

DOCTORAL THESIS

Development and application of liquid chromatography mass spectrometry methods for the analysis and toxicity study of polybrominated diphenyl ether metabolites

Lai, Yongquan

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**Development and Application of Liquid Chromatography
Mass Spectrometry Methods for the Analysis and Toxicity
Study of Polybrominated Diphenyl Ether Metabolites**

LAI Yongquan

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Principal Supervisor: Prof. CAI Zongwei

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Abstract

Polybrominated diphenyl ethers (PBDEs) are a class of ubiquitous environmental contaminants of great concern because of their potential to cause deleterious health effects to humans and wildlife. Existing evidence has shown that cytochrome P450s-dependent metabolism of PBDEs could generate some active metabolites, such as hydroxylated PBDEs (OH-PBDEs), dihydroxylated PBDEs (diOH-PBDE), bromophenols, bromohydroquinones and bromocatechols. Knowledge on the identity, persistence and toxicity of these PBDE metabolites is key to understanding the complete risks associated with PBDEs in humans and wildlife. This thesis focused on the analysis and toxicity study of PBDE metabolites by using high performance and ultra performance liquid chromatography (HPLC and UPLC) coupled with electrospray-ionization mass spectrometry (ESI-MS) that included ESI-ion trap-MS, ESI-triple quadrupole-MS and ESI-quadrupole-time of flight (QTOF)-MS.

Since OH-PBDEs exhibited potential endocrine-disrupting potency, identification of OH-PBDEs in biological samples is important. We described in the present thesis, a robust UPLC-ESI-triple quadrupole-MS/MS method for the rapid determination of OH-PBDEs in rat plasma. To evaluate the performance of the developed method, calibration linear range, sensitivity, recovery and repeatability were investigated. The developed method was applied for supporting the pharmacokinetic investigation of 6-OH-BDE-47 in two groups of Sprague-Dawley rats that received respectively a single dose of 0.60 mg/kg (high dose) and 0.15 mg/kg (low dose) by intravenous injection.

The detection of OH-PBDEs in humans and wild animals suggested that they are still persistent and lipophilic enough to be retained in body. The persistence of

contaminants is an important parameter for assaying their healthy risks and environmental fate. *In vitro* phase I and phase II metabolism were performed to evaluate metabolic stability of eleven OH-PBDEs that contain two to six bromine substituents. For phase I oxidative metabolism and phase II glucuronidation, OH-PBDEs were incubated with rat liver microsomes in the presence of coenzyme NADPH and UDPGA, respectively. *In vitro* metabolic half life was determined to investigate the metabolic persistence of OH-PBDEs. Effects of chemical structures of OH-PBDEs on metabolic behaviors were discussed. The most influential factor of OH-PBDEs persistence was determined to be the number of bromine substituents. Structural elucidation of metabolites was carried out by using HPLC–ESI–ion trap–MS/MS. The formation of hydroquinone and catechol metabolites were found to be the preferred metabolic pathway of OH-PBDEs.

The mechanisms for the estrogenic activity of OH-PBDEs is still unknown. It is possible that OH-PBDEs might exert part of their estrogenic activity by affecting estrogen metabolisms. In this thesis, we described the modulation of 17 β -estradiol (E2) oxidative metabolism and glucuronidation by OH-PBDEs. Inhibition studies were performed by incubation of the rat liver microsomes with E2 and OH-PBDEs. OH-PBDEs were found to significantly inhibit E2 metabolism with low micromolar IC₅₀ values. The noncompetitive inhibitory effects of OH-PBDEs on E2 metabolism was observed from enzyme kinetic analysis. The structure-activity relationship of OH-PBDEs was also examined, including the number of bromine atoms, existence of hydroxyl group and substitution pattern of hydroxyl and bromine group. The existence of hydroxyl group in OH-PBDE molecules was determined to be the most influential factor in their modulation of E2 metabolism.

The formation of DNA adducts is the first step of chemical carcinogenesis. For the first time, the mechanism of formation and structural characterization of PBDE-DNA adducts was investigated by using ESI-ion trap-MS, ESI-triple quadrupole-MS and ESI-QTOF-MS. The chemical structure of the DNA adducts was confirmed by accurate mass values, collision-induced fragmentation tandem mass spectra as well as isotopic patterns. The results indicated that PBDEs could covalently bind to DNA mediated by quinone metabolites, single ring bromobenzoquinones (BBQs) and double ring PBDE-quinones (PBDE-Qs). The reaction mechanism for the DNA adduction involved the Michael Addition, and some adducts were generated from the reaction of one dG molecule with two BBQ molecules. Lower reactivity of DNA adduction was also observed with the increasing number of bromine atoms on the phenyl ring in PBDE-Q molecules, indicative of DNA adduction depending on the chemical structures of quinone metabolites.

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