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Effects of triphenyl phosphate on growth, reproduction and transcription of genes of *Daphnia magna*

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ABSTRACT

The additive flame retardant triphenyl phosphate (TPHP) has been frequently detected in environments and biota. Evidences indicate that TPHP has potential risks to aquatic organisms. Seldom has been reported about its chronic effects to aquatic organism at low trophic levels, such as Cladocera. In the present study, < 12 h old *Daphnia magna* (*D. magna*) were exposed to 0, 5, 50 or 500 μg/L TPHP for 21 days to investigate the chronic effects of TPHP on body length, fecundity and survival. Meanwhile, *D. magna* PCR arrays were used to evaluate the transcriptional responses of 155 genes involved in 40 pathways. Exposure to 500 μg/L TPHP for 21 days significantly decreased the body lengths of both F0 and F1 generation and inhibited the fecundity of F0 generation. Results of RT-qPCR showed that the expressions of 76 genes involved in 15 pathways were significantly altered after exposure to 500 μg/L TPHP for 21 days. The significantly altered pathways related to genetic information processing, cellular process and metabolism might be responsible for the observed effects of TPHP. Overall, our results showed that chronic exposure to TPHP caused developmental and reproductive toxicities to *D. magna*.

1. Introduction

Triphenyl phosphate (TPHP), a typical organophosphate flame retardant (OPFR), was widely used in a variety of products, such as polyvinyl chloride (PVC), glues, foam, electronic equipment and casting resins (Bjorklund et al., 2004; Carlsson et al., 2000; Matsukami et al., 2015). Recently, the use of TPHP was increasing gradually since other flame retardants such as polybrominated diphenyl ethers (PBDEs) were phased out of use (van der Veen and de Boer, 2012; Wei et al., 2015). As an additive flame retardant without any connection with chemical bond, TPHP could easily leach out into the environments (Wang et al., 2014).

Results from a series of published reports indicated that TPHP was ubiquitous in various environmental media, especially in sediment and natural waters (Cristale et al., 2013a,b; Salamova et al., 2014; Sjodin et al., 2001; Stapleton et al., 2009; Tan et al., 2016; van der Veen and de Boer, 2012). For example, the concentrations of TPHP in sediments from the Pearl River Delta, China ranged from 5.6 to 253 ng/g dry weight (Tan et al., 2016). The detected concentrations of TPHP in rainwater collected in Rome were up to 20 ng/L (Bacaloni et al., 2008). In surface water of River Ruhr in Germany, the concentrations of TPHP were up to 40 ng/L (Andresen et al., 2004). And the maximum measured concentration of TPHP in river was 7900 ng/L in Denmark reported by The Danish Environmental Protection Agency (Lassen et al., 1999). Thus, TPHP was inevitably accumulated in aquatic organisms. For example, in fish samples from Swedish lakes and coastal areas, TPHP levels ranged from 21 to 180 ng/g (Sundkvist et al., 2010). The detection frequency of TPHP in fishes from Manila Bay, Philippines was 45%, and the concentrations ranged from 6 to 230 ng/g lipid weight (Kim et al., 2011). In addition, TPHP was even detected in drinking water, and it was reported to be one of the dominant OPFRs in tap and bottled water from eight cities in China (Li et al., 2014). Therefore, the potentially environmental and health risks of TPHP require further attention.

Results of previous toxicological studies suggested that TPHP could cause various toxicities in vertebrate (Du et al., 2015, 2016; Isales et al., 2015; Jarema et al., 2015; Kim et al., 2015; Lassen et al., 1999; Liu et al., 2013a,b; Saboori et al., 1991). Du et al. (2016) found that exposure to 0.05 mg/L TPHP for 7 days caused vacuolization and pyknotic nucleus of liver cells, and affected carbohydrate metabolism, lipid metabolism, and DNA damage repair system in the liver of zebrafish. In zebrafish embryos/larvae, Liu et al. (2013a) demonstrated that exposure to 100 or 500 mg/L TPHP decreased the rates of hatching and survival, and altered the expressions of genes involved in receptor-

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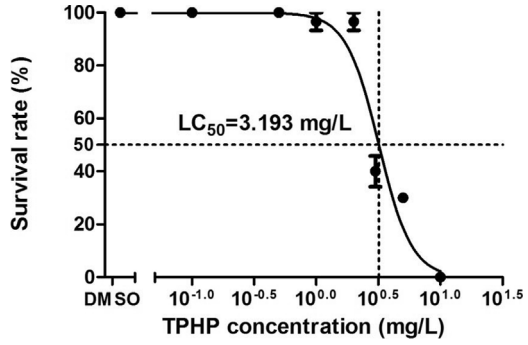

Fig. 1. Dose-dependent effect of triphenyl phosphate (TPHP) on the mortality of newborn (< 12 h) *D. magna* following a 48-h exposure. The concentrations were log-transformed and the survival rates were fitted to nonlinear regression curve (log(agonist) vs. normalized response) to determine the median lethal concentration(LC_{50}). Values represent mean ± SEM (n = 4).

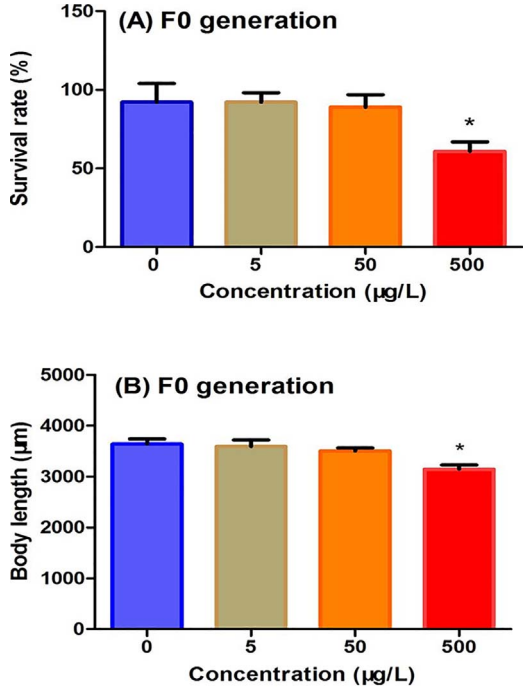

Fig. 2. Effects on the survival rate (A) and body lengths (B) of F0 generation after exposure to 0, 5, 50 or 500 μg/L TPHP for 21 days. Values represent mean ± SEM (n = 4). Significant difference is indicated by *$P < .05$.

centered gene networks. To the best of our knowledge, most of these studies reported focused on the acute instead of the chronic effects of TPHP, and most of organisms selected were vertebrate. However, environmental risks of TPHP on low-tropic organisms, such as *Daphnia magna* (*D. magna*), remain unknown.

Therefore, in the present study, *D. magna* was used and a 21-day standard reproductive test was performed to evaluate the effects of TPHP on growth and reproduction. Additionally, *D. magna* PCR arrays developed in a previous study were used to explore possible molecular mechanisms (Li et al., 2015).

2. Materials and methods

2.1. Materials and reagents

Triphenyl phosphate (TPHP, purity ≥ 99.0%) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and was dissolved in dimethyl sulfoxide (DMSO) as a stock solution. TRIzol reagent, reverse transcription and SYBR Green kits were purchased from Takara (Dalian, Liaoning, China). All the other reagents used in this study were of analytical grade.

2.2. Animal maintain and chemical exposure protocol

Daphnia magna (*D. magna*) were maintained in aerated tap water at a constant temperature of 22 ± 1 °C, with a 16 h light and 8 h dark cycle according to a previous study (Li et al., 2015). For exposure experiments, two parts were included. In the first part, a 48-h acute exposure experiment was performed to determine the median lethal concentration (LC_{50}) of TPHP. Briefly, < 12 h *D. magna* neonates were exposed to 0, 0.1, 0.5, 1, 2, 3, 5 or 10 mg/L TPHP for 48 h, and mortality was recorded daily. Four replicate glass beakers were included, and each beaker contained 80 mL exposure solution and 10 neonates. In the second part, a 21-day reproductive toxicity test was performed. Briefly, < 12 h *D. magna* neonates were exposed to 0, 5, 50 or 500 μg/L TPHP for 21 days. Each concentration contained 4 replicate glass beakers, and each beaker contained 800 mL exposure solution and 40 neonates. Exposure concentrations were selected based on the results of the acute toxicity test. During the exposure, *D. magna* from each beaker were fed with 4 mL mixture of *Chlorella pyrenoidosa* and *Scenedesmus obliquus* at a concentration of 9.35×10^8 cells/mL, and exposure solutions were renewed every day. Quantity of diet was doubled after the first production of offspring. Production of neonates was monitored daily. After exposure, lengths of F0 generation and F1 generation from the last three days of the exposure were measured. All exposure and control groups received 0.01% DMSO.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

The *D. magna* PCR arrays used in this study were developed in a previous study (Li et al., 2015). Quantitative real-time PCR was conducted according to the minimum information for publication of

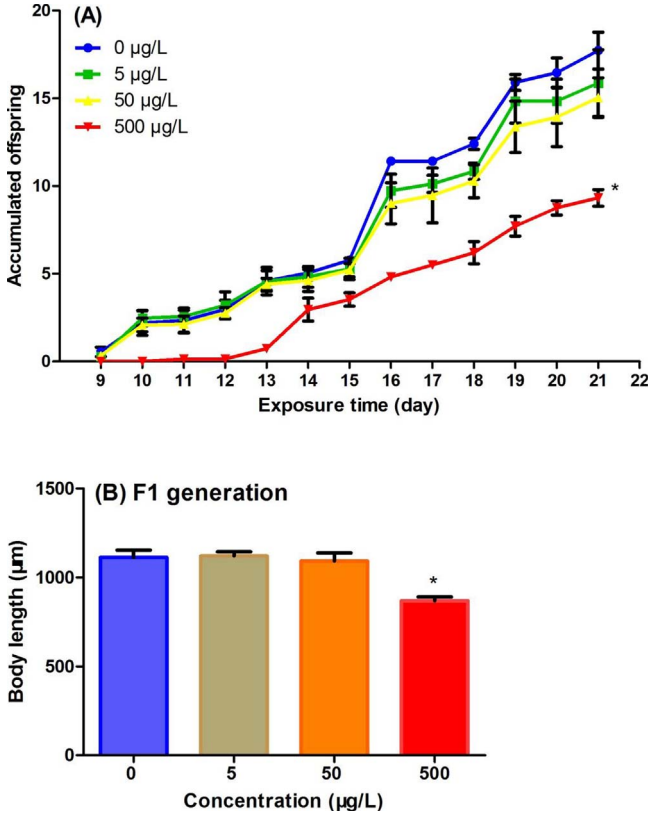

Fig. 3. Effects on accumulated number (A) and body lengths (B) of offspring in F0-generation *D. magna* exposed to 0, 5, 50 or 500 μg/L TPHP for 21 days. Values represent mean ± SEM (n = 4). Significant difference is indicated by *$P < .05$.