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Acute and Chronic Toxicity of Buprofezin on *Daphnia Magna* and the Recovery Evaluation

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Abstract The toxic effects of buprofezin on *Daphnia magna* after both chronic and acute exposures were evaluated according to OECD guidelines. A 48-h acute exposure of buprofezin resulted in daphnid immobility at an EC_{50} of 0.44 mg/L. In a 14 days chronic exposure of buprofezin (0, 0.025, 0.05, 0.10 and 0.15 mg/L), the development and reproduction of daphnids were all significantly affected and the body length was more sensitive than other observed parameters. However, the adverse effects of buprofezin on parental daphnids can be passed on to their offspring and cannot be recovered in a short time.

Keywords *Daphnia magna* · Chronic toxicity · Recovery test · Buprofezin

In recent years, there has been a growing concern for the toxicity of pesticides to both the natural environment and to humans (Li and Tan 2011; Wang et al. 2009). Buprofezin, an insect growth regulator pesticide with a specific mode of action and a good record of environmental safety, has risen in popularity for use in agriculture. In particular, buprofezin played an important role in the control of Lepidoptera pests and sucking insects since 1980s. Currently, Chinese production of technical buprofezin is about 4,500 tons/year. However, with the forbidden use of methamidophos on the rice planthopper and the low effectiveness of imidacloprid on the green rice leafhopper, buprofezin has risen as the preferred alternative to control these two pests. Therefore, the authors believe that large amounts of buprofezin will continue to be produced and applied in the near future. We can expect that the volume of buprofezin in aquatic environment is accumulating and with it, the aquatic ecosystems are at risk of exposure to buprofezin.

Though burprofezin is a widely used growth regulator, studies investigating the chronic toxicity to aquatic organisms were lacking (Anonymous 2010; NRA 2001). In order to gain a better understanding of the potential effects of burprofezin in aquatic system, we evaluate the acute and chronic toxicity of buprofezin using model organism *Daphnia magna*. Additionally, a recovery test with F_1 generation offspring was also carried out.

Materials and Methods

Buprofezin (95 % purity) was supplied by Jiangsu Pesticide Research Institute Co.LTD (China) and was dissolved in acetone to make a 0.20 g/L stock solution kept at 4°C.

Daphnia magna were originally obtained from the Chinese Academy of Protection Medicine Science (Beijing, China). The daphnid food (alga *Scenedesmus obliquus*) was cultured in house.

The acute immobilization test was performed according to OECD Guideline No.202 (OECD 2004). *D. magna* were maintained under a 16:8 light: dark cycle at a temperature of 20 ± 1°C. Three replicates of ten 6–24 h old *D. magna* neonates were transferred to 50 mL beakers without food containing 20 mL of test solution. The test concentrations were 0.10, 0.30, 0.50 0.70, 0.90 mg/L and a blank control without added any pesticide. Immobility was recorded after 48 h exposure.

The chronic toxicity of buprofezin was evaluated according to OECD guideline 211(OECD 1998) and have

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some adjustments. Based on acute toxicity results, daphnids were exposed to buprofezin for 14 days at sublethal concentrations (0.025, 0.050, 0.100 and 0.150 mg/L) with one blank control and one acetone control. For each treatment, twenty neonates (age < 24 h) were transferred into separate 50 mL beakers containing 30 mL of test solution. Alga *Scenedesmus obliquus* was supplied as food at a density of 5.0–6.0 $\times$ 10^5 cells/mL. Test solutions were renewed daily while the following parameters were recorded: longevity, reproduction (number of offspring per female) and developmental parameters (body length, number of molts). During the chronic test, the first generation offspring (F_1) neonates of the 1st and 3rd brood were collected randomly from each treatment and transferred to clean medium. The parameters as the chronic test were recorded for another 14 days.

The EC_{50} value for the acute test was determined using SPSS 13.0 based on the probit analysis method. Statistical significance between different treatments in the chronic and recovery studies were analyzed using a one-way ANOVA ($p < 0.05$) with SPSS 13.0.

Results and Discussion

The 48 h EC_{50} values of buprofezin for *D. magna* immobility was 0.44 ± 0.06 mg/L; which is classified as very toxic (≤1 mg/L) according to the European Chemicals Bureau and OECD guidelines(European Commission 1996; OECD 1998). This result agreed with the EFSA (Anonymous 2010) data which indicated buprofezin was very toxic to aquatic organisms.

Chronic toxicity was determined by measuring the adverse effect of buprofezin on *D. magna* development, survival, and reproduction. Results are delineated in Table 1. Test acceptability criteria for survival (>80 %) and reproduction (>60 neonates per female at the end of the test) were met. Mortality of acetone controls and the blank controls was less than 10 % in this study, and no difference was calculated between these two groups.

All monitored parameters were affected by buprofezin at sublethal concentrations during the chronic test. Survivorship of *D. magna* was not affected ($p > 0.05$) by buprofezin concentration up to 0.10 mg/L ($p < 0.05$). Most daphnids died at the 7th day with 0.15 mg/L exposure. The number of molts was reduced by 5 and the first brood was delayed by 2 days ($p < 0.05$) at concentration greater than 0.1 mg/L. Body length parameter was significantly affected at 0.05 mg/L compared to 0.10 mg/L for offspring per female, indicating that body length is more sensitive than fecundity in this study.

Table 2 shows the recovery indexes of the 1st brood daphnids. None of the parameters were completely restored after a 14 days recovery period and there was a notable decrease calculated for all parameters at 0.05 and 0.10 mg/L. At 0.1 mg/L, only 23 daphnids were observed for number of offspring per female compared to 122 for controls. Additionally, no offspring were produced at 0.15 mg/L. The number of molts (5 times) was also significantly smaller than that for controls (9 times). The body length and days to the first brood of the 1st brood offspring were inhibited and prolonged slightly but still significantly different from that in control.

Over all, the 3rd brood daphnids showed better recovery than the 1st brood offspring. As we can see in Table 3, the adverse effect of buprofezin can still transfer from the F_0 generation to F_1 (3rd brood) generation daphnids except day to first brood. However, 3rd brood daphnids showed higher fertility than their mothers. For example, F_0 daphnids exposed to 0.10 mg/L buprofezin produced only 26 offspring per female and the body length was 2.04 cm, whereas 68 neonates were produced in 3rd brood and the body length was 2.53 cm at this concentration.

For all the buprofezin concentrations measured, a phenomenon of reduced number of young per female and longevity was detected in the 1st brood compared to the F_0 generation, and then grow higher in the 3rd brood. Take the daphnids exposed to 0.1 mg/L buprofezin concentration and their offspring for example, the number of young per female and longevity in the F_0, 1st brood and 3rd brood were: 26, 23, 69; 10, 8, 12, respectively.

Table 1 Parameters evaluation of F_0 generation of *D. magna* in chronic test

Buprofezin (mg/L)	Body length (mm)	Longevity (d)	Number of molts	Day to first brood (d)	Number of young per female
Blank control	2.73 ± 0.22	12.83 ± 1.38	9.95 ± 1.43	5.47 ± 0.77	110.88 ± 5.98
Acetone control	2.68 ± 0.26	13.20 ± 1.32	9.38 ± 2.63	5.38 ± 0.51	110.56 ± 8.46
0.025	2.66 ± 0.27	12.67 ± 1.88	9.09 ± 0.97	5.00 ± 0.00	102.40 ± 5.72
0.05	2.35 ± 0.24*	12.33 ± 1.87	9.22 ± 1.48	5.44 ± 0.53	99.00 ± 22.66
0.1	2.04 ± 0.33*	9.88 ± 0.35*	7.08 ± 1.24*	6.14 ± 1.07*	26.14 ± 12.29*
0.15	1.81 ± 0.17*	7.60 ± 0.70*	4.42 ± 1.38*	7.57 ± 0.53*	4.57 ± 2.37*

Values are mean ± SD

* $p < 0.05$

Table 2 Parameters evaluation of F_1 (1st brood) offspring-generation of *D. magna*

Buprofezin (mg/L)	Body length (mm)	Longevity (d)	Number of molts	Day to first brood (d)	Number of young per female
Blank control	2.82 ± 0.14	13.72 ± 1.18	9.22 ± 0.55	5.22 ± 0.43	122.25 ± 14.51
Acetone control	2.77 ± 0.26	13.88 ± 0.33	8.88 ± 0.60	5.17 ± 0.38	122.88 ± 13.55
0.025	2.81 ± 0.14	13.78 ± 0.94	8.67 ± 0.69	5.17 ± 0.38	128.81 ± 13.08
0.05	2.68 ± 0.26	12.76 ± 2.31	7.59 ± 0.94*	5.35 ± 0.49	103.06 ± 18.67*
0.1	2.13 ± 0.33*	8.33 ± 2.79*	5.15 ± 1.14*	5.69 ± 0.48*	23.00 ± 11.76*
0.15	No offspring				

Values are mean ± SD

* $p < 0.05$

Table 3 Parameters evaluation of F1 (3rd brood) offspring-generation of *D. magna*

Buprofezin (mg/L)	Body length (mm)	Longevity (d)	Number of molts	Day to first brood (d)	Number of young per female
Blank control	2.81 ± 0.14	13.79 ± 0.92	9.16 ± 0.69	5.05 ± 0.23	131.11 ± 18.22
Acetone control	2.78 ± 0.14	13.59 ± 1.28	8.59 ± 0.94	5.12 ± 0.33	124.06 ± 20.84
0.025	2.73 ± 0.36	12.94 ± 2.26	8.00 ± 1.37*	5.17 ± 0.38	115.78 ± 31.12
0.05	2.68 ± 0.14	13.06 ± 1.52	7.76 ± 1.03*	5.29 ± 0.47	107.59 ± 26.17*
0.1	2.53 ± 0.26*	11.56 ± 3.69*	7.47 ± 1.51*	5.33 ± 0.49	68.93 ± 26.85*
0.15	No offspring				

Values are mean ± SD

* $p < 0.05$

In the chronic test of parental daphnids, reproduction began ($p < 0.05$) after 5.47 days in controls, but was delayed to 6.14 days at 0.10 mg/L and to 7.57 days at 0.15 mg/L. During the recovery period, F_1 (1st brood) daphnids took less time to reproduction than their maternal daphnids, but, they are still significantly delayed compared to the controls. However, after a 14 days period recovery, the F_1 (3rd brood) showed no significant differences between controls and treatments. This result indicated that, time to first brood was less sensitive to buprofezin than the other parameters.

The number of molts of F_0 daphnids was significantly reduced ($p < 0.05$) at 0.10 mg/L and higher. However, this phenomenon aggravated in the F_1 (1st brood) and F_1 (3rd brood) after a 14 days recovery period in a pesticide free medium as described above. As we can see in Table 2 and 3, number of molts of F_1 (1st brood) was significantly reduced ($p < 0.05$) at 0.05 mg/L, and all the concentration tested showed significantly difference to the blank control when it comes to F_1 (3rd brood). Therefore, we conclude that buprofezin had a significantly negative effect on molting of *D. magna* at concentrations higher than 0.10 mg/L, and molting recovery is difficult. Similar results were demonstrated when buprofezin was used against glassy-winged sharpshooters and cotton leafworm (Nasr et al. 2010; Prabhaker and Toscano 2007).

Reproductive output is always considered as the most sensitive parameter to toxicants (Villarroel et al. 2003; Zalizniak and Nugegoda 2006). However, in the present chronic toxicity study of buprofezin on *D. magna*, the body length (a development indicator) was more sensitive than reproduction. A more thorough investigation is needed to prove whether these development parameters could be equally important indicators for toxicity tests using other crustaceans.

Insect growth regulators were considered to be much safer to the non-target animals than the other pesticides (Nasr et al. 2010). However, the present study indicates that these kinds of pesticides may very toxic to aquatic animals such as *D. magna,* and the recovery is hard. Attention should be taken with regards to the adverse effects that buprofezin as well as other similar insecticides might have on non-target aquatic species.

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References

Anonymous (2010) Conclusion on pesticide peer review: conclusion on the peer review of the pesticide risk assessment of the active substance buprofezin. EFSA J 8:1624

European Commission (1996) Technical guidance document in support of commission directive 93/67/EEC on risk assessment

for new notified substances and commission regulation (EC) No 1488/94 on risk assessment for existing substances, part II. Office for Official Publications of the European Communities, Luxembourg

Li S, Tan Y (2011) Hormetic response of cholinesterase from *Daphnia magna* in chronic exposure to triazophos and chlorpyrifos. J Environ Sci 23:852–859

Nasr HM, Badawy MEI, Rabea EI (2010) Toxicity and biochemical study of two insect growth regulators, buprofezin and pyriproxyfen, on cotton leafworm *Spodoptera littoralis*. Pestic Biochem Physiol 98:198–205

NRA (National Registration Authority) (2001) Evaluation of the new active Buprofezin in the product applaud insecticide. National Registration Authority for Agriculture and Veterinary Chemicals, Canberra

OECD (Organization for Economic Cooperation and Development) (1998) *Daphnia magna* reproduction test. OECD guidelines for testing of chemicals, vol 211. Paris, France

OECD (Organization for Economic Cooperation and Development) (2004) *Daphnia sp.* acute immobilization test. OECD guidelines for testing of chemicals, vol. 202. Paris, France

Prabhaker N, Toscano NC (2007) Toxicity of the insect growth regulators, buprofezin and pyriproxyfen, to the glassy-winged sharpshooter, *Homalodisca coagulata* say (Homoptera: Cicadellidae). Crop Prot 26:495–502

Villarroel MJ, Sancho E, Ferrando MD, Andreu E (2003) Acute, chronic and sublethal effects of the herbicide propanil on *Daphnia magna*. Chemosphere 53:857–864

Wang C, Chen F, Zhang Q, Fang Z (2009) Chronic toxicity and cytotoxicity of synthetic pyrethroid insecticide cis-bifenthrin. J Environ Sci 21:1710–1715

Zalizniak L, Nugegoda D (2006) Effect of sublethal concentrations of chlorpyrifos on three successive generations of *Daphnia carinat*a. Ecotoxicol Environ Saf 64:207–214