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Bioaccumulation and maternal transfer of PBDE 47 in the marine medaka (*Oryzias melastigma*) following dietary exposure

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ABSTRACT

The bioaccumulation and maternal transfer of 2,2′,4,4′-tetrabromodiphenyl ether (PBDE 47) were investigated in the marine medaka (*Oryzias melastigma*) following dietary exposure, in which PBDE 47 was bioencapsulated into brine shrimp (*Artemia* sp.) and fed daily to male-female pairs of medaka. In the accumulation experiment, each 2-month-old (pre-breeding) medaka were provided with dietary PBDE 47 at 1.3 ± 0.2 μg/day for 21 days. Growth-corrected concentrations of PBDE 47 in the medaka increased over the 21 days of exposure and there were no significant differences between males and females at any of the sampling times. Final concentrations were similar for males and females after 21 days (230 ± 30 and 250 ± 30 μg g^{-1} wet weight, respectively), accounting for 84–100% of the PBDE 47 provided in the diet. In the maternal transfer experiment, 3-month-old (breeding) medaka were provided with dietary PBDE 47 at 1.2 ± 0.2 μg/day for 18 days, and reached body concentrations of 76 ± 3 (males) and 61 ± 6 (females) μg g^{-1} wet weight. Female growth-corrected PBDE 47 concentrations were significantly lower than males by day 12 ($P < 0.05$), and egg PBDE 47 concentrations were up to 25 ng/egg by day 18. Our results showed that maternal transfer is an important offloading mechanism for female fish. The fact that lipid normalized egg:female PBDE ratios did not significantly deviate from 1 further indicated that the maternal transfer of PBDE 47 is associated with lipid mobilization during egg production.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are manufactured as commercial mixtures, generically referred to as penta-BDE, octa-BDE and deca-BDE (La Guardia et al., 2006). They have been widely used as flame retardant chemicals, to prevent and/or slow down the flammability of electrical, construction, automotive and textile products (Alaee et al., 2003). Despite recent bans on the manufacture of penta-BDE and octa-BDE and the current phase out of deca-BDE mixtures, PBDEs are still found ubiquitously in marine environments and marine biota worldwide (Rahman et al., 2001; Yu et al., 2009; Zhang et al., 2010). Due to their lipophilic properties and resistance to breakdown, PBDEs bioaccumulate and biomagnify in aquatic food webs (Gobas et al., 1998; Streets et al., 2006; Tomy et al., 2008; Wan et al., 2008), and levels are typically orders of magnitude higher in biota, compared to water concentrations (de Wit, 2002). Therefore, dietary uptake is an important pathway for bioaccumulation of PBDEs in fish. PBDEs and their metabolites are known to have endocrine disrupting effects on humans and wildlife (Ding et al., 2007; He et al., 2008; Song et al., 2008), although their effects on fish remain poorly understood. An understanding of the accumulation and toxicokinetics of PBDEs in fish following dietary exposure is important in assessing the risk of these chemicals of emerging concern on aquatic biota and food webs.

The accumulation of organic chemicals (including PBDEs) in fish is a net product of exposure and elimination (biotransformation and excretion), and is dependent on the properties of the contaminant, the species and the environmental conditions (Newman and Unger, 2003). While many of these processes may be expected to be similar for male and female fish, adult females potentially have an additional pathway for elimination or sequestration of contaminants through maternal transfer to their eggs. This may result in sex-specific accumulation and tissue distribution of PBDEs that could lead to differential effects of these chemicals in male and female fish. More importantly, the maternal loading of toxic chem-

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icals to eggs may potentially affect embryonic development, and subsequently the survival and fitness of offspring. Maternal transfer of PBDEs has been documented in freshwater fish, and the limited results thus far appear to suggest that egg:female ratios of PBDE concentrations are species-, congener- and dose-specific (Nyholm et al., 2008; Zhang et al., 2010).

A number of studies have investigated the toxicokinetics of PBDEs and other brominated compounds in fish following dietary exposure (Gemmill et al., 2010; Nyholm et al., 2009; Tomy et al., 2004). However, investigations into the accumulation of PBDEs in fish are complicated by the fact that fish are generally exposed to mixtures of PBDEs in nature, and that deca-, hepta- and penta-PBDEs can be metabolized to less brominated congeners. For example, congeners 209, 153 and 99 have all been found to debrominate to PBDE 47 in the common carp, rainbow trout and Chinook salmon (Browne et al., 2009; Kierkegaard et al., 1999; Noyes et al., 2010; Stapleton et al., 2004a,b,c, 2006). However, due to the lack of any meta-substituted bromine atoms, the debromination of lower brominated PBDEs (e.g. PBDE 47) may occur to a lesser extent. In fact, Wan et al. (2009) found no significant metabolism of PBDE 47 in fish. The metabolism of higher brominated congeners combined with the stability of PBDE 47 and exposure to high levels of PBDE 47 from penta-BDE mixtures results in high accumulation of PBDE 47 in aquatic biota. Indeed, PBDE 47 is the generally the most abundant congener in aquatic biota (Christensen et al., 2002; Voorspoels et al., 2003), and is considered more toxic than the higher brominated congeners (Birchmeier et al., 2005). An understanding on the toxicokinetics and toxicodynamics of PBDE 47 is therefore essential in assessing the impact of PBDEs on fishes.

The marine medaka (*Oryzias melastigma*) has recently been proposed as a universal model for investigating toxicological effects in marine fish (Kong et al., 2008). They are easy to maintain in the laboratory, have a linear growth rate and short generation time, and produce eggs regularly once they reach sexual maturity (at approximately 3 months of age). In this study, we investigate the accumulation and maternal transfer of PBDE 47 in male and female *O. melastigma* following dietary exposure, with a view to understanding the toxicokinetics of lower brominated PBDEs in marine fish.

2. Materials and methods

2.1. Bioencapsulation of PBDE 47 in Artemia

A stock solution of 2,2′,4,4′-tetrabromodiphenyl ether (PBDE 47; 98.5% purity; Chem Service Inc., USA) was prepared in hexane (10 mg mL^{-1}) and 825 μL was added to a clean 150 mL conical flask. The hexane was evaporated and 100 mL of newly hatched *Artemia* (∼1400 nauplii/mL) were added to the conical flask. The *Artemia* culture was incubated under light aeration on a 12:12 h light:dark cycle for 30 h. To measure the rate of PBDE 47 accumulation by the *Artemia*, 3× 200 μL samples were removed after 1, 4, 8, 18, 24 and 30 h of incubation, rinsed with MilliQ water and analysed for PBDE 47. The remaining *Artemia* at the end of 30 h incubation was rinsed thoroughly and resuspended in MilliQ water (∼1400 nauplii/mL). A separate culture of PBDE 47 encapsulated *Artemia* was similarly prepared for use in the maternal transfer experiment, except *Artemia* were harvested after 24 h of incubation. Clean newly hatched *Artemia* (∼1400 nauplii/mL) were also prepared for feeding to the control medaka in both experiments. The entire amount of contaminated *Artemia* (and clean *Artemia*) required for each experiment was prepared in a single batch and distributed to clean glass tubes in amounts required for each day of exposure. All feed tubes were kept frozen (−20 °C) and defrosted each morning immediately prior to feeding to the medaka.

2.2. Dietary exposure of medaka to PBDE 47

Marine medaka (*O. melastigma*) were reared from a stock originally purchased from Interocean Industries (Taiwan). Glass tanks (15 cm × 15 cm × 15 cm), with a removable glass divider, were each filled with 2 L of filtered seawater (∼30 ppt), and placed in an environmental chamber (27 ± 1 °C) on a 12:12 h light:dark cycle with gentle aeration. Every second day, half of the water was changed in each tank and all waste was removed. Two separate experiments were performed: (1) an accumulation experiment: to investigate the accumulation of PBDE 47 in 2-month-old (pre-breeding) medaka over 21 days of exposure, and (2) a maternal transfer experiment: to investigate maternal transfer of PBDE 47 from 3-month-old (breeding) medaka to eggs over 18 days of exposure.

In the accumulation experiment, there were nine control tanks (sampled in triplicate on days 0, 7 and 21) and 18 exposure tanks (sampled in triplicate on days 1, 2, 4, 10, 14 and 21). In the maternal transfer experiment, there were 6 control tanks (sampled in triplicate on days 0 and 18) and 15 exposure tanks (5 tanks sampled on each of days 6, 12 and 18). One male and one female medaka were placed in each glass tank. For the accumulation experiment, the glass dividers were kept in place for the duration of the experiment. For the maternal transfer experiment, the glass dividers were only put in place for 30 min each day at the time of feeding. This ensured that each fish was given the same dose over the course of each experiment and that male and female fish were allowed to breed during the maternal transfer experiment only.

In both experiments, individual fish were fed 100 μL of either clean *Artemia* (controls) or PBDE 47 bioencapsulated *Artemia* (exposure) daily. This amount of *Artemia* was predetermined to be completely consumed by an individual 2- or 3-month-old medaka within 15 min. To maintain fish condition throughout the course of the study, the medaka were also fed hormone free flake fish food (AX5; Aquatic Ecosystems, USA) to satiation each afternoon and evening. Neither the clean *Artemia* nor the flake fish food were analysed for PBDE 47. Analysis of the control fish throughout the exposure period was performed to detect any possible accumulation of PBDE 47 from these food sources.

On each sampling day, 3 male and 3 female (uptake experiment) or 5 male and 5 female (maternal transfer experiment) medaka were removed from the tanks and euthanized in iced MilliQ water. Samples were collected approximately 24 h after the exposure to contaminated *Artemia* on the previous day. This ensured that there was no PBDE 47 bioencapsulated *Artemia* left in the digestive system of the fish, as determined by preliminary feeding experiments. In the maternal transfer experiment, eggs were collected daily and pooled for each female over three collection periods: days 0–6, 7–12 and 13–18. All fish and egg samples were kept at −80 °C until analysis for PBDE 47.

2.3. Chemical analysis of Artemia and medaka

PBDE 47 was extracted from *Artemia* and medaka eggs using a liquid-liquid extraction with hexane (Analytical Reagent; RCI Lab Scan, Thailand), following methods outlined in Muirhead et al. (2006). Prior to analysis, the number of *Artemia* nauplii were counted in 5× 50 μL aliquots from each sample time to establish mean density. To account for any losses of PBDE 47 from the *Artemia* due to cell bursting during freezing, the *Artemia* were defrosted, rinsed thoroughly and resuspended with MilliQ water to their original density. Three 200 μL *Artemia* aliquots, or 50–70 eggs, from each sampling time were collected and 50 μL of internal standard (^{13}C-BDE 47, ∼2.5 μg g^{-1} in ethanol) was accurately weighed (±0.0001 g) and added to each sample. The *Artemia*, or eggs, were homogenized in 2 mL MilliQ water using a mortar and