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Acute exposure to PBDEs at an environmentally realistic concentration causes abrupt changes in the gut microbiota and host health of zebrafish☆

Lianguo Chen [a, b], Chenyan Hu [c], Nelson Lok-Shun Lai [a], Weipeng Zhang [d], Jianghuan Hua [b], Paul K.S. Lam [a], James C.W. Lam [a, e, **], Bingsheng Zhou [b, *]

[a] State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong SAR, China
[b] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China
[c] School of Chemistry and Environmental Engineering, Wuhan Institute of Technology, Wuhan, 430072, China
[d] Division of Life Science, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong SAR, China
[e] Department of Science and Environmental Studies, The Education University of Hong Kong, Hong Kong SAR, China

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ABSTRACT

Contamination from lower brominated PBDEs is ubiquitous in the environments. However, their effects on gut microbiota and intestinal health have not yet been investigated. This study exposed adult zebrafish to an environmentally realistic concentration of pentaBDE mixture (DE-71) at 5.0 ng/L for 7 days, after which metagenomic sequencing of the intestinal microbiome was conducted and host physiological activities in the intestine and liver were also examined. The results showed that acute exposure to DE-71 significantly shifted the gut microbial community in a sex-specific manner. Certain genera (e.g., *Mycoplasma*, *Ruminiclostridium*, *unclassified Firmicutes sensu stricto*, and *Fusobacterium*) disappeared from the DE-71-exposed intestines, resulting in decreased bacterial diversity. Bacterial metabolic functions in guts were also affected by DE-71, namely those covering energy metabolism, virulence, respiration, cell division, cell signaling, and stress response. In addition, measurement of diverse sensitive biomarkers showed that the health of male intestines was remarkably compromised by the DE-71 exposure, as indicated by the disruption to its neural signaling (serotonin), epithelial barrier integrity (tight junction protein 2), inflammatory response (interleukin 1β), oxidative stress and anti-oxidant capacity, as well as detoxifying potential (ethoxyresorufin-O-deethylase activity). However, female intestines maintained intact physiological activities. Compared to the direct impact on intestines, a latent effect of DE-71 was observed in livers. Co-occurrence network analysis demonstrated that the gut bacteria vigorously interacted to establish the fittest community under DE-71 stress by promoting the reproduction of favorable genera, while diminishing the survival of unfavorable ones. Significant correlations between the zebrafish gut microbiota and physiological activities (e.g., oxidative stress, detoxification, neurotransmission, and epithelial integrity) were also observed. Overall, this study has demonstrated, for the first time, the high susceptibility of gut microbiota and intestinal health of zebrafish to DE-71, thus warranting more work to reveal its mode of toxicity.

Abbreviations: AhR, Aryl hydrocarbon receptors; CAT, Catalase; DMSO, Dimethyl sulfoxide; ELISA, Enzyme-linked immunosorbent assay; ER, Estrogen receptors; EROD, Ethoxyresorufin-O-deethylase; FA, Fatty acid; FAS, Fatty acid synthase; F/B, Firmicutes/Bacteroidetes; GPx, Glutathione peroxidase; GSH, Glutathione; HSI, Hepatosomatic index; IL1b, Interleukin 1β; K, Condition factor; PBDEs, Polybrominated diphenyl ethers; PCA, Principal component analysis; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TG, Triglyceride; TJP2, Tight junction protein 2.

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* Corresponding author.
** Corresponding author. State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong SAR, China.
E-mail addresses: jameslam@eduhk.hk (J.C.W. Lam), bszhou@ihb.ac.cn (B. Zhou).

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as additive flame retardants in many kinds of consumer products (e.g., furniture, textiles, appliances, and electronics), but have elicited much concern regarding their environmental pollution and adverse effects. Although the use of lower brominated congeners is now prohibited and replaced by the higher brominated BDE-209, the ecological risks from the lower brominated PBDEs are still prevalent because of their persistent nature and the dramatic debromination of BDE-209 (Stapleton et al., 2004, 2006; Feng et al., 2010). To this day, PBDEs containing fewer bromines are continuously detected, both widely and abundantly, in environmental samples taken from e-waste recycling sites in China (Liu et al., 2011; Zeng et al., 2013; Zheng et al., 2015; Zhao et al., 2016). Prior research has detected severe pollution from PBDEs that ranges from 0.34 ng/L to 68.0 ng/L in the environmental waters from the Pearl River Delta (Guan et al., 2007). Furthermore, although zebrafish *Danio rerio* are parentally exposed to BDE-209, mainly the lower brominated congeners are transferred to their offspring in large amounts, thus putting the developing embryos under greater risk from bioaccumulation and toxic threats (Chen et al., 2017a).

PBDEs, with lower brominated congeners being of particular concern, are known to disrupt the function of thyroid system and impair the development of central nervous system (Wiseman et al., 2011; Yu et al., 2011; Chen et al., 2012a). In addition, metabolic disorders and inhibited growth resulting from PBDE-related stresses are well documented in different animals, such as invertebrates (Ji et al., 2013; Chen et al., 2015), fish (Ghosh et al., 2013; Legradi et al., 2014), and rats (Nash et al., 2013; Kodavanti et al., 2015).

Gut microbiota are increasingly appreciated for playing crucial roles in the maintenance of host health by modulating various physiological activities, namely energy metabolism, immune response, neural signaling, and behavioral control (Holmes et al., 2011; Kinross et al., 2011; Semova et al., 2012; Tremaroli and Backhed, 2012; Portune et al., 2017). Alterations to the community of gut microbiota can dysregulate the normal physiological functions in host animals and trigger the onset of various diseases (e.g., obesity and diabetes) (Tilg and Kaser, 2011; Mathis and Benoist, 2012; Snedeker and Hay, 2012). Specifically in zebrafish, previous studies demonstrate that administration of probiotic recipe containing *Lactobacillus rhamnosus* changes gut microbiomic community, which subsequently modulates host lipid and glucose metabolism, activates the endocannabinoid system, controls immune response and inflammation, restructures intestinal epithelial organization, and eventually affects fish growth (Falcinelli et al., 2015, 2016; Gioacchini et al., 2017). In addition, the high susceptibility of gut microbiota makes them a particularly sensitive target of environmental pollutants. Previous studies have reported the dysbiosis of gut microbiota by various pollutants, such as antibiotics, heavy metals, and persistent organic pollutants (Snedeker and Hay, 2012; Jin et al., 2017).

PBDEs can be accumulated through feed and excreted through feces in animals (Huwe and Smith, 2007; Hakk et al., 2009; Chen et al., 2017b), thus subjecting gut microbes to their direct exposure. However, the effects of PBDEs on gut microbial dynamics are not yet understood. To this end, the current study acutely exposed adult zebrafish (*D. rerio*) to an environmentally realistic concentration (5.0 ng/L) of a pentaBDE mixture DE-71, containing the representative lower-brominated congeners that are highly persistent in biotic and abiotic environments. The duration of exposure lasted for just 7 days. Based on the metagenomic analysis of the gut microbiota and the monitoring of the host healthy indices, this study had two aims: (1) To determine whether such a short-term environmentally-realistic exposure of DE-71 is still able to impact gut microbial composition and metabolism, thus establishing the toxicological baseline about the susceptibility of gut microbiota; and (2) To provide clues about whether gut microbiota dysbiosis is relevant to host health under conditions of xenobiotic stress, thus finding potential microbial indicators of the toxicity from PBDEs.

2. Materials and methods

2.1. Chemicals

DE-71 with a >99.9% purity was obtained from Wellington Laboratories Inc. (Guelph, Canada). Stock solutions of DE-71 were prepared in dimethyl sulfoxide (DMSO) that was of high-performance liquid chromatography-grade (Sigma-Aldrich Corp., St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Fish maintenance and exposure

Four-month old adult zebrafish were cultured and exposed as previously described (Chen et al., 2012a), in a semi-static system that contained 20 L of charcoal-filtered and fully-aerated tap water under a stable ambient temperature ($28 \pm 0.5\,^{\circ}C$) and a light/dark cycle of 14/10 h. The fish were obtained from laboratory-raised strains after reproducing stably for many generations in order to minimize individual variation in gut microbiota for repeatable data (Roeselers et al., 2011). After two-weeks of acclimation, the fish were nominally exposed to 10 ng/L DE-71 for 7 days; only an equal volume of DMSO was added to the control tanks (<0.001% v/v). The exposure concentration was determined as environmentally realistic based on previous environmental monitoring data, which reports high levels of PBDEs (0.34–68.0 ng/L) in the water samples from the Pearl River Delta (Guan et al., 2007). Approximately 20 male and 20 female fish were randomly included in each tank. There were three replicate tanks for each group. During the exposure period, the exposure media were renewed daily, and the fish were fed twice daily with flake food and freshly-hatched *Artemia* nauplii. After the exposure, all the zebrafish were anesthetized using 0.03% MS222 (Sigma-Aldrich Corp.) and then exterior surfaces swabbed using pure ethanol (Roeselers et al., 2011). The intestines and livers were dissected using sterilized forceps, snap-frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ for the following metagenomic and biochemical analyses.

2.3. Measurement of PBDEs waterborne concentrations

PBDEs in the 5-mL exposure media were loaded into the pre-conditioned 200-mg OASIS HLB SPE cartridges (Waters, Milford, USA). After elution and reconstitution in 0.5 mL of methanol, the extracts were subjected to analysis on an Agilent 1290 Infinity LC coupled to an AB SCIEX QTRAP 5500 LC-MS/MS system with an Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm), as previously described by Chen et al. (2015). The procedural recovery of PBDEs from the exposure media was 70%.

2.4. Metagenomic sequencing and bioinformatic analyses

From each tank, 10 intestines of the same sex were pooled together and considered a biological replicate ($n = 3$ per treatment group). Genomic DNA was extracted with a DNeasy Blood & Tissue Kit following the manual's instructions (Qiagen, Hilden, Germany), and DNA quality was evaluated by an UV–Vis spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, USA) and by agarose gel electrophoresis. The obtained DNA was further