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Assessing the impacts of dimethoate on rotifers' reproduction through the pre-exposure history

Ruixin Guo, Jianqiu Chen *

Department of Environmental Science, China Pharmaceutical University, 210009 Nanjing, China

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ABSTRACT

Organism usually undergoes an exposure of environmental pollution after a maternal exposure before birth. Traditional toxicological studies often initiated with rotifer neonates derived from the unexposed mothers while ignoring the pre-exposure (maternal exposure). The present study assessed the effect of dimethoate on the reproduction of the rotifer *Brachionus calyciflorus*, considering how the pre-exposure occurred in the parental generation influenced the subsequent impact. The F_0 generation rotifers were exposed to the pesticide at five concentrations until the first F_1 generation rotifers were reproduced. The neonates (F_1 generation) were then exposed to the pesticide at the corresponding concentrations. The offspring reproduction, the time begins to reproduce, the duration of the reproductive period and the lifespan of the F_1 generation rotifers were evaluated. Our results indicated that dimethoate influenced the maturation and reproduction of the rotifers. The highest concentration (1.8 mg L^{-1}) of dimethoate caused an inhibition in the offspring reproduction, shortened the life span and reduced the duration of the reproductive period. In addition, of particular interest in our study was that reproduction is also accelerated by the lowest concentration (0.2 mg L^{-1}). However, the pre-exposure had a significant effect on the subsequent impact. The dimethoate pre-exposure increased the impacts when the F_1 generation rotifers were exposed to the substance, even at the same concentrations as in pre-exposure. It suggests that the maternal exposure history before birth is also important and has the long-lasting consequence from one generation to another.

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1. Introduction

Rotifer is the major component of zooplankton in freshwater, functioning as a grazer on phytoplankton and as prey for many larval fish and invertebrates such as crab, shrimp, cyclopoid and copepods (Nogrady et al., 1993). It often affects the algal species composition and water quality. With the high assimilation efficiency and rapid turnover rate, the organism plays an important role in the transfer of energy from primary producers to secondary and tertiary consumers in aquatic food webs (Wallace and Snell, 2001). It is also useful as a model in toxicity assessments and a standardized method exist for estimating acute and chronic toxicity of chemicals (APHA, 1998, ASTM, 1998, Snell and Janssen, 1995). There are several advantages of the rotifer that facilitates its use in ecotoxicological studies: (1) neonate rotifers hatch synchronously and in physiologically uniform condition, (2) hatching from resting eggs for elimination of the need to culture test animals, (3) short life cycle and rapid reproduction, (4) small body size, which need small cultured volume and amount of test compounds (Snell and Janssen, 1995). Thus, rotifers have been used to assess the toxicity of many substances including natural toxin, pesticides, heavy metal, antibiotics, ambient fine aerosols and hormone (Isidori et al., 2005; Marcial et al., 2005; Araujo and McNair, 2007; Arias-Almeida and Rico-Martinez, 2011a, b; Dahms et al., 2011; Verma et al., 2013; Garcia-Garcia et al., 2014). Pesticides usually pose some serious ecological problems because of their toxicities to both target and non-target organisms and their wide distribution in the environment (Wang et al., 2009; Loewy et al., 2011). They often interact with hormones and may exert adverse consequences as a result of their actions as endocrine disrupting chemicals (EDCs) (Frye et al., 2012). It is therefore important to clarify the reproductive disruption of rotifers by pesticides since their important roles in aquatic ecosystems. Many previous studies have been designed to investigate the effects of sub-lethal exposure to pesticides on the reproduction of rotifers. When exposed to lindane and 3.4-dichloro aniline, the generation time, the net reproductive rate and reproductive value of the rotifer *Brachionus calyciflorus* decreased with increasing toxicant concentrations (Ferrando et al., 1993). The effect of the pesticide aldrin on the life history characteristics of *B. calyciflorus* was

* Corresponding author. Fax: +86 25 86185190.
E-mail address: cjqalga@163.com (J. Chen).

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evaluated. Aldrin at relatively high concentrations (0.16–1.28 mg L^{-1}) could prolong the duration of the juvenile period while the relative lower concentrations (0.04–0.16 mg L^{-1}) prolonged the duration of reproductive period of the rotifer (Huang et al., 2007). Moreover, the organochlorine pesticide chlordecone significantly influenced the life history of the rotifer *B. calyciflorus*. 50 μg L^{-1} of the pesticide significantly prolonged the reproductive period and finally increased the lifetime egg production. It also prolonged the mean life span of the rotifers (Zha et al., 2007). In addition, the asexual and sexual reproduction of the rotifer were inhibited by thiophanate-methyl while were enhanced by other pesticide glyphosate. It also indicated that the sexual reproduction was more sensitive than the asexual reproduction to the two pesticides (Xi and Feng, 2004). The marine species *B. plicatilis* when was exposed to diazinon, fenitrothion, methoprene and isoprothiolane reproduced significantly fewer resting eggs. The hatching rate of the resting eggs under the four kinds pesticide was also significantly lower (Marcial et al., 2005). The previous study indicated that the pesticide chlorpyrifos, pentachlorophenol,methoprene and flutamide influenced the reproduction of rotifers as the potential environmental endocrine-disruptors (Preston et al., 2000).

Although the pesticides declined in water, aquatic animals are usually exposed to events of pollution (Ashauer et al., 2007), as a consequence of spray drift, surface runoff, drain flow and accidental spillage from factories or farms. Thus, the freshwater organisms are possibly exposed to the same pesticide several times during a period, especially in a periodic spraying of the given pesticide. Generally, the impact of environmental stresses which on the parental generation of the organism usually influenced the subsequent different life stages. Considering the varied exposure history, there should be different impacts among the first exposure and the subsequent several exposures. However, most common acute and chronic toxicity tests are only initiated with the rotifer neonates derived from the unexposed mothers, thus frequently failing to consider the effects of the pre-exposure to contaminants at the early life stage such as embryonic and hatching processes.

The previous study indicated the transgenerational and developmental exposure of Japanese medaka to ethinylestradiol (Foran et al., 2002). A recent work has also evaluated the effect of metal pre-exposure, which seemed to have mainly a negative effect on glutathione transfer activity in the gut of armyworm larvae (Kafel et al., 2014). Thus, the main purpose of the current study was to evaluate the impacts of a widely used organophosphorus pesticides (OP), dimethoate on the reproduction of the freshwater rotifer *Brachionus calyciflorus*. The pesticide was reported to inhibit acetylcholinesterase (AChE) and introduce endocrine disrupting action on vertebrates. We are especially interested in whether the maternal exposure of the rotifer as the pre-exposure influenced the subsequent exposure. We hypothesized that the reproduction of the rotifers to dimethoate impact would be different if they were pre-exposed to the pesticide or not.

2. Material and methods

2.1. Test organisms

The test rotifer *B. calyciflorus* was originally isolated from Sheshan reservoir in the southern suburb of Nanjing and was cultured in artificial freshwater medium (EPA medium, prepared daily by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$, and 4 mg KCl in 1 L of distilled water, the pH was adjusted to 7.5) at 25 ± 1 °C on the photoperiod 16:8 (*L*:*D*) with 4000 1x light. Cultures were established as a clone from a single female and maintained for more than three months before the experiment. We provided the single-cell green alga *Chlorella pyrenoidosa* as diet in pre-culture. The alga was obtained from freshwater algae culture collection of the Institute of Hydrobiology (FACHB-Collection), Chinese Academy of Sciences. Cells of the alga were cultured in BG-11 media, which including $NaNO_3$, $MgSO_4 \cdot 7H_2O$, Na_2CO_3, Ferric ammonium citrate, A_5+Co solution, $K_2HPO_4 \cdot 3H_2O$, $CaCl_2 \cdot 7H_2O$, citract acid and EDTA. Algae in exponential growth were centrifuged and then re-suspended in EPA medium before feeding rotifer (Zhao et al., 2009).

2.2. The chemical contaminant

The organophosphorus pesticide, dimethoate was obtained from Jiangsu Pesticides Research Institute. Toxicant test solutions were prepared by diluting specific volumes of a dimethoate stock solution in EPA media and adjusting pH to 7.5 (Andersen et al., 2006). The lethal effect was determined in our preliminary study. The concentrations chosen for the present toxicity testing were based on the preliminary results that the effects were observed but not to produce mortality. Based on our preliminary study, five concentrations (0.2, 0.6, 1.0, 1.4 and 1.8 mg L^{-1}) of the pesticide were tested for the rotifer reproduction disruption with an algal density of 0.5×10^6 cells mL^{-1} as food. The rotifers lacking toxic compound were measured as the control group.

Actual exposure concentrations were determined in triplicate for all treatments using a high-performance liquid chromatography (HPLC) technique. Dimethoate analytical standard sample (HPLC technical grade, 99.8% purity) was obtained from Sigma-Aldrich, U.S.A. The pesticide was separated and determined with a C18 ODS column. Acetonitrile/water (30:70) was used as mobile phases. Prior to use, the mobile phase was filtered through 0.45 μm filter paper with filtration assembly followed by sonication for 10 min for the complete removal of air bubbles/dissolved oxygen. The flow rate and column temperature were kept at 1 mL min^{-1} and 20 °C, respectively. The retention time is 9.45 min.

2.3. Experiment design

The pre-cultured rotifers from abundant ovigerous females were prepared in beakers (4 ind. mL^{-1}) before the experiment. The neonates reproduced by the rotifers were collected for the following test. The experiments were conducted in 24-well plate (Corning Inc. USA) and started with the introduction of one neonate (2 h old) into each well, which contained 1 mL of the test solution in a given concentration with 2.0×10^6 cells mL^{-1} of *C. pyrenoidosa*. The rotifers used in tests were performed in three parts (Group 1, Group 2 and control) as follows in Fig. 1. The pre-exposure process started with the neonate of the F_0 generation rotifer and lasted since the first neonate of the F_1 generation rotifer was reproduced. The reproduction toxic test started with the neonate of the F_1 generation rotifer and lasted since the F_1 generation rotifer died. In Group 1, the rotifers and their parental generation were both exposed to dimethoate at the corresponding concentrations as the pre-exposure treatment. In Group 2, the rotifers were exposed to dimethoate while their parental generation was not exposed to the pesticide as the no pre-exposure treatment. In control, the rotifers and their parental generation were not exposed to the pesticide.

At every 24-h interval, the F_1 generation rotifers were transferred to fresh EPA medium containing the appropriate toxicant–alga combination as before (Xi and Feng, 2004). Thus, the actual exposure concentrations of dimethoate at 0 and 24 h were determined respectively. The actual concentration was less than 10% lower than the nominal concentration. After 24 h, 43.9% of the pesticide degraded. Experiments were terminated when the last rotifer died. The rotifers in the reproduction toxic tests were

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