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Original Article

Residue Analysis of the Fungicide Benthiavalicarb-isopropyl and Its Degradation Products in Upland Field Soil

Hiroyuki MIZUTANI,* Junko SUZUKI, Yoshihiro SAITO,# Mitsumasa IKEDA and Akira YAGI

Life Science Research Institute, Kumiai Chemical Industry Co., Ltd., 276 Tamari, Kakegawa, Shizuoka 436–0011, Japan

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A method for residue analysis of benthiavalicarb-isopropyl, its diastereomer, and its four degradation products in soil by HPLC was established. The method of extraction under reflux with a mixture of acetone and ammonium chloride solution was optimal for most of the compounds. The limit of quantification in two soils was 0.01 to 0.02 mg/kg for the compounds, and the recovery rates of the compounds from the soils were 74 to 113%. But for the degradation product having an amino group, the rate of recovery from one soil only was good. The level of benthiavalicarb-isopropyl in two upland field soils decreased rapidly.

Keywords: benthiavalicarb-isopropyl, degradation products, analytical method in soil, HPLC, residue analysis in field soil.

INTRODUCTION

Benthiavalicarb-isopropyl (trade name; Mamorotto® water dispersible granule, isopropyl [(*S*)-1-[(*R*)-1-(6-fluorobenzothiazol-2-yl)ethylcarbamoyl]-2-methylpropyl]carbamate) is a novel fungicide developed by Kumiai Chemical Industry Co., Ltd. showing excellent efficacy against downy mildew and epidemics in fruit trees and vegetables. Since this compound has two asymmetric carbons, four optical isomers are present, but only benthiavalicarb-isopropyl is active.[1,2] The bulk agricultural form of the chemical contains benthiavalicarb-isopropyl and a trace amount of diastereomer, isopropyl [(*S*)-1-[(*S*)-1-(6-fluorobenzothiazol-2-yl)ethylcarbamoyl]-2-methylpropyl]carbamate (S-L), but not the other two enantiomers.

Benthiavalicarb-isopropyl is known to be degraded rapidly to 6-fluoro-2-hydroxybenzothiazole (M-1), 1-(6-fluoro-2-benzothiazolyl)ethyl alcohol (M-3), 6-fluoro-2-benzothiazolyl methyl ketone (M-4) and 1-(6-fluoro-2-benzothiazolyl)ethylamine (M-5) in indoor test soil, and then to carbon dioxide *via* these degradation products,[3] but the behavior of these compounds in field soil has not been studied.

In this report, in order to quantify the amount of benthiavalicarb-isopropyl, its diastereomer S-L and the main degradation products M-1, M-3, M-4 and M-5, in soil (Fig. 1), a method of analyzing these compounds in soil was established, and the dissipation of these chemicals in upland soil was investigated.

* To whom correspondence should be addressed.
E-mail: h-mizutani@kumiai-chem.co.jp
Present address: JA Zen-Noh Fukushima Agricultural R&D Center, 78–1 Aza-shimatogawara, Kanaya, Tamura-machi, Koriyama, Fukushima 963–0725, Japan.

MATERIALS AND METHODS

1. *Standard Materials and Reagents*

All standard materials, including benthiavalicarb-isopropyl, the diastereomer (S-L), M-1, M-3, M-4, M-5 and the acetylated form of M-5 (Ac-M-5), were synthesized at K.I. Chemical Research Institute Co., Ltd. The respective purity was not less than 99% (HPLC area ratio). The physicochemical properties of these standard substances are given below.

Benthiavalicarb-isopropyl; mol. formula: $C_{18}H_{24}FN_3O_3S$, mol. wt.: 381.46, white powder, mp 152.0°C and 169.2°C, vp $<3.0\times10^{-4}$ Pa, solubility (20°C): 13.14 mg/l in water, 41.7 g/l in methanol and 25.4 g/l in acetone, log P_{ow}: 2.52 (20°C), DT_{50}: one year in water at pH 4, 7 and 9 (25°C) and 301 days in water under xenon light (400 W/m^2, 300 to 800 nm). <S-L>; white powder, mp 231.0°C. <M-1>; pale yellow powder, mp 188.4°C. <M-3>; white powder, mp 74.9°C. <M-4>; pale yellow powder, mp 126.3°C, solubility in water (20°C): 30.0±0.8 μg/ml, vp (25°C) 1.5×10^{-3} Pa. <M-5>; pale yellow powder, mp 38.8°C, solubility in water (30°C): 13.1 g/l. <Ac-M-5>; acetylated M-5 (authentic standard M-5 for HPLC analysis, *N*-[1-(6-fluoro-2-benzothiazolyl)ethyl]acetamide); white powder, mp 149.0–152.0°C.

Benthiavalicarb-isopropyl and the S-L isomer labeled with ^{14}C at the benzene position of the benzothiazole ring

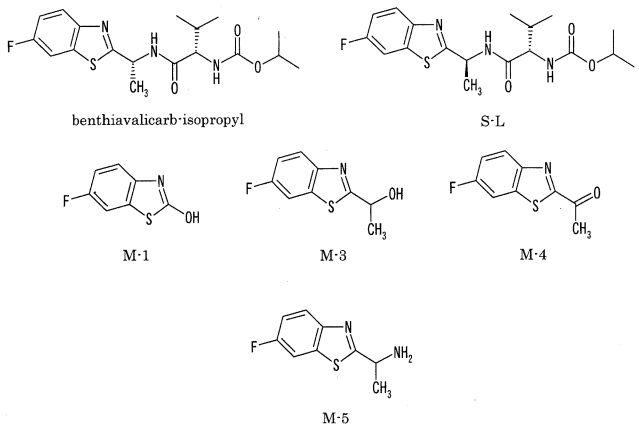

Fig. 1. Structures and abbreviated names of compounds.

(specific activity: 2.40 MBq/mg and 2.14 MBq/mg, respectively) synthesized by Daiichi Pure Chemicals Co., Ltd. were used for the extraction efficiency test.

Organic solvents of PCB-pesticide residue analysis grade (Wako Pure Chemical Industries, Ltd.), distilled water for high performance liquid chromatography (Wako Pure Chemical Industries, Ltd.), triethylamine of a special grade (Tokyo Kasei Kogyo Co., Ltd.), and other organic and inorganic reagents of special grade were used.

The packed solid phase extraction columns, including Supelclean™ ENVI™-Carb/LC-NH_2 (500 mg + 500 mg) and Supelclean™ ENVI™-Carb (500 mg), were purchased from Supelco. Bond Elut LRC® SI (500 mg), Mega Bond Elut® SI (1 g) and Mega Bond Elut® NH_2 (2 g) were obtained from Varian, Inc., and Sep-Pak® Plus Silica was purchased from Waters Corp.

2. *Test Soils*

Fresh soil samples were collected from upland fields of Kumiai Chemical Industry Co., Ltd. (Kakegawa soil), the Research Institute of Matsushiro of the Nagano Prefectural Plant Protection Association (Nagano soil) and the Research Institute of the Japan Plant Protection Association (Ushiku soil) in spring. The soils were passed through a sieve of 2 mm. Their properties are listed in Table 1.

3. *Analytical Procedures*

The analytical procedures used for benthiavalicarb-isopropyl, S-L, M-1, M-3, M-4 and M-5 are summarized in Fig. 2.

3.1. *Method of analysis of benthiavalicarb-isopropyl, S-L, M-1 and M-3*

3.1.1. *Extraction*

Forty grams of soil (dry weight) was placed in a 500-ml round-bottomed flask, 250 ml of a mixture of acetone and 0.2 M ammonium chloride solution (1 : 1, v/v) was added, and the above five compounds were extracted by refluxing at 80°C for 3 hr. The extract was filtered by suction. The washing obtained by rinsing the vessel with 100 ml of acetone was poured onto the residue on filter paper, and the residue was filtered by suction. The filtrates were combined and divided equally into Fraction A for the analysis of benthiavalicarb-isopropyl, S-L, M-1 and M-3 and Fraction B for the analysis of M-5. Subsequent analytical procedures were conducted using Fraction A.

3.1.2. *Liquid-liquid partition*

Fraction A was transferred into a 1000-ml round-bottomed flask and concentrated to about 50 ml under reduced pressure at below 40°C. The concentrate was extracted twice with 50 ml of a mixture of *n*-hexane and ethyl acetate (7 : 3, v/v). The extracts were combined and dehydrated through about 40 g of anhydrous sodium sulfate in a funnel. Next, 50 ml of fresh mixed solvent was passed through the anhydrous sodium sulfate. The filtrates were combined and concentrated to about 1 ml under reduced pressure at below 40°C, and the solvent was evaporated in a gentle stream of nitrogen.

3.1.3. *Purification with solid phase extraction columns*

The residue obtained from item *3.1.2.* was dissolved in 4 ml of acetonitrile and transferred onto the Envi-Carb/LC-NH_2 solid phase extraction column (preconditioned with 5 ml of acetonitrile). The vessel was washed twice with 3 ml of acetonitrile, and the washings were applied onto the column. Thirty milliliters of acetonitrile was passed through the column. The column eluates were combined and con-

Table 1. Properties of the test soils

Property	Location		
	Kakegawa	Nagano	Ushiku
Source	Upland Man-made	Alluvial	Volcanic ash
Soil texture	Clay loam	Light clay	Light clay
Main clay mineral	Chlorite	Montmorillonite	Allophane
Total carbon (%)	0.80[a)]	1.33	4.71
pH	7.0 (H_2O), 5.7 (KCl)	5.0 (H_2O), 4.15 (KCl)	6.71 (H_2O), 6.21 (KCl)
Phosphate adsorption coefficient (mg P_2O_5/100 g)	640	379	1680
CEC (meq/100 g)	7.9	21.74	37.8

[a)] Total organic carbon (%).

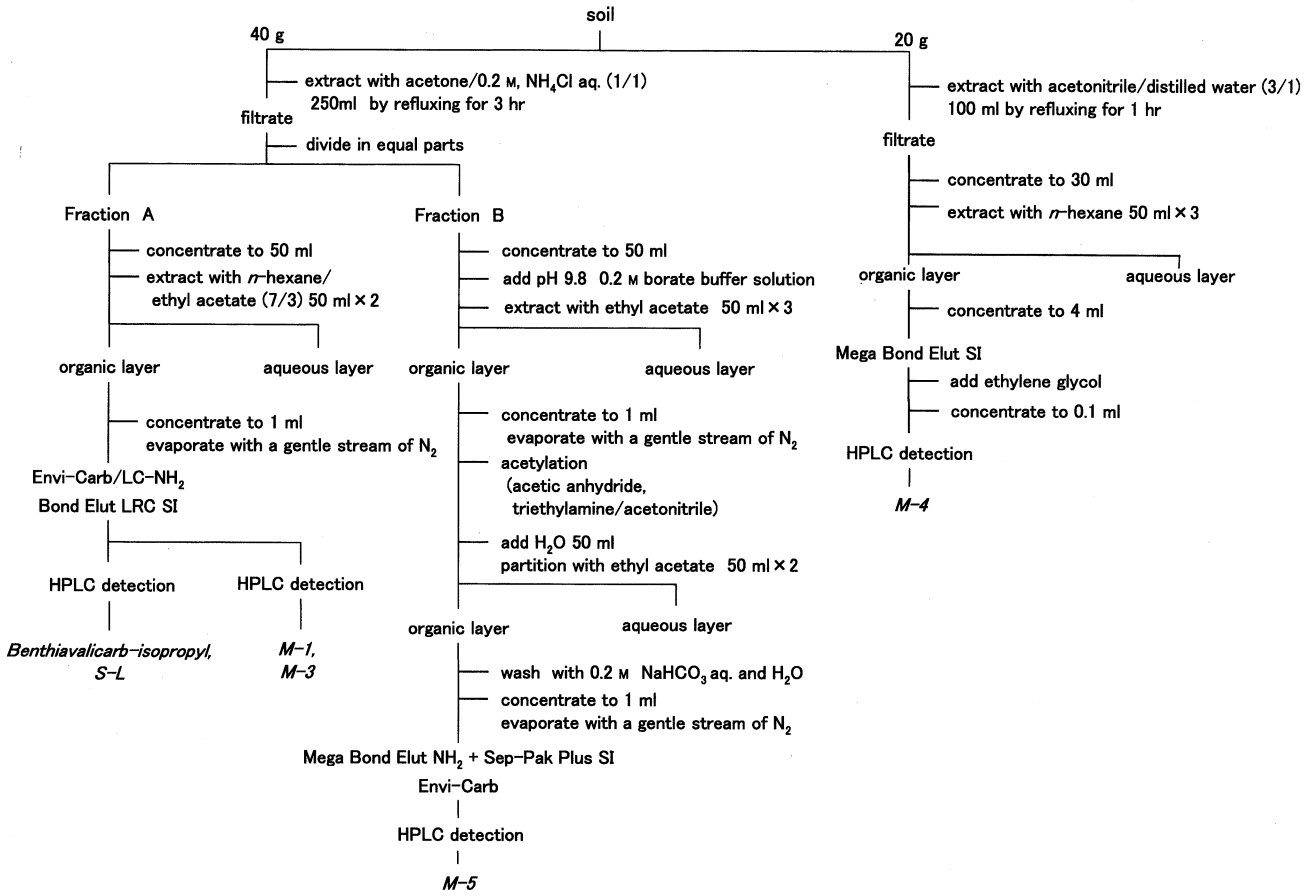

Fig. 2. Analytical scheme for benthiavalicarb-isopropyl, S-L and their degradation products in soil.

centrated under reduced pressure as described in *3.1.2.* to evaporate the solvent.

Then, the residue was dissolved in 4 ml of a mixture of *n*-hexane and ethyl acetate (19 : 1, v/v) and transferred onto the Bond Elut LRC SI solid phase extraction column (preconditioned with 10 ml of the mixed solvent). The vessel was washed twice with 3 ml of the solvent, and the washings were applied onto the column. Ten milliliters of the solvent was passed through the column. The column eluates were discarded. The degradation products, M-1 and M-3, were eluted with 40 ml of a mixture of *n*-hexane and ethyl acetate (9 : 1, v/v). Subsequently, benthiavalicarb-isopropyl and S-L were eluted with 40 ml of a mixture of *n*-hexane and ethyl acetate (7 : 3, v/v). The respective eluates were concentrated under reduced pressure as described in *3.1.2.* to evaporate the solvent. The respective residues were each dissolved in 4 ml of the HPLC mobile phase (a mixture of acetonitrile and water, 45 : 55, v/v).

3.2. Method for analysis of M-5

3.2.1. Extraction

Fraction B obtained by the method described in *3.1.1.* was subjected to the following tests.

3.2.2. Liquid-liquid partition

Fraction B was transferred into a 1000-ml round-bottomed flask and concentrated to about 50 ml under reduced pressure at below 40°C. The concentrate was mixed with approximately 30 ml of 0.2 M borate buffer solution (pH 9.8), and the pH was adjusted to about 9. In addition, 15 g of sodium chloride was added and dissolved. This compound was extracted three times with 50 ml of ethyl acetate. The extracts were combined and dehydrated with anhydrous sodium sulfate. The filtrates were combined and concentrated under reduced pressure to evaporate the solvent as described in *3.1.2.*

3.2.3. Acetylation

The residue was dissolved in 5 ml of acetonitrile. To this solution, 1 ml of triethylamine and then 1 ml of acetic anhydride were added, and the mixture was stirred and allowed to stand at room temperature for 2 hr. After the reaction, 50 ml of distilled water was added, and the mixture was extracted twice with 50 ml of ethyl acetate. The extracts were combined, partitioned with 50 ml of 0.2 M sodium hydrogen carbonate solution and a subsequent 50 ml of distilled water, and back washed. The extract was dehydrated with anhydrous sodium sulfate. The filtrates were combined and concentrated under reduced pressure as described in *3.1.2.* to evaporate the solvent.

3.2.4. Purification with solid phase extraction columns

The residue was dissolved in 4 ml of a mixture of *n*-hexane and ethyl acetate (7 : 3, v/v) and transferred onto a solid phase extraction column of Mega Bond Elut NH_2 connected to Sep-Pak Plus Silica (the Silica column at the elution port; preconditioned with 10 ml of the same mixture). The vessel was washed twice with 3 ml of the solvent, and the respective washings were applied onto the column. Ten milliliters of the solvent was passed through the column. The eluates were discarded. The column was eluted with 30 ml of a mixture of *n*-hexane and ethyl acetate (1 : 1, v/v) and 5 ml of ethyl acetate in this order. The eluates were combined and concentrated under reduced pressure as described in *3.1.2.* to evaporate the solvent.

In addition, the residue was dissolved in 4 ml of ethyl acetate and transferred onto the Envi-Carb solid phase extraction column (preconditioned with 5 ml of ethyl acetate). The vessel was washed twice with 3 ml of the solvent, and the respective washings were applied onto the column. Ten milliliters of ethyl acetate was passed through the column. The column eluates were combined and concentrated under reduced pressure as described in *3.1.2.* to evaporate the solvent. The resulting residue was dissolved in 4 ml of the HPLC mobile phase (a mixture of acetonitrile and water, 4 : 6, v/v).

3.3. Method for analysis of M-4

3.3.1. Extraction

Twenty grams of soil (dry weight) was placed in a 500-ml round-bottomed flask, 100 ml of a mixture of acetonitrile and water (3 : 1, v/v) was added, and M-4 was extracted by refluxing at 80°C for 1 hr. The extract was filtered by suction. The vessel was washed with 100 ml of acetonitrile, and the washing was poured onto the residue, which was filtered by suction. The filtrates were combined and concentrated to about 30 ml under reduced pressure at below 35°C.

3.3.2. Liquid-liquid partition

The concentrate was extracted three times with 50 ml of *n*-hexane. The extracts were combined and dehydrated with anhydrous sodium sulfate. The solvent was combined and concentrated to about 4 ml under reduced pressure at below 35°C.

3.3.3. Purification with solid phase extraction columns

The concentrate was transferred onto the Mega Bond Elut SI solid phase extraction column (preconditioned with 10 ml of *n*-hexane). The vessel was washed twice with 3 ml of the solvent, and the washings were applied onto the solid phase extraction column. Ten milliliters of a mixture of *n*-hexane and ethyl acetate (150 : 1, v/v) was passed through the column. The eluates were discarded. Subsequently, M-4 was eluted with 20 ml of a mixture of *n*-hexane and ethyl acetate (19 : 1, v/v). To this solution, 0.5 ml of a 1000 ppm ethylene glycol solution in acetone was added, and this mixture was concentrated to about 1 ml under reduced pressure at below 35°C. The concentrate was further concentrated to about 0.1 ml in a gentle stream of nitrogen. To this concentrate, the HPLC mobile phase (a mixture of acetonitrile and water, 4 : 6, v/v) was added to make exactly 4 ml.

4. Quantification by HPLC

4.1. HPLC operating conditions

A high performance liquid chromatograph (Shimadzu Co.) equipped with a pump (LC-10ADvp), a detector (SPD-10ADvp) and a column oven (CTO-10A) was used for quantitative analysis. The column oven was set at 35°C.

4.1.1. Benthiavalicarb-isopropyl and S-L

A mobile phase of acetonitrile and water (45 : 55, v/v) was delivered to an ODS-120A column (5 μm in particle size, 4.6 mm i.d. $\times$ 250 mm, Tosoh) at a flow rate of 1.0 ml/min, and these compounds were detected at a wavelength of 220 nm. The retention time was 16.6 min for benthiavalicarb-isopropyl and 17.8 min for S-L. The minimum detectable amount of the respective compounds was 0.5 ng.

4.1.2. M-1 and M-3

A mobile phase of acetonitrile and water (40 : 60, v/v) was delivered to an ODS-120A column (5 μm in particle size, 4.6 mm i.d. $\times$ 250 mm, Tosoh) at a flow rate of 1.0 ml/min, and the compounds were detected at a wavelength of 220 nm. The retention time was 8.3 min for M-1 and 9.1 min for M-3. The minimum detectable amount of the respective compounds was 0.5 ng.

4.1.3. M-4

A mobile phase of acetonitrile and water (50 : 50, v/v) was delivered to an Inertsil ODS-3 column (5 μm in particle size, 4.6 mm i.d. $\times$ 250 mm, GL Sciences Inc.) at a flow rate of 1.2 ml/min, and M-4 was detected at a wavelength of 305 nm. The retention time of this compound was 13.1 min, and the minimum detectable amount was 1.0 ng.

4.1.4. M-5; determined as an acetylated compound, Ac-M-5

A mobile phase of acetonitrile and water (40 : 60, v/v) was delivered to an Inertsil ODS-3 column (5 μm in particle size, 4.6 mm i.d. $\times$ 250 mm, GL Sciences Inc.) at a flow rate of 0.8 ml/min, and the compound was detected at a wavelength of 254 nm. The retention time of Ac-M-5 was 8.6 min, and the minimum detectable amount was 1.0 ng.

4.2. Preparation of a calibration curve

Specific amounts of authentic compounds were dissolved in the HPLC mobile phase to prepare 0.025 to 1.0 mg/l solutions for benthiavalicarb-isopropyl, S-L, M-1 and M-3, and 0.05 to 2.0 mg/l solutions for M-4 and Ac-M-5, and 20 μl each of these solutions was injected into the HPLC system under the conditions given in *4.1.* The peak heights (mAU) of the respective compounds on the chromatogram obtained were determined and plotted against the injected amounts (ng) to prepare the calibration curve.

4.3. Calculation of the concentration of residue

Twenty microliters of the final solution obtained as described in *3.1.* to *3.3.* was injected into the HPLC system under the conditions given in *4.1.* The peak heights (mAU)

of the respective compounds on the chromatogram obtained were determined, and the detected amounts (ng) were determined from the calibration curve. The concentrations were calculated from the equation shown below. For M-5 analyzed after derivatization to Ac-M-5, F [F=0.823, molecular weight (196.2) of M-5/molecular weight (238.2) of Ac-M-5] was multiplied by the calculation equation.

Concentration (mg/kg)=[Amount detected (ng) ×Final volume (ml)] / [Amount (g) of the test sample ×Volume injected (μl)]×F

5. Dissipation in the Upland Field

A 2000-fold diluted solution of benthiavalicarb-isopropyl water-dispersible granules (active ingredient of 15.0%) was applied three times (300 l/10 a/once) at one-week intervals to an upland field used to cultivate onions at the Research Institute of the Nagano Prefectural Plant Protection Association (Nagano soil) and the Research Institute of the Japan Plant Protection Association (Ushiku soil) in fiscal year 2002, and the dissipation of benthiavalicarb-isopropyl, S-L and the degradation products in the soils from immediately after to 120 days after the third application was examined. The concentration of benthiavalicarb-isopropyl detected after the third application was used as the initial concentration for calculating the dissipation rate. The application of pesticides and sampling of soil were conducted at the research institutes concerned.

RESULTS AND DISCUSSION

1. Extraction from Soil

1.1. Extraction efficiency

Samples of the Kakegawa soil and the Ushiku soil treated with an equal amount of a mixture of benthiavalicarb-isopropyl and the S-L form at 0.95 mg/kg and 0.77 mg/kg under upland conditions, respectively, were labeled with ^{14}C at the benzene position of the benzothiazole ring, and incubated at 30°C for 19 days and 26 days. The extraction of benthiavalicarb-isopropyl, S-L and their degradation products was quantified based on two-dimensional co-TLC radio-luminography. The extraction of these compounds was conducted with 3 types of 25% aqueous solvents (acetone, acetonitrile and methanol) and three extraction methods (refluxing at 80°C for 1 hr, shaking at 50 rpm for 30 min and sonication at 39 kHz for 30 min) (Table 2).

It was decided to use the acetone reflux extraction method in view of its significantly high rate of extraction for M-5 and generally good rates for benthiavalicarb-isopropyl, S-L, M-1 and M-3. This method, however, did not give satisfactory results for M-4. Therefore, the acetonitrile reflux method with which high rates of extraction were obtained particularly in the Ushiku soils was employed for M-4.

1.2. Effects of salts on the extraction of M-5 from soil

When Nagano soil spiked with M-5 at 1.0 mg/kg was treated with a mixture of acetone and water (1 : 1, v/v) with refluxing at 80°C for 3 hr, the recovery rate was as low as

Table 2. Extraction efficiency* of ^{14}C-benthiavalicarb-isopropyl, S-L and their degradation products from Kakegawa and Ushiku soil.

Soil	Compound	% of treated-^{14}C[a)]								
		Acetone+H_2O (3+1, v/v)			MeCN+H_2O (3+1, v/v)			MeOH+H_2O (3+1, v/v)		
		Reflux	Shake	Ultrasonic	Reflux	Shake	Ultrasonic	Reflux	Shake	Ultrasonic
Kakegawa	Benthiavalicarb-isopropyl	4.4	3.8	4	4.9	3.9	3.7	4	3.5	3.7
	S-L	1.7	1.6	1.6	1.8	1.4	1.4	1.7	1.4	1.3
	M-1	6.2	5.4	5.6	5.8	5.0	5.1	5.9	5.6	4.8
	M-3	1.8	1.7	1.8	1.9	1.8	1.8	1.9	2	1.7
	M-4	6.1	2.5	3.2	6.3	3.1	3.7	5	2.2	2.6
	M-5	9.1	8.8	6.1	6.2	5.5	3.2	7.1	4.3	4.1
	Total	29.3	23.8	22.3	26.9	20.7	18.9	25.6	19.0	18.2
Ushiku	Benthiavalicarb-isopropyl	6.1	4.2	4.2	7.3	3.9	4.7	6.9	3.6	4.1
	S-L	3.7	2.5	2.5	4.2	2.6	2.8	4.1	1.8	2.7
	M-1	10.9	9.1	8.5	10.4	8.6	8.6	9.8	7.3	6.8
	M-3	3.1	2.6	2.7	3.0	2.6	2.8	3.1	2.7	2.4
	M-4	6.1	4.0	8.9	10.2	4.7	4.3	7.3	1.9	3.3
	M-5	16.3	8.3	1.3	11.4	12.2	12.6	10.3	4.8	6.2
	Total	46.2	30.7	28.1	46.5	34.6	35.8	41.5	22.1	22.5

* An equal amount of ^{14}C-benthiavalicarb-isopropyl and the ^{14}C-S-L form was added to the Kakegawa and Ushiku soils at 0.95 mg/kg and 0.77 mg/kg under upland conditions, respectively, and incubated at 30°C for 19 days and 26 days. Using the two soil samples, the extraction efficiencies for benthiavalicarb-isopropyl, S-L and their degradation products were examined.

a) Average of duplicates.

Extraction method: refluxed at 80°C for 1 hr, shaken for 30 min, and sonicated for 30 min at 39 kHz.

42%. Since M-5 is a weak basic substance with amino groups having a p*K*a of 7.15,[4] ion-exchange adsorption by the soil particles was expected. Tokieda *et al.*[5] reported that the extraction of a degradation product of acetamiprid ion-exchange adsorption, *N*-methyl-(6-chloro-3-pyridyl)methylamine, from soil significantly increased with a mixture of methanol or acetone and ammonium chloride solution. The effects of adding salts such as ammonium to the water-containing acetone were examined using the Nagano soil, an alluvial soil. The recovery rate for M-5 increased with the use of any salt (0.5 M), ammonium chloride, ammonium acetate, ammonium phosphate, ammonium carbonate or calcium chloride, compared with no addition (Table 3). However, the recovery rate was highest after addition of ammonium chloride. In addition, a recovery rate of 67% was obtained using 0.2 M ammonia water instead of salts, which was similar to that obtained using ammonium carbonate.

Subsequently, the relationship between the concentration of ammonium chloride and the rate of extraction of M-5 was examined with the Nagano soil (alluvial soil) and the Ushiku soil (volcanic ash soil). The concentrations of ammonium chloride were 0.2 M and 1.0 M, the concentrations of M-5 were 0.02, 0.2 and 1.0 mg/kg, and the other conditions were as described previously (Table 4).

In the Nagano soil, when the concentration of ammonium chloride in the extracting solvent was low, the recovery rate was high at all concentrations of M-5. In the Ushiku soil, when the concentration of ammonium chloride was increased from 0.2 M to 1.0 M, the rate of recovery of M-5 markedly increased in the soil spiked with M-5 at 0.2 mg/kg and 1.0 mg/kg, but the increase in the soil spiked at 0.02 mg/kg was small. Tucker *et al.*[6] studied the nature of the adsorption of bipyridylium herbicides, diquat and paraquat by soil. These compounds show high water solubility and marked adsorption in soil. They reported that the compounds were desorbed with 18 N sulfuric acid at low concentrations in the soil, with a saturated ammonium chloride solution at slightly high concentrations in the soil, and even with water at higher concentrations.

The Freundlich soil adsorption coefficient, K_{oc}, of M-5 was very low, 495 to 790 (EU-Soil), compared with the soil adsorption coefficients of diquat and paraquat (K_d; >10,000 for both[7]). But the adsorption became stronger at low concentrations, and the slope from 0.73 to 0.79 was $1/n$.[4]

It was therefore assumed that M-5 at low concentrations, which could not be extracted from the Ushiku soil, was adsorbed to such an extent that it could not be desorbed with a mixture of acetone and ammonium chloride solution.

Furthermore, the addition of ammonium chloride to the extracting solvent increased the rate of recovery of M-5, but had no effect on the recovery rates of benthiavalicarb-isopropyl, S-L, M-1 and M-3. When Nagano soil spiked with M-5 at 0.02 mg/kg was treated with a mixture of acetone and 0.2 M ammonium chloride solution (1 : 1, v/v) with refluxing at 80°C, the recovery rate improved from 49% to 63% when the refluxing time was extended from 1 to 3 hr. Therefore, the extraction time for M-5 was set at 3 hr and similarly, 3 hr was used for benthiavalicarb-isopropyl, S-L, M-1 and M-3 because it is preferable that the same method of extraction be employed for all the compounds except M-4.

Table 3. Effect of salts on extraction of M-5 from Nagano soil

Salts	Recovery (%)[a]
—[b]	42
NH_4Cl	84
$AcNH_4$	74
$(NH_4)_2HPO_4$	70
$(NH_4)_2CO_3$	68
$CaCl_2$	57

Soil spiked with M-5 at 1.0 mg/kg.
Extraction; using a mixed solution of acetone and 0.5 M salt aqueous solution (1/1, v/v) and refluxed at 80°C for 3 hr.
[a] Average of duplicates.
[b] No salt added to extracting solvent (acetone/water, 1/1, v/v).

2. *Acetylation of M-5*

In the purification of M-5 with the solid phase extraction column (Bond Elut SCX) when the analysis was conducted directly without derivatization, the recovery rate was not constant, but changed from about 70% to 98%. In addition,

Table 4. Effect of NH_4Cl concentration on extraction of M-5 from Nagano and Ushiku soils

Soil	Spiked concentration of M-5 (mg/kg)	Recovery (%)[a]	
		0.2 M-NH_4Cl	1.0 M-NH_4Cl
Nagano (alluvial)	0.02	63	57
	0.2	69	64
	1.0	84	80
Ushiku (volcanic ash)	0.02	37	42
	0.2	42	77
	1.0	53	80

Extraction; using a mixture of acetone and NH_4Cl aqueous solution (1/1, v/v) and refluxed at 80°C for 3 hr.
[a] Average of duplicates.

it is desirable to delay the retention time of M-5 in HPLC in order to reduce the effects of the sample matrix. For these reasons, derivatization of M-5 was conducted.

Acetylation of M-5 in the triethylamine and acetic anhydride reaction system progressed rapidly at room temperature, the reaction rate with 0.4 to 20 μg of M-5 being 88 to 97%. The retention time of the acetylated form (Ac-M-5) of M-5 under the analytical conditions was 8.6 min which was considerably delayed compared with that of M-5, about 3.5 min.

3. *Quantification by HPLC*

Since benthiavalicarb-isopropyl, S-L and the degradation products M-1, M-3, M-4 and M-5, all have a benzothiazole ring in their structures, they could be detected by NP-FID GC. In the GC analysis, however, benthiavalicarb-isopropyl and S-L were partially transformed probably by the heat to a ring compound as shown in Fig. 3. The transformed compound could be detected with HP-1301 and HP-FFAP megabore GC columns. M-5 was mostly transformed into an acetone-attached form (confirmed by GC-MS) after injection of the acetone solution into the GC. In addition, since some M-5 was adsorbed by the microsyringe without inactivation, no reproducible GC peak could be detected.

As shown above, it was difficult to detect all compounds to be analyzed by GC. But each compound showed UV-absorption as they could be detected using the HPLC analysis. Therefore, as in the crop residue analysis of benthiavalicarb-isopropyl,[8] HPLC was used for soil residue analysis.

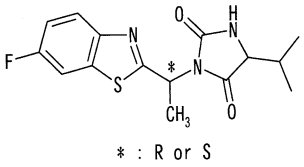

Fig. 3. Product of the thermal decomposition of benthiavalicarb-isopropyl and S-L obtained under GC-analysis.

4. *Recovery Test*

The rates of recovery of benthiavalicarb-isopropyl, S-L and the degradation products from the Nagano and Ushiku soils are listed in Table 5.

Benthiavalicarb-isopropyl, S-L, M-1, M-3 and M-4 showed good results in terms of both the recovery rate (74% to 113%) and the coefficient of variation (0.6% to 9.8%) in both soils. The limit of quantification was 0.01 mg/kg for benthiavalicarb-isopropyl, S-L, M-1 and M-3 and 0.02 mg/kg for M-4.

M-5 had a good rate of recovery (80% to 101%) and coefficient of variation (1.3% to 8.9%) in the Nagano soil. The limit of its quantification was 0.02 mg/kg. But in the Ushiku soil, while the coefficient of variation was 5.0% to 5.4%, the recovery rate was as low as 37% to 53%. The main reason for the low recovery rate in the Ushiku soil was assumed to be a low rate of the extraction from the soil as described in *1.2.* In addition, it was found that M-5 was partially degraded to M-4 (about 4% at maximum with the solvent without soil) during the reflux, which was considered another reason for the decreased recovery rate.

Separately, by HPLC analysis (CHIRAL PAK AD, 4.6 mm i.d. × 250 mm, Daicel Chemical Industries, Ltd., mobile phase: a mixture of *n*-hexane, 2-propanol and methanol (90 : 10 : 2, v/v/v), 1 ml/min, 254 nm, four optical isomers were separated into 4 peaks at retention times from 7.8 to 13.0 min) using the standard, it was confirmed that benthiavalicarb-isopropyl was not converted to the other optical isomers during the analysis.

None of the compounds used in the recovery tests showed

Table 5. Percent recoveries of benthiavalicarb-isopropyl, S-L and their degradation products from spiked Nagano and Ushiku soils

Compound	Spiked concentration (mg/kg)	Nagano soil		Ushiku soil	
		Recovery[a]	CV[b]	Recovery[a]	CV[b]
Benthiavalicarb-isopropyl	0.01	103	9.8	107	6.1
	0.5	110	1.4	103	0.6
S-L	0.01	95	1.6	96	2.6
	0.5	110	1.9	106	1.6
M-1	0.01	81	5.7	99	7.6
	0.5	97	1.8	99	1.0
M-3	0.01	74	5.4	113	3.7
	0.5	92	2.3	104	1.9
M-4	0.02	89	2.4	84	2.5
	1.0	76	1.9	82	4.3
M-5	0.02	101	8.9	37	5.4
	1.0	80	1.3	53	5.0

[a] The values were determined from the mean of triplicate samples.
[b] Coefficient of variation (%); calculated from the equation (SD/mean × 100) using triplicate samples.

interference on the respective HPLC chromatograms (Fig. 4).

5. *Dissipation in the Upland Field*

A 2000-fold diluted solution of benthiavalicarb-isopropyl water-dispersible granules was applied three times at one-week intervals to the upland fields at Nagano and Ushiku, and the dissipation of benthiavalicarb-isopropyl, S-L and the degradation products in the soils from immediately after to 120 days after the last application was examined. The concentration of each degradation product was expressed by multiplying by the correction coefficient (S-L; 1.0, M-1; 2.3, M-3; 1.9, M-4; 2.0, M-5; 1.9) corresponding to each degradation product as the value converted to benthiavalicarb-isopropyl (Fig. 5).

The parent compound, benthiavalicarb-isopropyl, disappeared rapidly from the upland soil: its half-life was estimated at 19 days in the Nagano soil and 41 days in the Ushiku soil, and the 90% decay time was estimated as 34 and 107 days, respectively. The initial concentrations of benthiavalicarb-isopropyl differed greatly between the Nagano soil (0.24 mg/kg) and the Ushiku soil (1.15 mg/kg), and the results of a study conducted in fiscal year 2000 showed a similar tendency (Nagano soil: 0.22 mg/kg, half-life of 15 days; Ushiku soil: 1.67 mg/kg, half-life of 26 days). The initial concentration in the Ushiku soil was slightly higher than the value of 0.82 mg/kg calculated from the bulk density (about 0.82) of soil estimated from the

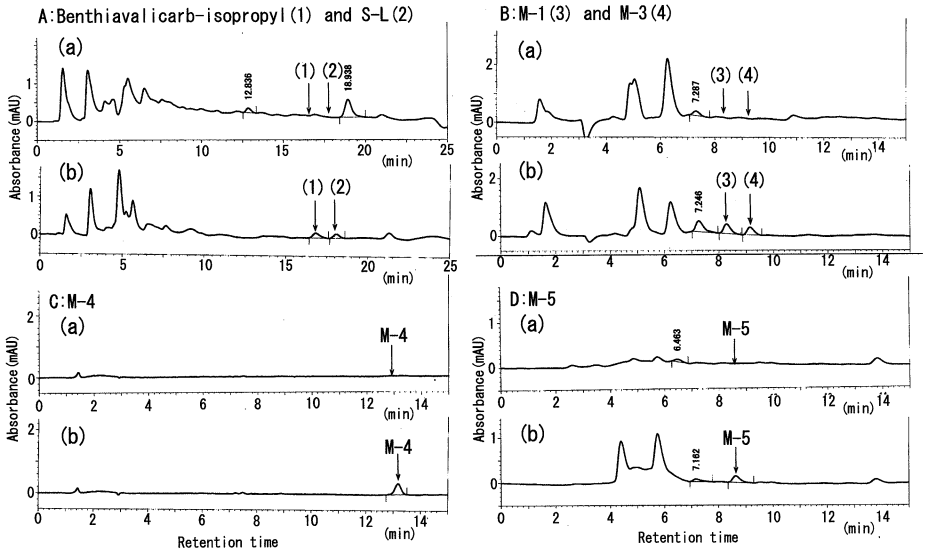

Fig. 4. Examples of HPLC chromatograms for Nagano soil samples.
(a) Nagano soil control, (b) Spiked sample at the limit of quantification.

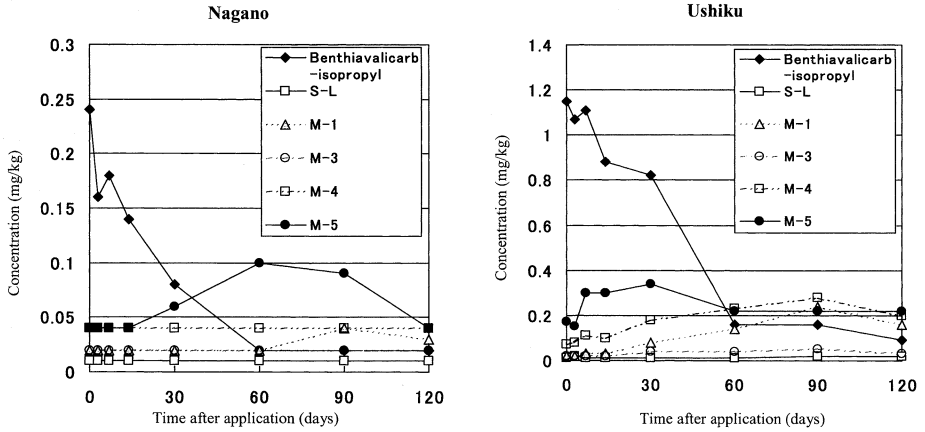

Fig. 5. Dissipation of benthiavalicarb-isopropyl, S-L and their degradation products in upland soils from Nagano and Ushiku.
The concentration of degradation products was converted on the basis of the concentration of benthiavalicarb-isopropyl. In the Nagano field, the concentration of S-L, M-3 and M-4 was below the limit of detection. Limit of detection: benthiavalicarb-isopropyl, S-L; 0.01 mg/kg, M-1, M-3; 0.02 mg/kg, M-4; 0.04 mg/kg.

specifications of the analytical sample (soil sample thickness of 10 cm and three applications).

The degradation of benthiavalicarb-isopropyl in soil in an indoor test was rapid (the half-life in the Ushiku soil at 30°C under the upland conditions was 7 days), and was markedly inhibited in sterilized soil.[3] In addition, since benthiavalicarb-isopropyl was resistant to hydrolysis and photodegradation in water and the rate of degradation of benthiavalicarb-isopropyl did not differ between under the irradiated condition and the shaded condition in the soil surface photodegradation test (no change to other optical isomers),[4] it is considered that the degradation of benthiavalicarb-isopropyl in the upland field soil is mainly attributable to degradation by microorganisms in the soil.

In the Ushiku soil, all degradation products were detected throughout the study period, and in amounts larger than in the Nagano soil. The degradation products, M-1, M-4 and M-5, showed maximum concentrations (0.24 to 0.34 mg/kg, equivalent to 20.9% to 29.6% converted to the R-L concentration at the start of the study) from 30 to 90 days after the application and tended to decrease thereafter. M-3 was detected in an amount about three times higher than the limit of quantification at maximum, and S-L was occasionally detected in an amount about twice the limit of quantification.

In the Nagano soil, almost no degradation product was detected, though M-1 and M-5 were occasionally detected at levels about three times higher than the limit of quantification at maximum. S-L was not detected.

The total residue concentration was determined by summing values for all the analytical compounds detected, including benthiavalicarb-isopropyl (values less than the limit of quantification were overestimated by employing the limit of quantification). The half-life of all analytical compounds in soil, which was estimated from the total residue concentration, was 105 days for the Nagano soil and 112 days for the Ushiku soil, a result showing that there was almost no long-term lingering of this pesticide in the soils from upland fields.

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