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Impact of Fipronil on Crustacean Aquatic Organisms in a Paddy Field-Fishpond Ecosystem

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Fipronil, a recently developed insecticide, has many advantages such as low rate of application, high efficiency, and killing a wide range of insects. In China, it is used as a substitute for highly toxic organophosphorus pesticides. However, Fipronil is highly toxic to many aquatic organisms (John E, 1998). Hazard to the environment and living organisms is associated with exposure concentration (Cai et al., 1997). In this study, we established a simulation paddy field-fishpond ecosystem (Lay et al., 1993; Li, 1999) to investigate the fate and effect of fipronil on shrimp (*Macrobrachium rosenbergii, Macrobrachium nipponensis*) and crab (*Eriocheir sinensis*) in order to provide a scientific basis for a safe application of fipronil.

MATERIALS AND METHODS

Five pencent fipronil suspended agent, the standard sample of parent fipronil and the metabolites, were provided by Aventis CropScience.

Macrobrachium rosenbergii, Macrobrachium nipponensis, and *Eriocheir sinensis* were provided by Taixing Aquiculture Farm, Jiangsu Province.

In the simulated ecological experiment, a paddy field and fishponds were built with cement, as shown in Figure 1. The paddy field had an area of 10 m^2 (4m × 2.5m), with a 25cm depth. Over the bottom was spread a 15cm-thick layer of earth which were collected from suburban of Nanjing city where the fipronil never be used. Glass partitions were set at a certain interval so that water would flow circuitously between the glass partitions when drained from the field into the treated fishpond. On one side of the field were two fishponds, each had dimension of 2 m (length) × 1.5 m (width) × 1.2 m (depth). At the bottom of both ponds were placed a certain amount of mud where some water grass was grown. One of the ponds was used for experimental treatment, and the other as a control. Before the experiment, rice plants were growing in the field as usual. In both ponds were placed two net-cages, where shrimp and crabs were separately bred so that the different sorts of organisms would not kill one another.

When test organisms in the fishpond had adapted themselves to the environment,

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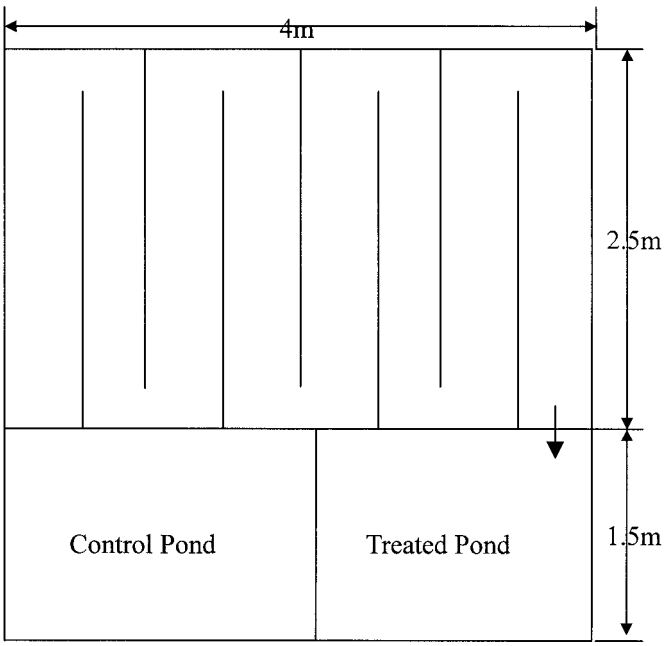

Figure 1. Paddy and fish-pond simulation system.

on June 12, 2000, the suspended agent of 5% fipronil was evenly sprayed over the experimental paddy field at a suggested rate of 45 g a.i./hm^2 and the two ponds were covered by plastic film to avoid pollution when fipronil was aprayed. From then on, water and soil samples were regularly taken from paddy field and fishpond to determine the fipronil content.

For examination of dynamics of fipronil in the paddy field soil, 21 culture dishes with a diameter of 9 cm were prepared, Each dish had 40 g of paddy soil that had been ground and passed through 1 mm mesh. Then, the culture dishes were placed at different points in the paddy field, and the soils in the culture dishes received the pesticides in water. After fipronil was sprayed over the field, the culture dishes at fixed time (3 dishes at one time) were collected to determine soil fipronil content.

For examination of dynamics of fipronil in the paddy field water, it was necessary to avoid having water drain from the paddy field to the fishpond. A glass container with dimensions of 50cm×40cm×23cm was buried in the paddy field to hold the water, just as with the rice field management. Water was regularly taken from the water container to determine fipronil content.

For examination of dynamics of fipronil in the fishpond water, 24 hr after fipronil spraying of the paddy field, water corresponding to half of the total paddy field water was drained from the field to the fishpond. Water was regularly taken from

the fishpond to determine the fipronil content.

Water samples were extracted with ether and soil samples with acetone. After being purified and concentrated, Fipronil concentration and that of its metabolic products were determined by Simadzu GC-14A Gas Chromatograph, with HP5 Capillary Chromatographic Column (30m×0.25mm×0.25μm). The minimum detection limit was 1 μg/L. The recovery rate of fipronil in water samples was 93.5-105.5%, and that of soil samples was 85.2-94.0%.

The effects of fipronil to *Macrobrachium rosenbergii, Macrobrachium nipponensis,* and *Eriocheir sinensis* in a simulation paddy field-field ecosystem were conducted. 80 *Macrobrachium rosenbergii,* 50 *Macrobrachium nipponensis,* and 80 *Eriocheir sinensis* (their average weight being 1.0 g, 2.3 g, and 1.0 g, respectively) were reared in the treated fishpond and the same amounts of test organisms were also reared in the control fishpond. Twenty-four hours after fipronil was applied to the paddy field, half the water in the field was drained into the fishpond to examine effects on the living organisms there. The mortalities and any observable abnormal behavioral responses were noted every 24 hr.

The toxicity of fipronil to *Macrobrachium rosenbergii, Macrobrachium nipponensis,* and *Eriocheir sinensis* was determined by the semi-static toxicity method. Aerated tap water was used as the test water, with pH 7.1. Test temperature was 20±1℃. Test containers were 25 L glass vats. In each of them was placed 20 L of test solution. A fresh working stocking solution of the pesticide was prepared. A range-finding test was conducted firstly, then a serious of range-finding concentration were prepared by adding measured volumes of stock solution to the water in the volumes of stock solution to the water in the containers and mixing throughly, an addition container with 100% dilution water served as control. 10 test organisms (their average weight being 1.0 g, 2.3 g, and 1.0 g for *Macrobrachium rosenbergii, Macrobrachium nipponensis,* and *Eriocheir sinensis*, respectively) were introduced into each container. Pesticide solution was renewed every 48 hr. At 24 hr, 48 hr, 72 hr and 96 hr after pesticide applied, the mortality and any observable abnormal behavioral responses of the test organisms were noted and recorded every 24 hours. The LC_{50} values and 95% confidence limits were calculated.

RESULTS AND DISCUSSION

After the fipronil application, samples were collected separately from the rice plant, the paddy field water, and the paddy field soil. Experimental results showed that the initial concentration of fipronil in rice plant was 2.4 mg/kg. According to rice plant biomass at that time, the amount of fipronil that stuck to rice plants was 22.81g a.i./hm^2, accounting for 50.7% of the sprayed pesticide. The initial concentration of fipronil in paddy field water was 31.5 μg/L, accounting for 38.5% of the pesticide applied. At the time, the fipronil concentration in the soil was very low. This indicates that most of the fipronil after application sticks to rice plants and dissolves in the paddy field water.

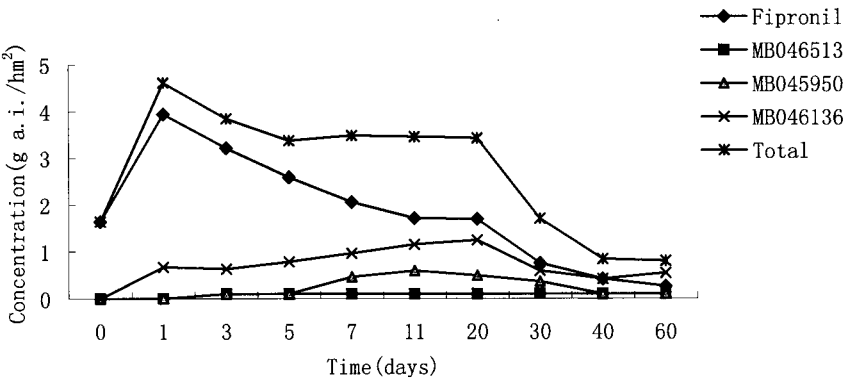

Figure 2.Degradation of fipronil in paddy soil.

In the initial stage of fipronil application, the fipronil concentration in the paddy field soil was 1.61 g a.i./hm^2, and 24 hr later that in the soil increased to 4.61 g a.i./hm^2. Then the fipronil content began to decline (Fig.2). Fipronil has several metabolic products (Camille MC, 1998), MB-046136 and MB-045950 were detected in soil, MB-046513 was negligible in amount. Parent fipronil and its metabolic products have similar toxicity to aquatic organisms (The 21day NOEC of fipronil, MB-046136 and MB-045950 to daphnia were 9.8, 13 and 1.5 μg/L respectly, the data was quoted from report of Aventis cropScience). The results showed that the metabolic products degraded more slowly than its parent, and they persist in soil for a longer time. The degradation half-life of fipronil in the paddy field soil is 21.7 d.

The initial residue of fipronil in the paddy field water was 32.6 μg/L (Fig.3). Fifteen days later, it decreased to 4.2 μg/L. The pesticide degraded fairly rapidly, the degradation half-life was 4.8d.At the 11th d, a small amount of its metabolic product MB-046136 was found, while the other two metabolic products were negligible in content.

The concentration of fipronil in the fishpond water was quite steady, the initial concentration was 3.48 μg/L (Fig.4). At the 30th d, the fipronil residue was still as much as 2.6 μg/L; the degradation half-life was 77.2 d. In the pond water only traces of metabolite MB-046136 were detected; other metabolic products were not found. The residue of fipronil persists for a longer time in the fishpond water than in the paddy field water.

The effect of fipronil on crustacean organisms bred in ecological pond: At 24 hr after fipronil was sprayed in the paddy field, the field water was drained into the fishpond. *Eriocheir sinensis* in the treated pond immediately responded to fipronil toxicity, crawling one after another from the bottom to the four walls of the net box, and giving an arch form of their bodies on eight legs, with symptoms of

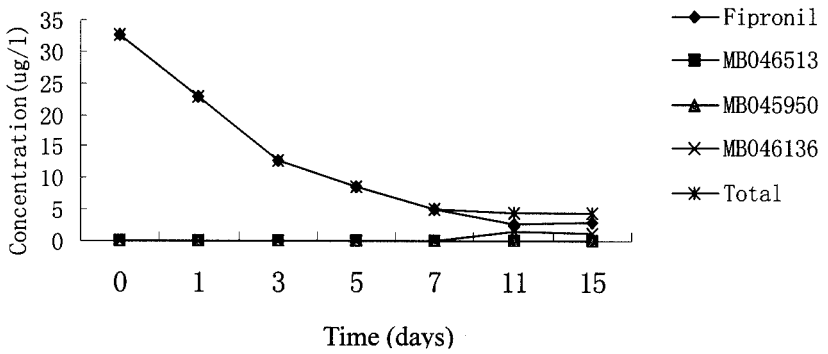

Figure 3. Degradation of fipronil in paddy field.

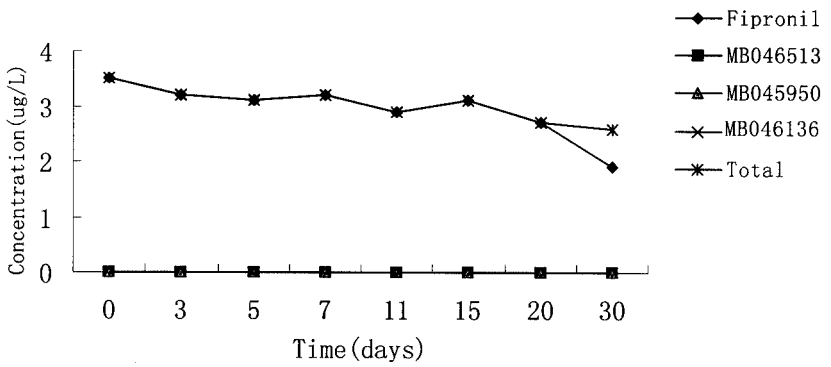

Figure 4. Degradation of fipronil in fishpond water

convulsion. *Macrobrachium rosenbergii* and *Macrobrachium nipponensis,* after exposure, dashed about in the net-cage and died in large numbers in the first 1-2 d after the field water drained into the pond. On the 3rd d, all *Macrobrachium rosenbergii* died in the treated pond, whereas the mortality in the control pond was only 13.8%. On the 7th d, all *Macrobrachium nipponensis* died in the treated pond, while the mortality in the control pond was 34.0%. On the 10th d, all *Eriocheir sinensis* died in the treated pond, the mortality in the control pond being 50.0%. The higher mortality of test organisms in control fishpond occurred in our experiments, it was because the test three organisms can fight and kill each other by their pincers, high mortality can occur even in natural condition.

Table 1. Effect of fipronil on test organisms in the simulated rice field ecosystem (mortality, %)

Time (d)	Treated pond			Control pond		
	E. sinensis	M. nipponensis	M. rosenbergii	E. Sinensis	M. nipponensis	M. rosenbergii
1	3.8	38.0	60.0	6.3	14.0	1.3
2	10.0	72.0	97.5	8.8	22.0	11.3
3	21.3	80.0	100	11.3	26.0	13.8
4	27.5	80.0		17.5	26.0	
5	33.8	96.0		20.0	28.0	
6	35.0	96.0		22.5	34.0	
7	42.5	100		27.5	34.0	
8	75.0			33.8		
9	92.5			42.5		
10	100			50.0		

The toxicity of fipronil to the three tested crustacean organisms was very high (Table 2), 96 hr LC_{50} of fipronil with *Macrobrachium rosenbergii* was 0.98 μg/L, and with *Macrobrachium nipponensis* and *Eriocheir sinensis* were 4.32 μg/L and 8.56 μg/L respectly. *Macrobrachium rosenbergii* was most sensitive to fipronil in three tested organisms, followed by *Macrobrachium nipponensis* and then *Eriocheir sinensis*. For all 3 test organisms, the longer the time of exposure, the longer the toxic effect of fipronil lasted. The death of the organisms mainly occurred on the 3rd to 4th d after pesticide application.

Table 2. The acute toxicity of fipronil to shrimp and crabs (LC_{50}, μg/L)

Organism	24 h	48 h	72 h	96 h
M. rosenbergii	6.41	2.24	1.63	0.98
M. nipponensis	>25.70	11.61	7.02	4.32
E. sinensis	>57.83	22.57	12.44	8.56

Our data showed that the three tested crustacean aquatic organisms were very sensitive to fipronil, their 96 hr LC_{50} value with *Macrobrachium rosenbergii, Macrobrachium nipponensis,* and *Eriocheir sinensis* was only 0.98 μg/L, 4.32 μg/L, and 8.56 μg/L, the results also showed that fipronil degradation was very slow in the environment, its degradation half-life time in the paddy field water, soil and fishpond water was 4.8 d、21.7 d and 77.2 d respectively, so fipronil could cause great hazard to crustacean aquatic organisms if it was widely used in rice or aquiculture areas.

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