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Ecological Risk Assessment Using High Resolution Analysis of Polychlorinated Biphenyls (PCBs)

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Abstract—Polychlorinated biphenyls (PCBs) are complex mixture of ubiquitous, persistent, bioaccumulative toxic substances. Congener specific determination of this mixture demands high resolution (against co-contaminants and coeluants), sensitivity and accuracy. Analytical methodology using high resolution cleanup and multi dimensional chromatography with electron capture detection (MDGC-ECD) was applied to archived samples of flat fish, a flightless bird (Penguin), a flying bird (Razorbill) and seal. Thus the determined concentrations, represented in pg/mg fat, ranged from as low as 88 to as high as 32,000. The actual levels (mean) were: flat fish: 152; penguin (n = 2): 321; razorbill (n = 2): 576 and seal (n = 7): 7645. Target organisms accumulated several of the toxic non-, mono-, and di-*ortho* Cl substituted congeners including the most toxic 3,3′,4,4′5-pentachlorobiphenyl (IUPAC = 126) in their body fat. Toxic Equivalents (TEQs) were calculated and reported as follows (fg/mg TEQ fat): flat fish: 70; penguin (n = 2): 68; razorbill (n = 2): 1600; and seal (n = 7): 635. Metabolic index for each PCB congener was calculated in seal based on X/153 ratio. This index revealed metabolism of several persistent congeners indicating induction of both Cytochrome P450 CYP IA and IIB enzymes. This congener-specific analysis of PCBs revealed the ecological stress in aquatic organisms from land based anthropogenic contamination.

Keywords: PCBs, multidimensional chromatography, metabolic stress, ecological indicators, ocean health, seals, birds, fish, Antarctic, North Atlantic, Baltic

INTRODUCTION

The objective of this study is to prove that high resolution determination of polychlorinated biphenyls (PCBs) in aquatic organisms, in combination with rigorous cleanup is advantageous not only for monitoring contaminants but also for understanding the effect of that contamination. PCBs are transformed in the environment both physically and biologically. This transformation is understood

properly if only the chemical analysis is accurate, unambiquitous and sensitive. Several such analytical methods have been developed for PCBs (Erickson, 1997). One of them from the Institute for Marine Research (IFM), Kiel, Germany fulfills several of the critical Quality Assurance and Quality Control (QA&QC) criteria (Petrick *et al*., 1988). The method includes several clean-up steps and high resolution determination using Multi Dimensional Gas Chromatography with Electron Capture Detection (MDGC-ECD) (Duinker *et al*., 1988). Body fat from several organisms was analyzed. PCBs bioaccumulate in fat tissues and their characterization will reveal the intricate biotransformation that occurred during metabolism. PCBs are well studied as model substances in understanding the biological enzyme induction in organisms, both in natural and in laboratory conditions (Tanabe *et al*., 1988; Boon *et al*., 1989, 1992; Safe 1994; Kannan *et al*., 1995). Unlike most gill breathing aquatic organisms, marine mammals cannot eliminate foreign substances to the surrounding water through equilibrium partitioning. Instead, they achieve this through metabolism.

The cytochrome P450 monooxygenase enzymes are responsible for this metabolism, whereby PCBs can act as inducers, inhibitors or substrates of the CYPIA and CYPIIB subfamilies of isozymes (Safe *et al*., 1985; McFarland and Clarke, 1989). Congeners lacking *ortho*-Cls, but having chlorine atoms at both *para* positions and at two, three or four *meta* positions are pure cytochrome P450 IA (Methylcholanthrine: MC-type) inducers. These molecules show the same induction pattern as 2,3,7,8-TCDD, as they can assume a planar configuration relatively easily. Molecules with structures derived from these pure MC-type inducers by substitution of one to four *ortho*-Cls show a progressively decreasing MC-type and increasing Phenobarbital: PB-type induction (McFarland and Clarke, 1989).

The structure-activity-relationship for the metabolism of PCBs depends on the presence of vicinal Hs in *m*,*p* positions (substrates for cytochrome P450 IIB) or *o*,*m* positions (substrates for cytochrome P450 IA) (Lewis *et al*., 1986). The number of *ortho*-Cls affects metabolism: cytochrome P450 IA and IIB isozymes show a high affinity for planar and globular molecules, respectively (Boon *et al*., 1992).

There is a qualitative difference among the species of marine mammals in metabolizing PCBs. Seals show PB-type activity (Tanabe *et al*., 1988; Boon *et al*., 1989; Nakata *et al*., 1995). Several authors reported a low or absence of PB-type activity in cetaceans (Tanabe *et al*., 1988; Watanabe *et al*., 1989). It cannot be excluded that analytical problems play a role in the reported differences as most of the relevant non-*ortho*, mono-*ortho* and di-*ortho* Cl PCBs co elute with other PCBs (Bruhn *et al*., 1995).

Hence, a thorough congener specific analysis for PCBs was undertaken from archived samples of flat fish, razorbill and seal from Northern Atlantic, Baltic sea and penguin from the Antarctic. The contamination status of the ecosystem was studied along with the toxic threat posed by PCB contamination as measured by Toxic Equivalency (TEQ) and Metabolic Index (MI).

MATERIALS AND METHODS

Body fat was analyzed from archived samples. The samples were stored in –20°C cold storage facility at the IFM, Kiel until analysis. Flat fish, Atlantic halibut (*Hippoglossus hippoglossus*), Razorbill (*Alca torda*), Harbour seal (*Phoca Vitulina*) and Adelie penguin (*Pygoscelis adeliae*) were analyzed. The animals were found dead or as by-catches of North Sea fisheries and brought to the Institute for archiving.

1–10 g of the tissues was homogenized with Na_2SO_4. Internal standards (CBs-40, -87, -207) were added. The sample was extracted with hexane in a modified soxhlet apparatus (Ehrhardt, 1987) for 3 h. The hexane extractable lipid content was determined in an aliquot of the extract. Another aliquot (about 10 mg lipid) was used for clean-up with alumina and a class separation of the organic compounds by High Pressure Liquid Chromatography (HPLC) (Petrick *et al.*, 1988) prior to measurement of PCBs by single and multidimensional GC-ECD. A Siemens SiChromat 2 GC was used for PCBs eluting as single peaks from a 60 m SE-54 column (phenyl silicone phase) (0.25 mm i.d.). For the co eluting pairs 66/95, 90/101, 77/110, 129/126/178, 132/105, 138/158, 151/82, 202/171/156 and for 187,180 and 146 MDGC-ECD analysis was performed with 30 m SE-54 (0.32 mm i.d.) and 60 m OV-210 (trifluoropropyl fluid) (0.25 mm i.d.) columns in series. The detection limits based on 10 mg lipid and an end volume of 100 ml were between 1 and 2 ng/g lipid (for example: 2 ng/g lipid for CB-52,1 for CB-153 and for CB-180).

72 individual or co eluting pairs of chlorobiphenyls were present at detectable levels. The CB contents were calculated on a lipid weight basis. CB patterns were compared by transforming contents into X/153 ratios. The behavior of an individual congener can be studied from the data in relation to the sum of all congeners measured or with reference to the most persistent congener. CB-153 (2,2,4,4′,5,5′-hexaCB) is an ideal candidate (biomarker) as it occurs often at the highest concentrations in biota and it can be analyzed accurately.

RESULTS AND DISCUSSION

The concentration of PCBs (pg/mg lipid) in target organisms is presented in Table 1. Among the organisms studied, seals (n = 7) recorded the highest concentration of 7645, followed by razorbill (n = 2) 576; penguin (n = 2) 321 and Atlantic halibut (flat fish) 152. Not all the 72 congeners were detectable in all specimens, however, most seals showed most of the congeners. The congeners ranged from trichloro to nonachloro biphenyls. The most prominent congeners in seals were PCB-153, 183, 187, 149 and 141/179. Heart-cut technique in MDGC-ECD made it possible to measure non-*ortho* Cl congeners without enrichment using a graphite column, as practiced by most other laboratories. Interestingly PCB-126 was measured in all specimens, PCB-169 in some and PCB-77 rarely. The absence of PCB-77 in our results, a congener which is invariably measured by most researchers is due to the fact that the co eluting PCB-110 is fully resolved in MDGC system. It was shown earlier that most carbon based methodologies do

Table 1. Concentration of PCBs (pg/mg lipid) in organisms.

(IUPAC #)	Flat fish	Penguin		Razorbill		Seal						
		1	2	1	2	1	2	3	4	5	6	7
20/33/53						2.8	0.8	1.1				
25						2.30	34	1.50				
28						4.5	4.9	3.7				
31						4.30		1.90				
36						1.30	1.10					
37		0.03			0.03	0.20	0.06	0.13				
41,64						4.9	3.1	3.6				
42		0.34	0.08	0.01	0.17	0.76	0	0.6	0.72	0.02	3.74	
44	1.9					8.70	3.30	5.80	2.90	1.30	39	3.18
46							6.90	27				
47/48/75						9.90	67	46				
49	1.1					6.60	16	15		0.30	49	4.43
51/22						0.90		2.00				
52	5.43					17	45	43	6.30	0.80	296	7.70
59	0.43	2.37	0.41	0.02	0.17	5.5	2.5	4.2	0.65	0.05	9.86	
60/56						3.9	3.5	3.9				
66/95	2.79					27	33	34		2.56		
70	0.87					13	7.10	7.90		0.46		
74	0.67					5.90	44	23		0.11		
77				0.07	0.4				0.05			
83						1.5	12	10				
87/115	1.64					25	29	26		0.23		
91						3.8	12	12				
92						11	38	33				
99	3.37					142	625	329		0.21		

(IUPAC #)	Flat fish	Penguin		Razorbill		Seal						
		1	2	1	2	1	2	3	4	5	6	7
100/67							2.60	3.40				
101/90	5.1					56	267	194			517	7.89
105		1.42	0.22		1.22	3.30	33	24	0.22	0.00	9.90	3.50
110		30	4.06	0.326	2.28	31	21	56	2.90	0.03	73	
114		0.86	0.19	3.26	0.18	1.80	45		34	0.00		
118	11	32	11	37	115	41	19	165	51	0.30	233	9.64
123	1.14	2.06	0.69	16.19	55	2.60	4.70	19	16	0.01	82	1.00
126	0.69	0.48	0.25	5.98	24	1.20	2.00	11	2.90	0.11	5.50	14
128						113	344	177		0.00	641	3.02
129	0.03	0.13	0.03	0.2	0.61	0.02	0.30	0.30	0.05	0.01	0.60	1.60
130						14	61	32				
131/122	0.62										544	
134						1.80	0.70	0.80				
135						9.00	46	39				
138	16.4					870	1978	1146	13	16	6776	101
141/179	0.47					20	29	30		0.47		
146	6.88					242	712	408	96	6.88	3278	6.08
149	3.57	18	5.28	9.86	16	53	220	239	190	0.30	1002	12
151/82						12	55.50	55				3.75
153	31	102	35	48	181	685	480	367	78	0.00	7910	54
156		188	170	256	0.5	52	110	110	32	0.56	295	
157	0.29	0.77	0.14	3.35	7.65	2.20	9.70	35	0.14	0.04		
167		7.05	7.1	96.17		2	5.20	4.50			133	
169				4		5.20		0.50			0.11	

Table 1. (continued).

(IUPAC #)	Flat fish	Penguin		Razorbill		Seal						
		1	2	1	2	1	2	3	4	5	6	7
170/190	2					367	483	341	1.70	2.00	1158	0.64
171		7.1	6.8	146	55	30	90	95	38	0.74	228	
172						42	103	78	1.80		209	
173		0.1	0.29	0.1			1.40	4.00	0.10			
174						15	51	41			78	
175						3.30	24	26				
176/137						33	197	103	13		442	
177	0.94					67	271	172		0.94	565	
180	12					652	827	668	20	12	2256	16
183	4.75					189	328	268	11	4.75	874	5.15
185							3.20	4.60				
187	24.1					403	801	489	29	24	2321	14
189				32	0.03	6.20	20	55	2.70	0.00	11	
191						8.00	25	21				
193	3					102	192	105		3.00	560	1.50
194	0.88					51	64	46		0.88	40	0.73
197	0.85					7.00	15	12		0.85		
198						5.90	11	8.90				
199	2.83					84	104	79	1.00	2.80	275	0.74
203/196	4.19					125	134	111	4.50	4.20	351	
202	1.41	3.55	3.1	33		418	0.00		141	0.55	280	
207						1.60	6.30	2.30				
208/195							20	11				
Sum	152	397	245	692	459	5130	9202	6489	790	88	31543	272

Table 2. TEQs (fg/mg lipid) of PCBs in North Atlantic Ecosystem.

PCBs	Flat fish	Penguin		Razorbill		Seal						
		1	2	1	2	1	2	3	4	5	6	7
Non-*ortho*												
77				0.007	0.04				0.005			
126	69	48	25	600	2400	120	200	1100	290	11	550	1400
169		21.9	21	120				15	3.3			
Mono-*ortho*												
105		0.042	0.0066		0.036	0.099	1.26	0.72	0.0066	0.003	0.297	0.105
114		0.0258	0.0057	0.099	0.0054	0.054	1.35		1.02			
118	0.33	0.96	0.33	1.11	3.6	1.23	0.57	4.95	1.53	0.009	6.9	0.288
156		5.7	5.1	7.8	0.015	1.95	4.5	3.3	0.96	0.0168	9	
157	0.0087	0.0231	0.0042	0.102	0.231	0.066	0.291	1.05	0.0042	0.0012		
167	0.0057	0.213	0.213	2.88	0	0.06	0.156	0.135	0		3.9	
189				0.96	0.0009	0.186	0.6	1.65	0.081		0.33	
Di-*ortho*												
101	0.14	0.44	1.02	0.00104	0.03	1.12	5.4	3.8	0	0	10.4	0.158
128	0.0044	0.36	0.132	0.038	1.32	2.2	6.8	3.6	0	0	12.8	0.06
129	0.00064	0.0026	0.00064	0.004	0.012	0.00044	0.0056	0.0054	0.001	0.00022	0.012	0.032
138	0.009	0.66	0.154	0.24	4.4	17.4	40	24	0.26	0.32	136	2
146	0.0066	0.132	0.038	0.0048	0.176	4.8	14.2	8.2	1.92	0.138	66	0.122
153	0.62	2	0.7	0.96	3.6	13.6	12.2	7.4	1.56	0.6	160	1.08
170		0.192	0.044			7.4	9.6	6.8	0.034	0.04	24	0.0128
172						1	2.8	2			4.2	
180	0.054	0.74		0.0018	0.086	13	16.6	13.4	0.4	0.24	46	0.32
194		0.078				1.02	1.28	0.92		0.0176	0.8	0.0146
Total	70	81	54	734	2414	185	318	1197	301	12	1031	1404

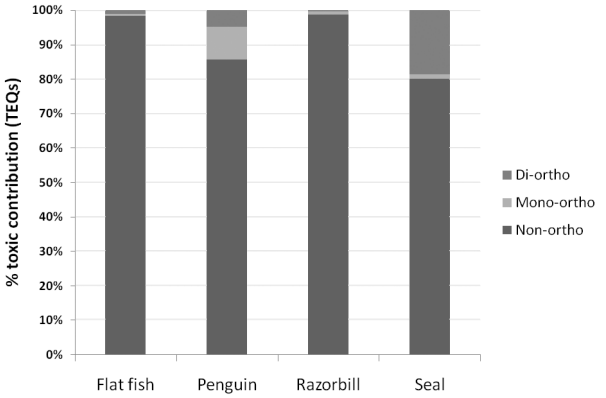

Fig. 1. Toxic contribution of PCBs in target species based on TEQs (WHO 2005).

not resolve this problem fully (Kannan *et al*., 1991) and a direct measurement (without carbon enrichment) using MDGC-ECD or in combination will resolve it satisfactorily (Kannan *et al*., 1992, 1993).

The concentration of toxic PCBs is customarily represented as Toxic Equivalents, after multiplying the concentration with TEF values (Bhavsar *et al*., 2008). Table 2 represents such an approach. The Toxic Equivalency (TEQs) (fg/mg lipid) in organisms are as follows: flat fish - 70; penguin ($n = 2$) - 68; razorbill ($n = 2$) - 1600; and seal ($n = 7$) - 635. The highest levels were recorded in Razorbill due to its high bioaccumulation of PCB-126. The percentage distribution of non-, mono and di-*ortho* Cl substituted PCBs in the target species is presented in Fig. 1. It is observable that in the Atlantic flat fish and Razorbill the entire toxicity arose from non-*ortho* Cl congeners, while in penguins and seals both mono- and di-*ortho* Cl PCBs contribute to toxicity.

There is another way to understand the toxic impact of PCBs in organisms. This is through the investigation of metabolism in organisms, facilitated through structure-biological activity-relations of PCBs (Boon *et al*., 1989; Kannan *et al*., 1995; Bruhn *et al*., 1995). The criteria are the presence or absence of vicinal hydrogen atoms in *meta*,*para* and/or *ortho*,*meta* positions as well as the number of *ortho*-Cls. These structural characteristics determine whether a molecule can be metabolized by the cytochrome P450IA and/or IIB isozymes. The PCBs are grouped as follows: Group I: congeners (153, 178, 180, 183, 187, 193, 194, 199, 201, 202) with no vic. Hs in *m*,*p* or *o*,*m* positions and having 2–4 Cls in the *ortho* position. These molecules are considered to be non-metabolizable and hence persistent. Group IIa consists of congeners (52, 92, 97, 101, 110, 129, 141) with 1-2 vicinal H atoms in the *m*,*p* or *o*,*m* positions with 2 Cls in the *ortho* position. This group has congeners that are either pure or weak PB type enzyme inducers such as CB-52 or 101. Group IIb (91, 95, 132, 136, 149, 151, 174, 179) has similar characteristics but with 3 to 4 Cls in the *ortho* positions. This group consists of

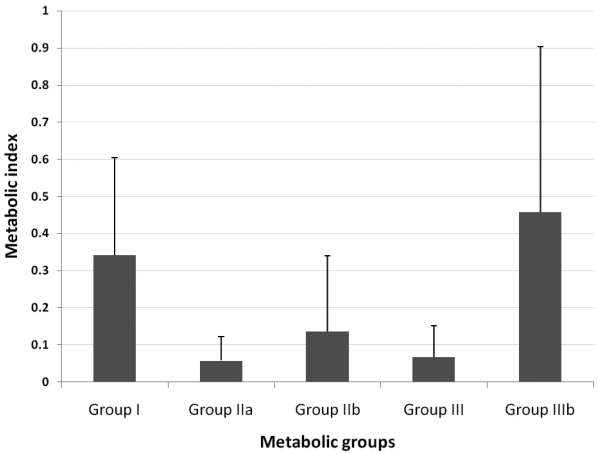

Fig. 2. Metabolic efficiency of Seals based on "metabolic index".

weak PB type inducers such CB-151. Group IIIa consists of congeners (66, 70, 74, 77, 105, 107, 118, 126, and 156) that have Cl substitution in the *meta-para* position and at least 1 to 2 vicinal hydrogen atoms at *ortho-meta* position. There is either no or 1 Cl substitution at *ortho* position. These congeners are strong inducers and substrate for MC type enzymes. Group IIIb (99, 128, 138, 170, 171, and 177) is similar with no vicinal H atoms at *meta-para* position, 1 or 2 vicinal H at *ortho-meta* position with 2 to 3 Cl substitution at *ortho* position. Thus, most PCBs in this group are MC type inducers but there are weak or pure PB type inducers as well (e.g. CB-99 and 177).

X/153 ratio was calculated for all detectable congeners in seal and listed according to the metabolic group. A metabolic index was derived by averaging the X/153 ratio in a group. The derived values are plotted in Fig. 2 according to metabolic groups. A metabolic index value of 1 represents, total non-metabolizability or persistence. 0 value or absence of a congener denotes possible metabolism or its absence in the diet. Thus group I should ideally have a value of 1 and group II and III should have a value ranging from 0 to 1 depending on the type of induction that occurred in the organism. This figure clearly indicates strong induction of both PB and MC type enzymes in Seal based on low metabolic index for Group IIa, b and IIIa. This is again derived from the absence of an ideal 1 value for any group indicating that even the most persistent congeners are metabolized in Seal. This is possible only if there is a strong induction of enzymes. Thus Fig. 2 points out the complicated pharmacokinetics of PCBs in Seal. MI value of less than 0.5 for all the 5 groups strongly points out that Baltic Seals were under great physiological stress. This raises concern for the health of these marine mammals. A recent study in Baikal seal (*Phoca sibirica*) not only confirms the bioaccumulation of non-*ortho* Cl PCBs but also the possibility of

Cytochrome P450 enzyme induction in those organisms (Tanabe *et al*., 1994, Nakata *et al*., 1995, 1997). Similarly, PCB contamination in Antarctic Penguins has also been reported earlier (Subramanian *et al*., 1986, 1987).

CONCLUSION

This study emphasizes the need for high resolution analytical techniques in the determination of complex environmental chemical mixtures such as PCBs for monitoring the contamination status and to assess the ecological health risks.

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