Chemical Activation of Microbially-Mediated PCB Dechlorination: A Field Study

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INTRODUCTION

Polychlorinated biphenyls (PCBs) were widely used for a variety of applications from 1929 until 1978. PCBs that were released to the environment persist in many aquatic sediments and are of concern because they tend to accumulate in biota and are potentially toxic. Microbial dechlorination of PCBs in several aquatic sediments has been documented [1-4]. This dechlorination is reported to detoxify PCBs by removing the *meta* and *para* chlorines of the "dioxin-like" congeners [1-3].

At other locations natural dechlorination has had less impact. PCB-contaminated sediment from the Housatonic River has accumulated in Woods Pond, an impoundment on the river located in Lenox, MA. The contaminant at this site is Aroclor 1260 which consists primarily of hexa- and heptachlorobiphenyls. Some dechlorination has occurred, but the PCBs from the most extensively dechlorinated samples show losses of only 13% of the *meta* and *para* chlorines and still have an average of 5.63 chlorines per biphenyl [5].

It has been hypothesized that PCB-dechlorinating microorganisms use PCBs as electron acceptors and may derive energy from dechlorination. Based on this hypothesis we reasoned that a brominated biphenyl might act as an alternate electron acceptor for the PCB-dechlorinating microorganisms and might be used to selectively "enrich" that microbial population. Subsequent laboratory experiments with Woods Pond sediments confirmed that an excess of 2,6-dibromobiphenyl (2,6-BB) would stimulate the indigenous microorganisms to dechlorinate the Aroclor 1260 in the sediment [6]. The dechlorination stimulated by 2,6-BB, known as Process N, selectively removes *meta* chlorines positioned next to other chlorines, particularly those on 2,3,4-, 2,4,5-, 2,3,4,5-, and 2,3,6-chlorophenyl rings [6]. Because these are the most common ring substituents in Aroclor 1260, this Aroclor is particularly susceptible to Process N dechlorination and the hexa- and heptachloro-biphenyls are largely dechlorinated to tetrachlorobiphenyls. In order to assess the feasibility of this process for remediation, we began a field experiment in Woods Pond on June 8, 1992. During the experiment we made no attempt to control the temperature, pH, or microbial predation.

Prior to the field study GE retained several consultants to assess whether the proposed use of 2,6-BB would present a risk to human health. Using known structure-activity relation-ships, the consultants concluded that 2,6-dibromobiphenyl and 2-bromobiphenyl are not

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acutely toxic, carcinogenic, promoters of two-stage carcinogenesis, mutagenic, or compara-ble to Firemaster BP-6, and that the proposed use of 2,6-dibromobiphenyl for an *in situ* test of PCB dechlorination at Woods Pond posed no significant risk.

EXPERIMENTAL

The field experiment was conducted in steel caissons (six feet in diameter) that were completely enclosed except for the bottoms which were driven seven feet into the clay subsoil. The depths of sediment and water enclosed by the caisson were 18 to 24 inches and 24 to 30 inches deep, respectively. The welded covers had four six inch sampling ports and additional utility ports for a thermocouple tree to measure water and sediment temperatures (at top, middle, and bottom), a water level monitor, and tubing and valves to introduce and vent gases. Each caisson was fitted with a pressure release valve set at 0.3 psi to prevent excessive buildup of methane.

Two caissons were used for the experiment: one control and one experimental. Each caisson contained approximately 400 Kg sediment (dry weight) and a total volume of 3500 liters (water and sediment). One month before the beginning of the field study a variable speed rotary mixer fitted with a 17 inch impeller positioned in the water column was used to briefly suspend and mix the sediment in order to make it as homogeneous as possible without disturbing the more compact clay subsoil. Initial sediment cores were taken from the experimental and control caissons on June 4, 1992. On June 8, which was designated T_0, the sediments were completely suspended again and the amendments were made to each caisson while mixing. Positive nitrogen flow was maintained throughout the procedure to prevent the introduction of oxygen. Each caisson received 17.5 L of disodium malate, pH 6.55, to yield a final concentration of 10 mM. In addition, the experimental caisson received 365 g of 2,6-BB (99.93% pure by GC/FID) dissolved in 17 L of acetone, whereas the control caisson received 17 L of acetone but no bromobiphenyl.

At each sampling sediment cores were collected from each of the four ports using a 2 inch diameter Lexan™ tube. A sampling pattern was designed to permit access to samples in three concentric circles of 7, 11, and 15 inch radii about each port center at the bottom of the sediment. This sampling pattern enabled us to take 38 core samples from each port while allowing 2 inches of space in all directions between adjacent samples. The cores were frozen, then cut into top, middle, and bottom sections, typically six to seven inches each. All sediment samples were kept frozen until they were analyzed.

We used two methods of extraction for our samples. Wet sediment samples were extracted for 20 to 24 h with 2,2,4-trimethylpentane using a Soxhlet extractor fitted with a Dean Stark trap. Following a rigorous cleanup these extracts were used for determination of PCB concentration, for monitoring dehalogenation of the 2,6-BB, and for congener-specific GC/MS analysis. In addition, congener-specific GC/ECD analysis was done using samples extracted by a one-step ether procedure. There were no significant differences in the congener distributions obtained by the two extraction methods.

Two to four samples from each level of sediment at each time point were analyzed. The dehalogenation of 2,6-BB to 2-BB and biphenyl was monitored by GC/MS in the selected ion mode. Congener-specific GC/MS and GC/ECD analyses were done using a 30 m DB-1 poly(dimethylsiloxane) capillary column. Previous experiments had demonstrated that many of the PCB congeners produced by dechlorination of Aroclor 1260 are either not present in Aroclors or are present in only minor quantities. In addition, some of these congeners coelute with less chlorinated PCB congeners that are more prominent in the Aroclors.

Hence it was apparent that we could not accurately quantify the dechlorinated PCBs using any single Aroclor or any mixture of Aroclors as a standard. We therefore developed a quantitative standard that included Aroclor 1260 plus known amounts of 38 congeners that we had identified in previous experiments as dechlorination products of Aroclor 1260. Weight percent values for congeners in Aroclor 1260 were determined as described [2,5]. We used a four point external calibration (219 ppb to 3509 ppb) with a quadratic fit forced through zero to quantify the PCBs in each sample, then calculated for each sample the mole percent amount for each individual peak, the *ortho, meta, para,* and total chlorines per biphenyl, and the PCB homolog distribution.

RESULTS AND DISCUSSION

The PCB concentration was 26.0 ppm (sediment dry weight) in the experimental caisson and 30.6 ppm in the control caisson and did not change throughout the experiment despite extensive dechlorination in the experimental caisson. The concentration of 2,6-BB at the beginning of the experiment was 880 ppm (sediment dry weight).

During the summer months the average air temperature was 17.6°C and the sediment temperature ranged from 15 to 17°C at the bottom to 16 to 19°C at the top. During the last three weeks of September the sediment temperatures dropped precipitously, then continued to drop slowly until February, reaching temperatures as low as 1°C for the uppermost sediments and 3°C for the bottom sediments before warming again in April. The pH of the sediments in the caissons fluctuated between 7.0 and 7.6.

Methane was detected two days after the amendments were made, and throughout the experiment. Dehalogenation of 2,6-BB to 2-BB and then biphenyl began within two weeks, and by 78 to 115 days 98 to 99% of the 2,6-dibromobiphenyl was converted to biphenyl at all sediment levels.

The PCBs in the control caisson showed no change in 373 days (Figure 1). In contrast, the PCBs in the experimental caisson were extensively dechlorinated and showed respective decreases of 70, 57, and 27% in the hexa-, hepta-, and octachlorobiphenyls in the top six inches of sediment after only 93 days (Figure 1). The hexa- through nonachlorobiphenyls in the top sediment layer decreased from an initial value of 68 mole percent of the total PCBs to 26 mole percent at day 93, 22 mole percent at day 212, and 17.6 mole percent at day 373. At the same time the tri- and tetrachlorobiphenyls increased from 17.6 to 63.1 mole percent. Despite dechlorination of most of the pentachlorobiphenyls that were originally present, the total amount of pentachlorobiphenyls increased from 14.9 to 19.2 mole percent due to the formation of specific pentachlorobiphenyls from the dechlorination of higher congeners.

The dechlorination progressed more slowly in the colder lower sediments, but continued longer into the cold months, probably because during the winter the bottom sediments were warmer than those at the top. By 373 days the hexa- through nonachlorobiphenyls had decreased by 74% in the top of the sediment and 69% in the bottom. The average number of chlorines per biphenyl dropped 21%, from 5.83 to 4.61, and the number of *meta* chlorines per biphenyl dropped 54%, from 2.14 to 0.99. The number of *para* chlorines per biphenyl dropped only 6%, from 1.31 to 1.23, and the number of *ortho* chlorines per biphenyl was constant at 2.39.

The dechlorination exhibited, Process N, was highly specific for the removal of chlorines from the *meta* position, but only those located adjacent to other chlorines. Tables 1 and 2
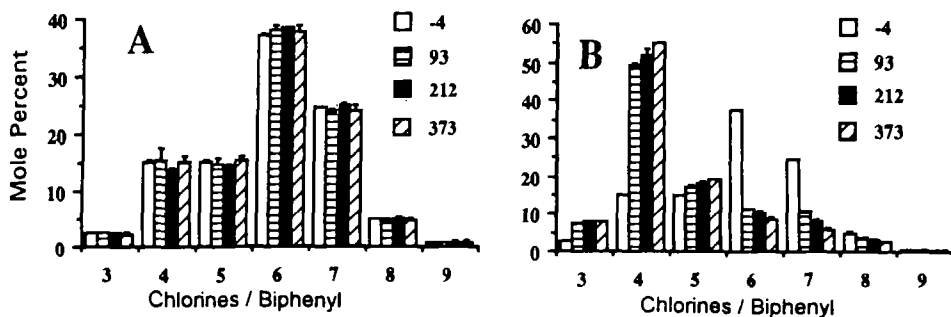
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Figure 1. PCB homolog distribution in the control (Panel A) and experimental (Panel B) caissons at - 4, 93, 212, and 373 days.

show the changes in key PCB congeners as a result of dechlorination. Many of the hexa- and heptachlorobiphenyls were dechlorinated to 2,4,2',4'-tetrachlorobiphenyl (24-24-CB), which increased by 18.71 mole percent. Other major products included 24-26-CB,24-25-CB, and 25-26-CB. A quantitative *mother-daughter* analysis of all major PCB congeners and their proposed dechlorination products yielded mass balances ranging from 95 to 119%, with an average mass balance of 104%. These results attest to the accuracy of our quantitation and interpretation of the dechlorination.

The dechlorination had a significant impact on the bioaccumulation potential of the sediment PCBs by *converting many* of the congeners reported to be most persistent in humans [7] to less persistent forms. We have used reported values for relative metabolic rates and human accumulations for all resolvable PCB components [7] to assess the relative persistence in humans of the sediment PCBs before and after the dechlorination stimulated by 2,6-BB. The PCB components with 10 to 100 year half-lives in humans decreased by 73%, from 33.2 to 9.0 mole percent, and those with 1 to 10 year half-lives decreased by 56%, from 14.6 to 6.4 mole percent. After the dechlorination 84.6% of the PCBs had half-lives in humans of less than 1 year.

We expect that the dechlorination also decreased any "dioxin-like" toxicity associated with the PCBs. "Dioxin-like" activity is attributed to only a few "coplanar" PCB congeners that are approximate stereoisomers of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin because they have three requisite structural features [8,9]: 1) no more than one *ortho* chlorine, 2) two *para* chlorines, and 3) at least two *meta* chlorines. Process N *dechlorination* removes these congeners by removing their *meta* chlorines.

The dechlorination has also made the PCBs more degradable. Aroclor 1260 is not degradable by aerobic bacteria because the high degree of chlorine substitution precludes or greatly hinders the initial ring oxidation by biphenyl dioxygenases. However, many of the dechlorinated PCBs including the major product, 24-24-CB, can be at least partially degraded by aerobic bacteria. We are currently studying ways to stimulate a second stage of dechlorination that will remove *para* chlorines thereby making the PCBs even more susceptible to microbial degradation.

We have demonstrated that it is possible to stimulate substantial microbial dechlorination of highly chlorinated PCBs *in situ* with a single addition of a chemical that is degraded to

Table 1. Dechlorination of Key Congeners in the Sediment PCBs

PCB Congener	Mole Percent			Percent Decrease	Proposed Dechlorination Pathway			
	Day -4	Day 373	Net Decrease					
236-25	2.00	0.00	2.00	100	→ 25-26			
245-24	1.85	0.34	1.51	82	→ 24-24			
236-34	2.76	0.51	2.25	81	→ 26-34	→ 26-4		
2356-25*	2.05	0.59	1.46	71	→ 236-25	→ 26-25		
236-245*	6.68	0.50	6.18	93	→ 236-24	→ 24-26		
245-34	1.47	0.11	1.36		→ 24-34	→ 24-4		
235-245	1.49	0.36	1.13	76	→ 235-24			
234-236	2.29	0.11	2.18	95	→ 236-24	→ 24-26		
245-245	8.51	0.78	7.73	91	→ 245-24	→ 24-24		
2345-25	1.28	0.00	1.28	100	→ 234-25	→ 24-25		
2356-236	1.11	0.61	0.50	45	→ 2356-26	→ 236-26	→ 26-26	
234-245*	5.73	1.14	4.59	80	→ 245-24	→ 24-24		
2356-34*	1.91	0.38	1.53	80	→ 2356-4	→ 236-4	→ 26-4	
2356-245	3.44	0.89	2.55	74	→ 2356-24	→ 236-24	→ 24-26	
2346-245	1.66	0.37	1.29	78	→ 245-246	→ 246-24		
2345-236	2.61	0.39	2.22	85	→ 236-245	→ 236-24	→ 24-26	
2356-234	1.61	0.26	1.35	84	→ 2356-24	→ 236-24	→ 24-26	
2345-34*	0.48	0.10	0.38	79	→ 245-34	→ 24-34	→ 24-4	
2346-234*	0.73	0.15	0.58	79	→ 234-246	→ 246-24		
2345-245	7.40	1.77	5.63	76	→ 245-245	→ 245-24	→ 24-24	
					→ 235-245	→ 235-24		
2345-234	2.70	0.57	2.13	79	→ 234-245	→ 245-24	→ 24-24	
2345-2356	1.18	0.52	0.66	56	→ 2356-245	→ 2356-24	→ 236-24	→ 24-26
2345-2346	0.48	0.23	0.25	52	→ 2345-246	→ 235-246		
23456-245	0.95	0.45	0.50	52	→ 2346-245	→ 245-246	→ 246-24	
					→ 2356-245	→ 2356-24	→ 236-24	→ 24-26
2345-2345	1.27	0.68	0.59	46	→ 2345-245	→ 245-245	→ 245-24	→ 24-24
					→ 2345-235	→ 245-235	→ 235-24	

Asterisks indicate pairs of coeluting congeners; the proportions of each congener were estimated.

Table 2. PCB Congeners Formed by Dechlorination

PCB Congener	Mole Percent		
	Day -4	Day 373	Net Increase
24-4	0.82	3.07	2.25
26-4	0.41	2.9	2.49
24-24	3.44	22.16	18.72
24-25	3.18	7.79	4.71
24-26	1.42	12.57	11.15
25-26	1.26	5.32	4.06
235-24*	0.35	4.63	4.28
236-24	1.36	3.61	2.25
246-24	0.42	3.54	3.12
2356-24	0.74	2.87	2.13

* This congener coelutes with a pentachlorobiphenyl that is dechlorinated. Amounts are based on calculations.

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innocuous products and without temperature control. Our results indicate that a single chemical intervention can have a long-term beneficial effect on PCB dechlorination by indigenous microorganisms even in a cold New England climate.

ACKNOWLEDGMENTS

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