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Parental transfer of tris(1,3-dichloro-2-propyl) phosphate and transgenerational inhibition of growth of zebrafish exposed to environmentally relevant concentrations☆

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ABSTRACT

Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is a re-emerging environmental contaminant that has been frequently detected at sub-ppb (<μg/L) concentrations in natural waters. The objective of this study was to evaluate effects of TDCIPP on growth in initial generation (F_0) zebrafish after chronic exposure to environmentally relevant concentrations, and to examine possible parental transfer of TDCIPP and transgenerational effects on growth of first generation (F_1) larvae. When zebrafish (1-month old) were exposed to 580 or 7500 ng TDCIPP/L for 240 days, bioconcentration resulted in significantly less growth as measured by body length, body mass, brain-somatic index (BSI) and hepatic-somatic index (HSI) in F_0 females but not F_0 males. These effects were possibly due to down-regulation of expression of genes along the growth hormone/insulin-like growth factor (GH/IGF) axis. Furthermore, residues of TDCIPP were detected in F_1 eggs after exposure of parents, which resulted in less survival, body length and heart rate in F_1 individuals. Down-regulation of genes in the GH/IGF axis (e.g., *gh*, *igf1*) might be responsible for transgenerational toxicity. This study provides the first known evidence that exposure of zebrafish to environmentally relevant concentrations of TDCIPP during development can inhibit growth of offspring, which were not exposed directly to TDCIPP.

1. Introduction

Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) is one of the primary organophosphate triesters used as flame retardants, which have been used extensively for decades in manufacturing of polymers, resins, latexes, products for infants and polyurethane foams (Dishaw et al., 2011; Stapleton et al., 2011, 2012). TDCIPP has been frequently detected in indoor and outdoor air, natural waters, sediments and aquatic species of fish, and is considered to be a re-emerging environmental pollutant (Sundkvist et al., 2010; van der Veen and de Boer, 2012). Concentrations of TDCIPP in natural waters have generally been reported at sub-per-billion (sub-ppb; <μg/L) concentrations. For example, in the Songhua River of China, concentrations of TDCIPP in water samples ranged from 2.5 to 40 ng TDCIPP/L (Cao et al., 2012; Wang et al., 2011). In seawater samples collected near the cities of Qingdao, Xiamen and Lianyungang, China, concentrations ranged from 24 to 377 ng TDCIPP/L (Hu et al., 2014).

Toxicological information has suggested that exposure to relatively great concentrations of TDCIPP can cause disruption of

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endocrine function (Crump et al., 2012; Liu et al., 2012, 2013a; Kojima et al., 2013; Wang et al., 2013; Zhang et al., 2014), neural toxicity (Dishaw et al., 2011, 2014; Ta et al., 2014; Wang et al., 2015c), developmental toxicity (Farhat et al., 2013, 2014; Fu et al., 2013; Li et al., 2015b; McGee et al., 2012; Wang et al., 2015a), and reproductive toxicity (Liu et al., 2013b; Wang et al., 2015b; Li et al., 2015a), and among these effects developmental changes might be the primary adverse effects. For example, exposure of zebrafish embryos to a large concentration of TDCIPP (1290 μg/L) inhibited rearrangement of cells at 4 h post-fertilization (hpf) and caused delay of epiboly at 5.7 and 8.5 hpf in zebrafish embryos and decreased masses of larvae (Fu et al., 2013). Exposure during early development to relatively small concentrations of TDCIPP (20 or 100 μg/L) resulted in significantly lesser body mass and body length of initial generation (F_0) zebrafish (Wang et al., 2015c). Furthermore, exposure of F_0 fish to TDCIPP (20 or 100 μg/L) resulted in transfer of TDCIPP to F_1 embryos and lesser body mass in F_1 larvae (Wang et al., 2015a).

Recently, studies of *Daphnia magna* (Li et al., 2015a, 2015b) and zebrafish (Zhu et al., 2015) demonstrated that exposure to environmentally relevant concentrations of TDCIPP causes significant growth inhibition. Specifically, treatment with 65 or 550 ng TDCIPP/L for 28 days significantly down-regulated expression of genes involved in synthesis of proteins, and expression of genes in the metabolism and endocytosis pathways, and decreased length of F_0 and first generation (F_1) *Daphnia magna* (Li et al., 2015a). While in zebrafish, exposure to 600 ng TDCIPP/L for 120 days resulted in bioconcentration of TDCIPP in tissues and lesser body length and body mass of females, and down-regulation of genes involved in production of hormones along the growth hormone/insulin-like growth factor (GH/IGF) axis was considered to be a possible mechanism of toxicity (Zhu et al., 2015). Therefore, results of the two studies demonstrated that changes in development might be critical toxic effects due to exposure to TDCIPP and suggested hazard to aquatic species.

Chronic exposure of larvae to environmentally relevant concentrations of TDCIPP due to transfer from the females during production of eggs might cause adverse effects on developing F_1 larvae. Although effects of transfer of TDCIPP from females to eggs were reported in a previous study of zebrafish, concentrations used in that study were greater than those reported in natural waters (Wang et al., 2015a). Whether exposure to environmentally relevant concentrations of TDCIPP can cause transgenerational toxicity remained unknown. To evaluate transgenerational toxicity and provide information required for assessment of hazard or risk of TDCIPP, zebrafish were exposed to environmentally relevant concentrations for 240-days. Bioaccumulation and maternal transfer of TDCIPP were evaluated, and effects on development of F_0 adult fish and F_1 larvae were examined. To elucidate possible mechanisms of development toxicity, gene expression patterns in GH/IGF axis were examined in both generations.

2. Materials and methods

2.1. Chemicals and reagents

TDCIPP was purchased from Sigma (St. Louis, MO, USA; purity: 95.7%), and was dissolved in dimethyl sulfoxide (DMSO). TDCIPP used as an analytical standard was from Tokyo Chemical Industry America (Portland, OR, USA; purity: 95%). Internal standards, d_{15}-TDCIPP and bis (1,3-dichloro-2-propyl) phosphate (BDCIPP) were purchased from Dr. Vladimir Below via Letcher Group-Organic Contaminants Research Laboratory (OCRL), NWRC (Ottawa, Canada), and purities of these two standards were >97%. MS-222 (3-aminobenzoic acid ethyl ester, methanesulfonate salt) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The TRIzol reagent and PrimeScript Reverse Transcription (RT) Reagent kits and SYBR Green kits were purchased from TaKaRa (TaKaRa, Dalian, China). All the other reagents used in this study were of analytical grade.

2.2. Maintenance and exposure of zebrafish to TDCIPP

Zebrafish (AB strain) were maintained according to a previously described method (Yu et al., 2011). One-month old zebrafish (fifteen fish in each of three replicated tanks for each concentration) were acclimated for 1 week in 15-L glass tanks then exposed to 0, 500 or 5000 ng/L TDCIPP for 240 days. The least concentration (500 ng/L), to which zebrafish were exposed, was comparable to that reported in natural waters along the coast of China near the city of Lianyungang (377 ng/L) (Hu et al., 2014). Exposure solutions were prepared with carbon-filtered water, and replaced daily with freshly prepared solutions containing corresponding concentrations of TDCIPP. Samples of exposure solutions were collected twice at the last day of the exposure, before and after renewal of water. Concentrations of TDCIPP and its metabolite (BDCIPP) were quantified. Both control and treated groups received 0.001% DMSO. During the exposure period, survival was recorded.

On the last day of the 240-day exposure, five females and five males from each tank were paired in clean water (without TDCIPP), and eggs were immediately collected for quantification of TDCIPP. Embryos were transferred to glass beakers containing clean water (without TDCIPP) to assess transgenerational toxicity. Hatching, survival, heart rate and growth were determined for F_1 larvae at 3-day post-fertilization (dpf) or 5-dpf. Thirty 5-dpf F_1 larvae were sampled randomly and frozen immediately in liquid nitrogen, and stored at −80 °C for the subsequent assay of gene expression. After that, fish were euthanized with 0.03% MS-222, and body length and body mass of female and male fish were recorded. Brains and livers were sampled and massed for brain-somatic index (BSI) and hepatic-somatic index (HSI) calculation, respectively. Additionally, brains and livers of females were also collected for quantitative real-time polymerase chain reactions (qRT-PCR). Since no significant effects of TDCIPP on growth in males were observed, expressions of genes in brains and livers of males were not investigated.

2.3. Quantification of TDCIPP and BDCIPP in exposure solutions, F_0 zebrafish and F_1 eggs

Concentrations of TDCIPP and BDCIPP in exposure solutions were directly measured by use of a Waters ACQUITY UPLC® I-Class system (UHPLC) coupled to Waters® Xevo™ TQ-S mass spectrometer (TQ-S/MS) (Milford, MA, USA) using electrospray ionization (ESI(+)) in the multiple reaction monitoring (MRM) mode. For more detailed information on instrumental parameters, please refer to previous publications (Su et al., 2014 and Su et al., 2015). During the analysis, decamethonium hydroxide was used as a dicationic derivatization reagent which was mixed with mobile phase post-LC separation at a constant rate of 10 μL/min with a "T" connector. TDCIPP and BDCIPP were quantified by use of transitions of m/z 430.9 > 99 and m/z 577.2 > 243.3, respectively. A 6-point calibration curve was run with each batch of samples to ensure instrumental response linearity. For the quantification of TDCIPP and BDCIPP in the exposure solutions, no background contamination was detectable, and thus the method limits of quantification were defined as a concentration that can generate instrumental response that is 10-fold greater than the signal-to-noise ratio. The method limits of quantification (MLOQs) of TDCIPP and BDCIPP were 0.01 and 0.015 ng/mL water, respectively.

Based on previous publications (Zhu et al., 2015), it was assumed