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# POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN GALAPAGOS SEA LIONS (*ZALOPHUS WOLLEBAEKI*)

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**Abstract**—Concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) were measured in muscle–blubber biopsy samples from 21 Galapagos sea lion (*Zalophus wollebaeki*) pups that were live captured in the Galapagos Islands (Ecuador) using gas chromatography/high-resolution mass spectrometry. Only traces of PBDEs were detected in one male pup, whereas PCDDs and PCDFs were not detected in any sample. The total concentration of PCBs (ΣPCB) in the pups averaged 104 μg/kg lipid (range, 49–384 μg/kg). No statistically significant differences in ΣPCB were observed among the four study sites in the Galapagos Islands. Concentrations of PCB congeners in Galapagos sea lion pups were dominated by low-molecular-weight congeners. These results suggest that global transport is the main source for PCBs in Galapagos sea lions. The ΣPCB levels were below immunotoxic and endocrine-disruption thresholds in pinnipeds, suggesting a limited risk of adverse health effects. The present study indicates that Galapagos sea lions can serve as a useful sentinel of pollutants with a long-range transport capacity and that Galapagos Islands are not exempt from the threats of global pollutants despite its remote locale.

**Keywords**—Polybrominated diphenyl ethers Polychlorinated biphenyls Galapagos sea lions Galapagos Islands Atmospheric transport

## INTRODUCTION

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) represent persistent, bioaccumulative, and toxic compounds of global concern. Whereas the legacy PCB, PCDD, and PCDF production or by-production has been curtailed, in part because of the global Stockholm Convention on persistent organic pollutants [1], PBDEs represent chemicals of emerging environmental concern [2–5].

In the industrialized world, PCBs were banned during the late 1970s as a result of concerns about their persistence, bioaccumulation, and toxicity to wildlife [1]. Polychlorinated biphenyls are found at relatively high concentrations in the northern hemisphere, but their presence in the tropics can be attributed, in part, to long-range transport [6–9]. Polychlorinated biphenyl concentrations in pinniped species have declined since the 1970s, as source control and regulations served to reduce inputs into the environment [10]. Before national controls, both PCDDs and PCDFs were formed as by-products of pulp and paper mill processes [11], but they also can be formed as by-products of combustion [12]. Assessing PCB, PCDD, and PCDF exposure is an important part of marine wildlife conservation, because these compounds have been associated with effects on the immune and endocrine systems of marine mammals [13–16], which can compromise survival and reproduction.

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in foams, textiles, coatings, furniture, construction materials, electronic devices (e.g., television sets, appliances, and computers), plastics, and paints since 1970 [2–4,17,18]. The production and use of PBDEs have been restricted in Europe and Canada, but the deca-PBDE formulation is still used extensively elsewhere [5]. The worldwide production of brominated flame retardants, including PBDEs, during the 1990s and the year 2000 was approximately 150,000 to 350,000 tons/year [2–4]. Similar to PCBs, a total of 209 PBDE congeners are possible, although commercial mixtures and environmental samples typically contain a small number of dominant PBDE congeners [4,19–21]. Polybrominated diphenyl ethers also have been detected in marine mammals, including polar bears (*Ursus maritimus*), seals, and cetaceans [22–25]. For example, PBDE concentrations in ringed seals (*Pusa hispida*) increased exponentially in the Canadian Arctic from 1981 to 2000 [26]. In Europe, however, declines in PBDE concentrations have resulted from the regulation of penta- and octa-formulations in 1998 [17,27,28]. Polybrominated diphenyl ethers are relatively persistent environmental contaminants that bioaccumulate in organisms and can undergo long-range transport to remote regions [6,29]. In addition, PBDEs can cause toxic effects,

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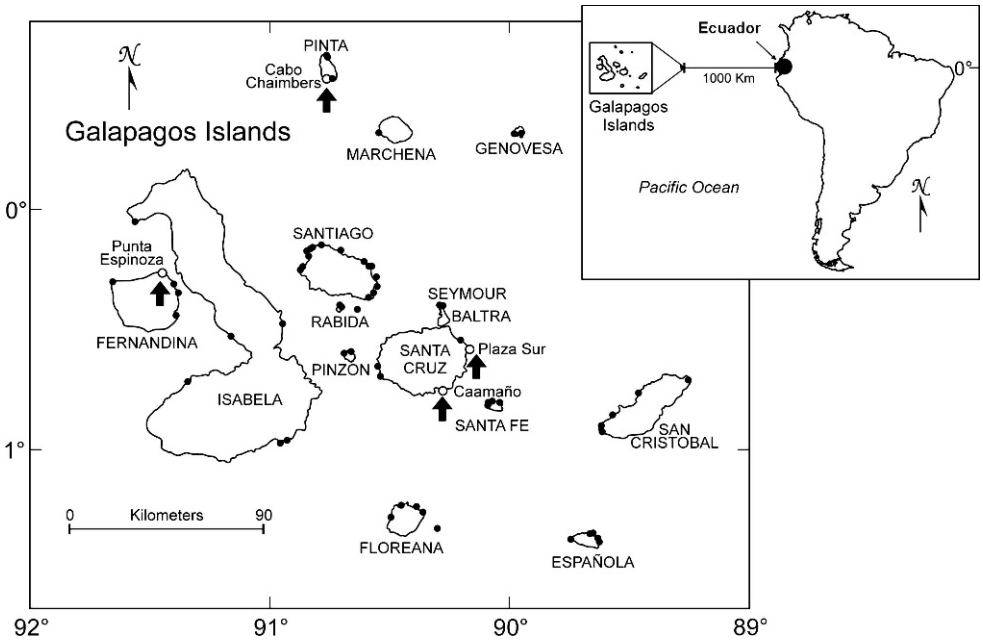


Fig. 1. Map of the Galapagos Islands in relation to Ecuador and South America showing sampling sites (white dots) indicated by black arrows and distribution of the Galapagos sea lion rookeries (small black dots).

including neurotoxicity, disruption of steroid and thyroid hormone regulation, teratogenicity, and carcinogenicity [30–32].

Evidence for the propensity of PCBs, PBDEs, PCDDs, and PCDFs to undergo long-range transport typically has been gauged by their occurrence in polar regions. The protection of peoples inhabiting Arctic regions from the adverse health effects of persistent organic pollutants is an integral component of the Stockholm Convention, which became international law on May 17, 2004 [1]. Long-range transport to tropical regions, however, is not receiving comparable attention. In the case of PBDEs and PCBs, no studies, to our knowledge, have been conducted in pinnipeds from equatorial or tropical areas.

Despite its protected status, the Galapagos sea lion (*Zalophus wollebaeki*) population decreased by 60% between the 1970s and the year 2000 [33]. Several hypotheses have been proposed to explain this decline. These include El Niño events, nutritional stress, fisheries interactions, illegal hunting, as well as diseases (e.g., *Leptospira* and *Morbillivirus* sp.) introduced by feral mammals, such as dogs [33,34]. As a result, the Galapagos sea lion is listed as ''threatened'' under the IUCN (World Conservation Union) endangered category [35]. To our knowledge, the potential impact of endocrine-disrupting persistent organic pollutants has not been investigated and could represent another factor contributing to the decline in Galapagos sea lions.

The present study measured PCB, PBDE, PCDD, and PCDF concentrations in Galapagos sea lions to characterize the presence of these priority contaminants in these pinnipeds and to evaluate any possible risks associated with exposure. The Galapagos Island Archipelago (Ecuador) is a United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Site that recently was listed as being at risk [36]. Understanding the fate and potential health effects of these contaminants on Galapagos sea lions is an important part of protecting biodiversity in this region and enhancing environmental stewardship.

## MATERIALS AND METHODS

### *Sampling*

Muscle–blubber biopsy samples (sample size, 100 mg; biopsy punch, 6 mm) of 21 Galapagos sea lion pups were obtained from four rookeries of the Galapagos Islands Archipelago (Fig. 1) at 1,000 km (600 miles) off the Ecuadorian continental coast, between 01°40′N and 01°25′S and 89°15′W and 92°00′W, during a field research expedition on March 13 to 21, 2005. Pups were sampled from the Caamaño ($n = 11$) and Plaza Sur ($n = 4$) colonies on Santa Cruz Island, from Punta Espinoza ($n = 3$) on Fernandina Island, and from Cabo Chaimbers ($n = 3$) on Pinta Island. Santa Cruz is a semiurbanized island, whereas Fernandina and Pinta islands are noninhabited, pristine environments. Nursing pups were chosen for the following reasons: They are readily accessible and easy to capture in most of the rookeries of the Galapagos Islands year-round; they are of approximately the same age, thus minimizing the influence of life history on contaminant concentrations; they are nursed by adult reproductive females with a similar diet item (i.e., milk); and they are in a high trophic position, feeding on mother's tissue (i.e., milk). Galapagos sea lions reproduce year-round. The main period of birth is between August and November, and the young are weaned after approximately 12 to 24 months [37,38]. The capture and immobilization of pups followed the field anesthesia methodology for studies of Galapagos sea lions and fur seals developed by Parás et al. [39]. Pups were selected based on observed nursing behavior, and estimated age based on size and weight which ranged from 2 to 12 months (i.e., less than two years). The animals' weight, length, and girth were measured. Further details on the pups' capture, determination of body condition index (i.e., Fulton condition factor), and immobilization are described in the *Supporting Information* (http://dx.doi.org/10.1897/08-331.S1).

Biopsy specimens were collected from the supraspinatus muscle, located right above the flipper, which had been cleaned previously with alcohol and betadine. Biopsy specimens were

wrapped in hexane-rinsed aluminum foil and placed into cryovials, which were stored in a cooler with ice during field work and then transferred in a freezer (−20°C) on board the expedition boat until transport to the laboratory. In the laboratory, the samples were stored at −80°C until chemical analysis.

*Chemical analysis*

The chemical analyses for all target contaminant classes (PCDDs, PCDFs, PBDEs, and PCBs) were performed by gas chromatography/high-resolution mass spectrometry (GC/HRMS) based analytical methodologies described elsewhere [40]. More details regarding the chemical analysis can be found in the *Supporting Information.* Briefly, the entire muscle–blubber biopsy sample (0.004–0.145 g) underwent extraction for the target contaminant classes. One biopsy sample contained some cartilaginous tissue in addition to blubber and exhibited a relatively low lipid content. The intact biopsy samples were spiked with a mixture of surrogate internal standards that contained all seventeen 2,3,7,8-chlorine–containing, $^{13}C_{12}$-labeled PCDDs and PCDFs (except octachlorodibenzofuran) as well as fifteen $^{13}C_{12}$-labeled PCBs and a suite of nine $^{13}C_{12}$-labeled PBDEs. All surrogate internal standards were purchased from Cambridge Isotope Laboratories. The spiked samples were homogenized with 20 g of $Na_2SO_4$ in a mortar, transferred quantitatively into an extraction column, and extracted with dichloromethane (DCM)/hexane (1:1, v/v). For some of the samples, the extract formed two layers/phases, a waxy-precipitate layer and the solvent layer. The solvent layer was transferred to a clean flask, and the waxy precipitate was treated with several aliquots of hexane and DCM. Each of these precipitates was then transferred to the flask that contained the solvent layer of the extract. Despite the treatment with additional volumes of hexane and DCM, vortexing, and pulverization, the waxy precipitate did not dissolve in the solvents used. As a result, it was not included in the corresponding sample extract that was used for lipid and contaminant determinations.

The DCM/hexane sample extracts were evaporated to dryness, and the residue was weighed to determine the total lipid content in the sample. Subsequently, the residue was resuspended in DCM/hexane (1:1) and divided quantitatively into two aliquots. The larger aliquot (75% of the extract) was subjected to sample cleanup for PBDE, PCB, PCDD, and PCDF determinations, whereas the remaining aliquot was stored for future contaminant determinations. Sample extracts were cleaned up by silica gel chromatography (with layers of basic, neutral, acidic, and neutral silica) and activated alumina chromatography and carbon fiber. Two fractions were collected on carbon fiber—namely, the PCB/PBDE fraction (in DCM/hexane) and the PCDD/PCDF fraction in toluene. Each fraction was concentrated to less than 10 µl and spiked with the corresponding $^{13}C$-labeled method performance standards before instrumental analysis. Details regarding the quality-assurance/quality-control (QA/QC) protocols followed, the amounts and composition of the surrogate internal and performance standards used, and the sorbents, solvents, and conditions used in all the cleanup steps are reported in detail elsewhere [40] and in the *Supporting Information.* The PCDD/PCDF fraction was analyzed by GC/HRMS for the corresponding analytes. The PCB/PBDE fraction was first analyzed by GC/HRMS for the target (mono- to hepta-)PBDE congeners. Subsequently, the PCB/PBDE fraction was combined with the PCDD/PCDF fraction and analyzed for full congener PCBs by GC/HRMS. For all analyses, the HRMS was operated at 10,000 resolution under positive conditions, and data were acquired in the single-ion resolving mode. The instrumental analyses conditions used for each of the three contaminant classes are provided in the *Supporting Information.* Tissue lipid contents were determined gravimetrically using 0.004 to 0.145 g (wet wt) of sample. Lipid contents were expressed as a percentage of the original wet tissue weight.

*Quality assurance/quality control*

Rigorous QA/QC protocols were applied for analysis of the Galapagos sea lion blubber samples. Biopsy samples were analyzed in batches of 12 samples, with each containing one or two procedural blanks that were used to determine the method detection limit (MDL), an in-house performance evaluation sample containing known concentrations of the analytes of interest or a certified reference material (CRM), and 9 or 10 biopsy samples. Analyte concentrations were calculated by the surrogate internal standard method using mean relative response factors determined from calibration standard runs before and after each batch of samples. Recoveries of individual internal standards were between 60 to 110% for all analyses. Concentrations of analytes were corrected for the recoveries of internal standards. Method blanks, consisting of $Na_2SO_4$, were extracted according to the same procedure as used for environmental samples and were analyzed with every batch of 12 samples to check for background contamination throughout the entire analytical procedure. Multipoint (for details, see *Supporting Information*) calibration curves were used to determine instrument detection limits, linearity in detector response, and dynamic range for each target analyte. Method accuracy and precision for all analytes was determined from the analyses of CRMs, participation in intercalibration studies, and analysis of in-house reference samples (spiked or natural matrices) analyzed repeatedly over long periods of time. The CRMs used for PCDD/PCDF and PCB method validation were EDF-2524, EDF-2525, and EDF-2526 (purchased from Cambridge Isotope Laboratories). The method accuracy and precision (i.e., % deviation from the mean or the certified value as applicable) established from analyses of these standards for all congeners of all four analyte classes was better than 20%.

Concentration analysis involved examining concentration data on a pg/sample basis (wet wt), because the amounts of sample weight for extraction available in the present study were 50- to 100-fold lower (5–50 mg) than those normally used. For PBDEs, the concentrations measured in these samples were close to the levels measured in the procedural blanks. Concentration data therefore were plotted as the mass of PBDEs measured in the sample as a function of sample weight (i.e., on a per-sample basis) to elucidate the contribution of background contamination to the total measured concentration. Measured concentrations of PCDDs, PCDFs, and PCBs were evaluated using the same approach.

Concentrations of all detected PCDDs, PCDFs, PBDEs, and PCBs were blank corrected using the MDL (i.e., MDL on a pg/sample basis), defined here as the mean response of the levels measured in three procedural blanks used plus threefold the standard deviation (SD) of the blanks ($MDL = Mean_{blanks} + 3SD_{blanks}$). The concentration of each congener was determined according to two methods, i.e., based on concentrations

Table 1. Life-history data for Galapagos sea lion (*Zalophus wollebaeki*) pups[a]

| | Male | Female | Welch's approximate *t* test (*p* value) |
|---|---|---|---|
| Sample size (*n*) | 8 | 13 | |
| Body weight (kg) | 20.6 ± 0.95 (18–25.6) | 66.9 ± 7.01 (14.4–98.4) | <0.0001* |
| Standard length (cm) | 102 ± 1.85 (96–109) | 155 ± 7.67 (87–177) | <0.0001* |
| FCF (corporal condition)[a] | 1.94 ± 0.03 (1.82–2.07) | 1.71 ± 0.06 (1.31–2.19) | 0.004* |
| Lipid (%) | 70.2 ± 9.34 (13.5–100) | 73.3 ± 3.92 (44.4–92.8) | NS |
| Log ΣPCB | 1.98 ± 0.10 (49.0–384)[b] | 1.90 ± 0.06 (53.2–353) | NS |
| Sample size for PBDEs (*n*) | (1)[c] | | |
| BDE 47 | 33.3 | | |
| BDE 49 | 0.87 | | |
| BDE 66 | 0.33 | | |
| BDE 183 | 0.63 | | |

[a] Data are reported as the arithmetic mean ± standard error (range) and as the log concentration of polychlorinated biphenyls (ΣPCB) and concentration of polybrominated diphenyl ethers (ΣPBDE; both concentrations μg/kg lipid wt; mean ± standard error). An asterisk indicates a significant difference. FCF = Fulton condition factor (weight × $10^5$/standard length$^3$; *Supporting Information,* http://dx.doi.org/10.1897/08-331.S1); NS = not significant.

[b] The range for ΣPCB values is presented in parentheses.

[c] Only a male pup from the South Plaza rookery (Santa Cruz Islands) exhibited detectable concentrations of PBDEs.

above the MDL only and based on all concentrations using half the MDL for those concentrations below the MDL. The total concentration of PCBs (ΣPCB) was determined as the sum of the concentrations of all 72 congeners using half the MDL for concentrations below the MDL. The total concentration of PBDEs (ΣPBDE) was calculated as the sum of the concentrations of four congeners (BDEs 47, 49, 66, and 183) above the MDL. Concentrations of contaminants were lipid normalized by dividing wet-weight concentration by the lipid content to account for the differences in lipid content among the muscle–blubber biopsies. The normality of the concentration data were explored and reported as geometric mean concentrations with asymmetric SDs.

### *Statistics*

Log-transformed morphometric data (i.e., length, weight, and body condition) of both sexes were compared using a Welch's modified two-tailed *t* test assuming unequal variances [41] to determine if any difference in life-history parameters existed between the sexes. To examine possible relationships between ΣPCB in sea lion tissues and age, length, girth, lipid content, and body condition index (i.e., Fulton condition factor [FCF]), correlation analyses were conducted among all variables and contaminant levels (results are presented in a correlation matrix). The occurrence of significant differences between ΣPCB concentrations in male and female pups was investigated using the Welch's approximate *t* test.

The Welch analysis of variance followed by a Tukey–Kramer multiple-comparisons test [41] was used to explore the occurrence of statistically significant differences in ΣPCB concentrations among sites, because the variances among sites were unequal (i.e., heteroscedasticity; Bartlett test, $p = 0.0187$). All statistical analyses were conducted using JMP 7.0 (SAS Institute) at a level for significance of $p < 0.05$.

### *Health risk assessment*

A preliminary hazard/effect assessment was based on the estimation of total toxic equivalent concentrations (TEQs, ng/kg lipid) using the most recent data on total equivalent factors for dioxin-like PCBs, including planar (non-*ortho*-)PCBs (sum of PCBs 77, 81, 126, and 169) and mono-*ortho*-PCBs (sum of PCBs 105, 114, 118, 123, 156, 157, and 167) reported by Van den Berg et al. [42]. Both PCDDs and PCDFs were not included in the TEQ calculations, because these compounds were not detected. The resulting TEQs were then compared to the TEQ threshold levels, including the no-observable-adverse effect level (NOAEL) and the lowest-observable-adverse effect level (LOAEL) for dioxin-like PCBs, derived from immunotoxic action and endocrine-disruption endpoints assessed in semicaptive harbor seals [13,43]. Total toxic equivalent concentrations for PBDEs were not assessed at this time, because total equivalent factors have yet to be determined for this group of organic contaminants.

## RESULTS AND DISCUSSION

### *Study animals*

The lipid content of our 21 Galapagos sea lion pup biopsy samples was of 72% ± 19% (mean ± standard error) (Table 1). The lipid content did not correlate with any of the life-history parameters (regression analysis for all body measurements, $p > 0.05$), including age, length, weight, girth, and corporal condition (Table 2). No significant differences were found in lipid content between female and male pups (Welch's approximate *t* test, $p = 0.550$) (Table 1). The ages of male and female pups (Welch's approximate *t* test, $p = 0.2350$) were similar, because biopsies were only performed on suckling pups (age, 2–12 months). In contrast, body weight and length of female pups were significantly greater than those of male pups (Welch's approximate *t* test, $p < 0.0001$ and $p < 0.0001$, respectively). Length and weight were highly correlated in these pups ($r = 0.98$, $p < 0.00001$) (Table 2). Similarly, girth showed a significant relationship with length and weight (regression analysis, $p < 0.0001$). The body condition index (i.e., FCF) of male pups was higher than that of female pups (Welch's approximate *t* test, $p = 0.004$), reflecting the generally higher body density of male otariid pups [44].

### *Concentrations of PCBs, PBDEs, PCDDs, and PCDFs*

Of a total of 207 PCB congeners included in the analysis, 72 congeners in Galapagos sea lion muscle–blubber biopsies were consistently detected at concentrations above the MDL. Lipid-normalized concentrations and MDLs for individual congeners detected are reported in the *Supporting Information* (Table S1, http://dx.doi.org/10.1897/08-331.S1). The sum of the mean PCB congener concentrations based only on detectable

Table 2. Correlation matrix presenting the correlation coefficients of the log total concentration of polychlorinated biphenyls (ΣPCB; μg/kg lipid) and all life-history parameters of Galapagos sea lion (*Zalophus wollebaeki*) pups analyzed in the present study[a]

| Variable | ΣPCB ($n$ =21) | % Lipid | Age | Girth | Weight | Standard length | FCF |
|---|---|---|---|---|---|---|---|
| ΣPCB | 1 | | | | | | |
| % Lipid | −0.76*** | 1 | | | | | |
| Age[b] | 0.24 | −0.32 | 1 | | | | |
| Girth | Male (0.76)* | −0.03 | 0.67* | 1 | | | |
| | Female (0.47) | | | | | | |
| Weight | Male (0.69) | 0.07 | 0.61* | 0.98*** | 1 | | |
| | Female (0.50) | | | | | | |
| Standard length | Male (0.50) | 0.09 | 0.58* | 0.96*** | 0.98*** | 1 | |
| | Female (−0.62)* | | | | | | |
| FCF | Male (0.14) | −0.28 | 0.19 | −0.51* | −0.53* | −0.69** | 1 |
| | Female (0.68)* | | | | | | |

[a] FCF = Fulton condition factor (weight × $10^5$/standard length$^3$; *Supporting Information,* http://dx.doi.org/10.1897/08-331.S1). *$p \leq 0.05$, **$p < 0.0005$, ***$p < 0.0001$.

[b] Lipid content and age were negatively correlated when the pup showing the lowest lipid content (13%) was included ($r = -0.59$; $p = 0.006$).

concentrations was 104 μg/kg lipid, and the geometric mean concentration of PCBs in the blubber samples using half the MDL for nondetectable PCB congener concentrations ($n$ =21) was 85 μg/kg lipid (lower geometric SD, 48 μg/kg lipid; upper geometric SD, 150 μg/kg lipid).

Among pups of the four different rookeries, ΣPCB concentrations were not significantly different (Welch analysis of variance, $p = 0.4964$; Tukey-Kramer test, $p > 0.05$), indicating a common environmental source for PCBs. This indicates that the majority of the Galapagos sea lions sampled were subject to the same degree of PCB exposure.

Most of the Galapagos sea lion samples did not contain PBDE concentrations that exceeded the MDL. Only one animal (PSP-03) out of 21 Galapagos sea lion pups exhibited detectable concentrations for four congeners, including BDEs 47, 49, 66, and 183 (Table 1 and *Supporting Information*, Fig. S1, http://dx.doi.org/10.1897/08-331.S1). To evaluate further whether background contamination interfered with the reporting of concentrations, correlations between the concentrations of PBDEs and PCBs were explored (*Supporting Information*, Fig. S2, http://dx.doi.org/10.1897/08-331.S1). A strong correlation was observed between concentrations (pg/sample) of PBDEs and PCBs ($r = 0.625$; $p = 0.0024$) (*Supporting Information*, Fig. S2). Such a correlation can occur naturally in animals exposed through similar routes (e.g., diet in female animals). As seen in the Figure S2 of the *Supporting Information,* the correlation between PBDE and PCB concentrations in procedural blanks has a much steeper slope than the correlation for biopsy samples, indicating a specific source of PBDE contamination in at least one of the blanks. The PBDE and PCB concentration correlations in the biopsy samples did not exhibit this steeper slope. This indicates that the samples with higher PBDE concentrations (e.g., PIP-01, -02, and -08) may not have been affected by this specific source of contamination. Hence, the PBDE concentrations in these samples may actually reflect detectable concentrations even though the concentrations are considered to be nondetectable based on the QA/QC rules regarding the MDL. On the other hand, sample PSP-03 contained an apparent high level of PBDE contamination that does not fit the general relationship between PCB and PBDE concentrations in biopsy samples. This concentration appears to be above the MDL following QA/QC rules but should be treated with caution, because the sample may have been inadvertently contaminated with PBDEs. Because only three procedural blanks were used, and because the procedural blanks suggest the possibility of significant PBDE contamination of these small samples, the PBDE concentration data should only be viewed in a qualitative way—that is, that the ΣPBDE concentration is low, with concentrations both within the range and below those measured in our procedural blanks.

Of 93 individual PCDD and PCDF congeners measured, none met the criteria for detectability (i.e., all were less than the MDL) in any of the samples examined. The highest MDL was 146 pg/g wet weight, for octachlorodibenzo-*p*-dioxin, whereas the lowest MDL was 51.4 pg/g wet weight, for 1,2,3,4,7,8-hexachlorodibenzofuran. The congener 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin exhibited a MDL of 67.0 pg/g wet weight, falling within these two values. Because no detectable concentrations were observed for any of the 93 target PCDD/PCDF congeners in any of the samples, it can be concluded that the exposure of Galapagos sea lions to PCDDs/PCDFs is very low.

### *Composition of PCBs*

The ΣPCB concentration was characterized by a dominant contribution of lower-chlorinated PCB congeners (i.e., di-, tri-, tetra-, and pentachloro-PCBs), which made up 56% of ΣPCB (Fig. 2). In most pinniped species from the northern hemisphere, hexa- and heptachloro-PCBs make up the majority of ΣPCB concentration (Fig. 3), revealing a different ΣPCB composition compared to our Galapagos sea lion pups and to southern elephant seals (*Mirounga leonina*) from Antarctica [45]. In the Galapagos sea lion pups, PCBs 5/8 (2.12%), 16/32 (1.24%), 85 (21.3%), 95 (1.55%), 99 (6.93%), 101 (5.49%), and 118 (2.87%) make up approximately 42% of ΣPCB concentrations, whereas PCBs 153 (7.00%), 138/163/164 (3.1.%), and 180 (19.4.%) contribute 30% of ΣPCB concentrations (Fig. 2). The finding of a light PCB signature suggests comparatively greater inputs from lower-molecular-weight and more volatile PCBs congeners that are more easily transported globally by atmospheric processes.

Similarly, the ΣPCB concentrations in southern elephant seals from Antarctica also contains a relatively high proportion of low-molecular-weight PCBs [45], with PCBs 18, 28, 31, 44, 49, and 74 contributing 22% of ΣPCB concentrations. In contrast, ΣPCB concentrations in northern elephant seal pups from California (USA) [46] and in harbor seal pups inhabiting industrialized regions from the Northeastern Pacific [47] contain a high proportion of heavier PCB congeners, resembling the composition of Aroclor 1260 [48] (Fig. 3). Global fractionation of PCB congeners may be playing an important

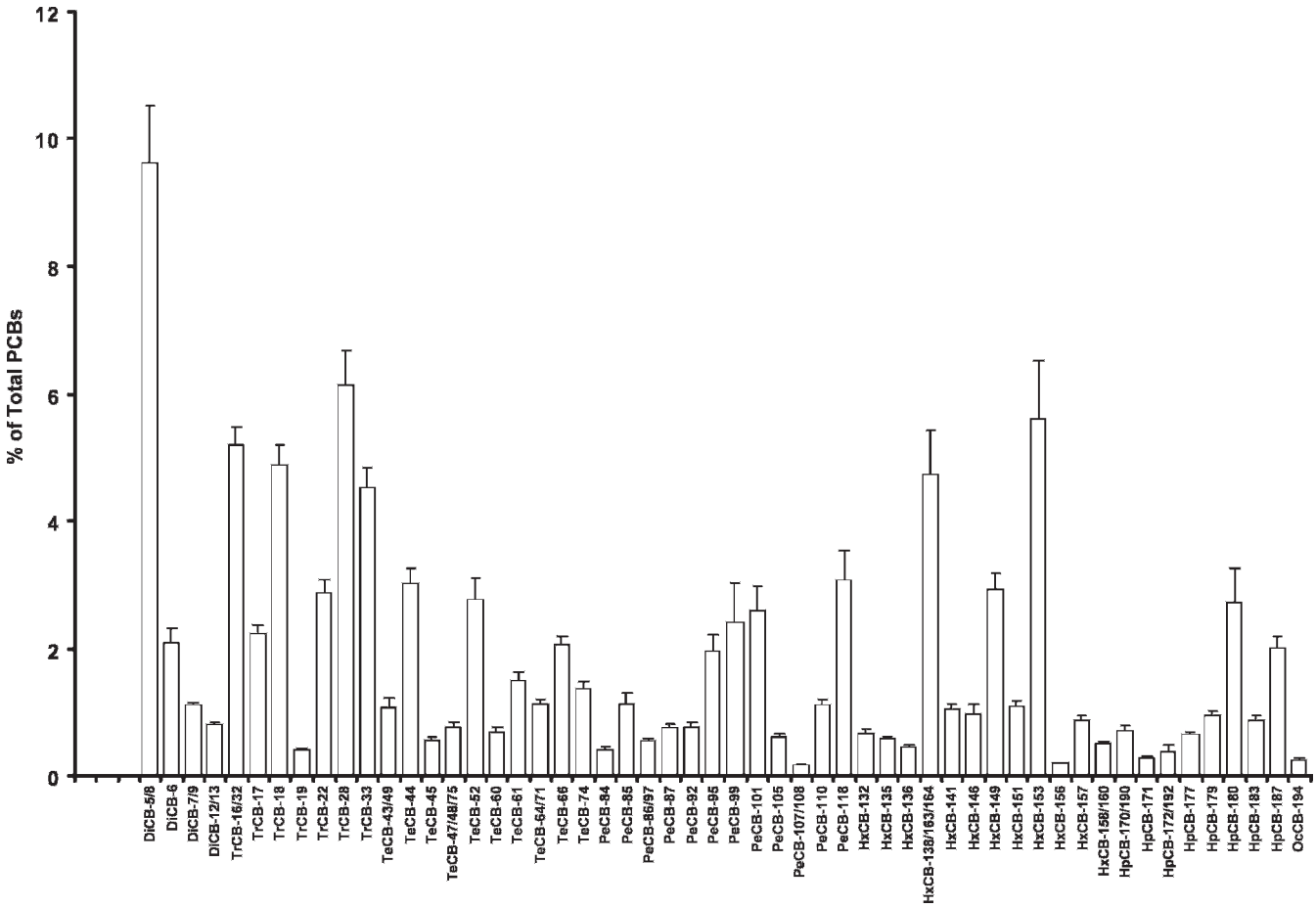


Fig. 2. Polychlorinated biphenyl (PCB) congener composition in pups of the Galapagos sea lion (*Zalophus wollebaeki*). Error bars indicate the standard error.

role in the PCB profile differences found among these pinniped species [7–9,49]. Low-molecular-weight PCB congeners tend to partition into the atmosphere to a greater extent than high-molecular-weight PCB congeners. These lower-molecular-weight PCBs therefore may be able to travel from their sources to remote locations faster than higher-molecular-weight congeners. The high partitioning tendency in air and the high transport rate in the atmosphere may cause the occurrence of PCB concentrations dominated by low-molecular-weight congeners in remote locations, such as the Galapagos Islands and Antarctica.

### *Life history and physiological factors as determinants of contaminant concentrations*

The influence of age and body condition on contaminant concentrations were minimized by collecting biopsy samples from similarly aged animals (age, one year or younger) at a time when pups were still nursing. Differences were observed in morphometric parameters between male and female pups, but no statistically significant differences in ΣPCB concentrations (Welch's approximate $t$ test, $p = 0.4927$) were found between sexes (Table 1). The lack of a difference in concentration between male and female pups may be caused by the similarity in prenatal and postnatal PCB exposure (i.e., milk) of male and female pups and by the lack of differences in the life histories of these young animals. Elimination of persistent organic pollutants via transplacental and milk transfer to their young ultimately will cause differences in PCB concentrations between males and females in sexually mature adult animals [50].

The ΣPCB concentrations in male pups did not show a statistically significant correlation with standard length, weight, or FCF (regression analysis of ΣPCB concentrations vs any of these morphometric parameters, $p > 0.05$) but was positively correlated with girth ($r^2 = 0.571$, $p = 0.030$). A negative relationship was found between the ΣPCB concentrations in biopsy samples of female pups and the standard length ($r^2 = 0.381$, $p = 0.025$), but a positive correlation was observed between ΣPCB concentrations in biopsy samples and FCF in female pups ($r^2 = 0.462$, $p = 0.011$) (Table 2). Other studies of marine mammals have found negative relationships or associations between contaminant concentration and length or age [50,51]. For instance, PCB concentrations in adult male Atlantic white-sided dolphins (*Lagenorhynchus acutus*) decreased with body length, possibly reflecting a growth dilution phenomenon [51]. Under the assumption of growth dilution, a young marine mammal receives a large initial contaminant load through lactation. After the pup is weaned, it experiences a period of growth coupled with a switch in food source from milk to less contaminated prey items. This produces a decline in PCB concentration over time after weaning. Because the sampling design of the present study was aimed at minimizing the effect of life-history factors on contaminant concentration, differences in weight, length, and body condition factors among the sampled animals are small. As a result, we do not associate specific significance to the apparent decrease of

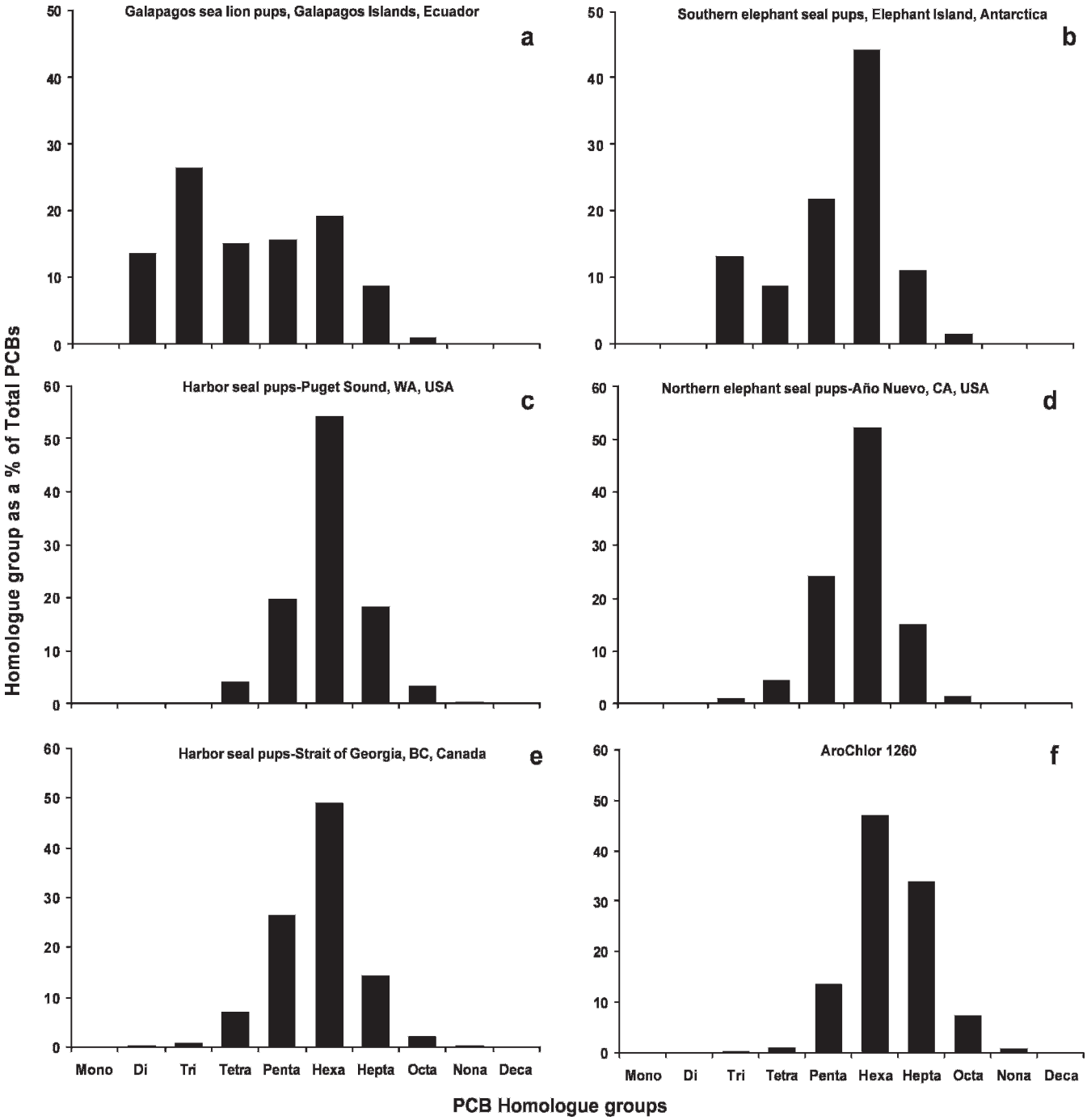


Fig. 3. Polychlorinated biphenyl (PCB) homologue composition in pups of various pinnipeds species from different locations in relation to that of Aroclor 1260: (**a**) The PCB pattern in Galapagos sea lions (*Zalophus wollebaeki*), (**b**) PCB congeners composition for pups of southern elephant seals (*Mirounga leonina*) from Antarctic [45], (**c**) harbor seal (*Phoca vitulina*) pups from Washington State (USA) [47], (**d**) northern elephant seal pups (*Mirounga angustirostris*) from California (USA) [46], (**e**) harbor seal pups from British Columbia (Canada) [47], and (**f**) Aroclor 1260 [48].

contaminant levels with length in female Galapagos sea lion pups.

The metabolic capacity of marine mammals can influence PCB patterns in these animals. Even though coplanar PCBs largely are retained by marine mammals, pinniped species (i.e., phocids) are able to metabolize most of the PCB congeners with *meta*- and *para*-vicinal-H atoms and two *ortho*-chlorines because of the enzymatic activity and induction of the cytochrome P450 enzymes CYP1A and CYP2B [52,53]. However, whereas planar (non-*ortho*-)PCBs were not detected in the samples, PCB congeners with *meta*- and *para*- as well as *ortho*- and *meta*-vicinal hydrogens were detected (*Supporting Information,* Table S1). These observations suggest a relatively poor metabolic capacity or lack of cytochrome P450 enzymatic induction, possibly resulting from the low level of PCB contamination in Galapagos sea lion pups.

Differences in foraging grounds and feeding behavior among female sea lions can influence PCB concentrations and the composition of ΣPCB. Lactating female Galapagos sea lions spent a significant proportion of time in other islands (i.e., multiple haul-out sites) other than their breeding colonies (i.e., rookery) during foraging trips [54]. This interisland

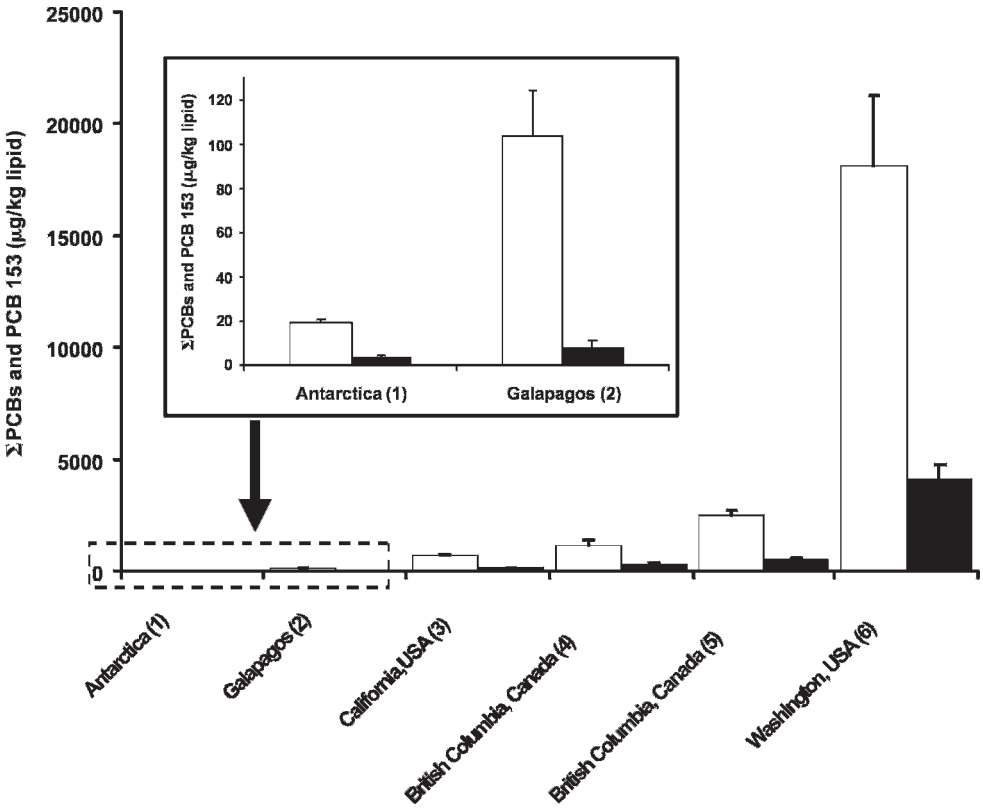


Fig. 4. Global comparisons of mean total polychlorinated biphenyls (ΣPCB; □) and PCB 153 (used here as a reference congener due to its recalcitrance nature) concentrations (µg/kg lipid; ■) in pups from pinnipeds species from different marine–coastal regions. Error bars indicate the standard error. All values are expressed on a lipid-weight basis (µg/kg lipid). For extended text of the legend, see *Supporting Information* (http://dx.doi.org/10.1897/08-331.S1).

movement may contribute to the similarity in PCB concentrations in Galapagos sea lions.

*Comparisons with other marine mammal species*

The ΣPCB concentrations in Galapagos sea lions is among the lowest PCB concentrations reported in pinniped species [46,47] (Fig. 4 and *Supporting Information*, Table S2, http://dx.doi.org/10.1897/08-331.S1). Only southern elephant seal pups from Antarctica [45] were found to have lower ΣPCB concentrations than Galapagos sea lion pups. Even if the recalcitrant PCB 153 is used as a measure of PCB contamination (to eliminate differences in concentrations resulting from differences in the number of congeners monitored and detected), the results are similar (Fig. 5).

Figure 5 and Table S3 (*Supporting Information,* http://dx.doi.org/10.1897/08-331.S1) show that the ΣPBDE concentrations in Galapagos sea lion pups (25.0 µg/kg wet wt or 35.2 µg/kg lipid) also are among the lowest reported concentrations in pinnipeds [24,55,56]. Northern fur seals (*Callorhinus ursinus*) from the Pacific coast of Japan [57] and ringed seals from the Canadian Arctic [26] also exhibited low ΣPBDE concentrations. The ΣPBDE concentrations detected in the Galapagos sea lion pups was lower than those measured in cetacean species, including harbor porpoises (*Phocoena phocoena*) from England and Wales [58], killer whales (*Orcinus orca*) from the Northeastern Pacific [59], beluga whales (*Delphinapterus leucas*) from the Arctic [23] and the St. Lawrence Estuary [60], and Atlantic white-sided dolphins [51].

It is difficult to compare PBDE and/or PCB concentrations directly across marine mammal species when gender, age, reproductive status, size and body condition, as well as differences in trophic position, feeding behavior/ecology, and bioenergetics vary. The comparisons made in the present study, however, place the degree of contamination of Galapagos sea lions in a global context.

*Health risks from exposure to contaminants*

The ΣPCB concentrations in Galapagos sea lion pups was less than the LOAEL threshold effect concentration of 1,300 µg/kg lipid for risk of immunotoxicity and endocrine disruption in harbor seals (L. Mos, M. Cameron, S.J. Jeffries, B. Koop, P.S. Ross, Institute of Ocean Sciences, Department of Fisheries and Ocean, Sidney, British Columbia, Canada, unpublished data). Because non-*ortho*-PCB congeners were not detected, they could not be included in the TEQ. Only mono-*ortho*-PCBs (i.e., PCBs 105, 118, 156, and 157) were detected, making up a total of 0.97 ng TEQ/kg lipid. The TEQ level in Galapagos sea lion pups was well below the LOAEL (286 ng TEQ/kg lipid) and NOAEL (90 ng TEQ/kg lipid) thresholds calculated from the lipid-normalized concentrations measured in harbor seals [43]. This suggests that these pups are not at risk of immunotoxicity and endocrine disruption as a result of PCBs. A lack of information regarding PBDE toxicity makes it difficult to assess the health risks associated with these flame retardants, but the very low concentrations observed in our Galapagos sea lions suggest limited risk. However, the endocrine-disrupting nature of these compounds has been demonstrated by in vitro studies and in vivo laboratory animal studies [30–32]. Despite the fact the Galapagos sea lion pups are less contaminated than other pinniped species from the northeastern Pacific Ocean, they may still be at risk for low-level, chronic exposure to PCBs,

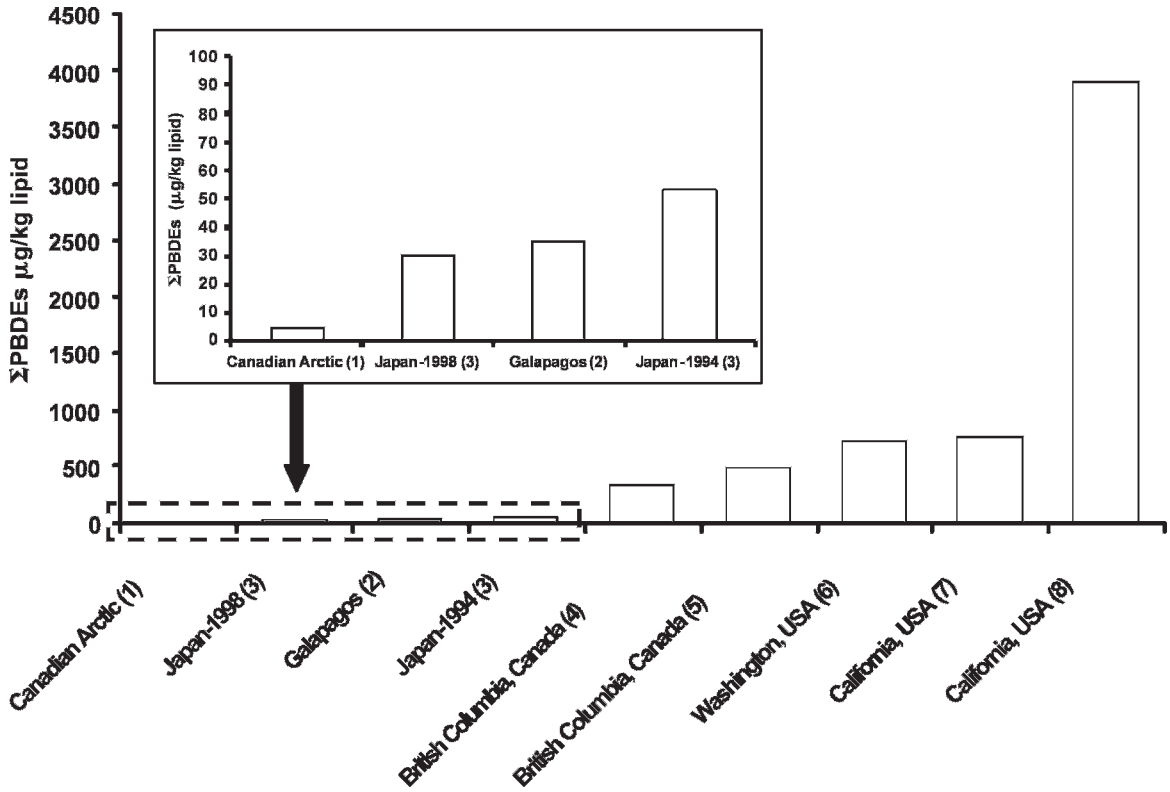


Fig. 5. Global comparisons of total polybrominated diphenyl ethers concentrations (ΣPBDE) measured in pinniped species from different marine regions (see also *Supporting Information*, Table S3, http://dx.doi.org/10.1897/08-331.S1). All values are expressed on a lipid-weight basis (µg/kg lipid). For extended text of the legend for this figure, see *Supporting Information*.

mainly during the weaning or postweaning fasting, a sensitive period when contaminants in the blubber (e.g., PCBs) can be released into the circulation [46].

*PCB and PBDE transport and fate in the Galapagos*

The lack of statistically significant differences in ΣPCB concentrations in Galapagos sea lions among the four sampling locations indicates a common source for these pollutants (Fig. 6). The remoteness of the Galapagos Islands and the long distances between sources and target organisms may be one of the key factors causing the low ΣPCB and ΣPBDE concentrations observed. Local sources, which can be expected to produce differences in concentrations between human-inhabited islands (e.g., Santa Cruz, which is a center of ecotourism) and uninhabited islands (e.g., Pinta), do not appear to be significant contributors to current concentrations of PCBs detected in Galapagos sea lion pups. The long-range transport capability of PCBs and PBDEs to remote areas of the world has been well documented [6–9,29]. Long-range environmental transport comprising both atmospheric and oceanic processes likely explains the route of entry of PCBs and PBDEs into the Galapagos sea lion food web. However, it cannot be ruled out that local sources from urbanized, human-inhabited islands (e.g., Santa Cruz and San Cristobal) may have contributed to the measured concentrations. Past burning of waste products (i.e., computer devices and furniture) containing flame-retardant formulation mixtures in open dumps without treatment can be potential sources of PBDEs. However, local waste management practices of municipal organic waste have improved over the last few years, and burning in open dumps close to harbors and coastal zones has been banned.

Based on the present results, it can be concluded that concentrations of PCBs and PBDEs in Galapagos sea lion pups are still fairly low and below toxicologically relevant concentrations. The present results also suggest that currently, local sources of PCBs and PBDEs likely are small compared to remote sources and that PCB and PBDE concentrations largely may be reflecting global rather than local contamination. The rapid increase in human population and development of the Galapagos Islands [36] presents an emerging risk for Galapagos sea lions, because land-based activities may increasingly release pollutants into coastal waters. However, global practices regarding the production, use, and disposal of these chemicals appear to be more important present determinants of PCB and PBDE concentrations in Galapagos sea lions compared with local practices. Both PCDDs and PCDFs were not detected in these samples, suggesting that a combination of low environmental concentration and/or metabolism prevent significant bioaccumulation in Galapagos sea lions.

Results of the present study suggest that whereas PCB, PBDE, and PCDD/PCDF concentrations are relatively low, the remote Galapagos Islands are not immune to the consequences of global environmental contamination. This means that in addition to remote polar regions, remote equatorial areas, such as the Galapagos Islands, deserve attention and consideration when contemplating the widespread use of commercial chemicals. Sea lions in the Galapagos Islands can serve as a useful sentinel of global pollution.

## SUPPORTING INFORMATION

**Table S1**. Concentrations (µg/kg lipid; mean ± standard error) of 72 polychlorinated biphenyl (PCB) congeners and

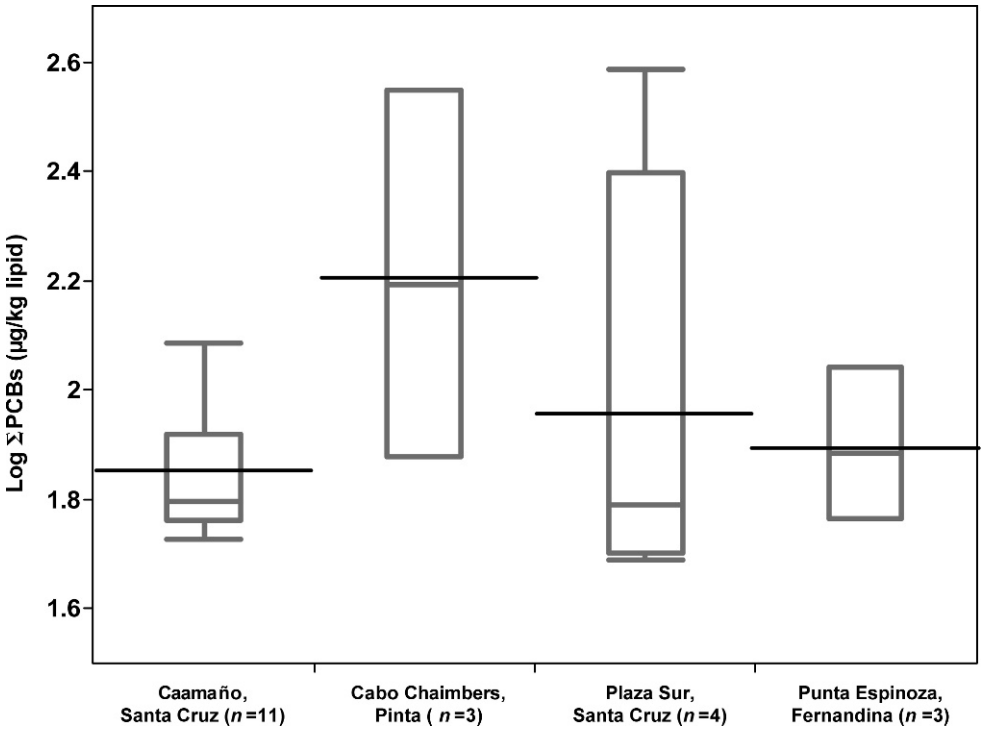


Fig. 6. Intersite comparisons showing box plots of log total polychlorinated biphenyl concentrations (ΣPCB) in sea lion (*Zalophus wollebaeki*) pups sampled from different rookeries of the Galapagos Islands (Ecuador). The internal lines across the boxes identify the median sample values, the ends of the boxes are the 25 and 75% quartiles, and the whisker bars are the minimum and maximum values. The external line crossing the middle on each box plot is the mean sample of log ΣPCBs of each rookery.

total PCB concentration (ΣPCB) in blubber samples of Galapagos sea lion (*Zalophus wollebaeki*) pups and the method detection limit (MDL; µg/sample). The mean of lipid content is 72% ($n$ = 21).

**Table S2**. Concentrations (mg/kg lipid; mean ± standard error) of polychlorinated biphenyls (PCBs) in blubber of pinnipeds from the Northeastern–Central Pacific Ocean and southern elephant seals (*Mirounga leonina*) from Antarctica (1971–2005).

**Table S3.** Comparisons of measured total polychlorinated biphenyl concentrations (ΣPCB), range of mean or geometric mean (SD) in µg/kg wet weight between the Galapagos sea lion (*Zalophus wollebaeki*) and other pinniped species of the world.

**Fig. S1**. Polybrominated diphenyl ether (PBDE) congener composition detected in one blubber sample of a Galapagos sea lion (*Zalophus wollebaeki*) pup.

**Fig. S2.** Relationship between the log total polybrominated diphenyl ether concentration (ΣPBDE) and log total polychlorinated biphenyl concentration (ΣPCB) in Galapagos sea lion (*Zalophus wollebaeki*) pups ($n$ = 21) on a pg/sample basis to explore the behavior of the laboratory blanks. Sample PSP-03 was the only one showing detectable concentrations of PBDEs. The regression line of procedural blank concentrations used during the laboratory analysis of both groups of contaminants also is shown as a dashed line.

All found at DOI: 10.1897/08-331.S1 (135 KB PDF).

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## REFERENCES

1. United Nations Environment Program. 2001. Final Act of the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic Pollutants, Stockholm, Sweden, May 22–23. Geneva, Switzerland.
2. de Boer J, Wester PG, Klamer HJ, Lewis WE, Boon J. 1998. Do flame retardants threaten ocean life? *Nature* 394:28–29.
3. de Wit CA. 2002. An overview of brominated flame retardants in the environment. *Chemosphere* 46:583–624.
4. Alaee M, Arias P, Sjödin A, Bergman Å. 2003. An overview of commercially used brominated flame-retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environ Int* 29:683–689.
5. Alcock RE, Sweetman AJ, Prevedouros K, Jones KC. 2003. Understanding levels and trends of BDE-47 in the UK and North America: An assessment of principal reservoirs and source inputs. *Environ Int* 29:691–698.
6. Shen L, Wania F, Lei YD, Teixeira C, Muir DCG, Xiao H. 2006. Polychlorinated biphenyls and polybrominated diphenyl ethers in the North American atmosphere. *Environ Pollut* 144:434–444.
7. Wania F, Mackay D. 1993. Global fractioning and cold condensation of low volatility organochlorine compounds in polar regions. *Ambio* 22:10–18.
8. Iwata H, Tanabe S, Sakai N, Tatsukawa R. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. *Environ Sci Technol* 27:1080–1098.

9. Jurado E, Jaward F, Lohmann R, Jones KC, Simó R, Dachs J. 2005. Wet deposition of persistent organic pollutants to the Global Oceans. *Environ Sci Technol* 39:2426–2435.
10. Blasius ME, Goodmanlowe GD. 2008. Contaminants still high in top-level carnivores in the Southern California Bight: Levels of DDT and PCBs in resident and transient pinnipeds. *Mar Pollut Bull* 56:1973–1982.
11. Hagen ME, Colodey AG, Knapp WD, Samis SC. 1997. Environmental response to decreased dioxin and furan loadings from British Columbia coastal pulp mills. *Chemosphere* 34:1221–1229.
12. Czuczwa JM, Hites RA. 1984. Environmental fate of combustion-generated polychlorinated dioxin and furans. *Environ Sci Technol* 18:444–450.
13. Ross PS, De Swart RL, Reijnders PJH, Van Loveren H, Vos JG, Osterhaus ADME. 1995. Contaminant-related suppression of delayed-type hypersensitivity and antibody responses in harbor seals fed herring from the Baltic Sea. *Environ Health Perspect* 103: 162–167.
14. Debier C, Ylitalo G M, Weise M, Gulland F, Costa DP, Le Boeuf BJ, de Tillesse T, Larondelle Y. 2005. PCBs and DDT in the serum of juvenile California sea lions: Associations with vitamins A and E and thyroid hormones. *Environ Pollut* 134:323–332.
15. Tabuchi M, Veldhoen N, Dangerfield N, Jeffries S, Helbing C, Ross P. 2006. PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging harbor seals (*Phoca vitulina*). *Environ Health Perspect* 114:1024–1031.
16. Mos L, Morsey B, Jeffries SJ, Yunker MB, Raverty S, De Guise S, Ross PS. 2006. Chemical and biological pollution contribute to the immunological profiles of free-ranging harbor seals. *Environ Toxicol Chem* 25:3110–3117.
17. Nylund K, Asplund L, Jansson B, Jonsson P, Litzen K, Sellstrom U. 1992. Analysis of some polyhalogenated organic pollutants in sediment and sewage sludge. *Chemosphere* 24:1721–1730.
18. van Esch GJ. 1994. Polybrominated diphenyl ethers. Environmental Health Criteria 162. World Health Organization, Geneva, Switzerland.
19. Rayne S, Ikonomou MG. 2002. Reconstructing source polybrominated diphenyl ether congener patterns from semipermeable membrane devices in the Fraser River, British Columbia, Canada: Comparison to commercial mixtures. *Environ Toxicol Chem* 21: 2292–2300.
20. Hale RC, Alaee M, Manchester-Neesvig JB, Stapleton HM, Ikonomou MG. 2003. Polybrominated diphenyl ether flame retardants in the North American environment. *Environ Int* 29: 771–779.
21. La Guardia MJ, Hale RC, Harvey E. 2006. Detailed polybrominated diphenyl ether (PBDE) congener composition of the widely used penta-, octa-, and deca-PBDE technical flame-retardant mixtures. *Environ Sci Technol* 40:6247–6254.
22. She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D. 2002. PBDEs in the San Francisco Bay area: Measurements in harbor seal blubber and human breast adipose tissue. *Chemosphere* 46:697–707.
23. Wolkers H, Van Bavel B, Derocher AE, Wiig O, Kovacs KM, Lydersen C, Lindstrom G. 2004. Congener specific accumulation and food-chain transfer of polybrominated diphenyl ethers in two Arctic food chains. *Environ Sci Technol* 38:1667–1674.
24. Hall AJ, Thomas GO. 2007. Polychlorinated biphenyls, DDT, polybrominated diphenyl ethers and organic pesticides in United Kingdom harbor seals (*Phoca vitulina*)—Mixed exposures and thyroid homeostasis. *Environ Toxicol Chem* 26:851–861.
25. Kelly BC, Ikonomou MG, Blair JD, Gobas FAPC. 2008. Bioaccumulation behavior of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. *Sci Total Environ* 40:60–72.
26. Ikonomou MG, Rayne S, Addison RF. 2002. Exponential increases of the brominated flame retardants, polybrominated diphenyl ethers, in the Canadian Arctic from 1981 to 2000. *Environ Sci Technol* 36:1886–1892.
27. Sellström U, Jansson B, Kierkegaard A, de Wit CA, Odsjö T, Olsson M. 1993. Polybrominated diphenyl ethers (PBDE) in biological samples from the Swedish environment. *Chemosphere* 26:1703–1718.
28. Meironyte D, Noren K, Bergaman A. 1999. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972–1997. *J Toxicol Environ Health A* 58: 329–341.
29. Ter Schure AFH, Larsson P, Agrell C, Boon JP. 2004. Atmospheric transport of polybrominated diphenyl ethers and polychlorinated biphenyls to the Baltic Sea. *Environ Sci Technol* 38:1282–1287.
30. Meerts IATM, Letcher RJ, Hoving S, Marsh G, Bergman Å, Lemmen JG, van der Burg B, Brouwer A. 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs, and polybrominated bisphenol A compounds. *Environ Health Perspect* 109:399–407.
31. Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. 2001. Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environ Health Perspect* (Suppl. 1):49–68.
32. Hallgren S, Darnerud PO. 2002. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and polychlorinated paraffins (CPs) in rats—Testing interactions and mechanisms for thyroid hormone effects. *Toxicology* 177:227–243.
33. Alava JJ, Salazar S. 2006. Status and conservation of Otariids in Ecuador and the Galapagos Islands. In Trites AW, Atkinson SK, DeMaster DP, Fritz LW, Gelatt TS, Rea LD, Wynne KM, eds, *Sea Lions of the World*. Alaska Sea Grant College Program, University of Alaska Fairbanks, Fairbanks, AK, USA, pp 495–519.
34. Merlen G, Salazar S. 2007. Estado y efectos antropogénicos en los mamíferos marinos de Galápagos. *Memorias, Taller de Trabajo sobre el Impacto de las Actividades Antropogénicas en Mamíferos Marinos en el Pacífico Sudeste*. CPPS/PNUMA, Bogotá, Colombia, Noviembre 28–29, 2006, pp 70–76.
35. Utreras V, Tirira D, Salazar S. 2001. The Galapagos sea lion (*Zalophus wollebaeki*). In Tirira D, ed, *The Red Book of the Mammals of Ecuador*. Simbioe, Ecocienia, Environmental Ministry, UICN, Quito, Ecuador, pp 66–67.
36. Watkins G, Cruz F. 2007. *Galapagos at Risk: A Socioeconomic Analysis of the Situation in the Archipelago*. Charles Darwin Foundation, Puerto Ayora, Province of Galapagos, Ecuador.
37. Trillmich F. 1986. Attendance behavior of Galapagos sea lions. In Gentry RL, Kooyman GL, eds, *Fur Seals: Maternal Strategies on Land and at Sea*. Princeton University Press, Princeton, NJ, USA, pp 196–208.
38. Trillmich F, Wolf JBW. 2008. Parent–offspring and sibling conflict in Galapagos fur seals and sea lions. *Behav Ecol Sociobiol* 62:363–375.
39. Páras A, Brousset DM, Salazar S, Aurioles-Gamboa D. 2002. Field anesthesia of two species of pinnipeds found in the Colon Archipelago or Galápagos (*Arctocephalus galapagoensis* and *Zalophus wollebaeki*). *Proceedings*, Annual Meeting of the American Association of Zoo Veterinarians, Milwaukee, WI, October 6–10, pp 431–433.
40. Ikonomou MG, Fraser TL, Crewe NF, Fischer MB, Rogers IH, He T, Sather PJ, Lamb RF. 2001. A comprehensive multiresidue ultratrace analytical method, based on HRGC/HRMS, for the determination of PCDDs, PCDFs, PCBs, PBDEs, PCDEs, and organohalogen pesticides in six different environmental matrices. *Can Data Rep Fish Aquat Sci* 2389:1–95.
41. Zar JH. 1999. *Biostatistical Analysis*, 4th ed. Prentice Hall, Upper Saddle River, NJ, USA.
42. Van den Berg M, Birnbaum L, Bosveld ATC, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem AR, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environ Health Perspect* 106:775–792.
43. Kannan K, Blankenship AL, Jones PD, Giesy JP. 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum Ecol Risk Assess* 6:181–201.
44. Luque SP, Aurioles-Gamboa D. 2001. Sex differences in body size and body condition of California sea lion (*Zalophus califonianus*) pups from the Gulf of California. *Mar Mammal Sci* 17:147–160.
45. Miranda-Filho KC, Metcalfe TL, Metcalfe CD, Metcalfe RB, Robaldo RB, Muelbert MMC, Colares EP, Martinez PE, Bianchini A. 2007. Residues of persistent organochlorine contaminants in southern elephant seals (*Mirounga leonina*) from Elephant Island, Antarctica. *Environ Sci Technol* 41:3829–3835.
46. Debier C, Le Boeuf BJ, Ikonomou MG, de Tillesse T, Larondelle Y, Ross PS. 2005. Polychlorinated biphenyls, dioxins, and furans

in weaned, free-ranging northern elephant seal pups from Central California, USA. *Environ Toxicol Chem* 24:629–633.
47. Ross PS, Jeffries SJ, Yunker MB, Addison RF, Ikonomou MG, Calambokidis JC. 2004. Harbor seals (*Phoca vitulina*) in British Columbia, Canada, and Washington State, USA, reveal a combination of local and global polychlorinated biphenyl, dioxin, and furan signals. *Environ Toxicol Chem* 23:157–165.
48. Schulz DE, Petrick G, Duinker JC. 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography–electron-capture detection. *Environ Sci Technol* 23:852–859.
49. Wania F, Su Y. 2004. Quantifying the global fractionation of polychlorinated biphenyls. *Ambio* 33:161–168.
50. Addison RF, Smith, TG. 1974. Organochlorine levels in Arctic ringed seals: Variation with age and sex. *Oikos* 25:335–337.
51. Tuerk KJS, Kucklick JR, McFee WE, Pugh RS, Becker PR. 2005. Factors influencing persistent organic pollutant concentration in the Atlantic white-sided dolphin (*Lagenorhynchus acutus*). *Environ Toxicol Chem* 24:1079–1087.
52. Tanabe S, Watanabe S, Kan H, Tatsukawa R. 1998. Capacity and mode of PCB metabolism in small cetaceans. *Mar Mamm Sci* 4: 103–124.
53. Boon JP, Van der Meer J, Allchin CR, Law RJ, Klungsoyr J, Leonards PEG, Spliid H, Starr-Hansen E, Mckenzie C, Wells DE. 1997. Concentration-dependent changes of PCB patterns in fish-eating mammals: Structural evidence for induction of cytochrome P450. *Arch Environ Contam Toxicol* 33:298–311.
54. Villegas-Amtmann S, Costa DP, Tremblay Y, Salazar S, Aurioles-Gamboa D. 2008. Multiple foraging strategies in a marine apex predator, the Galapagos sea lion (*Zalophus wollebaeki*). *Mar Ecol Prog Ser* 363:299–309.
55. Kalantzi OI, Hall AJ, Thomas GO, Jones KC. 2005. Polybrominated diphenyl ethers and selected organochlorine chemicals in grey seals (*Halichoerus grypus*) in the North Sea. *Chemosphere* 58:345–354.
56. Stapleton HM, Dodder NG, Kucklick JR, Reddy CM, Schantz MM, Becker PR, Gulland F, Porter BJ, Wise SA. 2006. Determination of HBCD, PBDEs, and MeO-BDEs in California sea lions (*Zalophus californianus*) stranded between 1993 and 2003. *Mar Pollut Bull* 52:522–531.
57. Kajiwara N, Ueno D, Takahashi A, Baba N, Tanabe S. 2004. Polybrominated diphenyl ethers and organochlorines in archived northern fur seal samples from the Pacific coast of Japan, 1972–1998. *Environ Sci Technol* 38:3804–3809.
58. Law RJ, Allchin CR, Bennett ME, Morris S, Rogan E. 2002. Polybrominated diphenyl ethers in two species of marine top predators from England and Wales. *Chemosphere* 46:673–681.
59. Rayne S, Ikonomou MG, Ross PS, Ellis GM, Barrett-Lennard LG. 2004. PBDEs, PBBs, and PCNs in three communities of free-ranging killer whales (*Orcinus orca*) from the Northeastern Pacific Ocean. *Environ Sci Technol* 38:4293–4299.
60. Lebeuf M, Gouteux B, Measures L, Trottier S. 2004. Levels and temporal trends (1988–1999) of polybrominated diphenyl ethers in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Canada. *Environ Sci Technol* 38:2971–2977.