TISSUE DISTRIBUTION OF PCBS AND ORGANOCHLORINE PESTICIDES IN ALASKAN NORTHERN FUR SEALS: COMPARISON OF VARIOUS CONGENER CLASSIFICATION SCHEMES

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Introduction

Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are believed to adversely affect reproduction and cause health problems in pinnipeds[1-4]. In this study, 145 PCB congeners and OCPs were analyzed in 10 juvenile male northern fur seals, *Callorhinus ursinus*, collected from Alaskan St. Paul Island. Tissue distribution of literature-proposed PCB congener grouping frameworks for human epidemiological studies was examined. The objectives are to understand the tissue distribution and potential risk associated with various chemical structures and toxicities of OCPs and particularly the PCBs. This study may not provide definitive explanations, since free ranging wild animals show large individual variation due to differences in exposure, habitat, and other confounding factors. Rather, the results might indicate avenues of research that could be fruitful to explore. Analyses of 145 PCB congeners and toxicity grouping evaluation in this study suggest that PCB contamination could cause the decrease in population of northern fur seals.

Materials and Methods

Alaskan northern fur seal tissue samples were collected from 10 juvenile males by subsistence hunters from St. Paul Island, Alaska during 2003 and 2004. The samples were lyophilized and extracted with accelerated solvent extraction and cleaned up with chromatographic columns. The cleaned-up extracts were analyzed on a Varian 3800 GC/Saturn 2000 ion trap mass spectrometer.[5]

Results and Discussion

Mean concentrations of PCBs and various OCPs in eight sets of fur seal tissues expressed both as wet weight (ww) and lipid weight (lw) are shown in Table 1. In all tissues analyzed, both the sum of PCBs and DDTs are the dominant pollutants with summed concentrations ranging from 4.8-578 ng/g ww and 7.7-798 ng/g ww, respectively. Concentrations of other groups of pollutants such as hexachlorocyclohexane (HCH), heptachlor and hexachlorobenzene (HCB) were relatively low in the tissues, ranging from non-detectable (nd) to 50.7 ng/g ww. For all pollutants analyzed, the highest concentrations were found in the blubber, lower levels (about 10% of that found in the blubber) in the heart, liver, and kidney, and the lowest levels were found in the reproductive tissue, muscle, brain, and lung. When the data were expressed in ng/g lw, considerable normalization among the tissues was observed with the exceptions of brain and lung (Table 1).

Several proposed groupings of individual PCBs based on their known toxic actions[6-10] were applied in this study. Despite the uncertainties of this approach, it yields greater insight to toxicity than merely observing the levels of the congeners without regard to their biological action.

∑PCB-TEQs, derived from 12 dioxin-like PCB congeners[6], ranged from 0.18 to 0.55 ng/g lipid weight (lw) in all analyzed tissues with the exception of brain and lung, where the levels were less than 0.01 ng/g lw (Table 2). The dominant contributors to ∑PCB-TEQs are CBs-105, 118, and 126. In the blubber samples, CB-126 contributes about 40% to ∑PCB-TEQs. In the other tissues, CB-118 becomes the dominant contributor to ∑PCB-TEQs with percentage contribution ranging from 35% to 69%. The remaining 197 PCB congeners have different modes of action underlying their toxic effects. One mode of action that has been shown for di-*ortho*-substituted non-coplanar PCBs is the interference with various intracellular signaling pathways dependent on

Ca^{2+} homeostasis and the resulting cellular, organ-level, and organismal effects. The *ortho*-substituted non-coplanar congeners produce other cellular or organ-level effects including changes in protein kinase C translocation, alteration of cellular dopamine uptake, formation of reactive oxygen species, and thyroid effects. A proposed neurotoxic equivalent (NEQ) scheme was applied for estimating these PCB mixtures' toxicity.[7] The highest PCB-NEQs were found in the blubber and liver while the lowest PCB-NEQs were found in the brain and lung tissues, at 17.7 ng/g lw and 34.5 ng/g lw, respectively (Table 2). Although the PCB-NEQ levels in the brain and lung are low compared to the other tissues, they are two times higher than the levels of Aroclor 1268 (16 ng/g), which had an effect on the intracellular Ca^{2+} buffering in rat cerebella granule cells[11]. The other tissues had much higher values than the brain and lung. The results indicated that PCBs could have cellular and biochemical effects on many seal tissues.

McFarland and Clarke[8] elected to group PCBs primarily according to enzymatic action related to induction of cytochrome P_{450} subfamilies, although other factors were also utilized (Figure 1). Group 1a included the three aryl hydrocarbon (Ah) agonists [3-methyl-cholanthrene-type CYP1A inducers, (3-MC)], and they are CBs-77, 126, and 169. Detectable levels of CBs-77 and 126 were found in the blubber, heart, kidney, and liver. Group 1b included PCBs that are mixed function oxidase inducers of both 3-MC and phenobarbital (PB) types (mixed-type inducers) that have been reported frequently in the environment and are relatively abundant, including CBs-105, 118, 128, 138, 156, and 170. Group 2 included known and predicted PB-type inducers, also known as constitutively active receptor agonists that are frequently reported in the environment and relatively abundant in tissues. This group included CBs-87, 99, 101, 153, 180, 183, and 194. Group 3 included CBs-18, 44, 49, 52, 70, 74, 151, 177, 187, and 201, which are weaker inducers or non-inducers but are found frequently in the environment. Group 4 included CBs-37, 81, 114, 119, 123, 157, 158, 167, 168, and 189, which are mixed-type inducers that are rarely found in the environment. For groups 1a and 4, lower but detectable levels were observed in the blubber, heart, kidney, liver, muscle, and reproductive tissue, and non-detectable levels in the brain and lung. Because these low levels are less likely to show an observable effect, they will not be discussed further. Group 1b occurs in high levels in all the tissues, and enzyme induction by this PCB group could be a factor in most tissues with exception of the brain and lung, which contain relatively low levels. Although groups 2 and 3 also occur in high concentrations, their potential for enzyme induction is rather low, and so they are less likely to cause problems than group 1b. Comparison of the relative abundance of groups 1b, 2, and 3 among tissues showed that while there are approximately two-fold higher concentrations in group 1b compared with those in group 3, this is not the case for reproductive tissue where similar concentrations were found for groups 1b and 3. Concentrations of group 2 generally are similar with those of group 3 among the tissues with exceptions of the blubber and reproductive tissues where relative concentrations between groups 2 and 3 were two-fold and two-thirds, respectively. This again provides strong evidence that the tissue distribution is specific for congeners and not totally explained by the lipophilicity of the congeners and the lipid content of the tissue.

Moysich et al.[9] divided all PCBs into low (Cl_{2-4}-PCBs), moderately (Cl_{5-7}-PCBs), and highly chlorinated (Cl_{8-10}-PCBs) groups. The results (data not shown) showed that the Cl_{5-7}-PCBs occur in the highest concentrations, while the Cl_{2-4}-PCB and Cl_{5-7}-PCB groups have lower concentrations in all the tissues. In the fur seal blubber, heart, kidney, liver, muscle and reproductive tissues, the levels of the Cl_{5-7}-PCB group are comparable with those in human adipose samples and blood samples collected from incident patients with non-Hodgkin lymphoma[12] and are two to thirteen times higher than those in human serum samples collected from patients with prostate cancer[13]. Wolff et al.[10] took into account the biological action of PCBs, assigning PCBs into three groups: estrogenic/neurotoxic (ER agonists), anti-estrogenic (Ah receptor agonists dioxin-like), and enzyme-inducing PB-type cytochrome P_{450}. The classification also incorporated prevalence in the environment based on major PCB peaks found in both human samples and household dust. This scheme was of special interest because the decline of seals could possibly be related to hormonal aberrations produced by PCBs.[1,2] The first group included potentially estrogenic PCBs and was divided into two subgroups. Group 1a consisted of non-persistent, estrogenic PCBs with weak PB-type-inducing activity. Group 1a included CBs-31, 44, 49, 52/69, and 70; Group 1b consisted of persistent PCBs with weak PB-type of activity. Group 1b included CBs-101/90, 174, 177, 187, and 201. Group 2 included PCBs with potentially dioxin-like, immunotoxic, and anti-estrogenic activity and was divided into two subparts. Group 2a consisted of persistent PCBs with dioxin-like activity; which included CBs-66, 74, 77, 105, 118, 126, 156, 167, and 169, thus including the most important dioxin-like congeners. Group 2b

consisted of persistent PCBs with limited dioxin-like activity, which included CBs-128, 138/163/164, and 170. Group 3 consisted of biologically persistent PB-type inducers, including CBs-99, 153, 180, 183, and 196/203, nearly identical to the McFarland and Clarke group 2 category. As can be seen in Figure 2, the tissue distribution is quite different from that observed in groupings that do not take toxicity into account. Although the blubber has high concentrations of PCBs, the other tissues (except brain and lung) show approximately equivalent amounts. Brain PCB concentrations are undoubtedly low because of the blood brain barrier. Lung tissue is highly perfused and normally shows little accumulation of lipophilic substances. Of particular interest is group 1a, which is significantly elevated in reproductive tissues where an estrogenic action could negatively impact reproduction in males. Although the data are insufficient to draw definite conclusions, the results do indicate this component of the classification may be of great interest in future studies.

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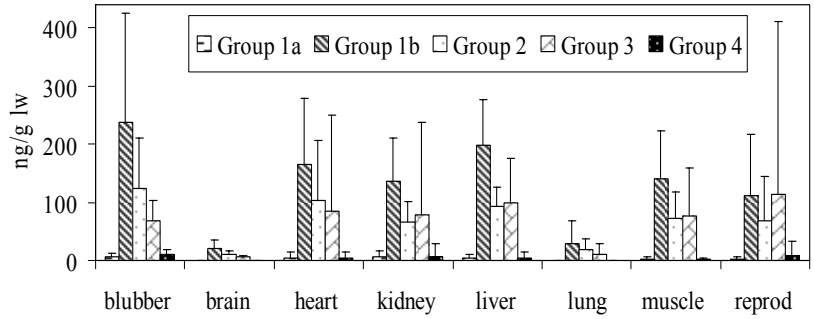

Figure 1. Concentrations (ng/g lw) of PCBs in Alaskan fur seal tissues, grouped according to McFarland and Clarke [8].

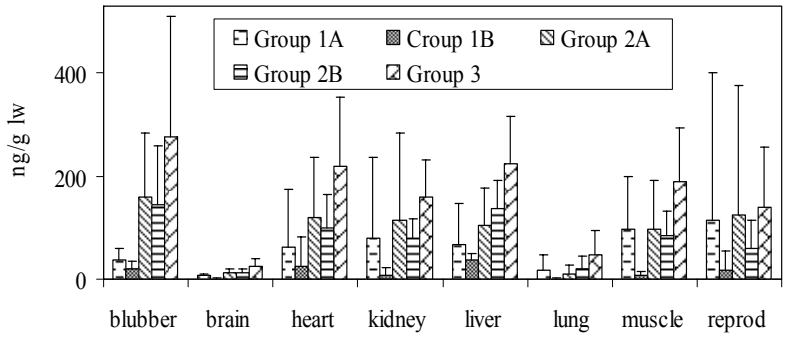

Figure 2. Concentrations (ng/g lw) of PCBs in Alaskan fur seal tissues, grouped according to their biological action and environmental prevalence proposed by Wolff et al.[10].

Table 1. Mean concentrations of PCBs and various OCPs in eight sets of Alaskan fur seal tissues expressed both as wet weight (ww) and lipid weight (lw)

Tissues	% lipid	ww or lw	Mean concentrations (ng/g)				
			∑PCBs	∑ DDT	∑HCH	Heptachlor	HCB
Blubber	73.1	ww	578.0	798.5	50.7	5.6	0.28
		lw	823.3	1094.7	72.1	13.0	0.42
Brain	11.8	ww	9.6	14.4	8.9	0.57	0.02
		lw	74.3	124.0	75.3	4.7	0.20
Heart	6.3	ww	46.9	54.3	6.3	1.3	0.03
		lw	732.0	875.3	89.6	19.3	0.38
Kidney	4.8	ww	31.2	36.6	4.1	1.1	0.02
		lw	586.7	714.6	80.3	21.4	0.47
Liver	4.6	ww	34.4	42.3	3.3	0.84	0.03
		lw	776.6	904.8	80.7	19.9	2.0
Lung	5.8	ww	4.8	7.7	0.68	0.44	nd[a]
		lw	128.2	284.4	22.2	15.0	nd
Muscle	2.0	ww	12.7	16.3	1.9	1.2	0.02
		lw	637.5	794.7	90.1	54.7	1.1
Reprod	2.4	ww	15.7	14.8	1.2	0.44	nd
		lw	645.7	589.2	52.9	18.3	nd

[a] Not detected.

Table 2. PCB-TEQs values in Alaskan fur seal tissues calculated as TEF[6] and PCB-NEQs as NEQ scheme[7] expressed both as wet weight (ww) and lipid weight (lw)

Tissue	Mean values of PCB-TEQs and PCB-NEQs			
	∑PCB-TEQs		∑PCB-NEQs	
	ng/g lw	ng/g ww	ng/g lw	ng/g ww
Blubber	0.5452	0.4590	193.1	134.0
Brain	0.0062	0.00087	17.7	2.1
Heart	0.4249	0.0326	172.4	11.0
Kidney	0.2435	0.0112	166.1	9.1
Liver	0.4156	0.0170	215.4	9.4
Lung	0.0003	0.000008	34.5	1.1
Muscle	0.2094	0.0046	177.1	3.5
Reprod	0.1792	0.0048	198.0	4.7