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Transgenerational impairments of reproduction and development of the marine invertebrate *Crepidula onyx* resulted from long-term dietary exposure of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47)☆

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ABSTRACT

Polybrominated diphenyl ethers have become ubiquitous in the environment and elevated concentrations have often been found in marine organisms. Using the gastropod *Crepidula onyx* as a study model, this multigenerational study sets out to test the hypotheses that 1) parental dietary exposure to environmentally realistic levels of 2,2′,4,4′-tetrabrominated diphenyl ether (BDE-47) would lead to transgenerational impairments on fitness traits of marine invertebrates, and 2) the organisms might develop adaptation/acclimation after exposure for one or more generations. F_0 generation of *C. onyx* was fed with the dinoflagellate *Isochrysis galbana* encapsulated with two concentrations of BDE-47 (1.78 and 16.0 ng million cells^{-1}, respectively), and half of the F_1 offspring from the higher concentration treatment was returned to control condition (transgenerational group), while the other half received BDE-47 treatment continuously (continuous treatment group). Bioaccumulation and maternal transfer of BDE-47 were evident in all life stages of the F_0 generation and in F_1 eggs, respectively. Exposure to BDE-47 reduced fecundity, delayed sexual maturity, and impeded embryonic development in F_0 to F_2. In particular, developmental toxicity of F_2 embryos was apparent in the transgenerational group, but not in the continuous treatment group, even when BDE-47 was not detected in the F_2 embryos nor in their mothers and they have never been exposed to the chemical. This study also suggested that the offspring might have developed adaptation/acclimation to the exposure of BDE-47 within two generations of exposure, and that the physiological alterations associated with acclimation/adaptation might have hindered the normal larval development under a stress free condition. These findings highlighted the need for long-term multigenerational studies in the ecological risk assessment of chemicals alike.

1. Introduction

Being widely used for three decades as flame retardants, polybrominated diphenyl ethers (PBDEs) are now ubiquitously found in the environment and are highly bioavailable to organisms in both freshwater (Hu et al., 2010) and marine ecosystems (Kelly et al., 2008). PBDEs are also known to cause negative impacts such as induction of liver neoplasm, altered thyroid hormone homeostasis, and damaged learning and memory functions in rodents (reviewed in Darnerud et al. (2001)). Environmental and public health concerns over the endocrine disrupting effects (reviewed in Legler (2008), Vonderheide et al. (2008), Yu et al. (2015)) and genotoxic and teratogenic impacts (reviewed in Lee and Kim (2015) and Yu et al. (2015)) of PBDEs have been rising in recent years.

There has been a growing knowledge on transgenerational effects, which are defined as the phenotypic alterations in unexposed offspring due to parental exposure to environmental stress. For example, transgenerational endocrine disrupting effects were found in the male offspring of female rats exposed to vinclozolin (Anway et al., 2005, 2006) and in the F_1 and F_2 offspring of marine medaka exposed to hypoxia (Wang et al., 2016). It is worthy to note that studies on the transgenerational consequences of persistent organic pollutants (POPs) on animal models other than fish and mammals are very scarce (Schwindt (2015), but see Winter et al. (2013)).

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While the toxic effects and mechanisms of PBDEs have been well-understood through numerous short-term, single-stage exposure experiments on model organisms such as fish (Chou et al., 2010; Yu et al., 2010, 2015) and rodents (Lee et al., 2010), there is a lack of study in which exposure lasts for multiple generations (e.g. F_0 to F_2). This would rightly be associated with the higher cost, effort and longer duration required for such studies, especially for animals with a long life-cycle (Ingersoll et al., 1998). Nonetheless, a few two-generation studies have been done to investigate the effects of PBDEs resulting from maternal transfer. Offspring of rodents and zebrafish exposed to PBDEs through maternal transfer during early developmental stages suffered from malformations (Berger et al., 2014; Blanco et al., 2012) and decreased motor and locomotion performance as their neurodevelopment was altered with lower AChE activity (Chen et al., 2012; He et al., 2011), respectively. The observed effects could be attributed to the parental transfer of chemical to germline or developing fetuses (mainly maternally), or by altered maternal investment in yolk content in stressed females (Verboven et al., 2009).

Marine invertebrates are not only valuable food and economic resources, but are also pivotal to regulating the structure and function of marine ecosystems, including coastal habitat maintenance, trophodynamics, energy fluxes, nutrient recycling and biogeochemical cycles. To date, knowledge about the impact of PBDEs on marine invertebrates remains virtually unknown. Using *Crepidula onyx* (Mollusca: Caenogastropoda) as a study model, this transgenerational study targeted to explore whether long-term dietary exposure to PBDEs may affect the Darwinian fitness traits (including survivorship, growth, development and fecundity) from F_0 to F_2 generations of marine invertebrates. The uptake and bioaccumulation of BDE-47 into the larvae, juveniles and adults of *C. onyx* via dietary intake and maternal transfer were also evaluated. *C. onyx* has a biphasic life cycle, well-studied embryonic development (Henry et al., 2010) and a wide distribution along the Pacific coast of North and South America, China, Japan and Hong Kong (Plutchak et al., 2006). *Crepidula* spp. have also been proposed as a good model for toxicity assessment of endocrine disrupting chemicals (EDCs) (Ingersoll et al., 1998).

2. Materials and methods

2.1. Culture and BDE-47 treatment of microalgae and C. onyx

The dinoflagellate *Isochrysis galbana* (clone T-ISO) has been shown to be able to take up a high loading of BDE-47 efficiently (Po et al., 2017) and thus, it was used to serve as the dietary carrier for the exposure experiment. *I. galbana* was cultured with Guillard's F2 medium (Guillard, 1975) prepared with artificial seawater (ASW; 30 ± 0.5‰, pH 8.1 ± 0.1) using Instant Ocean® Sea Salt (Blacksburg, VA, USA) and deionised water. BDE-47 (Chem Service Inc.; CAS#: 5436-43-1; 98% purity) stock solutions in dimethyl sulfoxide (DMSO) was spiked to the F2 medium to produce a concentration of 1 μg BDE-47 L^{-1} for the low concentration treatment (L) and 10 μg BDE-47 L^{-1} for the high concentration treatment (H). For control F2 medium, DMSO at the same concentration as the treatments (0.01%) was applied. The *I. galbana* cultures were harvested after 6–9 d of incubation (after reaching log-phase of growth) by centrifuge (2300 rcf; 12 min; 4 °C) and stored at 4 °C until use. Cell density was determined by Coulter Multisizer in triplicates.

Adult *C. onyx* had been collected from Victoria Harbour, Hong Kong, and cultured in the laboratory (25 °C, 14:10 day-night cycle) for over two years. Larvae hatched from these adults (F_0 generation) were cultured as previously described (Chiu et al., 2012). Briefly, they were cultured with 0.22 μm filtered ASW at a density of 1 larva mL^{-1} with daily change of medium. After 8 d when the larvae became competent, metamorphosis was induced by 15 mM potassium chloride in ASW. Juveniles that settled (140–190 juveniles (F_0) and 160 to 230 juveniles (F_1) in each tank in the beginning of the culture) were collected onto glass slides and cultured with continuous aeration and medium was changed twice per week. Post-larval culture density changed gradually from 1 mL individual^{-1} at juvenile stage to 30 mL individual^{-1} at adult stage.

C. onyx were fed with clean or BDE-47 encapsulated *I. galbana* daily at 2×10^5 cells mL^{-1} throughout larval to adult stages to produce control (C), low (L) and high (H) concentration treatments (Fig. 1). There were three replicates per treatment (i.e. nine groups/tanks in total). In addition, they were fed with untreated *I. galbana ad libitum* daily, and as females emerged, clean live *Artemia* nauplii (Premium Grade Brine Shrimp Eggs; Brine Shrimp Direct, USA) were supplemented *ad libitum* three times a week. Within week 34–45, after collection of the first batch of egg samples (within 2 d since oviposition) for BDE-47 accumulation analysis, the second batch of hatchings from F_0 C and H groups were used for the culture of F_1 generation. F_1 larvae from the H group were divided randomly into two groups: the transgenerational group which switched to clean *I. galbana* diet (HC), and the treatment group which continued to receive BDE-47 encapsulated *I. galbana* (HH). Same culture procedures of F_0 generation were applied for F_1 generation from larval to adult reproductive stages, and F_2 eggs were collected in the same way as F_1 eggs.

2.2. Growth, development and fecundity

Growth was determined by measuring the shell length to the nearest 0.05 mm using a dissection microscope (Zeiss; Stemi, 2000-C) equipped with an ocular micrometer for larvae or using a vernier calliper for adults. Measurements were taken once every 2 d (and 8 d) for F_0 (and F_1) larvae and every other month for F_0 adults. Sex of *C. onyx* was determined by examining the genital tract on ventral side where the presence of a penis or female genital papilla could be seen. Individuals at transitional state were identified by retraction or absence of the penis as well as the absence of female genital papilla. Presence of oviposition was checked under a dissection microscope two to three times a week since females emerged at week 22 (F_0) and week 24 (F_1). After 12 d, the whole broods were removed gently from the females and the larvae released were either cultured or counted and analysed.

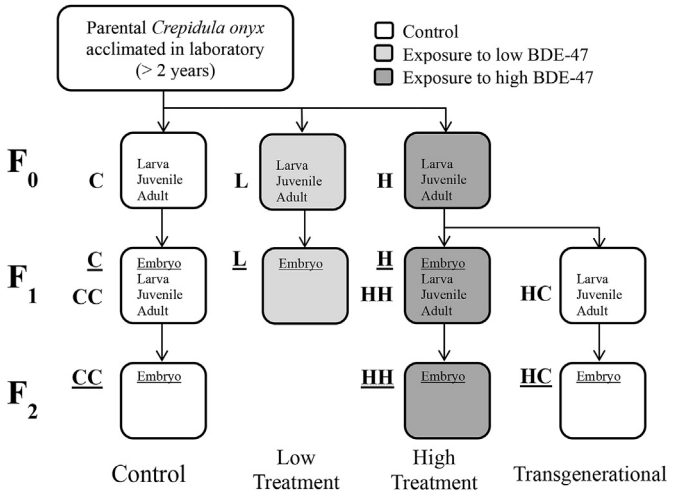

Fig. 1. Experimental design of the dietary exposure of BDE-47 in three generations of *Crepidula onyx*. Embryos of F_1 and F_2 generations were labeled as their parental generation (underlined).