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**Polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) in
harbor seal (*Phoca vitulina*) livers from San Francisco Bay, California and Gulf of
Maine**

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Abstract

Bioaccumulation of endocrine disruptors in marine mammals positioned at the top of the food chain is of toxicological concern. Stranded four pup and ten adult harbor seal (*Phoca vitulina*) livers were collected from San Francisco Bay and the Gulf of Maine and analyzed for polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs). We used GC-ECD and GC-NCI/MS to investigate the presence of 28 PCBs and 8 OH-PCB metabolites, respectively. Σ_{28} PCB concentrations (di- to octa-CBs) ranged from 1.81 to 35.9 $\mu\text{g/g}$ lipid with a median of 6.53 for the seal pups and 2.31 to 249 $\mu\text{g/g}$ lipid with a median of 28.9 for the adult seals. Σ_8 OH-PCB concentrations (penta- to hepta-OH-PCBs) ranged from 0.02 to 0.69 $\mu\text{g/g}$ lipid with a median of 0.04 for the adult seals, i.e., at much lower concentrations than those for PCBs. Ratios of OH-PCBs to PCBs (0.24% on average) were comparable to those in beluga whale, but were lower than ratios in human livers. The OH-PCB profiles were slightly different between SFB and GOM seal livers although similar PCB congener patterns were observed. Generally, 4-OH-CB107 was found predominantly in seal livers and was the only OH-PCB detectable in most of seal pup livers. This study provides information on OH-PCBs in seals, adding to the scarce exposure data for these chemicals.

Key words: hydroxylated PCB metabolites, polychlorinated biphenyls, harbor seal liver, San Francisco Bay, Gulf of Maine

1. Introduction

Polychlorinated biphenyls (PCBs) are known endocrine disruptors that are persistent and lipophilic, and biomagnify through the food chain (Wan et al., 2005). Since marine mammals such as seals are positioned at the top of the marine food web and have a relatively long life span (25-30 years), they are exposed to organochlorines (Neale et al., 2005a; Shaw et al., 2005) and are consequently vulnerable to endocrine disruptive effects such as reproductive impairment (Reijnders, 1986; Reddy et al., 2001), cancer (Martineau et al., 2002; Ylitalo, 2005), thyroid alteration (Sormo et al., 2005; Tabuchi et al., 2006), and immune suppression/infectious diseases (Ross, 2002; Beckmen et al., 2003; Hall et al., 2006; Hammond et al., 2005). PCBs are stored in the insulating blubber (lipid) and can be subsequently remobilized to the blood through lipid metabolism during the seasonal fasting (Debier et al., 2006), posing a health risk.

PCB metabolites; hydroxyl and methylsulfone PCBs (OH- and MeSO₂-PCBs) exert similar toxicological effects to PCBs in mammals, particularly thyroid alteration in laboratory rats (Meerts et al 2002), suggesting the need for their inclusion in risk assessment studies. Marine mammals, including seals, are capable of producing cytochrome P450 enzymes (Teramitsu et al., 2000) that biotransform PCBs to more soluble/excretable forms, i.e., OH-PCBs. Accordingly, 837 mono OH-PCB congeners can be theoretically formed in the body via the mechanisms of arene oxidation/1,2 shift and/or direct oxygen insertion (Letcher et al., 2000). While most of the OH-PCBs tend to be excreted (Letcher et al. 2000), some specific OH-PCBs have been retained predominantly in the blood of humans (Fängström et al., 2002; Park et al., 2007a) and

marine mammals (Hoekstra et al., 2003; Houde et al., 2006) due to their preferential binding to blood proteins (Letcher et al. 2000). OH-PCBs are also retained in the livers of rats (Bergman et al., 1994), humans (Guvenius et al., 2002), and beluga whales (McKinney et al., 2006), presumably by mechanisms similar to pentachlorophenol conjugation to liver fatty acids and/or liver protein/enzymes (Leighty and Fentiman, 1982). A set of structurally known OH-PCB metabolites preferentially retained in the body have an OH- group bound to a *para* carbon of a phenyl ring and chlorine atoms bound to carbon adjacent to the OH-group (Lans et al., 1993).

In this pilot research study we used liver samples collected from stranded, dead harbor seals from San Francisco Bay (SFB) and from an island off the Gulf of Maine (GOM). Although the causes of death of the harbor seals stranded on SFB and GOM shores were not known, it is possible that liver accumulation of PCBs and their metabolites may have contributed directly/indirectly to some causative effects, such as infectious disease and liver dysfunction. Thus, the circulation and bioaccumulation of these contaminants in the liver of seals is of concern.

Therefore, we investigated the distribution of possible PCB precursors and OH-PCB metabolites retained in liver tissues of harbor seals stranded in the SFB and the GOM coast where there are the scarce exposure data for OH PCBs in seals which often carry high PCB body burden (tens of ppm). The present study was also undertaken to provide data to compare contaminant exposure in other marine mammals and humans.

2. Materials and methods

2.1. Seal liver tissue sampling

Seal liver samples analyzed in this study were collected from SFB as part of a larger study that examined harbor seal population dynamics, health, contaminant residues and prey selection (Kopec and Harvey, 1995). Liver samples were collected from stranded, dead harbor seals (*Phoca vitulina*) between 1989 and 1998 found along the shores SFB, an urban/industrialized estuary that hosts a year-round resident population of about 500 harbor seals. Liver tissues were also collected from stranded harbor seals between 2002 and 2003 from Mt. Desert Rock, an isolated island 20 miles offshore of the central Maine coast. Seals hauling at Mt. Desert Rock are part of the larger regional seal population in the GOM numbering over 74,000 harbor seals. As a pilot study, a subset of four pup and five adult seal livers from SFB and five male seal livers from GOM were included in the present study and analyzed for PCBs and OH-PCBs, as summarized in Table 1. All harbor seal tissue collections were authorized by the National Marine Fisheries Service (NMFS) in coordination with local stranding network facilities (College of the Atlantic, Bar Harbor, Maine and Museum of Vertebrate Zoology, University of California, Berkeley, California). SFB samples consisted of males and females while all GOM livers were from males. All seal liver samples were collected during necropsy and were immediately placed in clean vials (I-Chem) at -20 °C prior to analysis (Kopec and Harvey, 1995). Seals were grouped by age class based on standard length (Bigg, 1969).

2.2. Chemicals and standards

113

114 The following OH-PCB standards were purchased from Wellington Laboratory
115 (TerraChem Inc., USA) and used as reference standards for the identification and
116 quantification of the analytes: 2,3,3',4',5-pentachlorobiphenyl-4-ol (4-OH-CB107),
117 2,2',3,4',5,5'-hexachlorobiphenyl-4-ol (4-OH-CB146), 2,2',3',4,4',5-hexachlorobiphenyl-
118 3-ol (3'-OH-CB138), 2,2',3,3',4',5-hexachlorobiphenyl-4-ol (4'-OH-CB130),
119 2,2',3,4',5,5',6-heptachlorobiphenyl-4-ol (4-OH-CB187), 2,2',3',4,4',5,5'-
120 heptachlorobiphenyl-3-ol (3'-OH-CB180), 2,2',3,3',4',5,5'-heptachlorobiphenyl-4-ol (4'-
121 OH-CB172). 2,2',4,4',5,5'-hexachlorobiphenyl-3-ol (3-OH-CB153) was provided by
122 Professor Åke Bergman (Stockholm University, Sweden). The numbering of PCBs and
123 OH-PCBs is based on that specified by Ballschmitter and Zell (1980) and Letcher et al
124 (2000), respectively. Internal surrogate standards for PCBs and OH-PCBs were CB-14,
125 65 and 166 for PCBs and 4'-OH-CB159 for OH-PCBs. Injection standards for PCBs and
126 OH-PCB analyses were CB-30 and 204, and CB-209, respectively. Diazomethane was
127 synthesized in hexane by using N-nitroso-N-methylurea (Sigma-Aldrich, USA) as
128 described elsewhere (Sandau, 2000). Other chemicals and solvents used for the analysis
129 include dichloromethane and hexane (trace analysis, Burdick & Jackson), methanol,
130 methyl-tert butyl ether, and water (HPLC grade, Fisher Sci., USA), 2-propanol (99.9%,
131 pesticide grade, Fisher Sci., USA), hydrochloric acid, sulfuric acid (98%), potassium
132 hydroxide, potassium chloride, sodium hydroxide, and ethyl alcohol (94-96%, 200 proof)
133 (Fisher Sci., USA), silica (200-400 mesh) (Sigma-Aldrich, USA). Organics in Cod liver
134 Oil (SRM1588b, National Institute of Standards and Technology, Gaithersburg, MD) was
135 used as a standard reference material for PCB analysis.

2.3. Analysis and clean up

The analyses were conducted at the ultra-clean laboratory of the Department of Toxic Substances Control, Berkeley, CA. The analytical method was modified from analyses of seal blubber (She et al., 2002) and blood serum (Park et al., 2007a). In summary, liver samples (0.4-3 g) along with a method blank (sodium sulfate), and a standard reference material (NIST 1588b) were homogenized, spiked with PCB and OH-PCB surrogate standards (1-5 ng and 1 ng, respectively), and extracted with a mixture of methyl-*tert*-butyl ether (MTBE):hexane (1:1, v/v) (75 mL) with 2-propanol (10 mL). The samples went through two more extractions by successive shaking and centrifuging with MTBE:hexane mixture. Lipid content was determined gravimetrically using 7% (v/v) of the extracts. The extracts were treated with 1% KCl wash and KOH phase separation. Only 7% (v/v) of the organic extracts were used for the PCB analysis to avoid saturation of the ECD. The phenolic compounds retained in the KOH solution were re-protonated by using 2M HCl (pH<2), extracted with MTBE:hexane (1:9, v/v) (4 mL), and derivatized overnight by using diazomethane. The organic extract was cleaned up by using concentrated sulfuric acid (98%). Further clean up and fractionation was conducted on the Pasteur pipette column packed with acidic silica gel (1:2 w/w, 0.6g) and activated silica (0.4g). The columns were eluted with 5 mL hexane and then 10 mL dichloromethane:hexane (1:1). The OH-PCB derivatives were cleaned up with concentrated sulfuric acid (98%), followed by passing through a Pasteur pipette column

packed with sulfuric acidic silica gel (0.5g) and activated silica (0.1g). Injection standards were added before GC analysis.

2.4. Instruments

Twenty eight PCB congeners (CB-28, 49, 52, 56, 66, 70, 74, , 99, 101, 105, 110, 113, 118, 137, 138, 146, 153, 156, 157, 170, 177, 180, 183, 187, 189, 190, 194, 203) were analyzed on a Varian 3800 gas chromatograph (GC) with ^{63}Ni electron capture detection (Varian Inc., Walnut Creek, CA) equipped with dual capillary columns; RTX-5MS (60m $\times$ 0.25 mm i.d., 0.25 μm thickness, Restek, Bellefonte, PA) and DB-XLB (60m $\times$ 0.25 mm i.d., 0.25 μm thickness, J&W Scientific, Folsom, CA). Carrier and make up gases were helium and nitrogen, respectively and set in constant flow mode. Injection (2 μL) was made in splitless mode with an injector temperature of 280 $^{\circ}\text{C}$. The initial GC temperature was set to 80 $^{\circ}\text{C}$ and held for 1.6 min followed by a 15 $^{\circ}\text{C}/\text{min}$ increase to 135 $^{\circ}\text{C}$, 1 $^{\circ}\text{C}/\text{min}$ to 261 $^{\circ}\text{C}$, 3 $^{\circ}\text{C}/\text{min}$ to 295 $^{\circ}\text{C}$ and 1 $^{\circ}\text{C}/\text{min}$ to 300 $^{\circ}\text{C}$ held for 3.5 min. Post run was set to 320 $^{\circ}\text{C}$ for 1 min.

Eight OH-PCBs were determined as methyl derivatives by using a Varian 3800 gas chromatograph equipped with a 1200L mass spectrometer (MS) (Varian Inc., Walnut Creek, CA). The MS was operated in negative chemical ionization (NCI) mode with electron energy of 70 eV and emission current of 300 μA using selected ion monitoring (SIM). The GC was equipped with a DB-5MS capillary column (60m $\times$ 0.25 mm i.d., 0.25 μm thickness, J&W Scientific, Folsom, CA, USA). Injection (2 μL) was made in split/splitless mode with an injector temperature of 250 $^{\circ}\text{C}$. The initial GC temperature

was set to 80 °C and held for 2 min followed by a 50 °C/min increase to 200 °C, 1 °C/min to 230 °C, and 30 °C/min to 300 °C and held for 4 min. Post run was set to 320 °C for 1 min. The temperatures for both ion source and quadrupole were set to 150 °C. We monitored the most intense ions, either molecular ion or fragmentation ion; $[M]^+$ for 4'-MeO-CB159, $[(M+2-CH_3)]^+$ for 4-MeO-CB187 and 4'-MeO-CB172, and $[(M-HCl)]^+$ or $[(M+2-HCl)]^+$ for the rest of the congeners. Carrier and reagent gases were helium and methane, respectively.

2.5. Quantification and QA/QC

All glassware were washed, rinsed with acetone and hexane, and baked at 550 °C for 8 hours. Each batch consisted of one procedural blank, one SRM, and six samples. The five points of external calibration curves were used for the quantifications of PCBs and OH-PCBs. CB-28, 49, 52,66, 70, 74, 101, 105, 110, 118, 138, 146, 153, 156, 157, 177, 180, 183, 187 and 194 were quantitated on the RTX-5MS column while CB-99, 170, 190, 199, and 203 were quantitated on the DB-XLB column. We tested precision for the PCB and OH-PCB analytical methods as well as the gravimetric lipid determination by using duplicate liver samples within and between batches. We tested accuracy by using the SRM samples. Both precision and accuracy were within reasonable error ranges ($\pm 25\%$). To monitor the performance of the experimental procedures, 2.00 ng of 4'-OH-CB159, which, to our knowledge, has not been detected in any animal liver to date, were added to all samples. We derivatized OH-PCB standards simultaneously with the sample extracts for more accurate quantification. Any values lower than the LOQ (~ 0.48 ng/g fat) were

replaced by LOQ/2 when calculating summary statistics. We conducted non-parametric tests (e.g., Spearman correlation) for PCB and OH-PCB data to assess their relationships by using Minitab statistical software.

The average ($\pm$ standard deviation) surrogate recoveries of CB-14, CB-65, and CB-166 for PCB analysis were $92\pm6\%$, $82\pm7\%$, and $82\pm19\%$, respectively. The recoveries from Standard Reference Materials (SRM 1588b Organics in Cod Liver Oil) ranged from 63% for CB-194 to 125% for CB-170. The average ($\pm$ standard deviation) recovery of the OH-PCB surrogate (4'-OH-CB159) was $77\pm15\%$.

3. Results and discussion

In Table 2 we report the ranges and medians of PCBs and OH-PCBs (on a lipid weight basis) measured in harbor seal liver tissues for four pups and five adults from SFB and five adult males from GOM.

3.1. PCBs

Concentrations of PCBs measured from SFB seal pup livers ranged from 1.81 to 35.9 $\mu\text{g/g}$ fat while adult seal livers showed a wider range (2.31-249 $\mu\text{g/g}$ fat). Because of the limited sample size we did not examine the impact of gender on SFB samples and geographical comparisons. In other study, females tend to have lower contaminant burdens than males since reproductively active females can transfer a significant portion

of their PCB body burden to their offsprings through gestation or nursing (Neale et al., 2005b). During the winter, GOM seals may migrate southward along the coast to urban/industrialized areas (e.g., Massachusetts Bay) (Waring et al., 2006) where they may be exposed to elevated PCB levels, while SFB seals reside in the Bay for most of the time.

CB-153 was the primary congener in both SFB and GOM adult seal livers, comprising 22 and 31% on average of Σ_{28} PCBs, respectively (Table 2) and was a good indicator for Σ_{28} PCBs ($r=0.98$, albeit the highest point was excluded). CB-153 together with CB-138, 187, and 180, comprised 67% and 66% on average of Σ_{28} PCBs in SFB and GOM, respectively. The nine PCB congeners presented in Table 2 accounted for more than 80% of Σ_{28} PCBs in SFB and GOM seal livers. The average concentrations of Σ_{28} PCBs measured in GOM harbor seal livers (28.3 ug/g fat) were comparable to Σ_{38} PCBs measured in beluga whale livers (31.9 ug/g fat) from the St. Lawrence River (McKinney et al., 2006). Although the number of congeners measured was different between the McKinney and our study, most PCB congeners predominantly found in the liver matrices, as well as the sample collection time, overlapped. In our study, 8 out of 14 seal livers (1 SFB male pup, 1 SFB female adult, 3 SFB male adults and 3 GOM male adults) showed PCB levels within or exceeding the estimated threshold level for adverse effects on immune/reproductive function (17-77 $\mu\text{g/g}$ blubber fat) in aquatic animals as summarized elsewhere (Shaw et al., 2005). Seal liver (this study) and blubber PCB concentrations (Park et al., unpublished data) showed correlation ($r=0.77$), which is consistent with other studies (Wolkers et al 2006). Thus, it is possible that some of the stranded harbor seals, particularly one SFB seal pup with high PCB liver accumulation,

might have suffered PCB-related adverse effects, such as infectious diseases and/or the conditions of the animals presented in Table 1.

3.2. OH-PCB metabolites

Although literature searches indicated that most of the OH-PCBs are retained in blood, we attempted to measure OH-PCBs in liver because major enzyme-mediated biotransformation of PCBs occurs in the liver. OH-PCBs were detected in almost all liver tissues of SFB and GOM harbor seals including SFB seal pups. 4-OH-CB107 was detected in almost all samples, followed by 4-OH-CB187 (67%), 4-OH-CB146 (60%), 3'-OH-CB138 (60%), and 3-OH-CB153 (47%). As shown in Figure 1, the two OH-PCB chromatograms analyzed as MeO-PCBs were presented for one of the calibration standards (top) and a SFB adult male seal liver (bottom). The standard chromatogram indicates that our GC temperature program resolved the possible co-elutions (e.g., 3-OH-CB153/4-OH-CB146, 3'-OH-CB138/4'-OH-CB130, and 3'-OH-CB180/4'-OH-CB172). Several unidentified OH-PCB peaks were present in the SFB seal liver chromatogram. Particularly, two peaks (a hexa and a hepta OH-PCB) observed between the peaks of 4'-OH-CB159 (internal standard) and 3'-OH-CB180 were fairly notable. When these unidentified OH-PCBs were quantified using response factors for 4-OH-CB107 and 4-OH-CB187 standards representing each hydroxyl homologue group, they comprised 16-27% (22% on average) of Σ OH-PCBs (sum of identified and unidentified).

The harbor seal liver concentrations of OH-PCB metabolites (0.020~0.693 μ g/g fat) in this study had a wider range than what has been reported for humans (0.007~0.175

273 $\mu\text{g/g}$ fat) (Guvenius et al., 2002) and beluga whales (<0.5 - 0.145 $\mu\text{g/g}$ fat) (McKinney et
274 al., 2006) due to one extreme value. Excluding that value, the range narrowed
275 (0.020 - 0.064 $\mu\text{g/g}$ fat) to previously reported levels. The $\Sigma_8\text{OH-PCB}$ levels identified in
276 the harbor seals were much lower (~ 0.59 % of $\Sigma_{28}\text{PCBs}$, 0.24% on average) than those of
277 parent PCBs. It should be noted that the sums of 28 PCBs includes congeners which are
278 not precursors of 8 OH-PCBs and that, in addition, the presence of many OH-PCB
279 congeners below the detection limits may bias their sum. These ratios were comparable
280 to data from beluga whales in St. Lawrence River, but they were lower than data in
281 human livers (usually between 1 - 10%). It is possible that these lower ratios in marine
282 mammals relative to humans may be due to a reduced capacity in marine mammals to
283 metabolize PCBs, or a higher Phase II conjugation process which rapidly depletes OH-
284 PCBs (McKinney et al., 2006). This OH-PCB/PCB ratio was even higher in human
285 blood (Park et al., 2007a,b) and marine mammal blood (Hoekstra et al., 2003; Houde et
286 al., 2006), indicating that OH-PCB metabolites preferentially bind to blood protein
287 relative to liver fatty acids and/or hepatic proteins and enzymes. Since we did not
288 measure lower chlorinated OH-PCBs (containing fewer than four chlorines) and also did
289 not include the ones detected but not identified, we may have underestimated the total
290 concentrations of OH-PCB metabolites in seal livers.

291 The OH-PCB profiles are presented in the bottom of Figure 2. This profile was
292 slightly different between SFB and GOM seal livers although similar PCB congener
293 patterns were observed in the two regions (Figure 2, top). This difference may result
294 from biological (e.g., nutritional/reproductive status, age, gender, polymorphism) and
295 environmental factors (e.g., temperature, migration) influencing enzyme activities and

thereby metabolite formation in seals. For example, Wolkers et al. (2008) reported that EROD activity negatively correlated to blubber content in seals, indicating contaminants get mobilized from blubber during fasting, and possibly induce the Phase I and II enzyme system. Seals also go through other seasonal lipid changes via molting and reproduction. However, the contributions from other various OH-PCB exposure pathways including diet intake/food chain accumulation (Campbell et al., 2003), and even the abiotic aquatic environments (Ueno et al., 2007) are probably minor, as observed in a recent study (Verreault et al., 2008).

Although the profiles of OH-PCB congeners varied from sample-to-sample, 4-OH-CB107 was the dominant congener, similar to the results from the St. Lawrence River beluga whales (McKinney et al., 2006). This was the only congener detected in three SFB seal pup livers in this study, which raises concern due to its relationship to thyroid dysfunction reported for the experimental rat fetus (Meerts et al., 2002). The known possible PCB precursors (CB-118 and CB-105) of 4-OH-CB107 in animals (Letcher et al., 2000) were also found in our samples, albeit they were not among the most dominant congeners. With 3-OH-CB153, 4-OH-CB146, 3'-OH-CB138, and 4-OH-CB187, they comprised 66 to 81% of Σ OH-PCBs (identified and unidentified penta, hexa, and hepta OH-PCBs). They are the OH-PCB congeners primarily found in the blood of humans (Park et al., 2007a,b) and wildlife (Park et al., 2008) since their chemical structure preferentially binds to the blood transthyretin (TTR) receptor protein. This binding involves a hydroxyl group in either the *para*- or *meta*-position of a biphenyl ring, adjacent to chlorine atoms on both sides (Lans et al., 1993; Letcher et al., 2000). This also seems to apply to the seal liver matrix, indicating interaction of those OH-PCBs with

liver enzyme/proteins as well as fatty acid conjugation. 4'-OH-CB130 was reported at considerable levels in human livers (Guvenius et al., 2002) while it was not detected or detected at trace levels in our seal livers.

The pairs of PCB precursors and respective OH-PCB metabolites were correlated. Since correlations could be driven by a single extreme value, that value was removed and the resulting unbiased correlations were: CB-153/3-OH-CB146 ($r=0.67$, $p<0.05$), CB-138/3-OH-CB138 ($r=0.74$, $p<0.05$), CB-187/4-OH-CB187 ($r=0.66$, $p<0.05$). However, the pairs of CB-118/4-OH-CB107 and CB-105/4-OH-CB107 showed weaker correlations ($r=0.24$ and 0.32 , respectively), possibly because 4-OH-CB107 is labile (Malmberg et al. 2004). In the body, PCBs are metabolized via the various transformation pathways mediated by the cytochrome P450 enzyme series (CYP1A, CYP2B, CYP3A). Preferential CYP450 enzyme mediated-metabolic capacity of marine mammals towards OH-PCBs depends on the number and positioning of the chlorine atoms around the biphenyl ring (Boon et al., 1997; Li et al., 2003; McKinney et al., 2004). For example, CB-118 and 105, having only ortho- and meta-unsubstituted sites and one or fewer ortho-Cl atoms, were subject to the CYP1A type enzyme-mediated metabolism, while CB-52, 49, 101 and 110, with meta- and para- vicinal H-atoms, were preferentially metabolized by CYP2B or CYP3A types of enzymes.

We here report several OH-PCBs retained in harbor seal livers in quantifiable amounts. This is one of a few studies available on OH-PCB metabolites detected in liver tissues; rats (Bergman et al., 1994; Haraguchi et al., 1998), humans (Guvenius et al., 2002), beluga whales (McKinney et al., 2006), glaucous gulls (Verreault et al., 2007), polar bears (Gebbink et al., 2008), and predatory birds (Jaspers et al., 2008). Data on seal

liver OH-PCB metabolites are especially valuable because of the difficulties in sampling, the rarity of the sample itself, and the complexity of liver analyses for OH-PCB metabolites.

There are no reports available to date on the toxicological index for hepatic OH-PCB metabolites. However, due to their endocrine disrupting properties, they should be included in the risk assessment of marine mammals' health.

4. Summary and conclusions

PCBs and OH-PCB metabolites were detected in livers of harbor seals from both the San Francisco Bay and the Gulf of Maine. OH-PCB metabolites were retained in seal liver, albeit at lower OH-PCBs to PCBs ratios compared to human liver, possibly due to a lower metabolic expression of CYP enzymes. The OH-PCB profiles were slightly different between SFB and GOM seal livers although similar PCB congener patterns were observed between the two regions. In general, 4-OH-CB107 was detected predominantly in seal livers and was the only OH-PCB detectable in most seal pup livers, raising toxicological concerns. These results should be treated with caution, as they are based on a small sample size. They do, however, highlight the need to include OH-PCB metabolites in the risk assessment of marine mammals, due to their potential endocrine disrupting properties. Further investigations are needed to better understand the relationship between exposure to these endocrine disruptors and possible health effects in seals.

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Table 1
Information of seal liver samples collected from San Francisco Bay (SFB) and Gulf of Maine (GOM)

Collection ID#	Area	Age	Sex	Condition	Season Collected	Stranding Location
AP01524	SFB	Fetus	F	poor	spring	Tiburon, SFB,
AP01531	SFB	Pup	F	poor	spring	Yerba Buena Island, SFB
AP01528	SFB	Fetus	M	poor	spring	Fremont, SFB
AP01530	SFB	Pup	M	good	spring	Berkeley, SFB
AP01527	SFB	AD	F	poor	fall	East Palo Alto, SFB
AP01526	SFB	AD	F	excellent	spring	Redwood Creek, SFB
AP01529	SFB	AD	M	good	spring	Alameda, SFB
AP01523	SFB	AD	M	good	spring	Marin Co. coast (previously captured in SFB)
AP01525	SFB	AD	M	good	fall	San Francisco, SFB
AP01533	ME	AD	M	good	summer	Mount Desert Rock, Maine
AP01536	ME	AD	M	good	summer	Mount Desert Rock, Maine
AP01535	ME	AD	M	moderate	summer	Mount Desert Rock, Maine
AP01534	ME	AD	M	moderate	summer	Mount Desert Rock, Maine
AP01537	ME	AD	M	excellent	summer	Mount Desert Rock, Maine

*length (cm)/weight (kg) to roughly quantify blubber thickness and general health.

Table 2
Concentrations of PCBs and OH-PCBs measured from livers of dead and stranded harbor seals along San Francisco Bay (SFB) and Gulf of Maine (GOM)

	San Francisco Bay Seals						Gulf of Maine Seals		
	Pups (N=4)			Adults (N=5)			Adults (N=5)		
	min	max	median	min	max	median	min	max	median
<i>PCBs ($\mu\text{g/g lipid}$)</i>									
PCB118	0.02	0.48	0.07	0.03	0.32	0.17	0.03	0.19	0.08
PCB146	0.03	1.52	0.24	0.05	8.88	1.36	0.24	2.84	0.89
PCB153	0.18	8.58	1.32	0.24	67.3	9.26	1.45	22.2	7.27
PCB105	0.001	0.15	0.03	0.01	0.12	0.06	0.003	0.07	0.04
PCB138	0.16	6.13	0.97	0.23	38.9	5.39	1.09	13.5	4.23
PCB187	0.09	3.45	0.68	0.62	38.6	4.53	0.37	6.73	2.25
PCB183	0.04	1.37	0.22	0.06	9.77	1.23	0.18	1.99	0.69
PCB180	0.11	4.00	0.60	0.15	42.3	3.86	0.39	4.58	2.39
PCB170	0.04	1.72	0.39	0.07	10.0	1.40	0.29	2.22	0.83
Σ_{28} PCBs	1.81	35.9	6.53	2.31	249	35.6	5.60	63.7	22.4
<i>OH-PCBs ($\mu\text{g/g lipid}$)</i>									
4-OH-CB107	<0.001	0.018	0.004	<0.001	0.123	0.026	0.008	0.033	0.017
3-OH-CB153	<0.001	<0.001	<0.001	<0.001	0.036	<0.001	<0.001	0.006	0.002
4-OH-CB146	<0.001	<0.001	<0.001	<0.001	0.218	0.002	0.003	0.018	0.008
3'-OH-CB138	<0.001	0.003	<0.001	<0.001	0.104	<0.001	0.002	0.013	0.005
4'-OH-CB130	<0.001	0.001	<0.001	<0.001	0.034	<0.001	<0.001	0.002	<0.001
4-OH-CB187	<0.001	0.011	<0.001	0.013	0.149	0.019	0.004	0.019	0.008
3'-OH-CB180	<0.001	<0.001	<0.001	<0.001	0.017	<0.001	<0.001	<0.001	<0.001
4'-OH-CB172	<0.001	<0.001	<0.001	<0.001	0.011	<0.001	<0.001	<0.001	<0.001
Σ_8 OH-PCBs	0.004	0.034	0.004	0.017	0.693	0.045	0.019	0.064	0.045

Figure captions

Fig. 1. GC chromatograms of calibration standard (top) and identified and unidentified OH-PCB metabolites from an adult harbor seal liver collected from San Francisco Bay (SFB) (bottom).

Fig. 2. Profiles of PCB precursors (top) and OH-PCB metabolites (bottom) observed in adult harbor seal livers from San Francisco Bay (SFB) and Gulf of Maine (GOM). Error bars indicate standard errors.