Effect of PCB-126 on the production of PBDEs by Atlantic tomcod (*Microgadus tomcod*) injected IP with technical DeBDE mix

Michel Lebeuf, Catherine M. Couillard, Benoit Légaré and Steve Trottier

Maurice Lamontagne Institute, Department of Fisheries and Oceans, Canada

Introduction:
Polybromo diphenyl ethers (PBDEs) are commonly used as flame retardants and typically produced in three commercial formulations of distinct degree of bromination, i.e. penta (PeBDE), octa (OcBDE) and deca (DeBDE) mixes[1]. The bioaccumulation of certain low brominated congeners, those predominantly present in the PeBDE mix, and their toxic effects to organisms have led to voluntarily withdraw or ban the use of PeBDE and OcBDE mixes in Europe and Japan[1,2]. In North America, the three mixes are still in use but the phase out production of the PeBDE and OcBDE mixes by the end of 2004 has been recently announced[3]. However, no phase out production is actually planned for the DeBDE mix, possibly because the deca bromo-substituted congener BDE-209, its main component (97-98%), is not efficiently transferred to organisms and exhibit low toxicity. The DeBDE mix represented more than 80% of world PBDE market demand in 2001[4].

The possible biotransformation of BDE-209 to potentially toxic low brominated congeners represents an important concern about the use of the DeBDE mix. A few studies have examined the capacity of fish to debrominate BDE-209. Rainbow trout (*Oncorhynchus mykiss*) were fed with a diet contaminated with the DeBDE mix[5]. A low transfer of BDE-209 to fish (<0.005%) was observed but concentrations of some PBDE congeners increased in fish tissues. It could not be determined if this increase resulted from the debromination of BDE-209 or the preferential accumulation of impurities in the DeBDE mix. In another study, juvenile carp (*Cyprinus carpio*) were exposed through their diet to BDE-209 originating from a standard solution of high purity (> 98%)[6]. The accumulation of several PBDE congeners was observed but the BDE-209 was not detectable in fish tissues. The presence of these low brominated PBDEs in the fish could not be explained by the contamination of the diet. In both studies, the production of PBDEs represented less than 0.5% of the BDE-209 accumulated in the fish tissues, which is less than the levels of impurities in the BDE-209 source products.

The objective of this study was to evaluate if the biotransformation of BDE-209 in fish was promoted by pre-treatment with a model cytochrome P450 inducer with a high potency to induce the liver detoxification system, the PCB-126. The DeBDE mix was intraperitoneally (IP) injected into the fish to maximize the uptake of BDE-209 by fish and to minimize the accumulation of impurities from the DeBDE mix. This paper reports preliminary results on the debromination of the DeBDE mix by a marine fish, Atlantic tomcod (*Microgadus tomcod*).

Materials and methods:

Sampling and laboratory exposure. Atlantic tomcods (175-250 mm) were captured in La Baleine in the St Lawrence Estuary in November and acclimated at a water temperature of 6-7 °C and a salinity of 30 ppm until the beginning of the experiment, in May. They were fed *ad libitum* twice a week with frozen capelin. After several months of acclimation, 200 fish were randomly distributed in groups of 25 fish and placed into eight 500-L fiberglass tanks. On day 0 of the experiment, fish from half of the tanks were anaesthetized in water containing tricaine methanesulfonate (75 mg/L) and were injected IP with a dose of 25 ng of PCB-126 per gram of fish in corn oil. Fish from the remaining tanks received a dose of corn oil proportional to their weight. After three weeks, fish from two of the four tanks that had received PCB-126 and fish from two of the four tanks that had received only corn oil were injected IP with a dose of 400 ng of the DeBDE mix par gram of fish in corn oil. Fish from the remaining tanks received a dose of corn oil proportional to their weight. After seven additional weeks, 7-8 fish from each tank were randomly sampled, weighed, measured and liver tissue was sampled for biochemical and chemical analyses. This resulted in sampling 15 fish from each of the four following treatments: (Co+Co) corn oil + corn oil, (Co+De) corn oil + DeBDE mix, (CB+Co) PCB-126 + corn oil and (CB+De) PCB-126 + DeBDE mix, in which 8, 10, 10 and 6 male fish were found, respectively.

Biochemical and chemical analyses. All male fish were analyzed for ethoxyresorufin-*O*-deethylase (EROD), encoded by the cytochrome P4501A1 and reflecting the liver metabolizing capacity. EROD activity in liver S9 fraction was measured at 22 ºC by a microplate spectrofluorometric assay[7] and expressed as pmol resorufin/min/mg protein. Only one male from treatment Co+De and five males from both treatments CB+Co and CB+De were selected for chemical analysis. PCB-126, BDE-209 and several low brominated PBDE congeners, including BDE-17, 28, 47, 49, 66, 75, 99, 100, 119, 153, 154 and 155, were analyzed in the liver. These chemicals were extracted from chemically dried liver samples, the extract cleaned up by GPC and purified on a Si/Al column. For PCB-126, a carbon column was also used. All analyses were done by GC-ion trap MS, operated in MS/MS mode, on a 30 m capillary column, except for BDE-209 for which a 15 m column was used. The hepatic concentration of each PBDE congener was normalized to the concentration of BDE-47 for each fish and the mean ratio (BDEx /BDE-47) of each congener was calculated for each treatment.

Results and Discussion:

Transfer of PCB-126 and BDE-209 to fish liver. On average 48±10% of the injected PCB-126 was transferred to the fish liver. The mean hepatic concentrations of PCB-126 did not differ between treatments CB+Co and CB+De (Fig.1A) and were equivalent to 1.5 ng/g TCDD TEQ, a level of contamination that has been reported in Atlantic tomcod from the Hudson River, NY[8]. Only one fish from treatment Co+De was analyzed for PCB-126 and the result, 0.06 ng/g ww, was in agreement with the values generally found in tomcod from the same location (Lebeuf, pers. com.). The efficiency of the PCB-126 transfer was further confirmed by the response of hepatic EROD activity, ten weeks after the IP injection. EROD was markedly induced in fish from treatments CB+Co and CB+De

compared to the other treatments (Fig.1B). Seven weeks after the IP injection of the DeBDE mix, hepatic concentrations of BDE-209 were 412 and 726±940 ng/g ww in fish from treatment Co+De and CB+De, respectively (Fig.1C). The efficiency of the transfer of the BDE-209 to the fish liver was on average 4.6±4.6%, which is low and more variable compared to PCB-126. Nevertheless, it represents an enrichment of BDE-209 in the liver compared to an even distribution of the injected BDE-209 within the fish. The penetration of BDE-209 into the liver of the fish was validated by analyzing separately the internal and external parts of the liver of two fish injected with BDE-209 and finding similar levels in both liver parts. Similar response of EROD in fish from treatments CB+Co and CB+De suggests that the presence of BDE-209 in liver tissue had not altered their hepatic metabolic activity.

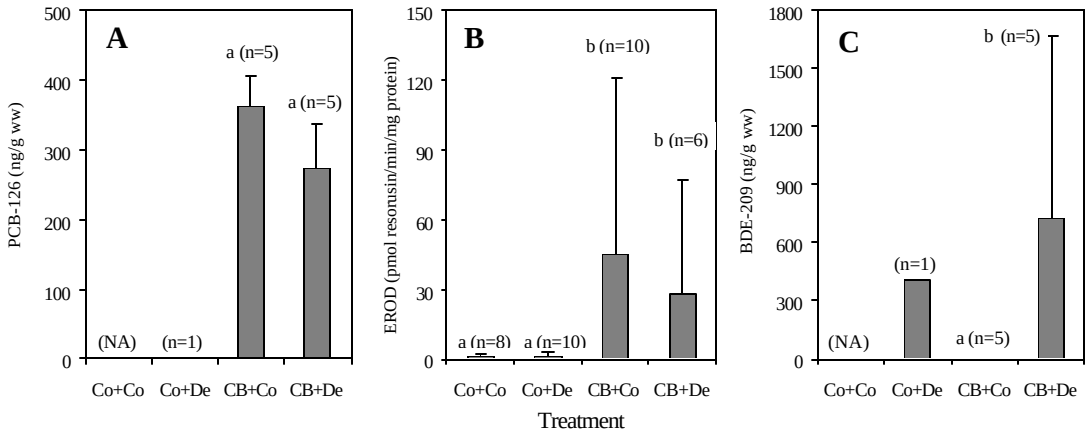

Figure 1. Concentrations of PCB-126 (A), EROD activities (B) and concentrations of BDE-209 (C) in fish liver according to treatments. Different letters indicate significant differences.

Enrichment of low brominated congeners. No significant difference in BDEx/BDE-47 ratios was observed for any of the PBDE congener examined between treatments CB+Co and CB+De, except for BDE-153 which was significantly higher in treatment CB+De (Fig.2). Enrichment of BDE-153 has been reported in rainbow trout fed with DeBDE mix[5]. Carp fed with BDE-209 show enrichments of BDE-154 and -155 but not of BDE-153[6]. Even with the presence of significant levels of BDE-209 into the liver, tomcod exhibited very limited capacity to produce PBDE congeners.

Contamination of the DeBDE mix. In contrast to dietary exposure, the administration of the DeBDE mix through an IP injection allows to determine precisely the total load of PBDE transferred to the fish. None of the PBDEs examined was detectable in a high concentration solution (1000 ng of DeBDE mix/ul). Based on our detection limits, it was possible to show that less than 1% of the BDE-153 in fish liver from treatment CB+De resulted from the contamination of the DeBDE mix injected into the fish. It cannot be excluded, however, that BDE-153 could result from the debromination of high brominated PBDEs, such as nona PBDEs, known to be present as impurities in the technical DeBDE mix[1].

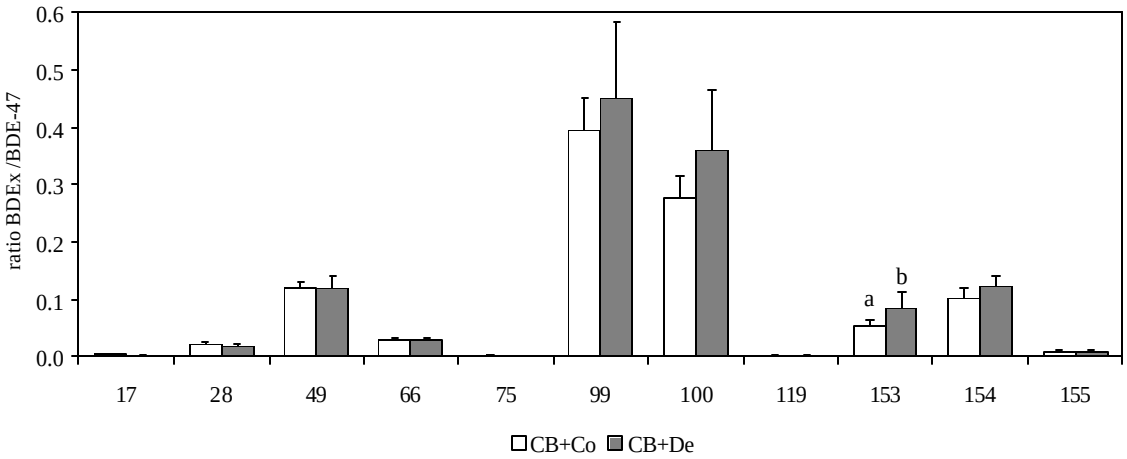

Figure 2. Reative ratio of PBDE congeners (BDEx/BDE-47) in fish from treatments CB+Co (n=5) and CB+De (n=5). Different letters indicate significant differences.

Conclusions:

The capacity of Atlantic tomcod to produce low brominated PBDEs was very limited, even after exposure through an IP injection optimizing the transfer of the DeBDE mix to the liver and pre-treatment with PCB-126 leading to induction of the hepatic metabolic enzymes activities. Compared to dietary exposure experiments, treatment via an IP injection improved our capacity to discriminate between the contribution of debromination and that of uptake of minor components from the source product of BDE-209. Most of the observed enrichment in PCB-153 in tomcod injected with BDE-209 resulted from debromination within the fish and not from uptake of impurities. Other pathways of environmental production of low brominated PBDEs from the DeBDE mix, such as the photolytic debromination of the BDE-209[9] and the thermodegradation of the BDE-209[10] must also be examined to support the safe use of the technical DeBDE mix.

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