Polychlorinated Biphenyls and Organochlorine Pesticides in Harbor Seal Pups from the Inland Waters of Washington State

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Blubber and liver samples from eight harbor seal (*Phoca vitulina*) pups, found dead at Puget Sound in 1990, have been analyzed for polychlorinated biphenyl (PCB) congeners including mono-*ortho* and non-*ortho* coplanar PCBs, hexachlorobenzene (HCB), *p,p′*-DDE, and mirex. Four of the seals were from Smith Island in the Strait of Juan de Fuca, and four were from southern Puget Sound primary at Gertrude Island. The levels of total PCBs, *p,p′*-DDE, and mirex are significantly higher in the seal samples from Gertrude Island than those from Smith Island. There was no significant difference in the level of HCB in seal samples between the two locations. PCB profiles were dominated by congeners 138, 153, and 180. The new PCB toxic equivalency factors (TEF) recommended by WHO (*1*), and the TEF for congener 81 from Harris *et al.* (*2*) was used for calculation of the contribution to dioxin-like toxicity from the PCB congeners. PCB congener 126 was the major contributor to PCB toxic equivalents (TEQs), followed by 156. The levels of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in marine mammals from Georgia Strait/Vancouver Island (3) were presented for reference purposes. Based on our studies of PCBs and PCDD/Fs data from the literature, the overall TEQs calculated for the monitored PCBs in Smith Island seals may contribute as much if not more dioxin-like toxicity as PCDD/Fs themselves.

Introduction

Polychlorinated biphenyl (PCB) congeners enter the food chain and are accumulated at most trophic levels (*4*−*6*). Marine mammals, that are top predators in the marine food chain, have residues of organochlorine compounds, such as PCBs, which are considered to be a possible cause of reproductive impairment (*7*−*9*). The levels of PCBs have been determined in marine mammal tissues for almost 3 decades. However, these analyses have generally been based on total PCB content. The PCBs in the marine mammals are accumulated via the food chain, and biotransformation of the original technical PCB mixtures has resulted in major changes of the congener composition (*6*). As each of the PCB congeners represents a unique combination of physical, chemical, and biological properties (*6*, *10*−*12*), the measurement of individual PCB congeners is important for evaluating the toxic potential of the residues.

Exposure to high levels of PCBs and other toxic chemicals reduces the immune competence of mammals (*13*−*15*). Beside immune suppression, PCBs also induce a variety of physiological dysfunctions and pathologies that may increase susceptibility to viral infection and impair the reproductive capacity (*8*, *16*−*18*).

It has been shown that PCB congener toxicity largely depends on the chlorine substitution pattern (*11*, *19*). The most toxic are the coplanar congeners, which are stereochemically similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). They exhibit biochemical activity and toxicity comparable to the activity of TCDD. From the limited amount of data available for individual PCB isomers, it appears that the environmental concentrations of biochemically active planar PCBs, especially the non-*ortho* and mono-*ortho* coplanar PCBs, are several orders of magnitude higher than those of TCDD (*19*). Hence, their environmental significance in terms of toxic potential is likely to be greater than that of TCDD (*20*).

The harbor seal is the most abundant species of marine mammal in Puget Sound. In addition, it breeds there and is the only species of marine mammal that resides there throughout the year (*21*−*23*). Because harbor seals feed at the top of the marine food chain, they are useful for monitoring the trophic transfer of chemical contaminants. Although past studies have found high concentrations of PCBs and *p,p′*-DDE in the blubber of harbor seals from several sites in Puget Sound (*24*−*27*), the concentrations of specific coplanar PCB congeners are unknown. The present study serves to fill this data gap. In addition, measurement of PCBs, HCB, *p,p′*-DDE, and mirex in the tissues of Puget Sound harbor seal pups provides an assessment of the level of bioaccumulation of these organochlorine compounds in mammals at the top level of the Puget Sound food web.

In the present study, congener-specific concentrations of PCBs including non-*ortho* and mono-*ortho* coplanar PCB congeners, HCB, *p,p′*-DDE, and mirex were determined in harbor seal pups from two sites located in different parts of the inland waters of Washington State. The 2,3,7,8-TCDD

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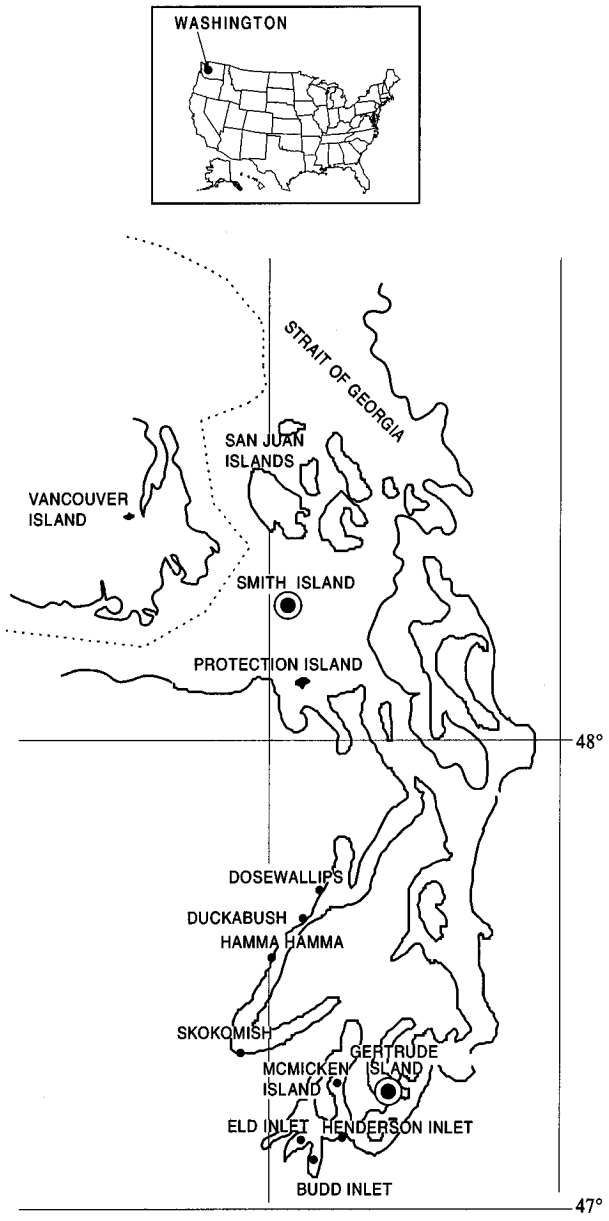

FIGURE 1. Collection sites of harbor seal pups.

toxic equivalents of non-*ortho*-, mono-*ortho*-, and di-*ortho*-substituted PCBs were estimated according to the toxic equivalent factors (TEF) proposed by WHO/IPCS (*1*).

Experimental Section

Reagents. All solvents used were nanograde from Burdick & Jackson, Muskegon, MI. The Aroclors and PCB congeners were obtained from AccuStand Inc., New Haven, CT.

Sampling. Harbor seal pups (generally ≤1 week old) were collected by Cascadia Research Collective (CRC) personnel in the summer of 1990 at two locations in the inland waters of Washington where harbor seals haul out on land (*27*). The two locations included Smith Island in the eastern Strait of Juan de Fuca (48° 19′ N, 122° 50′ W) and Gertrude Island in southern Puget Sound (47° 13′ N, 122° 39′ W) as shown in Figure 1. Beach searches for dead newborn seal pups were conducted at Smith and Gertrude Islands using the strategies and methods described in previous studies (*22*, *28*). Searches were timed to correspond with the pupping season. At Smith Island, searches were conducted on 9 days between June 13 and August 14, 1990. The eastern shore of Smith Island and all of Minor Island were searched on foot. However, portions of the beach could not be searched because seals were hauled out during some searches. At Gertrude Island, searches were conducted on 7 days between July 31 and September 13, 1990. All of Gertrude Island and the eastern shore of Still Harbor were searched on foot. The western shore of Still Harbor was searched from a boat.

Dead pups were selected for analysis and necropsied according to Puget Sound Estuary Program guidelines (*29*). Of the 43 dead pups (25 from Smith Island and 18 from Gertrude Island) found during field sampling, four were selected for the present study from each island. The eight pups analyzed were selected because they were completely intact (no scavenging), recently dead (little post-mortem degeneration of tissues), not emaciated, full-term, and generally 1 week old or less (with one exception). The carcasses of the eight pups were either necropsied at the collection site or kept cool on ice and returned unfrozen to the laboratory for necropsy. For each selected pup, weight, standard length, axillary girth, sex, age, and blubber thickness were determined. Biometric data of eight specimens analyzed are given in Table 1.

Blubber tissue was sampled from the mid-ventral region. These samples included the full thickness of the blubber layer. For liver samples, the posterior portions of the left two lobes were collected. Approximately 50−150 g of each kind of tissue was removed and immediately transferred to precleaned borosilicate glass jars with Teflon-lined lids. Samples collected in the field were stored on ice in a cooler and frozen upon return. Aliquots (10 g) were shipped to the Wadsworth Center for PCBs, HCB, *p,p′*-DDE, and mirex analyses. All samples were frozen at −20 °C until analysis.

Sample Extraction and Cleanup. Blubber (0.1−1 g) and liver (1−7 g) samples were weighed into 20-mL screw-top liquid-scintillation vials and mixed by spatula with 1−6 g of sodium sulfate. The samples and sodium sulfate were ground together with 10 mL of hexane in a Tekmar tissuemizer (Cincinnati, OH) for 1 min. Two more extractions with 10 mL of hexane each were performed using the tissuemizer and added to the Kuderna−Danish (K-D) evaporator.

The combined extracts were concentrated to approximately 1−2 mL in a K-D evaporator with a three-ball Snyder column on a steam bath. The extract was quantitatively transferred to a 1-cm diameter glass column containing 10 g of 4% deactivated Florisil and 2 g of sodium sulfate. The column was eluted with hexane, and the first 60 mL of the eluate was collected. The 60-mL fraction was concentrated to 1−2 mL with K-D apparatus and then further concentrated under a gentle stream of nitrogen (ultra-high purity) to ~100 μL and subsequently chromatographed on a porous graphitic carbon column (PGC).

Liquid Chromatography. The HPLC system consisted of a Model 6000A solvent-delivery pump, a U6K injector, and a Model 440 UV absorbance detector (Waters Associates Inc., Milford, MA). A porous graphitic carbon column (100 × 4.7 mm, 7-μm particle size, Hypercarb, Shandon Scientific, Ltd., U.K.) was used for the isolation of 12 non-*ortho* and mono-*ortho* coplanar PCBs (*30*, *31*). The PGC column was fitted with a 7040 Rheodyne switching valve to enable back-flushing of the column. An SP 4100 computing integrator (Spectra-Physics, Santa Clara, CA) was used. The column was eluted with hexane at 2 mL/min for 2 min and then back-flushed with the same solvent

TABLE 1

Characteristics of Harbor Seal Pups Selected for Chemical Analysis from Smith and Gertrude Islands

seal no.	sampling date[a]	sampling site	sex	age (wk)	standard length (cm)	axillary girth (cm)	weight	blubber thickness (cm)
CRC-344	July 4	S[b]	M	<1	85	48	11.5	1.5
CRC-347	July 6	S[b]	F	<1	80	49	9.3	1.0
CRC-349	July 11	S[b]	M	1	83	45	8.2	0.9
CRC-350	July 11	S[b]	M	1	85	56	13.5	1.8
CRC-375	Aug 21	G[c]	M	1	86	54	13.3	1.9
CRC-381	Sep 1	G[c]	F	~4	93	65	19	2.5
CRC-392	Sep 13	G[d]	M	1	87	51	12.6	1.2
CRC-393	Sep 13	G[d]	F	1	84	45	9.5	0.8

[a] All dates are 1990. [b] Smith Island. [c] Southern Puget Sound (put in the same vicinity as Gertrude Island). [d] Gertrude Island.

for 6 min. The nonplanar PCBs and pesticides were collected as the first fraction of 4 mL; mono-*ortho*- and non-*ortho*-substituted PCBs were collected as the second fraction of 12 mL by reverse elution. Both fractions were analyzed by GC/ECD. The detailed PGC methodology was described elsewhere (*30*).

Identification and Quantitation. The PCB fraction was chromatographed on an Ultra II (25 m × 0.2 mm × 0.33 μm film thickness, Hewlett Packard) capillary column using a Hewlett Packard 5890 gas chromatograph equipped with a ^{63}Ni electron-capture detector (ECD). PCB 77 was confirmed on an SB-octyl 50 capillary column (50 m × 0.2 mm × 0.25 μm film thickness, Lee Scientific, Salt Lake City, UI). The oven temperature schedule for the DB-5 column was as follows: initial temperature 100 °C for 2 min, then programmed at 10 °C/min to 160 °C, then 1 °C/min to 190 °C, 2 °C/min to 270 °C, and kept at 270 °C for 2 min. The oven temperature schedule for the SB-octyl 50 column was as follows: initial temperature 110 °C for 2 min, then programmed at 10 °C/min for 6 min, then 2 °C/min until 300 °C, and kept at 300 °C for 10 min. An equivalent mixture of Aroclors 1221, 1016, 1254, and 1260 with known PCB composition and content amended with mirex and *p*,*p*′-DDE at concentrations of 10 ng/mL each and hexachlorobenzene at 5 ng/mL was used as a calibration standard (external standardization) for the quantification of two to four *ortho*-substituted PCBs, HCB, *p*,*p*′-DDE, and mirex (*32*, *33*). A coplanar PCB standard that contained 10 ng/mL each of eight mono-*ortho*- and four non-*ortho*-substituted PCBs was used to quantitate coplanar PCBs (*30*, *31*). Total PCB concentrations were obtained by summing up the concentrations of individual isomers detected. These two standard mixes were used daily to check the instrument performance, reproducibility, and sensitivity.

Mean recoveries for HCB, *p*,*p*′-DDE, mirex, and PCB congeners including mono-*ortho*- and non-*ortho*-substituted PCBs averaged 93% and 91% and ranged from 89% to 107% and from 85% to 97% for three spiked blubber and three spiked liver samples, respectively.

Results and Discussion

Levels of Contaminants. Figure 2 shows the GC/ECD chromatograms of the first and second fraction from PGC chromatography of a blubber and a liver sample. The mean concentration and range of selected PCBs in blubber and liver samples from eight seals from the two different sites are given in Tables 2 and 3 (in ng/g wet weight). Some co-eluting pairs on the DB-5 column are indicated by a double identity. The first mentioned congener of each of these pairs was the major one. Congener 153 was the most abundant PCB in all samples analyzed. IUPAC numbers 52, 74 (94), 99, 101, 118, 128, 138, 146, 149, 153 (132), 170 (190), 180, and 187 (181) are the major PCBs (>2% of total

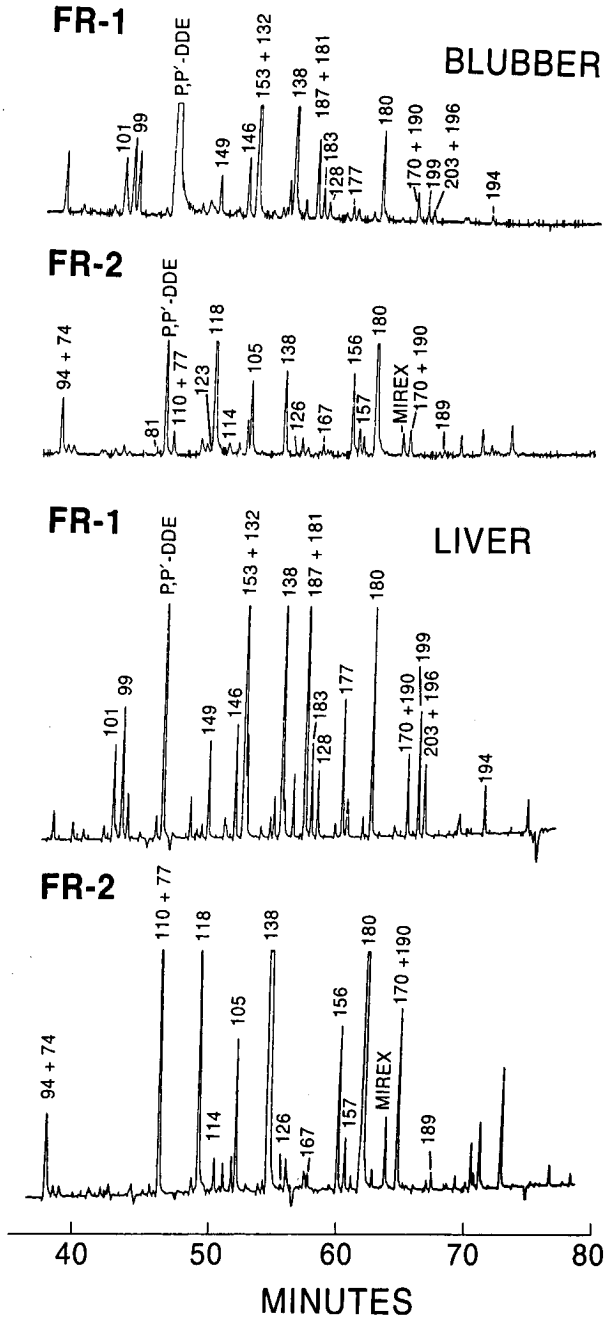

FIGURE 2. Chromatograms of fractions from porous graphitic carbon separation of a blubber sample and a liver sample.

TABLE 2

Mean Concentration (ng/g wet wt) of PCBs (≥1%) in Blubber of Seals from Two Sites

congener[a]	IUPAC No.	Gertrude Island mean	Smith Island mean
25/25	52	270 (226−367)	41 (36−55)
235/26 + 245/4	94 + 74	288 (233−365)	52 (30−73)
24/34	66	143 (102−209)	26 (19−30)
245/25	101	752 (546−1013)	98 (79−124)
245/24	99	730 (667−870)	94 (65−121)
236/34	110	248 (147−457)	33 (22−47)
236/245	149	348 (194−509)	47(43−54)
245/34	118	352 (201−449)	52 (32−91)
235/245	146	464 (337−578)	42 (32−55)
245/245 + 234/236	153 + 132	2410 (1780−3252)	236 (183−292)
234/34	105	239 (159−313)	43 (35−52)
234/235	130	285 (103−451)	27 (7.4−43)
234/245	138	1711 (1167−2195)	168 (116−209)
2346/34	158	287 (196−406)	26 (12−35)
2356/245 + 2345/246	187 + 181	549 (343−692)	49 (40−59)
2346/245	183	201 (125−268)	21 (15−28)
234/234	128	424 (324−495)	62 (427−108)
2345/34	156	150 (87−194)	19 (14−29)
2345/245	180	558 (322−856)	69 (67−94)
2345/234 + 23456/34	170 + 190	300 (191−422)	28 (19−37)
23456/245 + 2345/2346	203 + 196	210 (118−271)	26 (15−34)
2345/2345	194	192 (85−311)	20 (4.8−32)

[a] In GC elution order. Numbers in parentheses indicate the concentration range.

TABLE 3

Mean Concentration (ng/g wet wt) of PCBs (≥1%) in Liver of Seals from Two Sites

congener[a]	IUPAC No.	Gertrude Island mean	Smith Island mean
25/25	52	6.4 (4.9−11)	0.19 (0.13−0.23)
245/25	101	21 (11−40)	0.50 (0.27−0.84)
245/24	99	31 (15−66)	0.55 (0.35−0.82)
236/34	110	9.6 (6.8−13)	0.14 (0.1−0.2)
236/245	149	13 (7.6−23)	0.33 (0.21−0.54)
245/34	118	9.8 (4.5−20)	0.29 (0.1−0.59)
235/245	146	15 (7.6−32)	0.18 (0.11−0.28)
245/245 + 234/236	153 + 132	80 (39−177)	1.1 (0.59−1.58)
234/34	105	5.4 (2.2−11)	0.14 (0.05−0.27)
2346/236 + 2356/34	176 + 163	7.8 (5.6−14)	0.22 (0.12−0.43)
234/245	138	78 (44−166)	1.1 (0.69−1.8)
2356/245 + 2345/246	187 + 181	38 (26−53)	0.68 (0.49−0.87)
2346/245	183	9.6 (4.4−21)	0.09 (0.04−0.13)
234/234	128	14 (7.1−31)	0.20 (0.1−0.31)
2356/234	177	9.8 (6.6−13)	0.22 (0.14−0.33)
2345/245	180	29 (12−65)	0.27 (0.13−0.34)
2345/234 + 23456/34	170+190	14 (6.7−32)	0.23 (0.15−0.38)
2345/2356	199	9.8 (6.9−16)	0.10 (0.05−0.15)
23456/245 + 2345/2346	203 + 196	8.5 (4.1−18)	0.10 (0.03−0.19)

[a] In GC elution order. Numbers in parentheses indicate the concentration range.

PCB) detected in seals, which constituted ca. 60−70% of the total PCB content from the 73 congeners quantified in blubber and 54 congeners quantified in liver. Compounds that are often present in a moderate concentration (1−2% of total PCB) are 66, 105, 110, 130, 141, 156, 158, 176 (163), 177, 183, 194, 199, and 203 (196). A common feature of compounds detected in tissues is the 2,4,5-trichloro substitution on one of the phenyl rings and a *para*-chlorine on the second phenyl ring (i.e., congeners 74, 99, 118, 153, 156, 170, and 180). The most persistent PCB congeners are primarily penta-, hexa-, and heptachlorobiphenyls. Substitution at the 2, 3, and 4 positions is also common in the detected congeners (i.e., congeners 105, 138, 156, 170, 180, and 183). The pattern of the 26 major PCB congeners in both blubber and liver samples from two sites is shown in Figure 3. The figure shows that the distribution patterns of PCBs in different tissues, blubber and liver, were very similar. Boon et al. (*34*) has reported that the relative pattern of PCBs in different organs of harbor seals is virtually identical. The actual concentrations in each organ are different and depend primarily on the lipid levels in that tissue, but the ratio of the PCBs remains approximately constant. Although absolute distributions were 10 times higher at Gertrude Island relative to Smith Island, the congener distributions were very similar (see Figure 3). The less contaminated seals from Smith Island had more PCBs with lower degrees of chlorination than in the more highly contaminated samples from Gertrude Island. This agrees well with the findings of Heidmann et al. (*35*).

Theoretically, PCB patterns and concentrations in organisms are determined by the concentrations in the external environmental compartments such as water, sediment, and food and by internal physiological processes such as lipid metabolism and biotransformation. The gill-breathing species can take up their PCBs from water, sediments, and food; the main source for lung-breathing

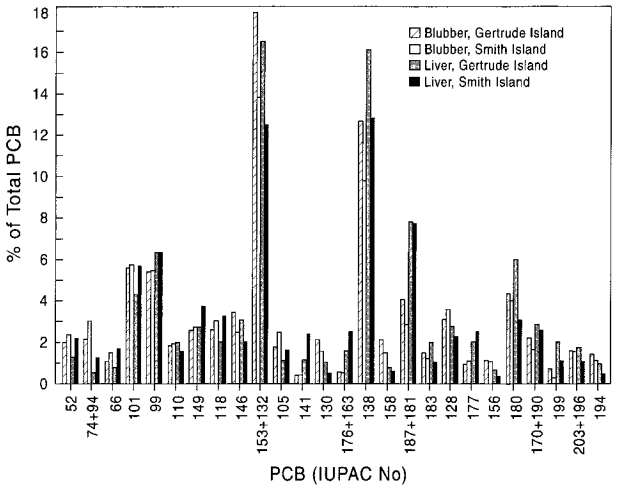

FIGURE 3. Relative PCB isomeric composition in blubber and liver of seals from Gertrude and Smith Islands.

animals is the diet. Since the PCB pattern of the food actually eaten by the seals is unknown in the present study, the relative ratio R_{rel} of individual congener in a given tissue of a seal in comparison to its diet can not be calculated.

Boon et al. (*36*) classified individual congeners into a number of structural groups based on the results of experimental study with seals (*37*) and a field study involving cetaceans (*20*): I, congeners without any vicinal hydrogen (H) atoms, e.g. IUPAC 146, 153, 180, 183, 187, 194, and 203; II, congeners with vicinal H atoms in the *ortho* and *meta* positions and ≥ 2 *ortho*-Cl atoms, e.g. IUPAC 99, 128, 130, 138, 158, and 170; III, congeners with vicinal H atoms in the *ortho* and *meta* positions and 1 *ortho*-Cl atom, e.g. IUPAC 74, 66, 105, 118, and 156; IV, congeners with vicinal H atoms in the *meta* and *para* positions and ≤2 *ortho*-Cl atoms, e.g. IUPAC 52, 101, and 110; V, congeners with vicinal H atoms in the *meta* and *para* positions and ≥3 *ortho*-Cl atoms, e.g. IUPAC 149. Boon et al. (*36*) concluded that biotransformation by the cytochrome P450-dependent monooxygenase system was the cause for values of $R_{rel} \ll 1$ (congeners belonging to groups III, IV, and V) and that groups I and II, containing congeners of which the pattern did not change from prey to predator ($R_{rel} \geq 1$), contained congeners that were highly persistent to biotransformation in seals. Lake et al. (*38*) reported that seals possess mixed function oxidase (MFO) systems which, when induced, metabolize PCB congeners. They also suggested that seals, like other marine mammals and fish, may lack phenobarbital (P450II-B) type enzymes (*20*). Similar distributions of congeners have been found in other seal studies (*36*, *38*–*42*).

HCB, *p*,*p*′-DDE, and mirex were detected in all eight seals. The range and mean concentrations of total PCB, total coplanar PCB, HCB, *p*,*p*′-DDE, and mirex, and *t*-value and *p*-value are shown in Table 4. PCB mean levels were two to five times higher than *p*,*p*′-DDE mean levels. Relatively low mean levels, ranging from 2.3 to 8.5 ng/g of HCB and from 7.5 to 60 ng/g of mirex, were found in the seal blubber (Table 4). Positive correlations between the levels of total PCB and total coplanar PCBs, *p*,*p*′-DDE, and mirex were found to be significant ($r = 0.99$, 0.86, and 0.93 for total coplanars, *p*,*p*′-DDE, and mirex, respectively; $n = 8$; $p < 0.05$). There also was a negative correlation between total PCBs and HCB in blubber ($r = 0.66$; $n = 8$; $p < 0.05$); the reason for this is unknown. The positive correlations

TABLE 4

Mean Concentration and Range of Total PCB, Total Coplanar (Non-, Mono-, and Di-*ortho*) PCB, *p*,*p*′-DDE, HCB, and Mirex in Blubber and Liver Tissue of Harbor Seal Pups from Gertrude and Smith Islands

	Gertrude Island		Smith Island			
	mean	range	mean	range	*t*	*p*
Blubber (μg/g wet wt)						
total PCB	13.1	9.2–16	1.7	1.3–2.1	7.44	0.005
total coplanar	1.75	1.1–2.3	0.23	0.17–0.32	5.7	0.01
HCB (ng/g)	3.5	2.3–5.3	5.9	4–8.5	1.95	0.1
p,*p*′-DDE	2.9	1.6–4	0.75	0.31–1.2	4.02	0.007
mirex (ng/g)	48	31–60	16	7.5–22	4.32	0.005
Liver (ng/g wet wt)						
total PCB	490	290–970	9	6–14	2.95	0.06
total coplanar	66	32–144	0.94	0.43–1.6	2.47	0.090
HCB	0.14	0.06–0.27	0.05	0.03–0.07	2.01	0.13
p,*p*′-DDE	77	28–190	2.4	1.2–3.9	1.96	0.15
mirex	2.5	1.3–4.3	1.1	0.46–1.7	1.93	0.10

between total PCB and *p*,*p*-DDE and mirex in all the samples analyzed probably reflects parallel accumulation of these substances in the food chain. Concentrations of PCBs and *p*,*p*′-DDE in the blubber of harbor seal pups from Puget Sound have declined significantly from the 1970s through 1990 (*27*).

The concentrations of total PCB, HCB, *p*,*p*′-DDE, and mirex in the blubber of seals may vary by blubber thickness and age of the pup. The ages of the pups in this study were generally 1 week old or younger (except that one seal was 4 weeks old). The 4-week-old seal from Gertrude Island did not contain higher concentrations of PCBs and pesticides compared with the others. The difference of blubber thickness between Gertrude and Smith seals was not significant with the mean value of 1.6 and 1.3 cm, respectively. These two factors (blubber thickness and age) were similar between the two sites. The PCBs observed in the pups at both sites are presumably the result of in-utero and lactational exposure. We cannot predict the relative contribution of these exposure pathways to the total PCB burden from the present data. However, it is likely that the pups PCB burden resulted primarily from in-utero exposure, since they could have nursed for only 1 week maximum (except one for 4 weeks). Given the pups early death, they may have failed to nurse at all. Regardless of the exposure pathway (i.e., transplacental or lactational), the PCB burden in the pups undoubtedly reflects their mothers' diet.

Dietary differences may also explain the variation in PCB burden between the two sites. The islands of northern Puget Sound are heavily encircled with kelp beds, attracting a variety of rockfish and transient pelagic fishes (*43*). In contrast, sandy, shallow-sloping, and cobble-laden bottoms are more common in southern Puget Sound, providing good habitat for benthic flatfish and shellfish (*43*). In contaminated waters, these species generally contain higher PCB levels than pelagic prey due to excessive uptake from the sediment interface (*44*). Thus, the local conditions between the Smith Island and Gertrude Island sites may predispose the seals to different PCB burdens because prey selectivity is restricted to species that are more (or less) contaminated. Alternatively, prey selected by the seals may be similar at the two sites, indicating that the reduced levels of PCBs from Smith Island pups are due to dilution of point-source

TABLE 5

Mean Concentrations and TEQ Values for Non-, Mono-, and Di-*ortho*-PCBs in Blubber of Harbor Seal Pups from Two Locations of Puget Sound

	mean concn (ng/g wet wt)			TEQ (pg/g wet wt)	
	Smith	Gertrude	TEF[a]	Smith	Gertrude
Non-*ortho*-PCBs					
345/4 (81)	1.9	4.8	0.004[b]	7.6	19.2
34/34 (77)	3.5	9.7	0.0005	1.8	4.9
345/34 (126)	0.6	7.7	0.1	60	770
345/345 (169)	<0.1	<0.1	0.01	<1	<1
Mono-*ortho*-PCBs					
345/24 (123)	2.9	9.3	0.0001	0.29	0.93
245/34 (118)	52	352	0.0001	5.2	35.2
2345/4 (114)	9.0	65	0.0005	4.5	32.5
234/34 (105)	43	239	0.0001	4.3	23.9
245/345 (167)	2.4	8.3	0.00001	0.024	0.083
2345/34 (156)	19	150	0.0005	9.5	75
234/345 (157)	2.5	41	0.0005	1.25	20.5
2345/345 (189)	0.9	4.8	0.0001	0.09	0.48
Di-*ortho*-PCBs					
2345/234 (170)	28	300	0.0001	2.8	30
2345/245 (180)	70	558	0.00001	0.7	5.6
total	236	1749		98.1	1019

[a] TEF, toxic equivalency factors from Ahlborg et al. (*1*). [b] TEF from Harris et al. (*2*). Numbers in parentheses indicate IUPAC No.

PCB contamination from southern Puget Sound. Tacoma has a large smelter and several chemical plants, and Everett and Tacoma have wood products industries (*44*). Seattle on the Duwamish River is an industrial area about 1 mi downstream from a point where over 200 gal of PCB-containing fluid had been spilled in September 1974 (*45*). PCB levels in cottids, mussels, and sediment were reported to be highest at or near the industrial areas of southern Puget Sound since municipal and industrial outfalls provide the major input of PCB into the environment (*44, 46*).

Mean levels of total PCB, *p,p′*-DDE, and mirex were significantly higher in the blubber tissue of seals from Gertrude Island as compared with the corresponding mean levels in seals from Smith Island (Table 4) ($p < 0.01$). No significant regional difference was found for HCB ($p > 0.1$) in seal samples. In liver samples, the trends in variations observed in PCB, *p,p′*-DDE, and mirex were similar to those found for the blubber sample ($p \leq 0.1$) (Table 4). The higher concentrations of PCB observed in seals from Gertrude Island as compared with those from Smith Island were consistent with the relative concentrations found in different age classes of seals from the two areas in past studies (*25*). This consistent difference between the two areas is likely the result of the closer proximity of Gertrude Island to some of the chemically contaminated urban bays of central and southern Puget Sound, where the concentrations of PCBs are generally elevated in environmental media. *p,p′*-DDE and mirex followed the geographical trend of total PCB. The geographical trend of HCB in seal blubber was different from that observed from the PCBs, which may reflect different sources of the compound.

Dioxin-like PCBs. The mean concentrations of non-, mono-, and di-*ortho*-PCB congeners in the blubber of seal pups are listed in Table 5. The concentrations of four non-*ortho* congeners, congener 81 ranged from 0.2 to 7.1 ng/g wet wt (mean 4.8 and 1.9 ng/g wet wt for Gertrude and Smith Island seals, respectively), congener 77 ranged from 0.6 to 17 ng/g wet wt (mean 9.7 and 3.5 ng/g wet wt for Gertrude Island and Smith Island seals, respectively), congener 126 varied between 0.3 and 10.5 ng/g wet wt (mean 7.7 and 0.6 ng/g wet wt for Gertrude and Smith Island seals, respectively), and congener 169 not detected in any sample, are given in Table 5. The concentrations of non-*ortho* coplanar PCBs (except congener 169, which was not detected) in blubber were found to be 3−4 orders of magnitude lower than total PCBs. The residual concentrations of mono- and di-*ortho* congeners were high, ranging (mean) from 0.9 to 350 ng/g wet wt for eight mono-*ortho* congeners, 20−860 ng/g wet wt for two di-*ortho* congeners.

3,3′,4,4′,5,5′-Hexachlorobiphenyl (no. 169) was not detected in any sample. Among the other three non-*ortho* coplanar PCBs, the mean residue levels in blubber were found to be in the order of nos 77 > 81 > 126 for Smith seals and nos 77 > 126 > 81 for Gertrude seals. Murk et al. (*47*) reported that 3,3′,4,4′-tetrachlorobiphenyl (no. 77), which possesses adjacent chlorinated *meta* and *para* positions, is metabolized *in vitro* by both the porpoise and the seal. The same goes for the conclusion of Boon et al. (*48*) that seals can metabolize PCBs with a planar configuration when there are adjacent H atoms present in the *ortho* and *meta* positions. Several other authors have determined non-*ortho*-substituted PCBs in marine mammals (*49, 50*). Their results show that marine mammals do possess a metabolic degradation capacity for PCBs 77 and 126. Kannan et al. (*51*) reported that the metabolism of three non-*ortho* planar PCBs is in the order IUPAC 77 (2 adjacent *o-m* H atoms) > IUPAC 126 (1 adjacent *o-m* H atoms) > IUPAC 169 (no *o-m* H atoms). The concentration of the more biodegradable PCB 77 remained highest despite the possibility that induced microsomal enzymes might have metabolized it substantially. However, the extent of exposure to congener 77 is higher because of its presence at 3−4 orders of magnitude greater than congeners 126 and 169 in commercial PCB preparations (*31*). Therefore, a higher exposure than elimination rate may be a plausible explanation for the maximum proportion of congener 77 despite enzyme induction.

Dioxin Toxic Equivalents (TEQs) of the Coplanar PCB Congeners. Several of the non- and mono-*ortho*-substituted PCBs induce biological effects similar to those caused by polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs). In analogy to what has been established for PCDDs and PCDFs, the toxicity of such PCB congeners can be expressed as a fraction of the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), resulting in a toxic equivalency factor (TEF). Different TEF values have been previously used by Safe (*11, 52*) and by a Nordic expert group (*12*). The discrepancy between the two models results mainly from the fact that Safe has given more weight to *in vitro* structure−activity relationships while the Nordic expert group has included *in vivo* data, which reflect to some extent the differences in toxicokinetics between the compounds. These TEFs proposed by the Nordic expert group are in most cases 5−10 times lower than the values derived by Safe. Recently, the WHO-European Center for Environmental and Health (WHO-ECEH) and the International Program on Chemical Safety (IPCS) have initiated a project to create a database containing information relevant to the setting of TEFs. TEFs were recommended for three non-*ortho*-, eight mono-*ortho*-, and two di-*ortho*-substituted PCBs by 12 experts from eight countries. In the present study, the internationally agreed TEFs for 13

PCBs proposed by WHO/IPCS and TEF for congener 81 estimated by Harris et al. (*2*) are used. Multiplication of the concentration of potent PCB by its assigned TEF value gives its concentration in terms of TEQs. The estimated TCDD toxic equivalents of four non-*ortho*-, eight mono-*ortho*-, and two di-*ortho*-PCBs in harbor seal blubber are presented in Table 5. Based on these TEF values, total TEQ for PCBs was 1019 pg/g wet wt from Gertrude Island and 98 pg/g wet wt from Smith Island for seal blubber. The coplanar PCBs 126, 156, 118, 114, 105, 81, 77, 170, and 157 are the major contributors to total TEQ of PCBs in all blubber samples. Non-*ortho* coplanar PCB 126 contributed the greatest percentage to the TEQ in the blubber of harbor seals from Gertrude Island and Smith Island at 76% and 61%, respectively; followed by mono-*ortho* coplanar PCB 156, at 7% and 10%, respectively. Di-*ortho* coplanar PCBs 170 and 180 contributed about 3.5% to total PCB TEQs in seal blubber.

Polychlorinated dioxins and dibenzofurans (PCDD/Fs) are contributors to TEQ. We did not test for these compounds. However, levels of PCDD/Fs were reported in killer whale, harbor porpoise, and false killer whale from Georgia Strait and Vancouver Island, which is located north of Smith Island (*3*). The calculated dioxin TEQs in these marine mammals ranged from 2.4 to 21.2 pg/g. When these ∑TEQ values for the PCDD/Fs are compared with the ∑TEQ originating from non-*ortho*-, mono-*ortho*-, and di-*ortho*-PCBs in the present study, it is clear that the ∑TEQ originating from the dioxin-like PCB congeners in Smith Island seals is about 10 times higher than ∑TEQ originating from the PCDD/Fs in marine mammals at Georgia Strait and Vancouver Island. In other words, the coplanar PCBs may contribute as much if not more dioxin-like toxicity to harbor seals from this location as PCDD/Fs themselves.

Acknowledgments

Funding for the collection of samples was from the U.S. Environmental Protection Agency (Region 10) under subcontract to PTI Environmental Services. The U.S. Fish and Wildlife Service, Washington Department of Corrections, and the San Juan Islands National Wildlife Refuge provided permits to conduct research at either Smith or Gertrude Islands. The authors wish to thank Ms. Ju Xiao for her assistance with the chemical analysis and Mr. Joseph Evenson and Mr. Brian McLaughlin for their assistance in tissue collection.

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Received for review April 18, 1995. Revised manuscript received October 3, 1995. Accepted October 3, 1995.[⊗]

ES950270V

[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1995.