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Metabolic pathways of decabromodiphenyl ether (BDE209) in rainbow trout (*Oncorhynchus mykiss*) via intraperitoneal injection

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ABSTRACT

Decabromodiphenyl ether (BDE209) was of great concern due to its biotransformation in different organisms. However, most studies devoted to the metabolic intermediates of BDE209, less has been done on the metabolic pathways *in vivo*, especially on the relationships among debrominated-BDEs, OH-BDEs and MeO-BDEs. In this study, the metabolic pathways and intermediates of BDE209 in rainbow trout (*Oncorhynchus mykiss*) were investigated, and the time-dependent transformations of the metabolites were also examined. The primary debrominated metabolites were BDE47, 49, 99, 197, 207; the main MeO-BDEs were MeO-BDE47, MeO-BDE68 and MeO-BDE100; OH-BDEs were primarily composed of OH-BDE28 and OH-BDE42. From the time-dependent and dose-effect relationships, the debromination should be followed by hydroxylation, and then by methoxylation. The increasing in body burden of MeO-BDEs corresponded to the decreasing of OH-BDEs, which could indirectly prove the inter-conversion between OH-BDEs and MeO-BDEs. This study would motivate the future research of toxicological mechanisms of BDEs.

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are additive flame retardants that are applied in a variety of consumer products, such as plastics and electronic castings. PBDEs can accumulate in various species, especially in fish, and exposure to some PBDE congeners is associated with health problems such as reproductive and developmental effects, neurobehavioral toxicity and thyroid hormone disruption (Dingemans et al., 2011; Costa et al., 2014; Guyot et al., 2014; Buratovic et al., 2014). Decabromodiphenyl ether (BDE209), which is the principal component of commercial PBDEs, comprises approximately 80% of the world market demand for PBDEs (de Wit, 2002). BDE209 has unique physicochemical properties, such as extremely low water solubility, large molecular size and very

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low bioavailability. Therefore, it is characterized as an environmentally stable and inert product that is hard to degrade in the environment. However, elevated concentrations of BDE209 have been detected in different organisms and environmental media (Van den Steen et al., 2007; Crosse et al., 2012; Wang et al., 2014; Nelson et al., 2015). Previous studies have shown that BDE209 could accumulate in various species, especially in fish (Darnerud et al., 2001).

Many studies have shown that the toxicity of BDE209 itself is relatively lower, while the toxicity might be due to the metabolites, such as hydroxylated BDEs (OH-BDEs) and methoxylated brominated diphenyl ethers (MeO-BDEs). For example, it is reported that OH-BDEs have thyroxine-like and estrogen-like chemical structures that can bind competitively with transthyretin (TTR), a transport protein of thyroid hormones (Meerts et al., 2001). The competitive binding of OH-BDE to TTR is considered to be potentially responsible for the disruption of thyroid homeostasis (Li et al., 2010). However, whether BDE209 could be metabolized to toxic intermediates and which metabolic pathways that govern the metabolism in biota have not been clearly addressed. Some toxicological studies have shown that lower brominated diphenyl ethers, which might be a metabolite of BDE209 in biota, are more toxic than higher brominated diphenyl ether (Hooper and McDonald, 2000, Xie et al., 2014). It has also been suggested that deiodinase enzymes play a role in the debromination of BDEs in fish owing to the structural similarity of BDEs to thyroid hormones (Stapleton et al., 2004a; Stapleton et al., 2006). A preliminary study revealed slow but measurable uptake of BDE209 via dietary exposure, with several lower brominated diphenyl ether metabolites being detected in rainbow trout (Kierkegaard et al., 1999). Several published papers have demonstrated the biotic debromination of BDE209 in fish (Roberts et al., 2011). In addition to the debrominated diphenyl ether metabolites, MeO-BDEs and hydroxylated metabolites were also detected in many species (Mörck et al., 2003; Valters et al., 2005; Sun et al., 2013, Wen et al., 2015). However, the majority of research on the occurrence of MeO-BDEs and OH-BDEs in biota samples has attributed its presence to natural biogenetic formation. For example, MeO-BDEs and OH-BDEs were believed to be produced by marine sponges and algae (Teuten et al., 2005), but only limited studies have investigated the metabolism of these compounds from parent BDEs (Malmvärn et al., 2005; Marsh et al., 2006; Wan et al., 2010; Wang et al., 2012). Basically, previous studies of BDE metabolism indicated that metabolism of BDEs in humans and other mammals typically occurred via oxidative pathways, producing OH-BDEs and brominated phenols (Chen et al., 2006). Nevertheless, there was no direct evidence that could prove that exposure to BDEs would undergo oxidative process in fish, leading to the oxidative metabolites of BDEs. Rather, studies about BDEs metabolism in fish mainly focused on the reductive process, with debrominated BDEs being produced (Stapleton et al., 2004b; Stapleton et al., 2004a). Moreover, although the different kinds of BDE209 metabolites were reported, little was known about the metabolic pathway of BDE209 in organisms. The majority of those studies about BDE209 metabolism mainly elaborated the metabolites, including their concentration levels, tissue distribution characteristics and specific congener types. It was still not clear whether its metabolites, e.g., OH-BDEs and MeO-BDEs, could be formed through metabolism in fish *in vivo* when exposed to environmentally relevant concentrations of parent BDE209.

The present study was conducted (i) to propose the specific metabolic pathways of BDE209 in rainbow trout based on the distribution profiles of its metabolites; (ii) to present the time sequences of BDE209 metabolites; and (iii) to investigate the inter-conversion relationship of OH-BDEs and MeO-BDEs *in vivo* during the exposure experiment.

2. Materials and methods

2.1. Standards and reagents

Dichloromethane (DCM), hexane and acetone, which were used for extraction and clean up of the extract, were HPLC grade purchased from Mallinckrodt Baker Inc. (USA). BDE209 used for the exposure experiment were kindly provided by the Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences (CAS), China. BDE and MeO-BDE standards were purchased from Wellington Laboratories (Ontario, Canada). BDE mixture standards included BDE209 and 26 potential debrominated BDE congeners (BDE3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184, 191, 196, 197, 206, 207). MeO-BDE standards were purchased from Wellington Laboratories (Ontario, Canada), including eight tetra- to penta- congeners. OH-BDE standards were obtained from AccuStandard®, Inc. (USA), containing eight di- to penta- congeners. ^{13}C-labeled 2,2′,4,4′-tetrabromo-6-methoxy diphenyl ether (6-MeO-BDE47) and ^{13}C-labeled 2,2′,4,4′,6-pentabromo-6′-methoxy diphenyl ether (6′-MeO-BDE100) were used as recovery surrogates for MeO-BDE congeners. ^{13}C-labeled-2,2′,4,4′-tetrabromo-6-hydroxy diphenyl ether (6-OH-BDE47) and ^{13}C-labeled-2,2′,4,4′,6-pentabromo-6′-hydroxy diphenyl ether (6′-OH-BDE100) were used as recovery surrogates for OH-BDE compounds. ^{13}C-labeled -2,2′,3,4,4′,5′-hexabromodiphenyl ether (^{13}C-BDE138) was used as an internal standard for MeO-BDE determination during GC/MS analysis.

2.2. Experimental design

Juvenile rainbow trout (about 4 months old), approximately 20 cm in length and 100 g in weight, were purchased from a local fish farm. Fish were randomly stocked in 250 L glass tanks that included control and treatment groups. These fish were maintained in aerated de-chlorinated tap water (using an activated carbon filter) at a constant temperature (15 ± 2 °C), under a 16 h: 8 h (light: dark) photoperiod, which was close to their optimal temperature range in the natural environment. Fish were acclimated for one week prior to the beginning of the experiment.

BDE209 was dissolved in corn oil to prepare a stock solution with a concentration of 0.25 g/L. Intraperitoneal injection was carried out for fish exposure. The fish were weighed before injection to determine the volume of dosage per gram body mass of each fish. The intraperitoneal injection process was listed in detail as follows: for the treatment groups, BDE209 was injected with injection syringe. The needle of the syringe