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Metabolic Fate of Phenothrin in Plants and Soils

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(+)–*Trans* and (+)–*cis* isomers of phenothrin [3–phenoxybenzyl (±)–*cis, trans*-chrysanthemate] labeled with ^{14}C at the methylene group of the alcohol moiety disappeared rapidly from the treated leaves of bean and rice plants with half-lives of less than one day under greenhouse conditions. On and/or in these plants, both isomers underwent ozonolysis at the isobutenyl double bond to yield ozonides of phenothrin isomers which were rapidly decomposed to the corresponding aldehydes and carboxylic acids. These ester products were further metabolized *via* cleavage of the ester linkage, hydroxylation at 2′– and 4′–positions of the alcohol moiety, and oxidation of benzyl alcohols to benzoic acids. The resulted alcohols and carboxylic acids were subsequently conjugated with sugars. Bean plant seedlings planted in Kodaira soil, Katano soil and Muko sand treated with 1.0 ppm of ^{14}C–phenothrin isomers took up very little ^{14}C into shoots, and pods and seeds, whereas roots retained 0.21–3.48 ppm of ^{14}C. No parent compounds were detected in shoots. ^{14}C–Phenothrin isomers were rapidly decomposed in Kodaira and Katano soils with half-lives of 1 to 2 days under upland conditions. On the other hand, degradation of both isomers was much slower under flooded conditions, and half-lives were 2 to 4 weeks and 1 to 2 months for the (+)–*trans* and (+)–*cis* isomers, respectively. Degradation of phenothrin isomers in the soils proceeded *via* cleavage of the ester linkage, hydroxylation at 4′–position of the alcohol moiety, cleavage of the diphenyl ether linkage and oxidation of benzyl alcohols to benzoic acids. These products were not persistent in the soils under both conditions and the labeled carbon was finally decomposed to $^{14}CO_2$.

INTRODUCTION

Pyrethrins and other chrysanthemates are useful for control of many insect pests of man, livestock and stored grains, but not of crops, because of their instability in air and light. The substitution of the unstable alcohol moiety with 3–phenoxybenzyl alcohol expanded the potential use areas of pyrethroids. Phenothrin [3–phenoxybenzyl (±)–*cis, trans*–chrysanthemate] is very effective in controlling agricultural pest insects as well as vectors.[1] In connection with the practical use of this insecticide, it is important to evaluate the metabolic fate in mammals and in the environment.

Metabolism of (+)–*trans*– and (+)–*cis*–phenothrin was established in rats.[2,3] On exposure to daylight in England indoors and outdoors, phenothrin decomposed rapidly with half-lives of 4 to 7 days.[4] On irradiation of 270–370 nm light on glass, phenothrin degraded with the half-life of one day.[5] Barlow *et al.*[5] also reported that phenothrin decomposed on a laterite from Uganda with half-lives of 40 to 150 days.

This report deals with the metabolic fate of the (+)–*trans* and (+)–*cis* isomers of phenothrin in plants and soils, and uptake of soil residues into plants.

MATERIALS AND METHODS

1. Spectroscopy

Infrared spectra (IR) were recorded on a Hitachi 285 spectrometer. Samples were analyzed as a thin film on NaCl crystals. Nuclear magnetic resonance spectra (NMR) were determined in deuteriochloroform ($CDCl_3$) using tetramethylsilane as internal standard on a Hitachi R–40 spectrometer at 90 MHz.

Electron impact mass spectra (MS) were obtained with a Shimadzu–LKB 9000 mass spectrometer. Samples were analyzed at 12 and 70 eV by means of a direct insertion probe.

2. Chemicals

(+)–*Trans*– and (+)–*cis*–phenothrin labeled with ^{14}C at the benzyl–methylene carbon of the alcohol moiety were prepared by Hazue and Kamata[6] of Sumitomo Chemical Co. (+)–*Trans* and (+)–*cis* are abbreviated as *t* and *c*, respectively. Each ^{14}C–preparation has a specific activity of 7.3 mCi/mmole and a radiochemical purity of greater than 98% as determined by silica gel thin-layer chromatography (*tlc*). The *t*– and *c*–phenothrin preparations were 94.4% and 93.6% pure, respectively, relative to their *trans-cis* isomer content as determined by *tlc*.

The following unlabeled chemicals were prepared in this research department:[2,7] *t*–phenothrin, *c*–phenothrin, 3–phenoxybenzyl alcohol [PBalc], 3–phenoxybenzoic acid [PBacid], 3–(2′–hydroxyphenoxy)benzyl alcohol [2′–HO–PBalc], 3–(4′–hydroxyphenoxy)benzyl alcohol [4′–HO–PBalc], 3–(2′–hydroxyphenoxy)benzoic acid [2′–HO–PBacid] and 3–(4′–hydroxyphenoxy)benzoic acid [4′–HO–PBacid]. 3–Hydroxybenzyl alcohol [3–HO–Balc] was purchased from Aldrich Chemical Co., Milwaukee, Wis., and 3–hydroxybenzoic acid [3–HO–Bacid] was obtained from Wako Pure Chemical Co., Osaka, Japan. 3–(4′–Methoxyphenoxy)benzyl (+)–*trans*–chrysanthemate [4′–CH_3O–*t*–phenothrin] was prepared as in the following. *t*–Chrysanthemoyl chloride (112 mg) was dropwise added to a stirring solution of 3–(4′–methoxyphenoxy)benzyl alcohol (110 mg) and pyridine (46 mg) in anhydrous toluene at room temperature, and stirring was continued overnight. The reaction mixture was extracted with ether and the ether extract was concentrated. The obtained crude product was purified by preparative *tlc* in *n*–hexane–acetone (4 : 1). The structure of this compound was confirmed by IR, NMR and MS. IR ν_{max}^{direct} cm^{-1}: 1,730 (ester), 1,590 and 1,500 (aromatic ring); NMR δ (ppm): 1.12 (3H, s), 1.26 (3H, s), 1.44 (H, d, J=7 Hz), 1.71 (6H, s), 2.09 (H, dd, J=7,8 Hz), 3.79 (3H, s), 4.92 (H, d, J=8 Hz), 5.08 (2H, s), 6.80–7.40 (8H, m); MS *m/e*: 380 (M), 213, 123. Each of the following three compounds was also prepared from the corresponding acid chloride and alcohol in the same manner as mentioned above. 3–(4′–Methoxyphenoxy)benzyl (+)–*cis*–chrysanthemate [4′–CH_3O–*c*–phenothrin]; IR ν_{max}^{direct} cm^{-1}: 1,730 (ester), 1,590 and 1,500 (aromatic ring); NMR δ (ppm): 1.19 (3H, s), 1.23 (3H, s), 1.68 (3H, s), 1.74 (3H, s), 1.60 (H, d, J=8 Hz), 1.90 (H, dd, J=8,8 Hz), 3.80 (3H, s), 5.05 (2H, s), 5.38 (H, d, J=8 Hz), 6.80–7.40 (8H, m); MS *m/e*: 380 (M), 213, 123. 3–Methoxybenzyl (+)–*trans*–chrysanthemate [CH_3–desphenyl–*t*–phenothrin]: IR ν_{max}^{direct} cm^{-1}: 1,720 (ester), 1,580 and 1,490 (aromatic ring); NMR δ (ppm): 1.12 (3H, s), 1.28 (3H, s), 1.45 (3H, d, J=7 Hz), 1.71 (6H, s), 2.09 (H, dd, J=7,8 Hz), 3.79 (3H, s), 4.92 (H, d, J=8 Hz), 5.10 (2H, s), 6.80–7.40 (4H, m); MS *m/e*: 288 (M), 123. 3–Methoxybenzyl (+)–*cis*–chrysanthemate [CH_3–desphenyl–*c*–phenothrin]: IR ν_{max}^{direct} cm^{-1}: 1,730 (ester), 1,590 and 1,490 (aromatic ring); NMR δ (ppm): 1.19 (3H, s), 1.28 (3H, s), 1.68 (3H, s), 1.75 (3H, s), 1.70 (H, d, J=7 Hz), 1.95 (H, dd, J=7,8 Hz), 3.80 (3H, s), 5.07 (2H, s), 5.42 (H, d, J=8 Hz), 6.80–7.40 (4H, m); MS *m/e*: 288 (M), 123. 3–Phenoxybenzyl (+)–2,2–dimethyl–3–*trans*–formyl–cyclopropane–1–carboxylate [formyl–*t*–phenothrin] was prepared by ozonolysis of *t*–phenothrin. Ozonized oxygen gas was gently bubbled in chloroform solution of *t*–phenothrin (1.01 g) for 15 min at 0°C and then the reaction solution was purged by nitrogen gas. The chloroform solution was concentrated and the obtained crude product was purified by repeated *tlc* in the following solvent systems; *n*–hexane–acetone (4 : 1) and *n*–hexane–ether (5 :1). The structure of this compound was confirmed by IR, NMR and MS; IR ν_{max}^{direct} cm^{-1}: 2,740 (aldehyde), 1,730 and 1,710 (ester and aldehyde), 1,590 and 1,490 (aromatic ring); NMR δ (ppm): 1.29 (3H, s), 1.32 (3H, s), 2.50 (2H, m), 5.11 (2H, s), 6.90–7.50 (9H, m), 9.60 (H, m); MS *m/e*: 324 (M), 183, 97. 3–Phenoxybenzyl (+)–2,2–dimethyl–3–*cis*–formyl–cyclopropane–1–carboxylate [formyl–*c*–phenothrin] was similarly prepared from *c*–phenothrin; IR ν_{max}^{direct} cm^{-1}: 1,720

and 1,700 (ester and aldehyde), 1,580 and 1,490 (aromatic ring); NMR δ (ppm): 1.26 (3H, s), 1.53 (3H, s), 1.85 (H, dd, J=9,9 Hz), 2.17 (H, d, J=9 Hz), 5.13 (2H, s), 6.90–7.50 (9H, m), 9.78 (H, d, J=9 Hz); MS *m/e*: 324 (M), 183, 97. 3–Phenoxybenzyl (+)–2,2–dimethyl–3–*trans*–carboxyl–cyclopropane–1–carboxylate [carboxyl–*t*–phenothrin] was prepared from formyl–*t*–phenothrin by hydrogen peroxide oxidation. Hydrogen peroxide solution (30%, 5 ml) was dropwise added to a stirring solution of formyl–*t*–phenothrin (0.50 g) in 10 ml of acetic acid at 60°C over 40 min and then 10 ml water was added to the reaction mixture, which was then extracted with ether and the ether extract was concentrated. The obtained crude product was purified by repeated *tlc* in the following solvent systems; *n*–hexane–toluene–acetic acid (3 : 15 : 2) and *n*–hexane–acetone (2 : 1). The structure of this compound was confirmed by IR, NMR and MS; IR ν_{max}^{direct} cm^{-1}: 3,050 (OH), 1,730 and 1,700 (ester and carboxylic acid), 1,590 and 1,490 (aromatic ring); NMR δ (ppm): 1.29 (3H, s), 1.32 (3H, s), 2.28 (2H, s), 5.11 (2H, s), 6.90–7.50 (9H, m), 8.0–9.0 (H, br); MS *m/e*: 340 (M), 183, 113. 3–Phenoxybenzyl (+)–2,2–dimethyl–3–*cis*–carboxyl–cyclopropane–1–carboxylate [carboxyl–*c*–phenothrin] was similarly prepared from formyl–*c*–phenothrin; IR ν_{max}^{direct} cm^{-1}: 3,030 (OH), 1,730 and 1,700 (ester and carboxylic acid), 1,580 and 1,480 (aromatic ring); NMR δ (ppm): 1.26 (3H, s) 1.37 (3H, s), 1.97 (2H, s), 5.13 (2H, s), 6.90–7.50 (9H, m), 7.5–8.5 (H, br); MS *m/e*: 340 (M), 183, 113.

β–Glucosidase (almond) and cellulase (*Aspergillus niger*) were purchased from Sigma Chemical Co., St. Louis, Mo.

3. *Radioanalysis and Radioautography*

Procedures for liquid scintillation counting (*lsc*), combustion analysis and *tlc* radioautography were reported previously.[8] For counting the radioactivity in organosoluble extracts of plants, aliquots were absorbed on to pieces of filter paper which were then combusted prior to *lsc*. Edible portions of plants were dried over P_2O_5 in a vacuum desiccator and then subjected to combustion. For radioautograms of whole plants, harvested bean plants were each pressed between sheets of filter paper for about two weeks and then exposed to X-ray films for two weeks at 4 to 8°C.

4. *Tlc*

Precoated silica gel 60F–254 chromatoplates (20×20 cm, 0.25 mm thickness, E. Merck) were used for analysis and preparation of degradation products. The following solvent systems were used; A, *n*–hexane–toluene–acetic acid (3 : 15 : 2); B, benzene saturated with formic acid–ether (10 : 3); C, chloroform–ethyl acetate (2 : 1); D, toluene; E, *n*–hexane–acetone (7 : 1); F, *n*–hexane–ether (5 : 1); G, *n*–hexane–ether (50 : 1); H, *n*–hexane–acetone (4 : 1); I, chloroform–acetic acid (10 : 1). The *tlc Rf* values for phenothrin and related compounds are given in Table 1. Solvent systems for two-dimensional development are illustrated for example as follows: (A×2, B) indicates development in the first direction twice with solvent system A and the second direction with solvent system B. For co-chromatography, spots of unlabeled standards were visualized under ultraviolet light and then compared with radioactive spots detected by radioautography.

5. *Treatments of Plants*

Kidney bean plants (*Phaseolus vulgaris* L.) and rice plants (*Oryza sativa* L.) were grown in a greenhouse at 25±2°C. Aliquots of methanol solution of ^{14}C–labeled *t*– and *c*–phenothrin were each applied evenly to the upper surface of two primordial leaves of 14-day old bean seedlings at the rate of 50 μg per 20 cm^2 on November 1, 1977 or three leaves of the third to fourth leaves from frag leaves of 3-month old rice seedlings at the rate of 48 μg per 20 cm^2 on June 20, 1978. These dosages were equivalent to practical application rates of 2.5 g a.i./are for vegetable crops and 2.4 g a.i./are for rice plants.

At various time intervals, two seedlings were harvested and sectioned into three portions; treated leaves, shoots and roots except the treated leaves, and edible portions (pods and seeds of bean plants, and unhulled rices at a milk ripe stage). The treated leaves, shoots and roots were frozen in liquid nitrogen

Table 1 *Tlc Rf* values for phenothrin isomers, their metabolites and derivatives.

Compound	*Rf* values with indicated solvent systems										
	A	A×2	B	B×2	C	D	E	F	G×7	H	I
c–Phenothrin	0.61	0.82	0.68	0.83	0.65	0.48	0.48	0.46	0.47	0.54	0.90
t–Phenothrin	0.59	0.80	0.68	0.83	0.64	0.46	0.48	0.45	0.43	0.54	0.90
c–Phenothrin Ozonide	0.45	0.74		0.81							
t–Phenothrin Ozonide	0.44	0.69		0.81							
Formyl–*c*–Phenothrin	0.36	0.59	0.55	0.73						0.28	0.82
Formyl–*t*–Phenothrin	0.38	0.63	0.57	0.75						0.31	0.83
Carboxyl–*c*–Phenothrin	0.30	0.43	0.40	0.51						0.08	0.62
Carboxyl–*t*–Phenothrin	0.32	0.48	0.45	0.58						0.09	0.64
4′–HO–*c*–Phenothrin	0.29										
4′–CH_3O–*c*–Phenothrin	0.56					0.37	0.39	0.34			
4′–HO–*t*–Phenothrin	0.29										
4′–CH_3O–*t*–Phenothrin	0.56					0.34	0.39	0.32			
Desphenyl–*c*–Phenothrin	0.29										
CH_3–Desphenyl–*c*–Phenothrin	0.56					0.35	0.44	0.38			
Desphenyl–*t*–Phenothrin	0.29										
CH_3–Desphenyl–*t*–Phenothrin	0.53					0.33	0.44	0.36			
PBalc	0.26	0.38	0.35	0.51	0.39						
2′–HO–PBalc	0.12	0.16	0.24		0.21						
4′–HO–PBalc	0.06	0.10	0.22		0.18						
3–HO–Balc	0.04	0.07	0.16		0.12						
PBacid	0.34	0.54	0.46	0.60							
2′–HO–PBacid	0.22	0.33	0.34								
4′–HO–PBacid	0.15	0.22	0.29								
3–HO–Bacid	0.12	0.19	0.28								

immediately after harvest, ground to a fine powder with a mortar and pestle, and then extracted three times with methanol (5 ml/g) by homogenization with a Waring blender (Nihon Seiki Co., Tokyo, Japan). The homogenate was filtered to separate extract residues from the methanol solution. The methanol-insoluble residues of treated leaves of rice plants were further extracted three times with water–methanol (1 : 1) (5 ml/g) by homogenization with a Polytron (Kinematica GmbH, Luzern, Steinhofhalde, Switzerland). The combined methanol and water–methanol extracts of plants were analyzed for ^{14}C. After concentration of the extracts the residues were dissolved in 2 ml of methanol and 50 μl aliquots were applied to *tlc* plates 3 cm in width together with authentic standards for analysis of phenothrin and its metabolites. By the combination of *tlc*, radioautography and *lsc*, the minimum detection limit of radiocarbon was 200 dpm, being equivalent to 4.4 ng of phenothrin isomers. Therefore, when 10 g and 3 g of the treated leaves of bean and rice plants were used for analysis, respectively, the detection limit of phenothrin isomers was 0.02 ppm in bean plants and 0.06 ppm in rice plants. The recovery of *t*– and *c*–phenothrin from the treated leaves of both plants immediately after foliar application was 96.5% and 95.2% in bean plants and 95.3% and 94.9% in rice plants, respectively.

6. Uptake of Soil Residues by Bean Plants

Kodaira light clay soil, Katano sandy loam soil and Muko sand were used in this experiment. These soils were kept at 0–4°C in the dark and passed through a 2-mm sieve prior to use. Characteristics of these soils were reported previously.[8] Kodaira and Katano soils (300 g), and Muko sand (600 g) were each treated with 0.1 mg of ^{14}C–labeled *t*– or *c*–phenothrin in 1 ml acetone at the rate of 1.0 ppm on a dry weight basis and then placed into a plastic pot (11 cm i.d. × 7 cm ht.). Immediately after soil treatment, a seedling of 14-day old bean plants was transplanted to the pot. The plants were held in a greenhouse at 25±2°C. About 30–50 ml of water for Kodaira and Katano soils or Hoagland solution[20] for Muko sand were applied every day. After 30 days, the plants were carefully pulled out of soils and the roots were thoroughly washed with 200 ml of water. The harvested seedlings were sectioned into roots, shoots and edible portions and these portions were analyzed for ^{14}C and/or metabolites. The soils were also extracted with methanol and the methanol extracts were analyzed for ^{14}C and degradation products as described in the following section.

7. Treatment of Soils

Kodaira light clay and Katano sandy loam soils were used. Each soil equivalent to 20 g on a dry weight basis was placed into a 50 ml beaker. Under upland conditions, soils were moistened to 50% of the maximum water holding capacity. Under flooded conditions, 16–23 ml of distilled water was added to soils, covering the soils to a depth of approximately 1.5 cm. The soils were incubated at 25±2°C in the dark for a week. After preincubation, ^{14}C–labeled *t*– or *c*–phenothrin (20 μg in 100 μl methanol) was added to each soil at the rate of 1.0 ppm on a dry weight basis by a microsyringe, and then the soils were mixed thoroughly. The beakers were covered with an aluminium foil which allowed any volatile products formed to escape and then held at 25±2°C in the dark for up to 6 months. The soil moisture was readjusted to their original values by the addition of distilled water every two weeks.

At various time intervals, the treated soils were each extracted three times with 60 ml methanol by shaking (10 min) with a KM-shaker (Iwaki Co., Tokyo, Japan). The combined methanol extracts were assayed for ^{14}C and then concentrated with a rotary vacuum evaporator. The residues were dissolved in 0.2 ml of methanol and 40 μl aliquots were applied to *tlc* plates 2 cm in width together with authentic standards for analysis of phenothrin and its degradation products. By the combination of methanol extraction, *tlc* analysis, radioautography and *lsc*, 0.0011 ppm or over of phenothrin isomers in soil was quantitatively analyzed. The recovery of *t*– and *c*–phenothrin from soils immediately after application at the rate of 0.1 ppm was more than 92%.

In a separate experiment, volatile ^{14}C–products evolved from the labeled compounds, including CO_2, were trapped with a polyurethane foam plug and 0.5 N NaOH solution linked in series, as reported previously.[8] The traps were sampled periodically for monitor of ^{14}C. The amounts of $^{14}CO_2$ was determined by addition of 1 ml of 1 N $BaCl_2$ to 10 ml of the NaOH solution containing trapped $^{14}CO_2$, precipitation of the formed $Ba^{14}CO_3$ by centrifugation, and measurement of ^{14}C in supernatant fractions and the NaOH solution. After 30 days, the experiment was stopped and the radioactivity remaining in the soil was determined to complete the balance.

Soils sampled at 6 months after treatment with ^{14}C–phenothrin isomers which had been extracted with methanol were fractionated into fulvic acid, humic acid and humin, according to the Method of Robert and Standen.[9] Degradation products in the fulvic acid fraction were extracted with ethyl acetate and then analyzed by *tlc*.

8. Leaching

Leaching of phenothrin isomers and their degradation products in soils was tested using soil columns. Kodaira light clay soil, Katano sandy loam soil and Muko sand were used for the test. About 70 g of Kodaira soil, 144 g of Katano soil or 144 g of Muko sand on a dry weight basis was packed uniformly to a height of 20 cm in a glass column of 2.5 cm i.d. and 30 cm. The bulk density of the packed soils in columns was almost equivalent to that of the field soil. Onto the soil column, 40 g of each soil on a dry weight basis was placed immediately after treatment with either 1.0 ppm of ^{14}C–labeled *t*– or *c*–phenothrin and 2 weeks after incubation of the treated soils at 25±2°C in the dark under upland conditions. Then, about 300 ml of distilled water was added to each column at a flow rate of 30 to 40 ml/day using a Perista mini pump (Atto Co., Tokyo, Japan). This flow rate was equivalent to rainfall of 60 to 80 mm/day. During leaching test, the columns were held at 25±2°C in the dark. After leaching, the soil was pushed out of the glass column and divided transversely into five fractions (the treated soil, 0–5 cm, 5–10 cm, 10–15 cm and 15–20 cm downward). These fractions and effluents were also extracted three times with ether at pH 1–2 and then the extracts were analyzed for degradation products by *tlc*.

9. Characterization of Metabolites

The organosoluble fractions from the treated leaves of plants and soils were each subjected to preparative *tlc* in solvent system A×2 or A. Radioactive gel regions were divided into several fractions which were each extracted with methanol. The isolated products were tentatively identified by one- or two-dimensional *tlc* cochromatography with authentic standards. Some degradation products thought to be carboxylic acids and phenols were methylated by reaction with diazomethane (0–4°C, overnight) to the corresponding methyl esters and ethers, which were subjected to *tlc* cochromatography. The products retaining the ester linkage were subjected to alkaline hydrolysis (1 N NaOH, 100°C, one hour). The hydrolysis products were extracted with ether at pH 1–2 and analyzed by *tlc*. Some of the degradation products were subjected to MS analysis for identification.

The fraction remaining at the origin of *tlc* plate developed with solvent system A×2 or A was designated as "polar products". The fraction of polar products from bean and rice plants was dissolved in 2 ml of 0.2 M acetate buffer, pH 5.0, and incubated with a mixture of β–glucosidase (6 mg) and cellulase (10 mg) at 37°C for 48 hr. Then, the incubation mixtures were adjusted to pH 1 to 2 with conc. hydrochloric acid and extracted with ether (8 ml×3).

Liberated aglycons were cochromatographed with authentic standards in solvent systems A and B. Unextractable residues of the treated leaves were also subjected to enzyme hydrolysis. About 100 mg of the dried residues was dissolved in 10 ml of 0.2 M acetate buffer, pH 5.0, and then incubated with a mixture of β–glucosidase (30 mg) and cellulase (50 mg) at 37°C for 48 hr. The incubation mixtures were each separated into supernatant and residual fractions by centrifugation. The supernatant was analyzed as described above.

RESULTS

1. Metabolism in Bean and Rice Plants

After foliar application of ^{14}C–phenothrin isomers to bean and rice plants, the recovery of total radiocarbon decreased gradually with time (Tables 2 and 3). After 30 days, 72 to 81% of the applied radiocarbon were still recovered from both plants. Most of the radiocarbon was present in the treated leaves of both plants, whereas only 1 to 3% of the applied radiocarbon was found in the other parts. The whole-body radioautograms of bean plants harvested at 14 days after treatment showed that most of the applied ^{14}C remained at the treated leaves (Fig. 1). These findings indicate that very little ^{14}C moved from the application site to the other parts of both plants. The *tlc* analysis of organosoluble fractions from the treated leaf, and the shoot and root portions indicated that the parent compounds were detected in the treated leaves but not in the shoot and root portions of both plants. The edible portions of both plants contained very small amounts of ^{14}C so that these were not subjected to analysis of the parent compounds.

The residue levels of *t*– and *c*–phenothrin in the treated leaves of bean and rice plants are determined as shown in Fig. 2. Both isomers disappeared rapidly from the treated leaves of both plants with half-lives of less than one day, and the residue levels in both plants after 30 days were 0.04–0.28 ppm for *t*–phenothrin and 0.10–0.30 ppm for *c*–phenothrin.

Products in organosoluble fractions of the treated leaves of both plants were separated into nine fractions by preparative *tlc* in solvent system A×2. Fraction I (Rf=0.80–0.82) was identified as phenothrin isomers which were about 94% pure relative to their *trans-cis* isomer content as determined by *tlc* in solvent system G×7. The purity of products was the same as those of the used ^{14}C–labeled preparations, indicating that *trans-cis* isomerization did not occur on and/or in the plant leaves. Fractions II (Rf=0.74) and III (Rf=0.69) gave essentially the same MS, which were suggestive of an ozonide of phenothrin as in the followings; m/e 398 (M, phenothrin+O_3), 340 (M–C_2H_6CO), 324 (M–C_2H_6COO), 279, 183 (3–phenoxybenzyl), 167, 113, 58 (C_2H_6CO). On alkaline hydrolysis, both products gave PBalc which was identified by two-dimensional *tlc* cochromatography with the authentic standard in solvent system (A, B). In addition, a portion of fraction II was

(a) (b)

Fig. 1 Whole-body radioautograms of bean seedlings harvested at 14 days after foliar treatment with a) ^{14}C–*t*–phenothrin and b) ^{14}C–*c*–phenothrin.

Table 2 Amounts of ^{14}C, phenothrin and its metabolites in bean plants after foliar application of ^{14}C–phenothrin isomers at the rate of 50 μg per 20 cm^2.

	% of the applied ^{14}C									
	t–Phenothrin					*c*–Phenothrin				
	Days after application									
	1	3	7	14	30	1	3	7	14	30
Treated leaves	96.1	91.4	88.2	82.4	76.7	97.3	93.5	91.5	82.4	76.0
Extractable ^{14}C	95.0	88.3	83.8	75.7	68.6	95.8	90.3	86.9	76.1	68.3
Phenothrin	15.4	3.7	2.1	0.4	0.2	10.2	4.6	2.5	0.6	0.4
t–Phenothrin Ozonide	9.1	7.2	5.8	1.2	0.7	<0.1	<0.1	<0.1	<0.1	<0.1
c–Phenothrin Ozonide	<0.1	<0.1	<0.1	<0.1	<0.1	12.1	9.3	9.9	2.0	2.9
Formyl–*t*–Phenothrin	24.7	9.4	6.8	4.5	2.0	1.7	1.9	1.8	0.4	0.9
Formyl–*c*–Phenothrin	2.3	1.4	2.3	1.6	1.1	43.9	21.6	17.2	12.3	7.2
Carboxyl–*t*–Phenothrin	15.1	27.6	33.1	37.6	36.6	1.7	2.6	3.0	3.3	3.0
Carboxyl–*c*–Phenothrin	0.9	2.2	3.3	3.2	2.5	7.8	22.6	22.1	30.2	26.7
PBalc	0.8	0.9	0.9	0.8	1.1	0.6	0.8	1.1	0.9	1.5
Polar products	17.1	27.3	21.8	17.1	14.3	11.3	18.9	19.9	19.4	16.6
Other products	9.6	8.6	7.7	9.3	10.1	6.5	8.0	9.4	7.0	9.1
Unextractable ^{14}C	1.1	3.1	4.4	6.7	8.1	1.5	3.2	4.6	6.3	7.7
Shoots and roots except the treated leaves	0.02	0.11	0.44	1.04	1.77	0.01	0.06	0.66	1.62	2.98
Extractable ^{14}C	0.02	0.11	0.41	0.91	1.36[a)]	0.01	0.06	0.59	1.33	1.99[a)]
Unextractable ^{14}C	<0.01	<0.01	0.03	0.13	0.41	<0.01	<0.01	0.07	0.29	0.99
Pods, ^{14}C					0.02					0.02
Seeds, ^{14}C					<0.01					<0.01
Total ^{14}C	96.1	91.5	88.6	83.4	78.5	97.3	93.6	92.2	84.0	79.0

a) No phenothrin isomers were detected in this fraction.

Table 3 Amounts of ^{14}C, phenothrin and its metabolites in rice plants after foliar application of ^{14}C–phenothrin isomers at the rate of 48 μg per 20 cm^2.

	% of the applied ^{14}C									
	t–Phenothrin					*c*–Phenothrin				
	Days after application									
	1	3	7	14	30	1	3	7	14	30
Treated leaves	95.6	90.5	84.6	81.2	70.9	94.7	91.2	87.8	86.5	79.9
Extractable ^{14}C	94.6	87.7	78.0	68.2	51.6	93.9	89.6	84.3	77.0	62.6
Phenothrin	11.8	2.8	0.8	0.6	0.3	6.3	1.8	1.2	0.7	0.3
t–Phenothrin Ozonide	4.5	3.0	1.6	0.6	0.2	<0.1	<0.1	<0.1	<0.1	<0.1
c–Phenothrin Ozonide	<0.1	<0.1	<0.1	<0.1	<0.1	1.5	1.1	0.9	0.6	<0.1
Formyl–*t*–Phenothrin	38.5	31.4	12.7	5.2	1.2	4.2	3.4	2.0	1.4	0.6
Formyl–*c*–Phenothrin	4.4	4.8	2.7	1.5	0.8	49.8	42.2	24.2	13.1	3.2
Carboxyl–*t*–Phenothrin	4.7	10.2	13.3	14.6	11.2	0.8	1.3	1.9	1.9	2.3
Carboxyl–*c*–Phenothrin	<0.1	4.7	4.4	1.2	1.1	6.5	7.0	12.1	9.8	9.5
PBalc	0.2	1.6	1.9	1.3	0.9	0.1	0.2	0.2	2.3	1.5
Polar products	7.6	13.6	23.5	27.8	23.5	6.0	7.2	15.2	21.7	26.5
Other products	22.9	15.6	17.1	15.4	12.4	18.7	25.4	26.6	25.5	18.7
Unextractable ^{14}C	1.0	2.8	6.6	13.0	19.3	0.8	1.6	3.5	9.5	17.3
Shoots and roots except the treated leaves	0.06	0.14	0.28	0.52	1.08	0.07	0.12	0.25	0.55	0.91
Extractable ^{14}C	0.04	0.08	0.16	0.22	0.40[a]	0.05	0.08	0.15	0.32	0.36[a]
Unextractable ^{14}C	0.02	0.06	0.12	0.30	0.68	0.02	0.04	0.10	0.23	0.55
Unhulled rices,[b] ^{14}C					0.07					0.06
Total ^{14}C	95.7	90.6	84.9	81.7	72.1	94.8	91.3	88.1	87.1	80.9

[a] No phenothrin isomers were detected in this fraction.

[b] Rices were at a milk ripe stage.

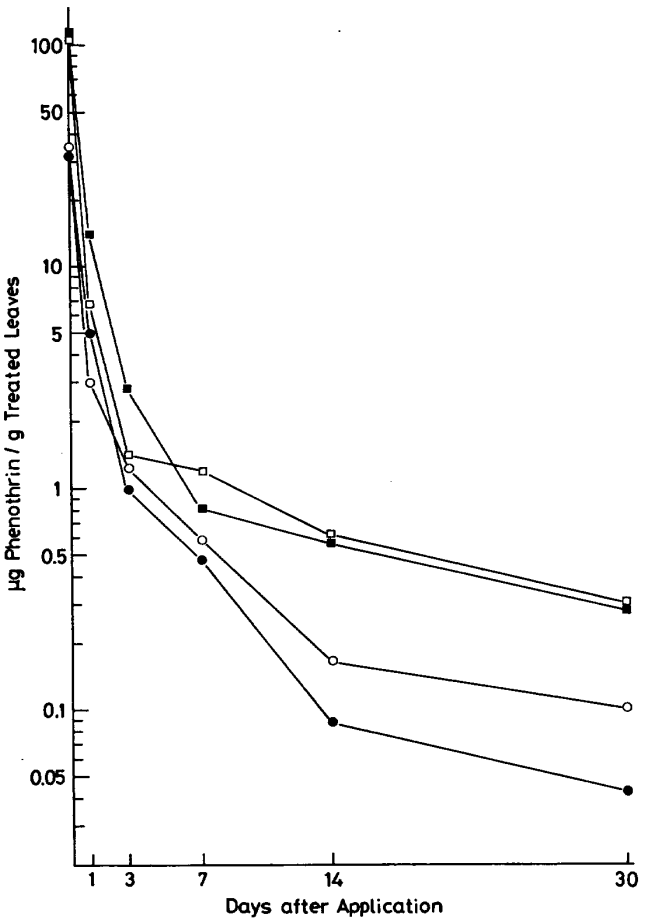

Fig. 2 Residue levels of phenothrin isomers in the treated leaves of bean and rice plants.
■: *t*–Phenothrin, Rice, □: *c*–Phenothrin, Rice
●: *t*–Phenothrin, Bean, ○: *c*–Phenothrin, Bean

decomposed to formyl–*c*–phenothrin and carboxyl–*c*–phenothrin, and fraction III also produced formyl–*t*–phenothrin and carboxyl–*t*–phenothrin during developing with acidic solvent systems. The produced formyl– and carboxyl–phenothrin isomers were each identified by *tlc* cochromatography with the authentic standards and MS analysis, as mentioned below. On the basis of these data, it seems to be reasonable to presume that fraction II is *c*–phenothrin ozonide and fraction III is *t*–phenothrin ozonide, although authentic standards were not available. Fractions IV (Rf=0.63) and V (Rf=0.59) were identified as formyl–*t*–phenothrin and formyl–*c*–phenothrin, respectively, by *tlc* in solvent systems (A×2, B×2) and (A, H). The MS of both products were identical with those of the corresponding authentic standards. Fractions VI (Rf=0.48) and VII (Rf=0.43) were identified as carboxyl–*t*–phenothrin and carboxyl–*c*–phenothrin, respectively, by *tlc* in solvent systems (A×2, B×2) and (A, I). Also, both products showed essentially the same MS of the corresponding authentic standards. Formyl-phenothrin and carboxyl-phenothrin obtained from *t*–phenothrin contained the corresponding *cis*–isomers to extents of 9–40% and 3–16%, respectively, and those from *c*–phenothrin included 2–32% and 10–20% of the *trans*–isomers, respectively, indicating that *trans*/*cis* interconversion occurs to some extents during formation of formyl– and carboxyl–phenothrin. Fraction VIII (Rf=0.38) was identified as PBalc by *tlc* in solvent system (A×2, B×2).

As shown in Tables 2 and 3, the ozonides of phenothrin isomers were detected in the treated leaves immediately after treatment and decreased rapidly thereafter. Instead, formyl-phenothrin was produced in large amounts in both plants shortly after treatment and decreased gradually with time. With decrease of both ozonide and formyl–phenothrin, amounts of carboxyl–phenothrin increased and reached maximum levels after 7 to 14 days. Thereafter, amounts of carboxyl–phenothrin tended to decrease slowly. In addition to carboxyl–phenothrin, amounts of polar metabolites increased gradually with time. Larger amounts of polar products were obtained from rice plants as compared with bean plants. Most of polar products (Fraction IX, Rf=0.0) were hydrolyzed by incubation with a mixture of β–glucosidase and cellulase to liberate aglycons which were identified by *tlc* in solvent systems A×2 and B (Table 4). The identified aglycons were as follows; carboxyl–*t*–phenothrin, carboxyl–*c*–phenothrin, PBalc, 2′–HO–PBalc, 4′–HO–PBalc, PBacid, 2′–HO–PBacid and 4′–HO–PBacid. The amount of each aglycon was 4% or less of the applied total radiocarbon. In addition to the polar metabolites, unextractable residues increased gradually and reached maximum levels of 7–8% and 17–19% in bean and rice plants, respectively. Approximately 40% of the unextractable ^{14}C from bean plants was released by incubation with a mixture of β–glucosidase and cellulase, whereas that from rice plants was hardly

Table 4 Amounts of pehnothrin metabolites obtained from polar products and unextractable residues of the treated leaves of bean and rice plants after hydrolysis with a mixture of β-glucosidase and cellulase.

	% of the applied ^{14}C											
	Bean plant						Rice plant					
	Polar Product				Unextractable residue		Polar Product				Unextractable residue	
	t-Phen.		*c*-Phen.		*t*-Phen.	*c*-Phen.	*t*-Phen.		*c*-Phen.		*t*-Phen.	*c*-Phen.
	Days after application											
	7	30	7	30	30	30	7	30	7	30	30	30
Hydrolyzable products	18.9	11.9	13.8	12.8	3.4	3.0	16.6	13.3	10.9	14.9	0.5	0.5
Carboxyl-*t*-Phen.-conj.	0.3	0.2	0.8	0.2	0.2	<0.1	3.9	2.9	0.2	0.3	0.1	<0.1
Carboxyl-*c*-Phen.-conj.	0.3	0.1	1.7	0.3	<0.1	0.2	0.4	0.2	0.3	2.6	<0.1	<0.1
PBalc-conj.	3.0	1.8	1.3	1.2	0.4	0.2	1.5	1.1	1.0	1.0	0.1	0.1
2′-HO-PBalc-conj.	1.3	1.1	0.6	2.0	0.3	<0.1	0.3	0.2	1.2	0.9	<0.1	<0.1
4′-HO-PBalc-conj.	1.1	0.8	0.7	0.9	0.2	0.5	0.5	0.6	0.5	1.6	<0.1	<0.1
PBacid-conj.	0.7	0.5	1.1	0.7	0.2	0.1	1.6	1.0	0.3	1.2	0.1	0.1
2′-HO-PBacid-conj.	1.2	0.8	0.3	0.6	0.4	0.2	0.4	0.6	0.2	0.5	<0.1	<0.1
4′-HO-PBacid-conj.	1.1	1.0	0.3	0.4	0.4	0.1	0.5	0.5	0.2	0.4	0.1	0.1
Other conjugates	9.9	5.6	7.0	6.5	1.3	1.7	7.5	6.2	7.0	6.4	0.1	0.2
Nonhydrolyzable products	2.9	2.4	6.1	3.8	4.7	4.7	6.9	10.2	4.3	11.6	18.8	16.8
Total	21.8	14.3	19.9	16.6	8.1	7.7	23.5	23.5	15.2	26.5	19.3	17.3

released by the enzyme system. The aglycons liberated from the unextractable residues of bean plants were the same as those from the polar metabolites. The amount of each aglycon was 0.5% or less of the applied ^{14}C.

2. Uptake of Soil Residues into Bean Plants

After 30 days cultivation of bean plants in Kodaira and Katano soils treated with 1.0 ppm of ^{14}C–phenothrin isomers, the ^{14}C content in roots was in the same range as those in the soils, whereas the roots in Muko sand contained several times larger amounts of ^{14}C relative to that in the sand (Fig. 3). The radiocarbon in the roots appears to be in part due to contamination with the soils. The shoots contained much less amounts of the radiocarbon as compared with the roots. The ^{14}C content in pods and seeds was 0.01 to 0.03 ppm and much less than those in the shoots. Analysis of the roots and shoots indicated that major constituents were polar metabolites and unextractable residues and no parent compound was detected in shoots and roots, although a small amount of the parent compound was present in the root in Muko sand. No further analysis was conducted with the pods and seeds. In addition to bean plants, the soils contained a small amount of the parent compound, more amounts of its degradation products and bound ^{14}C.

3. Degradation in Soil

After application of ^{14}C–phenothrin isomers

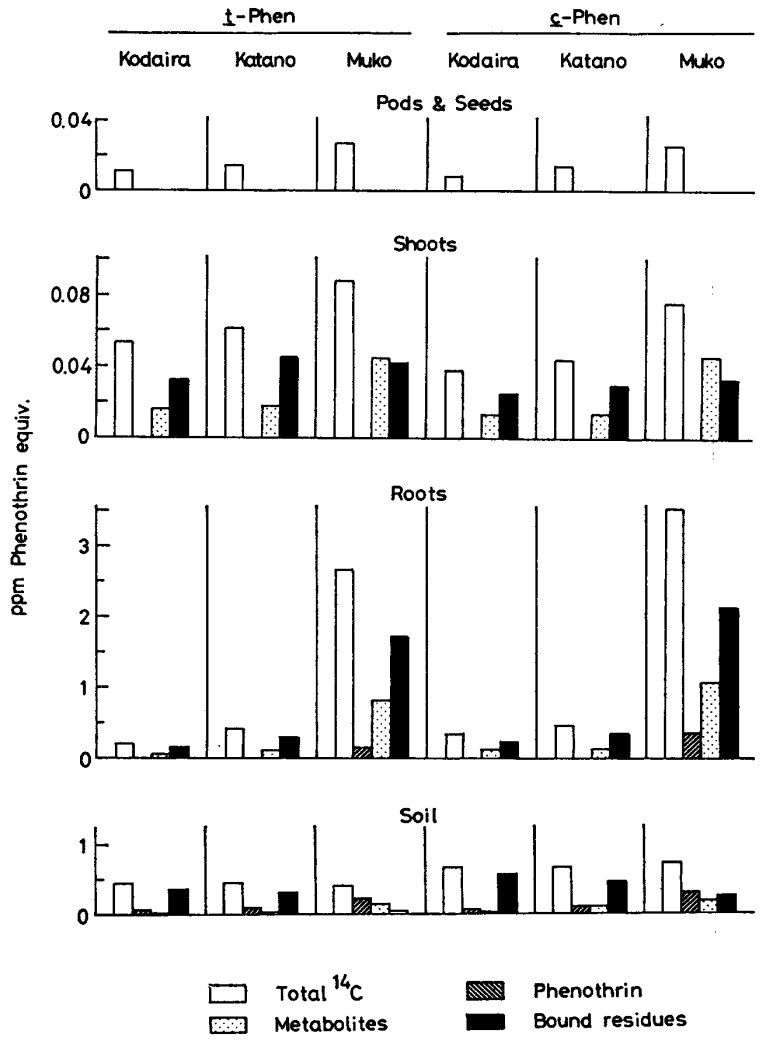

Fig. 3 Uptake and distribution of ^{14}C in bean plants at 30 days after treatment of soils with ^{14}C–phenothrin isomers at the rate of 1.0 ppm.

The fraction of metabolites includes 4′–HO–phenothrin, desphenyl–phenothrin, PBalc, PBacid and polar products.

Table 5 Amounts of ^{14}C, phenothrin and its degradation products after treatment of soils with ^{14}C–phenothrin isomers at the rate of 1.0 ppm under upland conditions.

	% of the applied ^{14}C													
	t–Phenothrin													
	Kodaira soil							Katano soil						
	Days after application													
	1	3	7	14	30	60	180	1	3	7	14	30	60	180
Extractable ^{14}C	71.2	37.2	14.4	7.5	3.4	2.3	1.2	76.4	37.2	23.8	8.3	4.0	2.7	1.4
Phenothrin	57.6	29.7	10.5	5.1	1.5	0.7	0.6	62.0	29.7	17.5	4.9	1.7	0.6	0.5
4′–HO–Phen., Desphenyl–Phen.	5.5	0.6	0.7	0.8	0.6	0.7	<0.1	1.5	0.6	0.3	0.7	0.2	0.3	<0.1
PBalc	2.4	2.1	0.8	<0.1	0.2	0.1	<0.1	3.6	2.1	1.7	<0.1	0.2	0.1	<0.1
4′–HO–PBalc, 3–HO–Balc	0.3	0.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	<0.1	<0.1	<0.1	<0.1	<0.1
PBacid	0.7	0.5	0.3	0.1	0.1	0.1	<0.1	1.0	0.5	1.0	0.3	0.2	0.1	<0.1
4′–HO–PBacid, 3–HO–Bacid	0.3	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	0.7	0.2	0.4	0.1	<0.1	<0.1	<0.1
Other products	4.4	3.7	2.1	1.5	1.0	0.7	0.6	7.6	3.7	2.9	2.3	1.7	1.6	0.9
Unextractable ^{14}C	17.9	32.5	34.6	33.5	35.6	32.8	31.7	10.7	32.5	30.9	37.6	30.8	27.4	20.3
Total ^{14}C	89.1	69.7	49.0	41.0	39.0	35.1	32.9	87.1	69.7	54.7	45.9	34.8	30.1	21.7

	% of the applied ^{14}C													
	c–Phenothrin													
	Kodaira soil							Katano soil						
	Days after application													
	1	3	7	14	30	60	180	1	3	7	14	30	60	180
Extractable ^{14}C	82.5	61.5	31.5	12.6	5.6	3.3	1.6	85.5	68.3	44.1	25.3	7.5	4.2	2.4
Phenothrin	48.3	37.1	19.3	6.8	2.3	0.5	0.7	55.0	42.2	26.2	12.3	2.8	0.5	0.8
4′–HO–Phen, Desphenyl–Phen.	12.8	5.1	0.7	2.5	0.4	0.7	0.1	7.3	2.5	0.9	4.2	0.4	0.4	0.3
PBalc	6.0	7.6	5.3	0.3	0.7	0.2	<0.1	8.4	12.9	9.0	0.4	1.2	0.3	<0.1
4′–HO–PBalc, 3–HO–Balc	<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
PBacid	8.1	3.9	1.1	0.7	0.4	0.4	<0.1	4.8	2.3	1.2	0.6	0.3	0.3	0.1
4′–HO–PBacid, 3–HO–Bacid	1.1	1.3	0.9	0.4	0.1	0.1	<0.1	2.0	1.7	0.7	1.5	0.5	0.2	<0.1
Other products	6.2	6.5	4.2	1.9	1.7	1.4	0.8	8.0	6.7	6.1	6.3	2.3	2.5	1.2
Unextractable ^{14}C	12.3	21.7	43.4	54.9	54.0	49.0	45.8	7.3	15.8	24.8	34.6	45.6	39.0	31.5
Total ^{14}C	94.8	83.2	74.9	67.5	59.6	52.3	47.4	92.8	84.1	68.9	59.9	53.1	43.2	33.9

Table 6 Amounts of ^{14}C, phenothrin and its degradation products after treatment of soils with ^{14}C–phenothrin isomers at the rate of 1.0 ppm under flooded conditions.

	% of the applied ^{14}C													
	t–Phenothrin													
	Kodaira soil							Katano soil						
	Days after application													
	3	7	14	30	60	120	180	3	7	14	30	60	120	180
Extractable ^{14}C	89.4	73.1	63.4	56.5	45.6	26.3	16.3	93.8	86.9	82.7	70.3	54.2	16.7	9.6
Phenothrin	80.3	57.1	53.5	39.6	29.4	19.8	14.0	81.6	69.4	65.6	51.3	36.9	10.9	5.9
4′–HO–Phen., Desphenyl–Phen.	1.2	6.6	2.7	7.7	9.3	1.9	0.2	0.4	1.8	1.5	2.4	4.0	0.5	0.2
PBalc	1.9	2.4	1.7	2.9	1.4	1.3	0.2	1.4	3.2	3.9	4.7	3.0	1.0	0.3
4′–HO–PBalc, 3–HO–Balc	<0.1	<0.1	<0.1	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	0.3	<0.1	<0.1	<0.1
PBacid	1.6	2.7	2.2	2.9	2.5	1.3	1.0	4.9	7.1	7.1	7.5	5.9	2.3	1.3
4′–HO–PBacid, 3–HO–Bacid	0.4	0.5	0.3	0.5	0.3	0.1	<0.1	0.7	0.8	0.7	0.9	0.9	0.2	0.1
Other products	4.0	3.8	3.0	2.4	2.7	1.9	0.9	4.8	4.6	3.6	3.2	3.5	1.8	1.8
Unextractable ^{14}C	9.9	13.9	26.3	30.4	32.8	41.9	43.3	4.3	7.5	11.9	20.5	27.4	29.5	27.6
Total ^{14}C	99.3	87.0	89.7	86.9	78.4	68.2	59.6	98.1	94.4	94.6	90.8	81.6	46.2	37.2

	% of the applied ^{14}C													
	c–Phenothrin													
	Kodaira soil							Katano soil						
	Days after application													
	3	7	14	30	60	120	180	3	7	14	30	60	120	180
Extractable ^{14}C	90.3	82.6	77.2	65.6	55.4	39.2	25.6	98.5	94.7	87.2	87.0	73.2	36.3	17.9
Phenothrin	74.4	61.7	62.1	48.6	29.6	30.3	22.2	84.3	77.8	73.3	72.3	53.1	27.1	14.0
4′–HO–Phen., Desphenyl–Phen.	5.7	7.3	3.7	5.3	11.5	2.5	0.7	5.6	5.3	3.7	3.8	5.3	2.0	0.6
PBalc	3.0	4.1	4.0	4.3	2.7	0.8	0.5	2.4	2.9	3.1	3.4	3.7	0.6	0.3
4′–HO–PBalc, 3–HO–Balc	<0.1	<0.1	<0.1	0.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
PBacid	2.8	4.0	2.1	2.8	5.0	1.4	0.4	1.9	2.7	1.9	3.2	4.6	1.7	0.6
4′–HO–PBacid, 3–HO–Bacid	0.8	1.0	0.5	0.7	1.8	0.5	<0.1	1.1	1.0	0.9	0.8	0.8	0.7	0.3
Other products	3.6	4.5	4.8	3.4	4.8	3.7	1.8	3.2	5.0	4.3	3.5	5.7	4.2	2.1
Unextractable ^{14}C	6.7	11.4	14.0	23.7	31.8	38.3	39.5	3.0	5.5	7.7	9.3	16.0	27.7	32.7
Total ^{14}C	97.0	94.0	91.2	89.3	87.2	77.5	65.1	101.5	100.2	94.9	96.3	89.2	64.0	50.6

to Kodaira and Katano soils, recovery of the total radiocarbon decreased gradually under upland conditions (Table 5). After 6 months, 22–47% of the applied radiocarbon were recovered from both soils. Also, the radiocarbon in methanol extracts decreased rapidly and only 1–2% was recovered after 6 months. In turn, the radiocarbon in unextractable residues increased rapidly and reached maximum levels after 14 to 30 days. Thereafter, it tended to decrease slowly. Under flooded conditions, decrease of the recovered radiocarbon was much slower as compared with under upland conditions (Table 6). About 37–65% of the applied radiocarbon were recovered after 6 months. The radiocarbon in methanol extracts also decreased readily with time. In contrast, the amounts of unextractable residues increased gradually with time, amounting to 28–43% after 6 months. Under both upland and flooded conditions, much more radiocarbon was recovered from Kodaira soil than Katano soil, and from *c*–phenothrin than from *t*–phenothrin in both soils. Loss of the radiocarbon appears to be mainly due to evolution of $^{14}CO_2$, as mentioned below.

A balance study was conducted for up to 30 days to determine the nature and amount of volatile products formed in the soils. The amount of radiocarbon trapped in polyurethane plugs was less than 0.1% to 0.3% of the applied during 30 days period. The trapped radiocarbon largely consisted of the parent compounds. The radiocarbon trapped in 0.5 N NaOH solution was proved to be due to $^{14}CO_2$ by the addition of $BaCl_2$ solution to form $Ba^{14}CO_3$. Figure 4 shows that increase of $^{14}CO_2$ evolution is almost linear with time for up to 30 days. Larger amounts of $^{14}CO_2$ were evolved from both soils under upland conditions than under flooded conditions and from *t*–phenothrin than from *c*–phenothrin. In this study, the recovery of the total radiocarbon was 94.2–97.6%.

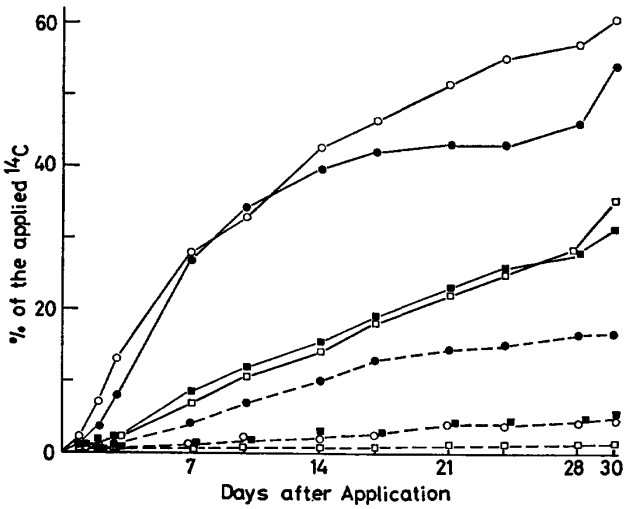

Fig. 4 Evolution of $^{14}CO_2$ from soils treated with ^{14}C–phenothrin at the rate of 1.0 ppm under upland and flooded conditions.

● *t*–Phenothrin, Kodaira soil ■ *c*–Phenothrin, Kodaira soil ○ *t*–Phenothrin, Katano soil □ *c*–Phenothrin, Katano soil —— Upland conditions ····· Flooded conditions

Figure 5 shows the residue levels of *t*– and *c*–phenothrin in two types of soil under upland and flooded conditions. Under upland conditions, both isomers rapidly disappeared with half-lives of 1 to 2 days, and the residue levels after 6 months were 0.005 to 0.008 ppm. Under flooded conditions, both isomers decreased much more slowly as compared with upland conditions. The half-lives were 2 weeks to 1 month and 1 to 2 months for *t*– and *c*–phenothrin, respectively. The residue levels after 6 months were 0.06–0.14 ppm for *t*–phenothrin and 0.14–0.22 ppm for *c*–phenothrin. Thus, *t*–phenothrin decreased at faster rates than *c*–phenothrin in both soils.

Degradation products in methanol extracts were separated into five fractions by preparative *tlc* in solvent system A. Frantions I (Rf=0.34) and III (Rf=0.26) were identified as PBacid and PBalc, respectively, by *tlc* in solvent system (A, B). On methylation with diazomethane, fraction II (Rf=0.29) derived from ^{14}C–*t*–phenothrin produced 4′–CH_3O–*t*–phenothrin and CH_3–desphenyl–*t*–phenothrin which were tentatively identified by *tlc* in solvent systems (D, E) and (E, F). Similarly, fraction II derived from ^{14}C–*c*–phenothrin gave 4′–CH_3O–*c*–phenothrin and CH_3–desphenyl–*c*–phenothrin which were also identified in the same manner as with the *trans* isomers. Therefore it appears that fraction II is a mixture of 4′–HO–phenothrin and desphenyl–phenothrin. Fraction IV (Rf=0.12–0.15) consisted of a mixture of 3–HO–Bacid and 4′–HO–PBacid which were tenta-

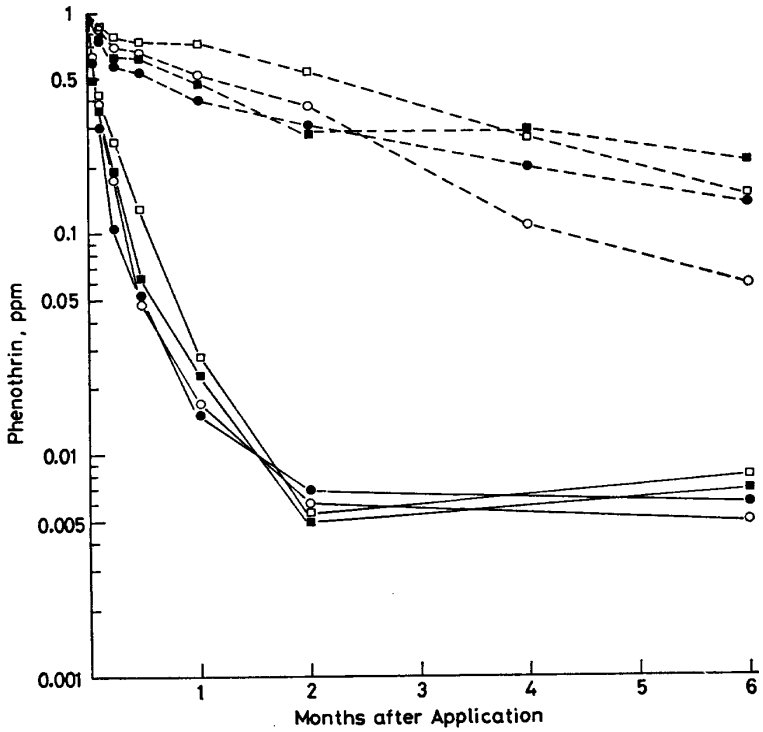

Fig. 5 Residue levels of phenothrin isomers in soils under upland and flooded conditions.
● *t*–Phenoththrin, Kodaira soil ■ *c*–Phenothrin, Kodaira soil
○ *t*–Phenothrin, Katano soil □ *c*–Phenothrin, Katano soil
—— Upland conditions ····· Flooded conditions

Table 7 Analysis of bound residues after 6-month incubation of soils with ^{14}C–phenothrin isomers under upland and flooded conditions.

Fraction	% of the applied ^{14}C							
	t–Phenothrin				*c*–Phenothrin			
	Kodaira soil		Katano soil		Kodaira soil		Katano soil	
	Upland	Flooded	Upland	Flooded	Upland	Flooded	Upland	Flooded
HCl prewash	1.9	1.7	1.6	2.4	2.1	1.7	2.1	2.4
Fulvic acid	5.5	8.1	8.2	11.2	8.0	7.8	13.7	12.2
Ethyl acetate extract	2.0	6.4	2.6	6.7	3.4	5.1	5.5	6.2
PBalc	<0.1	0.3	0.2	0.7	<0.1	0.4	0.4	1.4
4′–HO–PBalc, 3–HO–Balc	0.3	0.5	0.6	0.5	2.2	1.3	2.5	1.6
PBacid	0.2	3.7	0.1	3.9	<0.1	1.6	<0.1	1.4
4′–HO–PBacid, 3–HO–Bacid	<0.1	0.8	<0.1	1.0	<0.1	<0.1	<0.1	<0.1
Other products	1.5	1.1	1.7	0.6	1.2	1.8	2.6	1.8
Water extract	3.5	1.7	5.6	4.5	4.6	2.7	8.2	6.0
Humic acid	15.1	22.0	5.8	5.8	23.8	15.9	8.6	7.7
Humin	9.2	11.5	4.7	8.2	11.9	14.1	7.1	10.4
Total ^{14}C	31.7	43.3	20.3	27.6	45.8	39.5	31.5	32.7

tively identified by *tlc* in solvent systems A×2 and B. Fraction V (Rf=0.04–0.06) was proved to contain 3–HO–Balc and 4′–HO–PBalc by *tlc* in solvent systems A×2, B and C. These identified products were obtained under both upland and flooded conditions, although the ratio of these products varied under both conditions. The amount of these

degradation products in both soils were very small at 6 months after treatment and much smaller under upland conditions than under flooded conditions. Thus, it appears that phenothrin degradation products are not persistent in the soils and more rapidly decomposed in the soils under upland conditions than under flooded conditions. The fraction presented as "other products" included a number of minor polar products which were remaining at the origin of *tlc* plates developed with solvent system A.

Kodaira and Katano soils sampled at 6 months after treatment which had been extracted with methanol were fractionated into fulvic acid, humic acid and humin fractions (Table 7). The radiocarbon was distributed through these fractions of the soil organic matter and only small amounts of free ^{14}C were present in the HCl prewash fraction. Larger amounts of radiocarbon were associated with humic acid fraction in Kodaira soil and fulvic acid fraction in Katano soil. The fulvic acid fractions contained PBacid, PBalc, 4′–HO–PBacid, 3–HO–Bacid, 4′–HO–PBalc and 3–HO–Balc. There was no difference in the ^{14}C distribution of these fractions from both *t*– and *c*–phenothrin.

4. *Leaching*

Table 8 shows the degree of leaching of phenothrin isomers and their degradation products through soil columns. Immediately after treatment of soils with ^{14}C–labeled phenothrin isomers, the radiocarbon was hardly eluted from the treated soil in Kodaira light clay and Katano sandy loam soils. In contrast, a small amount of the radiocarbon moved from the treated soil to lower layers in Muko sand and 5–9% of the applied radiocarbon was found in effluents. This effluent contained polar products including PBacid, 3–HO–Balc, 4′–HO–PBalc, 3–HO–Bacid and 4′–HO–PBacid. After 14-days incubation of the treated soils, trace amounts of the radiocarbon were eluted from the treated Kodaira and Katano soils. In the leaching study, relatively low recovery of the total radiocarbon seems to be due to evolution of $^{14}CO_2$ from the soils during incubation and percolation.

Table 8 Leaching of ^{14}C from soils immediately or 14 days after application of ^{14}C–phenothrin isomers at the rate of 1.0 ppm.

	% of the applied ^{14}C									
	Without incubation						14-day incubation			
	t–Phenothrin			*c*–Phenothrin			*t*–Phenothrin		*c*–Phenothrin	
	Kodaira	Katano	Muko	Kodaira	Katano	Muko	Kodaira	Katano	Kodaira	Katano
Soil column										
Treated soil	54.6	37.5	62.8	93.7	80.1	63.8	42.2	31.8	65.1	59.6
0–5 (cm)	1.4	3.1	6.2	1.3	3.9	2.6	—[a)]	0.7	0.4	2.1
5–10	0.3	0.7	0.5	—	0.3	1.0	—	—	—	0.5
10–15	—	0.5	0.8	—	—	0.5	—	—	—	—
15–20	—	0.5	0.7	—	—	0.4	—	—	—	—
Effluent	—	0.5	8.6	—	—	5.2	—	1.3	0.6	1.4
PBacid			(1.5)			(1.4)				
3–HO–Balc, 4′–HO–PBalc			(0.3)			(0.3)				
3–HO–Bacid, 4′–HO–PBacid			(0.5)			(0.2)				
Total	56.3	42.8	79.6	95.0	84.3	73.5	42.2	33.8	66.1	63.6

a) —; <0.3%

DISCUSSION

After foliar treatment, both *t*– and *c*–phenothrin disappeared rapidly from bean and rice plants with half-lives of less than one day. Under the same conditions, *t*– and *c*–permethrin were decomposed in bean plants with half-lives of about 7 days and 9 days, respectively.[10] In another study, the half-lives of both *t*– and *c*–permethrin in bean plants were one to two weeks.[11] Topically-applied decamethrin disappeared from cotton plants with the half-life of 1.1 weeks under greenhouse conditions.[12] From these results, degradation of phenothrin in plants appears to be much more rapid than the other pyrethroids with 3–phenoxybenzyl moiety.

Figure 6 shows the proposed metabolic pathways for phenothrin isomers on and/or in plants. Both *t*– and *c*–phenothrin underwent ozonolysis at the isobutenyl double bond probably by the action of air and/or light, as reported in photodecomposition studies of pyrethrin I, allethrin, phthalthrin and dimethrin.[13] The life of the ozonides on and/or in plants was very short, being decomposed rapidly to formyl–phenothrin which was further oxidized to carboxyl–phenothrin. These ester products were subsequently metabolized *via* hydrolysis of the ester linkage, hydroxylation at 2′– and 4′–positions of the phenoxy moiety, oxidation of the benzyl alcohols to the benzoic acids.

Metabolism of (+)–*trans*–phenothrin in rats proceeded rapidly *via* hydrolysis of the ester linkage and hydroxylation at 4′–phenoxy position of the alcohol moiety.[2] Also, (+)–*cis*–phenothrin was metabolized in rats to give three ester products which resulted from hydroxylation at 4′–phenoxy position of the alcohol moiety, oxidation of *trans*–isobutenyl methyl group and hydroxylation of *cis*–geminal–dimethyl group of the acid moiety.[3] Phenothrin ozonide, formyl– and carboxyl–

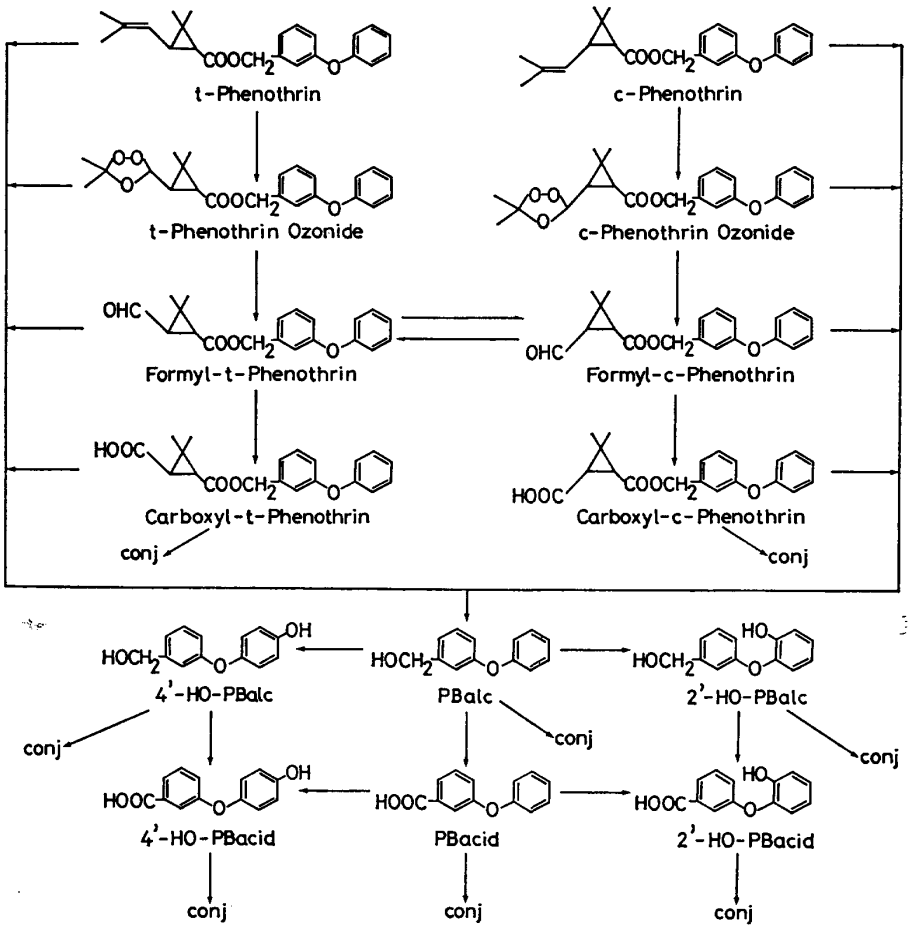

Fig. 6 Proposed metabolic pathways for *t*– and *c*–phenothrin on and/or in plants.
conj; conjugates

phenothrin were not found in rats, whereas major constituents of the residues in plants were polar metabolites and unextractable bound residues. The bound residues are not likely to be bioavailable and may be excreted mainly into the feces when administered to mammals, whereas the conjugates appears to be readily absorbed and metabolized in mammals.[14]

While phenothrin residues in soil were taken up to some extents into the roots of bean plants, very little was transferred from roots to shoots and edible portions. In addition, the residues absorbed into the plants may be metabolized easily, because no parent compounds were detected in shoots. Therefore, there seems to be little effect of phenothrin residues in soils on rotational crops in the field.

Phenothrin isomers were decomposed rapidly in soils under upland conditions with half-lives of 1 to 2 days. The degradability of some pyrethroids with 3–phenoxybenzyl moiety was also studied under similar conditions. The half-life was 6–12 days[15] and 3 weeks[16] for permethrin, 2–4 weeks for cypermethrin,[9] 4–>16 weeks for fenpropanate[17] and 15 days–3 months,[8] 7 weeks[16] for fenvalerate. From these results, the degradation of phenothrin isomers in soils appears to be much faster than these pyrethroids tested under upland conditions. Under flooded conditions, the degradation rate of phenothrin isomers was much slower as compared with under upland conditions. This tendency was also reported with other pyrethroids including permethrin,[18] cypermethrin,[9] fenpropanate[17] and fenvalerate.[8] Also, degradation of *trans*–isomer was faster than that of the *cis*–isomer in soils under flooded conditions. The same tendency was reported with permethrin isomers[15,18] and cypermethrin isomers.[9] Degradation of these pyrethroids in soil appears to be mainly due to microbial action.[8,18]

Figure 7 shows the proposed degradation pathways for phenothrin isomers in soils. Both isomers underwent hydrolysis of ester linkage, cleavage of the diphenyl ether linkage, hydroxylation at 4′–position of the phenoxy group, oxidation of the benzyl alcohols to the benzoic acids and decarboxylation of the benzoic acids. Among these reactions, hydrolysis of the ester linkage was the most predominant reaction and faster with the *trans*–isomer than the *cis*–isomer. Hydroxylation at 4′–position of the phenoxy group was also found with permethrin,[15] cypermethrin[9] and fenvalerate.[8] Although cleavage of diphenyl ether linkage was found with fenvalerate,[8] this reaction may occur following hydroxylation at 2′–position of the phenoxy group, because 2′–hydroxy–3–phenoxybenzoic

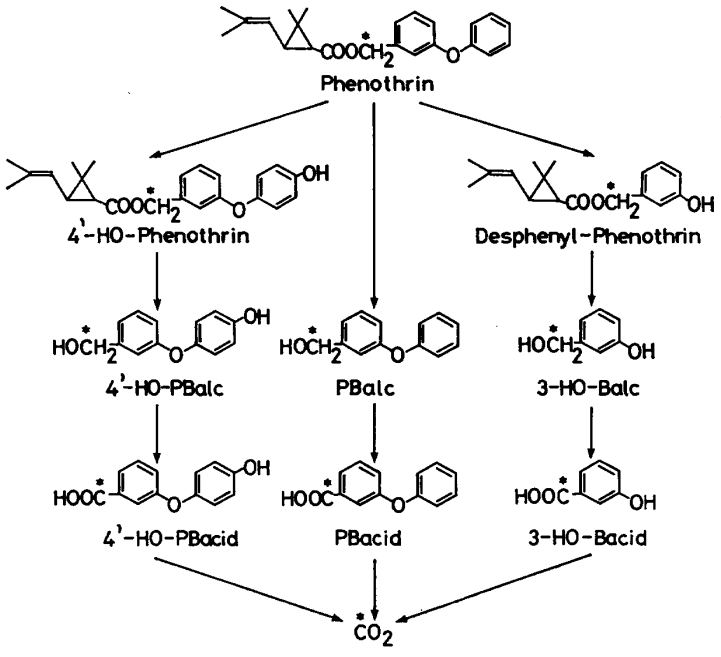

Fig. 7 Proposed degradation pathways for phenothrin isomers in soils.

acid was unstable under acidic conditions to decompose to 3–hydroxybenzoic acid easily as reported previously.[19] The products retaining the ester linkage were also hydrolyzed to give the corresponding benzyl alcohols which were oxidized to the corresponding benzoic acids. Under upland conditions, the benzoic acids were very rapidly decarboxylated to yield $^{14}CO_2$. However, under flooded conditions, decarboxylation proceeded much more slowly as compared with under upland conditions. Although 3–phenoxybenzoic acid derived from cypermethrin[9] and fenpropanate[16] was rather persistent in waterlogged soils under anaerobic conditions, this product was not accumulated under the conditions tested in the present study.

The ^{14}C bound residues are distributed into fulvic acid, humic acid and humin fractions of soil organic matter. The pattern of ^{14}C distribution varied with soil types, as reported with permethrin,[18] cypermethrin[9] and fenpropanate.[17] The humic acid fraction from Kodaira light clay soil and the fulvic acid fraction from Katano sandy loam soil contained larger amounts of ^{14}C. The fulvic acid fraction from both soils included PBacid and 4′–HO–PBacid which were also found in the same fraction with cypermethrin.[9]

As reported with permethrin[15] and fenvalerate,[8] phenothrin isomers were also adsorbed tightly on to soil particles and were hardly eluted with water, although the polar products such as PBacid were eluted from Muko sand.

When exposed to light, phenothrin decomposed more rapidly than permethrin but more slowly than bioresmethrin.[4] Under the tested conditions, decomposition of phenothrin was more rapid than that of permethrin. Therefore, when phenothrin is applied to the field, this compound is likely to stay at the application sites, not to move other sites and to decompose gradually on and/or in plants and soils, and by light.

REFERENCES

1) K. Fujimoto, N. Itaya, Y. Okuno, T. Kadota & T. Yamaguchi: *Agric. Biol. Chem.* **37**, 2681 (1973)
2) J. Miyamoto, T. Suzuki & C. Nakae: *Pestic. Biochem. Physiol.* **4**, 438 (1974)
3) T. Suzuki, N. Ohno & J. Miyamoto: *J. Pesticide Sci.* **1**, 151 (1976)
4) M. Elliott, A. W. Farnham, N. F. Janes, P. H. Needham, D. A. Pullman & J. H. Stevenson: Proceedings 7th British Insecticide and Fungicide Conference, p. 721, 1973
5) F. Barlow, A. B. Hadaway, L. S. Flower, J. E. H. Grose & C. R. Turner: *Pestic. Sci.* **8**, 291 (1977)
6) M. Hazue & T. Kamata: Unpublished
7) H. Ohkawa, H. Kaneko & J. Miyamoto: Unpublished observations
8) H. Ohkawa, K. Nambu, H. Inui & J. Miyamoto: *J. Pesticide Sci.* **3**, 129 (1978)
9) T. R. Roberts & M. E. Standen: *Pestic. Sci.* **8**, 305 (1977)
10) H. Ohkawa, H. Kaneko & J. Miyamoto: *J. Pesticide Sci.* **2**, 67 (1977)
11) L. C. Gaughan & J. E. Casida: *J. Agric. Food Chem.* **26**, 525 (1978)
12) L. O. Ruzo & J. E. Casida: *J. Agric. Food Chem.* **27**, 572 (1979)
13) Y.-L. Chen & J. E. Casida: *J. Agric. Food Chem.* **17**, 208 (1969)
14) T. C. Marshall & H. W. Dorough: *J. Agric. Food Chem.* **25**, 1003 (1977)
15) H. Kaneko, H. Ohkawa & J. Miyamoto: *J. Pesticide Sci.* **3**, 43 (1978)
16) I. H. Williams & M. J. Brown: *J. Agric. Food Chem.* **27**, 130 (1979)
17) T. R. Roberts & M. E. Standen: *Pestic. Sci.* **8**, 600 (1977)
18) D. D. Kaufman, S. C. Haynes, E. G. Jordan & J. A. Kayer: *ACS. Symp. Ser.* **42**, 147 (1977)
19) L. C. Gaughan, T. Unai & J. E. Casida: *J. Agric. Food Chem.* **25**, 9 (1977)
20) D. R. Hoagland & T. C. Broyer: *Plant Physiol.* **11**, 471 (1936)

要　　約

フェノトリンの植物および土壌における代謝・分解

南部健二，大川秀郎，宮本純之

アルコール側のメチレン基を ^{14}C で標識したフェノトリンの (+)-*trans* および (+)-*cis* 体はおのおのインゲンマメまたはイネの葉面に処理すると，半減期1日以下の速度で急速に減少した．両異性体は酸側のイソブテニル基のオゾン酸化を経て相当するアルテヒドおよびカルボン酸体に変換された．これらの分解物はさらにエステル結合の開裂，アルコール側の 2′ および 4′ 位の水酸化，ベンジンアルコールの安息香酸への酸化，ならびに

生成するアルコール類とカルボン酸類は糖類との抱合などを受けた．

^{14}C-フェノトリンを 1.0 ppm の割合で処理した小平土壌，交野土壌および武庫砂土にインゲンマメ幼苗を移植し，植物体への ^{14}C の吸収・移行性を調べた結果，地上部や可食部には ^{14}C がほとんど認められなかったが，根には 0.21-3.48 ppm の ^{14}C が認められた．地上部には親化合物はまったく検出されなかった．

^{14}C-フェノトリンは小平および交野土壌中，畑地条件下で半減期 1-2 日の速度で急速に減少した．それにくらべて，水田条件下での分解はずっと遅く，(+)-*trans* および (+)-*cis* 体の半減期はおのおの 1-2 週間および 1-2 カ月であった．両異性体は土壌中でエステル結合の開裂，アルコール側の 4′ 位の水酸化，ジフェニルエーテル結合の開裂およびベンジルアルコールの安息香酸への酸化を経て分解された．これらの分解物は土壌中に長く残留することなく，標識炭素は最終的には $^{14}CO_2$ にまで分解された．