

## Behavioral Responses of *Daphnia Magna* to Stresses of Chemicals with Different Toxic Characteristics

Zongming Ren · Zhiliang Li · Mei Ma ·  
Zijian Wang · Rongshu Fu

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**Abstract** Behavior of an organism is affected by exposure to toxic chemicals. However, less has been known about behavioral responses of an organism to stresses of toxic chemicals with different toxic characteristics. In present work, *Daphnia magna* Straus was exposed to gradient concentrations of deltamethrin, chlorothalonil and nitrofen and the behavioral changes of *Daphnia magna* under different stress were examined. The results showed that the behavioral responses of *Daphnia magna* to the tested chemicals were affected in general by exposure concentration, rather than toxic characteristics of the chemicals. The duration of avoidance response (DAR) was in a power regression relationship with the toxic unit (TU), defined as the ratio of exposure concentration of the tested chemical to its  $LC_{50-48}$ . DAR was independent of the toxic characteristics of chemicals. However, significant behavior adjustment could be observed after exposure to deltamethrin while only step-by-step decrease in behavior strength could be observed when exposed to chlorothalonil and nitrofen. It was suggested from the observation that

avoidance behaviors of *Daphnia magna* to exposures of chemicals with different toxic characteristics could be similar, while their specific response could be different.

**Keywords** Behavioral responses · *Daphnia magna* · Toxic characteristics

Deltamethrin, chlorothalonil and nitrofen are extensively used in agriculture, silviculture, and urban settings and can enter surface waters through rainfall runoff, spray drift, or atmospheric deposition subsequently impacting aquatic biota. The ways that they exert their toxicity are different, though they all cause toxic effects on aquatic organisms. Deltamethrin is a type II pyrethroid (Soderlund and Bloomquist 1989) which has been encouraged to use in intensive agriculture as replacement for more toxic insecticides such as organophosphates that exert their toxicity by inhibiting acetyl cholinesterase (AChE) of organisms (Moore and Waring 2001; Duquesne 2006). Its principal molecular mode of action is the modification of the sodium channel kinetics leading to hyperexcitation of the nervous system (Narahashi et al. 1992). It induces neurotoxicity and the effects of deltamethrin on nervous, respiratory, and hematological systems in organisms are reported (Wiles and Jepson 1994; Şener Ural and Sağlam 2005; Pimpão et al. 2007). Chlorothalonil is a widely applied organochlorine nonsystemic fungicide in rural and urban environments. It inhibits important cellular enzymes (i.e., NADPH oxidase) by binding to the sulphhydryl groups altering metabolic functions in cellular respiration (Caux et al. 1996). It also depletes glutathione (GSH), a non-enzymatic reducing agent involved in cell detoxification (Tillman et al. 1973). The effects of chlorothalonil on the survival of *Daphnia magna* (Ernst et al. 1991; Fernández-Alba et al.

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Z. Ren · Z. Li · M. Ma · Z. Wang (✉)  
State Key Laboratory of Environmental Aquatic Chemistry,  
Research Center for Eco-Environmental Science, Chinese  
Academy of Science, Shuangqing Rd 18, Haidian District,  
P.O. Box 2871, Beijing 100085, China  
e-mail: wangzj@rcees.ac.cn

Z. Ren  
Yantai Institute of Coastal Zone Research for Sustainable  
Development, Chinese Academy of Sciences, Yantai 264000,  
China

Z. Ren · Z. Li · R. Fu  
College of Life Science, Shandong Normal University,  
Jinan 250014, China

2002) and the reproduction of the meiobenthic estuarine copepod *Amphiascus tenuiremis* (Bejarano et al. 2005) are reported. Nitrofen, which is a diphenyl ether herbicide, interferes with thyroid hormone and/or retinoic acid signaling and induces organ damage (Manson 1986; Brandsma et al. 1994; Mascrez et al. 1998; Greer et al. 2003) and it produces a spectrum of fetal abnormalities in rodents (Kling et al. 2005).

The freshwater cladoceran *Daphnia magna* Straus are normally considered as sensitive to contaminants (Sturm and Hansen 1999; Rosa et al. 2006). The species have often been used in bioassays and environmental monitoring of aquatic systems due to the ease and economy of maintaining cultures (Tomasik and Warren 1996; Martínez-Jerónimo et al. 2005; Heckmann et al. 2007). The initial response of an individual to contaminant exposure is a possible change in its behavior, due to visual or chemoreception (olfaction, taste) damage (Scarfe et al. 1983; Riddell et al. 2005; Mandrillon and Saglio 2007). Changes in the movement behavior of organisms can therefore be used as a suitable indicator in ecotoxicological risk assessment. According to previous research (Steele et al. 1989; Saglio and Trijasse 1996; Eriksson Wiklund et al. 2006), avoidance of the organism to contaminants is an adaptive behavior that may reduce exposure to harmful conditions and failure to avoid exposure may result in reduced fitness and survival, eventually leading to detrimental effects. The movement patterns of *Daphnia magna* have many different components and the behavioral responses of *Daphnia magna* to different contaminants are reported (Untersteiner et al. 2003; Reynaldi et al. 2006).

The current study was undertaken to assess whether different contaminants (Deltamethrin, Chlorothalonil and Nitrofen) with different toxic characteristics would induce different effects on the behavioral responses of *Daphnia magna* or not. Meanwhile, avoidance responses as the first behavior modulation of *Daphnia magna* to try to escape from the polluted environment were investigated to discuss the relationship between the duration of avoidance response (DAR) and their  $LC_{50-48}$  based toxic unit (TU).

## Materials and Methods

The experimental *Daphnia magna* (24-h young) were obtained from the Chinese Environmental Science Academy and cultured in our laboratory for more than 3 generations. The general culture of *Daphnia magna* was maintained in the Standard Reference Water (SRW) (ISO 1996a), at  $20 \pm 2^\circ\text{C}$  with 16L: 8D photoperiod. *Daphnia magna* were fed with a suspension of batch-cultured green algae (*Scenedesmus obliquus*). Before the exposure experiments, the gravid female *Daphnia magna* were taken

out and cultured individually in 50-mL glass beakers of SRW until they oviposited.

Healthy neonates (about 24 h) from the second and following clutches were applied in the study. During the individual culture of female *Daphnia magna* with eggs, the growth medium was renewed regularly and *Daphnia magna* were fed with *Scenedesmus obliquus* algae three times a week. Before feeding *Daphnia magna*, the culture medium of *Scenedesmus obliquus* was filtered and then diluted by SRW until the concentration reached  $1 \times 10^5$  cells/mL. The quantity of the algae was about 1% beaker volume.

Deltamethrin, chlorothalonil and nitrofen were purchased from J&K Chemical Ltd (Beijing). All compounds were technical grade (>95% purity). Stock solutions (stored at  $4^\circ\text{C}$  until use) with proper concentration of each chemical were prepared in dimethyl sulfoxide (DMSO, analytical pure) with appropriate aliquots used to make each test solution of a specific concentration. The concentration of DMSO in water was less than 0.5%. A study has shown that DMSO of such concentration would neither lead to acute toxicity to *Daphnia magna* nor affect the mobility of *Daphnia magna* (Sandbacka et al. 2000).

Preliminary acute toxicity test was conducted in order to obtain the 48-h median lethal concentrations ( $LC_{50-48}$ ) to *Daphnia magna* for the three contaminants, following the standard protocol for *Daphnia magna* acute test (ISO 1996b). Death of *Daphnia magna* was defined as the inability to swim for more than a few strokes within 15 s after gentle agitation of the test vessel (Villegas-Navarro et al. 1999).

In this study,  $LC_{50-48}$  was taken as one toxic unit (1 TU) and the behavioral responses of *Daphnia magna* to five concentration gradients, i.e., 0.1 TU, 1 TU, 2 TU, 5 TU, and 10 TU, were evaluated using a biomonitoring device (Gerhardt et al. 2002). The device was made up of eight test chambers (2 cm long, 1.5 cm in diameter), which were closed off on both sides with nylon nets (250  $\mu\text{m}$ ) and matched with the size of the tested organisms. Five healthy neonates about 24 h of *Daphnia magna* were selected at random and placed in each test chamber. In the device, a pair of electrodes at the walls of the test chambers emits a high frequency signal of alternating current, which was received by a second pair of non current-carrying electrodes. The movement of test organism will cause signal alternation, which was transformed by the A/D transformer and the signal changes were analyzed automatically. Behavior strength, which was defined as the intensity of behavior parameters representing motility, was detected directly by the system, and full behavior strength of tested organisms was regarded as 1 unit. The monitoring data are analyzed

using functions of “alarm generation”, which is based on the alarm algorithm in an ARIMA-model. The data were sampled automatically every 10 min and 6 data records in 1 h were used to calculate the average. The averages within 48-h exposure were used to assess the behavioral responses. Controlled the flow rate of each test chamber about 2 L per hour, and it was proved to have no effect on the motility of *Daphnia magna* (Ren et al. 2006).

During the studies, laboratory conditions were kept the same as in the culturing room. All determinations were repeated three times. Control groups were tested in a solvent solution (SRW 99.5% + 0.5% DMSO). No food was added during the experiments.

The LC<sub>50-48</sub> values were calculated with linear interpolation using SPSS 10.0. One-way analysis of variance (ANOVA) of SPSS 10.0 was performed to compare the differences of behavioral responses of *Daphnia magna* exposed to different contaminants with the control with a

95% confidence limit. Data were presented by mean of the three repetitions and curve fitting was performed with Origin 7.0.

## Results and Discussion

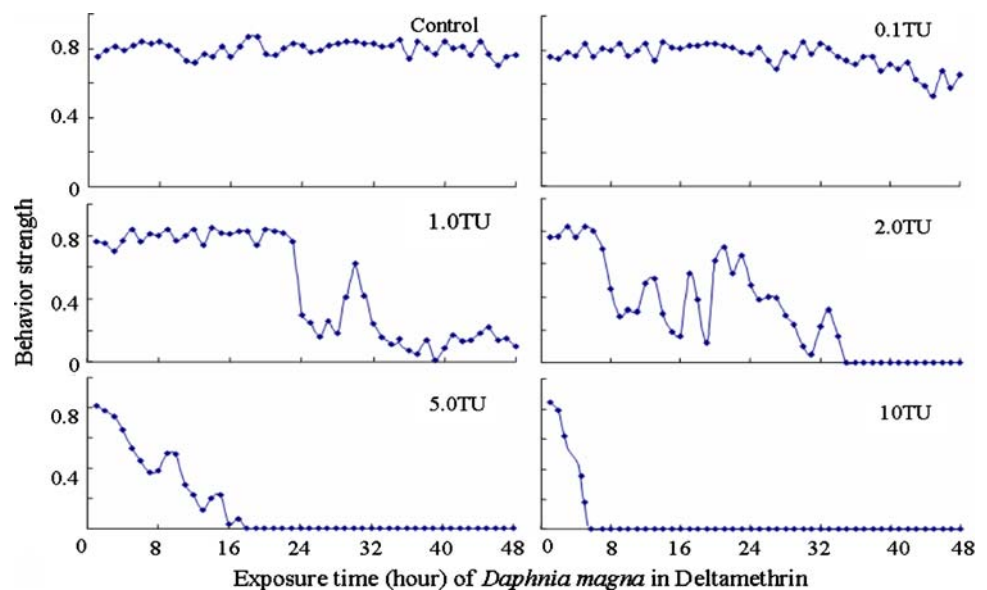
The LC<sub>50-48</sub> for *Daphnia magna* was 0.45 µg/L for deltamethrin, 75 µg/L for chlorothalonil, and 70 µg/L for nitrofen, respectively (Table 1). Though these results were close to previous research, pollutant levels of these chemicals to *Daphnia magna* were not measured here. Therefore, the exposure doses were relative.

Figures 1, 2, 3 showed the effects of deltamethrin, chlorothalonil and nitrofen on the behavioral responses of *Daphnia magna* in 48-h exposure. Within the control group, no significant changes in behavioral responses were detected. In the case of deltamethrin exposure, behavior

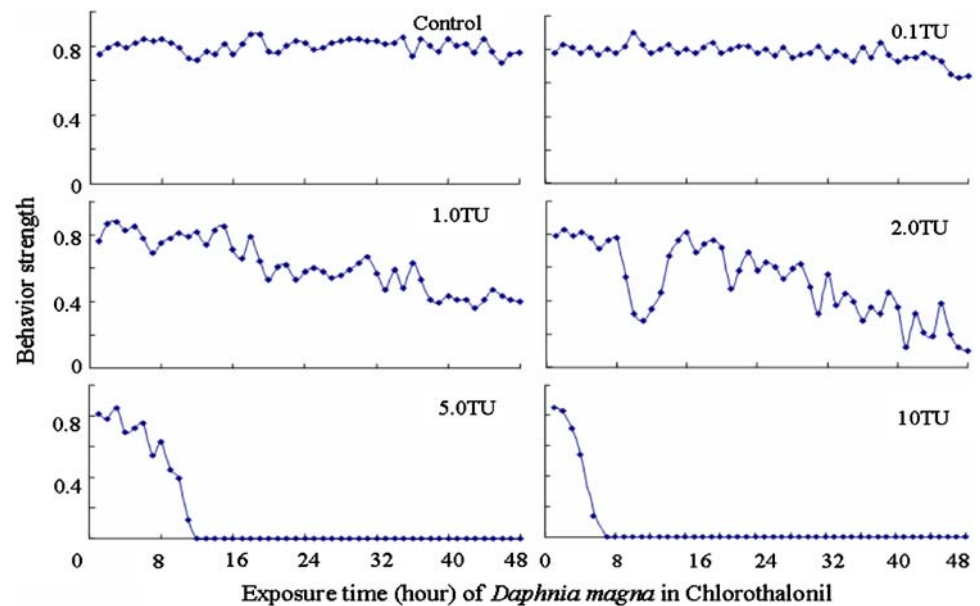
**Table 1** The acute toxic effects of deltamethrin, chlorothalonil and nitrofen on different organisms

Chemicals	Test organisms	LC <sub>50</sub> values (µg/L)			References
		LC <sub>50-24</sub>	LC <sub>50-48</sub>	LC <sub>50-96</sub>	
Deltamethrin	Fish			0.4–2.0	WHO (World Health Organization) (1990)
	<i>Oncorhynchus</i>	3.19	1.66	0.70	Şener Ural and Sağlam (2005)
	<i>Daphnia magna</i>		0.45		Author
Chlorothalonil	<i>Daphnia magna</i>		28		Fernández-Alba et al. (2002)
			81–113		Ernst et al. (1991)
			75		Author
	Oysters	108.5			Baier-Anderson and Anderson (2000)
	<i>Amphiascus tenuiremis</i>			59.4	Bejarano et al. (2005)
Nitrofen	<i>Daphnia magna</i>		70		Author

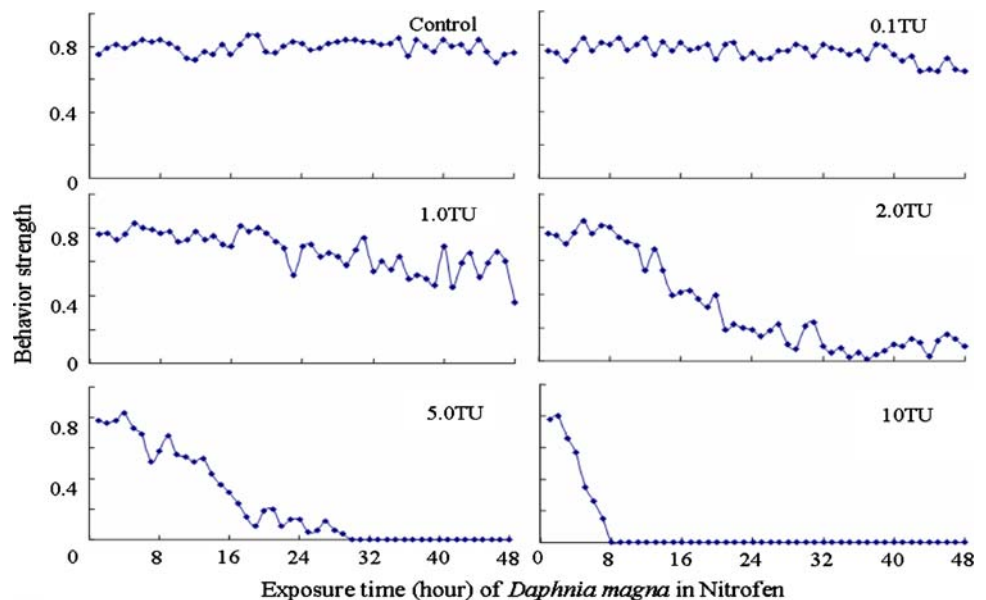
**Fig. 1** The effects of deltamethrin on the behavioral responses of *Daphnia magna* in 48-h exposure (\* $p \leq 0.05$ )



**Fig. 2** The effects of chlorothalonil on the behavioral responses of *Daphnia magna* in 48-h exposure ( $*p \leq 0.05$ )



**Fig. 3** The effects of nitrofen on the behavioral responses of *Daphnia magna* in 48-h exposure ( $*p \leq 0.05$ )

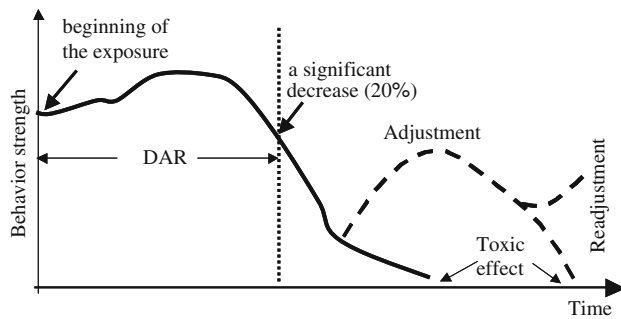


strength changed with exposure time and concentration. Higher concentrations resulted in a greater degree of the behavioral responses, as well as a shorter response time for the first phase behavioral response, which was regarded as avoidance behavior (Sager et al. 2000; Erik et al. 2005; Eriksson Wiklund et al. 2006). Even in the sublethal concentrations (0.1 TU), the first phase behavioral response could be detected.

Though the trends of behavioral responses were similar to each other, there were still some differences among the three contaminants, which may be due to the different ways they exert their toxicity. In the case of deltamethrin exposure, shortly after decrease in behavior strength, there

were regulatory responses evidently. The toxicity of deltamethrin was mainly attributed to the neurotoxic stressors, which was similar to the effects of organophosphorous pesticides. The behavior adjustment after exposure to deltamethrin resulted in similar behavioral responses as exposed to organophosphorous pesticides (Ren et al. 2007). However, for chlorothalonil and nitrofen exposures, behavior strength decreased step by step without significant behavior adjustment. Since both chlorothalonil and nitrofen are cytotoxic chemicals, the impairment to animal may develop gradually.

Behavior changes for the general adaptation of organisms in relationship to environment stress have been

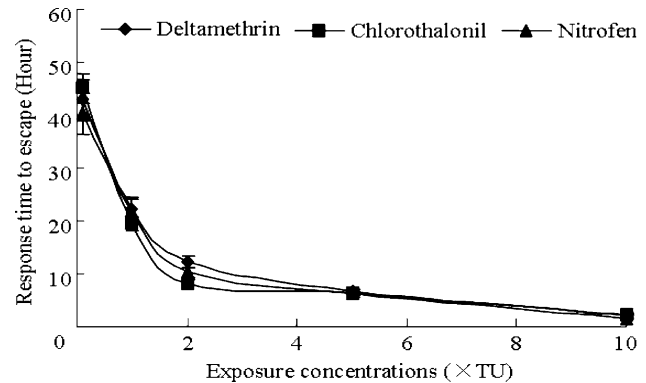


**Fig. 4** A generalized behavior response of *Daphnia magna* under exposure to different chemicals. \*Broken line stood for the effects of deltamethrin on the behavioral responses of *Daphnia magna*, in which there were regulatory responses evidently

reported in previous work (Selye 1973; Gerhardt 2001) and could be seen from Figs. 1, 2, 3.

For deltamethrin, except the 10 TU exposure, the dose-effect relationship included no effect, stimulation, acclimation, adjustment (readjustment at 0.1 TU, 1.0 TU and 2.0 TU), and intoxication. The step-wise response was summarized in Fig. 4. For chlorothalonil and nitrofen exposure, the movement behavior in almost all the exposures (except 2.0 TU chlorothalonil) decreased step by step until intoxication. No significant behavior adjustment has been observed. In comparison, chlorothalonil exposure caused a gradually decreased movement behavior followed by a regulatory response, while deltamethrin exposure followed a different adjustment/readjustment pattern. Avoidance that varied with time and concentration upon organisms is an important defense mechanism in organisms, and higher concentrations of contaminants would lead to increased avoidance (Pedder and Maly 1985). Many studies on avoidance response that was regarded as one of the most important behavioral responses of organisms to different contaminant stress had been reported (West and Ankley 1998; Lefcort et al. 2004; Eriksson Wiklund et al. 2006). There was hardly discusses on the on the duration of avoidance response (DAR), which was defined as time between beginning of the exposure and a significant decrease (20%) in behavior strength (Fig. 4). Therefore, the duration of avoidance behavior, which was mainly affected by toxic unit of the chemical, was investigated.

Figure 5 showed the effects of different contaminant stresses on DAR of *Daphnia magna* and the relationship was described in Eq. 1. In the equation, Y represented DAR and X represented the TUs of different chemicals. The equation showed that there was power relationship between DAR and the TU of different contaminants, i.e., the increase of TU would result in a significant decrease in DAR ( $p < 0.05$ ). From Fig. 5, it could be seen that the DAR-TU relationships for three chemicals were quite similar ( $R = 0.95$ ).



**Fig. 5** The duration of avoidance response (DAR) of *Daphnia magna* in different contaminant stress ( $\square$ , showed by  $M \pm S.D.$ ;  $*p \leq 0.05$ )

**Table 2** The equation parameters in different contaminant stress

Chemicals	Coefficient (a)*	Exponent (b)*	Correlation coefficient (R)
Deltamethrin	$13.87 \pm 1.03$	$-0.63 \pm 0.06$	0.95
Chlorothalonil	$12.85 \pm 1.40$	$-0.69 \pm 0.06$	0.97
Nitrofen	$12.46 \pm 0.93$	$-0.75 \pm 0.04$	0.94

\* Showed as  $M \pm S.D$

$$Y = 13.11X^{-0.71} \quad (1)$$

$$R = 0.95$$

The parameters, i.e., the coefficient (a) and the exponent (b) in equation 1, were calculated for three chemicals (Table 2). There were no significant differences among these parameters for different chemicals, indicating that DAR should be mainly affected by exposure concentration, rather than the toxic characteristics. Therefore, the avoidance behavior of *Daphnia magna* under exposure of toxic chemical could be used as the early warning thresholds of different toxic chemicals, even though they had different toxic characteristics.

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