UPTAKE, METABOLISM AND DEPURATION OF POLYBROMINATED DIPHENYL ETHERS (PBDES) BY THE COMMON CARP, (*CYPRINUS CARPIO*)

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Introduction

Polybrominated diphenyl ethers (PBDEs) are quickly becoming a contaminant of emerging concern as evidence continues to build suggesting humans and the environment are being exposed to exponentially increasing levels. [1,2] This increasing trend is alarming and has resulted in a voluntary ban on their use and production in the European Union, with government restrictions expected in the near future [3]. Exposure to PBDEs can lead to a variety of detrimental effects including changes in intragenic recombination in mammalian cells [4] and significant effects upon thyroid hormone homeostasis. [5,6,7]

Little is currently known about the accumulation and metabolism of PBDEs by aquatic species. A few recent studies have found that some benthic fish residing close to suspected point sources of PBDEs displayed elevated concentrations of highly brominated diphenyl ethers. [8,9] In all of these studies, carp (*Cyprinus carpio*) displayed an unusual pattern of PBDE accumulation in which BDE 99 (2,2',4,4', 5-pentabromodiphenyl ether) was significantly depleted in carp tissues relative to other fish collected from the same area, indicating that either carp possess the ability to eliminate, presumably via metabolism, specific PBDE congeners, or they do not assimilate these congeners. The current study was initiated to gain a better understanding of the uptake, elimination and fate of PBDE homologue groups within the common carp.

Methods and Materials

In a preliminary study, juvenile carp, approximately 11cm in length, were kept in static tanks in the laboratory and exposed to a suite of PBDE compounds through spiked food pellets for a period of 25 days. The food pellets (homogenized blood worms) were spiked with PBDE congeners 47, 99, 100, 153, 154 and 183 at a concentration of 100 ng/g wet weight each. After 25 days fish were fed unspiked food pellets for 7 days to monitor depuration. Fish were fed 1 gram of food/day/fish. Every five days two fish were sacrificed, their length and mass recorded and their livers removed. Whole body and liver samples were homogenized and analyzed for PBDEs and lipid content, using standard laboratory extraction procedures.[9] All samples were quantified for PBDEs using a GC/MS and electron impact ionization.

A larger scale experiment was set up to monitor the same end points but incorporating a 60 day exposure period followed by a 30 day depuration period. Eight tanks were set up in a water flow-through system with twelve juvenile carp in each tank. Six of the tanks were used as exposure tanks for two different treatments (3 replicates for each treatment) and two tanks were used as controls. Fish were again fed 1 gram spiked food/day/fish. One fish was sacrificed from each tank on days 0, 5, 10, 20, 30, 45, 60, 70, 80 and 90. The two food treatments were food pellets spiked solely with PBDE 209

at a level of 1000 ng/g wet weight, and a cocktail spiked with 100 ng/g wet weight of each of the following: BDE 28, 47, 99, and 153 in addition to PCB congeners 52, 153 and 180. Upon sacrifice, the fish's length and mass were recorded, their livers removed, and a small blood sample taken. As in the preliminary experiment, homogenized whole body and liver tissues were analyzed for PBDE and PCB congeners. A small sub-sample of the liver was frozen in liquid nitrogen and analyzed for EROD activity. Blood samples were centrifuged and the serum removed for the analysis of possible PBDE metabolites as well as thyroid hormone analysis (T4 and T3 levels).

A second experiment was conducted in which carp were fed food pellets spiked solely with PBDE 183 at a concentration of 100 ng/g wet weight for 25 days to investigate possible debromination of PBDE 183 by the carp. Fish were fed 1 gram of food/day/fish and one fish was sacrificed every 5 days, its length and mass recorded and their livers removed. As in the preliminary experiment, whole body and liver samples were extracted and quantified for PBDE content.

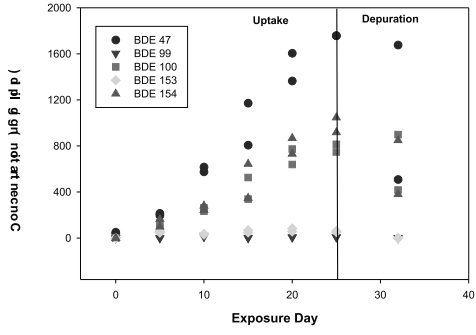

Figure 1. Accumulation of PBDEs over time in the common carp.

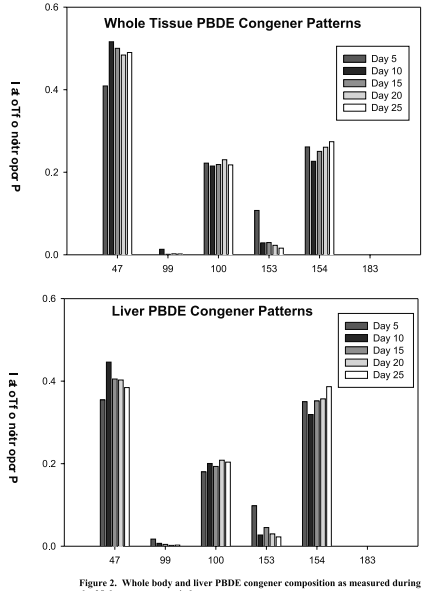

Figure 2. Whole body and liver PBDE congener composition as measured during the 25 day exposure period.

Results and Discussion

Results from the preliminary experiment indicate that uptake of most PBDEs occurred within the first five days of exposure (Figure 1). BDE 47 displayed the most rapid uptake with an uptake rate of approximately 70 ng/ g lipid/day. However, BDE 183 was not detected in the fish tissue during the 25day exposure period. Aditionally, accumulation of BDE 99 and BDE 153 was minimal in fish tissues, similar to reported field results, [10,11] again suggesting that these two congeners are either efficiently metabolized by carp or not assimilated. Calculated depuration rate coefficients for BDE 47, 100 and 154 were 0.32, 0.23 and 0.28day $^{-1}$, respectively.

Interestingly, the congener composition was different within whole body tissue versus liver tissue (Figure 2). Within whole body tissues, BDE 47 was the dominant congener and comprised 48 ± 4 % of the total PBDE burden, similar to many field studies. [10] However, the liver displayed elevated levels of BDE 154 relative to whole body tissue. Within the liver tissue, BDE 154 comprised 35 ± 2 % of the total PBDE burden, while BDE 47 comprised 39 ± 3 % of the total. This suggests a kinetic limitation in the equilibrium partitioning of these congeners among fish tissues.

Similar uptake rates for BDE 100 and BDE 154 were observed. This was surprising considering the different molecular weights and bromination of these compounds. In previous laboratory studies, uptake rates and efficiencies of hexa-bromodiphenyl ethers were lower than the uptake of penta-bromodiphenyl ethers. [11,12] Considering the bromine substitution pattern in BDE 154 is very similar to the substitution pattern in BDE 183, the debromination of BDE 183 to BDE 154 was considered. An experiment in which carp were fed food pellets spiked solely with BDE 183 confirmed this hypothesis. In another 25day exposure, no detectable traces of BDE 183 were observed, however, increasing levels of BDE 154 and an unknown hexaBDE congener were observed over time. Ion ratios confirmed the identity of an unknown hexabromodiphenyl ether present in the fish tissues. This indicates that BDE 183 does indeed break down into BDE 154, however, it is not known whether BDE 183 was biotransformed or debrominated previous to fish uptake. A more detailed experiment is needed to discover the route by which BDE 183 is debrominated. Further results of the 60day exposure study will be presented at the meeting.

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