02-0200

FIRST QUARTERLY STATUS REPORT

HOUSATONIC RIVER
VELOCITY & SEDIMENTATION CONTROL
PILOT STUDY

GENERAL ELECTRIC COMPANY
PITTSFIELD, MASSACHUSETTS

AUGUST 1988

BLASLAND & BOUCK ENGINEERS, P.C.
5793 WIDEWATERS PARKWAY
SYRACUSE, NEW YORK 13214

TABLE OF CONTENTS

		Page
SECTION 1 - BACKGROUND		
1.1	Objective of This Report	1-1
1.2	General Description of the Pilot Study	1-2
1.3	Background Information	1-3
SECTION 2 - VELOCITY & SEDIMENTATION MONITORING PROGRAM		
2.1	Initial Baseline Monitoring Program	2-1
2.2	Results of Initial Monitoring Activities	2-2
SECTION 3 - ANALYSIS OF WOODS POND SEDIMENT		
3.1	Description of Sampling Program	3-1
3.2	Selective PCB Resampling Analysis	3-2
3.3	PCB Biodegradation Evaluation	3-5
SECTION 4 - CONCLUSIONS AND RECOMMENDATIONS		
4.1	Status of Pilot Study	4-1
4.2	Preliminary Conclusions	4-1
4.3	Future Activities	4-3
REFERENCES		
TABLES		
1-1	Pilot Study Schedule	
1-2	Housatonic River Project Chronology	
2-1	Sediment Collection Summary - October 1987	
3-1	Comparative Sediment Resampling Program	
3-2	Cesium 137 Analysis	
FIGURES		
1	Site Plan	
2	October 1987 Velocity Monitoring Results	
3	Housatonic River Sediment Cores - Location Plan	
4	Housatonic River Sediment Data - Core 18KI-2	
5	Housatonic River Sediment Data - Core 18H-3	
6	Housatonic River Sediment Data - Core 17E-15	
7	Housatonic River Sediment Data - Core 17E-11	
8	Housatonic River Sediment Data - Depositional Patterns	

TABLE OF CONTENTS

(Cont'd.)

APPENDICES

A. Letter from General Electric Corporate Research and Development; Re: Sediment Dechlorination.
B. Annual Status Report; GE Research and Development Program for the Destruction of PCBs; June 1988

SECTION 1 - INTRODUCTION

1.1 Objective of This Report

The objective of this Report is to demonstrate compliance by the General Electric Company with the requirements of the United States Environmental Protection Agency (USEPA) and the Massachusetts Department of Environmental Quality Engineering (DEQE) regarding the Housatonic River Velocity and Sedimentation Control Pilot Study. Specifically, this report responds to approval letters from the USEPA and DEQE dated October 23, 1987, and October 26, 1987, respectively (References 1 and 2). These approval letters were in response to a proposed scope of study entitled, "Housatonic River; Velocity and Sedimentation Control Pilot Study, Work Plan; June 1987 (Reference 3) as prepared by Blasland & Bouck Engineers, P.C. This Work Plan had been provided to the USEPA and the DEQE by James Thayer, P.E. of the General Electric Company on July 15, 1987.

The Pilot Study Work Plan was designed to assess whether modifications to the Woods Pond Dam and Raceway channel would reduce sediment transport beyond the dam without significantly impacting sedimentation rates in Woods Pond itself. Concurrently, research regarding PCB biodegradation is also being examined to assess whether this phenomenon can be considered for remediations of the PCB-containing sediments.

This document represents the first of a series of quarterly status reports related to the Pilot Study activities which will be prepared and submitted to the USEPA and DEQE. This report also includes a presentation of related activities associated with the Housatonic River Program including the results of ancillary sampling and analysis activities in the Woods Pond

area; and an updated presentation of information regarding PCB-biodegradation as it pertains to this program.

1.2 General Deposition of the Pilot Study

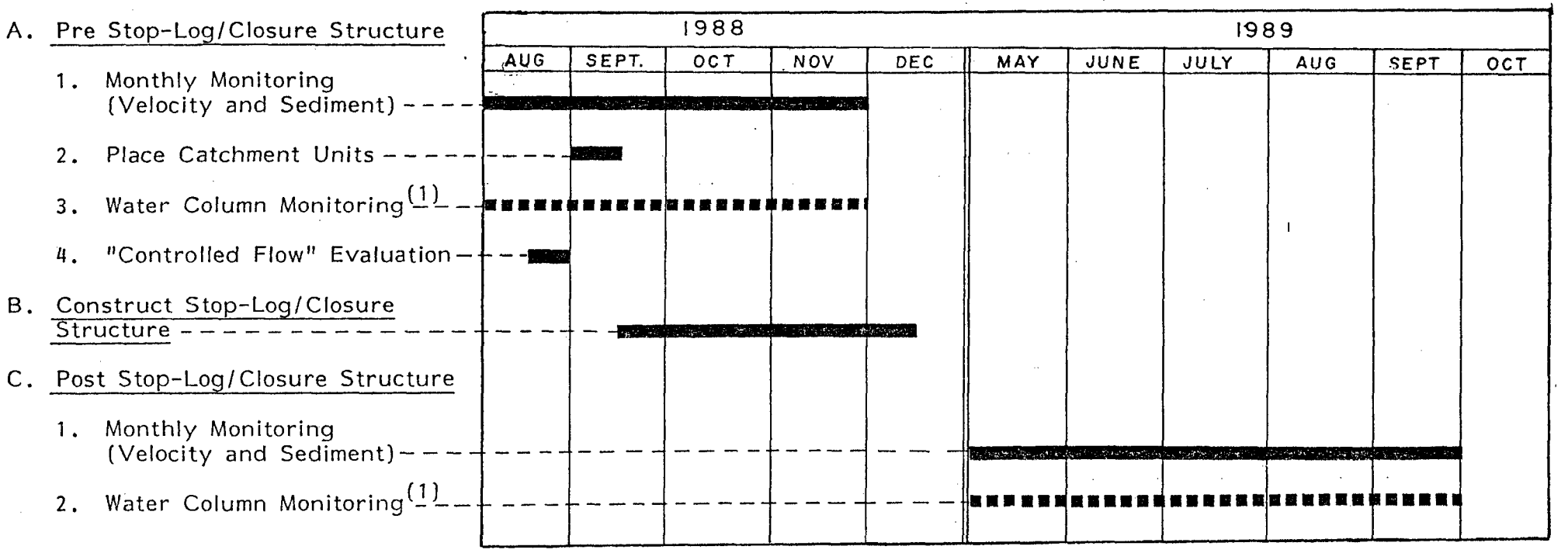
The approved "Housatonic River Velocity and Sedimentation Control Pilot Study" Work Plan presents the methodologies to be utilized to determine the deposition of suspended sediments in Woods Pond and the Woods Pond Dam raceway channel under a range of river flow conditions, and also to determine the influence of the stop-log closure structure installation on this process. In accordance with the Work Plan, this will be accomplished by performing velocity, sedimentation and water column monitoring at various locations in the river channel and raceway.

Velocity versus depth profiles have been determined at six cross sections, two located in the raceway and four in the river channel. Sediment and sedimentation is to be monitored by collecting core samples at the quarter points of each of the cross sections, and installing sediment catchment devices in each of the cross sections. Sediment samples are to be described through analyses of particle size, settleabiltiy, scour velocity and polychlorinated biphenyl concentration. Water quality samples are to be collected at a point downgradient of the sluice gates. The velocity and water column monitoring will also be performed after the stop-logs have been installed, with the stop-logs opened and the sluice gates at two settings, opened and closed and with the sluice gates opened and the stop-logs at two settings, opened and closed. Water column monitoring is proposed at high river discharge flows in excess of 700 cubic feet per second. The schedule for completion of the Pilot Study is shown in Table 1-1.

TABLE 1-1

HOUSATONIC RIVER
VELOCITY & SEDIMENTATION CONTROL
PILOT STUDY

PILOT STUDY SCHEDULE



(1) Water Column Monitoring to be conducted in accordance with the June 1987 Pilot Study Work Plan sediment monitoring (when river flow exceeds 700 cfs).

AUGUST 1988
101.15.01

1.3 Background Information

The Pilot Study Work Plan represents a continuation in the series of efforts which describe, define and address concerns associated with PCB-containing sediments in the Housatonic River. Table 1-2 presents a chronology of events associated with the Housatonic River Program beginning with the signing of a Consent Decree between General Electric, the DEQE and the EPA on May 25, 1981. As indicated by this chronology, a series of studies and reports was prepared by numerous parties which led to the current project status. A summary of the more significant activities is presented below.

In 1982, Stewart Laboratories, Inc. issued a report entitled, "Housatonic River Study" (Reference 4). This Report (the "Stewart" Report) provided a comprehensive survey of the occurrence, distribution and transport of PCBs in the bottom sediments of the Housatonic River. In addition to documenting the presence of PCBs in the river system, the Stewart Report served to provide solid baseline data from which further studies and/or additional work efforts could be defined.

Following issuance of the Stewart Report, General Electric initiated a series of extensive studies to identify and assess various remedial alternatives concerning the presence of PCBs in the river sediments. In May 1985, a report entitled "135-Day Interim Report, Housatonic River Study, Assessment of Remedial Alternatives" (Reference 5), was prepared by Blasland & Bouck Engineers, P.C. and others for the General Electric Company.

The "135-day Report" refined a series of previous reports prepared by Blasland & Bouck and others which presented information regarding the various potential remedial alternatives for the Housatonic River (References 6,

TABLE 1-2

HOUSATONIC RIVER
VELOCITY & SEDIMENTATION CONTROL
PILOT STUDY

HOUSATONIC RIVER PROJECT CHRONOLOGY

Date	Event
5/25/81	GE signs Consent Order with DEQE and EPA
12/82	GE issues Housatonic River Study (by Stewart) covering 1980 and 1982 investigations
12/82	Frink Study issued documenting PCB transport and distribution in Housatonic River
2/83	Harza prepares report documenting September 1982 inspection of dam during repairs by Kimberly Clark Corporation
1984	Gay & Frimpter (USGS) issue report demonstrating no impact of Woods Pond sediments on adjacent aquifer
3/84	GE issues report entitled, "Report on Initial Screening of Housatonic River Remedial Alternatives"
4/84	Stewart Labs issues report documenting additional investigations performed during 1983
5/84	GE issues report entitled, "Report on Proposed Engineering Evaluation of Selected Housatonic River Remedial Alternatives"
10/84	GE issues 45-Day Interim Report evaluating potential sediment disposal sites
2/85	GE issues 90-Day Interim Report evaluating river channelization, in-situ impoundment, and flow and sedimentation control
4/85	GE issues Notice of Intent Permit Applications to Towns of Lee and Lenox to allow for Pilot Study activities
5/85	GE issues 135-Day Interim Report evaluating sediment removal and local disposal, river channelization, in-situ impoundment, flow and sedimentation control, and biodegradation
5/85	GE accepts bids for construction of stop log baffle system at location of existing slots in raceway channel
6/12/85	Town of Lee Conservation Commission issues Order of Conditions providing local approval of Pilot Study. Town of Lenox does not respond

Table 1-2

HOUSATONIC RIVER PROJECT CHRONOLOGY
(Cont'd.)

7/1/85	DEQE authorizes GE to proceed with Pilot Study
7/1/85	GE prepared to initiate Pilot Study, however, access issues prevent study from starting.
9/85	Berkshire County Regional Planning Commission retains Malcolm Pirine to evaluate wet dredging techniques
10/85	GE issues Revised Notice of Intent document incorporating comments from DEQE regarding possible air emissions
5/23/86	EPA and DEQE issue Conditional Approval of 135-Day Interim Report
6/86	GE responds to DEQE Conditional Approval with conceptual descriptions of monitoring plan
8/86	GE prepares contract for construction of stop log baffle system at existing sluice gate structure in raceway channel
8/86	EPA requests additional details on monitoring plan for Pilot Study
8/86	GE issues 135-Day Interim Report Addendum responding to EPA 5/23/86 comments (more details on dredging, biodegradation and resampling plan)
9/86	GE issues letter indicating schedule delay due to possible dam stability concerns, and failure to reach agreement with pipeline owners and property owner
10/86	GE retains diver to evaluate timber crib dam abutement
10/86	GE issues report which documents dam stability concerns
10/86	GE proposes relocation of stop logs to sluice gate structure
11/86	USEPA commissions ACOE to perform Dam Stability review
2/87	GE collects additional sediment cores for CS-137 dating and biodegradation assessment. Also, 4 cores obtained from areas samples in 1982 to define changes (if any)
3/87	GE transmits to EPA revised report "Velocity Control and Sediment Control Alternatives - Stop Log Baffle System Monitoring Study Overview"

Table 1-2

HOUSATONIC RIVER PROJECT CHRONOLOGY
(Cont'd.)

4/87	EPA issues PRC Report which identifies potential local sediment disposal sites
4/87	EPA issues letter outlining comments on draft monitoring plan for Pilot Study
6/87	GE issues Work Plan for Housatonic River - Velocity and Sedimentation Control Pilot Study
9/87	ACOE issues Woods Ponds Dam, Phase I Inspection Report
10/23/87	USEPA authorizes GE to proceed with Pilot Study Baseline activities
10/87	GE collects preliminary baseline data for Velocity and Sedimentation Pilot Study
6/88	GE collects second round of baseline data
8/88	First quarterly status report issued

7 and 8). The five remediation alternatives addressed in detail by these reports were as follows:

1. Sediment Removal and Local Disposal;
2. River Channelization;
3. In-situ Impoundment;
4. Flow and Sedimentation Control; and
5. Biodegradation.

In review of these alternatives, the best approach to remediation of the PCB-containing sediments appeared to be that of "containing" the sediments in place and allowing for natural or enhanced biodegradation. It was determined that reducing the transport of PCB-containing sediments past the Woods Pond Dam by enhancing sedimentation upstream of the dam would result in greater containment of these materials and an increased opportunity for biodegradation. Biodegradation should then result in reducing the quantity of PCBs present in the sediments as well as potential threat to public health and the environment from these materials.

The conclusions presented in the 135-Day Reports therefore indicated that the remedial alternatives of "Flow and Sedimentation Control" and "Biodegradation" were significant and worthy of additional evaluation. The other remediation alternatives considered (sediment removal and disposal, in-situ impoundment and river channelization) were not "discarded" but were considered as backup alternatives should the "Flow and Sedimentation Control" and/or "Biodegradation" alternatives prove inappropriate.

The 135-Day Report also concluded that to allow for further assessment of the Flow and Sedimentation Control Alternative, Pilot Study activities would be required. The report further included the scope of the Pilot Study as it was then envisioned. The stated goal of the Pilot Study was to allow

for assessment of two potential hydraulic modifications to be the Woods Pond Dam site which could be made and result in decreased sediment transport past the dam. These included reduction in the "pulling" of sediments around the dam in the existing raceway channel and the modification of the existing dam spillway to reduce the velocity of water passing over the crest of the dam. These hydraulic modifications could be studied by the placement of a new stop-log structure in the existing raceway channel with study activities before and after placement of the stop-logs .

Reducing sediment transport past the Woods Pond dam site should result in enhanced sedimentation in the Woods Pond area. This obvious goal of this remedial alternative has the potential disadvantage of accelerating the "silting in" of Woods Pond with attendant concerns over increased eutrophication. As indicated by the 135-Day Report, the rate of increased sedimentation is expected to be low such that an increase in either "silting-in" of the Pond or measurably increased eutrophication would not be expected. As confirmation, the Pilot Study includes establishing the historical sedimentation rates in Woods Pond and setting a baseline deposition rate against which changes brought about by the Flow and Sedimentation Control Alternative can be compared.

By letter of May 23, 1986, the EPA approved the 135-Day Report subject to six conditions pertaining to requests for additional information. Condition #1 specifically applied to the Pilot Study activities, and indicated, "Within thirty (30) days of receipt of this letter, General Electric shall submit to EPA for review and approval a report detailing the measures which will be taken by General Electric to mitigate adverse environmental effects attributable to the Pilot Study. In particular, this report must address the removal of sediments which may accumulate behind the stop-logs and dam as a result of

the stop-log installation. EPA will not require the implementation of the Drawdown Study portion of the Pilot Study at this time."

The requested information was subsequently supplied to the USEPA and the DEQE as a result of continued correspondence and dialogue between the parties.

At this point in the program, a schedule delay was incurred due to property ownership disputes with the dam owner and technical concerns over the stability of the embankment between the dam and the raceway channel. In parallel with resolution of these delays; the Pilot Study Work Plan was updated and revised to reflect discussions between General Electric, the USEPA and the DEQE. The revised Pilot Study Work Plan was submitted to both the USEPA and the DEQE in June of 1987 (Reference 3).

Letters dated October 23, 1987, from the USEPA (Reference 1) and October 26, 1987, from the DEQE (Reference 2) provided the necessary approval to begin initiation of the Pilot Study activities.

In parallel with the Pilot Study activities; and as required by the approval of the 135-day report, General Electric Company has continued its assessment of PCB Biodegradation as it pertains to the Housatonic River. This research is being performed independently by the GE Corporate Research and Development Group in Schenectady, New York, as well as independent researchers at various organizations and universities across the United States.

SECTION 2 - VELOCITY & SEDIMENTATION MONITORING PROGRAM

2.1 Initial Baseline Monitoring Program

In October 1987, field investigations were conducted for the area within and upgradient of the raceway channel to establish initial baseline data for the Pilot Study. A total of six river locations were selected for monitoring. Four of the river sections were located upstream of Woods Pond Dam and downstream of the point where the river channel exits Woods Pond. The remaining two locations were positioned within the length of the raceway channel. The six locations were graphically identified in the June 1987 Work Plan, and are included in this Status Report as Figure 1. The stretch of river subject to study has been historically characterized as containing minimal sediment deposition (Reference 4). By modifying the water velocities in this area, sediment deposition is expected to occur in this area and in other upstream areas.

The scope of activities encompassed by this initial phase of the Pilot Study involved the generation of river cross sections, measurement of river velocities and the collection of available sediment samples.

The calculation of flow rates and velocities for each river cross section required that accurate river bed contours be developed. Therefore, contour mapping was performed to determine actual river bed elevations across each location. To facilitate the identification of the six river locations during future study activities, steel rods were installed on each edge of the river bank and documented using the existing horizontal and vertical control survey for the area.

At each cross section location, velocity measurements were obtained using the methods presented in the June 1987 Pilot Study Work Plan. Each

cross section was divided into a series of horizontal sections, the spacing of which was a function of river width at the specific location. Velocity measurements were taken at one-foot depth intervals, down to within six inches of the river bed bottom. The total flow within a given section was calculated by summing the product of the individual velocity measurement and the associated cross sectional area within the cross section.

In addition, during the October 1987 sampling round sediment probing was performed and core samples collected when the quantity of sediment allowed. These samples will be beneficial in providing an assessment and characterization of sediment deposition patterns prior to the construction of the stop-log baffle system/closure structure. A second data collection round was initiated in June of 1988 to provide further baseline information. While velocity data was collected at this time, it has proven to be of limited value for reasons described below.

2.2 Results of Initial Monitoring Activities

The October 1987 data collection period resulted in the generation of accurate river bed contours at each of the selected cross sections, an initial set of river velocity data, and the identification and collection of river sediments when available.

The flow rate of water across each river cross section was calculated by multiplying and summing the recorded velocity measurements and the associated flow areas. In turn, the overall average river flow for the river was obtained by averaging the calculated flow at each of the river cross sections. Similarly, the flow in the raceway channel was calculated by averaging the flow monitored at each of the raceway cross-sections. A summary of this information is presented below:

River Stage Elevation	2.42 ft
Average River Flow	267.9 cfs
Average Raceway Flow	2.4 cfs

During the October 1987 monitoring effort, the existing sluice gates were essentially closed, as can be confirmed by the near zero average velocity readings and low flow readings for the data at Cross Section No. 1 (i.e. the cross section location nearest to the sluice gate.) Presented in Figure 2 is a summary of the velocity data collected at each river cross section for this collection period. This information is presented in terms of the horizontal stationing for each cross section and the average velocity measurement for each one-foot depth increment.

The importance of the information presented in Figure 2 is the data presenting the vertical distribution of the river velocities within each cross section. Although it is premature to form conclusions from this baseline data, the data tends to show the expected curve of velocities between the river bottom and the water surface.

Core samples of river/raceway channel sediment were attempted during the October 1987 collection period at the quarter points of each cross section location. Cores were attempted utilizing lexan core tubing as defined in the June 1987 Pilot Study Work Plan. In general, very little sediment was encountered at the six cross sections. A summary of each sediment location and description from this data collection period is presented in Table 2-1. The two sediment samples which were collected have been placed in frozen storage for future analysis, if desired. The sediments encountered include sand and silt as well as black, organic material with a "slimy" appearance.

TABLE 2-1

HOUSATONIC RIVER
VELOCITY & SEDIMENTATION CONTROL
PILOT STUDY

SEDIMENT COLLECTION SUMMARY - OCTOBER 1987

Section	Sample Location Sample # (STA.)	Sample Depth	Comments
1	--	--	No sediments encountered.
2	--	--	No sediments encountered.
3	3-1 (0 + 39)	--	No sediment.
3	3-2 (0 + 78)	--	Sediments less than 0.3' of thick "slimy" material. Could not recover sample.
3	3-3 (1 + 17)	0.8'	Fine brown sand over "slimy" black organic material.
4	4-1 (0 + 38)	--	Sediments less than 0.4' of thick "slimy" material. Could not recover sample.
4	4-2 (0 + 76)	--	No sediment.
4	4-3 (1 + 14)	--	Sediments less than 0.4' of thick "slimy" material. Could not recover sample.
5	5-1 (0 + 57.5)	1.5'	Fine sand & silt material. Some organics present.
5	5-2 (1 + 04)	--	No sediment encountered.
5	5-3 (1 + 50.5)	--	No sample--small amounts of sand & gravel present (less than 0.5').
6	6-1 (0 + 28)	--	Rock Bottom; no sample
6	6-2 (0 + 49)	--	Sediments less than 0.3' of black "slimy" material. Could not recover sample.
6	6-3 (0 + 70)	--	Sediments less than 0.5' of black "slimy" material. Could not recover sample.

Similar sediment characterizations including references to the black organic material were made by Stewart Laboratories for sediments within Woods Pond itself (Reference 4). It is believed that the "slimy" appearance of these sediments is the result of naturally decayed vegetation within the sediment deposits.

As indicated above, a second baseline monitoring event was performed in June of 1988. Unfortunately, the data collected is of limited use due to the amount of flow passing through the raceway channel at the time of the monitoring activities. Apparently, either the existing sluice gates had been slightly opened (by unknown parties) or excessive leakage through the gates was occurring. This unanticipated situation resulted in the collection of baseline velocity data which is not comparable to either the October 1987 data or to future rounds of baseline data collection.

Prior to initiation of the next round of data collection, efforts will be directed at removing submerged limbs, leaves and other debris that is positioned within the raceway channel abutting the sluice gates. Field observations made during 1987 noted the presence of this debris. It is unknown as to the quantity of this debris and its effect on the flow through the raceway channel; however, it is considered important that flow through the channel cross sections remains unobstructed so as not to potentially hinder the results of the baseline monitoring program.

SECTION 3 - ANALYSIS OF WOODS POND SEDIMENT

3.1 Description of Sampling Program

It was estimated in the Stewart Report that a large portion of the total PCB "load" in the river system is positioned upstream of the Woods Pond Dam. Work efforts associated with the Pilot Study have focused on the portion of the river immediately upgradient of the Dam. This area includes Woods Pond and backwater areas, where the nature of river flow enhances sediment deposition.

To provide further baseline information concerning the Woods Pond area, a sediment sampling program was initiated to gather information to be used in the evaluation of the potential remedial alternatives. This sediment sampling program, conducted in early 1987, consisted of the collection of a total of eighteen sediment core samples from Woods Pond (Figure 3). The locations chosen for core sampling were selected in an attempt to duplicate the sampling program conducted in 1982 (as part of the Stewart Report).

To date, six of the eighteen core samples have undergone analysis as presented below. The remaining 12 samples have been placed in frozen storage for future use, if desired. Four of the six samples analyzed thus far were selected for the purpose of duplicating a special PCB analysis conducted by Stewart Laboratories which included the sampling and analysis of sediments in 2-inch depth increments. The duplicate sampling program conducted in early 1987, also analyzed the sediment cores (taken from approximately the same four locations) in 2-inch depth increments for a comparative PCB analysis.

Two of these samples, and a total of four other samples were also selected for radioisotope analysis for the purpose of evaluating the "age" of

the sediment at each location. Presented below is a list of the 6 samples that have undergone analysis, and the type of analysis conducted for each sample:

Sample No.	PCB Analysis	Isotope Dating
17E-11	X	X
17E-15 *	X	X
17E-20 *	X	
18KI-2 *	X	X
18KI-7 *	X	
18H-3	X	X

* Represents duplicate samples of 1982 Stewart Report PCB analysis.

Presented below is a discussion of each phase of the Woods Pond sediment analysis as it pertains to the Pilot Study.

3.2 Selective PCB Resampling and Analysis

One reason for duplicating the locations of the sample locations used in the Stewart Report was to determine if PCBs present during previous sampling activities (i.e. the 1982 Stewart Report) are still present and to what degree. Table 3-1 is a comparison of the 1987 sampling program and the sampling conducted in 1982. As can be seen from Table 3-1, the results of the recent sampling efforts are in the same order of magnitude those presented in the Stewart Report from 1982. Although the results are not in exact correlation, they are of the same magnitude, and certain variances (sample location, handling, preparation, etc.) may account for the differences.

The data tends to support the belief that resuspension or redistribution of PCBs in the Woods Pond area has not occurred since the extensive study of the area in 1982.

TABLE 3-1

HOUSATONIC RIVER
VELOCITY & SEDIMENTATION CONTROL
PILOT STUDY

COMPARITIVE SEDIMENT RESAMPLING PROGRAM

	Location 17E-15				Location 17E-20				Location 18K1-2			Location 18K1-7		
Depth (inches)	1982 Initial Core Sampling	1982 Special Study		1987 Core Sampling	1982 Initial Core Sampling	1982 Special Study		1987 Core Sampling	1982 Special Study		1987 Core Sampling	1982 Special Study		1987 Core Sampling
0-2		57	58	59		59	62	62	23	24	33	37	43	59
2-4	160	94	160	150	140	79	114	41	36	38	30	67	59	100
4-6		216	222	250		147	112	67	63	54	58	96	62	74
6-8		244		120		103		89	170	110	130		76	64
8-10	28			48	75			30			130			140
10-12														
12-14				24				3.1						
14-16	0.7													
16-18					19									
18-20				0.21				< 0.05						
20-22														
22-24				0.1	0.21			0.06						
24-26														

Note: PCB concentrations in dry weight parts per million.

6/22/88

3.3 Sediment Deposition Rates

Select core samples taken from Woods Pond have been geo-chronologically dated to determine the sediment depositional pattern for the Woods Pond area. The dating techniques used for this phase of the Pilot Study have been successfully utilized by the United States Geological Survey (USGS) in a number of similar applications. Specifically, four samples selected for analysis from the 1987 Woods Pond sampling program were analyzed in 1-inch depth increments for radioisotopes Cesium 137 and Lead 210. Both of these elements (Cs-137 and Pb-210) are radioisotopes that decay exponentially over time. Pb-210 occurs naturally in the environment and may co-precipitate with silt in certain waterways. Cs-137 is present in sediment due to fallout activity from open-air nuclear testing that occurred during the 1950's and 1960's. Cs-137 usually can be identified in sediments after 1955, when open air nuclear testing was initiated.

The "dating" analyses were designed to provide an approximate measure of the rate of historic sediment deposition in the Woods Pond area, and will serve to provide both a projected sediment deposition rate and a comparative value for potential future dating analysis. This will allow the determination of any additional sedimentation impacts on the Woods Pond area resulting from installation of the stop-log baffle/closure structure system beyond those anticipated from previous sedimentation rates.

Analysis of the four core samples indicated that the Lead 210 dating method was inconclusive due to a lack of quantifiable trends in the sediments. However, the results of the Cesium 137 analysis proved to be more conclusive. Cs-137 should begin to appear after 1955, the first year of extensive atmospheric nuclear testing. Thus, a sudden transition of Cs-137 values in sediment from measurable to non-detectable (or low) Cs-137 levels

TABLE 3-2

HOUSATONIC RIVER
VELOCITY & SEDIMENTATION CONTROL
PILOT STUDY

CESIUM 137 ANALYSIS

Core	Depth, in.	CS-137, pCi/g (dry)
18K1-2	3-4	0.74 ± 0.24
	5-6	0.72 ± 0.18
	9-10	LT 0.4
	11-12	LT 0.1
	17-18	LT 0.4
	23-24	LT 0.2
18H-3	3-4	0.84 ± 0.49
	5-6	0.30 ± 0.11
	11-12	1.9 ± 0.4
	17-18	1.6 ± 0.5
	18-19	2.65 ± 0.34
	19-20	1.88 ± 0.36
	21-22	1.54 ± 0.25
	22-23	LT 0.3
	23-24	LT 0.7
17E-15	3-4	2.0 ± 0.6
	5-6	0.14 ± 0.10
	9-10	LT 0.2
	11-12	LT 0.4
	17-18	LT 0.3
	23-24	LT 0.3
17E-11	3-4	1.2 ± 0.2
	5-6	1.3 ± 0.2
	11-12	1.8 ± 0.3
	17-18	1.0 ± 0.3
	19-20	0.18 ± 0.16
	21-22	0.24 ± 0.17
	22-23	0.91 ± 0.33
	23-24	LT 0.3

LT - Less Than

as the sediment depth increases can be interpreted as the 1955 "horizon" in the sediment bed. Presented in Table 3-2 are results of Cs-137 analysis by depth for each selected sample location.

The data presented in Table 3-2 is plotted in Figures 5 through 9. Also shown on each graph are the extrapolated dates of known or assumed activities. Specifically, the surface of the sediments is taken to represent sediment deposition in 1987, and the interface at the bottom of the sediments with underlying native materials is taken to be 1890 (the approximate year that the Schweitzer Dam was constructed). The upper graph in each figure shows Cs-137 activity as a function of depth, with each point representing 1-inch of sediment depth. Error bars show 95% confidence intervals or, in the case of values below the detection limit, the maximum range of activities (i.e. "less than 0.2" is shown as a bar extending between 0 and 0.2).

The data collected in this study provides valuable information regarding sediment age, PCB concentration, and their relationship. The Cs-137 data provides information concerning the approximate sediment level for the year 1955 in each core. In core 18K1-2, the location appears to fall between the 5 to 6-inch depth and the 9 to 10-inch depth. In core 18H-3, sediment from 1955 is located near the 22-inch depth. In core 17E-15, the CS-137 activity falls off between the 3 to 4-inch and the 5 to 6-inch depths. Data from core 17E-11 is not as clear as the other results. Cesium 137 activity declines between 18 and 19 inches, but rises again in the 22 to 23-inch section. Possibly the Cs-137 activity reported for the 22 to 23-inch section is incorrect, which would mean that 1955 falls between 18 and 19 inches. Another possible explanation is that this core represents a more dynamic flow environment than the other locations. That is, older sediments (pre-1955)

from upstream may have been resuspended and deposited on top of more recent sediments (post-1955).

The annual sediment deposition rates presented on the figures are summarized below:

Core Designation	Approximate Deposition Rate
Core 18K1-2	0.22 inches/year
Core 18H-3	0.20-0.72 inches/year
Core 17E-15	0.12-0.23 inches/year
Core 17E-11	0.69 inches/year

3.3 PCB Biodegradation Evaluation

The PCB laboratory results for the samples described in Section 3.2 were provided to the General Electric Corporate Research & Development (CR&D) Group (Specialized Biological Sciences Branch) in Schenectady, New York, for their review. The purpose of this review was:

1. Identify any noticeable signs of natural dechlorination and biodegradation of PCBs in the sediment; and
2. Provide control data against which future analytical results can be compared.

Among the items provided to CR&D were certification of laboratory analysis; PCB chromatograms for each sample; and the sample "extracts" associated with each sample. These materials were analyzed by GR&D in comparison with reference data for non-degraded PCBs. In a letter report (Appendix A) from Dr. John F. Brown of the Specialized Biological Services Branch of CR&D; Dr. Brown concludes:

> "What I can say at the moment is that (a) the PCBs in Woods Pond have undergone a partial dechlorination; (b) the dechlorination system involved is clearly different from those found in Silver Lake (Systems F and G), and similar, though not identical, to one of those seen in marine sediments. (System h); (c) that this dechlorination has thus far effected a roughly 50% loss of the

chemically (and toxicologically) more reactive congeners; and (d) that it has also effected a 10-15% decline of the hptachlorobiphenyls that are the most persistent in people."

Based upon this conclusion, and supporting research data from similar sites, biodegradation continues to be considered as a leading sediment remediation technology for Housatonic River sediments.

The 1988 Status Report entitled "GE Research and Development Program for the Destruction of PCBs, Seventh Progress Report, for the period 6/1/87-5/31/88, June 1988" is provided in Appendix B. This report presents the current status of research by GE and others on the biodegradation process as it relates to destruction of PCBs.

SECTION 4 - CONCLUSIONS AND RECOMMENDATIONS

4.1 Status of Pilot Study

This report presents the following information regarding the "Pre-Stop Log Structure Installation" phase of the Pilot Study:

1. A general discussion of pertinent background information to summarize the historical references contained in the June 1987 "Housatonic River Velocity & Sedimentation Control Pilot Study Work Plan."
2. Presentation and discussion of baseline data resulting from the completion of initial Pilot Study activities. These include:
   a) The generation of river velocities of select locations with the sluice gates closed;
   b) The occurrence and location of river sediments upstream of the Woods Pond Dam; and
   c) Historical sediment deposition rates within Woods Pond.
3. An assessment of PCB Biodegradation within the site area.

4.2 Preliminary Conclusions

In general terms, several conclusions may be drawn from the completion of initial activities as presented in this report. These items are as follows:

1. Baseline information has been generated for the subject river system at one flow condition with the existing sluice gates in the fully closed position;
2. Historical rates of sedimentation have been established for select locations within Woods Pond. These rates vary between 0.12 and 0.72 inches per year;

3. Sediment resampling in 1987 demonstrated similar sediment PCB concentrations as indicated in 1982. The four locations sampled in the Woods Pond area support this conclusion; this data supports the belief that resuspension or redistribution of PCBs in the Woods Pond area has not occurred since the extensive study of the area in 1982; and

4. Biodegradation of PCB-laden sediment within Woods Pond is occurring naturally.

The baseline velocity data generated in October 1987 indicates that the river is subject to predictable velocity variations and distributions. It should be noted that the velocity variations within the raceway channel due to the closed position of the sluice gates were virtually non-existent and in the anticipated range given the low volume of water leaking through the sluice gates. As larger volumes of water are passed through the sluice gates during the upcoming velocity monitoring events, it is anticipated that significant velocity variations will be observed.

An estimation of historic sedimentation rates within Woods Pond has been calculated. Based on the geochronological "dating" analysis performed, a range of deposition rates between 0.12-0.72 inches per year was determined with the range reflecting the four different sampling locations in Woods Pond. This data will be useful in predicting an average sediment depth that may be expected at each location during future duplication sampling. This rate should reflect natural increased deposition. If additional location-specific sedimentation in excess of that expected is identified, it may indicate the effects due to Pilot Study activities.

The basis for conclusions regarding the natural biodegradation of PCBs in the sediments of Woods Pond have been provided by the GE Corporate

Research & Development Group. As stated, differences between reference PCB data and the available PCB data for analyses of Woods Pond sediment has indicated that PCB degradation has been initiated, thus reinforcing the potential application of this alternative to remediation of the sediments in this area.

4.3 Future Activities

Activities to date have been dedicated to the collection of baseline velocity data and initial sediment monitoring activities. The immediate upcoming activities associated with the Pilot Study are presented in Table 1-1 and will include the installation of sediment catchment units at locations identified during the first velocity monitoring event, the continued regular velocity profiling activities and the "controlled flow velocity monitoring" activities. Based on the presence of some black organic material and measurements of "low" velocity identified during initial monitoring activities, the following locations have been selected for placement of sediment trap and suspended sediment trap apparatus.

River Section	Station Location
3	1 + 17
5	0 + 57.5

In addition, it is recommended that a sediment trap and suspended sediment trap also be placed at Station 2 + 15 for Cross-Section Location No. 2 (just above the proposed stop-log baffle system/closure structure) to monitor the future buildup (if any) of sediments in this area.

As outlined in the June 1987 Work Plan, the two different sediment catchment units will be placed side-by-side at each of the above three select

locations to determine the most effective apparatus. These units will be retrieved in the spring of 1989.

Also to be initiated over the course of the next several velocity/sediment monitoring periods will be the initiation of water column monitoring as dictated by the June 1987 Pilot Study Work Plan. Samples will be taken for high river discharge rates, corresponding to a storm event with flows in excess of 700 cfs at the Schweitzer Bridge. These samples will be collected every two hours for a duration of eight hours in order to characterize the high stream flow conditions. Water samples will be analyzed for total suspended solids and PCB concentration (filterable and nonfilterable).

Ancillary activities to the Pilot Study have been initiated and may have an effect on the timing of the various planned study efforts. Of greatest significance is the proposed construction of a new dam to replace the existing Schweitzer Dam. Construction activities for the proposed dam will be performed in 1989, pending permit approvals. Following confirmation and more definitive details of this schedule, modifications to the Pilot Study may be proposed and documented in subsequent Interim Reports.

It is also recommended that continued research be performed into the Biodegradation Alternative, to build upon the positive conclusions drawn to date regarding this alternative.

REFERENCES

1. October 23, 1987 Letter to Mr. Ronald Desgroseilliers, General Electric Company from Mr. Gerald Levy, United States Environmental Protection Agency.

2. October 26, 1987 Letter to Mr. Ronald Desgroseilliers, General Electric Company from Mr. Stephen F. Joyce, Massachusetts Department of Environmental Quality Engineering.

3. Blasland & Bouck Engineers, P.C., Housatonic River Velocity and Sedimentation Control Pilot Study - Work Plan; June 1987.

4. Stewart Laboratories, Inc., December 1982 Housatonic River Study - 1980 and 1982 Investigations, Volume I and II prepared for General Electric Company.

5. Blasland & Bouck Engineers, P.C., Housatonic River Study, 135-Day Interim Report; Assessment of Remedial Alternatives; May 1985.

6. Eresco Reports, Report on Initial Screening of Housatonic River Remedial Alternatives, March 6, 1984, and Report on Proposed Engineering Evaluation of the Selected Housatonic Remedial Alternatives; May 1984.

7. Blasland & Bouck Engineers, P.C., Housatonic River Study; 45-Day Interim Report; Remedial Alternatives Evaluation; Sediment Disposal Sites; October 1984.

8. Blasland & Bouck Engineers, P.C., Housatonic River Study, 90-Day Interim Report; Remedial Alternatives Evaluation; River Channelization, In-Situ Impoundment, and Flow and Sedimentation Control; February 1985.

Figures

Area Environmental & Facility Programs
General Electric Company
100 Woodlawn Avenue, Pittsfield, MA 01201

August 17, 1988

Ms. Dorothy Allen
U.S. Environmental Protection Agency
J.F. Kennedy Federal Building
HPR-CAN2
Boston, MA 02203-2211

Mr. Stephen F. Joyce
Deputy Regional Environmental Engineer
Air, Solid and Hazardous Materials
Department of Environmental Quality Engineering
436 Dwight Street
Springfield, MA 01103

Dear Ms. Allen and Mr. Joyce:

This letter confirms my transmittal of the First Quarterly Report of the Housatonic Velocity and Sedimentation Control Pilot Study, dated August 1988.

Please call me if you have any questions regarding this report.

Yours truly,

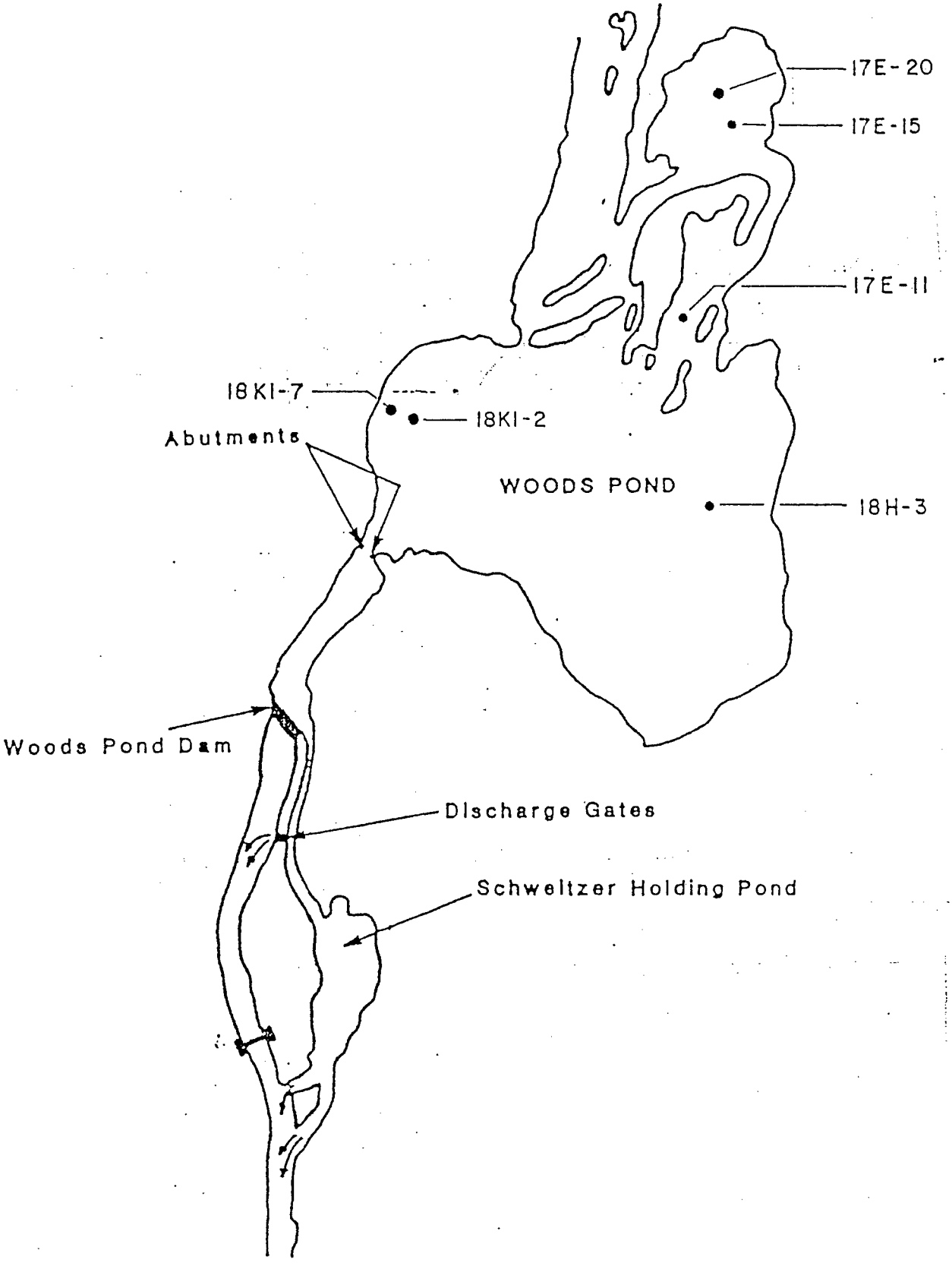
G. G. Bowman

G. Grant Bowman
Manager - Environmental Engineering

/ljr

Enclosure

FIGURE 3
HOUSATONIC RIVER SEDIMENT CORES
LOCATION PLAN

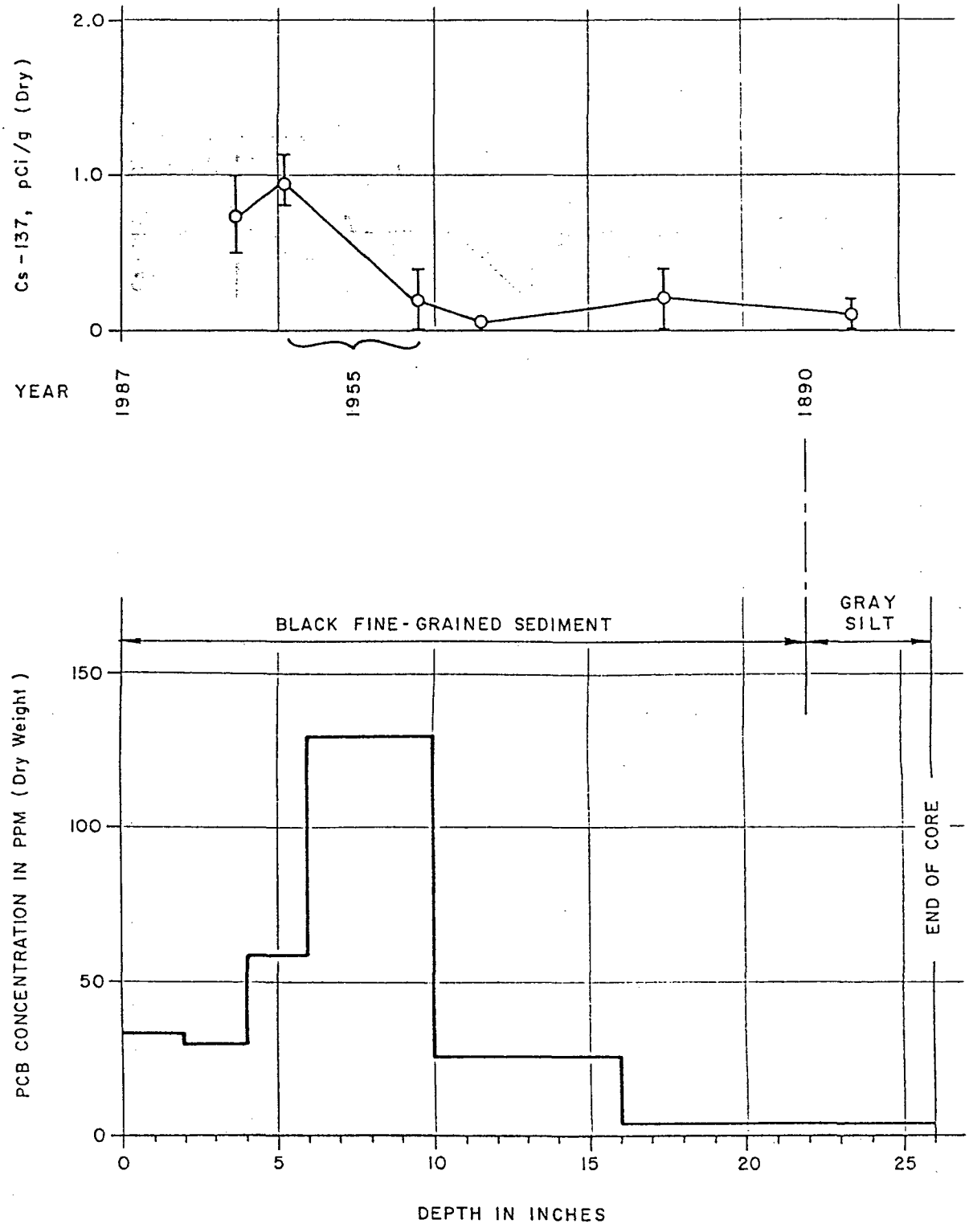


AUGUST 1988
01.15.01

REFERENCE: FIGURE 3-8 OF 1982 STEWART REPORT

BLASLAND & BOUCK ENGINEERS, P.C.

FIGURE 4
HOUSATONIC RIVER SEDIMENT DATA
CORE 18K1-2

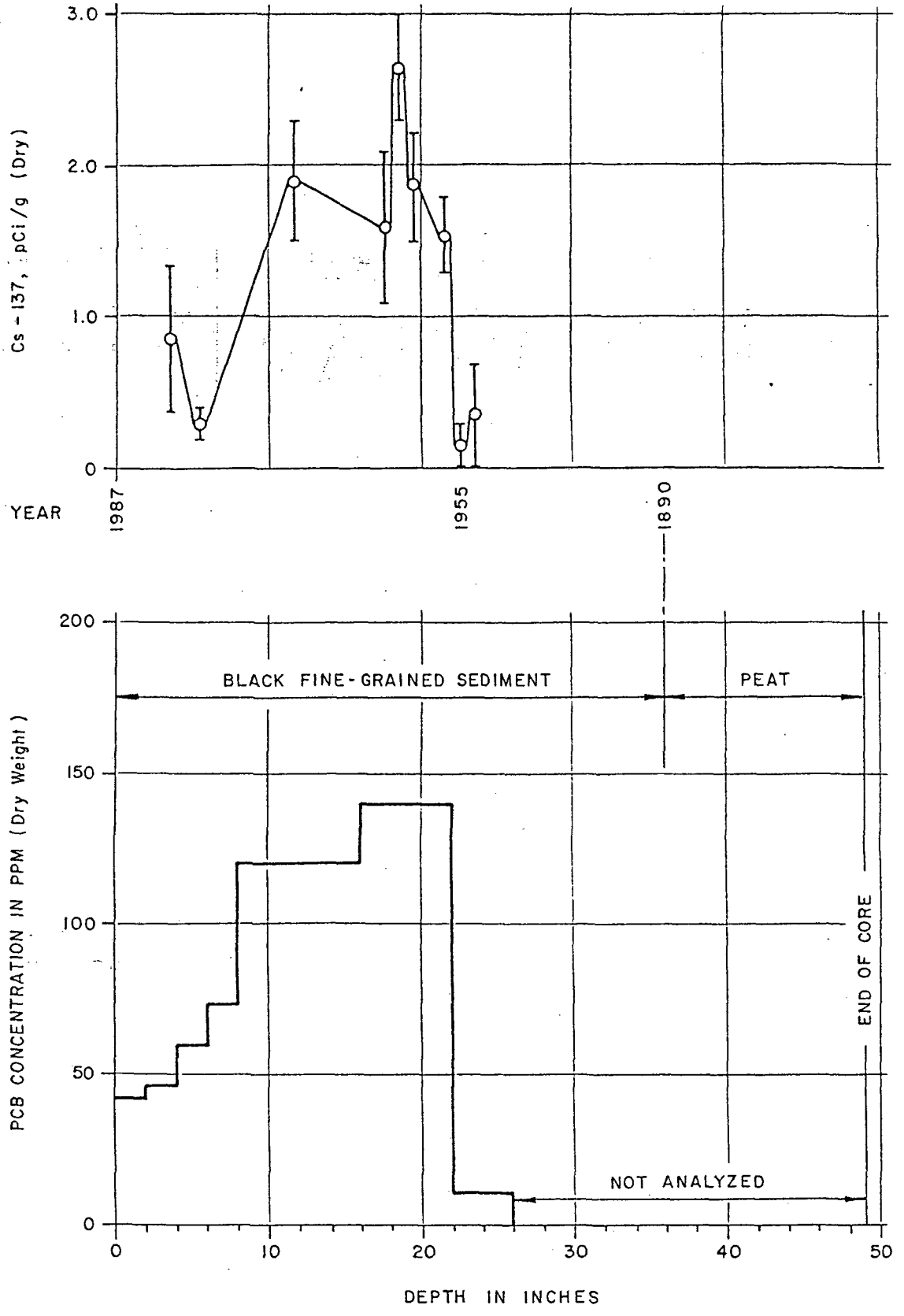


AUGUST 1988
101.15.01

BLASLAND & BOUCK
ENGINEERS, P.C.

FIGURE 5

HOUSATONIC RIVER SEDIMENT DATA
CORE 18H-3

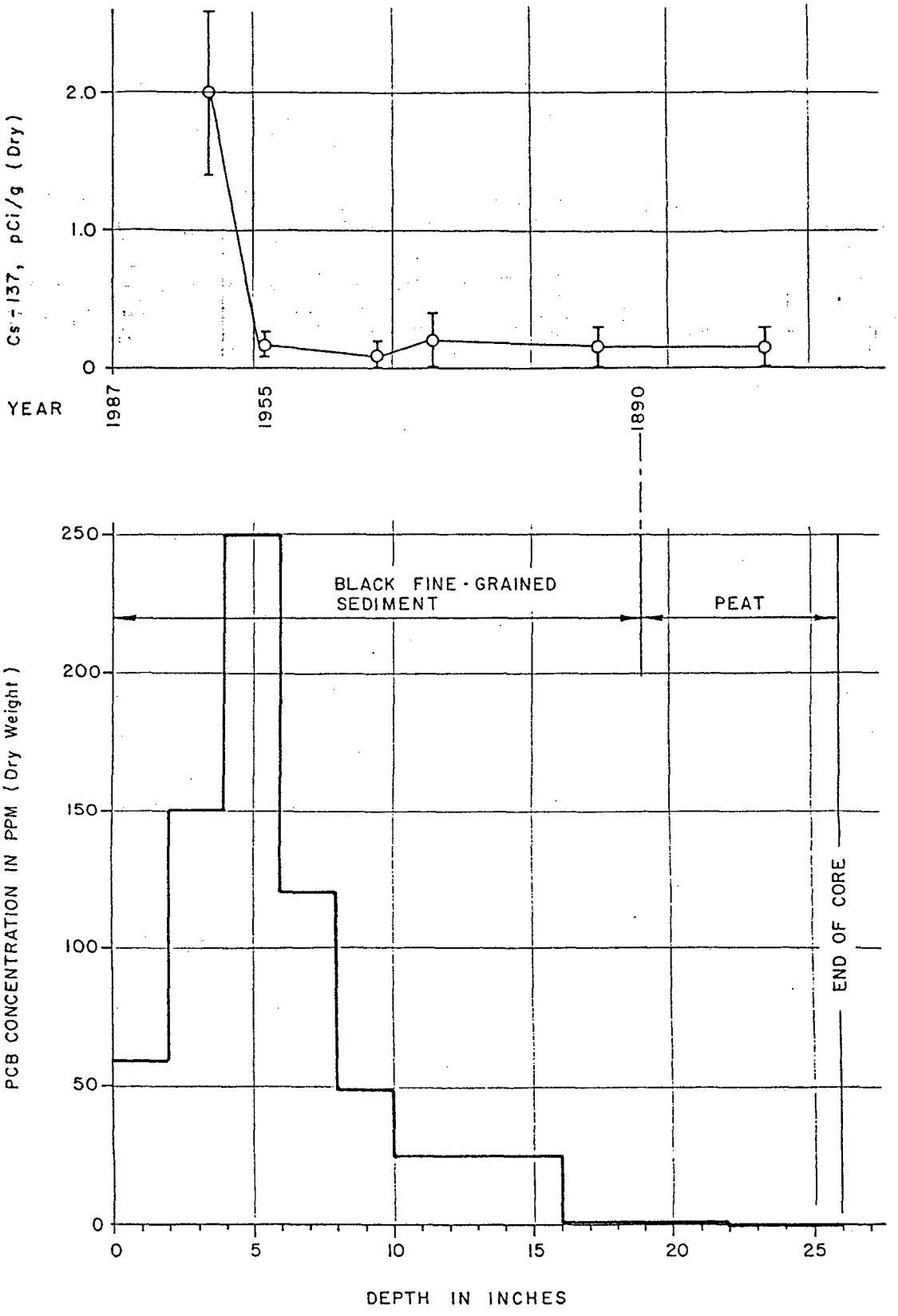


AUGUST 1988
101.15.01

BLASLAND & BOUCK
ENGINEERS, P.C.

FIGURE 6

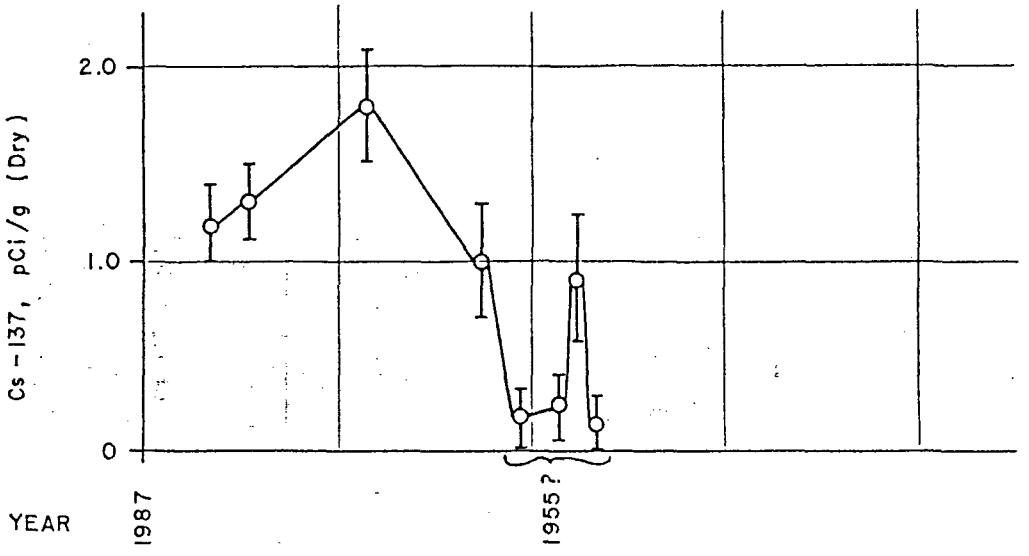
HOUSATONIC RIVER SEDIMENT DATA
CORE 17E-15

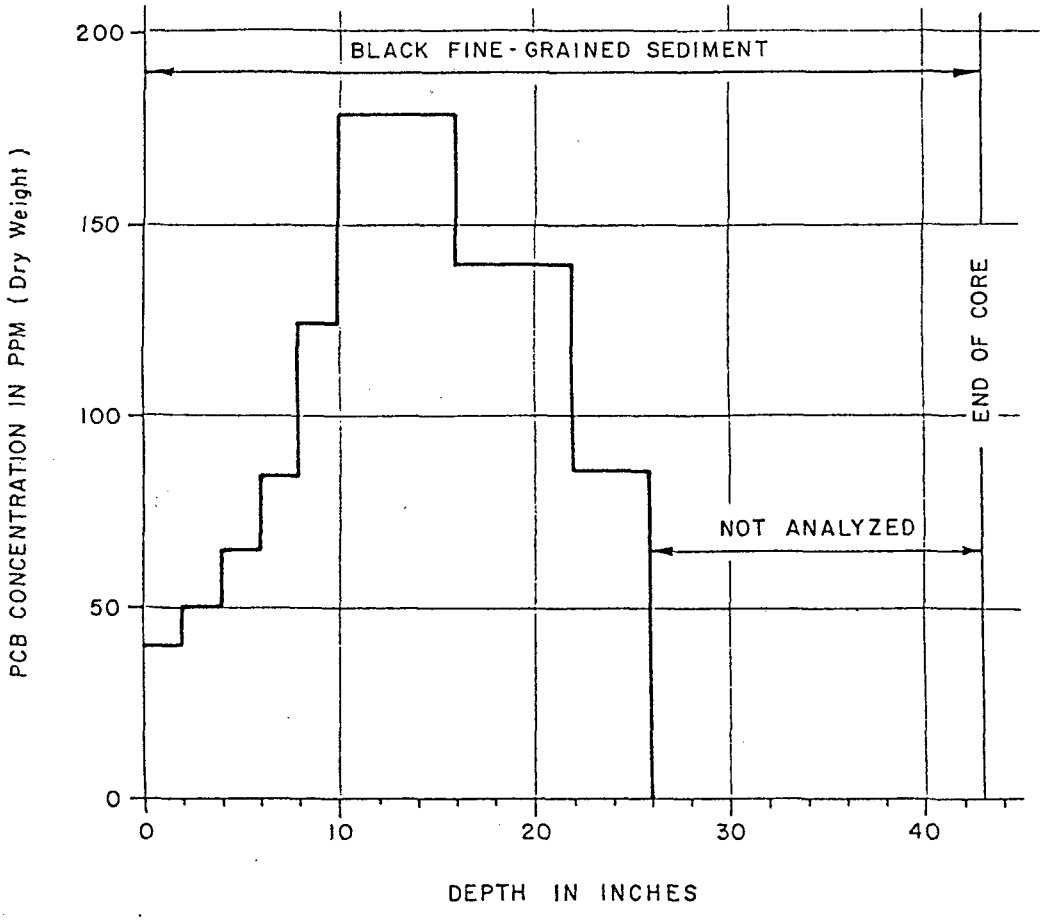


AUGUST 1988
01.15.01

BLASLAND & BOUCK
ENGINEERS, P.C.

FIGURE 7
HOUSATONIC RIVER SEDIMENT DATA
CORE 17E-11



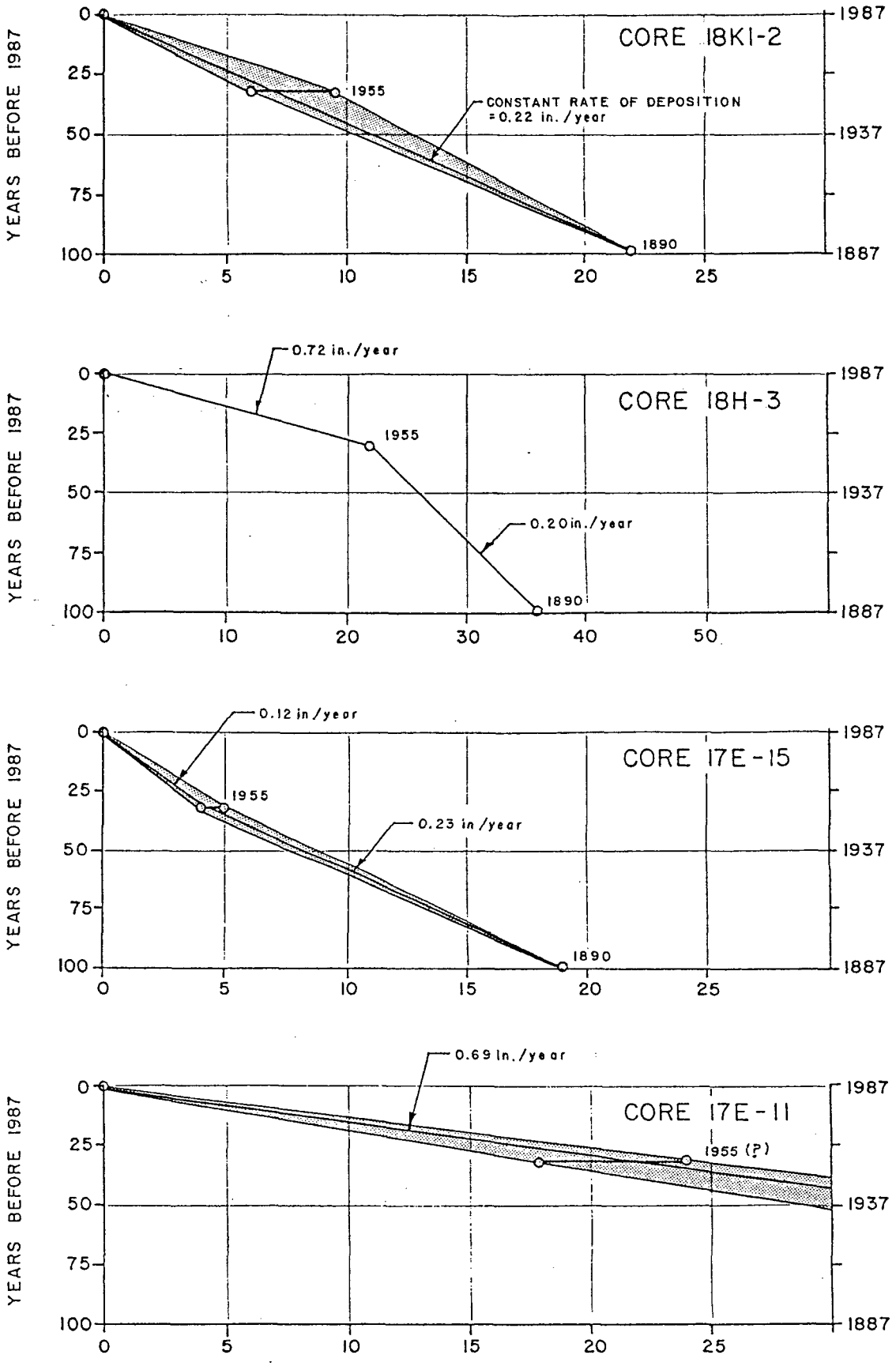


AUGUST 1988
101.15.01

BLASLAND & BOUCK
ENGINEERS, P.C.

FIGURE 8

HOUSATONIC RIVER SEDIMENTS
DEPOSITIONAL PATTERNS



AUGUST 1988
11.15.01

BLASLAND & BOUCK
ENGINEERS, P.C.

Appendices

APPENDIX A

MAY 18, 1987 LETTER FROM GENERAL ELECTRIC

CORPORATE RESEARCH AND DEVELOPMENT

GENERAL ELECTRIC

CORPORATE RESEARCH AND DEVELOPMENT
GENERAL ELECTRIC COMPANY • RESEARCH AND DEVELOPMENT CENTER • P.O. BOX 8 • SCHENECTADY, NEW YORK 12301 • (518) 387-5000

May 18, 1987

Mr. J. H. Thayer
Defense Systems Division
General Electric Company
100 Plastics Ave., Bldg. 42-306
Pittsfield, MA

Subject: PCB Dechlorination in Wood's Pond Sediments

Dear Jim:

On April 17, 1987 I received from Robert K. Goldman, P.E., of Blasland and Bouck Engineers, P.C., the following:

1. Certification of Analysis and chromatograms for Housatonic River sediment samples as provided by IT Analytical Services. These materials represent the analysis of sediment samples collected by Blasland and Bouck in late February, 1987.

2. Sample extracts associated with item 1 above. (Extracts packaged in cooler accompanying letter.)

3. Blasland and Bouck summary chart entitled "Housatonic River Study--Sediment Resampling Program--Comparison of 1982 and 1987 Data" dated April 10, 1987.

4. IT Corp. Chain of Custody Records (#36371, 36372, 36373).

The IT Analytical Service chromatograms referred to had been run on a 6' x 4mm packed column filled with SP2250 and SP2401 as in EPA Method 608. Such chromatograms provide somewhat better resolution than the packed columns of a decade ago, but still much less than can be obtained with modern capillary gas chromatography, (GC).

My review of the 29 packed column analytical chromatograms provided by Goldman indicated a marked similarity among them; all showed the peaks expected of mixtures of Aroclors 1260, 1254, and 1242 that were predominantly Aroclor 1260 with only very small and quite variable levels of somewhat biodegraded Aroclor 1242 present. In addition, all showed changes in relative peak height that suggested the occurrence of dechlorination in a single pattern of change.

In order to further characterize these changes, I selected one sample for detailed examination by capillary gas chromatography using a DB-1 (polydimethylsiloxane-coated) capillary. This sample (Sample No. AA8740, from sampling site 18 K1-2, 8-10" depth) was selected mainly because it showed a relatively low level of Aroclor 1242-derived trichlorobiphenyls in proportion to those derived by dechlorination, indicating a minimal need to consider contributions from Aroclor 1242 in interpreting the GC pattern. This sample was

reported by IT Analytical Services to contain <9, 12, and 120 ppm of PCBs quantifiable as Aroclors 1242, 1254, and 1260, respectively, for a total Aroclor level of 130 ppm, suggesting a composition that was 85-90% Aroclor 1260 and about 10% Aroclor 1254, with a trace of Aroclor 1242. However, when we compared the DB-1 GC of the sample with standards prepared by mixing Aroclors 1260 and 1254 in the proportions 100:0, 95:5, 90:10, 80:20, and 67:33 we got the best overall peak match with the last of these standards, suggesting that the original proportions of Aroclor 1260 to 1254 had been approximately 2:1. I attach copies of the CB-1 GC's of both Sample AA8740 and the 67:33 1260:1254 standard.

If you examine these two GC's closely you will note that although almost half the peaks show about the same relative heights in both chromatograms, the remainder do not. Some of these altered peaks (including those very small trichlorobiphenyl peaks associated with the trace of Aroclor 1242 present) are stronger in the sample; many are weaker. The differences are almost all a bit subtle, since the peaks that show a large proportionate change are almost always those that were relatively weak to start with, whereas the changes in the major peaks are generally relatively small. It is only when you look at the chemical identities of the PCB congeners responsible for the peaks undergoing change that it becomes fully evident that something other than random error, perhaps associated with an incorrect selection of reference standard, has occurred.

The chemical story is quite simple. Aside from the few very weak peaks of the Aroclor 1242, and a probable small enhancement of the 2,5,2',5'-tetrachlorobiphenyl peak (hereinafter abbreviated 25-25 CB) arising from dechlorination of 245-25 and 245-245 CB, all of the peaks showing an increased level contain a PCB congener carrying a 3-, 3,5-, 2,3-, 2,4-, 2,3,5-, or 2,4,6- chlorobiphenyl group, and all of the peaks showing a decrease contain a congener carrying a 3,4-, 2,3,4-, 3,4,5-, 2,3,4,5-, 2,3,4,6-, or 2,3,4,5,6-chlorophenyl group. I enclose a Table of DB-1 peak assignments, so that you can check these relationships out for yourself, if you wish. The overall pattern of change was similar, though not quite identical, to that produced by dechlorination system H, which we have observed in (marine) sediments from the upper Acushnet Estuary (New Bedford, MA), Escambia Bay (Pensacola, FL), and certain New York harbor fish.

Evidently, Woods Pond contains a dechlorination system that is capable of effecting on PCB congeners the following transformations of the chlorophenyl groups present therein: 3,4- to 3- (except when 2',6'-substitution present); 2,3,4- to (probably mainly) 2,4- and (some) 2,3-; 3,4,5- to 3,5-; 2,3,4,5- to 2,3,5-; 2,3,4,6- to 2,4,6-; 2,3,4,5,6- to an unidentified product, probably 2,3,5,6-; and (probably somewhat more slowly) 2,4,5- to 2,5-. These dechlorinative conversions, like those of the upper Hudson, of Waukegan Harbor, and also of the marine sites mentioned, but unlike those occurring in Silver Lake, evidently involve losses of chlorines from only the meta and para (3,4, and 5) positions on the phenyl ring. Also like the other meta/para dechlorinations, but unlike those of Silver Lake, the rate of dechlorination is somewhat retarded by increased substitution on the opposite ring.

As for the actual extent of this dechlorination in Sample No. AA8740, I would estimate the conversions of 23-34, 24-34, and 25-34 to 23-3, 24-3, and 25-3 CB; of 234-25, 234-24, and 234-23 to 24-25, 23-25, 24-24, 24-23, and 23-23; of 345-25 and 345-24 to 35-25 and 34-24; and of 2345-25 and 2345-23 to 235-25 and 235-23 CB to have proceeded to roughly 50% completion. The conversions of the major chlorobiphenyl 234-245 to 245-24, with a little 245-23, and of the minor congeners 2346-34 (to 246-34) and of 23456-25 (to an uncertain product) had proceeded to about 20% of completion. Conversions in the 10-15% range occurred in the major hepachlorobiphenyls: 2345-245 (going to 235-245), 2345-234 (going to 235-234 and/or 235-24), 2345-236 (going to 235-236), and 2346-245 (going to 245-246 CB). Judging from the extent of 25-25 CB formation, the conversions of the major penta- and hexachlorobiphenyls containing 245-chlorophenyls as the only reactive group were probably in the 0-5% range.

In short, Woods Pond contains a dechlorination system that is somewhat more selective than any of the others that we've discovered to date, but still fairly similar to the System H of the marine sediments in its overall pattern of congener selectivity. The Woods Pond System, interestingly enough, still provides for preferential removal of the congeners exhibiting significant toxicity in laboratory animals, all of which contain 4-, 3,4-, 2,3,4-, 3,4,5-, or 2,3,4,5-chlorobiphenyl groups in combinations providing the presence of two para (4-) chlorine substituents, at least two meta (3- or 5-) chlorines, and no more than one ortho (2-) chlorine. Thus, this dechlorination system will effect PCB detoxication as it procedes. It will also, somewhat more slowly, remove the hexa- and heptachlorobiphenyls that are persistent in Aroclor-exposed humans.

The extent of this conversion in the sample examined (50% decline in the more reactive group of congeners) is lower than we have seen in other sediments, generally those taken closer to the original release point, yet still higher than the 0% change usually seen in lightly contaminated sediments that are distant from known sites of bulk PCB release. At the moment, I cannot say why the dechlorination is not more advanced. One possibility would be that seeding with the agent responsible for the dechlorination (presumably an anaerobic bacterium) did not occur until fairly recently. This hypothesis could be tested by a careful comparison of the 1982 and 1987 analytical (packed column) chromatograms, which were run by the same laboratory using substantially the same GC columns and programs. An alternative hypothesis would be that the dechlorination rate is low because of poor availability in the Woods Pond sediments of some critical nutrient needed by the PCB-dechlorinating microbes. This possibility will take longer to evaluate. We don't yet know what nutrients are required to support any of the PCB-dechlorinating bacteria. John Quensen and Steve Boyd in Professor Tiedje's lab at Michigan State have started to work on precisely this problem, but the rate of progress in studies of biodegradative processes that take years in the natural environment tends to be slow.

What I can say at the moment is that (a) the PCBs in Woods Pond have undergone a partial dechlorination; (b) the dechlorination system involved is clearly different from those found in Silver Lake (Systems F and G), and similar, though not identical, to one of those seen in marine sediments (System

H); (c) that this dechlorination has thus far effected a roughly 50% loss of the chemically (and toxicologically) more reactive congeners; and (d) that it has also effected a 10-15% decline of the heptachlorobiphenyls that are the most persistent in people.

In the interests of brevity, I have omitted references to the prior literature. You will find many of them, however, in my paper on "PCB Dechlorination in Aquatic Sediments," which was published in last week's Science magazine. If you need more background information, please don't hesitate to call.

Sincerely,

John F. Brown Jr.

John F. Brown, Jr.
Manager - Health Research
Biological Sciences Branch

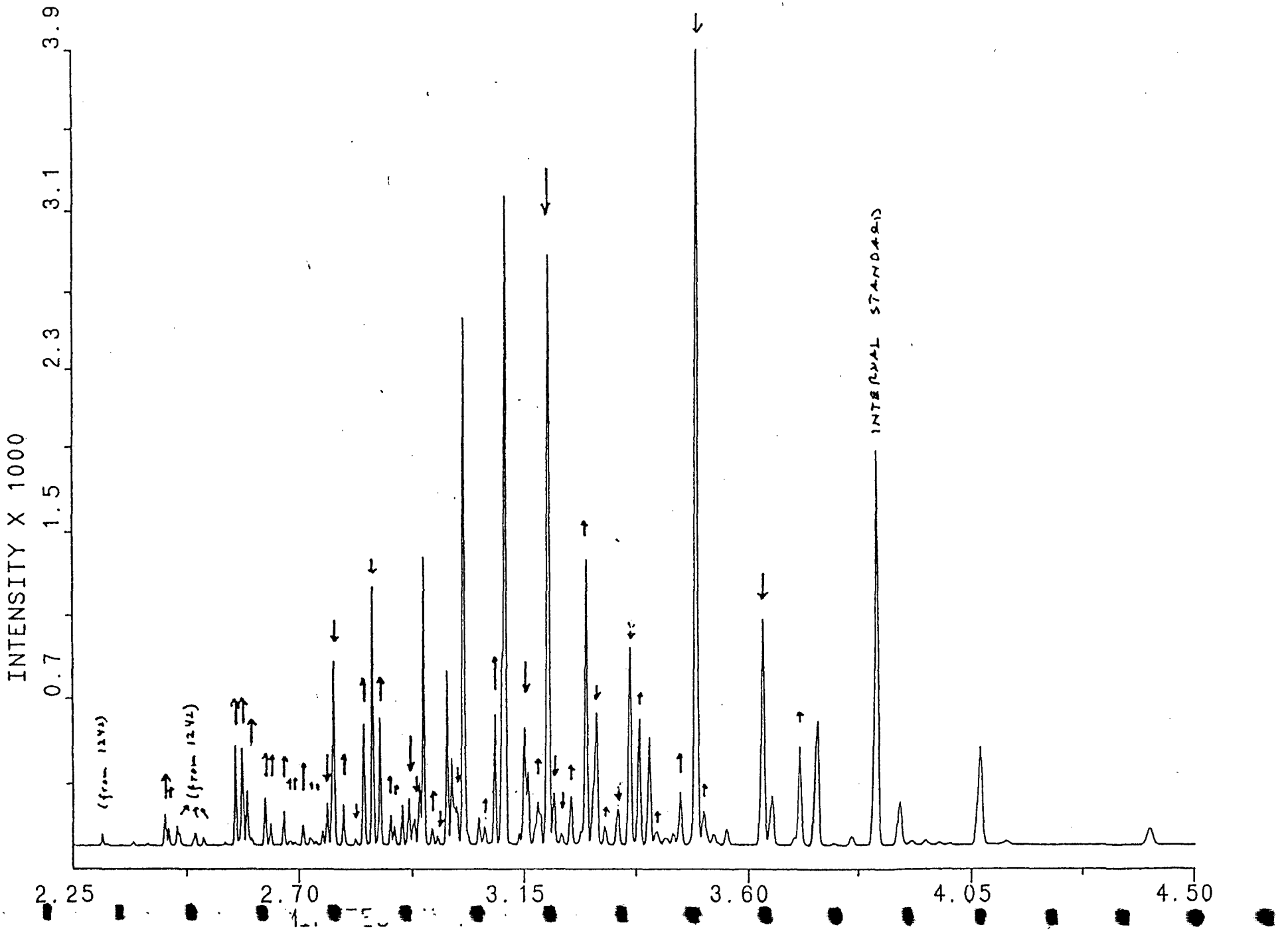
JFB/jg

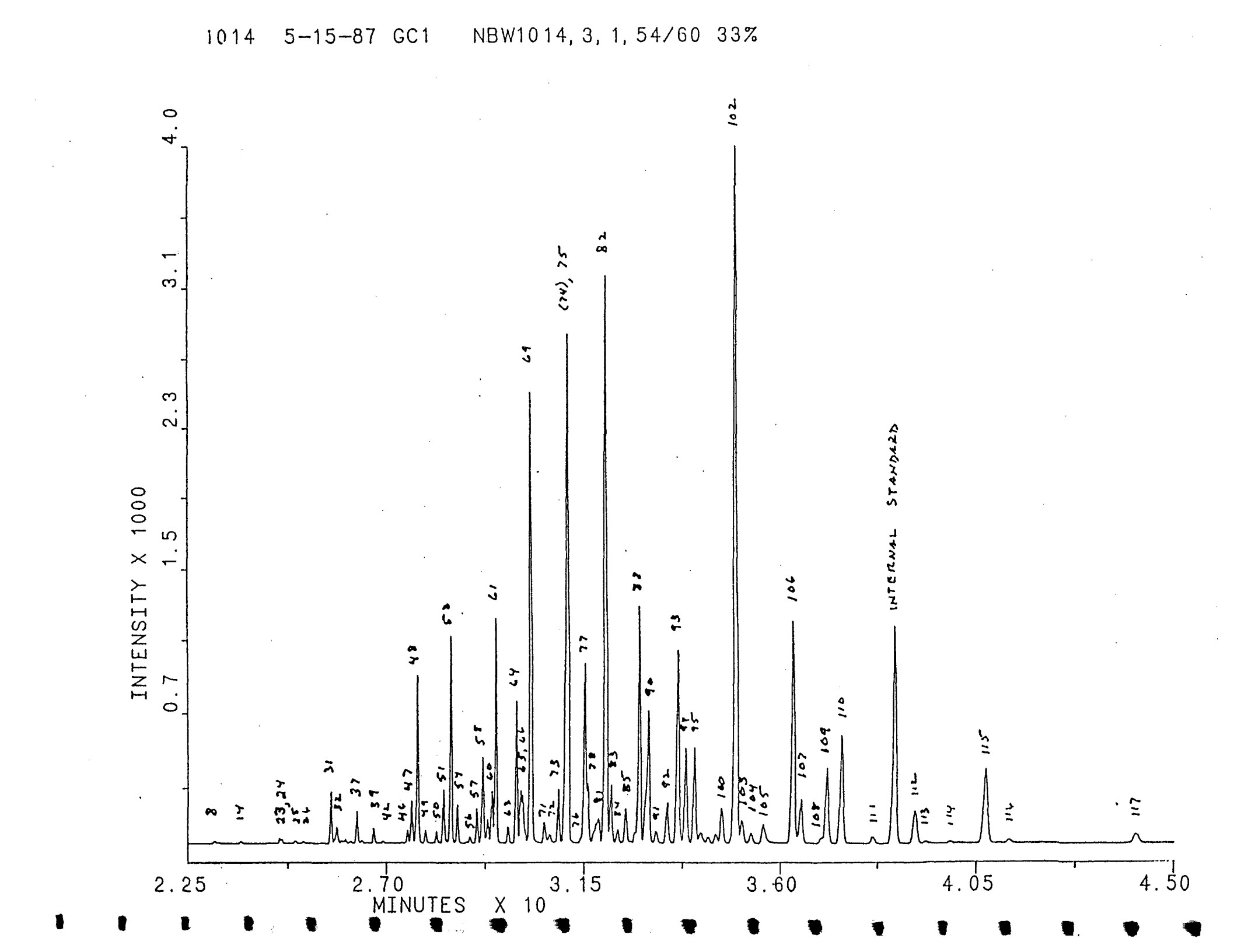
encl. GC of AA8740
GC of 2:1 Aroclor 1260:1254 mix
DB-1 peak assignment table
Xerox of Science paper

cc: SB Hamilton, Fairfield
JH Claussen, Fairfield
HL Finkbeiner, CRD
RD Unterman, CRD
RE Wagner, CRD

1009 5-14-87 GC1 NBW1009, 3, 1, #8740

Arrows indicate direction of change relative to 67:33 Aroclor 1260:1254 standard




1014 5-15-87 GC1 NBW1014, 3, 1, 54/60 33%
INTENSITY X 1000
0.7
1.5
2.3
3.1
4.0
2.25
2.70
3.15
3.60
4.05
4.50
MINUTES X 10
INTERNAL STANDARD
102
82
(74), 75
69
49
53
71
44
77
90
93
106
110
115
117

IDENTIFICATION OF DB 1 CAPILLARY GC PEAKS FOR PCBs

PEAK#	RLT. TIME	T-CL:0-CL			RRT	CONGENERS	WEIGHT %	MOLE %
1	46.96	0:0			.8997	BIPHENYL	0.0000	0.0000
2	46.72	1:1		001	.1844	2	0.0000	0.0000
3	50.26	1:0		002	.1937	3	0.0000	0.0000
4	50.62	1:0		003	.1975	4	0.8142	1.4134
5	52.35	2:2		004	010 .2295	22' : 26	0.0000	0.0000
6	54.78	2:1		007	009 .2566	24 : 25	0.0211	0.0310
7	55.61	2:1		006	.2709	23'	0.0431	0.0633
8	56.12	2:1		005	008 .2785	28 : 29'	0.2032	0.2943
9	57.99	2:0		014	.2973	25	0.0591	0.0866
10	57.78	3:3		019	.3045	22'6	0.0475	0.0684
11	58.99	3:2		018	.3165	296	0.0073	0.0093
12	59.21	3:2		011	.3238	33'	0.0000	0.0000
13	59.46	3:2		012	013 .3297	34 : 39'	0.0000	0.0000
14	59.84	3:2	2:0	016	015 .3347	22'5 : 44'	6.4331	8.5740
15	60.05	3:2		017	.3396	22'4	0.1864	0.2397
16	60.75	3:2		024	027 .3508	286 : 23'6	0.0331	0.0421
17	61.07	3:2		016	022 .3625	22'3 : 29'6	0.1976	0.2514
18	62.17	3:1		023	.3778	235	0.0128	0.0152
19	62.46	3:1	0:0	034	034 .3800	2'35 : 22'66'	0.0000	0.0000
20	62.78	3:1		029	.3820	245	0.0000	0.0000
21	63	3:1		026	.3911	23'5	0.0479	0.0618
22	64.2	3:1		025	.4037	25'4	0.0316	0.0402
23	63.64	3:1		031	.4024	24'5	0.3313	0.4215
24	64.77	3:1	0:0	028	050 .4031	244' : 22'46	0.2381	0.2919
25	64.55	3:1	0:0	021	033 .4170	233' : 234 : 22'56'	0.2749	0.3434
26	65.09	3:1	0:0	022	051 .4267	234' : 22'46'	0.1859	0.1279
27	65.53	3:1		045	.4334	22'36	0.0713	0.0799
28	65.92	3:1		036	.4379	33'5	0.0203	0.0256
29	66.12	3:1		046	.4450	22'56'	0.0000	0.0000
30	66.45	3:1		039	.4486	34'5	0.0000	0.0000
31	66.64	3:2		052	073 .4554	22'55' : 23'5'6	5.2173	5.8533
32	67	3:2		049	.4610	22'45	1.2727	1.4278
33	67.24	3:2		047	.4639	22'46'	0.2175	0.2440
34	67.39	3:2		048	075 .4651	22'45 : 24'6	0.1372	0.1540
35	67.55	3:2		065	062 .4665	2346 : 2356	0.1284	0.1350
36	67.8	3:2		035	.4738	33'4	0.0320	0.0407
37	68.19	3:2	0:2	104	048 .4852	22'446' : 22'35'	2.1934	2.4584
38	68.48	3:2	0:2	037	042 .4870	344' : 22'34' : 233'6	0.3513	0.3975
39	69.17	3:2		064	071 .4990	23'34 : 234'6 : 23'4'6 +	0.7526	0.8443
40	69.25	3:1		066	.5040	24'45' 7	0.0000	0.0000
41	69.41	3:1		096	.5057	22'346'	0.0835	0.0638
42	69.72	3:2		068	.5102	22'33'	0.2104	0.2361
43	70.16	3:2	0:1	103	057 .5155	22'45'6 : 233'5	0.0997	0.0545
44	70.54	3:3	0:1	108	067 .5212	22'44'6 : 23'4'5	0.8404	0.8423
45	70.85	3:1		058	063 .5267	233'5' : 234'5	0.8806	0.8905
46	71.16	3:1	3:3	074	094 .5340	244'5 : 22'354'	0.7848	0.8214
47	71.4	3:1		070	061 .5407	23'4'5 : 2'345 : 24457	3.4077	3.8251
48	71.73	3:1	3:3	066	095 .5467	23'44' : 22'356 : 22'35'6	5.8139	5.6643
49	72.22	3:3	0:1	091	098 .5549	22'34'6 : 22'3'46 : 235'4	1.0562	1.0752
50	72.64	3:1		054	060 .5676	235'4' : 2344'	0.8098	0.9085
51	73.28	3:2	3:3	155	084 .5666	22'44'66' : 22'33'61 22'355'	3.4769	3.4703
52	73.49	3:3		089	.5779	22'346'	0.0000	0.0000
53	73.7	3:2		101	090 .5814	22'34'5 : 22'455'	6.2099	6.2399
54	74.08	3:2		099	.5880	22'44'5	2.8965	2.9071
55	74.65	3:2	3:2	150	112 .5969	22'34'66' : 233'56 : 23'44'6	0.1491	0.1496
56	74.83	3:2		086	109 .6029	22'33'5 : 233'44	0.7625	0.7653
57	75.29	3:2	3:2	152	097 .6062	22'3544' : 22'345 : 22'3'45	2.1686	2.1565
58	75.54	3:2		087	111 .6175	22'345' : 245'35' : 2344'6	3.9043	3.9186
59	75.87	3:2		085	116 .6224	22'344' : 234547	1.3197	1.3245
60	76.1	3:3		136	.6257	22'33'66'	0.7114	0.6960
61	76.3	3:0	3:2	077	110 .6295	33'44' : 233'4'6	0.8120	0.8442
62	76.77	3:3		154	.6349	22'44'56'	0.8716	0.8834
63	77.05	3:2		082	.6456	22'33'4	0.9930	0.9967
64	77.52	3:3		151	.6479	22'355'6	0.8857	0.8039
65	77.74	3:3	3:1	135	129 .6563	27'32'56' : 2'344'5	1.1147	1.0417
66	77.9	3:3		144	.6586	22'345'6	0.0000	0.0000
67	78.01	3:1	3:3	107	100 .6626	233'4'5 : 233'45' : 22'34'56	0.8463	0.8252
68	78.25	3:1		123	.6658	2'344'5	0.0000	0.0000
69	78.42	3:3	3:1	149	118 .6672	22'34'5'6 : 23'44'5 : 233'45	10.2404	9.5690
70	78.55	3:3		139	140 .6707	22'344'6 : 22'344'6'	0.0000	0.0000
71	79.2	3:3	3:1	134	145 .6796	22'33'56' : 22'3456'! 2344'5	0.7865	0.7727
72	79.5	3:1	3:3	122	131 .6871	2'33'45! 22'33'46!22'33'55'+	0.2922	0.2813
73	80	3:2		146	141 .6955	22'34'55' : 233'45'6	0.7998	0.7260
74	80.3	3:3	3:1	132	105 .7035	22'33'44' : 233'44'	3.8915	3.8439
75	80.5	3:2		153	.7036	22'44'55'	5.6128	5.0949
76	81	3:2		148	.7066	23'44'5'6	0.0154	0.0141
77	81.5	3:2		141	.7206	22'3455'	0.8357	0.7586
78	81.7	3:4		179	.7205	22'33'544'	0.0000	0.0000
79	82	3:2		130	.7284	22'33'45'	0.7575	0.6876
80	82.2	3:2		137	.7329	22'344'5	0.7258	0.6570
81	82.55	3:4		176	.7385	22'33'446'	0.0000	0.0000
82	87.7	3:2		138	163 .7403	22'344'5' : 233'45'6 : +2	6.5382	5.9349
83	83	3:2		156	.7429	233'44'6	0.9707	0.8792
84	83.4	3:2		129	.7501	22'33'45	0.7188	0.6452
85	84	3:3		178	.7537	22'33'55'6	0.0000	0.0000
86	84.2	3:2		166	.7572	2344'36	0.8344	0.8313
87	84.5	3:3		175	.7611	22'33'45'6	0.0257	0.0213
88	84.7	3:3		187	182 .7656	22'34'55'6 : 22'344'56'	0.2355	0.1952
89	85.1	3:2		128	.7761	22'33'44'	1.3466	1.2243
90	85.4	3:3		183	.7728	22'344'5'6	0.0000	0.0000
91	85.7	3:1		167	.7814	23'44'35'	0.6041	0.5484
92	86.4	3:3		185	.7846	22'3455'6	0.8346	0.8279
93	87	3:3		174	161 .7965	22'33'456' : 22'344'56	0.3841	0.2526
94	87.5	3:3		177	.8031	22'33'4'56	0.2213	0.1639
95	88	3:3	3:1	171	156 .8105	22'33'44'6 : 233'44'5	1.1820	1.0431
96	88.44	3:4		202	.8089	22'33'55'66'	0.0000	0.0000
97	88.5	3:1		157	.8104	233'44'5'	0.1443	0.1310
98	88.91	3:3		173	.8152	22'33'456	0.0000	0.0000
99	89.4	3:4		206	204 .8197	22'33'55'66' : 22'344'566'	0.0847	0.0836
100	89.6	3:2		172	192 .8276	22'33'455' : 233'455'6	0.1229	0.1018
101	90.2	3:4		197	.8293	22'33'44'66'	0.0000	0.0000
102	90.4	3:2		180	.8362	22'344'55'	0.5996	0.4969
103	91	3:2		193	.8397	233'4'55'6	0.0946	0.0839
104	91.4	3:2		191	.8447	233'44'5'6	0.8177	0.8146
105	92.2	3:4		199	.8494	22'33'4544'	0.8226	0.8173
106	93.9	3:2		170	.8748	22'33'44'5	0.4532	0.3756
107	94.3	3:2		190	.8748	233'44'56	0.1144	0.0946
108	95.5	3:3		198	.8845	22'33'455'6	0.8183	0.8139
109	95.9	3:3		201	.8875	22'33'4'56'6	0.8388	0.8290
110	96.8	3:3		196	203 .8935	22'33'44'5'6 : 22'344'55'6	0.8422	0.8321
111	98.6	3:1		169	.9142	233'44'55'	0.8196	0.8163
112	101.2	3:3		195	.9321	22'33'44'56	0.8116	0.8068
113	101.87	3:4		208	.9328	22'33'455'66'	0.0000	0.0000
114	103.29	3:4		207	.9423	22'33'44'566'	0.0000	0.0000
115	105.3	3:2		194	.9628	22'33'44'55'	0.8347	0.8265
116	106.77	3:2		205	.9678	233'44'55'6	0.0000	0.0000
117	114.1	3:3		206	1.010	22'33'44'55'6	0.0000	0.0000
118	126.35	3:3		209	1.050	22'33'44'55'66'	0.0000	0.0000

Polychlorinated Biphenyl Dechlorination in Aquatic Sediments

JOHN F. BROWN, JR., DONNA L. BEDARD, MICHAEL J. BRENNAN, JAMES C. CARNAHAN, HELEN FENG, ROBERT E. WAGNER

The polychlorinated biphenyl (PCB) residues in the aquatic sediments from six PCB spill sites showed changes in PCB isomer and homolog (congener) distribution that indicated the occurrence of reductive dechlorination. The PCB dechlorinations exhibited several distinct congener selection patterns that indicated mediation by several different localized populations of anaerobic microorganisms. The higher (more heavily chlorinated) PCB congeners that were preferentially attacked by the observed dechlorination processes included all those that are either pharmacologically active or persistent in higher animals. All the lower (less heavily chlorinated) PCB congeners formed by the dechlorinations were species that are known to be oxidatively biodegradable by the bacteria of aerobic environments.

DESPITE GREAT PUBLIC AND REGULATORY concern over the accumulation of polychlorinated biphenyls (PCBs) in the environment, little is known about their actual fate in specific environmental niches (*1*). Recently, however, we found that agents capable of attacking PCBs may leave residues that exhibit characteristic signatures in their capillary gas chromatographic (GC) patterns. These characteristic patterns occur because all the PCBs that were used commercially were complex mixtures of isomers and homologs (congeners) that were produced in fixed relative proportions by the chlorination process used and because each physical, chemical, or biological alteration process exhibits its own set of relative activities toward the individual PCB congeners. Thus many strains of aerobic bacteria that oxidize PCBs were found to exhibit, at least under laboratory conditions, PCB congener depletion patterns that were clearly distinguishable from each other (*2*) and from the more familiar patterns shown by animals that have mixed function oxidase systems based on cytochrome P-450 (*3–5*).

General Electric Research and Development Center, Schenectady, NY 12301.

To see whether such characteristic transformation signatures were present in environmental samples, we have reviewed several hundred chromatograms of the PCB residues in soils, sediments, and water. In the soil and water samples the alterations in the GC patterns, if any, could be readily related to known types of transformation processes such as simple evaporation from dry soils or aerobic microbial degradation in rivers or groundwater. Alterations of a different type, however, were seen in aquatic sediments from several PCB spill sites.

PCB mapping and transport studies have indicated that the upper Hudson River contained 134 metric tons of PCB in 1977, with much of it concentrated at depths of 15 to 30 cm in areas of low hydrodynamic shear as "hot spots" that have PCB concentrations greater than 50 ppm (*6*). Our sediment analyses and existing plant records indicate that this PCB was originally almost entirely Aroclor 1242 that was released from capacitor manufacturing operations at Hudson Falls and Fort Edward, New York, between 1952 and 1971. For PCB transformation studies we collected and sectioned sediment cores from four "hot spots" distributed around river reach 8 (the stillwater that is located immediately below Fort Edward village and that extends from 4 to 12 km below the major PCB release point) as well as 15 "surface grab" sediment samples distributed around the same section of the river (*7*). Analyses were performed as previously described (*8*) with a DB-1 polydimethylsiloxane-coated capillary GC column that was capable of resolving environmental PCB mixtures into 118 distinct peaks.

The chromatograms showed congener distributions that generally tended toward one of four major limiting patterns, which have been designated A, B, B′, and C (*8*) and are illustrated in Fig. 1. Pattern A looked similar to that of Aroclor 1242 except for some modest quantitative differences. Patterns B, B′, and C all showed markedly lower levels of most tri-, tetra-, and pentachlorobiphenyls and increased levels of mono- and dichlorobiphenyls. They were most easily distinguished from each other by the presence of three, two, or one strong dichlorobiphenyl peaks, respectively (Fig. 1). Two minor variants (not illustrated) were pattern D, which showed enhancement of two trichlorobiphenyls (*8*), and pattern E, which exhibited several distinctive alterations among the penta-, hexa-, and heptachlorobiphenyls.

To determine how representative these patterns might be, we reviewed the numerically reduced data for 2000 upper Hudson River samples analyzed during the 1977 New York State survey (*6*) and about 100 of the original packed-column chromatograms (*9*). All of the PCB-containing sediment specimens that were collected between Fort Edward and Troy, New York (a river distance of 69 km), exhibited patterns that resembled A, B-B′, or C. (The resolution of the older chromatograms was not sufficient to distinguish B from B′ or to detect the variant patterns D or E.) Pattern A was typically associated with lightly contaminated but extensive surface deposits, which have been estimated to contain a total of 57 metric tons of PCBs (*6*), whereas patterns

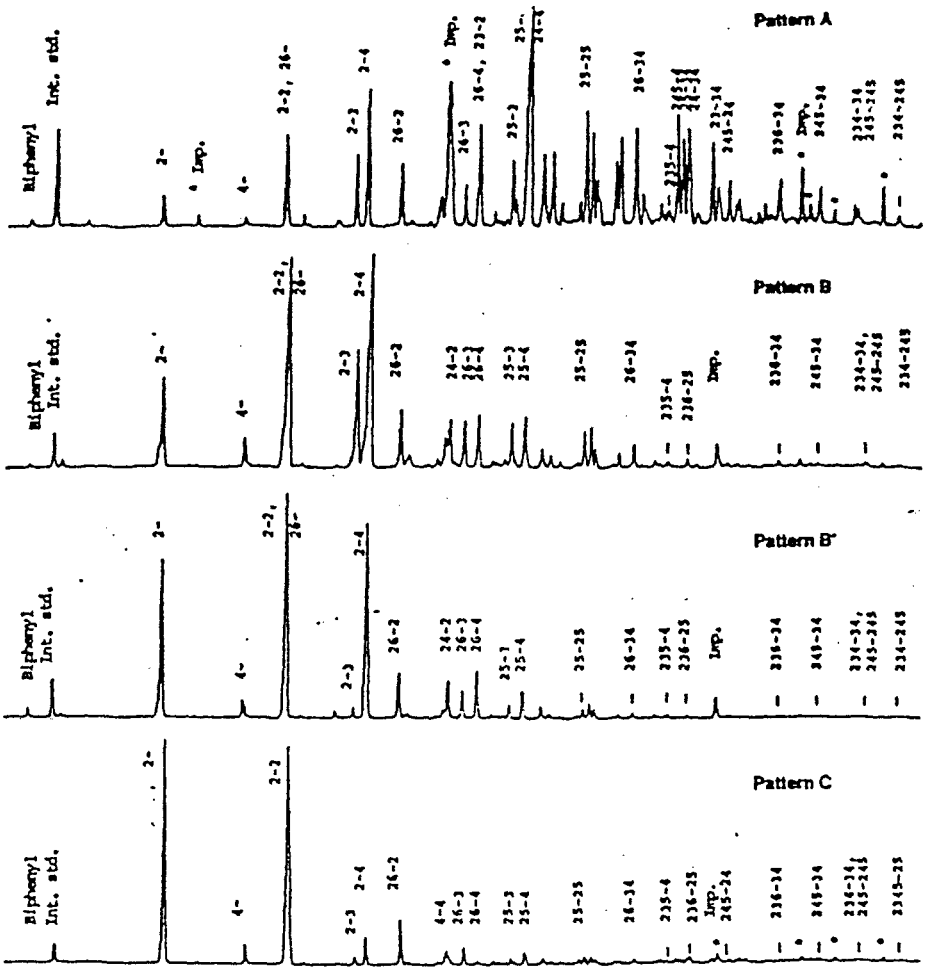


Fig. 1. DB-1 capillary gas chromatograms (plots of detector response versus elution time) of upper Hudson River sediments that show surface pattern A (largely unchanged Aroclor 1242) and subsurface patterns B, B', and C. A flame ionization detector was used so that the PCB peak response was nearly *proportional to molar concentration; however, non-PCB impurities in the samples also produced* observable peaks (designated * and Imp.). The major PCB congeners responsible for the observed peaks are designated by the numbers that correspond to the position of chlorines on each of the two phenyl rings; thus 2-2 and 24-4 indicate 2,2'-dichlorobiphenyl and 2,4,4'-trichlorobiphenyl, respectively. Internal standard peaks are designated Int. std.

B' and C were associated with the deeper "hot spots," which have been estimated to contain 77 metric tons of PCBs (*6*).

Quantitation of the individual capillary GC peaks indicated that the levels of most tri- and tetrachlorobiphenyls were depressed relative to those in Aroclor 1242 in all classes of upper Hudson River sediments, but particularly in those that showed patterns B, B', or C. Summary data for 2,5,4'-plus 2,4,4'-chlorobiphenyl (CB) and for 2,5,3',4'-CB (which are representative of congeners with lesser or greater responsiveness to dechlorination, respectively) are shown in Table 1. Conversely, in all sediment classes the levels of the 2,6,2'- and 2,6,3'-CBs and those of all dichlorobiphenyls were increased two- to sixfold, and the levels of the monochlorobiphenyl 2-CB increased 7- to 70-fold, with the largest changes observed in the samples that showed patterns B, B', or C (Table 1). The increases in the mono- and dichlorobiphenyls occurred despite their greater tendency to elute into the river water or undergo aerobic biodegradation. Thus it was evident that in the upper Hudson River as a whole a massive (40 to 70 metric tons) conversion of tri-, tetra-, and higher chlorobiphenyls to mono-, di-, and 2,6,X'-trichlorobiphenyls (X' = 2, 3, or 4) had occurred, particularly in the subsurface (15- to 30-year-old) portion of the sediments.

The sediments of Silver Lake, a 10-ha urban pond in Pittsfield, Massachusetts, contain an estimated 29 metric tons of PCBs (*10*), which are believed to have originally been almost entirely Aroclor 1260 released from adjacent transformer-manufacturing operations before 1972. A mapping and

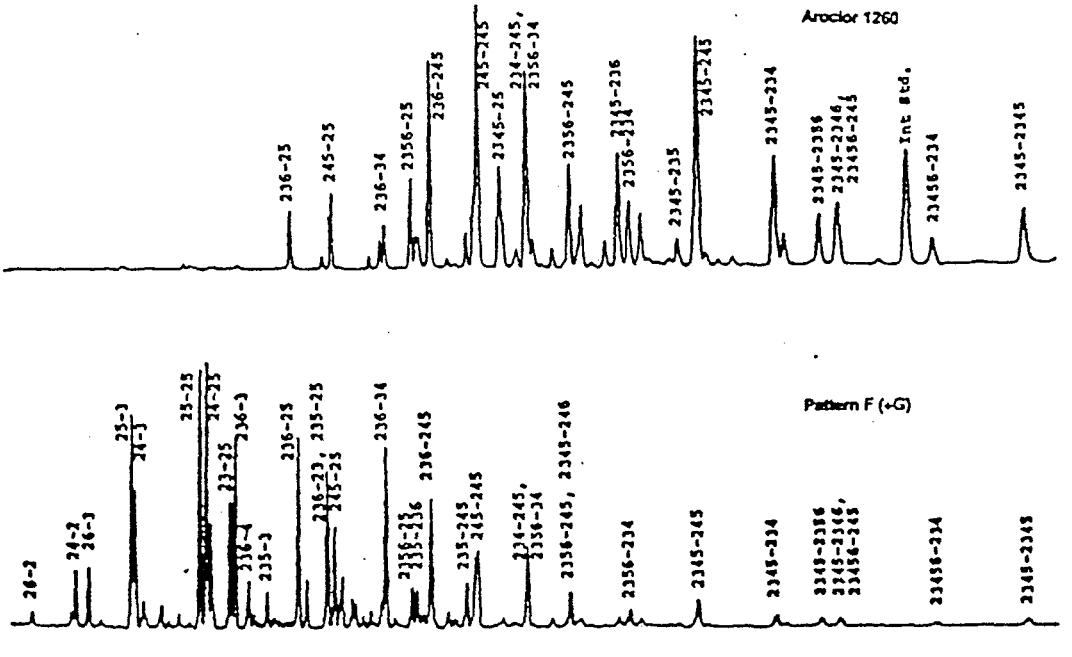


Fig. 2. DB-1 capillary gas chromatograms (plots of detector response versus elution time) of Aroclor 1260 and of the Aroclor 1260 residue extracted from a Silver Lake sediment composite that showed mainly pattern F (with some pattern G, which contributed the three small peaks on the left). These chromatograms were obtained with an electron capture detector. Such detectors give a stronger response with the more heavily chlorinated PCB congeners, a weaker response with the less heavily chlorinated ones, and little or no response with unchlorinated impurities. The major PCB congener peaks and the internal standard are designated as in Fig. 1.

transport study (*10*) indicated that there has been no significant movement of the PCB deposits. Half of the sediment sections collected (40 out of 80) gave packed-column GC patterns resembling those of mixtures of Aroclor 1260 and Aroclor 1254, indicating that only limited alteration had occurred (*10, 11*). The other half, which included specimens from all sectors of the lake, showed extensive alteration: 90 to 98% loss of the hexa- and heptachlorobiphenyls originally present and their replacement in the distribution by tetra- (31 to 50%), tri- (26 to 32%), and lower chlorobiphenyls, all of which are virtually absent (<1%) in the original Aroclor 1260. The chromatograms that showed extensive alteration exhibited two limiting patterns, F and G. In pattern F, the trichlorobiphenyls that were formed consisted solely of the 2,5,3′- and 2,4,3′-isomers, which were not present at more than trace levels in any commercial product. In pattern G, the trichlorobiphenyls that were formed consisted of these same isomers plus the 2,6,2′-, 2,6,3′-, and 2,4,2′-CBs. Figure 2 shows a capillary GC for a composite specimen that exhibited both patterns. Evidently, massive conversions of higher to lower PCB congeners have occurred in the Silver Lake sediments but with congener selectivity patterns that are different from those of the upper Hudson River.

There is limited evidence for dechlorination at other sites as well. Chromatograms of sediments from the Hoosic River (North Adams, Massachusetts), the Sheboygan River (Sheboygan, Wisconsin), and the Acushnet Estuary (New Bedford, Massachusetts) sent to us by other investigators all showed enhanced levels of the unusual 2,5,3′- and 2,4,3′-trichlorobiphenyls. A single study (*12*) has presented without comment Apolane C-87 capillary GC patterns and congener distributions for five sediment samples from Waukegan Harbor, Illinois, which had received large releases of Aroclor 1248. These patterns showed various degrees of removal for most of the tri-, tetra-, and pentachlorobiphenyls originally present, for example, losses of 47 to 98.5% for 3,4,3′,4′-CB, 6 to 71% for all tetrachlorobiphenyls, 15 to 89% for 2,4,5,3′,4′- and 2,3,4,3′,4′-CBs, and 26 to 83% for all pentachlorobiphenyls. These alterations apparently occurred in only one congener selection pattern, which we label pattern W. Corresponding increases appeared in the levels of several lower chlorobiphenyl peaks, particularly those that were identified (*12*) as 2,2′-, 2,3′-, 2,4′-, 4,4′-, 2,4,2′- (plus possibly 2,6,3′-), 2,6,4′-, 2,4,3′-, and 2,4,2′,4′-CBs.

The dechlorination of some PCBs by upper Hudson River sediments, like the analogous position-selective dechlorinations of chlorobenzoates (*13*) and chlorophenols (*14*), has recently been shown to occur under anaerobic culturing conditions in the laboratory and to be arrested by sterilization, which indicates that the process is microbially mediated (*15*). Simple chemical reducing agents that are present in anaerobic sediments and sludges do not attack chlorinated aromatics (*1, 3*), although they are capable of dechlorinating some aliphatic chlorine compounds (*16*). Thus the localized subsurface agents responsible for PCB dechlorination according to selection patterns B, B′, C, E, F, G, and W would appear to be separate strains of as yet unidentified anaerobic bacteria. Detailed chemical descriptions of these characteristic dechlorination patterns will be presented elsewhere (*17*).

We found that all of the lower PCB congeners formed by the observed reductive dechlorinations are biodegradable by one or more of the aerobic PCB-degrading bacteria that have been isolated from soils and sediments (*2, 8, 18*). These congeners are also degraded by eukaryotes that have P-450 cytochromes (*1, 3–5*). Thus a two-step sequence of dechlorination in aquatic sediments followed by oxidative biodegradation in aerobic environments will eventually effect total PCB destruction.

The dechlorination step alone, however, has significant toxicological consequences. The PCB residues in subsurface sediments from the upper Hudson River, Silver Lake, and Waukegan Harbor all showed preferential loss of 3,4,3′,4′-, 2,3,4,3′,4′-, and 2,4,5,3′,4′-CBs, and other higher PCB congeners that have chlorine atoms in positions 4 and 4′ [Figs. 1 and 2; (*12*)]. The relative disappearance rates for these congeners in the Hudson River were generally similar to that of 2,5,3′,4′-CB (Table 1). This group of PCB congeners includes all those that are either persistent in man (*5*), inducers of P-448–type cytochromes (*19*), or thymotoxic in rats (*19*). Thus, although anaerobic dechlorination does not immediately reduce the total mass of chlorinated biphenyl in an environmental deposit, it can accomplish detoxication.

Our sampling of archival GC data indicates that environmental PCB patterns that show the distinctive features of either aerobic microbial biodegradation (*1, 2*) or reductive dechlorination must have been observed hundreds of times during the past decade and yet have not been reported in the open literature. Instead, analysts have routinely reported observed PCB concentrations in terms of whichever commercial Aroclor had about the same average chlorine level. This practice of misrepresenting observed environmental PCB compositions can lead to appreciable quantitative errors (*4*). More significantly, it has left concealed not only the extent of PCB degradation in nature but also the diversity of the microbiological processes that are involved.

Table 1. Proportions of selected chlorobiphenyl (CB) congeners in Aroclor 1242 (released into the upper Hudson River from 1952 to 1971) and in the PCBs isolated from upper Hudson River sediments (patterns A, B, B′, and C). One hundred fifty sediment surface-grab samples and core sections were collected from known PCB "hot spot" areas (*6, 7*) that were distributed around river reach 8 (river miles 188.6 to 193.3). All samples were analyzed by DB-1 capillary gas chromatography to determine pattern type and total PCB content (ratio of the weight of PCB to the dry weight of the sediment). The numbers of samples of each type that contained at least 2 ppm total PCB and their concentration ranges were as follows: A, 28 samples, 5 to 165 ppm; B, 34 samples, 2 to 2604 ppm; B′, 11 samples, 2 to 2091 ppm; C, 18 samples, 60 to 619 ppm. Of the 28 pattern A samples, 5 also exhibited pattern D (*8*), and 28 of the 63 samples that showed patterns B, B′, or C also exhibited pattern E (*17*). Almost all specimens that showed pattern A were surface (0 to 10 cm) samples; almost all specimens that showed patterns B, B′, or C were subsurface (below 10 cm) specimens from core sections. All of the 70 samples that contained less than 2 ppm PCB (results not included in the table) showed patterns B or B′, but the congener distribution measurements were considered unreliable; most of these samples were from deep strata below the heavily contaminated zone.

CB congener	Observed range of CB congener (% by weight)				
	Aroclor 1242	Pattern A	Pattern B	Pattern B′	Pattern C
2-	0.7	5–25	10–17	28–52	13*–43
2,2′- (+2,6-)	2.6	3–10	12–19	14–27	30–41
2,3′-	1.3	2.8–3.2	4–9	0.3–0.9	0.7–1.6
2,4′- (+2,3-)	6.2	9–13	15–18	6–16	2.5–4.6
2,6,2′-	0.9	2.1–2.8	2.8–4.1	2.9–3.7	5.1–5.4
2,6,3′-	0.8	2.2–2.5	2.8–4.3	2.6–3.5	2.6–3.1
2,5,4′- + 2,4,4′-	14.4	11–13	3.1–8.4	1.9–4.2	1.6–3.1
2,5,3′,4′-	3.3	1.1–1.5	0.1–0.4	0.0–0.5	0.1–0.8

*This value was seen in a single surface-grab sample taken from a mid-channel area that was subject to scouring.

REFERENCES AND NOTES

1. K. Furukawa, in *Biodegradation and Detoxification of Environmental Pollutants*, A. M. Chakrabarty, Ed. (CRC Press, Boca Raton, FL, 1982), chap. 2.
2. D. L. Bedard *et al.*, *Appl. Environ. Microbiol.* 51, 761 (1986).
3. G. Sundström, O. Hutzinger, S. Safe, *Chemosphere* 5, 267 (1976).
4. R. W. Lawton, J. F. Brown, Jr., M. R. Ross, J.

Feingold, *Arch. Environ. Health* 40, 29 (1985).
5. S. Safe, L. Safe, M. Mullin, *J. Agric. Food Chem.* 33, 24 (1985).
6. E. G. Horn, L. J. Hetling, T. J. Tofflemire, *Ann. N.Y. Acad. Sci.* 320, 591 (1979).
7. One frozen 1977 upper Hudson River sediment core and assistance in our 1982–84 sediment collections were provided by T. J. Tofflemire, New York State Department of Environmental Conservation, Albany.
8. J. F. Brown, Jr., *et al.*, *Northeast. Environ. Sci.* 3, 167 (1984).
9. Gas chromatograms of 1977 upper Hudson River sediment samples were provided by D. R. Hill, O'Brien & Gere Engineering, Syracuse, NY.
10. *The Housatonic River Study 1980 and 1982 Investigations Final Report* (Stewart Laboratories, Inc., Knoxville, TN, 1982).
11. Gas chromatograms of 1982 Silver Lake sediment cores were provided by J. Hall and A. Yoakum, IT Laboratories, Knoxville, TN.
12. 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).
13. J. M. Suflita, A. Horowitz, D. R. Shelton, J. M. Tiedje, *Science* 218, 1115 (1982).
14. S. A. Boyd, D. R. Shelton, D. Berry, J. M. Tiedje, *Appl. Environ. Microbiol.* 46, 50 (1983).
15. J. F. Quensen III, S. A. Boyd, J. M. Tiedje, personal communication.
16. G. M. Klecka and S. J. Gonsior, *Chemosphere* 13, 391 (1984).
17. J. F. Brown, Jr., *et al.*, *Environ. Toxicol. Chem.*, in press.
18. D. L. Bedard, R. E. Wagner, M. J. Brennan, M. L. Haberl, J. F. Brown, Jr., *Appl. Environ. Microbiol.* 53, 1094 (1987).
19. A. Parkinson *et al.*, *J. Biol. Chem.* 258, 5967 (1983).

5 December 1986; accepted 16 March 1987

APPENDIX B

ANNUAL STATUS REPORT

GENERAL ELECTRIC RESEARCH AND DEVELOPMENT PROGRAM FOR THE DESTRUCTION OF PCBs

GE RESEARCH AND DEVELOPMENT PROGRAM FOR THE DESTRUCTION OF PCBs

Seventh Progress Report

For the Period

June 1, 1987 through May 31, 1988

June 1988

Submitted by

Herman L. Finkbeiner and Stephen B. Hamilton

CONTRIBUTORS

Donna L. Bedard, John A. Bergeron, Lawrence H. Bopp, Michael J. Brennan,
Ronald E. Brooks, John F. Brown, Jr., Frank J. Mondello,
Ronald Unterman, and James R. Yates

Biological Sciences Laboratory
GE Research and Development Center
Schenectady, New York

David P. Mobley, John B. McDermott, Kenneth M. Carroll, and David K. Dietrich

Chemical Process Laboratory
GE Research and Development Center
Schenectady, New York

Robert E. Wagner, James C. Carnahan, Steven B. Dorn, and Carl L. Chalek

Materials Characterization and Engineering Support Operation
GE Research and Development Center
Schenectady, New York

Richard W. Lawton, M.D.

Consultant
GE Research and Development Center
Schenectady, New York

James M. Tiedje, Stephen A. Boyd, and John F. Quensen

Department of Crop and Soil Sciences
Michigan State University
East Lansing, Michigan

Sandra Woods and Edmond Low

Department of Civil Engineering
Oregon State University
Corvallis, Oregon

TABLE OF CONTENTS

Section		Page
	Executive Summary	1
1	Bioremediation of PCBs in Soil	7
2	Studies of a PCB-Contaminated Industrial Sludge	17
3	Physical Chemistry Aspects of PCB "Uptake" and Metabolism by *Pseudomonas putida* LB400	23
4	PCB Biodegradation by Genetically Engineered Bacteria	33
5	Reductive Dechlorination of PCBs in Anaerobic Microbial Communities	43
6	Reductive Dechlorination of PCBs in Upflow Anaerobic Filter Reactors	51
7	Biotransformation of PCBs by Hydrogen-Oxidizing Bacteria	57
8	Identification of PCB Transformation Processes Occurring in Nature	61
9	Process Modeling: Surfactant Extraction of PCBs from Soil	73
10	Research Plans	79
	References	83

LIST OF ILLUSTRATIONS

Figure		Page
1-1	Time course of PCB biodegradation at drag strip field trial.	9
1-2	Drag strip field trial – control plot.	10
1-3	Effect of temperature on rate of PCB biodegradation by LB400.	10
1-4	Differential congener specificity of MB1 and LB400 biodegradation of drag strip soil PCBs using an "in situ" protocol.	12
1-5	Effect of sequential dosing of drag strip soil with LB400 and MB1.	14
2-1	DB-1 capillary gas chromatograms of the extract of the sludge, the extract of the sludge following washing with 0.1 N HCl, and an Aroclor 1242 standard.	18
2-2	Oxidation of 2,3,2′-CB (50 μM) and formation of 2,3-Cba by resting cells of *A. eutrophus* H850 in the absence or presence of a 150-fold excess of di(2-ethylhexyl) phthalate.	20
3-1	Distribution of 2,4′-CB between solution, suspension, and adsorbed.	24
3-2	Distribution of 2,4,5,2′5′-CB between solution, suspension and adsorbed.	25
3-3	Exchange of 2,4′-CB between solution in the aqueous medium and "killed" LB400.	26
3-4	Disappearance of 2,4′-CB for initial concentrations within the soluble range for two concentrations of LB400.	28
3-5	Lineweaver-Burk plots for 2,4′-CB in the soluble range for two concentrations of LB400.	29
3-6	Comparison of the kinetics of disappearance of 2,4′-CB at the limit of solubility and with a large excess in suspension.	30
3-7	Initial kinetics of 2,4,5,2′5′-CB disappearance for two nominal concentrations greatly above the limit of solubility.	31
3-8	Kinetics of 2,4,5,2′5′-CB disappearance over 24 h for two initial nominal concentrations and several concentrations of LB400.	32
4-1	Arrangement of *Eco*RI restriction fragments in pGEM410.	34
4-2	Genetic map of the region encoding PCB metabolism in pGEM410.	35
4-3	Degradation of Aroclor 1242 (10 ppm) by *Pseudomonas* strain LB400 and *E. coli* FM4560 in a 24-h resting cell assay.	38
4-4	Hybridization of a probe (pGEM415) containing the *bph* cluster of *Pseudomonas* strain LB400 to genomic digests of a variety of PCB-degrading bacteria.	40
5-1	Capillary gas chromatograms showing the anaerobic dechlorination of 700-ppm Aroclor 1242 after 16 weeks incubation.	44

LIST OF ILLUSTRATIONS (Cont'd)

Figure		Page
5-2	Changes in PCB congener distribution over time for the 700-ppm Aroclor live treatment.	45
5-3	Decrease in the average number of chlorines by position at three Aroclor concentrations.	46
6-1	Upflow anaerobic filter detail.	52
6-2	Effluent concentration of the five feed PCBs.	53
6-3	DB-5 chromatographs of the formation of the unknown metabolite vs time at RT 24.15 min.	54
6-4	Emergence of metabolite at RT 24.15 min.	55
6-5	Emergence of 2,3,4,5,6- and 2,3,5,6-CB in reactor 3.	56
7-1	Biotransformation of PCBs by hydrogen-oxidizing strain R2B-2A.	60
8-1	Relationship between numbers of *ortho* and non-*ortho* chlorine atoms in commercial Aroclors.	71
9-1	Soil extraction process schematic.	74
9-2	Flow diagram of four-stage, bench-scale extraction process with performance data for laboratory-spiked Oakland soil.	75
9-3	PCB extraction rate data.	76
9-4	Absorbances of soil suspensions as a function of centrifugation time.	78

LIST OF TABLES

Table		Page
1-1	Congener Specificity of MB1 and LB400 Biodegradation of Drag Strip Soil ...	13
4-1	PCB Degradation in Recombinant Strains relative to *Pseudomonas* Strain LB400	36
4-2	PCB-Degradative Competence	37
5-1	Percentage of 2,3,4,5,6-Pentachlorobiphenyl Dechlorinated by *Microorganisms From* Various Sources	47
5-2	Effects of Amendments of 2,3,4,5,6-CB Dechlorination by Hudson River Microorganisms After 32 Weeks Incubation	48
7-1	PCB Congener Mixture for Assaying Dechlorinase Activity	58
8-1	PCB Homolog Distributions in Aroclor 1260, in the Estimated Metabolic Losses, and in the Unmetabolized Residue Approximately 25 Years After Exposure To Aroclor 1260	62
8-2	Mean Rates of PCB Congener Loss from the Sera of Occupationally Exposed Capacitor Workers and Yusho Patients	63
8-3	Effects of Environmental Alteration Processes on the Mean Levels of *Ortho* and Non-*Ortho* Chlorines per Biphenyl	70
9-1	Distribution of PCB Between Fine and Coarse Soil Fractions	76

EXECUTIVE SUMMARY

The activity this year reflected a period of transition from research in the laboratory to practical application. At the same time as the initial site test was conducted based on the previously acquired knowledge of and capability for aerobic biodegradation of PCBs, the laboratory research was expanded on complementary biological mechanisms. In addition to kinetic, biochemical, and genetic studies concerned with improving aerobic biodegradation, research intensified on reductive dechlorination under anaerobic conditions and studies began of PCB transformations by aerobes in a hydrogen-enriched atmosphere. The description and analysis of patterns of PCB transformations in nature also continued. Finally, process modeling for surfactant extraction of PCBs from soil continued in the bench-scale phase.

Field Trial of Aerobic Bioremediation of PCB in Soil

This was the first known field trial of a biotreatment for PCB contamination. The test site was a former drag racing strip where PCB oils had been used for dust control. At 525 ppm, the PCB contamination resembled the composition of Aroclor 1248. The experimental area, enclosed within a transparent tent, had an experimental and a control plot. After preparation by rototilling, each plot was divided in two, one half subsequently left undisturbed and the other half tilled regularly. After characterization of the PCB concentration by sampling on a 1×1-ft grid, the experimental and control plots were dosed three times a week with either biphenyl-grown *P. putida* LB400 or buffer, respectively. Progressive PCB destruction was observed (June through October 1987), but the rate was less than previously obtained with the same soil and protocol in pots in the laboratory at 25 °C. A major contributor to this difference was the extreme temperature variation at the site. Both the rate of biodegradation and organism survival are temperature dependent. The results encourage further development. A better understanding of the variables that determine the early rate of PCB degradation by the organism and those that affect the survival and establishment of the organism in this soil will be needed to achieve the goal of 90 to 99% destruction.

Aerobic Biodegradation of PCBs in Soil

The previous laboratory model of the basic drag strip experiment (soil in pots, no mixing, percolative addition of organisms three times a week, 25 °C) was used to examine the prospect for improved biodegradation of PCB by dosing with aerobic organisms that have complementary patterns of congener preference. The organisms were *P. putida* LB400, used in the initial site test, and *Corynebacterium* sp. MB1. When applied separately, the observed pattern of PCB degradation exhibited the expected congener preference. When dosing was sequential, LB400 then MB1, the breadth and degree of degradation improved compared with LB400 alone. A "dual culture" strategy is feasible. Until a genetically engineered organism with all of the desirable characteristics becomes available, the use of complementary organisms offers a reasonable compromise. This use of complementary competence could include sequential anaerobic and aerobic phases as effective anaerobic dechlorinating strains become available.

Aerobic Biodegradation of PCBs in an Industrial Sludge

PCB contamination exists in locations that may differ greatly in biologically important variables. A single bioremediation procedure may not be appropriate for all applications. Sludge that is contaminated at 500 ppm with PCBs and that also contains a variety of other organic compounds and metals was obtained from an industrial settling tank. In initial experiments, the PCBs in the sludge were resistant to biodegradation by *P. putida* LB400, even after long incubation with high cell densities. A similar resistance was observed with *A. eutrophus* H850. The aqueous phase separated from the sludge did not inhibit PCB degradation by H850. Di(2-ethylhexyl) phthalate (DEHP) oil was found to be present in the sludge at about 150 times the PCB content, and when such a mixture of DEHP:PCB was assayed with H850, the PCB biodegradation was greatly inhibited. In separate experiments, DEHP did not inhibit the growth of H850, and therefore it was not toxic. The inhibition by DEHP of PCB biodegradation by LB400 and H850 might reflect the reduced availability of PCB to the organisms, caused by its partitioning into a DEHP phase. Understanding and learning how to mitigate the inhibition observed in this case could prove useful for bioremediation at other sites also.

Kinetics of PCB "Uptake" and Aerobic Metabolism

The insolubility of PCBs in water presents a special problem for kinetic analysis. By selecting congeners of greatly differing solubility and studying the interaction of both "killed" and live LB400 with either dissolved or dissolved plus excess suspended PCB, it was shown that the interaction between the cells and PCBs is mediated by the dissolved PCB. With killed cells the exchange was reversible and rapid. Partitioning between phases accounted satisfactorily for "uptake." For dissolved 2,4′-CB, the kinetics of degradation by the 2,3-dioxygenase pathway was consistent with the classical Michaelis-Menten description of a monomolecular enzyme catalyzed reaction. When 2,4′-CB was present in excess, the rate of solution of the excess suspended PCB determined the kinetics. 2,4,5,2′5′-CB, a substrate for the putative 3,4-dioxygenase, was too insoluble to be studied except with suspended excess. The observed kinetics at short time were consistent with expectation from the above. The kinetics changed over 24 hours in a way suggestive of some form of product inhibition. These experiments demonstrated the importance of the rate of PCB solution and transport to the organisms in determining the kinetics and the maximum rates of degradation attainable. Because the nominal PCB concentrations of concern in environmental contamination greatly exceed solubility, process variables that affect PCB availability are expected to be important for bioremediation.

Genetic Engineering of Aerobic PCB Biodegradation

Cloning and preliminary characterization of the genes for aerobic PCB degradation in LB400 were reported last year. Several recombinant plasmids were isolated that conferred on an *E. coli* host the ability to convert a variety of dechlorinated PCBs to the corresponding dichlorobenzoic acids. The plasmids contained the genes for the initial four enzymes of PCB/biphenyl metabolism (*bph* A-D) and functioned in the heterologous host. The genes now have been mapped on the recombinant plasmids pGEM410 and pGEM456. The PCB-degradative competence of the recombinant strain FM4560 was found to be similar to LB400

in resting cell assays with congener mixes 1B, 2B, and Aroclor 1242. Comparative genetic studies with DNA:DNA hybridization strongly suggested a close genetic relationship between the genes encoding for aerobic PCB metabolism in LB400 and H850. These strains exhibit similar PCB metabolism but are assigned to different genera. There was no such similarity with a variety of other organisms that exhibit differing PCB competence. It can be inferred that in nature genes for superior aerobic PCB metabolism are exchanged and expressed in different bacteria.

Reductive Dechlorination of PCBs in Anaerobic Microbial Communities

The altered congener distribution patterns first observed in Hudson River sediment samples, and now recognized in other sites, signaled congener-specific dechlorination of the original Aroclors by a previously unrecognized and unknown anaerobic biological mechanism. Last year initial evidence for reductive dechlorination in the laboratory was reported. Those results have been confirmed and extended with microorganisms obtained from one of those sites. The organisms were eluted from contaminated sediments and transferred to serum bottles containing cleaner sediment and spiked with PCBs. When incubated under anaerobic conditions, Aroclor 1242 was dechlorinated relatively rapidly, producing primarily *ortho*-only substituted congeners. The resulting congener distribution matched closely the Pattern C previously described for the same site (H7). All observed dechlorination was primarily from the *meta*- and *para*-positions. The process occurred more rapidly at the higher PCB concentrations tested. The biological basis of the process was indicated by sensitivity to autoclaving, by transfer with a technique known to elute microorganisms, by congener specificity, and by the sites of origin. Dechlorination is regarded as environmentally beneficial because the less chlorinated PCBs can be regarded as less toxic and are more aerobically biodegradable.

Reductive Dechlorination of PCBs in Upflow Anaerobic Filter Reactors

Anaerobic filter reactors offer the possibility of a continuous process to treat PCB-contaminated water. In prior experiments in an anaerobic reactor of different design (sludge blanket), PCB-dechlorination products were not observed after prolonged operation with inocula from several Hudson River sites. When four anaerobic upflow filter reactors, each started with a different inoculum (two each from Silver Lake and the Hudson River), were fed progressively increasing concentrations of a mix of five PCBs, a dechlorination product was observed with three of the reactors. The product, identified as 2,3,5,6-CB, was the same in each reactor. The only congener in the mix that could have produced this product by a simple dechlorination was 2,3,4,5,6-CB. The removal efficiency of the anaerobic filters exceeded 99.9% of the added PCBs. Sorption to the bacteria could account for most of the removal but biological processes other than the observed reductive dechlorination may also be involved.

PCB Transformation by Aerobic Hydrogen-Oxidizing Bacteria

Anaerobic reductive dechlorination (see above) occurs in the environment and has been demonstrated in the laboratory with inocula from such sites. There are reports in the literature of aerobic dechlorination of some aromatic compounds. It seemed likely that aerobic

dechlorinases for PCBs might also exist and that the hydrogenases of hydrogen-oxidizing bacteria might perform this function. The hypothesis has been examined with well-characterized organisms from culture collections and new isolates from PCB-contaminated soils and sediments. The isolates were grown autotrophically on minimal plates in an atmosphere of 10% CO_2 (the carbon source), 5% O_2, and 85% hydrogen. A mixture of five PCB congeners was selected for the assay. 2,6,2′6′-CB was selected as an internal standard on the basis of resistance to both known aerobic biodegradation and the *m,p*-dechlorination occurring in the Hudson River. The other congeners were known to be sensitive to the *m,p*-dechlorination system active in the Hudson River sediments. With five of the isolates and one organism from the culture collection, all four of the congeners were depleted but to different extents. Two patterns were observed. Unexpectedly, the internal standard also was affected. To date only one metabolite has been observed. It has not been definitively identified, but it did not correspond to any expected dechlorination product. It is clear that these isolates are metabolizing PCBs, but the nature of the transformations is not understood. Despite considerable growth in knowledge of aerobic and anaerobic PCB metabolism, these surprising results with hydrogen-oxidizing bacteria suggest that much still remains to be learned before we have a comprehensive view of the mechanisms by which bacteria can attack PCBs.

Identification of PCB Transformations in Nature

Determination of environmental changes requires careful analysis of the alterations in congener distribution exhibited by the environmental PCB residues. Use of such procedures over the past four years led to the discovery of a number of distinguishable types of reductive dechlorination occurring in aquatic sediments. In many regions this represents the most important form of PCB biodegradation and detoxification. This year we found that the PCB metabolism in higher organisms can occur via either of two distinguishable forms of biooxidation: a 3,4-monooxidation by cytochrome isozymes of the P450 (i.e., phenobarbital-induced) type, and a 2,3-monooxidation by isozymes of the P448 (i.e., methylcholanthrene-induced) type. Metabolism by P450-like enzymes appears to be the dominant form of PCB degradation in humans, mammals, crustaceans, and in some unidentified component of the food chain in the Great Lakes. A less extensive metabolism by P448-like enzymes appears to occur in mammals exposed to methylcholanthrene or chloracnegens and in fish. However, the most important alteration seen in the residual PCBs of the fish of the lower Hudson River is neither of these oxidation processes, but instead is a reductive dechlorination. This particular type of dechlorination is also seen in sediments from New Bedford (Massachusetts), Escambia Bay (Florida), the Housatonic River (Massachusetts and Connecticut), the mid-Hudson River (New York), and the Sheboygan River (Wisconsin).

We have found that the net extent of PCB alteration in environmental samples may be quantified via the relative proportions of chlorine atoms in *ortho* vs non-*ortho* (i.e., *meta* plus *para*) positions in the biphenyl ring system. Since the relationship between these two parameters is linear in the commercial Aroclors, any deviation from the original level of non-*ortho* chlorines can be determined, even when the relative proportions of the various Aroclors in the original release is unknown, thus making it possible to determine the probable composition of the original Aroclor.

Process Modeling of Surfactant Extraction of PCBs From Soil

The current phase concerned a bench-scale process comprising a countercurrent extraction unit and a surfactant/PCB precipitation unit. The design was based on the results of the previously reported Phase 1 study. The study was conducted with soil from the Oakland site contaminated with 100 to 200 ppm Aroclor 1260. The control was a subsurface Oakland soil spiked in the laboratory with 1000 ppm Aroclor 1260. The two soil samples had similar mineralogy. For the control, the process reduced the soil from 1000 to about 40 ppm. The PCB concentration in the waste water was only 1.8 ppb, about the level of solubility. For the environmental sample, the process reduced the soil from 100-168 ppm to 18-24 ppm, a low value but not proportionate to the 25-fold ratio attained for the control. A closer examination revealed several other differences between the control and environmental samples. The process used for spiking affected both particle size distribution and extraction rate. At this time it is not possible to determine whether there is a barrier to PCB extraction for low-level contamination of Oakland soil.

Chapter 1

BIOREMEDIATION OF PCBS IN SOIL

Ronald Unterman, Michael J. Brennan, Ronald E. Brooks and Frank J. Mondello
Biological Sciences Laboratory

David P. Mobley, John B. McDermott and David K. Dietrich
Chemical Process Laboratory

Robert E. Wagner
Organic and Polymer Characterization Unit

GE Research and Development Center
Schenectady, New York

INTRODUCTION

For the past several years we have been conducting research on the bacterial degradation of PCBs bound to soils. This work has employed three of our most competent PCB-degrading strains (MB1, H850, and LB400) and has involved several matrices (PCB-spiked sand and "clean" soil, and actual environmental samples) [GE Reports, 1985-1987; Unterman et al., 1987a, 1987b, 1988a, 1988b]. The major portion of our more recent work has been with an environmental sample from the site of a former drag racing strip where PCB oils were used for dust control. This soil contains 525 ppm of a transformed Aroclor 1242. It is depleted in the di- and trichlorobiphenyls and therefore appears similar in composition to Aroclor 1248.

Laboratory resting cell assays (high cell concentration, shaking, 30 °C) with this soil and LB400 demonstrated substantial and rapid biodegradation (approximately 50% in 3 days [GE Report, 1986]). In subsequent laboratory experiments using experimental conditions more suitable to an in situ application of bacteria (percolative application of LB400 to standing soils, fewer cells, no shaking, 25 °C) it took approximately 100 days to achieve 50% biodegradation in the topmost soils [GE Report, 1987]. These studies were done in preparation for an actual field test application of LB400 at the drag strip. This test was conducted in the summer of 1987 and the results are discussed below. In addition to the 1987 field test, we have continued our laboratory research to further investigate the factors influencing soil PCB biodegradation, as well as the feasibility of using two complementary PCB-degrading bacterial strains (MB1 and LB400) to degrade a wider range of PCB congeners more rapidly.

RESULTS AND DISCUSSION

Drag Strip Site Test

The site used for this test was the former drag racing strip discussed in prior reports [GE Reports, 1986, 1987]. The Aroclor contamination was limited to the top 6 in. of a relatively

sandy soil. This shallow contamination with a lower chlorinated Aroclor mixture (1248-like) on soil with moderately good percolation made this an ideal site for a biodegradation test using LB400.

The site for the test was prepared by rototilling a 17- by 40-ft area to a depth of approximately 20 cm in an effort to create a vertically homogeneous PCB distribution, which ranged from 50 to 500 ppm across the test site. A transparent tent was erected over the area to protect the site from the elements and to provide some solar heating. (This tent later proved to be as much of a handicap as an asset because it resulted in overheating of the test site.) Two 3- by 3-m test plots were marked off for the test, one for the biodegradation trial, the other for a control. The experimental and control plots were dosed three times a week with 200 l of LB400 (2×10^9 cells/ml) and 200 l of buffer solution, respectively. Prior to each dosing, half of each plot was rototilled to determine the effects of soil mixing on a larger scale. Samples were collected weekly for analysis of bacterial population and PCB concentration.

The test began in June 1987 and the last dosing occurred on October 21, 1987. Before the test began, the soil was sampled using a 1- by 1-ft grid configuration throughout the entire tent area; a final soil sampling in November was configured similarly. While the analysis of these grid samples (plus some test borings) is not yet complete, the analysis of the weekly samples has been completed and provides some results for evaluation.

PCB biodegradation was first detectable after 6 to 8 weeks. Figure 1-1 shows that at 6 weeks only the more readily degraded congeners, characteristic for LB400, were slightly degraded: peak 4 (2,5,4′-CB), peak 10 (2,5,2′5′-CB), peak 14 (2,5,2′3′-CB), and peak 17 (2,3,2′3′-CB). After 16 weeks the soil in the top centimeter of the unstirred portion of the test plot showed substantially more degradation. PCB peaks 4 and 17 (Figure 1-1) were almost completely degraded and many of the other di-, tri-, and tetrachlorinated congeners (peaks 1, 2, 6, 7, 10, 11) were significantly depleted. However, little activity was seen on the most highly chlorinated congeners, peaks 20 through 33. After 19 weeks, approximately 26% of the PCB had been degraded from the top centimeter of the unmixed portion. The soil from the lower depths of the unmixed soil, which received fewer bacteria, displayed less, but significant, PCB destruction. The mixed half showed approximately 19% destruction, but most significantly, throughout the complete 15-cm mixing depth. No PCB destruction was evident in any of the soil samples from the control plot (see Figure 1-2).

The rate of biodegradation at the drag strip (approximately 25% in 100 days) was about one-half the rate seen in the corresponding laboratory experiments. A large portion of the rate reduction can probably be attributed to the poor control of soil temperature and moisture content. This was particularly a problem during the exceptionally hot weather experienced during July. The air temperature in the tent often exceeded 50 °C and the soil temperature exceeded 43 °C at the surface, thus drying the soil and overheating and desiccating the bacteria. LB400 cell counts on the soil two days after dosing were virtually zero during this period. The converse was true for the months of September and October, which together were the wettest (little solar heating) on record for this area. This often resulted in temperatures of 10 to 15 °C. As can be seen in Figure 1-3, high temperatures (50 °C), as well as low, dramatically influence the rate of PCB biodegradation. Although the tent was constructed to

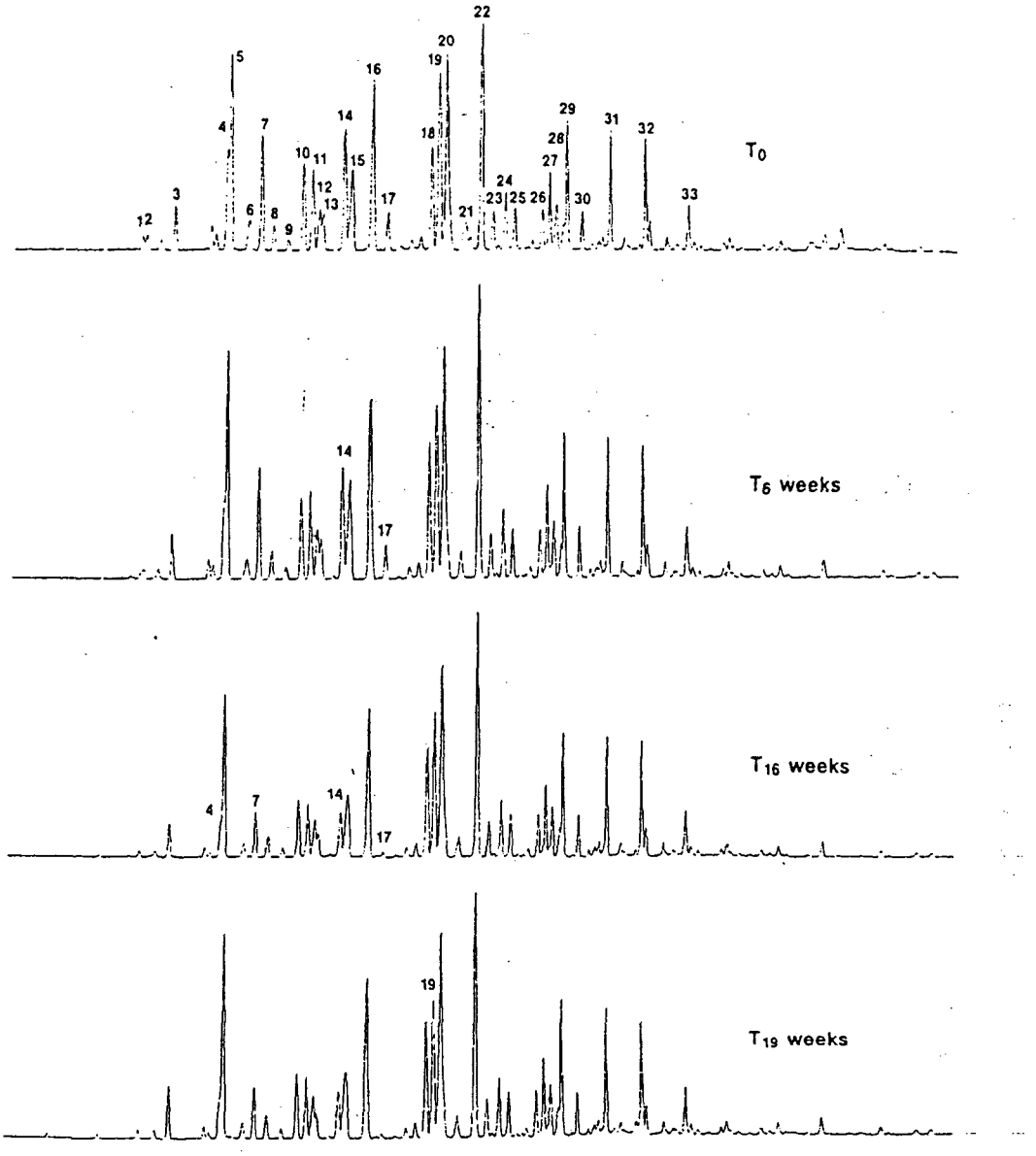


Figure 1-1. Time course of PCB biodegradation at drag strip field trial. Soil samples were taken weekly, extracted, and analyzed (GC) for PCB composition. Samples shown here were from the top centimeter of the unmixed, experimental plot (LB400 dosed).

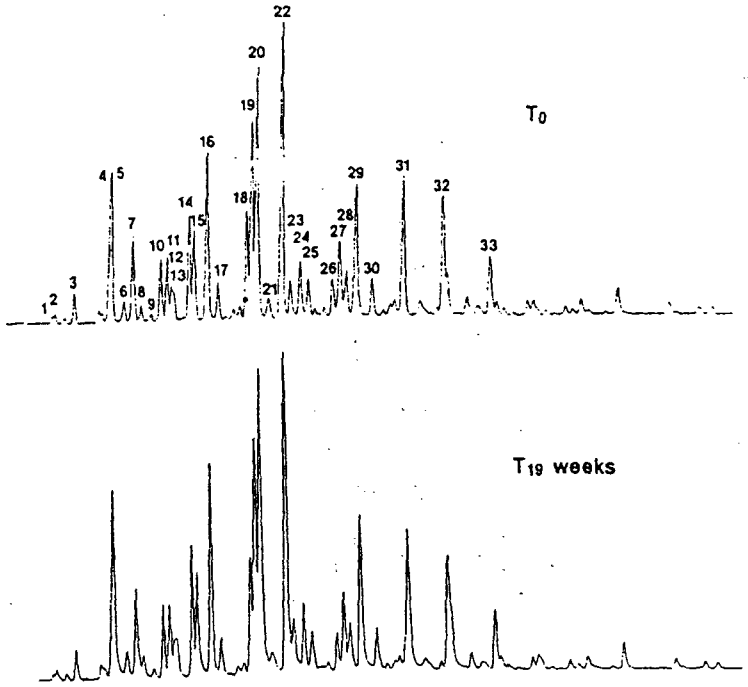


Figure 1-2. Drag strip field trial – control plot. Samples were analyzed as in Figure 1-1, except these samples were taken from the top centimeter of the unmixed control plot (buffer dosed).

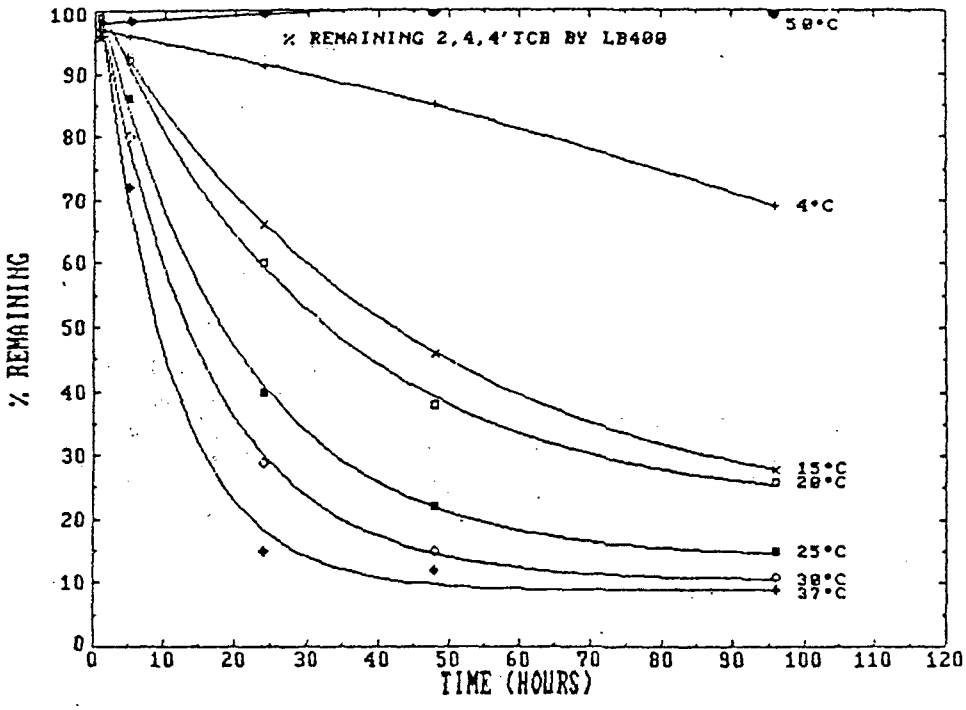


Figure 1-3. Effect of temperature on rate of PCB biodegradation by LB400. Depicted are the rates of 2,4,4´-CB degradation using a standard Mix 1B resting cell assay [Bedard, et al., 1986] performed at various temperatures.

provide solar heating for optimizing the rate of PCB degradation (see Figure 1-3, 37 °C), control of overheating was an important problem. The lower temperatures of autumn (exceptional or not) are part of the reality of field trials in northern regions. This experiment was the first in situ PCB biodegradation field test ever conducted. The results were modest compared with laboratory tests, but still very encouraging; a great deal has been learned about the difficulties likely to be encountered during field applications of this type.

Ultimately the best biodegradation strategy will involve a microorganism that remains viable for an extended period of time in the environment (therefore requiring few dosings) and has a very high level of activity. Toward this goal, we have isolated the genes responsible for the PCB-degrading capabilities of LB400 and have cloned them into *E. coli* (see Chapter 4). This clone has recently demonstrated PCB activity similar to LB400. With the genes now isolated from LB400, one can begin to engineer a superior, environmentally optimized bacterium for in situ treatment of PCB-contaminated soil.

Mixed Culture Laboratory Studies

As we have discussed before, our collection of bacterial strains exhibit two distinct classes of congener specificity as exemplified by MB1 and H850 (or LB400) [GE Reports, 1984 through 1987; Bedard et al., 1984, 1986, 1987b; Unterman et al., 1988a]. MB1 is better able to degrade double-*para*-substituted PCBs (e.g., 4,4′-CB) and H850 (and LB400) are better able to degrade congeners with blocked 2,3 positions (e.g., 2,5,2′5′-CB). We have previously demonstrated this specificity, and the advantages of dual cultures, for PCB biodegradation on soils [GE Report, 1986, Figure 7-1, p. 41; Unterman et al., 1988a]. However, these initial studies were conducted at low PCB concentration (50 ppm Aroclor 1242), with spiked "clean" soils, and using a resting cell assay (high cell concentration, shaking, 30 °C). In an effort to more realistically model an in situ approach to dual culture PCB bioremediation, we have now conducted mixed culture experiments with an environmental sample (drag strip soil, ~500 ppm Aroclor 1248-like), and using an "in situ" protocol (percolative application of LB400, no mixing, 25 °C).

In the first of these experiments, 300 g of drag strip soil was dosed three times a week with 40 ml (~10^9 CFU/ml) of either MB1 or LB400, allowed to percolate, and left at ambient temperature (25 °C). As shown in Figure 1-4 and Table 1-1, each organism again exhibited its specific congener depletion pattern.

It is clear from the results shown in Table 1-1 that MB1 preferentially degrades double-*para*-substituted congeners (X,4,4′-CB, etc.), but LB400 better degrades those congeners with a 2,5-substituted phenyl ring. (Peak 12 – 2,4,2′4′-CB – is an exception to this pattern of activity for which we as yet have no complete explanation.) Thus, the use of these complementary bacterial strains in mixed culture or sequential in situ bioremediation should likewise provide a greater extent of PCB destruction, as first demonstrated for the resting cell case described previously [GE Report, 1986, p. 41].

To test this strategy we conducted a sequential dosing of LB400 and MB1 (130 ml, three times weekly) on 1-kg drag strip soil samples using the in situ protocol described above. Two samples were first dosed with LB400 for 37 weeks, then the dosing changed for the subse-

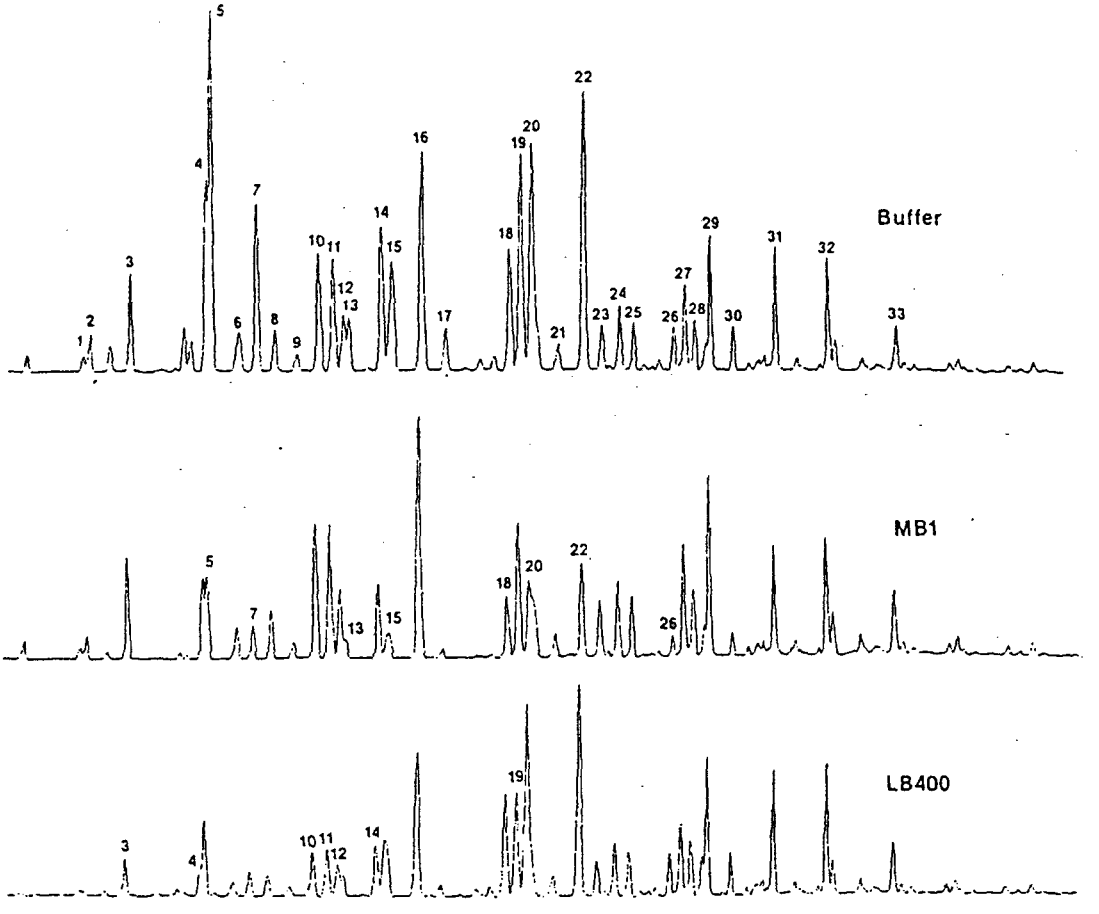


Figure 1-4. Differential congener specificity of MB1 and LB400 biodegradation of drag strip soil PCBs using an "*in situ*" protocol. 300 g of soil were dosed thrice weekly with 40 ml of buffer or bacterial culture ($\sim 10^9$ CFU/ml) for 34 weeks. Samples were extracted and analyzed using GC.

Table 1-1

CONGENER SPECIFICITY OF MB1 AND LB400 BIODEGRADATION OF DRAG STRIP SOIL

Peak No.	PCB[a] Congener(s)	Preferentially Degraded by MB1	LB400
4	2,5,4′		+
5	2,4,4′	+	
10	2,5,2′5′ [b]		+
11	2,5,2′4′		+
12	2,4,2′4′		+
14	2,3,2′5′		+
15	3,4,4′ [b]	+	
18	2,4,5,4′	+	
19	2,5,3′4′		+
20	2,4,3′4′ [b]	+	
22	2,3,3′4′ and 2,3,4,4′	+	

[a] Data tabulated from experiment described in Figure 1-4
[b] Major component of coeluting congeners

quent 34 weeks, one getting buffer (Figure 1-5, panel B), the other getting MB1 (Figure 1-5, panel C). A third sample was dosed with buffer for both periods (37 + 34 weeks) and, as expected, showed no degradation (Figure 1-5, panel A). The pattern of congener specificity seen in panel B is characteristic for LB400, as discussed above: note the depletion of peaks 10, 11, 12, 14, and 19, and little or no activity on peaks 15, 18, 20, 22, 26, and 30. This was the same pattern seen when initially analyzed following the first 37-week dosing, with no further changes during the 34-week buffer dosing because of the very short survival rate of LB400 on these soils. This is evident by comparing panel C with panel B, where one sees little or no continued degradation of congeners that MB1 is unable to degrade (peaks 10, 11, 12, 14, and 19). Conversely, a comparison of panel C with B illustrates that MB1-degradable congeners that were essentially untouched by LB400 during the first 37-week dosing were now degraded by this complementary organism (see peaks 15, 18, 20, 22, 26, 30, and 31).

Thus we have now been able to demonstrate that sequential dosing with two complementary strains, using an in situ protocol, can effect a greater extent and breadth of PCB congener degradation on an environmental sample. We believe that this approach of exploiting the different biochemical capabilities of our two classes of PCB-degrading organisms will ultimately be the most successful approach to aerobic bioremediation, whether with mixed cultures or, alternatively, with a genetically engineered strain combining both activities.

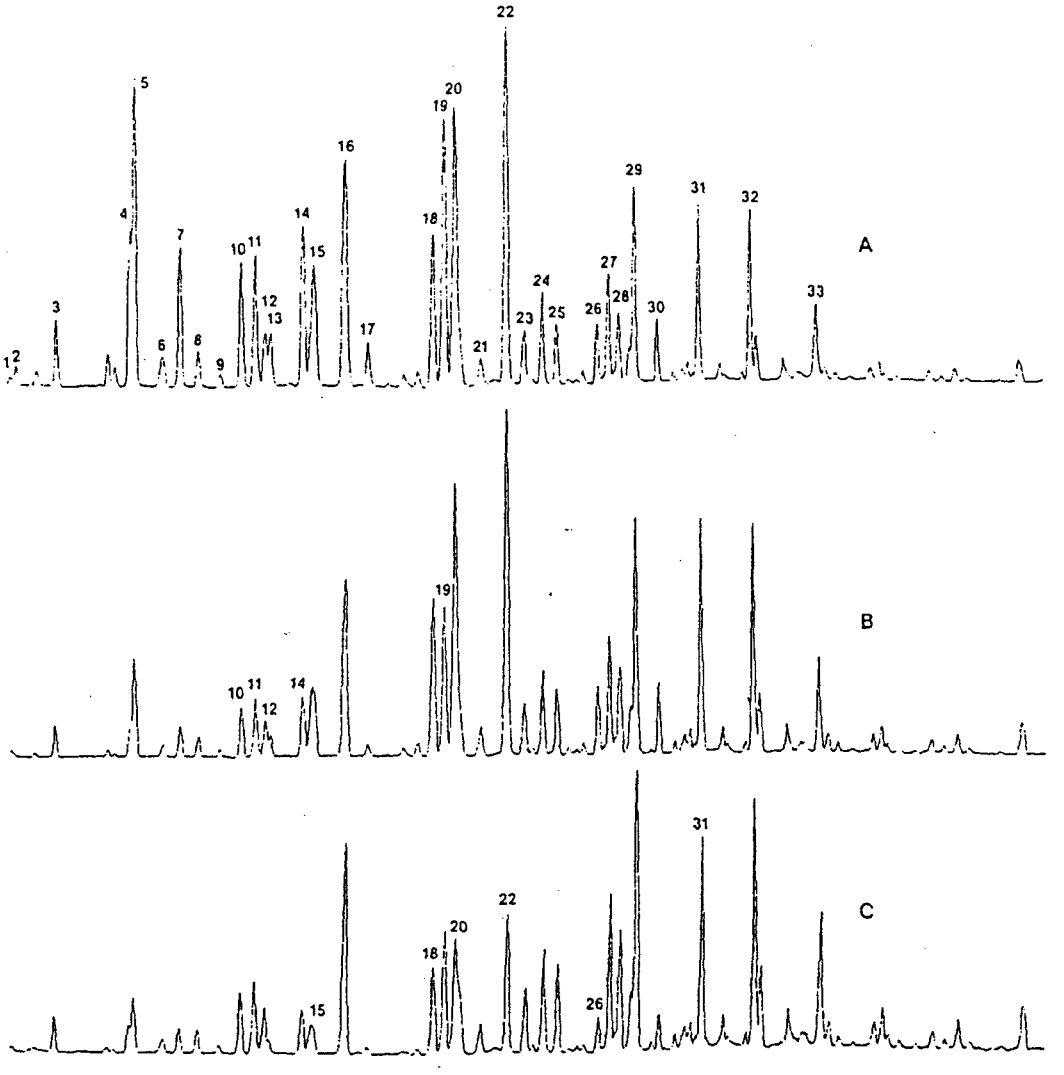


Figure 1-5. **Effect of sequential dosing of drag strip soil with LB400 and MB1. See text for details. Panel A, buffer/buffer; panel B, LB400/buffer; panel C, LB400/MB1.**

SUMMARY AND CONCLUSIONS

Our major effort this year was the first recorded field trial of a biotreatment approach to environmental PCB contamination. The results showed modest but significant PCB destruction, illustrate the feasibility of this technology, and encourage further development. Laboratory studies with two complementary cultures (MB1 and LB400) have demonstrated the benefits of using a dual culture strategy to effect greater PCB destruction. However, the many variables affecting organism viability, establishment, competition, and rates of degradation must be better understood if we are to achieve our goal of 90 to 99% destruction.

FUTURE PLANS

We will continue to study both in situ and reactor approaches to PCB bioremediation. The drag strip soil studies with MB1 will be continued in order to further assess its microbial stability and congener specificity. Research will be carried out to address such critical variables as (1) PCB availability (shaking rate, surfactants, solvent redeposition, ball milling, oil phase partitioning), and (2) factors affecting bacterial activity (temperature, cell number, oxygen availability, moisture content, soil pH, inhibitors). Some of these laboratory studies will be conducted with our recombinant DNA strains of PCB-degrading bacteria as a first step toward the eventual use of future generations of high-activity, engineered PCB degraders. Other environmental samples containing Aroclors 1242 and 1248, in addition to drag strip soil, will also be included in this aerobic work. In parallel to the in situ approaches, we will pilot a kilogram-scale reactor technology for rapid PCB biodegradation using dual cultures. And finally, if our first efforts at developing anaerobic cultures to dechlorinate PCBs are successful, we would like to assay their capability for biotreating Aroclor 1260 contaminated soils and sediments, for example, those at Oakland, California and Pittsfield, Massachusetts.

Chapter 2

STUDIES OF A PCB-CONTAMINATED INDUSTRIAL SLUDGE

Donna L. Bedard and John A. Bergeron
Biological Sciences Laboratory
GE Research and Development Center
Schenectady, New York

INTRODUCTION

Recently we began research to determine whether microbial biodegradation is a viable option for removing (or significantly lowering the concentration of) PCBs in sludge from a settling tank at an industrial site. The sludge is composed of oily, coarse sand containing ~500 ppm PCB. The pH is about 7 and the sludge is anaerobic (0.2 ppm O_2) at the bottom. Based on plant use, the bulk of the PCB was believed to be Aroclor 1242 with small amounts of Aroclors 1221, 1016, and 1254. Additional organic compounds expected to be in the sludge include trichlorobenzenes, di(2-ethylhexyl) phthalate (DEHP), mineral oil, kerosene, and No. 2 fuel oil. Heavy metals are also present. In preliminary experiments conducted by Ron Unterman, the PCB was refractory to microbial degradation despite a prolonged incubation with a high cell density of *P. putida* LB400. Heavy metal toxicity was suspected.

RESULTS AND DISCUSSION

Figure 2-1 shows a gas chromatogram of an ether extract of the sludge and compares it to the profile of Aroclor 1242. Although the electron capture detector (ECD) primarily detects halogenated compounds, several nonhalogenated compounds have been tentatively identified. The peak designated S_8 is most likely elemental sulfur, a common component of sediments. The peak labeled DEHP has been tentatively identified as di(2-ethylhexyl) phthalate, based on coelution with authentic DEHP on two different columns. The three peaks on the left in the top two panels (following the injection peak) are probably trichlorobenzenes. Several other peaks were water soluble and could be removed by washing the sludge with dilute HCl (compare the top and middle panels). Following removal of these components the PCB profile of the sludge extract looks very much like unaltered Aroclor 1242 containing some Aroclor 1016 and some Aroclor 1254 (right end of the chromatogram).

Our initial experiments were designed to determine whether a water soluble constituent was inhibiting biodegradation of the PCBs in the sludge. Fifty ml of sludge was washed three times with four volumes of either glass-distilled water or 0.1 N HCl and allowed to settle. The sludge and the washes were analyzed for the presence of heavy metals. The results revealed that the sludge contained zinc, aluminum, magnesium, tin, and iron in amounts ranging from 1000 to 3000 ppm, but only zinc (22 ppm), iron (22 ppm), and tin (5 ppm) were elevated in the acid leachate indicating that most of the metals are present in a poorly soluble form. Lead and copper were also elevated in the sludge (238 and 111 ppm, respectively) as were manganese (59 ppm), barium (26 ppm), strontium (32 ppm), arsenic (18 ppm), and vanadium

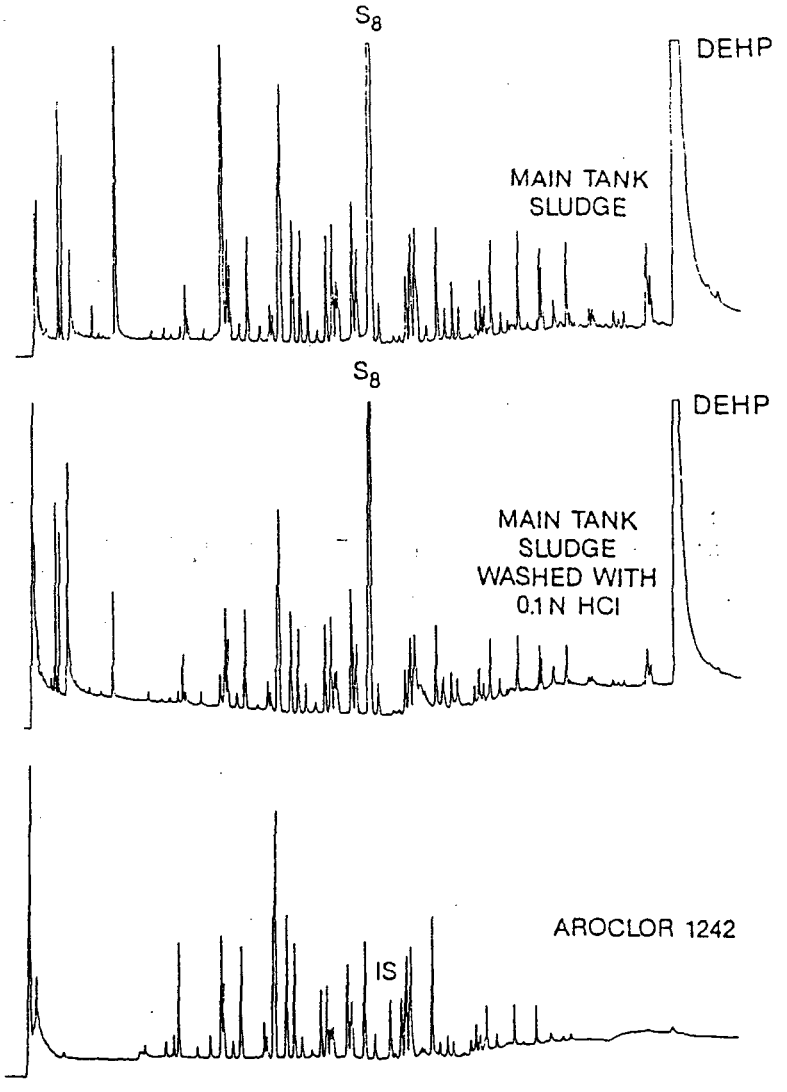


Figure 2-1. DB-1 capillary gas chromatograms of the extract of the sludge (top), the extract of the sludge following washing with 0.1 N HCl (middle), and an Aroclor 1242 standard (bottom). IS refers to an internal standard, 2,4,6,2′4′-CB, that has been added to the Aroclor 1242.

(19 ppm). However, in the leachates, all except magnesium (4 ppm) and strontium (3 ppm) were less than 1 ppm. These results show that the metals are present mainly in poorly soluble form.

Each sample of washed sludge was diluted with phosphate buffer and incubated with resting cells of *Alcaligenes eutrophus* H850 in two separate experiments (2 OD/ml for 7 days and 5 OD/ml for 2 days). The final PCB concentration was ~50 ppm. No biodegradation was

seen in either case, indicating that something in the washed sludge was still blocking degradation. In order to determine whether there was a water soluble inhibitor in the sludge, we collected the water phase from the two original unwashed sludge samples. In each case a concentrated cell suspension of H850 and concentrated phosphate buffer were added to 10 to 11 volumes of the water phase from the sludge to yield a suspension of resting cells at 1 $OD_{615\ nm}$ in 0.05 M sodium phosphate buffer, pH 7.5 (standard assay conditions). Aroclor 1242 was added at two final concentrations, 10 ppm and 100 ppm, and the samples were incubated for 68 h. The PCBs were extracted and analyzed by capillary GC. The results showed that the water phase had no effect on the cells' ability to oxidize PCBs. Based on area (ECD) 50% of the PCB was degraded in the 10-ppm samples containing the water phase from the sludge vs 63% in a standard Aroclor 1242 assay, and 30 to 34% of the PCB was degraded in the 100-ppm samples containing the water phase from the sludge vs 27% in the assay without leachate. These experiments demonstrated that the agent responsible for blocking PCB degradation in the sludge is not in the water phase.

Since there was no evidence of a water-soluble inhibitor, we asked whether the oil component of the sludge was blocking biodegradation. Aroclor 1242 has a very low solubility in water (288 ppb) but is readily soluble in oil (the octanol:water partition coefficient for Aroclor 1242 is 196,500) [Table 1, Chou and Griffin, 1986]. The partitioning of PCB into an oil phase would be expected to depress the availability for biodegradation. Because di(2-ethylhexyl) phthalate is the most readily identifiable oil component in the sludge, we tested its effect on PCB biodegradation. The ratio of DEHP to PCB in the sludge was estimated at 150:1 on the basis of GC analysis; therefore, we compared the biodegradation of Aroclor 1242 (10 ppm) by H850 in the presence and absence of a 150-fold excess of DEHP. The sample without DEHP was 78% degraded in 72 h, but no degradation of the PCB was detected in the presence of DEHP. Subsequently, we compared the degradation of 2,3,2′-CB (50 μM) to 2,3-Cba in the presence and absence of a 150-fold excess of DEHP. This congener was completely degraded to the chlorobenzoic acid in 8 h when DEHP was not present, but was only 16% degraded after 72 h in the presence of DEHP (Figure 2-2). Similar results were obtained for the biodegradation of 2,4′-CB (1 mM) to 4-Cba in the presence or absence of a 75-fold excess of DEHP. Sixty-five percent of the 2,4′-CB was degraded to 4-Cba in 8 h when DEHP was not present vs 15% after 72 h in the presence of DEHP. In the presence of DEHP there was an initial period of 8 to 12 h before biodegradation was detectable; thereafter it proceeded at a fairly constant rate until the incubation was terminated at 72 h.

It is apparent from these experiments that DEHP, at a ratio of 150:1 had a strong negative effect on the biodegradation of PCBs. There are several possible explanations: (1) the DEHP might be toxic, (2) the DEHP might interfere with transport of the PCB into the cell, (3) the DEHP might compete with PCB for, or otherwise affect, the PCB-degrading enzymes, or (4) the DEHP might sequester the PCB and make it unavailable for biodegradation. We have now determined that H850 can utilize DEHP as a growth substrate, hence it does not appear to be toxic. Further experiments will be necessary to elucidate the exact nature of the inhibition exerted by DEHP, but it is clear from these data that there is a stringent kinetic limitation on PCB biodegradation in the presence of DEHP.

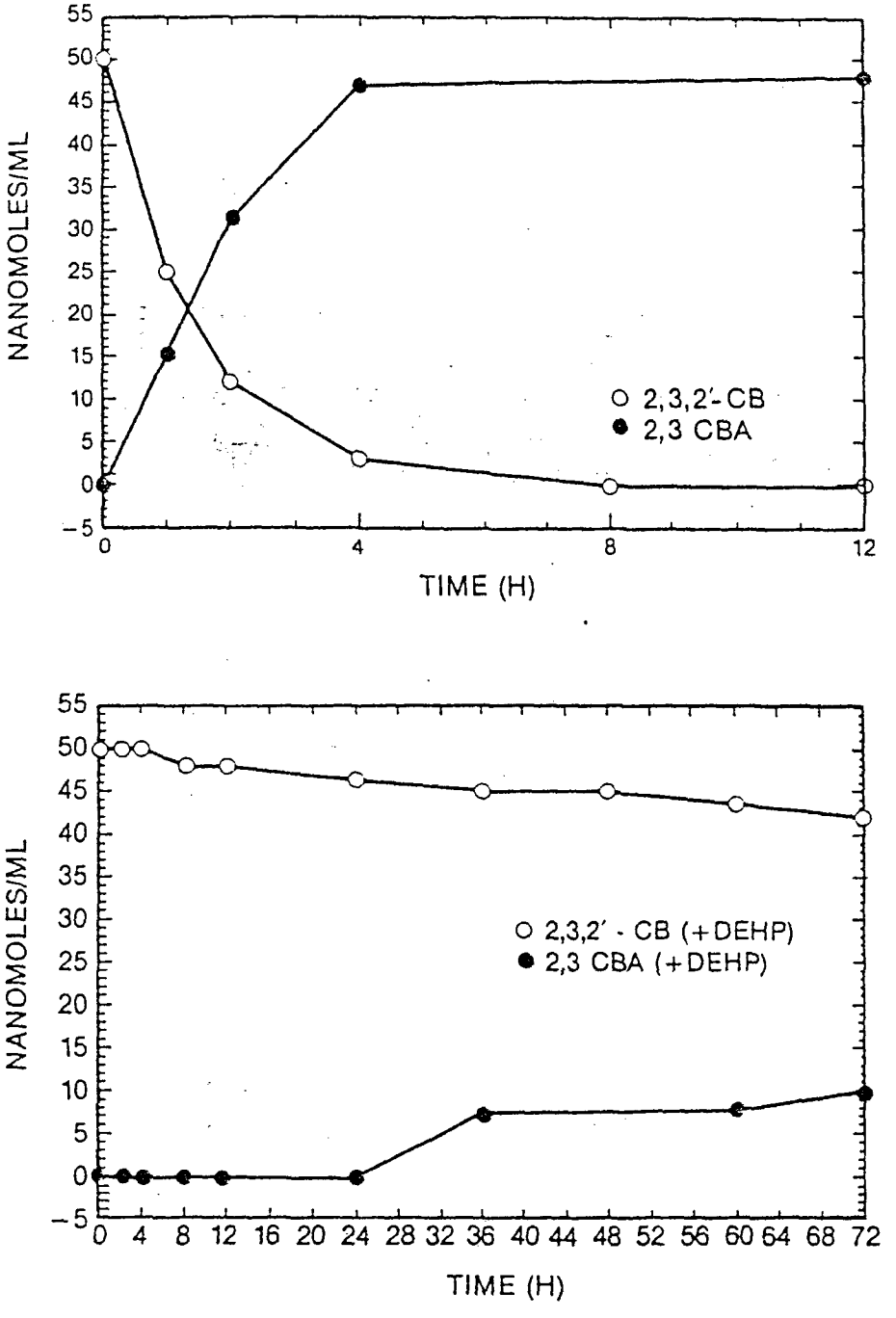


Figure 2-2. Oxidation of 2,3,2′-CB (50 μM) and formation of 2,3-Cba by resting cells of *A. eutrophus* H850 in the absence (top) or presence (bottom) of a 150-fold excess of di(2-ethylhexyl) phthalate.

SUMMARY AND CONCLUSIONS

We have conducted several experiments to determine whether bioremediation is a viable option for removing the PCB in sludge from an industrial settling tank. The results indicate that direct treatment of this sludge with bacteria is ineffective. We have determined that the water phase of the sludge is not inhibitory and we have identified one component of the sludge, di(2-ethylhexyl) phthalate, that has a strong negative effect on the degradation of PCBs but is not toxic to H850.

FUTURE PLANS

Future experiments will be designed to determine whether the effect of DEHP on PCB biodegradation is biochemical, physical, or both. The sludge will be characterized to determine what other components are present, particularly oils, and we will test the effects of these components on PCB biodegradation.

Chapter 3

PHYSICAL CHEMISTRY ASPECTS OF PCB "UPTAKE" AND METABOLISM BY *PSEUDOMONAS PUTIDA* LB400

John A. Bergeron and Lawrence H. Bopp*
Biological Sciences Laboratory
GE Research and Development Center
Schenectady, New York

INTRODUCTION

A better understanding of the kinetics of microbial attack on PCBs may be needed for the development of a practical microbiological process for PCB destruction. The insolubility of PCBs in water is a barrier to kinetic analysis and understanding. Assays of PCB transformation by bacteria commonly employ single congeners, defined mixes thereof, or commercial mixtures (Aroclors) at initial concentrations which, *as a practical necessity,* greatly exceed the solubility in water. Such assays have provided this program [GE Reports, 1982-1987] with measures of the competence of different bacteria for PCB destruction and of the susceptibility to attack of different PCBs. They have also served for studying metabolic pathways. Unfortunately, such assays are not sufficient, per se, for kinetic analysis. Because the system is heterogeneous, the measured PCB concentration is actually the sum of PCB in solution, in suspension, and adsorbed, averaged over the volume assayed (usually the entire reaction volume and vessel surface). Kinetic analysis requires knowledge of the changes in the concentration of the actual reactant or substrate species. In addition, since the slowest process controls overall kinetics and, in the heterogeneous system, could be the transfer (1) from one physical state to another, (2) between phases, (3) from the medium into the cell, or (4) the subsequent interaction with an intracellular enzyme, it is necessary to simplify or dissect the heterogeneous system in order to analyze the kinetics of PCB disappearance.

The experimental strategy chosen to deal with the insolubility of PCBs has been (1) to define the relative proportions of the PCB in solution, in suspension, and adsorbed, when equilibrated in the assay buffer at differing initial nominal PCB concentrations; (2) to measure and characterize the interaction of dissolved or suspended PCB with "killed" (no metabolism) cells, and (3) to study separately the kinetics of disappearance with live cells for dissolved PCB and for dissolved with excess suspended and adsorbed PCB.

The organism used was biphenyl-grown *P. putida* LB400. Based on the range of PCBs degraded and comparative activity based on optical density, it is overall our most effective PCB degrader. In addition, the breadth and depth of knowledge about PCB metabolism, the enzymes involved, and the underlying genetics is arguably the best for LB400 [GE Reports, 1982-1987].

---

* Now located at New York State Department of Health, Wadsworth Center for Laboratories and Research, Albany, New York

Two PCBs were selected for the initial kinetic studies: 2,4′- and 2,4,5,2′5′-CB. Both are attacked effectively by LB400, but by different pathways (2,3- vs putative 3,4-dioxygenase and possibly a monooxygenase). With respect to solubility, the two span much of the range exhibited by PCBs. According to the literature [S.F.J. Chou and R.A. Griffen, 1986] 2,4′-CB is water soluble at 1,260 ppb and the octanol-water partition coefficient (Kow) is 45,708; 2,4,5,2′5′-CB is water soluble at 31 ppb and the Kow is 691,830.

RESULTS AND DISCUSSION

Relation Between Nominal PCB Concentration and Proportion Dissolved, Suspended, and Adsorbed

Because PCBs are denser than water (~1.3 to 1.5 g/ml) even fine dispersions in buffer sediment relatively easily. It is therefore possible to estimate the proportions of PCB in solution, suspended and adsorbed with a simple protocol. Beginning with a known volume of buffer at a given nominal concentration of PCB that has been equilibrated (72 h, mixed by inversion, 20 rpm) the initial aliquot represents both dissolved and suspended PCB, a second aliquot after centrifugation represents dissolved, and the vessel with the remaining volume represents adsorbed, dissolved, and sedimented. After solvent extraction and PCB analysis by gas chromatography, a mass balance can be calculated for allocation of the PCB to solution, suspension, and adsorbed. For 2,4′-CB (Figure 3-1) the limit of solubility was about 4.5 μM.

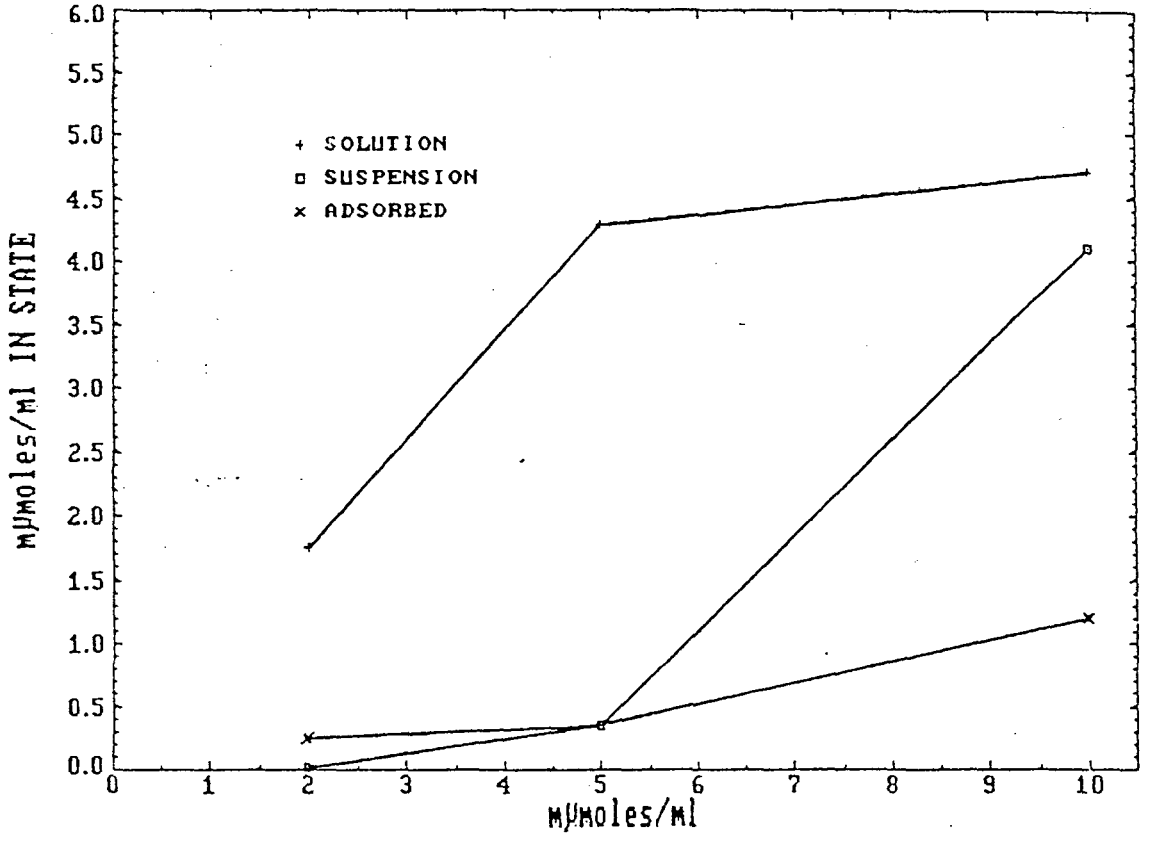


Figure 3-1. **Distribution of 2,4′-CB between solution, suspension, and adsorbed.**

As the nominal concentration approached and exceeded 5 μM the major change was in the proportion in suspension. The amount adsorbed was never a major fraction and appeared to increase linearly. For 2,4,5,2′5′-CB (Figure 3-2), the limit of solubility was very much lower, about 0.1 μM; hence, the amount in solution was only 2 to 10% over the nominal concentration range of 1.0 to 5.0 μM. The amount adsorbed was also small. For 2,4,5,2′5′-CB, the nominal concentration approximated the amount in suspension.

The solubility of 2,4′-CB was adequate (see below) to allow studies of PCB exchange with killed cells, and studies of the kinetics of degradation, both within and beyond the soluble range, permitting the strategy outlined above. The limited solubility of 2,4,5,2′5′-CB restricted studies to the condition when almost all of the PCB was in suspension.

PCB Interaction With Killed Bacteria

In order to examine the interaction of PCB with cells in the absence of metabolism, the bacteria were "killed" by glutaraldehyde, a good preservative of ultrastructure. This bifunctional reagent cross-links protein via amino groups but is comparatively unreactive with other cellular constituents. Cells, harvested and washed by centrifugation in assay buffer, were resuspended in buffered 10 wt% glutaraldehyde, mixed for 24 h at room temperature, harvested, and washed by centrifugation (three times), and stored in the refrigerator.

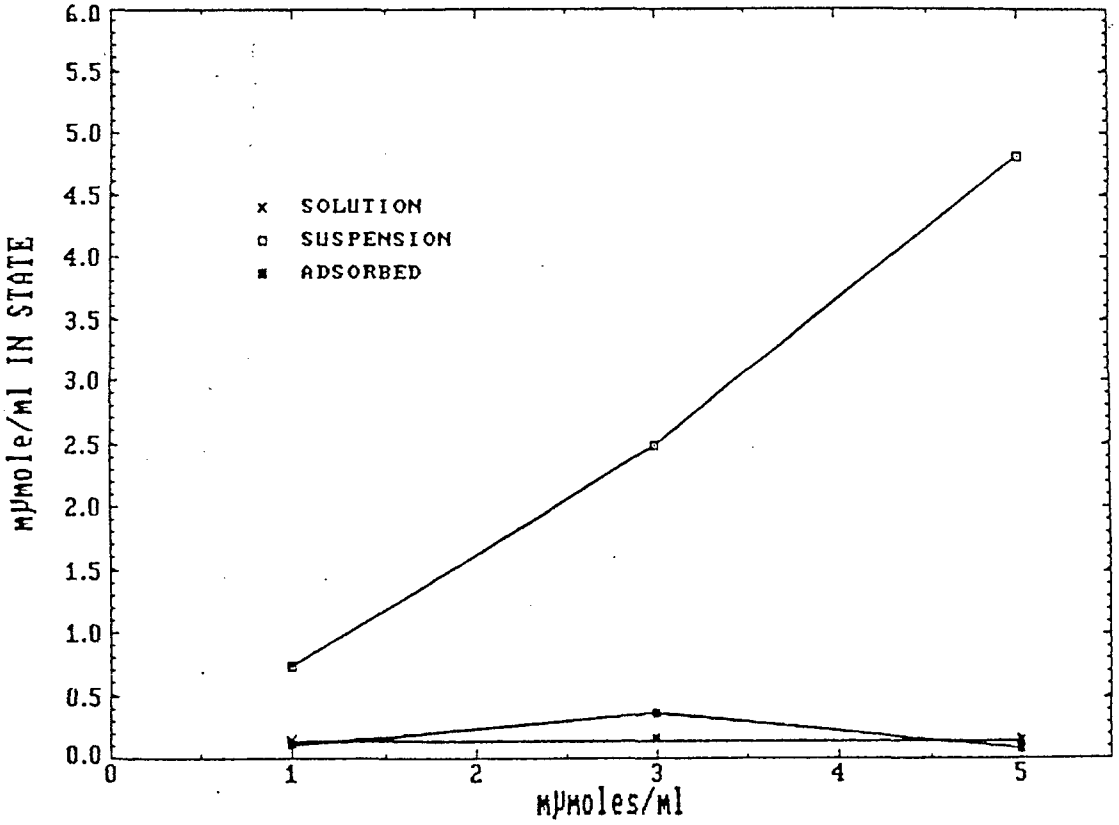


Figure 3-2. **Distribution of 2,4,5,2′5′-CB between solution, suspension and adsorbed.**

Dissolved PCB. 2,4′-CB was added to assay buffer in glass vials to a nominal initial concentration of 5 μM. The system was allowed to equilibrate (72 h). Glutaraldehyde-fixed cells from the concentrated stock were added to known final optical density (OD). The vials, now containing fixed cells, were equilibrated, then centrifuged. An aliquot of the supernatant was analyzed for PCB. A separate group of vials equilibrated at 0.1 OD were diluted by the addition of assay buffer to new OD and allowed to equilibrate (a third time), then centrifuged, and an aliquot of the supernatant was assayed. The relationship between cell concentration, expressed as OD, and the equilibrium concentration of dissolved 2,4′-CB is shown in Figure 3-3 for both the initial equilibration and the new equilibrium attained when additional buffer was added to vials with 0.1 OD cells.

The dissolved 2,4′-CB transferred from the medium into the cells, establishing a new, lower concentration in solution which was dependent upon cell concentration. When such a system was diluted with buffer, the new equilibrium concentration was higher than would be expected just from the dilution; thus, dissolved PCB exchanged reversibly between the external medium and the cells. Separate experiments showed that the exchange was rapid; the equilibrium value was attained within the shortest contact time allowed by the experimental requirement to centrifuge the cells (15 min) out of the medium. The observations fit the calculation from partition theory. The relationship is total PCB = (equilibrium concentration in

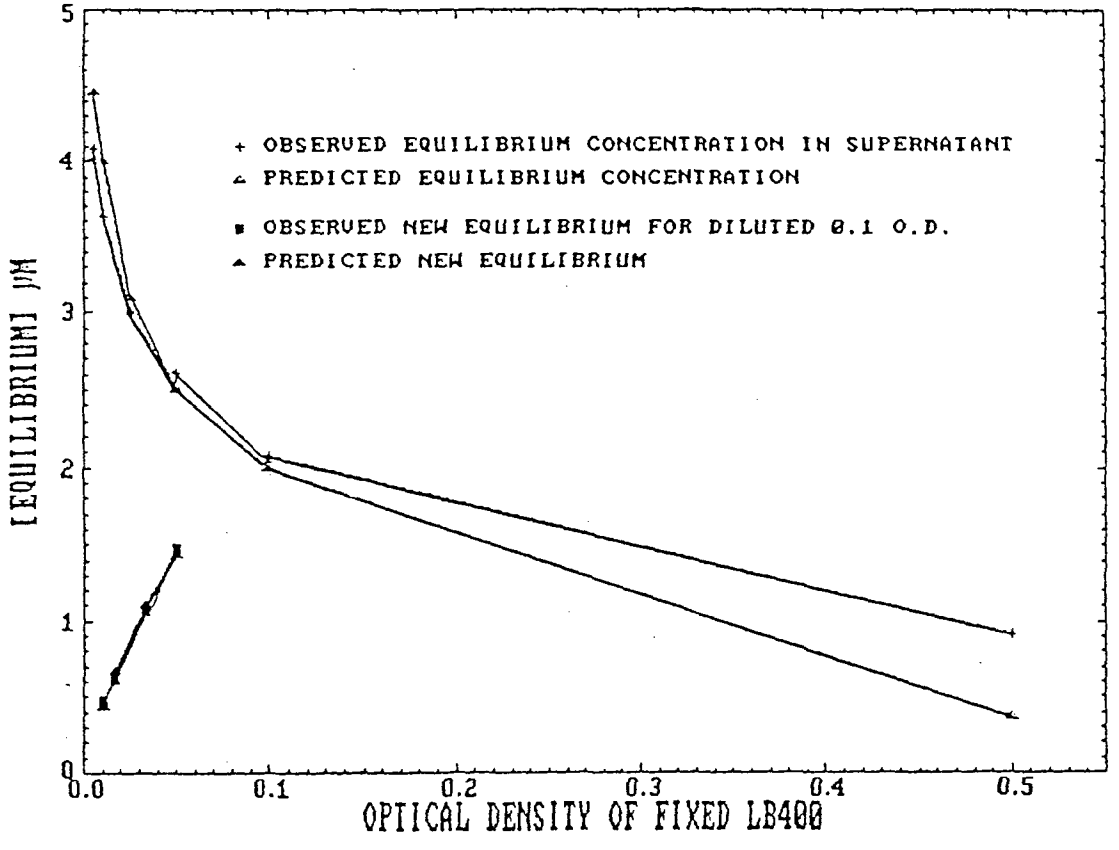


Figure 3-3 Exchange of 2,4′-CB between solution in the aqueous medium and "killed" LB400.

the medium x volume of the medium) + (equilibrium concentration in the medium x Kow x volume of octanol equivalent to the OD of cells) The entire curve was generated from the calculated octanol volume equivalence of 0.1 OD cells by assuming proportionality between octanol volume equivalence and cell concentration. The same relationship predicted the observed new equilibrium concentration after dilution of the medium (Figure 3-3).

Partitioning adequately describes the interaction between dissolved 2,4′-CB and the cells. To the extent that this molecule is representative of other dissolved PCBs and *P. putida* LB400 of other bacteria, the relationship may be generally applicable.

Suspended PCB. When "fixed" cells were added to equilibrated 2,4,5,2′5′-CB in the nominal concentration range of 1 to 5 μm and centrifuged, very little PCB was found in the supernatant; hence, without the knowledge given above that PCB suspensions readily sediment in the same centrifugal field as bacteria, one might conclude that the suspended PCB became cell-associated. The lack of association was demonstrated by experiments with both pH and divalent cation induced flocculation of cells alone and of PCB suspensions. For conditions that preferentially induced cell flocculation with slow sedimentation on standing, the 2,4,5,2′5′-CB concentration in the supernatant above the floc was about the same as before the addition of flocculating agent. It was concluded that suspended 2,4,5,2′5′-CB did not bind appreciably to the bacteria; otherwise, it would have sedimented with the flocculated cells.

From the experiments with killed cells, it can be concluded that PCB interaction with cells is mediated primarily by dissolved PCB and that, in the absence of metabolism, the exchange rapidly approaches an equilibrium caused by partitioning into the cells.

Kinetics of PCB Disappearance

Dissolved 2,4′-CB. There are two experimental options for studying the time course of PCB disappearance: timed aliquots from a reaction vessel, or multiple vessels with timed arrest of the reaction. When adequate sampling is difficult because of heterogeneity and adsorption, the latter approach is preferable and is routinely used in this laboratory. For studies with 2,4′-CB in the soluble range, particularly when multiple short-interval samples were required, the former approach was selected.

Within the range of solubility and for cell concentrations between 0.003 and 0.03 OD, the change in concentration of 2,4′-CB with time was linear when plotted in semilogarithmic coordinates (Figure 3-4). The slope was proportional to cell concentration. At a given cell concentration, the reaction velocity was directly proportional to the dissolved PCB concentration and therefore was first order in PCB. When the relationship between initial velocity and initial PCB concentration was examined as for an enzyme-catalyzed reaction by the double reciprocal (Lineweaver-Burk) plot (Figure 3-5), a straight line was obtained. The intercepts indicate both the maximum velocity for the given enzyme concentration and an apparent K_m (Michaelis constant). The physical meaning of this K_m (~8 μM) is not clear. Since the enzyme(s) involved are intracellular rather than dissolved in the medium and many steps, including transfer into the cells, precede formation of an enzyme-substrate complex, the apparent K_m may be a kinetic (steady state) rather than a thermodynamic (equilibrium) constant.

For dissolved 2,4′-CB, the observed kinetics were consistent with a single substrate enzyme reaction as described by the Michaelis-Menten equation. The integral form of the equation fit the experimental observations satisfactorily (not shown).

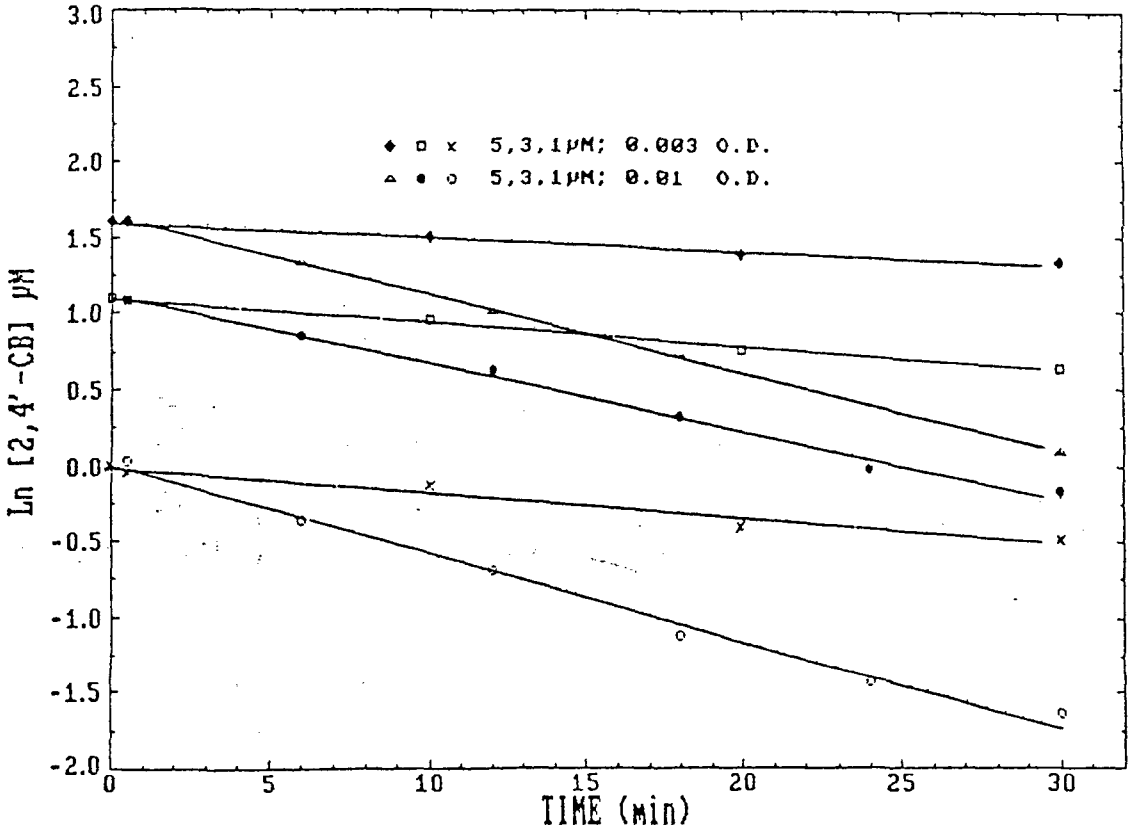


Figure 3-4. **Disappearance of 2,4′-CB for initial concentrations within the soluble range for two concentrations of LB400.**

2,4′-CB at Nominal Concentration Well Above Solubility. It was shown above that for nominal 2,4′-CB concentration above solubility, the concentration in solution at equilibrium is 4 to 5 μM, with the remainder mainly in suspension but with some adsorbed to the vessel wall.

2,4′-CB was added to assay buffer in vials to a nominal concentration of 20 μM and equilibrated. The supernatant from centrifugation was transferred to fresh vials for study of the rate of degradation in the absence of excess suspended 2,4′-CB. An uncentrifuged aliquot was extracted and assayed for the dissolved plus suspended PCB. In parallel kinetic runs, cells were added to an OD of 0.03, and timed aliquots were removed from both vials (dissolved vs suspended, dissolved and adsorbed) and transferred to acidified solvent for extraction and analysis. The residual volumes were acidified and extracted by addition of solvent to the reaction vessel to obtain a mass balance.

The reaction in the supernatant (4 μM) proceeded as described above for soluble 2,4′-CB. The disappearance was essentially complete at 90 min, but with some residual adsorbed PCB (Figure 3-6). The reaction was first order.

The reaction in the heterogeneous system (nominal 14 μM; 4 μM dissolved, 10 μM suspended, ~0.7 nmol adsorbed/ml) was linear over time (independent of nominal concentration) until the concentration in the aliquot fell to the limit of solubility then declined rapidly (Figure 3-6). The PCB recovered from the vessel wall corresponded with the amount calculated by difference to be present initially.

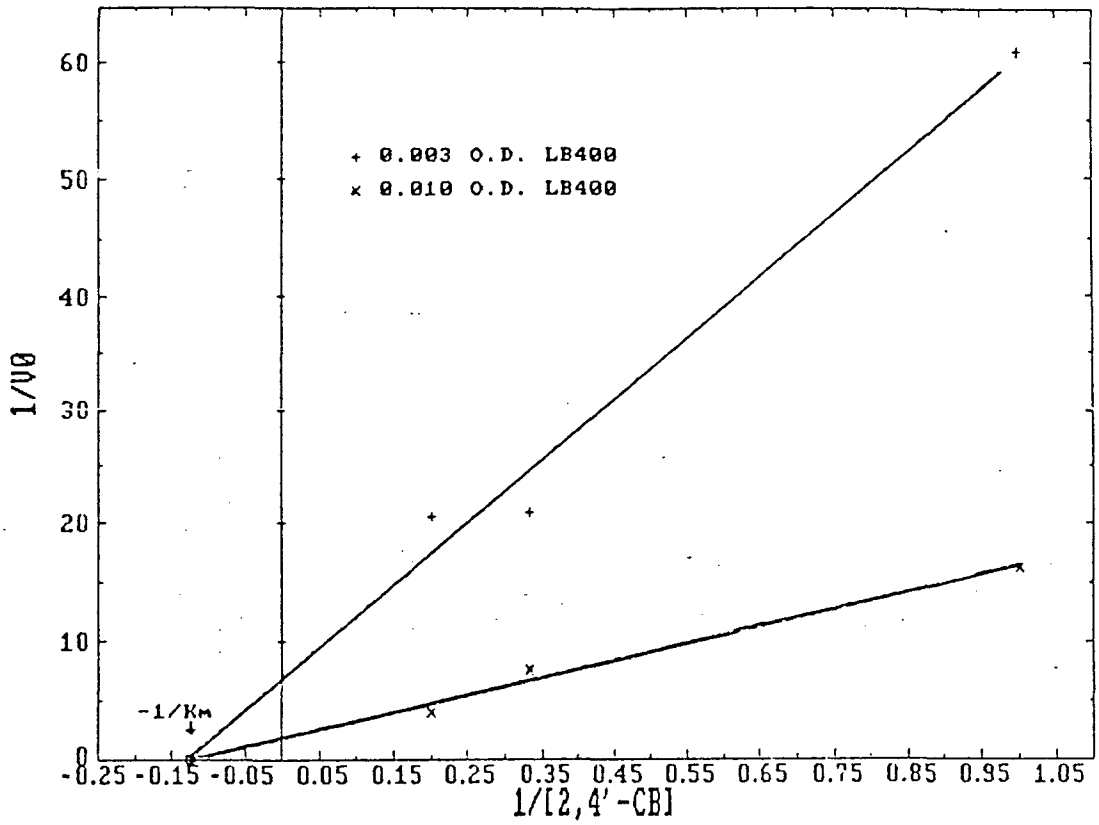


Figure 3-5. **Lineweaver-Burk plots for 2,4′-CB in the soluble range for two concentrations of LB400.**

The results with the heterogeneous, well-mixed system showed that the initial kinetics were determined by the rate at which the excess suspended PCB went into solution. The observed velocity was constant as if the concentration in solution were buffered near the limit of solubility until the suspended excess had dissolved.

2,4,5,2′5′-CB in Suspension and Solution. Since the solubility (~0.1 μM) of 2,4,5,2′5′-CB was too low for studies within the soluble range to be practical, all of the studies were performed an nominal concentrations (1 to 10 μM) where about 90 to 99% of the PCB was in suspension. The assays were performed on timed aliquots as described above. While 2,4,5,2′5′-CB is readily attacked by LB400 and disappears almost quantitatively, the reaction is slower than for 2,4′-CB; hence, longer times and higher concentrations of organism were utilized. With 1 OD cells and 3.0 and 10.0 μM (nominal) 2,4,5,2′5′-CB, the decrease was linear over 60 to 90 min and independent of nominal PCB concentration (Figure 3-7); that is, apparent zero order in PCB. Over long time (24 h), the disappearance for nominal initial concentrations of 1.0 to 10 μM for a broad range of cell concentration (0.1 to 3.0 OD) was apparent second order (Figure 3-8). The plot of the ratio of initial nominal concentration (C0) to nominal concentration at given time (C1) was linear with a slope proportional to cell concentration and inversely proportional to initial nominal PCB concentration.

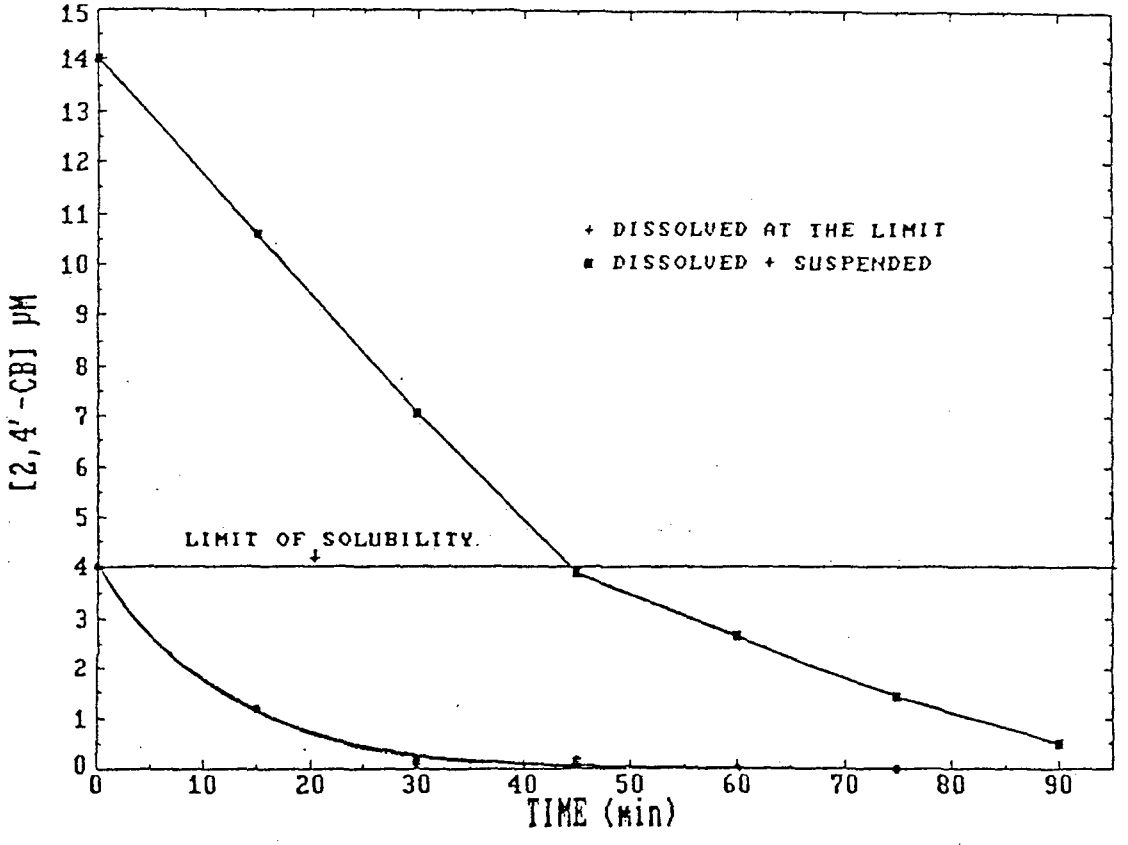


Figure 3-6. **Comparison of the kinetics of disappearance of 2,4′-CB at the limit of solubility and with a large excess in suspension.**

The reaction at short time is consistent with the experience with the 2,4′-CB suspensions: the stirred suspension dissolves sufficiently rapidly to buffer near the limit of solubility, so the initial rate of disappearance at given cell concentration is linear and independent of the nominal PCB concentration. The initial velocity is still proportional to enzyme (cell) concentration because the reaction is sufficiently slow over the range of cell concentration examined (0.10 to 3.0 OD) to be saturated even by the low (~0.1 μM) concentration of dissolved 2,4,5,2′5′-CB. The reason why the rate of reaction declines and exhibits second order kinetics over 24 h is not known but may reflect a form of product inhibition.

SUMMARY AND CONCLUSIONS

The insolubility of PCBs presents a special problem for kinetic analysis. The experiments show that the interaction between bacterial cells and PCBs is mediated by the dissolved PCB and the exchange seems rapid. The kinetics of disappearance of dissolved 2,4′-CB via the 2,3-dioxygenase pathway is consistent with a classical Michaelis-Menten description for a monomolecular enzyme catalyzed reaction. In well-stirred PCB suspensions the rate of solution of the suspended PCB determines the kinetics. Under the conditions used in these experiments, the rate of disappearance was near the value expected if the solution were buffered at the limit of solubility. For 2,4,5,2′5′-CB, a substrate without open 2,3 sites, the kinetics over short time are consistent with the above description for suspensions but change with time suggestive of some form of product inhibition.

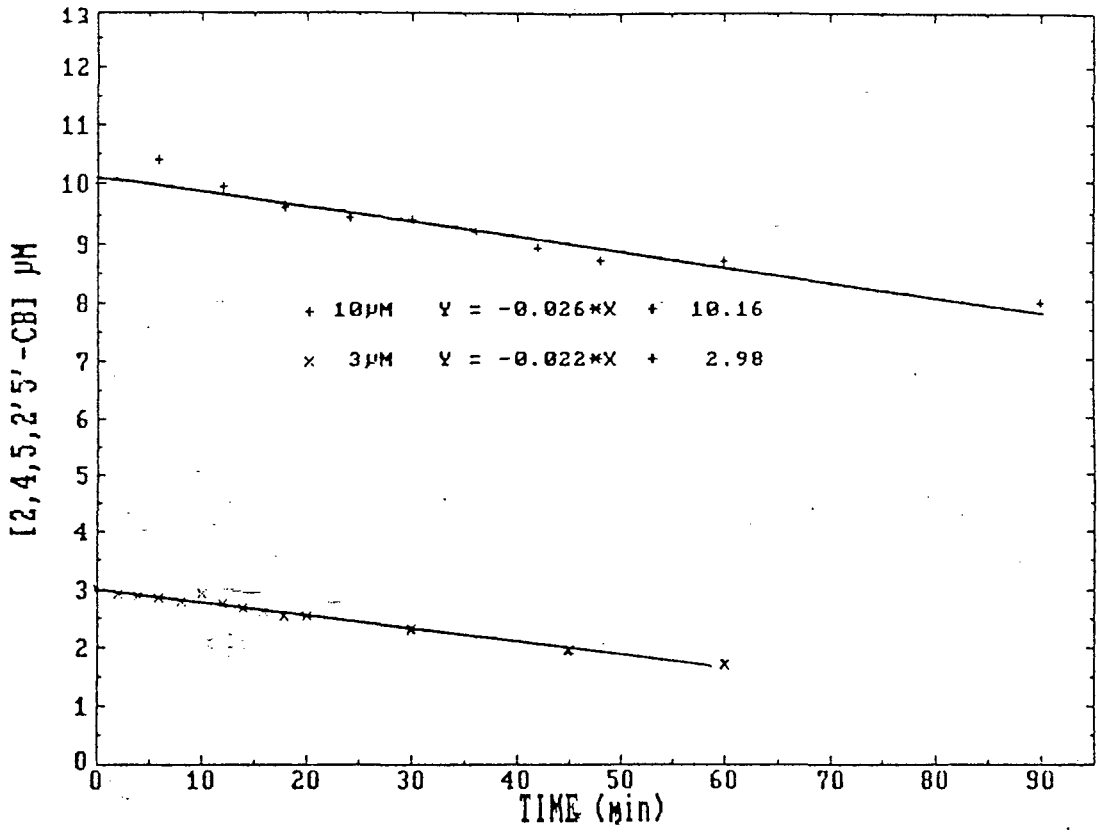


Figure 3-7. Initial kinetics of 2,4,5,2′5′-CB disappearance for two nominal concentrations greatly above the limit of solubility.

FUTURE PLANS

1. Examine more closely the nature of the apparent product inhibition observed with 2,4,5,2′5′-CB and check whether it may be characteristic of substrates without unchlorinated 2,3 sites.
2. Test the generality of the observations on 2,4′-CB with another substrate for 2,3-dioxygenase that also is sufficiently soluble to be studied at concentrations below the limit of solubility.
3. Attempt to determine the origin and potential significance of the apparent K_m (~8 μM) observed for 2,4′-CB.

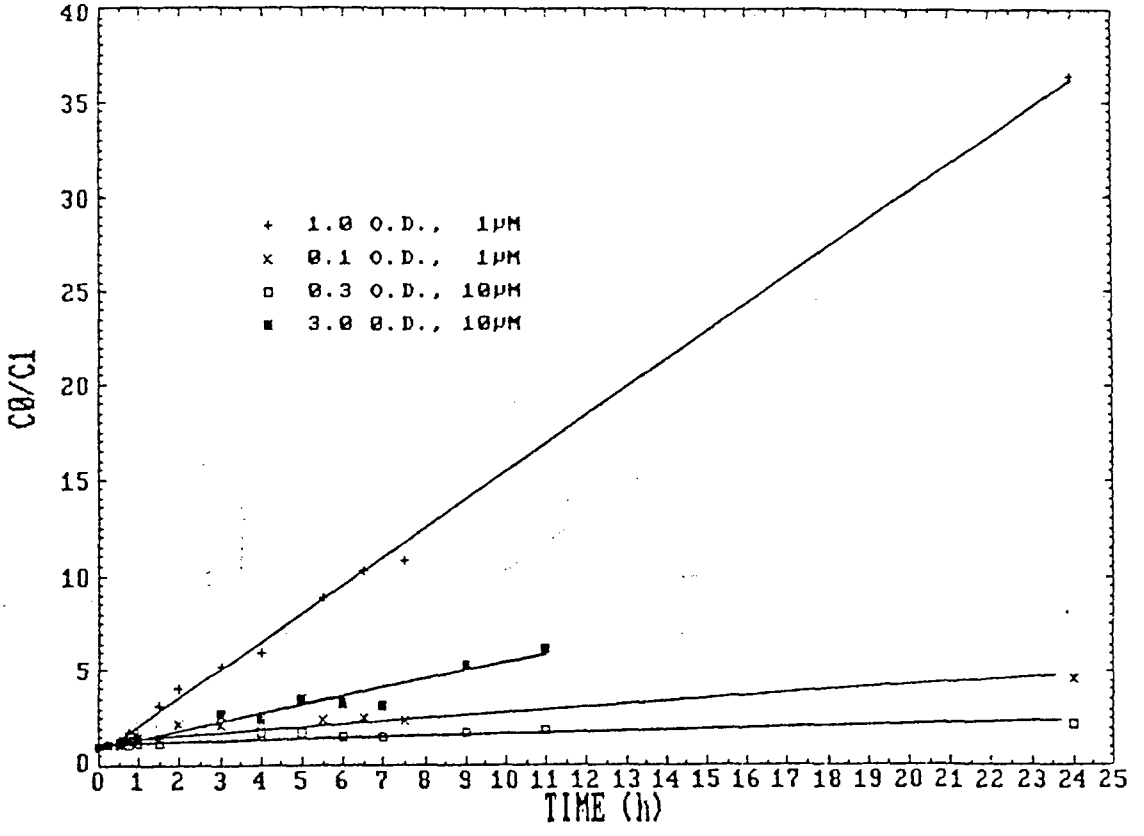


Figure 3-8. Kinetics of 2,4,5,2´5´-CB disappearance over 24 h for two initial nominal concentrations and several concentrations of LB400.

Chapter 4

PCB BIODEGRADATION BY GENETICALLY ENGINEERED BACTERIA

Frank J. Mondello and James R. Yates
Biological Sciences Laboratory
GE Research and Development Center
Schenectady, New York

INTRODUCTION

Last year we reported the cloning and preliminary characterization of the genes involved in PCB metabolism from *Pseudomonas* strain LB400. In those studies, several recombinant plasmids were isolated that conferred on an *Escherichia coli* host the ability to convert a variety of dichlorinated PCBs to their corresponding dichlorobenzoic acids. The results demonstrated conclusively that these plasmids contained the genes encoding the initial four enzymes of PCB/biphenyl metabolism (*bph* A-D) and that these genes could function in a heterologous host [GE Report, 1987].

Recent studies have concentrated on the further characterization of recombinant plasmid pGEM410 as well as the modified derivative pGEM456. This work involved the construction of restriction endonuclease and genetic maps to establish the location of the PCB degradative genes within the cloned DNA fragment, and the examination of the PCB competence of recombinant strains relative to that of the wild type.

RESULTS AND DISCUSSION

Mapping Studies

In order to determine more precisely the location of the PCB degradative genes within pGEM410, and to facilitate additional subcloning studies, a restriction endonuclease map of this plasmid is being constructed.

The digestion of pGEM410 with the endonuclease *Eco*RI results in the formation of eight DNA fragments with sizes of approximately 15.8, 6.65, 6.1, 2.9, 2.3, 2.1, 0.9, and 0.5 kilobases (kb). The relative positions of these fragments was determined by generating a series of subclones from an incomplete *Eco*RI digest of pGEM410 DNA. These subclones contained overlapping multi-fragment segments of the original recombinant plasmid. Extrachromosomal DNA from the subclones was isolated, cut with *Eco*RI, and analyzed using agarose gel electrophoresis. By examining the fragment content of a large number of these clones, the relative position of *Eco*RI sites in pGEM410 was determined (Figure 4-1).

Previous subcloning experiments demonstrated that biphenyl dioxygenase activity requires the presence of both a 2.9- and 6.65-kb *Eco*RI fragment. As predicted, the *Eco*RI map of pGEM410 confirms that these two DNA fragments are adjacent to each other in this plasmid.

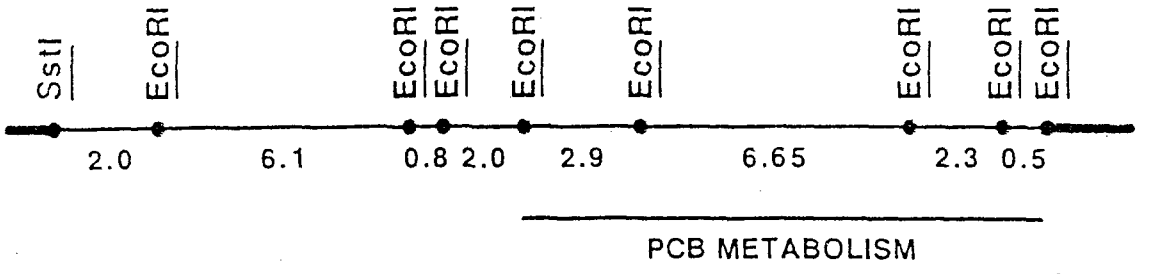


Figure 4-1. Arrangement of *Eco*RI restriction fragments in pGEM410.

The region containing the PCB-degradative genes in pGEM410 has been found to extend from within the 2.9-kb *Eco*RI fragment to a point inside the 0.5-kb fragment. The location of the individual genes within this 12.35-kb region is shown in Figure 4-2.

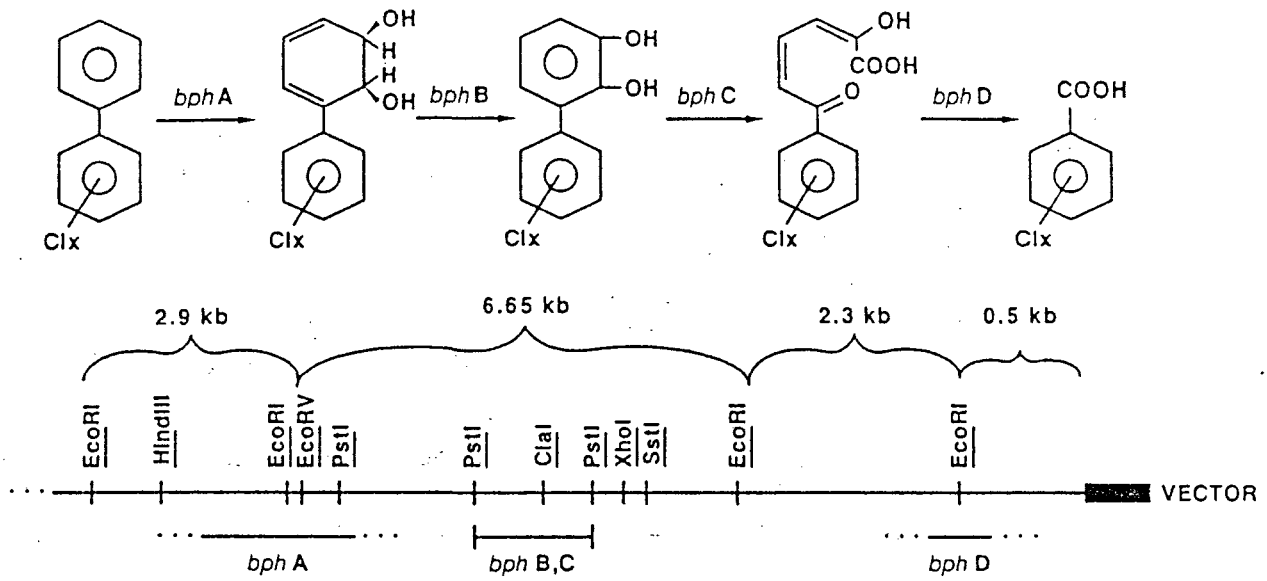
PCB Degradation by Recombinant Strains

An examination of the PCB metabolic capacity of the recombinant strains was conducted so that a comparison could be made to the wild-type strain. The strains used in this study were FM4100 and FM4560. FM4100 consists of *E. coli* strain HB101 containing the original recombinant plasmid pGEM410. FM4560 consists of the *E. coli* strain TB1 containing plasmid pGEM456. This plasmid is a derivative of pGEM410, in which the 2.9- and 6.65-kb *Eco*RI fragments (encoding the initial three enzymes of the *bph* pathway) have been cloned onto the expression vector pUC-18.

The abilities of strains FM4100 and FM4560 to metabolize PCB mixes 1B and 2B were determined by resting cell assay [Bopp 1986]. The recombinant strains were grown using succinate as the carbon and energy source because they are unable to grow on biphenyl. The results of this study are shown in Table 4-1. The ability of FM4100 to metabolize the two PCB mixtures was found to be significantly lower than that of LB400. Unlike the wild-type strain, FM4100 was unable to completely deplete each of the tetrachlorinated biphenyls tested, and was almost totally inactive against the pentachlorinated PCBs and those containing chlorine atoms at both *para* ring positions.

The PCB-degradative competence of the recombinant strain FM4560 was found to be much greater than that of FM4100 for the congeners in the two mixtures. FM4560 demonstrates significant degradative activity against a wide variety of tetra-, penta,- and double-*para*-substituted PCBs. Although the competence of this strain is not as great as that of LB400, it is very similar both in the range of PCBs attacked and in the extent of their depletion.

A comparison of the PCB-degradative competence of the recombinant strain FM4560 with those previously reported [GE Report, 1984] for a variety of environmental isolates is shown in Table 4-2. Based on mix 1B, 2B assays [Bopp, 1986], it is apparent that the recombinant organism is superior both in substrate range and extent of congener depletion to many of the "natural" strains examined. The pattern of activity found for FM4560 is clearly more similar to that of LB400 and the other "high-competence" strains, *Alcaligenes eutrophus* H850 and *Pseudomonas* strain LB410.



bph A - Biphenyl / PCB Dioxygenase

bph B - Dihydrodiol Dehydrogenase

bph C - 2,3-Dihydroxybiphenyl Oxygenase

bph D - Hydrase

Figure 4-2. Genetic map of the region encoding PCB metabolism in pGEM410.

The increased PCB-degradative ability of FM4560 relative to that of the original recombinant strain FM4100 may be due to a variety of different factors. One of the more likely factors is that cells of FM4560 contain more copies of the *bph* gene cluster than do those of FM4100. Vector pUC-18 (used in pGEM456) is usually present in ~60 to 80 copies per cell, compared with 15 to 20 for vector pMMB34 (used in pGEM410). Therefore, each FM4560 cell may have over three times the number of PCB genes (and perhaps enzymes) as a cell of FM4100. Similarly, the increased PCB competence of FM4560 may be a result of more efficient expression of the PCB genes in pGEM456 by a promoter in the vector. Another possibility is that the absence of the hydrase enzyme in FM4560 prevents the accumulation of chlorinated benzoic acids, which may inhibit PCB metabolism by FM4100.

In order to obtain high levels of PCB degradation by LB400, the organism must be grown using biphenyl as the sole source of carbon and energy. Growth of the organism on other carbon sources (e.g., succinate), is known to severely decrease the competence of the strain (Table 4-1). Under these conditions, the PCB competence of LB400 is very similar to that of FM4100 and is inferior to that of FM4560. It is possible that the PCB competence of FM4560 and FM4100 is much greater than actually observed, but is limited because these cells are unable to grow on biphenyl. For the wild-type as well as the recombinant strains, the addition of biphenyl to growth media containing succinate does not increase the activity of the organisms.

Table 4-1

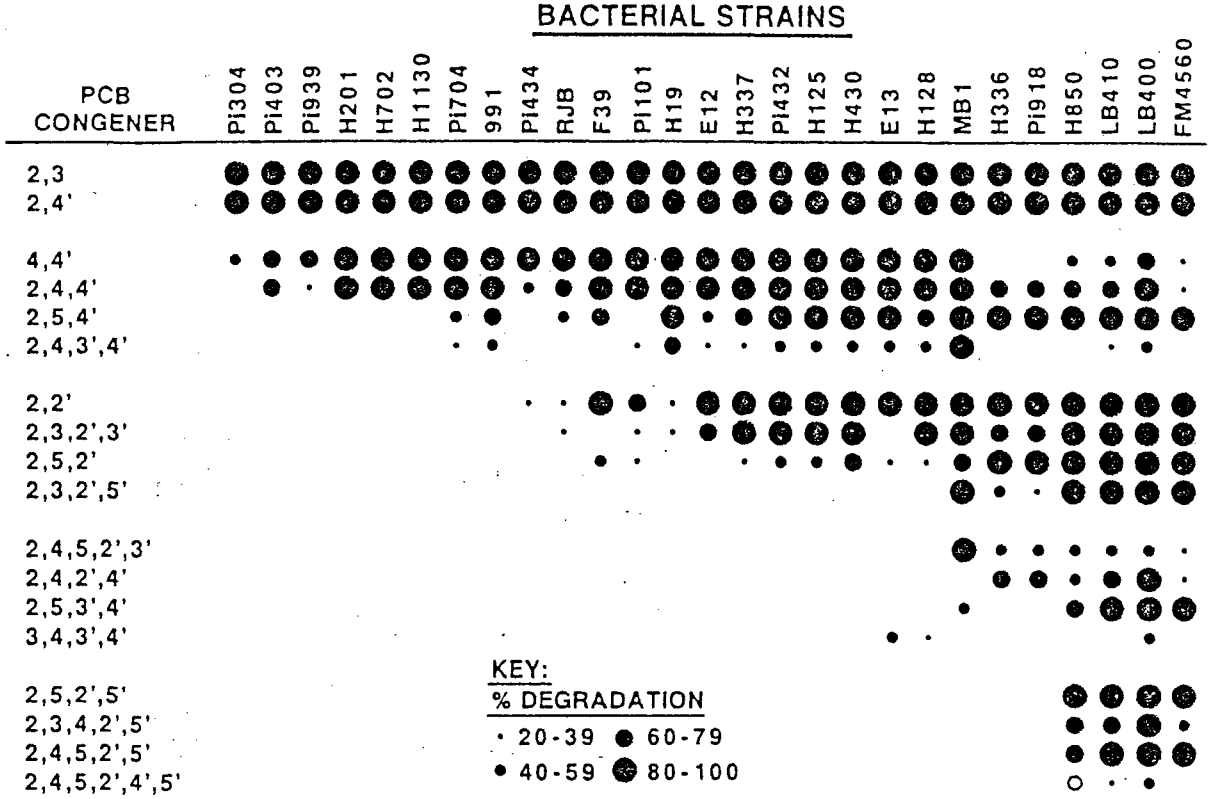
PCB DEGRADATION IN RECOMBINANT STRAINS RELATIVE TO *PSEUDOMONAS* STRAIN LB400

PCB Congener	Percent Activity[a]			
	LB400 (Biphenyl)	FM 4100	FM 4560	LB400 (S)
2,3	100	100	100	100
2,2′	100	100	100	100
2,4′	100	100	100	100
2,5,2′	100	100	100	62
2,5,4′	100	100	100	52
2,3,2′3′	100	45	100	91
2,3,2′5′	100	50	100	88
2,5,3′4′	100	40	100	61
2,5,2′5′	100	62	100	75
2,4,5,2′5′	100	10	87	55
2,3,4,2′5′	100	0	45	25
2,4,5,2′3′	100	0	57	8
4,4′	100	0	34	0
2,4,4′	100	5	85	1
2,4,3′4′	100	0	33	0
2,4,2′4′	100	0	28	0
3,4,3′4′	100	0	0	0
2,4,5,2′4′5′	100	0	50	0

[a] Indicated as the percent of degradation compared with biphenyl-grown LB400
Resting cell assay: Mix 1B, 2B
FM4100: HB101 (pGEM410)
FM4560: TB1 (pGEM456)
LB400 (S): grown using succinate

Table 4-2

PCB-DEGRADATIVE COMPETENCE



The level of PCB-degradative activity in FM4560 makes it a possible candidate for laboratory study on contaminated soils. In fact, the organism may provide several advantages over LB400 in such tests. For example, while FM4560 does not have as high a level of PCB-degradative ability as biphenyl-grown LB400, it does not appear to exhibit the competence instability found in that organism, and may therefore behave more predictably in any future biodegradation process. FM4560 has been found to retain both its viability and PCB activity for much longer periods on laboratory media. If the same is true on soil samples, it may not be necessary to dose as often with the recombinant organism. In addition, *E. coli* strains have a higher optimum growth temperature and tolerance, a faster growth rate, and the ability to grow under anaerobic conditions. All of these factors could contribute to increased PCB metabolism in a soil test.

Degradation of Aroclor 1242

Because the vast majority of environmental PCB contamination involves the commercial mixtures known as Aroclors, we have begun to examine the ability of the recombinant strain FM4560 to metabolize these materials in resting cell assays. Preliminary results indicate that

in a 24-h incubation with 10- and 100-ppm Aroclor 1242, FM4560 was able to degrade approximately 85 and 25% of the PCB, respectively. LB400 was found to degrade 91 and 53% of 10- and 100-ppm Aroclor 1242, respectively. As can be seen in Figure 4-3, the patterns of depletion demonstrated by the two strains appear nearly identical. These early data are promising in that they demonstrate the ability of the recombinant strain to degrade a PCB mixture that is an environmental contaminant.

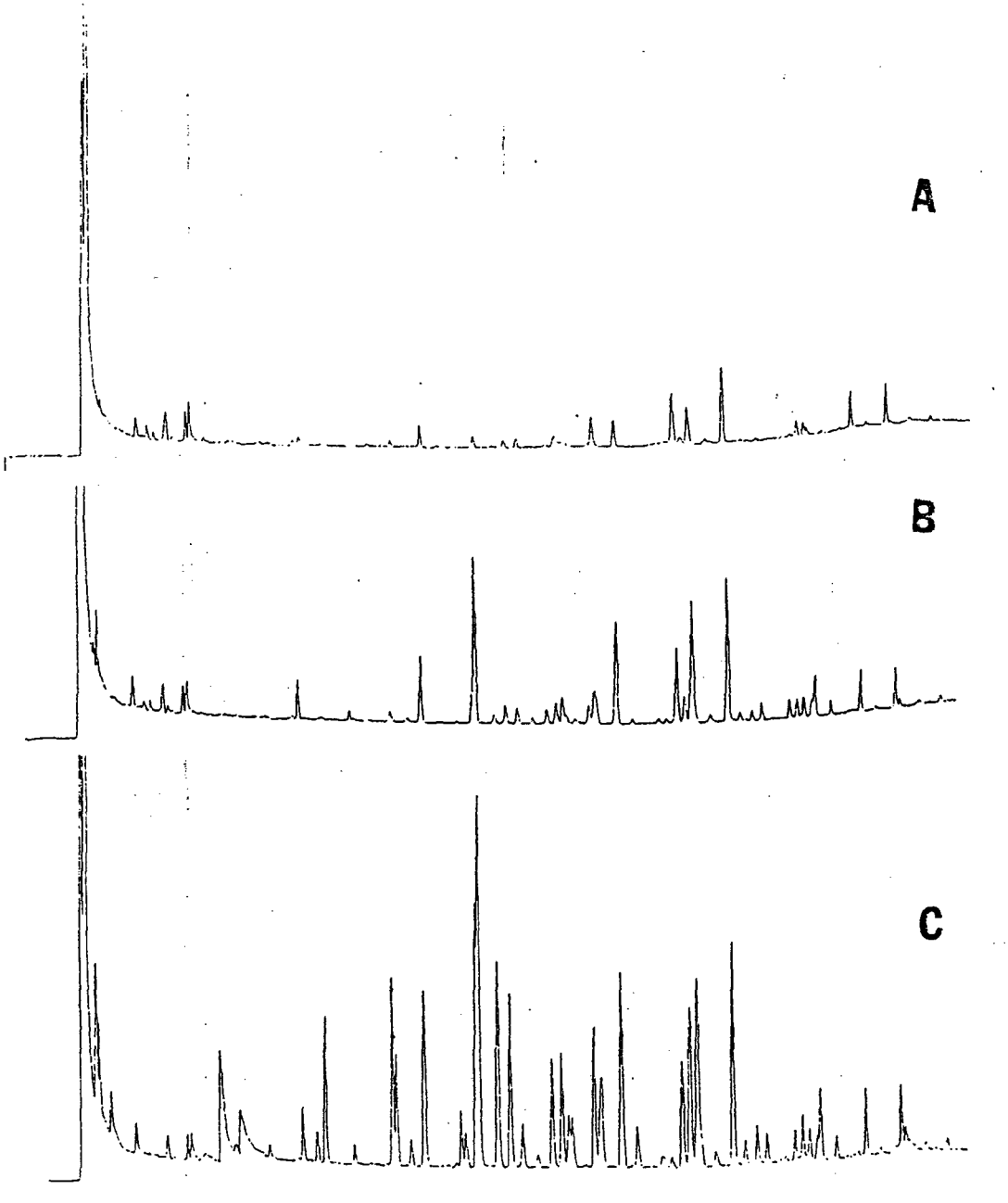


Figure 4-3. Degradation of Aroclor 1242 (10 ppm) by *Pseudomonas* strain LB400 and *E. coli* FM4560 in a 24-h resting cell assay. Panel A, LB400; panel B, FM4560; panel C, mercury-killed control.

DNA Homology Studies

The PCB-degradative competences of *Pseudomonas* strain LB400 and *Alcaligenes eutrophus* H850 are significantly different from those of other bacteria. This is reflected in the wide range of congeners they can metabolize and in their possession of 34-dioxygenase activity [GE Report, 1985]. These biochemical differences could be the result of some minor alterations in a single class of PCB-degradative genes, or may indicate the existence of a fundamentally different set of PCB genes possessed by LB400 and H850. The availability of pGEM410 and its subclones has made it possible to compare the genetic information responsible for PCB metabolism in LB400 to that of a variety of other bacteria.

DNA:DNA hybridization is the method of choice to make these comparisons. This technique is based on the ability of closely related segments of DNA to "hybridize," or bind to each other. DNA from the organisms to be examined are fragmented and fixed to an inert support (a nylon membrane). Plasmids containing the PCB-degradative genes from LB400 were radioactively labeled and used as a "probe." The probe will bind to the DNA on the nylon membrane only if the DNA contains sequences that are closely related to those of the probe. Autoradiography is used to visualize the regions of similarity.

Genomic DNA from nine different bacterial strains were tested for their ability to hybridize to a recombinant plasmid containing the *bph* genes of LB400. The strains selected for study had various degrees of PCB competence and contained representatives from four different genera and from six species (including *A. eutrophus*, *A. faecalis*, *P. cepacia*, and *P. testosteroni*). As shown in Figure 4-4, the only bacterial genome demonstrating any detectable homology to probe DNA was that of *A. eutrophus* strain H850. In addition, the genomic fragments of H850 that bound to the *bph* genes were found to be identical in size to those in LB400. These data strongly suggest a close genetic relationship between the genes encoding PCB metabolism in LB400 and H850, and explain the similar degradative abilities of these organisms.

It is interesting to speculate as to how such diverse organisms as a *Pseudomonas* strain and an *Alcaligenes eutrophus* both came to possess such closely related PCB-degradative genes. Since it is virtually impossible that the *bph* clusters of LB400 and H850 evolved in these organisms independently, they must have been acquired by some form of genetic transfer. It therefore appears that, in the environment, the genes encoding superior PCB-degradative ability can be acquired by, and expressed in, a wide variety of bacterial strains.

Sequencing of the Genes for PCB Metabolism

Determining the nucleotide sequence of the LB400 genes encoding PCB metabolism would provide information critical in obtaining proper gene placement in high-expression vectors. In addition, it would supply a great deal of important data regarding both the structure of the enzymes and the regulation of gene expression. Studies are presently being conducted to identify the nucleotide sequence of the *bph* cluster of LB400.

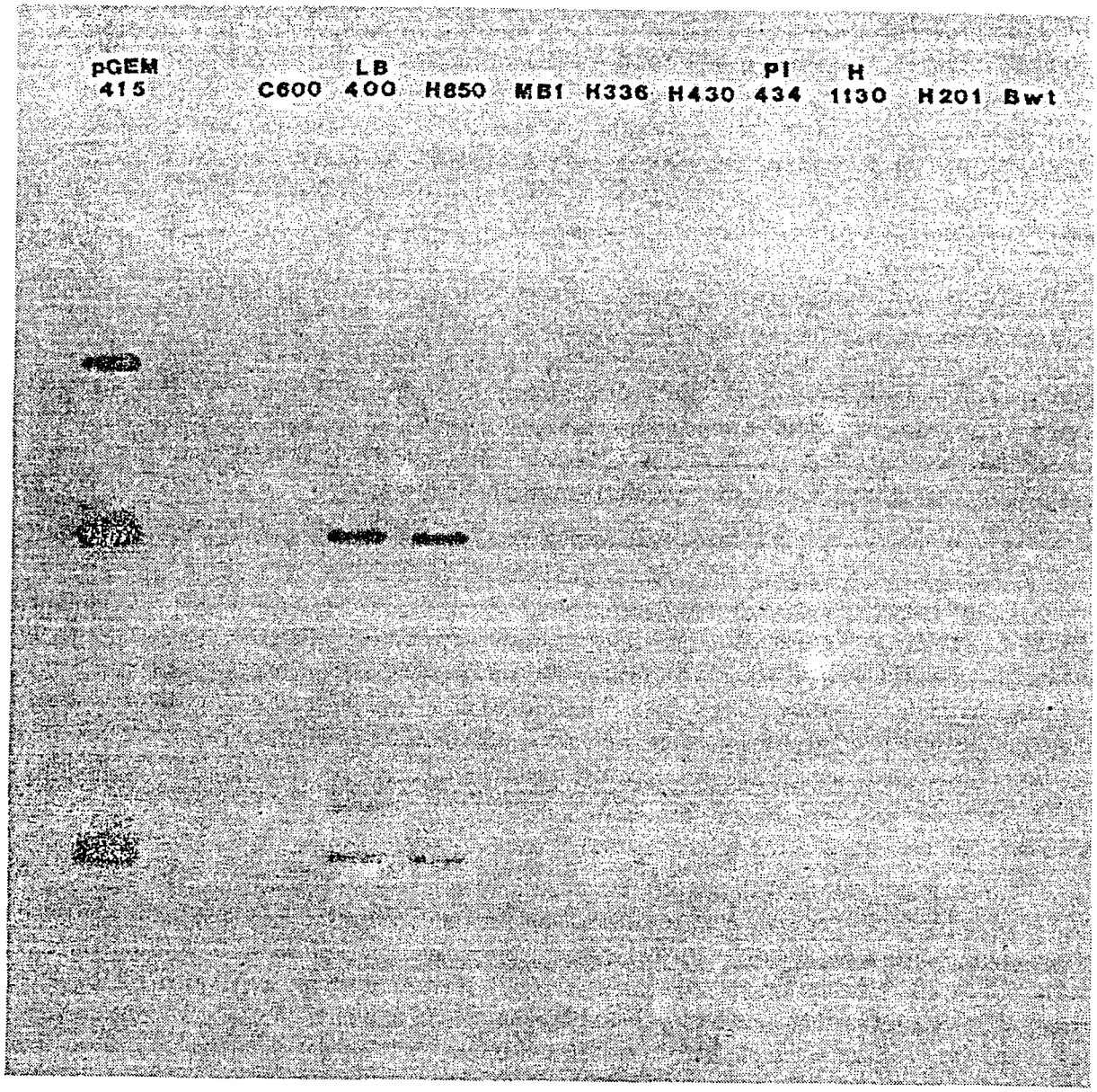


Figure 4-4. Hybridization of a probe (pGEM415) containing the *bph* cluster of *Pseudomonas* strain LB400 to genomic digests of a variety of PCB-degrading bacteria. *Escherichia coli* C600 (C600) included as a negative control. PCB-degradative competences of the strains are shown in Table 4-2.

SUMMARY AND CONCLUSIONS

The location of the genes encoding the PCB-degradative pathway have been mapped on pGEM410 and pGEM456. The DNA region encoding the initial four enzymatic steps extends from near the *Eco*RI site of the vector to a point located approximately 12.3 kb within the DNA insert. DNA homology studies using the *bph* cluster of LB400 as a probe have demonstrated genetic similarities between the PCB genes of LB400 and *A. eutrophus* H850.

The PCB-degradative competence of the recombinant strain FM4560 was found to be similar to that of LB400 as measured by resting cell assays on mixes 1B, 2B, and Aroclor 1242.

FUTURE PLANS

The PCB competence of a variety of existing subclones of pGEM410 will be examined to determine if any of these are superior to FM4560. In addition, new subclones will be created for use in "soil-adapted" strains. Our goal would be to introduce the PCB genes from LB400 into an organism indigenous to a contaminated soil. In this way we might produce a strain with better survival characteristics on soil than LB400. In addition, the use of a soil bacterium closely related to *Pseudomonas* strain LB400 may offer increased expression of the cloned genes over that found in *E. coli*.

Chapter 5

REDUCTIVE DECHLORINATION OF PCBs IN ANAEROBIC MICROBIAL COMMUNITIES

James M. Tiedje, Stephen A. Boyd, and John F. Quensen
Department of Crop and Soil Sciences
Michigan State University
East Lansing, Michigan

INTRODUCTION

The altered polychlorinated biphenyl (PCB) congener distribution patterns first observed in sediment samples from the upper Hudson River suggest that reductive dechlorination of PCBs may occur in anaerobic environments [Brown et al., 1984]. Similarly altered PCB congener distribution patterns have since been noted for other sites including Waukegan Harbor (Illinois), and Silver Lake and the Acushnet River (Massachusetts) [Brown and Wagner, 1987; Brown et al., 1987a, b; GE Report, 1987; Stalling, 1982]. In all cases the dechlorination was interpreted to be the result of a biological process because congener selectivity was observed and no known nonbiological mechanisms of aromatic dechlorination are operable under the conditions found in these sediments [see Hutzinger et al., 1974; Wade et al., 1969]. However, controlled experiments were necessary to prove a biological process is actually responsible.

In last year's report we provided the first such experimental evidence for biologically mediated reductive dechlorination of PCBs. We observed the dechlorination of (i) 2,3,4,5,6- to 2,3,4,6- and/or 2,3,5,6-CB (coeluting isomers), (ii) of 2,3,4,6,2′4′5′- to 2,4,5,2′4′6′-CB, and (iii) an apparent loss of 2,4,3′4′-CB added to Hudson River sediments collected from a PCB-contaminated site (river mile 193.3, west bank) [GE Report, 1987]. The reliable detection of dechlorination products from the 24-34-CB was not possible because of the high background levels of lesser chlorinated PCBs already present in these sediments.

To avoid the problem of detecting dechlorination products against these high background PCB levels in subsequent experiments, we developed a "transfer" technique in which microorganisms were eluted from contaminated sediments and transferred to serum bottles containing cleaner Hudson River sediments. Two different PCB solutions were added to these sediments in separate experiments. The first was an acetone solution of a PCB mixture (3,4,3′4′-, 2,3,4,5,6-, 2,4,6,2′4′6′-, 2,3,4,6,2′4′5′-, and 2,3,4,5,2′3′4′5′-CB) and the second was Aroclor 1242. The transferred organisms were always maintained under anaerobic conditions. The bottles were sealed with Teflon-coated rubber stoppers and incubated for various times before sampling and analyzing for dechlorination products by capillary gas chromatography.

We have used this technique to evaluate Aroclor 1242 dechlorination by Hudson River microorganisms. Aroclor 1242 was added to give final concentrations of 14, 140, and 700 ppm

on a sediment dry weight basis. We have also used the transfer technique and the PCB mixture above (final concentration of 1 ppm each) to evaluate the PCB dechlorinating activity of microorganisms from other sites and to test the effects of various amendments on dechlorination by the Hudson River microorganisms.

RESULTS AND DISCUSSION

Aroclor 1242 Dechlorination

Dechlorination of the Aroclor was evident from a visual inspection of the chromatograms (Figure 5-1). Early eluting peaks, corresponding to the lesser chlorinated congeners, increased with time in the live samples but not in the autoclaved controls. There was a corresponding decrease in the later-eluting, more highly chlorinated congeners. Most notable was the accumulation of chlorobiphenyls substituted only at the *ortho-* position. 2-CB increased from 0 to 63% in the live treatments receiving 700 ppm Aroclor, and 2,2′- and/or 2,6-CB (coeluting isomers) increased from 1 to 14% of the total PCBs. 2,6,2′-CB also increased from 0.4 to 2%.

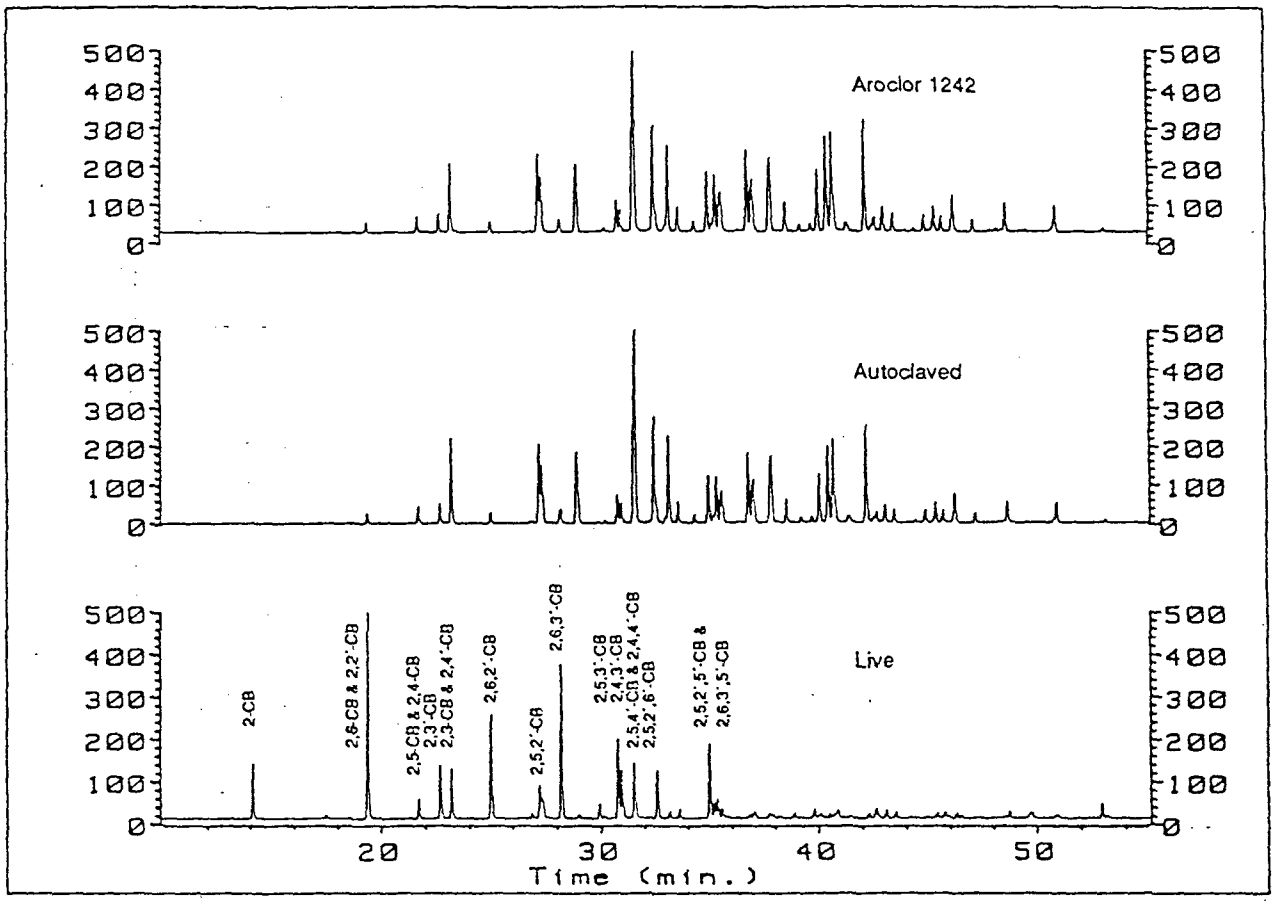


Figure 5-1. Capillary gas chromatograms showing the anaerobic dechlorination of 700-ppm Aroclor 1242 after 16 weeks incubation.

The progressive nature of the dechlorination process was evident from the relative proportions of mono-, di-, tri-, tetra-, penta-, and hexa-CBs at each sampling time (Figure 5-2). Aroclor 1242 contains predominantly tri- and tetra-CBs; these were progressively dechlorinated to di- and mono-CBs in the live treatments receiving 140 and 700 ppm Aroclor, but there was no appreciable change over time in the autoclaved controls.

PCB dechlorination occurred primarily from the *meta-* and *para-* positions and appeared to be concentration dependent (Figure 5-3). Dechlorination was most extensive at the highest PCB concentration. In the 700-ppm treatment the *meta-* plus *para-* chlorines decreased from an average of 1.98 to 0.31 per biphenyl after 16 weeks, but decreased to only 1.19 in the 140-ppm treatment. At 14-ppm PCB there was no observable difference between the live and autoclaved controls after 16 weeks.

The same Aroclor 1242 experiment was performed with microorganisms eluted from non-PCB-contaminated Hudson River sediments. These have shown no evidence of PCB dechlorination after 16 weeks of incubation.

Sediment Survey

We have also observed dechlorination of 2,3,4,5,6-CB in a second series of experiments using microorganisms eluted from PCB-contaminated sediments from the Hudson River (New York); Silver Lake (Massachusetts); the Pine River (Michigan), contaminated with polybrominated biphenyls, hexabromobenzene, and DDT; and the Red Cedar River (Michigan),

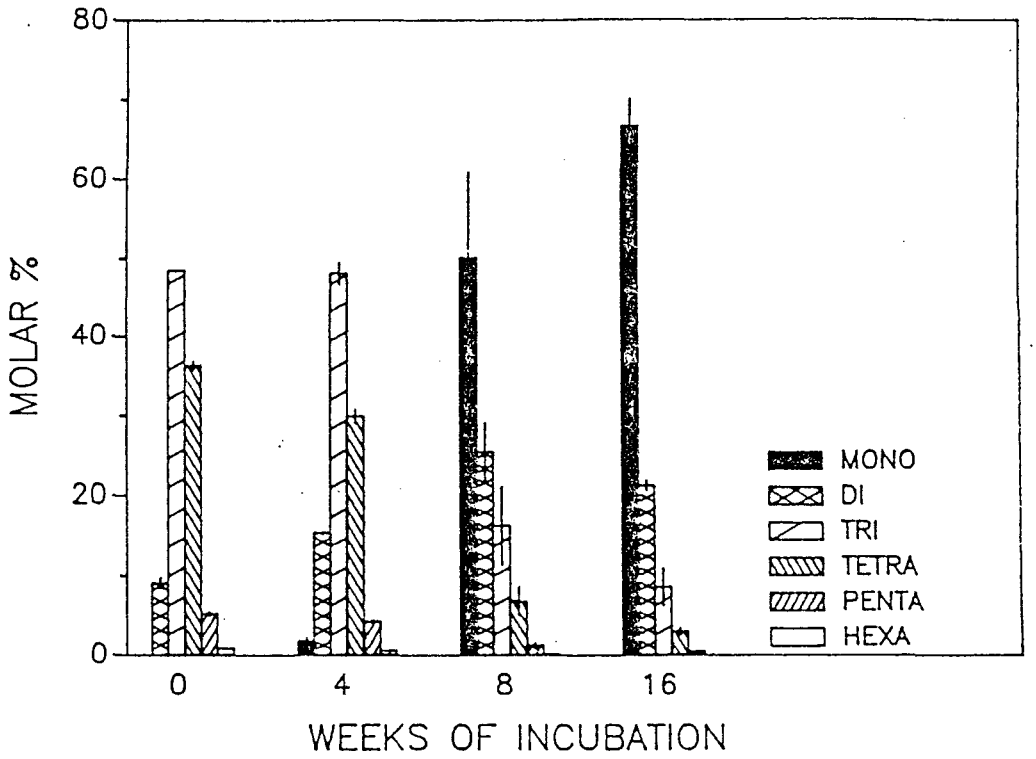


Figure 5-2. **Changes in PCB congener distribution over time for the 700-ppm Aroclor live treatment.**

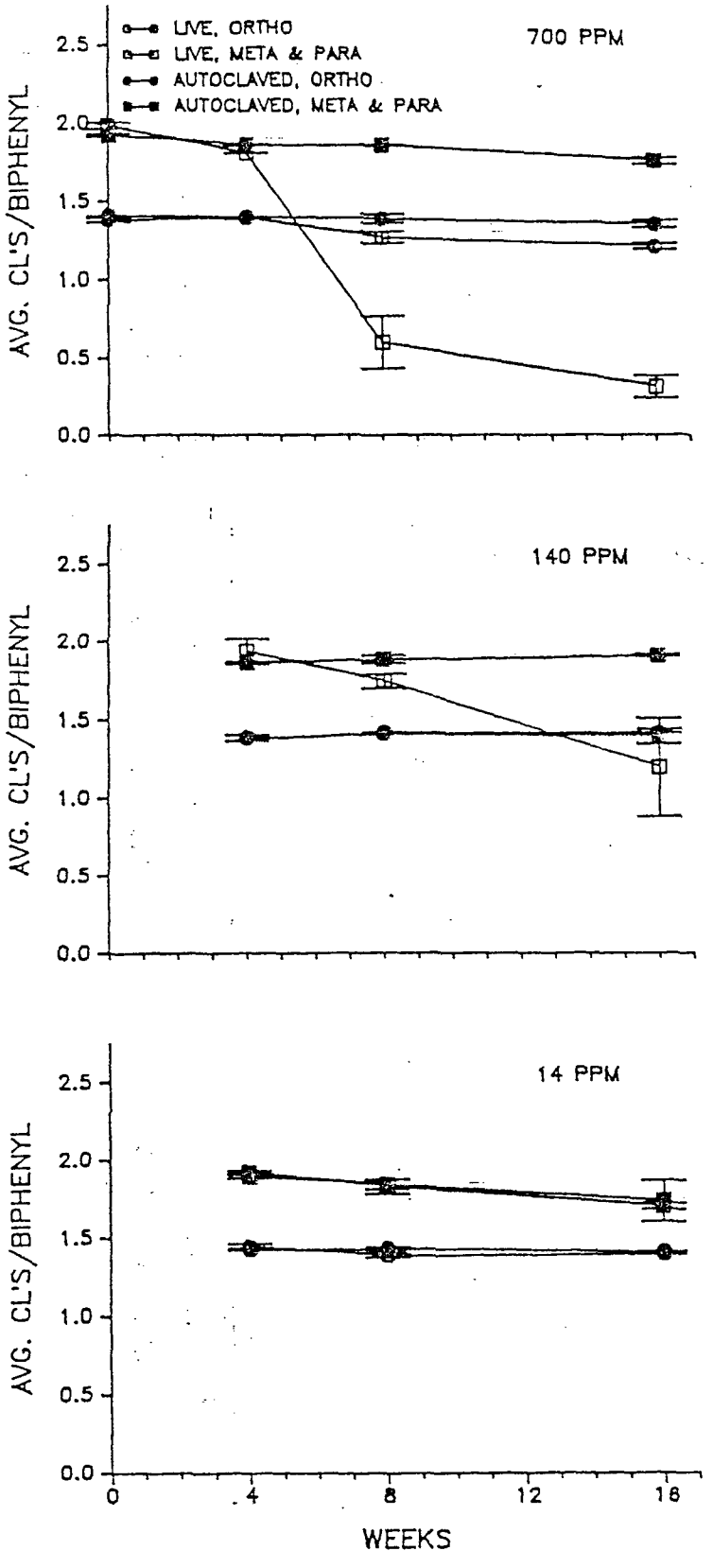


Figure 5-3. Decrease in the average number of chlorines by position at three Aroclor concentrations.

with no known history of halogenated biphenyl contamination. The Pine River cultures showed the earliest evidence of dechlorination of the 2,3,4,5,6-CB. Approximately 3% of this PCB congener was dechlorinated by the end of four weeks by the Pine River microorganisms (Table 5-1). The cultures from the three PCB-contaminated sites exhibited longer lag times before dechlorination was observed, but showed greater amounts of dechlorination by the end of the experiment. The Red Cedar River sediments showed only 1.3% dechlorination of 2,3,4,5,6-CB at the end of 32 weeks. There was little or no evidence of the dechlorination of the other PCB congeners added.

Table 5-1

PERCENTAGE OF 2,3,4,5,6-PENTACHLOROBIPHENYL DECHLORINATED BY MICROORGANISMS FROM VARIOUS SOURCES

Source	Treatment		Week					
			0	4	8	16	24	32
Pine River	Live	$\bar{X}$	0.0	2.9	4.9	7.2	10.3	10.2
		$S_{\bar{x}}$	0.0	0.2	0.3	0.3	2.0	0.7
	Dead	$\bar{X}$	0.0	0.0	0.0	0.0	0.0	0.3
		$S_{\bar{x}}$	0.0	0.0	0.0	0.0	0.0	0.3
Silver Lake	Live	$\bar{X}$	0.0	0.0	0.0	4.6	11.8	13.1
		$S_{\bar{x}}$	0.0	0.0	0.0	0.2	0.6	1.0
	Dead	$\bar{X}$	0.0	0.0	0.0	0.0	0.0	0.0
		$S_{\bar{x}}$	0.0	0.0	0.0	0.0	0.0	0.0
Red Cedar River	Live	$\bar{X}$	0.0	0.0	0.0	0.2	1.5	1.3
		$S_{\bar{x}}$	0.0	0.0	0.0	0.2	0.5	0.3
	Dead	$\bar{X}$	0.0	0.0	0.0	0.0	0.0	0.0
		$S_{\bar{x}}$	0.0	0.0	0.0	0.0	0.0	0.0
Thompson Island[a]	Live	$\bar{X}$	0.0	0.0	2.2	7.4	17.7	18.1
		$S_{\bar{x}}$	0.0	0.0	0.3	0.4	2.0	1.2
	Dead	$\bar{X}$	0.0	0.0	0.0	0.0	0.0	0.0
		$S_{\bar{x}}$	0.0	0.0	0.0	0.0	0.0	0.0
H7[a]	Live	$\bar{X}$	0.0	0.0	2.2	6.8	13.9	12.7
		$S_{\bar{x}}$	0.0	0.0	0.1	0.0	0.8	0.2
	Dead	$\bar{X}$	0.0	0.0	0.0	0.0	1.3	2.8
		$S_{\bar{x}}$	0.0	0.0	0.0	0.0	1.3	2.8

[a] Thompson Island and H7 are PCB-contaminated sites in the Hudson River, New York (river miles 188.6 and 193.3, respectively)

Amendments

Using the transfer technique and the suite of five PCB congeners at a final concentration of 1 ppm each, various amendments were tested for their effect on PCB dechlorination by the Hudson River microorganisms. These amendments were acetate, sewage sludge supernatant, yeast extract, a H_2/CO_2 head space, and Triton X-305. The first three are various carbon sources, the H_2/CO_2 supports methanogens (but inhibits dechlorination of metachlorobenzoate by DCB-1 [Linkfield, 1985]), and the Triton should improve PCB solubility. Only the acetate amendment resulted in a statistically significant increase in the dechlorination of 2,3,4,5,6-CB observed after 32 weeks incubation (Table 5-2).

SUMMARY AND CONCLUSIONS

Aroclor 1242 was dechlorinated relatively rapidly by microorganisms collected from a PCB-contaminated site, producing primarily *ortho*-only substituted congeners. The chromatographic pattern observed matched closely the Pattern C previously described for the deeper sediments at this same site [Brown et al., 1984]. It is therefore probable that the same microorganisms dechlorinated Aroclor in the laboratory experiment and in situ, and that these microorganisms are still present and viable in the Hudson River.

All observed dechlorination was primarily from the *meta-* and *para-* positions. This results in products that are both less toxic and more aerobically biodegradable [Bedard et al., 1987; Safe, et al., 1982]. Hence dechlorination is viewed as an environmentally beneficial process.

Dechlorination appeared to be concentration dependent, occurring much more quickly at higher PCB concentrations. This may be the result of a greater PCB availability to the dechlorinating microorganisms at higher concentrations. The results of both the sediment survey and the Aroclor 1242 experiment (two sediments) suggest that prior (environmental) exposure to PCBs selects for PCB dechlorinating activity. Acetate enhanced dechlorination

Table 5-2

EFFECTS OF AMENDMENTS OF 2,3,4,5,6-CB DECHLORINATION BY HUDSON RIVER MICROORGANISMS AFTER 32 WEEKS INCUBATION

Amendment	%Dechlorination ($\bar{X}$ + S. E.)[a]
None	10.3 + 1.2
Acetate	16.2 + 0.5
Sludge supernatant	14.4 + 0.1
Yeast extract	12.9 + 1.9
Hydrogen	14.6 + 1.9
Triton X-305	13.4[b]

[a] For two observations
[b] For only one observation

during 32 weeks of incubation. We believe the observed PCB dechlorination is biologically mediated because it is sensitive to autoclaving, transferable by a technique known to elute and transfer microorganisms, restricted to sites of high PCB concentration (implying selection for PCB dechlorination has occurred), and is congener-specific, as would be expected if enzyme system(s) are involved.

FUTURE PLANS

The successful demonstration of Aroclor 1242 dechlorination has suggested several new objectives as well as new approaches to previous objectives. Our proposed objectives for the next two years are

1. To evaluate the PCB dechlorinating capability of various sediments using 500 ppm of Aroclor 1242 rather than the previously used suite of five pure congeners at 1 ppm each.
2. To continue to examine various environmental factors to determine their influence on PCB dechlorination rate using Aroclor 1242.
3. To test for the dechlorination of Aroclor 1260.
4. To evaluate the dechlorination of the more toxic congeners (3,4,3′4′-, 2,3,4,3′4′-, and 2,4,5,3′4′-CB) using higher concentrations or Aroclor mixtures fortified with these congeners.
5. To determine which congeners are dechlorinated and relative congener-specific rates of dechlorination.
6. To further characterize the biology of the process and to attempt to identify the physiological group of microorganism(s) responsible for PCB dechlorination; if possible we will isolate pure culture(s).
7. To determine the bioavailability of PCBs to the dechlorinating microorganisms in historically contaminated soil and/or sediment.

Chapter 6

REDUCTIVE DECHLORINATION OF PCBs IN UPFLOW ANAEROBIC FILTER REACTORS

Sandra Woods and Edmond Low
Department of Civil Engineering
Oregon State University

INTRODUCTION

Four anaerobic filters with recycle (Figure 6-1) were operated to treat a synthetic wastewater containing five chlorinated biphenyls. The major distinguishing feature between the reactors was the source of bacterial inocula used in each reactor. The reactors were fed increasing concentrations of each of the five parent compounds and were monitored for the appearance of metabolic products.

The reactors were constructed of threaded chromatography glassware and had a volume of 300 ml with a 50-ml settling chamber. They were fed semicontinuously by daily feed additions yielding a 10-day hydraulic retention time. The glass components were coupled together with Teflon fittings. The reactor was packed with 6×6-mm Rasching rings to provide 3000-cm^2 surface area and a 250-ml void volume. The filter had three sampling ports: reactor off gases were collected from a septum on the gas outflow line, effluent samples were collected off the recycle line at the top of the reactor, and reactor sediments were collected from the port at the bottom of the reactor. Recycle was provided by a peristaltic pump that delivered between 450 and 500 ml/day. All fluid lines were 1/8-in. OD FEP Teflon tubing.

The sources of inocula were samples C2 and F3 from Silver Lake (reactors 1 and 2), and H7 and TI from the Hudson River (reactors 3 and 4). The reactors were inoculated and then fed a synthetic wastewater containing 200 mg/l acetate, 140 mg/l methanol, 100 mg/l glucose, and 80 mg/l acetone, as well as inorganic nutrients and vitamins. After this stabilization period, the reactors were fed increasing concentrations of five chlorinated biphenyls (2,4,3′4′-; 2,3,4,5,6-; 2,4,6,2′4′6′-; 2,3,4,6,2′4′5′-; 2,3,4,5,2′3′4′5′-CB) and the reactor effluent and reactor solids were monitored for the appearance of metabolic products.

PCBs were dissolved in acetone and injected into the reactor, followed by addition of the synthetic wastewater. The acetone concentration in the reactor influent (in the synthetic wastewater) varied as the PCB concentration was increased from 50 μg/l to 1000 μg/l over several months of operation.

RESULTS AND DISCUSSION

All reactors had an initial period of complete PCB removal (influent PCB concentrations were below levels of detection). Effluent concentrations of the parent compounds for reactor 3, which was inoculated with Hudson River sediments, are shown in Figure 6-2. However,

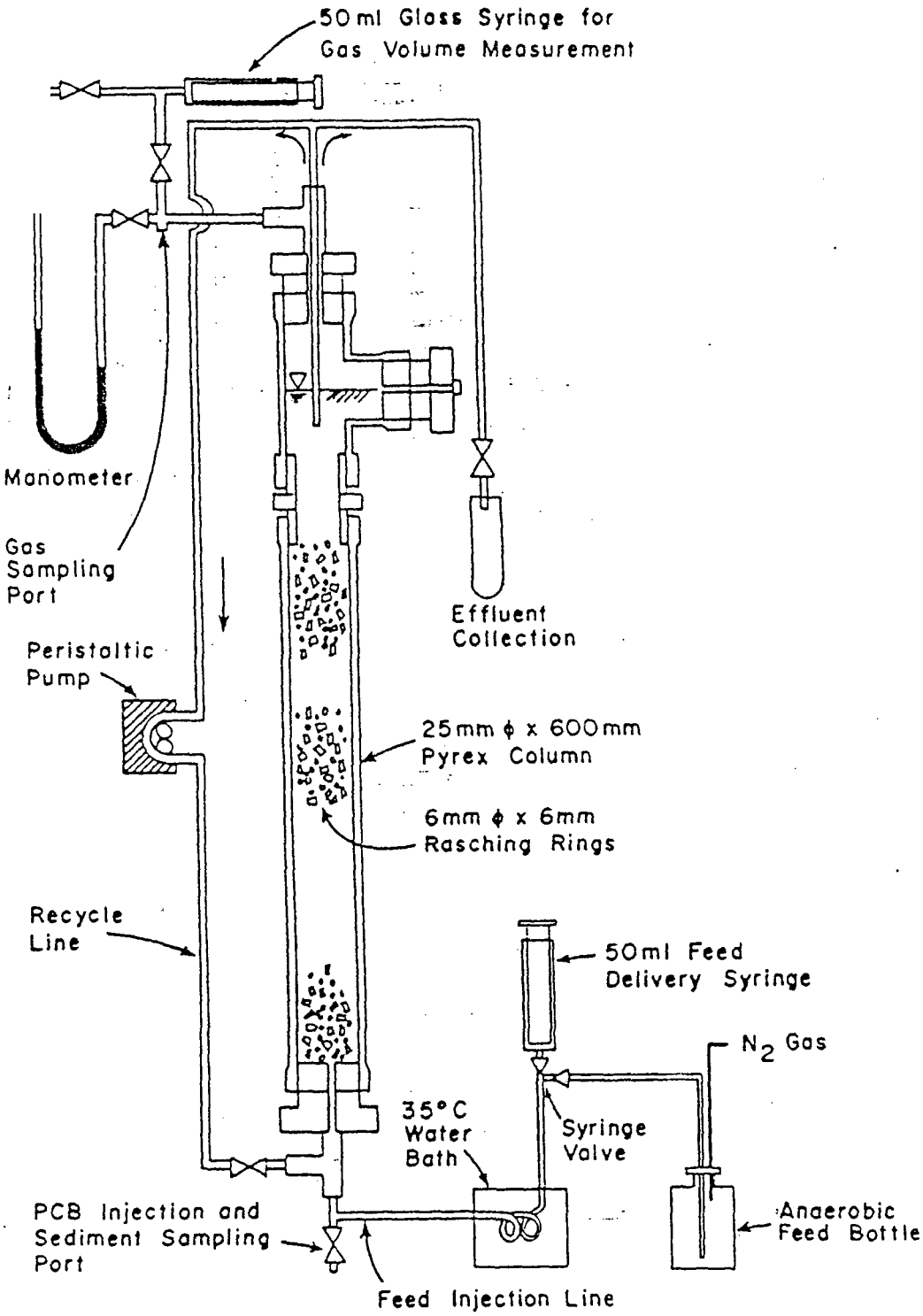


Figure 6-1. Upflow anaerobic filter detail.

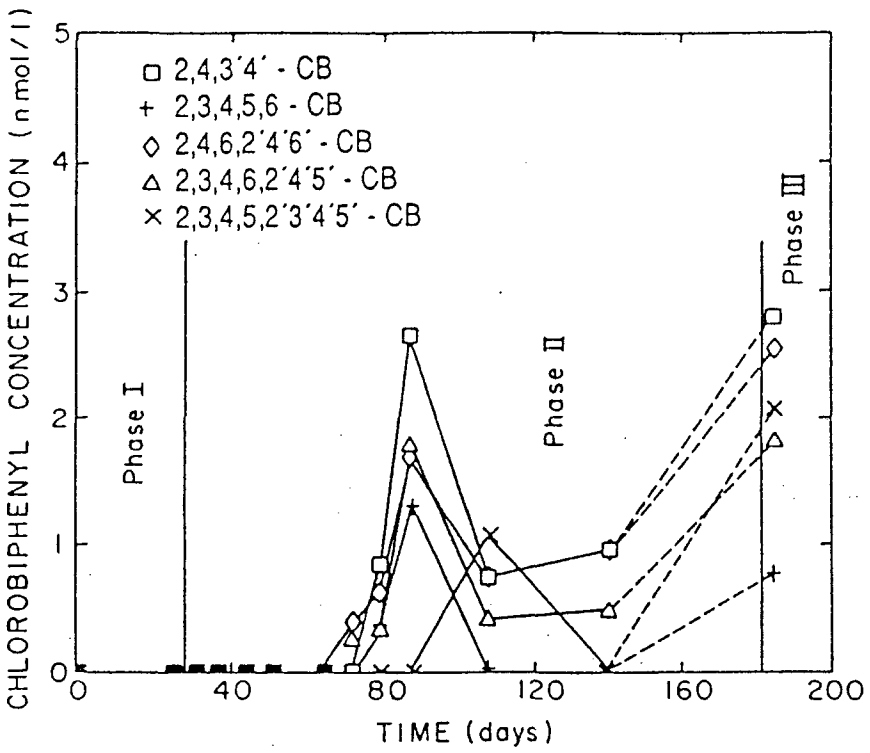


Figure 6-2. Effluent concentration of the five feed PCBs during Phases I, II, and III.

each reactor behaved similarly. Breakthrough occurred at 60 ± 10 days in all reactors for all congeners except 2,3,4,5,2'3'4'5'-CB. The octachlorobiphenyl first appeared in all reactors between 90 and 110 days. Throughout the experiment, the removal efficiency of the anaerobic filters exceeded 99.9%.

Characterization of the reactor solids suggested the formation of a potential PCB degradation product in reactors 1, 3, and 4. The unknown compound was detected by observing the formation of a new peak at a retention time of 24.15 min in sediment hexane extracts analyzed by GC/ECD. The unknown peak was first detected in reactor 3 at 93 days, at the base of the tetrachloronaphthalene internal standard (Figure 6-3). The peak continued to increase until it dominated the internal standard peak (Figure 6-3d). Figure 6-3a represents the absence of the peak at 24.15 min in the initial sediment inocula.

Analyses of data from reactors 1, 3, and 4 were performed by normalizing the unknown raw peak areas to the area of internal standard and the mass of solids extracted, and examining the data for the emergence of new peaks. Once a peak was identified, concentrations were determined. The increase in concentration of the unknown compound with time for the four reactors is shown in Figure 6-4.

The unknown compound was identified by gas chromatography with mass spectrometric detection (GC/MS) as a tetrachlorobiphenyl. The unknown compound was then positively identified by cochromatography as 2,3,5,6-tetrachlorobiphenyl. Confirmation of this identification was made by M.D. Mullin at the U.S. EPA Laboratory (Large Lakes Research Station, Grosse Ile, Michigan).

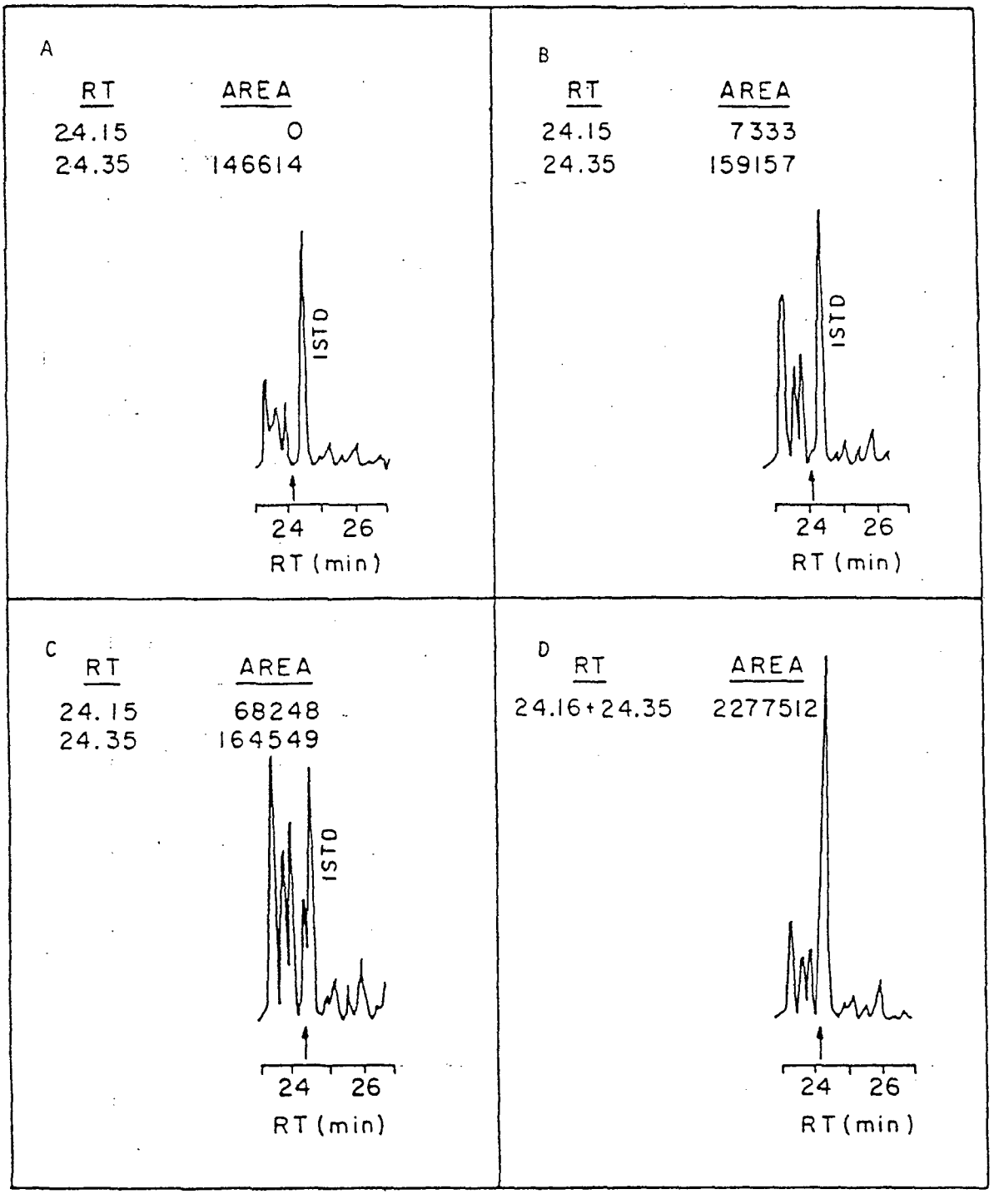


Figure 6-3. DB-5 chromatographs of the formation of the unknown metabolite vs time at RT 24.15 min: (a) 8/24/87, 0.016 g TVS; (b) 12/7/87, 0.015 g TVS; (c) 2/3/88, 0.027 g TVS; (d) 3/7/88, 0.082 g TVS.

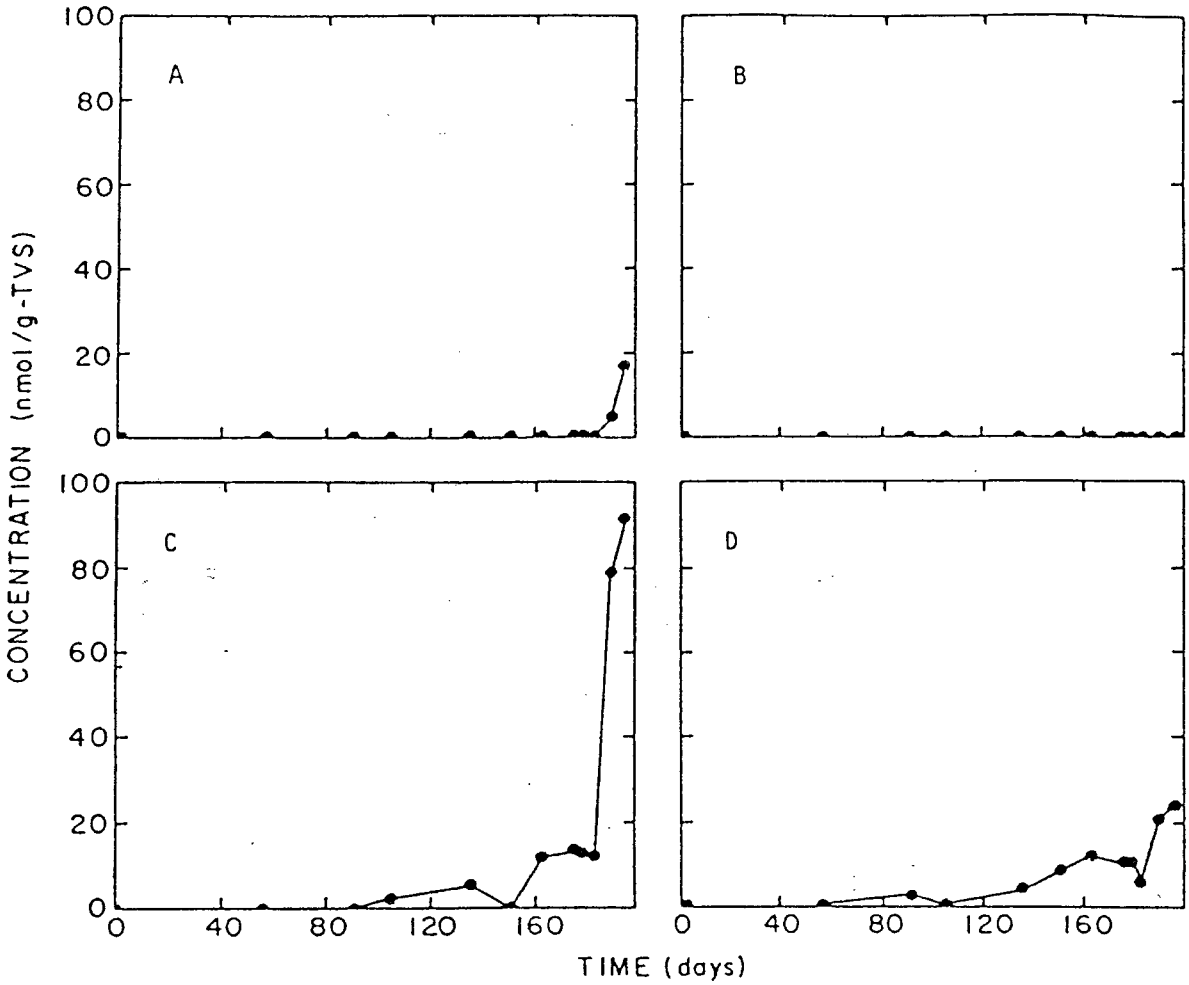


Figure 6-4. Emergence of metabolite at RT 24.15 min: (a) reactor 1, C2; (b) reactor 2, F3; (c) reactor 3, H7; (d) reactor 4, TI.

Based on the position of chlorines on the biphenyl molecule, only 2,3,4,5,6-CB could could have produced 2,3,5,6-CB by a simple dechlorination reaction. Therefore 2,3,4,5,6-CB was identified as the parent compound. Concentrations of 2,3,5,6-CB and 2,3,4,5,6-CB in the reactor solids in reactor 3 are shown in Figure 6-5. These data suggest that the pentachlorobiphenyl was reductively dechlorinated at the para position.

SUMMARY AND CONCLUSIONS

Upflow anaerobic filters were successful in removing over 99.9% of five chlorinated biphenyls. Although much of this removal was due to sorption of chlorobiphenyls to the reactor and reactor biomass, three reactors were successful in reductively dechlorinating 2,3,4,5,6-CB at the para position to produce 2,3,5,6-CB. A complete mass balance for each of the parent compounds could not be performed; therefore, other mechanisms of biodegradation may have ocurred.

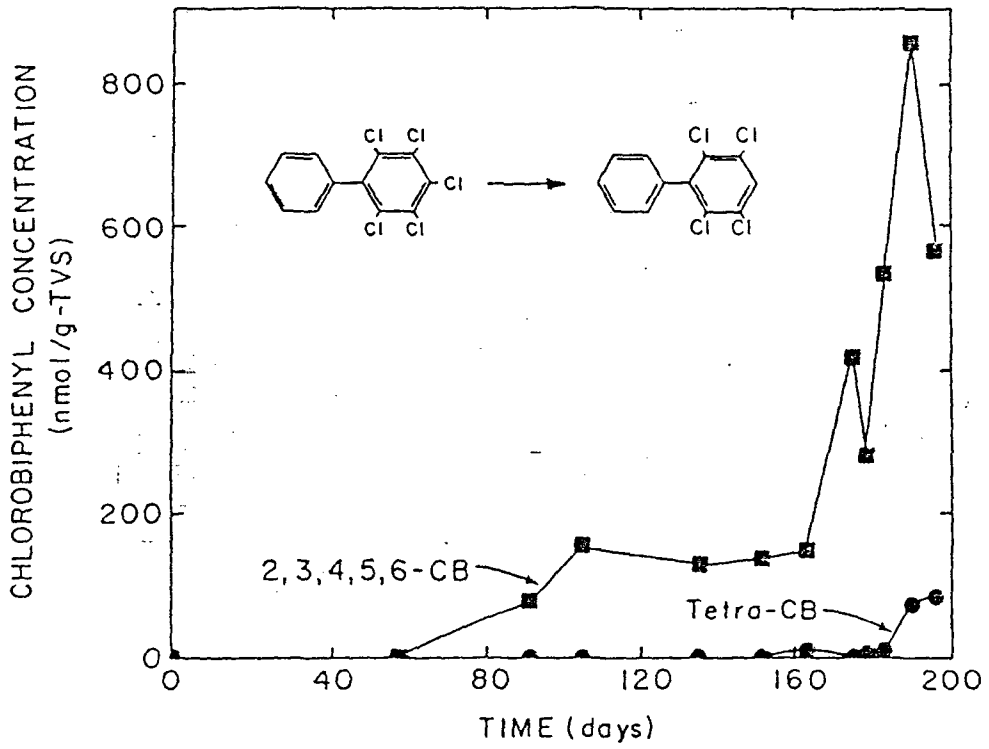


Figure 6-5. Emergence of 2,3,4,5,6- and 2,3,5,6-CB in reactor 3.

The dechlorinated metabolic product appeared first in the Hudson River sediments (reactors 3 and 4), but also appeared in reactor 1 (Silver Lake, C2) after approximately five months. It is expected that the metabolic product will also appear in reactor 2 (Silver Lake, F3).

FUTURE PLANS

Studies are continuing with reactors 1 through 4. Concentrations of the parent compounds in the reactor influent will be increased and the reactor effluent and solids will continue to be monitored for the appearance of metabolic products. Based on these products, degradation mechanisms will be inferred.

A fifth reactor has been constructed and will be inoculated with effluent from reactors 1 through 4. In this way, the fifth reactor will act much like an enrichment culture. The objectives of the fifth reactor are to develop a large biomass of acclimated organisms within the reactor and to eliminate much of the background contamination. By reducing the background contamination, we may better evaluate the appearance of metabolic products. More importantly, the selection of an acclimated, active population should increase the rate of PCB biodegradation.

Chapter 7

BIOTRANSFORMATION OF PCBs BY HYDROGEN-OXIDIZING BACTERIA

Donna L. Bedard and John F. Brown, Jr.
Biological Sciences Laboratory
GE Research and Development Center
Schenectady, New York

INTRODUCTION

Aroclors 1254, 1260, and 1268 are quite resistant to bacterial oxidation because they are composed of highly chlorinated PCB congeners (penta- through octachlorobiphenyls). Partial dechlorination of these Aroclors would render them more susceptible to degradation via dioxygenases. It has now been demonstrated that anaerobic consortia can reductively dechlorinate PCBs (see Chapter 5). It has also been demonstrated that aerobic bacteria can reductively dechlorinate several chloroaromatic compounds: trichlorohydroquinone, mono- and dichlorotrihydroxybenzene [Apajalahti and Salkinoja-Salonen, 1987], and 2,4-dichlorobenzoic acid [van den Tweel et al., 1987]. From these reports of novel bacterial dehalogenases it seems likely that some aerobic bacteria may be capable of dechlorinating PCBs. The hydrogenases of hydrogen-oxidizing bacteria would appear to be good candidates for aerobic PCB dechlorinases. These enzymes enable the bacteria to use hydrogen gas as an electron donor and as an energy source. They contain nickel at the active site and a hydride seems to be involved in their reaction mechanisms [Cammack and Yates, 1986]. It is known that hydrided (Raney) nickel and nickel-hydrogen combinations can catalyze the hydrodechlorination of PCBs [LaPierre et al., 1978; Dennis et al., 1979.]

To investigate the potential of hydrogenases as dechlorinating agents, we are testing two strains of hydrogen-oxidizing bacteria obtained from culture collections, H16 and 17741, as well as several isolates obtained from PCB-contaminated soils and sediments. H16 is a strain of *Alcaligenes eutrophus* (the same species as H850) that contains two different hydrogenases [Schneider and Schlegel, 1976; Schink and Schlegel, 1979 and 1980]. Both hydrogenases have been well-characterized biochemically, and both are encoded on a transmissible plasmid that could be mobilized into a PCB-oxidizing strain such as H850. Therefore, if these hydrogenases were capable of dechlorinating PCBs it would be possible to construct a strain that can both dehalogenate and oxidize PCBs. Strain 17741 is a *Paracoccus denitrificans* [Beijerinck and Minkman, 1910; Davis et al., 1969]. The new isolates have not been characterized taxonomically.

RESULTS AND DISCUSSION

We collected samples from three sites along Reach 8 of the Hudson River: a soil sample from the dredge spoils (site H8) that had originally yielded *Alcaligenes eutrophus* H850, and

sediment samples from two sites (H7 and R2) that had shown evidence of both reductive dechlorination and oxidation of PCBs at the interface of the aerobic and anaerobic zones. Saline dilutions of these samples were plated directly on minimal medium without a carbon source and grown autotrophically in an atmosphere of 5% O_2, 10% CO_2, and 85% H_2. After approximately 3 weeks of incubation individual colonies were picked and streaked on Luria plates. After restreaking as necessary for colony purification, colonies were again streaked onto minimal plates and grown autotrophically. At this time we have obtained and purified seven isolates from the R2 site and two from the H8 site. None of these isolates has been taxonomically identified. Hydrogenase activity in the isolates was verified in three out of four isolates that were tested using an assay based on triphenyltetrazolium chloride [Aragno and Schlegel, 1981].

We devised a mixture of five PCB congeners (Table 7-1) to assay for dechlorination activity. The congeners were chosen from those that are most readily dechlorinated by the *meta-, para*-dechlorination systems that are active in Hudson River sediments [Brown et al., 1987b]. The potential dechlorination products can be easily resolved from each other and from the parent compound by gas chromatography. We included 26-26-CB as an internal standard because it is recalcitrant to both dioxygenase degradation and *meta-, para*-dechlorination. Preliminary experiments were conducted using the two culture collection strains (H16 and 17741) and five isolates: R2B-1, R2B-2A, R2B-2B, R2C-5B, and H8A-3. Cells were grown autotrophically on minimal plates, then harvested and resuspended in 0.05-M sodium phosphate buffer (pH 7.5) at an optical density ($OD_{615\ nm}$) of 1.0. One-ml aliquots of cells were transferred to 2-dram vials and 10 μl of a concentrated (100X) stock of the PCB mixture (in acetone) were added. The final concentration of each PCB congener was 5 μM. The vials were flushed for 30 s with H_2, sealed with foil-lined screw caps, and incubated at 30 °C in a gyratory shaker. Controls were heat-killed (70 °C, 20 min) prior to the addition of PCBs. After 48 to 72 h the incubations were terminated and the PCBs were extracted and analyzed by gas chromatography as previously described [Bedard et al., 1986].

Table 7-1

PCB CONGENER MIXTURE FOR ASSAYING DECHLORINASE ACTIVITY

PCB Congener	Retention Time	Potential Dechlorination Products	
2,6,2′6′ [a]	12.45		
3,4,2′	13.80	2,3′	2,4′
2,3,2′5′	20.13	2,5,2′	2,3,2′
2,3,4,2′3′4′	36.51	2,3,4,2′4′	2,3,4,2′3′

[a] Internal standard

All five of the isolates and *A. eutrophus* H16 depleted all four congeners, albeit to different extents. *Paracoccus denitrificans* 17741 had no effect on the PCBs. Two different patterns of biotransformation were seen. Strains H16, H8A-3 and R2C-5B depleted 3,4,2′- and 2,6,2′6′-CB to the greatest extent and 2,3,2′5′- and 2,3,4,2′3′4′-CB to a lesser extent. For these strains the depletion of 3,4,2′- and 2,6,2′6′-CB ranged from 20% for R2C-5B to 50% for H16. The remaining congeners were depleted by approximately 30% in strains H16 and H8A-3 and by about 5% in strain R2C-5B. No metabolites were seen for any of these cultures. Strains R2B-1, R2B-2A, and R2B-2B showed a different pattern of biotransformation (Figure 7-1). These three strains differ from each other in color, morphology, and growth characteristics, yet all three depleted 3,4,2′-CB to a greater extent than 2,6,2′6′-CB and all produced a metabolite that eluted with a GC retention time 28.95. This metabolite ran in the region of the chromatogram, where potential pentachlorobiphenyl dechlorination products of 2,3,4,2′3′4′-CB would elute. However, subsequent capillary chromatography showed that the metabolite did not coelute with any of the pentachlorobiphenyls (including 2,3,4,3′4′-CB) expected from dechlorination of the hexachlorobiphenyl. GC-mass spectrometry confirmed that the metabolite is not a pentachlorobiphenyl, but the sample was too small to permit identification of the compound.

It is clear that the hydrogen-oxidizing isolates are transforming PCBs, but the nature of the biotransformation is not understood. The depletion of 2,6,2′6′-CB was not anticipated. This congener is not oxidized by any of the more than 30 strains of bacteria that have been described in the literature, nor would it be dechlorinated by the *meta*-, *para*-dechlorination systems that are active in Hudson River sediments. However, this congener is dechlorinated by the unusual *ortho*-, *meta*-, *para*-dechlorination system that exists in Silver Lake (Pittsfield, Massachusetts) [Brown et al., 1987b] and would probably be metabolized by eukaryotic mixed function oxidase systems based on cytochrome P-450 [Saeki et al., 1983]. Although unidentified, the PCB metabolite seen (Figure 7-1) underscores the difference between this biotransformation and those seen in other bacteria to date. The hydroxylated intermediates produced by mono- and dioxygenase attack can be silylated with N,O-*bis*(trimethylsilyl)trifluoroacetamide, but this metabolite could not.

Encouraged by these results, we transferred the isolates to liquid culture in order to obtain larger amounts of cells. We have succeeded in growing seven of our nine isolates, as well as the two culture collection strains, on liquid media under autotrophic conditions (23% O_2, 10% CO_2, and 67% H_2). Using CO_2 as the sole carbon source and H_2 as the sole energy source, these cultures have been carried for more than a month. The strains vary considerably in the extent of growth. H16 grew to an $OD_{615\ nm}$ of 13.5, strains 17741, R2B-1, and R2B-2A to an OD between 4.0 and 5.4, and the remaining strains to an OD ranging from 0.5 to 2.3. Two strains, R2B-3 and H8A-1, could not be grown under the conditions used.

SUMMARY AND CONCLUSIONS

We have succeeded in isolating and growing autotrophically nine strains of hydrogen-oxidizing bacteria. Five of these strains, as well as two culture collection strains, *A. eutrophus* H16 and *Paracoccus denitrificans* 17741, have been assayed for the ability to metabolize PCBs. All except strain 17741 metabolize PCBs, but the congener selectivity pattern and metabolite characteristics do not conform to those previously observed for bacterial oxidation of PCBs or for dechlorination of the type seen in anaerobic sediments [Bedard et al., 1986, 1987a, 1987b; Brown et al., 1987a, 1987b].

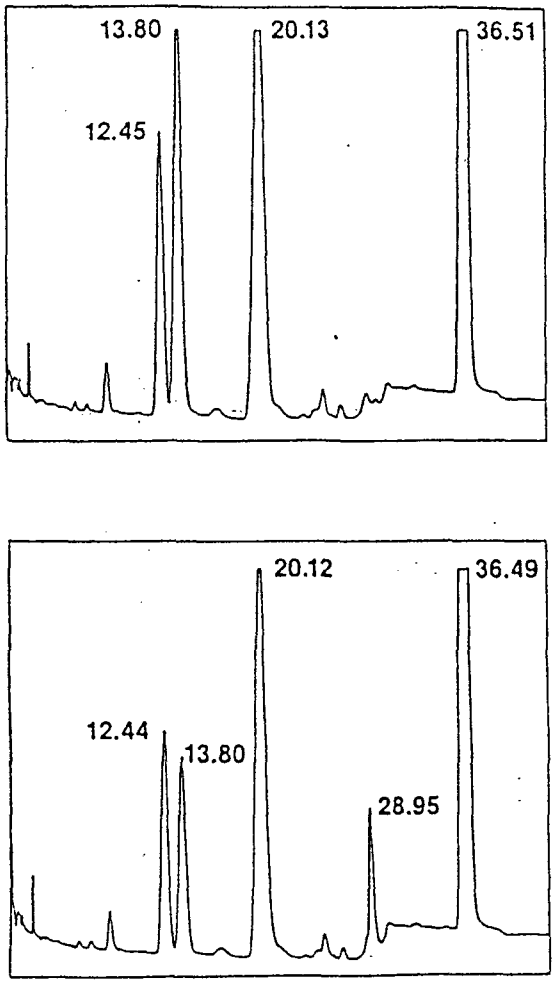


Figure 7-1. **Biotransformation of PCBs by hydrogen-oxidizing strain R2B-2A. GC profile of PCBs extracted from heat-killed control (top) and from live cells (bottom).**

FUTURE PLANS

Identification of the PCB metabolite produced by three of the hydrogen-oxidizing isolates should provide some information regarding the nature of the biotransformation that is occurring. We will attempt to determine which of the PCBs in the mixture is metabolized to the unknown product. We will then try to optimize growth and assay conditions in order to obtain enough of this metabolite to characterize by GC-MS. The direction of further research will be determined by the information provided by these experiments.

Chapter 8

IDENTIFICATION OF PCB TRANSFORMATION PROCESSES OCCURRING IN NATURE

John F. Brown, Jr.
Biological Sciences Laboratory

Robert E. Wagner, James C. Carnahan, Steven B. Dorn, and Carl L. Chalek
Materials Characterization and Engineering Support Operation

Richard W. Lawton, M.D.
Consultant

GE Research and Development Center
Schenectady, New York

INTRODUCTION

About four years ago we began to realize that PCBs carry in their gas chromatographic (GC) tracings a coded record of their origins and transformation histories. These records appear because each type of environmental process alters the distribution of components in the commercially used PCB mixtures according to its own congener selectivity pattern. Since then, we have been learning how to read these codes by assembling a chromatographic library of Aroclor alteration patterns and using it to identify the known or unknown PCB alteration processes occurring in specific environmental compartments. A particularly significant process discovered in this way was reductive dechlorination, which now appears to be widespread in anaerobic aquatic sediments [GE Reports, 1984-1987; Brown et al., 1984, 1987a, 1987b] and even duplicable in the laboratory (see Chapter 5 of this report).

During the past year we have been concerned not only with the transformations occurring in sediments, but also with those occurring in aerobic environments, higher animals, and man. We also have been attempting to develop simpler and more comprehensible indexes for reporting the changes in composition brought about by any of these environmental transformation processes.

RESULTS AND DISCUSSION

Persistence of Aroclor 1260 Components in Man

During a review of serum PCB analytical data for occupationally exposed transformer workers, we identified an individual who had a heavy exposure to Aroclor 1260 in the past (estimated from job history as 25 to 30 years before the time of sampling), and had no significant exposure to other Aroclors subsequently. Three very similar Apolane C87 capillary gas chromatograms (GCs) had been run on serum samples from this individual by L.S. Sheldon and E.D. Pellizzari of the Research Triangle Institute, and we ran a somewhat higher resolution DB1 capillary GC in our own laboratory to resolve some coeluting congeners and to complete the range of peak identifications. Inspection showed that several major congeners, notably 2,3,4,5,3′4′-, 2,3,4,5,2′4′5′-, 2,3,4,5,2′3′4′-, 2,3,4,5,2′3′5′6′-, and 2,3,4,5,2′3′4′5′-CB

were present in about the same relative proportions as in Aroclor 1260; the relative levels of all other peaks were lower (except for a few small ones that will be discussed later). Accordingly, we concluded that metabolic loss in this "2,3,4,5-tetrachlorophenyl" group of PCB congeners was probably near zero, and that we therefore could use the congener 2,3,4,5,2′3′4′5′-CB as an internal standard for judging the relative extent of metabolic loss of all other congeners.

This procedure showed that some 18 to 20 congeners had undergone less than 50% metabolic loss after about 25 years in the subject's body. These highly persistent congeners constituted 31% of the original Aroclor 1260 and 74% of the residue left after about 25 years. The overall metabolic loss was estimated at 66.5% of the original 1260, and was distributed over the various isomer groups (Table 8-1). It is noteworthy that losses occurred in all isomer groups, and that the homolog distribution in the subject's PCB residue peaked at the heptachloro, rather than the usual (background-exposed human) hexachloro, level of chlorination.

Refinement of the measurements of the extent of loss of the various individual Aroclor 1260 congeners is still incomplete. However, intercomparisons among the preliminary estimates of relative congener loss rates suggested that the relative tendencies of chlorophenyl groups to persist fell in the order 2,3,5,6 > 2,3,4,5; 3,4,5; 2,3,5 > 2,3,4,5,6 > 2,4,5 > 2,3,4,6; 2,3,4 > 3,4 > 2,4, 4 > > 2,3,6; 2,5; 2,3; 2,6; 3; 2; 0.

Persistence of Aroclor 1242 and 1254 Components in Man

Many published GC studies have demonstrated that human exposure to the usual commercial PCB products (e.g., Aroclors 1242, 1248, or 1254, or their European or Japanese equivalents) results in the accumulation of a characteristic "normal" profile of at least moderately persistent PCB congeners [e.g., Wolff et al., 1982; Lawton et al., 1985a]. However, this GC profile is altered to a characteristic "Pattern A" [Masuda et al., 1974] in persons who

Table 8-1

PCB HOMOLOG DISTRIBUTIONS IN AROCLOR 1260, IN THE ESTIMATED METABOLIC LOSSES, AND IN THE UNMETABOLIZED RESIDUE APPROXIMATELY 25 YEARS AFTER EXPOSURE TO AROCLOR 1260

Chlorination Level	Weight Percent Distribution 1260	Est. Loss[a]	Est. Remaining[a]	Final Distribution
Tetra	1.8	1.7 (97%)	0.1	0.2
Penta	11.1	10.5 (95%)	0.5	1.5
Hexa	41.2	32.8 (80%)	8.3	24.9
Hepta	38.1	18.7 (49%)	19.4	57.8
Octa	7.1	2.5 (35%)	4.7	13.9
Nona	0.8	0.2 (25%)	0.6	1.7
Total	100.0	66.5	33.5	100.0

[a]Observed decline relative to peak for 2,3,4,5,2′3′4′5′ octachlorobiphenyl, which was presumed to have undergone no metabolic alteration.

consumed mixtures of PCB and PCDF, and subsequently exhibited symptoms of PCDF poisoning (chloracne, "yusho disease"). The only published study of PCB congener clearance rates in man [Chen et al., 1982] dealt with a population of yusho disease patients who were followed over periods of 11 to 17 months. This study reported clearance rates for only two congeners, but did provide raw data and chromatograms from which we could calculate or estimate the clearance rates of several others. Meanwhile, we had been conducting a longitudinal study on a population of capacitor workers who had extensive exposure to Aroclors 1254, 1242, and 1016 over the period 1946 to 1977, and had complete medical examinations, including determinations of serum PCB levels, in 1976, 1979, and 1983, with the next examinations planned for the fall of 1988. Thus far, this population has exhibited "normal" GC profiles and health status [Lawton et al., 1985b]. When it became evident from capillary GC studies of the sera that most of the peaks observed in the original low-resolution packed column analytical GCs represented single congeners, and hence could be used to follow the changes in such congeners over time, an effort was made to recover the analytical GCs from the analyst and remeasure the individual peaks. Thus far, we have located matched pairs of GCs run on 1976 and 1983 sera for 39 individuals, and have used them to estimate the mean clearance rates for the seven largest congeners present. The results, along with comparable figures calculated from Chen's data, are shown in Table 8-2.

First-order rate constants, like those tabulated, can be converted into half-time ($t_{1/2}$) values by dividing them into 0.693 (the natural log of 2). Thus, the estimated $t_{1/2}$ for 2,4,4′-trichlorobiphenyl clearance is 1.43 years, and that for 2,4,5,2′4′5′-hexachlorobiphenyl is 12.4 years. The latter figure is exactly what was also calculated for the 1260-exposed transformer worker discussed in the previous section, assuming a 25-year clearance time.

Table 8-2

MEAN RATES OF PCB CONGENER LOSS
FROM THE SERA OF OCCUPATIONALLY EXPOSED
CAPACITOR WORKERS AND YUSHO PATIENTS

PCB Congener	IUPAC No.	Mean Rate of Loss (yr^{-1}) Capacitor Workers[a]	Yusho Patients	Ratio
2,4,4′	028	0.49	-	-
2,4,5,4′	074	0.22	~0.62	2.9
2,4,5,2′4′	099	0.21	0.03	0.14
2,4,5,3′4′	118	0.12	0.85	7.3
2,3,4,3′4′	105	0.18	1.24	7.0
2,4,5,2′4′5′	153	0.056	0.014	0.25
2,3,4,2′4′5′	138	0.089	0.025	0.28

[a]Based on two observations 7.75 years apart. However, use of Aroclor 1016, and hence direct exposure to 2,4,4′-CB and a little 2,4,5,4′-CB, continued for about 1.4 years after the first examination. We assume the time interval over which the observed PCB decay occurred was 6.3 to 6.5 years for 2,4,4′- and 2,4,5,4′-CB and 7.7 years for the other congeners.

Table 8-2 clearly shows the kinetic origin for PCB congener retention Pattern A in yusho patients. Three of the major congeners are cleared three to seven times as fast as in normal persons, while another three are cleared three to seven times more slowly. Evidently, the clearance processes operative in the two populations are different.

Using the peaks listed in Table 8-2 as internal standards, we have estimated the relative clearance rates for many minor congeners in the capacitor worker population. This permitted the congeners to be grouped into sets according to whether they were cleared very rapidly ($t1/2$ < 1 year), slowly ($t1/2$ 1 to 25 years), or very slowly or not at all ($t1/2$ >25 years). It was found that all lower (<6 Cl/BP) congeners lacking in 4,4′-substitution fell into the first group, while most Aroclor 1254 constituents having 4,4′-substitution fell into the second.

Identification of Oxidase Responsible for Normal Human PCB Metabolism

To determine which oxidase(s) might be responsible for human PCB metabolism according to the observed patterns, we examined two studies of the clearance of PCB congeners by individual rat liver oxidases. One of these [Kaminsky et al., 1981] reported the relative reactivities of ten dichlorobiphenyls with reconstituted, purified cytochromes P450 PB-B or BNF-13, which are the major terminal oxidase isozymes resulting from induction by phenobarbital (PB) or β-naphthoflavone (BNF), respectively. We shall refer to these enzymes generically as species of the P450- and P448-type, respectively, the latter also being assayable as aromatic hydrocarbon hydroxylase (AHH) or ethoxyresorufin O-demethylase (EROD) activity. The other study [Saeki et al., 1983] reported the relative reactivities of all resolvable mixed Kanechlor peaks toward rat hepatic microsomal enzymes induced by PB or 3-methylcholanthrene (MC). At the time, these authors were not able to make complete PCB congener identifications for all 101 resolved peaks. However, their column used the same stationary phase as ours, so we were able to use our peak identifications [Brown et al., 1987b] on their chromatograms.

Saeki et al. reported their observations in terms of $t1/2$ values in their experimental system, with all of the longer times reported as simply ">200 minutes." Thus, the relative reactivities of the more persistent peak components (37 of the 101 for P450, 80 of the 101 for P448) could not be assessed. However, PCB congener reactivity patterns for the two enzyme preparations were quite different. Comparing them with the reactivity pattern observed in the capacitor worker population, we found a close correlation between those congeners observed to be cleared rapidly in man and those cleared with $t1/2$ under 200 min by the PB-induced rat liver P450. Accordingly, we conclude that the enzyme normally involved in human PCB metabolism must be the human analog of rat P450.

Structure-Activity Relationships for PCB Metabolism by P450

Upon examining data from the transformer worker, the capacitor workers, or the PB-induced rat liver, a remarkably simple overall structure-activity relationship appeared: if either ring of the PCB molecule carries a pair of adjacent hydrogen atoms at *meta* and *para* (i.e., 3- and 4- or 4- and 5-) positions on the ring, that congener will be cleared rapidly in the human and show a $t1/2$ less than 200 min in the Saeki system. If neither ring carries a hydrogen atom at a *meta* (3- or 5-) position, it will be cleared either very slowly or not at all. If the congener has a ring carrying a *meta* but not a *para* hydrogen, it will be cleared slowly, with the exact rate depending strongly upon the substitution pattern in the opposite ring.

It has long been recognized [Sundström et al., 1976] that the metabolism of PCBs in mammals proceeds via epoxidation; i.e., they attack at a pair of adjacent carbon atoms. The observed selectivity pattern now further indicates that in both the rat and the normal human, this attack probably occurs only at the bond between positions 3 and 4. The attack occurs most readily when no chlorine is present at either position, but it may proceed slowly when the 4-position is chlorinated. The improbability of P450 attack at the 2,3-position is indicated both by the observations of chlorine atom rearrangements during oxidations of biphenyls chlorinated in the 4-position [Sundström et al., 1976] and our observation that Aroclor 1260 congeners carrying 2,3,4,6-chlorophenyl groups are about as easily attacked as the corresponding congeners carrying 2,3,4-chlorophenyl groups.

The effect of opposite ring chlorination patterns on the attack of 4-substituted-3-unsubstituted rings is quite marked. 4,4′-dichlorobiphenyl, although more slowly attacked than the other dichlorobiphenyls [Kaminsky et al., 1981], is not noticeably persistent in man. However, 2,4,4′-, 2,4,5,4′-, and 2,3,4,5,4′-CB are increasingly resistant to metabolism. The pattern of relative ability to impede attack on 4-, 2,4-, 3,4-, 2,3,4-, or 2,4,5-chlorophenyl groups appears to be 2,3,4,5 > 2,3,5,6 > 2,4,5 > 2,4. This may help to explain why the "highly persistent" ($t1/2$ >25 years) species observed in the 1260-exposed transformer worker contained about 75% PCB congeners carrying 2,3,4,5-chlorophenyl groups, 20% those carrying 2,3,5,6-chlorophenyls, and 5% with 2,3,4,5,6-chlorophenyls.

Evidence for PCB Dechlorination in Man

The above set of structure-activity relationships predicts that all congeners carrying only groups lacking in open *meta* positions (i.e., 2,3,4,5,6-, 2,3,4,5-, 2,3,5,6-, 3,4,5-, and 2,3,5-chlorophenyl groups) should be equally persistent in man. This is not quite in accord with the pattern of congener retention observed in the transformer worker's serum. There, several congeners carrying 2,3,5,6-chlorophenyl groups appeared at 10 to 50% higher levels (relative to 2,3,4,5,2′3′4′5′-CB) than in Aroclor 1260, and all congeners carrying 2,3,4,5,6-chlorophenyls were somewhat depressed. Moreover, there was a 25% overall loss in non-achlorobiphenyls, all of which carry 2,3,4,5,6-chlorophenyl groups (Table 8-1).

These findings suggest that a very slow dechlorination of pentachlorophenyl to 2,3,5,6-tetrachlorophenyl groups can occur in the human body. The requisite electron donor could be either the reduced form of the cytochrome, some other heme protein, or even another redox system; many examples of dechlorinations of freons, DDTs, chlorinated solvents, and other non-aromatic chlorine compounds by such biochemical agents are known. Moreover, the pentachlorophenyl group is known to have a reduction potential some 120 mv lower than that of any other chlorophenyl group and to give mainly 2,3,5,6-tetrachlorophenyl groups upon reduction [Farwell et al., 1975], so that a weakly active electron donor might well be expected to carry out only this one type of dechlorination.

In ordinary Aroclor-exposed humans, this presumptive dechlorination does not make a significant contribution to PCB metabolism: the reactive congeners constitute only tiny fractions of the original Aroclors, and the reaction half-time is about 50 years. However, it may be more important in other species. For example, it could account for the fairly rapid clearance of 3,4,5,3′4′5′-CB (and other hexachlorobiphenyls) that has been observed in the Japanese quail [Sparling and Safe, 1980].

Identification of Oxidase Responsible for PCB Metabolisms in Yusho Patients

Table 8-2 shows that in patients with yusho disease the normal P450 PCB-metabolizing activity is partially suppressed, and that a new activity has appeared. It is well known that the polychlorinated dibenzofurans (PCDFs), which are the primary causative agents of yusho disease, are also powerful inducers of P448 cytochromes [Safe, 1987], and that P448 cytochrome can attack PCBs with a selectivity pattern different from that of P450 [Saeki et al., 1983]. Unfortunately, Saeki's data do not extend to the reactivity range covered by the congeners of Table 8-2, so we do not have direct evidence that the enhanced metabolism of 2,4,5,3′4′- and 2,3,4,3′4′-CB in the yusho patients can be attributed to P448. However, we have noted some remarkable correlations that can be used as indirect evidence.

In many respects, the PCB congener selectivity pattern of P450 parallels that of the aerobic bacteria *Pseudomonas putida* LB400 and *Alcaligenes eutrophus* H350, which have been extensively described in earlier reports of this series. Both the P450 and the cited aerobes preferentially attack congeners with open 3,4-positions and are reluctant to attack those that are 4,4′-substituted, although slow attack on species like 2,4,5,2′4′5′-CB is possible. For both, the order of relative reactivity toward symmetrical dichlorobiphenyls is 2,2′- >> 3,3′- >> 4,4′-CB [Kaminsky et al., 1981; Bedard et al., 1987a]. Likewise, there appear to be parallels between the selectivity patterns of P448 and *Corynebacterium* MB1, which also has been extensively described in this series. Both appear to be generally reluctant to attack congeners having 2,2′-substitution, but easily attack those substituted 4,4′. For both, the order of relative reactivity toward symmetrical dichlorobiphenyls is 3,3′ >> 4,4′- >> 2,2′-CB [Kaminsky et al., 1981; Bedard et al., 1987a].

Within the pentachlorobiphenyl series, MB1 can oxidize 2,4,5,3′4′- and 2,3,4,3′4′-CB but not 2,4,5,2′4′-. This is precisely the pattern seen in the last column of Table 8-2. Thus, if the parallelism between the MB1 and P448 selectivity patterns observed for the lower PCB congeners can be extended to the pentachlorobiphenyls, what we are seeing in the yusho patients is indeed the pattern that would be expected for PCB oxidation by P448.

We believe that the reactivity parallelisms just noted have a simple basis: in attacking biphenyl derivatives the MB1 and P448 mixed function oxidases behave as 2,3-dioxygenases and 2,3-monooxygenases, respectively, and the H850/LB400 and P450 oxidases behave as 3,4-dioxygenases and 3,4-monooxygenases, respectively; and it is the orientation of the attack, rather than the mode of oxygen addition, that is responsible for much of the congener selectivity pattern. We must note, however, that the parallelism is not total. Both monooxygenases are far more reactive to congeners with 2,6-substitution than the dioxygenases, and the total range of congeners attacked by P450 is considerably greater than that attacked by LB400.

The range of PCB congeners attacked by P448 as a 2,3-monooxygenase appears to be considerably smaller than the range of those attacked by P450 [Saeki et al., 1983], just as the range of congeners attacked by the ordinary 2,3-dioxygenase-carrying bacteria is smaller than those for attack by H850 or LB400 [Bedard et al., 1986]. However, certain relatively persistent congeners, notably 2,4,5,3′4′- and 2,3,4,3′4′-CB, are more rapidly attacked (Table 8-2). We suggest that the accelerated removal of these congeners (relative to 2,4,5,2′4′, for example) may be a useful indicator of past P448 induction or chloracnegic disease in human or wildlife specimens.

Evidence of P450 Activity in Environmental Systems

Having learned from the extensive human and more limited rat data what the patterns for P450-degraded Aroclors looked like, we have begun an examination of some available environmental PCB gas chromatograms to see where other such alterations may be occurring in nature.

The chromatograms of the PCBs in Hudson River brown bullheads and striped bass generally resembled those of Aroclor mixtures that had been partially dechlorinated, usually by System H/H′, and then considerably depleted in mono-, di-, and trichlorobiphenyls. It was not immediately apparent whether the latter feature resulted from 2,3-dioxygenase attack by aerobic bacteria in the water, from 2,3-monooxygenase attack by P448 cytochromes in the fish, or both. The congener distribution pattern in the tetra- through heptachlorobiphenyl range showed little or no evidence of P450-mediated metabolism. This was expected, in view of literature reports that fish generally don't show much PCB-degradative capacity [Niimi and Oliver, 1983] and what little there is (e.g., metabolism of 3,3′- and 3,4,3′4′-CB) strongly suggests mediation by P448 rather than P450.

By contrast, the available GCs of New Bedford lobsters and crabs [e.g., Farrington et al., 1986] showed well-advanced PCB metabolism proceeding along the same lines as in humans, rats, and other mammals. Evidently the crustaceans, unlike mollusks and most fish, can use a P450-like system to metabolize PCBs at rates well above those for equilibration with their environment.

An unexpected result appeared in an unpublished report from the Columbia National Fisheries Laboratory [Stalling, 1982] that included chromatograms of a variety of Great Lakes fish (lake trout, bloater, yellow perch, carp, northern pike, walleye). The chromatograms, which were all quite similar, showed evidence of extensive P450-type biodegradation. At the moment, we do not know what component of the Great Lakes ecosystem (e.g., algae, fungi, protozoa) is responsible for this biodegradation or whether this process is the basis for the rather remarkable rate of decline of PCB levels that has been observed in Great Lakes biota [Hillman, 1988].

Additional Riverbottom Areas Showing PCB Dechlorination

In order to determine what changes might be occurring in the PCBs of the mid-Hudson, sediments were collected from deposits located a little below the low-tide line at various sites near Troy, Albany, Catskill, and Kingston, New York. The locations sampled included, but were not restricted to, those described in Richard Bopp's PhD thesis [Bopp, 1979] and subsequent publications. The sediments were collected as cores 16 to 24 in. long, which were divided into two or three 6- to 10-in. sections for analysis. Analyses on air-dried samples were done as previously described [Brown et al., 1984; 1987b].

The PCB levels found in the samples from the Albany-Troy area ranged between 0 and 42 ppm, with the average being about 6 ppm. The Catskill and Kingston samples showed only a few parts per million, if any.

All samples from the Troy-Albany area containing more than 5 ppm total PCB appeared to have originally been predominantly Aroclor 1242, unless some unusually extensive removal of more highly chlorinated congeners had occurred. However, two samples did contain residual Aroclor 1254 in the 15 to 20% range, three contained 3 to 5% residual Aroclor 1260, one

about 9% Aroclor 1268 (a wax extender that once had a wide variety of minor uses), and one was >50% Aroclor 1270, a long-obsolete product once used as an investment casting wax.

There was evidence of dechlorination at all sites that presented more than just traces of PCBs. At two sites, one in Troy and one in Albany, the dechlorination patterns looked similar, although not identical, to Pattern B of the Thompson Island Pool [Brown et al., 1987a,b]. At other sites in the Troy-Albany area and at all sites at Catskill and Kingston where there was enough PCB to get a good GC pattern, the dechlorination pattern resembled Patterns H or H′ found in New Bedford Harbor (Massachusetts), Escambia Bay (Pensacola, Florida), Woods Pond (Massachusetts), the Housatonic River (Connecticut), and the Sheboygan River and harbor (Wisconsin) [GE Report, 1987].

It would now appear that this alteration pattern was first seen (in low-resolution analytical packed column GC tracings), by Richard Bopp during his thesis work, but was initially interpreted as indicating the presence of Aroclor 1016 [Bopp, 1979; Bopp et al., 1981, 1984]. Even at low resolution, however, the match with an Aroclor 1016 pattern is unimpressive; the only feature in common is the diminution of the two mono-*ortho* tetrachlorobiphenyl peaks. These peaks were removed from Aroclor 1242 by the fractional distillation process used to produce Aroclor 1016, but are also among those most susceptible to reductive dechlorination. In higher resolution GCs the distinction between Aroclor 1016 and H/H′-dechlorinated 1242 is unambiguous: a whole series of peaks are removed by the System H dechlorination, and another series of new peaks are formed. As a matter of fact, we have not seen Aroclor 1016 in any sediment sample examined to date, despite diligent search. At the time this Aroclor was introduced (1971), there was already considerable concern about the presence of PCBs in the environment, and it would appear that releases were minimal.

We have also received several fairly well-resolved packed column GCs of Sheboygan harbor sediments for examination. Most showed the Aroclor 1248 that was released there at various stages of Pattern H/H′ dechlorination. One showed a different pattern, more similar to upper Hudson Pattern B or Waukegan (Illinois) harbor Pattern W, indicating more extensive dechlorination.

New Indexes for Characterizing Environmental PCB Alteration

Thus far, our searches for the types of PCB alteration that can occur in natural systems have identified about ten generic types of processes (2,3- and 3,4-dioxygenase oxidation, 2,3- and 3,4-monooxygenase [P448, P450] oxidation, low- and high-potential *m,p*-dechlorination, a possible very low-potential eucaryotic dechlorination process, low-potential *o,m,p*-dechlorination, photolytic dechlorination, and phase-transfer partitioning) and many species-specific process variants. For most of these processes, we now have representative chromatograms in hand that portray the changes in GC patterns induced by that particular alteration process, and usually we have a chemical rationale that explains why some groups of peaks disappear faster than others. However, a particular pattern of change within a group of several dozen peaks is not a simple concept to describe, communicate, or quantify. Accordingly, we have been exploring ways to provide summary measures of the overall level of PCB alteration that would avoid the complexities of talking about patterns of change within all 140 measurable GC and GC-MS PCB peaks.

One summary measure that is currently being used by a few others is to report the observed distribution of PCB congeners according to total chlorine number. (The measurement

of this distribution can be quite easily made with moderate accuracy by GC-MS, even without knowing any congener identifications.) This sort of distribution can be used to estimate the original Aroclor composition of the release (assuming that no environmental alteration has occurred), but the reported data, like older reports of total Aroclor levels, conceal, rather than reveal, any evidences for environmental change that may have been in the original GC-MS tracing.

It occurred to us that by counting not just the total chlorines per PCB molecule in aggregate, but instead the chlorines in *ortho* and non-*ortho* (i.e., *meta* plus *para*) positions separately, we might have some more useful indexes, even if we looked only at the average *ortho* and non-*ortho* chlorination levels for all PCB congeners present.

To evaluate this possibility, we devised a computer program that could be applied to our existing GC records, along with certain inputs from GC-MS measurements on peaks given by non-isomeric coeluting congeners. This program calculates not only the PCB congener distribution according to total chlorine number, but also the total numbers of *ortho* and non-*ortho* chlorine atoms per biphenyl residue.

Having such data on some 200 PCB specimens, our first concern was to establish that we were indeed measuring a consistent PCB property. To do this, we collected a series of Aroclor 1242 specimens from capacitors with a range of manufacturing dates from 1950 to 1971. Good consistency in the homolog distributions and excellent consistency (most values within 1% of the mean) in the differential chlorine levels were observed.

Representative data for Aroclor 1242 and 1260 specimens that had undergone one or more of the known types of environmental alterations are shown in Table 8-3. From these data, several conclusions can be drawn.

First, simple phase transfer processes, such as evaporation or extraction into water, do not change the level of *ortho* chlorination, even though these processes have the expected result of increasing the total chlorine level in the PCBs in the residual phase and decreasing that of those in the volatilized or dissolved phase. Thus, a heavily evaporated 1242 still had the *ortho* chlorine content of 1242 rather than that of 1248 or 1254.

Second, the *meta*, *para*-selective dechlorination processes, such as the upper Hudson processes B,B′ and C, the Waukegan harbor process W (data not shown), and the widespread H/H′ processes, do indeed remove only the non-*ortho* chlorines, again leaving the *ortho* chlorination level unchanged. *Ortho* chlorine loss (thus far, seen only in Silver Lake, Pittsfield, Massachusetts) is effected, however, by process F and also by process G, if the latter is indeed a single process and not simply a combination of F with a *m,p*-selective dechlorination.

Third, metabolism by the eucaryotic P450 monooxygenase results in a considerable increase in the non-*ortho* (*meta* plus *para*) chlorination level. Calculations show that during this process the levels of *ortho* chlorines should be quite rapidly reduced initially, and then more slowly increased, so that after sufficient time (e.g., 15 to 25 years in the human) the *ortho* chlorine level would be back at its original value.

Fourth, biodegradation by the ordinary environmental mixtures of PCB-degrading aerobic bacteria, which appear to carry 2,3-dioxygenases with activities largely limited to the mono-*ortho* di- and trichlorobiphenyls, results in increases in both the *ortho* and non-*ortho* chlorine

Table 8-3

EFFECTS OF ENVIRONMENTAL ALTERATION PROCESSES ON THE MEAN LEVELS OF *ORTHO* AND NON-*ORTHO* CHLORINES PER BIPHENYL

Original Composition	Alteration Process	Cl Atoms per Biphenyl Residue *ortho*	*m* + *p*	Total
Aroclor 1242	None	1.41	1.81	3.22
Aroclor 1242	31.4% evaporation	1.40	2.06	3.46
Aroclor 1242	93.4% evaporation	1.42	2.80	4.26
Aroclor 1242	Extraction into water	1.43	1.07	2.50
Aroclor 1242	Extraction into water plus 23-DO[a]	1.96	1.19	3.15
Aroclor 1242	Dechlor, plus 23-DO[b]	1.67	2.33	3.96
Aroclor 1242	Evap., plus 23-DO[c]	1.60	2.36	4.00
Aroclor 1242 (+1254)	P450 metab., man[d]	1.25	2.87	4.12
Aroclor 1242 (+1254)	Dechlor., Pattern B[e]	1.45	0.87	2.32
Aroclor 1242 (+1254)	Dechlor., Pattern B'[e]	1.40	0.42	1.82
Aroclor 1242 (+1254)	Dechlor., Pattern C[e]	1.47	0.30	1.77
Aroclor 1260	None	2.28	4.05	6.33
Aroclor 1260	P450 metab., man[d]	2.22	4.63	6.83
Aroclor 1260	Dechlor., Pattern F[f]	1.80	2.10	3.90
Aroclor 1260	Dechlor., Pattern G[f]	1.80	1.20	3.00

[a] PCB in ground near Aroclor 1242 dumpsite; subjected to water extraction plus aerobic microbial biodegradation of 2,3-dioxygenase type.

[b] PCB in riverside deposits in strata lying just at aerobic-anaerobic interface.

[c] PCB from South Glens Falls dragstrip.

[d] PCB in either capacitor workers, 5 years after exposure to a 1254, 1242, 1016 sequence, or in a transformer worker, 25 years after exposure to 1260.

[e] Approximately 95:5 1242-1254 mixture dechlorinated by upper Hudson *m,p*-dechlorinase systems.

[f] PCB, presumed originally all 1260, dechlorinated by Silver Lake *o,m,p*-dechlorinase systems.

levels, but mainly in the former. Such increases are more prominent in PCB specimens having high contents of the mono-, di-, and trichlorobiphenyls, such as those of groundwater or dechlorination sites, and less so in specimens where the lower PCB congeners are depleted by evaporation.

When the *ortho* and non-*ortho* chlorine levels for the individual Aroclors were plotted against each other, a striking correlation emerged. As shown on Figure 8-1, all of the points for the Aroclor 1221-1268 series fell on a straight line. This means, of course, that the point given by any mixture of these Aroclors would also have to fall on that same line. Thus, we immediately have one simple indication of whether an environmental PCB mixture has undergone alteration, and one that can be applied without any prior knowledge of the composition of the original release: if the *ortho*, non-*ortho* point falls off the trend line shown in Figure 8-1,

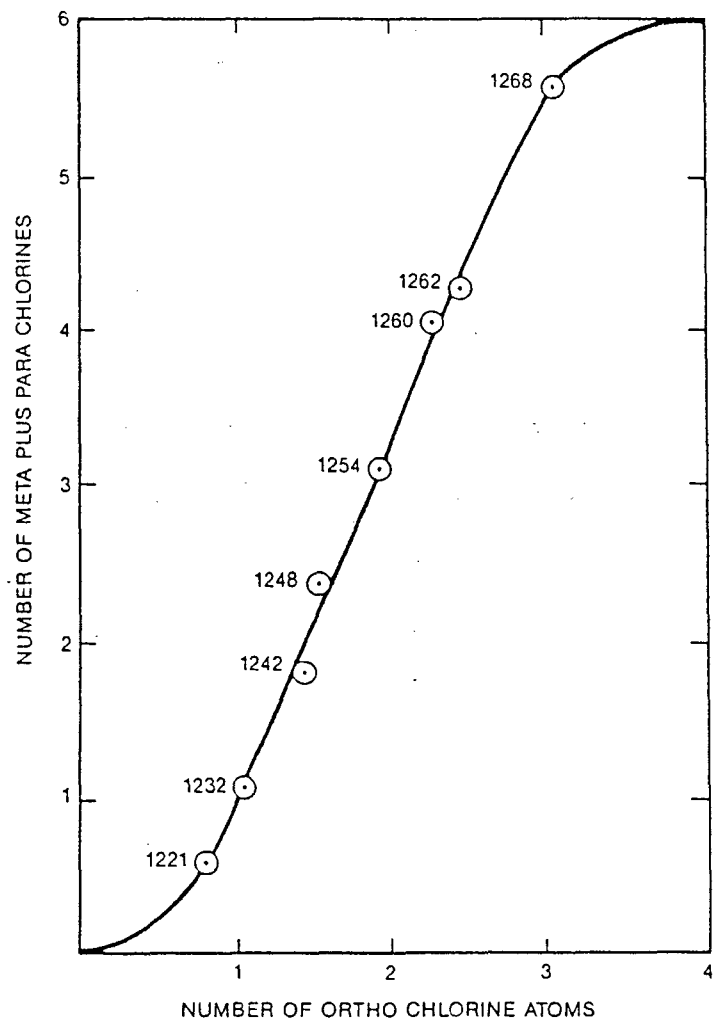


Figure 8-1. **Relationship between numbers of *ortho* and non-*ortho* chlorine atoms in commercial Aroclors.**

some kind of alteration must have occurred, and the net extent of the alteration may be indicated by the departure from the line. For most specimens, an upward departure will indicate the aggregate extent of evaporation, elution, and oxidative biodegradation; a downward departure, the net extent of anaerobic dechlorination; and the position along the *ortho* chlorine axis, the approximate level of chlorination in the original Aroclor mixture.

SUMMARY AND CONCLUSIONS

We have characterized the overall rates of human PCB clearance for the seven major persistent congeners of Aroclor 1242/1254, and relative rates for many components of Aroclors 1242, 1254, and 1260. PCB metabolism in humans and other animals was found to be normally mediated by cytochrome P450 isozyme(s) of the phenobarbital-induced type, although contributions by P448-type isozyme(s) may occur in chloracne patients. A minor contribution to

human PCB metabolism may be made by dechlorination of congeners carrying pentachlorophenyl groups. The PCBs in the Great Lakes appear to have undergone a P450-mediated biooxidation; those of mid-Hudson River (Troy to Kingston, New York) sediments have undergone a Pattern H/H′-type dechlorination. The net result of any of the known types of environmental PCB transformation can be summarized in terms of their effects on the levels of *ortho* and non-*ortho* chlorination in the residue. For the commercial Aroclors of the 1200 series, a linear relationship exists between these two parameters, so that the net effect of all alteration processes operating on an environmental PCB specimen can be measured as a departure from that relationship, even when the original composition of the release is unknown.

FUTURE PLANS

During the coming year we plan to carry out further analysis of the GC data resulting from our 1976-to-1988 clinical studies of workers who had past PCB exposure. From this analysis we will obtain more precise clearance rate data on a broader range of PCB congeners, and can assess the extent of variability in human PCB clearance. We also plan to apply our new indexes of PCB alteration states to analytical data arising from a survey of the PCBs in the tidal Hudson River (Troy to New York City), to develop maps of current PCB sources and transformation sites in the river.

Chapter 9

PROCESS MODELING: SURFACTANT EXTRACTION OF PCBs FROM SOIL

David P. Mobley, John B. McDermott,
David K. Dietrich and Kenneth M. Carroll
Chemical Process Laboratory
GE Research and Development Center
Schenectady, New York

INTRODUCTION

This chapter presents research into the use of aqueous surfactant solutions for the extraction of PCB from soil. This past year we have concentrated our efforts on modeling a soil extraction process on a small scale in the laboratory. This bench-scale process comprised a countercurrent extraction unit and a surfactant/PCB precipitation unit. Selection of the equipment and the operating conditions was based on previously reported Phase I investigations [GE Reports 1985, 1986]. Although an actual soil extraction process would include more units and operations, these two units are the most important in determining the overall efficacy of this process.

Figure 9-1 is a schematic of the complete soil extraction process. The soil is excavated, and then subjected to mechanical breakup and size separation to remove large stones and debris. This oversize material, with its low specific surface area, is returned to the site after minimal treatment. The bulk of the soil then goes to the countercurrent washing operation where the PCB is extracted using an aqueous solution of sodium dodecylbenzenesulphonate (SDBS, an anionic surfactant). An additional water wash of the soil to remove excess surfactant is optional. After dewatering, the soil is returned to the site. The aqueous process stream exiting the washing operation, containing PCB, is mixed with $CaCl_2$ to precipitate the surfactant, PCB, and any soil fines. The waste water is a brine solution with a minimal amount (<2 ppb) of PCB. This waste stream could be treated with activated carbon to further reduce the PCB content. The result of the process is soil suitable for returning to the site and a concentrated PCB and surfactant precipitate that could be incinerated for final PCB destruction.

RESULTS AND DISCUSSION

Bench-Scale Process

The surfactant extraction and precipitation units have been demonstrated with a small bench-scale process composed of four mixing stages, a laboratory centrifuge, and a precipitation vessel. The process was conducted with Oakland subsurface soil spiked with 1000 ppm Aroclor 1260 and soil from the contaminated portion of the Oakland site which had 100 to 200 ppm Aroclor 1260; these two soil samples have similar mineralogy. In this discussion, these soils are referred to as spiked soil and native contaminated soil, respectively.

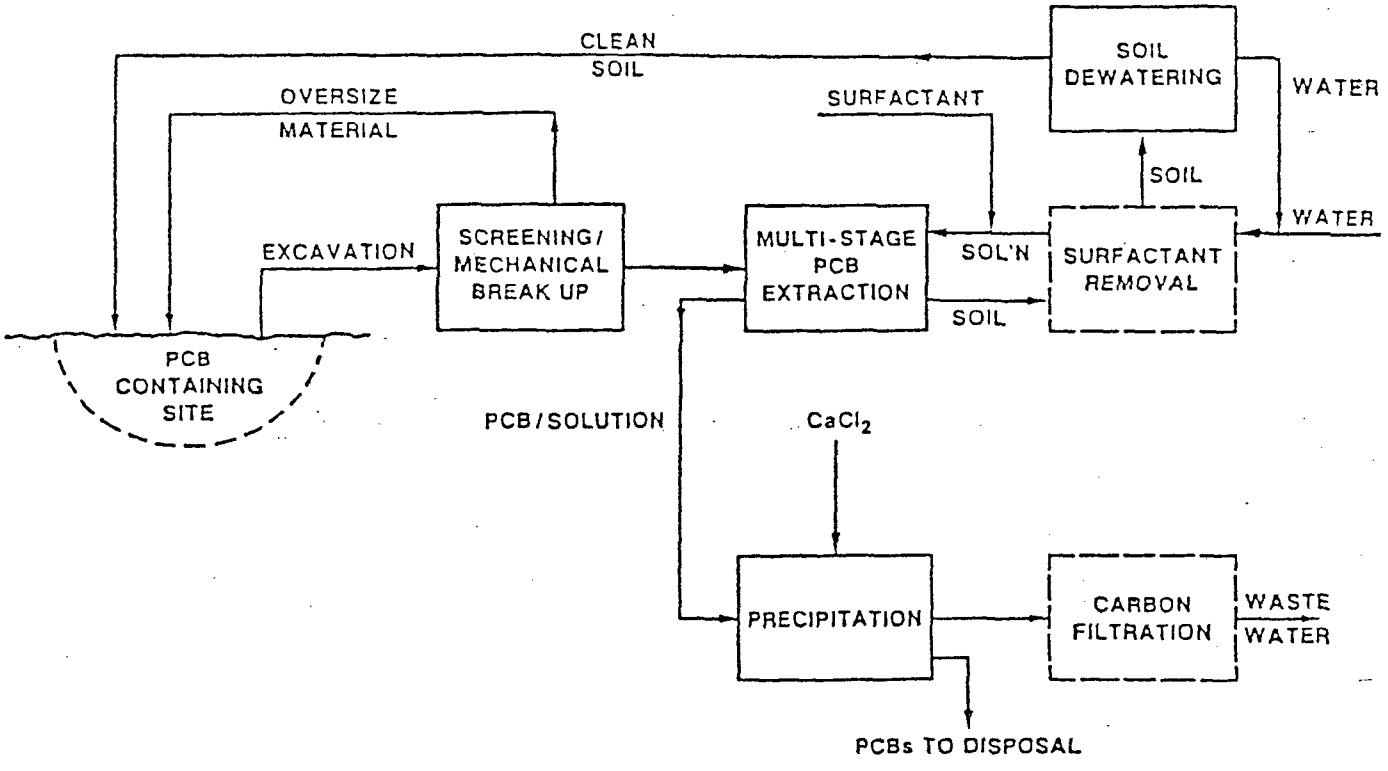


Figure 9-1. Soil extraction process schematic.

The material was processed in batches and countercurrently. The stage capacity was 40 g of soil and 200 ml of a 1% SDBS surfactant solution. A 2-in. marine propeller mixed the soil and surfactant at 300 rpm for 20 min. Liquid and solids were separated by centrifuging 20 min at 1000 × g, which resulted in an underflow of 50% solids. The solid:liquid feed ratio was 1:4.

Figure 9-2 is the schematic of the bench-scale process and the performance data for each stage for the spiked soil trial. The soil, initially at 1000 ppm, was cleaned to approximately 40 ppm. This represents a 25-fold reduction in PCB concentration. The PCB balance around the extraction process was 100%; some PCB was unaccounted for in the precipitation process as the result of some losses during the extraction and analysis of the precipitate material. The true precipitate PCB concentration was higher than the 2.6% found by analysis and reported in the figure. The waste water exiting the precipitation unit had a PCB concentration of 1.8 ppb, approximately the solubility of Aroclor 1260 in water.

The performance of the extraction process with the native contaminated soil was rather different. The contamination of the feed soil ranged from 100 to 168 ppm Aroclor 1260 (ranges must be given as a result of the heterogeneity of the site soil); the product soil exiting the process had a PCB concentration ranging from 18 to 24 ppm. While this represented a cleanup to well under 50 ppm PCB, the degree of extraction was not nearly as good as that for the laboratory spiked soil.

The native contaminated soil also behaved differently from the laboratory spiked soil in the process in another way: approximately 10% of the native contaminated soil was lost to the precipitate as fines, but the loss with the laboratory spiked soil was virtually zero. This greatly increases the amount of material that would be sent to incineration. A centrifuge with a higher g-force would reduce that loss.

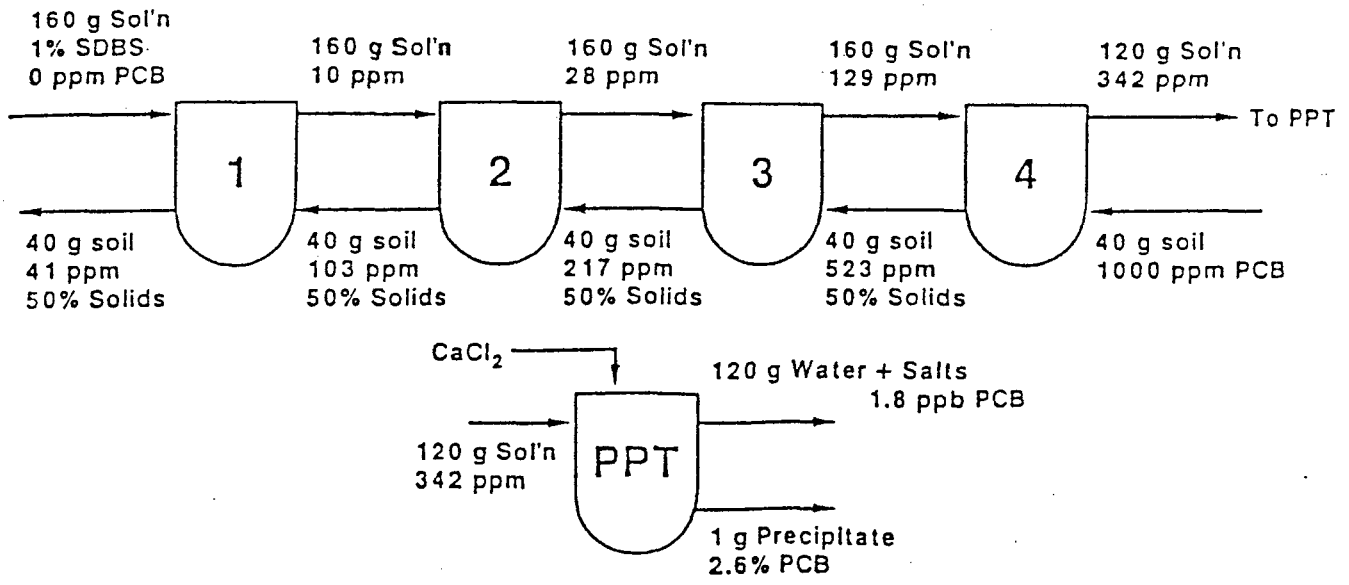


Figure 9-2. Flow diagram of four-stage, bench-scale extraction process with performance data for laboratory-spiked Oakland soil.

Spiked vs Native

The native contaminated soil differed from the laboratory spiked soil in several ways: the age of contamination, the mode of contamination, and the level of contamination. The following experiments were conducted to discover how these differences might affect the extraction efficiency.

Clay is one of the more difficult soil components from which to remove organic contaminants. To investigate the possibility that the distribution of PCB between fine (clay-silt) and coarse (sand) material was different for the native soil and the spiked soil, the soil samples were size-fractionated and then analyzed for PCB content. The fractionation was accomplished by forming a water/soil slurry and then letting the slurry settle for approximately 1 min. The slowly settling material still in suspension was removed from the material that settled rapidly. The coarse material (rapidly settling) was repeatedly slurried with water and allowed to settle until no more fines were suspendable. Despite the vast difference in contamination age and level, there was little difference in the distribution of PCB between fine and coarse material for the two soil samples (Table 9-1).

A series of extraction rate experiments were performed to determine if a lower rate was responsible for the lower extraction efficiency of the native contaminated soil. The experiments were conducted by mixing 40 g of soil with 200 ml of a 1% SDBS solution. Samples of the slurry were taken periodically and quickly centrifuged at 16,000 × g to separate the solids from the supernatant. The supernatant was then analyzed for PCB content.

Figure 9-3 contains the temporal variations of the PCB concentration in the supernatant for the four cases investigated: spiked Oakland soil, native contaminated soil, native contaminated soil at elevated temperature (60 °C), and native contaminated soil using ultrasound agitation instead of a marine propeller. The concentrations are normalized to the long-time

Table 9-1

DISTRIBUTION OF PCB BETWEEN FINE AND COARSE SOIL FRACTIONS

	Wt%	PCB (ppm)	% of PCB
Spiked soil			
Fine	33	2550	89
Coarse	67	155	11
Native contaminated soil			
Fine	39	375	86
Coarse	61	39	14

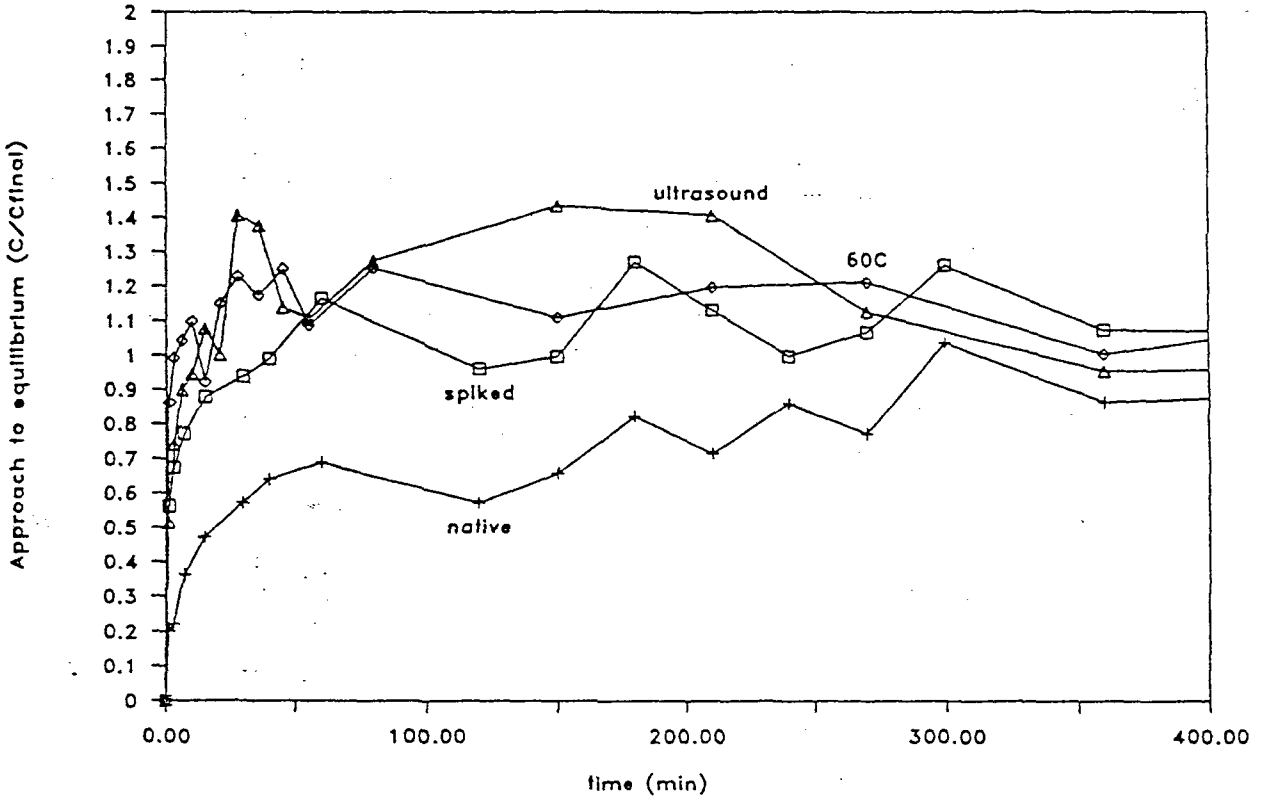


Figure 9-3. PCB extraction rate data.

equilibrium value; i.e., the system is at equilibrium when the concentration is equal to one. Unfortunately, the data contain a fair amount of scatter because soil fines were accidentally included in some of the supernatant samples. This inclusion of fines increases the apparent PCB concentration and results in some of the normalized concentrations being greater than 1. Despite the scatter in the data, some general conclusions can be made. The approach to equilibrium for the Oakland spiked soil is significantly more rapid than it is for the native contaminated soil when the extraction is conducted at room temperature. The extraction rate for the native contaminated soil when exposed to elevated temperature or ultrasound agitation increased to approximately the same rate as the spiked soil. Because the ultrasound also increased the temperature of the mixture, it is impossible to determine whether the increased rate was a result of the agitation or the increased temperature.

Closer examination of the data for the ultrasound experiment shows a decrease in the PCB concentration of the supernatant at longer times. This might be the result of the scatter in the data or it could indicate that the ultrasound increased the surface area of the soil particles by fracturing or delaminating the particles. A significant increase in the surface area could alter the adsorption equilibrium since adsorption is a surface phenomenon.

The extraction rate experiment also led to another observation. The soil slurries for the spiked soil and the native contaminated soil were allowed to settle for several weeks on the laboratory bench. The spiked soil had settled to a much denser mass, indicating that the particle size distribution was shifted toward larger particles. To investigate this further, the spiked soil, the native contaminated soil and a sample of clean Oakland soil that had not been spiked with PCB were analyzed with a Horiba Centrifugal Particle Analyzer. This apparatus measures the light absorbance of a suspension of particles as they settle in an accelerated field ($2{,}200 \times g$). As the particles settle, the suspension clears, causing the absorbance to decrease. At the high centrifugation speeds used for this analysis, only the slowly settling fraction (small particle size) was observed. The absorbance for each of the soil suspensions is plotted as a function of centrifugation time in Figure 9-4. The data for the native contaminated soil and the clean Oakland soil coincide fairly well, with average particle sizes of 0.21 and 0.17 μm, respectively. The spiked Oakland soil, which we made by applying PCB to the same clean Oakland soil used in this experiment, differed considerably from the other two soils and had an average particle size of 0.39 μm. This much smaller average particle size of the native contaminated soil corresponds with the slower settling observed in the bench-scale process. The soil fractionation data does not contradict this data because the division between fine and coarse occurred at such a relatively large particle size that the difference in the size distribution of very small particles seen in this analysis was undiscernible.

The change in particle size as the result of spiking with PCB cannot be explained at this point. There is some loss of small particles during the spiking process resulting from dusting but not enough material is lost to account for the change. The spiking process does effectively dry the soil and this might affect the colloidal character of the clay particles.

SUMMARY AND CONCLUSIONS

A bench-scale process using an aqueous surfactant solution cleaned laboratory spiked soil and native contaminated soil to less than 50 ppm PCB. The extraction efficiency for the native contaminated soil was much lower than for the spiked soil.

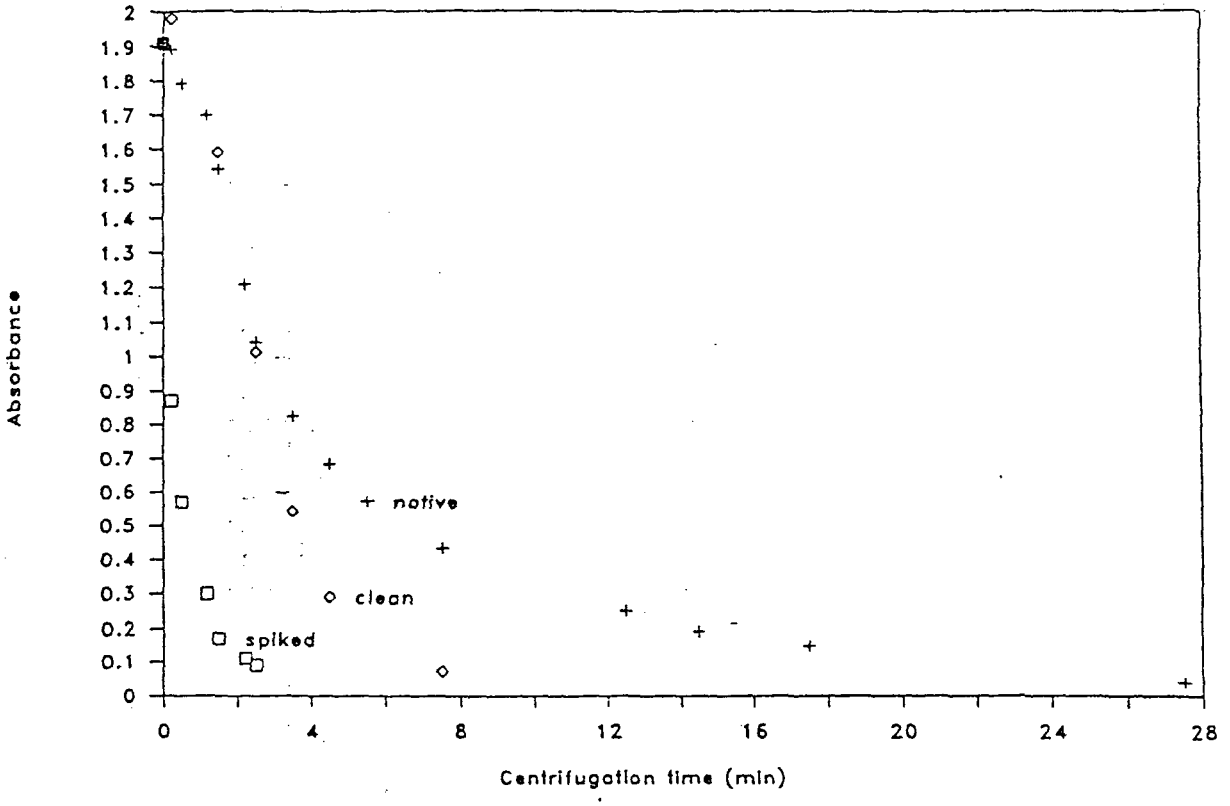


Figure 9-4. Absorbances of soil suspensions as a function of centrifugation time.

The process used for spiking the PCB onto the soil affects the particle size distribution and the extraction rate. Two methods have been identified that improve the extraction rate of PCB from native contaminated soil and might improve the extraction efficiency of the process.

The possibility of a barrier to extraction at low PCB concentrations still exists. In order to determine whether the level of contamination has an effect on the extraction efficiency, it is necessary to conduct experiments with site soil that has a much higher PCB concentration. We have made arrangements for Oakland soil with a higher PCB content to be sent to us and expect to receive it shortly.

FUTURE PLANS

Our immediate plans are aimed at determining the feasibility of using this process for the Oakland site. With the more heavily contaminated soil we will be better able to determine which factors have the greatest influence on the extraction efficiency and test the strategies for improvement noted above. If the process proves feasible technically, the next area of investigation will be reduction of the process costs to improve the economic practicality.

Chapter 10

RESEARCH PLANS FOR 1988-1989

The biological research in this program, both intra- and extramural, has focused on two general mechanisms of microbial attack on PCBs: oxidation and cleavage of the ring, and reductive dechlorination. Both lines of research have produced promising results and will be continued. The integration of both modes of microbial attack into a single, two-step process could lead to the biodegradation of all PCB molecules, including the more highly chlorinated congeners of Aroclor 1260.

Plans for next year for each of the continuing projects are presented below. Because of the very significant results from the laboratories of Tiedje, Boyd, and Woods, the research on microbial dechlorination will be expanded. Several new projects (mainly at universities) will begin. These will include attempts to enrich and characterize novel PCB dechlorinating cultures from other sites (e.g., marine sediments from Sheboygan Harbor, Wisconsin; Pittsfield, Massachusetts; and Waukegan Harbor, Illinois) and other microbial niches (e.g., denitrifying strains) and culture collections (e.g., pentachlorophenol or benzene degrading anaerobes). Additional engineering studies with anaerobic PCB degraders will also begin. A more detailed description of these "new starts" is provided under separate cover in the proposed long-term research plan.

Biodegradation of PCBs in Soil

Both in situ and reactor approaches to aerobic PCB bioremediation will continue. Laboratory studies will be carried out to address such critical variables as (1) PCB availability (shaking rate, surfactants, solvent redeposition, ball milling, oil phase partitioning), and (2) factors affecting bacterial activity (temperature, cell number, oxygen availability, moisture content, soil pH, inhibitors). Some of these studies will involve our recombinant DNA strains of PCB-degrading bacteria as a first step toward the eventual use of future generations of high-activity, engineered PCB degraders. In parallel to the in situ approaches we will pilot a kilogram-scale reactor technology for rapid PCB biodegradation using dual cultures. And finally, if the efforts at developing anaerobic cultures to dechlorinate PCBs are successful, we will assay their capability for biotreating Aroclor 1260 contaminated soils and sediments (for example, those at Oakland, California and Pittsfield, Massachusetts).

Aerobic Biodegradation of PCBs in an Industrial Sludge

Experiments will be performed to determine whether the just-discovered inhibitory effect of DEHP on PCB biodegradation is biochemical, physical, or both. The sludge will be characterized more fully to determine what other components are present, particularly oils, and we will test the effects of these components on PCB biodegradation.

Kinetics of PCB "Uptake" and Aerobic Metabolism

Studies will continue of the physical chemistry aspects of aerobic PCB metabolism; notably, the relationship between the very low water solubility of PCBs and kinetics. The physical significance of the apparent K_m observed for 2,3-dioxygenase activity with 2,4′-CB is not known; it may be a kinetic (steady state) constant. The generality of the observations on 2,4′B will be examined with other substrates for 2,3-dioxygenase, which can be studied in the soluble range. The origin and generality of the long-time (24-hour) second-order reaction observed with a substrate for the putative 3,4-dioxygenase (2,4,5,2′5′-CB) will also be examined.

Genetic Engineering of Aerobic Biodegradation

The PCB competence of a number of existing subclones of pGEM410 will be examined to determine if any of these are superior to FM4560. In addition, new subclones will be created for use in "soil-adapted" strains. The goal would be to introduce the PCB genes from LB400 into an organism indigenous to a contaminated soil. In this way we might produce a strain with better survival characteristics on soil than LB400. In addition, the use of a soil bacterium closely related to *P. putida* LB400 may offer increased expression of the cloned genes over that found in *E. coli*.

Reductive Dechlorination of PCBs in Anaerobic Microbial Communities

The successful demonstration of Aroclor 1242 dechlorination has suggested several new objectives as well as new approaches to previous objectives. Our goals include (1) evaluating the PCB dechlorinating capability of various sediments using an elevated concentration (500 ppm) of Aroclor 1242 rather than the previous suite of five pure congeners at 1 ppm each, (2) examining various environmental factors to determine their influence on the PCB dechlorination rate of Aroclor 1242, (3) testing for the dechlorination of Aroclor 1260, and (4) identifying and attempting to isolate the microorganism(s) responsible for PCB dechlorination.

Reductive Dechlorination of PCBs in Upflow Anaerobic Filter Reactors

Research will continue with the four upflow anaerobic filter reactors now established. The concentration of the present suite of PCB congeners in the influent will be increased and the reactor effluent and solids will be monitored for the appearance of metabolic products. Based on the products, degradation mechanisms in the reactor(s) will be inferred. A new reactor of the same type will be inoculated with the effluent of the other four reactors. This reactor is designed to accumulate a large mass of acclimated organisms and to eliminate much of the background contamination. The acclimated active population should increase the rate of biodegradation.

PCB Transformation by Aerobic Hydrogen-Oxidizing Bacteria

The identification of the PCB metabolite produced by three of the hydrogen-oxidizing isolates should provide some insight about the type of biotransformation that is occurring. Toward this end we will first attempt to determine which of the PCBs in the mixture is metabolized to the unknown product. We will then try to optimize growth and assay conditions in order to obtain enough of this metabolite to characterize by GC-MS. The direction of further research will be determined by the information provided by these experiments.

PCB Transformations in Nature

Analysis of the GC data from the 1976-1988 clinical studies of workers with a history of PCB exposure will continue. The data should provide more precise clearance rates on a broader range of PCB congeners and a better characterization of the variability in human PCB clearance. The new indexes of PCB alteration state will be applied to analytical data arising from a survey of the PCBs in the tidal Hudson River (Troy, New York to New York, New York). The objective is to develop maps of current PCB sources and transformation sites in the river.

Process Modeling of Surfactant Extraction of PCBs From Soil

The immediate plans are aimed at determining the feasibility of using this process for the Oakland site. With the more heavily contaminated soil from the site we will be better able to determine which factors have the greatest influence on the extraction efficiency and test alternatives for increasing the rate of extraction. If the process proves feasible technically, the next area of investigation will be the reduction of process costs to improve the economic practicality.

REFERENCES

Apajalahti, J.H.A., and M.S. Salkinoja-Salonen. 1987. Complete dechlorination of tetrachlorohydroquinone by cell extracts of pentachlorophenol-induced *Rhodococcus chlorophenolicus*. J. Bacteriol. *169*:5125-5130.

Bedard, D.L. and M.J. Brennan. 1984. Degradation of polychlorinated biphenyls by *Alcaligenes eutrophus* strain H850. Abstracts of the 84th Annual Meeting of the American Society for Microbiology, Q51, p. 213.

Bedard, D.L., M.J. Brennan, and R. Unterman. 1984. Bacterial degradation of PCBs: evidence of distinct pathways in *Corynebacterium* sp. MB1 and *Alcaligenes eutrophus* H85O, p. 4-101 to 4-118. In Proceedings of the 1983 PCB Seminar; G. Addis and R. Komai, ed.; Electrical Power Research Institute. Palo Alto, CA.

Bedard, D.L., R. Unterman, L.H. Bopp, M.J. Brennan, M.L. Haberl, and C. Johnson. 1986. Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Appl. Environ. Microbiol. *51*:761-768.

Bedard, D.L., M.L. Haberl, R.J. May, and M.J. Brennan. 1987a. Evidence for novel mechanisms of polychlorinated biphenyl metabolism in Alcaligenes eutrophus *H850. Appl. Environ. Microbiol. 53*:1103-1112.

Bedard, D.L., R.E. Wagner, M.J. Brennan, M.L. Haberl and J.F. Brown, Jr. 1987b. Extensive degradation of Aroclors and environmentally transformed polychlorinated biphenyls by *Alcaligenes eutrophus* H850. Appl. Environ. Microbiol. *53*:1094-1102.

Beijerinck, M. and D.C.J. Minkman. 1910. Bildung und verbrauch von Stickoxydul durch Bakterien. Centralblatt fur Bakteriologie, Parasitenkunde und Infektionskrankheiten, Abt. 2 *25*:30-63.

Bopp, L.H. 1986. Degradation of highly chlorinated PCBs by *Pseudomonas* strain LB400. J. Ind. Microbiol. *1*:23-29.

Bopp, R.F., H.J. Simpson, and B.L. Deck. 1985. Release of polychlorinated biphenyls from contaminated Hudson River sediments, final report. Lamont-Doherty Geological Observatory of Columbia University, Palisades, NY 10964.

Bopp, R.F., H.J. Simpson, B.L. Deck, and N. Kostyk. 1984. The persistence of PCB components in sediments of the lower Hudson. Northeast Environ. Sci. *3*:180-190.

Bopp, R.F., H.J. Simpson, C.R. Olsen, and N. Kostyk. 1981. Polychlorinated biphenyls in sediments of the tidal Hudson River, New York. Environ. Sci. Technol.*15*:210-216.

Bopp, R.F. 1979. The geochemistry of polychlorinated biphenyls in the Hudson River. PhD Thesis, Columbia University.

Boyd, S.A. and D.R. Shelton. 1984. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Appl. Environ. Microbiol. *47*:272-277.

Boyd, S.A., D.R. Shelton, D. Berry, and J.M. Tiedje. 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Appl. Environ. Microbiol. *46*:560-54.

Brown, J.F., Jr. and R.W. Lawton. 1984. Polychlorinated biphenyl (PCB) partioning between adipose tissue and serum. Bull. Environ. Contam. Toxicol. *33*:277-280.

Brown, J.F., Jr. R.E. Wagner, D.L. Bedard, M.J. Brennan, J.C. Carnahan, and RJ. May. 1984. PCB transformations in upper Hudson sediments. Northeastern Environmental Science *3*:167-179.

Brown, J.F., Jr., R.E. Wagner, D.L. Bedard, M.J. Brennan, J.C. Carnahan, R.J. May, and T.J. Tofflemire. 1985. PCB dechlorination in upper Hudson sediments. Preprint Extended Abstracts, Division of Environmental Chemistry. American Chemical Society, 25(1):35-37.

Brown, J.F., Jr., D.L. Bedard, M.J. Brennan, J.C. Carnahan, H. Feng, and R.E. Wagner. 1987a. Polychlorinated biphenyl dechlorination in aquatic sediments. Science *236*:709-712.

Brown, J.F., Jr., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan and Ralph J. May. 1987b. Environmental dechlorination of PCBs. Environ. Toxicol. Chem. *6*:579-593.

Brown, J.F., Jr., and R.E. Wagner. 1987. PCB dechlorination by marine sediments. Abstract No. 36, 8th Annual Meeting, Society of Environmetal Toxicology and Chemistry, November 9-12, 1987, Pensacola, Florida.

Cammack, R. and M.G. Yates. 1986. Hydrogenases: From chemistry to legume growth. Nature *319*:182.

Catelani, D., A. Colombi, C. Sorlini, and V. Treccani. 1973. Metabolism of biphenyl. 2-Hydroxy-6-oxo-6-phenylhex-2,4-dienote: the *meta*-cleavage product from 2,3-dihydroxybiphenyl by *Pseudomonas putida*. Biochem. J. *134*:1063-1066.

Chen, P.H., M.L. Luo, C.K. Wong, and C.J. Chen. 1982. Comparative rates of elimination of some individual polychlorinated biphenyls from the blood of PCB-poisoned patients in Taiwan. Fd. Chem. Toxic. *20*:417-425.

Chou, S.F.J. and R.A. Griffen. 1986. Solubility and soil mobility of polychlorinated biphenyls. Chapter 5 in PCBs and the Environment; J.S Waid, ed.; CRC Press, Boca Raton, Florida.

Davis, D.H., M. Doudoroff, R.Y. Stanier and M. Mandel. 1969. Proposal to reject the genus *Hydrogenomonas*: Taxonomic implications. Int. J. Systemat. Bacteriol. *19*:375-390.

Dennis, W.H. Jr., Y.H. Chang, and W.J. Cooper. 1979. Catalytic dechlorination of organochlorine compounds. V. Polychlorinated biphenyls - Aroclor 1254. Bull. Environ. Contam. Toxicol. 22:750-753.

Dodge, R.H., C.E. Cerniglia, and D.T. Gibson. 1979. Fungal metabolism of biphenyl. Biochem. J. *178*:223-230.

Dolfing, J. and J.M. Tiedje. 1987. Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic culture. Arch. Microbiol. In press.

Farrell, R. and A.M. Chakrabarty. 1979. Degradative plasmids: molecular nature and mode of evolution. In Plasmids of Medical, Environmental, and Commercial Importance; K.N Timmis and A. Puhler, ed.; Elsevier/North-Holland Biomedical Press, New York, p. 97-109.

Farrington, J.W., A.C. Davis, B.J. Brownawell, B.W. Tripp, C.H. Clifford, and J.B. Livramento. 1986. The biogeochemistry of polychlorinated biphenyls in the Acushnet River Estuary, Massachusetts. In Organic Marine Geochemistry; Mary L. Sohn, ed.; ACS Symposium Series No. 305, American Chemical Society, Washington, DC.

Farwell, S.O., F.A. Beland, and R.D. Geer. 1975. Reduction pathways of organohalogen compounds, part II. Polychlorinated biphenyls. J. Electroanal. Chem. Interfacial Electrochem. *61*:315-324.

Finette, B.A., V. Subramanian and D.T. Gibson. 1984. Isolation and characterization of *Pseudomonas putida* mutants defective in the toluene dioxygenase enzyme system. J. Bacteriol. *160*:1003-1009.

Friello, D.A., J.R. Mylroie, D.T. Gibson, J. Roger, and A.M. Chakrabarty. 1976. XYL. A nonconjugative xylene-degradative plasmid in *Pseudomonas* Pxy. J. Bacteriol. *127*:1217-1224.

General Electric Company, 1981. Phase II Study, Final Report. General Electric Company Plant Site, Oakland, California.

General Electric Company Research and Development Program for the Destruction of PCBs, First Progress Report. 1982. General Electric Corporate Research and Development, Schenectady, New York.

General Electric Company Research and Development Program for the Destruction of PCBs, Second Progress Report. 1983. General Electric Corporate Research and Development, Schenectady, New York.

General Electric Company Research and Development Program for the Destruction of PCBs, Third Progress Report. 1984. General Electric Corporate Research and Development, Schenectady, New York.

General Electric Company Research and Development Program for the Destruction of PCBs, Fourth Progress Report. 1985. General Electric Corporate Research and Development, Schenectady, New York.

General Electric Company Research and Development Program for the Destruction of PCBs, Fifth Progress Report. 1986. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Sixth Progress Report, 1987. General Electric Corporate Research and Development, Schenectady, NY.

Gibson, D.T., R.L. Roberts, M.C. Wells, and V.M. Kobal. 1973. Oxidation of biphenyl by a *Beijerinckia* species. Biochem. Biophys. Res. Commun. *50*:211-219.

Gibson, D.T., W-K. Yeh, T-N. Liu, and V. Subramanian. 1982. Toluene dioxygenase: a multicomponent enzyme system from *Pseudomonas putida*. In Oxygenases and Oxygen Metabolism; M. Nozaki, S. Yamamoto, Y. Ishimura, M.J. Coon, L. Ernster and R.W. Estabrook, ed.; Academic Press, New York, p. 51-62.

Haigler, B.E., M.J. Schocken, L.P. Wackett, and D.T. Gibson. 1985. Napthalene dioxygenase: evidence for dioxygenase, monoxygenase, and desaturase activities. Abstracts Ann. Mtg. Am. Soc. Microbiol. K165.

Hileman, B. 1988. The Great Lakes cleanup effort. Chem. Engrg. News, February 8, p. 22-39.

Horowitz, A., J.M. Suflita, and J.M. Tiedje. 1983. Reductive dehalogenations of halobenzoates by anaerobic lake sediment microorganisms. Appl. Environ. Microbiol. *45*:1459-1465.

Hutzinger, O., S. Safe, and V. Zitko. 1974. The Chemistry of PCBs. CRC Press, Cleveland, p. 23.

Jerina, D.M., H. Selander, H. Yagi, M.C. Wells, J.F. Davey, V. Mahadevan, and D.T. Gibson. 1976. Dihydrodiols from anthracene and phenanthrene. J. Am. Chem. Soc. *98*:5988-5966.

Kaminsky, L.S., M.W. Kennedy, S.M. Adams, and F.P. Guengerich. 1981. Metabolism of dichlorobiphenyls by highly purified isozymes of rat liver cytochrome P450. Biochemistry *20*:7379-7384.

LaPierre, R.B., L. Guczi, W.L. Kranich, and A.H. Weiss. 1978. Hydrodechlorination of polychlorinated biphenyl. J. Catalysis *52*:230-238.

Lawton, R.W., J.F. Brown, Jr., M.R. Ross, and J. Feingold. 1985a. Comparability and precision of serum PCB measurements. Arch. Environ. Health. *40*:29-37.

Lawton, R.W., M.R. Ross, J. Feingold, and J.F. Brown, Jr. 1985b. Effects of pcb exposure on biochemical and hematological findings in capacitor workers. Environ. Health Perspect. *60*:165-184.

Linkfield, T.G. 1985. Anaerobic reductive dehalogenation: The lag period preceeding haloaromatic dehalogenation, enrichment of sediment activity and the partial characterization of a dehalogenating organism, strain DCB-1. Ph.D. Thesis, Michigan State University.

Masuda, Y., R. Kagawa, and M. Kuratsune. 1974. Comparison of polychlorinated biphenyls in yusho patients and ordinary persons. Bull. Environ. Contam. Toxicol. *11*:213-216.

Mikesell, M.S. and S.A. Boyd. 1985. Reductive dechlorination of the pesticies 2,4-D, 2,4,5-T and pentachlorophenol in anaerobe sludge. J. Environ. Qual. *14*:337-340.

Nadim, L., M.J. Schocken, F.J. Higson, D.T. Gibson, D.L. Bedard, L.H. Bopp, and F.J. Mondello. 1987. Bacterial oxidation of polychlorinated biphenyls. Proceedings of the 13th Annual Research Symposium on Land Disposal, Remedial Action, Incineration, and Treatment of Hazardous Waste. In press.

Niimi, A.J. and B.G. Oliver. 1983. Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairderi*). J. Fish Aquat. Sci. *40*:1388-1394.

Saeki, S., S. Tanabe, Y. Nakagawa, and R. Tatsukawa. 1983. Biotransformation of polychlorinated biphenyl composition in rat hepatic microsomal enzymes induced by phenobarbital and 3-methylcholanthrene. Nippon Nogeikagaku Kaishi *57*:1219-1226.

Safe, S, L.W. Robertson, and L. Safe. 1982. Halogenated biphenyls: molecular toxicology. Can. J. Physiol. Pharmacol. *60*:1057-1064

Schink, B. and H.G. Schlegel. 1979. The membrane-bound hydrogenase of *Alcaligenes eutrophus*. I. Solubilization, purification, and biochemical properties. Biochim. Biophys. Acta *567*:315-324.

Schink, B. and H.G. Schlegel. 1980. The membrane-bound hydrogenase of *Alcaligenes eutrophus*. II. Localization and immunological comparison with other hydrogenase systems. Antonie van Leeuwenhoek *46*:1-14.

Schneider, K. and H.D. Schlegel. 1976. Purification and properties of soluble hydrogenase from *Alcaligenes eutrophus* H16. Biochim. Biophys. Acta *452*:66-80.

Shelton, D.R.and J.M. Tiedje. 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. *48*:840-848.

Sparling, J. and S. Safe, 1980. The effects of ortho chlorine substituents on the retention of PCB isomers in rat, rabbit, Japanese quail, guinea pig, and trout. Toxicol. Lett. 7:23-28.

Stalling, D.L. 1982. Isomer specific composition of PCB residues in fish and sediments from Waukegan Harbour and other Great Lakes fish. Columbia National Fisheries Research Laboratory, Columbia, MO 6520l. Unpublished report.

Suflita, J.M., A. Horowitz, D.R. Shelton, and J.M. Tiedje. 1982. Dehalogenation: A novel pathway for the anaerobic biodegradation of haloaromatic compounds. Science *218*:1115-1117.

Suflita, J.M., J.A. Robinson, and J.M. Tiedje. 1983. Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. Appl. Environ. Microbiol. *45*:1466-1473.

Suflita, J.M., J. Stout, and J.M. Tiedje. 1984. Dechlorination of (2,4,5-trichlorophenoxy) acetic acid by anaerobic microorganisms. J. Agric. Food Chem. *32*:218-221.

Sundström, G., O. Hutzinger, and S. Safe. 1976. The metabolism of chlorobiphenyls - a review. Chemosphere *5*:267-298.

Tiedje, J.M., S.A. Boyd, and B.Z. Fathepure. 1987. Anaerobic degradation of chlorinated aromatic hydrocarbons. Dev. Industr. Microbiol. *27*117-127.

Tiedje, J.M., B.Z. Fathepure, and S.A. Boyd. 1987. Studies on reductive dechlorination of hexachlorobenzenes in anaerobic digestor sludge. Environ. Toxicol. Chem. Submitted.

Unterman, R., D.L. Bedard, L.H. Bopp, M.J. Brennan, C. Johnson, and M.L. Haberl. 1985. *Microbial degradation of polychlorinated biphenyls.* Proceedings: International conference on new frontiers for hazardous waste management. U.S. Environmental Protection Agency, Cincinnati, Ohio, p. 481-488.

Unterman, R., D.L. Bedard, M.J. Brennan, C. Johnson, and J.R. Yates. 1984. Degradation of polychlorinated biphenyls by a *Corynebacterium* species. Abstracts of the 84th Annual Meeting of the American Society for Microbiology, Q50, p. 212.

Unterman, R., R.E. Brooks, and C. Johnson. 1986. Modeling a PCB biodegradation process. Abstracts of the 86th Annual Meeting of the American Society for Microbiology Q60, p. 74.

Unterman, R., M.J. Brennan, R.E. Brooks, and C. Johnson, 1987a. Biological degradation of polychlorinated biphenyls. Proc. Intl. Conf. on Innovative Biological Treatment of Toxic Wastewaters; R.J. Scholze et al. ed.; US Army, CERL, Champaign, IL, N-87/12, p. 379-389.

Unterman, R., F.J. Mondello, M.J. Brennan, R.E. Brooks, D.P. Mobley, J.B. McDermott, and C.C. Schwartz, 1987b. *Bacterial treatment of PCB-contaminated soils: Prospects for the application of recombinant DNA technology.* Proc. Second Intl. Conf. on New Frontiers for Hazardous Waste Management, U.S. Environmental Protection Agency, Cincinnati, OH, EPA/600/9-87/018F, p. 259-264.

Unterman, R., D.L. Bedard, M.J. Brennan, L.H. Bopp, F.J. Mondello, R.E. Brooks, D.P. Mobley, J.B. McDermott, C.C. Schwartz, and D.K. Dietrich. 1988a. Biological approaches for PCB degradation. Reducing Risks from Environmental Chemicals Through Biotechnology; G.S. Omenn et al., ed.; Plenum Press, New York, p. 253-269.

Unterman, R., M.J. Brennan, R.E. Brooks, D.P. Mobley, J.B. McDermott, and C.C. Schwartz, 1988b. Bacterial degradation of PCBs in soil. Proc. Symp. on Bioaugmentation as a Means to Enhance Waste Treatment. The Lewis Publishers, in press.

van den Tweel, W.J.J., J.B. Kok, and J.A.M. de Bont. 1987. Reductive dechlorination of 2,4-dichlorobenzoate to 4-chlorobenzoate and hydrolytic dehalogenation of 4-chloro, 4-bromo-, and 4-iodobenzoate by *Alcaligenes denitrificans* NTB-1. App. Environ. Microbiol. *53*:810-815.

Wade, R.S., R. Harlin, and C.E. Castro. 1969. The oxidation of iron (II) by organic molecules. J. Am. Chem. Soc., *91*:7530.

Waid, J.S., ed. 1986. PCBs and the environment. CRC Press, Boca Raton, Florida.

Wolff, M.S., J. Thornton, A.S. Fischbein, R. Lilis, and I.J. Selikoff. 1982. Disposition of polychlorinated biphenyl congeners in occupationally exposed persons. Toxicol. Appl. Pharmacol. *62*:294-306.