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Toxicity of cypermethrin to *Daphnia magna* HB

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Abstract: The acute toxic effect of the pesticide cypermethrin to *Daphnia magna* HB was examined. *D. magna* HB was exposed to cypermethrin at concentrations of 0, 1, 3, 5, 7, and 9 mg/L for 24 h. Data showed that the 24 h-LC_{50} of cypermthrin on *D. magna* HB was 4.81 mg/L. In contrast, the 24 h-LC_{50} of $K_2Cr_2O_7$ (the national standard toxicant) to *Daphnia magna* was 0.38 mg/L in the current study. Results indicated that the *Daphnia magna* was very sensitive to pesticides. In addition, the effects of the culture condition (such as hardness, temperature and DO etc.) on *Daphnia magna* HB was also studied.

Keywords: cypermethrin; *Daphnia magna* HB; toxicity

Introduction

The studies of the toxicity of pesticides on non-target organisms set very important models for the evaluation of the impact of pesticides on aquatic environment. *D. magna* is a necessary hinge in substance cycling and energetic floating of limnetic ecosystem and are were very sensitive to the toxicant. *D. magna* has been used as a model to assess the toxicity of chemical products, monitor the water pollution and constitute the standard of water quality (Cai, 1999; Qu, 1989; Tan, 1994; Blayock, 1985; Buikema, 1980). Although the toxicity of cypermethrin is low in some non-target organisms such as mammal, it has been reported that cypermethrin was highly toxic on aquatic animals such as fish (Qu, 1989). This study examined the acute toxic effect of cypermethrin on *Daphnia magna* HB, providing information for risk assessment of pesticides in the aquatic environment.

1 Materials and methods

1.1 Culture

1.1.1 Culture water preparation

Culture water was prepared by applying regular tap water through an active carbon column. The active carbon column was 80 cm in length, 4 cm in diameter, 100 ml/min in flowing speed, and it should be kept in dark in order to avoid generating algae, the exit of the column should be wrapped by a double-layer nylon fabric, diameter was 64 μm. Residual chlorine and impurity should be removed, blowing oxygen into the column, at last placed quietly at least 1 d.

1.1.2 Foods

Fresh *Scenedesmus obliquus* was used as bait. The cultured *S. obliquus* was collected by natural sediment method. Condensed *S. obliquus* was easily to decay when the temperature was above 20℃, therefore, it should be shaked frequently (2/d in our study). The culture water (HB-4) was renewed regularly. All glassware was sterilized.

1.2 Test materials and methods

Test animals: *D. magna* HB was parthenogenetic which was long-term cultured in laboratory. The offspring of a dam daphnia were used after 24 h pre-culture. They are high temperature tolerance type which was first isolated and kept in Insitute of Hydrobiology, Chinese Academy of Science (Xun, 1991).

Pesticides: Cypermethrin, purity was 96.4%; $K_2Cr_2O_7$, its purity was 99.8%.

Physical and chemical parameters of culture water: pH: 6.53—7.71; dissolved oxygen: 2.3—3.5 mg/L; hardness: 5.42 (Germany hardness); conductance rate: 140 us/om; redox potential: 58 mV.

NOEC (no observed effect concentration) of solvent's: Cypermethrin was only slightly dissolved in water, thus, acetone was chosen as its solvent. However, acetone was toxic to *D. magna* HB, so the NOEC of acetone should be firstly studied. Acetone was added to culture water with concentration at 0.1%, 0.15%, 0.20%, 0.50% and 1% respectively. After 96 h, compared with the control group, acetone under 0.50% had no effect on *D. magna* HB, when acetone concentration at 0.50% to 1%, the growth of daphnia had been inhibited significantly. The NOEC of acetone on *D. magna* HB was 0.15%. From above, it could be seen that the concentration of acetone should be lower than 0.15% in order to avoid the effect of solvent.

Test process: Five concentrations of cypermethrin (0, 1, 3, 5, 7, and 9 mg/L) were used, and the experiment was repeated 3 times. 10 *D. magna* HB cleaned by diluted water was put into a beaker (25 ml) containing 20 ml cypermethrin sollution. Then the tested daphnia was placed into the bio-incubator, the temperature was controlled at 21 ± 2℃. During test period, daphnia was fed on nothing for 24 h. The results were analyzed with linear regression equation with one unknown in order to gain LC_{50} (Zhou, 1989).

D. magna HB death could be identified directly with naked eyes. Although its antennae, gill and gut were still moving, the distance within 15 s was no longer than its length, the daphnia was considered to be dead.

2 Results

2.1 The toxicity of $K_2Cr_2O_7$ on *D. magna* HB

From Table 1, it could be seen the 24 h-LC_{50} was 0.363 mg/L. This obtained 24 h-LC_{50} was higher than the international criterion (0.9—2.0 mg/L), suggesting that this species *Daphnia magna* HB was very sensitive. In European countries, $K_2Cr_2O_7$ was used as standard toxicant when testing the daphnia sensitivity (National Environmental Protection Bureau, 1993). It was stipulated by ISO (1982) that 24 h-LC_{50} of *D. magna* HB must be between 0.9 and 2.0 mg/L. However, it was hard to fit for this criterion in China through many researches, and it is necessary to use another standard toxicant to evaluate the sensitivity of *Daphnia magna* HB.

Table 1 Effects of different $K_2Cr_2O_7$ concentration on *D. magna* HB

(Conditions: 10/20 ml, 24 h, 23℃)

No.	CK	1	2	3	4	5
Concentration C, mg/L	0.0	0.1	0.2	0.25	0.3	0.4
Death rate P, %	0	10	23.3	16.7	46.7	56.7
Linear equation	$P = 146.78C - 3.35$ $r = 0.9775$					
LC_{50}	0.363 mg/L					

2.2 Toxicity of cypermethrin to *D. magna* HB

The results of different concentration of cypermethrin on *D.*

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magna HB are shown in Table 2. According to this assay, the 24 h-LC_{50} of cypermethrin on *D. magna* HB was 4.81 ± 0.40 mg/L. The toxicity of cypermethrin on *D. magna* HB in laboratory is in the middle level according to the previous reports(National Environmental Protection Bureau, 1993).

Table 2 Effects of different concentration of cypermethrin on *D. magna* HB

(Conditions: 10/20 ml, 24 h, 24℃)

No.	CK	1	2	3	4	5
Concentration, mg/L	0	1	3	5	7	9
Death rate (*P*1), %	0	13.3	20	26.7	60	76.7
Death rate (*P*2), %	0	16.7	23.3	60	76.7	86.7
Death rate (*P*3), %	0	26.7	40	63.3	73.3	83.3
Concentration effect curve	Relative quotiety, *r*		LC_{50}, mg/L		Mean LC_{50}, mg/L	
*P*1 = 6.98*C* + 13.45	*r* = 0.9700		5.23		4.81 ± 0.40	
*P*2 = 9.97*C* + 2.37	*r* = 0.9826		4.77			
*P*3 = 8.75*C* + 11.26	*r* = 0.9703		4.43			

3 Discussion

3.1 Toxicity of cypermethrin on *D. magna* HB

Sun *et al.*(Cai, 1999) had already studied the acute toxicity of Γ-benzene hexchloride and furadan on *D. carinata*, the results showed that each 48 h-LC_{50} of these four kinds of pesticides on *D. carinata* was 0.000123, 0.013, 0.075 and 0.76 mg/L respectively. Chen *et al.* (Cai, 1999) had differentiated the standing crop of plankton in the pond including *Cladocera*(*Daphnia*, *Diaphanosoma*); *Copepod*(*Diaphomus*, *Cyclops*); *Rotifer* (*Epiphanes*, *Phildina*, *Brachionus*) and protist (*Amoeba* sp., *Paramaecium* and *Stylonychia*), the results showed that cypermethrin was very toxic to plankton and the sensitivity sequence was *Cladocera* > *Copepoda* > protist > *Rotifer*. The toxicity was enhanced with the concentration increasing, and dose response effect was obvious. Under the simulative condition in the field, the LC_{50} (using standing crop as index) of cypermethrin on these four kinds of plankton : *Cladocera*, *Copepoda*, *Rotifer* and protist was 0.18, 0.30, 2.00, 0.66 μg/L respectively. It was demonstrated that different pesticide had different toxicity on plankton, which means that the sensitivity of *D. magna* could be caused by different pesticides. But generally, the sensitivity of *D. magna* to pesticides was very high. The result of this assay was in accordance with some researches to some extent. This was related to the type and the purity of pesticides, and also was related to the species and the breeding of *D. magna*. The relationship between predatory and prey was very complicated in water body, the food chain might be destroyed by pesticides inducing and then the balance of ecosystem was disturbed. *D. magna* was very important plankton, main bait for fish, and a necessary hinge in substance and energetic cycling of aquatic ecosystem. Therefore, it was necessary to study the environmental impact of pesticides.

3.2 Effect of test condition on the toxicity of *D. magna*

3.2.1 Test water

Chlorin should have been removed completely when using tap water. The self-made test water was recommended to use in this test in order to avoid pollution. Because the toxicity was affected by water hardness, it was suggested that different test water should be used for different test organisms.

3.2.2 Physical and chemical parameters

Temperature: It was shown in many studies that 20—25℃ was suitable for culturing and testing *D. magna*. The sudden change of temperature should be avoided in test period, and the temperature variation should be 1℃.

Dissolved oxygen: According to many studies, the growth and generation of daphnia might be inhibited by oxygen shortage. So sufficient dissolved oxygen should be provided in the water in order to keep daphnia growing normally.

pH: Daphnia could not survive at pH 1—4 and above 12, pH 5—6 and 11 was its tolerance limitation, it could survive well at pH 8—10. So pH 9 was suggeted for *D. magna*'s culture.

Illumination: It was suggested that toxicity test should be carried on under the natural light and ratio of day:night should be 10 h:14 h.

The hardness of water: The distribution of daphnia was affected by the hardness of water. In general, the higher the hardness of water, the lower the toxicity of pesticides on daphnia was. In fact it was not clear about the effect of water hardness on daphnia toxic assay.

3.3 Standardization of daphnia bioassay

APH, ASTM, ISO, DIN, OECD all had its own recommended standard approach on daphnia bioassay respectively. There were also different standard approaches in other countries(such as the Netherlands and Japan etc.)(National Environmental Protection Bureau, 1993). Feardo(Feardo, 1996) had pointed out that the methods for culturing and testing daphnia were diverse, and the species and breeding of daphnia were also various. In order to compare the results in different laboratories, the important experimental parameter should be defined and some regulation should be pointed out or these parameters should be standardized. Thus, it is necessary to standardize international daphnia bioassay.

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