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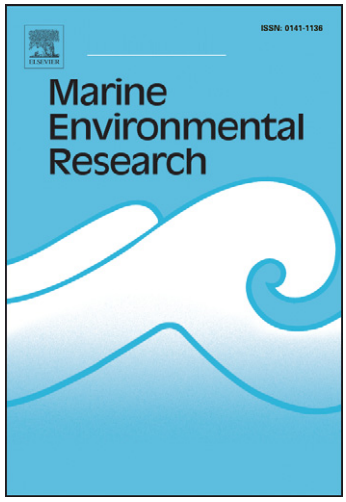

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Polychlorinated biphenyls (PCBs) and their hydroxylated metabolites (OH-PCBs) in harbor seal (*Phoca vitulina*) livers from San Francisco Bay, California and Gulf of Maine

June-Soo Park[1], Olga Ioanna Kalantzi[1], Dianne Kopec[2], Myrto Petreas[1]

[1]Department of Toxic Substances Control, California Environmental Protection Agency, Berkeley, California 94710, USA; [2]Department of Biological Sciences, University of Maine, Orono, Maine 04469, USA

*Corresponding author. Email address: jpark@dtsc.ca.gov (J.S. Park)

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 μg/g lipid with a median of 6.53 for the seal pups and 2.31 to 249 μg/g lipid with a median of 28.9 for the adult seals. Σ_8OH-PCB concentrations (penta- to hepta-OH-PCBs) ranged from 0.02 to 0.69 μg/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 $MeSO_2$-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

90

91 *2.1. Seal liver tissue sampling*

92

93 Seal liver samples analyzed in this study were collected from SFB as part of a larger
94 study that examined harbor seal population dynamics, health, contaminant residues and
95 prey selection (Kopec and Harvey, 1995). Liver samples were collected from stranded,
96 dead harbor seals (*Phoca vitulina*) between 1989 and 1998 found along the shores SFB,
97 an urban/industrialized estuary that hosts a year-round resident population of about 500
98 harbor seals. Liver tissues were also collected from stranded harbor seals between 2002
99 and 2003 from Mt. Desert Rock, an isolated island 20 miles offshore of the central Maine
100 coast. Seals hauling at Mt. Desert Rock are part of the larger regional seal population in
101 the GOM numbering over 74,000 harbor seals. As a pilot study, a subset of four pup and
102 five adult seal livers from SFB and five male seal livers from GOM were included in the
103 present study and analyzed for PCBs and OH-PCBs, as summarized in Table 1. All
104 harbor seal tissue collections were authorized by the National Marine Fisheries Service
105 (NMFS) in coordination with local stranding network facilities (College of the Atlantic,
106 Bar Harbor, Maine and Museum of Vertebrate Zoology, University of California,
107 Berkeley, California). SFB samples consisted of males and females while all GOM livers
108 were from males. All seal liver samples were collected during necropsy and were
109 immediately placed in clean vials (I-Chem) at -20 ^{o}C prior to analysis (Kopec and Harvey,
110 1995). Seals were grouped by age class based on standard length (Bigg, 1969).

111

112 *2.2. Chemicals and standards*

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

136

137 *2.3. Analysis and clean up*

138

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

158 packed with sulfuric acidic silica gel (0.5g) and activated silica (0.1g). Injection
159 standards were added before GC analysis.
160
161 *2.4. Instruments*
162
163 Twenty eight PCB congeners (CB-28, 49, 52, 56, 66, 70, 74, , 99, 101, 105, 110, 113,
164 118, 137, 138, 146, 153, 156, 157, 170, 177, 180, 183, 187, 189, 190, 194, 203) were
165 analyzed on a Varian 3800 gas chromatograph (GC) with ^{63}Ni electron capture detection
166 (Varian Inc., Walnut Creek, CA) equipped with dual capillary columns; RTX-5MS (60m
167 × 0.25 mm i.d., 0.25 μm thickness, Restek, Bellefonte, PA) and DB-XLB (60m × 0.25
168 mm i.d., 0.25 μm thickness, J&W Scientific, Folsom, CA). Carrier and make up gases
169 were helium and nitrogen, respectively and set in constant flow mode. Injection (2 μL)
170 was made in splitless mode with an injector temperature of 280 ^{o}C. The initial GC
171 temperature was set to 80 ^{o}C and held for 1.6 min followed by a 15 ^{o}C/min increase to
172 135 ^{o}C, 1 ^{o}C/min to 261 ^{o}C, 3 ^{o}C/min to 295 ^{o}C and 1 ^{o}C/min to 300 ^{o}C held for 3.5 min.
173 Post run was set to 320 ^{o}C for 1 min.
174 Eight OH-PCBs were determined as methyl derivatives by using a Varian 3800 gas
175 chromatograph equipped with a 1200L mass spectrometer (MS) (Varian Inc., Walnut
176 Creek, CA. The MS was operated in negative chemical ionization (NCI) mode with
177 electron energy of 70 eV and emission current of 300 μA using selected ion monitoring
178 (SIM). The GC was equipped with a DB-5MS capillary column (60m × 0.25 mm i.d.,
179 0.25 μm thickness, J&W Scientific, Folsom, CA, USA). Injection (2 μL) was made in
180 split/splitless mode with an injector temperature of 250 ^{o}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 (±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

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

207 The average (± standard deviation) surrogate recoveries of CB-14, CB-65, and CB-
208 166 for PCB analysis were 92±6%, 82±7%, and 82±19%, respectively. The recoveries
209 from Standard Reference Materials (SRM 1588b Organics in Cod Liver Oil) ranged from
210 63% for CB-194 to 125% for CB-170. The average (± standard deviation) recovery of
211 the OH-PCB surrogate (4'-OH-CB159) was 77±15%.

212

213

214 **3. Results and discussion**

215

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

219

220 *3.1. PCBs*

221

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

227 of their PCB body burden to their offsprings through gestation or nursing (Neale et al.,
228 2005b). During the winter, GOM seals may migrate southward along the coast to
229 urban/industrialized areas (e.g., Massachusetts Bay) (Waring et al., 2006) where they
230 may be exposed to elevated PCB levels, while SFB seals reside in the Bay for most of the
231 time.

232 CB-153 was the primary congener in both SFB and GOM adult seal livers,
233 comprising 22 and 31% on average of Σ_{28}PCBs, respectively (Table 2) and was a good
234 indicator for Σ_{28}PCBs (r=0.98, albeit the highest point was excluded). CB-153 together
235 with CB-138, 187, and 180, comprised 67% and 66% on average of Σ_{28}PCBs in SFB and
236 GOM, respectively. The nine PCB congeners presented in Table 2 accounted for more
237 than 80% of Σ_{28}PCBs in SFB and GOM seal livers. The average concentrations of
238 Σ_{28}PCBs measured in GOM harbor seal livers (28.3 ug/g fat) were comparable to
239 Σ_{38}PCBs measured in beluga whale livers (31.9 ug/g fat) from the St. Lawrence River
240 (McKinney et al., 2006). Although the number of congeners measured was different
241 between the McKinney and our study, most PCB congeners predominantly found in the
242 liver matrices, as well as the sample collection time, overlapped. In our study, 8 out of
243 14 seal livers (1 SFB male pup, 1 SFB female adult, 3 SFB male adults and 3 GOM male
244 adults) showed PCB levels within or exceeding the estimated threshold level for adverse
245 effects on immune/reproductive function (17-77 μg/g blubber fat) in aquatic animals as
246 summarized elsewhere (Shaw et al., 2005). Seal liver (this study) and blubber PCB
247 concentrations (Park et al., unpublished data) showed correlation (r=0.77), which is
248 consistent with other studies (Wolkers et al 2006). Thus, it is possible that some of the
249 stranded harbor seals, particularly one SFB seal pup with high PCB liver accumulation,

250 might have suffered PCB-related adverse effects, such as infectious diseases and/or the
251 conditions of the animals presented in Table 1.
252
253 *3.2. OH-PCB metabolites*
254
255 Although literature searches indicated that most of the OH-PCBs are retained in blood,
256 we attempted to measure OH-PCBs in liver because major enzyme-mediated
257 biotransformation of PCBs occurs in the liver. OH-PCBs were detected in almost all
258 liver tissues of SFB and GOM harbor seals including SFB seal pups. 4-OH-CB107 was
259 detected in almost all samples, followed by 4-OH-CB187 (67%), 4-OH-CB146 (60%), 3'-
260 OH-CB138 (60%), and 3-OH-CB153 (47%). As shown in Figure 1, the two OH-PCB
261 chromatograms analyzed as MeO-PCBs were presented for one of the calibration
262 standards (top) and a SFB adult male seal liver (bottom). The standard chromatogram
263 indicates that our GC temperature program resolved the possible co-elutions (e.g., 3-OH-
264 CB153/4-OH-CB146, 3'-OH-CB138/4'-OH-CB130, and 3'-OH-CB180/4'-OH-CB172).
265 Several unidentified OH-PCB peaks were present in the SFB seal liver chromatogram.
266 Particularly, two peaks (a hexa and a hepta OH-PCB) observed between the peaks of 4'-
267 OH-CB159 (internal standard) and 3'-OH-CB180 were fairly notable. When these
268 unidentified OH-PCBs were quantified using response factors for 4-OH-CB107 and 4-
269 OH-CB187 standards representing each hydroxyl homologue group, they comprised 16-
270 27% (22% on average) of ΣOH-PCBs (sum of identified and unidentified).
271 The harbor seal liver concentrations of OH-PCB metabolites (0.020~0.693 μg/g fat)
272 in this study had a wider range than what has been reported for humans (0.007~0.175

μg/g fat) (Guvenius et al., 2002) and beluga whales (<0.5-0.145 μg/g fat) (McKinney et al., 2006) due to one extreme value. Excluding that value, the range narrowed (0.020~0.064 μg/g fat) to previously reported levels. The Σ_8OH-PCB levels identified in the harbor seals were much lower (~0.59 % of Σ_{28}PCBs, 0.24% on average) than those of parent PCBs. It should be noted that the sums of 28 PCBs includes congeners which are not precursors of 8 OH-PCBs and that, in addition, the presence of many OH-PCB congeners below the detection limits may bias their sum. These ratios were comparable to data from beluga whales in St. Lawrence River, but they were lower than data in human livers (usually between 1-10%). It is possible that these lower ratios in marine mammals relative to humans may be due to a reduced capacity in marine mammals to metabolize PCBs, or a higher Phase II conjugation process which rapidly depletes OH-PCBs (McKinney et al., 2006). This OH-PCB/PCB ratio was even higher in human blood (Park et al., 2007a,b) and marine mammal blood (Hoekstra et al., 2003; Houde et al., 2006), indicating that OH-PCB metabolites preferentially bind to blood protein relative to liver fatty acids and/or hepatic proteins and enzymes. Since we did not measure lower chlorinated OH-PCBs (containing fewer than four chlorines) and also did not include the ones detected but not identified, we may have underestimated the total concentrations of OH-PCB metabolites in seal livers.

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

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

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

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

322 The pairs of PCB precursors and respective OH-PCB metabolites were correlated.
323 Since correlations could be driven by a single extreme value, that value was removed and
324 the resulting unbiased correlations were: CB-153/3-OH-CB146 ($r=0.67$, $p<0.05$), CB-
325 138/3-OH-CB138 ($r=0.74$, $p<0.05$), CB-187/4-OH-CB187 ($r=0.66$, $p<0.05$). However,
326 the pairs of CB-118/4-OH-CB107 and CB-105/4-OH-CB107 showed weaker correlations
327 ($r=0.24$ and 0.32, respectively), possibly because 4-OH-CB107 is labile (Malmberg et al.
328 2004). In the body, PCBs are metabolized via the various transformation pathways
329 mediated by the cytochrome P450 enzyme series (CYP1A, CYP2B, CYP3A).
330 Preferential CYP450 enzyme mediated-metabolic capacity of marine mammals towards
331 OH-PCBs depends on the number and positioning of the chlorine atoms around the
332 biphenyl ring (Boon et al., 1997; Li et al., 2003; McKinney et al., 2004). For example,
333 CB-118 and 105, having only ortho- and meta-unsubstituted sites and one or fewer ortho-
334 Cl atoms, were subject to the CYP1A type enzyme-mediated metabolism, while CB-52,
335 49, 101 and 110, with meta- and para- vicinal H-atoms, were preferentially metabolized
336 by CYP2B or CYP3A types of enzymes

337 We here report several OH-PCBs retained in harbor seal livers in quantifiable
338 amounts. This is one of a few studies available on OH-PCB metabolites detected in liver
339 tissues; rats (Bergman et al., 1994; Haraguchi et al., 1998), humans (Guvenius et al.,
340 2002), beluga whales (McKinney et al., 2006), glaucous gulls (Verreault et al., 2007),
341 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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549

550 Table 1
551 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

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

553 Table 2
554 Concentrations of PCBs and OH-PCBs measured from livers of dead and stranded harbor seals along San
555 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
PCBs (μg/g lipid)									
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
OH-PCBs (μg/g lipid)									
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_8OH-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.