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Non-, Mono-, and Di-*o*-Chlorobiphenyl Concentrations and Their Toxic Equivalents to 2,3,7,8-Tetrachlorodibenzo[*p*]dioxin in Aroclors® and Digestive Glands from American Lobster (*Homarus americanus*) Captured in Atlantic Canada

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Polychlorinated biphenyls (PCBs) are contaminants of concern in coastal marine fisheries. Much of their toxicity is associated with chlorobiphenyls (CBs) that can assume a planar configuration, the non-*o*-chlorinated ones; 3,3',4,4',5-pentachlorobiphenyl (IUPAC No. 126) and 3,3',4,4',5,5'-hexachlorobiphenyl (IUPAC No. 169) being the most toxic (Kannan et al. 1993). These and their mono- and di-*o*-chlorinated analogs are similar in structure and toxicity to 2,3,7,8-tetrachlorodibenzo[*p*]dioxin (Hühnerfuss et al. 1995; Kannan et al. 1993) and are generally present in environmental materials at higher concentrations than the chlorinated dioxins (de Voogt et al. 1990). The toxic equivalent factors (TEFs) of selected non-, mono-, and di-*o*-chlorinated CBs to 2,3,7,8-tetrachlorodibenzo[*p*]dioxin that were originally estimated by Safe (1990), have been modified later (Hühnerfuss et al. 1995), and subjected to a panel opinion (Ahlborg et al. 1994).

Due to its high fat content, PCBs are found in the digestive glands of American lobsters (*Homarus americanus*). We have investigated concentrations of CBs in lobster digestive gland and the Aroclors®. The Aroclors were analyzed to assist in the identification of possible sources of the CBs found in lobster samples.

MATERIALS AND METHODS

Market-sized lobsters (usually 10) were captured with commercial gear in: Halifax (large industrialized harbor), Sydney (site of a massive coal tar dump), and Petit-de-Grat (site of a large fish plant) Harbors, Nova Scotia; at the dredge spoil dump at Saint John Harbor (New Brunswick); and in St. Margarets Bay, Nova Scotia (Control). The digestive glands were removed and two pools (usually 5 animals per pool) from each site prepared and stored frozen. PCBs were extracted into isooctane from saponified tissue and cleaned up by gel-permeation chromatography and treatment with sulfuric acid. Non-o-chlorinated CBs (IUPAC Nos. 37, 77, 126, and 169 - Fraction 2) were separated from the rest (Fraction 1) by Florisil:carbon chromatography and analyzed by capillary gas chromatography-selected ion mass (SIM) spectrometry (King et al. 1996). CB standards were obtained from Ultra Scientific, Kingstown, Rhode Island., CB standard mixtures, CLB1A-D, from The

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National Research Council of Canada, Halifax, Nova Scotia, and Aroclors® from Monsanto, St. Louis, Missouri. All solvents were pesticide grade (distilled in glass; BDH, Toronto, Ontario). An operational blank (no sample) was analyzed with each batch of 4 samples along with one spiked sample. Recoveries of non-*o*-chlorinated CBs (spikes ranged 20-5000 $pg \cdot g^{-1}$) averaged 84.5±6.5%. The detection limits (3 times noise level) were 20 $pg \cdot g^{-1}$ wet wt. for IUPAC Nos. 37, 81, and 126 and 50 $pg \cdot g^{-1}$ wet wt. for IUPAC No. 169. Recoveries of other added CBs averaged 88.8±10.1% with a detection limit set at 50 $pg \cdot g^{-1}$ wet wt.

RESULTS AND DISCUSSION

Figure 1 shows SIM-total ion chromatograms: A. Sydney Harbor digestive gland extract (Fraction 1 off Florisil:carbon); B. Fraction 2 of the same sample; and, C. SIM-multiple ion chromatogram of B. Tables 1 and 2 contain data for 34 CBs found and measured in digestive gland (only concentrations >0.1 $ng \cdot g^{-1}$ wet wt. are shown) and Aroclors® (only concentrations >0.5 $\mu g \cdot g^{-1}$ wet wt. are shown), the total CB sum (ΣCB) in each, and the sum (ΣnCB) of the non-, mono-, and di-*o*-CBS in each. Two CBs listed in a row are possible identities, within the limits of our capability. However, de Voogt et al. (1990) report that, of the pair 138/158, CB 138 is more prevalent in environmental samples, and, of the pair 166/167, CB 166 has not been found in environmental samples. Standards were not available for all “toxic” CBs considered in the toxicological review of Ahlborg et al. (1994), namely CBs 123 and 157; however, we identified all significant peaks present in our chromatograms. A few unknown peaks were present in our chromatograms at intensities just above baseline. This suggests that insignificant concentrations of these CBs were present in our lobster samples and the Aroclors®. Our CB concentrations in the Aroclors® are comparable to those given in Schwartz et al. (1993) and in Hong et al. (1993).

The relative contributions of the CBs to the total CB concentration are also given in Table 1. Only percentages greater than 1.0% are shown for readability and to indicate major bioaccumulated CBs in contrast to their relative percentages in the Aroclors®. IUPAC Nos. 153, 138, 118, and 180 account for 65-67% of the total summed CBs in lobsters, but only 18, 44, and 48% for Aroclors® 1248, 1254, and 1260, respectively. Contributions of these four individual CBs to the summed CB total in lobsters are more variable (Table 1) than implied by their totals. Di- and trichlorobiphenyls are present in lobster digestive gland samples and Aroclors®. The ΣnCB/ΣCB ratios (Tables 1 & 2) in lobster suggests that Aroclors® 1254 and 1260 were the source of the contamination, because this ratio in these two Aroclors® (56% for Aroclor® 1254 and 1260) approach the ratios present in the lobsters (73.3-84.2%). However, the lobster ratios consistently exceed the ratios present in the Aroclors®, showing general enrichment of the “planar” CBs. Notably, the relative percentages of IUPAC No. 126 found in all lobster samples, the Sydney Harbor sample in particular, are higher than its relative percent in any of the Aroclors®. Selective enrichment of this planar CB and other “toxic” CBs raises toxicological concern with respect to the lobsters themselves and their predators.

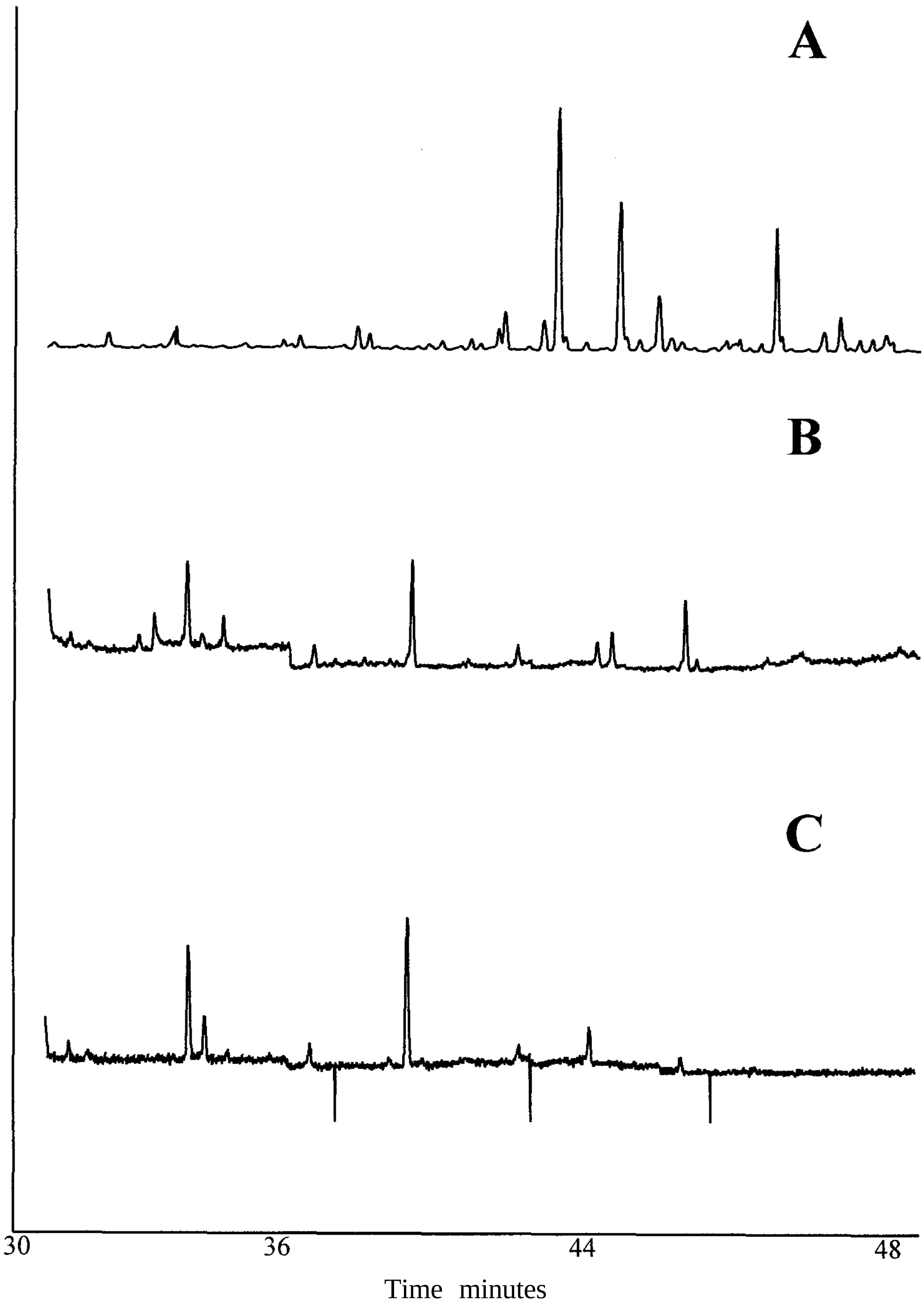

Figure 1. SIM-total ion chromatograms: A. Sydney Harbor digestive gland extract (Fraction 1 off Florisil-carbon); B. Fraction 2 of the same sample; and, C. SIM-Multiple ion chromatogram of B.

Table 1. Chlorobiphenyl Concentrations (only concentrations >0.1 $ng \cdot g^{-1}$ wet wt. are shown) in Lobster Digestive Glands from Various Maritime Harbors. (ΣCB - sum of all CBs, ΣnCB - sum of planar, mono- and di-*o*-chlorobiphenyls [* in table]. Values [only > 1% are shown] in parentheses are percent relative contributions to ΣCB).

IUPAC No.	CB Class	Halifax Harbor	Saint John Dump Site	Petit-de-Grat	Sydney Harbour	Control
1	mono					
2	mono					
3	mono					
5/8	di	0.2		1.0		
15	di	1.1	0.3	1.9		0.2
28/31	tri	22(1.2)	3.6(1.5)	15(1.0)	5.3	2.6(1.7)
37*	tri	3.4(2.0)	0.1	1.4	0.4	0.2
49	tetra	15	1.5	7.5	3.4	1.8(1.2)
77*	tetra	7.0	0.4	2.7	0.6	0.3
81*	tetra	0.2		0.1		
87	penta	18	0.1	31(2.1)	3.1	1.5
101	penta	93(5.0)	2.8(1.1)	210(14)	16(1.0)	2.9(1.8)
105*	penta	152(8.0)	9.5(3.8)	80(5.3)	14	6.5(4.1)
114*	penta	8.7	1.9	6.6	0.9	0.3
118*	penta	330(17)	25(10)	240(16)	38(2.0)	18(11)
126*	penta	1.1	0.2	0.7	0.2	0.1
138/158*	hexa	420(22)	22(8.9)	320(21)	340(21)	34(21)
141	hexa	6.2	0.7	7.3	11	
151	hexa	5.4	0.5	0.8	14	0.5
153*	hexa	350(18)	88(35)	330(22)	380(24)	39(24)
156	hexa	38(2.0)	2.6(1.0)	19(1.3)	4.1	2.1(1.3)
166/167*	hexa	51(2.7)	2.5(1.0)	26(1.7)	14	6.0(3.8)
169*	hexa			0.1		
170*	hepta	41(2.2)	8.7(3.5)	18(1.2)	64(4.0)	3.8(2.4)
180*	hepta	160(8.4)	31(12)	89(5.9)	320(20)	14(8.8)
183	hepta	18	6.6(2.6)	14	35(2.2)	
187	hepta	56(2.9)	26(10)	49(3.0)	160(10)	10(6.3)
189*	hepta	1.7	0.30	0.94	2.9	0.2
194*	octa	14	2.2	7.3	34(2.1)	2.4(1.5)
195	octa	4.4	2.5(1.0)		11	0.5
196	octa	6.2	0.74	7.5	32	2.1(1.3)
201	octa	11	1.1	4.5	10	1.0
203	octa	8.7	2.1	6.7	23(1.4)	1.9(1.2)
206	nona	6.1	1.3	10	6.6	3.2(2.0)
ΣnCB		1600(84.2)	200(80)	1100(73)	1200(75)	130(81)
ΣCB		1900	250	1500	1600	160

Table 2. Chlorobiphenyl Concentrations (only concentrations >0.5 $\mu g \cdot g^{-1}$ wet wt. are shown) in Aroclors®. (ΣCB - sum of all CBs, ΣnCB - sum of planar, mono- and di-*o*-chlorobiphenyls [* in table]. Values [only > 1% are shown] in parentheses are percent relative contributions to ΣCB).

IUPAC	Aroclor®						
No.	1016	1221	1232	1242	1248	1254	1260
1	1.8	160(83)	82(36)	1.5			
2		6.3(3.3)	3.9(1.7)				
3		24(13)	14(6.1)				
5/8	73(33)	0.8	37(16)	71(29)	5.1(2.8)		
15	21(9.4)	0.5	9.0(3.9)	19(7.7)	3.5(1.9)	0.5	
28/31	120(54)	1.5	64(28)	120(49)	62(34)	2.6	1.2
37*	3.4(1.5)	0.4	1.1	2.1	2.0(1.1)	0.2	0.2
49	3.6(1.6)		2.0	3.8(1.5)	7.3(4.0)	5.4(1.5)	
77*			0.75	1.1	2.0(1.1)		
81*							
87			3.1(1.4)	4.4(1.8)	16(8.8)	30(8.5)	5.5(1.1)
101			6.3(2.8)	11(4.5)	24(13)	69(19)	42(8.1)
105*			1.8	3.7(1.5)	12(6.6)	24(6.8)	
114*					1.2	1.4	
118*			2.3(1.0)	4.9(2.0)	11(6.1)	59(17)	5.1(1.0)
126*							
138/158*				1.2	1.4	31(8.7)	79(15)
141					3.2(1.8)	8.4(2.4)	23(4.4)
151					3.1(1.7)	6.1(1.7)	59(11)
153*			0.6	0.9	12(6.6)	51(14)	95(18)
156*					0.6	8.6(2.4)	2.4
166/167*						2.6	1.6
169*							
170*						5.3(1.5)	25(4.8)
180*					8.6(4.8)	16(4.5)	71(14)
183					1.7	19(5.4)	28(5.4)
187					4(2.2)	8(2.3)	46(8.9)
189*							0.9
194*					0.6	1.3	8.9(1.7)
195						0.5	16(3.1)
196					0.8	2.0	7.2(1.4)
201							3.4
203					0.5	0.8	8.1(1.6)
206							2.9
ΣnCB	3.4(2)	0.4(<1)	7(3)	14(6)	51(28)	200(56)	290(56)
ΣCB	220	190	230	250	180	360	520

Table 3. Non-, Mono-, and Di-*o*-chlorobiphenyl Toxic Equivalent Concentrations (TEQs - based on their TEFs to 2,3,7,8-tetrachlorodibenzo[*p*]dioxin [Ahlborg et al. 1994]) in Lobster Digestive Gland ($pg \cdot g^{-1}$ wet wt. Only TEQs ≥0.05 $pg \cdot g^{-1}$ wet wt. are shown) and Aroclors ($\mu g \cdot g^{-1}$. Only TEQs ≥0.01 $\mu g \cdot g^{-1}$ wet wt. are shown).

		Lobster Digestive Gland				
IUPAC No.	TEF	Halifax Harbor	Saint John Dump Site	Petit-de-Grat	Sydney Harbor	Control
Non-ortho						
77	0.0005	3.5	0.20	1.3	0.31	0.15
126	0.1	110	19	74	23	8.0
169	0.01			0.8		
Mono-ortho						
105	0.0001	15	1.0	8.0	1.4	0.65
114	0.0005	4.4	0.95	3.3	0.45	0.15
118	0.0001	33	2.5	24	4.0	2.0
156	0.0005	20	1.3	9.5	2.0	1.0
189	0.0001	0.17			0.30	
Di-ortho						
170	0.0001	4.0	0.9	1.8	6.4	0.40
180	0.00001	1.6	0.31	0.89	3.2	0.14
SUM		**192**	**26.2**	**124**	**41.1**	**12.49**

				Aroclor®			
IUPAC No.	1016	1221	1232	1242	1248	1254	1260
Non-ortho							
77	0.04	0.03	0.37	0.55	1.0	0.22	0.09
126			0.90	2.0	7.0	12	0.3
169							
Mono-ortho							
105			0.18	0.37	1.2	2.4	0.03
114					0.6	0.7	
118			0.23	0.49	1.1	5.9	0.51
156			0.02	0.03	0.3	4.3	1.2
189						0.02	0.09
Di-ortho							
170						0.53	2.5
180					0.09	0.16	0.71
SUM	**0.04**	**0.03**	**1.7**	**3.4**	**11.3**	**26.2**	**5.4**

Toxic Equivalent Factors (TEFs) for selected "planar" CBs (determined by WHO for human health, taken from Table 1 of Ahlborg et al. 1994) were used to calculate toxicological equivalent concentrations (TEQs) to 2,3,7,8-tetrachloro-dibenzo[*p*]dioxin (Table 3) for the non-, mono-, and di-*o*-CBs found in lobsters and the Aroclors®. None of the lobster ΣCB values exceed the present Canadian tolerance for PCB in fish tissue of 2 $mg \cdot kg^{-1}$ wet wt.; however, when expressed in

TEQs, the four in-harbor samples exceed the 20 ng·kg^{-1} wet wt. Canadian tolerance for 2,3,7,8-tetrachlorodibenzo[*p*]dioxin (Health Canada 1993).

The summed TEQs for the ten toxic CBs found in lobster digestive gland ranged 12.5 (Control) to 192 (Halifax Harbor) ng·kg^{-1} wet wt, with IUPAC Nos. 126, and 118 contributing most to the summed TEQs, i.e., the same CBs that account for most of the summed TEQs for the Aroclors®. However, we note that Safe (1990) has pointed out that the calculated TEFs for Aroclors 1242, 1248, 1254, and 1260 significantly overestimated the immunosuppresive effects of the Aroclors and suggested antagonism among the CBs comprising the mixtures.

The presence of di- and trichlorobiphenyls, having a planar configuration, in lobster warrants further investigation, especially in light of their apparent relative enrichment. Contamination of lobsters by the aromatic organics that require mixed function oxidase for their metabolism and ultimate excretion is particularly worrisome because lobsters do not appear capable of metabolising such compounds. For example, Uthe and Musial(1986) found that polycyclic aromatic hydrocarbon concentrations in the digestive gland decreased very slowly in relation to the water solubilities of the compounds during holding of live lobsters in filtered seawater for one year.

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