HEPATIC RETENTION OF POLYCHLORINATED BIPHENYLS (PCBS) AND THEIR HYDROXLATED METABOLITES (OH-PCBS) IN HARBOR SEAL (*PHOCA VITULINA*) FROM CALIFORNIA AND MAINE

Park JS[1], Kalantzi, O[2], Kopec, D[3], Petreas, M[1]

[1]Department of Toxic Substances Control, California Environmental Protection Agency, Berkeley, California 94710, USA; [2]Department of Chemistry, National and Kapodistrian University of Athens, Panepistimioupoli Zografos, 15771 Athens, Greece; [3]Department of Biological Sciences, University of Maine, Orono, Maine 04469 , USA

Abstract

Bioaccumulation of endocrine disruptors in marine mammals positioned at the top of the food chain is of toxicological concern. We investigated the presence of 28 PCBs and 8 OH-PCB metabolites in livers of harbor seals (*Phoca vitulin)* from California and Maine by using GC-ECD and GC-NCI/MS, respectively. We found that seals were still highly exposed to PCBs and that specific OH-PCB metabolites retained in their liver tissues were at much lower levels (0.24% on average) than levels of PCBs. Ratios of OH-PCBs to PCBs were comparable to those in beluga whale livers, but lower than those in human livers. Although the concentrations of both the sum of PCBs and the sum of OH-PCBs (those identified) measured in adult seal livers were not significantly different between the two U.S. coastal areas ($p>0.05$), the profiles of possible PCB precursors of OH-PCBs varied, possibly due to different exposure pathways (e.g., diet) or variable individual biological metabolic capacity. Generally, 4-OH-CB107 was the predominant metabolite in seal livers and was the only OH-PCB detectable in most of seal pup livers. Further investigations are needed to better understand the relationship between exposure to these endocrine disruptors and possible health outcomes in seals.

Introduction

Polychlorinated biphenyls (PCBs) are known endocrine disruptors that are persistent and lipophilic, and consequently bioaccumulate/biomaginfy through the food web. Since marine mammals, such as seals, are positioned at the top of the marine food chain and have relatively long life span (25-30 years), they are still highly exposed to organochlorines and consequently vulnerable to endocrine disruptive effects such as reproductive impairment [1], cancer [2], thyroid alteration [3], and immune suppression/infectious diseases [4]. Their metabolites (e.g., OH-PCBs, $MeSO_2$-PCBs) also exert similar toxicological effects, particularly thyroid alteration [5,6], suggesting that they should be included in risk assessments, since they may be contributing to health effects. Marine mammals including seals are capable of producing cytochrome P450 enzymes [7] that biotransform PCBs to more soluble/excretable forms, i.e., polychlorobiphenylols (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 [8]. While most of the OH-PCBs are excreted, some specific OH-PCBs have been retained in the blood due to their preferential binding to blood proteins. OH-PCBs are also retained in the livers of rats [9], humans [10], and beluga whales [11], presumably by being conjugated to liver fatty acids and/or liver protein/enzymes [12]. Thus, the circulation and bioaccumulation of these contaminants in the liver of marine mamals is of concern, and may have implications for other mammals, including humans. Therefore, we investigated the distribution of possible PCB precursors and OH-PCB metabolites retained in liver tissues of harbor seals stranded in the San Francisco Bay of California and along the Maine coast.

Materials and Methods

The liver tissues were collected from stranded, dead harbor seals (*Phoca vitulina*) found along the shores of San Francisco Bay (SFB), a highly industrialized estuary, between 1989 and 1998. Four pup and 5 adult seal livers were analyzed for PCBs and OH-PCBs. These were males and females. In addition, liver tissues from stranded male harbor seals were collected in 2002 and 2003 from Mt. Desert Rock, an isolated island 20 miles offshore of the central Maine coast (GOM). All collections were authorized by the NMFS in coordination with local stranding networks. All seal liver samples were collected during necropsy using clean techniques and stored in glass I-Chem vials at -20 ^{o}C prior to analysis. The OH-PCB standards were

purchased from Wellington Laboratory (TerraChem Inc., USA) or donated from Stockholm University, Sweden. Other chemicals and solvents of the purest analytical grades were used for the analysis. The analyses were conducted at the clean laboratory of the Department of Toxic Substances Control, Berkeley, CA. The analytical method was modified from analyses of seal blubber [13] and blood serum [14]. In summary, liver samples (0.4-3 g) were homogenized and extracted with MTBE:hexane (1:1, v/v), and treated with 1% KCl wash and KOH phase separation. Only 7% (v/v) of the organic extracts were used for the analysis of PCBs to avoid saturation of the ECD. The phenolic compounds retained in KOH solution were re-protonated by using 2M HCl (pH<2), extracted with MTBE:hexane (1:9, v/v), and derivatized using diazomethane overnight. The sample was initially cleaned up with H_2SO_4 and then with Pasteur pipette column chromatography packed with acidic silica gel/activated silica gel. Injection standards were spiked (CB-30 and 204 for PCB analysis and CB-209 for OH-PCB analysis) before GC analysis. Twenty eight PCB congeners were analyzed on a Varian 3800 GC-ECD (Varian Inc., Walnut Creek, CA) equipped with dual columns; RTX-5MS capillary column (60m × 0.25 μm i.d., 0.25 μm thickness, Restek, Bellefonte, PA) and DB-XLB (60m × 0.25 μm i.d., 0.25 μm thickness, J&W Scientific, Folsom, CA) and nine OH-PCB metabolites including an internal standard were determined as methyl derivatives using a Varian 1200 GC-NCI/MS (Varian Inc., Walnut Creek, CA) equipped with (60m × 0.25 μm i.d., 0.25 μm thickness, J&W Scientific, Folsom, CA, USA). The recoveries of surrogates and Standard Reference Materials (SRM), and the accuracy and precision of PCBs, OH-PCBs, and lipid measurements were within reasonable error ranges.

Results and Discussion

Lipid content of seal livers ranged from 2.4 to 35%. Concentrations of PCBs measured from SFB seal pup livers ranged from 1.81 to 35.9 μg/g fat, while adult seal livers showed a wider range (2.31-249 μg/g fat). Although we didn't have mother-pup pairs, all seal pup livers showed lower concentrations than those of adult seals, with one exception. The concentrations of PCBs were not statistically different between SFB and GOM adult seal livers ($p>0.05$). The higher mean PCB and OH-PCB concentrations observed in SFB seals are probably driven by one SFB seal liver sample with very high concentrations, rather than any actual temporal or geographic differences. GOM seals may migrate southward along the coast to urban/industrialized areas (e.g., Massachusetts Bay) during the winter [15] where they might be exposed to PCBs while SFB seals reside in the bay for most of the time. The average concentrations of Σ_{28}PCBs measured in GOM harbor seal livers (28.3 μg/g fat) were comparable to Σ_{38}PCBs measured in beluga whale livers (31.9 ug/g fat) from the St. Lawrence River [11]. The GOM seals showed different PCB congener profiles, indicating not only that they might have different exposure pathways, but also different metabolic biotransformations of PCBs resulting in the variation of OH-PCBs in their tissues. We here report several OH-PCBs retained in harbor seal livers in quantifiable amounts. This is one of a few studies on OH-PCB metabolites detected in liver tissues. OH-PCBs were detected in almost all liver tissues of SFB and GOM harbor seals including seal pups from SFB. The unidentified OH-PCBs, comprised 16-27% (22% on average) of Σ_{17}OH-PCBs (sum of identified and unidentified). Possible reasons for the retention of OH-PCBs in the liver may be partly be attributed to: 1) fatty acid conjugation, 2) interaction with hepatic protein and enzyme, and/or 3) residual blood. The levels of OH-PCBs were much lower (~0.59 % of Σ_{28}PCBs, 0.24% on average) than those of PCBs, and did not show any spatial variation. These ratios are comparable to data from beluga whales in St. Lawrence River [11], however, higher ratios of OH-PCBs to PCBs (1-10%) have been reported for human livers [10] and even higher for blood of humans [14] and wildlife [16,17]. Generally, the elevated hepta-PCB homologue in SFB seal livers is mainly attributable to CB-187 and 180 (Figure 1a). This might have resulted in the slightly different profiles of the OH-PCB distributions in GOM seals (Figure 1b), in addition to some environmental factors (e.g., temperature, stress, change of habitats, diets) affecting environmental exposure and/or biotransformation capacity. Although the profiles of OH-PCB congeners varied from sample-to-sample, 4-OH-CB107 was detected predominantly in 77% of seal liver samples from both SFB and GOM, similar to the results from the St. Lawrence River beluga whales [11]. This was the only congener detected in three SFB seal pup livers, which becomes of concern due to its relationship to thyroid dysfunction reported for rat fetus [6]. The known possible PCB precursors (CB-118 and CB-105) of 4-OH-CB107 in experimental rats [8] were also found in our samples, albeit they were not among the most predominant congeners. With 3-OH-CB153, 4-OH-CB146, 3'-OH-CB138, and 4-OH-CB187, they comprised 66 to 81% of Σ_{17}OH-PCBs (sum of identified and unidentified). Those congeners are also dominant in the blood of humans and wildlife. 4'-OH-CB130 was reported in human livers [10] while it was not detected or detected in trace levels in our seal livers. We suggest that OH-PCB

metabolites found in the liver be included 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 outcomes in seals.

Acknowledgement and Disclaimer
The authors would like to thank Joginder Dhaliwal and Dr. Reber Brown of the Department of Toxic Substances Control for their invaluable analytical expertise. The ideas and opinions expressed herein are those of the authors and do not necessarily reflect the official position of the California Department of Toxic Substances Control.

References

1. Reijnders PJH. *Nature* 1986; 324:456.
2. Ylitalo GM. *Mar Pollut Bull* 2005; 50(1):30.
3. Tabuchi M, Veldhoen A, Dangerfield N, Jeffries S, Helbing CC, Ross PS. *Environ Health Perspect* 2006; 114(7):1024.
4. Hammond JA, Hall AJ, Dyrynda EA. *Aquat Toxicol* 2005; 74(2):126.
5. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, Bergman A, Visser TJ. *Toxicol Ind Health* 1998; 14(1-2):59.
6. Meerts I, Assink Y, Cenijn PH, van den Berg JHJ, Weijers BM, Bergman A, Koeman JH, Brouwer A. *Toxicol Sci* 2002; 68(2):361.
7. Teramitsu I, Yamamoto Y, Chiba I, Iwata H, Tanabe S, Fujise Y, Kazusaka A, Akahori F, Fujita S. *Aquat Toxicol* 2000; 51(2):145.
8. Letcher RJ, Klasson WE, Bergman A. In: Handbook of Environ Chemistry. New Types of Persistent Halogenated Compounds, Vol. 3, Part K (Paasivirta J, ed). Berlin: Springer-Verlag. 2000.
9. Bergman A, Klassonwehler E, Kuroki H. *Environ Health Perspect* 1994; 102(5):464.
10. Guvenius DM, Hassanzadeh P, Bergman A, Noren K. *Environ Toxicol Chem* 2002; 21(11):2264.
11. McKinney MA, De Guise S, Martineau D, Beland P, Lebeuf M, Letcher RJ. *Environ Toxicol Chem* 2006; 25(5):1246.
12. Leighty EG, Fentiman AF. *Bull of Environ Contam Toxicol* 1982; 28(3):329.
13. She JW, Petreas M, Winkler J, Visita P, McKinney M, Kopec D. *Chemosphere* 2002; 46(5):697.
14. Park JS, Linderholm L, Charles MJ, Athanasiadou M, Petrik J, Kocan A, Drobna B, Trnovec T, Bergman A, Hertz-Picciotto I. *Environ Health Perspect* 2007; 115(1):20.
15. Waring, GT, Gilbert JR, Loftin, J, Cabana. N. *Northeastern Naturalist* 2006; 13:1.
16. Hoekstra PF, Letcher RJ, O'Hara TM, Backus SM, Solomon KR, Muir DCG. *Environ Toxicol Chem* 2003; 22(11):2650.
17. Sandala GM, Sonne-Hansen C, Dietz R, Muir DCG, Valters K, Bennett ER, Born EW, Letcher RJ. *Sci Tot Environ* 2004; 331(1-3):125.

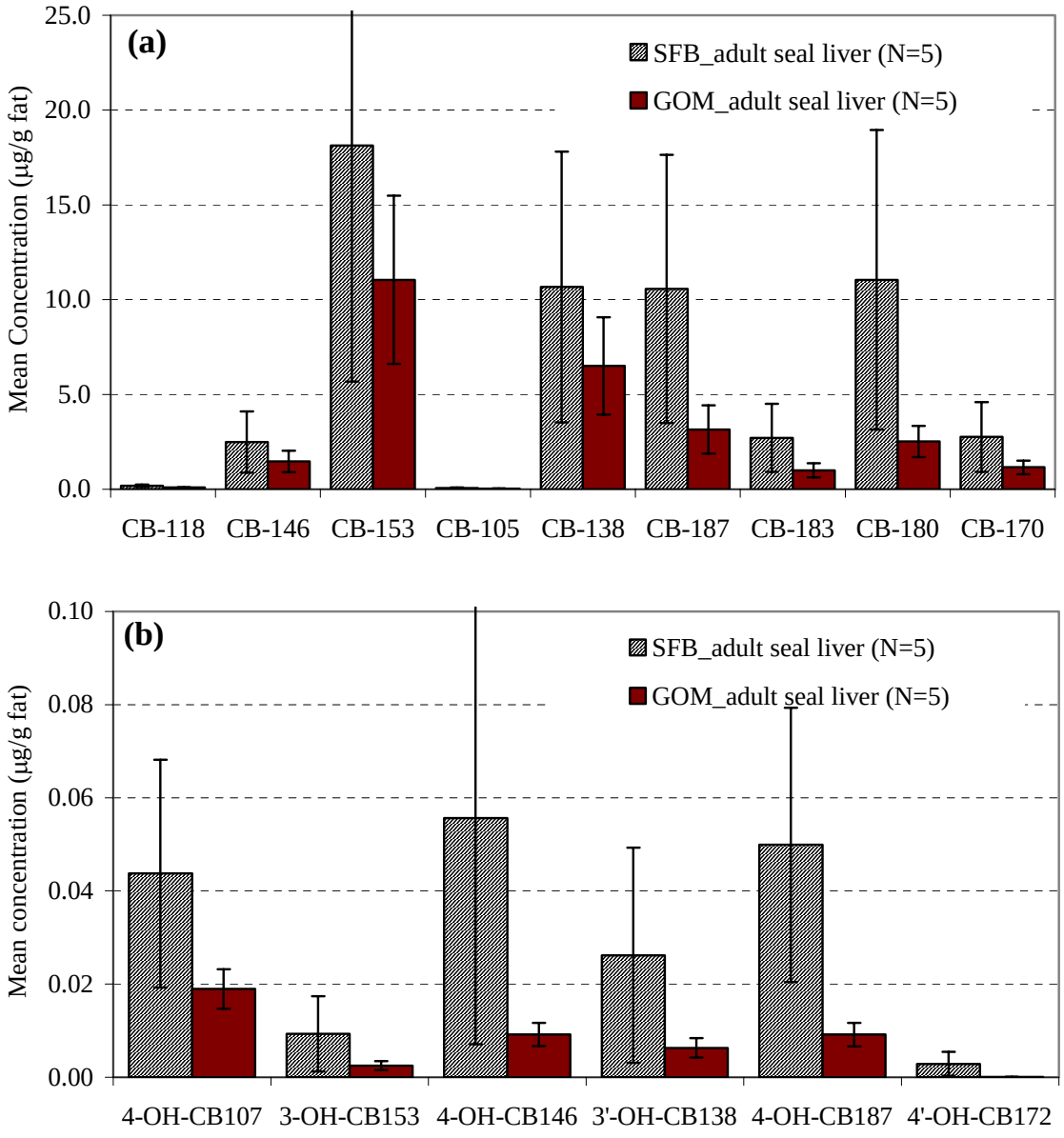

Figure 1. Distributions of possible PCB precursors (a) and their OH-PCB metabolites (b) retained in the livers of harbor seals stranded along the coastal areas of San Francisco Bay (SFB) and Gulf of Maine (GOM). Error bars indicate the standard errors.