ベンチアバリカルブイソプロピルの土壌中での分解

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Original Article

Degradation of the Fungicide Benthiavalicarb-isopropyl in Soils

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The degradation of benthiavalicarb-isopropyl (BVI) in soils was studied in the laboratory using two ^{14}C-compounds labeled at the benzene ring and the valine-moiety. The half-life of BVI in two kinds of soils (Ushiku soil and Kakegawa soil) was less than 11 days, under both upland and flooded conditions. Five major degradation products, in addition to $^{14}CO_2$, were identified. It is presumed that the degradation begins initially with hydrolytic cleavage of the amide bond, followed by several processes resulting in the final production of $^{14}CO_2$. © Pesticide Science Society of Japan

Keywords: degradation, half-life, fungicide, benthiavalicarb-isopropyl, metabolism in soil, BVI.

INTRODUCTION

Benthiavalicarb-isopropyl (Isopropyl [(*S*)-1-[(*R*)-1-(6-fluorobenzothiazol-2-yl)ethylcarbamoyl]-2-methylpropyl]carbamate, BVI) is a fungicide under development by Kumiai Chemical Industry Co., Ltd., showing excellent protection against late blight caused by *Phytophthora infestans*, and downy mildew caused by *Plasmopara viticola*, *Pseudoperonospora cubensis* and *Peronspora parasilica* in potatoes and tomatoes.[1,2] In general, pesticides applied to farm fields disappear via chemical and/or biological degradation. However, it has been reported that BVI was slow to hydrolyze (half-life time: one year in water at pH 4, 7 and 9, 25°C) or photodegrade (half-life time: 301 days in water under xenon light, 400 W/m^2, 300 to 800 nm),[3] and there was little metabolic transformation in plants.[4] The degradation by soil microorganisms is the primary factor in the fate of BVI in the environment. In this paper, the rate and routes of degradation of BVI in soils are clarified in the laboratory by using two ^{14}C-compounds labeled at different positions.

MATERIALS AND METHODS

1. Chemicals

1.1. Labeled compounds

Two ^{14}C-compounds were used: one was labeled uniformly at the benzene-ring of the benzothiazole of BVI ([Bz-^{14}C]BVI) and the other was labeled at the 2-position of valine ([Val-^{14}C]BVI), as shown in Fig. 1. Both compounds were synthesized by Daiichi Pure Chemicals Co., Ltd. The specific radioactivity of [Bz-^{14}C]BVI was 2.40 MBq/mg and [Val-^{14}C]BVI was 2.23 MBq/mg. The radiochemical purity, as determined by thin-layer chromatography (TLC), was more than 99.9% for both compounds.

1.2. Authentic compounds

BVI and the following authentic compounds were used: BVI, white powder, mp 152.0°C and 169.2°C, vp $<3.0\times10^{-4}$ Pa; 6-Fluoro-2-hydroxybenzothiazole (M-1), pale yellow powder, mp 188.4°C; 1-(6-fluoro-2-benzothiazolyl)ethyl alcohol (M-3), white powder, mp 74.9°C; 6-fluoro-2-benzothiazolyl methyl ketone (M-4), pale yellow powder, mp 126.3°C; 1-(6-fluoro-2-benzothiazolyl)ethylamine (M-5), pale yellow powder, mp 38.8°C; *N*-[1-(6-fluoro-2-benzothiazolyl)ethyl]acetamide (M-8), white powder, mp 149.0–152.0°C. They were synthesized and supplied by K·I Chemical Research Institute Co., Ltd., and their purities were above 98%. The TLC R_f values, NMR data and MS data of these compounds are shown in Table 1.

2. Soils

Ushiku upland field soil (sampled from the Research Institute of the Japan Plant Protection Association, Ibaraki Prefecture) and Kakegawa upland field soil (sampled from Life Science Research Institute, Kumiai Chemical Industry Co., Ltd., Shizuoka Prefecture) were used. The soils were passed through a 2 mm mesh sieve and stored at 4°C in the dark for about 2 months before use. The properties of these soils are listed in Table 2.

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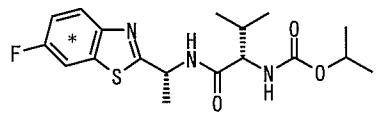
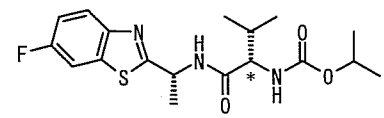


Benzene ring-^{14}C benthiavalicarb-isopropyl
([Bz-^{14}C]BVI)



Valyl-2-^{14}C benthiavalicarb-isopropyl
([Val-^{14}C]BVI)

Fig. 1. Structural formula of benthiavalicarb-isopropyl and ^{14}C-labeled positions (*).

Table 1. TLC R_f values, ^{1}H-NMR data and GC-MS data

Compounds	R_f values in solvent systems[a]			^{1}H-NMR (δ_H, ppm in $CDCl_3$)[b]	GC-MS m/z[c]
	A	B	C		
benthiavalicarb-isopropyl (BVI)	0.42	0.30	0.51	0.95 (6H, dd, J=6.7 and 16.7 Hz), 1.12–1.19 (6H, m), 1.63 (3H, d, J=7.1 Hz), 2.13–2.17 (1H, m), 4.07–4.10 (1H, m), 4.80–4.83 (1H, m), 5.30–5.33 (1H, m), 5.39–5.46 (1H, m), 7.11–7.16 (1H, m), 7.24–7.27 (1H, m), 7.40–7.42 (1H, m), 7.28–7.86 (1H, m)	381 (M^+), 321 (M^+-55), 222 (M^+-159), 195 (M^+-186), 180 (M^+-201), 158 (M^+-223)
M-1	0.45	0.37	0.41	6.98–7.18 (3H, m), 9.8 (1H, br.)	169 (M^+), 141 (M^+-CO), 114 (M^+-55),
M-3	0.35	0.30	0.55	1.71 (3H, d, J=8.8 Hz), 3.31 (1H, s), 5.19–5.27 (1H, m), 7.20 (1H, ddd, J=3.4 and 11.9 Hz, J_{HF}=11.9 Hz), 7.56 (1H, dd, J=3.4 Hz, J_{HF}=10.7 Hz), 7.88–7.93 (1H, m)	197 (M^+), 182 (M^+-CH_3), 154 (M^+-43)
M-4	0.62	0.58	0.71	2.81 (3H, s), 7.33 (1H, ddd, J=3.4 and 11.9 Hz, J_{HF}=11.9 Hz), 7.65 (1H, dd, J=3.4 Hz, J_{HF}=10.7 Hz), 8.12–8.17 (1H, m)	195 (M^+), 180 (M^+-CH_3), 167 (M^+-28), 153 (M^+-42)
M-5	0.08	0.06	0.26	1.61 (3H, d, J=9.0 Hz), 2.04 (2H, br.), 4.49 (1H, q, J=9.0 Hz), 7.19 (1H, ddd, J=3.4 and 11.9 Hz, J_{HF}=11.9 Hz), 7.56 (1H, dd, J=3.4 Hz, J_{HF}=11.0 Hz), 7.88–7.92 (1H, m)	196 (M^+), 181 (M^+-CH_3), 154 (M^+-42)
M-8	0.14	0.06	0.29	1.70 (3H, d, J=6.8 Hz), 2.12 (3H, s), 5.46–5.53 (1H, m), 6.75–6.77 (1H, br.), 7.24 (1H, ddd, J=2.7 and 9.0 Hz, J_{HF}=8.8 Hz), 7.55 (1H, dd, J=2.7 Hz, J_{HF}=8.0 Hz), 7.92–7.96 (1H, m)	238 (M^+), 195 (M^+-43), 154 (M^+-84)

[a] Solvent systems (Silica gel 60F_{254} chromatoplate). A) ethyl acetate : chloroform (1 : 1, v/v), B) ethyl acetate: *n*-hexane (1 : 1, v/v), C) isopropyl ether: acetonitrile: 28% ammonia solution (60 : 30 : 1, v/v/v).

[b] ^{1}H-NMR spectra were measured on a JEOL JMN-LA-400 (400 MHz) spectrometer.

[c] Mass spectra were measured on a JEOL JMS-Automass II-50 mass spectrometer. Column: HP-5 Trace Analysis, 30 m×0.32 mm I.D.×0.25 μm (Hewlett Packard), Oven Temp.: 100–290°C, Ionization: EI-Posi, 70 eV, Carrier gas: He, 1.5 ml/min.

3. *Degradation Study of BVI in Soils*

3.1. *Soil conditioning and treatment*

The soil samples (equivalent to 30 g on an oven-dry basis) were weighed in 100-ml Erlenmeyer flasks. Distilled water was added to adjust the water content of the soil to 55% of the maximum water-holding capacity (MWHC) for the upland soils and to a water depth of 1.5 cm for the flooded soils. The flasks were covered with aluminum foil, and preincubated at 30°C for 2 weeks in the dark. Upland soils were mixed with 50 μl (54 kBq) of an acetone solution of [Bz-^{14}C]BVI or 50 μl (50 kBq) of an acetone solution of [Val-^{14}C]BVI, and the flooded soil was mixed with 50 μl (54 kBq) of an acetone solution of [Bz-^{14}C]BVI. After mixing, each soil was incubated under the same conditions used for the preincubation. The

Table 2. Properties of the soils used

Parameters	Soils	
	Ushiku	Kakegawa
Soil origin	Volcanic ash	Upland man-made
Soil texture	Light clay	Clay loam
Particle-size distribution (%)		
Sand	33.6	51.5
Silt	29.6	29.9
Clay	26.4	18.6
Total organic carbon (%)	4.7	0.8
pH (H_2O)	6.7	7.0
Cation exchange capacity (me/100 g)	37.8	7.9
Maximum water-holding capacity (g/100 g)	107	72
Phosphate adsorption coefficient (mg P_2O_5/100 g)	1680	640
Main clay mineral	Allophane	Chlorite

initial concentration of BVI in the soil was 0.75 ppm within the oven-dried samples. The oxidation-reduction potential (Eh) ranged from 130 to 270 mV just before [Bz-^{14}C]BVI was added under flooded conditions.

A vial containing 5 ml of 2 M NaOH was hung in the flask to trap the $^{14}CO_2$ generated. The sample weight was measured regularly during the incubation period, and the water lost was replaced with sterilized distilled water to keep the water content at the initial level. The $^{14}CO_2$-trapping solution was changed every 2 weeks.

After preincubation, the soil assigned to the sterilized group was autoclaved at 120°C for 20 min, twice at 2-day intervals. The soil was then treated with one of the ^{14}C-labeled compounds in a clean bench and incubated at 30°C in the dark.

3.2. Extraction and fractionation

The upland soil was mixed with 50 ml of distilled water, 100 ml of a mixture of acetone and acetonitrile (1/1, v/v) and 50 μg of M-5 (50 μl of the 1000 ppm acetonitrile solution), which was added to prevent degradation during extraction and thin layer chromatography. The soil was refluxed at 85°C for 1 hr. Procedures used for flooded soils were the same as those used for upland soils, except for the addition of distilled water. The soil was filtered by suction. The residue on the filter paper (TOYO ROSHI No. 5C, 90 mm) was rinsed with 60 ml of the solvent mixture and filtered again. The soil residues were stocked for analysis of the non-extractable residues. The filtrates were combined and concentrated to about 50 ml under reduced pressure. The concentrate was extracted with 50 ml of a mixture of hexane and ethyl acetate (4/1, v/v). The aqueous solution was mixed with 6 ml of a Tris-phosphate buffer solution (pH 7.7, 1 M) to make the extract alkaline and extracted twice with 50 ml of the same solvent mixture. The remaining aqueous solution was designated as the aqueous fraction. The extracts were combined and dehydrated with anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (the ethyl acetate fraction).

The radioactivity was determined with a liquid scintillation counter (LSC; TRI-CARB 2750TR/LL, Packard) using AQUASOL®-2 (Packard) for the ethyl acetate fraction and the aqueous fraction, and HIONIC-FLUOR® (Packard) for the $^{14}CO_2$-trapping alkaline solution as the scintillators. The radioactivity in the non-extractable residue was measured by LSC following combustion using a sample oxidizer (Model 307, Packard).

3.3. Determination of degradation products

BVI and its degradation products were identified by comparison with authentic compounds by thin layer chromatography and mass spectrometry. The ethyl acetate fraction and authentic compounds were applied onto silicagel 60F_{254} chromatoplates (20×20 cm, 0.25 mm thick, E. Merck), which were developed with a mixture of ethyl acetate and chloroform (1/1, v/v) in 1st dimension and, after being dried, with a mixture of ethyl acetate and hexane (1/1, v/v) in 2nd dimension. The TLC plate, after thoroughly drying, was cut into 2 pieces along the horizontal line of the R_f value of about 0.2 in 1st dimension. Then, the piece containing the origin spot was developed in the same direction as two-dimension with a mixture of isopropyl ether, acetonitrile and 28% ammonia water (60/30/1, v/v/v) to separate the polar degradation spots (Fig. 3). The authentic compounds were detected under UV irradiation. The radioactive spots were detected and measured by autoradiography with a Bio-imaging Analyzer (Fujix BAS1000, Fuji Photo Film Co., Ltd.).

4. Degradation Study of M-5 and M-4

The degradation rates and degradation products of M-5 and M-4 in soil were examined. A 0.2 ml portion of a 3000 ppm acetonitrile solution of M-5 or M-4 was added to the Ushiku and Kakegawa upland soils (equivalent to 30 g of oven-dry soil), which had been preincubated for 9 days, as described in section 3.1 (a concentration of 20 ppm on an oven-dry basis). The soils were incubated again under preincubation conditions. The soils were sampled on the scheduled days. They were analyzed by acetone extraction by shaking for 30 min, partitioning into ethyl acetate, purifying with a Sep-pack® Plus Silica cartridge column (Waters, eluted with 10 ml of ethyl acetate), and measuring the parent compounds and their degradation products by GC-MS (detected ion: *m/z* 141 for M-1, 154 for M-3, 195 for M-4, 154 for M-5 and 195 for M-8). The operating conditions for GC-MS are shown in Table 1.

5. Non-Extractable Residue

5.1. Extraction of non-extractable residue

A non-extractable residue sample (5 g) was shaken with 25 ml of 0.5 M NaOH at room temperature for 24 hr to extract radioactive materials, and centrifuged (3000 rpm, 10 min) to separate the supernatant and the precipitate (humin fraction). The supernatant was adjusted to pH 2 with HCl and centrifuged (3000 rpm, 10 min) to separate the supernatant (fluvic acid fraction) and the precipitate (humic acid fraction). Then, the supernatant was extracted with ethyl acetate to fractionate radioactive components into the ethyl acetate-soluble and the water-soluble fractions according to the method of Ikeda *et al.*[5]

5.2. Degradation study of non-extractable residue

The non-extractable residue from the [Bz-^{14}C]BVI-treated soil incubated for 56 days under the upland conditions (Ushiku soil: 28 kBq/28 g soil, Kakegawa soil: 24 kBq/24 g) was mixed with fresh soil (equivalent to 100 g of oven-dry soil), which was preincubated at 28°C for 7 days under upland conditions prior to being mixed. The mixture was incubated at 28°C in the dark. The $^{14}CO_2$ generated was trapped with 3 M NaOH.

RESULTS

1. Degradation in Soils

1.1. Upland soils

The distribution of radioactivity in the soils treated with [Bz-^{14}C]BVI under upland conditions is shown in Fig. 2. The soils were analyzed immediately after and 3, 7, 14, 28 and 56 days after the application, while the sterilized soils were analyzed 28 and 56 days post-application. The total rate of recovery of radioactivity ranged from 82% to 104%. Most of the radioactivity extracted from soils was partitioned into the ethyl acetate fraction, with less than 5% in the aqueous fraction. The radioactivity in the ethyl acetate fraction gradually decreased to 21% of that applied in the Ushiku soil and 7% in the Kakegawa soil after 56 days. In contrast, the radioactivity remaining in the soil increased. The non-extractable residue contained 55% of the applied radioactivity in the Ushiku soil 56 days and 58% in the Kakegawa soil 28 days after the application. In addition, generation of $^{14}CO_2$ was observed, and the amounts accumulated 56 days post-application were 6% in the Ushiku soil and 18% in the Kakegawa soil.

[Bz-^{14}C]BVI and its degradation products were identified and measured by co-TLC with authentic compounds (Fig. 3). The half-life values of [Bz-^{14}C]BVI, computed by regression analysis assuming first-order kinetics ($Y=Ae^{-\lambda t}$), were 6.9 days for the Ushiku soil (correlation coefficient, $r=0.998$) and 2.8 days for the Kakegawa soil ($r=0.999$). M-1, M-3, M-4, M-5 and M-8 were identified as major degradation products by co-TLC with authentic compounds and their dissipation is shown in Fig. 4. The maximal amounts of degradation products were 17.8% for M-5, 8.6% for M-4, 6.3% for M-1 and 3.9% for M-3 in the Ushiku soil, and 20.1% for M-5, 4.6% for M-4, 4.6% for M-1 and 1.9% for M-3 in the Kakegawa soil. All the degradation products reached a maximum from 7 to 28 days after the application and then decreased. Within the aqueous fraction, analyzed 28 days post-application, most of the radioactivity was attributed to very polar substances remaining at the origin of the TLC plate, except for a small amount of M-5 that was detected.

In the sterilized soils, 88.1% of [Bz-^{14}C]BVI was detected in the Ushiku soil and 98.8% in the Kakegawa soil 56 days after the application. Most of the remaining radioactivity was detected in the non-extractable residue fraction (Ushiku soil; 7.4% and Kakegawa soil; 2.2%, 56 days post-application). The amounts of parent compound detected in both sterilized soils were very large compared to those in unsterilized soils, and very slow degradation occurred under the sterilized conditions.

The distribution of radioactivity and the dissipation of [Val-^{14}C]BVI after its application are shown in Fig. 5. The half-

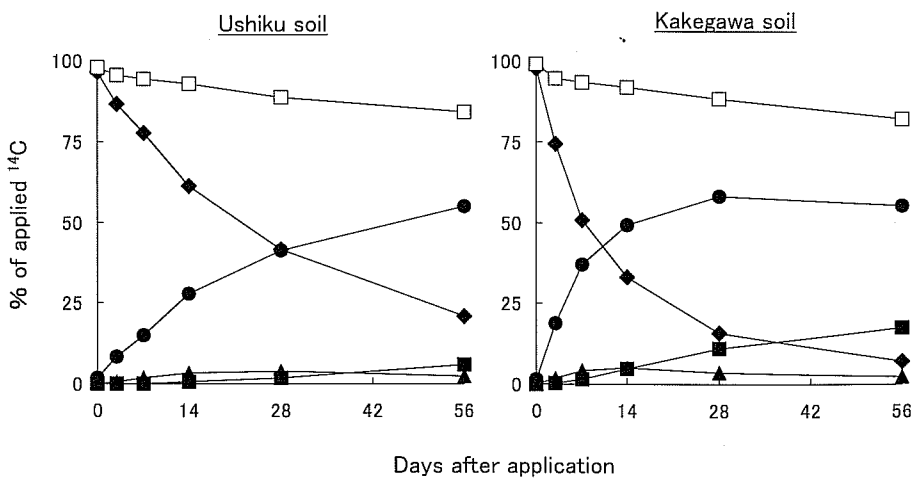


Fig. 2. Distribution of radioactivity in Ushiku and Kakegawa soils treated with [Bz-^{14}C]BVI under upland conditions. □: Total, ◆: ethyl acetate fraction, ●: non-extractable residue, ▲: aqueous fraction, ■: $^{14}CO_2$.

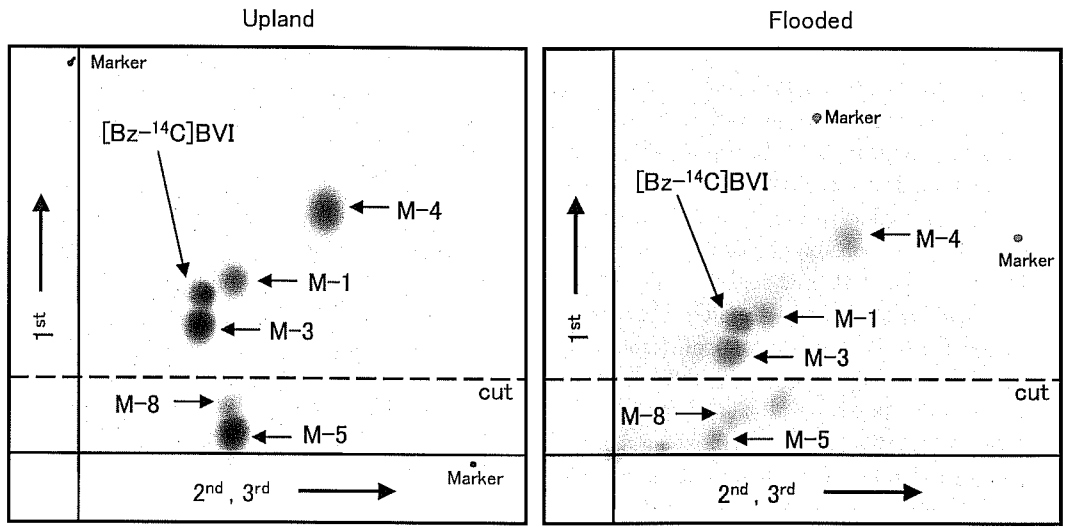


Fig. 3. Typical TLC radioluminograms of [Bz-^{14}C]BVI and its degradation products identified in Ushiku soil treated with [Bz-^{14}C]BVI under upland and flooded conditions. TLC plate: Merck Silicagel 60F_{254}, solvent system: 1st) chloroform/ethyl acetate (1 : 1); 2nd) hexane/ethyl acetate (1 : 1); 3rd) isopropyl ether/acetonitrile/28% ammonia solution (60 : 30 : 1).

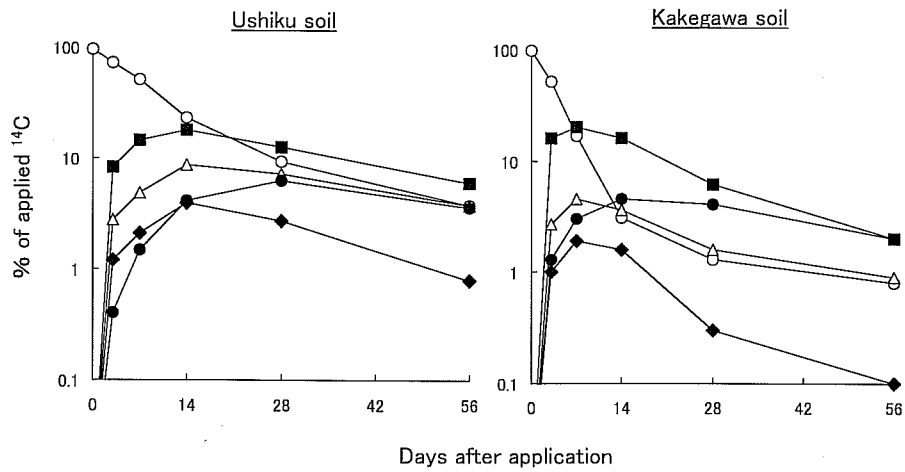


Fig. 4. The amount of [Bz-^{14}C]BVI and its degradation products in Ushiku and Kakegawa soils treated with [Bz-^{14}C]BVI under upland conditions. ○: [Bz-^{14}C]BVI, ●: M-1, ◆: M-3, △: M-4, ■: M-5.

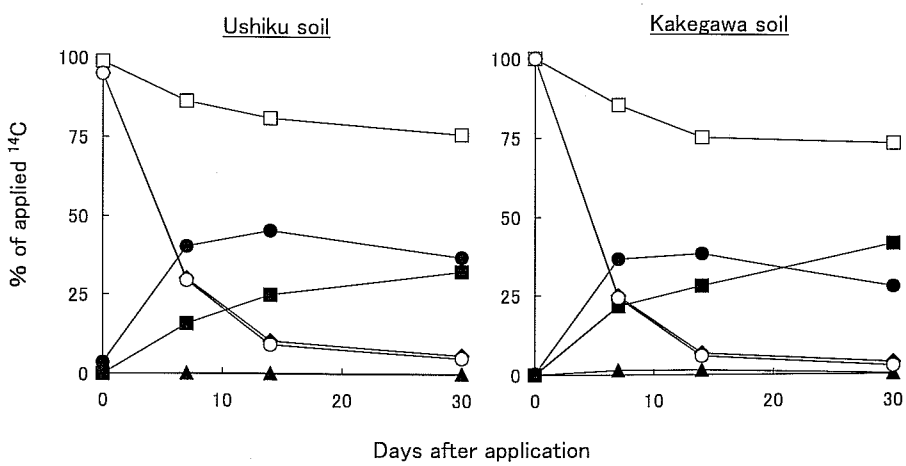


Fig. 5. Distribution of radioactivity and dissipation of [Val-^{14}C]BVI in Ushiku and Kakegawa soils under upland conditions. □: Total, ○: [Val-^{14}C]BVI, ◆: ethyl acetate fraction, ●: non-extractable residue, ▲: aqueous fraction, ■: $^{14}CO_2$.

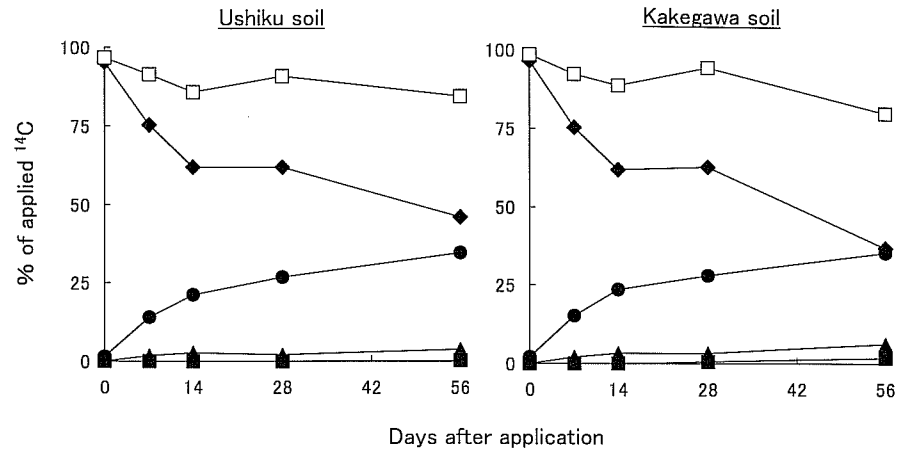


Fig. 6. Distribution of radioactivity in Ushiku and Kakegawa soils treated with [Bz-^{14}C]BVI under flooded conditions. □: Total, ◆: ethyl acetate fraction, ●: non-extractable residue, ▲: aqueous fraction, ■: $^{14}CO_2$.

life values of [Val-^{14}C]BVI, determined by first-order kinetics, were 4.2 and 3.4 days in the Ushiku soil (r=1.00) and the Kakegawa soil (r=1.00), respectively. These values were nearly the same as those of [Bz-^{14}C]BVI. The radioactivity in the non-extractable residue and in the trapped $^{14}CO_2$ increased with a decrease of [Val-^{14}C]BVI. The maximal level in the non-extractable residue was 45% of the treated amount in the Ushiku soil and 39% in the Kakegawa soil 14 days after the application. The amount of $^{14}CO_2$ accumulated 30 days post-application reached 33% in the Ushiku soil and 42% in the Kakegawa soil. The radioactive substance trapped in the alkaline solution was $^{14}CO_2$. This was confirmed by precipitation of ^{14}C-barium carbonate, formed by adding $BaCl_2$ solution into the trapped sample, and measuring radioactivity at less than 0.1% of the initial level in the supernatant (example: Ushiku soil; 7072 Bq/6.4 ml to 3 Bq/8.3 ml and Kakegawa soil; 10931 Bq/6.3 ml to 9 Bq/8.2 ml 7 days post-application under upland conditions). Most of the radioactivity in the ethyl acetate fraction was attributed to [Val-^{14}C]BVI, and the proportion in degradation products was less than 1%.

1.2. Flooded soils

The distribution of radioactivity in the soil treated with [Bz-^{14}C]BVI under flooded conditions is shown in Fig. 6. The total rates of recovery throughout the study were 80% to 108%. The radioactivity in the ethyl acetate fraction decreased to 46% of that applied in the Ushiku soil and 37% in the Kakegawa soil 56 days after the application. In contrast, that in the non-extractable residue increased. The maximum amount in the non-extractable residue was 35% in the Ushiku soil and 35% in the Kakegawa soil 56 days post-application. The radioactivity in the aqueous fraction was below 6%. The $^{14}CO_2$ accumulated 56 days after the application was as much as 0.3% of the treated amount in the Ushiku soil and 1.8% in the Kakegawa soil.

The half-life values of [Bz-^{14}C]BVI under flooded conditions, determined by the first-order kinetics, were 11 days for the Ushiku soil (r=0.982) and 6 days for the Kakegawa soil (r=0.979). M-1, M-3, M-4, M-5 and M-8 were identified as the major degradation products and their dissipation is shown in Fig. 7. The maximal amounts were 20.8% for M-5, 13.7% for M-3 and 8.7% for M-4 in the Ushiku soil, and 32.0% for M-5 and 16.7% for M-3 in the Kakegawa soil. All the degradation products reached a maximum between 7 to 28 days after the application and then decreased. These degradation products detected under flooded conditions corresponded to those detected under upland conditions. Many minor degradation products were also detected (Fig. 3).

In the sterilized soils, [Bz-^{14}C]BVI was detected at 95% of the treated amount in the Ushiku soil and at 90% in the Kakegawa soil 56 days after the application. Most of the remaining radioactivity was detected in the non-extractable residue fraction (Ushiku soil; 6.7% and Kakegawa soil; 7.6% 56 days post-application). The amounts of parent compound detected in both sterilized soils were very large, compared to those of unsterilized soils (less than 10% of the treated amount), indicating that very slow degradation occurred under sterilized conditions.

2. Degradation of M-5 and M-4 in Soils

The degradation of M-5 under upland conditions is shown in Fig. 8. M-5 decreased with a half-life of about 5 days in both Ushiku and Kakegawa soils. The degradation products M-1, M-3, M-4 and M-8 were identified by GC-MS. The main degradation products were M-4 and M-3, with maximal amounts of 14% and 20% in the Ushiku soil and 27% and 20% in the Kakegawa soil, respectively.

The degradation of M-4 under upland conditions is shown in Fig. 9. M-4 decreased with a half-life of about 8 days in both Ushiku and Kakegawa soils. M-1 and M-3 were identified as products of the degradation of M-4 by GC-MS. The main degradation product was M-3, with a maximum of 27% in the Ushiku soil and 27% in the Kakegawa soil.

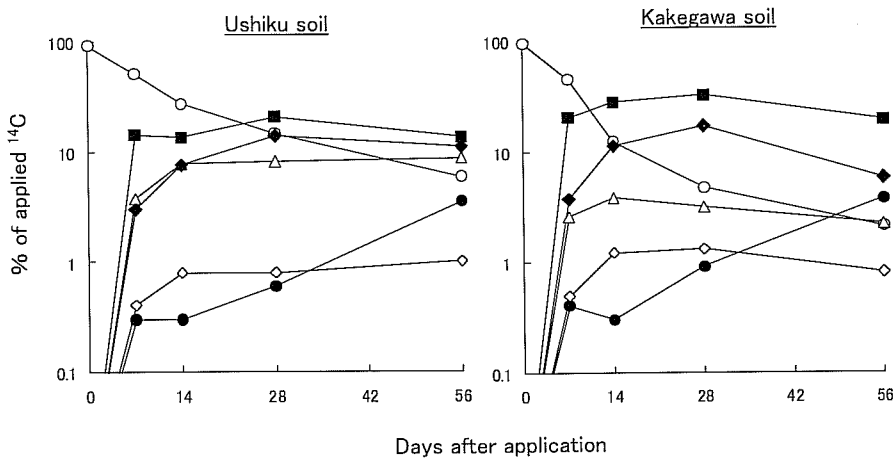


Fig. 7. The amount of [Bz-^{14}C]BVI and its degradation products in Ushiku and Kakegawa soils treated with [Bz-^{14}C]BVI under flooded conditions. ○: [Bz-14C]BVI, ●: M-1, ◆: M-3, △: M-4, ■: M-5, ◇: M-8.

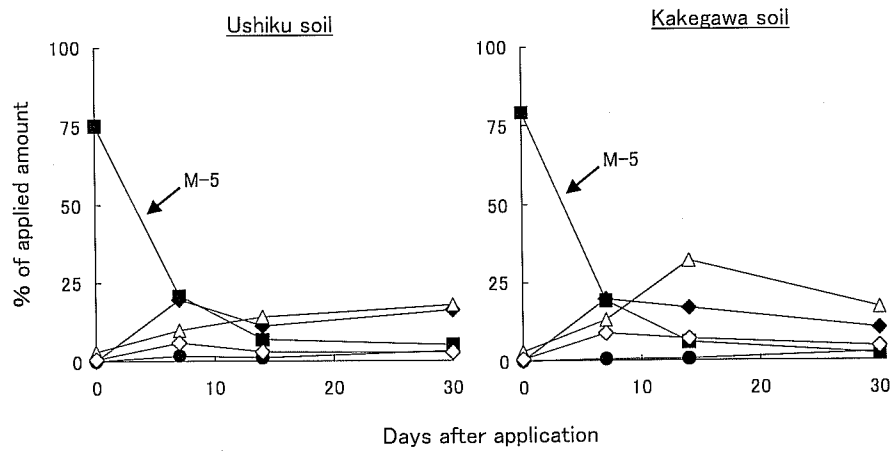


Fig. 8. The amount of M-5 and its degradation products in Ushiku and Kakegawa soils treated with M-5 under upland conditions. ■: M-5, ●: M-1, ◆: M-3, △: M-4, ◇: M-8.

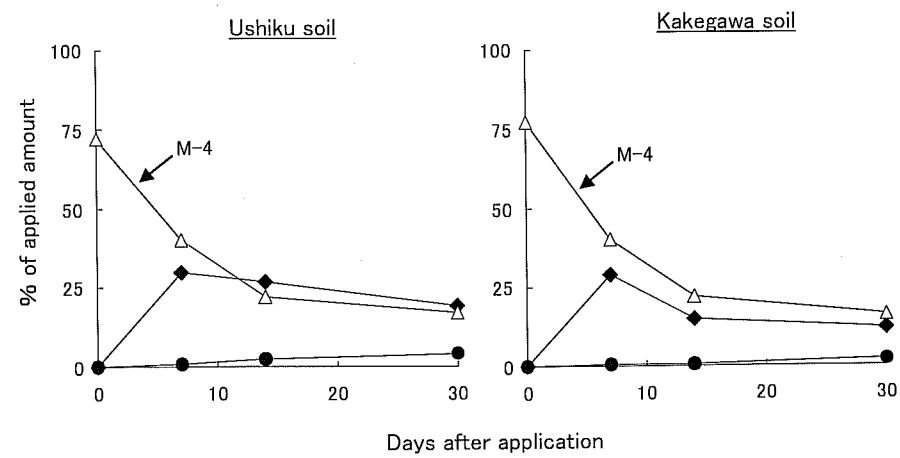


Fig. 9. The amount of M-4 and its degradation products in Ushiku and Kakegawa soils treated with M-4 under upland conditions. △: M-4, ●: M-1, ◆: M-3.

Table 3. Fractionation of radioactivity in non-extractable residue from [Bz-^{14}C]BVI 56 days post-application

Fractions	% of applied ^{14}C			
	Upland		Flooded	
	Ushiku	Kakegawa	Uiku	Kakegawa
Humin	23.7	24.1	14.5	19.3
Humic acid	14.5	12.9	12.9	2.2
Fluvic acid	16.6	18.2	8.7	14.5
Ethyl acetate-soluble	(5.1)	(4.4)	(5.2)	(7.0)
Water-soluble	(11.5)	(13.8)	(3.5)	(7.5)
Non-extractable residue	54.8	55.2	36.1	36.0

3. Extraction and Degradation of Non-Extractable Residue

Table 3 shows the results for non-extractable samples treated with 0.5 M NaOH. Forty to 50% of the radioactivity was distributed into the humin fraction under both upland and flooded conditions. When the ethyl acetate soluble fraction was analyzed, an unidentified compound was detected in relatively large amounts (about 1% of the applied radioactivity in the upland sample and 3% in the flooded sample). The parent compound, M-1, M-3 and M-4 were detected in negligible amounts. In the sterilized soils, the same compounds were also observed, although their amounts were small.

When the non-extractable residue was mixed and incubated with fresh soil for 30 days, additional $^{14}CO_2$ was produced at a rate of 1.2% and 3.9% in the Ushiku soil and the Kakegawa soil, respectively. The non-extractable residue slowly degraded to CO_2.

DISCUSSION

1. Degradation Rate

When the degradation of BVI in soil was examined at 30°C under upland conditions, the half-life values (DT_{50}) were found to be 4 to 7 days in the Ushiku soil and about 3 days in the Kakegawa soil, indicating a rapid degradation in both soils, and minimal difference due to soil type. The DT_{50} of BVI under flooded conditions was 11 days in the Ushiku soil and 6 days in the Kakegawa soil, which was slightly longer than that under the upland conditions.

Since the degradation in the sterilized soil was significantly retarded under both conditions, we conclude that the degradation was attributable to soil microorganisms.

2. Degradation Route

Degradation products of BVI in soil were examined using two kinds of ^{14}C-labeled BVI, [Bz-^{14}C]BVI and [Val-^{14}C]BVI, comparing radioactive products with synthesized authentic compounds. Five degradation products, *i.e.*, M-1, M-3, M-4, M-5 and M-8 (shown in Fig. 10), in addition to $^{14}CO_2$, were identified when [Bz-^{14}C]BVI was applied. Degradation products were common under both upland and flooded conditions, although the amount produced differed. From these results, the same degradation routes were presumed under both conditions. On the other hand, $^{14}CO_2$ was the major degradation product of [Val-^{14}C]BVI under upland conditions and the amount produced ranged from 32 to 42% after 30 days. Based on these results, it is suggested that BVI was hydrolyzed to M-5 and an intermediate (2-isopropoxycarbonyl-amino-3-methyl-butyric acid) which was rapidly degraded to CO_2. This cleavage may be the first step of degradation for BVI. Hennebole[6] reported that the main degradation product of iprovalicarb (SZX0722) was *p*-methyl-phenethylamine, produced by the cleavage of the amide bond.

In order to clarify the degradation routes of BVI, separate experiments were conducted on two degradation products, M-5 and M-4. When M-5 was applied to soils, M-4 and M-3 were the main products, and when M-4 was applied, M-3 was the main product. This suggests that M-5 might undergo oxidative deamination,[7,8] to produce M-4, which is reduced to M-3, and then M-3 is transformed into M-1.

The non-extractable residue was detected in large amounts under both upland and flooded conditions, and the radioactivity was incorporated mainly into the humin fraction. On the other hand, based upon the small amount of parent compound in the ethyl acetate fraction of the non-extractable residue and the minimal amount of non-extractable residue (<10%) in sterilized soils, it can be assumed that most of the non-extractable residue was formed through degradation products. Mizutani *et al.*[3] reported that M-5 in soil samples was efficiently extracted by adding ammonium chloride in the solvent for extraction. Rouchaud *et al.*[9] reported that 2-aminobenzothiazole, produced from methabenzthiazuron (MBT) in soil, existed mainly as soil-bound residue. Based on these findings, M-5 might be easily bound to organic matter in soil.

Benzothiazole compounds are generally used as rubber additives and are released into the environment.[10] Several studies have reported their degradation reactions.[11] Waver *et al.*[12]

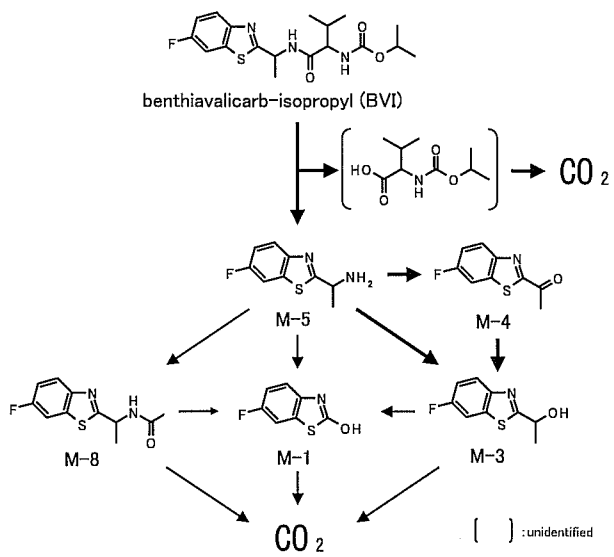


Fig. 10. Proposed degradation pathways of benthiavalicarb-isopropyl in soil.

reported that 2-hydroxybenzothiazole was oxidized to dihydroxy derivatives by a *Rhodococcus erythropolis* strain, and Haroune *et al.*[13] also reported that 2-hydroxybenzothiazole was oxidized by *Rhodococcus pyridinovarans* strain PA to dihydroxy derivatives and then cleaved to the corresponding diacid. Cheng *et al.*[14] reported that $^{14}CO_2$ was slowly generated from benzothiazolyl-2-^{14}C-labeled MBT in soils. In our study, since $^{14}CO_2$ was slowly generated from [Bz-^{14}C]BVI and additional $^{14}CO_2$ was also produced after mixing the non-extractable residue with fresh soils, it is likely that the benzothiazole ring is finally degraded to carbon dioxide.

The possible routes of degradation of BVI in soils are shown in Fig. 10.

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