These bound $^{14}$C residues may be distributed among the various soil organic matter fractions (Roberts and Standen, 1977, Sakata et al., 1986). The bound $^{14}$C residues in HG soil constituted mainly 3PBacid and an unknown product. However, the RYL soil contained 3PBacid and 3PBald in addition to an unknown compound in the form of bound residues. These bound residues resulting from cypermethrin treatment of the two Brazilian soils may not be extracted when methods commonly used for residue analysis of this insecticide are employed. The biological or ecological significance of 3PBacid and 3PBald present in the soils in the form of bound residues is not known. However, it has been shown that microbiological processes, changes in soil chemistry due to environmental changes, or changes in agricultural practices, may release these bound residues in soil (Calderbank, 1989). Harris et al (1981) reported that cypermethrin was not inactivated when applied to soils and a higher fungitoxicity for 3PBacid and 3PBald was observed (Straton & Corke, 1982).

Acknowledgments. This study was undertaken during the training fellowship program of the senior author (S.B.O.) sponsored by the International Atomic Energy Agency, Vienna, Austria.

## REFERENCES

Calderbank A (1989) The occurrence and significance of bound pesticide residues in soil. Rev Environ Contam Toxicol 108:71-103

Harris CR, Chapman RA, Harris C (1981) Laboratory studies on the persistence and behavior in soil of 4 pyrethroid insecticides. Can Entomol 113:684-694

Khan SU, Hamilton HA (1980) Extractable and bound (nonextractable) residues of promethryn and its metabolites in an organic soil. J Agric Food Chem 28:126-132

Musumeci MR, Tomita RY, Silva MCD, Sampaio MRFD (1992) Formacao E biodisponibilidade de residuos de piretroides - $^{14}C$ ligados ao solo. Pesq Agropec Bras 27:265-270

Robert TR, Standen ME (1977) Degradation of the pyrethroid cypermethrin NRDC 149 (±) -∝-cyano-3-phenoxybenzyl (±)-cis, trans-3-(2,2-dichloro-vinyl-2,2 dimethyl cyclopropane-carboxylate and the respective cis-NRDC 160) and the trans- (NRDC 159) isomers in soils. Pestic Sci 8:305-319

Robert TR, Standen ME (1981) Further studies of the degradation of pyrethroid insecticide cypermethrin in soils. Pestic Sci 12:285-296

Sakata S, Mikami N, Matsuda T, Miyamoto J (1986) Degradation and leaching behavior of the pyrethroid insecticide cypermethrin in soils. J Pestic Sci 11:71-79

Stratton GW, Corke CT (1982) Comparative fungi toxicity of the insecticide permethrin and ten degradation products. Pestic Sci 13:679-685