J. Pestic. Sci., **32**(4), 393–401 (2007)
DOI: 10.1584/jpestics.G07-14

Original Article

Aerobic metabolism and adsorption of pyrethroid insecticide metofluthrin in soil

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(Received May 11, 2007; Accepted August 23, 2007)

Metofluthrin [2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-((1*EZ*)-prop-1-enyl)cyclopropanecarboxylate] was rapidly degraded in two aerobic US soils with first-order half-lives of 2.3–3.5 days primarily *via* ester cleavage to give the corresponding acid and alcohol without any epimerization and geometrical isomerization. The rapid oxidation proceeded either at the prop-1-enyl group of the acid to moiety form the diacid derivative or the benzyl carbon to finally give the terephthalic acid derivative. These metabolites were finally mineralized to carbon dioxide with partial formation of bound residues. The soil adsorption coefficients (K_{oc}) of the *Z* isomer, the main component of metofluthrin, in three German soils were determined to be 3553–6124 (ml/g o.c.) by the batch equilibrium method. The screening groundwater simulation model SCI-GROW using the metabolic half-lives and K_{oc} values clearly indicates that metofluthrin is most unlikely to contaminate groundwater even in the unrealistic worst case. ©Pesticide Science Society of Japan

Keywords: metofluthrin, soil metabolism, soil adsorption.

Introduction

The new pyrethroid insecticide metofluthrin [SumiOne®; 2,3,5,6-tetrafluoro-4-(methoxymethyl)benzyl (1*R*,3*R*)-2,2-dimethyl-3-((1*EZ*)-prop-1-enyl)cyclopropanecarboxylate] is now under worldwide development for environmental health use.[1,2)]

Metofluthrin has extremely high knockdown activity, especially against mosquitoes, and its relatively high volatility (1.96×10^{-3} Pa at 25°C) as well as low mammalian toxicity[2)] made it possible to develop new products such as a fan vaporizer, and paper and resin emanators, in addition to the existing coil and liquid vaporizer. Household and public hygiene usage restricts its contamination of the environment at minimum but indirect deposit on soil when metofluthrin is used outdoors may be envisaged due to its high volatility as compared with other pyrethroids.[3)] The environmental profiles of various pyrethroids for agricultural use have been extensively studied and their moderate biodegradability in aerobic soil with low mobility due to their high adsorptivity are well known.[3,4)] In contrast to these pyrethroids, metofluthrin has a unique chemical structure both in acid and alcohol moieties and therefore, its metabolism in soil together with soil adsorptivity should be examined from the viewpoint of environmental safety. Although its contamination of soil is very limited, a risk assessment of the unexpected contamination in outdoor usage should be conducted. Pyrethroids usually undergo microbial hydrolysis of ester linkage together with hydroxylation of aromatic rings[4)] but the degradation pathway, especially on the chrysanthemic acid moiety, is only predicted according to known microbial reactions.[5)] The most probable oxidation of the isobutenyl moiety was confirmed in plant metabolism for phenothrin[6)] but not its soil metabolism. Paingankar *et al.*[7)] have recently reported dehydrogenation of the acid moiety of allethrin by *Acidomonas* sp. but the oxidative pathway was not reported. The microbial degradation of a polyfluorinated aromatic moiety has been less examined, except for tefluthrin with an analogous alcohol moiety.[8)]

The objective of this study was to determine the metabolic and adsorptive profiles of metofluthrin using typical agricultural soils. Metofluthrin has two optical centers and geometrical isomerism in the acid moiety, which results in possible eight isomers. Among them, two biologically more active isomers having 1*R-trans* isomerism at the cyclopropyl ring and *E*/*Z* isomerism in the propenyl side chain (abbreviated "*RTZ*" and "*RTE*") are the active ingredients, and their *E*/*Z* composition is approximately 1 : 8,[2)] therefore, two isomers individually ^{14}C-labeled in acid and alcohol moieties were subjected

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to aerobic soil metabolism and their soil adsorptivity was examined for the major isomer *RTZ*.

Materials and Methods

1. Chemicals

RTZ and *RTE* isomers of metofluthrin, separately labeled with ^{14}C at the α-position of the 2,3,5,6-tetrafluoro-4-methoxymethylbenzyl group [Alc-^{14}C] and carbonyl carbon [Acid-^{14}C], were synthesized in our laboratory (Fig. 5). The specific activities and radiochemical purities of both isomers were 6.19 MBq/mg and 99.3–99.4%, and 6.11 MBq/mg and 98.9–99.9%, respectively. The non-radiolabeled isomers of metofluthrin, the corresponding alcohol moiety (I) and chrysanthemic acid derivatives, **IV** and **V**, whose chemical structures are shown in Fig. 5, were synthesized in our laboratory.[1)] Compound **VI** was synthesized from (1*R*)-*trans*-norchrysanthemic acid. The corresponding *tert*-butyl ester prepared in the usual manner was oxidized in aqueous butanol by $KMnO_4/NaIO_4$ in the presence of K_2CO_3. The resulting mono-ester was hydrolyzed by TsOH in toluene under heating. **VI**: MS *m/z*: 157 (M−H); ^{1}H-NMR δ_H (CD_3OD): 1.30 (6H, s, CH_3), 2.09 (2H, s, cyclopropyl-H). The corresponding acid of **I** was synthesized from 2,3,5,6-pentafluorobenzaldehyde. Another chrysanthemic acid derivative **VII** was synthesized from methyl (1*R*)-*trans*-chrysanthemate *via* oxidation similarly as **VI**; **VII**: MS *m/z*: 173 (M+H); ^{1}H-NMR δ_H ($CDCl_3$): 1.32 (6H, dd, J=1.5 Hz, CH_3), 2.25 (2H, t, 1.5 Hz, cyclopropyl-H), 3.61 (3H, d, J=1.5 Hz, CH_3O). The benzaldehyde was treated with lithium bromide under heating, which led to the formation of 4-bromo-2,3,5,6-tetrafluorobenzaldehyde, which was then reduced in methanol with sodium borohydride. The resulting alcohol was methylated by dimethyl sulfate in dioxane and carboxylated at the 4-position by treatment with *n*-butyl lithium under bubbling with carbon dioxide. **II**: MS *m/z*: 237 (M−H), 193 (M−CO_2H); ^{1}H-NMR δ_H ($CDCl_3$): 3.41 (3H, s, CH_3-O-CH_2), 4.61 (2H, s, CH_3-O-CH_2). 2,3,5,6-Tetrafluoroterephthalic acid **III** was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and used without further purification. The chemical purity of each standard was determined to be >96% by high-performance liquid chromatography (HPLC).

2. Radioassay

The radioactivity in organic and aqueous fractions by the extraction of soils, bound residues and trapping media was individually determined by liquid scintillation counting (LSC) with a Packard Model 2000CA liquid scintillation spectrometer equipped with an automatic external standard in low potassium glass vials, using 10 ml of Packard Emulsifier Scintillator Plus™. The unextractable soil-bound residues were powdered after drying in a vacuum desiccator and a portion was subjected to combustion analysis using a Packard Model 307 sample oxidizer under ^{14}C recovery of >95%. Details of the radioassay have been previously reported.[9)]

3. Chromatography

Aliquots from each soil extract were analyzed by reversed-phase HPLC for either quantitation of metofluthrin and its degradates or their chemical identification. A Hitachi L-7100 pump equipped with a Sumipax ODS A-212 column (5 μm, 6-mm i.d.×15 cm, Sumika Chemical Analysis Service, Ltd., Osaka) was operated at a flow rate of 1 ml min^{-1} using mobile phase stepwise changing as follows: 0 min, %A (methanol)–%B (0.1% trifluoroacetic acid), 5 : 95; 0–35 min, linear, 90 : 10 at 35 min, 35–40 min, isocratic, 90 : 10 at 40 min, 40–41 min, linear, 5 : 95 at 41 min, 41–50 min, isocratic, 5 : 95 at 50 min. The isomeric contents of metofluthrin isomers were analyzed using chiral columns. A Hitachi L-6200 pump equipped with SUMIPAX DI-NO_2 (5 μm, 4-mm i.d.×25 cm, Sumika Chemical Analysis Service, Ltd., Osaka) and CHIRALCEL OD-H (5 μm, 4.6-mm i.d.×25 cm, Daicel Chemical Industries, Ltd., Tokyo) columns linked in series was isocratically operated using hexane/ethanol=1000/0.5 (v/v) as the mobile phase at a flow rate of 1 ml min^{-1}. The radioactivity of column effluent was monitored with a Flow Scintillation Analyzer 150TR (Packard) equipped with a 500-μl liquid cell using Ultima-Flo AP® (Packard) as the scintillator. Major ^{14}C peaks were identified by HPLC co-chromatography with the corresponding non-radiolabeled authentic standards being detected at 270 nm with a Hitachi model L-7400 UV detector. Two-dimensional thin-layer chromatography (TLC) was conducted using pre-coated silica gel $60F_{254}$ thin-layer chromatoplates (20×20 cm, 0.25-mm layer thickness, E. Merck) with the solvent systems of A; chloroform/methanol=9/1 (v/v) and B; toluene/ethyl formate/formic acid (5/7/1, v/v/v). Autoradiograms were prepared by exposing a TLC plate to a BAS-III_s Fuji imaging plate (Fuji Photo Film Co., Ltd.) for several days, and the radioactivity in each spot and diffuse region was quantified using a Typhoon 9200 Variable Mode Imager (Amersham Biosciences). Non-radiolabeled reference standards were detected by ultraviolet light at 254 nm. The typical retention times (R_t) and R_f values of metofluthrin and its related compounds are listed in Tables 1 and 2.

4. Spectroscopy

LC/MS spectra of metabolites were measured for their chemical identification using a Hitachi M-1000 mass spectrometer equipped with an APCI interface at 280°C (acetonitrile/0.1% formic acid, 5/95 (v/v); 1 ml min^{-1}) and a Thermofinnigan TSQ Quantum mass spectrometer equipped with an ESI interface at 290°C and Cadenza CD-C18 column (150×4.6 mm, Imtakt) (acetonitrile/0.1% acetic acid; 60/40 (v/v); 0.2 ml min^{-1}). Fourier-transfer nuclear magnetic resonance (FT-NMR) spectra were measured in dilute solutions of chloroform-d_1 and/or methanol-d_4 using tetramethylsilane (TMS) as an internal standard (δ=0.00 ppm) with a Varian Unity 300 NMR spectrometer at 300 MHz.

Table 1. Chemical structures and chromatographic properties of metofluthrin and its related compounds

Compound	R_t (min)[a]	R_f values [b]	
		A	B
Metofluthrin	39.4	0.78	0.71
I	23.5	0.55	0.47
II	20.4	0.08	0.37
III	5.9	0.05	0.22
IV	31.0	0.49	0.59
V	31.5	0.49	0.59
VI	17.0	–	–

[a] Typical HPLC retention time. [b] TLC R_f values with indicated solvent systems. A, chloroform/methanol (9/1, v/v); B, toluene/ethyl formate/formic acid (5/7/1, v/v/v).

Table 2. HPLC retention times of metofluthrin isomers

Metofluthrin isomer[a]	Retention time (min)
RTE	30.6
RTZ	29.3
RCE	24.5
RCZ	36.1
STE	27.1
STZ	24.5
SCE	22.1
SCZ	25.6

[a] *RT*, *RC*, *ST* and *SC* mean 1*R-trans*, 1*R-cis*, 1*S-trans* and 1*S-cis* isomerism on the cyclopropenyl ring, respectively. *E* and *Z* mean geometrical isomerism at the propenyl side chain.

5. *Aerobic soil metabolism*

US sandy loam soils were collected from the experimental farms of Valent USA Corporation in California and Mississippi, and the air-dried soils were passed through a 2-mm sieve prior to use to remove stones and plant debris. The physical and chemical properties of the soils are listed in Table 3. Each soil, equivalent to 20 g on a dry-weight basis, was placed in a 30-ml cylindrical glass beaker (3.5-cm diameter), moistened with distilled water to achieve 75% field moisture capacity (pF 2.5) at 1/3 bar and incubated at 25±1°C in darkness for 17–32 days to stimulate microbial activity.

Since the application rate of metofluthrin in the field could not be simply defined because of its environmental health use, it was determined to be 431 g a.i./ha by assuming the worst-case scenario that 4-g aerosol of the water-based formulation containing 0.1% (w/w) of metofluthrin in a typical commercial can was sprayed once onto a square-foot field. By assuming uniform mixing with soil at 0–6 inches in depth and a bulk density of 1.50, the application rate was calculated as 0.19 μg/g of dry soil. Although the isomeric content of *RTZ* and *RTE* in the active ingredient is approximately 8 : 1, the metabolism study was conducted at the same application rate of 1 μg/g of dry soil. It was about 5-fold the expected rate of metofluthrin in order to obtain sufficient amounts of metabolites to be determined. After pre-incubation, a 50-μl aliquot of acetonitrile solution of each [^{14}C] isomer (400 mg/liter) was dropwise added to each soil using a microsyringe and mixed well by spatula. The treated soil samples were placed in a 3-liter glass jar and incubated at 25±1°C in darkness. The incubation period was 14 days for [Acid-^{14}C]labels due to their rapid degradation in both soils, while longer incubation was conducted for [Alc-^{14}C]labels in California (2 months) and Mississippi (4 months) soils due to slower degradation of the

Table 3. Characteristics of soils

	California	Mississippi	German 2.1	German 2.2	German 2.3
Soil texture (%)					
Sand	77	49	90	75	58
Silt	14	48	8	17	33
Clay	9	3	2	8	9
Soil classification (USDA)	Sandy loam	Sandy loam	Sand	Loamy sand	Sandy loam
Organic matter content (%)	1.0	0.5	0.5	2.3	1.3
Cation exchange capacity (meq/100 g dry soil)	7.7	6.7	5	11	10
pH (H_2O)	7.3	7.1	5.5	5.8	6.5
Bulk density (g/cm^3)	1.3	1.3	–	–	–
1/3 bar moisture (%)	11.0	9.0	–	–	–

–: not available

alcohol moiety. Humidified CO_2-free air was passed over soil samples into two gas-washed bottles each containing 350 ml of ethylene glycol and 0.5 M NaOH solution in sequence to trap volatile ^{14}C. The soil moisture content was adjusted to its original level by the addition of distilled water once a month.

At appropriate intervals, soil samples were taken in duplicate and subsequently extracted three times with 50 ml of methanol and then 50 ml of conc. HCl–methanol (1/100, v/v) by mechanical shaking for 10 min, followed by centrifugation at 5000 rpm for 10 min. A 1-ml aliquot of each extract was radioassayed in duplicate by LSC and the extract was individually concentrated to dryness *in vacuo* for HPLC and TLC analyses. In order to confirm the chemical identity of metofluthrin isomers, the concentrated solution was first developed on TLC with chloroform/methanol (9/1, v/v). The corresponding band to metofluthrin was scraped off the TLC plate and the collected gel was eluted with hexane/ethyl acetate (20/1, v/v). After further purification by HPLC with the ODS column, the isolated isomers were subjected to chiral HPLC analysis. The unextractable soil-bound residues were dried *in vacuo* and a portion (approximately 300 mg) was combusted using a sample oxidizer prior to LSC.

6. *Soil adsorption*

The HPLC retention time of a chemical is known to be conveniently used to estimate its soil adsorption coefficient, as described in OECD guideline 121.[9] Since *RTZ* and *RTE* isomers were most likely to have almost the same hydrophobicity as demonstrated by their same retention times in HPLC with the ODS column, the soil adsorption coefficients (K_{oc}) of metofluthrin were conveniently examined using the *RTZ* isomer by the batch equilibrium method recommended by OECD.[11] Three German standard soils were used and their characteristics are listed in Table 3. The soils were supplied by Landwirtschaftliche Untersuchungs und Forschungsantalt Speyer (Germany). Autoclaving at 121°C for 20 min was undertaken to estimate adsorption profiles of the *RTZ* isomer which was found to be very susceptible to biotic degradation during equilibration. The soil-to-solution ratio was adjusted to 1 : 20 (w/v). Each sterile soil sample (1.0 g) was added to glass centrifuge tubes with a Teflon-lined screw cap containing sterile 0.01 M $CaCl_2$ (20 ml), and the tubes were mechanically shaken at 20±1°C in darkness for 16 hr. After pre-equilibration, the appropriate volume of stock solution of [^{14}C]*RTZ* (0.539 μg/μl) was aseptically added in duplicate to give nominal concentrations of 0.005, 0.01, 0.05, 0.1, and 0.5 mg/L. After shaking for 4 hr at 20±1°C in darkness, the glass tubes were centrifuged at 2500 rpm for 10 min. A 1-ml aliquot of the supernatant separated by decantation was radioassayed in duplicate. The remaining soil was extracted and analyzed similarly as a metabolism study.

The Freundlich adsorption coefficient (K_F) was estimated by the following equation, $\log C_s(eq) = \log K_F + 1/n \times \log C_{aq}(eq)$, where $C_s(eq)$ is the concentration of the *RTZ* isomer adsorbed on soil at equilibrium (μg/g), $C_{aq}(eq)$ is that in the aqueous phase (μg/ml) and n is the constant. K_F and 1/n values were estimated by least-squares regression of a $\log C_{aq}(eq)$ *vs.* $\log C_s(eq)$ plot. The K_{oc} value was calculated by normalizing the K_F value to the content of soil organic carbon (% o.c.) by the equation, $K_{oc} = K_F \times 100/\%$ o.c.

7. *Calculation*

The first-order rate constant and half-life (DT_{50}) of each *RTZ* and *RTE* isomer were calculated by the usual least-squares approximation method. These calculations were performed with Microsoft Excel 2000 (Version 9.0.8950 SP-3). The degradation rate constants of the main metabolites in the aerobic soils were estimated using the Model-Maker® program (version 4, SB Technology) based on the proposed metabolic pathways. In order to estimate the physico-chemical profiles of metofluthrin, the EPI (Estimation Programs Interfaces) Suite™ (Version 3.12)[12] was utilized as a screening level model. The chemical structure of metofluthrin without any isomerism was used as input data in SMILES notation. Furthermore, the possibility of groundwater contamination was concisely assessed based on DT_{50} and K_{oc} values of metofluthrin by using the SCI-GROW (Screening Concentration in Ground Water) simulation model provided by the Office of Pesticide Programs (OPP) in the Environmental Protection Agency (EPA).[13]

Results

1. *Aerobic soil metabolism of metofluthrin*

The extractable radioactivity from soils treated with [Acid-^{14}C]*RTZ* and *RTE* rapidly decreased with a concomitant increase of bound residues that was larger in California soil, as summarized in Table 4. Good ^{14}C recovery, 98.4–104.1% of the applied ^{14}C, was obtained through the study. As with incubation, significant mineralization proceeded and the evolved $^{14}CO_2$ amounted to 80.9–81.7% (California soil) and 58.6–64.6% (Mississippi soil) after 14 days. Almost the same ^{14}C distribution was observed for both *RTZ* and *RTE* isomers but with slight differences between the soils. Similar distribution profiles with slower transformation were obtained for the [Alc-^{14}C]-labels, as summarized in Table 5 (data from 30 to 120 days are not shown). At the end of incubation, the extractable ^{14}C decreased to 10.5–12.7% (California soil after 60 days) and 39.9–61.8% (Mississippi soil, after 120 days) of the applied ^{14}C with significant mineralization of 87.4–88.5% (California soil) and 47.8–56.0% (Mississippi soil). The bound fraction was much less (2.9–6.4%) than those in the [Acid-^{14}C]-labels. By applying first-order kinetics to the disappearance of *RTZ* and *RTE* isomers determined by HPLC analysis, the half-lives of each isomer were estimated to be 2.3–3.5 days, as listed in Table 6. The dissipation of each isomer in two US soils is shown in Fig. 1. Chiral HPLC analyses of the isolated *RTZ* and *RTE* isomers by TLC and HPLC gave a single peak corresponding to each isomer at any point of

Table 4. Soil metabolism of [Acid-^{14}C]*RTE* and *RTZ* under aerobic conditions[a)]

	Days after application (day)															
	California soil								Mississippi soil							
	1		3		7 or 8		14		1		3		7 or 8		14	
	RTE	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*
Volatiles ^{14}C	5.4	5.3	25.7	26.1	68.9	66.6	82.0	81.1	2.6	2.5	12.3	11.8	36.1	32.8	59.2	65.1
$^{14}CO_2$	5.4	5.3	25.6	26.0	68.6	66.5	81.7	80.9	2.6	2.5	12.3	11.8	35.9	32.6	58.6	64.6
Others	<0.1	<0.1	0.1	0.1	0.2	0.2	0.2	0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.2	0.6	0.5
Extract ^{14}C	94.2	92.5	67.2	66.9	16.9	17.7	7.7	6.3	100.0	97.9	85.5	86.1	56.8	58.1	30.3	24.7
RTZ	nd	81.3	nd	39.4	nd	11.1	nd	3.4	nd	88.3	nd	59.2	nd	25.9	nd	5.7
RTE	82.1	nd	42.6	nd	9.1	nd	4.2	nd	92.8	nd	63.4	nd	27.9	nd	6.4	nd
IV	nd	5.7	nd	21.9	nd	3.0	nd	nd	nd	4.0	nd	12.8	nd	8.4	nd	nd
V	6.2	nd	15.0	nd	2.0	nd	nd	nd	3.6	nd	6.5	nd	6.4	nd	0.5	nd
VI	2.3	1.8	4.9	2.1	1.3	2.6	nd	nd	1.3	0.6	4.1	2.9	3.9	6.2	6.6	6.4
VII[b)]	1.1	1.8	2.6	1.4	4.6	1.0	nd	nd	1.5	2.0	6.1	5.2	9.6	9.4	10.0	6.8
VIII[b)]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.4	1.8	4.6	3.3	2.1	1.6
Others	2.5	1.8	2.2	1.7	nd	nd	nd	nd	0.9	3.0	4.0	4.4	4.5	5.4	4.8	4.2
Bound ^{14}C	2.8	2.4	7.0	5.7	13.6	14.1	14.3	14.2	1.4	1.5	4.1	4.0	7.5	8.1	11.4	11.6
Total ^{14}C	102.4	100.2	99.6	100.2	99.4	98.4	104.1	101.7	104.1	101.9	101.8	101.9	100.4	99.1	100.9	101.4

[a)] Percentage of applied ^{14}C [b)] These products are confirmed to be artifacts from VI through extraction by acidic methanol.

Table 5. Soil metabolism of [Alc-^{14}C]*RTE* and *RTZ* under aerobic conditions[a)]

	Days after application (day)															
	California soil								Mississippi soil							
	1		3		7 or 8		14		1		3		7 or 8		14	
	RTE	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*	*RTE*	*RTZ*
Volatiles ^{14}C	0.5	0.4	1.8	1.6	8.9	6.3	17.6	17.9	0.3	0.3	1.0	1.2	3.3	3.1	5.9	7.0
$^{14}CO_2$	0.5	0.4	1.8	1.6	8.9	6.3	17.6	17.9	0.3	0.3	1.0	1.2	3.2	3.1	5.7	7.0
Others	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1	0.2	<0.1
Extract ^{14}C	102.4	103.4	101.0	99.8	92.9	96.6	80.1	79.7	103.3	102.1	102.5	101.2	98.5	96.2	94.5	94.2
RTZ	nd	73.8	nd	34.3	nd	8.5	nd	1.7	nd	81.3	nd	51.2	nd	15.9	nd	1.8
RTE	74.4	nd	33.4	nd	8.5	nd	2.7	nd	86.0	nd	59.5	nd	25.5	nd	5.3	nd
I	0.5	1.1	nd	nd	nd	nd	nd	nd	0.5	1.2	nd	nd	nd	nd	nd	nd
II	23.2	24.3	59.0	56.3	65.2	72.4	52.7	58.2	13.7	17.8	35.8	43.3	61.6	69.0	69.1	76.0
III	1.2	1.8	6.5	6.9	16.8	14.8	23.2	19.0	0.8	nd	3.4	4.7	10.8	10.8	20.1	15.5
Others	3.2	2.4	2.2	2.3	2.4	0.9	1.4	0.8	2.2	2.0	3.8	2.0	0.6	0.5	nd	0.8
Bound ^{14}C	0.4	0.4	0.8	0.7	1.9	1.6	2.1	2.5	0.2	0.2	0.4	0.4	0.8	0.8	1.3	1.1
Total ^{14}C	103.4	104.2	103.6	102.2	103.7	104.4	99.8	100.0	103.7	102.7	103.5	102.8	102.6	100.2	101.6	102.4

[a)] Percentage of applied ^{14}C

Table 6. Half-lives of *RTE* and *RTZ* isomers of metofluthrin

Label	Isomer	Half-life (day)[a]	
		California soil	Mississippi soil
Acid-^{14}C	*RTZ*	2.8 (0.972)	3.3 (0.991)
	RTE	2.9 (0.964)	3.5 (0.988)
Alc-^{14}C	*RTZ*	2.3 (0.983)	2.4 (0.997)
	RTE	2.6 (0.977)	3.3 (0.988)

[a] Values in parentheses are coefficients of correlation by regression analysis assuming first-order kinetics using the results of 0–14 days for calculation.

sampling from every incubation, indicating that neither epimerization nor geometrical isomerization in the acid moiety proceeded during the soil metabolism of metofluthrin.

HPLC analysis of the first methanol extracts of California soil treated with [Acid-^{14}C]labels showed that the main components were *RTZ* and *RTE* isomers with the corresponding acid isomers **IV** and **V**. **VI** was only detected in the acid extracts.

As with the decrease of *RTZ* and *RTE*, **IV** and **V** individually increased to 21.9% and 15.0% of the applied ^{14}C after 3 days but subsequently decreased with trace amounts of unknown metabolites (<2.5%). The amount of **VI** peaked (2.6–4.9%) at 3–8 days. The other product in the acid extracts with HPLC retention time of 24 min was detected either from *RTZ* or *RTE* isomer and 2D-TLC confirmed formation of the same products from two isomers. ESI-LC-MS analysis of the ^{14}C peak in negative ion mode gave peaks at *m/z* 173 (M−H), 144 (M−CH_3O+H) and 119. When authentic **VI** was incubated in methanol/conc. HCl (100/1, v/v) at room temperature overnight, it was mostly transformed to a product with a HPLC retention time of 24 min, which was assigned to **VII** by HPLC and 2D-TLC co-chromatography with the authentic standard (Fig. 2). In the case of Mississippi soil treated with [Acid-^{14}C]labels, acidic extraction gave another product whose HPLC retention time was 28 min, showing the more

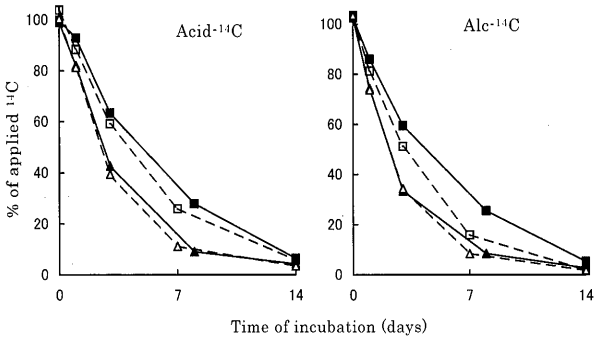

Fig. 1. Degradation of [^{14}C]metofluthrin in each soil system. ▲, *RTE* isomer in California soil; △, *RTZ* isomer in California soil; ■, *RTE* isomer in Mississippi soil; □, *RTZ* isomer in Mississippi soil.

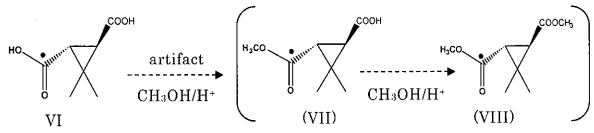

Fig. 2. Proposed structures of artifacts in acidic extraction.

hydrophobic character of this product. From *RTZ* and *RTE* isomers, this product was formed at the levels of 1.6–3.3% and 1.4–4.6% of the applied ^{14}C, respectively. When authentic **VI** was similarly incubated in acidic methanol for 5 days, most **VI** was transformed to this product. Its APCI-LC-MS spectrum in positive ion mode gave peaks at *m/z* 187 (M+H), 173 (M−CH_2), 155 (M−CH_2O) and 127 (M−$COOCH_3$), indicating the methyl ester of **VII**. The ^{14}C-peak at 28 min was co-chromatographed with this product both in HPLC and 2D-TLC, and therefore, its chemical structure was assigned to **VIII** (Fig. 2). Since similar methylation in acidic methanol has been previously reported,[17] **VII** and **VIII** were considered to be artifacts during acidic methanol extraction; therefore, additional study using [Acid-^{14}C]*RTE*, which gave more **VII** and **VIII**, was repeated for the Mississippi soil under the same conditions for 15 days to examine the artificial formation of **VII** and/or **VIII** through acidic extraction. The soil sample was first extracted with acetonitrile instead of methanol, and HPLC analysis showed that the main component in this fraction was RTE. Half of the soil residue was then further extracted with acetonitrile/conc. HCl (100/1, v/v) and the other with methanol/conc. HCl (100/1, v/v). The extracts were separately analyzed by HPLC. Figure 3 clearly

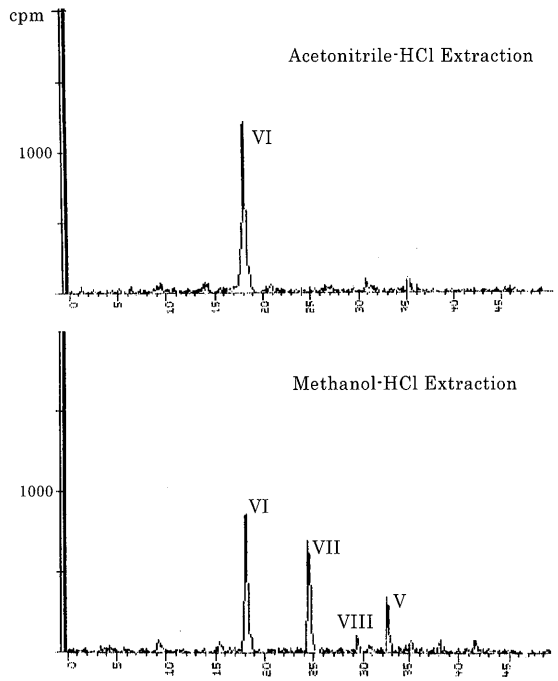

Fig. 3. HPLC chromatogram of extracts from 15-day soil samples with different acidic solvents. Half of the soil residue after extracting with acetonitrile was further extracted with acetonitrile/conc. HCl (100/1, v/v) and the other with methanol/conc. HCl (100/1, v/v).

demonstrated no formation of **VII** and **VIII** through acidic acetonitrile extraction, while their formation was clearly reproduced in acidic methanol extraction. As a result, **VI** gradually increased *via* oxidation of **IV** and **V**, and peaked after 3–14 days with more formation in Mississippi soil (18.7–18.9%) than California soil (3.6–7.5%). There were no significant differences in formation and decline patterns of **IV**, **V** and **VI** between *RTZ* and *RTE* isomers.

From both [Alc-^{14}C]isomers, the extraction gave three same metabolites in addition to the corresponding isomer. HPLC and 2D-TLC co-chromatographies with authentic standards identified them as **I** (0.5–1.2%), **II** (65.2–76.0%) and **III** (21.8–57.2%). The formation and decline patterns are shown in Fig. 4. In both soils, the primary metabolite **I** *via* ester cleavage of *RTZ* and *RTE* isomers was detected in trace amounts but with the significant formation of **II** following in the early period of incubation. As with the decrease of **II**, **III** gradually increased and its slower dissipation was observed in the Mississippi soil. These results showed that the main degradation pathway of the alcohol moiety is stepwise oxidation at both benzyl carbons to **III**, which was finally mineralized to carbon dioxide.

2. *Degradation rates of metabolites*

Based on the metabolism study, the metabolic pathways of metofluthrin are proposed in Fig. 5. Each path in ester hydrolysis followed by oxidation was simplified as below and used for kinetic analysis by the ModelMaker® program. The "Others" compartment means the sum of carbon dioxide and bound residues.

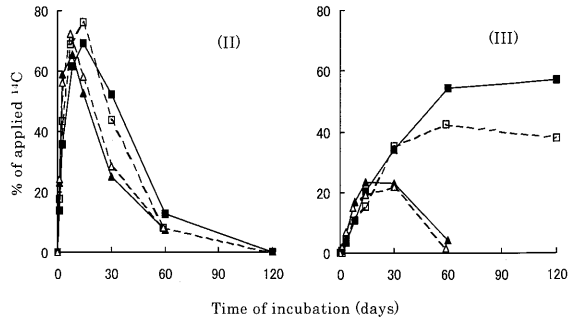

Fig. 4. Distribution of the degradates of [^{14}C]metofluthrin in each soil system. ▲, *RTE* isomer in California soil; △, *RTZ* isomer in California soil; ■, *RTE* isomer in Mississippi soil; □, *RTZ* isomer in Mississippi soil.

RTZ or *RTE* → (II) → (III) → Others
RTZ or *RTE* → (IV) or (V) → (VI) → Others

Since the primary metabolite **I** showed a transient profile in trace amounts, it was conveniently ignored in kinetic analysis, and the coefficient of correlation ranged from 0.971 to 0.993. The degradation rates of *RTZ* and *RTE* isomers were estimated to be 0.149–0.329 day^{-1}, corresponding to the half-lives of 2.1–4.6 days, which are in good accordance with those estimated by first-order kinetics. The metabolites originating from the acid moiety showed very short half-lives of less than 2 days. In contrast, the half-lives of **II** and **III** were estimated to be around 1–2 weeks in the California soil and

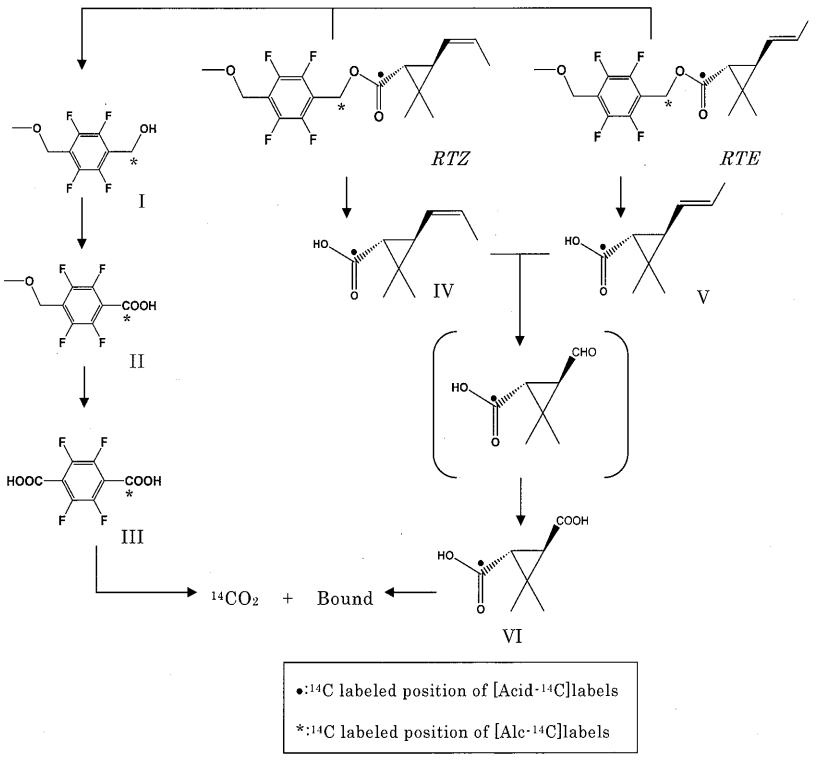

Fig. 5. Proposed degradation pathways of metofluthrin *RTE* and *RTZ* isomers in aerobic soil. The metabolite shown in parentheses was not observed in this study.

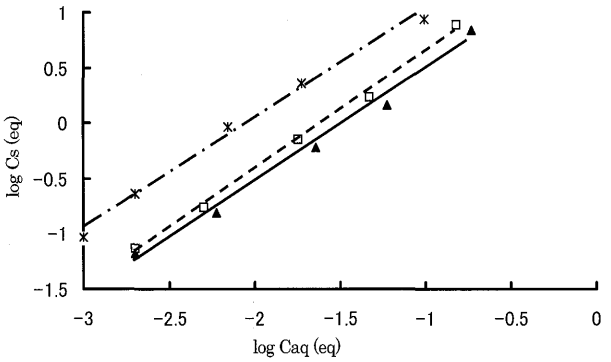

Fig. 6. Freundlich adsorption isotherm for *RTZ* isomer of [^{14}C]metofluthrin in each soil. ▲, German standard soil 2.1: $\log C_s(eq)=1.49+1.021\times\log C_{aq}(eq)$; *, German standard soil 2.2: $\log C_s(eq)=1.91+0.920\times\log C_{aq}(eq)$; □, German standard soil 2.3: $\log C_s(eq)=1.68+1.034\times\log C_{aq}(eq)$.

longer in the Mississippi soil.

3. *Adsorption of metofluthrin in soil*

Since the rapid metabolism of metofluthrin was demonstrated in aerobic soils, its stability in each intact soil/0.01 M $CaCl_2$ (1/2, w/v) suspension was first examined by mechanical shaking for 4 hr. Radioactivity in the aqueous phase amounting to 15–33% of the applied ^{14}C was analyzed by HPLC. The recovered *RTZ* was 87.6, 43.5, and 82.2% from the aqueous phase of German standard soils 2.1, 2.2, and 2.3 suspensions, respectively. In contrast, autoclaving each soil prior to the adsorption study improved the stability of *RTZ*, as demonstrated by HPLC analysis of both supernatant and soil extract where 92.1–94.3% and 94.1–96.4% of radioactivity were recovered as *RTZ* from each phase, with unextractable soil residues amounting to less than 0.3%. No isomerization of *RTZ* isomer was observed by chiral HPLC analysis. Since the stability of *RTZ* in sterilized soils could be confirmed, ^{14}C concentrations in the equilibrated aqueous and soil phases determined by LSC and combustion analysis were conveniently used as those of *RTZ* in the calculation of soil adsorption coefficients. The adsorbed amount of ^{14}C under sterile conditions was periodically monitored for up to 24 hr and the system was found to reach equilibrium after 4 hr for all soils (data not shown). The adsorbed % of ^{14}C to German standard soils 2.1, 2.2, and 2.3 was found to be 66.6, 85.1, and 71.4%, respectively. The adsorption isotherm was found to follow the Freundlich equation for all soils with correlation coefficients of 0.991–0.998, as shown in Fig. 6. Freundlich adsorption coefficients (K_F and K_{oc}) were calculated to be 31.23 ml/g and 6124 ml/g o.c., 81.00 ml/g and 3553 ml/g o.c. and 47.70 ml/g and 3613 ml/g o.c. for German standard soils 2.1, 2.2, and 2.3, respectively.

Discussion

The EPI Suite^{TM} program gave estimated water solubility and vapor pressure of metofluthrin to be 0.1–1.8 ppm and 2.01×10^{-5} mmHg (2.68×10^{-3} Pa, Modified Grain method), respectively, which were in good agreement with the experimental values (0.73 ppm and 1.96×10^{-3} Pa).[2] High soil adsorption was also estimated by this program to be 1.82×10^{4}, larger than the experimental values by a factor of 3–5. The basic physico-chemical properties of *RTZ* were well estimated and the level III fugacity model indicated that metofluthrin emitted to the environment could be dominantly distributed in soil (47.9%) and sediment (47.1%). As demonstrated by the AOP program in EPI Suite^{TM}, *RTZ* and *RTE* isomers in air are most likely to be rapidly degraded with half-lives of less than 2 hr by reaction with hydroxyl radical or ozone. The present metabolism study clearly shows the rapid degradation of metofluthrin if it unexpectedly reaches the soil environment. Metofluthrin, irrespective of its geometrical isomerism in the acid moiety, undergoes cleavage of ester linkage similarly as reported for other pyrethroids[6,14–18] but at much higher rates. The retarded degradation of *RTZ* by autoclaving soil shows the involvement of a microbial process, which has been demonstrated for other pyrethroids by using microbes isolated from soils and sediments.[7,19,20]

The primary metabolite originating from the acid moiety of metofluthrin was **IV** or **V** but they also underwent rapid degradation *via* oxidation of the propenyl side chain to form **VI**. Although significant mineralization of the chrysanthemic acid moiety of other pyrethroids with the formation of bound residues has been reported,[16–18] this study clearly demonstrated the oxidative degradation process through **VI**.

Metofluthrin has a unique perfluorinated alcohol moiety but it was also biodegraded. Different from the structurally analogous tefluthrin where the aryl methyl group was oxidized with retention of the ester linkage,[4,8] both *RTZ* and *RTE* isomers primarily gave **I** *via* ester cleavage and oxidation at either benzyl carbons proceeded to form **II** and **III**. Significant mineralization was observed for [Alc-^{14}C]-labels, indicating liberation of the ^{14}C-labeled benzyl carbon from these metabolites. In the analogy of aerobic degradation of fluorobenzoic acids,[21] these metabolites are considered to be first dioxygenated to form the corresponding fluorocatechols which subsequently undergo ring cleavage and oxidation with liberation of fluoride.

As for the potential mobility of pesticides in the soil environment, the obtained K_{oc} values for *RTZ*, a major component of metofluthrin (3553–6124 ml/g o.c.), were slightly lower than those of other pyrethroids ($>10^5$ ml/g o.c.),[3,4,22] and metofluthrin can be classified into the "Slight mobility or Immobile" category.[23] Concerns about groundwater contamination by metofluthrin were conveniently examined. SCI-GROW (Screening Concentration in Ground Water),[13] the tier-1 screening simulation model developed by US EPA, was utilized for this purpose. The worst-case application rate per year was assumed to be 9.10 lb/acre (1020 mg/m^2), about 5-fold higher than that in the present metabolism study, by assuming that the content of one commercial can containing the highest amount of metofluthrin, 0.4% (w/w) in 255 g of

aerosol, is sprayed once onto a square-meter field. Since the K_{oc} value is known to be less independent of soil texture and the obtained values ranged within a factor of 2, they were used as good surrogates of those from US soils. The average K_{oc} value (4430 ml/g o.c.) and first-order half-life (2.9 days) were used as input data. The groundwater screening concentration of metofluthrin was calculated to be 0.01 μg/L even in the unrealistic worst-case scenario, clearly indicating no possible contamination of groundwater. A metabolism study showed longer persistency of **III** than other metabolites in some soils and the contamination of groundwater due to its hydrophilicity is anticipated. Incidentally, the field dissipation study of ^{14}C-tefluthrin giving **III** as one of the main metabolites under aerobic conditions has clearly shown that no radioactivity would be detected below 20 cm from the ground surface[8]; therefore, the contamination of groundwater by **III** is most unlikely.

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