The effects of PBB153 on the levels of E2, T and Vtg in zebrafish

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Abstract. Polybrominated biphenyls (PBBs) are a class of industrial chemicals with the characteristics of persistent organic pollutant, which can cause pathological effects on the endocrine system such as the thyroid and adrenal glands. At the present study, the experiment was designed to obtain the effects of PBB153 on the levels of E2, T and Vtg in zebrafish. The zebrafish were exposed to increasing concentrations of PBB153 solution (0, 0.1 mg/L, 0.2 mg/L, 0.5 mg/L) for 21 days. The results showed that PBB153 had a certain inhibitory effect on the secretion of E2, T and Vtg in zebrafish. However, there are relatively few studies on the harm of PBBs and others environmental chemical pollutants to the endocrine system of aquatic organisms, and further research is needed.

1. Introduction

Hexabromobiphenyls (HexaBBs), which belong to one of PBBs, are the main additive component of FireMaster®BP-6 and FireMaster®FF-1 products in history, and PBB153 accounts for more than 53% of the total PBBs[1]. Studies have shown that PBBs could have pathological effects on the thyroid[2-3], adrenal glands[4-5] and reproductive system[6-7] of humans and animals. In recent years, HexaBBs can be detected in different environmental media such as soil[6], water birds[9] and aquatic products[10-11] with the characteristics of long-distance transport and persistent residue of HexaBBs, which may have a disruptive effect on the endocrine system of aquatic organisms. In 2016, PBBs were listed by the International Agency for Research on Cancer as a category 2A substance that may cause cancer to the human body[12].

Zebrafish is one of the most popular model organisms in vertebrate biology, which have been used as aquatic biological models to study the hazards of environmental chemical pollutants, such as polychlorinated biphenyls (PCBs)[13], organochlorine pesticides[14] and triclocarban[15] et al, these compounds can interfere with the function of fish steroid hormone system, thyroid and other tissues[16-17]. PBBs and PCBs were both halogenated cyclic compounds with similar structures, PBB153 had been detected many times in aquatic organisms and other samples[9-11], but there were few studies on the endocrine toxicological effects of HexaBBs on aquatic organisms. HexaBBs may also participate in the regulation of the endocrine system of aquatic organisms like PCBs, disturb the dynamic balance of
hormones in the endocrine system, and ultimately affect the growth and development of aquatic organisms\textsuperscript{[13],[18]}.

In order to study the estrogenic disrupting effects of PBB153 on aquatic organisms, this study used zebrafish as a model organism to reveal whether PBB153 would have any effects on the levels of sex hormones (17\textbeta-estradiol (E2), testosterone (T)) and vitellogenin (Vtg) in zebrafish. It was of great significance to explore the interference effects of PBB153 and other PBBs on the endocrine system of aquatic organisms.

2. Experimental

2.1 Reagents and materials
PBB153 (2,2',4,4',5,5'-hexabromobiphenyl) was purchased from Toronto Research Chemicals Inc. (trc-Canada); E2, T and Vtg kits were supplied by Shanghai Enzyme-linked Biotechnology Co., Ltd.; Dimethyl sulfoxide (DMSO) was obtained from Shanghai Aladdin Biochemical Technology Co., Ltd.; Multiskan MK3 microplate reader was purchased from Thermo Company USA.

2.2 Test organisms and experimental methods
Wild-type (AB strain) adult zebrafish were purchased from China Zebrafish Resource Center under a 16 h:8 h light: dark photoperiod cycle (temperature: 28 ± 0.5 °C, pH 7.54 ± 0.08), feed fresh brine shrimp (Artemia) once every morning and evening. The zebrafish were exposed to increasing concentrations of PBB153 solution (0, 0.1 mg/L, 0.2 mg/L, 0.5 mg/L) for 21 days following the OECD guideline 229\textsuperscript{[19]}. The selected concentrations of PBB153 were determined based on the in vitro measurements before this study and other published reports data from relevant literature, which included environmental exposure levels and rat LD\textsubscript{50}. Three parallels containing 15 zebrafish were set for each PBB153 exposure level and solvent control (0.005% (vol/vol) DMSO). Each replicate was placed in 3 L glass tank under semi-static conditions. Half of the exposure solution was updated to a newly prepared exposure solution of the same mass concentration every two days. After the exposure, all zebrafish were anesthetized with MS-222 and their heads and tails were mixed and weighed, and store them frozen at -80 °C for research and analysis.

2.3 Determination of E2 T and Vtg
The collected samples were placed in a 15 mL centrifuge tube, homogenized with a tissue homogenizer in ice bath, added 9 times the mass of phosphate buffered saline solution (10 mmol/L PBS, pH 7.2-7.4) and mixed well; at 4 °C centrifuge for 15 min (10,000 r/min), and ultimately draw the supernatant under the fat layer for the determination of E2, T and Vtg.

2.4 Statistical analysis
All experimental data were expressed as mean ± standard deviation (mean ± SD). Data were analyzed by one-way analysis of variance followed by Tukey's multiple comparisons test was used to test statistical significances between the solvent control and the treatment groups. Statistical significance was set at the P<0.05 level. Statistical analysis was performed using IBM SPSS 25.

3. Results and analysis

3.1 Determination results of E2, T and Vtg in zebrafish
The E2, T and Vtg levels of zebrafish were measured according to the double antibody sandwich method provided by the instructions in the kits. The effect of PBB153 on E2 is shown in Figure 1, which can be obtained that PBB153 caused the decrease of E2 level in zebrafish after exposure to increasing concentrations of PBB153 for 21 days. The E2 level of female zebrafish decreased significantly at concentration levels of PBB153 were 0.2 and 0.5 mg/L (P<0.05), and there was a certain dose-effect
relationship. The data of male zebrafish was the same result at the concentration of PBB153 was only 0.5mg/L (P<0.05).

Figure 1 The effect of PBB153 on E2 in zebrafish homogenate

Figure 2 shows the effect of PBB153 on T in zebrafish. The T contents of female zebrafish did not change significantly at the first two exposure levels of PBB153, and a significant reduction was observed in T levels of female zebrafish exposed to 0.5 mg/L PBB153 (P<0.05). The T levels of male zebrafish were significantly lower than those in the blank solvent control at concentration levels of PBB153 were 0.2 mg/L and 0.5 mg/L after 21 days of exposure (P<0.05).

Figure 2 The effect of PBB153 on T in zebrafish homogenate

The Vtg levels of zebrafish are shown in Figure 3. The Vtg contents of female zebrafish gradually decreased with the increasing of PBB153 exposure concentrations, showing a dose-effect relationship, and the significant reduction were observed in Vtg levels of female zebrafish exposed to 0.2 mg/L and 0.5 mg/L PBB153 (P<0.05) when compared to the solvent control. The Vtg contents of male zebrafish were lower overall, which was significant compared with the solvent control at the concentration of PBB153 was only 0.5 mg/L (P<0.05). And this phenomenon was consistent with the above-mentioned effects of PBB153 on E2 in zebrafish.

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Sex hormones play an important role in the differentiation of biological sexual characteristics, development of gonads, and maintaining the balance of the endocrine system. Environmental chemical pollutants are mostly ring structures, which are similar to the structure of sex hormones, and may participate in the regulation of sex hormones in the organism together with endogenous sex hormones, and ultimately affect the growth and development of the organisms[13].

This study showed that PBB153 inhibited the secretion of E2 and T in zebrafish after exposure to increasing concentrations of PBB153. The gonad is the main place for organisms to synthesize E2, and CYP19 and CYP11A1 are the rate-limiting enzymes in the process of synthesizing E2[20]. Reports indicated that some environmental chemical pollutants can inhibit the activity of these key enzymes to produce anti-estrogen effects[21]. Ankley et al.[22] evaluated the effects of prochloraz and fenarimol on the reproductive endocrine function of the fathead minnow and found that both prochloraz and fenarimol can not only inhibit the activity of CYP19 in the brain and ovarian homogenates of fathead minnow, but also competitively bind to the T receptors in male fathead minnow, resulting in a significant decrease in the levels of E2, T and Vtg. Shilling et al.[23] also obtained the same results in the study of rainbow trout, and found that letrozole can reduce the activity of CYP19 by 90% in a dose-dependent manner. Skolness et al.[21] explored the effects of prochloraz on the endocrine system of female fathead minnow and found that the expression level of 17α-hydroxylase mRNA, which belongs to the CYPs family and involves in multiple processes of E2 synthesis, was reduced after 6 h of exposure. This study showed that PBB153 inhibited the secretion of E2 and T in zebrafish, which may be because PBB153 affected the activity of the corresponding key enzymes. However, there are relatively few studies on the endocrine toxicity of PBBs on aquatic organisms, and this conclusion needs further research to confirm.

3.3 The effects of PBB153 on the level of Vtg in zebrafish

Through this study found that PBB153 inhibited the secretion of Vtg in zebrafish, especially for female zebrafish. Vtg has been used as a biomarker in the liver and plasma of juvenile and male fish to assess the risk of environmental chemical pollutants such as dioxins and PCBs that interfere with the endocrine system of organisms[18]. Quintaneiro et al.[13] evaluated the potential endocrine effects of PCB77 on zebrafish gonadal differentiation and found that the Vtg levels of juvenile fish decreased after exposure to PCB77, and the Vtg levels and gonadal index (GSI) of female zebrafish decreased after 3 months, indicating that PCB77 inhibited the production of yolk in juvenile zebrafish, and continued to adulthood. PCB77 is an AhR agonist with anti-estrogen activity, which can interact with AhR to interfere with ER-mediated signaling pathways and exhibit anti-estrogen effects[13][24]. Calò et al.[18] studied the effects of PCB126 and PCB153 on the Vtg expression of Sparus aurata and found that the expression of Vtg in juvenile fish reached the highest level after 12 h of exposure to PCB126, PCB153 or a mixture of both,
showing an estrogenic effect. However, an anti-estrogenic activity was detected after 24 h of exposure, which may be due to the defense mechanism against endogenous hormones or exogenous hormones, and the expression of Vtg was inhibited by AhR.

In this study, the Vtg levels of zebrafish were significantly reduced after 21 days of exposure to PBB153. Studies have shown that the synthesis of Vtg is mainly regulated by the steroid hormone E2[25], the decrease of Vtg levels may be induced the synthesis of cytochrome P450 (P4501A1, P4501B1) after the combination of PBB153 and AhR, which can enhance the oxidative metabolism of E2 in zebrafish[26], and ultimately lead to the decrease of E2 and Vtg levels in zebrafish.

4. Conclusions
The main purpose of this study was to explore the effects of PBB153 on the levels of E2, T and Vtg in zebrafish. Research results showed that PBB153 had a certain inhibitory effect on the secretion of E2, T and Vtg in zebrafish. This may be due to PBB153 inhibited the activity of certain enzyme rate-limiting enzymes or the combination of PBB153 and AhR in zebrafish. However, there are relatively few studies on the harm of PBBs and other environmental chemical pollutants to the endocrine system of aquatic organisms, and further research is needed.

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References
[1] Silberhorn E M, Glauert H P, Robertson L W. Carcinogenicity of polyhalogenated biphenyls : PCBs and PBBS[J]. Critical Reviews in Toxicology, 1990, 20(6): 440-496.
[2] Jacobson M H, Darrow L A, Barr D B, et al. Serum polybrominated biphenyls (PBBs) and polychlorinated biphenyls (PCBs) and thyroid function among michigan adults several decades after the 1973-1974 PBB contamination of livestock feed[J]. Environmental Health Perspectives, 2017, 125(9): 097020: 1-15.
[3] Bahn A K, Mills J L, Snyder P J, et al. Hypothyroidism in Workers Exposed to Polybrominated Biphenyls[J]. New England Journal of Medicine, 1980, 302(1): 31-33.
[4] Meserve L A, Murray B A, Landis J A. Influence of maternal ingestion of aroclor 1254® (PCB) or firemaster BP-6® (PBB) on unstimulated and stimulated corticosterone levels in young rats[J]. Bulletin of Environmental Contamination and Toxicology, 1992, 48(5): 715-720.
[5] Graceli J B, Dettogni R S, Merlo E, et al. The impact of endocrine-disrupting chemical exposure in the mammalian hypothalamic-pituitary axis[J]. Molecular and Cellular Endocrinology, 2020, 518: 110997.
[6] Small C M, Murray D, Terrell M L, et al. Reproductive outcomes among women exposed to a brominated flame retardant in utero[J]. Archives of Environmental & Occupational Health, 2011, 66(4): 201-8.
[7] Greeson K W, Fowler K L, Estave P M, et al. Detrimental effects of flame retardant, PBB153, exposure on sperm and future generations[J]. Scientific Reports, 2020, 10(1): 8567.
[8] Zhao G F, Wang Z J, Dong M H, et al. PBBs, PBDEs, and PCBs levels in hair of residents around e-waste disassembly sites in Zhejiang Province, China, and their potential sources[J]. Science of the Total Environment, 2008, 397(1-3): 46-57.
[9] Luo X J, Zhang X L, Liu J, et al. Persistent halogenated compounds in waterbirds from an e-waste recycling region in south China[J]. Environmental Science & Technology, 2009, 43(2): 306–311.
[10] Gieron J, Grochowalski A, Chrzaszcz R. PBB levels in fish from the Baltic and North seas and in selected food products from Poland[J]. Chemosphere, 2010, 78(10): 1272-1278.
[11] Shen H Q, Main K M, Andersson A-M, et al. Concentrations of persistent organochlorine compounds in human milk and placenta are higher in Denmark than in Finland[J]. Human Reproduction, 2007, 23(1): 201-210.
[12] Lauby-Secretan B, Loomis D, Grosse Y, et al. Carcinogenicity of polychlorinated biphenyls and polybrominated biphenyls[J]. The Lancet Oncology, 2013, 14(4): 287-288.
[13] Quintaneiro C, Soares A M V M, Costa D, et al. Effects of PCB-77 in adult zebrafish after exposure during early life stages[J]. Toxic/Hazardous Substances and Environmental Engineering, 2019, 54(5): 478-483.
[14] Rahman M S, Islam S M M, Haque A, et al. Toxicity of the organophosphate insecticide sumithion to embryo and larvae of zebrafish[J]. Toxicology Reports, 2020, 7: 317-323.
[15] Shi Q, Zhuang Y, Hu T, et al. Developmental toxicity of triclocarban in zebrafish (Danio rerio) embryos[J]. Journal of Biochemical & Molecular Toxicology, 2019, 33(5): e22289.
[16] Simmons D B D, Mcmaster M E, Reiner E J, et al. Wild fish from the Bay of Quinte Area of Concern contain elevated tissue concentrations of PCBs and exhibit evidence of endocrine-related health effects[J]. Environmental International, 2014, 66: 124-137.
[17] Arukwe A, Olufsen M, Cicero N, et al. Effects on development, growth responses and thyroid-hormone systems in eyed-eggs and yolk-sac larvae of Atlantic salmon (Salmo salar) continuously exposed to 3,3',4,4'-tetrachlorobiphenyl (PCB-77)[J]. Journal of Toxicology Environmental Health Perspectives, 2014, 77(9-11): 574-586.
[18] Calò M, Alberghina D, Bitto A, et al. Estrogenic followed by anti-estrogenic effects of PCBs exposure in juvenile fish (Sparus aurata)[J]. Food and Chemical Toxicology, 2010, 48(8): 2458-2463.
[19] OECD. Test NO. 229: Fish short term reproduction assay[M]. Section 2. Paris: OECD Publishing, 2012: 1-40.
[20] Chumsri S, Howes T, Bao T, et al. Aromatase, aromatase inhibitors, and breast cancer[J]. The Journal of Steroid Biochemistry and Molecular Biology, 2011, 125(1): 13-22.
[21] Skolness S Y, Durhan E J, Garcia-Reyero N, et al. Effects of a short-term exposure to the fungicide prochloraz on endocrine function and gene expression in female fathead minnows (Pimephales promelas)[J]. Aquatic Toxicology, 2011, 103(3-4): 170-178.
[22] Ankley G T, Jensen K M, Durhan E J, et al. Effects of two fungicides with multiple modes of action on reproductive endocrine function in the fathead minnow (pimephales promelas)[J]. Toxicological Sciences, 2005, 86(2): 300-308.
[23] Shilling A D, Carlson D B, Williams D E. Rainbow trout, Oncorhynchus mykiss, as a model for aromatase inhibition[J]. The Journal of Steroid Biochemistry and Molecular Biology, 1999, 70(1): 89-95.
[24] Skjetne Mortensen A, Tolfsen C C, Arukwe A. Gene expression patterns in estrogen (nonylphenol) and aryl hydrocarbon receptor agonists (PCB-77) interaction using rainbow trout ( Oncorhynchus mykiss ) primary hepatocyte culture[J]. Journal of Toxicology Environmental Health Part A, 2006, 69(1-2): 1-19.
[25] Czarny K, Szczukocki D, Krawczyk B, et al. The impact of estrogens on aquatic organisms and methods for their determination[J]. Critical Reviews in Environmental Science Technology, 2017, 47(11): 909-963.
[26] Spink D C, Johnson J A, Connor S P, et al. Stimulation of 17 beta-estradiol metabolism in MCF-7 cells by bromochloro- and chloromethyl-substituted dibenzo-p-dioxins and dibenzofurans: correlations with antiestrogenic activity[J]. Journal of Toxicology Environmental Health Perspectives, 1994, 41(4): 451-466.