

DOCTORAL THESIS

GC/ion trap MS method development and applications for the analysis of polybrominated diphenyl ethers in environmental and biota samples

Luo, Qian

Date of Award:
2008

[Link to publication](#)

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

**GC/ion Trap MS Method Development and Applications
for the Analysis of Polybrominated Diphenyl Ethers in
Environmental and Biota Samples**

LUO Qian

A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principle Supervisor: Prof. CAI Zongwei

Hong Kong Baptist University

March 2008

ABSTRACT

Investigations on the concentrations and congener pattern distributions of polybrominated diphenyl ethers (PBDEs) in environmental samples (sediment, ash, and soil) and fish samples collected from Guiyu were conducted. Guiyu is a traditional rice-growing village located in southeastern Guangdong, China. In recent years, however, the village has turned into an intensive electronic-waste (e-waste) recycling and dumping site. Incomplete combusting of e-waste, dismantling and dumping of processed materials have been identified as the major sources of various toxic chemicals including PBDEs. Analytical methods were developed and applied for the determination of PBDEs in the environmental and biota samples. Samples were prepared by using Soxhlet extraction and cleaned-up with multiple-step chromatographic columns. PBDEs were analyzed by gas chromatography coupled with ion trap mass spectrometry for mono- to hepta-BDEs and quadrupole mass spectrometry for deca-BDE. The method performance was evaluated with the analyses of quality control samples and certified reference material with different calculation range, and with the recoveries of ^{13}C -labeled internal standards. The obtained recoveries ranged from 75 to 125% with a relative standard deviation of lower than 10% for the targeted PBDE congeners.

Ash from e-waste burning field is one of the contamination sources because ash could be deposited in local soil and river or transported to remote locations. Total PBDE concentrations were detected with the range from 2379 to 6238 $\mu\text{g}/\text{kg}$ (dry weight) in the ash samples collected from a largest open burning site and from 247 to 1025 $\mu\text{g}/\text{kg}$ in soil samples collected from different locations close to the open burning site. BDE-209, -183, -153, -99, and -47 were detected with the highest

concentrations in the ash and soil samples. The data of the PBDEs concentrations and congener patterns in both ash and soil samples indicated that PBDEs contamination in soil might be resulted from the e-waste burning.

Total PBDEs concentrations ranged from 4.43 to 16.1×10^3 $\mu\text{g}/\text{kg}$ and 55 to 445 $\mu\text{g}/\text{kg}$ (dry weight) in bank and bottom sediments, respectively, from Nanyang River where e-wastes were disposed. PBDEs were detected at levels of 51 to 365 $\mu\text{g}/\text{kg}$ in bottom sediment from Lianjiang River (Guiyu) that is located next to a residential area, compared to the PBDEs concentrations of 16 to 21 $\mu\text{g}/\text{kg}$ in the bottom sediment samples collected from Lo Uk Tsuen (Hong Kong) that received the wastewater discharged from a vehicle repairing workshop. No PBDEs were detected in sediment from Mai Po Marsh Pond (Hong Kong) that was served as a reference site. The obtained results indicated that open burning and dumping of e-waste are the major causes of PBDEs contamination. Different congener pattern profiles were observed in sediments from various sampling sites, with BDE-47 as the predominant congener, followed by BDE-99, -153, -183, and -209.

The mean concentrations of total PBDEs in mixed muscles of tilapia (*Oreochromis spp*) from Lianjiang River were 115 ng/g wet weight (ww) and 4.1 ng/g ww in fishes from Lo Uk Tsuen. The highest PBDEs concentrations were obtained in liver tissues (2.70×10^3 ng/g ww), followed by abdomen muscle (1.09×10^3 ng/g ww) of bighead carp (*Aristichthys nobilis*) collected from Nanyang River. The total PBDEs concentrations in fishes showed the following trend: grass carp < mud carp < crucian carp < silver carp < carp. PBDEs concentrations in the abdomen, back and tail muscles of carp were 766, 458 and 530 ng/g ww, and 53, 52, 45 ng/g ww in grass carp, respectively. The PBDEs congener concentrations in muscles correlated well with their lipid contents. BDE-47 and BDE-28 were the

most abundant congeners followed by BDE-17, -15, -66, -154 and -153 detected in the fishes collected from Guiyu. A significant correlation of PBDEs concentrations between sediment and muscle of fish was observed.

Estrogenic affect were used to investigate for the individual PBDEs including BDE-28, -47, -99, -153, -154, -183, -209 and the commercial product DE-71. Results indicate PBDEs may have weak or moderate binding affinity to receptor (estrogenic or EROD) or weak inducing the *umu* operon. PBDEs were found inactive at all stages of signal transduction and expression. No significant mutagenicity and carcinogenicity for individual PBDEs were observed in SOS/*umu* assays.

Because BDE-209 may be debrominated to more toxic low brominated congeners, its absorption, metabolism and toxicokinetics in Japanese medaka (*Oryzias latipes*) were studied. Japanese medaka of d-rR strain was continually exposed to BDE-209 (from 1 ng/L to 100µg/L) for 15, 30 and 60 days by using a flow-through system. Upon the completion of experiments, the fish weights were measured and the PBDEs concentrations were determined. In general, the final weights of the whole fish, the liver of the fishes treated with BDE-209 decreased. The gonads of male fishes also had the higher weight, while the female gonads had lower weight. PBDEs concentrations exponentially increased for the fishes exposed to 10 and 100 µg/L. Total concentrations of PBDEs (tri- to hepta-BDEs) ranged from 42.4 to 697 ng/g ww and BDE-209 was 8.19 to 354 ng/g ww in muscle tissues, indicating that significant debrominated metabolism occurred during the BDE-209 exposure experiments. BDE-209, -155, -47, and -99 were identified as the predominant congeners. Toxicokinetic study indicated that the half-life of BDE-209 ranged from 16.5 to 19.4 days under the continually flow-through condition.

TABLE OF CONTENTS

Declaration.....	i
Abstract.....	ii
Acknowledgements.....	v
Table of Contents.....	vi
List of Tables.....	xii
List of Figures.....	xiv
Abbreviations and Acronyms.....	xvii

CHAPTER I. INTRODUCTION

1.1 Overview.....	1
1.1.1 Physicochemical Properties.....	2
1.1.2 Commercial PBDEs Mixtures.....	3
1.2 Analytical Methodology.....	5
1.2.1 Pretreatment.....	5
1.2.2 Extraction Methods.....	5
1.2.2.1 Soxhlet Extraction.....	5
1.2.2.2 Microwave Assisted Extraction and Accelerated Solvent Extraction.....	6
1.2.2.3 Solid-Phase Extraction and Solid-Phase Microextraction.....	7
1.2.3 Cleanup.....	7
1.2.4 Gas Chromatographic Analysis.....	8
1.2.4.1 Injection Techniques.....	8
1.2.4.2 GC Capillary Columns.....	9
1.2.5 Detection.....	10
1.2.5.1 Electron Capture Negative Ionization-Mass Spectrometry.....	11
1.2.5.2 Electron Ionization-Mass Spectrometry with Low Resolution.....	11

1.2.5.3	Electron Ionization–Mass Spectrometry with High Resolution	12
1.2.5.4	Electron Ionization–Tandem Mass Spectrometry	12
1.2.5.5	Electron Capture Detector.....	13
1.3	Environmental Occurrence of PBDEs.....	13
1.3.1	Air and Dust.....	14
1.3.2	Sediment and Soil.....	14
1.3.3	Water.....	16
1.3.4	Sewage Sludge and Sewage Treatment Plant.....	16
1.3.5	Electronic Waste	17
1.4	PBDE Levels in Fish.....	18
1.5	Human Exposure of PBDEs.....	19
1.6	Degradation.....	21
1.6.1	Microbial Degradation.....	21
1.6.2	Photolytic Degradation.....	22
1.6.3	Pyrolysis Degradation.....	22
1.6.4.	Metabolism.....	23
1.7	Toxicity.....	23
1.8	Spatial and Temporal Trends.....	24
1.9	Objectives of This Study.....	25

CHAPTER II. DEVELOPMENT OF ANALYTICAL METHODS FOR THE DETERMINATION OF PBDES

2.1	Introduction.....	27
2.2	Materials and Methods.....	29
2.2.1	Chemical Reagents and Standard Solutions.....	29
2.2.2	Sohxlet Extraction.....	31
2.2.3	Cleanup.....	31

2.2.3.1	Multi-layered Silica Gel Column.....	31
2.2.3.2	Florisil Column.....	32
2.2.3.3	Alumina Column.....	32
2.2.3.4	Active Carbon Column.....	33
2.2.4	Chromatographic Separation.....	33
2.2.4.1	Analytical Instruments.....	33
2.2.4.2	Capillary Columns.....	33
2.2.4.3	Instrumental Parameters.....	34
2.2.5	Mass Spectrometric Detection.....	34
2.3	Results and Discussion.....	36
2.3.1	Soxhlet Extraction Method Development.....	36
2.3.2	Development of Sample Cleanup Procedure.....	39
2.3.2.1	The Use of Multi-layered Silica Gel Column.....	39
2.3.3.2	The Use of Acid Alumina and Florisil Columns.....	42
2.3.3.3	The Uses of Combined Columns.....	42
2.3.3	GC/MS and GC/MS/MS Analyses.....	45
2.3.4	Analysis of the Spiked Sea Sand Samples.....	51
2.3.5	Method Validation with Certified Reference Material of Fish Tissue.....	51
2.4	Conclusion.....	54

CHAPTER III. PBDES CONGENER DISTRIBUTIONS AND LEVELS IN THE ENVIRONMENT POLLUTED BY ELECTRONIC-WASTE

3.1	Introduction.....	55
3.2	Materials and Method.....	58
3.2.1.	Sampling Site.....	58

3.2.2	Chemicals and Reagents.....	62
3.2.3.	Extraction and Cleanup.....	62
3.2.4	GC/MS and GC/MS/MS Analyses	63
3.2.5	Quality Assurance/Quality Control.....	64
3.3	Results.....	65
3.3.1	PBDEs Concentrations and Congener Patterns in Sediments.....	65
3.3.2	PBDEs Concentrations and Congener Patterns in Ash.....	72
3.3.3.	PBDEs Concentrations and Congener Patterns in Soil.....	76
3.4	Discussion.....	80
3.5	Conclusion.....	88

CHAPTER IV. PBDES IN FISHES FROM RIVERS LOCATED IN ELECTRONIC-WASTE REGION

4.1	Introduction.....	89
4.2	Materials and Method.....	91
4.2.1.	Chemicals and Reagents.....	91
4.2.2.	Sample Preparation.....	91
4.2.3.	Extraction and Cleanup.....	92
4.2.4.	GC/MS and GC/MS/MS Analyses.....	93
4.2.5.	Quality Assurance/Quality Control.....	93
4.3	Results.....	95
4.3.1	GC/MS Analyses.....	95
4.3.2	Concentrations of PBDEs Congeners in the Fish Samples	95
4.3.3	Congener Distributions of PBDEs in Fish.....	100
4.4	Discussion.....	103
4.4.1	Analytical Method.....	103
4.4.2	PBDEs Congener Levels and Distribution Profiles in Fish.....	104

4.4.3	Relationships between PBDEs Concentration and Lipid Content..	109
4.4.4	Relationship between Sediment and Fish.....	111
4.5	Conclusion.....	115

CHAPTER V. ABSORPTION, METABOLISM AND TOXICOKINETICS OF DECA-BDE IN JAPANESE MEDAKA (*ORYZIAS LATIPES*) USING A FLOW-THROUGH SYSTEM

5.1	Introduction.....	116
5.2	Materials and Methods.....	119
5.2.1	Chemicals.....	119
5.2.2	Experimental Fish.....	119
5.2.3	Exposure and Experimental Design.	121
5.2.4	Biological Endpoints.....	125
5.2.5	Analyses by GC/MS and GC/MS/MS.	125
5.2.6	Toxicokinetic Method.	126
5.3	Results.....	127
5.3.1	Biological Endpoints.....	127
5.3.2	PBDEs Congener Profile and Concentration.....	132
5.4	Discussion.....	140
5.4.1	Biological Endpoints.....	140
5.4.2	Chemical Analyses.....	142
5.4.3	Toxicokinetics of BDE-209.....	146
5.5	Conclusion.....	147

CHAPTER VI. TOXICITY TESTS OF PBDES USING RECOMBINANT YEAST, EROD AND SOS/*UMU* ASSAYS

6.1	Introduction.....	148
------------	--------------------------	------------

6.2 Materials and Methods	151
6.2.1 Chemicals.....	151
6.2.2 Recombinant Yeast Assay.....	152
6.2.3 Ethoxyresorufin-O-deethylation Assays.....	153
6.2.4 SOS/ <i>umu</i> Assays.....	156
6.3 Results and Discussion	158
6.3.1 Recombinant Yeast Assay.....	158
6.3.2 Ethoxyresorufin-O-Deethylation Assays.....	161
6.3.3 SOS/ <i>umu</i> Assays.....	165
6.4 Conclusion	169

CHAPTER VII. GENERAL DISCUSSION AND CONCLUSIONS

7.1 Introduction	170
7.2 Development on Analytical Method of PBDEs	172
7.3 PBDEs Contamination Source and Transport Pathway in E-Waste Recycling Activity Region	172
7.4 Absorption and degradation of BDE-209 in Medaka	177
7.5 Future Research	178

APPENDICES

A1: References	181
A2: List of Papers Published	211
A3: List of Oral Presentation	212
A4: Curriculum Vitae	213