Bioaccumulation and Debromination of BDE-209 in Japanese medaka (*Oryzias Latipes*) under continuously exposure

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Introduction

Occurrence of Polybrominated diphenyl ethers (PBDEs) and their metabolites in aquatic environment have been ubiquitously founded and widely reported[1-3]. PBDEs have bioaccumulation and biomagnification capacity via food web[4]. BDE-209 showed a minimal accumulation, hexa- to nona-BDEs showed higher tendency of accumulation in fish muscle by dietary exposure[5]. Penta-BDE is considered generally more toxic than Octa-BDE, whereas BDE-209 is less toxic to invertebrates. Penta-BDE may interfere with thyroid and estrogen hormone systems in fish[6] and delay their embryo hatching[7]. It was proposed that hydroxylated PBDEs are structurally similar to thyroxine (T4) or triiodothyronine (T3), and was involved in the regulation of thyroid function in hypothalamo-pituitary-thyroid axis through relevant genes expressions on development[8]. At high doses, BDE-209 could cause changes in the thyroid glands in Fathead Minnows (Pimephales promelas), where it was metabolized to reductive products ranging from penta- to octa-BDEs[3]. PBDE congeners (BDE-28, -47, -99, -100, -153, and -209) could be accumulated in Common sole (*Solea solea L.*) and debrominated congeners of BDE-209 were detected in fish tissues[9], leading to more toxic debromination or hydroxylation products than their precursor PBDEs[10].

Many studies have been conducted on the metabolic degradation process of BDE-209 in fishes, and most of them were carried out in static exposure conditions with single administration dose. However, fish lives in the continuous flow of waters and is exposed to a consistent concentration of toxicant. It has been well documented that the thresholds or even toxicology of toxicant derived in static exposure differs significantly from those in a flowing-through exposure design[11]. Thus, the objective of this study was to elucidate the process of accumulation and debromination of BDE-209 in Japanese medaka when continuously exposed to environmental relevant concentrations.

Materials and methods

Reagents and standards: BDE-209 was purchased from Tokyo Kasei (Tokyo, Japan). The appropriate amount of BDE-209 stock solution was diluted in the distilled water and used in the exposure experiments. The nominal PBDEs concentrations in exposure setups were 0 (as control), 1, 10, 100 and 1000 ng/L, respectively. The measured concentration of BDE-209 was determined daily by SPE-GC/MS when the test solution was freshly prepared. No significant differences were observed between nominal and measured concentrations.

Experimental fish and flow-through exposure protocol: Japnanes medaka (*Oryzias latipes*) may be used as a test organism because it is relatively sensitive to compounds such as endocrine disrupting chemicals[11]. Japanese medaka (*Oryzias latipes)* of the d-rR strain was provided by the Laboratory of Freshwater Fish Stock in Bioscience Center, Nagoya University, Japan. The d-rR strain contains a Y-chromosome linked gene coding for a red body color phenotype, allowing the simple determination of sex genotype: males have red phenotype, while females have white phenotype. Sexually mature medaka (about 4 months of age) was the offspring bred

from the same pair of brood stock. Forty males and forty females were then randomly assigned to control and each treatment groups. The body weights and lengths were 281-345mg and 47.3±3.6mm, respectively. These fish were kept in the following-through de-chlorine tap water of 25 ± 2 °C with a photoperiod of 16:8 h (light: dark). The previous experiment[11] indicated the 0.01% DMSO did not affect the dissolved in water were not significantly different ($p>0.05$) from those exposed to clean water.

Chemical analysis: The analytical protocols used for the analysis of PBDEs in fish muscle were based on the procedure described by Luo et al[12]. The GC/MS analysis was performed on Agilent 6890 GC/5975 MS (Agilent Technologies, Madrid, Spain) and selected ion monitoring using a short DB-5ms capillary column (10m or 15m) was used for the determination of deca-BDEs (BDE-209, nona-BDEs and octa-BDEs) Under the GC-MS/MS conditions, the molecular ions ($[M]^+$ or $[M+2]^+$) and fragment ions resulting from the loss of Br_2 ($[M-Br_2+2]^+$ or $[M-Br_2+4]^+$) were selected as the precursor ions for tandem mass spectrometric analysis.

Results and discussion:

No BDE-209 or its metabolic congeners could be detected in fish muscle of the control group (distilled water with 0.01% DMSO). BDE-209 was detected in all treatment groups and the concentration levels ranged from 29.3±1.02 ng/g to 53.6±2.54 ng/g wet weight (ww) at 15 days, 11.2±0.42 to 33.4±1.58 ng/g ww at 30 days, and 8.19±0.32 to 22.9±1.04 ng/g ww at 60 days, respectively. Comparing to the previous work, the concentrations of BDE-209 in the muscle of medaka under continuous exposure were obviously higher than in rainbow trout (*Oncorhynchus mykiss*) which was 5.3±3.0 ng/g ww at the end of the 5 months after oral exposure of 7.5-10 µg of deca-BDE/kg per day[9].

Deferent fish species has different metabolic potencies to debrominate BDE-209[3,5] and the lower brominated BDEs could be accumulated in fish tissues[8,9,13]. Several hexa-BDE congeners were detected in the muscle of medaka, indicating BDE-209 debromination. The two frequent hexa-BDEs congeners (BDE-154 and BDE-153) that are the major components in commercial penta-BDE were detected at relative lower levels of 3.19±0.19 and 12±0.58 ng/g ww, respectively only after 30 days exposure to 1000 ng/L BDE-209. It indicated that BDE-154 and BDE-153 as intermediates in fish under continuous exposure were negligible, unlike previous work where BDE-154 was found to be the most accumulative metabolite of BDE-209 in juvenile fathead minnows[3], in juvenile rainbow trout and common carp[5], as well as in harbor seals along the northwest Atlantic[14].

It was unexpected that BDE-155, the hexa-BDEs congener that has rarely been reported in fresh fish[15, 16] and marine mammals[17] was detected in present work. Concentration level of BDE-155 ranged from several ng/g ww to a maximum of 178±8.2 ng/g ww and was obviously higher than that of BDE-154. Since BDE-155 is present in commercial penta-BDE mixtures[18] (about 0.2-0.7%), the occurrence of BDE-155 in medaka suggested that it might be also excreted into environmental media as an intermediate of biological transformation from BDE-209. Congeners of penta-BDEs (BDE-100 and BDE-99) were detected at concentration levels of 46.4±2.1 and 9.9±0.58 ng/g ww in exposure group (100 ng/L) at 30 days, while BDE-183 was not detected in present study, as it could rarely detected in other fish samples[19]. Previous work has shown that BDE-183 could easily debrominated to BDE-154 and another unidentified hexa-BDE in carp tissue, which may explain the absence of this congener in fish. The congener pattern of PBDEs showed only slight difference among treatment groups (Fig. 1) illustrating independent of dose. Obviously, this pattern was not the same as those found in other biota or commercial mixtures of BDEs. For sample, the ratio of BDE-99 to BDE-47 is nearly 2:1 in the commercial penta-BDE mixtures[20], about 1:2 in human milk[21], and only 1:0.65 in medaka. It was an indication that lower brominated BDEs may have slower elimination rates in fish.

Accumulation and debromination of PBDEs in fish muscles depended on the exposure concentration and time. The BDE-209 concentration profile in fish muscle (Fig. 2a) showed an exponential increase with increasing exposure concentrations from 1 to 100 ng/L, but reached a steady state from 100 to 1000 ng/L. On the other hand, highest concentration of BED-209 in fish muscle occurred at 15days, followed by decreasing to a constant level (Fig. 2b). The dose-dependent BDE-209 concentration profile in fish muscle (Fig. 2c) was different from that of BDE-209. At 15 days and 60 days, BDE-155 had an exponential increase at lower doses and decrease at higher doses. However at 30 days, BDE-155 showed constant increase from 1 to 1000 ng/L. From Figure 2d, it could be seen that the body burden was higher before 30days and decreased to a constant level, as the same as that for BDE-209. The results indicated that BDE-155 might be a transitional metabolite of BDE-209 and its body burden was adjusted by certain accumulation/elimination mechanism. The muscle concentration profile of BDE-47 was noticeably different from those of BDE-209 and BDE-155. After 60 days, concentration of BDE-47 decreased with the time under the exposure of BDE-209 at 100 ng/L (Fig. 2e and 2f), indicating that debromination and elimination of BDE-47 in fish might have reached a steady state after 60 days. This observation was similar to those of previous work, indicating that BDE-47 might be persistent spices in fish muscle[22]. In general, BDE-209 appeared to be more readily metabolized to lower brominated congners, but its metabolic process and persistent species in fish might be different from other terrestrial species[9, 23] and the time sequence could be seen by comparing Fig. 2b, 2d and 2f.

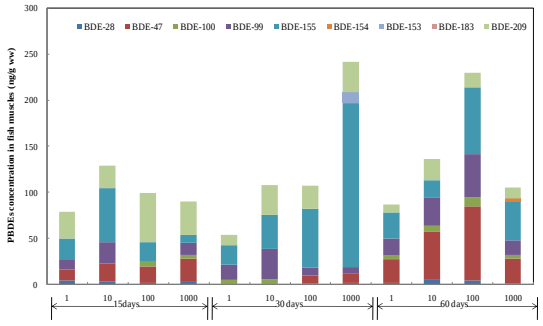

Fig. 1

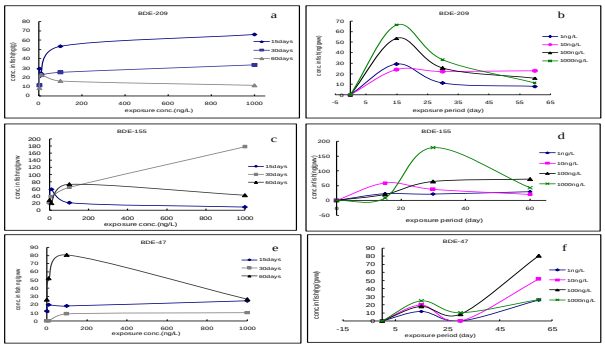

Fig. 2

The time dependent accumulation of BDE-209 in the medaka was fitted to a one-compartment clearance-volume toxicokinetic model usingan iterative, nonlinear least-squares regression program: $C_t = \left[e^{-K_{e\lim}\cdot t} - e^{-K_{abs}\cdot t}\right] K_{abs} FC / (K_{abs} - K_{e\lim})$ where Ct: BDE-209 concentration in the muscle at time t; Kabs: first-order absorption rate constant; Kelim: first-order elimination rate constant; F: bioavailability; C: exposure concentration. Secondary parameters used for calculation included the area under the whole-body concentration-time curve extrapolated to infinity and the terminal elimination half-life (t1/2). The half-life of a chemical from two concentration levels separated by a time interval was calculated according to the equation: $t_{1/2} = 0.693 / K$

When absorption and elimination of BDE-209 (other congeners) reached to steady state in the flow-through condition, the following equation was used: $K = (\ln C_{ending} - \ln C_{beginging}) / t_{\text{interval}}$

The highest concentration of BDE-209 was detected in the fish muscle tissues at 30dwhen the absorption and elimination of BDE-209 reaching a steady state. The result was in line with the predication of uptake and elimination of BDE-209 based on the one-compartment toxicokinetic model that was used for BDE-47[24]. Results of preliminary analysis indicated that a one-compartment model provided a better fit with the data compared to a two-compartment model. The BDE-209 half-life of 16.5 to 19.4 days was reported based for medaka under the

flowing-through exposure condition, which was different from those for rat and gray seals that were experimentally orally dosed, i.e., 2.5 days[25] and 8 to 13 days[26] or 6.8 to 15 days in human serum in occupationally exposed workers[36].

We conclude that BDE-209 was accumulated and debrominated to lower congeners in Japanese medaka. Predominant debrominated congeners found in medaka muscles included BDE-47, -99, and -155. The body burden of BDE-209 and BDE-155 increased with the increase of exposure time and reached to a steady state, while that of BDE-47 reached the highest at end of the exposure (60 days). The half-life of BDE-209 in the medaka was 16.5 to 19.4 days. This study suggested that fish may have a different bioaccumulation capacity and metabolic pattern from other species, either because of species difference or the manner of exposures.

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