

PATTERNS AND TRENDS OF PCBs AND PCDD/Fs IN NW ATLANTIC HARBOR SEALS: REVISITING TOXIC THRESHOLD LEVELS USING THE NEW TEFS

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Abstract

Harbor seals (*Phoca vitulina concolor*) inhabiting the northwestern Atlantic are closely associated with polluted near-shore environments and are highly contaminated by persistent organic pollutants (POPs). The population is experiencing recurring epizootics, and it can be speculated their high body burdens of immunotoxic chemicals may be playing a contributory role in these events. Here we report, for the first time, the levels, patterns, and trends of PCBs, dioxins and furans (PCDD/Fs) in harbor seals from this region. PCB concentrations in blubber were relatively high, especially in the younger seals (56800 – 60500 ng/g lw). PCDD/Fs were detected at trace concentrations (<5 pg/g lw), likely reflecting a species specific degradative capacity for these compounds. No temporal trend was found in concentrations between 1991 and 2005, suggest a continuous input of PCBs in the northwestern Atlantic. Highest concentrations were found in seals in the southern region (New York to Massachussets) compared with those in eastern Maine, indicating that the main sources are near the highly industrialized population centers in the northeastern US. WHO-TEQs of 12 dioxin-like PCBs and PCDD/Fs calculated using the most recent TEFs were two to four times lower than those calculated using 1998 TEFs, implying that the estimated threshold value for adverse effects in the species should be reassessed.

Introduction

Harbor seals (*Phoca vitulina concolor*) inhabiting the northwestern Atlantic are closely associated with polluted near-shore environments and have been shown to be highly contaminated by persistent organic pollutants (POPs).¹⁻⁴ In our previous work, we reported that body burdens of polychlorinated biphenyls (PCBs) in these seals are similar to levels reported in seals from polluted regions of Europe and Asia, and exceed the estimated threshold levels for adverse reproductive and immune system effects in the species.^{1,5} Moreover, altered *in vitro* immune responses in free-ranging adult harbor seals were found to be significantly associated with plasma levels of dioxin-like compounds.⁶ While the health implications of this finding are unclear, it is consistent with the body of evidence suggesting that harbor seals may be particularly vulnerable to adverse effects on immune function following exposure to compounds that act by binding the cytosolic aryl hydrocarbon (*Ah*) receptor, e.g., PCBs, dioxins and furans (PCDD/Fs).⁷⁻¹⁰ Since the early 1970s, the northwestern Atlantic harbor seal population has increased to an estimated 99,000 animals, but these seals have experienced at least four major epizootics since 1980, suggesting that they are susceptible to disease.¹¹⁻¹⁵ In fall 2006, an epizootic of unknown etiology resulted in the deaths of more than 400 animals along the southern Maine coast. Whereas influenza A and phocine distemper virus (PDV) were confirmed as the cause of two earlier mass mortalities, no specific causal agent has been identified in the current mortality event which is still ongoing. In view of their high body burdens of immunotoxic POPs, it can be speculated that chemical stress, in concert with other factors, may be playing a contributory role in these events.

The objective of the present study was to assess the concentrations, patterns, and trends of dioxin-like compounds including PCBs and PCDD/Fs in northwestern Atlantic harbor seals, and to examine the potential toxicological implications of their exposure to these

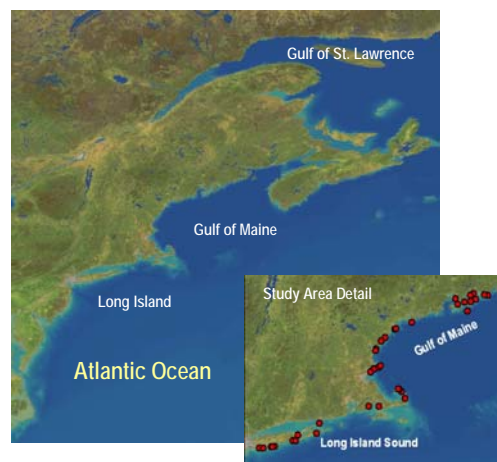


Figure 1. Map of NW Atlantic showing harbor seal stranding locations

compounds using the most recent international toxic equivalency factors (TEFs).¹⁶ To our knowledge, this is the first extensive analysis of dioxin-like PCBs and PCDD/Fs in seals from this region.

Materials and Methods

Samples. Blubber samples were collected from 42 harbor seals (7 adult males, 8 adult females, 14 yearlings, 13 pups) that stranded along the northwestern Atlantic coast from the eastern coast of Maine to Long Island, New York between 1991 and 2005 (Figure 1). Seals were weighed, and standard length and axillary girth were measured. Age was estimated based on body size. Condition indices were calculated by dividing axillary girth/standard length and body weight/standard length. Blubber samples were stored in a freezer at -40°C until analysis.

Chemical Analysis. Blubber samples were analyzed for 146 PCB congeners (including 8 mono- and 4 non-ortho PCBs) and 17 PCDD/Fs following the isotope dilution quantification method as previously described.² Analyses of PCBs congeners were performed by HRGC-LRMS (HP 6890GC with DB-XLB column, 30 m × 0.25 mm i.d. × 0.25 μm film thickness coupled to an HP 5972 mass spectrometer) and monitored by selected ion monitoring (SIM) at the two most intensive ions in the molecular ion or M-2Br ion cluster. PCB concentrations were calculated using the internal standard method and were corrected by surrogate recoveries. Congener concentrations below the level of detection were treated as if half the detection limit. WHO-TEQs were calculated for the dioxin-like PCBs and PCDD/Fs using the most recent international WHO-TEFs.¹⁶

Statistics. Variables were log-transformed to reduce skewness prior to statistical analysis using SPSS 14.0. Comparisons between age classes and between regions were made with transformed data using non-parametric tests. Concentrations below the level of detection were calculated by treating the result as if half the detection limit.

Results and Discussion

Mean Σ PCB concentrations (sum of 120 congeners) were highest in the younger seals (60479 and 56799 ng/g, lipid weight, lw in yearlings and pups, respectively). Mean PCB concentrations in adult females (14047 ng/g, lw) were less than half those in the adult males (36685 ng/g, lw), but the differences were not statistically significant due to wide variances. This pattern is consistent with that observed for most lipophilic organohalogenated compounds in which females transfer a large proportion of their body burden to pups during gestation and lactation, while an age-dependent accumulation is observed in male seals.

PCB congener profiles were dominated by the hexaCBs 153 and 138, followed by the pentaCB 99 in the younger seals and the heptaCB 180 in the adults. Examination of PCB homolog profiles revealed significant differences in patterns by age class (Figure 2). In pups and yearlings, the lower chlorinated congeners (tetra- and pentaCBs) contributed proportionally more to the total than in the adult seals ($p < 0.01$), whereas the adults retained a greater proportion of the hepta- ($p < 0.05$), octa- and nonaCBs ($p < 0.01$). Although we did not examine mother-pup pairs, this pattern is consistent with other studies in which substantially lower concentrations of the higher chlorinated and hydrophobic compounds are found in blubber of pups than in maternal blubber, reflecting selective partitioning of persistent PCB congeners into milk over the course of lactation.¹⁷⁻²⁰ The same pattern was observed in our yearling seals, although they retained more of the octa- and nonaCBs than the pups ($p < 0.01$), reflecting the gradual accumulation of higher chlorinated CBs through feeding.

Of 17 PCDD/Fs analyzed in seal blubber, only two isomers were detected in at least 30% of samples and these contributed ~2% to the total TEQ. 1,2,3,6,7,8-HxCDD was detected in 36% of samples and 2,3,7,8-TCDF was detected in 61% of samples. Mean PCDD/F concentrations were extremely low ranging from 0.6 pg/g lw in the adult females to 3.9 pg/g lw in the pups, and

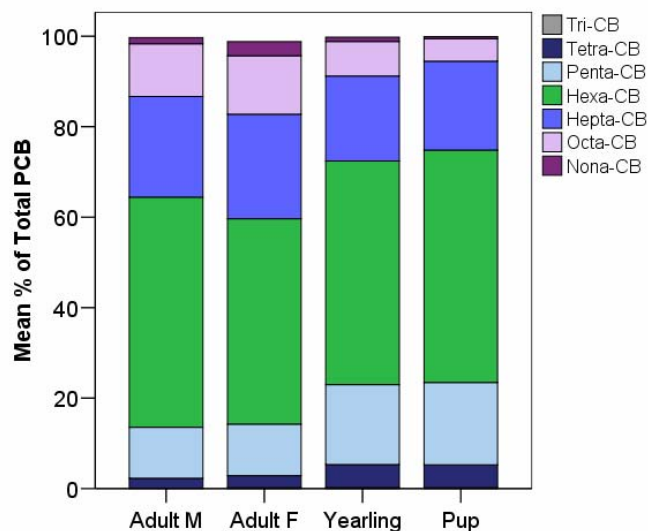


Figure 2. PCB homolog profile in harbor seals by age

could reflect the absence of major local sources but are more likely due to a species specific degradative capacity for these compounds. Several studies have reported a lack of significant bioaccumulation of PCDD/Fs in seals with known exposure, implying that they may be more readily metabolized than the PCBs.^{7, 21, 22}

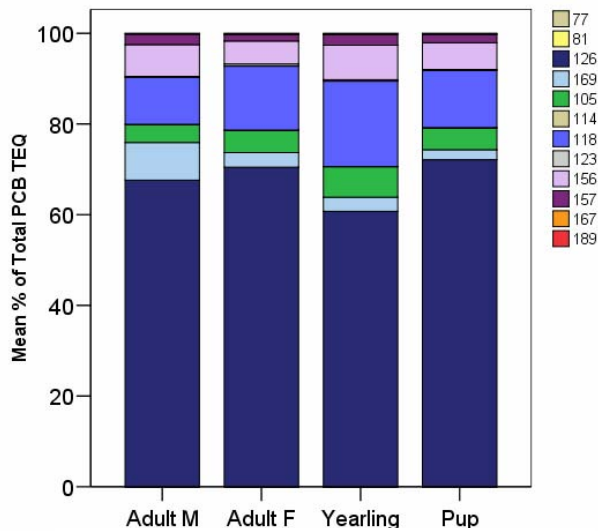


Figure 3. PCB TEQ profile by age

WHO-TEQs calculated using the most recent TEFs¹⁶ were highest in the pups (191 pg/g, lw) and yearlings (128 pg/g, lw), followed by the adult males (55 pg/g, lw) and females (25 pg/g, lw). Differences between the younger seals and adult females were significant ($p < 0.05$). PCB TEQ congener profiles were fairly similar (with one exception) and were dominated by the non-ortho CB 126 (61-72%), followed by the mono-ortho CBs 118 (10-19%) and 156 (5-8%) (Figure 3). In the adult males, the non-ortho CB 169 contributed more to the total than in the adult females, yearlings or pups ($p < 0.05$).

In addition to age and gender, the influence of condition on concentrations was examined. Adult seals were in better condition (weight/length) than the younger animals ($p < 0.01$), but condition indices were not correlated with PCB concentrations and were only weakly correlated with WHO-TEQs of dioxin-like PCBs in blubber ($R^2 = 0.15$, $p = 0.02$ for weight/length). Similarly, we found no correlations between concentrations of PCBs or PCB TEQs

and body weight, length or girth. Lipid content in our samples was positively correlated with both condition indices (weight/length $R^2 = 0.17$, girth/length $R^2 = 0.23$, $p < 0.02$), and negatively correlated with total PCB ($R^2 = 0.34$, $p < 0.01$) and PCB TEQ ($R^2 = 0.54$, $p < 0.01$). Overall lipid content averaged 59%, indicating that some seals were in poor nutritive condition. However, when those animals were removed from the analysis, mean concentrations in the blubber still remained relatively high (PCBs 24,900 ng/g lw, range 4412 – 90172).

Temporal Trends. Concentrations of PCBs and dioxin-like compounds in blubber showed no temporal variation between 1991 and 2005, suggesting a continuous input of PCBs into the northwestern Atlantic environment.¹ A similar lack of a temporal trend was reported in seals from industrialized areas of Europe and the northwestern Canadian Arctic, where PCB levels have remained constant or declined only slightly since the 1980s.²²⁻²⁴

Spatial Trends. PCB concentrations varied significantly by region among adult male harbor seals ($p = 0.03$) (Figure 4). From north to south a significant decreasing spatial trend was observed, with seals in the southern region (from New York to Massachusetts) having five times higher levels than those in the north (eastern Maine), suggesting that the main sources are near the industrialized and densely populated centers in the northeastern US compared to rural Maine. This trend is consistent with that previously reported for lower trophic organisms (blue mussels) along the northwestern Atlantic.²⁵

PCB congener patterns were similar across the region but differences were found in the profiles of dioxin-like PCBs (Figure 5). From north to south, a decreasing trend was observed in the non-ortho PCBs, whereas the opposite trend was observed for mono-ortho PCBs. Since mono-ortho PCBs may be derived from technical PCB products, this pattern may reflect the presence of more historical sources of these

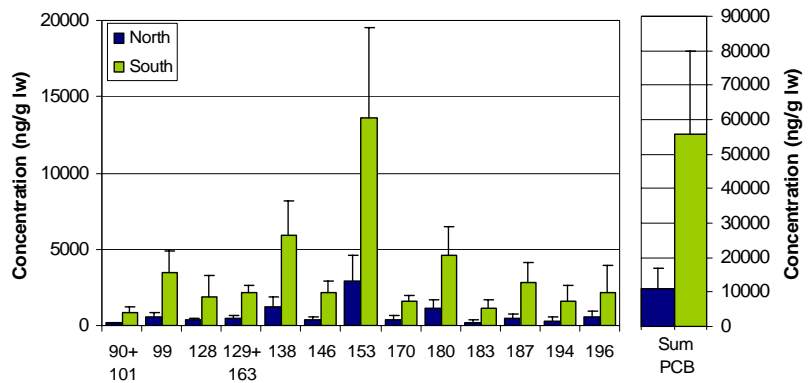


Figure 4. Mean \pm SD concentration of PCB congeners and total PCBs in adult males from northern (Maine) and southern (New York to Mass) regions

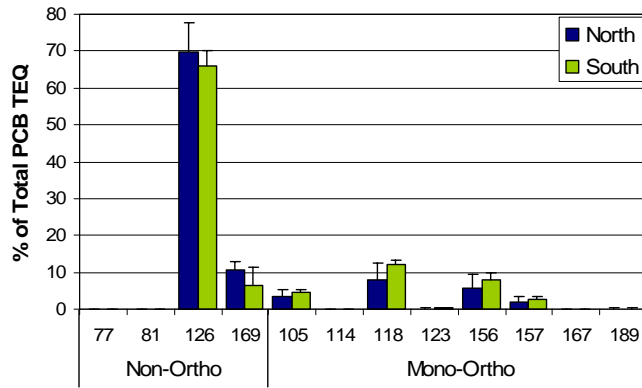


Figure 5. PCB TEQ profiles of adult males from northern (ME) and southern (New York-Mass) regions

disruption in harbor seals based on results of a captive feeding experiment.^{5,7} However, it should be noted that TEQ values calculated for dioxin-like PCBs and PCDD/Fs in the feeding experiment used the 1998 TEFs.²⁵ Recalculation of these values using the most recent TEFs¹⁶ would yield total TEQ concentrations at least 50% lower (~100 pg/g, lw). Alternatively, calculation of the WHO-TEQs in our samples using the 1998 TEFs yields values that are two to four times higher (584, 490, 145, and 60 pg/g lw in pups, yearlings, adult males and females, respectively) than those we report here, meaning that the levels in the pups and yearlings in this study would exceed by two to three-fold the estimated threshold value published in the literature for adverse effects in pinnipeds including immunotoxic effects. These observations underscore the need to recalculate the estimated threshold value for adverse effects using the new TEFs. Moreover, in view of the recurring epizootics affecting the northwestern Atlantic harbor seal population, further research is needed to elucidate the real risks of dioxin-like compounds and the numerous other immune-and endocrine-disrupting POPs to which these seals are exposed.

Acknowledgments

The authors thank Kirk Trabant, Marine Environmental Research Institute, and members of the NOAA/NMFS Northeast Region Stranding Network for providing harbor seal tissues for this study. This work was supported by the National Oceanographic and Atmospheric Administration (NOAA).

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compounds in the industrialized southern regions compared to the less populated northern areas where atmospheric transport may be relatively more important.

Toxicological Implications. Mean concentrations of PCBs found in northwestern Atlantic harbor seals are relatively high on a global scale. PCB concentrations in the younger seals (~50-60 µg/g lw) and adult males (~37 µg/g lw) are two to four-fold higher than the estimated threshold level of 17 µg PCB/g lw for adverse effects in the species.⁵ The total WHO-TEQ values of dioxin-like PCBs and PCDD/Fs in blubber of the pups (mean 191 pg/g, lw) are approaching the estimated threshold level of 209 pg/g, lw for immune suppression and endocrine

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