

Stimulation of Microbial *para*-Dechlorination of Polychlorinated Biphenyls That Have Persisted in Housatonic River Sediment for Decades

DONNA L. BEDARD,*
STEPHEN C. BUNNELL,[†] AND
LYNN A. SMULLEN

Environmental Laboratory, GE Research and Development
Center, P.O. Box 8, Schenectady, New York 12301

We added 2,5,3',4'-tetrachlorobiphenyl (25-34-CB) (350 μ M) to slurries of Aroclor 1260-contaminated sediment from Woods Pond (Lenox, MA) in an attempt to stimulate dechlorination of the PCBs. The slurries were incubated under methanogenic conditions at 23–25 °C and analyzed by GC/ECD and GC/MS. The 25-34-CB was stoichiometrically converted to 25-3-CB and stimulated a selective *para*-dechlorination, which we designate Process P, that decreased the penta- through heptachlorobiphenyls containing 2,3,4- (234-), 245-, or 2345-chlorophenyl groups by up to 83% in 12 weeks. The products were tetra- and penta-chlorobiphenyls containing 23-, 25-, and 235-chlorophenyl groups, especially 25-25-CB, 23-25-CB, 24-25-CB, 235-25-CB, and 235-23-CB. Mass balances for key parent congeners and their dechlorination products ranged from 80 to 115%. No dechlorination was detected in autoclaved controls or in live controls that were not amended with 25-34-CB. We propose that the 25-34-CB selectively enriched a population of PCB-dechlorinating microorganisms that can use it as an electron acceptor and thus "primed" the PCB dechlorination. This research demonstrates for the first time a successful strategy for stimulating rapid microbial dechlorination of PCBs that have persisted in sediments for decades and lays a foundation for the development of *in situ* methods for bioremediation of PCBs.

Introduction

Polychlorinated biphenyls (PCBs) were manufactured by catalytic chlorination of biphenyl to produce complex mixtures, each containing 60–90 different PCB molecular species or congeners. In the United States, PCB mixtures were manufactured by Monsanto under the tradename

Aroclor and were widely used as dielectric fluids in capacitors and transformers from 1929 to 1978. PCBs are widespread contaminants of aquatic sediments and continue to be a focus of environmental concern because they tend to accumulate in biota and are potentially toxic.

The discovery that microbial dechlorination of PCBs was occurring in many aquatic sediments (1, 2) brought the hope that this process would provide a natural means of remediation. Dechlorination decreases the bioaccumulation potential of PCBs by making them more degradable and is expected to decrease the potential toxicity of PCBs (1–7). Extensive microbial dechlorination of PCBs has occurred in some aquatic sediments including those of the Hudson River (1, 2) and Silver Lake (Pittsfield, MA) (ref 2 as reinterpreted in ref 6). At other locations such as Woods Pond (Lenox, MA), far less PCB dechlorination has occurred. It would be desirable to accelerate microbial dechlorination of PCBs in such sites.

The sediments of the upper Housatonic River are contaminated with PCBs from a transformer manufacturing operation located in Pittsfield, MA. Some of the PCB-contaminated sediments have accumulated in Woods Pond, a shallow impoundment formed by a dam on the river 11 mi downstream of Pittsfield. The pond sediments are contaminated with an unidentified hydrocarbon oil (5–32 mg/g sediment dry weight) and with a PCB mixture (15–180 μ g/g sediment dry weight) composed of tri- to octachlorobiphenyls, the residue from Aroclor 1260 that was used at the Pittsfield transformer operation from 1934 to 1973 and that has been partially dechlorinated by the loss of *meta* and *para* chlorines (7). Our objective was to determine whether the microorganisms responsible for this dechlorination were present in Woods Pond sediment and, if so, to determine whether they could be stimulated to further dechlorinate the PCBs in the sediment.

This laboratory previously demonstrated that anaerobic microorganisms from sediments in Woods Pond could remove *ortho* and *meta* chlorines from 2356-CB (8). Here we report the presence of a microbial population that carries out a highly selective *para* dechlorination of PCBs that has not been observed elsewhere. We also demonstrate for the first time a strategy for stimulating rapid microbial dechlorination of PCBs that have accumulated in sediments for decades. This work was previously presented at the 1991 General Meeting of the American Society for Microbiology in Dallas (9) and at the 1992 ASM Conference on Anaerobic Dehalogenation and its Environmental Implications in Athens, GA (10).

Materials and Methods

Sediment Collection and Storage. Sediments were collected from 12 locations in Woods Pond, six each along the eastern and western shores. Sediment samples were collected by repeated coring using a Lexan tube (5 cm diameter) and were then transferred to glass jars, topped with site water, sealed, and stored at 4 °C.

Preparation of Slurries and Incubation. Slurry preparation and sampling were carried out in an anaerobic chamber in an atmosphere of nitrogen and 3–5% hydrogen. Methanogenic slurries were prepared by mixing wet sediment (2 vol) with revised anaerobic mineral medium

* Corresponding author e-mail address: bedardd@crd.ge.com.

[†] Present address: Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138.

(RAMM) (3 vol) (11) and L-cysteine-HCl (0.1%). The slurries (30 mL each) were dispensed into serum bottles, and 2,5,3',4'-tetrachlorobiphenyl (25-34-CB) or other congeners (99% purity, AccuStandard, New Haven, CT) were generally added from a concentrated stock solution (70 mM in acetone) to give a final concentration 100 μ g/mL or 350 μ M, roughly 700 μ g/g (sediment dry weight). For one experiment, the same amount of PCB was added by preweighing into the serum bottles 55 mg of floated silica powder (about 240 mesh, Fisher Scientific, S-153) coated with 25-34-CB (57 μ g/mg). This was prepared by dissolving the PCB in a 1:1 mixture of acetone and toluene and then removing the solvent under reduced pressure using a rotary evaporator. The bottles were crimp-sealed with Teflon-lined butyl rubber septa (Wheaton). To prepare sterile controls, slurries were pasteurized (75 °C, 20 min), then briefly incubated (23–25 °C, 24 h), and finally autoclaved (121 °C, 3 h). Duplicate samples and controls were incubated in the dark at 23–25 °C without shaking.

Sample Extraction and Analysis. Aliquots (1 mL) of the slurries were sampled weekly and extracted by vigorous shaking (24 h) with anhydrous ether (5 vol) and elemental mercury (1/4 vol, to remove sulfur) in vials with Teflon-lined foam-backed screw caps. Samples were analyzed and visually inspected by gas chromatography (GC) using a Ni⁶³ electron capture detector (ECD) and a DB-1 poly(dimethylsiloxane) capillary column (J & W Scientific, 30 m \times 0.25 mm i.d. \times 0.25 μ m) as previously described (12). The 25-3-CB formed as a dechlorination product of 25-34-CB was initially identified by matching GC retention times with that of an authentic standard (99% purity, AccuStandard) and was later confirmed by GC/mass spectrometry (GC/MS).

The dechlorination of 25-34-CB was quantified with a Hewlett Packard 5890/5971A GC/MS operated in the selected ion mode. We monitored for all possible *meta*- and *para*-dechlorination products of 25-34-CB and for biphenyl. Potential products included 2-CB, 3-CB, 4-CB, 2-3-CB, 2-4-CB, 25-CB, 25-3-CB, 25-4-CB, and 34-2-CB (all 99% purity, AccuStandard). The GC temperature was held at 70 °C for 2 min, then raised to 180 °C at 20°/min, then to 270 °C at 4°/min, then to 270 °C at 15°/min, and then held for 13 min.

The masses monitored (each with a dwell time of 300 ms) for the parent congener and each of the possible products were the two largest ions of the molecular cluster and the characteristic ion formed by the loss of two chlorines. These were as follows: *m/z* 292, 290, and 220 (tetra-CB); *m/z* 258, 256, and 186 (tri-CB); *m/z* 224, 222, and 152 (di-CB); and *m/z* 190, 188, and 152 (mono-CB). For biphenyl, we monitored the two largest ions of the molecular cluster (*m/z* 154 and 152) and the characteristic ion formed by the loss of a phenyl group (*m/z* 76). We constructed a three-point linear calibration curve from standards composed of 25-34-CB and each of the possible products described above at individual concentrations of 5, 50, and 200 μ M in hexane. The total concentration of 25-34-CB and its products in our GC samples was in the range of 70 μ M.

For selected samples quantitative GC/ECD congener-specific PCB analysis was done at Northeast Analytical, Schenectady, NY. These samples were Soxhlet extracted with a 1:1 mixture of hexane/acetone for 18–20 h. Sulfur was removed by shaking with mercury, and polar compounds were removed by treatment with concentrated

sulfuric acid followed by filtration on a Florisil Sep-pak column (Waters Associates, Inc., Milford, MA). Aroclors 1242, 1254, and 1260, each at a concentration of 4 μ g/mL, were analyzed separately and used to generate relative response factors for all peaks. Prior to capillary analysis on a Varian 3400 GC equipped with a DB-1 capillary column, each sample extract was analyzed by packed column GC for an estimate of total PCB concentration. The volume of each extract was then adjusted to bring the final PCB concentration to 2–4 μ g/mL. The data were collected and processed with Waters Maxima 820 data acquisition software. Congener and homolog distributions for each sample were calculated and reported in units of mole percent after subtracting the values for 25-34-CB, 25-3-CB, and 24-3-CB. (The latter was not resolved from the large amount of 25-3-CB formed by dechlorination.) Further details of the analysis are described elsewhere (2, 7, 12).

Results and Discussion

Dechlorination of 25-34-CB. Our previous studies (7) demonstrated that microbial dechlorination of the Aroclor 1260 in Woods Pond sediments has removed as much as 13.7% of the *meta* and *para* chlorines, but it is not clear when the dechlorination occurred or whether it occurred upstream in the Housatonic River or in the pond. The only way to determine if the microorganisms responsible for the PCB dechlorination were present in the pond sediments was to demonstrate dechlorination in these sediments in controlled laboratory experiments. In our first experiments, we added high concentrations (100 and 500 μ g/mL of slurry) of a 1:1 mixture of Aroclors 1254 and 1260 to sediment slurries incubated under methanogenic conditions at 23–25 °C. Others have reported substantial PCB dechlorination of such high concentrations of Aroclors added to sediment slurries within 12–16 weeks (3, 13, 14). However, in our slurries there was little evidence of PCB transformation after 20 weeks using this approach.

Subsequently we elected to add a high concentration of a single PCB congener. Dechlorination of a single congener provides a very sensitive assay since only a few dechlorination products are formed in high amounts. Furthermore, if PCBs can act as electron acceptors as has been proposed (2, 3), a high concentration of a PCB congener that is a good dechlorination substrate might selectively increase the population of PCB-dechlorinating microorganisms. We selected 25-34-CB as a trial dechlorination substrate because it is a fairly prominent component of several Aroclors and because its dechlorination has been reported in many PCB-contaminated sediments (1, 2, 15).

When 25-34-CB (350 μ M) was added to slurries of Woods Pond sediment, we detected the formation of 25-3-CB from loss of the *para* chlorine after only 3 weeks of incubation at 23–25 °C. During the next 13 weeks about 90% of the tetrachlorobiphenyl was stoichiometrically converted to 25-3-CB (Figure 1). No other dechlorination products of 25-34-CB were detected by either GC/ECD or GC/MS.

Dechlorination of Aroclor 1260 Associated with the Sediment. Beginning at 7 weeks, when 45–60% of the 25-34-CB had been dechlorinated to 25-3-CB, changes in the PCB congener distribution of the Aroclor 1260 that had persisted in the sediment for decades revealed that it was being dechlorinated. The dechlorination was selective and progressed with time. Figure 2 compares the PCB congener distributions for a typical sample at the start of the experiment and after a 12-week incubation. Decreases of

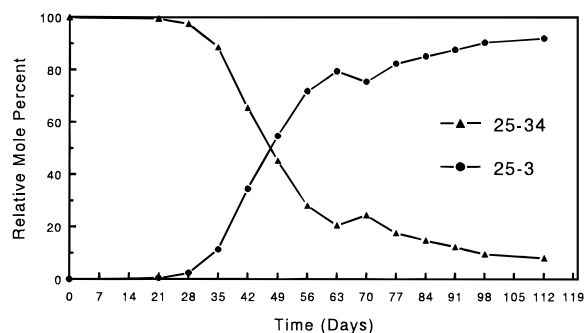


FIGURE 1. Conversion of 25-34-CB to 25-3-CB as a function of time. Quantitation was by GC/MS.

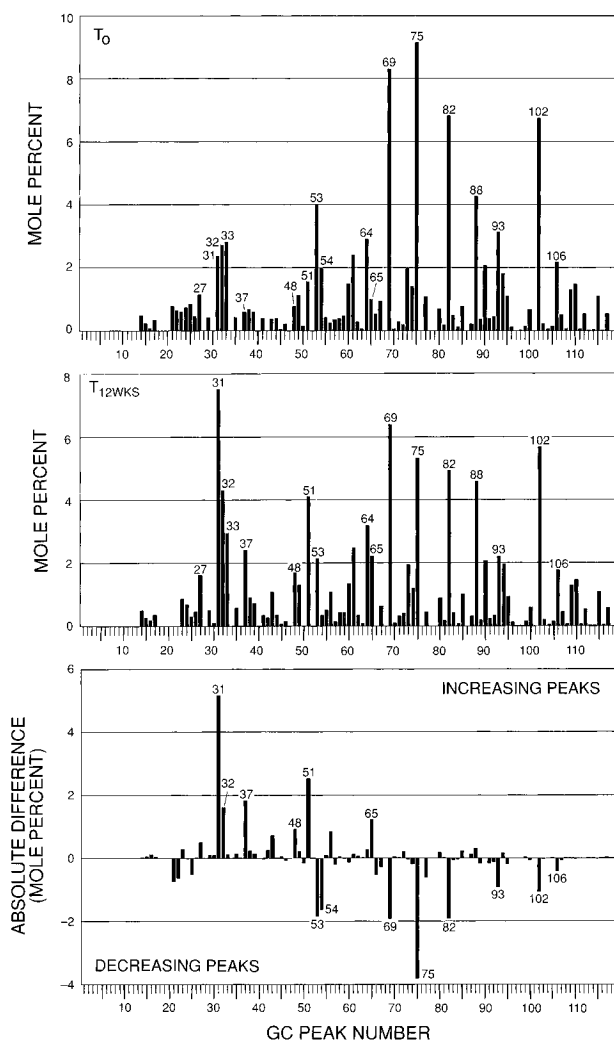


FIGURE 2. Change in the PCB congener distribution in Woods Pond sediment after priming with 25-34-CB.

40–80% occurred in several major penta- and hexachlorobiphenyls: notably 245-25-CB, 245-24-CB, 245-245-CB, and 2345-25-CB (peaks 53, 54, 75, and 77, respectively). Most other major hexa- and heptachlorobiphenyls, 236-245-CB, 234-245-CB, 2345-236-CB, 2345-245-CB, and 2345-234-CB (peaks 69, 82, 93, 102, and 106, respectively), showed decreases of 17–31%. At the same time, large increases appeared in some of the tetra- and pentachlorobiphenyls, especially 25-25-CB, 24-25-CB, 23-25-CB, and 235-25-CB, (peaks 31, 32, 37, and 51, respectively).

To determine if the acetone used as the carrier for adding PCBs influenced or was necessary for this dechlorination, we coated fine silica with 25-34-CB and added the 25-34-

CB to the sediment slurries by preweighing the PCB-coated silica into the serum bottles. The results were the same as reported above for 25-34-CB added in acetone. Experiments with no addition or with acetone alone (no added PCBs) showed no dechlorination. Hence the 25-34-CB stimulated the dechlorination of the PCBs associated with the sediment and the acetone was not necessary.

Two other PCB congeners, 24-34-CB and 234-CB, stimulated the same pattern of PCB dechlorination when added to sediment slurries. These congeners were also dechlorinated from the *para* position to form 24-3-CB and 23-CB, respectively.

Quantitation of PCB Dechlorination. Table 1 identifies the PCB congeners in each peak shown in Figure 2 and gives the mole percent contribution for each peak before and after incubation with 25-34-CB. The values for samples from the duplicate slurries matched closely. The data were analyzed by a two-sample, two-tailed *t*-test with unequal variance (16) to test whether the changes observed in the mole percent contributions of the various congeners were significant. More than half of the 86 PCB peaks detected in these sediments showed a significant change ($P < 0.05$). The *P* values for each of the significant changes are listed in Table 1.

Significant changes were split almost evenly between decreases and increases (24:21). Most of the penta-, hexa-, and heptachlorobiphenyls that decreased had a 245-, 234-, or 2345-chlorophenyl ring, and most of the PCBs that increased, primarily tetra- and pentachlorobiphenyls, had a 25-, 23-, or 235-chlorophenyl ring. These data indicate that PCB congeners having 245-, 234-, and 2345-chlorophenyl groups lost the *para* chlorine, yielding products carrying 25-, 23-, and 235-chlorophenyl rings, respectively.

Table 2 lists the PCB congeners formed in the highest amounts and shows how they might have been formed by *para*-dechlorination. The expected increase for each of these congeners was calculated from the observed decrease(s) in the proposed parent congener(s). This quantitative mother–daughter analysis yields an excellent mass balance (Table 2) and further supports the conclusion that the 245-, 234, and 2345-chlorophenyl groups of PCBs were *para*-dechlorinated in this experiment.

Specificity of the Dechlorination. In addition to dechlorination of congeners bearing 235-, 234-, and 2345-chlorophenyl groups, there were also a few instances where congeners bearing 345- or 23456-chlorophenyl groups were dechlorinated to form congeners bearing 35- and 2356-chlorophenyl groups, respectively. For example, the 0.16 mol % decrease in 245-345-CB (peak 91) was balanced by an increase of 0.13 mol % in the expected product of *para*-dechlorination, 25-35-CB (peak 39). Similarly, the 0.13 mol % decrease in 23456-25-CB (peak 92) partly accounted for an increase of 0.23 mol % in 2356-25-CB (peak 64). Most likely the *para* chlorines of 34-chlorophenyl groups were also removed to form 3-chlorophenyl groups as was the case for 25-34-CB and 24-34-CB. In contrast, 4-, 24-, and 246-chlorophenyl groups were not dechlorinated. Hence, only *para* chlorines flanked by at least one chlorine were susceptible to this type of dechlorination.

There were indications that the dechlorination was sensitive to steric hindrance. Except for 2346-34-CB (peak 83), 23456-25-CB (peak 92), and 23456-34-CB (peak 107), congeners chlorinated in both *ortho* positions of either ring, i.e., at carbons 2 and 6, were not dechlorinated. For example, 246-34-CB (peak 55), 236-34-CB (peak 61),

TABLE 1

Quantitative Changes in Individual PCB Congeners after Priming with 25-34-CB

DB-1 peak no.	IUPAC No.	PCB congener identification ^a	mol % of total PCBs					significant changes		
			0 weeks		12 weeks		av difference	increases	decreases % change	T-test P value
			A	B	A	B				
1		biphenyl								
2	1	2 ^b								
3	2	3 ^b								
4	3	4 ^b								
5	4, 10	2-2, 26 ^b								
6	7, 9	24, 25 ^b								
7	6	2-3 ^b								
8	5, 8	23, 2-4 ^b								
9	14	35 ^b								
10	19	26-2 ^b								
11	30	246 ^b								
12	11	3-3 ^b								
13	12, 13	34, 3-4 ^b								
14	18, 15	25-2, 4-4	0.44	0.50	0.47	0.48	0.00			
15	17	24-2	0.23	0.23	0.26	0.25	0.03			
16	27, 24	26-3, 236	0.00	0.16	0.19	0.19	0.11			
17	32	26-4	0.34	0.33	0.36	0.36	0.02			
19	34, 23, 54	35-2, 235, 26-26 ^b								
20	29	245 ^b								
21	26	25-3 ^c								
22	25	24-3 ^c								
23	31	25-4	0.58	0.62	0.85	0.90	0.28	+		0.017
24	28	24-4	0.74	0.70	0.69	0.67	−0.04			
25	53	25-26 ^d	0.71	0.78	0.27	0.25	−0.48			
26	51	24-26	0.41	0.40	0.40	0.40	−0.01			
27	45	236-2 ^b								
28	36	35-3 ^b								
29	46	23-26	0.43	0.40	0.46	0.52	0.07			
30	39	35-4	0.00	0.00	0.00	0.19	0.09			
31	52	25-25	2.46	2.34	7.54	7.63	5.19	+		0.000
32	49	24-25	2.77	2.71	4.30	4.36	1.59	+		0.001
33	47	24-24	2.87	2.84	2.96	2.96	0.10			
34	48, 75	245-2, 246-4 ^b								
35	62, 65	2346, 2356 ^b								
36	35	34-3 ^b								
37	44	23-25	0.60	0.58	2.33	2.47	1.81	+		0.022
38	42, 59	23-24, 236-3	0.63	0.61	0.83	0.84	0.21	+		0.011
39	71, 64, 41, 72	26-34, 236-4, 234-2, 25-35	0.59	0.59	0.71	0.72	0.13	+		0.008
40/41	68, 96	24-35, 236-26 ^e	0.38	0.39	0.33	0.34	−0.05		14	0.025
42	40	23-23	0.00	0.00	0.25	0.26	0.25	+		0.020
43	57, 103	235-3, 246-25	0.32	0.33	0.96	1.01	0.66	+		0.019
44	100	246-24	0.39	0.30	0.33	0.33	−0.02			
45	63	235-4	0.00	0.07	0.07	0.08	0.04			
46	94, 74	235-26, 245-4	0.20	0.19	0.14	0.14	−0.06		29	0.035
47	70	25-34 ^c								
48	66, 95, 102	24-34, 236-25, 245-26	0.77	0.70	1.58	1.65	0.88	+		0.003
49	91	236-24	1.12	1.10	1.31	1.31	0.20	+		0.045
50	56, 60	23-34, 234-4	0.16	0.15	0.00	0.00	−0.16		100	0.021
51	92, 84	235-25, 236-23	1.59	1.60	4.04	4.22	2.54	+		0.023
52	89	234-26 ^b								
53	101, 90	245-25, 235-24	4.04	4.03	2.17	2.10	−1.90		47	0.008
54	99	245-24	2.00	2.00	0.36	0.30	−1.67		83	0.012
55	119, 150	246-34, 236-246	0.40	0.40	0.49	0.49	0.09	+		0.006
56	83, 112	235-23, 2356-3	0.23	0.24	1.04	1.11	0.84	+		0.022
57	97, 152	245-23, 2356-26	0.33	0.36	0.14	0.13	−0.21		61	0.041
58	87, 111	234-25, 235-35	0.38	0.40	0.43	0.42	0.03			
59	85	234-24	0.47	0.47	0.44	0.44	−0.03		7	0.020
60	136, 120, 77	236-236, 245-35, 34-34	1.52	1.49	1.36	1.36	−0.14			
61	110	236-34	2.38	2.30	2.43	2.41	0.09			
62	154	245-246	0.27	0.27	0.31	0.33	0.05			
63	82	234-23	0.05	0.05	0.06	0.07	0.01			
64	151	2356-25	2.96	2.95	3.19	3.17	0.23	+		0.007
65	135, 124	235-236, 345-25	1.00	1.01	1.80	1.89	0.84	+		0.032
66	144	2346-25	0.57	0.55	0.51	0.46	−0.08			
67	147, 109, 107	2356-24, 235-34, 234-35	0.91	0.93	0.63	0.61	−0.30		33	0.002
68	123	345-24 ^b								
69	149, 118	236-245, 245-34	8.29	8.15	6.35	6.14	−1.97		24	0.007
70	140	234-246, 2346-24	0.05	0.05	0.09	0.09	0.04	+		0.001
71	114, 134	2345-26, 2356-23	0.29	0.29	0.32	0.31	0.02			
72	131, 133	2346-23, 235-235	0.19	0.19	0.37	0.38	0.19	+		0.023
73	146	235-245	1.99	1.98	1.90	1.90	−0.09			

Table 1 (Continued)

DB-1 peak no.	IUPAC No.	PCB congener identification ^a	mol % of total PCBs					significant changes		
			0 weeks		12 weeks		av difference	increases	decreases % change	T-test P value
			A	B	A	B				
74	105, 132	234-34, 234-236	1.16	1.61	1.07	1.28	-0.21			
75	153	245-245	9.31	9.31	5.51	5.20	-3.95		42	0.025
76	168	246-345 ^b								
77	141	2345-25	1.20	1.21	0.45	0.43	-0.77		64	0.001
78	179	2356-236	2.03	1.95	2.34	2.25	0.30	+		0.035
79	137	2345-24 ^b								
80	130	234-235	0.67	0.70	0.85	0.87	0.18	+		0.024
81	176	2346-236	0.18	0.18	0.17	0.17	-0.01			
82	138, 163	234-245, 2356-34	6.99	6.88	5.03	4.86	-1.99		29	0.005
83	158	2346-34	0.50	0.49	0.42	0.41	-0.08		17	0.010
84	129	2345-23	0.11	0.11	0.06	0.06	-0.05		44	0.002
85	178	2356-235	0.77	0.77	0.96	1.04	0.23			
87	175	2346-235	0.18	0.21	0.31	0.30	0.11	+		0.047
88	187, 182	2356-245, 2345-246	4.32	4.32	4.57	4.63	0.28			
89	128	234-234	0.36	0.35	0.19	0.18	-0.17		48	0.023
90	183	2346-245	2.09	2.10	2.06	2.07	-0.03			
91	167	245-345	0.36	0.39	0.22	0.21	-0.16		43	0.038
92	185	23456-25	0.46	0.44	0.33	0.32	-0.13		28	0.008
93	174	2345-236	3.14	3.17	2.24	2.15	-0.96		31	0.016
94	177	2356-234	1.86	1.86	1.95	1.97	0.10	+		0.044
95	156, 171	2345-34, 2346-234	1.11	1.12	0.92	0.90	-0.21		19	0.028
96	202, 157	2356-2356, 234-345	0.11	0.12	0.11	0.11	-0.01			
98	173	23456-23	0.02	0.02	0.01	0.00	-0.01			
99	201	2346-2356	0.14	0.13	0.14	0.16	0.01			
100	172	2345-235	0.68	0.67	0.59	0.59	-0.09		12	0.009
101	197	2346-2346	0.02	0.01	0.02	0.02	0.00			
102	180	2345-245	6.83	6.83	5.71	5.60	-1.17		17	0.029
103	193	2356-345	0.22	0.22	0.19	0.18	-0.04			
104	191	2346-345	0.05	0.05	0.05	0.04	-0.01			
105	200	23456-236	0.14	0.14	0.15	0.13	0.00			
106	170	2345-234	2.21	2.18	1.79	1.72	-0.44		20	0.017
107	190	23456-34	0.52	0.52	0.45	0.44	-0.07		13	0.036
108	198	23456-235	0.05	0.06	0.06	0.06	0.01			
109	199	2345-2356	1.32	1.31	1.29	1.29	-0.03			
110	196, 203	2345-2346, 23456-245	1.48	1.49	1.45	1.46	-0.03			
111	189	2345-345	0.05	0.06	0.05	0.05	-0.01			
112	195	23456-234	0.53	0.53	0.52	0.52	-0.01			
113	208	23456-2356	0.04	0.02	0.02	0.03	0.00			
114	207	23456-2346	0.02	0.03	0.03	0.03	0.00			
115	194	2345-2345	1.12	1.11	1.08	1.09	-0.03		3	0.040
116	205	23456-345	0.02	0.03	0.03	0.03	0.01			
117	206	23456-2345	0.53	0.54	0.58	0.56	0.03			
118	209	23456-23456 ^b								

^a The order in which congeners are reported indicates the relative contributions we believe they make to the peak in Aroclor 1260 or in dechlorinated Aroclor 1260. ^b These congeners were never detected. ^c The values for 25-34-CB, the congener added to the sediment, and 25-3-CB, its observed dechlorination product in these experiments, were subtracted before calculating mole percents. The amount of 24-3-CB could not be quantified because of interference from the large peak at 25-3-CB. ^d The value for 25-26-CB reported for T_0 samples here is inconsistent with the value determined for the same sediment in other experiments. Therefore, we believe that the value reported here represents a non-PCB contaminant and not 25-26-CB. ^e These congeners are not substrates for this dechlorination. Even though the T-test showed this decrease to be real, it most likely is not.

2346-25-CB (peak 66), 2345-26-CB (peak 71), 2356-245-CB/2345-246-CB (peak 88), and 2346-245-CB (peak 90) were not dechlorinated although each of these congeners bears at least one chlorophenyl ring that was a substrate in other congeners. This steric hindrance is also a likely explanation for the reduced activity against heptachlorobiphenyls and the lack of activity against octachlorobiphenyls. Many of the former and all but one of the latter have *ortho* chlorines in both positions 2 and 6. Surprisingly two *ortho* chlorines positioned on opposite rings, i.e., at carbons 2 and 2', apparently did not inhibit dechlorination since many of the congeners that were dechlorinated had *ortho* chlorines in these positions. We have designated this type of dechlorination Process P and have summarized the major transformations that characterize it in Figure 3.

Impact of Process P Dechlorination on Aroclor 1260.

Table 3 shows the effect of Process P dechlorination on the homolog distribution of the PCBs in the sediment. The

hexa- and heptachlorobiphenyls showed significant decreases ($P < 0.05$) of approximately 22 and 8%, respectively. The formation of new pentachlorobiphenyls such as 235-25-CB (peak 51) and 235-23-CB (peak 56) from the dechlorination of hexa- and heptachlorobiphenyls was roughly equivalent to the losses from dechlorination and explains the small net change in pentachlorobiphenyls. The tetrachlorobiphenyls increased substantially, from 12.0 to 21.2 mol % of the total PCBs. However, the overall impact of this dechlorination was rather limited. Only 16.5% of the *para* chlorines were removed, and the average number of chlorines per biphenyl dropped only slightly, from 5.90 to 5.67. This is not surprising given the limited specificity and the apparent sensitivity to steric hindrance exhibited by Process P dechlorination.

Comparison of Dechlorination Process P with Other Microbial Dechlorination Processes That Remove *para* Chlorines from PCBs. Process P dechlorination has not

TABLE 2

Quantitative Mass Balance of Dechlorination of Key Congeners

observed dechlorination product		proposed parent congener and route of dechlorination			mol % of total PCBs			mass balance (%)
peak no.	congener	peak no.	parent congener	pathway	observed decrease	expected total increase ^a	observed increase	obs. increase/exp. increase
31	25-25	53	245-25	→ 25-25	1.90			
		75	245-245	→ 245-25 → 25-25	3.95	5.85	5.19	89
32	24-25	54	245-24	→ 24-25	1.67	1.67	1.59	95
37	23-25	82	234-245 ^b	→ 234-25 → 23-25	1.59 ^b	1.59	1.81	114
51	235-25 ^c	77	2345-25	→ 235-25	0.77			
		102	2345-245	→ 2345-25 → 235-25	1.17	2.03	2.29 ^c	113
		73	235-245	→ 235-25	0.09			
56	235-23	106	2345-234	→ 234-235 → 235-23	0.44			
		84	2345-23	→ 235-23	0.05	0.96	1.02 ^e	106
		107	23456-34	→ 2356-34 → 2356-3	0.47 ^{b,d}			
65	235-236 ^c	93	2345-236	→ 235-236	0.96	0.96	0.77 ^c	80

^a Based on the assumption that the observed decreases in the proposed parent congeners should correspond to the observed increases in product(s). ^b Congeners 234-245-CB and 2356-34-CB coelute, but 234-245-CB is present in a much higher proportion and is more easily dechlorinated. We have assumed that 80% of the decrease in peak 82 is due to 234-245-CB. The observed decrease has been adjusted accordingly. ^c This congener is estimated to constitute 90% of this peak. The observed increase has been adjusted accordingly. ^d This value includes observed decreases in the intermediate as well as the parent congener. ^e Includes an observed increase of 0.18 in the intermediate 234-235-CB.

	23-34	→	23-3
	24-34	→	24-3
	25-34	→	25-3
	234-4	→	23-4
	245-4	→	25-4
	245-24	→	24-25
	245-34	→	25-34 → 25-3
	234-234	→	234-23 → 23-23
	234-236	→	236-23
	234-245	→	234-25 → 23-25
	236-245	→	236-25
	245-245	→	245-25 → 25-25
	2345-34	→	235-34 → 235-3
	2345-234	→	234-235 → 235-23
	2345-245	→	235-245 → 235-25
		→	2345-25 → 235-25
	2345-236	→	235-236
	23456-34	→	2356-34 → 2356-3
Specificity:	Flanked <i>para</i> Cl, doubly flanked <i>para</i> Cl		
Reactive Groups:	34, 234, 245, 345, 2345, 23456		
	Poor activity against 2346		
Unreactive <i>para</i> Cl:	No activity on 4, 24, 246		
Comments:	Most active on tetra- through hexa-CB		

FIGURE 3. Transformations and characteristics observed for Process P dechlorination.

previously been described, but a similar type of dechlorination, Process H, has been observed in sediments from New Bedford Harbor (Massachusetts) and some regions of the Hudson River (15) (New York) and in laboratory incubations of Aroclor 1260 using inocula from Hudson River sediment (13). Like Process P, Process H preferentially removes flanked *para* chlorines from 34-, 245-, 2345-, and 23456-chlorophenyl groups. The unflanked *para* chlorines on 4-, 24-, and 246-chlorophenyl rings are not substrates for either process. The key differences between the two processes are that Process H is less sensitive to steric hindrance (13) and that Process H removes the doubly flanked *meta* chlorines from 234- and 2346-chlorophenyl groups (6) whereas Process P is restricted to *para*-dechlorination. It has been suggested that the New Bedford Process H dechlorination may actually result from two or

possibly three independent activities that usually occur together, namely, *para*-dechlorination of 34- and 245-chlorophenyl rings and *meta*-dechlorination of 234-chlorophenyl rings (6, 17). This raises the question of whether the *para*-dechlorination component(s) of Process H might be the same as Process P. We do not think this is the case. Both the substrate range and the ability to *para*-dechlorinate 234-chlorophenyl groups both distinguish Process P from Process H (6, 13, 17). Furthermore, all evidence suggests that Process P is a single activity.

Another type of *para*-dechlorination, Process Q, has been observed in incubations of sediments from the Hudson River with Aroclor 1242 (3). Process Q apparently removes all *para* chlorines from mono-, di-, and trichlorophenyl groups of lower chlorinated PCBs and is not limited to removal of flanked *para* chlorines as are Processes H and P (6). However, it is not clear whether Process Q can dechlorinate congeners with six or more chlorines.

Role of Process P Dechlorination in Woods Pond. The sediment PCBs display evidence of various combinations of two different dechlorination processes (7). Process P is responsible for a modest *para*-dechlorination of the PCBs, but it accounted for no more than 25% of the *in situ* dechlorination and removed a maximum of 6.9% of the *para* chlorines (7). The second major dechlorination process active in Woods Pond, Process N, is responsible for most of the *in situ* dechlorination (7). Process N preferentially removes *meta* chlorines and will be described elsewhere (18).

The addition of 25-34-CB stimulated Process P dechlorination of the PCBs in sediments taken from all 12 locations in the pond, demonstrating that the microorganisms responsible for the dechlorination are present and are widely distributed throughout Woods Pond. Hence, it is not clear why its activity in the sediment has been so limited. Even in the laboratory we have only detected dechlorination of the PCBs in the sediment after adding a high concentration (350 μ M) of a halogenated biphenyl. The addition of RAMM medium with or without various carbon sources, but without the addition of PCBs, stimulated methanogenesis but did not stimulate dechlorination of the sediment

TABLE 3

Quantitative Changes in the Homolog Distribution (Mol %) and Chlorine Distribution of Aroclor 1260 after Priming Dechlorination with 25-34-CB

	incubation time				av difference	significant changes	
	0 weeks		12 weeks			% change	<i>T</i> -test <i>P</i> -value
	A	B	A	B			
PCB homolog ^a							
trichlorobiphenyls	2.32	2.54	2.81	3.03	0.50		
tetrachlorobiphenyls	12.08	11.89	21.01	21.39	9.21	76.9	0.003
pentachlorobiphenyls	17.05	16.94	17.35	17.53	0.44		
hexachlorobiphenyls	36.63	36.80	28.94	28.44	−8.02	−21.9	0.011
heptachlorobiphenyls	26.38	26.30	24.40	24.12	−2.08	−7.9	0.029
octachlorobiphenyls	4.95	4.95	4.86	4.87	−0.08	−1.7	0.007
nonachlorobiphenyls	0.59	0.59	0.63	0.61	0.03		
chlorine distribution							
<i>ortho</i> Cl per biphenyl	2.40	2.39	2.40	2.40	0.01		
<i>meta</i> Cl per biphenyl	2.24	2.24	2.22	2.22	−0.02		
<i>para</i> Cl per biphenyl	1.26	1.26	1.06	1.04	−0.21	−16.5	0.016
total Cl per biphenyl	5.90	5.90	5.68	5.66	−0.23	−3.8	0.025

^a No mono-, di-, or decachlorobiphenyls were detected.

PCBs. These data imply that the slow dechlorination of PCBs in Woods Pond is not due to the lack of an essential nutrient or to inhibition by any toxic compound. A more likely explanation for the slow dechlorination in Woods Pond is that partitioning of the PCBs into both the highly organic sediment and the hydrocarbon oil associated with it may limit the growth and activity of the PCB-dechlorinating microbial population. Nevertheless, decreases of up to 83% in some of the susceptible congeners clearly demonstrate that the PCBs were bioavailable over the course of the experiment.

Proposed Explanations for the Priming Effect of 25-34-CB. Others have proposed that the anaerobic bacteria responsible for dechlorination of PCBs might use the PCBs as electron acceptors and may therefore benefit from PCB dechlorination (2, 3). Our findings are consistent with this hypothesis. The addition of a PCB congener in high concentration might selectively stimulate the growth of PCB-dechlorinating microorganisms by providing them with a readily accessible electron acceptor in an environment where electron acceptors are limiting. The resulting higher numbers of PCB-dechlorinating bacteria could then dechlorinate the PCBs in the sediment. In our experiments the PCB congener added was an ideal substrate for dechlorination and was used at a final concentration of approximately 700 $\mu\text{g/g}$ (sediment dry weight), roughly 7–14-fold higher than the PCB concentration in the sediments.

Further support for the hypothesis that PCBs act as electron acceptors comes from the demonstration that daily addition of high concentrations (1–2 mM) of 236-CB to a PCB-dechlorinating enrichment culture increased the volumetric rate of dechlorination of 236-CB to 26-CB more than 100-fold and decreased the rate of methanogenesis by nearly 80% (19). Thus, the proportion of electrons channeled to dechlorination increased relative to the proportion channeled to methanogenesis (6).

We favor the hypothesis that the 25-34-CB selectively increased the PCB-dechlorinating microbial population by acting as an electron acceptor, but our data do not rule out other hypotheses. An alternative explanation is that the 25-34-CB may have induced the synthesis of the appropriate PCB-dechlorinating enzyme(s).

Implications of Priming PCB Dechlorination. This work demonstrates for the first time a strategy for stimulating indigenous microorganisms to rapidly dechlorinate PCBs that have persisted in aquatic sediment for decades. The narrow specificity of dechlorination Process P makes this particular dechlorination unsuitable for PCB remediation. Furthermore, it is neither desirable nor acceptable to add PCBs to the environment. Nevertheless, the strategy of adding a PCB congener to stimulate microbial dechlorination of the PCBs in contaminated sediment can be used to determine if other dechlorination processes that effect more extensive dechlorination can be stimulated (10). This strategy, which we call priming, is based on the premise that it is possible to stimulate the microorganisms that carry out a particular dechlorination process by adding a halogenated compound that is a preferred dehalogenation substrate and electron acceptor for that particular dechlorination process. Once it has been established that the indigenous microbial population can extensively dechlorinate the PCBs, other compounds that stimulate the same dechlorination process can be sought. Thus, this work lays a foundation for the development of methods for *in situ* PCB bioremediation.

Acknowledgments

We thank Robert Wagner and Scott O'Neil of Northeast Analytical, Inc., for expert technical analysis; Ralph May for development of computer programs for data processing; Heidi Van Dort for technical assistance; John Bergeron and Carolyn Morgan for help in statistical analysis; and Blasland and Bouck Engineers, P.C., for collecting sediment samples.

Literature Cited

- (1) Brown, J. F., Jr.; Bedard, D. L.; Brennan, M. J.; Carnahan, J. C.; Feng, H.; Wagner, R. E. *Science* **1987**, *236*, 709–712.
- (2) Brown, J. F., Jr.; Wagner, R. E.; Feng, H.; Bedard, D. L.; Brennan, M. J.; Carnahan, J. C.; May, R. J. *Environ. Toxicol. Chem.* **1987**, *6*, 579–593.
- (3) Quensen J. F., III; Tiedje, J. M.; Boyd, S. A. *Science* **1988**, *242*, 752–754.
- (4) Abramowicz, D. A. *Res. Microbiol.* **1994**, *145*, 42–46.
- (5) Brown, J. F. *Environ. Sci. Technol.* **1994**, *28*, 2295–2305.
- (6) Bedard, D. L.; Quensen, J. F. Microbial reductive dechlorination of polychlorinated biphenyls. In *Microbial Transformation and*

Degradation of Toxic Organic Chemicals; Young, L. Y., Cerniglia, C., Eds.; Wiley-Liss Division, John Wiley & Sons, Inc.: New York, 1995; pp 127–216.

- (7) Bedard, D. L.; May, R. J. *Environ. Sci. Technol.* **1996**, *30*, 237–245.
- (8) Van Dort, H. M.; Bedard, D. L. *Appl. Environ. Microbiol.* **1991**, *57*, 1576–1578.
- (9) Bedard, D. L.; Bunnell, S. C.; Van Dort, H. M. Reductive dechlorination of PCBs (Aroclor 1260) in methanogenic sediment slurries. *Abstracts of the 91st General Meeting of American Society of Microbiology*; American Society for Microbiology: Washington, DC, 1991; Q-36, p 282.
- (10) Bedard, D. L.; Van Dort, H. M.; Bunnell, S. C.; Principe, J. M.; DeWeerd, K. A.; May, R. J.; Smullen, L. A. In *Anaerobic Dehalogenation and Its Environmental Implications*; Abstracts of 1992 American Society for Microbiology Conference, Athens, GA; Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency: Athens, GA, 1993; pp 19–21.
- (11) Shelton, D. R.; Tiedje J. M. *Appl. Environ. Microbiol.* **1984**, *47*, 850–857.
- (12) Bedard, D. L.; Wagner, R. E.; Brennan, M. J.; Haberl, M. L.; Brown, J. F., Jr. *Appl. Environ. Microbiol.* **1987**, *53*, 1094–1102.
- (13) Quensen, J. F., III; Boyd, S. A.; Tiedje, J. M. *Appl. Environ. Microbiol.* **1990**, *56*, 2360–2369.
- (14) Abramowicz, D. A.; Brennan, M. J.; Van Dort, H. M.; Gallagher, E. L. *Environ. Sci. Technol.* **1993**, *27*, 1125–1131.
- (15) Brown, J. F., Jr.; Wagner, R. E. *Environ. Toxicol. Chem.* **1990**, *9*, 1215–1233.
- (16) Satterthwaite, F. W. *Biometrics Bull.* **1946**, *2*, 110–114.
- (17) Lake, J. L.; Pruett, R. J.; Osterman, F. A. *Mar. Environ. Res.* **1992**, *33*, 31–47.
- (18) Bedard, D. L.; Van Dort, H. M. Manuscript in preparation.
- (19) Boyle, A. W.; Blake, C. K.; Price, W. A., II; May, H. D. *Appl. Environ. Microbiol.* **1993**, *59*, 3027–3031.

Received for review June 29, 1995. Revised manuscript received August 18, 1995. Accepted August 18, 1995.[®]

ES950463I

[®] Abstract published in *Advance ACS Abstracts*, November 15, 1995.