China **2**, 57 (1977).
9. J. Byerlee, *Pure Appl. Geophys.* **116**, 615 (1977).
10. K. Hoshino, H. Koide, K. Inami, S. Iwamura, S. Mitsui, *Geol. Surv. Jpn. Rep.* **244**, 1 (1972).
11. J. Suppe and J. H. Wittke, *Petrol. Geol. Taiwan* **14**, 11 (1977).
12. E. R. Oxburgh, in *Physics of Magmatic Processes*, R. B. Hargraves, Ed. (Princeton Univ. Press, Princeton, NJ, 1980), pp. 161–199.
13. S. P. Clark, *Geol. Soc. Am. Mem.* **97**, 459 (1966).
14. ———, Z. E. Peterman, K. S. Heier, *ibid.*, p. 521.
15. C. R. Lee and W. T. Cheng, paper presented at the Fourth Circum-Pacific Energy and Mineral Resources Conference, Singapore, 17 to 22 August 1986.
16. T. K. Liu, *Proc. Geol. Soc. China* **25**, 22 (1982).
17. W. G. Ernst, in *Mountain Building Processes*, K. J. Hsü, Ed. (Academic Press, London, 1982), pp. 247–256.
18. J. G. Liou, *Mem. Geol. Soc. China* **4**, 551 (1981).
19. *The Europa Yearbook* (Europa, London, 1986), p. 744.
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Reductive Dechlorination of Polychlorinated Biphenyls by Anaerobic Microorganisms from Sediments

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Microorganisms from Hudson River sediments reductively dechlorinated most polychlorinated biphenyls (PCBs) in Aroclor 1242 under anaerobic conditions, thus demonstrating PCB dechlorination by anaerobic bacteria in the laboratory. The most rapid dechlorination was observed at the highest PCB concentration used; at 700 parts per million Aroclor, 53 percent of the total chlorine was removed in 16 weeks, and the proportion of mono- and dichlorobiphenyls increased from 9 to 88 percent. Dechlorination occurred primarily from the meta and para positions; congeners that were substituted only in the ortho position (or positions) accumulated. These dechlorination products are both less toxic and more readily degraded by aerobic bacteria. These results indicate that reductive dechlorination may be an important environmental fate of PCBs, and suggest that a sequential anaerobic-aerobic biological treatment system for PCBs may be feasible.

PRIOR TO THE 1970s, PCBs WERE widely used for a variety of industrial purposes, including fluid-filled capacitors and transformers, hydraulic fluids, heat transfer fluids, plasticizers, and carbonless copy paper. The commercial mixtures (called Aroclors in the United States) used were complex mixtures of many homologs and isomers (congeners). In general, PCBs are considered to be highly persistent in natural environments such as soils and sediments. Their biological degradation under aerobic conditions is generally limited to the congeners with five or fewer chlorines and at least two adjacent unsubstituted carbon atoms (*1–3*). Recently, however, the altered PCB congener distribution patterns found in anaerobic sediment samples from the upper Hudson River have been interpreted to be the result of biologically mediated reductive dechlorination (*4, 5*).

Although the main PCB input to the upper Hudson River between 1951 and 1973 is reported to have been Aroclor 1242 (*4, 5*), subsurface sediment samples now show depletion of the tri- and higher chlorinated congeners present in Aroclor 1242 and a corresponding increase in the proportion of mono- and dichlorobiphenyls substituted only in the ortho position or positions (*4, 5*). Similar but generally less pronounced differences between known PCB inputs and analyzed sediment samples taken several years later have also been observed for Waukegan Harbor (Illinois) (*6*), and Silver Lake (*5*) and the Acushnet River (both in Massachusetts) (*7*). In all of these cases a biological process was presumed to be responsible for the difference in congener distribution patterns, because congener selectivity was observed and a strictly abiologic reduction of PCBs has not been demonstrated under the conditions found in anaerobic sediments (*8*). Attempts to chemically dechlorinate aromatic compounds under similar conditions with reduced iron porphyrins have not succeeded (*9*). However, other workers have suggested that the observed enrichment of mono- and dichlorobiphenyls in the Hudson River sediments is the result of selective partitioning (*10*). The controversy over the mechanism responsible for the observed congener profiles in the upper Hudson River led to a recent exchange of letters in *Science* (*11*). Thus laboratory experiments to directly demonstrate biological dechlorination of PCBs are important to clarify the mechanism. We report the successful demonstration of biologically mediated reductive dechlorination of an Aroclor mixture.

We assessed the ability of microorganisms from PCB-contaminated Hudson River sediments (60 to 562 ppm PCBs) (*12*) to dechlorinate Aroclor 1242 under anaerobic conditions by eluting microorganisms from the PCB-contaminated sediments (*13*) and transferring them to a slurry of reduced anaerobic mineral medium (*14*) and PCB-free sediments (*15*) in tightly stoppered serum bottles (*16*). This procedure reduced the PCB background, enabling us to quantify the dechlorination of freshly added Aroclor 1242. Three concentrations of Aroclor were used corresponding to 14, 140, and 700 ppm on a sediment dry-weight basis.

Dechlorination of the Aroclor by the eluted organisms was evident from a simple visual inspection of the chromatograms of PCBs extracted after 16 weeks of incubation (Fig. 1). Early eluting peaks, corresponding to the lesser chlorinated congeners, increased with time in the biologically active (live) treatments but not in the autoclaved controls. There was a corresponding decrease in the later eluting, more highly chlorinated congeners. Most notable was

Table 1. Changes in PCB homolog distribution over time for the 700-ppm live treatment. Values (in mole percent of the PCBs recovered) are the means of two replicates ± the standard deviation of the mean.

Congeners	Distribution of homologs (mol %) for week			
	0	4	8	16
Mono-	0.0 ± 0.0	1.7 ± 0.5	50.1 ± 10.7	66.7 ± 3.4
Di-	9.1 ± 0.7	15.3 ± 0.2	25.5 ± 3.7	21.3 ± 0.6
Tri-	48.5 ± 0.1	48.2 ± 1.3	16.2 ± 4.9	8.5 ± 2.3
Tetra-	36.3 ± 0.6	30.0 ± 0.9	6.8 ± 1.8	3.0 ± 0.4
Penta-	5.2 ± 0.2	4.2 ± 0.2	1.3 ± 0.4	0.5 ± 0.1
Hexa-	0.9 ± 0.0	0.6 ± 0.0	0.2 ± 0.1	0.0 ± 0.0

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the accumulation of chlorobiphenyls substituted only at the ortho positions. The 2-chlorobiphenyl (2-CB) concentration increased from 0 to 63% (*17*) of the total PCBs in the biologically active treatments that received 700 ppm Aroclor, and 2,2′-CB, 2,6-CB, or both (coeluting isomers) increased from 1 to 14%. The concentration of 2,2′,6-CB also increased from 0.4 to 2%.

The progressive nature of the dechlorination process was evident from changes in the relative proportions of mono-, di-, tri-, tetra-, penta-, and hexachlorobiphenyls at each sampling time (Table 1). Aroclor 1242 contains predominantly tri- and tetrachlorobiphenyls; these were progressively dechlorinated to di- and monochlorobiphenyls in the biologically active treatments that received 140 and 700 ppm Aroclor, whereas there was no appreciable change over time in the autoclaved controls.

PCB dechlorination in the biologically active treatments occurred primarily from the meta and para positions with no significant loss of chlorines in the autoclaved controls (Fig. 2). Dechlorination was most extensive at the highest PCB concentration. In the 700-ppm treatment, the average number of meta plus para chlorines per biphenyl decreased from an average of 1.98 to 0.31 after 16 weeks, but decreased to only 1.19 in the 140-ppm treatment (*18*). At 14 ppm there was no observable difference between the live samples and autoclaved controls after 16 weeks. The dependence of dechlorination on PCB concentration may be related to PCB bioavailability. Higher concentrations in the sediment would result in higher solution concentrations according to partition equilibria between these two phases (*19*), and it is probably only the PCBs in solution that are available for uptake by the dechlorinating microorganisms (*20*). Meta and para chlorines were estimated together because it was not always possible to distinguish the two classes when coeluting isomers were involved. Dechlorination of both positions occurred because meta and para chlorines occur in nearly equal proportions (5:4 ratio) in Aroclor 1242. The observed decrease in meta plus para chlorines from

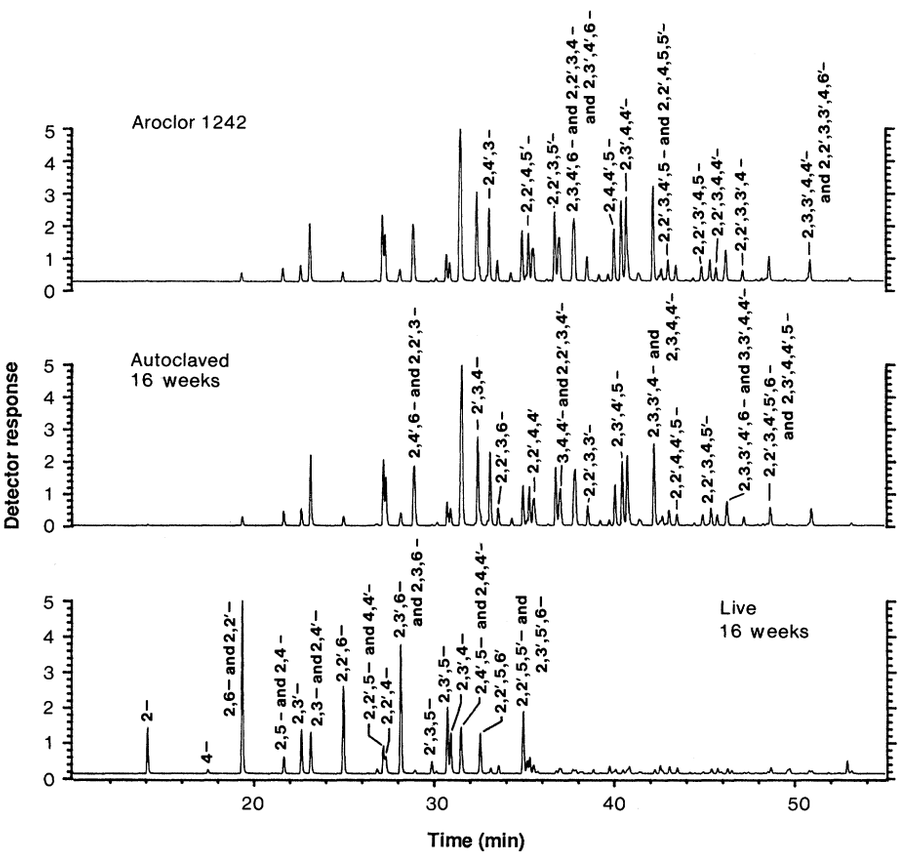

Fig. 1. Capillary gas chromatograms showing the anaerobic dechlorination of Aroclor 1242 by Hudson River microorganisms. All chromatograms were normalized so that the highest peak had a height of 5. An electron capture detector was used.

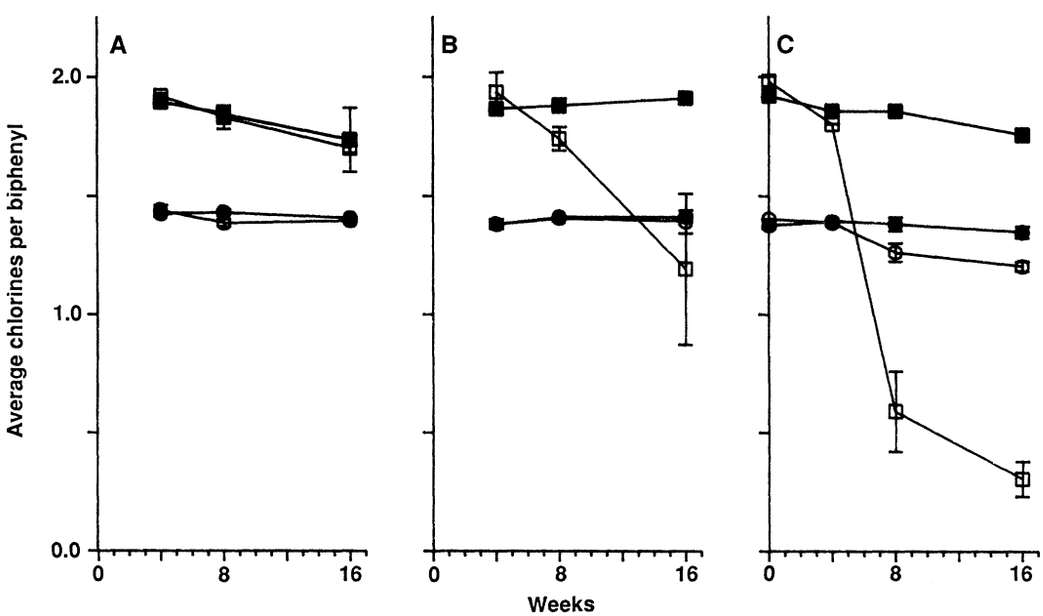

Fig. 2. Removal of chlorines by position at three Aroclor 1242 concentrations. Dechlorination was almost exclusively from the meta and para positions in the biologically active (live) treatments only. Zero time data for the 14- and 140-ppm treatments were not reliable because an insufficient amount of material was sampled, and it is not plotted. The vertical bars represent ± one standard deviation of the mean based on two independent determinations. Aroclor 1242 is at the following concentrations: (**A**) 14 ppm; (**B**) 140 ppm; and (**C**) 700 ppm. ○, Live, ortho chlorines; □, live, meta + para chlorines; ●, autoclaved, ortho chlorines; and ■, autoclaved, meta + para chlorines.

1.98 to 0.31 cannot be accounted for unless dechlorination occurred at both positions.

The experiment described above has also been performed with microorganisms from an Aroclor 1242–free site in the Hudson River (*15*). No dechlorination of 700-ppm Aroclor 1242 was observed in 16 weeks. Perhaps the long-term exposure (>15 years) to high PCB concentrations at the PCB-contaminated site selected for this dechlorinating activity. A deficiency of electron acceptors limits microbial growth in most anaerobic environments. Thus any microorganisms that could use PCBs as terminal electron acceptors would have an advantage in anaerobic sediments (*5*); they may obtain energy from the dechlorination step itself. By analogy, the 3-chlorobenzoate–dechlorinating strain DCB-1 may derive energy from the exergonic aryl dechlorination ($\Delta G' = -112$ kJ/mol) as the growth yield for the consortium is higher when grown on chlorobenzoate than on benzoate (*21*).

The PCB-contaminated Hudson River sediment used in this experiment was collected from below Fort Edward, New York. The PCB congener profile observed in our laboratory experiment is similar to the pattern C profile previously described for environmental samples from this region of the river (*4*). Thus the indigenous sediment organisms that were responsible for the dechlorination products found in the laboratory probably also accounted for dechlorination of PCBs in situ.

The removal of meta and para chlorines, although not decreasing the molar concentration of PCBs, can be expected to decrease the mammalian toxicity of the PCB residues and make them more readily degradable by aerobic bacteria. The PCBs with the greatest dioxin-like toxicity are those with at least one meta and a para chlorine on each ring and no more than one ortho chlorine (for example, 3,3′,4,4′-CB, 2,3,3′,4,4,′-CB, or 2,3′,4,4′,5-CB) (*22*). Removal of the meta and para chlorines from these congeners should eliminate their toxicity. Because of the potential of this process for detoxication, an assessment of PCB dechlorination should be made on a case-by-case basis before deciding on the appropriate remedial action for PCB-contaminated sediments.

The most commonly known initial step in the aerobic degradation of PCBs is catalyzed by a dioxygenase; this requires two adjacent unsubstituted carbons [at either the ortho and meta positions (2,3-dioxygenase) (*3*) or the meta and para positions (3,4-dioxygenase) (*1*)]. Removal of the meta and para chlorines should make any ring with no more than one ortho chlorine subject to 2,3-dioxygenase attack, and any biphenyl ring subject to 3,4-dioxygenase attack. Whereas it is true that congeners with two ortho chlorines on the same ring are degraded more slowly by the 3,4-dioxygenase system, the aerobic degradation of all PCB congeners accumulating from the dechlorination of Aroclor 1242 in this experiment has been demonstrated by one or more strains of bacteria (*2*). Hence it is likely that most PCBs can be biodegraded by a suitable sequential anaerobic-aerobic process.

REFERENCES AND NOTES

1. D. L. Bedard, M. L. Haberl, R. J. May, M. J. Brennan, *Appl. Environ. Microbiol.* **53**, 1103 (1987).
2. D. L. Bedard, R. E. Wagner, M. J. Brennan, M. L. Haberl, J. F. Brown, Jr., *ibid.*, p. 1094.
3. K. Furukawa, N. Tomizuka, A. Kamibayashi, *ibid.* **38**, 301 (1979).
4. J. F. Brown, Jr., *et al.*, *Northeast. Environ. Sci.* **3**, 167 (1984).
5. J. F. Brown, Jr., *et al.*, *Environ. Toxicol. Chem.* **6**, 579 (1987); J. F. Brown, Jr., *et al.*, *Science* **236**, 709 (1987).
6. D. L. Stalling, *Isomer Specific Composition of PCB Residues in Fish and Sediment from Waukegan Harbour and Other Great Lakes Fish* (Columbia National Fisheries Research Laboratory, Columbia, MO, 1982).
7. J. F. Brown, Jr., and R. E. Wagner, paper presented at the Eighth Annual Meeting of the Society of Environmental Toxicology and Chemistry, Pensacola, FL (November 1987).
8. F. A. Beland, S. O. Farwell, A. E. Robocker, R. D. Geer, *J. Agric. Food Chem.* **24**, 753 (1976).
9. R. S. Wade, R. Havlin, C. E. Castro, *J. Am. Chem. Soc.* **91**, 7530 (1969); G. M. Klecka and S. J. Gonsior, *Chemosphere* **13**, 391 (1984).
10. B. Bush, L. A. Shane, M. Wahlen, M. P. Brown, *Chemosphere* **16**, 733 (1987).
11. M. P. Brown, B. Bush, G. Y. Rhee, L. Shane, *Science* **240**, 1674 (1988); J. F. Brown, Jr., R. E. Wagner, D. L. Bedard, *ibid.* **240**, 1676 (1988).
12. PCB-contaminated sediments were collected from near the west bank of the Hudson River at River Mile 193.3. This corresponds to site H7 in (*4*).
13. We have not been successful at isolating the PCB-dechlorinating members of the consortium by conventional enrichment procedures. This is consistent with the difficulty of obtaining dechlorinating isolates on other halogenated compounds. The difficulty arises in part because the halogenated compounds are usually not used as carbon sources by the dehalogenating microorganisms.
14. D. R. Shelton and J. M. Tiedje, *Appl. Environ. Microbiol.* **47**, 850 (1984).
15. Sediments free of Aroclor 1242 residues (PCB-free) were collected upstream from the site in (*12*) at River Mile 215. Aroclor 1260–like residues were detected (1 to 2 ppm), but these did not interfere with the analysis of the Aroclor 1242 added or its dechlorination products.
16. Sieved, air-dried sediment from a PCB-free Hudson River site (*15*) was added to each of 12 160-ml serum bottles. Reduced anaerobic mineral medium (RAMM) (*13*) and 50 μl of ethanol were added while flushing with filter-sterilized O_2-free N_2:CO_2 (80:20, v/v), and the bottles were sealed. Microbial oxidation of the ethanol depleted any residual oxygen in the bottles. The bottles were incubated until methane production was detected to indicate that anaerobic conditions were established. All bottles were then autoclaved. The microorganisms were eluted from PCB-contaminated Hudson River sediment (*12*) by shaking a slurry of equal volumes of sediment and RAMM and then allowing the slurry to settle for 15 min. Supernatant (50 ml) from this slurry was used to inoculate each serum bottle. After inoculation, 6 of the 12 bottles, which served as controls, were autoclaved twice with a 3-day interval between. Aroclor 1242 in 100 μl of acetone was added in three different amounts (0.7, 7, and 35 mg per bottle) resulting in two biologically active and two autoclaved bottles at each concentration. These amounts of Aroclor correspond to concentrations of 14, 140, and 700 μg/g (ppm) on a sediment dry-weight basis. The bottles were sealed with Teflon-lined stoppers after the Aroclor additions, and the bottles were shaken for 30 min after the PCB addition and for 10 min prior to each sampling event. Incubation was in the dark at 25°C. Samples (approximately 2 ml of slurry) were removed with sterile pipettes while flushing with filter-sterilized O_2-free N_2:CO_2 (80:20, v/v), and bottles were resealed after sampling. The samples were frozen until extracted.

    We extracted the samples by shaking once with 10 ml of acetone (to remove water) and twice with hexane:acetone (9:1, v/v). The acetone was extracted with 2% NaCl in distilled water, and the hexane extract was cleaned with mercury and sulfuric acid (to remove sulfur) and a miniature Florisil column eluted with hexane (to remove polar compounds). The samples were then analyzed on a capillary gas chromatograph equipped with a Hewlett-Packard HP-5 capillary column (25 m, 0.2-mm inside diameter, 0.11-μm film thickness) and an electron capture detector. The HP-5 liquid phase is 5% diphenyl and 95% dimethyl polysiloxane and is the equivalent of SE-54 or DB-5. The chromatographic conditions were as follows: split injection (20:1 ratio), inlet 220°C, detector 300°C, oven 100° to 240°C at 2°C per minute, and an He linear flow rate of 28 cm/s.

    Peak identifications were based on matching retention times to standards (2-CB and 4-CB) or published relative retention indices [M. D. Mullin *et al.*, *Environ. Sci. Technol.* **18**, 468 (1984)] for the other Aroclor 1242 peaks. Quantitation of 2-CB and 4-CB was based on pure standards. Quantitation of other congeners was based on data on the composition of Aroclor 1242 provided by J. F. Brown, Jr., and R. E. Wagner [see (*4*) for a discussion of their method].

    The percentage of the total PCBs (on a molar basis) represented by each of 60 resolvable peaks was calculated, and the data were summarized by homolog class (mono, di, and so forth), and the average number of ortho versus meta plus para chlorines per biphenyl. Several simplifying assumptions were made in performing these calculations. For peaks with coeluting congeners with different numbers of chlorines, the proportion of each was estimated from the average molecular weight for that peak as determined by mass spectroscopy and provided by R. E. Wagner and J. F. Brown, Jr. For peaks with coeluting isomers it was assumed that they occurred in equal proportions. It was further assumed that all of the PCBs in the same peak increased or decreased to the same extent as a result of the dechlorination.
17. The remaining 4% monochlorobiphenyl reported in Table 1 was 4-chlorobiphenyl.
18. There is some variation in the estimate of the number of chlorines per biphenyl introduced by the extraction and cleanup procedure. The number of meta plus para chlorines per biphenyl for Aroclor 1242 standards was 1.81 to 1.83, but varied between 1.73 and 1.93 for autoclaved controls at 700-ppm Aroclor 1242.
19. C. T. Chou, L. J. Peters, V. H. Freed, *Science* **206**, 831 (1979).
20. Two compartment models of this type have been shown to explain 2,4-D degradation rates in sediments; see A. V. Ogram, R. E. Jessup, L. T. Ou, P. S. C. Rao, *Appl. Environ. Microbiol.* **49**, 582 (1985).
21. J. Dolfing and J. M. Tiedje, *Arch. Microbiol.* **149**, 102 (1987).
22. S. Safe, L. W. Robertson, L. Safe, *Can. J. Physiol. Pharmacol.* **60**, 1057 (1982).

23. We thank R. Unterman and J. F. Brown, Jr., for providing the Hudson River sediments and R. E. Wagner and J. F. Brown, Jr., for providing the congener-specific analysis of Aroclor 1242 on which our calibration method is based. This research was supported by grants from General Electric Company and the State of Michigan Research Excellence Fund. Published as Journal Article No. 12754 of the Michigan Agricultural Experiment Station and of the Center for Environmental Toxicology.

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