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Effect of pH on the degradation of imidacloprid and fipronil in paddy water

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(Received December 14, 2012; Accepted August 4, 2013)

This study investigated the degradation of imidacloprid and fipronil in paddy water at pH 7 and pH 10. Both imidacloprid and fipronil degraded faster at pH 10, and fipronil degraded faster than imidacloprid at pH 10. The half-life estimates of imidacloprid were 182 and 44.7 days at pH 7 and pH 10, respectively, and those values of fipronil were 87.9 and 13.2 days, respectively. Fipronil sulfone was found in the degradation process of fipronil. © Pesticide Science Society of Japan

Keywords: degradation, pH, imidacloprid, fipronil, paddy water.

Introduction

Imidacloprid and fipronil are two systemic insecticides that are widely used in Japan. They were used for nursery-box application to protect rice plants against various insects in the early period of rice cultivation in the paddy field.¹⁾ Dissipation of imidacloprid^{1–5)} and fipronil^{4,6,7)} in actual paddy fields as well as in lysimeter has been reported. The dissipation rate was varied and depended on many parameters such as physical and chemical properties of pesticides, water management, and environmental conditions. Understanding major factors that influence the dissipation of pesticide in the paddy environment is an important task for ensuring the safe use of pesticide and for reducing its negative impact on adjacent aquatic environments.

In the rice paddy field, pesticide was always submerged in paddy water, thus its degradation pathways, such as photolysis, hydrolysis, and biochemical degradation dependent on the water chemistry, are very important in determining its environmental fate. Photolysis of imidacloprid and fipronil in paddy water has been reported by Thuyet *et al.*⁸⁾ Other degradation pathways, such as hydrolysis of those pesticides in water, were also well documented.^{9,10)} However, the pH dependence on the

biochemical degradation of the above pesticides in paddy water has been rarely reported. The environmental fate of some organic pesticides can be dramatically affected by water pH.¹¹⁾ Singh *et al.*¹²⁾ reported that pH variations in water during hydrolysis have a major role in the breakdown and degradation of pesticides. In paddy water, pH varied from neutral (7.0) to alkaline (9.9) depending on time and field condition.^{5,6)} In order to accurately evaluate the fate of imidacloprid and fipronil under a realistic paddy environment, effect of pH on the degradation processes of those insecticides in paddy water is required. The objective of this study is to investigate the effect of pH on biochemical degradation of imidacloprid and fipronil in paddy water.

Materials and Methods

Imidacloprid and fipronil standards, acetic acid, and acetonitrile solvents at analytical grade (Wako, Osaka, Japan) were used in this study along with a Milli-Q Water Purification System (Millipore, Billerica, MA, USA), syringes (TERUMO 5 mL), a disk filter (0.2 μ m), C18 ENVI cartridge, a pH meter (D-23; Horiba, Kyoto, Japan), an Eh meter (EHS-120; Fujiwara Scientific), and temperature sensors connected to data loggers (UIZ3633; UIZIN, Tokyo, Japan).

Since imidacloprid and fipronil are sensitive to solar radiation,^{8,13,14)} degradation of those pesticides was examined at pH 7 and pH 10 in the dark to eliminate the effect of photodegradation. All experiments were conducted separately for imidacloprid and fipronil and performed in duplicate. Pesticide-free paddy water used in this study was collected from rice paddy field at the university farm (FM Honmachi) of Tokyo University of Agriculture and Technology in Tokyo, Japan on March 25, 2011, three days prior to the experiment. Imidacloprid and fipronil had not previously been applied to the plots. Chemical analysis also confirmed that imidacloprid and fipronil were not detected in the water samples. The paddy water had pH 7.4 ± 0.2 , Electrical conductivity (EC) $38.7 \pm 0.3 \mu\text{S/cm}$, and Redox potential (Eh) $351.5 \pm 3.4 \text{ mV}$. The paddy water was passed through a 1.2 μ m glass fiber filter (GF/C; Whatman, Maidstone, UK), and then its pH was adjusted to pH 7 and pH 10 by using buffer solutions.

The phosphate buffer solution was prepared by adding an appropriate volume of 0.1 M HCl to 1 L solution of 0.1 M dipotassium hydrogen phosphate K_2HPO_4 (17.42 g/mol) to achieve a pH 7 solution.¹⁵⁾ The borate buffer solution was prepared as 0.1 M H_2BO_3 in 0.1 M KCl, then the solution was adjusted by 0.1 M NaOH to attain pH 10.¹⁵⁾ The paddy water was mixed with buffer solution at a ratio of 50 mL/500 mL of paddy water. The solution was then adjusted to pH 7 and pH 10 by 0.1 M HCl and 0.1 M NaOH, respectively, and 400 mL of each was transferred into 900-mL glass bottles. A total of 10 bottles was prepared for each pesticide.

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Published online October 3, 2013

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An appropriate amount of pesticide standard solution was added to the paddy water in the bottles to achieve the initial desired concentrations of imidacloprid and fipronil, 60 and 3 µg/L, respectively. Those concentrations were similar to the maximum concentrations observed in paddy water in actual paddy fields after application of imidacloprid and fipronil.^{2,3,6)}

All bottles were placed in a constant temperature room at $18.2 \pm 0.4^\circ\text{C}$ and kept in the dark. The bottles were uncapped, and water samples could freely contact the air. The pH and water level in each bottle were monitored every two days and adjusted to the initial values, if necessary. Eh was also monitored on each sampling day. Previous studies on the dissipation of imidacloprid and fipronil in actual paddy fields were conducted 35 days after the pesticide applications when the pesticide concentrations in the paddy water were relatively high,^{2,3,6)} thus a monitoring period of 35 days was also chosen for this study.

Water samples were collected 0, 3, 7, 21, and 35 days after pesticide treatment (DAT). All water samples were analyzed to determine the concentrations of imidacloprid, fipronil, and fipronil sulfone by solid-phase extraction coupled with reversed-phase HPLC and with a photodiode array detector using the methods of Thuyet *et al.*³⁾ and Thuyet⁶⁾ on the same sampling day. Briefly, 400 mL of the water sample was first passed through a 1.2-µm glass fiber filter (GF/C; Whatman, Maidstone, UK), and then it was loaded to a solid-phase ENVI C18 Super-clean cartridge (500 mg/6 mL, Supelco; Sigma-Aldrich, St. Louis, MO, USA) at a flow rate of 3 mL/min for the extraction of pesticides. Pesticide was eluted by 10 mL acetonitrile. The eluate was blown down to 1 mL under a gentle stream of nitrogen gas. The samples were passed through a 0.2-µm filter (Whatman, Maidstone, UK) and kept for HPLC analysis. Analyses were performed on a Shimadzu HPLC with a photodiode array detector and a C-18 column (150 mm × 4.6 mm × 4.6 µm) (Shimadzu Corporation, Kyoto, Japan). The mobile phase used for analysis of imidacloprid was acetonitrile/water (20:80, v/v) and that for fipronil and fipronil sulfone was acetonitrile/water (60:40, v/v). The flow rate of both the mobile phase was 1 mL/min with isocratic mode. The column was kept at a constant temperature of 40°C. The detector monitored a wavelength of 270 nm for imidacloprid and 280 nm for fipronil and fipronil sulfone. The retention times of imidacloprid, fipronil, and fipronil sulfone were 7.8, 7.8, and 11.2 min, respectively.

Results and Discussion

The degradation of imidacloprid in paddy water at pH 7 and pH 10 is shown in Fig. 1. The concentration of imidacloprid decreased for the first 7 days, and afterward, the concentration was rather stable at pH 7. Imidacloprid degraded faster at pH 10 than at pH 7. After 35 days in paddy water, concentrations of imidacloprid dropped by 12% and 48% of the initial concentration at pH 7 and pH 10, respectively (Fig. 1a).

Causes of the dissipation of imidacloprid in the dark could include hydrolysis, biochemical degradation, and volatilization. The major hydrolysis product of imidacloprid was reported to

Fig. 1. Degradation of imidacloprid (a) and fipronil (b) in paddy water at pH 7 and pH 10; error bars indicate standard deviations from the mean of the duplicates.

be 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidone.¹⁶⁾ In general, pH can affect the hydrolysis of pesticides. Imidacloprid has a $-\text{C}=\text{N}-$ bond that couples with a strong electron-withdrawing group ($-\text{NO}_2$) and an imidazolidine ring, thus the bond has a small positive charge.¹⁶⁾ The positive charge can interact with the OH^- ion in the solution. A higher pH solution has a higher concentration of OH^- that increases the hydrolysis rate of imidacloprid.¹⁶⁾ Zheng and Liu¹⁶⁾ reported that 1.5% and 20% of imidacloprid was lost after three months in deionized water at pH 7 and pH 9, respectively. The volatilization of imidacloprid could be negligible because imidacloprid has a low vapor pressure of 1.0×10^{-7} mmHg and a low Henry's law constant of 6.5×10^{-11} atm m³/mol.¹³⁾

The degradation of imidacloprid can be described by the first-order kinetics ($r^2 \geq 0.7$) (Table 1). The extrapolated degradation half-lives (DT_{50} s) of imidacloprid were 182 and 44.7 days at pH 7 and pH 10, respectively. The increase of pH resulted in the decreased DT_{50} s of imidacloprid. The hydrolysis DT_{50} of imidacloprid was reported to be greater than 30 days at pH 7 and 25°C .¹³⁾ A similar trend was also reported by Zheng and Liu.¹⁶⁾ The DT_{50} s of imidacloprid in deionized water were 20 and 2.9 days at pH 10.8 and 11.8, respectively.¹⁶⁾ On the other hand, a study on the persistence of formulated imidacloprid in HPLC-grade water in amber colored glass bottles observed that the DT_{50} of imidacloprid increased with the increase of pH.¹⁷⁾ Note that Sarkar *et al.*¹⁷⁾ used imidacloprid in the form of liquid and

powder formulations; thus, the effect of pH on the degradation of formulated imidacloprid may differ from that of pure imidacloprid. The DT_{50} of imidacloprid in HPLC-grade water at pH 4, pH 7, and pH 9 ranged from 31 to 46.3 days.¹⁷⁾ As compared to the hydrolysis of imidacloprid in water,¹³⁾ the DT_{50} of biochemical degradation in this experiment was not smaller but still in the reported range.

In actual paddy fields, the dissipation of imidacloprid involved the integration of multiple factors such as photolysis, hydrolysis, biochemical degradation, sorption/desorption, water movement, *etc.*³⁾ The dissipation DT_{50} of imidacloprid in paddy water was 2.5–4.7 days and 2.0–2.4 days in the micro paddy lysimeter⁵⁾ and the actual paddy field,³⁾ respectively. Imidacloprid concentration at 35 DAT was about 1–3% of its maximum concentration at 1 DAT.³⁾ Moreover, Thuyet *et al.*⁸⁾ found that imidacloprid in paddy water was very sensitive to solar radiation, and its photolysis DT_{50} was about 1.0 day. Liu *et al.*¹⁸⁾ also reported that the hydrolysis of imidacloprid was lower than its photolysis. Although pH was varied in paddy water from a neutral 7.0 pH to an alkaline 9.9 pH^{5,6)} and the change of the pH affected the degradation of imidacloprid, as found in this study, the effect of pH on the dissipation of imidacloprid in paddy water was relatively small as compared to that of photolysis and other dissipation pathways in actual paddy fields.

In the same pH condition, fipronil tended to dissipate faster than imidacloprid did. The dissipation rate of fipronil was faster at pH 10 than at pH 7. Fipronil losses accounted for 23% and 84% of the initial fipronil mass at pH 7 and pH 10, respectively, at 35 DAT (Fig. 1b). Similarly to imidacloprid, volatilization of fipronil was very slow, and its experimental Henry's law constant was $6.60 \times 10^{-6} \text{ m}^3 \text{ atm/mol}$.¹⁹⁾ The dissipation of fipronil *via* volatilization can be ignored in this study. The degradation processes of fipronil in the dark could include hydrolysis and biochemical degradation. Fipronil has a nitrile group ($-\text{C}\equiv\text{N}$) in its molecular structure, thus it could interact with the OH^- ion in an alkaline condition to form fipronil amide.²⁰⁾ Since this reaction rate is dependent on the concentration of OH^- ions, fipronil degraded faster at pH 10 than at pH 7 in this study.

The degradation of fipronil in paddy water followed first-order kinetics ($r^2 \geq 0.9$) (Table 1). The DT_{50} was 13.2 days at pH 10, and its extrapolated DT_{50} was 87.9 days at pH 7 (Table 1). These DT_{50} values are close to the DT_{50} s reported in aquatic environments in recent literature. Bobe *et al.*²⁰⁾ reported that the hydrolysis DT_{50} of fipronil in HPLC-grade water containing

2.5% methanol was >100 days, 32 days, and 4.8 days at pH 7, pH 9, and pH 10, respectively, at an ambient temperature of $22 \pm 2^\circ\text{C}$. This result indicates that the contribution of biochemical degradation to the dissipation of fipronil in this study seems to be obvious at pH 7 but not clear at a higher pH. Chopra and Kumari²¹⁾ examined the dissipation of fipronil in canal water placed in amber-colored bottles in laboratory conditions, indicating that the DT_{50} of fipronil was 20.6 days. The different DT_{50} s of fipronil in paddy water, HPLC-grade water, and river water indicate that the contribution of biochemical degradation to the dissipation of fipronil at a neutral pH really depends on water characteristics; therefore, examination of the biochemical degradation is necessary to ensure the environmental fate of fipronil in the studied aquatic environments.

Fipronil dissipated as fast as imidacloprid in paddy water in actual paddy fields.⁶⁾ The dissipation of fipronil in actual paddy fields was also attributed to a combination of photolysis, hydrolysis, biodegradation, sorption/desorption, water movement, *etc.*⁶⁾ The degradation DT_{50} of fipronil at pH 10 in this study was close to its dissipation- DT_{50} range in actual paddy fields. The dissipation DT_{50} s of fipronil in paddy water were 0.9–3.1 days in actual paddy fields⁶⁾ and 5.4 days in the micro paddy lysimeter.⁷⁾ The photolysis DT_{50} of fipronil in paddy water was 1.5 days.⁸⁾ Regarding the effect of pH on the degradation of fipronil in this study, the DT_{50} of fipronil obviously decreased from 88 days at pH 7 to 13 days at pH 10, thus, pH could play an important role in the dissipation of fipronil in paddy water in actual paddy fields, in addition to photolysis.

Fipronil sulfone was reported as a major metabolite formed in the oxidation of fipronil in water.²²⁾ It is the most concerning metabolite of fipronil in aquatic environments because of its high toxicity in nature. The toxicity of fipronil sulfone to many species is usually equal to or higher than that of fipronil.¹⁴⁾ Fipronil sulfone was found in paddy water during the degradation process of fipronil in this study, probably because of the oxidative condition of the paddy water. Paddy water maintained an oxidative condition at a measured Eh of $358 \pm 29 \text{ mV}$ by exchanging oxygen with ambient air during the monitoring period. Bobe *et al.*²⁰⁾ reported that the $-\text{SOCF}_3$ group of fipronil could be oxidized to $-\text{HSO}_3$ to form fipronil sulfone under oxidative conditions. The maximum concentrations of fipronil sulfone were $0.3 \mu\text{g/L}$ at 7 DAT (pH 7) and $0.7 \mu\text{g/L}$ at 3 DAT (pH 10) (Fig. 1b). At the end of the monitoring period (35 DAT), those concentrations had declined to $0.2 \mu\text{g/L}$ in pH 7

Table 1. Degradation kinetics of imidacloprid and fipronil in paddy water at pH 7 and pH 10

Insecticide	pH	Equation ^{a)}	r^2 ^{b)}	DT_{50} ^{c)}	Lower 95% ^{d)}	Upper 95% ^{d)}
Imidacloprid	7	$y = -0.0038x + 4.05$	0.7	182	123	353
	10	$y = -0.0155x + 4.01$	0.9	44.7	35.5	60.6
Fipronil	7	$y = -0.0079x + 1.09$	0.9	87.9	68.1	124
	10	$y = -0.0524x + 1.07$	1.0	13.2	12.4	14.1

^{a)} y is a natural logarithm of pesticide concentration $\ln(c)$, and x is the day after pesticide application. ^{b)} r^2 is the square of the correlation coefficient of y and x . ^{c)} DT_{50} is degradation half-lives of pesticide (day). ^{d)} Lower and upper 95% confidence intervals of DT_{50} values.

Fig. 2. Mass balance of fipronil in paddy water, % of the initial mass of fipronil.

and $0.3 \mu\text{g/L}$ in pH 10 (Fig. 1b). Similar results were also observed in actual paddy fields. The concentration of fipronil sulfone in paddy water peaked at 3 DAT, and its maximum concentration ranged from 0.4 to $0.9 \mu\text{g/L}$.⁶⁾ It indicates that a similar oxidation process of fipronil in this study also occurs in actual paddy fields. The volatilization of fipronil sulfone has not been reported;⁹⁾ however, it is not expected to be volatilized because its molecular structure is similar to that of fipronil.

Figure 1b shows that the decline of fipronil correlates well with the increase of fipronil sulfone in paddy water. In the first 3 days, the fast degradation of fipronil associated with the fast formation of fipronil sulfone indicates that pH 10 promoted the transformation of fipronil to fipronil sulfone in the early period of fipronil degradation. Although hydrolysis of fipronil was faster at pH 10 than at pH 7, the mechanism of how pH could promote the oxidation of fipronil in paddy water is still unknown. pH could act as a catalyst in the oxidation of fipronil in paddy water; however, this mechanism requires further investigation. In studies of the degradation of the insecticide methylparathion in wastewater treatment by ozonation, Usharani *et al.*²³⁾ also found that the oxidation of methylparathion is more effective at pH 9 than at pH 7 or pH 3.

The mass balances on fipronil and fipronil sulfone are presented in Fig. 2. Fipronil's mass balances in paddy water at 35 DAT were 77% and 16% of the initial fipronil mass at pH 7 and pH 10, respectively. There was a similar transformation trend at both pH 7 and pH 10. Fipronil was first transformed to fipronil sulfone then to other products. The total mass of fipronil and degraded fipronil, which was transformed to fipronil sulfone, was conserved approximately 100% until 7 DAT and 3 DAT at pH 7 and pH 10, respectively. This indicates that fipronil sulfone was the solely formed metabolite in the early period of monitoring at both pH 7 and pH 10. Since the total mass was conserved until 3 DAT at pH 10 and 7 DAT at pH 7, fipronil may be degraded or transformed to other metabolites (see blank bars in Fig. 2) after the corresponding days. Hydrolysis could be the major degradation pathway of fipronil during those periods as discussed in the previous paragraph, and hydrolysis probably proceeded at a slower rate as compared to the rate of oxidation. Oxidation of fipronil still occurred after 7 DAT at pH 7 and after 3 DAT at pH 10; however, the mass of fipronil sulfone tended to decrease at 21 DAT and slightly increase at 35 DAT (Fig. 2). This indicates

the possibility of compensation between the formation and the degradation of fipronil sulfone after 7 DAT at pH 7 and after 3 DAT at pH 10.

Conclusion

In conclusion, pH is one of the important factors that affect the degradation of imidacloprid and fipronil in paddy water. Both imidacloprid and fipronil were relatively stable at pH 7. The degradation rate was increased at pH 10. The degradation DT_{50} s of imidacloprid and fipronil at pH 10 were 44.7 days and 13.2 days, respectively. Fipronil sulfone was found in the degradation of fipronil in paddy water.

Acknowledgements

This work was supported in part by the Heiwa Nakajima Scholarship Foundation and a Grant-in-Aid for Biological Studies on Wildlife under the Framework ExTEND2005 in Japan. Special thanks to Dr. H. Saito for providing experimental facilities in Tokyo University of Agriculture and Technology.

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