Behavior of isoprothiolane and fipronil in paddy water, soil, and rice plants after nursery-box or submerged applications

Keiya Inao,*, Takashi Iwafune,§ Takeshi Horio# and Ikuko Kitayama*

National Institute for Agro-Environmental Sciences, Kannondai, Tsukuba, Ibaraki 305–8604, Japan
*Present affiliation: Institute for Agro-Environmental Sciences, NARO
§Present address: Food Safety Commission Secretariat, Akasaka, Minato-ku, Tokyo 107–6122, Japan

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We investigated the behavior of isoprothiolane and fipronil after nursery-box application and that of isoprothiolane after submerged application in an experimental paddy field. The concentrations of the pesticides and their metabolites were monitored in paddy water, soil, and rice plants. The distribution profile for isoprothiolane mass in the field differed greatly between the nursery-box and submerged applications. The nursery-box-applied pesticides were mostly distributed in soil near the transplanted rice seedlings (root zone), versus little distribution in paddy water and rice plants (<1.1 and <0.3% of the applied mass, respectively). The residual levels in rice plants were similar to those in the root-zone soil. To estimate the soil pesticide mass, we defined a key parameter: the ratio of the root-zone area to the total area of the paddy field estimated to be 0.1 to 0.15. This parameter is important when evaluating the concentrations of nursery-box-applied pesticides in soil and rice plants.

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Keywords: environmental fate of pesticides, paddy conditions, nursery box, submerged application, root zone.

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Introduction

Nursery-box applications of fungicides and insecticides, in which pesticides are applied to the plants while they are still growing in the nursery, have become popular during rice cultivation in Japan. These pesticides have systemic and residual activity, and they are widely used to protect against diseases (e.g., rice blast) and insect pests during the early and middle growth stages of paddy rice. However, pesticides used in paddy fields can flow into rivers via drainage canals due to spray drift, surface runoff, or lateral seepage loss from paddy fields. Many rice pesticides and their metabolites have been detected at high frequencies and concentrations in river water during the cultivation season.18,19) The metabolites of some pesticides (e.g., organophosphorus insecticides) may be more toxic to aquatic organisms than the parent compound. Clear peaks of some nursery-box-applied pesticides and their main metabolites have been observed annually at rice- transplanting time.7,9) Thus, for assessing and managing the ecological risks of the pesticides in river systems, it is important to evaluate the behavior of rice pesticides, including nursery-box-applied pesticides, and their metabolites in paddy fields.

Nursery-box application of pesticides has been used to reduce pesticide runoff and drift from paddy fields. However, field studies to clarify the difference in pesticide behavior in paddy fields between nursery-box and submerged applications (in which pesticides are applied in the flooded paddy) are limited.10,11) Several studies of the behavior of nursery-box-applied pesticides in paddy fields have been reported.12–16) In these previous reports, pesticide concentration changes were examined in paddy soil and rice plants12,13) or in the paddy water and soil.14,16) There was a large difference in the residual level of the pesticides in surface soil samples between the root-zone and inter-plant areas.16 However, the mass distribution of the nursery-box-applied pesticides in the paddy water, soil, and rice plants has not been investigated. Recently, high-density seedlings in nursery boxes and sparse planting, which are new rice-cultivation methods, have become popular because they have a low cost and save labor. There are concerns about less effective plant protections under these methods as a result of the decreasing application dose of the nursery-box-applied pesticides to paddy fields.17 Therefore, it is important to evaluate the mass distribution of the nursery-box-applied pesticides in paddy fields, including rice plants, for optimal protection against diseases and insect pests under various rice-cultivation methods.

* To whom correspondence should be addressed.
E-mail: keinao@affrc.go.jp

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The objective of this study was to investigate the simultaneous behavior of the rice pesticides (isoprothiolane and fipronil), including their major metabolites, in paddy water, soil, and rice plants and to compare the behavior of isoprothiolane between nursery-box and submerged applications. We also discuss how the key factors that determine mass distribution of the pesticides in the root-zone and inter-plant areas could be used to improve the previously developed PADDY model\cite{18-20} for simulating the behavior of nursery-box-applied pesticides in paddy fields.\cite{21}

**Materials and Methods**

1. **Target pesticides**
   In this study, the fungicide isoprothiolane (di-isopropyl 1,3-dithiolan-2-ylidenemalonate) and the insecticide fipronil \([((\pm)-5\text{-amino}-1\text{-}(2,6\text{-dichloro}\text{-}\alpha,\alpha,\alpha\text{-trifluoro}\text{-p-toly})\text{-}4\text{-trifluoromethylsulfinyl}ypyrazole-3\text{-carbonitrile})]\) were monitored in an experimental paddy field in 2008 and 2009. These pesticides are widely used for nursery-box applications in Japan.\cite{22} Isoprothiolane is also used for submerged or foliage applications to protect against rice blast disease before heading or harvesting. In addition, isoprothiolane sulfoxide \([\text{dipropan-2-yl 2-(1-oxo-1,3-dithiolan-2-ylidene)propanedioate}]\) and fipronil sulfone \([5\text{-amino}-1\text{-}[2,6\text{-dichloro-4-(trifluoromethyl)phenyl}]\text{-}4\text{-}(trifluoromethylsulfonyl)pyrazole-3\text{-carbonitrile}]\) were monitored in 2009. Isoprothiolane sulfoxide is one of the metabolites formed in rice plants and in soil under flooded conditions.\cite{23} Fipronil sulfone, a metabolite found in both paddy water and soil,\cite{16} has a toxicity similar to or higher than that of the parent fipronil to aquatic invertebrates.\cite{24}

2. **Experimental field conditions**
   A field experiment was conducted in a paddy plot (10 m × 50 m) at the National Institute for Agro-Environmental Sciences in Tsukuba, Ibaraki, Japan from May 12 to Aug. 12, 2008 and from May 13 to Aug. 19, 2009 (Fig. 1). The soil texture in the plot is light clay (clay 47%, silt 19%, sand 34%) with an organic carbon content of 1.8% w/w. The plot was surrounded by a concrete bund. Irrigation water was supplied from five irrigation pipes installed in the plot. A drainage gate was established at a height of 6–7 cm above the soil surface. During the study period, the water depth was kept at around 5 cm by means of intermittent irrigation management to avoid excess water. Two aluminum walkways (3 m × 0.3 m) were installed just after the transplanting to aid sampling in the central part of the plot in 2009.

   The rice seedlings (‘Koshihikari’) were grown in nursery boxes (30 cm × 60 cm) filled with ca. 3 cm of bed soil until about the three-leaf stage. They were then transplanted into the plot using a rice-planting machine at a spacing of 16 cm × 30 cm on May 12, 2008 and May 13, 2009 (Fig. 2). The transplanting depths in 2008 and 2009 were ca. 2.5 and 3.5 cm, respectively. The number of rows was 33 in both years, for a total of ca. 9300 hills per plot in both 2008 and 2009. Midsummer drainage was carried out from June 19 to 26, 2008 and from June 30 to July 13, 2009. After the midsummer drainage, the plot was reflooded from June 27 to Aug. 12, 2008 and from July 14 to Aug. 19, 2009.

3. **Pesticide application**
   We used both pesticide application methods in the same plot, but at different times: the first application was in the nursery boxes before transplanting, and the second was when the

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Fig. 1. Layout of the experimental paddy plot.

Fig. 2. Time schedule of the field experiments. (○) Sampling of paddy water; (▲) sampling of paddy water and soil; (●) sampling of paddy water, soil, and rice plants. All numbers on the timeline represent days after transplanting (DAT). Paddy water samples on the day of both pesticide applications were collected before and 3–6 hr after the applications in 2008 and 2009. Soil and rice plants were also sampled before the applications on May 13 and July 15, 2009.
paddy was reflooded after the midsummer drainage period. In the nursery-box application, a granular formulation, Fuji-One Prince (1.0% w/w fipronil and 12.0% w/w isoprothiolane; Nihon Nohyaku Co., Ltd., Tokyo, Japan), was uniformly applied to the surface of the nursery boxes described in the preceding section at the recommended rate of 50 g per box on May 12, 2008 and May 13, 2009 (Fig. 2). The pesticide-treated rice seedlings were transplanted just after the pesticide application. The actual application rates to the plot were 8.7 kg/ha (87 g a.i./ha fipronil and 1044 g a.i./ha isoprothiolane) in 2008 and 8.5 kg/ha (85 g a.i./ha fipronil and 1020 g a.i./ha isoprothiolane) in 2009. In the submerged application, the granular fungicide Fuji-One (12.0% w/w isoprothiolane) was uniformly applied to the same plot at the recommended rate of 30 kg/ha (3600 g a.i./ha isoprothiolane) using a manual granule applicator under flooded conditions on July 1, 2008 and July 15, 2009 (Fig. 2).

4. Observation of hydrological conditions
Paddy water depth, irrigation amount, and vertical percolation were measured during the experimental periods. Water depth was measured at five locations near the water sampling spots in the plot (Fig. 1). The volume of irrigation water was estimated by measuring the difference in water level before and after irrigation and multiplying this difference by the area of the plot. The daily vertical percolation was estimated by measuring the daily fall in water level inside two stainless steel cylinders (30 cm i.d. × 50 cm length) with lids buried to a depth of ca. 30 cm in the soil (Fig. 1). Reference crop evapotranspiration (ET₀) was obtained from an agro-meteorological database coupled with a crop model (MeteoCrop DB)²⁵,²⁶ and was calculated using the FAO Penman-Monteith method²⁷ based on meteorological data observed in Tsukuba (Tateno) at a distance of about 3 km from the study site.²⁸ The crop evapotranspiration (ET₅₀) was calculated by multiplying ET₀ values by the crop coefficient (Kc) of 1.1 during the initial growth stage of paddy rice and 1.2 during the midseason stage.²⁹ Daily percolation and discharge of paddy water were calculated from a water-balance equation³⁰ using the measured water-balance components.

5. Sampling
Figure 2 shows the field management and the sampling schedule for the paddy water, soil, and rice plants. Paddy water samples on the day of both pesticide applications were collected before and 3–6 hr after the applications in 2008 and 2009. Soil and rice plants were also sampled before the applications on May 13 and July 15, 2009. All of the samples were extracted on the sampling day using the analytical method described in Section 6. Paddy water samples (ca. 200 mL) were collected at a depth of 1 to 2 cm below the water surface at five locations in the plot in both 2008 and 2009 (Fig. 1) using a water sampler composed of a manual suction pump and a 1000 mL brown glass bottle connected to a Tellon tube. A composite water sample from the five locations (ca. 1000 mL) was used for the pesticide analysis. The pH of the composite water samples was measured using a pH sensor (pHTester10, AS ONE, Osaka, Japan). Soil samples were collected in 2009 using disposable polypropylene syringes (2.8 cm i.d., 50 mL volume; Terumo Corporation, Tokyo, Japan) comprised a plunger and a cylindrical tube with its head removed. The syringe was driven into the soil to a depth of about 5 cm, and the top 3 cm of the soil (ca. 30 g) was sampled. The two soil samples were randomly taken at each of five locations between the rice plants near the water sampling spots (i.e., inter-plant soil sample) shown in Fig. 1. A composite soil sample from the five locations (ca. 300 g) was used for pesticide analysis. In addition, two soil samples from both sides of the rice plants (foot-zone soil sample) were taken using the same approach during the early experimental period (between 0 and 42 days after transplanting [DAT]) in 2009 (Fig. 1). To obtain the pesticide concentrations in rice plants, the whole shoot of the rice plant above the water surface was randomly sampled at 10 locations in the plot in 2009. A composite sample was then used for analysis.

6. Pesticide analysis
Analyses of the pesticide contents in the water, soil, and plant samples were carried out using solid-phase extraction (SPE) followed by high-performance liquid chromatography linked with tandem mass spectrometry (LC-MS/MS; Waters, Milford, MA, USA). The procedures are described here briefly. A 20 mL aliquot of the water samples, in which 1 g of sodium chloride was dissolved for salting out, was loaded onto an SPE diatomite column (CE 1020; Agilent Technologies Inc., Santa Clara, CA, USA). The SPE column was eluted with 100 mL of ethyl acetate. The elute was evaporated to ca. 0.5 mL with a rotary evaporator (R-210, Buchi, Flawil, Switzerland) and then dried under a nitrogen stream. The residue was dissolved in 2 mL of an acetonitrile–water mixture (1 : 1 v/v). A 20 g aliquot of the wet soil sample was weighed into a 200 mL conical flask with a stopper, and 5 mL of water and 100 mL of acetone were added to the sample. The sample mixture was shaken vigorously for 30 min on a shaker (SR-2s, Taitec, Koshigaya, Japan) and then suction-filtered through a glass fiber filter (60 mm in diameter, GF-B filter, Whatman, Maidstone, UK). The solid residue on the filter was washed with 50 mL of acetone. All of the extracts were combined and then evaporated to approximately 10 mL with the rotary evaporator. After the evaporated extract was dissolved, as described earlier in this section for salting out, the eluate was evaporated with the rotary evaporator to ca. 0.5 mL and dried under a nitrogen stream; the residue was then reconstituted in 2 mL of acetone. The resulting solution was loaded onto a graphitized carbon black SPE cartridge (0.25 g, ENVI-Carb SPE, Supelco, Bellefonte, PA, USA) that had been preconditioned with 10 mL of acetone. The SPE cartridge was eluted with 20 mL of acetone. The eluate was evaporated to ca. 0.5 mL with the rotary evaporator and then dried under a nitrogen stream. The residue was reconstituted in 5 mL of an acetonitrile–water mixture (1 : 1 v/v), and the resulting solution was filtered using a syringe-driven polytetrafluoroethylene membrane filter unit (0.45 µm, GL Sciences Inc., Tokyo, Japan).
Plant samples (5 g fresh weight) were weighed into a blender cup and homogenized in a mixture of 10 mL of water and 100 mL of acetone. The sample mixture was blended for 10 min at high speed with a blender (CM-100, AS ONE, Osaka, Japan) and then suction-filtered through a GF-B glass fiber filter. The solid residue on the filter was washed with 50 mL of acetone. This extraction procedure was repeated twice. The collected extract was evaporated to approximately 10 mL with a rotary evaporator, and cleanup of the extract with an SPE diatomite column was carried out as described above for the water samples. The eluate was evaporated to ca. 0.5 mL with the rotary evaporator and dried under a nitrogen stream; the residue was then reconstituted in 2 mL of a toluene–acetonitrile mixture (1:3 v/v). The resulting solution was loaded onto a graphitized carbon black and aminopropyl silica gel SPE cartridge (0.5 g/0.5 g, ENVI-Carb/LC-NH₂ SPE) that had been preconditioned with 10 mL of a toluene–acetonitrile mixture (1:3 v/v). The SPE cartridge was then eluted with 20 mL of a toluene–acetonitrile mixture (1:3 v/v). The eluate was evaporated using the rotary evaporator and then dried under a nitrogen stream. The residue was reconstituted in 10 mL of an acetonitrile–water mixture (1:1 v/v), and the resulting solution was filtered with a syringe-driven polytetrafluoroethylene membrane filter unit.

All samples were analyzed by means of LC-MS/MS using the multiple-reaction monitoring mode under the analytical conditions shown in Supplemental Tables S1 and S2. A recovery test of the compounds was conducted with triplicate pure water samples (20 mL) spiked using an analytical-grade standard (Wako Pure Chemicals Industries, Osaka, Japan, or Hayashi Pure Chemical Industries, Osaka, Japan) at 5 µg/L, with four replicates of the field soil samples (20 g) spiked at 2 µg/kg and four replicates of the plant samples (5 g) spiked at 10 µg/kg. Recoveries from the water, soil, and plant samples ranged from 84.1 to 108.2%, from 94.7 to 102.2%, and from 74.9 to 101.5%, respectively, but the recovery of fipronil sulfone from plant samples was poor (4.3%). The coefficients of variation were less than 5.0% for all compounds except fipronil sulfone from the soil and plant samples, as shown in Supplemental Table S3. The limits of quantification for the compounds in the water, soil, and plant samples were 0.01–0.05 µg/L, 0.025–0.125 µg/kg, and 0.2–1 µg/kg, respectively.

Results and Discussion

1. Hydrological conditions

The pH of the paddy water samples during the experimental periods in 2008 and 2009 ranged from 6.5 to 8.0 and from 6.2 to 7.5, respectively. The measured water depth ranged from 3 to 7 cm, and the measured vertical percolation rates (homogeneous water flow) were about 0.1 cm/day during the experimental period in both years. The water-balance components in the experimental plot are shown in Fig. 3 and Table 1. The calculated average percolation, including both vertical and horizontal components, under the conditions with no cracks before the midsummer drainage in 2008 and 2009 was 0.3 cm/day (Table 1). Therefore, the horizontal percolation rate was estimated to be 0.2 cm/day in both years. The average percolation after the midsummer drainage was larger than that before the drainage in both years (Table 1). This was probably due to preferential water flow through cracks formed on the soil surface during the midsummer drainage. The average percolation after the midsummer drainage in 2009 (0.8 cm/day) was larger than that in 2008.

![Fig. 3](image-url) Changes in the daily water-balance components in the experimental paddy plot in 2008 and 2009. Horizontal dashed lines represent the height of the drainage gate above the soil surface during the flooded periods. Table 1 summarizes these data for the two study periods in each year. ET<sub>C</sub> represents the estimated crop evapotranspiration.
(0.4 cm/day) (Table 1). This was caused by faster preferential flow as a result of the development of longer and deeper cracks in 2009 because of the longer midsummer drainage in 2009 (13 days, versus 7 days in 2008). The total discharge in 2009 was larger than that in 2008 during both periods because of the larger total rainfall in 2009 during the experimental period (Table 1). The largest discharge event (2.5 cm) occurred at 8 DAT in 2008 and resulted from high rainfall of 6.2 cm/day (Fig. 3). However, only two small discharge events (<1 cm/day) occurred subsequently in 2008. In contrast, five and one major discharge events (>1 cm/day) occurred before and after the midsummer drainage in 2009, respectively (Fig. 3).

2. Behavior of isoprothiolane and fipronil in the paddy field

2.1. Paddy water

The concentrations of nursery-box-applied isoprothiolane in paddy water increased gradually after transplanting and reached a maximum of 74.3 µg/L at 7 DAT in 2008, versus 31.0 µg/L at 3 DAT in 2009 (Fig. 4a). Subsequently, the concentrations decreased gradually. The concentration of fipronil showed a pattern different from that of isoprothiolane (Fig. 4b). In 2008, the fipronil concentration increased steadily until 28 DAT; in 2009, the concentration increased until 7 DAT, remained roughly constant until 21 DAT, and then decreased slowly. The maximum concentrations of fipronil were 1.98 µg/L at 28 DAT in 2008 and 0.55 µg/L at 21 DAT in 2009.

During the later experimental period (July 1 to Aug. 12, 2008 and July 15 to Aug. 19, 2009), the isoprothiolane concentration increased dramatically immediately after submerged application and reached a maximum of 3200 µg/L at 2 days after application in 2008 and 3680 µg/L at 1 day after application in 2009 (Fig. 4a). The concentrations then decreased rapidly to 2.8 and 17.6 µg/L at 14 days after application in 2008 and 2009, respectively. These results were comparable to those of Kanauchi et al., who found that the isoprothiolane concentration in paddy water peaked between 3000 and 7000 µg/L within 1 to 3 days after submerged application (4800 g a.i./ha) and then decreased rapidly. The dissipation patterns of the submerged isoprothiolane were similar to those of other granular rice herbicides under sub-

Table 1. Summary of the water-balance components in the experimental paddy plot during two periods (before and after the mid-summer drainage). Figure 3 shows the time course of these data during the study period.

|                  | 2008        | 2009        |
|------------------|-------------|-------------|
|                  | May 12–June 9 | July 1–Aug. 12 | May 13–June 24 | July 15–Aug. 19 |
| Inputs (cm)      | Total<sup>a</sup> | Ave.<sup>b</sup> | Total<sup>a</sup> | Ave.<sup>b</sup> | Total<sup>a</sup> | Ave.<sup>b</sup> |
| Irrigation       | 2.9         | 0.1         | 24.6         | 0.6         | 8.4         | 0.2         |
| Precipitation    | 17.6        | 0.6         | 5.3          | 0.1         | 24.2        | 0.6         |
| Total            | 20.5        | 0.7         | 29.9         | 0.7         | 32.6        | 0.8         |
| Outputs (cm)     |             |             |              |             |             |             |
| Discharge        | 3.7         | 0.1         | 0.0          | 0.0         | 9.9         | 0.2         |
| Percolation<sup>c</sup> | 9.5        | 0.3         | 15.1         | 0.4         | 12.2        | 0.3         |
| Evapotranspiration<sup>d</sup> | 7.0         | 0.3         | 15.5         | 0.4         | 10.6        | 0.3         |
| Total            | 20.2        | 0.7         | 30.6         | 0.7         | 32.8        | 0.8         |

<sup>a</sup> Total water depth during each experimental period (cm).
<sup>b</sup> Daily average water depth (cm/day).
<sup>c</sup> Sum of vertical and horizontal percolation. During the period after the mid-summer drainage (July 1 to Aug. 12, 2008 and July 15 to Aug. 19, 2009), preferential water flow was contained in the values.
<sup>d</sup> Crop evapotranspiration (ET<sub>C</sub>) calculated by multiplying reference crop evapotranspiration (ET<sub>0</sub>) values by the crop coefficient (K<sub>C</sub>).
merged applications. In contrast, fipronil during the later period remained at a low concentration of 0.06 \( \mu g/L \) until 92 DAT in 2008 (Fig. 4b). In 2009, fipronil was not detected in the paddy water during the later period.

Tsuyet et al. reported that the maximum concentrations of nursery-box-applied pesticides in paddy water were 10 to 30% of the levels after submerged application of the pesticides. In our study, the maximum isoprothiolane concentrations after the nursery-box applications in 2008 and 2009 were only 2.3% and 0.8%, respectively, of those after the submerged application. These results indicate that nursery-box applications are beneficial for reducing pesticide runoff from paddy fields because of their relatively low concentrations in paddy water, but the maximum concentrations of nursery-box-applied pesticides varied among the studies.

Several studies of the behavior of nursery-box-applied fipronil under paddy conditions have been reported. The reported concentrations in paddy water varied widely. Hayasaka et al. reported that the maximum concentration of fipronil used at the same application rate as in our study was ca. 1.0 \( \mu g/L \) within 1 day after treatment and then declined rapidly in experimental paddy mesocosms. Thuyet et al. reported that the maximum concentrations of fipronil using two nursery-box treatments (before transplanting and at the time of sowing) ranged from 0.9 to 2.5 \( \mu g/L \) at 1 day after transplanting. These are similar to the levels in the present study. In addition, they found that the variation in peak concentrations depended on the degree of direct dissolution of the granular pesticides dropped around the rice seedlings on the soil surface or buried in the paddy soil through the transplanting holes. These differences resulted from differences in nursery-box treatment methods, transplanting practices (planting depth), and soil preparation conditions (puddling and leveling of the soil).

In our study, the maximum concentrations of nursery-box-applied isoprothiolane and fipronil in 2009 were lower than those in 2008 during the early period, and clear peaks were not observed just after the transplanting. The findings of Thuyet et al. showed that the concentration of the pesticides in the paddy water varied widely. Hayasaka et al. reported that the maximum concentrations of nursery-box-applied pesticides in paddy water were 10 to 30% of those after the submerged application. The concentration change in the inter-plant soil samples (solid lines) was similar to that in the root-zone soil sample, but the maximum concentration of 559 \( \mu g/kg DW \) at 1 DAT was lower than that in the root-zone soil sample. The peak concentrations of fipronil in the root-zone and inter-plant soil samples (dashed and solid lines, respectively, in Fig. 5b) were 573 \( \mu g/kg DW \) at 7 days and 8.9 \( \mu g/kg DW \) at 1 day, respectively. The fipronil concentrations in the root-zone soil were 20 to 300 times those in the inter-plant soil during the early experimental period. These results are similar to those of Thuyet et al., who found that fipronil persisted mainly in the soil near the transplanted rice seedlings, with fipronil concentrations in the top 5 cm of the root-zone soil about 10 times those in the inter-plant soil. The larger ratios in the present study probably resulted from the different soil sampling methods (sampling location and depth). An unexpected increase of the concentrations in the inter-plant soil sample was observed at 42 DAT (solid lines in Fig. 5). This was probably due to contamination of the samples with high concentrations of the pesticides during the soil sampling.

During the later period (July 15 to Aug. 19, 2009), the isoprothiolane concentration in the inter-plant soil increased immediately after the submerged application and gradually reached a maximum of 8840 \( \mu g/kg DW \) at 7 days after application; then
the concentration decreased gradually (Fig. 5a). This pattern was similar to that in previous submerged applications of granular rice herbicides.\textsuperscript{18,19} Isoprothiolane has low to moderate mobility in soil, with a soil adsorption constant ($K_{oc}$) of 196 to 2300.\textsuperscript{23} Uchida \textit{et al.}\textsuperscript{32} reported that >80% of the applied isoprothiolane was distributed in the top 2 cm of the soil by 15 days after application under conditions with no vertical percolation, and they found that the leaching loss of isoprothiolane increased with the increasing vertical percolation. In our study, most of the isoprothiolane after the submerged application was probably distributed in the upper soil because the daily vertical percolation rate (except for preferential water flow) was very low (ca. 0.1 cm/day) during the experimental period. The nursery-box-applied fipronil was detected at 2.0 µg/kg DW at 84 DAT (Fig. 5b). This result is comparable to that of Hayasaki \textit{et al.}\textsuperscript{31} who found that fipronil remained in the soil at 0.5 to 3.0 µg/kg DW at the end of the rice cultivation period.

2.3. Rice plants

The concentrations (fresh weight basis, FW) of the nursery-box-applied isoprothiolane in the shoots of rice plants reached a maximum of 13,000 µg/kg FW at 7 DAT and then declined to 191 µg/kg FW at 42 DAT (Fig. 6a). The concentration trends for fipronil were similar to those for isoprothiolane. The peak concentrations of fipronil were 678 µg/kg FW at 7 DAT, and they declined steadily thereafter (Fig. 6b). The residual levels of the two pesticides in the rice plants were similar to those in the root-zone soil (14,000 µg/kg DW for isoprothiolane and 573 µg/kg DW for fipronil at 7 DAT). The ratios of the concentration in rice plants to that in the root-zone soil were 0.9 for isoprothiolane and 1.3 for fipronil at 7 DAT. This result indicates that the nursery-box-applied pesticides in the root-zone soil were taken up efficiently by the rice roots and were transported into the aboveground tissues of the rice plants.

In contrast, the isoprothiolane concentration increased gradually after the submerged application, and the residual level during the first two weeks (224 to 510 µg/kg FW) was much lower than that (12,400 to 13,000 µg/kg FW) under the nursery-box application (Fig. 6a). Kanauchi \textit{et al.}\textsuperscript{30} reported that the concentration of isoprothiolane in rice shoots reached a maximum of 4000 to 7000 µg/kg FW within 3 to 11 days after a submerged application. In our study, the maximum concentration of 511 µg/kg FW was about 10% of these previously reported concentrations. As mentioned above, the vertical percolation rate (except for preferential water flow) was very low during our experimental period, so the concentrations of isoprothiolane in the root-zone soil to a depth of around 10 cm, corresponding to the rice rhizosphere at the time of the submerged application, may have been lower than those in the top 3 cm of the soil (3080 to 8840 µg/kg DW). Consequently, the concentration of isoprothiolane in the rice plants would be lower than the previously reported values.\textsuperscript{30}

3. Behavior of isoprothiolane sulfoxide and fipronil sulfone in the paddy field

Isoprothiolane sulfoxide and fipronil sulfone in the inter-plant soil samples were detected at 2.1 to 9.3 µg/kg DW and 6.0 to 8.8 µg/kg DW, respectively, from 0 to 28 DAT in 2009 (Fig. 5). However, they were also detected at similar levels (5.3 µg/kg DW for isoprothiolane sulfoxide and 5.0 µg/kg DW for fipronil sulfone) in the samples just before transplanting. This was due to the presence of residue from the use of their parent compounds in 2008.

The concentration changes of isoprothiolane sulfoxide in the paddy plot were similar to those of isoprothiolane, but the residue levels were remarkably lower (Figs. 4a, 5a, 6a). The maximum concentrations of isoprothiolane sulfoxide in the water, soil, and rice plants after the nursery-box application were 1.97 µg/L, 47.6 µg/kg DW (root-zone soil sample), and 149 µg/kg FW, respectively, and the corresponding values under submerged application were 70.2 µg/L, 20.8 µg/kg DW, and 47.2 µg/kg FW, respectively. The concentration ratios of isoprothiolane sulfoxide to the parent compound in paddy water, soil, and rice plants were 0.01–0.19, 0.002–0.019, and 0.01–0.11, respectively. This is comparable to a previous result,\textsuperscript{33} in which the maximum contents of isoprothiolane sulfoxide in flooded soil and in rice plants were 0.9 and <10% of the treated isoprothiolane, respectively.

Fipronil sulfone in the paddy water was detected at low concentrations (<0.05–0.06 µg/L; Fig. 4b). Thuyet \textit{et al.}\textsuperscript{33} found that fipronil sulfone is a minor photodegradation product in natural water and that the detected mass was 5.2% of the applied fipronil 79 hr after application. Thuyet \textit{et al.}\textsuperscript{16} also reported that the maximum concentrations of fipronil sulfone in two nursery-box treatments were 0.5 to 0.9 µg/L at 3 DAT. Therefore, our results agree with these previous results. Fipronil sulfone persisted in the root-zone soil at almost the same level (15.4–28.0 µg/kg DW) until 28 DAT (Fig. 5b). Similar results were reported by Thuyet \textit{et al.},\textsuperscript{16} who found that fipronil sulfone remained sta-
ble until the end of their experimental period. In the rice plant samples, fipronil sulfone was also found at a maximum of a few dozen µg/kg FW (data not shown); we have not discussed this result because of the low recovery of fipronil sulfone (4.3%) from the plant samples. In a subsequent study of the recovery test (data not shown), good recovery of fipronil sulfone from rice plant samples (92.4%) was obtained using the same extraction method as in the present study, but under dark conditions.

4. Mass distribution of pesticides in the paddy field

4.1. Nursery-box-applied pesticides

The mass distributions of isoprothiolane, fipronil, and their metabolites in the paddy plot under the nursery-box application were calculated from the measured concentrations in the paddy water, soil, and rice plants in 2009. The masses of the metabolites were converted into the corresponding weights of their parent compounds by multiplying the concentrations by 0.948 for isoprothiolane sulfoxide and by 0.965 for fipronil sulfone. The recovered mass of the two pesticides from both paddy water and rice plants was very low from 0 to 42 DAT (Supplemental Table S4). The maximum mass of isoprothiolane, including its metabolites, in the paddy water and plants at this time was 1.1% (3 DAT) and 0.3% (14 DAT) of the applied mass, respectively, and the corresponding values for fipronil were 0.3% (21 DAT) and 0.2% (42 DAT) of the applied mass, respectively. This result indicates that the nursery-box-applied pesticides were mainly distributed in the soil.

The recovered masses of isoprothiolane and fipronil, including their metabolites, calculated from the measured peak concentrations in the root-zone soil sample (7 DAT) were only 8.9 and 4.6% of the applied mass, respectively (Supplemental Table S4). However, the estimated mass was probably much lower than the actual residual mass because the transplanting depth of 3.5 cm was deeper than the sampling depth of 3 cm in this study, and the soil samples corrected from the root-zone area accounted for a small portion of the entire region that contained the nursery-box-applied granules. This result is comparable to that of Thuyet et al., who found that the mass of fipronil recovered from the soil sample collected to a depth of 5 cm in the root-zone area was only about 10% of the applied mass.

To estimate the initial mass distribution of the nursery-box-applied pesticides in the soil in 2009, we defined the ratio of the root-zone area to the total area of the paddy plot as \( f_{RZarea} \) (dimensionless) and made the following assumptions: (1) the pesticides are distributed up to a depth of 4.5 cm from the soil surface in the root-zone area, corresponding to the transplanting depth plus 1 cm, with the concentrations observed in the root-zone soil samples to a depth of 3 cm; and (2) in the inter-plant area, pesticides are distributed in the top 3 cm of the soil, and the distribution is based on the concentrations observed in the inter-plant soil samples. The masses of isoprothiolane and fipronil, including the corresponding metabolites, in the soil of the root-zone and inter-plant area were calculated from the concentrations at 7 DAT and the different values of \( f_{RZarea} \). The total amount of isoprothiolane in the soil (sum of the masses in the root-zone and inter-plant area) at \( f_{RZarea} = 0.1 \), 0.15, and 0.2 was estimated to be 66, 95, and 124% of the applied mass, respectively (Fig. 7a). This result indicates that the root-zone area accounted for 10–15% of the paddy plot in this study. However, the recovered mass of fipronil in the soil at \( f_{RZarea} = 0.1 \), 0.15, and 0.2 was only 33, 48, and 63% of the applied mass, respectively (Fig. 7b). The water solubility of fipronil, at 3.78 mg/L, is much lower than that of isoprothiolane (48.5 mg/L) and fipronil has a higher soil adsorption constant \( (K_{oc} = 548 \text{ to } 1720, \text{ average } = 1035) \) than isoprothiolane \( (K_{oc} = 196 \text{ to } 2300, \text{ average } = 759) \). Therefore, fipronil may be distributed in a smaller region near the rice roots because of its relatively low mobility in the soil as compared to isoprothiolane. Consequently, the fipronil concentrations observed in the root-zone soil samples did not adequately represent the actual concentrations in the main part of the soil that contained the fipronil.

4.2. Distribution of isoprothiolane after the submerged application

Table 2 shows the mass distribution of isoprothiolane, including its sulfoxide metabolite, in the paddy plot after the submerged application in 2009. These values were corrected by subtracting the residue mass just before pesticide application. Isoprothiolane was mainly distributed in the paddy water for 3 days after the submerged application (27.3 to 35.9% of the applied mass). The isoprothiolane mass was much larger than that after the nursery-box application in 2009 (1.1% of the applied mass at
the root-zone and inter-plant areas, as well as those in the rice concentrations of nursery-box-applied pesticides in the soil of area of the paddy plot. This parameter is important to assess the percentage of the initial applied mass (180 g a.i./500 m²) just before pesticide application from the gross residue mass at each time.

3 DAT). However, the isoprothiolane mass in the soil increased to 43.4% of the applied mass at 7 days after application, with the amount of isoprothiolane in the paddy water decreasing due to adsorption onto soil particles. The distribution patterns after the submerged isoprothiolane application were similar to those for the submerged application of granular rice herbicides in previous research. The maximum mass of isoprothiolane in rice plants was 0.6% of the applied mass at 35 days after application. This result was roughly equivalent to that after the nursery-box application in 2009 (a maximum of 0.3% of the applied mass at 14 DAT).

## Conclusion

The present results suggest that the distribution of pesticides in the experimental paddy field differed greatly between the nursery-box and submerged applications and that the masses of the nursery-box-applied pesticides were mostly distributed in the soil near the transplanted rice seedlings and were present only at low levels in the paddy water and rice plants. In addition, the residual levels in the rice plants were similar to those in the root-zone soil rather than to those in the inter-plant soil. To estimate the mass of pesticides in the soil, we defined a key parameter (ʃᵣᵣₐᵣ) that equaled the ratio of the root-zone area to the total area of the paddy plot. This parameter is important to assess the concentrations of nursery-box-applied pesticides in the soil of the root-zone and inter-plant areas, as well as those in the rice plants. These findings will enable us to improve the parameterization of the previously developed PADDY model.

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## References

1. S. Maru: Spec. Bull. Chiba Agric. Exp. Stn. 18, 1–62 (1991) (in Japanese).
2. A. Numabe, T. Inoue and S. Ebise: Jpn. Soc. Water Environ. 15, 662–671 (1992) (in Japanese).
3. K. Nakamura: Bull. Saitama Agric. Exp. Stn. 46, 61–69 (1993) (in Japanese).
4. H. Mitobe, T. Ibaraki, A. Tanabe, K. Kawata, M. Sakai and I. Kifune: J. Environ. Chem. 9, 311–320 (1999) (in Japanese).
5. M. Sudo, T. Kunimitsu and T. Okubo: Water Res. 36, 315–329 (2002).
6. S. Ishihara, M. Ishizaka, T. Horio, Y. Kobara and M. Ueki: J. Weed Sci. Tech. 51 (2006) (in Japanese).
7. T. Iwafune, K. Inao, T. Horio, N. Iwasaki, A. Yokoyama and T. Nagai: J. Pestic. Sci. 35, 114–123 (2010).
8. T. K. Phong, K. Yoshino, K. Hiramatsu, M. Harada and T. Inoue: Paddy Water Environ. 8, 361–369 (2010).
9. T. Iwafune, A. Yokoyama, T. Nagai and T. Horio: Environ. Toxicol. Chem. 30, 1834–1842 (2011).
10. K. Tsukiji: Iwate Agricultural Research Center Research Executive Summary, 2001, http://www.pref.iwate.jp/~hp2088/seika/h13/b55.pdf (Accessed 22 February, 2018) (in Japanese).
11. K. Tsukiji: Proceedings of the 18th Symposium on Environmental Science of Pesticides, 19–26 (2001) (in Japanese).
12. S. Asaka, K. Kawachi, S. Koyama and K. Emura: J. Pestic. Sci. 3, 305–310 (1978) (in Japanese).
13. S. Koyama, K. Emura and A. Kojima: J. Pestic. Sci. 8, 183–191 (1983) (in Japanese).
14. T. K. Phong, D. T. Nhung, T. Motobayashi, D. Q. Thuyet and H. Watanabe: Water Air Soil Pollut. 202, 3–12 (2009).
15. D. Q. Thuyet, H. Watanabe and T. Motobayashi: J. Pestic. Sci. 36, 9–15 (2011).
16. D. Q. Thuyet, H. Watanabe, T. Motobayashi and J. Ok: Agric. Ecosyst. Environ. 179, 69–77 (2013).
17. http://www.jppa.or.jp/symposium/data/S290914.pdf (Accessed 11 January, 2018) (in Japanese).
18. K. Inao and Y. Kitamura: Pestic. Sci. 55, 38–46 (1999).
19. K. Inao, Y. Ishii, Y. Kobara and Y. Kitamura: J. Pestic. Sci. 26, 229–235 (2001).
20. K. Inao, H. Mizutani, Y. Yogo and M. Ikekda: J. Pestic. Sci. 34, 273–282 (2009).
21. K. Inao, T. Iwafune and T. Horio: J. Pestic. Sci. 36, 219–226 (2011).
22. Japan Plant Protection Association (ed.): “Noyaku Yoran 2010,” Japan Plant Protection Association, Tokyo, 2010 (in Japanese).
23. http://www.fsc.go.jp/fsciis/evaluationDocument/show/kya20120521508 (Accessed 1 June, 2017) (in Japanese).
24. A. S. Gunasekara, T. Truong, K. S. Goh, F. Spurlock and R. S. Tjeerdema: J. Pestic. Sci. 32, 189–199 (2007).
25. http://meteocrop.dc.affrc.go.jp/real/ (Accessed 1 June, 2017) (in Japanese).
26. T. Kuwagata, M. Yoshimoto, Y. Ishigooka, T. Hasegawa, M. Utsumi, M. Nishimori, Y. Masaki and O. Saito: J. Agric. Meteorol. 67, 297–306 (2011).
27. R. G. Allen, L. S. Pereira, D. Raes and M. Smith: “Crop Evapotranspiration, Guidelines for Computing Crop Water Requirements,” FAO Irrigation and Drainage Paper 56, Food and Agriculture Organization of the United Nations, Rome, Italy, 293 pp., 1998.
28. http://www.data.jma.go.jp/obd/stats/etrn/index.php (Accessed 1
June, 2017) (in Japanese).
29) S. H. Vu, H. Watanabe and K. Takagi: Agric. Water Manage. 76, 195–210 (2005).
30) M. Kanauchi, M. Uchida and K. Tsuchiya: J. Pestic. Sci. 7, 377–383 (1982).
31) D. Hayasaka, T. Korenaga, K. Suzuki, F. Saito, F. Sánchez-Bayo and K. Goka: Ecotoxicol. Environ. Saf. 80, 355–362 (2012).
32) M. Uchida, M. Kanauchi and K. Hashimoto: J. Pestic. Sci. 5, 249–254 (1980).
33) D. Q. Thuyet, H. Watanabe, K. Yamazaki and K. Takagi: Bull. Environ. Contam. Toxicol. 86, 548–553 (2011).
34) http://www.fsc.go.jp/fsciis/evaluationDocument/show/kya20151013447 (Accessed 1 June, 2017) (in Japanese).