The Avoidance Responses of *Daphnia magna* to the Exposure of Organophosphorus Pesticides in an On-Line Biomonitoring System

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Abstract In this study, avoidance behavior of the freshwater cladoceran *Daphnia magna* Straus was used as indicator to assess the early stress of accidental organophosphorus pesticide (OP) contamination. The movement behavior was detected by a multi-species biomonitoring system. There was obvious concentration–response relationship between the OP stress and the behavioral response even at sublethal exposure. A rising OP stress resulted in a significant decrease of response time to escape (RTE; $p < 0.05$). In comparison of different OPs, it was found that there was a power regression between RTE and the toxic unit of OPs. Therefore, the avoidance behavior of *D. magna* was a sensitive indicator of sublethal OP stress, and the power relationship could be used to predict the early warning thresholds of more OPs in the on-line biomonitoring system.

Keywords Avoidance behavior · *Daphnia magna* · Organophosphorus pesticide (OP) · Response time to escape (RTE) · Toxic unit (TU)

1 Introduction

The widely used organophosphorus pesticides (OPs) exert their toxicity by inhibiting acetyl cholinesterase (AChE) of organisms [3, 6]. Therefore, extensive use and discharges of OPs in environment may impair biological communities and the accidental release into source water may cause unexpected human intoxication.

The initial response of an individual to contaminant exposure is a possible change in its behavior due to visual or chemoreception (olfaction, taste) damage [22, 26]. Changes in the movement behavior of organisms can therefore be used as a suitable indicator in ecotoxicological risk assessment. According to previous research [8, 9, 19, 28], avoidance of 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. AChE inhibition may cause hyperactivity, loss of coordination, convulsions, paralysis, and other kinds of behavioral changes. Hence, it may be appropriate to assess OP toxicological impact by detecting the avoidance behavioral changes in a non-target invertebrate species. Invertebrate behavioral studies are most commonly developed [2, 13, 16]. The freshwater cladoceran *Daphnia magna* Straus are normally considered as sensitive to contaminants [23, 29], and behavioral responses to contaminants were reported [11, 21, 30].

The current study was undertaken to assess the effects of four OPs on the avoidance behavior of *D. magna*. The correspondence between the response time to escape (RTE) of *D. magna* and different OP stress, after 48-h exposure to dichlorvos, malathion, parathion, and methyl parathion, was investigated using the movement signal detected by a multi-

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species biomonitoring system to show whether the avoidance behavior was feasible as indicator to evaluate early stress of accidental OP contamination.

2 Materials and Methods

2.1 Device

The movement behavior of test organism in this study was detected by a multi-species biomonitoring system equipped with eight test chambers [10]. The measurement principle is based on quadropole impedance technique. Test organisms are placed in flow-through test chambers (2 cm long, 1.5 cm in diameter) of the system, which is closed off on both sides with nylon nets (250 μm). One pair of electrodes at the walls of the test chambers sends a high frequency signal of altering current, which is received by a second pair of non current-carrying electrodes. The on-line movement signal of test organisms is transformed by the A/D transformer. Translated by discrete Fast Fourier Transform, on-line movement signal is changed into time series data simultaneously, and the original data can be only exported in movement frequency type that changes from 0.5 Hz (–0.5) to 8.5 Hz (–8.5). The signal changes formed by the A/D transformer are analyzed automatically. The on-line monitoring data are analyzed using functions of “alarm generation,” which is based on the alarm algorithm in an ARIMA-model.

2.2 Test Species

The experimental *D. magna* (24-h young) were obtained from the Chinese Environmental Science Academy and cultured in our laboratory for more than three generations. The general culture of *D. magna* was maintained in the standard reference water (SRW) [15], at $20\pm 2^\circ\text{C}$ with 16L/8D photoperiod. *D. magna* were fed with a suspension of batch-cultured green algae (*Scenedesmus obliquus*).

Before the exposure experiments, the gravid female *D. magna* were taken out and cultured individually in 50-ml glass beakers of SRW until they oviposited. As *D. magna* neonates from the first brood are usually the smallest [18] and much more sensitive to different growth media, healthy neonates (about 24 h) were always taken from the second and following clutches as recommended [1]. During the individual culture of female *D. magna* with eggs, the growth medium was renewed regularly, and *D. magna* were fed with *S. obliquus* algae three times a week. Before feeding *D. magna*, the culture medium of *S. obliquus* was filtered and then diluted by SRW until the concentration reached 1×10^5 cells/ml. The quantity of the algae was about 1% beaker volume.

2.3 Test Chemicals

Dichlorvos, malathion, parathion, and methyl parathion were purchased from J&K Chemical Ltd. All compounds were technical grade (>95% purity). Stock solutions (stored at 4°C until use) with proper concentration of each chemical were prepared in dimethyl sulfoxide (DMSO) with appropriate aliquots used to make each test solution of a specific concentration. All solvents were of analytical grade.

The concentration of DMSO in water was less than 0.5% in all experiments. A study has shown that DMSO of such concentration would neither lead to acute toxicity to *D. magna* nor affect the mobility of *D. magna* [25].

2.4 Experimental Setup

According to previous works [4, 12, 17] and the Pesticide Ecotoxicity Database of US Environmental Protection Agency [31], the 48-h median lethal concentrations (LC_{50-48}) for *D. magna* (24 h young) were 0.20 (0.14–0.27) $\mu\text{g/l}$ for dichlorvos, 2.2 (0.7–3.5) $\mu\text{g/l}$ for malathion, 5.0 (2.7–7.5) $\mu\text{g/l}$ for parathion, and 8.7 (6.4–11.0) $\mu\text{g/l}$ for methyl parathion. In this study, LC_{50-48} of different OP was taken as one toxic unit (1TU) and acute 48-h toxicity tests under flow-through conditions were performed for seven concentration gradients, i.e., 0.1TU, 0.5TU, 1TU, 2TU, 5TU, 10TU, and 20TU, to evaluate the effects of different OPs on the avoidance behavior of *D. magna*. Control groups were tested in a solvent solution (SRW99.5%+0.5% DMSO). No food was added during the experiments.

Although the behavior strength had been applied to assess the effects of environment stressor on the behavior of some organisms [5, 14], there was not a precise concept yet. In this study, behavior strength is defined as the intensity of behavior parameters representing motility. It is an integrative reflection of all instantaneous motions, e.g., swimming velocity, behavioral frequency, and movement extent, and so on. Full behavior strength was regarded as 1 U.

Five healthy neonates about 24 h of *D. magna* were selected at random and placed in test chamber. Controlling the flow rate of each test chamber about 2 l/h, it was proved to have no effect on the motility of *D. magna* [20]. The data were sampled automatically every 10 min, and six data records in 1 h were used to calculate the average. The averages within 48-h exposure were used to assess the behavior strength changes. The experiment was repeated three times for each 48-h exposure.

2.5 Statistical Analyses

The average values of the three repetitions of exposure experiments were presented. One-way analysis of variance of Statistical Package for the Social Sciences 10.0 software

was performed to compare the effects of OP stress on the behavior strength of *D. magna* with the controls. Curve fitting was performed with Origin 7.0.

3 Results and Discussion

3.1 The Effects of OP Stress on the Behavior Strength

Figures 1, 2, 3, and 4 showed the effects of different OPs on the behavior strength of *D. magna* in 48-h exposure. Within the control group, no significant changes in behavior strength were detected. In the case of OP exposures, whether exposed in high or low concentrations OPs, the movements of *D. magna* changed in a similar manner. Comparison of behavioral responses of *D. magna* to different OPs with the results from 48-h exposure experiments indicated that there were obvious concentration–response relationships between OPs and the behavioral changes. Higher concentrations of the OPs resulted in a greater degree of the behavioral changes, as well as a shorter response time for the first behavioral response. One TU of OPs usually exceeded avoidance threshold concentrations and even in the sublethal concentrations of OPs (0.1TU), the behavioral changes were evident. These results advised that the behavior strength was time-dependent (<http://www.skleac.ac.cn/Publications.asp>).

Besides these changes, the behavioral responses of *D. magna* to different OPs in the same concentration levels were similar. All the behavioral changes showed in Figs. 1, 2, 3, and 4 were in accordance with the general adaptation of organisms in relationship to environment stress according to the description of Selye [27]. Shortly, upon continued exposure to OPs stress, no organism could be maintained continuously in a state of alarm. If the stress was so drastic

Fig. 2 The effects of Malathion on the behavior strength of *Daphnia magna* in 48-h exposure. * $P \leq 0.05$

that continued exposure became incompatible with life, the organism lost its mobility during the alarm reaction (Threshold 1) within the first behavioral response phase in a short time (in 10TU and 20TU concentration levels of different OPs). If the organism could survive, this initial reaction was necessarily followed by a stage of resistance. After still more exposure to the stress (in 1TU, 2TU, and 5TU concentration levels of different OPs), the acquired adaptation is lost again (Threshold 2). The organism entered into a third phase, the stage of exhaustion. Under constant stress, the toxic effects happened. Once behavior adaptation had occurred, and ample energy was available, resistance based on the movement behavior should go on indefinitely (in 0.1TU concentration levels of different OPs). The consequential reactions of *D. magna* in a certain behavior under OP stress included the categories of No effect, Stimulation, Acclimation, Adjustment, Toxic effect,

Fig. 1 The effects of Dichlorvos on the behavior strength of *Daphnia magna* in 48-h exposure. * $P \leq 0.05$

Fig. 3 The effects of Parathion on the behavior strength of *Daphnia magna* in 48-h exposure. * $P \leq 0.05$

Fig. 4 The effects of Methyl Parathion on the behavior strength of *Daphnia magna* in 48-h exposure. * $P \leq 0.05$

and Readjustment, as shown in Fig. 5. As the time length of different behavior categories was decided by OP stress, the lasting time of the time-dependent reactions of *D. magna* in a certain behavior under OP stress was highly variable, and it was normality if the lasting time of a certain behavior reactions was very short or even zeros (Figs. 1, 2, 3, and 4).

Different behaviors of different species had different toxicant sensitivity, but the first behavioral response to the stress, by which organisms tried to escape from the pollution source with higher behavior strength, was the general behavior adaptation of organisms, and it was defined as “avoidance behavior” [7, 8, 24]. Therefore, according to the results of the behavioral responses of *D. magna* to OP stress, avoidance behavior was mainly made up of no effects and stimulation actions in Fig. 5, and the RTE could be regarded as a feasible indicator in early warning of accidental OP contamination.

3.2 The Relationships Between RTE and OP Stress

Avoidance is an important defense mechanism in organisms, and no organism could maintain avoidance behavior at all

Fig. 5 The theoretical reactions of *Daphnia magna* in a certain behavior under OP stress

times. In this study, once the decrease of behavior strength was significant in the first behavioral response of stress, it was regarded that the avoidance responses of test organisms to contaminations terminated. Therefore, RTE was determined by strength changes of the first behavioral response. And the first significant decrease of behavior strength was observed while differences between average values of first and second six data records were more than 20%.

Figure 6 showed the changes of RTE in different OP stress. The relationship between RTE and the TU of OPs is defined as

$$Y = 12.12X^{-0.70} \quad (1)$$

$$R^2 = 0.86$$

where Y represents RTE and X represents the TU of OPs. The equation suggested that it was power relationship between RTE and TU of OPs, and a rising OP stress induced a significant decrease of RTE ($p < 0.05$).

According to the RTE Eq. 1, in the on-line monitoring accidental OP contamination, the measurement and alarm system were designed as follows.

Among the eight test chambers, two were used as control, three were used for test solutions, and the rest three were used for the 1:1 mixture of control and test solutions. Therefore, the relationships between RTE and OP stress could be described as

$$\begin{cases} Y = 12.12X^{-0.70} \\ Y = 12.12(X/2)^{-0.70} - T \end{cases} \quad (2)$$

where T represents the difference of RTE between test solutions and 1:1 mixture of control and test solutions, and it could be showed directly by the system.

According to the RTE Eq. 2, if an accidental aquatic OP contamination happened, the biomonitoring system could detect the time when the accidental contamination happened (Y value) and the relative toxicity (based on TU) of OPs for test organisms (X value) in theory. Therefore, the

Fig. 6 The response time to escape (RTE) of *Daphnia magna* in different OP stress. Symbols Showed by mean $\pm$ S.D. * $P \leq 0.05$

relationships between RTE and OP stress could be applied in early warning of accidental aquatic OP contamination.

4 Conclusions

This study proved behavioral responses of the freshwater cladoceran *D. magna* Straus to be sensitive bioindicators to OP stress. Changes in the behavioral parameter “behavior strength” can be used as early stress responses for acute aquatic OP contamination as part of ecological risk assessment.

The results from the present study showed that a rising OP stress induced a significant decrease of RTE ($p < 0.05$), which seems to be a very sensitive biological endpoint. An intense association was observed between the endpoint and avoidance; furthermore, avoidance was determined to be much more sensitive than lethality. Therefore, RTE should be recommended as a complementary tool in on-line monitoring accidental OP contamination.

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