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Acute and Chronic Effects of Pulsed Phenol Exposure to *Moina macrocopa*

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Abstract. The toxicity of phenol to aquatic biota has been studied extensively for a long time. However, most studies have used a constant concentration aqueous exposure of phenol to an invertebrate species. This research was designed to characterize the toxicity effects of pulsed phenol (C_6H_5OH) exposure to *Moina macrocopa*. The *M macrocopa* was exposed to 2.51, 3.98, 6.31, 10.0 mg C_6H_5OH l^{-1} at 6 h phenol pulses in this experiment. Our study demonstrated that the increase of pulsed concentration in the exposure of the parent generation (F_0) led to the decrease of EC_{50} and an increase of 21-day mortality of the first generation of offspring (F_1). The cumulative reproduction (21-day CR) of F_1 was significantly higher than this of F_0 when they experienced the same pulse.

Introduction

Pollution incidents of the aquatic environment often occur as pulses, such as discharge of storm water, overflow from sewage treatment plants, spraying of pesticides, industrial discharges, and runoff/drainage from fields during rain events [1, 2]. Some studies have shown that such pulse discharges may result in even more pronounced effects than continuous discharges, but in the literature, most information regarding the aquatic toxicity of organic compounds is still based on continuous exposures [1, 3]. Today, however, we still know little about the short-term and long-term toxic effects on phenol compounds during pulse exposures. Meanwhile, the offspring of exposed organisms may be affected during times of pulse and their responses are important to conduct more complete ecological risk assessment. The aim of the present study therefore was to investigate short-term (48 h) and long-term (21 d) effects of pulse exposures with phenol (C_6H_5OH) on mobility and reproduction in a model *M macrocopa*. We propose that results obtained in this way be applied in effect assessments for intermittent releases, especially in an ecological or field context.

Materials and methods

M macrocopa, an important and cosmopolitan group of zooplankton in freshwater, were used in the present study. The cladoceran *M. macrocopa* clone (from the Chinese Academy of Sciences, Wuhan, PR China) used in this study had been cultured in our laboratory for more than 5 years. *M. macrocopa* were fed a suspension of the unicellular green alga *Pseudokirchneriella subcapitata* three times a day. The feeding rate was 2×10^5 cells/ml/d. Algae were grown in the medium described in the International Organization for Standardization (IOS) guideline 8692[4].

The 48 h acute toxicity tests using *M. macrocopa* were conducted according to the OECD standard operating procedure 202 [5]. Based on acute toxicity tests, appropriate concentrations were selected for the pulse-exposure tests. Two-days-old *M. macrocopa* were used in the experiment. Pulse-exposure time was 6 h. After exposure, organisms in each beaker were transferred to control culture media in clean beaker and maintained until the test termination (21-d). Offspring and dead organisms were removed daily.

Water chemistry was characterized at the start and end of exposures and weekly during each test according to OECD standard methods. The pH and dissolved oxygen concentration, hardness, and alkalinity in all test waters were 7.39±0.06, 8.24±0.03 mgl^{-1}, 106±5 mg/l as $CaCO_3$, and 65±3 mg/l as $CaCO_3$, respectively.

To ensure agreement between nominal and actual phenol concentrations in the aquaria, water samples were analyzed during the experimental period by LC–MS/MS. Water samples were collected from the test aquaria after 1 h and 24 h of renewing the test solutions.

The statistical analysis was performed using a one-way ANOVA (Origin 8.0 Corporation, USA). The 48-h EC_{50} and their 95 percent confidence limits were calculated. Student t test was applied to study the relationship between the different concentration samples. A $p < 0.05$ was considered to be significant.

Results

Acute toxicity tests： The EC_{50} values generated by phenol to the F_1 generation are presented in Fig. 1. The parents were abbreviated to F_0. The first generation was abbreviated to F_1. On the scale of test, the EC_{50} of F_1 declined continuously with the increase of pulsed concentrations of F_0. As shown in Fig.1, all EC_{50} values were lower than control. Specially, the EC_{50} values of 6.31 and 10.0mg $C_6H_5OHl^{-1}$ were significantly decreased compared with the control ($p < 0.05$)

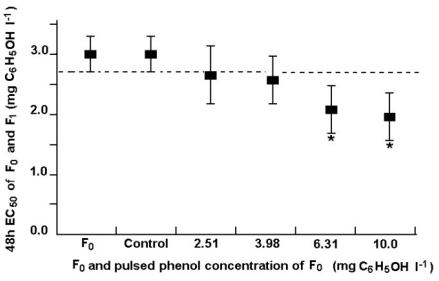

Fig.1 48 h EC_{50} values with 95% confidence intervals (bars) of F_0 and F_1（the production of offspring after F_0 generation pulse exposure）for phenol. Asterisks (*), above a bar mean, indicates a value that was significantly different from control ($p < 0.05$).

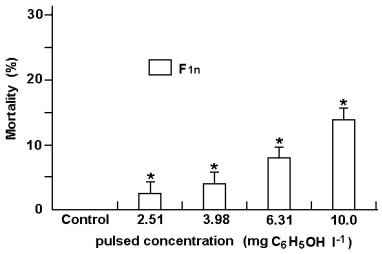

Fig 2 21-days mortality with standard deviation (error bars) of F_{1n}(no pulsed F_1 was abbreviated to F_{1n}). F_0 generation was pulsed at 2.51, 3.98, 6.31 and 10.0mgl^{-1} phenol. Asterisks (*), $p<0.05$

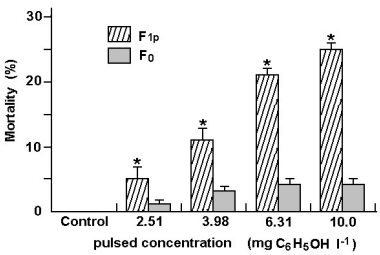

Fig 3 21-days mortality with standard deviation (error bars) of F_0 and F_{1p} (pulsed F_1 was abbreviated to F_{1p}) at 2.51,3.98,6.31,10.0 mg C_6H_5OH l^{-1} ,*$p<0.05$

21-days mortality：In the present study 21-days mortality of no pulsed F_1 (no pulsed F_1 was abbreviated to F_{1n} in Fig. 2) was affected by pulsed phenol exposure of F_0 generation. 21-days mortality of F_1 increased continuously with the enhancement of pulsed concentrations of F_0 (Fig. 2). After a single pulse of phenol, the 21-day mortality of F_0 generation was all less than five percent at 2.51, 3.98, 6.31 and 10.0mg$C_6H_5OHmgl^{-1}$ exposure and differences between control and treatment groups were not obvious (Fig.3). But for pulsed F_1 generation (pulsed F_1 was abbreviated to F_{1p} in Fig. 2b), the 21-day mortality is 5 percent, 11 percent, 21 percent and 25 percent at the four phenol treatments, respectively.

Reproduction： Effects of pulsed phenol exposure on the reproduction of *M. macrocopa* are presented in Table 1. Pulsed phenol exposure on the reproduction consisted of four concentrations, ranging from 2.51 to 10.0 mg l^{-1}. There is no significant difference between phenol treatments and control of F_0 on time to first brood (TFB， age at which organism produce the first neonate generation) and 21-d CR (cumulative reproduction). Pulsed phenol exposure decreased 21-d CR of F_{1n} at the highest treatment concentration 10.0mg l^{-1} ($p < 0.05$). However, this reduction 21-d CR of F_{1n} did not resulted in a change on the corresponding TFB ($p > 0.05$). A significant effect on 21-d CR of F_{1p} occurred in 3.98mgl^{-1} and higher, but no significant inhibition on TFR occurred at concentration below 10.0 mg l^{-1}. ($p > 0.05$).

Table 1 Reproduction of *M. macrocopa* pulsed to phenol in the control group and exposed groups (2.51, 3.98, 6.31 and 10.0mg C_6H_5OH l^{-1}). All values are means± SD (n = 4). Asterisks (*) represent $p < 0.05$ (Significantly different from control).

Concentration （mg C_6H_5OH l^{-1}）	control	2.51	3.98	6.31	10.0
Time to first brood (TFB) (d)					
F_0	8.6±0.8	9.6±2.0	8.0±1.3	10.0±3.0	9.0±1.2
F_{1n}	8.0±0.1	8.8±2.2	9.0±3.5	10.0±3.2	8.4±2.4
F_{1p}	8.8±0.3	9.5±2.5	10.5±0.6	9.8±0.6	16.0±3.1*
21-d cumulative reproduction (21-d CR)					
F_0	90±30	93±27	104±45	87±27	96±19
F_{1n}	130±19	101±13	98±30	112±32	81±24*
F_{1p}	108±25	84±51	46±29*	55±36*	30±18*

* Significantly different from control.

Discussion

EC_{50} and 21-Days mortality： Delayed effect which most often was represented by latent mortality of exposed organism themselves has been as a predictor of pulse exposure [6-8]. The response of offspring to pulse exposure, however, remains largely unknown. In the present study, the survival state of F_0 was little affected after pulsed four concentrations, 6 h phenol exposure and latent mortality was less than five percent in 21 days of observation. But the EC_{50} of F_1 dropped continuously as the pulsed copper and phenol exposure of F_0 added. As shown in Fig.1, the EC_{50} values was significantly lower with 6.31 and 10.0 phenol mgl^{-1} than in the control in our experiments ($p < 0.05$). The offspring of *M. macrocopa* which were exposed 6.31, 10.0 mgC_6H_5OH l^{-1} should distinctly reduce tolerance to phenol compared to control. Our data show that the reduction of EC_{50} to F_1 is closely correlated with the pulsed phenol exposure of F_0. Perhaps for trans-generation effect the moment that pulsed exposure of F_0 occurred might need to be considered more comprehensive.

Reproduction： There is no significant difference on TFB and 21-d CR between phenol treatments and control *M. macrocopa* of F_0 generation in this study. Hoang et al. reported that growth and reproduction of surviving *D. magna* were not affected by single pulsed Se exposure [8]. The result is similar to our experiment data. Hosmer et al. found there were no significant effects on AFR of first and second generation *D. magna* in any age group[9], but he did not investigate the effect on 21-CR. Our experiments show that there is no difference in the TFB of F_{1n} (no pulsed F_1 was abbreviated to F_{1n}), compared to control, but the 21-CR of the C_6H_5OH10.0 mgl^{-1} were lower than the control (Table 1 $p < 0.05$). In absence of compensatory processes, stressful environmental conditions would be expected to negatively impact growth, energy storage and reproduction [10, 11].

The toxic effects of continuous phenol exposures have been documented. However, virtually no information on the toxicity of pulsed phenol exposures on *M. macrocopa* exists, despite such exposures being expected to occur frequently in the field from anthropogenic sources such as effluent discharges and pesticide applications [12]. There is also a lack of research on the chronic effects of pulsed copper exposures. So far, the limited data made by researchers are insufficient to answer the reproduction toxicity effects of pulsed phenol (C_6H_5OH) exposure to *Moina macrocopa*. A significant inhibition on 21-d CR of F_{1p} occurred at concentrations of 3.98 mg C_6H_5OH l^{-1} and higher, but no significant inhibition on TFB occurred at concentration below 10.0 mg C_6H_5OH l^{-1}(Table 1).

Intermittent, fluctuating and pulsed contaminant discharges result in organisms receiving highly variable contaminant exposures [12]. We propose that results obtained in this way be applied in effect assessments for intermittent releases, especially in an ecological or field context.

Conclusions

Our study demonstrated trans-generation effects of pulsed C_6H_5OH exposure on *M. macrocopa*. Based on the results, the mortality of first filial generation (F_1) was significantly higher level than

parent generation (F_0) when they experienced the same phenol pulse. The increase of pulsed phenol concentration of parent generation (F_0) can lead to the decrease of EC_{50} and the increase of 21-day cumulative mortality in first filial generation (F_1). The decline in EC_{50} and the increase of 21-day cumulative mortality in first filial generation (F_1) can be caused by increased pulse concentration of phenol to parent generation (F_0).

Acknowledgements

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