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Prolonged exposure of di(2-ethylhexyl) phthalate induces multigenerational toxic effects in *Caenorhabditis elegans*

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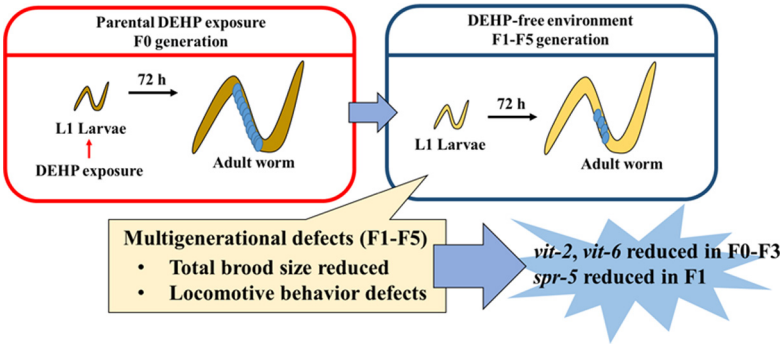
HIGHLIGHTS

- Multigenerational toxicity caused by DEHP (F0) exposure might cause potential risk.
- After prolonged exposure to DEHP (F0), locomotive behaviors decreased (F0 to F5).
- After prolonged exposure to DEHP (F0), total brood size decreased (F0 to F5).
- The multigenerational toxicity might relate to vitellogenin and H3Kme2 demethylase.

GRAPHICAL ABSTRACT



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ABSTRACT

The plasticizer di(2-ethylhexyl) phthalate (DEHP) is an emerging organic contaminant that has represented a risk for organisms present in the environment. However, there is still limited information regarding DEHP-induced multigenerational toxicity and the underlying mechanisms. In this study we investigated the multigenerational toxic effects including locomotive behaviors and reproduction upon prolonged DEHP exposure (from larval L1 to adult) and the underlying mechanisms in the nematode *Caenorhabditis elegans*. The multigenerational effects were examined over 6 generations (F0–F5) with only parental *C. elegans* (F0) was exposed to DEHP from larval L1 to adults (72 h), and the subsequent offsprings (F1–F5) were grown under DEHP-free conditions. The results showed that prolonged exposure (72 h) to various concentrations of DEHP caused dose-dependent locomotive impairments and reproduction defects in *C. elegans* and that a concentration of 0.2 mg/L DEHP was enough to cause such sublethal effects. The results showed that after prolonged exposure to DEHP in the F0 generation, abnormal locomotive behaviors such as reduced body bends and head thrashes were observed from generations F0 to F5. Additionally, prolonged exposure to DEHP (20 mg/L) in F0 significantly reduced total brood size in F0, and this parental exposure was sufficient to cause multigenerational reproductive toxicity in the offspring generations (F1–F5) as well. Furthermore, the expressions of reproduction-related genes such as *vit-2* and *vit-6* were down-regulated by about 20% until F3, and the expression of H3Kme2 demethylase, *spr-5*, was downregulated in F1 by about 40%. Results from this study demonstrate that prolonged exposure to DEHP only at F0 adversely affected reproduction and locomotive behaviors in *C. elegans* across generations and might be associated with inadequate vitellogenin production and malfunction of H3Kme2 demethylase. This study implies that parentally prolonged exposure to DEHP caused multigenerational defects in both reproduction and locomotive behaviors raising the potential health and ecological risk.

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1. Introduction

The plasticizer di(2-ethylhexyl) phthalate (DEHP) is the most widely used plasticizer and was originally synthesized to increase the flexibility of plastics such as polyvinyl chloride (PVC). DEHP is used in PVC containers, food packaging, toys, medical care products, and other consumer products (Magdouli et al., 2013). DEHP might be leaked from plastic products into the environment or even to food, which makes it a potential risk on the environment and human health (Magdouli et al., 2013). DEHP has been widely found in different ecological compartments (Magdouli et al., 2013). DEHP concentrations have been found in surface water within the range of 0.33–97.87 μg/L (Khan and Jung, 2008). Because DEHP is lipophilic in nature, DEHP would accumulate in fish tissue and adsorb to suspended particles which might cause ecological risk in aquatic environment (Adeniyi et al., 2011). In addition to environmental concern, DEHP might cause potential risk to human health. It has been reported that DEHP levels ranged from not detected to 3.41 mg/kg in foods collected during 2011–2012 in China (Sui et al., 2014). One study reported that the average concentration of DEHP metabolites was 398.6 μg/L and 333.7 μg/L in the urine of 2 and 5 year-old Taiwanese children, respectively, which was higher than that of other countries (Lin et al., 2011). Also, DEHP exposure is prominent in the United States, urinary metabolites of several phthalates including DEHP have been widely detected in the U.S. population (Zota et al., 2014).

DEHP has been considered an endocrine-disrupting chemical (EDC) with higher toxicity than other plasticizers (Biemann et al., 2012). DEHP exerts endocrine disruption effects on reproduction system in male mice at the concentration about 10 mg/kg/day by interfering the Leydig cell which is the only cell with luteinizing hormone receptor in testis, regulating the production of androgen (Akingbemi et al., 2004). Accumulated studies have shown that the high exposure frequency of DEHP causes reproduction and behavior defects, specifically on vulnerable infants (Ipapo et al., 2017; Kim et al., 2017; Wen et al., 2017). Toxic effects including neurotoxicity and reproductive toxicity induced by DEHP have been reported (Tseng et al., 2013; Pocar et al., 2017).

The endocrine disrupting effects of DEHP could be observed at even lower concentration at ng/L to μg/L level in invertebrates (Oehlmann et al., 2009). In addition, toxic effects including neurotoxicity with reduced thermosensing ability and locomotion impairments and reproductive toxicity induced by excessive ROS production by DEHP have been reported in environmental relevant organism, *C. elegans*, at 2–20 mg/L and mice model (Tseng et al., 2013; Pocar et al., 2017). Another environmental relevant exposure condition has been reported that chronic exposure to DEHP (1 ng per week) reduces egg-laying rate in queen ants (Cuvillier-Hot et al., 2014). Additionally, the reproduction rate of rotifer is affected by DEHP with alteration of gene expression at the concentration of 2 mg/L (Cruciani et al., 2016). Many invertebrates in the environment are also sensitive to DEHP contamination adversely affecting the reproduction (Oehlmann et al., 2009). Therefore, the population decline of some invertebrate species caused by prolonged exposure to DEHP might be potentially harmful to the stability or fitness of the ecosystem.

Some recent studies have indicated that exposure to DEHP caused transgenerational reproductive dysfunctions (Rattan et al., 2017; Pocar et al., 2017; Quinnies et al., 2017). Parental exposures to DEHP have been reported to promote the transgenerational inheritance of reproductive dysfunctions and social behaviors in mice (Rattan et al., 2017; Pocar et al., 2017; Quinnies et al., 2017). However, the underlying mechanisms associated with DEHP exposure-induced transgenerational reproductive toxicity are not clear. Particularly, there is a growing concern about the long-term impact of DEHP exposure and whether the toxic effects persist even after removal of DEHP exposure. It is worrisome that DEHP-induced long-term multigenerational toxicity might cause reduction of population or fitness for offsprings in human and other species. Therefore, this study hypothesizes that parentally prolonged exposure to DEHP would cause multigenerational defects persisted to following generations leading to the potential risk in ecosystem and human health.

Hence, in order to address the possible risk of multigenerational effects, this study aims to examine the multigenerational toxicity of DEHP on locomotive behaviors and reproduction in the nematode *C. elegans*. The environmentally relevant nematode *C. elegans* is abundant in the soil and sediment of fresh water system. As a multicellular organism, *C. elegans* can represent both toxicity response and bioavailability with various endpoints. Moreover, *C. elegans* has been suggested as a bioindicator for environmental contaminant (Leung et al., 2008). In the present study, the multigenerational toxic effects including locomotive behaviors and reproduction upon prolonged DEHP exposure (from larval L1 to adult) were examined over 6 generations (F0–F5), the parental *C. elegans* (F0) being exposed to DEHP (20 mg/L) from larval L1 to adults (72 h), and the subsequent offsprings (F1–F5) were grown under DEHP-free conditions. In addition, the potential underlying mechanisms associated with the multigenerational locomotive behaviors and reproductive toxicities of parentally prolonged DEHP exposure were dissected.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA) unless otherwise stated. DEHP was prepared from a serial dilution with dimethyl sulfoxide (DMSO) to reach a nominal concentration.

2.2. Experimental nematodes and cultured conditions

Wild-type N2 *C. elegans* was incubated on nematode growing medium (NGM) agar plates (51 mM NaCl, 25 mM KH_2PO_4, 1.7% agar, 0.25% peptone, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mg/L cholesterol) that were seeded with a lawn of *Escherichia coli* OP50 as a food source in a 20 °C incubator. Synchronization of *C. elegans* was achieved by treating gravid hermaphrodite nematodes with bleaching medium (0.45 M NaOH, 2% HOCl) to collect the synchronized eggs. Subsequently, the eggs were resuspended in an appropriate amount of S-basal (100 mM NaCl, 50 mM KH_2PO_4, 5 mg/L cholesterol) for hatching worms to the first larval stage (L1-stage) overnight at 20 °C (Sulston and Hodgkin, 1988).

2.3. Locomotive behavior assays

2.3.1. Exposure setup

Locomotive behavior assays including body bends and head thrashes were performed based on a previous study (Tsalik and Hobert, 2003). Synchronized wild-type L1 nematodes were incubated in S-basal with various concentrations of DEHP (0.2, 2, 20, 100 mg/L) or 0.1% DMSO (solvent control) and fed with *E. coli* OP50 (10^9 cells/mL) under 20 °C for 72 h before the observation of locomotive behaviors.

2.3.2. Body bending

Before conducting the body bending assay, worms were washed with K-medium (32 mM KCl, 53 mM NaCl) 3 times and then transferred to clean NGM plates to evaluate the number of body bends in an interval of 20 s by visual inspection with a dissecting microscopy. A body bend was defined as a change in direction of part of the worm corresponding to the posterior bulb of the pharynx along the Y-axis assuming that the worm was traveling along the X-axis. This assay was performed for 4 independent trials with at least 20 worms in each treatment.