Original Article

Degradation of Buprofezin in Flooded and Upland Soils under Laboratory Conditions

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[Phenyl ring- ^{14}C] buprofezin, 2-*tert*-butylimino-3-isopropyl-5-phenyl-perhydro-1,3,5-thiadiazin-4-one, gradually decomposed in soils under flooded and upland conditions, with the half lives of 104 and 80 days, respectively. After 150 days, five degradation products were identified by thin-layer cochromatography: 2-*tert*-butylimino-5-(4-hydroxyphenyl)-3-isopropyl-perhydro-1,3,5-thiadiazin-4-one, 3-isopropyl-5-phenyl-perhydro-1,3,5-thiadiazin-2,4-dione, 1-*tert*-butyl-3-isopropyl-5-phenyl-biuret, 1-isopropyl-3-phenylurea and phenylurea. As minor products, 2-*tert*-butylimino-5-phenyl-perhydro-1,3,5-thiadiazin-4-one or buprofezin sulfoxide were found in the flooded or the upland soil. [^{14}C]Carbon dioxide and bound ^{14}C residue accounted for 23–24% and 13–21% of the applied radioactivity, respectively. The degradation of buprofezin remarkably delayed in sterile soils. Since neither formation of $^{14}\text{CO}_2$ nor ring hydroxylation was observed in the sterile soils, buprofezin seems to have undergone complete mineralization in the soils under both flooded and upland conditions through biological transformation by soil microorganisms.

INTRODUCTION

Buprofezin (Applaud®, 2-*tert*-butylimino-3-isopropyl-5-phenyl-perhydro-1,3,5-thiadiazin-4-one) is an insect growth regulator that effectively controls harmful insect pests including the brown rice planthopper, *Nilaparvata lugens* STÅL, and the greenhouse whitefly, *Trialeurodes vaporariorum* WESTWOOD.^{1,4)} *N. lugens* nymphs treated with buprofezin exhibit abnormalities in their ability to shed the exuviae and finally die at the time of molting.³⁾ The compound was recently demonstrated to act on the insects by inhibiting the integumentary cuticle deposition and chitin biosynthesis.^{5,6)} Although no lethal effect of buprofezin on adult insects has been observed, its strong suppression on the egg-laying of *N. lugens* female adults is indicated.⁷⁾ More recently, the similar effects

on the twenty-eight-spotted ladybird, *Henosepilachna vigintioctopunctata* FABRICIUS, was reported.^{7,8)}

Buprofezin is not highly toxic to mammals: acute oral LD_{50} is over 2000 mg/kg for rats and mice.¹⁾ Because of the high hydrophobicity ($\log P=4.31$), buprofezin is easily adsorbed with soil particles.⁹⁾ Strong soil adsorption is sometimes contributed to persistence of the compound in soils. Therefore, the degradability of buprofezin in soils has been studied at our institute and is discussed in the present paper.

MATERIALS AND METHODS

1. Compounds

[Phenyl ring- ^{14}C] buprofezin was synthesized from [^{14}C]aniline hydrogen sulfate (Amersham International, Buckinghamshire, UK) by reacting the intermediate, *N*-chloro-

Table 1 Authentic compounds and their *R_f* values.

Compound No.	Structure				<i>R_f</i> values
	Solvent system used ^{a)}				
	R ₁	R ₂	X	Y	A	B	C	D	E
1 ^{b)}	CH(CH ₃) ₂	N-C(CH ₃) ₃	H	S	0.65	0.56	0.64	0.50	0.88
2	CH(CH ₃) ₂	N-C(CH ₃) ₃	OH	S	0.31	0.38	0.41	0.18	0.61
3	H	N-C(CH ₃) ₃	H	S	0.16	0.00	0.06	— ^{c)}	0.28
4	CH(CH ₃) ₂	O	H	S	0.62	0.42	0.56	0.31	—
5	CH(CH ₃) ₂	N-C(CH ₃) ₃	H	SO	0.51	0.31	0.51	0.18	0.71

	<i>R_f</i> values				
	R ₃	R ₄	A	B	C	D	E
6	CH(CH ₃) ₂	CONHC(CH ₃) ₃	0.64	0.47	0.60	0.41	—
7	CH(CH ₃) ₂	H	0.46	0.32	0.43	0.15	0.53
8	H	H	0.34	0.04	0.10	0.01	0.12

^{a)} Solvent systems used; A: benzene/ethyl acetate/acetic acid (6/2/1), B: diisopropyl ether/acetone (9/1), C: heptane/ethyl acetate/ethanol/28% NH₄OH (25/25/5/1), D: hexane/ethyl acetate (2/1), E: diisopropyl ether/acetonitrile (9/1).

^{b)} Buprofezin.

^{c)} Not determined.

methyl-*N*-[phenyl-¹⁴C]phenylcarbamoyl chloride, with 1-*tert*-butyl-3-isopropylthiourea.^{1,10)} Its specific activity was 6.9 mCi/mmol and radiochemical purity more than 98%. Authentic compounds in Table 1 were used for identification of degradation products from buprofezin (**1**). Compound **2** was obtained from 2-*tert*-butylimino-3-isopropyl-5-(4-methoxyphenyl)-perhydro-1,3,5-thiadiazine-4-one by reacting with hydroiodic acid.¹⁰⁾ Compound **3** and 2-(1,1-dimethyl-2-hydroxyethyl)imino-3-isopropyl-5-phenyl-perhydro-1,3,5-thiadiazine-4-one (**9**) were synthesized from *N*-chloromethyl-*N*-phenyl-carbamoyl chloride and the corresponding thioureas. The reaction of compound **9** with hydrochloric acid gave compound **4**. Compounds **5** and **6** were prepared from buprofezin (**1**) by reacting with perbenzoic acid and chlorine, respectively. Compounds **7** and **8** were obtained from the appropriate amines and phenyl isocyanate.¹¹⁾

2. TLC and Radioassay

TLC and radioassay were performed according to the methods reported previously.¹²⁾ Solvent systems and obtained *R_f* values for

authentic compounds **1–8** are listed in Table 1.

3. Soils and Incubation

Soils were collected from a paddy and an upland field in Ehime Prefecture. They were passed through a 1-mm sieve and then stored at −20°C until use. The soil analysis data are summarized in Table 2. Each soil (50–250 g of dry weight) was put into a 250-ml wide-mouthed flask with water. The water content in the soil was adjusted once a week to maintain a flooded condition (with water 1.5 cm deep) or an upland condition (60% of the maximum water holding capacity).¹³⁾ In order to obtain a sterile condition, the soils were autoclaved at 120°C for 30 min, and the following procedures such as [¹⁴C]buprofezin application, aeration and adjustment of water content were performed under the sterile condition. After preincubation at 25°C for 2 weeks, [¹⁴C]buprofezin was added to the flasks to give a final concentration of 1.6 ppm (0.53 μCi/flask) in the flooded soil or 2.5 ppm (0.82 μCi/flask) in the upland soil. The treated soils under the flooded or upland condition were incubated at 25°C in the dark. Carbon

Table 2 Analysis data of soils used.

Soil (origin)	Texture	Sand (%)	Silt (%)	Clay (%)	Carbon (%)	Cation exchange capacity (meq/ 100 g)	pH		Maximum water holding capacity (%)
							H ₂ O	KCl	
Ehime (Paddy, alluvial)	Silty clay loam	23.0	63.1	13.9	3.8	11.6	6.4	4.7	72.4
Ehime (Upland field, dilluvial)	Sandy loam	55.3	22.6	22.1	2.4	11.0	7.0	6.3	43.0

dioxide-free air was gently introduced into the flasks and the air from the outlets was passed through urethane foam and 2 M NaOH to trap volatile ^{14}C -products and $^{14}\text{CO}_2$, respectively. These traps and the soils were sampled at various intervals during the 150-day incubation.

4. Extraction and Analysis of Degradation Products

The paddy water covering the soil under the flooded condition was separated by decantation. The obtained water was extracted twice with ethyl acetate (100 ml). The soils under the flooded and upland conditions were extracted three times with acetone (100 ml) and then twice with methanol (100 ml). The extracts were combined, concentrated *in vacuo* and then redissolved in 100 ml of water. The resulting aqueous solution was extracted twice with ethyl acetate (100 ml) at pH 7 and then twice at pH 1. The radioactivity remaining in the soils was finally extracted with 100 ml of methanolic 2 M NaOH (methanol/water, 1/3) at 40°C overnight. The methanolic alkaline extracts were partitioned with diethyl ether (100 ml) at pH 14 and 1 and then combined with the ethyl acetate extracts. The urethane foam traps were extracted with 50 ml of methanol. The radioactivity in the methanol extracts was attributed to [^{14}C]buprofezin by thin-layer cochromatography, accounting for 2–7% of the applied ^{14}C . The amount of [^{14}C]buprofezin in the methanol extract was registered as that of buprofezin in the soils. Small portions of the obtained organic or aqueous solutions were subjected to radioassay or TLC analysis. The unextractable radioactivity in soils was determined by using a sample oxidizer and liquid

scintillation counters.¹³⁾

The degradation products of buprofezin in the flooded and the upland soils were separated and identified by thin-layer cochromatography with appropriate authentic compounds. Those on TLC plates were analyzed by scraping the autoradiographically detected regions into counting vials and subjected to the subsequent liquid scintillation counting. The $^{14}\text{CO}_2$ in NaOH traps was determined by acidifying with conc. HCl and trapping the reforming $^{14}\text{CO}_2$ with 10 ml of Oxisorb- CO_2 (New England Nuclear, Boston, USA), followed by liquid scintillation counting.¹³⁾

RESULTS AND DISCUSSION

1. Disappearance of [^{14}C]Buprofezin and $^{14}\text{CO}_2$ Formation in Soils

Under the flooded condition, [^{14}C]buprofezin gradually decomposed in the soil as shown in Fig. 1. After 150 days, 42% of the applied buprofezin remained unchanged. From the first-order decreasing line ($\ln Y = -0.0060354X + 4.54023$, $r = 0.966$), the half life of buprofezin in the flooded soil was estimated as 104 days. Correspondingly, the $^{14}\text{CO}_2$ trapped in 2 M NaOH increased with time and finally accounted for 24% of the applied radioactivity after 150 days.

Sterilization of the flooded soil retarded buprofezin decomposition (the extrapolated half life, 432 days) and completely inhibited the evolution of $^{14}\text{CO}_2$ (Fig. 1). Even after 150 days, 80% of [^{14}C]buprofezin was recovered from the flooded soil and the $^{14}\text{CO}_2$ trapped in 2 M NaOH was negligible.

[^{14}C]Buprofezin also decreased gradually in the soil under the upland condition (Fig. 1), showing a half life of 80 days (calculated from

Fig. 1 [Phenyl-¹⁴C]buprofezin remaining unchanged in Ehime soils under flooded and upland conditions, and the cumulative ¹⁴CO₂ in 2 M NaOH trap.

More than 97% of applied [¹⁴C]buprofezin was recovered from both soils on the 1st day of incubation according to the methods described in the text.

the first-order decreasing line: $\ln Y = -0.0074596X + 4.50517$, $r=0.988$). The formation of ¹⁴CO₂ was observed to be similar to that

under the flooded condition, accounting for 23% of the applied radioactivity after 150 days.

Here again, soil sterilization retarded buprofezin degradation in the soil (the extrapolated half life, 673 days) and completely inhibited the evolution of ¹⁴CO₂ under the upland condition (Fig. 1).

2. Analysis of Degradation Products

The degradation products identified by thin-layer cochromatography and their abundance in the soils are shown in Table 3. Most of the extractable radioactivity was attributed to [¹⁴C]buprofezin even after 150 days. The identified products from the unsterile soils were compounds **2-8** under the flooded condition or compounds **2** and **4-8** under the upland condition. Minor unidentified products were also detected in the soils under both conditions but only accounted for 2-3% of the applied radioactivity as a whole. The aqueous ¹⁴C (8.5 or 7.3% of the radioactivity applied to the flooded or upland soil, respectively) has not been identified yet. The major products were ¹⁴CO₂ and bound ¹⁴C residue which accounted for 24.4 and 12.7% or 23.2 and 20.5% of the radioactivity applied to the flooded or upland

Table 3 Buprofezin and its degradation products, and their abundance in soils under flooded and upland conditions.

Buprofezin and products	Abundance (% of applied radioactivity) after 150 days in soils under			
	Flooded condition		Upland condition	
	Unsterile	Sterile	Unsterile	Sterile
Buprofezin (1)	41.84	80.45	36.26	85.90
Compound 2	0.61	— ^{a)}	0.08	—
3	0.06	—	—	—
4	0.22	0.83	0.18	trace
5	trace	—	0.15	0.16
6	0.23	0.42	0.75	trace
7	0.80	1.32	1.20	0.93
8	0.15	0.05	0.13	0.06
Unidentified organic soluble	3.15	2.37	3.36	2.05
Aqueous	8.50	2.92	7.30	1.73
¹⁴ CO ₂	24.47	—	23.21	—
Bound ¹⁴ C residue	12.67	6.24	20.53	6.16
Total	92.70	94.60	93.15	96.99

^{a)} Less than 0.01%.

Fig. 2 Proposed pathways of buprofezin degradation in soils under flooded and upland conditions.

* shows the ^{14}C labeled position.

soil, respectively (Table 3). The extractable identified and unidentified products were found after 10 or 30 days but did not increase thereafter. Therefore, these products of buprofezin seems to have degraded further to $^{14}\text{CO}_2$ or bound ^{14}C residue.

Under the sterile condition, compounds **1** and **4-8** were detected in both the flooded and upland soils (Table 3). However, no formation of compound **2** and $^{14}\text{CO}_2$ was observed throughout the experiments under the flooded and upland conditions. These products must have been formed through the biological transformation by soil microorganisms. The aromatic ring-opening process by microorganisms is well established to be operated by a series of oxidative ring hydroxylation reactions.¹⁴⁾ The mineralization of buprofezin to $^{14}\text{CO}_2$ also seems to undergo through oxidative ring hydroxylation by soil microorganisms, in which compound **2** may be the important intermediate.

3. Pathways of Buprofezin Degradation in Soils

From the identified degradation products, the pathways of buprofezin degradation in soils under the flooded and upland conditions can be proposed as shown in Fig. 2. As already mentioned, ring hydroxylation occurred only under the unsterile condition, which may

be the key step for $^{14}\text{CO}_2$ formation. Buprofezin also underwent cleavage of thiadiazinone ring presumably through thiadiazindione (**4**) or sulfoxide (**5**). Phenylurea herbicides have been hydrolyzed to their corresponding anilines in soils.^{15,16)} Although phenylureas (**7** and **8**) were detected as the minor products of buprofezin, no aniline was found in the soils so far.

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要 約

Buprofezinの湛水および畑地条件下の容器内土壌における分解

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[Phenyl 環- ^{14}C]buprofezin は土壌(愛媛県で採取)中で徐々に分解し, その半減期は湛水および畑地条件下でそれぞれ 104 および 80 日と推定された. 同定された 7 分解物は, phenyl 環の水酸化, イオウの酸化, 脱 *tert*-butylimino 化および thiadiazinone 環の開裂により生成すると考えられた. 150 日後には, $^{14}\text{CO}_2$ および結合型残留物が, 施用 ^{14}C のそれぞれ 23~24% および 13~21% を占めた. 土壌をあらかじめ滅菌しておく, buprofezin の半減期は著しく延長し (それぞれ 432 および 673 日, 外挿値), phenyl 環の水酸化および $^{14}\text{CO}_2$ の生成が認められなくなった. したがって, buprofezin の $^{14}\text{CO}_2$ への分解には土壌微生物が関与すると考えられた.