DOCTORAL THESIS

Synthesis and Determination of Dihydroxylated Polybrominated Diphenyl Ethers and Investigation of Developmental Toxicity for Early Life-Stages Zebrafish

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs) are a series of halogenated flame retardants that have been widely used in wire insulation, furniture, textiles and electronic devices. About 2.3 million tons of e-waste are produced every year in China. The recovery and recycling process of electronic waste could be the mainly way to release PBDEs. In this study, air and dust samples were taken from e-waste recycling plants for the recycling of waste TVs, washing machines, refrigerators, and printed wiring. Σ_7PBDEs concentrations were measured and the exposure risk for workers at e-waste recycling plants were evaluated. Σ_7PBDEs in air and dust samples were in the ranges of 55.28-369.66 ng/m^3 and 158.07-669.81 μg/g, respectively. BDE-209 was the main PBDEs workers who exposed to and accounted for more than 85%. The hazard quotient (HQ) levels ranged from 0.01-0.18, thus indicating that no adverse health effects are expected for occupational exposure. Further study showed that the toxicity of PBDEs related to the metabolites of PBDEs, especially the hydroxylated metabolites.

The biological effects and toxicity of dihydroxylated PBDEs (diOH-PBDEs) should be investigated owing to their potentially high bioavailability and structural similarity to hydroxylated PBDEs (OH-PBDEs). Toxicity of OH-PBDEs were widely studied. However, research on diOH-PBDEs is limited because of the lack of reference standards. Therefore, hydroxylated metabolites of PBDEs were synthesized as follows:

6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6OH-BDE47), 3,5-dihydroxy-2,2',4-tribromodiphenyl ether (3,5-diOH-BDE-17), 6,6''-dihydroxy-2,2',4,4'-tetrabromodiphenyl ether (6,6'diOH-BDE47),

3,5-dihydroxy-2,3',4,5'-tetrabromodiphenyl ether (3,5-diOH-BDE-51), and 3,5-dihydroxy-2,2',4,4',6-pentabromodiphenyl ether (3,5-diOH-BDE-100).

In the present study, a method was established to determine the OH-PBDEs and diOH-PBDEs in marine fish samples. The recovery of this method ranged from 47.82% to 87.42% for the spiking levels of 40 ng. The equipment detection limits were in the range of 0.31 to 2.78 pg/μL, and the limits of detection for this method were in the range of 5.07 to 38.74 pg/g wet weight. The concentrations of diOH-PBDEs in the marine fish samples were in the range of 32.43 to 1528.63 pg/g wet weight. There were similar compositions of OH-PBDEs/diOH-PBDEs within the same family of marine fish.

Acute toxicity tests for OH-PBDEs/diOH-PBDEs in zebrafish embryos showed that the 6,6'-diOH-BDE-47 was the most toxic of the diOH-PBDEs (LC_{50} = 516 nM) but still less than the 6-OH-BDE-47 (LC_{50} = 235 nM). Severe developmental delays and morphological deformities were observed in zebrafish larvae at high exposure concentrations. At exposure levels of 1 to 50 nM, the concentration of accumulated 6-OH-BDE-47 and 6,6'-diOH-BDE-47 in the larvae ranged between 226–2279 nmol/g and 123–539 nmol/g, respectively. Significantly increased thyroid hormone levels were recorded for 6-OH-BDE-47 and 6,6'-diOH-BDE-47 exposure. Exposure to 6-OH-BDE-47 and 6,6'-diOH-BDE-47 induced significant upregulation of thyroglobulin (TG) expression in the thyroid gland, thus indicating that exposure might exert adverse effects associated with the HPT axis.

An *in silico* study was performed to reveal the underlying mechanism by which the TRs of zebrafish bind to the diOH-PBDEs. Three-dimensional (3D) structural models of zebrafish, TRα and TRβ, were successfully constructed by a homology modelling

method. Ramachandran plots of TRα and TRβ were within the allowable range. The TRα and TRβ zebrafish conformations were evaluated by molecular dynamics (MD) simulation. The binding energies of the OH/diOH-PBDEs with TRα and TRβ ranged from -9.66 to −7.56 kcal/M. The OH/diOH-PBDEs did not bind to the TRs as strongly as T3. Disruption effects of OH/diOH-PBDEs on thyroid hormones evaluated by competitive binding with TRα and TRβ. In the thyroid hormone SRC2-2 recruitment assay, only the 6-OH-PBDEs showed significant recruitment of SRC2-2. None of the diOH-PBDEs showed the recruitment of SRC2-2 at 0.32–25000 nM. Meanwhile, 3,5-diOH-BDE-100, and 6,6'-diOH-BDE-47 showed antagonistic activity against the thyroid hormones. It indicated that the agonistic or antagonistic activities of the OH/diOH-PBDEs were induced by subtle conformational changes in the protein.

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