THE RELATIONSHIP BETWEEN AGE AND LEVELS OF POLYBROMINATED DIPHENYL ETHERS IN BELUGA WHALES FROM THE ST LAWRENCE ESTUARY, CANADA

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Introduction

Polybrominated diphenyl ether (PBDEs) flame-retardants are used to reduce the risk of fire in a wide range of manufactured products such as computers, furniture and automobiles. Since the 1980s, the demand for PBDEs has increased in many countries and continues to rise[1]. Combustion and recycling of waste products of flame-retardants appear to be among the main sources of PBDEs to the environment. As a result, PBDEs are now ubiquitous in the marine environment[1].

The properties of PBDEs are comparable to those of other lipophilic persistent organic pollutants (POPs) such as PCBs and DDTs. Therefore, levels of PBDEs in adult marine mammals are expected to be higher in males than in females, as observed for several POPs[2,3,4]. The sex difference in POP levels in adult marine mammals is generally attributed to the transfer of contaminants from the mother to the young, via the placenta and the mother's milk. In addition, the relationship between age and levels of POPs in adult marine mammals also differs between genders. For instance, concentrations of POPs are usually positively correlated with the age of male animals whereas levels of these contaminants reach a plateau in adult females. Unexpectedly, however, we have measured similar concentrations of PBDEs in both male and female adult beluga whales (*Delphinapterus leucas*) collected in the St Lawrence Estuary (SLE) between 1997 and 1999[5]. In order to explain these observations, we examined the relationship between the age of beluga whales from the SLE and the concentrations of PBDEs.

Materials and Methods

Samples. Blubber samples were obtained from stranded adult male (n=14) and female (n=15) beluga whales found on the shores of the SLE between 1997 and 1999. A sample of blubber extending the depth of the blubber layer was taken from a larger piece of blubber collected from the dorso-lateral region of each individual. Blubber samples were either placed in a solvent rinsed glass jar, or wrapped in solvent rinsed aluminium foil and placed in a sealed plastic bag, and then stored at –20°C until analysis.

Analysis of BDPEs. Prior to the analysis, the skin was removed from the blubber. Blubber samples (0.5-1 g wet weight) were then chemically dried with sodium sulphate before being transferred to a glass column. A single $^{13}C_{12}$ PCB was added to the column before the extraction procedure. Lipids and lipophilic compounds were extracted from the sample with dichloromethane-hexane (50:50). The extraction solution received a mixture of four $^{13}C_{12}$ PBDEs and was prepared for purification. Lipids were removed from the remaining extract by gel permeation chromatography. The extract was further cleaned by elution through a two-layer column packed with neutral silica and alumina.

The final extract was reduced in volume and spiked with an instrument performance solution containing two $^{13}C_{12}$ PCBs.

Forty individual PBDEs for which we have authentic standards were measured in the samples (Table 1). Since the chemical structure of PBDEs resembles that of PCBs, the same numbering system is used. Quantification of individual PBDEs was performed using a Varian 3400CX series gas chromatograph equipped with a Varian Saturn IV ion trap, a Varian 1078 split/splitless programmable injector (5 µl injection volume) operated in splitless mode, and a Varian 8200CX autosampler. Chromatographic separation of the contaminants was achieved using a 30m DB-5MS column (0.25 mm ID, 0.25 µm film thickness) with helium as the carrier gas. The ion source was operated in electron impact ionisation mode and the ion trap in MS/MS mode. Concentrations of PBDE congeners were calculated using relative response factors (RRFs) determined from a three-point calibration curve. The accuracy of the method was assessed by measuring the recoveries of known quantities of native congeners added to a blank solution, which subsequently underwent all

Table 1. List of PBDE congeners included in the standard solution.

2-MoBDE (1)	2',3,4-TrBDE (33)	2,3,4,5,6-PeBDE (116)
3-MoBDE (2)	2,4,4'-TrBDE (28)	2,2',3,4,4'-PeBDE (85)
4-MoBDE (3)	3,3',4-TrBDE (35)	3,3',4,4',5-PeBDE (126)
2,6-DiBDE (10)	3,4,4'-TrBDE (37)	2,3,3',4,4'-PeBDE (105)
2,4-DiBDE (7)	2,4,4',6-TeBDE (75)	2,2',4,4',6,6'-HxBDE (155)
2,4'- / 3,3'-DiBDE (8+11)	2,2',4,5'-TeBDE (49)	2,2',4,4',5,6'-HxBDE (154)
3,4-DiBDE (12)	2,3',4',6-TeBDE (71)	2,2',4,4',5,5'-HxBDE (153)
3,4'-DiBDE (13)	2,2',4,4'-TeBDE (47)	2,2',3,4,4',6'-HxBDE (140)
4,4'-DiBDE (15)	2,3',4,4'-TeBDE (66)	2,2',3,4,4',5'-HxBDE (138)
2,4,6-TrBDE (30)	3,3',4,4'-TeBDE (77)	2,3,4,4',5,6-HxBDE (166)
2,4',6-TrBDE (32)	2,2',4,4',6-PeBDE (100)	2,2',3,4,4',5,6-HpBDE (181)
2,2',4-TrBDE (17)	2,3',4,4',6-PeBDE (119)	2,2',3,4,4',5',6-HpBDE (183)
2,3',4-TrBDE (25)	2,2',4,4',5-PeBDE (99)	2,3,3',4,4',5,6-HpBDE (190)

the analytical steps. The average recovery of the tetra to hepta-bromodiphenyl ether congeners was 98 ± 12 %.

Results and Discussion

Arithmetic mean (± standard deviation) concentrations of PBDEs, expressed as the sum of forty congeners, in females (665 ± 457 ng/g wet weight) and males (466 ± 230 ng/g wet weight) collected between 1997 and 1999 were not significantly different ($p = 0.15$). The similar levels of PBDEs in these animals cannot be explained by a difference in mean age between the sexes since the ages of males (17.5 ± 5.5 years) and females (20.7 ± 7.4 years) were also not significantly different ($p = 0.20$). In addition, the relationship between age and concentrations of PBDEs was similar (Figure 1). As expected, however, mean concentrations of PCBs and DDTs measured in the same animals were respectively 3-4 times and 5-6 times higher in males than in females. Furthermore, concentrations of PCBs and DDTs, increase with the age in males whereas not significant age-related trend was observed in females. These results indicate that PBDEs are not accumulated in beluga whales from the St Lawrence Estuary as did PCBs and DDTs.

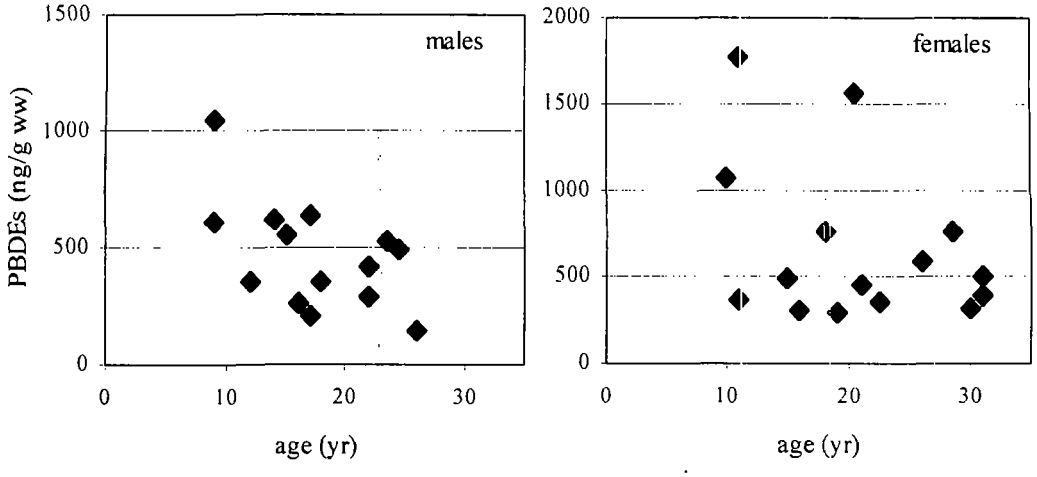

Figure 1: Age-related variation of PBDEs in beluga whales from the SLE.

There are a few possible explanations that can be put forward to describe these observations. For instance, the transfer of BPDEs from females to calves may not be as important for PBDEs when compared to that of PCBs and DDTs and could explain why males and females are similarly contaminated by PBDEs. If the transfer of PBDEs from females to calves was negligible, levels of PBDEs would be expected to increase with the age of the animals. However, levels of PBDEs in both males and females tended to decrease with the age of the animals (Figure 1). Moreover, the results of a recent study on long-finned pilot whales from the Faeroe Islands[6] suggest that post-natal transfer of PBDEs in juveniles is important.

Another possible explanation is that males could be more efficient than females in transforming PBDEs. This last assumption is in agreement with the relatively lower capacity of female mammals to transform contaminants, as reported by Aguilar[7]. The presence of elevated levels of POPs in male beluga whales is expected to activate their detoxification system and help them to transform and eliminate at least some PBDE congeners. However, the relative distribution pattern of PBDEs, based on the forty congeners measured (Table 1), is very similar in male and female belugas[8]. This suggests that the capacity to transform these compounds is similar in both sexes.

However, the elevated levels of PBDEs in beluga whales from the SLE are a relatively recent phenomenon, which can very likely explain why concentrations of these compounds in males and females are similar. Concentrations of PBDEs in blubber samples of male and female belugas collected between 1997 and 1999 were approximately 20 times higher than those in samples collected between 1988 and 1990[5]. The increase of PBDE levels in St Lawrence beluga whales during the last 10-15 years is in agreement with temporal trends reported in other studies, such as those for lake trout from the Great Lakes[9], and for beluga whales from the SE Baffin and Cumberland Sound[10]. However, the increase of PBDEs observed in belugas from the SLE is at least two times higher than those reported in the other studies during the same time period. This recent and significant increase of PBDEs in belugas from the SLE could possibly explain the lack of difference in concentrations of PBDEs between males and females. We suggest that at the

present time these compounds are tending to be accumulated in both female and male beluga whales rather than be eliminated. The overall contamination of the beluga whales is due in large part to their recent accumulation of PBDEs. Therefore, the trends observed for the bioaccumulation of persistent organochlorinated compounds such as PCBs and DDTs, which have reached their maximum contamination in the SLE, are not directly comparable with those of PBDEs. However, differences in concentrations and age-related variations of PBDEs between male and female beluga whales are expected to resemble to those observed for PCBs and DDTs upon a regulation of the inputs of these compounds into the SLE.

In summary, the growing use of PBDEs as flame-retardants has resulted in an increasing contamination of beluga whales from the St. Lawrence Estuary. Unlike the bioaccumulation trends observed for other persistent organic compounds, levels of PBDEs as well as the relationship between age and levels of PBDEs in male and female belugas are similar. These results can very likely be explained by the relatively recent and important increase of BPDEs in beluga whales from the St Lawrence Estuary.

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