HALOGENATED PHENOLIC CONTAMINANTS AND METABOLITES IN THE BLOOD OF KILLER WHALE (*ORCINUS* ORCA)

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Introduction

On the west coast of Canada and northwest coast of the United States there are two sympatric populations of killer whale (*Orcinus orca)*. There are resident and transient communities where the resident population has been divided into two sub-communities (northern and southern).[1] The resident population spend the majority of the year in coastal waters with a diet mainly consisting of fish. On the other hand, the transient populations mainly consume pinnipeds and cetaceans.[2] It was recently shown these populations are highly contaminated with polychlorinated biphenyls (PCBs) and other lipophilic polyhalogenated aromatic hydrocarbon (PHAH) contaminants.[1,3] Sum (S) PCB concentrations range from about 10 to 250 ppm (lipid wt. basis) in blubber biopsies from male and female killer whales from resident and transient populations. In comparison, in blubber biopsies from St. Lawrence beluga whale (*Delphinapterus leucas*), S-PCB concentrations in males and females average about 10 ppm, but 128 ppm levels have been documented.[4,5]

It has been shown that polyhalogenated aromatics hydrocarbon (PHAH) contaminants are potentially toxic and accumulate in the large lipid reserves of mammals, especially marine mammals.[6] However, PHAHs have the potential to be biotransformed to retained or persistent metabolites, in some cases with appreciable biological half-lives, in PHAH-exposed organisms. This ability to metabolize PHAHs is a function of the activation, substrate selectivity and profile of Phase I, cytochrome P450 monooxygenase (CYP) enzymes and Phase II conjugation-mediating enzyme systems.[7] For example, persistent aryl sulfone PCB ($MeSO_2$-PCB) and retained hydroxylated PCB (OH-PCB) have been reported. These metabolites have been shown to elicit biological and toxicological effects in exposed organisms, and *in vitro* cell systems, including estrogenic and thyroidogenic effects.[7] The present study aims to determine the levels of PHAH contaminants, biotransformation, and the characterization and levels of PHAH metabolites in a single blood sample collected from a killer whale.

Materials and Methods

A single blood sample (10 mL) was collected, and made available for this study, from an adult female killer whale, held in captivity at the Vancouver Aquarium Marine Science Centre, British Columbia, Canada, and stored immediately at -4 ºC. The killer whale had been fed a diet of wild Pacific herring (*Clupea Pallasi*). The whole blood sample was thawed and the liquid portion (serum with lysed red blood cells) was separated from the coagulated mass for extraction. Approximately 5.0 grams of the sample was extracted and four contaminant fractions were separated, i.e., PCBs, organochlorine pesticides (OCs), aryl methylsulfones ($MeSO_2$) and hydroxylated (OH) compounds. The procedures of Letcher *et al.*[4] and Sandau *et al.*[8] were used with minor modifications.[9] All four fractions were analyzed using gas chromatography/electron capture detection (GC/μECD). Gas chromatography/electron impact mass spectrometry (GC/MS(EI)) and/or GC/MS with electron capture

negative ionization (ECNI) was used for conformation of $MeSO_2$- and OH-containing compounds. An external standard quantification approach was used for PCBs (40 congeners including coelutions, Figure 1) and OCs (*p,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, α-HCH, ß-HCH, γ-HCH, oxychlordane, *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor, heptachlor epoxide and octachlorostyrene (OCS)). CB-83 and CB-122 were used as OCB and OC internal standards. GC/ECD quantification for the $MeSO_2$- and OH-containing compounds were based on a $MeSO_2$-internal standard (3-$MeSO_2$-2-Me-2',3',4,5,5'-pentachlorobiphenyl) and OH-PCB-internal standards (4-OH-CB72 and 4'-OH-CB159), respectively. ECD responses and retention times were compared to authentic standards (i.e., 16 $MeSO_2$-PCBs, 3-$MeSO_2$-*p,p'*-DDE, 14 OH-PCBs and 4-OH-heptachlorostyrene (4-OH-HpCS) and pentachlorophenol (PCP)). Extraction efficiencies for PCBs, OCs and aryl sulfones were >70 % while OH-PCBs were 30 to 50 %. The OH method was originally developed for plasma, and extraction efficiencies have also been evaluated using spiked blood, serum and plasma from other organisms (blood<serum<plasma).[9]

Results and Discussion

The levels of contaminants analyzed in the killer whale blood sample were not lipid normalized (Table 1). The lysed red blood cells in the sample prevented low lipid determination using a colorimetric method. Of the analytes detected, PCBs were the major contaminant followed by DDTs and OH-PCBs (PCB metabolites). The PCB congener pattern in blood (Figure 1) was almost identical to the patterns found in blubber biopsies from west coast resident and transient killer whales despite dietary differences (i.e., wild Pacific herring versus salmonids (residents) versus pinnipeds and cetaceans (transients).[1] Ross *et al.*[1] reported similar Σ-PCB (Table 1) for congeners of 52, 101, 118, 153, 138 and 180 (almost 50 % of the Σ-PCBs) in both the resident and transient killer whale blubber biopsies. These data support that there is a specific retention of certain PCB congeners independent of the killer whales diet, and blood is a good surrogate of the body burden profile of PCBs. PCB metabolites and other phenolic compounds reported here are likely compared to blood metabolite profiles in free-ranging Pacific killer whale.

Table 1. Sum (Σ) PCBs, $MeSO_2$-PCBs, OH-PCBs, DDTs, Chlordanes and DDTs and other neutral and phenolic compounds in killer whale blood.

Compound Class Σ-Levels	Killer Whale ng/g (w/w)	Compound Class Individual Levels	Killer Whale ng/g (w/w)
Σ-PCB	12.50	CB-153	1.425
Σ-precursor-PCB	3.867	HCB	0.711
Σ-$MeSO_2$-PCB	N.D.	OCS	0.073
Σ-OH-PCB	2.041	4-OH-HpCS	0.670
Σ-DDTs	2.866	PCP	0.322
Σ-Chlordanes	0.486	Heptachlor Epoxide	0.006
Σ-HCHs	0.451	Dieldrin	0.007

$MeSO_2$-PCB concentrations are not reported, even though an apparent congener pattern was observed, because the levels were near the limit of detection (<0.002 ppm (w.w.)). It was hypothesized that these PCB metabolites would have been present at detectable levels. Other species of (odontocete) cetaceans (beluga whale, sperm whale, and pygmy sperm whale) have demonstrated an ability to form these metabolites.[4,10] Also, there is more evidence for this metabolic capacity because of the 47 PCB

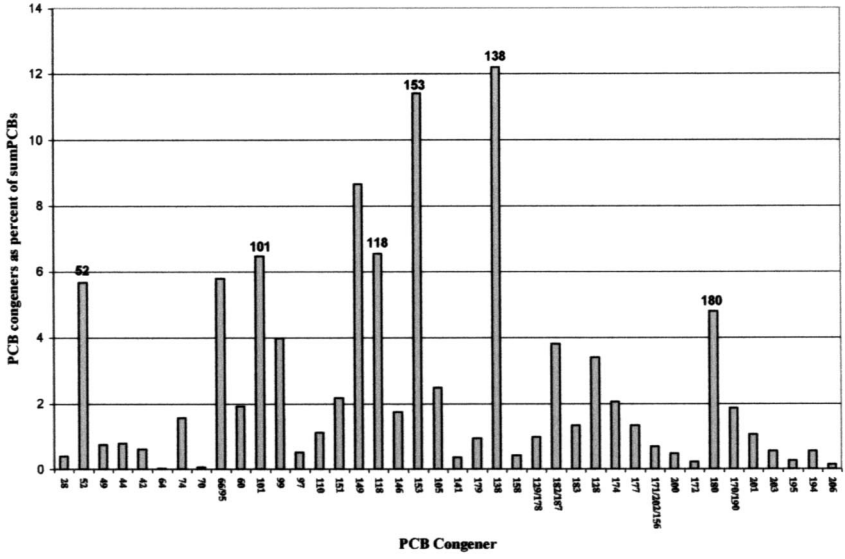

Figure 1. PCB congener pattern in killer whale blood, expressed as a percentage of the Σ-PCB concentration (labeled congeners also found to be dominant by Ross *et al.*[1] in blubber biopsies).

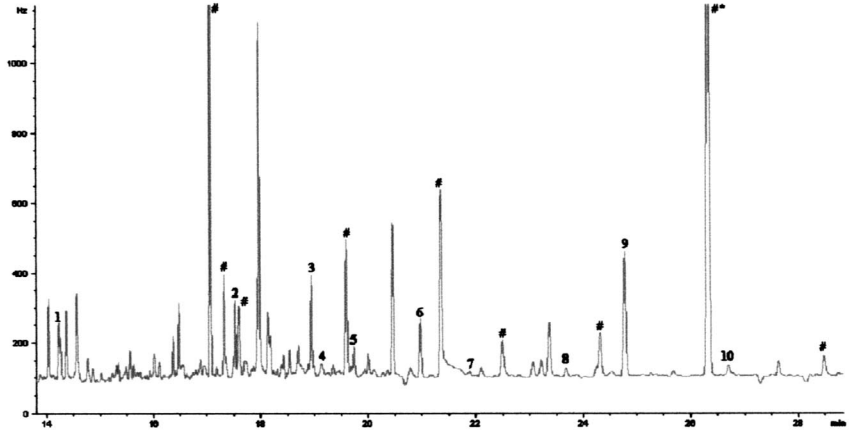

Figure 2. GC/ECD chromatogram of OH-PHAHs (derivatized to MeOH-compounds) in killer whale blood (1= PCP; 2= 4-OH-HpCS; 3= 4-OH-CB72, ISTD; 4= 4'-OHCB-104; 5= 4'-OHCB-121; 6= 3'-OHCB-85, ISTD; 7= 4-OHCB-112; 8= 3'-OHCB-138; 9= 4'-OHCB-159, ISTD; 4= 4-OHCB-193; #= unidentified hydroxylated compounds; #*= unidentified, but major phenolic compound.

congeners (including 5 coeluting congeners) analyzed, 9 congeners were precursors for $MeSO_2$-PCBs (*meta-para*, non-chlorinated substitutions).

These *meta-para*-PCBs were also relatively dominant in blubber biopsies of St. Lawrence beluga whale.[4,5] Blubber biopsies from St. Lawrence beluga contained an average PCB:precursor ratio of 38 %, whereas in the present study the ratio is approximately 31 %.[4] This similarity suggested that odontocete killer whales have the potential to form aryl sulfone PCBs. These low levels of MeSO2-PCBs in killer whale blood may be due to several factors such as species-specific metabolism, enzyme induction, the blood sample size being too small, blood rather than blubber samples, the age of the whale (23 yrs), and the time held in captivity (>20 yrs).

The identified OH-PCBs and Σ-OH-PCB concentration are presented in Table 1 and Figure 2. The Σ-OH-PCB level was less than Σ-PCBs, similar to Σ-DDTs, and much higher than Σ-Chlordanes and Σ-HCHs. Furthermore, the Σ-OH-PCB/Σ-PCB ratio was intermediate to ratios of about 2.00 and 0.002 for polar bear and ringed seal plasma, respectively, from the Canadian Arctic.[8] 4-OH-HpCS, an apparent metabolite of OCS (a byproduct of electrolysis industries), was recently identified in Canadian arctic marine mammals.[7] The 4-OH-HpCS level and 4-OH-HpCS/OCS ratio (9.2) in the present killer whale was again intermediate to polar bear and ringed seal plasma.[7] Major phenolic peaks were detected at about 17 min and 26.5 min (Figure 2). GC/MS(ECNI) fullscan (50-600 m/z) of the later eluting peak (not shown) indicated a phenolic compound containing 3 bromine and 2 chlorine atoms. To our knowledge, this is the first report on PCB and other PHAH metabolites in a cetacean species, and in the blood of a cetacean. Assuming there is no accumulation from the fish diet, which is unlikely, killer whale are shown to be able to form appreciably high levels of potential, endocrine-disrupting OH-PCB and 4-OH-HpCS metabolites, and possibly of $MeSO_2$-PCB metabolites. Further studies are underway to fully identify the unknown phenolic compounds. Moreover, more studies are required to assess the food web dynamics and health impacts.

References

1. Ross PS, Ellis GM, Ikonomou MG, Barrett-Lennard LG and Addison RF (2000) *Mar. Pollut. Bull.*, 40(6): 504-515.
2. Ford JKB, Ellis GM, Barrett-Lennard LG, Morton, AB, Palm RS and Balcomb KC (1998) *Can. J. Zoo.*, 76: 1456-1471.
3. Hayteas DL and Duffield DA (2000) *Mar. Pollut. Bull.*, 40(6): 558-561.
4. Letcher RJ, Norstrom RJ, Muir DCG, Sandau C, Koczanski K, Michaud R, De Guise S and Beland P (2000) *Environ. Tox. Chem.*, 19(5): 1378-1388.
5. Hobbs KE, Muir DCG, Michaud R, Béland, Letcher RJ and Norstrom RJ (2002) *Environ. Pollut.*, In Press.
6. Ross PS (2002) *Human and Ecological Risk Assessment*, 8(2): 277-292.
7. Letcher RJ, Klasson-Wehler E and Bergman Å (2000) in: Anthropogenic Compounds: New Types of Persistent Compounds (Paasivirta J and Hutzinger O, Eds.), Springer-Verlag
8. Sandau CD, Meerts IATM, Letcher RJ, McAlees AJ, Chittim B, Brouwer A and Norstrom RJ (2000) *Environ. Sci. Technol.* 34(18): 3871-3877.
9. Bennett ER, Li H, Sandela G, Hauschild K and Letcher R.J. (2002) In preparation.
10. Hansler RJ, Moisey J, Montie E, Norstrom RJ, Boon JP, van den Berg M, Seinen W and Letcher RJ (1999) *Organohalogen Compounds*, 42: 197-200.