

## 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.  $\Sigma_7$ PBDEs concentrations were measured and the exposure risk for workers at e-waste recycling plants were evaluated.  $\Sigma_7$ PBDEs in air and dust samples were in the ranges of 55.28-369.66 ng/m<sup>3</sup> and 158.07-669.81  $\mu$ g/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/ $\mu$ L, 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, TR $\alpha$  and TR $\beta$ , were successfully constructed by a homology modelling

method. Ramachandran plots of TR $\alpha$  and TR $\beta$  were within the allowable range. The TR $\alpha$  and TR $\beta$  zebrafish conformations were evaluated by molecular dynamics (MD) simulation. The binding energies of the OH/diOH-PBDEs with TR $\alpha$  and TR $\beta$  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 TR $\alpha$  and TR $\beta$ . 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.

## Table of Contents

DECLARATION.....	i
ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	v
Table of Contents.....	viii
List of Tables.....	xiv
List of Figures.....	xvi
List of Schemes.....	xxi
List of Abbreviation.....	xxii
Chapter 1 Introduction.....	1
1.1 Occurrence of diOH-PBDEs.....	1
1.1.1 Usage of PBDEs.....	1
1.1.2 Formation of dihydroxylated-PBDEs (diOH-PBDEs).....	2
1.2 PBDEs and their metabolites in environmental samples and biosamples.....	5
1.3 Thyroid hormone production regulation.....	9
1.4 Thyroid hormone toxicity of PBDEs and their metabolites.....	14
1.5 OH-PBDEs and thyroid hormone activity in early life stages of zebrafish....	16
1.6 In vitro study with respect to thyroid hormone toxicity.....	18

1.7 In silico study about the thyroid hormone toxicity.....	20
1.8 Objective of this project.....	22
Chapter 2 PBDEs emission from e-waste recycling workshops and workers' exposure characteristics in China.....	25
2.1 Introduction.....	25
2.2 Material and methods.....	27
2.2.1 Sampling.....	27
2.2.2 Sample extraction, clean-up, and analysis.....	28
2.2.3 Exposure level and health hazard assessment.....	30
2.3 Results and discussion.....	32
2.3.1 PBDE concentrations in air and dust.....	32
2.3.2 PBDE composition in air and dust.....	38
2.3.3 PBDEs exposure and risk assessment.....	41
2.4. Conclusion.....	45
Chapter 3 Synthesis of Dihydroxylated Polybrominated Diphenyl Ethers - Potential Polybrominated Diphenyl Ether Metabolites.....	47
3.1 Introduction.....	47
3.2 Experimental Procedures.....	48

3.2.1	General procedure for synthesis of diaryliodonium salts.....	48
3.2.2	General procedure for synthesis of methoxy bromophenols.....	49
3.2.3	Synthesis of MeO-PBDEs via brominated diphenyliodonium salts.....	56
3.2.4	Demethylation of MeO-PBDEs with boron tribromide.....	60
3.3	Conclusion.....	67
Chapter 4	Determination of newly synthesized dihydroxylated polybrominated diphenyl ethers in marine fish via gas chromatography-tandem mass spectrometry...	69
4.1	Introduction.....	69
4.2	Materials and methods.....	71
4.2.1	Standards and reagents.....	71
4.2.3	Sample treatment, extraction and clean-up.....	75
4.2.4	Instrument analysis.....	79
4.2.5	Method validation.....	81
4.3	Results and discussion.....	82
4.3.1	Optimization of sample pretreatment.....	82
4.3.2	Method performance.....	86
4.3.3	Method application.....	90
4.4	Conclusion.....	95

Chapter 5	Toxicity and Accumulation of 6-OH-BDE-47 and Newly Synthesized 6,6'-diOH-BDE-47 in Early Life-Stages of Zebrafish ( <i>Danio rerio</i> ).....	96
5.1	Introduction.....	96
5.2	Materials and methods.....	98
5.2.1	Chemicals.....	98
5.2.2	Zebrafish maintenance and experimental design.....	99
5.2.3	Bioconcentration analysis.....	100
5.2.4	Thyroid hormones (T <sub>3</sub> /T <sub>4</sub> ) extraction and detection.....	102
5.2.5	RNA isolation and quantitative reverse expression polymerase chain reaction (qRT-PCR).....	102
5.3	Results.....	106
5.3.1	Acute toxicities.....	106
5.3.2	Accumulation of 6-OH-BDE-47 and 6,6'-diOH-BDE-47.....	111
5.3.3	T <sub>3</sub> and T <sub>4</sub> levels in the whole body.....	112
5.3.4	Expression profiles of zebrafish larvae HPT genes.....	114
5.4	Discussion.....	116
5.5	Conclusion.....	119
Chapter 6	Homology modeling of thyroid hormone receptor for zebrafish and	



insights into diOH-PBDEs binding through molecular docking and molecular dynamics simulations studies.....	121
6.1 Introduction.....	121
6.2 Materials and methods.....	122
6.2.1 Swiss-model homology modeling.....	122
6.2.2 Molecular docking with the Autodock.....	122
6.2.3 Molecular dynamics (MD) simulation with GROMACS.....	123
6.2.4 Interaction of OH/diOH-PBDEs toward TR.....	124
6.3 Results and discussion.....	124
6.3.1 Homology modeling for zebrafish TR $\alpha$ and TR $\beta$ .....	124
6.3.2 Molecular docking for TR $\alpha$ and TR $\beta$ to T3.....	133
6.3.3 Molecular dynamics simulation for ligand binding stable of TR-T3/4134	
6.3.4 Binding affinity and molecular dynamics simulation for TR-OH/diOH-PBDEs.....	137
6.4 Conclusion.....	145
Chapter 7 In vitro and in silico competitive binding of diOH-PBDEs with human thyroid hormone receptors.....	146
7.1 Introduction.....	146

7.2 Materials and Methods.....	147
7.2.1 Chemicals.....	147
7.2.2 Coactivator recruitment assay.....	148
7.3 Results and discussion.....	149
7.3.1 TR disrupting activity assays with SPR-based biosensor.....	149
7.3.2 Binding affinity and molecular dynamics simulation for TR-OH/diOH-PBDEs.....	155
7.4 Conclusion.....	161
Chapter 8 Conclusion and future perspectives.....	163
List of References.....	166
Outcome of the Thesis Work.....	206
Curriculum Vitae.....	错误! 未定义书签。

## List of Tables

Table 1. 1 Composition by weight of commercial penta-, octa-, and decaBDEs.....	2
Table 2. 1 Concentrations of PBDE congeners in air and dust at domestic e-waste recycling workshops treating printed wiring boards (PWBs), TVs, washing machines, and refrigerators.....	34
Table 2. 2 Concentrations of selected PBDEs in dust and air samples from e-waste recycling workshops in previous studies and in this research.....	35
Table 2. 3 Inhalation exposure dose ( $ADD_{inh}$ , pg/kg/d) and dust ingestion exposure dose ( $ADD_{ing}$ , pg/kg/d) of PBDEs at PWB, TV, washing machine, and refrigerator recycling workshops.....	42
Table 4. 1 Characterization and MRM parameters of methoxylated OH-PBDEs/diOH-PBDEs.....	73
Table 4. 2 Recovery of the OH-PBDEs/diOH-PBDEs partitioned with a KOH-solution (n=3).....	84
Table 4. 3 Method performance for the analysis procedure of OH-PBDEs/diOH-PBDEs in fish samples.....	86
Table 4. 4 Response of 20 ng/mL methxyled OH-PBDEs/diOH-PBDEs (n=5) and matrix effect (ME) evaluation.....	88
Table 4. 5 Concentrations (pg/g wet weight) of OH-PBDEs/diOH-PBDEs detected in the market sea fish muscle.....	93
Table 5. 1 Gene expressions/proteins related to the zebrafish ( <i>Danio rerio</i> )	

hypothalamic- pituitary -thyroid axis.....	105
Table 6. 1 Amino acid sequences of TR $\alpha$ (Q98867) and TR $\beta$ (Q9PVE4) for zebrafish.....	125
Table 6. 2 Amino acids ratio for TR $\alpha$ and TR $\beta$ .....	126
Table 6. 3 Molecular length and docking results of T3 and OH-PBDEs obtained for TR $\alpha$ and TR $\beta$ .....	140
Table 7. 1 Molecular length and docking results of T3 and OH-PBDEs obtained for TR $\alpha$ and TR $\beta$ .....	158

## List of Figures

Figure 1. 1 Metabolites or degradation products of the PBDEs.....	3
Figure 1. 2 The thyroid gland secretes T3 and T4 into the bloodstream.....	9
Figure 1.3 T3 binds to the thyroid hormone receptors (TR) to modulate gene expression in the nucleus.....	12
Figure 1.4 The hypothalamus-pituitary-thyroid (HPT) axis AB adjust thyroid hormone level.....	13
Figure 2. 1 Air and dust sampling sites in the (A) PWB, (B) TV, (C) Washing machine and (D) Refrigerator recycling workshops.....	28
Figure 2. 2 Distribution of PBDE congeners in air (a) and dust (b) of PWB, TV, washing machine, and refrigerator recycling workshops.....	40
Figure 2. 3 Hazard quotient (HQ) values for inhalation (a) and dust ingestion (b) exposure of PBDEs at PWB, TV, washing machine and refrigerator recycling workshops.....	43
Figure 2. 4 Hazard quotient (HQ) distribution of BDE-47, BDE-99, BDE-153, and BDE-209 caused by inhalation exposure (n=10) and dust ingestion exposure (n=10).....	45
Figure 3.1 Proton NMR Spectrum for 2-bromo-3,5-dimethoxy-phenol (2).....	51
Figure 3. 2 Proton NMR Spectrum for 2,6-dibromo-3,5-dimethoxy-phenol (3)..	52
Figure 3. 3 Proton NMR Spectrum for 2,3,6-tribromo-3,5-dimethoxy-phenol (4)	53

Figure 3. 4	Proton NMR Spectrum for 4,6-dibromo-2-methoxy-phenol (5).....	54
Figure 3. 5	ChemNMR H-1 estimation for (A) 4,6-dibromo-2-methoxy-phenol and (B) 3,5-dibromo-2-methoxy-phenol .....	55
Figure 3. 6	Activating and deactivating groups in electrophilic aromatic substitution (Taken from Organic Chemistry (Mc Murry) 8th ED).....	55
Figure 3. 7	Proton NMR Spectrum for 3,5-diMeO-BDE-17 (8).....	57
Figure 3. 8	Proton NMR Spectrum for 3,5-diMeO-BDE-51 (9).....	58
Figure 3. 9	Proton NMR Spectrum for 3,5-diMeO-BDE-100 (10).....	59
Figure 3. 10	Proton NMR Spectrum for 3,5-diMeO-BDE-100 (11).....	60
Figure 3. 11	Proton NMR Spectrum for 3,5-diOH-BDE-17 (14).....	62
Figure 3. 12	Proton NMR Spectrum for 3,5-diOH-BDE-51 (15).....	63
Figure 3. 13	Proton NMR Spectrum for 3,5-diOH-BDE-100 (16).....	64
Figure 3. 14	Proton NMR Spectrum for 6-OH-BDE-47 (17).....	65
Figure 3. 15	Proton NMR Spectrum for 6,6'-diOH-BDE-47 (18).....	66
Figure 3.16	Mass spectra of the methoxylated (A) 3,5-diOH-BDE-17, (B) 6,6'-diOH-BDE-47, (C) 3,5-diOH-BDE-51 and (D) 3,5-diOH-BDE-100....	67
Figure 4. 1	Optimized extraction and cleanup procedure for the determination of OH-PBDEs/diOH-PBDEs in fish samples.....	76
Figure 4. 2	Chromatograms of the methoxylated OH-PBDEs/diOH-PBDEs present in (A) Total Ion Chromatogram (TIC, all the quantifier and qualifier signal combined) mode and (B), (C) Multiple Reaction Monitoring (MRM)	

mode in 10 ng/mL.....	81
Figure 4. 3 Influence of the solvents on the (A) extraction recoveries of OH-PBDEs/diOH-PBDEs (n=3) by the pressurized liquid extraction with no matrix included (B) and elution recoveries of OH-PBDEs/diOH-PBDEs (n=3) by florisil cartridges cleanup procedure with no matrix included.....	85
Figure 4. 4 Chromatograms of the methoxylated OH-PBDEs/diOH-PBDEs in white-spotted spinefoot fish sample: (A) Total Ion Chromatogram (TIC, all the quantifier and qualifier signal combined) mode and (B), (C) Multiple Reaction Monitoring (MRM) mode.....	92
Figure 5. 1 Mortality of zebrafish larvae. The 96 hpf LC <sub>50</sub> value of (A) 6OH-BDE47, (B) 3,5-diOH-BDE-17, (C) 6,6'-diOH-BDE-47, (D) 3,5-diOH-BDE-51 and (E) 3,5-diOH-BDE-100 presented as a fitted sigmoidal dose-response curve. Each exposure experiment was replicated three times with 40 fish embryos.....	107
Figure 5. 2 (A) Hatching rates of zebrafish larvae in different exposure concentrations of 6-OH-BDE-47 and 6,6'-diOH-BDE-47 at 48 hpf and (B) photomicrographs of the zebrafish larvae in control, 6-OH-BDE-47 (800 nM), and 6,6'-diOH-BDE-47 (1075 nM) exposure groups at 48 hpf.....	109
Figure 5. 3 Photomicrographs demonstrating changes in morphology at 96 hpf following zebrafish embryo exposure to different concentrations of (A) 6-OH-BDE-47 and (B) 6,6'-diOH-BDE-47.....	110
Figure 5. 4 (A) Chemicals concentration and (B) BCF factors of 6-OH-BDE-47 and 6,6'-diOH-BDE-47 measured in zebrafish larvae after exposure to 1, 10, 20, and 50 nM of the two chemicals in water for 96 hpf.....	112

Figure 5. 5 Whole body levels of T <sub>4</sub> and T <sub>3</sub> in zebrafish exposed to 0, 1, 10, 20, and 50 nM of 6-OH-BDE-47 and 6,6'-diOH-BDE-47. ....	113
Figure 5. 6 Gene expression analysis shows a fold change compared with the controls and normalised to β-actin expression. Embryos were exposed from 2-96 hpf to (A) 6-OH-BDE-47 and (B) 6,6'-diOH-BDE-47. Three replicates with 40 fish embryos each dish were used in the experiment. ....	115
Figure 6. 1 Comparison of TR $\alpha$ and TR $\beta$ to their templates sequence human thyroid hormone alpha and human thyroid hormone beta .....	127
Figure 6. 2 3D structures of predicted (A) TR $\alpha$ and (B) TR $\beta$ .....	129
Figure 6. 3 Local quality plot of predicted (A) TR $\alpha$ and (B) TR $\beta$ , generated by the SWISS-MODEL tool.....	130
Figure 6. 4 Comparison of predicted TR $\alpha$ 3D structure to its template human thyroid hormone alpha (THRA) and TR $\beta$ to its template human thyroid hormone beta (THRB).....	132
Figure 6. 5 Ramachandran Plots for the 3D structure prediction of TR $\alpha$ and TR $\beta$ .....	136
Figure 6. 6 The interactions of T <sub>3</sub> with TR $\alpha$ and TR $\beta$ .....	133
Figure 6. 7 The TR $\alpha$ and TR $\beta$ protein conformation before (red colour) and after (green colour) molecular dynamics simulation.....	134
Figure 6. 8 Root mean square deviation (RMSD) of TR $\alpha$ and TR $\beta$ backbone in the molecular dynamics simulation with GROMACS.....	135
Figure 6. 9 Root mean square deviation (RMSD) of T <sub>3</sub> in the TR $\alpha$ and TR $\beta$	



molecular dynamics simulation.....	136
Figure 6. 10 Radius of gyration of TR $\alpha$ and TR $\beta$ molecular dynamics simulation	137
Figure 6. 11 T3 and OH/diOH-PBDEs (A) TR $\alpha$ and (B) TR $\beta$ binding complex.	138
Figure 6. 12 The root-mean-square of deviation (RMSD) of carbon atoms (backbone) of (A) TR $\alpha$ and (B) TR $\beta$ OH/diOH-PBDEs monitored during a 10 ns MD simulation trajectory.....	142
Figure 6. 13 The root-mean-square of deviation (RMSD) of OH/diOH-PBDEs in the (A) TR $\alpha$ and (B) TR $\beta$ monitored during a 10 ns MD simulation trajectory. .....	144
Figure 7. 1 (A) TR $\alpha$ and (B) TR $\beta$ TR-FRET coactivator assay with serial dilution of agonist T3 for 2-hour incubation.....	151
Figure 7. 2 OH/diOH-PBDEs (A) TR $\alpha$ and (B) TR $\beta$ TR-FRET coactivator assay.	157
Figure 7. 3 T3 and OH/diOH-PBDEs competitive binding (A) TR $\alpha$ and (B) TR $\beta$ in the TR-FRET coactivator assay.....	153
Figure 7. 4 Merged images of T3/OH/diOH-PBDEs with (A) TR $\alpha$ and (B) TR $\beta$ .	156
Figure 7. 5 RMSD of carbon atoms (backbone) of (A) TR $\alpha$ and (B) TR $\beta$ OH/diOH-PBDEs monitored during a 10 ns MD simulation trajectory.....	160
Figure 7. 6 RMSD of OH/diOH-PBDEs in the (A) TR $\alpha$ and (B) TR $\beta$ monitored during a 10 ns MD simulation trajectory.....	161

## List of Schemes

Scheme 3. 1 Synthesis of diaryliodonium salts.....	49
Scheme 3. 2 Synthesis of methoxy bromophenols.....	50
Scheme 3.3 Synthesis of MeO-PBDEs via brominated diphenyliodonium salts..	56
Scheme 3. 4 Demethylation of MeO-PBDEs with boron tribromide (BBr <sub>3</sub> ).....	61