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Dechlorination of Individual Congeners in Aroclor 1248 as Enhanced by Chlorobenzoates, Chlorophenols, and Chlorobenzenes

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Previous investigations showed that three classes of haloaromatic compounds (HACs; chlorobenzoates, chlorophenols, and chlorobenzenes) enhanced the reductive dechlorination of Aroclor 1248, judging from the overall extent of reduction in Cl atoms on the biphenyl. In the present study, we further investigated the kind of polychlorinated biphenyl (PCB) congeners involved in the enhanced dechlorination by four isomers belonging to each class (2,3-, 2,5-, 2,3,5-, and 2,4,6-chlorobenzoates; 2,3-, 3,4-, 2,5-, and 2,3,6-chlorophenols; and 1,2-, 1,2,3-, 1,2,4-, and penta-chlorobenzenes). Although the PCB congeners involved in the enhanced dechlorination varied with the HACs, the enhancement primarily involved *para*-dechlorination of the same congeners (2,3,4'-, 2,3,4,2'- plus 2,3,6,4'-, 2,5,3',4'- plus 2,4,5,2',6'-, and 2,3,6,2',4'-chlorobiphenyls), regardless of the HACs. These congeners are known to have low threshold concentrations for dechlorination. To a lesser extent, the enhancement also involved *meta* dechlorination of certain congeners with high threshold concentrations. There was no or less accumulation of 2,4,4'- and 2,5,4'-chlorobiphenyls as final products under HAC amendment. Although the dechlorination products varied, the accumulation of *ortho*-substituted congeners, 2-, 2,2'-, and 2,6-chlorobiphenyls, was significantly higher with the HACs, indicating a more complete dechlorination of the highly chlorinated congeners. Therefore, the present results suggest that the enhanced dechlorination under HAC enrichment is carried out through multiple pathways, some of which may be universal, regardless of the kind of HACs, whereas others may be HAC-specific.

Keywords: Chlorobenzene, chlorobenzoate, chlorophenol, dechlorination, polychlorinated biphenyl

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Polychlorinated biphenyls (PCBs) are a class of 209 discrete chemical compounds, called congeners, which differ in the number and position of chlorines. PCBs were commercially produced as complex mixtures for various industrial applications, including dielectric fluids in capacitors and transformers. The major PCB producer in the United States marketed them under the trade name Aroclor from 1930 to 1977 [5]. The Aroclor mixtures typically contained 60–90 different PCB congeners, and Aroclor 1248 included an approximately 48 weight% of chlorine [5, 7]. Owing to their chemical and physical stability, PCBs became ubiquitous and recalcitrant pollutants in the environment, primarily in aquatic sediments. PCBs have a strong tendency of bioaccumulation and biomagnification in the food chain, and are known to be toxic to humans and other living organisms [5, 8].

Evidence of the reductive dechlorination of PCBs is widespread in many contaminated sediments [14]. This microbial process, which takes place in the anaerobic layers of sediments, is especially important for highly chlorinated congeners that are resistant to aerobic microbial degradation [11, 12].

Microbial reductive dechlorination has been observed in many haloaromatic compounds (HACs). There is evidence to suggest that the same microorganism may be involved in the dechlorination of more than one HAC. For instance, an enrichment culture containing a PCB-dechlorinating bacterium also dechlorinated polychlorinated benzenes [16], *Dehalococcoides ethenogenes* strain 195 was shown to be able to dechlorinate certain PCBs and chlorodibenzo-*p*-dioxin congeners, as well as chlorobenzenes [6], and anaerobic mixed cultures enriched with trichlorobenzene and chlorophenol were demonstrated to carry out PCB dechlorination [2, 10]. However, it is unclear whether the same enzyme was involved in the dechlorination of different compounds. In *Desulfitobacterium* strain PCE1, reductive dechlorination of tetrachloroethene and chlorophenols was

found to be carried out by two distinct enzyme systems [15].

A recent study [3] has shown that HACs not only enhance PCB dechlorination, but also enrich PCB-dechlorinating microorganisms in PCB-free sediments. A study of PCB-dechlorinating microorganisms initially indicated that PCBs were needed for their growth [9]. However, further investigations showed that these microorganisms can also grow in sediments containing chlorobenzoates, chlorobenzenes, and chlorophenols [3]. When sediment microorganisms from PCB-contaminated sediments in the St. Lawrence River were enriched in PCB-free sediments with 14 isomers of these three compounds, the enrichment cultures were able to dechlorinate Aroclor 1248, whereas no PCB dechlorination was found in cultures similarly grown in HAC-free sediments. Furthermore, when PCB-contaminated sediments were amended with the same HAC compounds, PCB dechlorination was significantly enhanced, judging from the overall decrease of Cl atoms on the biphenyl ring.

In earlier studies, the current authors classified the PCB congeners in Aroclor 1248 into three groups based on their threshold level of dechlorination [4]. The first group (group A) consisted of congeners with low threshold concentrations for dechlorination to occur, and was first to be dechlorinated. This group accounts for approximately 53 mol% of the total in Aroclor 1248. The second group (group B) comprised congeners requiring high threshold concentrations. Their chlorination levels are generally lower than those of the group A congeners, and this group includes some intermediate dechlorination products of group A and other highly chlorinated congeners. The concentration of this group is about half of group A at approximately 30 mol% of the total. Finally, the third group (group C) consisted of the residual products of dechlorination, mainly low-molecular-weight congeners rich in *ortho*-chlorines. These congeners accumulate over time with no further dechlorination, and in fresh Aroclor 1248, this group represents approximately 17 mol% of the total.

Accordingly, the present study investigated the enhancement effect of HACs on the dechlorination of individual PCB congeners in each kinetic group in Aroclor 1248. The results showed that the HAC-enhanced dechlorination of PCBs involved many of the same congeners, regardless of the HACs, although HAC-dependent variations were found with certain congeners.

Materials and Methods

Sediment Slurry Preparation

All the experiments were carried out using stringent anaerobic techniques. For the sediment slurry preparation, uncontaminated sediments collected from the Grasse River, a tributary of the St. Lawrence River (NY, U.S.A.), were air-dried, sifted through a sieve with 150-μm openings, and analyzed to confirm the absence of PCBs. These sediments were then spiked with Aroclor 1248 in hexane to yield a concentration of 300 μg/g of sediment on a dry weight basis. The PCB-spiked sediments (10 g dry weight) were made into slurries by adding 45 ml of a reduced synthetic mineral medium [1] in 100-ml serum vials under an atmosphere of N_2:H_2:CO_2 (85:10:5) in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI, U.S.A.). The vials were capped with Teflon-coated butyl rubber stoppers and aluminum crimp seals in the anaerobic chamber and autoclaved at 121°C for 40 min on three successive days. The sediment vials were then amended individually with each of the non-PCB HACs (2,3-, 2,5-, 2,3,5-, and 2,4,6-chlorobenzoates; 2,3-, 3,4-, 2,5-, and 2,3,6-chlorophenols; and 1,2-, 1,2,3-, 1,2,4-, and penta-chlorobenzenes) in acetone stock solutions (0.5%, w/v) to yield a final concentration of 200 μg/g of sediment. Thereafter, the vials were inoculated with microorganisms eluted from St. Lawrence River sediment contaminated mostly by Aroclor 1248 [14]. Following the inoculation, the vials were incubated statically at room temperature for 42 weeks. All the experiments were performed in duplicate. HAC-free sediments spiked with Aroclor 1248, with or without sediment microorganisms, served as the control.

PCB Analysis

The sediment extraction and congener-specific analysis of the PCBs were performed as described previously [13, 14]. Briefly, the sediments were extracted using an Accelerated Solvent Extractor system (Dionex, Sunnyvale, CA, U.S.A.) according to the U.S. Environmental Protection Agency Method 3545. After removing the sulfur using a tetrabutylammoniumhydrogen sulfite reagent, the hexane extracts were cleaned using a Florisil column. The congener-specific analysis was then carried out on a Hewlett-Packard 5890 II gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) equipped with a ^{63}Ni electron capture detector, an autosampler, a splitless injector, an Rtx-5 capillary column (Restek, Bellefonte, PA, U.S.A.), and a computerized data acquisition system (ChromPerfect, Justice Innovations, Mountain View, CA, U.S.A.). The gas chromatography conditions were as previously described [13]. The PCB congeners were identified and quantitated using a calibration standard containing a 1:1:1:1 mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 μg/ml of each in hexane). The peaks were identified and calibrated according to the response factors [7, 13].

A paired *t* test was used to determine the difference in the congener levels between the HAC-free and HAC-amended sediments, and between these two sediments and the initial values in fresh Aroclor 1248. The PCB congener numbering system in the text uses a slash to represent the ring separation and permit an easier visualization of the chlorination substitution pattern (e.g., 2,4,5,4'-chlorobiphenyl (CBP) appears as 245/4-CBP), and co-eluting congeners are connected with "+".

Results

Overall Dechlorination by Congener Groups

No dechlorination was evident in the autoclaved controls up to 42 weeks of incubation. Meanwhile, in all the inoculated Aroclor 1248 sediments, the dechlorination reached a plateau by 27 weeks of incubation, as no

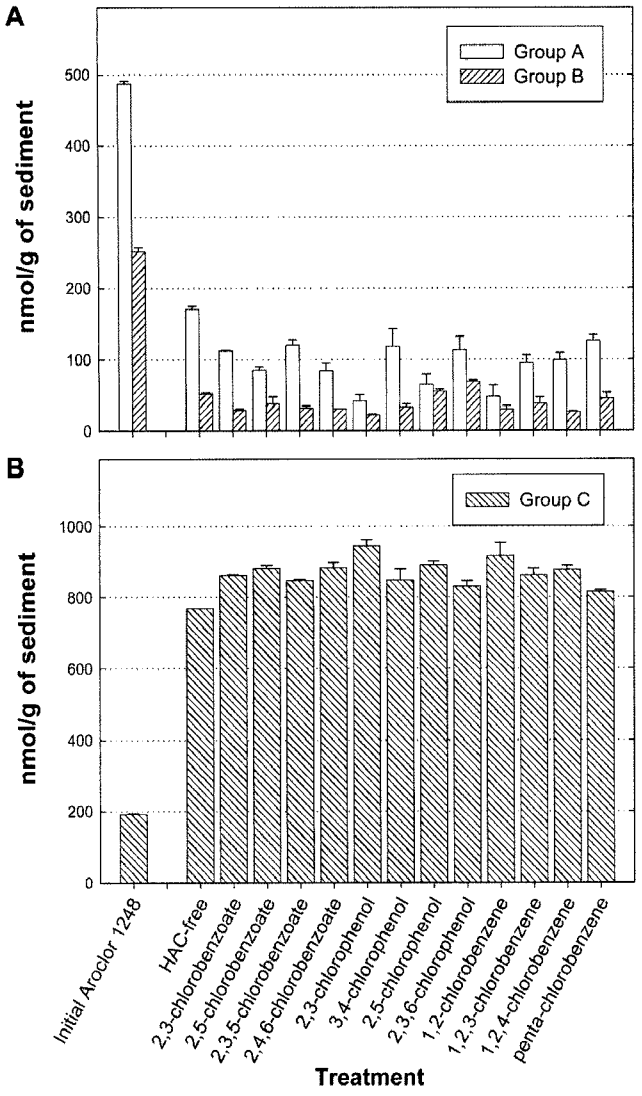

Fig. 1. Total congener concentrations (nmol/g of sediment) for groups A and B (**A**), and group C (**B**) in initial Aroclor 1248 (300 μg Aroclor 1248/g of sediment, 0 week), and in HAC-free and HAC-amended sediments at plateau (27-42 weeks).
Bars indicate standard deviation.

significant changes were observed after this time. Thus, the difference in the dechlorination with the 12 HACs was examined on a congener basis at this plateau. The group A congeners in the HAC-free sediments were reduced to 171 nmol/g of sediment from an original concentration of 487 nmol/g of sediment, representing a reduction of 316 nmol/g of sediment or 65% from the initial concentration (Fig. 1A, Table 1). In the HAC-amended sediments, the dechlorination of the group A congeners was much greater, ranging from 361 (74%, penta-chlorobenzene) to 445 nmol/g of sediment (91%, 2,3-dichlorophenol), amounting to approximately 114% to 141% of the level in the HAC-free sediments.

The dechlorination of the group B congeners in the HAC-free sediments also decreased from 251 to 52 nmol/g of sediment, representing a reduction of about 80% (Fig. 1B, Table 1). Although this percentage was higher than the 65% for group A, the absolute amount was much lower, as the initial concentration of group A was nearly twice as high. Enrichment with 2,3-dichlorophenol was the most effective on the overall dechlorination of the group B congeners, as with group A, with the level of dechlorination increasing to 115% of the value in the HAC-free sediments (Figs. 1A and 1B, Table 1). On the other hand, 2,5-dichlorophenol, 2,3,6-trichlorophenol, and penta-chlorobenzene showed little enhancement of the group B dechlorination (Fig. 1B, Table 1).

The concentration of group C in the HAC-free sediments increased from the initial level of 192 to 770 nmol/g of sediment, an increase of more than 300%, and the level was even higher in the presence of the HACs (Fig. 1C, Table 1). The accumulation was highest with 2,3-dichlorophenol at 945 nmol/g of sediment, an increase of more than 491% of the initial concentration and 123% of the level in the HAC-free sediments (Fig. 1C, Table 1). This increase mirrored the decrease in groups A and B. The next highest accumulation was in the 1,2-dichlorobenzene sediments at 916 nmol/g of sediment, a 477% increase from the initial concentration and 119% of the value in the HAC-free control (Fig. 1C, Table 1). The values in the presence of the other HACs ranged from 424% (penta-chlorobenzene) to 464% (2,5-dichlorophenol) over the initial value, or 106% to 116% over the value in the HAC-free sediments (Fig. 1C, Table 1).

The dechlorination of Aroclor 1248 reduced the molar concentrations of groups A and B, and increased the concentration of group C. The sum of their molar concentrations was greater after dechlorination than before (Fig. 1, Table 1).

HAC Effect on Group A Congeners

HAC-enhanced dechlorination was observed for the following congeners: 23/4- (chromatographic peak #23), 234/2-+236/4- (#34), 25/34-+245/26- (#39), 24/34-+245/26- (#40), 236/24- (#41), 23/34-+234/4-+235/25- (#42), 236/23-CBPs (#43), 245/25-+235/24-+234/26- (#44), and 245/24-CBPs (#45) (Table 2). One or more of the HACs were involved in the enhancement. The dechlorination of 234/2-+236/4-CBP (peak #34) was significantly greater in all the HAC-amended sediments. Congeners 23/4- (#23), 25/34-+245/26- (#39), and 236/24-CBPs (#41) also showed significantly greater dechlorination with most of the HACs. Among the tested HACs, 2,3-dichlorophenol was the most effective, and in addition to the above four peaks, it also increased the dechlorination of #40, #42, #43, and #44 (Table 2).

Unlike the other HACs, 2,5-dichlorophenol had no significant effect on #23 and #39 (Table 2), yet it significantly increased the dechlorination of #40, #42, and #43, which

Table 1. Total congener concentrations of groups A, B, and C in initial Aroclor 1248, and in HAC-free and HAC-amended sediments at plateau.

	Concentrations (nmol(±SD)[a])			
	Group A	Group B	Group C	Groups A+B+C
Initial Aroclor 1248	487(±4)	251(±6)	192(±2)	931(±4)
HAC-free	171(±4)	52(±1)	770(±0)	993(±5)
2,3-Chlorobenzoate	**112(±1)**[b]	**29(±2)**	**862(±3)**	1,003(±4)
2,5-Chlorobenzoate	**85(±5)**	38(±1)	**881(±9)**	1,004(±4)
2,3,5-Chlorobenzoate	**120(±8)**	**32(±3)**	**848(±3)**	1,000(±8)
2,4,6-Chlorobenzoate	**84(±11)**	**31(±0)**	**883(±16)**	998(±4)
2,3-Dichlorophenol	**42(±9)**	**22(±1)**	**945(±16)**	1,009(±8)
3,4-Dichlorophenol	118(±25)	**33(±5)**	849(±30)	1,000(±0)
2,5-Chlorophenol	**65(±14)**	55(±3)	**891(±12)**	1,010(±5)
2,3,6-Chlorophenol	113(±19)	**69(±2)**	**831(±16)**	**1,014(±1)**
1,2-Chlorobenzene	**48(±16)**	**30(±5)**	**916(±37)**	995(±16)
1,2,3-Chlorobenzene	**95(±11)**	38(±9)	**863(±18)**	996(±1)
1,2,4-Chlorobenzene	**99(±10)**	**26(±1)**	**877(±12)**	1,001(±1)
Penta-chlorobenzene	**126(±8)**	45(±8)	**816(±5)**	988(±5)

[a]Sum of congener concentrations (nmol/g of sediment) in 300 μg Aroclor 1248/g of sediment.
[b]Underlined bold face indicates a significant difference ($P<0.05$, paired t test) between HAC-free and HAC-amended sediments.

most of the other HACs had no effect on. Similarly, 2,3,6-trichlorophenol had no effect on #39. However, unlike 2,5-dichlorophenol, there was no dechlorination specific to 2,3,6-trichlorophenol, and since #39 accounted for 24% of group A, 2,3,6-trichlorophenol was among the least effective on group A. 2,4,6-Trichlorobenzoate and penta-chlorobenzene showed no significant effect on peak #41.

HAC-Enhanced *para*-Dechlorination

The seven peaks (#23, #34, #39, #40, #42, #44, and #45) that showed a significant increase in dechlorination with the HAC enrichments (Table 2) together represented approximately 70% of group A and more than 33% of Aroclor 1248 (Table 3). It is interesting that all the peaks have *para*-substitutions (Table 3). Two other peaks (#41, #43) without *para*-substitution also exhibited an increased

Table 2. Concentrations of group A congeners in initial Aroclor 1248, and in HAC-free and HAC-amended sediments at plateau.

Congeners	Peak No.	Initial conc.[a]	HAC-free[b]	Chlorobenzoates[c]				Chlorophenols[c]				Chlorobenzenes[c]			
				23-	25-	235-	246-	23-	34-	25-	236-	12-	123-	124-	penta-
23/4	#23	14.9	19.9	**3.3**[e]	**6.6**	**4.7**	**5.2**	**0.9**	**4.1**	5.1	**9.1**	**1.0**	**4.2**	**2.5**	**6.6**
234/2+236/4	#34	37.6	***23.6***[d]	**12.2**	**13.2**	**13.8**	**4.7**	**11.6**	**12.9**	**8.9**	**10.9**	**12.2**	**13.7**	**10.3**	**16.7**
245/4	#38	80.3	***30.3***	46.6	20.6	47.6	21.1	11.0	39.6	14.3	23.1	8.7	27.4	36.4	35.9
25/34+245/26	#39	115.2	***45.6***	**10.4**	**11.4**	**10.6**	**15.4**	**1.5**	**15.3**	16.9	30.7	**5.0**	**11.0**	**12.3**	**12.7**
24/34+236/25	#40	50.6	***11.9***	7.5	7.8	7.4	10.7	**3.0**	9.3	**1.4**	9.3	**2.3**	6.8	6.4	10.0
236/24	#41	4.4	2.7	**1.1**	**1.6**	**0.9**	1.4	**0.6**	**1.1**	**0.4**	**0.9**	**1.0**	**1.1**	**1.0**	1.6
23/34+234/4+235/25	#42	70.3	***10.1***	4.2	5.8	5.5	5.4	**2.9**	5.4	**2.6**	3.6	6.9	6.0	4.9	7.1
236/23	#43	8.5	***3.1***	0.9	1.1	0.8	1.1	**0.3**	1.0	**0.5**	0.8	0.8	1.0	0.8	2.2
245/25+235/24+234/26	#44	16.9	***4.2***	2.7	2.8	2.9	2.9	**1.8**	3.8	2.2	3.2	2.2	3.2	2.1	3.6
245/24	#45	16.2	***8.5***	7.7	6.0	6.5	6.3	3.4	7.3	6.6	7.7	**2.7**	6.3	4.9	7.5
236/34+34/34	#52	21.5	***8.1***	5.4	4.0	5.8	3.4	2.4	6.7	2.0	3.8	3.2	5.9	3.5	8.7
245/34	#57	28.8	***3.2***	8.7	2.9	11.2	6.3	2.4	11.2	3.1	9.9	1.5	6.4	8.6	8.8
234/236+234/34	#64	21.9	***0.0***	1.0	1.0	1.9	0.6	0.2	0.3	0.7	0.3	1.0	1.8	5.1	5.0
Sum		487.2	***171.0***	**111.8**	**84.9**	**119.7**	**84.5**	**41.9**	117.9	**64.6**	113.2	**48.5**	**94.9**	**98.9**	**126.4**

[a]Initial congener concentrations (nmol/g of sediment) at 0 weeks in 300 μg Aroclor 1248/g of sediment.
[b]Congener concentrations (nmol/g of sediment) at plateau (27-42 weeks) in HAC-free sediments.
[c]Congener concentrations (nmol/g of sediment) at plateau (27-42 weeks) in sediments amended with 200 μg HACs/g of sediment.
[d]Bold italic font indicates significant difference ($P<0.05$, paired t test) from initial congener concentration.
[e]Underlined bold face indicates significant difference ($P<0.05$, paired t test) from congener concentration in HAC-free sediments.

Table 3. Group A congeners exhibiting enhanced dechlorination by HACs.

Peak No.	Congeners	Concentrations[a]	% of the peak
Congeners enhanced by most HACs			
23	23/4	15.5	100
34	234/2+236/4	7.9+30.9	20.3+79.6
39	25/34+245/26	74.8+1.8	97.7+2.4
41	236/24	5.8	100
Congeners enhanced by 2,3- and 2,5-chlorophenols			
40	24/34+236/25	60.0+18.0	76.9+23.1
42	23/34+234/4+235/25	32.5+19.0+3.5	59.1+34.6+6.4
43	236/23	11.6	100
44	245/25+235/24+234/26	20.4+0.0+1.8	91.8+0.0+8.2
45	245/24	13.5	100
Congeners not enhanced by HACs			
38	245/4	46.2	100
52	236/34+34/34	25.1+0.0	100+0.0
57	245/34	21.6	100
64	234/236+234/34	0.0+16.7	0.0+100

[a]nmol/g of sediment in 300 μg Aroclor 1248/g of sediment, based on reference [7].

dechlorination, yet only comprised about 4% of group A or less than 2% of the initial Aroclor. Therefore, it appeared that the HAC-enhanced dechlorination of group A congeners primarily involved the removal of *para*-substituted chlorines.

HAC Effect on Group B Congeners

The effect of the HACs on the individual group B congeners varied widely, unlike the pattern observed in group A (Table 4). It also appeared that certain HACs prevented the dechlorination of congeners. For example, the dechlorination of 25/2-CBP (#11) was significantly enhanced by 2,3-, 2,3,5-, and 2,4,6-chlorobenzoates, 2,3- and 3,4-chlorophenols, and 1,2,4- and penta-chlorobenzenes, whereas 2,5-chlorobenzoate, and 2,5- and 2,3,6-chlorophenols had no effect (Table 4). In the case of peak #21 (23/3-+25/26-+2/34-CBPs), 2,3-dichlorophenol was the most effective, yet its dechlorination was significantly lower (*e.g.*, inhibition) with 2,5- and 2,3,6-chlorophenols than without, and with 2,3,6-chlorophenol there was even a net accumulation (25%) above its level in the fresh Aroclor 1248, which contributed to an increase in the total concentration of group B (Table 4). It is interesting to note that the enhancement of dechlorination in this group largely involved congeners with *meta*-substitution such as peaks #11, #21, #26, and #27.

In the case of the other group B congeners, such as 23/26- (#25), 23/25- (#31), 236/3-+23/24-+34/4- (#32), and 23/23-CBPs (#35), the dechlorination in the HAC-free sediments reduced 83–98% of the initial concentration, leaving little margin for HAC enhancement. Most of the HACs showed no effect on the dechlorination of these congeners.

Accumulation of Group C Congeners

In the HAC-free sediments, the level of group C showed an increase of more than 300% from the initial concentration. The level was significantly higher in sediments with all the HACs, except for 3,4-dichlorobiphenyl. The higher concentration in the HAC-amended sediments was primarily due to the greater accumulation of 2- (#2), 2/2-+26- (#5), 2/4-+23- (#8), 26/2- (#9), and 24/2-+4/4-CBPs (#12) (Table 5). The accumulation of *ortho*-substituted congeners (#2, #5, and #9) was especially pronounced in the presence of the HACs (Table 5). The concentration of #2 in the HAC-free sediments was 4 nmol/g of sediment, whereas it ranged from 17 to 72 nmol/g of sediment in the HAC-treated sediments. The higher accumulation of #5 was significant with all the HAC amendments, except for

Table 4. Concentrations of group B congeners in initial Aroclor 1248, and in HAC-free and HAC-amended sediments at plateau.

Congeners	Peak No.	Initial conc.[a]	HAC-free[b]	Chlorobenzoates[c]				Chlorophenols[c]				Chlorobenzenes[c]			
				23-	25-	235-	246-	23-	34-	25-	236-	12-	123-	124-	penta-
25/2	# 11	51.2	***9.4***[d]	**1.8**[e]	16.5	**3.0**	**2.7**	**3.2**	**3.1**	9.0	11.5	4.5	4.9	**2.6**	**5.0**
23/3+25/26+2/34	# 21	38.5	***20.3***	14.8	**7.0**	13.9	11.2	**5.7**	15.3	**36.4**	**49.8**	**9.0**	14.1	**12.6**	18.6
23/26	# 25	3.6	***0.3***	1.0	2.1	0.7	1.0	0.3	**0.8**	0.1	0.1	0.4	0.9	0.6	**1.6**
25/25	# 26	32.8	***3.9***	**2.1**	2.7	2.6	2.7	**2.0**	2.8	**1.5**	1.4	2.5	3.0	**1.6**	3.6
24/25+235/2	# 27	35.2	***13.5***	6.3	**5.1**	7.8	7.4	**6.8**	**6.9**	**5.4**	4.8	7.4	9.5	**4.3**	10.2
23/25	# 31	41.2	***1.0***	0.9	1.8	1.7	2.1	**1.5**	1.3	0.8	0.9	1.4	1.9	1.1	**2.0**
236/3+23/24+34/4	# 32	35.7	***1.9***	**1.0**	1.4	1.8	3.5	0.9	1.9	1.0	**0.8**	2.5	1.9	2.0	1.8
23/23	# 35	13.2	***1.5***	0.9	1.4	0.4	0.0	1.9	1.1	0.4	0.1	2.6	2.0	1.2	2.2
Sum of congeners		251.3	***51.7***	**28.8**	37.9	**31.9**	**30.6**	**22.2**	**33.2**	54.7	**69.4**	**30.2**	38.0	**25.9**	44.9

[a]Initial congener concentrations (nmol/g of sediment) at 0 weeks in 300 μg Aroclor 1248/g of sediment.
[b]Congener concentrations (nmol/g of sediment) at plateau (27-42 weeks) in HAC-free sediments.
[c]Congener concentrations (nmol/g of sediment) at plateau (27-42 weeks) in sediments amended with 200 μg HACs/g of sediment.
[d]Bold italic font indicates significant difference ($P<0.05$, paired t test) from initial congener concentration.
[e]Underlined bold face indicates significant difference ($P<0.05$, paired t test) from congener concentration in HAC-free sediments.

Table 5. Concentrations of group C congeners in initial Aroclor 1248, and in HAC-free and HAC-amended sediments at plateau.

Congeners	Peak No.	Initial conc.[a]	HAC-free[b]	Chlorobenzoates[c]				Chlorophenols[c]				Chlorobenzenes[c]			
				23-	25-	235-	246-	23-	34-	25-	236-	12-	123-	124-	penta-
2	# 2	0.0	***4.2***[d]	**46.5**[e]	52.6	**39.4**	**19.6**	**64.0**	42.3	16.7	28.7	**41.1**	**52.4**	**71.5**	17.6
2/2+26	# 5	4.2	***125.3***	**195.3**	**202.1**	**186.0**	143.3	**218.2**	**194.3**	**201.3**	**151.8**	**177.9**	**182.3**	**205.0**	**156.0**
24+25	# 6	2.1	***16.2***	23.1	32.6	12.6	6.7	7.6	19.1	2.9	10.4	7.5	14.2	22.8	14.9
2/3	# 7	2.1	***34.1***	29.5	**42.3**	30.5	36.5	**39.5**	31.8	**9.1**	**19.2**	46.3	40.8	26.2	39.2
2/4+23	# 8	13.7	***60.8***	75.0	88.3	68.7	**129.8**	**145.4**	73.8	**246.3**	185.3	**152.9**	84.6	88.1	56.3
26/2	# 9	3.3	***30.0***	**50.0**	33.0	47.3	39.4	**62.5**	**47.4**	38.6	18.3	**66.7**	51.6	59.7	44.1
24/2+4/4	# 12	32.7	***128.9***	**167.4**	123.5	166.2	164.1	**196.4**	**172.4**	183.0	147.2	**188.8**	148.2	157.9	152.0
236+26/3	# 13	2.1	***16.6***	15.6	19.4	14.4	22.2	**36.0**	14.8	20.2	14.2	**35.3**	15.6	20.2	15.4
23/2+26/4	# 14	24.0	***57.3***	**48.3**	53.3	48.4	**44.8**	**51.3**	**47.6**	62.5	58.0	58.6	**49.3**	**51.4**	48.6
25/3	# 17	7.7	***47.6***	20.1	31.2	25.6	22.9	15.8	24.7	**3.1**	**6.6**	16.8	39.7	18.2	50.4
24/3	# 18	3.5	***37.2***	34.9	29.6	35.5	**15.6**	22.6	35.5	**1.4**	**5.0**	**12.2**	32.4	28.3	42.9
25/4	# 19	41.3	***85.8***	**54.8**	**71.3**	**52.3**	93.8	**37.1**	**43.9**	**59.4**	72.1	**61.8**	**59.5**	**31.9**	82.2
24/4	# 20	39.5	***101.0***	**60.7**	**75.0**	**81.6**	106.3	**20.8**	**61.8**	**27.6**	98.6	**16.1**	**58.9**	**58.0**	**61.7**
24/26	# 22	0.7	0.6	**1.7**	0.5	**1.7**	1.0	**4.4**	**1.6**	1.5	0.4	**3.4**	**1.5**	**1.8**	1.7
24/24+246/4	# 28	15.5	***24.8***	38.9	26.2	**38.3**	**36.8**	23.3	**37.7**	17.4	**15.4**	30.6	32.1	**35.7**	33.3
Sum		192.4	***770.3***	**861.9**	**880.8**	**848.4**	**882.8**	**945.1**	848.7	**891.2**	**831.2**	**915.9**	**863.1**	**876.6**	**816.2**

[a]Initial congener concentrations (nmol/g of sediment) at 0 week in 300 μg Aroclor 1248/g of sediment.
[b]Congener concentrations (nmol/g of sediment) at plateau (27-42 weeks) in HAC-free sediments.
[c]Congener concentrations (nmol/g of sediment) at plateau (27-42 weeks) in sediments amended with 200 μg HACs/g of sediment.
[d]Bold italic font indicates significant difference ($P<0.05$, paired t test) from initial congener concentration.
[e]Underlined bold face indicates significant difference ($P<0.05$, paired t test) from congener concentration in HAC-free sediments.

2,4,6-trichlorobenzoate. The concentration of #9 was also higher in the sediments amended with 2,3- and 3,4-dichlorophenols, and 1,2-dichlorobenzene.

Although it is difficult to trace the production pathways of these congeners, the current results indicated that the *meta*- and *para*-dechlorination of the PCB congeners was more complete in the presence of the HACs, and that the dechlorination enhancement did not involve *ortho*-chlorines. The concentration of #8 was especially high in the 2,5-dichlorophenol-amended sediments at 246 nmol/g of sediment, comprising 28% of the total group C concentration (Table 5). With 2,3-dichlorophenol, 2/3- (#7), 236-+26/3- (#13), and 24/26-CBPs (#22) also showed a significant increase in addition to the above five peaks.

Group C Congeners with No or Less Accumulation

It is interesting to note that the two major peaks of this group, #19 (25/4-CBP) and #20 (24/4-CBP), were lower for most of the HAC-amended sediments than for the HAC-free sediments (Table 5). In the HAC-free control, the concentration of these two congeners increased from 41 to 86 nmol/g of sediment (#19) and 40 to 101 nmol/g of sediment (#20), increases of more than 100% for both (Table 5). On the other hand, the level of the same peaks was lower with all the HACs, except for 2,4,6-trichlorobenzoate and 2,3,6-trichlorophenol, when compared with the level for the control, ranging from 32 to 82 nmol/g of sediment (#19) and from 16 to 82 nmol/g of sediment (#20) (Table 5). Nonetheless, when compared with the initial values, the values for #19 and #20 were still higher in most of the HAC-amended sediments, with a few interesting exceptions. Peak #19 was lower with 2,3-dichlorophenol and 1,2,4-trichlorobenzene (Table 5), whereas 2,3- and 2,5-dichlorophenols and 1,2-dichlorobenzene reduced #20 to a level below its initial level. Hence, the lower or no accumulation of these two *para*-substituted congeners in most of the HAC sediments was a strong indication that the enhanced dechlorination of the group A congeners by the HACs involved primarily *para*-dechlorination. However, it should be noted that the lower or no accumulation does not necessarily mean the absence of any production of these congeners, because it is possible that dechlorination occurred concurrently with their production as intermediate products of the dechlorination of other highly chlorinated congeners.

Possible Pattern of Dechlorination

A pattern for the HAC-enhanced dechlorination emerged when possible parent congeners for #19 and #20 were traced. In the HAC-free sediments, #19 could be produced from the dechlorination of #38 (245/4-CBP) at the *para* position on the first ring, 25/34-CBP (in #39) at the *meta* position on the second ring, and #57 (245/34-CBP) at the *para* and *meta* positions on the first and second ring, respectively. Among these three, only #39 showed HAC-enhanced ($P<0.05$) dechlorination (Table 1), and more than

97% of this peak comprised 25/34-CBP (Table 3). Thus, the difference in #19 between the HAC-free and HAC-amended sediments reflected an enhancement of *para*-dechlorination on the second ring of 25/34-CBP. In the HAC-enhanced dechlorination with no or less accumulation of #19, various pathways can be speculated. The first possibility is that *para*-dechlorination occurred. The second is that although 25/4-CBP was produced, it was further dechlorinated. Finally, 25/34-CBP could have been dechlorinated through unknown pathways that did not result in the production of 25/4-CBP.

In the HAC-free sediments, the potential sources of #20 accumulation included 245/4-(#38), 24/34- (in #40), 234/4- (in #42), and 234/34-CBPs (in #64), since *meta*-dechlorination can produce 24/4-CBP. Among the peaks, the concentrations of #40 and #42 were lower in the presence of the HACs than in their absence (Table 1). The 24/34- and 234/4-CBPs accounted for approximately 77% and 35% of the corresponding peaks, respectively (Table 3). Therefore, as in the case of #19, the lower level of #20 in most of the HAC-amended sediments may have stemmed from HAC-enhanced *para*-dechlorination in these congeners.

DISCUSSION

The present study showed that the HAC-enhanced dechlorination of PCBs involved many of the same congeners, regardless of the HACs, although HAC-dependent variations were observed with certain congeners. Interestingly, the enhanced dechlorination with most HACs primarily involved an increase in *para*-dechlorination, regardless of the type of HACs. This conclusion is supported by the fact that the increased dechlorination among the group A congeners was mainly related to the *para*-rich congeners, whereas there appeared to be no or less accumulation of 25/4- and 24/4-CBPs in group C above their levels of HAC-free control, suggesting that the HACs, regardless of their type, enriched the same dechlorinating populations that removed *para* chlorines from certain group A congeners. Furthermore, since the concentrations of 25/4- (#19) and 24/4-CBPs (#20) were not decreased below their initial levels with most of the HACs, the *para*-dechlorinating enzymes of the group A congeners were mostly ineffective for these two *para*-substituted congeners, suggesting a stereospecificity of dehalogenases. Alternatively, it is possible that these congeners have higher threshold concentrations for dechlorination [4].

For the individual Aroclor 1248 congeners, the extent of enhanced dechlorination varied widely according to the HACs used. This variation was seen in both group A and group B. Differences among the HACs were also reflected in the varying accumulation of the group C congeners, indicating that the HACs enriched dechlorinators other than those that enhanced *para*-dechlorination. Although it is unclear whether the same PCB-dechlorinating microorganism was also involved in HAC utilization, in the HAC-amended sediments, the PCB dechlorination lagged until the HAC concentration became very low [3], whereas in the HAC-free sediments, there was no such a lag. Therefore, it would appear that the same microorganism was involved in the degradation of both HACs and PCBs, although it is also possible that the lag was due to the competitive inhibition of PCB-dechlorinating microorganisms by HAC utilizers.

Since there was no evidence of *ortho*-dechlorination, the sum of groups A, B, and C should have been the same before and after dechlorination. However, the total turned out to be greater after dechlorination (Table 1). This was probably because the dechlorination analysis excluded congeners that comprised less than 1% of the Aroclor, consisting of congeners that were mostly heavier than pentachlorobiphenyls and representing approximately 10% of the total. The dechlorination products of these congeners may then have become members of group A, B, or C, making their sum greater after dechlorination than before.

This study indicates that PCB dechlorination in natural sediments can vary if the site is also contaminated by non-PCB HACs. A limit was also found for the microbial dechlorination of PCBs in contaminated sediments in the St. Lawrence River. Whereas it was possible to increase the dechlorination of the congeners in groups A and B, other congeners in group C were accumulated. Most of the congeners in group C are mono- to trichlorobiphenyls. Although rich in *ortho*-chlorine, they also have *meta*- and *para*-chlorines. Therefore, the key to the successful bioremediation of PCBs is to reduce the group C congeners through aerobic degradation or complete dechlorination.

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