Original Article

Degradation of Organophosphorus Insecticide Salithion in Soils

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Degradation of salithion (2-methoxy-4*H*-1,3,2-benzodioxaphosphorin-2-sulfide) in two types of Japanese soils was studied by using a ^{14}C preparation labeled at the phenyl ring. Salithion disappeared rapidly with a half-life of less than 3 days under aerobic upland conditions, finally degrading to $^{14}\text{CO}_2$ and unextractable bound residues. The degradation pathways included cleavages of *P-O*-aryl and *P-O*-aralkyl linkages, demethylation, and oxidative desulfuration of the thiophosphoryl moiety. The degradation rate retarded under sterilized conditions, suggesting microbial degradation in the soils. Non-biological formation of the bound residues also caused the disappearance of salithion in the Ushiku soil (Andosol).

INTRODUCTION

Salithion (2-methoxy-4*H*-1,3,2-benzodioxaphosphorin-2-sulfide) is an organophosphorus insecticide with a unique cyclic ester structure. It has been widely used for control of a broad spectrum of insect pests in orchards and vegetable gardens.¹⁾ The metabolic fate of salithion in mammals and plants,²⁾ and its photodegradation in water solution and on soil surface³⁾ have been reported, but its degradation in soil has not yet been extensively examined. From the standpoint of environmental safety, it is important to clarify the fate of salithion in soil. This paper deals with the degradation of ^{14}C -salithion in Japanese soils under aerobic upland conditions, together with comparative studies using sterilized soils to clarify the chemical *versus* microbial degradation processes of salithion in the soils.

MATERIALS AND METHODS

1. Chemicals

^{14}C -Salithion labeled uniformly at the phenyl ring was synthesized at our laboratory. The specific activity was 42.5 mCi/mmol and the radiochemical purity was more than 99.9%,

as measured by silica gel TLC with solvent systems A and C shown in Table 1.

Unlabeled salithion (**I**) and 2-hydroxybenzoic acid (**VI**), and 2-hydroxybenzyl alcohol (**V**) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka) and Kanto Chemical Co., Inc. (Tokyo), respectively, and used without further purification. The following compounds were synthesized as authentic standards according to a previous report²⁾: 2-methoxy-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (**II**), 2-hydroxy-4*H*-1,3,2-benzodioxaphosphorin-2-sulfide (**III**), and 2-hydroxy-4*H*-1,3,2-benzodioxaphosphorin-2-oxide (**IV**). The TLC *R_f* values of each compound with the solvent systems are listed in Table 1.

2. Radioassay

Radioactivity in the gel regions scraped off of silica gel chromatoplates, organosoluble and aqueous solutions were measured with a Packard Model 460-CD liquid scintillation spectrometer (LSC), by using 10 ml of Emulsifier Scintillator 299 (Packard). Radioactivity in unextracted soil residues was measured by combustion of a sample (300 mg) with a Packard Model 306 sample oxidizer

Table 1 Thin-layer chromatographic properties of salithion and its degradation products.

Compound	<i>R_f</i> values with indicated solvent systems ^{a)}				
	A	B	C	D	E
I	0.73	0.71	0.66	0.51	0.32
II	0.67	0.57	0.42	0.18	0.14
III	0.06	0.41	0.13	0.01	0.00
IV	0.01	0.19	0.07	0.01	0.00
V	—	0.65	0.42	0.32	—
VI	0.48	0.65	0.54	0.11	0.18

^{a)} A, chloroform/methanol(9/1); B, toluene/ethyl acetate/2-propanol/acetic acid (8/12/5/3); C, toluene/ethyl formate/formic acid (5/7/1); D, toluene/acetic acid (7/1); E, *n*-hexane/acetone (5/2).

Table 2 Characteristics of the soils used.

	Ushiku	Noichi
Soil group	Andosol	Gray lowland soil
Soil texture	Loam	Sandy clay loam
Sand (%)	51.0	72.5
Silt (%)	40.8	8.1
Clay (%)	8.2	19.4
Clay mineral	Allophane	Kaolinite
Organic matter (%) ^{a)}	4.1	2.4
CEC (meq/100 g) ^{a, b)}	19.6	8.8
pH (H ₂ O)	6.4	5.7
MWHC (%) ^{a, c)}	126.3	71.4

^{a)} Dry soil basis. ^{b)} Cation exchange capacity.

^{c)} Maximum water-holding capacity.

followed by LSC measurements.

3. TLC

Extracts from soils were spotted on pre-coated silica gel 60F₂₅₄ chromatoplates (20×20 cm, 0.25 mm thickness, E. Merck), which were developed in two directions, first with solvent system B and secondly with solvent system C. Radioactive spots were detected by autoradiography using Kodak SB-5 X-ray films, and unlabeled authentic compounds by UV light fluorescence quenching.

4. Soils

Two types of soils collected from upland fields in Japan (Ushiku, Ibaraki Pref. and Noichi, Kochi Pref.) were used. The characteristics of the soils are listed in Table 2. Soil samples were passed through a 2-mm sieve before use.

5. Degradation Study in Soils

5.1 Soil treatment

The soil equivalent to 30 g on a dry weight basis was taken into a 50-ml beaker and moistened with distilled water to 50% of the maximum water-holding capacity. ¹⁴C-I (7.39 μ Ci) in dimethyl sulfoxide (50 μ l) was put on the soils after 2 weeks of incubation at 25°C in the dark. The treated soils were mixed thoroughly to a concentration of 1.25 ppm, placed in a 3-l brown glass jar covered with aluminum foil and incubated at 25°C for 60

days. CO₂-free air was passed through each jar connected with a polyurethane foam plug and a gas-washing bottle containing 400 ml of 0.5 N NaOH solution linked in series at a flow rate of 25–30 ml/min, to collect volatile ¹⁴C including ¹⁴CO₂. The polyurethane foam and alkaline solution were renewed every two weeks. The soil moisture was adjusted periodically to its original level by adding distilled water.

A similar application was done to autoclaved soils under sterilized conditions (120°C for 1 hr, twice every other day). Beakers containing the treated soils were covered with aluminum foil and kept aseptically at 25°C for 14 days.

5.2 Characterization of volatile ¹⁴C

The polyurethane foam plug was washed with methanol (30 ml×3) to collect volatile ¹⁴C. To measure the ¹⁴CO₂ dissolved in the NaOH solution, radioactivity was separately assayed in the alkaline solution (10 ml) and in the supernatant fraction (10 ml) separated by centrifugation from Ba¹⁴CO₃ after the addition of 10 ml of 2 N BaCl₂ solution to 30 ml of the NaOH solution.

5.3 Extraction and analysis of ¹⁴C

Each of the treated soils (30 g) was extracted three times with 80 ml of a mixture of ethyl acetate and 0.1 N HCl (7:1) by mechanical shaking for 10 min. After a sample (1 ml) of the combined ethyl acetate layers was radioassayed, the remaining portion was washed

with brine and concentrated under reduced pressure. The extracts were analyzed by TLC co-chromatography together with the authentic standards. The TLC and LSC analysis showed that more than 99% of **I** was recovered from each soil through extraction immediately after the application at two concentrations (1.25 and 0.124 ppm). The unextracted soil residues were dried under reduced pressure. An aliquot (300 mg) of the soil sample was packed with filter paper and combusted to $^{14}\text{CO}_2$ for radioassay.

RESULTS

1. Dissipation in Soils

Extractable **I** disappeared rapidly in the both soils under the aerobic upland conditions as shown in Fig. 1. The half-lives of disappearance were 3 and 1.5 days in the Ushiku and Noichi soils, respectively. Less than 3% of the applied ^{14}C -**I** was recovered after 28 days of aerobic incubation. The disappearance of **I** retarded under the sterilized conditions, compared with under the non-sterilized conditions. The half-life of disappearance was 9 days in the Ushiku soil, and it was estimated to be 29 days in the Noichi soil by the least-square approximations method.

2. Degradation Products in Soils

The amounts of **I** and its degradation products in the Ushiku and Noichi soils are listed in Tables 3 and 4, respectively. Under the aerobic conditions, most of the radioactivity in the extracts was derived from **I**. **V** was a major degradation product at 2.4% (Noichi soil) on the first day, but rapidly disappeared thereafter. **III**, **IV**, and **VI** were minor degradation products at earlier sampling times, each amounting to less than 0.5% after 3 days of incubation. The majority of the applied ^{14}C was mineralized to $^{14}\text{CO}_2$, amounting to 38.8–44.9% after 60 days of incubation. Other volatile ^{14}C s were less than 2% of the applied ^{14}C . The bound residues gradually increased up to 48.4–58.5% of the applied ^{14}C between 7th and 14th day with a concomitant decrease of the extractable ^{14}C , but slowly decreased thereafter.

Under the sterilized conditions, most of the extractable ^{14}C was derived from **I**. Degradation

Fig. 1 Decline curves of salithion under aerobic and sterilized upland conditions.

—, aerobic upland conditions; ·····, sterilized upland conditions; ●, Ushiku soil; ○, Noichi soil.

tion product **V** increased with time up to 2.0–4.3% of the applied ^{14}C during 14 days of incubation, in contrast with the rapid disappearance under the non-sterilized conditions. The amount of bound ^{14}C under the sterilized conditions varied depending on the soil. After 14 days of incubation, the bound residues increased steadily up to 53.1% of the applied ^{14}C in the Ushiku soil. This proportion of the bound ^{14}C was almost equal to the 55.4% under the non-sterilized conditions. In contrast, the bound ^{14}C in the Noichi sterilized soil amounted to 14.1% of the applied ^{14}C , compared with 41.8% under the non-sterilized conditions.

DISCUSSION

It is generally known that organophosphorus pesticides are less persistent than organochlorines in soil, and that they dissipate, with some exceptions, within a few weeks after application.⁴⁻⁶⁾ Since **I** dissipated from the soils in a short time as other organophosphorus pesticides did, **I** seemed to be only slightly residual in the soil environment.

Pesticides are thought to degrade by chemical and/or microbial processes in soil, and many studies have been done to examine each process.⁷⁻⁹⁾ Sterilization of soil is one method to clarify the relative importance of these processes. Degradation in sterilized soil is considered to be non-biological, and a decrease of degradation rate by sterilization is attributable to the participation of micro-

Table 3 Degradation of ¹⁴C-salithion in Ushiku soil under non-sterilized and sterilized upland conditions.

	% of the applied ¹⁴ C (days after application)									
	Non-sterilized conditions						Sterilized upland conditions			
	1	3	7	14	28	60	1	3	7	14
Volatile ¹⁴ C	2.7	8.3	18.7	24.1	32.0	39.5	— ^{a)}	—	—	—
Polyurethane plug	<0.1	0.1	0.1	0.1	0.1	0.1	—	—	—	—
NaOH soln. trap ¹⁴ CO ₂	2.7	8.2	18.6	24.0	31.9	39.4	—	—	—	—
Others	<0.1	<0.1	<0.1	0.3	0.3	0.6	—	—	—	—
Extractable ¹⁴ C	69.4	50.3	28.7	12.2	3.4	1.6	89.3	75.4	59.5	41.9
I	68.7	49.8	28.5	12.1	3.3	1.6	88.8	74.2	56.9	37.4
II	nd ^{b)}	nd	nd	nd	nd	nd	<0.1	nd	nd	nd
III	0.1	<0.1	nd	nd	nd	nd	0.1	0.1	0.1	0.2
IV	<0.1	nd	nd	nd	nd	nd	<0.1	nd	nd	nd
V	0.5	0.4	0.1	0.1	0.1	nd	0.4	1.1	2.5	4.3
Others	0.1	0.1	0.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Bound ¹⁴ C	24.8	36.5	44.5	58.5	57.0	55.4	11.3	23.0	35.8	53.1
Total ¹⁴ C	96.9	95.1	92.0	94.8	92.4	96.5	100.6	98.4	95.3	95.0

a) Not tested. b) Not detected.

Table 4 Degradation of ¹⁴C-salithion in Noichi soil under non-sterilized and sterilized upland conditions.

	% of the applied ¹⁴ C (days after application)									
	Non-sterilized conditions						Sterilized upland conditions			
	1	3	7	14	28	60	1	3	7	14
Volatile ¹⁴ C	6.8	18.8	31.2	35.8	41.4	46.4	— ^{a)}	—	—	—
Polyurethane plug	0.1	0.1	0.1	0.1	0.1	0.1	—	—	—	—
NaOH soln. trap ¹⁴ CO ₂	6.7	18.7	31.1	35.7	41.3	46.3	—	—	—	—
Others	0.1	0.4	0.6	0.7	0.8	1.4	—	—	—	—
Extractable ¹⁴ C	64.8	33.3	9.2	3.6	2.0	1.2	98.0	92.1	87.0	75.9
I	60.3	31.5	7.7	3.5	1.8	1.2	97.8	91.2	85.0	71.4
II	nd ^{b)}	nd	nd	nd	nd	nd	<0.1	nd	nd	nd
III	1.4	0.4	<0.1	nd	nd	nd	<0.1	0.2	0.3	0.7
IV	0.1	<0.1	0.2	nd	nd	nd	<0.1	<0.1	<0.1	0.1
V	2.4	1.2	1.1	0.1	0.1	nd	0.1	0.5	1.2	2.0
VI	0.4	0.2	nd	nd	nd	nd	nd	nd	nd	0.2
Others	0.2	<0.1	0.2	<0.1	0.1	<0.1	0.1	0.2	0.5	1.5
Bound ¹⁴ C	22.4	38.3	48.4	47.8	44.5	41.8	3.1	6.8	8.5	14.1
Total ¹⁴ C	94.0	90.4	88.8	87.2	87.9	89.4	101.1	98.9	95.5	90.0

a) Not tested. b) Not detected.

organisms. On the basis of the above considerations, it is likely that malathion and diazinon dissipate by both chemical and microbial hydrolysis of the *P-O*-aryl linkage,⁸⁻¹⁰⁾ whereas parathion degrades mainly either by hydrolysis or by reduction of the

nitrogroup by soil microorganisms.⁹⁻¹¹⁾

The dissipation rates of **I** under the sterilized conditions were approximately 1/3 and 1/18 of those under the non-sterilized conditions in the Ushiku and Noichi soils, respectively. This retardation under the sterilized conditions and the production of $^{14}\text{CO}_2$ up to 38.8–44.9% under the non-sterilized conditions clearly suggested the participation of microorganisms in the disappearance of **I**. Bound residues, up to 53.1% (Ushiku soil) and 14.1% (Noichi soil), were observed under the sterilized conditions for 14 days, showing that **I** also disappeared by non-biological formation of bound residues. Since degradation product **V** slightly increased with time to 2.0–4.3% for 14 days under the sterilized conditions, chemical decompositions such as cleavage of ester linkages of **I** seemed to proceed slowly in the both soils.

From the above observations, the disappearance of **I** seemed to proceed in the following ways: in the Ushiku soil **I** disappeared both by non-biological adsorption and by microbial degradation, as shown by a large amount of bound ^{14}C rapidly formed under the sterilized conditions (a non-biological process) and $^{14}\text{CO}_2$ generated up to 38.8% for 60 days under the non-sterilized conditions (microbial mineralization), whereas in the Noichi soil **I** was present in forms available to **I**-degrading microorganisms, as shown by slight transformation of **I** to bound ^{14}C . These observations suggest that **I** dissipated mostly by microbial degradation. The amount of bound residues was larger under the non-sterilized conditions than under the sterilized

conditions. This is probably attributed to that the degradation products of **I** were adsorbed to the soil. Soils with a higher organic-matter content and CEC adsorb organophosphorus pesticides to a greater extent.^{12,13)} The bound ^{14}C formed in the Ushiku soil is likely to be larger in amount due to the higher organic-matter contents and CEC derived from the allophane component^{14,15)} in the soil.

From the identified degradation products, it has been confirmed that **I** is degraded finally to carbon dioxide in aerobic upland soils *via* cleavages of *P-O*-aryl and *P-O*-aralkyl linkages, demethylation, and oxidative desulfuration of the thiophosphoryl group, like other organophosphorus pesticides.^{11,16)} Since the degradation products of **I** in the extractable ^{14}C immediately disappeared, the major degradation pathway was not specified. Although almost all of the degradation products identified in this experiment have been also found in mammals and plants,²⁾ **VI** was characteristic of the degradation of **I** in the soils used. **V** was excreted as the final metabolic product in mammals, and was stored in the form of conjugates with glucose in plants.²⁾ In contrast, in this experiment **V** was further oxidized to form **VI** in the soils. On the basis of the accumulated evidence on the microbial degradation of aromatic compounds, reports say that **VI** undergoes further oxidation to catechol with subsequent *meta* or *ortho* cleavage of the phenyl ring and is finally mineralized to CO_2 by soil microorganisms.^{17,18)} Degradation pathways for **I** under aerobic upland conditions are proposed as shown in Fig. 2 based on the experiment.

Fig. 2 Proposed degradation pathways for salithion in soils.

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要 約

有機リン殺虫剤サリチオンの畑地土壌における分解

井藤和人

サリチオン (2-メトキシ-4*H*-1,3,2-ベンゾジオキサホスホリン-2-スルフィド) のフェニル環を ^{14}C で標識した化合物を用いて, 2種類の日本の土壌中における分解について検討した. サリチオンは好氣的畑地条件下において, 3日以内の半減期で速やかに消失し, 最終的には $^{14}\text{CO}_2$ および bound ^{14}C となった. おもな分解経路は *P-O*-アリアル結合および *P-O*-アラルキル結合の開裂, 脱メチル化および *P=S* から *P=O* への酸化であった. 滅菌条件下においてはサリチオンの分解が抑制されたことから, サリチオンの土壌中における分解には微生物が関与していることが示唆された. また, 牛久土壌 (黒ボク土) におけるサリチオンの消失には非生物的な bound ^{14}C の形成も関与していることが示唆された.