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Residue Analysis Method of Pyributicarb and Its Related Compounds in Soil

Hideo MORINAKA, Mitsuyuki MURAKAMI,*
Hiromi TAKESADA, Hiroaki TENMA
and Kenji TSUZUKI

Nan-yo Research Laboratories, Tosoh
Corporation, Kaisei-cho, Shin-nan-yo
746, Japan

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INTRODUCTION

Pyributicarb [*O*-3-*tert*-butylphenyl 6-methoxy-2-pyridyl(methyl)thiocarbamate, (I)] is a thiono-type thiocarbamate herbicide developed by Tosoh Corporation. It has excellent herbicidal activity against annual paddy weeds, especially barnyardgrass, and it shows almost no phytotoxicity to transplanted young rice seedlings. Herbicidal activities of pyributicarb under flooded condition were reported by Tsukuda *et al.*¹⁻³⁾

Technical grade of pyributicarb contains a small amount of 4-*tert*-butylphenyl isomer [*O*-4-*tert*-butylphenyl 6-methoxy-2-pyridyl(methyl)thiocarbamate, (II)]. On the other hand, *O*-demethyl compound [*O*-3-*tert*-butylphenyl 6-hydroxy-2-pyridyl(methyl)thiocarbamate, (III)] was reported as one of the main metabolite in soil and the only major soil metabolite maintaining thiocarbamate skeleton.⁴⁾

This paper describes an analytical method for the residues of pyributicarb, 4-*tert*-butylphenyl isomer and *O*-demethyl metabolite, all of which have thiocarbamate skeleton, in soil.

MATERIALS AND METHODS

1. Reagents

Analytical standards of pyributicarb (mp 85.7–86.2°C), 4-*tert*-butylphenyl isomer (mp 87–88°C) and *O*-demethyl metabolite (mp 188–189°C) were prepared in our laboratory and all their purities were over 99.5%. Acetone, methanol, hexane, benzene, dichloromethane and ethyl acetate were pesticides residue analysis grade,

acetonitrile was HPLC grade and all these solvents were purchased from Wako Pure Chemical Industries, Ltd. Silica gel (Wakogel C-100) for column chromatography and other inorganic chemicals (guaranteed reagent) were also purchased from Wako.

2. Apparatus

The GC analysis was carried out on a Hewlett-Packard HP-5890A with an NPD using a 2 mm i.d. × 4 ft. glass column packed with 3% Silicone Gum SE-30 on 60/80 mesh Chromosorb W-HP. Column, injection and detector temperatures were 205, 220, 250°C, respectively. The flow rates of nitrogen (carrier), hydrogen and air were 20, 3 and 110 ml/min, respectively. The injection volume of the sample solution was 4 µl.

The HPLC system was composed of a CCPD (computer control dual pump, Tosoh) and a UV-8 model II (UV detector, Tosoh) using a TSK-gel ODS-120T column (4.6 mm i.d. × 25 cm, Tosoh). A mixture of acetonitrile/water (3/1, v/v) was used as the mobile phase at a flow rate of 1.0 ml/min (column pressure=about 70 kg/cm²). The injection sample volume was 100 µl and UV 300 nm was used for monitoring.

3. Soil Samples

Three different paddy field soil samples, Ibaraki, Osaka and Yamaguchi soil, were used in this experiment. Properties of these soils are shown in Table 1. Soil samples were passed through a 4.75 mm sieve prior to use.

4. Extraction and Purification

Soil sample (30 g in dry weight) was placed in an Erlenmeyer flask, then 100 ml of methanol/water (3/7, v/v)⁵⁾ was added. The flask was stoppered and shaken for 30 min by a mechanical shaker, then the mixture was filtered with suction. The extraction procedure was repeated again for the residue using a fresh solvent and the methanol was evaporated from the combined filtrate. After adding 50 ml of saturated aqueous sodium chloride solution and 100 ml of water, the concentrate was extracted twice with 100 ml portions of benzene. The combined extract was dehydrated over anhydrous sodium sulfate, then concentrated to about 1 ml.

The residue was purified by silica gel column chromatography using a 1.5 cm i.d. × 40 cm chromatographic glass column and 10 g of silica

* Present address: Naruto Research Center, Otsuka Chemical Co., Ltd., Satoura-cho, Naruto 772, Japan

Fig. 1 Structures of pyributicarb (I), 4-*tert*-butylphenyl isomer (II) and *O*-demethyl metabolite (III).

Table 1 Recovery of pyributicarb (I), 4-*tert*-butylphenyl isomer (II) and *O*-demethyl metabolite (III) from paddy field soil samples.

Soil sample (City)	Soil properties					Fortification		Recovery ^{a)} (%)
	Soil source (Texture)	pH (H ₂ O)	Total carbon (%)	Clay (%)	C.E.C. (meq/100 g soil)	Com- pound	ppm	
Ibaraki soil ^{b)} (Ushiku)	Volcanic ash (Light clay)	5.61	6.19	25.8	35.9	(I)	1.5	97
							0.15	92
						(II)	1.5	98
							0.15	89
						(III)	0.5	96
							0.05	88
Osaka soil ^{c)} (Habikino)	Diluvial (Clay loam)	6.0	1.6	24–25	9.5	(I)	1.5	92
							0.15	92
						(II)	1.5	94
							0.15	86
						(III)	0.5	94
							0.05	88
Yamaguchi soil ^{d)} (Tokuyama)	Alluvial (Clay loam)	5.9	1.64	16.8	9.1	(I)	1.5	95
							0.15	96
						(II)	1.5	91
							0.15	98
						(III)	1.0	89
							0.10	85

^{a)} Values are the average of duplicates.

^{b)} Data of soil properties were provided from Research Institute of the Japan Association for Advancement of Phyto-Regulators.

^{c)} Data of soil properties were provided from Osaka Prefectural Agricultural and Forestry Research Center.

^{d)} Data of soil properties were obtained at Research Center of Palyno Survey Corporation.

gel. The column was developed with 40 ml of benzene followed by 80 ml of ethyl acetate/benzene (3/7, v/v). The initial 40 ml was collected (benzene eluent, fraction No. 1), the next 40 ml was discarded, and the last 40 ml was collected again (fraction No. 2). Pyributicarb and 4-*tert*-butylphenyl isomer were eluted in fraction No. 1 and *O*-demethyl metabolite was eluted in fraction No. 2. Fraction No. 1 was evaporated to dryness and the residue was dissolved in acetone to attain a fixed volume over

2 ml for GC analysis. On the other hand, fraction No. 2 was evaporated to dryness and the residue was dissolved in the mobile phase to attain a fixed volume over 10 ml for HPLC analysis.

5. Calibration

Standard plots of peak height *vs.* nanogram of test compounds obtained from the standard solutions were used for the determination of the amounts of each compounds. Solutions of pyri-

buticarb and 4-*tert*-butylphenyl isomer in acetone ranging from 0.5 to 2.5 ppm and solutions of *O*-demethyl metabolite in the mobile phase ranging from 0.08 to 1.9 ppm were used for GC and HPLC, respectively. These plots were linear over the range of the weights of the test compounds injected.

RESULTS AND DISCUSSION

1. Extraction from Soil Samples

Pyributicarb, 4-*tert*-butylphenyl isomer and *O*-demethyl metabolite could be extracted easily from soil with both acetone/water (8/2, v/v) and methanol/water (7/3, v/v). Since a number of interfering components, however, were extracted with acetone/water, methanol/water was selected for extraction solvent.

2. Purification

After the evaporation of methanol, the three compounds were extracted from the residual aqueous solution. To avoid troublesome operations, we selected a solvent which could extract the three compounds at the same time. Pyributicarb and 4-*tert*-butylphenyl isomer could be extracted with various kinds of solvents, hexane, benzene, dichloromethane *etc.* But in the case of *O*-demethyl metabolite, hexane was not adequate, and benzene, dichloromethane and ethyl acetate could be used for the extraction. From the stand point of recovery and purification, benzene was selected for an extraction solvent of the three compound.

Pyributicarb and the isomer could be easily purified by various conditions of silica gel column chromatography, and benzene was suitable for the eluent. On the other hand, *O*-demethyl metabolite could be eluted from the silica gel column with 200 ml of ethyl acetate/benzene (2/8 or 3/7, v/v). Good recovery and reproducibility were obtained under the column condition but purification was not enough for HPLC analysis. Then, fractionation of the column chromatography using these solvent systems was investigated. As the results, when mixing proportion of ethyl acetate and benzene was 2/8, a large amount of the solvent was required to obtain good recovery, and 3/7 was selected for an eluent of the *O*-demethyl metabolite. Finally, we established the procedure to analyze the residues of the three compounds as described above.

3. Detection Limits and Recoveries

The detection limits of pyributicarb, 4-*tert*-butylphenyl isomer and *O*-demethyl metabolite

in soil were determined to be 0.005, 0.005 and 0.01 ppm as dry soil weight basis, respectively.

Known amounts of the three compounds were added to sieved soil sample, then the recoveries were determined by the proposed procedure and the results are shown in Table 1. No significant difference in recovery was observed on differences in soil types and fortified levels. Average recoveries ranged more than 90% for pyributicarb, more than 85% for the isomer and the metabolite. Representative chromatograms are shown in Figs. 2 and 3 and no troublesome interference was detected.

Fig. 2 Typical gas chromatograms of extracts of soil samples fortified and unfortified with pyributicarb (I) and 4-*tert*-butylphenyl isomer (II).

(A) Soil blank. (B) Soil + 0.15 ppm (I) + 0.15 ppm (II).

Fig. 3 Typical high performance liquid chromatograms of extracts of soil samples fortified and unfortified with *O*-demethyl metabolite (III).

(A) Soil blank. (B) Soil + 0.5 ppm (III).

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要 約

土壌におけるピリブチカルブおよび関連化合物の残留分析法

森中秀夫, 村上充幸, 竹貞博美

天満浩章, 続木建治

土壌中におけるピリブチカルブ, 4-tert-ブチルフェニル異性体および土壌中代謝物であるO-脱メチル体の残留分析法を確立した。4.75 mmの篩を通した土壌試料から, 30%含水メタノールで振とう抽出した。溶媒を減圧溜去した後, 飽和食塩水および水を加えてベンゼンで抽出した。抽出物はシリカゲルカラムクロマトグラフィーで精製し, 二つの画分を得た。ピリブチカルブおよび4-tert-ブチルフェニル異性体はGC(NPD)で, O-脱メチル体はHPLC(UV)で定量した。3種類の土壌で検討した結果, 検出限界および回収率にともに違いは認められず, 検出限界はピリブチカルブおよび4-tert-ブチルフェニル異性体が0.005 ppm, O-脱メチル体が0.01 ppmであり, 回収率はピリブチカルブが90%以上, 4-tert-ブチルフェニル異性体およびO-脱メチル体が85%以上であった。