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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

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Abstract—The harbor seal (*Phoca vitulina*) can serve as a useful indicator of food web contamination by persistent organic pollutants (POPs) because of its high trophic level, wide distribution in temperate coastal waters of the Northern Hemisphere, and relative ease of capture. In 1996 through 1997, we live-captured 60 harbor seal pups from three regions, spanning remote (Queen Charlotte Strait, BC, Canada), moderately industrialized (Strait of Georgia, BC, Canada), and heavily industrialized (Puget Sound, WA, USA) marine basins straddling the Canada–United States border. Biopsy samples of blubber were taken and analyzed for congener-specific polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) by using high-resolution gas chromatography–high-resolution mass spectrometry. Harbor seals in Puget Sound were heavily contaminated with PCBs, whereas seals from the Strait of Georgia had relatively high concentrations of PCDDs and PCDFs. Pattern evaluation and principal components analysis suggested that proximity to sources influenced the mixture to which seals were exposed, with those inhabiting more remote areas being exposed to lighter PCB congeners (those with lower Henry's law constant and K_{OW}) that disperse more readily through atmospheric and other processes. Total toxic equivalents to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for the PCBs, PCDDs, and PCDFs suggest that Puget Sound seals are at greatest risk for adverse health effects, and that PCBs represent the class of dioxinlike contaminants of greatest concern at all sites.

Keywords—Harbor seal *Phoca vitulina* Polychlorinated biphenyls Polychlorinated dibenzo-*p*-dioxins Polychlorinated dibenzofurans

INTRODUCTION

Despite the restrictions on the discharges of persistent organic pollutants (POPs) in many industrialized nations, contamination of the global environment with POPs continues to present health risks to wildlife and humans. Fish-eating wildlife, including marine mammals, are particularly vulnerable to such contamination given their long lives, high trophic level, relative inability to metabolize many POPs, and the bioaccumulation of these contaminants in aquatic food chains. The characterization of the factors that affect the transport and fate of POPs in the environment and their dynamics in aquatic food webs is important to identifying sources and implementing appropriate mitigative strategies. Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs or dioxins), and polychlorinated dibenzofurans (PCDFs or furans) represent three structurally related classes of POPs that have been extensively studied. The 419 theoretically possible PCB, PCDD, and PCDF congeners have a range of physicochemical characteristics [1–3], which profoundly affect their persistence, environmental distribution, and bioaccumulation in aquatic food chains [4,5].

The harbor seal (*Phoca vitulina*) is a small pinniped that is widely distributed throughout the temperate coastal waters of the Northern Hemisphere. The physiology, biology, and ecology of this marine mammal have been extensively studied as a result of its relative abundance and ease of capture. As with ringed seals (*Phoca hispida*) for the Arctic and herring gulls (*Larus argentatus*) for the Great Lakes (North America), harbor seals are increasingly viewed as an indicator species, providing an integrated measure of the contamination of coastal environments [6]. In British Columbia (BC), Canada, and Washington State, USA, harbor seals are common and nonmigratory, with adult ranges of approximately 20 km^2 [7]. Approximately 108,000 harbor seals are present in the coastal waters of British Columbia and 15,000 are present in the inland coastal waters (Puget Sound, Hood Canal, San Juan Islands, and Juan de Fuca Strait) of Washington State [8,9].

The transboundary waters of northern Washington State and southern British Columbia are not immune to problems of environmental contamination. Local fisheries closures in British Columbia in the late 1980s followed the discovery of high concentrations of PCDDs and PCDFs formed through chlorine bleaching processes by pulp mills and the use of pentachlorophenol as a wood preservative [10]. Studies of sediments and fish suggest that Puget Sound is highly contaminated with PCBs [11]. Elevated levels of PCBs, DDT, and other POPs have been detected in fish-eating birds from southern British Columbia [12]. Recently, we demonstrated that the southern resident and transient killer whales (*Orcinus orca*) of the northeastern (NE) Pacific Ocean are highly contaminated with PCBs, and can now be considered among the most contaminated marine mammals in the world [13].

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Table 1. Samples were collected from 60 young harbor seals inhabiting three basins (remote, moderately industrialized, and heavily industrialized) in British Columbia (BC, Canada), and Washington State (WA, USA), stretching from sites adjacent to northern Vancouver Island (Queen Charlotte Strait, [QCS], Canada) through the Strait of Georgia (Canada) and into southern Puget Sound (WA, USA). No significant intersite differences were found in body weight (analysis of variance)

Region	Basin	Site	Latitude (N)	Longitude (W)	*n*	Body wt (kg)
BC	QCS	Holford Island	50°44′	126°49′	5	22.8 ± 2.8
BC	Strait of Georgia	Hornby Island	49°29′	124°39′	8	21.3 ± 1.6
		Crofton	49°03′	123°43′	10	17.7 ± 0.7
		Vancouver	49°11′	123°12′	13	20.4 ± 1.2
		Victoria–Sidney	48°23′	123°29′	7	22.3 ± 2.0
WA	Puget Sound	Gertrude Island	47°13′	122°40′	17	20.6 ± 0.8

Although local sources of POPs used historically undoubtedly contribute to the contamination of coastal food webs in British Columbia and Washington, the long-range atmospheric transport and deposition of chemicals used in other parts of the world also likely play a role. Recent studies have detected PCBs and organochlorine pesticides in biota from remote parts of the North Pacific Ocean, including albatross (*Diomedea* sp.) from Midway Atoll, sea otters (*Enhydra lutris*) from the Aleutian Islands, and sockeye salmon (*Oncorhyncus nerka*) returning to Alaska (USA) [14–16]. We have speculated that POPs in salmon returning from the open Pacific Ocean may partly explain the contamination of NE Pacific killer whales because resident populations have a diet that is estimated to consist almost entirely of these anadromous fishes [13]. Such observations, coupled with recent evidence of rapid atmospheric delivery (5–8 d) of dust particles and pollutants from Asia to the west coast of North America [17], increasingly point to the NE Pacific Ocean as a sink for POPs and again underscore the global nature of POP dispersion.

The contamination of coastal food webs with POPs inevitably integrates multiple sources and processes, and reflects both the global background and a local signal that has been modified to some degree by atmospheric and aquatic fractionation. Here we evaluate the utility of the harbor seal as a high–trophic-level sentinel of coastal contamination by measuring PCB, PCDD, and PCDF concentrations and patterns in blubber biopsies from healthy, free-ranging harbor seal pups at several sites in British Columbia and Washington.

MATERIALS AND METHODS

We live-captured 60 harbor seal pups, estimated to be three to six weeks of age (according to age–body wt equations established previously [7]), from several locations in British Columbia and Washington State in 1996 to 97 (Table 1). Blubber and skin biopsy samples were obtained as part of three field projects. The first of these involved the 1996 live-capture of 22 harbor seals from three sites (Vancouver, Hornby Island, and Crofton) in the Strait of Georgia in southern British Columbia. Animals were transported to a temporary holding facility for a health assessment study before being released; blubber and skin biopsies were taken within 24 h of capture. The second project involved the 1997 live-capture of 21 seal pups from sites in the Strait of Georgia (Vancouver) and adjacent areas north (Queen Charlotte Strait) and southwest (Victoria–Sidney) of this basin. These animals were released immediately after taking blubber and skin biopsy samples. The third project involved the 1996 blubber and skin biopsy sampling of 17 harbor seal pups from Gertrude Island in south Puget Sound, Washington State.

Seals were live-captured as described elsewhere [7,18]. In all circumstances, capture stress and holding time were minimized. Blubber biopsies provided an integrated sample of the blubber layer to near the muscle by using an Acu-Punch 6-mm-diameter biopsy sampler (Acuderm, Ft. Lauderdale, FL, USA) after appropriate cleansing of the site and application of a local anesthetic as described in detail elsewhere [19]. Samples were wrapped in hexane-rinsed aluminum foil, placed in cryovials, and stored at −20°C until analysis. All procedures were carried out under the auspices of the respective animal care committees and scientific research permits for researchers in British Columbia (Fisheries and Oceans Canada Animal Care Committee with guidelines from the Canadian Council on Animal Care; Scientific Research Permit) and Washington State (U.S. Marine Mammal Protection Act Permit 835).

Blubber samples ranging from 0.1 and 0.3 g were analyzed for congener-specific PCBs, PCDDs, and PCDFs, as well as lipid content, after a dichloromethane–hexane extraction. Briefly, thawed blubber samples were ground and spiked with a mixture of $^{13}C_{12}$-labeled PCBs, PCDDs, and PCDFs (Cambridge Isotope, Andover, MA, USA) and processed by using procedures described previously [13,20]. Sample extracts were analyzed for PCDDs, PCDFs, mono-ortho, di-ortho, and non-ortho (planar) PCBs by using high-resolution gas chromatography–high-resolution mass spectrometry. Details on the chromatographic and mass spectrometric conditions, the criteria used for identification and quantitation, and quality assurance and quality control are also described elsewhere [13,20].

Although 160 PCB peaks were quantified (out of a theoretical total of 209 congeners), many congeners were not detectable in samples of harbor seal blubber. Total PCB concentrations were calculated as the sum of the concentrations of the 109 peaks that were detectable in at least 70% of the seal samples from all sites. Where congeners were undetectable, the detection limit was substituted. Congeners that were detected in less than 70% of the samples were not included in calculations. All results are expressed on a lipid weight basis. Total toxic equivalents (hereafter referred to as TEQ_{98}) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin were calculated for all dioxinlike mono-ortho and nonortho PCBs and 2,3,7,8-chloro-substituted PCDDs and PCDFs by using the most recent international mammalian toxic equivalency factors [21].

Principal components analysis (PCA) helped to elucidate the PCB, PCDD, and PCDF composition differences in seals from different regions. Both PCDD and PCDF concentration data were available for all 2,3,7,8-substituted congeners and the non–2,3,7,8-homologue totals. As in previous work [10,22], a non–2,3,7,8-isomer total was obtained for each isomeric group by subtracting the 2,3,7,8-isomer total from the isomer total. The PCDD and PCDF and PCB congeners and non–2,3,7,8-PCDD and non–2,3,7,8-PCDF isomer totals that were undetectable in fewer than 30 samples (50% of the 60-sample total) were removed from the data set after preliminary PCA. Undetectable values were replaced by a random number between zero and the limit of detection. The purpose here was to insert values that would not artificially skew the model, and a random number was expected to influence the PCA algorithm less than would an arbitrary, across-the-board, substitution (such as the detection limit, or one half of the detection limit). On average, 120 out of the 124 PCBs, PCDDs, and PCDFs used for PCA were detectable.

To avoid negative bias with normalized data, we employed the centered log ratio procedure. This method produces a normalized data set that is not affected by closure. Data were normalized to the concentration total followed by the geometric mean, and then were log transformed and autoscaled before PCA [23,24]. Finally, a Varimax rotation was applied to the first three principal components; this rotation maximizes or minimizes the loading of each variable on each principal component while preserving trends. One outlier was removed from the PCA model to give a final sample size of 59. Geometric mean regression [25] was used to quantify the relationships between Henry's law constant–log K_{OW} and PCA projections.

Prevailing wind patterns were plotted to provide insight into the atmospheric processes that may contribute to the dispersion of contaminants in the coastal region of British Columbia and Washington State (Fig. 1 inset). This wind reanalysis was carried out by using National Centers for Environmental Prediction/National Center for Atmospheric Research reanalysis data from the National Oceanographic and Atmospheric Administration–Cooperative Institute for Research in Environmental Sciences Climate Diagnostics Center (Boulder, CO, USA), from their Web site (http://www.cdc.noaa.gov/).

RESULTS AND DISCUSSION

Harbor seals occupy a high trophic position in the coastal food chains of British Columbia and Washington State. Although they are prone to accumulating elevated levels of many of the POPs, numerous factors influence the concentrations and patterns of contaminants to which they are exposed, including a multitude of partitioning processes in the environment, as well as physiological processes at different levels of the marine food chain and in seals. In our study, we compared concentrations and patterns of PCB, PCDDs, and PCDFs in biopsies from 60 young, healthy harbor seals of comparable age, body weight and condition from different sites in British Columbia and Washington (Table 1), effectively eliminating the major factors that often confound the interpretation of contaminant data in marine mammals.

Total concentrations of PCBs, PCDDs, and PCDFs in harbor seals

Concentrations of PCBs were particularly high in seals from Puget Sound (Fig. 1 and Table 2), likely reflecting the historical use of this persistent chemical in urban and industrial areas. Polychlorinated biphenyl contamination of Puget Sound has been exacerbated by the semienclosed nature of this basin, which is characterized by low sedimentation, and, hence, low-burial rates [26]. Levels of PCBs were considerably lower in seals from the Strait of Georgia and were lowest in seals from Queen Charlotte Strait, concurrent with increasing distance from urban and industrial areas. Concentrations of PCBs in the harbor seals in this study were at the upper end of, or exceeded, those reported in adult arctic ringed seals of varying ages [27], but were generally lower than those reported in stranded European harbor seals [28].

In contrast, concentrations of PCDDs and PCDFs were lower in seals from Puget Sound than in seals from the Strait of Georgia (Fig. 1 and Table 2). The high concentrations of PCDDs and PCDFs in seals from the Strait of Georgia likely reflect the persistence of pulp and paper industry pollutants that were introduced to parts of coastal British Columbia before the implementation of source controls in the late 1980s and early 1990s [29]. Elemental chlorine was extensively used as a pulp bleaching agent and pentachlorophenol was used as a wood preservative by pulp and paper mills in British Columbia, with many mills serving as a source of PCDDs and PCDFs for the Strait of Georgia (Fig. 1).

Patterns of PCBs, PCDDs, and PCDFs in seals

The composition of total PCBs in seals varied among basins, as evidenced by the greater proportion of the more heavily chlorinated homologue groups in Puget Sound, and the greater proportion of the less chlorinated homologue groups in Queen Charlotte Strait (Fig. 2). Patterns in the Strait of Georgia were intermediate to those of Puget Sound and Queen Charlotte Strait.

Principal components analysis further differentiated seals on the basis of the interbasin variation in PCB, PCDD, and PCDF congener proportions (Fig. 3a). Although seals from different sites within the Strait of Georgia are effectively indistinguishable from each other, those from Queen Charlotte Strait and Puget Sound are clearly different from those from the Strait of Georgia. The third principal component (7.6% of the variance) shows much the same sample distribution, with one Puget Sound outlier and a small separation of a few Crofton samples (not shown). Because a direct source correspondence exists between the positions of PCB, PCDD, and PCDF congener variables in a loadings plot and the positions of the seal blubber samples in a scores plot, the PCA results can be used to ascertain the relative inputs of the different contaminants to different animals (Fig. 3b). Seals from Puget Sound project to the upper left corner of the samples plot and have high proportions of the heavily chlorinated PCBs (6–10 Cl). The hexachloro PCB (143/134) that projects to the right of the *y*-axis is undetectable in 50% of the samples (the highest percentage of any variable) but it has been retained in the model because concentrations are well above the detection limit when it is present. Seal samples from Queen Charlotte Strait project on the lower right and have the highest proportion of the lower chlorinated PCBs (2–4 Cl). Seals from the Strait of Georgia project on the center right beginning to the left of the axis center, indicating a predominance of PCBs of intermediate chlorination (4–6 Cl) along with high proportions of PCDDs and PCDFs. The pentachloro PCBs are distributed relatively evenly about the *x*-axis in the variables plot, whereas the PCDDs and PCDFs project in a small cluster in the south-eastern quadrant.

Two basic input patterns emerge from the contaminant concentration results and the PCA. First, pulp and paper mills appear to be associated with the high total concentrations of PCDDs and PCDFs in seals sampled in the Strait of Georgia, and in the vicinity of Crofton (BC) in particular. Second, PCB

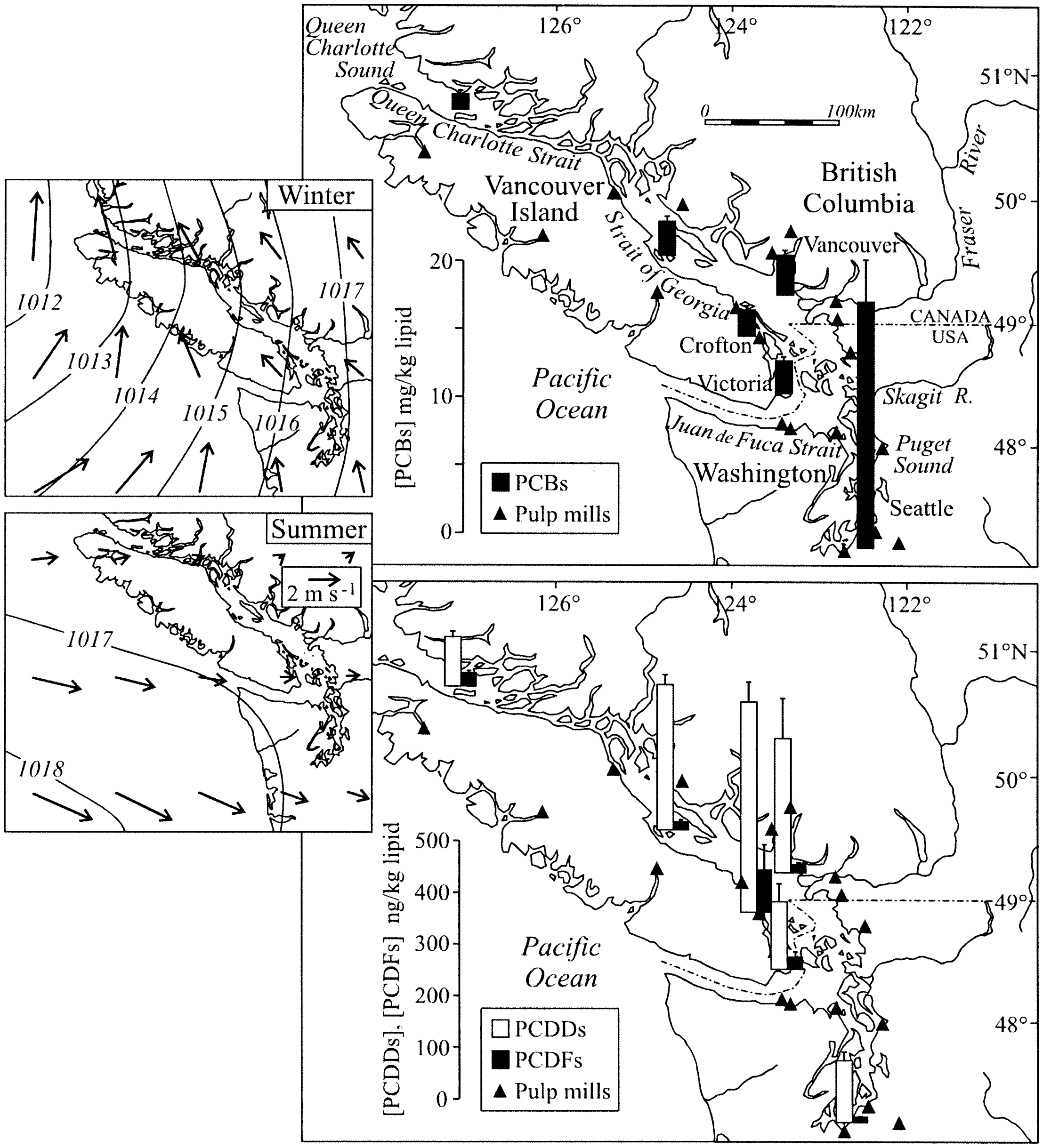

Fig. 1. Total mean polychlorinated biphenyl (PCB) concentrations (mg/kg lipid) (**above right**) and total polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) concentrations (ng/kg lipid) (**below right**) measured in blubber biopsies collected from healthy harbor seal pups (aged three to six weeks) from different sites in British Columbia (Canada) and the state of Washington (USA). Pulp and paper mill sites, representing sources of PCDDs and PCDFs before source controls, are indicated by triangles. Inset maps (**left**): A plot of reanalysis of winds provides an integrated overview of air currents for the study area at 10 m and sea level pressure (kPa) during cool (October to March) and warm (April to September) seasons and averaged over the 20-year period 1980 to 2000 (see *Materials and Methods*).

pattern profiles in seals exhibit a gradient away from the industrialized Puget Sound, suggesting a spatial fractionation process associated with the physicochemical characteristics of different congeners. The log of total PCB and total PCDD/PCDF concentrations are both significantly correlated ($p < 0.001$, $v = 57$) with t1 (the sample scores of the first principal component), but the correlation is much stronger with the PCBs ($r^2 = 0.820$) than with the PCDD/PCDFs ($r^2 = 0.168$). A significant correlation also is observed between the log of the Henry's law constant (log H; as calculated from the planar total surface area in the study by Hawker [30]) for the PCBs and p1 (the variable loadings of the first principal component; $p < 0.001$, $v = 112$; $r^2 = 0.610$; Fig. 4). The log H constant for each PCB is a fundamental parameter that describes the partitioning between surface waters and the atmosphere. In addition to planar total surface area, log H is also functionally related to other physical and chemical properties such as log K_{OW}, and thereby integrates a wide range of properties for the congeners in question [1,30]. Log H and log K_{OW} do not fully describe the fate of complex POP mixtures in a high–trophic-level species, but can serve as proxies to characterize important aspects of the fractionation and partitioning of PCB congeners among different environmental compartments (e.g., air, water, particulate, and lipid).

Although intersite differences in dietary preferences may exist, harbor seals are largely piscivorous and have a strong preference for Pacific herring (*Clupea harengus*) and hake (*Merluccius productus*) in the NE Pacific Ocean [31]. Our current research into food web contamination reveals that harbor seal food baskets (weighted composite seal prey samples)

Table 2. Concentrations of total polychlorinated biphenyls (PCBs) (μg/kg), polychlorinated dibenzo-*p*-dioxins (PCDDs) (ng/kg), and polychlorinated dibenzofurans (PCDFs) (ng/kg) in blubber biopsies collected from healthy harbor seal pups from three basins in British Columbia (BC, Canada) and the State of Washington (USA).[a] All values are expressed on a lipid weight basis

	QCS (A)	Strait of Georgia (B)	Puget Sound (C)	ANOVA *p* value	Tukey's HSD
ΣPCBs	1,143 ± 262	2,475 ± 174	18,135 ± 3,082	0.000	AC, BC
Σ2,3,7,8-PCDDs	72 ± 6	256 ± 31	119 ± 11	0.003	AC, BC
ΣPCDDs	96 ± 10	279 ± 32	119 ± 16	0.002	BC
Σ2,3,7,8-PCDFs	22 ± 2	16 ± 2	10 ± 1	0.080	—
ΣPCDFs	26 ± 4	25 ± 13	10 ± 1	0.458	—

[a] Total PCB concentrations did not differ among Strait of Georgia (Canada) sites, leading us to pool seals from this basin. Minor differences were noticed among Strait of Georgia sites for PCDDs and PCDFs. Data are expressed on a lipid weight basis as mean ± standard error of the mean. Interbasin differences were assessed by using analysis of variance (ANOVA); where significant, letters (A, B, C) indicate which two regions differ (Tukey's post hoc honestly significant difference [HSD] test). QCS = Queen Charlotte Strait (Canada).

from Puget Sound are dominated by more heavily chlorinated PCBs compared to the Strait of Georgia [32]. These findings are consistent with our observations in harbor seals, and indicate that the patterns of contaminants in lower trophic levels (i.e., seal prey) differ among basins. Collectively, these results support the idea of a preferential dispersion of lighter (hence, low log H and K_{OW}) PCB congeners away from sources and into more remote areas, where they become incorporated into local food chains.

The relative extent to which POPs are dispersed through the coastal environment of British Columbia and Washington via the movement of air and water masses, or migrating biota (e.g., fish), is unclear. However, atmospheric transport represents the major mechanism for the delivery of POPs into remote environments [3], with the more volatile compounds or congeners traveling greater distances [33]. Lesser chlorinated PCB, PCDD, and PCDF congeners increasingly dominate the total composition of each contaminant class away from source, reflecting their greater tendency to volatilize and travel great distances in the vapor phase [34]. More heavily chlorinated PCB, PCDD, and PCDF congeners, on the other hand, are less volatile and are more likely to be scavenged by particles, leading to a more rapid deposition into (and retention by) sediments in areas adjacent to sources. The increasingly light contaminant signature in our study seals inhabiting more remote areas (e.g., Queen Charlotte Strait) is consistent with the notion of atmospheric transport from distant sources. The light or remote signals observed in these seals, in turn, reflect a multitude of POP origins, including local, regional, and global sources. The heavy signature observed in seals from Puget Sound, on the other hand, is more typical of a local source signal, where the more chlorinated congeners are retained and dominate the composition of PCBs. Atmospheric influences were thought to partly explain the lighter PCB pattern in bald eagle eggs collected from northern Vancouver Island (Johnstone Strait), compared to those from the Strait of Georgia, although differences in dietary selection were also suspected of playing a role [35]. Although we cannot rule out a possible influence of different industrial PCB product applications on patterns observed in seals at different locations, the PCA results do not indicate any anomalous shifts of individual seals that might suggest different product uses in different areas.

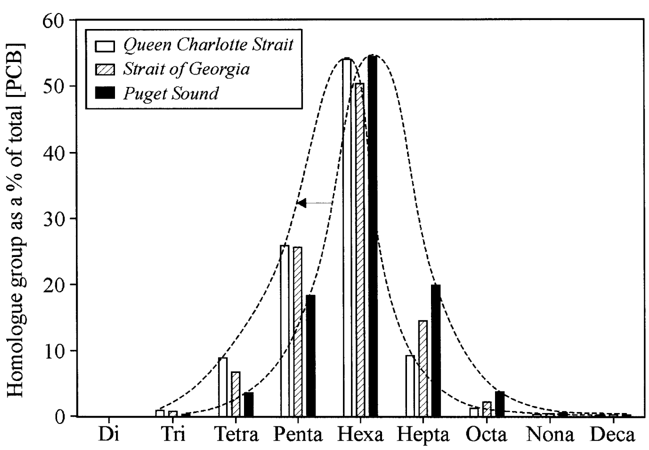

Fig. 2. Variation in basic polychlorinated biphenyl (PCB) patterns in harbor seals is first evident as differences in the contributions of homologue groups to total PCB concentrations among the three basins studied. Basic patterns of PCBs shifted (emphasized here by dotted bell curves and an arrow indicating direction of shift) from the more heavily chlorinated PCB homologue groups in Puget Sound (WA, USA) (e.g., hepta-, octa-, and nona-chloro PCBs) to the less chlorinated PCB homologue groups in seals from the remote Queen Charlotte Strait (Canada) (e.g., tri-, tetra-, and pentachloro PCBs).

Physiological factors affecting PCBs, PCDDs, and PCDFs in seals

In addition to the environmental processes that may differentially influence the concentrations and patterns of POPs in seals among sites, certain physiological factors also can play a role. Concentrations of many POPs in marine mammals increase with age in males, and decrease as a consequence of reproduction and lactation in mature females [13,36]. Condition of animals sampled represents another confounding factor that can affect the interpretation of contaminant analyses [37]. In our study, we minimized age-, sex-, and condition-associated variation in POP concentrations by collecting blubber biopsy samples from healthy, free-ranging harbor seal pups of approximately the same age (as inferred by body weight and defined pupping seasons at the respective sites; Table 1). Our three- to six-week age estimate suggests that seals from all sampling locations obtained their contaminant burdens almost entirely, if not exclusively, from their mothers through transplacental and milk transfer (harbor seals are weaned at ~32 d in this region, and undergo a subsequent fast [7]). It is not possible to describe the individual prey preferences of female seals (i.e., the mothers of our study pups), nor is it possible to fully characterize the distribution and feeding ecology of

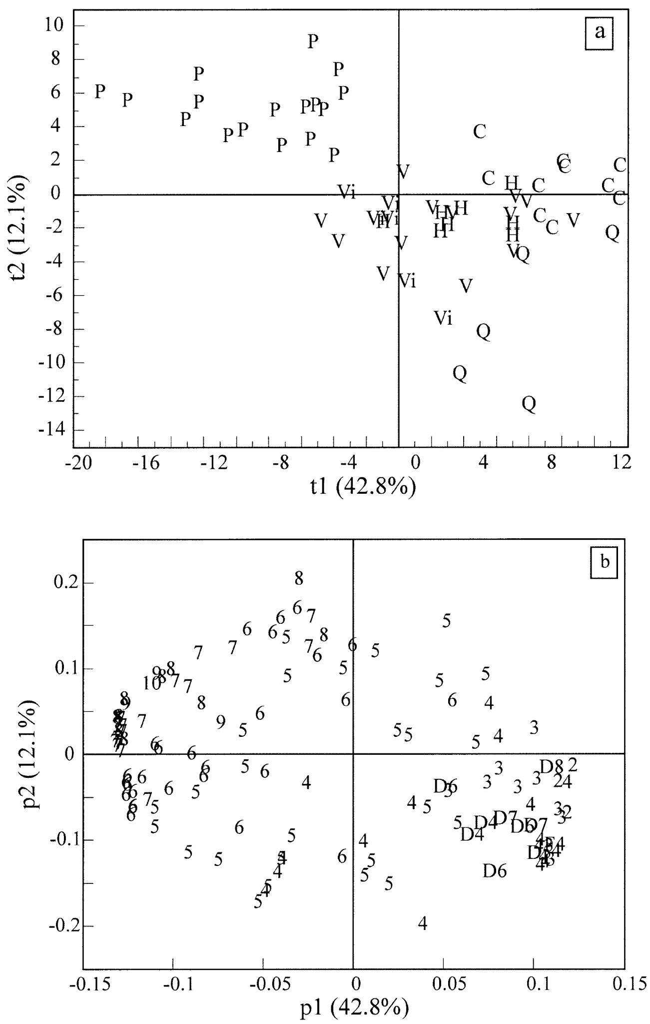

Fig. 3. Principal components analysis (PCA) model for the first two principal components where the variance accounted for by each principal component is shown in parentheses after the axis label. (**a**) Patterns of polychlorinated biphenyl (PCB), polychlorinated dibenzo-*p*-dioxin (PCDD), and polychlorinated dibenzofuran (PCDF) congeners in harbor seals differed among study sites, as demonstrated here by the sample scores plot (t1 and t2) of 59 seals. Symbols represent individual seals from the state of Washington (USA) (P = Puget Sound) and from British Columbia (Canada) (V = Vancouver; Vi = Victoria and Sidney; C = Crofton; H = Hornby Island; Q = Queen Charlotte Strait. (**b**) Prinicipal components analysis loadings plot (p1 and p2) for individual PCB, PCDD, and PCDF congeners based on measurements made in these same 59 harbor seals. Plotted numbers represent the chlorine number, with dioxins indicated by the prefix D, furans by F, and PCBs by no prefix.

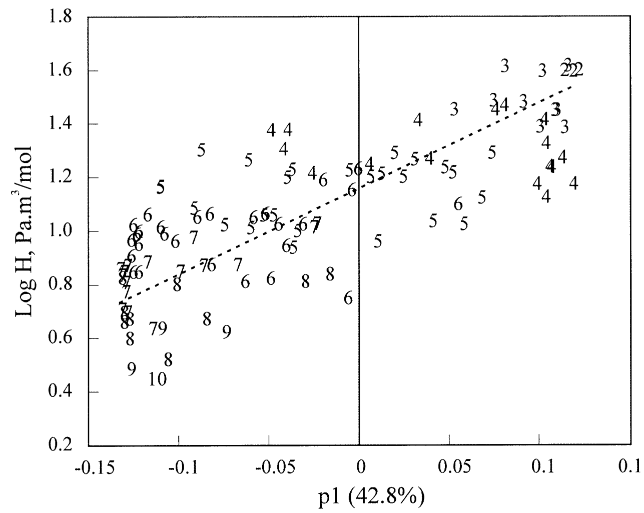

Fig. 4. The first principal component is tightly correlated ($r^2 = 0.610$; $p < 0.001$) with the Henry's law constant (log H) for polychlorinated biphenyl (PCB) congeners, suggesting that seals from more remote sites are exposed to increasingly light PCB mixtures, consistent with atmospheric signals, whereas seals from the industrialized Puget Sound (WA, USA) are exposed to heavier PCB mixtures. This suggests that log H represents an important factor influencing the transport pathways and partitioning of PCB mixtures in the environment, thereby affecting the ultimate pattern observed in harbor seals.

the prey species themselves. However, the 20-km² feeding range described for adult harbor seals in British Columbia [7] does provide a general indication of the spatial bounds for the contaminant signal measured in our study animals.

In addition to factors affecting the total concentrations of contaminant classes, the profiles or patterns of POPs in marine mammals can be altered by disease or fasting-associated alterations in blubber chemistry [37]. Nursing pups have lower proportions of the more heavily chlorinated PCB congeners relative to their mothers, reflecting an apparent barrier between females and their pups to congeners that are larger or have higher log K_{OW} values [38]. This may be the result of a physical barrier at the level of the placenta or mammary glands, or may reflect a differential congener-specific mobilization from the mother's blubber for milk production. Although we attempted to eliminate age and condition as confounding factors, even slight variations in age and body weights of our seals may have influenced the deposition or mobilization of different congeners from lipid stores. However, body weights of our seals did not differ among sites (Table 1; single factor analysis of variance, $p = 0.183$). In addition, no significant relationships were found between PCB patterns and body weights within sites (body wt vs homologue group measured as a proportion of total PCB concentration; regression analyses for all homologue groups $p > 0.05$; results not shown). This suggests that seals from different sites were of comparable age and condition.

The metabolic removal of certain congeners associated with the induction of detoxifying enzymes in the liver of the more contaminated pups sampled (or their mothers) represents another factor that could influence contaminant patterns within or among sites. The cytochrome P450 group of enzymes can be induced to preferentially eliminate PCBs with adjacent pairs of *meta*- and *para*-positions with no chlorine substituents [39,40]. The planar 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related congeners also have been shown to be rapidly eliminated by harbor seals [41]. A dose-dependent elimination of metabolizable congeners may therefore occur, with a resulting change in the composition of retained PCBs [40]. The PCA analysis within PCB homologue groups for each of the tetra- through heptachloro congeners (i.e., groups with similar physicochemical characteristics) revealed no differences between metabolizable (i.e., adjacent unsubstituted pairs at *meta*- and *para*- positions) and nonmetabolizable congeners, suggesting that metabolism is not contributing overtly to the pattern differences observed in seals among sites (results not shown). Further research is needed to characterize the role of metabolism in pattern changes of POPs in marine mammal food chains.

Table 3. Toxic equivalents (TEQ_{98}) to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from polychlorinated biphenyl (PCB), polychlorinated dibenzo-*d*-dioxin (PCDD), and polychlorinated bienzofuran (PCDF) classes in blubber biopsies collected from harbor seals in Queen Charlotte Strait (QCS, Canada), the Strait of Georgia (Canada), and Puget Sound (WA, USA)[a]. Italicized lines indicate breakdowns of the former parent line (ΣPCB TEQ_{98})

	QCS (A)	St. of Georgia (B)	Puget Sound (C)	ANOVA *p* value	Tukey's HSD
ΣPCB TEQ_{98}	12.5 ± 2.1	32.8 ± 2.1	158.1 ± 21.4	0.000	AC, BC
Σnon*ortho* PCBs TEQ_{98}	*4.3 ± 0.7*	*10.7 ± 0.6*	*36.9 ± 3.9*	*0.000*	*AC, BC*
Σmono-*ortho* PCBs TEQ_{98}	*8.2 ± 1.4*	*22.2 ± 1.5*	*121.2 ± 18.0*	*0.000*	*AC, BC*
ΣPCDD TEQ_{98}	10.5 ± 1.6	17.4 ± 1.4	12.2 ± 1.0	0.024	NS
ΣPCDF TEQ_{98}	2.2 ± 0.2	1.5 ± 0.2	2.0 ± 0.2	0.309	—
ΣTEQ_{98}	25.2 ± 3.9	51.7 ± 2.6	172.3 ± 22.4	0.000	AC, BC

[a] Data are expressed on a lipid weight basis as mean (ng/kg) ± standard error of the mean. Interbasin differences were assessed by using analysis of variance (ANOVA); where significant, letters (A, B, C) indicate which two regions differ Tukey's posthoc honestly significant difference [HSD] test). QCS = Queen Charlotte Strait. NS = not significant.

Health risks from exposure to contaminants

Toxic equivalency patterns suggest that, of the PCBs, PCDDs, and PCDFs, PCBs contribute the most to the dioxinlike toxic risk to seals from British Columbia and Washington. The TEQ_{98} values were highest in seals from Puget Sound, reflecting the overwhelming contribution of PCBs (91%) to the total TEQ_{98} (Table 3). A steady decrease occurred in the relative contribution of PCBs to total TEQ_{98} with distance from Puget Sound, with PCBs in seals from the Strait of Georgia and Queen Charlotte Strait contributing 64% and 49%, respectively, to the total TEQ_{98}.

The lack of detailed dose–response studies makes it exceedingly difficult to estimate risks for adverse health effects in free-ranging marine mammal populations; however, previous studies of both captive and free-ranging harbor seals do provide guidance for conservationists and managers. Our previous observation that vitamin A physiology in seals in southern British Columbia and Washington was disrupted by exposure to environmental contaminants highlights the potential for other health effects, because vitamin A is a nutrient that is essential to normal growth, development, and immune function [19]. Total mean TEQ_{98} in harbor seals from Puget Sound also approaches the threshold for immunotoxicity (~209 ng/kg) demonstrated in captive feeding studies of juvenile harbor seals [28,42], suggesting that even young seals from this region (i.e., before a lifetime accumulation) may be more vulnerable to diseases resulting from reduced immunocompetence. Although carefully controlled studies under laboratory or semifield conditions are often necessary to elucidate mechanistic effects of POPs on wildlife, evidence suggests that POPs continue to present health risks to populations of fish-eating birds and marine mammals around the world.

Harbor seals as sentinels of marine food chain contamination

The interbasin differences in PCB, PCDD, and PCDF patterns observed in harbor seals from British Columbia and Washington State largely reflect a combination of proximity to source and the physicochemical characteristics of the different congeners. In addition to the abiotic processes that cause a fractionation of PCBs, PCDDs, and PCDFs away from sources in our study areas, partitioning into the lipid fraction of marine food chains and subsequent bioaccumulation entail numerous processes, including partitioning between water and lipids, uptake and loss via gills of invertebrates and fish, uptake via diet by invertebrates, fish, and marine mammals, and metabolic removal by these organisms at different trophic levels. The physicochemical characteristics of the congeners in question are of great importance, with POP pattern changes at each trophic level reflecting congener-specific uptake and loss efficiencies, metabolic removal processes, and retention by lipid [43]. The ultimate mixture (pattern) to which seals are exposed through dietary intake will essentially integrate all of the abiotic and biotic interactions between emission (source) and uptake by their prey.

Although temporal trend analyses carried out on wildlife in the northern hemisphere indicate a rapid decline in the concentrations of many legacy POPs directly after the implementation of source and regulatory controls in the industrialized world, recent evidence is less encouraging [4,44]. Polychlorinated biphenyls persist in the environment as a consequence of leakage from contaminated sites, their persistence and cycling in the environment, and their delivery via atmospheric processes from distant regions where regulatory controls may not yet be in place. The global transport of semivolatile legacy and new POPs is likely to continue to present health risks for high–trophic-level biota and subsistence-oriented humans in the NE Pacific Ocean and elsewhere. Understanding how such complex mixtures fractionate among abiotic and biotic components of the environment represents an important aspect of characterizing the transport and fate of POPs, and provides a critical foundation for risk assessments on a regional and global scale.

Our research indicates that harbor seals can play a useful role in the understanding of contaminant accumulation in coastal food chains of the NE Pacific Ocean, and provide insight into the relative importance of regional versus international sources of persistent, bioaccumulative, and toxic compounds. In this context, our results provide insight into contaminant hot spots that may partly explain the high degree of PCB contamination in killer whales, another important high–trophic-level species in the NE Pacific Ocean [13].

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