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Decrease of Herbicide Bromobutide and its Debromo Metabolite in Paddy Field Soil during 24 weeks After Application

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Abstract Variations in concentrations of herbicide bromobutide (RS)-2-bromo-*N*-(α,α-dimethylbenzyl)-3,3-dimethylbutyramide, and its metabolite bromobutide-debromo, *N*-(α,α-dimethylbenzyl)-3,3-dimethylbutyramide were investigated in soils from three paddy fields used for rice farming at 24 weeks after application. The bromobutide concentration was maximum within 24 h after application. That of bromobutide-debromo was maximum within 5–7 days of application. Each gradually decreased to below detection limits at 12–22 weeks after application. Bromobutide was detected up to 76–104 days after application in the paddy soils, whereas bromobutide-debromo was detected up to 125 days after application. The bromobutide composition was higher than 90 % within 6 days of application, decreasing to less than 5 % by 125 days of application. The decrease of bromobutide amount in the soil was inferred as the first-order reaction. The bromobutide half-life was calculated as 12–21 days (16 days mean) during 18–104 days following application.

Keywords Herbicide · Metabolite · Paddy field · Soil · Half-life

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Throughout the world, paddy field farming has played an important role in rice production. Current agricultural practices rely heavily on pesticides for rice production. In Japan, paddy fields cover a total area of 2.5 million ha, accounting for 54 % of all cultivated area (4.6 million ha) in FY2010. Approximately 170 pesticides have been used to control paddy field weeds and fungi, and pests such as insects and nematodes. Among the approximately 70 herbicides applied to paddy fields, bromobutide (RS)-2-bromo-*N*-(α,α-dimethylbenzyl)-3,3-dimethylbutyramide, is a commonly used herbicide in Japan. Bromobutide, an amide-type herbicide possessing strong herbicidal activity toward perennial weeds and annual weeds in paddy fields (Isobe et al. 1984; Takahashi et al. 1985), has water solubility and logarithm of the octanol–water partition coefficient (log P_{OW}) reported respectively as 3.54 mg/L and 3.46 (Food Safety Commission 2008). Bromobutide is well known to degrade to bromobutide-debromo, *N*-(α,α-dimethylbenzyl)-3,3-dimethylbutyramide via a photochemical reaction (Takahashi et al. 1985) and metabolism (Isobe et al. 1984).

We previously investigated variations in the bromobutide and bromobutide-debromo concentrations in paddy field water and soil during the initial 18 days following application. The authors evaluated the runoff ratios of bromobutide from the paddy fields to drainage channels as 28 % ± 16 % (Morohashi et al. 2012). Therefore, adsorption into the paddy soil was the main route of the residual bromobutide, just as it is for herbicides thiobencarb, mefenacet, pyrazosulfuron-methyl, and dymron (Amano et al. 2001; Ishii et al. 2004), as well as the fungicide phthalide (Iwashita et al. 2008).

Ishii et al. (2004) reported the half-lives of three herbicides (mefenacet, pyrazosulfuron-methyl, and dymron) based on paddy field experiments conducted during

12 weeks after application. For bromobutide, the laboratory experimental half-lives have been reported as 25–54 days (Food Safety Commission 2008) and 31–64 days (Kanazawa 1996). However, few reports in the relevant literature have described bromobutide degradation behavior in paddy fields after application. We previously reported the half-life of bromobutide in paddy soils during the initial 18 days following application as 6.9 ± 2.6 days (Morohashi et al. 2012), but the long-term degradation behavior of bromobutide was not reported in paddy fields. Herein, we describe the variations in the bromobutide and bromobutide-debromo concentrations in paddy field soils during 24 weeks after application. The authors also evaluate the rate of bromobutide decrease in the paddy fields.

Materials and Methods

The three investigated paddy fields 1–3 were located in a rice cultivation area in Niigata City, Niigata, Japan (Morohashi et al. 2012). Bromobutide was applied 180 g to paddy field 1, with an area of 2,700 m^2, 240 g to paddy field 2 (4,030 m^2), and 120 g to paddy field 3 (1,740 m^2) on May 23, 2009 (day 0). The paddy water layers remained from the day of application to June 10 (18 days after application) at 1–5 cm depth. Soils were sampled during May 23–November 5, 2009 at four locations in paddy fields 1 and 3, and at six locations in paddy field 2. Soil samples were collected from the 0–4 cm surface layer (Mine 1987) from a paddy field at each location. Then equal weights of these soils were combined and mixed as samples. Results for soil samples collected during May 23–June 10 were described in an earlier report together with results of paddy waters (Morohashi et al. 2012). The Water contents of paddy soils 1, 2, and 3 were 55.0 % ± 6.36 %, 57.2 % ± 5.46 % and 44.7 % ± 6.68 %, respectively. The soil densities were 1.29 ± 0.12 g/cm^3 (paddy soil 1), 1.28 ± 0.093 g/cm^3 (paddy soil 2) and 1.22 ± 0.10 g/cm^3 (paddy soil 3); the silt and clay contents were 36.5 % (paddy soil 1), 44.8 % (paddy soil 2) and 12.9 % (paddy soil 3). The organic contents were estimated as the loss upon ignition (550°C, 15 h) and calculated as the percent dry weight (Iwashita et al. 2008). The organic contents of paddy soils 1, 2 and 3 were 7.21 % ± 1.34 %, 7.90 % ± 2.03 % and 4.31 % ± 0.86 %, respectively. All samples were stored in the dark at 5°C. They were analyzed within 24 h after collection.

A gas chromatograph – mass spectrometer (GC/MS, Finnigan POLARIS Q; Thermo Electron, Waltham, MA, USA) equipped with an auto-injection system (Combi Pal; CTC Analytics, Zwingen, Switzerland) was used for the quantitative analyses. A 30 m × 0.25 mm i.d. (0.25 μm film thickness) fused-silica InertCap 5 ms/Sil column (GL Sciences, Tokyo, Japan) was used for the gas chromatographic separation. Reagents including the standard pesticides were purchased from GL Sciences, Kanto Kagaku (Tokyo, Japan) and from Wako Pure Chemical Industries (Osaka, Japan). 1,4-Diiodobenzene and 9-bromoanthracene were used as the internal standard. Standard solutions of bromobutide and bromobutide-debromo (1,000 and 50 μg/mL) and an internal standard solution (50 μg/mL) were prepared in acetone. The purified water was from a Milli-Q system (Millipore, Bedford, MA, USA).

Determination of bromobutide and bromobutide-debromo from the soil samples was conducted according to a method reported previously (Kawata et al. 2005; Morohashi et al. 2012). The procedure is as follows. A 10 mL portion of acetone was added to a 10 g soil sample. The mixture was ultrasonicated for 15 min and then shaken at 2,500 strokes/min for 15 min. The slurry was centrifuged at 3,000 rpm (1,700×*g*) for 10 min. The supernatant solvent phases were filtered through a glass-fiber filter. The extraction procedure was repeated twice. The extracts were combined and evaporated to 5 mL at 30°C. The solution was added to 10 mL hexane. Then the resulting solution was washed four times with 2 mL purified water. After the hexane layer was dried over anhydrous sodium sulfate, the solution was concentrated to 1 mL under a stream of pure nitrogen gas. A 10 μL aliquot of the internal standard solution was added to the concentrated solution. All samples were stored at 4°C until the GC/MS analysis.

A 1-μL aliquot of the resulting solution was analyzed using GC/MS. The GC/MS conditions were as follows: column temperature, programmed from 50 (held for 1 min) to 280°C (held for 10 min) at a rate of 10°C/min; injector temperature, 250°C; injection mode, splitless; helium carrier gas flow rate, 1.0 mL/min; MS transfer temperature, 290°C; ion source temperature, 250°C; ionization mode, electron impact; ionization energy, 70 eV; mass scan range, *m/z* 50–450. The ions used for the quantitation and confirmation were *m/z* 120 and 232 for bromobutide, and *m/z* 120 and 233 for bromobutide-debromo, respectively. Ions for the internal standards were *m/z* 330 for 1,4-diiodobenzene and *m/z* 256 for 9-bromoanthracene. 1,4-Diiodobenzene and 9-bromoanthracene were used as internal standards for bromobutide and bromobutide-debromo, respectively. The ratios of the peak area of the ions to that of the internal standard were used for quantification of the target compounds. The concentrations of the target compounds in soil were calculated on a dry basis.

Results and Discussion

Variations in the bromobutide and bromobutide-debromo concentrations in the paddy soils are presented in Fig. 1.

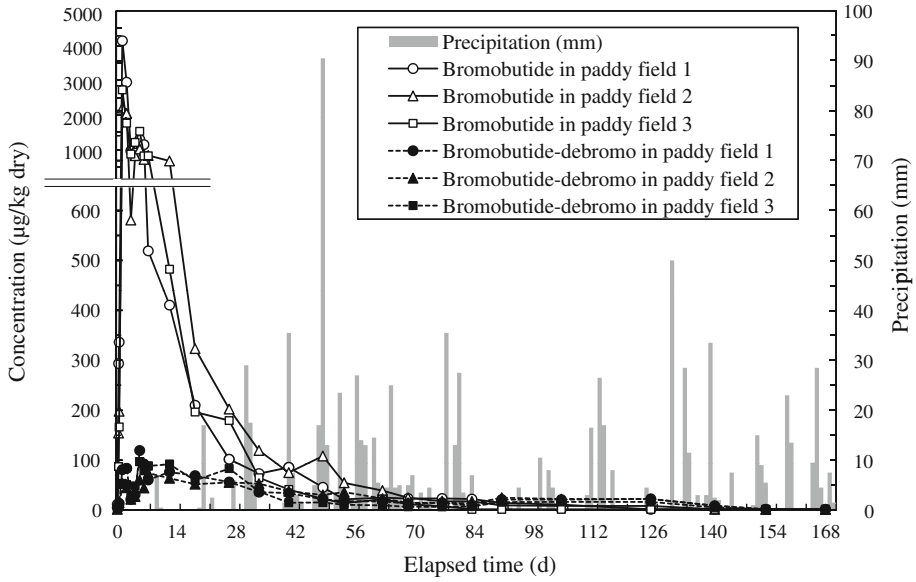

Fig. 1 Concentrations of bromobutide and bromobutide-debromo in paddy soil

The maximum concentrations of bromobutide were observed within 24 h after application, as reported previously (Morohashi et al. 2012). The bromobutide amounts in the paddy soils decreased to 20 %–36 % of the applied amounts at 7 days after application, and to 0.5 %–0.9 % at 68 days after application. Bromobutide was detected on 76 days after application (paddy field 3) and at 104 days after application (paddy fields 1 and 2), although it was not detected at 83 days of application (paddy field 3) or at 125 days after application (paddy fields 1 and 2). Amounts of thiobencarb and mefenacet in paddy soil were reported for 21 days after application, respectively. The amounts reached maximum levels (about 20 % of the applied amounts) at 3 and 4 days after application, respectively (Amano et al. 2001). We previously investigated variations in the concentrations of a fungicide, phthalide, in two paddy field soils at 137 days after application (Iwashita et al. 2008). Phthalide was detected at maximum levels within 24 h after application, and was observed for 112 days after application in both paddy fields. The behavior of bromobutide was more similar to that of phthalide than that of either thiobencarb or mefenacet when measured 20 days after application. However, bromobutide had decreased remarkably compared to phthalide, as described later. These different behaviors are attributable mostly to differences in properties of the pesticides and differences in the paddy field conditions.

Maximum concentrations of bromobutide-debromo were observed at 5–7 days after application (Morohashi et al. 2012). The bromobutide-debromo amounts in the paddy soils were 3.7–5.0 % of the applied amounts at maximum. They subsequently decreased to 0.4–0.7 % by 68 days after application. Bromobutide-debromo was detected on 125 days after application, although it was not detected on 140 days after application in any of the three paddy fields. The distinct variations in bromobutide-debromo concentrations were attributable predominantly to the degradation of bromobutide to bromobutide-debromo in paddy fields.

Figure 2 presents variation in the mean molar compositions of bromobutide and bromobutide-debromo in paddy soils. The bromobutide composition was greater than 90 % within 6 days of application. Then the composition decreased to 60 % by 33 days after application, to 20 % by 90 days after application, and to less than 5 % by 125 days after application. The decline was caused by bromobutide degradation to bromobutide-debromo and other metabolites in paddy fields via metabolism (Isobe et al. 1984) and a photochemical reaction (Takahashi et al. 1985).

The decrease of the bromobutide amounts (mmol) in the soil is shown in Fig. 3. The decrease can be interpreted using first-order reaction kinetics (Qin et al. 2004; Iwashita et al. 2008) as Eq. (1):

$$\ln A = kt + C \tag{1}$$

where A signifies the amount of bromobutide in the paddy soil (mmol), k denotes the decreasing rate (/d), t is the elapsed time after application (d), and C represents a constant. We previously reported that the natural logarithm of the bromobutide amount in paddy soils 1–3 significantly correlated ($p < 0.01$) with the elapsed time within 1–18 days of application (Morohashi et al. 2012). Therefore, we evaluated the relation between the natural logarithm of the bromobutide amount during 18–104 days after application and the elapsed time. The calculated k and C values and the correlation coefficients (r) are presented in Table 1. The decreasing plots of paddy field soils were fitted significantly to the first-order reaction ($p < 0.01$),

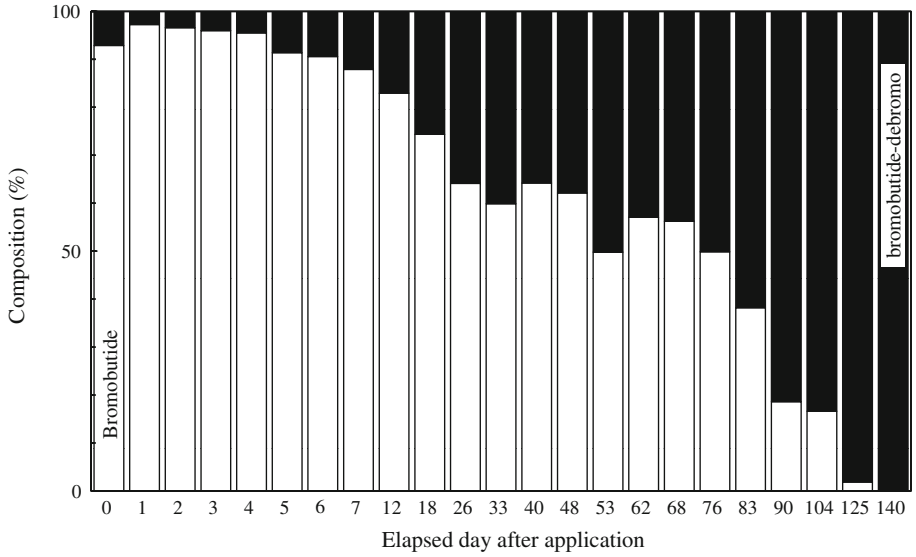

Fig. 2 Mean compositions of bromobutide and bromobutide-debromo in paddy soil

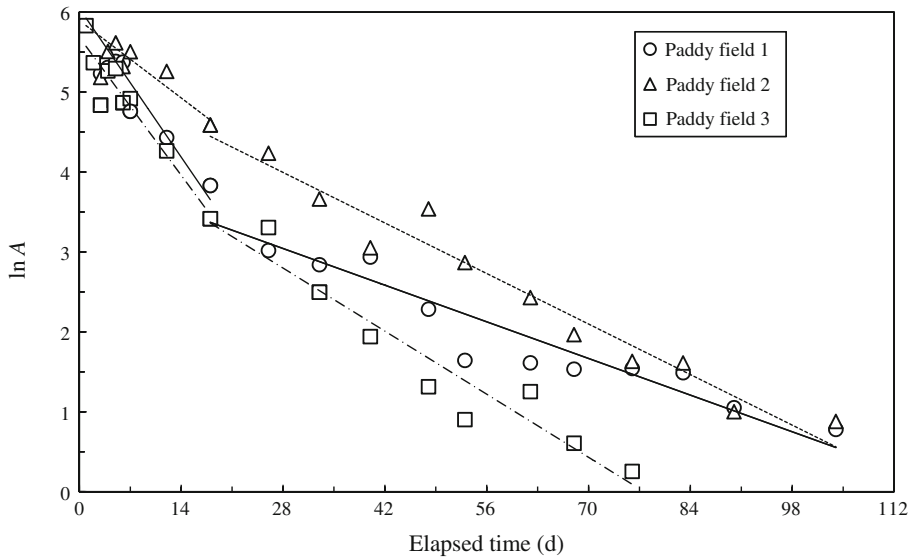

Fig. 3 Natural logarithm of bromobutide amounts (mmol) in paddy soil versus elapsed time with regression lines by the least-squares method

Table 1 Kinetic parameters of the first-order decrease the model estimated for bromobutide in paddy soils during the 18–104 days after application

Paddy field	k (/d)	C	n	r	p	$t_{1/2\ S}$ (d)
1	−0.0327	3.960	12	−0.950	< 0.01	21
2	−0.0452	5.260	12	−0.980	< 0.01	15
3	−0.0564	4.376	9	−0.965	< 0.01	12
			Mean ± standard deviation			16 ± 4.5

given as Eq. (1). The regression lines inferred using the least-squares method are also presented in Fig. 3. Regarding bromobutide-debromo, no significant correlation was found between the natural logarithm of the bromobutide-debromo amount in paddy soil and the elapsed time. Therefore, we infer that the metabolite concentrations were affected by the decrease of the compound itself and the increase according to the decomposition of bromobutide.

The half-life of bromobutide in the soil ($t_{1/2\ S}$) during 18–104 days after application was estimated using the established relations (Table 1). We previously reported estimates of $t_{1/2\ S}$ values within 1–18 days of application as 6.9 ± 2.6 days (Morohashi et al. 2012), whereas the

laboratory experimental half-lives of bromobutide were reported as 25–54 days after application (Food Safety Commission 2008) and 31–64 days after application (Kanazawa 1996). The marked decrease of bromobutide within 1–18 days of application was attributable to the distribution of bromobutide between water and soil because paddy water was present during the period. Moreover, the decrease was attributable to the runoff of bromobutide from the paddy field according to the drainage of paddy water within 4 days of application (Morohashi et al. 2012). However, the $t_{1/2\ S}$ value during the 18–104 days of application was estimated as 16 ± 4.5 days (Table 1). The obtained value was 2.4 times as large as that obtained within 1–18 days of application. This difference reflects the absence of the paddy water layer and the lack of a runoff event during the period. Although the value in this study was smaller than the reported half-life value of 25–64 days (Food Safety Commission 2008; Kanazawa1996), the difference in the half-life values is attributable to the properties of the investigated conditions as well as those of the soils used. The half-lives of herbicides based on the paddy field experiments were reported as 12 and 21 days for mefenacet, 4.6 and 11 days for pyrazosulfuron-methyl, and 30 days for dymron (Ishii et al. 2004). The $t_{1/2\ S}$ values of bromobutide in this study were the same as those of mefenacet.

The half-lives might depend on soil matrix characteristics including the organic matter content, the clay content, and water content. Qin et al. (2004) reported that the half-life of a nematicide, fosthiazate, was correlated with the organic matter content and clay content of soil. In contrast, the half-life of phthalide in the paddy soil with the higher organic content was shorter than that in the paddy soil with lower organic content (Iwashita et al. 2008). The $t_{1/2\ S}$ values of bromobutide (21 days in paddy soil 1, 15 days in paddy soil 2) with organic contents of 7.21 % ± 1.34 % and 7.90 % ± 2.03 %, respectively, were larger than those (12 days) with contents of 4.31 % ± 0.86 % in paddy soil 3; the $t_{1/2\ S}$ values in paddy soils 1 and 2 with silt and clay contents of 36.5 % and 44.8 %, respectively, were larger than that with the content of 12.9 % in paddy soil 3. These results for bromobutide agree with those of fosthiazate. García-Valcárcel and Tadeo (1999) reported that the half-lives of two herbicides, hexazinone and simazine, decreased with increased soil moisture content. In contrast, the half-life of phthalide in paddy soil with higher water content was longer than that in the paddy soil with the lower water content (Iwashita et al. 2008). The $t_{1/2\ S}$ values of bromobutide (21 days in paddy soil 1, 15 days in paddy soil 2) with respective water contents of 55.0 % ± 6. 36 % and 57.2 % ± 5.46 %, were longer than the $t_{1/2\ S}$ value of bromobutide (12 days) with the content of 44.7 % ± 6.68 % in paddy soil 3. This result for bromobutide agreed with that for phthalide. These different tendencies between the half-lives and soil properties are attributable to the properties of the investigated pesticides and the investigated conditions.

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