The Dissipation of Pyrimorph in Lake Water/Sediment Systems

ZHUONAN LI, WENBI GUAN, YONGQIANG MA*, HENG ZHAO AND ZHAOHAI QIN**
College of Science, China Agricultural University , Beijing 100193 , P.R. China
mayongqiang@cau.edu.cn*, qinzhaohai@263.net*

(Received on 22nd June 2012, accepted in revised form 19th June 2013)

Summary: A study into the environmental behavior of pyrimorph in lake water/sediment systems has been carried out. The residue of pyrimorph in water and sediment for aerobic and anaerobic tests was determined, respectively. For aerobic test, the dissipation half-life of pyrimorph was 11.6 days in lake water of water/sediment system and 96.3 days in lake water of control treatment. For anaerobic test, the dissipation half-life of pyrimorph was 5.5 days in lake water of water/sediment system and 42.3 days in lake water of control treatment. Also, the pyrimorph concentration increased gradually in sediment of water-sediment system. The results showed that the dissipation of pyrimorph in water resulted mainly from sediment adsorption of pyrimorph and O_2 inhibited the dissipation of pyrimorph. The degradation product of pyrimorph has been detected by HPLC-MS/MS. In the dissipation process, pyrimorph lost morpholine ring and formed an aldehyde compound.

Keywords: pyrimorph, dissipation, lake, water/sediment system, aerobic test, anaerobic test.

Introduction

Pyrimorph is a novel systemic carboxylic acid amide (CAA) fungicide [1] with the chemical structure shown in Fig. 1. Pyrimorph is discovered by China Agriculture University and is widely used in China. Bioassay tests proved that pyrimorph has high antifungal activity in inhibiting the mycelium growth of Phytophthora infestans, Phytophthora capsici and Rhizoctonia solani and in inhibiting the zoosporangia germination of Pseudoperonospora cubensis[1]. Recent study has shown that the toxicity of pyrimorph is low. Acute oral LD_{50} and acute percutaneous LD_{50} for rats is more than 5000 mg/kg and more than 2000 mg/kg, respectively, Also, there is no rabbits skin and eye irritation. Sub-chronic (acute) toxicity to rats: maximal no-effective dose is 30 mg/kg.bw/d. For mutagenicity ames test, micronucleus or bone marrow cell chromosome aberration and dominant lethal germ cell chromosome aberrations: in vitro mammalian cell gene mutation tests were negative [2].By now, several researches on pyrimorph have been reported, such as inhibitory effects and action mechanism against phytophthora capsici [3], acute toxicity and bioconcentration in zebrafish, brachydanio rerio [1], residue dynamic on tomatoes, cucumbers and soil [4]. Adsorption and leaching behavior of pyrimorph in soil has been studied by Wang Jing *et al*. [5]. But no study has been reported on dissipation of pyrimorph in aquatic environments. Therefore, fundamental research on the elimination of pyrimorph, especially in natural river systems, is particularly needed.

Once pesticides are introduced into surface water, they may also undergo physico-chemical reactions, hydrolysis, biodegradation and adsorption to sediment. In the process of pesticides adsorption to sediment, we can determine the concentration of pesticides in water and sediment to study its behavior in the aquatic environment [6]. This study set out to determine the environmental behavior of pyrimorph in the water-sediment system of a natural lake on a laboratory scale. Also, the degradation product of pyrimorph has been detected by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS).

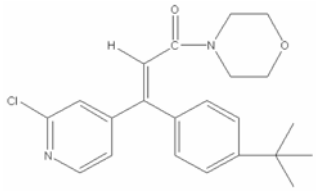

Fig. 1: Chemical structure of pyrimorph.

Results and discussion

Method Validation

Standard Curve: Known pesticide amounts were added to the representative blank extracts (lake water and sediment of lake) to obtain the final working standard solution of concentrations 0.1-10 mg/L. Good linear relationships and coefficients of determination (R^2> 0.999) were achieved over the concentration range of 0.1-10 mg/L.

Accuracy and Precision: To evaluate the accuracy and precision of the sample pretreatment

*To whom all correspondence should be addressed.

method, the recovery experiment was conducted. All matrices (lake water and sediment of lake) were spiked at three different levels (0.5, 1.0 and 5.0 mg/kg). A total of five replicate measurements were performed for each fortified level. The recoveries were from 74.4 to 107.5% for the four matrices. The relative standard deviation values (RSD, %) ranged from 1.7-11.9%. The results showed in Table-1.

Table-1: Precision and accuracy of the method for determining in lake water and sediment of lake (intra-day, n=5)

	lake water		Sediment of lake	
Spiking level (mg/kg)	**Mean recovery (%)**	**RSD (%)**	**Mean recovery (%)**	**RSD (%)**
0.5	**90.2±4.9**	**5.4**	**74.4±8.8**	**11.9**
1.0	**96.7±2.4**	**2.5**	**107.5±4.6**	**4.3**
5.0	**92.6±3.6**	**3.9**	**104.9±9.3**	**8.9**

Dissipation of Pyrimorph

The results of pyrimorph dissipation data are shown in Table-2, Fig. 2 and Fig. 3. The water and sediment samples were analysed for transformation or degradation metabolites in lake water and sediment. For aerobic test, the dissipation half-life of pyrimorph was 11.6 days in lake water of water/sediment system and 96.3 days in lake water of control treatment. For anaerobic test, the dissipation half-life of pyrimorph was 5.5 days in lake water of water/sediment system and 42.3 days in lake water of control treatment. Pyrimorph dissipated very fast in water/sediment system and very slow in control treatment. From the dissipation half-lives ($T_{1/2}$) of pyrimorph in water for aerobic and anaerobic tests, pyrimorph dissipated much faster in water-sediment system than in control treatment. There is no sediment in control treatment and we determined the pyrimorph concentration in sediment of water-sediment system to demonstrate effect of sediment on the dissipation of pyrimorph. With the reduction of pyrimorph in water of water-sediment system, pyrimorph concentration increased gradually in sediment of water-sediment system (Fig. 2 and 3). It could be concluded that pyrimorph translated fast from water into sediment and the dissipation of pyrimorph in water resulted mainly from sediment adsorption of pyrimorph. To test whether O_2 has effect on dissipation of pyrimorph, aerobic test and anaerobic test have been designed. No matter in water-sediment system or in control treatment, pyrimorph dissipated much faster in anaerobic test than in aerobic test. It could be concluded that O_2 inhibited the dissipation of pyrimorph.

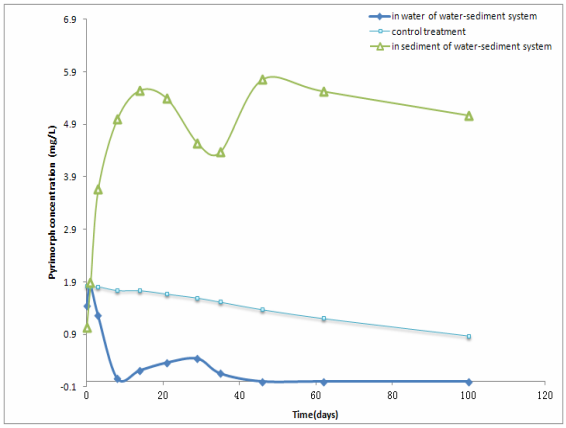

Fig. 2: The dissipation of pyrimorph in the aerobic test.

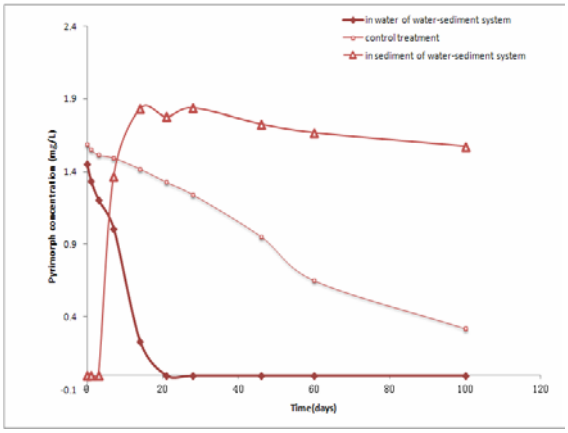

Fig. 3: The dissipation of pyrimorph in the anaerobic test.

Degradation Product of Pyrimorph

The degradation product of pyrimorph in lake water was monitored by HPLC-MS/MS which was operating in the positive scan mode. A [M+H] molecular peak at m/z 279.40 was detected and there was an ion of one-third-height at m/z 281.44. We speculated that pyrimorph lost morpholine ring in the dissipation process to form an aldehyde compound. The degradation route is shown in Fig. 4.

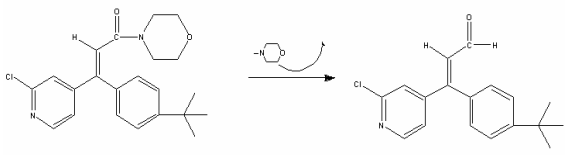

Fig. 4: The degradation route of pyrimorph.

Table-2: Dissipation parameters and half-lives of pyrimorph (spiked at 2 mg/L) under different treatments in lake water

condition		pyrimorph dissipation kinetics	R^2	$T_{1/2}$(d)
aerobic test	water-sediment system	$c = 1.3486e^{-0.06t}$	0.7346	11.6
	control treatment	$c = 1.8879e^{-0.0072t}$	0.9798	96.3
anaerobic test	water-sediment system	$c = 1.6726e^{-0.1269t}$	0.9065	5.5
	control treatment	$c = 1.783e^{-0.0164t}$	0.9755	42.3

Experimental

Chemicals and Apparatus

Standard of pyrimorph (95.0% purity) was provided by China Agriculture University. Stock standard solution of pyrimorph (1000 mg/L) was prepared in methanol and stored at -20 °C. The stock standard solution was diluted with methanol as required. HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Milli-Q pure water (Millipore, Bedford, MA) was used during the whole analysis. Analytical reagent grade anhydrous sodium chloride (NaCl), formic acid (98%), acetic acid (36%), acetone, dichloromethane and anhydrous sodium sulfate (Na_2SO_4) were purchased from Sinopharm Chemical Reagent Co. Ltd., China. Syringe filter (MCE, 0.45 μm) was purchased from PeakSharp. Rotary evaporator (EYELA-1000, Japan) was used during the experiment.

Sediment and Water Collection

Water and sediment samples were collected from West Lake (Zhejiang Province, China).Sediment samples were taken from the sediment layer at a depth of 5-15 cm. The sediment had total organic carbon content (TOC) of 21.3 mg/L and pH 6.80. The organic matter content of sediment was 3.08 g/kg. The water samples were taken from the lake water layer at a depth of 6-10 cm. It had oxygen concentration of 4.9 mg/L and pH 7.49. Water and sediment samples were kept in the dark at 4°C during sampling procedure. The samples were immediately transported to the lab and stored in the dark at 4°C until being pretreated within 24 h. Sediment samples were homogenized and wet sieved to less than 2 mm. There is no pyrimorph residue in the collected samples.

Test Design

Aerobic Test: Sediment of the lake (80 g dry weight) was put into 500 mL beakers. 240 mL of lake water was added into the beakers. The combined water and sediment were at a volume ratio of 3:1. Certain amounts of water and sediment consist of the water-sediment systems of a natural lake. Prior to spiking with pyrimorph, the water-sediment systems were acclimated to the test condition for at least 7 d. As following, the supernatant was spiked at initial concentration of 2 mg/L with stock standard solution of pyrimorph (200mg/L). The systems were incubated in the dark at 25 ± 1 °C. As J.O. Lalah's experiment [7], we set control treatment in our experiment to study the dissipation of pyrimorph in lake water only (no sediment). So in the control treatment, there is no sediment and only water was added in the water-sediment systems. Water samples and sediment samples in the water-sediment system were collected (0, 1, 3, 7, 14, 21, 28, 35, 46, 62 and 100d) for 100 days, respectively. Two replicates of the tests were run for each series. The residue of pyrimorph in water and sediment of the water-sediment system was analyzed using HPLC. The degradation product of pyrimorph was detected using HPLC-MS/MS.

Anaerobic Test: The beakers were saturated with N_2 (purity 99.9%) during the incubation period. Other experiment process was the same as aerobic test.

Analytical Procedure

Sample Pretreatment

Water: After collection, samples of water were filtered into an autosampler vial with 0.45 μm syringe filters and then analyzed by HPLC without cleanup steps.

Sediment: Sediment samples (20.0 g) were put into two 50mL centrifuge tubes and mixed with 10 mL water and 30 mL acetone. The tube was shaken in a reciprocating shaker for 1 h and centrifuged for 5 min at relative centrifugal force (RCF) 3802×g. 20 mL of supernatant water/acetone layer was transferred from the upper layer into glass stopped separating funnels of 250 mL. 5 mL of 10% NaCl water solution was added into separating funnels and supernatant water/acetone layer was extracted with 20 mL of dichloromethane for two

times. The organic extract was collected and evaporated to dryness in vacuum at 40°C water bath temperature. The dry residue in the flask was redissolved with 5 mL methanol and then analyzed by HPLC.

HPLC Analysis

The chromatographic separation was achieved using an Agilent 1100 HPLC series (Agilent technologies, USA) containing AichromBond-AQ C18 (5 μm×4.6 mm×250 mm) column (Agilent technologies, USA). The mobile phase was the mixture of acetonitrile-methanol-water (35/40/25, V/V/V) and the injection volume was 20 μL. The column temperature was maintained at 25 °C with a flow rate of 1.0 mL/min. UVD detection was set at 240 nm.

HPLC-MS/MS Analysis

The chromatographic separation was achieved using an Agilent 1200 HPLC series (Agilent technologies, USA) consisting of a G1322A degasser, a G1311A quaternary pump, a G1316A TCC, a G1329A ALS and a 1.8 μm reversed phase HT C18 (2.1 mm×50 mm) column (Agilent technologies, USA). The mobile phase was the mixture of acetonitrile-0.1% formic acid (70/30, V/V) and the injection volume was 5 μL. The column temperature was maintained at 30 °C with a flow rate of 0.3 mL/min. The effluent from the LC system was introduced into an Agilent 6410B triple-quadrupole mass spectrometer (Agilent technologies, USA), equipped with an electrospray ionization interface, operating in the positive scan mode (ESI+). The source temperature was 100 °C and desolvation gas temperature was 300 °C. The desolvation gas and nebulizer gas (N_2) were set at 10.0 L/min and 35.0 psi, respectively. For instrument control, masshunter workstation software data acquisition for triple quad B.02.01 (B2043.12) and qualitative analysis version B.03.01/build 3.1.346.0 were used for data acquisition and processing.

Dissipation Kinetics of Pyrimorph

The pyrimorph dissipation kinetics were simulated and found to be a well-fitted first order model:

$$C=C_o e^{-kt}$$

$$T_{1/2} =\ln(2)/k$$

where, C is pyrimorph concentration (mg/kg) at time t (d), C_o is pyrimorph initial concentration (mg/kg), and k is the first-order rate constant (d^{-1}) independent of C_o. Based on the logarithmic plot of normalized concentration against time, linear regressions were calculated to determine the dissipation rate constant (k) for pyrimorph using Excel 2007.

Conclusions

The environmental behaviors of pyrimorph in Lake water/sediment systems have been studied, such as the dissipation of pyrimorph and degradation product of pyrimorph. Pyrimorph dissipated very fast in water/sediment system and very slow in control treatment. The dissipation of pyrimorph in water resulted mainly from sediment adsorption of pyrimorph. For aerobic test and anaerobic test, it could be concluded that O_2 inhibited the dissipation of pyrimorph.

References

1. C. Q. Zhao, B. Q. Liu, J. Wang, N. Li, Z. H. Qin and L. H. Qiu, *Pest Management Science*, **67**, 1178 (2011).
2. Pyrimorph. *Pesticide Science and Administration*, **32**, 59 (2011).
3. X. J. Yan, W. C. Qin, L. P. Sun, S. H. Qi, D. B. Yang, Z. H. Qin and H. Z. Yuan. *Journal of Agriculture and Food Chemistry*, **58**, 2720 (2010).
4. J. Wang, L. L. Zhao, X. S. Li, Y. P. Jiang, N. Li, Z. H. Qin and C. P. Pan. *Bulletin of Environmental Contamination and Toxicology*, **86**, 326 (2011).
5. J. Wang, L. L. Zhao, Z. S. Liu, Z. H. Qin and N. Li. *Journal of Agro-Environment Science*, **29**, 2128 (2010).
6. B. J. Xu, D. Q. Mao, Y. Luo and L. Xu. *Bioresource Technology*, **102**, 7069 (2011).
7. J. O. Lalah, K. W. Schramm, B. Henkelmann, D. Lenoir, A. Behechti, K. Gunther and A. Kettrup. *Environmental Pollution*, **122**, 195 (2003).