SPECIFIC ACCUMULATION OF POLYBROMINATED DIPHENYL ETHERS INCLUDING DECA-BDE IN TISSUES OF HARBOR SEALS FROM THE NORTHWEST ATLANTIC

Shaw, S D[1], Berger M L[1], Weijs, L[2,3], Roosens, L[2] and Covaci, A[2,3]

[1] Marine Environmental Research Institute, P.O. Box 1652, Blue Hill, ME 04614, USA
[2] Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
[3] Ecophysiology, Biochemistry and Toxicology, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

Abstract
To examine tissue-specific accumulation of PBDEs, this study analyzed tri- through deca-BDEs in liver samples of harbor seals (n=56) from the northwest Atlantic region, and compared concentrations and patterns with those detected previously in blubber. Hepatic concentrations of ΣPBDEs ranged from 35 to 19547 ng/g lw (overall mean 2671 ng/g lw) and were similar to the concentrations in blubber (overall mean 2403 ng/g lw). Hepatic ΣPBDE concentrations were highest in the male pups (mean 4397 ng/g lw); their levels were significantly higher than those in the female pups. Tissue distribution of PBDEs differed markedly among male and female pups, suggesting possible gender differences in metabolism and elimination/retention of PBDEs among young seals. Congener profiles were dominated by BDE-47, indicating exposure to penta-BDE mixtures. Congener dominance in the pups was in the order: BDE-47>-99>-100>-153>-154>-155>, whereas hexa-BDEs surpassed penta-BDEs in the adult male profiles. Hepta- and octa-BDEs and BDE-209 were detected in seal liver, suggesting recent exposure to the octa- and deca-BDE formulations and/or BDE-209 debromination processes. Although detection frequency was low, BDE-209 levels in liver samples with detection (range:10 to 40 ng/g lw) were up to five times higher than those detected in blubber, implying that liver may be a preferential tissue for BDE-209 accumulation in harbor seals. Moreover, hepatic concentrations of BDE-209 in the seals were up to ten times higher than those reported in fishes that are harbor seal prey. These results suggest that BDE-209 can biomagnify in seal tissues and is subject to placental and/or lactational transfer, possibly placing pups at risk for adverse developmental effects.

Introduction
Polybrominated diphenyl ethers (PBDEs) are a class of synthetic halogenated compounds used in numerous polymer-based commercial and household products such as textiles, furniture, and electronics to increase their flame ignition resistance and meet fire safety standards[1]. PBDEs are structurally similar to thyroxine, and laboratory studies indicate that PBDE exposure may be associated with endocrine-disrupting and reproductive/neurodevelopmental effects in animals[2, 3]. Temporal studies indicate that PBDE levels are increasing in wildlife and humans, particularly in North America[4], where usage of the penta-BDE formulation dominated the global market over three decades[5]. Following bans on the penta- and octa-BDE products, deca-BDE (>97% BDE-209) now dominates the global market for PBDEs and has only recently begun to be regulated in the United States and Europe[6]. Substantial releases of deca-BDE from industrial sources directly to the environment have been reported[7]. In addition, concerns have been raised about the abiotic and biotic debromination of BDE-209, the major constituent of deca-BDE, to more bioaccumulative and potentially toxic congeners[8-11].

PBDEs enter coastal and marine waters from multiple sources and readily biomagnify in marine food webs[12]. PBDEs have been detected at relatively high concentrations in harbor seals *(Phoca vitulina concolor),* apex predators in the northwest Atlantic marine ecosystem[13]. Congeners found in harbor seals and their fish prey (tri- through deca-BDEs) are suggestive of both direct exposure to the penta-BDE mixture and indirect exposure through abiotic and/or metabolic breakdown of deca-BDE[11]. BDE 209 was detected at low levels in seal blubber and whole fish samples[11], indicating that this congener is bioavailable and can be accumulated through the marine food chain. Whereas tetra- through hexa-BDEs were highly biomagnified from fish to adult seal blubber (BMFs 17-76), the biomagnification potential for BDE-209 was low (BMF≤1)[11]. Gray seals (*Halichoerus grypus)* dosed with deca-BDE accumulated similar low levels of BDE-209 in blubber[14]. Whereas a short half-life (8 to 13 days) was indicated for BDE-209 in gray seal serum, suggesting rapid clearance of this compound in seal blood, BDE-209 remained in blubber stores long after cessation of exposure (months), indicating that once BDE-209 partitions into blubber, it is relatively stable. Whether the lack of biomagnification of BDE-209 is a result of a low uptake rate for this large molecule into blubber or efficient debromination processes in fish is unclear. It is also possible that BDE-209 may be partitioning to tissues other than blubber in the harbor seal. Studies in rats and fish have shown that BDE-209 preferentially binds to blood proteins and migrates to highly perfused tissues, such as the liver[15-17]. Thus,

concentrations measured in blubber may underestimate the body burden of BDE-209 in seals. In view of the widespread use of deca-BDE, there is a clear need for more information on the kinetics and accumulation of BDE-209 and its derivatives in tissues. Therefore, the aim of this study was to investigate the accumulation of PBDEs including BDE-209 and other highly brominated congeners in harbor seal liver samples, and to compare concentrations and congener patterns in liver with those previously analyzed in blubber. Most of the animals investigated were pups (n=50), providing an opportunity to examine tissue distribution of PBDEs resulting from placental and lactational transfer. To assess possible tissue differences in the biomagnification potential of PBDEs, biomagnification factors (BMFs) were calculated for sum PBDEs and individual congeners from marine fishes to liver of adult male seals and compared with those previously reported in blubber.

Materials and Methods

Samples. Liver samples were collected between 2001 and 2006 from 56 harbor seals (6 adult males, 22 male pups, and 28 female pups) that stranded along the northwest Atlantic coast from the eastern coast of Maine to Long Island, New York (Figure 1). Seals were weighed, and standard length and axillary girth were measured. Age was estimated based on body size. Liver samples were stored in a freezer at -40°C until analysis.

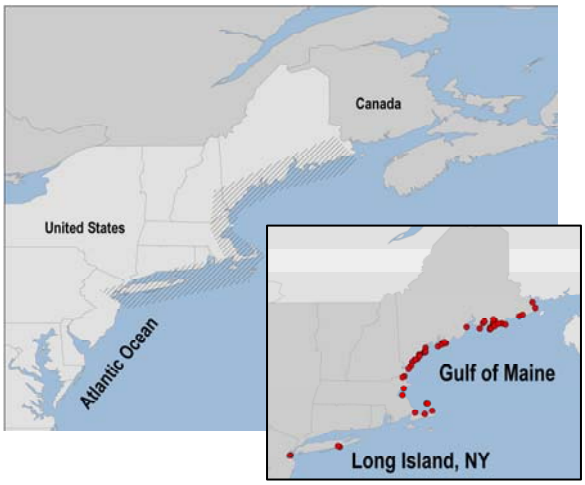

Figure 1. Map of the northwest Atlantic showing stranding locations of harbor seals

Sample preparation. Seal liver samples (2 - 2.5 g) were ground with sodium sulfate and spiked with internal standards (BDE 77, BDE 128, CB 143, ^{13}C-BDE 209 and ^{13}C-HBCDs). Samples were extracted for 2 hours by hot Soxhlet with a mixture of acetone/hexane (1/3, *v/v*). The extract was evaporated and cleaned by passing through 8 g of acid silica (H_2SO_4, 44%), from which pollutants were eluted with 20 ml hexane and 15 ml DCM[18,19]. Minor adaptations were required as PBDEs and PCBs were analysed by GC-ECNI/MS or GC-EI/MS and HBCDs by LC-MS/MS. The cleaned extract was evaporated to dryness, redissolved in 0.5 ml hexane and eluted from pre-packed silica cartridges with 6 ml hexane (for GC analysis) and 6 ml DCM (for LC analysis). Both fractions were evaporated to dryness and redissolved in 100 µl iso-octane or methanol, respectively.

GC Analysis. The determination of PBDEs was performed with an Agilent 6890GC-5973MS equipped with a 15 m x 0.25 mm x 0.10 µm DB-5 capillary column and operated in electron capture negative ionisation (ECNI) mode. The ion source, quadrupole and interface temperatures were 250, 150 and 300 °C, respectively. The electron multiplier voltage was set at 2200 V. The MS was operated in SIM mode (*m/z* 79 and 81 were monitored for the entire run, *m/z* 487 and 495 were monitored for BDE-209 and ^{13}C-BDE 209, respectively). Dwell times were set to 40 ms. Helium was used as carrier gas at constant flow (1.0 mL/min), while methane as moderating gas. One µL of the extract was injected in solvent vent mode (initial injector temperature at 90 °C, stay 0.03 min, then heated at 700 °C/min to 300 °C, vent time 0.03 min, vent flow 75 mL/min, splitless time 1.50 min). The temperature of the DB-5 column was programmed from 90°C, kept for 1.5 min, then increased with 15°C/min to 295°C, kept for 15 min.

For confirmation of lower PBDEs, the extracts were injected into a GC/MS operated in electron ionization (EI) mode and equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. The mass spectrometer was used in SIM mode with the two most intense ions (typically from the molecular cluster) acquired for each homologue group or isomer. One µL of the extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) then to 300 °C at 700 °C/min, pressure pulse 25 psi, pulse time 1.50 min, splitless time 1.50 min). Helium was used as carrier gas at constant flow (1 mL/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, holding for 20 min.

Quality Assurance and Quality Control. QA/QC was performed through the analysis of procedural blanks, a replicate sample and a standard reference material (SRM 1945, PBDEs in whale blubber). For the replicate and SRM 1945, the relative standard deviations (RSD) were < 10 % for most analytes. Additionally, the method

performance was assessed through successful participation to interlaboratory studies organized by NIST (PBDEs and PCBs in marine mammals). Procedural blanks of PBDEs were consistent (RSD < 20 %) and therefore the mean value of each analyte in the procedural blanks was used for subtraction. Method quantification limits (LOQs) for individual PBDE congeners were based on procedural blanks (3x SD) and the amount of sample taken for analysis. LOQs for tri-hepta PBDEs range between 1 and 2 ng/g lipid weight (lw). LOQ for BDE 209 was variable and ranged between 5 and 35 ng/g lw.

Statistics. Values below the detection limit were replaced with ½ LOQ for calculation of means and totals. Concentrations were log transformed prior to statistical analysis with 1 or 2-way ANOVAs. The level of significance was set at p=0.05. All concentrations are reported on a lipid weight (lw) basis.

Results and discussion

PBDE concentrations in harbor seal tissues. PBDE congeners detected in liver of harbor seals (n=56) included: BDEs-28, -47, -49, -85, -99, -100, -153, -154, -155, -181, -183, -184, an unidentified hepta-, -191, -197, and -209. BDEs-66, -196, and -203 were not detected. ΣPBDE (tri-deca) concentrations in seal liver samples ranged from 35 to 19547 ng/g lw, with an overall mean of 2671 ± 3566 ng/g lw. Similar concentrations of ΣPBDE (mono-hexa-BDEs) were previously detected in blubber samples of harbor seals from this region (overall mean 2403 ± 5406; range 80 to 25720 ng/g lw; n=42)[13]. These concentrations are an order of magnitude higher than those reported in harbor and gray seals from Europe[20,21], reflecting the greater usage of penta-BDE in North America. Higher PBDE levels were reported in California sea lions (*Zalophus californianus)*[22] and harbor seals from San Francisco Bay[23].

Influence of age and gender on tissue distributions. Comparisons using one-way ANOVA with log-transformed concentrations revealed that the male pups had two-fold higher hepatic ΣPBDE concentrations (mean 4397 ± 4868 ng/g lw; n=22) than the female pups (1680 ± 1807 ng/g lw; n=28) ($F_{2,55}$=4.6, p=0.02). The highest PBDE level in liver (19,546 ng/g lw) was observed in a male pup from southern Maine. In addition, hepatic concentrations of the BDE congeners -28, -47, -49, -99, and -153 were significantly higher in the male pups (Figure 2). As the figure illustrates, the tissue distribution pattern for PBDEs (sum PBDE and major congeners) differed dramatically between the male and female pups, implying that male pups may preferentially retain PBDEs in liver whereas female pups may accumulate higher concentrations in blubber. The first weeks of life represent a period of rapid growth, during which harbor seal pups almost triple their birth weight and lay down layers of blubber prior to weaning. The exact ages of the pups were unknown, thus we used biometric data to examine the possible influence of growth on hepatic PBDE concentrations. No correlations between PBDEs and body weight, body length, or lipid content of the samples were found for male or female pups. Nevertheless, this finding was interesting, and is suggestive of possible gender differences in metabolism and elimination/tissue sequestration of PBDEs among young seals.

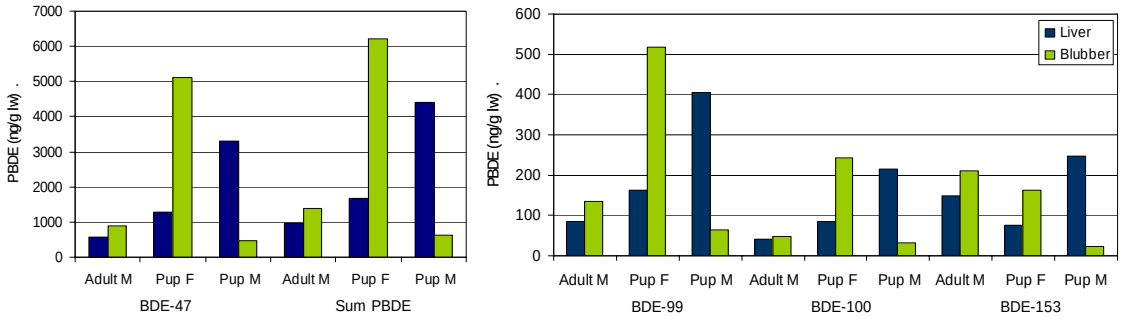

Figure 2. Mean concentrations of ΣPBDEs and BDE congeners in harbor seal liver and blubber. Note the higher y-axis scale for ΣPBDEs and BDE-47.

ΣPBDE concentrations in the adult males (mean 968 ±775 ng/g, lw; n=6), although nearly two- to four-fold lower, were not significantly different than levels in the pups. This accumulation pattern is similar to that observed in blubber and shows that the highest lifetime exposure to lipophilic contaminants may result from maternal transfer *in utero* and during nursing, placing pups at risk for the adverse developmental effects of PBDEs.

Congener profiles. Eight PBDE congeners (BDEs- 28, -47, -49, -99, -100, -153, -154, and -155) contributed 81–99.9% of the total PBDE content in seal liver and 91–99.6% in blubber (Figure 3). BDE-47 was the dominant congener, contributing 62-75% of the total in liver and blubber. BDE profiles in the pup liver and blubber were similar and followed the order: BDE-99>100>153>154>155>28>49. In contrast, the highly persistent and bioaccumulative hexa-BDEs -153, -154, and -155 were prominent in both liver and blubber profiles of the adult males. These differences likely reflect the different exposure pathways between adults and pups, as well as age-related differences in the ability to metabolize and eliminate BDE congeners. Whereas the profiles in the males reflect uptake and accumulation through feeding on teleost fishes, the pattern in the pups suggests efficient placental and lactational transfer of BDE-47 and to a lesser degree, BDEs-99, and -100, but very limited transfer of the hexa-BDEs. In grey seals, maternal transfer efficiency was shown to decline with increasing degree of bromination of the molecule, as a function of increasing K_{ow} values[24]. This may be a consequence of molecular size of higher BDEs, which may limit diffusion and lipid/water partitioning in females during lactation.

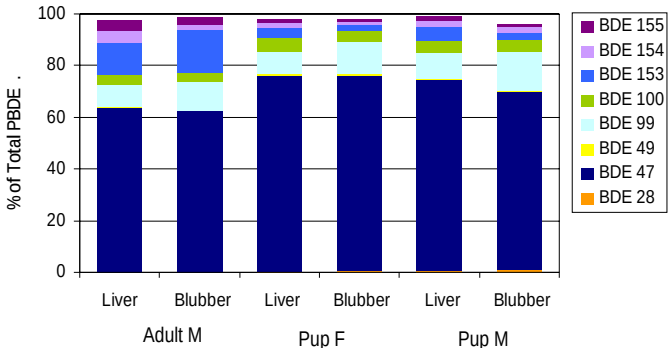

Figure 3. PBDE congener profiles in harbor seal tissues

Spatial and temporal trends. Considered relatively non-migratory, northwest Atlantic harbor seals nevertheless make seasonal movements along the coast from New Jersey to Maine[25], following an urban/industrial-rural-remote gradient from south to north. However, no significant differences were found for ΣPBDE concentrations in male/female pups from the more industrialized southern area compared with those in the north. This lack of a spatial trend for PBDEs was also found in seal blubber samples[13], and probably reflects the presence of diffuse local sources (e.g., waste water treatment plants, sewage sludge applications, landfill leachate) across the harbor seal range. To assess possible temporal trends, concentrations in pups were plotted against the year of sampling (Figure 4). Results show a lack of a temporal trend for ΣPBDE concentrations in male or female pups from 2001 to 2006, which is consistent with our findings in blubber[13], and with the lack of a trend observed in other studies over the same time period[22,23,26]. Collectively, the data suggest that PBDE levels were increasing in marine mammals between the 1970s and the mid-1990s, but may have stabilized or reached equilibrium over the past decade.

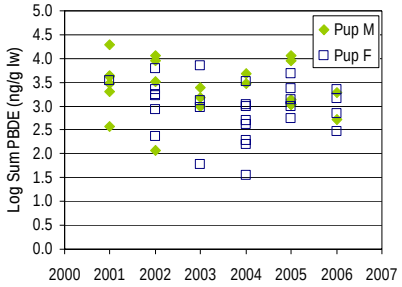

Figure 4. Lack of a temporal trend in hepatic concentrations of ΣPBDEs from 2001 to 2006 for male (◊) and female pups (□)

Higher brominated congeners in seal tissues. Several hepta- and octa-BDEs were detected at low levels in seal liver including BDE-183 (ND – 13 ng/g lw), BDE-197 (ND – 4.7 ng/g lw), and an unidentified hepta- BDE (ND – 17 ng/g lw). BDEs- 181, -184, and -191 were detected at trace levels in liver. Hepta-and octa-BDEs were previously reported at slightly higher concentrations in seal blubber samples[13]: BDE-183 (ND – 45 ng/g lw), BDE-197 (ND –

57 ng/g lw) and an unidentified hepta- (ND – 15 ng/g lw) and octa-BDE (ND – 84 ng/g lw). BDE-209 was also detected in seal liver samples; the range of detected values was 14 to 40 ng/g lw. While the presence of tetra- to hexa-BDEs in seal tissues indicates exposure to components of the penta-BDE mixture, the occurrence of hepta- and octa-BDEs suggests recent exposure to the octa- and deca-BDE mixtures and/or BDE-209 debromination processes, since a short half-life (months) is indicated for higher-BDE congeners[27].

Detection frequency for BDE-209 was low, but among the samples with detection, BDE-209 concentrations (range: 14 to 40 ng/g lw) were up to five times higher in harbor seal liver than those previously detected in blubber (1-8 ng/g lw)[13], implying that liver may be a preferential tissue for BDE-209 accumulation in harbor seals. While preliminary, this finding is consistent with results of laboratory studies showing preferential accumulation of BDE-209 in liver of rats and fish[15-17], and with studies showing selective hepatic retention of this compound in terrestrial wildlife such as red foxes *(Vulpes vulpes)*[28], and Japanese raccoon dogs[29].

Our previous work demonstrated that PBDEs (tri- through hexa-BDEs) readily biomagnify through the northwest Atlantic marine food web, with BMFs for ΣPBDEs from prey fishes to adult male harbor seal blubber ranging, on average, from 17 to 76[11]. In contrast, biomagnification of BDE-209 was not observed from fish to blubber (BMF≤1)[11]. In the present study, calculation of BMFs from fish to liver of the adult male seals revealed a similar biomagnification potential for the lower brominated congeners (with BMFs ranging from 14 to 54 for tri- to hexa-BDEs in liver). However, BDE-209 levels in liver of harbor seals were up to 10 times higher than those in the fish, suggesting that BDE-209 can biomagnify to a greater extent in seal tissues than previously thought. Moreover, most of the seals in this study were pups, implying that BDE-209 is subject to placental and/or lactational transfer, possibly placing pups at risk for developmental neurotoxicity and other adverse effects. Given the growing reservoirs of deca-BDE in marine food webs, the biomagnification of BDE-209 in marine mammals is of concern.

Acknowledgements
Susan Shaw and Michelle Berger acknowledge financial support from the National Oceanographic and Atmospheric Administration (NOAA) and the Britton Fund. Adrian Covaci is financially supported by a postdoctoral fellowship from the Research Scientific Foundation - Flanders (FWO). Liesbeth Weijs acknowledges financial support from the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT). The authors thank Kirk Trabant and the NOAA National Marine Fisheries Service Northeast Region Stranding Network for providing harbor seal liver samples for this study. This work was partly supported by the University of Antwerp.

References

1. Alaee M., Arias P., Sjodin A. and Bergman Å. *Environment International* 2003; 29: 683-689.
2. Birnbaum L. S. and Staskal D. F. *Environmental Health Perspectives* 2004; 112: 9-17.
3. Darnerud P. O. *Environment International* 2003; 29: 841-853.
4. Hites R. A. *Environmental Science & Technology* 2004; 38: 945-956.
5. Bromine Science and Environmental Forum (BSEF), 2003. <www.bsef.com>.
6. Betts K. *Environmental Health Perspectives* 2008; 116: A210-A213.
7. Hale R. C., La Guardia M. J., Harvey E., Gaylor M. O. and Mainor T. M. *Chemosphere* 2006; 64: 181-186.
8. Stapleton H. M. and Dodder N. G. *Environmental Toxicology & Chemistry* 2008; 27: 306-312.
9. Stapleton H. M., Letcher R. J. and Baker J. E. *Environmental Science & Technology* 2004; 38: 1054-1061.
10. La Guardia M. J., Hale R. C. and Harvey E. *Environmental Science & Technology* 2007; 41: 6663-6670.
11. Shaw S. D., Berger M. L., Brenner D., Kannan K., Lohmann N. and Päpke O. *Science of the Total Environment* 2009; 407: 3323–3329.
12. de Wit C. A. *Chemosphere* 2002; 46: 583-624.
13. Shaw S. D., Brenner D., Berger M. L., Fang F., Hong C.-S., Addink R. and Hilker D. *Chemosphere* 2008; 73: 1773-1780.
14. Thomas G. O., Moss S. E. W., Asplund L. and Hall A. J. *Environmental Pollution* 2005; 133: 581-586.
15. Mörck A., Hakk H., Örn U. and Wehler E. K. *Drug Metabolism and Disposition* 2003; 31: 900-907.
16. Huwe J. K, Hakk H., and Birnbaum L. S. *Environmental Science & Technology* 2008; 42: 7018-7024.
17. Stapleton H. M., Brazil B., Holbrook R. D., Mitchelmore C. L., Benedict R., Konstantinov A. and Potter D. *Environmental Science & Technology* 2006; 40: 4653-4658.
18. Voorspoels S, Covaci A, Schepens P. *Environmental Science & Technology* 2003; 37: 4348–57.
19. Covaci A, Losada S, Roosens L, Vetter W, Santos FJ, Neels H, Storelli A, Storelli MM. *Environmental Science & Technology* 2008; 42: 8657-8660.

20. Weijs L., Gheorghe A., Dirtu A. C., Das K., Reijnders P. J. H., Neels H., Blust R. and Covaci A. *Organohalogen Compounds* 2007; 69: 825-828.
21. Kalantzi O. I., Hall A. J., Thomas G. O. and Jones K. C. *Chemosphere* 2005; 58: 345-354.
22. Stapleton H. M., Dodder N. G., Kucklick J. R., Reddy C. M., Schantz M. M., Becker P. R., Gulland F., Porter B. J. and Wise S. A. *Marine Pollution Bulletin* 2006; 52: 522-531.
23. She J., Petreas M., Winker J., Visita P., McKinney M. and Kopec D. *Chemosphere* 2002; 46: 697-707.
24. Ikonomou M. G. and Addison R. F. *Marine Environmental Research* 2008;
25. National Marine Fisheries Service (NMFS). 2007. <http://www.nmfs.noaa.gov/pr/sars/species.htm>.
26. Tuerk K. J. S., Kucklick J. R., Becker P. R., Stapleton H. M. and Baker J. E. *Environmental Science & Technology* 2005; 39: 692-698.
27. Geyer H. J., Schramm K.-W., Darnerud P. O., Aune M., Feicht E. A., Fried K. W., Henkelmann B., Lenoir D., Schmid P., McDonald T. A. *Organohalogen Compounds* 2004; 66: 3867-3872.
28. Voorspoels, S., Covaci, A., Lepom, P., Escutanaire, S., Schepens, P. *Environmental Science & Technology* 2006; 40: 2937-2943.
29. Kunisue, T., Takayanagi, N., Isobe, T., Takahashi, S., Nakatsu, S., Tsubota, T., Okumoto, K., Bushisue, S., Shindo, K., and Tanabe, S. *Environmental Science & Technology* 2008; 42: 685-691.