Debromination of Decabromodiphenyl Ether (BDE 209) in Human and Fish Liver Microsomes

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Introduction

Polybrominated diphenyl ethers (PBDEs) are flame retardant chemicals that are now considered a new class of persistent organic pollutants. Over the past thirty years, the levels of PBDEs have been increasing rapidly in a variety of environmental compartments and in human tissues (Hites 2004). Presently, two of the three commercial mixtures of PBDEs (pentaBDE and octaBDE) are either banned from use or voluntarily phased out of production and it is therefore anticipated that levels of PBDEs will decrease in the environment. However, use of the third commercial mixture, decaBDE, remains unrestricted. Several studies have demonstrated that decaBDE can be reductively dehalogenated to congeners present in the pentaBDE and octaBDE commercial mixtures via photodegradation (Eriksson et al. 2004; Soderstrom et al. 2004; Ahn et al. 2006), biodegradation (Gerecke et al. 2005; He et al. 2006) and biotransformation (Kierkegaard et al. 1999; Stapleton et al. 2004; Tomy et al. 2004).

In a previous study, we observed biotransformation of BDE 209, the primary component of decaBDE, in rainbow trout (Stapleton et al. 2006). Biotransformation occurred primarily via debromination to hexa- through nonaBDE congeners and was observed *in vivo* and *in vitro*. A comparison of the biotransformation of BDE 209 in rainbow trout and common carp liver microsomal cell fractions found significant species specific differences. The primary enzymes system responsible for the debromination is still unclear but is hypothesized to be either deiodinase (DI) enzymes or glutathione S-transferases (GSTs). These observations raise questions about the potential for debromination of BDE 209 in humans. A recent study observed significant accumulation of octa- and nonaBDE congeners in electronic dismantling workers exposed to the decaBDE commercial mixture (Thuresson et al. 2005). Their observations, and the measured half-lives of these congeners in their blood stream, suggests debromination of BDE 209 does occur in humans (Thuresson et al. 2006). The present study was undertaken to investigate the potential for debromination of BDE 209 in humans using commercially available pooled human liver microsomes. The objective of this study was to determine if biotransformation of BDE 209 occurs in human liver microsome fractions and to compare the rate of transformation to those observed in the previous fish microsome experiments.

Materials and Methods

Pooled human liver microsomes were purchased from In Vitro Technologies in Baltimore, MD (USA). The pooled microsomes were prepared from tissues donated to a human tissue bank and contained a 1:1

ratio of female to male donors. Fish liver microsomes were prepared based on the procedures of Nilsen (Nilsen 1998). Briefly, livers were homogenized while on ice with 3 to 5 times (w/v) of homogenization buffer (0.1 M potassium phosphate buffer containing 0.15 M KCl, 1mM EDTA, 10 mM DTT, and 20% v/v glycerol) at pH 7.4. Samples were centrifuged once at 12,000g for 20 minutes at 4°C. The supernatant was transferred to a new tube and centrifuged at 100,000g for 70 minutes (4°C). Cytosol was decanted and the microsomal pellet re-homogenzied in a resuspension buffer containing 0.1 M potassium phosphate, 1 mM EDTA, 10 mM DTT, and 20% glycerol (v/v) buffer (reuspension buffer, pH 7.4). The homogenate was re-centrifuged at 100,000g for 40 minutes (4°C) and the resulting supernatant decanted and the microsomal pellet resuspended in a 1:1 w/v ratio of resuspension buffer. A 22-1 ½ gauge syringe was used to obtain a homogenous mixture of the microsomes. The microsomal suspension was transferred to cryotubes and stored in liquid nitrogen until utilized for *in vitro* assays. Protein concentrations were determined using the BCA (bicinchoninic acid) assay (Pierce, Rockford, IL) using bovine serum albumin to generate the standard curve.

Human microsomes were incubated with approximately 100 ng of BDE 209 delivered in 5 microliters of acetone. Incubations were conducted in a water bath at 37 °C for 24 hours to exhaust all enzyme systems and allow for maximum biotransformation. The reaction mixture had a total volume of 1 mL which contained the microsomal fraction at 1 mg/mL protein concentration. The incubation buffer consisted of 0.1 M NaH_2PO_4, 1 mM EDTA, 10 mM DTT, and 100 µM NADPH buffer (pH 7.4). Afterwards, the reaction was stopped by the addition of 1 mL of ice cold methanol. Samples were snap frozen in liquid nitrogen and stored at -20°C until extraction and analysis. Heat inactivated microsomes and buffer spikes were used as negative controls and to assess background contaminants. To avoid potential UV breakdown of BDE-209 test-tubes were wrapped in foil during the incubation.

In vitro incubations were extracted using a liquid/liquid extraction technique. Samples were spiked with ^{13}C labeled 209 (30 ng) as an internal standard prior to extraction. Hexane (5 ml) was added to the microsome solution and the mixture was vortexed for 30 seconds. Samples were centrifuged at 3000 RPM for 20 minutes and the organic layer removed. This process was repeated twice. The combined organic extracts were treated with approximately 1.0 ml of sulfuric acid, vortexed for 30 seconds and centrifuged again. The organic layer was removed and reduced in volume to 1.0 ml and cleaned using gel permeation chromatography. The collected extract was again reduced in volume, transferred to hexane and reduced to 0.1 ml for analysis using GC/ECNI-MS.

Results and Discussion

Huaman liver microsomes were incubated with three different buffers (designated alpha, beta and gamma) to examine cofactors necessary for biotransformation. The alpha buffer was comprised of our optimized buffer used in the fish microsome experiments and contained 10 mM dithiothreitol (DTT) and 100 µM NADPH as cofactors. Buffers labeled beta contained neither DTT nor NADPH and the gamma buffer consisted of 100 µM of NADPH but no DTT. The preliminary data from our first experiment is presented in Figure 1. Biotransformation of BDE 209 was observed in microsomes incubated with the alpha buffer. Approximately 10% of the BDE 209 was metabolized after 24 hours of incubation in the alpha buffer. Both octa- and nonabrominated congeners were observed following incubations. BDE 207 and BDE 197 were found at greater concentrations in the microsomes incubated in the alpha buffer. The concentration

of BDE 197 was significantly higher ($p<0.05$) in the microsomes incubated with the alpha buffer relative to the two other buffers. The formation of BDE 207 and 197 both occur via removal of *meta* substituted bromine atoms. These two congeners have also been observed to accumulate in the liver of rats exposed to BDE 209 in a laboratory study (Huwe and Smith, 2006) and in cows receiving exposure to BDE 209 from silage (Kierkegaard et al., 2006). These results, and the results from our in vitro studies, highly suggest BDE 209 can be debrominated in mammalian species.

From these observations it appears that DTT is necessary for the biotransformation of BDE 209, whereas the metabolism is independent of NADPH. This observation supports the involvement of DI enzymes in the metabolism as DTT is a necessary cofactor for DI activity. However, we cannot rule out the possibility that other enzymes are required. These observations also suggest cytochrome P450 enzymes are not involved in the biotransformation as NADPH is required for their activity.

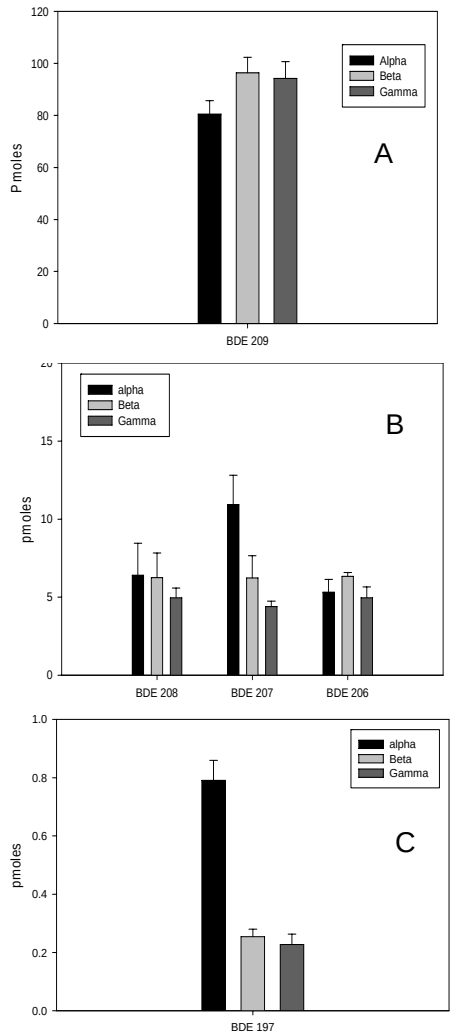

Figure 1. Concentration of BDED 209 (a), nonaBDEs (b) and BDE 197 (c), after 24 hour incubation with human liver Microsomes and 100 ng of BDE 209.

The BDE 209 biotransformation rate in the human liver microsome incubations are less than the rate observed in both rainbow trout and carp liver microsomes from our earlier experiments (Stapleton et al. 2006). A greater proportion of the BDE 209 mass was metabolized to hexa- through nonaBDE congeners in the fish liver microsomes (as much as 65% after 24 hours). Without knowing the exact enzymes involved in this biotransformation it is difficult to determine if this observation is driven by a greater enzyme activity in fish relative to humans or due to other factors. Because these human microsomes were purchased from a commercial supplier, differences in the preparation of the microsomes could have led to different enzyme activity. Further studies using inhibitors and competitors will be conducted to determine the influence of various enzyme systems present in the microsomal fraction on the metabolism of BDE 209. In addition, further studies will be carried out using cryogenically frozen hepatocytes to investigate differences in metabolism between microsomal cell fractions and whole cells.

The results from this study support the likelihood that BDE 209 can be debrominated to lower brominated congeners *in vivo* in humans. BDE 209 has been detected in human breast milk and blood suggesting it is bioavailable. However, few studies have measured the concentrations of octa- and nonaBDE congeners and it is difficult to make any assessments on the potential for BDE 209 debromination *in vivo*. Because debromination of BDE 209 can lead to more persistent and potentially more toxic BDE congeners, it will be important to determine whether this process is occurring in the human population.

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