PCB Related Effects Thresholds As Derived through Gene Transcript Profiles in Locally Contaminated Ringed Seals (Pusa hispida)

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ABSTRACT: Causal evidence linking toxic injury to polychlorinated biphenyl (PCB) exposure is typically confounded by the complexity of real-world contaminant mixtures to which aquatic wildlife are exposed. A local PCB “hotspot” on the Labrador coast provided a rare opportunity to evaluate the effects of PCBs on the health of a marine mammal as this chemical dominated their persistent organic pollutant (POP) burdens. The release of approximately 260 kg of PCBs by a military radar facility over a 30 year period (1970–2000) contaminated some local marine biota, including the ringed seal (Pusa hispida). The abundance profiles of eight health-related gene transcripts were evaluated in liver samples collected from 43 ringed seals in the affected area. The mRNA transcript levels of five gene targets, including aryl hydrocarbon receptor (Ahr), interleukin-1 β (Il1b), estrogen receptor α (Esr1), insulin like growth factor receptor 1 (Igf1), and glucocorticoid receptor α (Nr3c1) correlated with increasing levels of blubber PCBs. PCB threshold values calculated using best-fit hockey-stick regression models for these five genes averaged 1,680 ± 206 ng/g lw, with the lowest, most conservative, being 1,370 ng/g lw for Il1b. Approximately 14% of the seals in the region exceeded this threshold. The dominance of PCBs in the seals studied enabled an assessment of the effects of this chemical on gene transcripts involved in regulating the health of a highly mobile predator, something that is rarely possible in the world of complex mixtures.

INTRODUCTION

While marine mammals occupying high trophic levels in arctic food webs have been found to be contaminated with moderately high concentrations of persistent organic pollutants (POPs),1,2 it remains unclear whether current levels represent a risk to their health. Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) in the Arctic have been largely attributed to long-range transport through atmospheric processes, although some point sources do exist (e.g., military radar stations).3,4

The highly complex mixtures of POPs to which marine mammals are exposed in Arctic and sub-Arctic regions render it difficult to ascribe cause-and-effect relationships to any single contaminant. However, PCBs are widely considered a priority contaminant at the top of food webs with impacts on the biology of a variety of northern wildlife species. For example, endocrine disruption, reproductive impairment, and immunotoxicity in polar bears (Ursus maritimus) have been associated with PCBs.5–9 An association between thyroid hormone disruption and some PCB congeners was found in beluga whales (Delphinapterus leucas) from Svalbard, Norway.10 Phase I enzyme and glutathione-S-transferase (GST) activities, which biotransform group III and IV PCBs, were positively associated with PCBs in ringed seals (Pusa hispida) from the Baltic Sea.11 Furthermore, PCBs were considered to be the cause of uterine occlusions which resulted in reproductive failure in grey seals (Halichoerus grypus) in the Baltic Sea.12,13 More recently, changes in hepatic and circulatory vitamin A levels and hepatic Ahr and Cyp1a1 mRNA levels in beluga whales from the western Canadian Arctic have been associated with PCBs.14,15

Received: July 3, 2014
Revised: October 2, 2014
Accepted: October 6, 2014
Published: October 6, 2014
In southern, more contaminated areas, chemical detoxification enzymes and altered endocrine and immune functions in several marine mammal populations have also been associated with PCBs. In all these cases, however, correlations between health effects and PCB concentrations are based on the premise that PCBs are either the putative contaminant driving the relationship, or represent a proxy for the other cooccurring POPs.

A PCB point source associated with a military radar station in Sagleq Bay, Labrador, Canada, has contaminated the adjacent marine food web. This radar facility has been in operation since the late 1950s; however, it was not until 1996 that extensive contamination was discovered in three areas (Site Summit, Antenna Hill, and beach area) at the site, and that PCBs were found to have entered the marine environment. Very high PCB concentrations were measured in the local marine sediments, the benthic food web, and ringed seals. Results of an ecological risk assessment indicated that shorthorn sculpin (Myoxocephalus scorpius) and black guillemot (Cepphus grylle) nestlings from the area were at increased risk of impaired reproduction or death. Untill now, no studies have assessed health risks to ringed seals, despite the "another marine mammal species, the arctic beluga whale."15 Levels have been associated with changes in feeding ecology in between health effects and PCB concentrations are based on the premise that PCBs are either the putative contaminant driving the relationship, or represent a proxy for the other cooccurring POPs.

Contaminant Analysis. Ringed seal blubber samples were analyzed by the Great Lakes Institute for Environmental Research’s organic analytical laboratory (Windsor, ON) (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certification) for concentrations of 62 PCB congeners and organochlorine pesticides (OCPs): α-, β-, γ-hexachlorocyclohexane, α- and γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, heptachlor epoxide, dichlordiphenyldichloroethane [pp'-DDE], dichlordiphenyldichloroethylenel [pp'-DDE], pp'-DDT, dieldrin, and hexachlorobenzene (HCB). The detailed methodology for extraction, cleanup, and quantification of target analytes has been reported elsewhere. Briefly, homogenized wet tissue (0.5–1 g), anhydrous sodium sulfate, and surrogate standard were ground with motor and pestle and then extracted following a microextraction technique. Samples were analyzed for individual PCB congeners and OCPs by gas chromatography electron capture detection (GC-ECD). Percent lipid was determined using gravimetric lipid determination by weight of extract method with dichloromethane. For each batch of six samples, an in-house reference homogenate tissue, method blank, and the external PCB-34 recovery standard were analyzed for 62 PCB congeners. All PCB congeners and OCPs that were detected in 90% of the samples were included in the data analysis, in samples where an individual congener was not detected it was replaced with a random number between the detection limit (0.011 to 0.150 ng/g) and zero. Recoveries of individual PCB congeners in the homogenate reference tissue with each sample batch run were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts. Recovery efficiencies for the PCB34 standard were 99 ± 0.95% (mean ± standard error). Procedural method blanks (n = 11) were below detection for all PCB congeners and OCPs. All study samples were recovery corrected for PCB congener and OCP concentrations.

Hereafter, ∑PCBs refers to the sum of the 62 PCB congeners, ∑HCH refers to the sum of α- and γ-hexachlorocyclohexane, ∑chlordanes refers to the sum of α- and γ-chlordane, cis-nonachlor, trans-nonachlor, oxychlordane, heptachlor epoxide, and ∑DDT refers to the sum of pp'-DDE, pp'-DDE, and pp'-DDT. ∑PCBs was used in the data analysis since both coplanar and noncoplanar PCBs have been shown to elicit toxic effects. Further, less than half (42%) of the dioxin-like PCBs were analyzed in the present study using GC-ECD.

Hepatic RNA Isolation and cDNA Synthesis. Detailed procedures on total RNA extraction and cDNA synthesis are described elsewhere. Briefly, each sample was homogenized in a 1.5 mL microcentrifuge tube using a Retsch MM301 mixer mill (Thermo Fisher Scientific, Ottawa, ON, Canada) following the addition of 700 μL TRIzol reagent (Thermo Fisher Scientific) and a 3 mm diameter tungsten-carbide bead. Samples were homogenized in two 3 min intervals at a frequency of 20 Hz with a cooling period of 2–3 min on ice and 180° rotation of the mixing chambers between intervals. Isolated hepatic total RNA was resuspended in 40 μL of diethyl pyrocarbonate-treated distilled deionized water and stored at −80 °C. RNA purity and concentration were assessed by spectrophotometry at A260 and A280 and 1 μg of each sample was subsequently used to prepare cDNA with the High

Materials and Methods

Sample Collection. All tissue samples from adult (≥6 years) and subadult (<6 years) ringed seals (n = 43) were obtained from Inuit hunters in four marine inlets (Nachvak Fjord, Sagleq Fjord, Okak Bay, and Anaktalik Bay) along the northern Labrador coast during the fall season (September and October) from 2009 to 2011 (see Figure 1 in ref. 23). Males/females ratio was 10/11 and 11/11 for subadult and adult seals, respectively. Sex, weight, length, girth, and blubber thickness (at the sternum) were recorded for each ringed seal. Ages were determined at Matson’s Laboratory, U.S.A., by longitudinal thin sectioning a lower canine tooth and counting annual growth layers in the cementum using a compound microscope and transmitted light. Samples used for stable isotopes (muscle) and organochlorines (blubber) were stored at −20 °C prior to the analyses. Carbon (δ13C) and nitrogen (δ15N) isotope signatures were evaluated because altered mRNA transcript levels have been associated with changes in feeding ecology in another marine mammal species, the arctic beagle whale. Liver samples (~1 g) collected for the measurement of mRNA abundance profiles were preserved directly in the field in RNAlater tissue preservation solution as per the manufacturer’s instructions (Applied Biosystems, Foster City, CA, U.S.A.) and stored at −20 °C until isolation of total RNA. All tissue samples were obtained within 1 h of harvesting.
Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, U.S.A.). Each cDNA sample was diluted 40-fold with PCR-grade water prior to gene-specific analysis.

**Quantitative Real Time Polymerase Chain Reaction (qPCR) Assay.** Eight genes were selected to provide an overview of the biological consequences of exposure to PCBs, based in large measure on past mechanistic studies of this chemical class. These include aryl hydrocarbon receptor (Ahr), thyroid hormone receptor α (Thra), estrogen receptor α (Esr1), thyroid stimulating hormone β (Tshb), retinoic acid receptor α (Rara), interleukin-1 β (Illiib), insulin like growth factor receptor 1 (Igfl1), and glucocorticoid receptor α (Nr3c1). These protein-encoding genes play critical roles in detoxification pathways, immune and endocrine systems, and the regulation of growth, development, and metabolism. Three additional transcripts were chosen as normalizers for correction of input variation and assessment of sample quality: ribosomal protein L8 (Rpl8), β-like 2 actin (Actb1l2), and eukaryotic translation elongation factor-1 alpha (Efrl1a).

Expressed gene sequences were isolated from frozen seal muscle tissue (0.5–1 g) was freeze-dried and homogenized. Lipid was extracted from all samples using a chloroform/methanol extraction and then dried for analysis. Carbon and nitrogen isotopic analyses were performed using Continuous Flow Ion Ratio Mass Spectrometer (CFIR-MS) (Finnigan MAT Delta plus, Thermo Finnigan, San Jose, CA, U.S.A.). Detailed methodology on the procedure has been reported elsewhere. Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) using the following equation:

$$\delta_{\text{sample}}\%e = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where $R$ is the ratio of heavy to light isotope ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample and standard. The standards used for carbon and nitrogen analyses were Pee Dee Belemnite limestone formation and atmospheric nitrogen, respectively. Precision based on two standards (bovine muscle (NIST 8414) and tilapia fish muscle internal laboratory standard; $n = 65$ for each) were <0.16‰ and <0.08‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. Accuracy of isotope analysis, based on the NIST standards sucrose (NIST 8542) and ammonium sulfate (NIST 8547) analyzed during the present study ($n = 3$ for each) were within <0.1‰ of certified $\delta^{15}N$ and $\delta^{13}C$ values.

**Data Analysis.** Unless otherwise stated, univariate statistical analyses were performed in SPSS Version 20.0 (IBM, Armonk, NY, U.S.A.). Data were log-transformed when necessary to meet the normality assumptions for parametric analyses. Linear and nonlinear “hockey-stick” regression analyses were used to determine relationships between blubber PCB concentrations and hepatic mRNA abundance. Hockey-stick regression analyses were carried out using R version 2.9.0 (http://cran.r-project.org/bin/windows/base/old/2.9.0/). The “hockey-stick” regression assumes a constant background mRNA abundance up to a threshold tissue concentration of PCBs, above which mRNA abundance increases in concert with PCBC concentrations of contaminants.

The best variable or combination of variables (biological: age, sex, year, weight, length, girth, blubber thickness, $\delta^{15}N$ and $\delta^{13}C$; contaminant: $\sum$PCBs, $\sum$DDTs, $\sum$chlordane, $\sum$HCH, HCB, dieldrin) to describe the mRNA abundance profile of each gene was selected using the lowest Akaike information criteria (AIC) (SYSTAT Version 13, Systat Software Inc., San Jose, CA, U.S.A.). The Akaike differences ($\Delta$AIC) and normalized Akaike weights ($wi$) were calculated to select the best variable or variables. The models with a ΔAIC of zero and up to two were considered to have the most support. Significant relationships were further evaluated using Principal Component Analysis (PCA) (Pirouette, Infometrix, Bothell, WA, U.S.A.). Gene transcript fold change values were autoscaled (scaled to variable mean and standard deviation) before PCA. The PCA scores from axis one and two were regressed by each of the biological or contaminant variables.
Results and Discussion

Contaminant Concentrations in Ringed Seals. Concentrations of \(\sum\)PCBs, \(\sum\)DDTs, and \(\sum\)chlordanes were higher in the blubber of adult male ringed seals than in subadult and adult female ringed seals (\(p < 0.05\); Table 1). No differences (\(p > 0.05\)) were found between subadult males and females for \(\sum\)PCBs and the 5 OCPs (\(\sum\)DDT, \(\sum\)chlordanes, \(\sum\)HCHs, dieldrin, and HCB) measured. No differences (\(p > 0.05\)) were found between subadult and adult female ringed seals for \(\sum\)PCBs and the 5 OCPs. Such results are typical for pinnipeds, where females reduce their POP burden by transferring fat-soluble contaminants to offspring via placental and lactational transfer. The \(\sum\)HCHs, HCB, and dieldrin concentrations did not vary (\(p > 0.05\)) between sexes or age classes. Similar results between sexes have been observed previously in ringed seals for these contaminants.

While average \(\sum\)PCB and OCP concentrations for ringed seals at the Labrador sites fell within the range observed across the Canadian Arctic, three features stood out in the present study. First, some individuals (14%) exhibited much higher PCB levels than expected for ringed seals in the Arctic, something we previously attributed to feeding in the contaminated Sagleka Bay. Second, PCBs accounted for 58\% of the total POP profile in Labrador ringed seals (Table 1), which far exceeds the contribution observed in ringed seals elsewhere in the Canadian Arctic (24-40%). DDT was consistently ranked as the second most prevalent POP, with some variation (\(\sum\)Chlordanes or \(\sum\)HCHs) across the three age/sex categories for the third POP (Table 1). This variation is likely due to differing biological status related to seal age and sex that influence POP uptake and metabolism. Third, some seals in the area were previously shown to have heavier PCB concentrations in their liver compared to background levels in more industrialized regions. While none of the adult female seals exceeded proposed PCB effects thresholds for marine mammals, nearly 46% of the adult males and 5% of the subadults (<1 yr) exceeded endocrine and immune thresholds. Overall, these results suggest that ringed seals inhabiting the northern Labrador coast are at increased risk for endocrine and immune disruption from PCBs compared to seals inhabiting other areas in the Arctic. This increased risk can be attributed to the exposure to the "local" PCB source at Sagleka Bay.

PCB-Related Changes in Hepatic mRNA Abundance. We know of no other case of a marine mammal population that has been solely exposed to a PCB point source, such that this study afforded us with the opportunity to investigate the association between liver mRNA abundance profiles and PCB concentrations.

A correlation was observed between 5 of the 8 gene transcripts assessed (Aryl hydrocarbon receptor (Ahr), interleukin-1 \(\beta\) (Il1b), estrogen receptor \(\alpha\) (Esr1), insulin like growth factor receptor 1 (Igf1) and glucocorticoid receptor \(\alpha\) (Nrf3c1)) and \(\sum\)PCB concentrations; with both linear and nonlinear regressions being significant (SI Table S3; Figure 1; Table 2; \(p < 0.05\)). Coefficients of determination (\(r^2\)) were highest for the nonlinear regressions compared with the linear regressions. We therefore present the nonlinear "hockey stick" regressions (Figure 1; Table 2). Modeling of data using a "hockey-stick" regression has been previously used to establish sediment quality thresholds and effects thresholds in biota. Furthermore, this approach provided a model that incorporates a change point representative of a contaminant level (threshold) below which an effect is not expected. The correlation between hepatic Ahr, Il1b, Esr1, Igf1, and Nrf3c1 mRNA abundance and \(\sum\)PCB concentrations and their associated effects thresholds for ringed seals from Labrador (Table 2) suggest that contaminant-associated responses in molecular end points can be detected at levels considerably lower (10- to 20-fold) than those currently observed in ringed seals from the area.
contaminated Baltic Sea where increased phase 1 enzyme activity and endocrine effects have recently been reported\(^{11,43}\) and where a history of reproductive and developmental anomalies exists.\(^ {13}\)

For ringed seals, \(\sum PCB\) threshold values estimated for the five genes averaged 1,680 ± 206 ng/g lw, with the lowest being 1,370 ng/g lw for \(I\!l\!1b\) (Table 2). Three of the five genes (\(Ahr\), \(I\!l\!1b\), and \(N\!r\!3c1\)) had a threshold similar to those derived for endocrine and immune effects in harbor seals (1,300 ng/g lw,\(^ {25}\)) and vitamin A disruption in beluga whales (1,600 ng/g lw,\(^ {14}\)), but lower than most other thresholds reported for marine mammals.\(^ {40,41}\) In contrast, \(Esr1\) (2,460 ng/g lw \(\sum PCBs\)) and \(I\!g\!1\) (1,740 ng/g lw \(\sum PCBs\)) had the highest thresholds, suggesting that these parameters may be less sensitive to PCBs compared with the other molecular end points. Thus, the most conservative threshold value identified (1,370 ng/g lw \(\sum PCBs\)) may be considered as the most protective among those thresholds for ringed seals.

Adult males had the highest \(\sum PCB\) levels and exceeded our proposed threshold concentration of 1,370 ng/g lw (Table 1; Figure 1). One subadult seal had a PCB concentration (1,390 ng/g lw) that just slightly exceeded the effects threshold concentration. This is consistent with previous findings where the majority of adult males and a minority of subadult ringed seals exceeded the effects threshold (1,300 ng/g lw,\(^ {25}\)) for immunotoxicity and endocrine disruption in harbor seals.\(^ {23}\)

The ligand-induced aryl hydrocarbon receptor (AHR) mediates the metabolism of many POPs, including dioxin-like PCBs.\(^ {44}\) Such compounds bind to the AHR and modulate the activity of the transcriptional regulator which, in turn, induces expression of phase 1 and 2 xenobiotic detoxification enzymes, including cytochrome P450 enzymes (\(Cyp1a1\), \(Cyp1a2\), and \(Cyp1b1\)) and UDG-glucuronosyltransferases (\(Ugt1a1\)).\(^ {45}\) Consistent with the results of the present study, hepatic \(Ahr\) mRNA levels were correlated with non-ortho planar PCB concentrations in Baikal seals (\(Pusa sibirica\))\(^ {46}\) and with total PCB concentrations in arctic beluga whales (\(Delphinapterus leucas\)).\(^ {15}\) In addition, positive relationships between blubber \(Ahr\) and PCB concentrations have been observed in heavily contaminated killer whales (\(Orcinus orca\)) from the Northeastern Pacific,\(^ {20}\) striped dolphins (\(Stenella coeruleoalba\)) from the Mediterranean,\(^ {47}\) and fin whales (\(Balaenoptera physalus\)) from the Mediterranean Sea and Gulf of California.\(^ {48}\)

A weight of evidence suggests that elevated PCBs and other POPs (e.g., polychlorinated dibenzo-p-dioxins and furans) may have contributed to marine mammal epizootics through reduced immunocompetence.\(^ {49}\) \(I\!l\!1b\) encodes a pro-inflammation...
This confirms that PCB concentrations was the best variable to explain the variations of hepatic mRNA levels for the five genes which correlated with blubber PCB concentrations in ringed seals. Models with $\Delta \text{AIC}_c$ below 2 are presented. $^6AIC_c = \text{second order AIC log (no } ) + 2K$ bias adjusted AIC for small sample size = $AIC + (2 \delta K)$, $\delta = \text{sample size}$. $^7\delta = \text{sample size}$.

The glucocorticoid receptor (NR3C1) is a steroid hormone receptor involved in regulating growth, development, metabolism, and apoptosis. Contaminant-related effects on NR3C1 mRNA levels have not been reported previously in marine mammals. However, there is evidence that PCBs can reduce the number of brain glucocorticoid receptors and that some PCB metabolites can bind competitively to glucocorticoid receptors. In addition, a reduced or delayed cortisol response was observed in fish experiencing stress in heavily polluted waters, suggesting that PCBs and other organochlorine contaminants may alter cortisol secretion. Further, grey seal (Halichoerus grypus) and ringed seal populations in the heavily polluted Baltic Sea suffered from a disease syndrome which is thought to have been caused by increased glucocorticoid hormones concentrations.

The remaining 3 gene transcripts (Thyroid hormone receptor $\alpha$ (Thra), Retinoic acid receptor $\alpha$ (Rara), Thyroid-stimulating hormone $\beta$ (Tshb)) in ringed seals exhibited no relationship with PCBs. These transcripts are involved in hormone signaling pathways, regulation of growth and metabolism, and development. These results differ from those of more contaminated marine mammals, which may reflect the lower PCB dose to which our Labrador ringed seals were exposed.

Longitudinal liver samples from the Baltic Sea were associated with increased hepatic PCB concentrations. $^9$PCBs exhibit both estrogenic and antiestrogenic activity. For example, PCBs can interfere with endocrine signaling by mimicking endogenous hormone action through binding to estrogen receptors and modulating their function with resultant impact on estrogen-dependent processes, such as steroid metabolism. A few studies have looked for a possible link between disruption of estrogen-dependent processes and/or estrogen levels and PCB concentrations in marine mammals. Female polar bears did not show a significant relationship between estradiol and PCB levels in plasma although a borderline negative relationship was observed between estradiol and PCB-118 in females with offspring. Harbor seals fed PCB/DDE-contaminated fish from the Wadden Sea, The Netherlands, exhibited a perturbation in the estrus cycle consistent with the reduced reproductive success of seal populations from this geographic area. This latter observation is consistent with the results of the present study. Further, previous studies have reported a decrease in circulating levels of estrogen and PCBs in marine mammals from contaminated areas.

$Igfl$ plays an important role in regulating cellular differentiation and proliferation, as well as a number of tissue-specific functions. It is also involved in the development of a number of diseases, including cancer, diabetes, and growth disorders. Previous studies have suggested that exposure to environmental contaminants, such as PCBs and other aromatic hydrocarbons, may alter $Igfl$ homeostasis in rats and humans. Contaminant-related variation in $Igfl$ mRNA levels has not been reported previously in marine mammals.

**Table 3. Akaika Information Criterion (AIC) in Combination with Backwards Stepwise Regression**

<table>
<thead>
<tr>
<th>gene</th>
<th>predictors</th>
<th>$r^2$</th>
<th>p-value</th>
<th>AIC</th>
<th>AIC$_c$</th>
<th>$\Delta$AIC$_c$</th>
<th>$w_i$</th>
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<tbody>
<tr>
<td>$Ahr$</td>
<td>PCBs, age, log$_{10}$weight</td>
<td>0.49</td>
<td>&lt;0.001</td>
<td>256.5</td>
<td>264.9</td>
<td>0</td>
<td>0.71</td>
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<tr>
<td></td>
<td>PCBs, age, log$_{10}$weight, HCH</td>
<td>0.50</td>
<td>&lt;0.001</td>
<td>257.8</td>
<td>260.3</td>
<td>1.3</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>PCBs, age, log$_{10}$weight, HCH, chlordanes</td>
<td>0.52</td>
<td>&lt;0.001</td>
<td>258.2</td>
<td>262.9</td>
<td>1.7</td>
<td>0.11</td>
</tr>
<tr>
<td>$Il1b$</td>
<td>PCBs, age</td>
<td>0.26</td>
<td>0.005</td>
<td>178.3</td>
<td>179.4</td>
<td>0</td>
<td>0.48</td>
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<td></td>
<td>PCBs, age, log$_{10}$blubber</td>
<td>0.26</td>
<td>0.014</td>
<td>179.3</td>
<td>181.2</td>
<td>1.1</td>
<td>0.28</td>
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<tr>
<td>$Esr1$</td>
<td>PCBs, HCH</td>
<td>0.27</td>
<td>0.007</td>
<td>112.8</td>
<td>114.2</td>
<td>0</td>
<td>0.25</td>
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<td>PCBs, HCH, log$_{10}$weight</td>
<td>0.30</td>
<td>0.012</td>
<td>113.6</td>
<td>115.7</td>
<td>0.8</td>
<td>0.17</td>
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<tr>
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<td>PCBs, HCH, log$_{10}$weight, age</td>
<td>0.33</td>
<td>0.014</td>
<td>114.3</td>
<td>119.9</td>
<td>1.5</td>
<td>0.12</td>
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<tr>
<td>$Igfl$</td>
<td>PCBs, sex</td>
<td>0.24</td>
<td>0.007</td>
<td>328.3</td>
<td>329.5</td>
<td>0</td>
<td>0.42</td>
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<td></td>
<td>PCBs, sex, age</td>
<td>0.25</td>
<td>0.014</td>
<td>329.6</td>
<td>331.4</td>
<td>1.3</td>
<td>0.53</td>
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<tr>
<td></td>
<td>PCBs, sex, age, HCH</td>
<td>0.28</td>
<td>0.017</td>
<td>329.9</td>
<td>332.4</td>
<td>1.5</td>
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<td>$Nr3c1$</td>
<td>PCBs, sex, log$_{10}$weight, $\delta^{13}C$</td>
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<td>0.009</td>
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<td>151.5</td>
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<td>0.29</td>
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<td>0.015</td>
<td>149.3</td>
<td>154.0</td>
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variation in the five end points. In addition, age, sex, and weight (a proxy for age) contributed to the final model for 3, 2, and 2 of the 5 significant gene targets, respectively (Table 3). These statistical results build on the observations above, which showed that the adult males were most contaminated and were driving the up-regulation of the five PCB-correlated molecular end points (Figure 1).

**Age and Sex-Class vs Vulnerability to PCB Effects.** The females in the present study were far less contaminated than the adult males, which can likely be attributed to transfer of PCBs through the placenta and nursing to their young.\(^{33,34}\) In this way, our findings that the females and subadults exhibited no relationship \((p > 0.05)\) with PCBs, while the adult males did for 3 of the 5 significant gene transcripts \((Ahr: r^2 = 0.76; p = 0.007; \beta = 0.62; p = 0.004; Igf1: r^2 = 0.45; p = 0.025)\) illustrates the way in which the contaminant burdens of our study animals straddle our derived effects threshold. The lack of relationship between \(Esr1\) and \(Nr3c1\) and \(\sum\)PCBs in adult males may be due to the small sample size \((n = 11)\) and increased variation for these two transcripts. The previously demonstrated endocrine and immune threshold of 1,300 ng/g lw\(^{35}\) is remarkably similar to the inflection point of our most conservative effects threshold \((1,370 \text{ ng/g lw})\), providing strong support for an effects threshold in phocid seals for PCBs in this range. In transcriptomic studies of more contaminated marine mammal populations, PCBs drive gene expression response with secondary, limited contributions from age and sex\(^{20,43}\) further substantiating our threshold derivation for relatively low levels of PCBs.

PCA was used to further explore the factors underlying these patterns in adult males (Figure 2). Forty-three percent of variance in mRNA levels was explained by the first PCA factor (Figure 2a). The PCA variables plot of mRNA transcripts revealed a divergence of the three mRNA gene transcripts \((Ahr, \beta, \text{ and } Igf1)\) which correlated with \(\sum\)PCBs and the other 5 gene transcripts (Figure 2b). \(\sum\)PCBs were correlated \((r^2 = 0.42; p = 0.04)\) with the sample scores of the first principal component \((t1)\) (Figure 2c). None of the other biological variables correlated with \(t1\) or \(t2\) \((p > 0.05)\). Despite the lack of relationship between \(Nr3c1\) and \(\sum\)PCBs for adult males, \(Nr3c1\) was positioned relatively close to these three transcripts, suggesting that it too may be affected by \(\sum\)PCBs. These observations corroborate with our univariate findings for adult males with mRNA transcript levels for three of the eight genes being driven by \(\sum\)PCBs. Collectively, our findings suggest that while some Labrador ringed seals have low PCB levels which do not elicit detectable effects, others, notably the adult males are affected by elevated PCBs.

This present study provides a unique opportunity to evaluate the effects of PCBs on the health of a wild marine mammal, as this chemical class dominated tissue residues over other POPs. Despite declining PCB concentrations and associated effects in bottom-feeding fish (shorthorn sculpin) and seabirds (black guillemots) at Sagkeleg Bay following remedial action\(^{68}\), we show here protracted effects in a long-lived high trophic level pinniped.

### ASSOCIATED CONTENT

#### Supporting Information

Table S1 provides qPCR primer pair sequences for genes used in ringed seal \((Pusa hispida)\) liver. Table S2 provides primers and isolated ringed seal expressed gene sequences not submitted to NCBI GenBank due to the minimum length requirement of GenBank. Table S3 shows the linear regression relationships between five mRNA transcripts \((Ahr, \beta, Esr1, Igf1, \text{ and } Nr3c1)\) and \(\sum\)PCBs. This material is available free of charge via the Internet at http://pubs.acs.org/.

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**Figure 2.** (a) Principal component analysis (PCA) of hepatic mRNA transcript levels of adult male Labrador ringed seals \((n = 11)\) each circle on the scores plot represents an adult male seal. (b) Eight mRNA gene transcripts were included in the PCA; a divergence between three gene transcripts \((Ahr, \beta, \text{ and } Igf1)\) which correlated with \(\sum\)PCBs in the blubber and the other 5 gene transcripts was observed along the variable loadings of the first principal component \((p1)\) (c) Sample scores of the first principal component \((t1)\) was correlated with \(\sum\)PCBs.
Acknowledgments

Funding and support were provided by the Northern Contaminants Program of Aboriginal Affairs and Northern Development Canada, Fisheries and Oceans Canada, the Tornaght Joint Fisheries Board, the Director General Environment of the Department of National Defence, Raincoast Conservation Foundation, Natural Sciences and Engineering Research Council of Canada (NSERC) (awards to T.M.B.), and the ArcticNet Canadian Network of Centres of Excellence, ArcticNet (Project ArcticNet Nunatsiavut Nuluak, funding to K.J.R., P.S.R., A.T.F.). We thank Taka-Aki Ichu and Dr. Mary Lesperance for assisting with statistical analyses. Finally, the authors gratefully acknowledge Joey Angnatok and the crew of the “What’s Happening” for their steadfast support, expertise, and active participation in the field.

References


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