MAY 11 1992

M. Peter Lanahan, Jr.
Manager, Mid-Atlantic Region

State Government Relations
General Electric Company
12 Sheridan Avenue
Albany, NY 12207
518 462-4537

May 5, 1992

Mr. Doug Blasey, Counsel
U.S. Environmental Protection Agency
Region II
26 Federal Plaza
New York, N.Y. 10278

Dear Doug:

To follow up on our discussion Friday about the results of Dr. Dan Abramowicz' Hudson River research, I have enclosed a copy of the final report on the field test.

The following major conclusions are reported here:

- Aerobic PCB destruction is occurring naturally in the Hudson River. This aerobic biodegradation of lightly chlorinated PCBs complements the extensive anaerobic dechlorination that has already been documented.

- Aerobic PCB biodegradation can be stimulated in Hudson River sediments *in situ*. Through the addition of various nutrients and oxygen, up to 55 percent of the PCBs contained in six experimental caissons were destroyed by microorganisms present in the river during our ten-week experiment.

- Earlier laboratory research into aerobic biodegradation produced results that are consistent with what was observed under actual river conditions.

Dr. Abramowicz and members of the Research and Development staff are now focusing on other aspects of our ongoing PCBs research. We would be happy to brief you on this work at your convenience.

Sincerely,

M. Peter Lanahan, Manager
State Government Relations

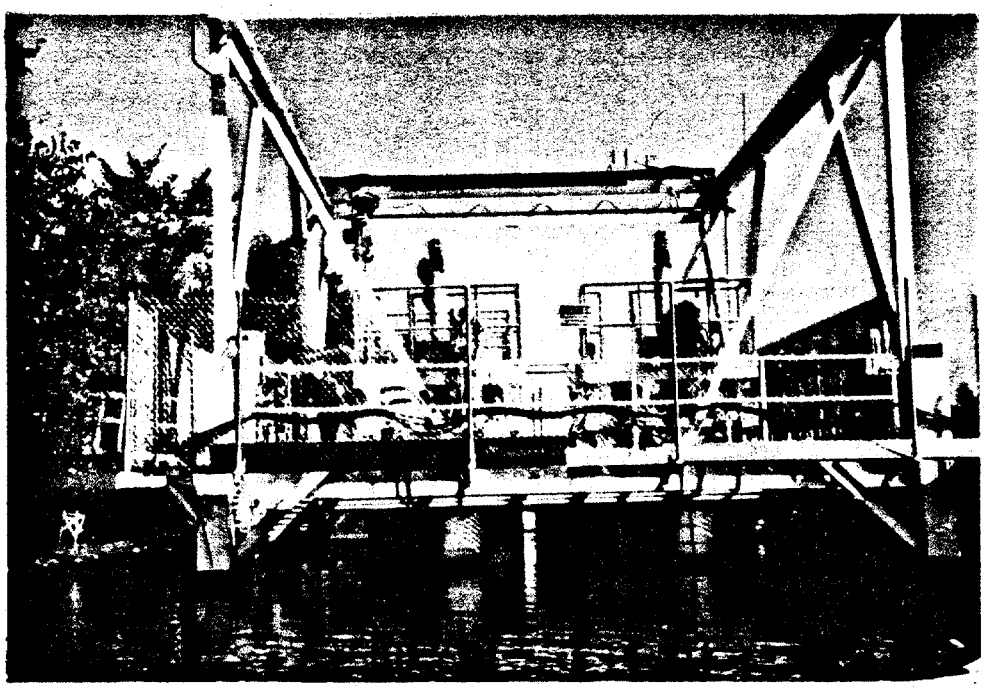
314491

1991 *In Situ* Hudson River Research Study :

A Field Study on Biodegradation of PCBs in Hudson River Sediments

Final Report

February 1992



Prepared by

General Electric Company
Corporate Research and Development

1991 *In Situ* Hudson River Research Study :

A Field Study on Biodegradation of PCBs in Hudson River Sediments

Final Report

February 1992

Submitted by

Daniel A. Abramowicz, Mark R. Harkness, John B. McDermott, and Joseph J. Salvo

CONTRIBUTORS

Daniel A. Abramowicz, Angelo A. Bracco, Michael J. Brennan, Kim A. DeWeerd, Mark R. Harkness, Kenneth M. Fish, George M. Frame, John T. Jackman, David T. Lin, John H. Lobos, Frank J. Mondello, Joseph J. Salvo, and Mark L. Stephens

Bioremediation Laboratory
GE Corporate Research and Development
Schenectady, NY

Kenneth W. Carroll, John B. McDermott, Greg L. Warner, and Paul W. Wilson

Process Engineering and Chemistry Laboratory
GE Corporate Research and Development
Schenectady, NY

William P. Flanagan and Ralph J. May

Chemical/Bio Reactions Laboratory
GE Corporate Research and Development
Schenectady, NY

David K. Dietrich and William L. Gately

Environmental Technology Laboratory
GE Corporate Research and Development
Schenectady, NY

Carolyn B. Morgan

Management Science and Statistics Program
GE Corporate Research and Development
Schenectady, NY

John G. Haggard

GE Corporate Environmental Programs
Albany, NY

ACKNOWLEDGEMENTS

GE would like to acknowledge the contribution of the following people to the successful completion of the 1991 *In Situ* Hudson River Research Study. Joan Blake of US EPA (TSCA), for permission to undertake the study. Paul Ammann and Bruce Ostar of the Brattle Group, for their contribution to the conceptual design of the HRRS. David Hale of O'Brien and Gere Engineers, for coordinating site sampling and surveying. Michael Hill of Covington and Burling, Gail Port of Proskauer, Rose, Mendelson, and Getz, and Suzanne Howard of Goldman Environmental Consultants, for assistance with the permitting. William Wright and Robert Scherer of GE RECO, for field support during the installation, operation, and demobilization of the HRRS. Paul Flathman of OHM Corporation, for many helpful discussions leading up to the field study. Pat Woodhull of OHM Corporation, for coordinating the final design. Jan Evans of OHM Corporation, for invaluable equipment support during the field study. William Flanagan, Frank Mondello, Mark Stephens, and Linda Prest of GE CRD for assistance with final editing and manuscript preparation.

GE would also like to acknowledge the contribution of OHM Corporation (Findlay, Ohio) for assistance in the design, construction, and installation of the HRRS facility.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

EXECUTIVE SUMMARY

This report contains the results of a study on the *in situ* aerobic biodegradation of polychlorinated biphenyls (PCB) in Hudson River sediments. The experiments were performed in the Upper Hudson River (mile point 193.5) at the GE Hudson River Research Station (HRRS) for approximately ten weeks from August 9 to October 21, 1991. The HRRS facility consisted of six self-contained caissons (six feet in diameter) driven into the river bottom to isolate the natural biota and sediment from the river environment. The PCB distribution at this site consisted of primarily lightly chlorinated PCBs (approximately 62-73% mono- and dichlorobiphenyls) due to the widespread natural anaerobic dechlorination of the original Aroclor 1242 (approximately 17% mono- and dichlorobiphenyls) deposited in Upper Hudson River sediments. Appendix A-2 contains a more detailed discussion of this anaerobic microbial dechlorination activity in the Hudson River.

The present study had three major objectives: 1) to demonstrate that aerobic PCB biodegradation could occur under actual field conditions, based on prior laboratory investigations, 2) to identify the key variables that influence the rate and extent of PCB biodegradation in Hudson River sediments, and 3) to investigate the potential for natural aerobic biodegradation to occur in these sediments via indigenous PCB-degrading microorganisms.

Six major conclusions can be drawn from these field studies.

1) Indigenous PCB-degrading microorganisms are present in these sediments, and these organisms can degrade the lightly chlorinated PCBs typically present in Upper Hudson sediments.

2) PCB biodegradation observed in the field correlated well with the results of previous laboratory research.

3) Aerobic PCB biodegradation can be accelerated in Hudson River sediments *in situ* by the addition of nutrients and supplemental oxygen.

4) Inoculation of extensively dechlorinated sediments with large numbers of a purified PCB-degrading organism resulted in little benefit over the degradation achieved via stimulating the growth of the indigenous microflora.

5) Rapid biodegradation of chlorobenzoates indicates that PCBs can be fully metabolized through these intermediates by indigenous microflora present in Hudson River sediments.

6) Evidence from this field study and preliminary river surveys suggests that aerobic PCB destruction is occurring naturally in the Hudson River. This aerobic biodegradation of lightly chlorinated PCBs complements the extensive anaerobic dechlorination previously documented.

The specific details supporting these conclusions are included in the body of this report. A range of 38-55% biodegradation was achieved in the experimental caissons in ten weeks, the designed duration of the experiment. No extensive PCB biodegradation occurred in either control caisson where oxygen levels were limited. This compares favorably with PCB biodegradation observed in corresponding laboratory studies with Hudson River sediments. Moreover, the transient accumulation of chlorobenzoic acids, intermediates in the biodegradation of PCBs, was detected only in the caissons where significant PCB biodegradation occurred. Furthermore, these intermediates correlated in congener distribution to the PCBs present in the caissons, and were quickly degraded by the microorganisms within the caissons. No significant PCB volatilization occurred during the experiment, as confirmed by the presence of only trace amounts of PCB in vapor traps on the caissons, and PCB losses via Fenton chemistry were determined to be insignificant. These data firmly establish the successful demonstration of *in situ* PCB biodegradation in the field. Importantly, no acute toxicity to the test organism *Ceriodaphnia dubia* was associated with the river sediments sampled from the caissons either before or after the field study.

In general, PCB-biodegradation rates in the field were 2-3 fold slower than results with much smaller scale laboratory experiments. Typical

laboratory experiments were performed with ≤ 0.1 kg of sediment compared to the 500 kg of PCB-contaminated sediments contained within each caisson. The observed difference in PCB-degradation rate is likely due to the effect of increased length scale on mass transfer and mixing, along with lower experimental temperatures encountered in the field.

The extent of PCB biodegradation in both laboratory and field experiments was limited by the characteristic bioavailability of the PCBs in the sediments. This phenomenon is commonly observed to varying degrees whenever the biodegradation of hydrophobic contaminants like oil and PCB is carried out in the presence of soils or sediments. At PCB concentrations typical for this Hudson River hotspot (20-40 ppm), only about 50% of the target compounds were shown to be readily available for the bacteria to degrade over the time course of the experiment. This PCB fraction was generally degraded in the experiment. The remaining resistant fraction was not available to the PCB-degrading bacteria and is unlikely to be available to other organisms, as well. This may have important implications for establishing risk estimates. The resistant PCB fraction is expected to become available over long periods of time (months-years), although physical, chemical, and natural aerobic biodegradative processes should mitigate the effects of this slowly desorbing material.

Future research efforts will focus on establishing the natural rate and extent of aerobic PCB biodegradation occurring in Hudson River sediments, investigating the role of nutrients in the process, and understanding the ramifications of bioavailability. It is important to note that the bioavailability issue is more pronounced at low PCB concentrations. At higher concentrations (e.g. approximately 200 ppm) as much as 75% of the contamination should be available for biodegradation, with a greater percentage available at even higher concentrations. But at the low concentrations typically found in Hudson River sediments, significantly less total PCB is available for accelerated aerobic biodegradation or bioaccumulation through the food chain.

In summary, aerobic PCB biodegradation has been successfully demonstrated *in situ* with Hudson River sediments, and preliminary

evidence suggests that this process also occurs naturally. The bioavailable PCB was extensively degraded during the 10 week experiment, removing the fraction that represents the greatest potential for transport into the food chain. In addition, widespread natural anaerobic PCB dechlorination has already resulted in a significant reduction of the more highly chlorinated PCB congeners throughout Hudson River sediments. Thus, the combined action of anaerobic PCB dechlorination and aerobic PCB biodegradation can significantly reduce both the amount and availability of PCBs in Hudson River sediments.

CHAPTER 1
INTRODUCTION

On August 9, 1991, the General Electric Company (GE) initiated a 10-1/2 week field experiment to investigate the *in situ* biodegradation of PCBs in Hudson River sediment. This study utilized only aerobic bacteria native to the upper Hudson River. Six caissons were installed at the Hudson River Research Station (HRRS) to isolate sections of river bottom for this experiment. *In situ* biodegradation was stimulated by the addition of oxygen and nutrients to the sediments. Other variables in the investigation were mode of agitation and microbial inoculation. This report is submitted in fulfillment of the conditions of the US EPA (TSCA) permit obtained to carry out this study.

Background

Polychlorinated biphenyls, (PCBs), are a group of structurally related compounds (congeners) consisting of a biphenyl nucleus carrying from 1 to 10 chlorines. There are 209 possible PCB congeners which differ in the number and position of chlorines on the biphenyl ring. The vast majority of PCBs were used as complex commercial mixtures containing 60-80 different PCB congeners. In the United States, these mixtures were manufactured under the trade name Aroclor (Monsanto, St. Louis, MO). Aroclor 1242, present in Upper Hudson River sediments, is comprised of mainly tri- and tetrachlorobiphenyls. Individual PCB congeners are denoted by the position of the chlorines on each of the rings, generally by first indicating the chlorinated positions on one ring and then those on the other. For convenience, the name is usually abbreviated, e.g. 25-25-tetrachlorobiphenyl is represented as 25-25-CB.

The physical and chemical properties of polychlorinated biphenyls (PCB) led to their widespread use. Commercial PCB mixtures are generally liquids at room temperature, with low vapor pressures, low water solubilities, and desirable dielectric properties. Chemical properties include stability to

oxidation, flame retardance, and relative inertness. Because of these properties, PCBs were widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, plasticizers, flame retardants, organic diluents, and dielectric fluids [Hutzinger, et al., 1974].

In a 50-year period approximately 1.4 billion pounds of PCBs were produced. Disposal and release of these chemically and thermally stable compounds have resulted in widespread distribution [Buckley, 1982; Jensen, 1966; Tanabe, et al., 1983]. It is estimated that several hundred million pounds have entered the environment worldwide [Hutzinger & Veerkamp, 1981]. The lipophilic nature of PCBs contributes to their tendency to accumulate in fatty tissues and results in biomagnification in the food chain [Safe, 1980].

Two GE plants located in Fort Edward and Hudson Falls, New York have manufactured capacitors for approximately fifty years. From 1946 to 1977, most of the capacitors were filled with pyranol, a PCB-containing solution, which served as a dielectric fluid. In 1977, PCB usage at both plants ceased. It is estimated that greater than 95% of the PCB discharged from the plants since the 1940's consisted of Aroclors 1016 and 1242, with the balance made up by Aroclor 1254.

For over ten years, GE has studied PCB biodegradation at its Corporate Research and Development Center (CRD) in Schenectady, New York [reviewed in Abramowicz, 1990; Bedard, 1990]. These compounds, formerly believed to be non-biodegradable, have repeatedly been shown to biodegrade under a variety of conditions.

Two distinct classes of bacteria with PCB-degrading capabilities have been identified: 1) anaerobic bacteria, which live in oxygen-free environments such as river sediments and 2) aerobic bacteria, which live in oxygenated environments such as water columns in river systems and surficial sediment layers. The naturally occurring anaerobic PCB-degrading bacteria typically attack the higher chlorinated PCB congeners through reductive dechlorination [Brown et al., 1984], a process which removes chlorines while leaving the biphenyl rings intact. Essentially all sediment samples collected above the Federal Dam in Troy, New York displayed a significant reduction in

higher chlorinated PCB congeners, relative to Aroclor 1242, with a commensurate increase in lower chlorinated congeners [Appendix A-2, this report]. The observed selective shift in PCB congener distribution is evidence of microbial anaerobic dechlorination [Brown et al., 1984; Quensen et al., 1988; Quensen et al., 1990].

The less chlorinated PCB congeners resulting from this anaerobic dechlorination are suitable substrates for oxidative degradation by a wide range of aerobic organisms [Bedard, 1986]. This latter process is responsible for destroying these compounds. The complementary nature of the anaerobic and aerobic systems provides the capability to completely biodegrade PCBs to carbon dioxide, water, and chloride ion.

Since the PCBs at the study site had been extensively dechlorinated by the indigenous anaerobic bacteria, the site was well suited for a study of oxidative degradation by aerobic bacteria. Therefore the experiment at the HRRS focused on stimulating or enhancing *in situ* aerobic PCB biodegradation in Hudson River sediment.

Aerobic biodegradation of PCBs has been observed and reported by many investigators since the early 1970's [e.g. Ahmed and Focht, 1973; Sundstrom and Hutzinger, 1976; Furukawa et al., 1978; Furukawa, 1982; Rochkind et al., 1986]. The enzymatic pathway for oxidative PCB biodegradation is generally well understood in a number of bacterial systems. The predominant mechanism occurs via a 2,3-dioxygenase attack [Ahmed and Focht, 1973; Nadim et al., 1987], as shown in Figure 1-1. The hydroxylated product is then further metabolized, resulting in ring cleavage after attack via a second dioxygenase. The ultimate product of this enzymatic pathway is the corresponding chlorobenzoic acid. While the chlorobenzoic acids are readily degraded by other aerobic bacteria and do not persist in the system [Focht and Hickey, 1990; Hernandez et al., 1991], the transitory presence of the corresponding chlorobenzoic acid is clear evidence for the aerobic biodegradation of PCB.

A number of aerobic bacteria that display a wide range of PCB-degrading capabilities have been isolated from local PCB contaminated areas.

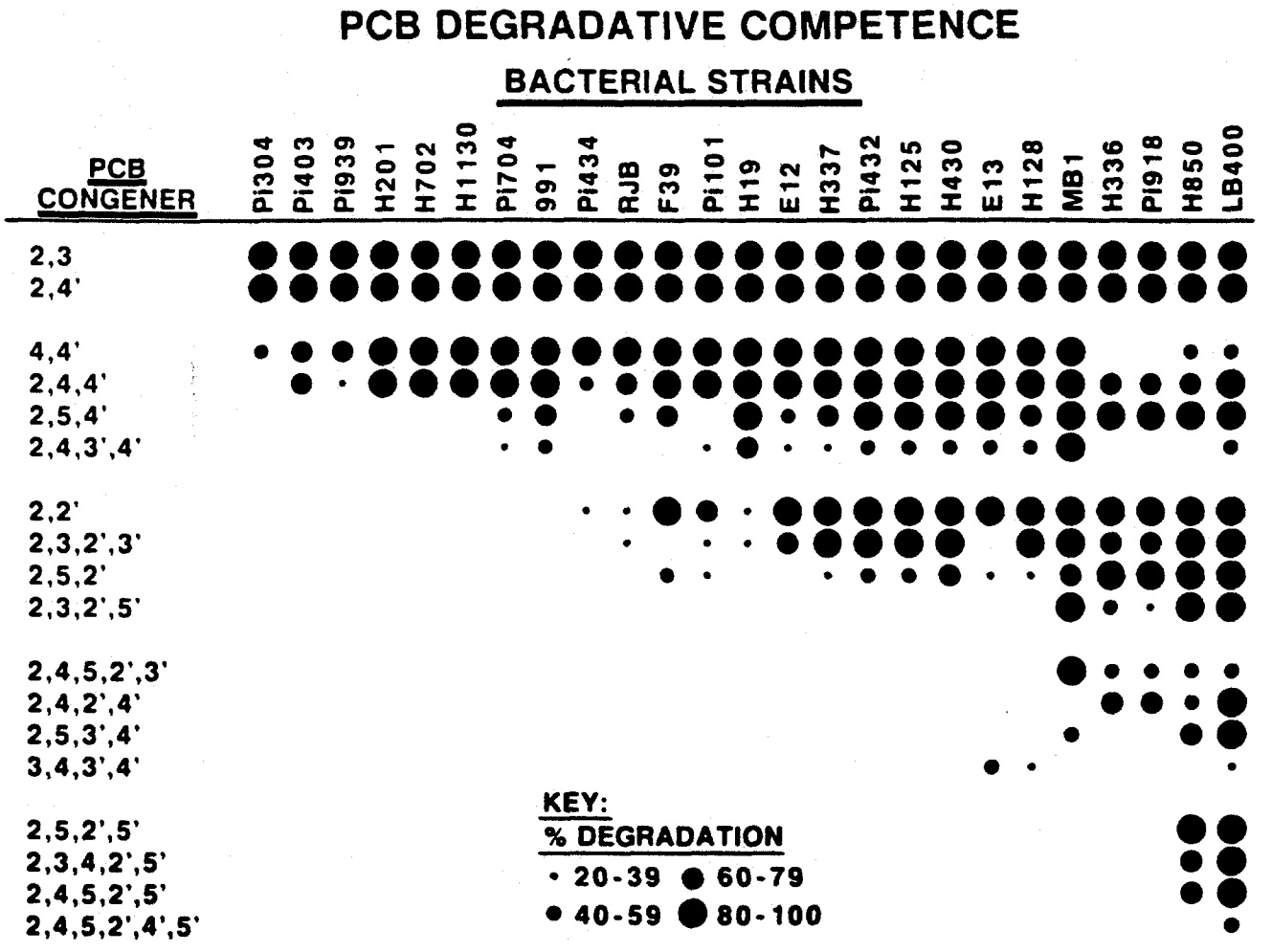


Table 1-1. Extent of biodegradation after 24 hour resting cell assays with aerobic bacteria.

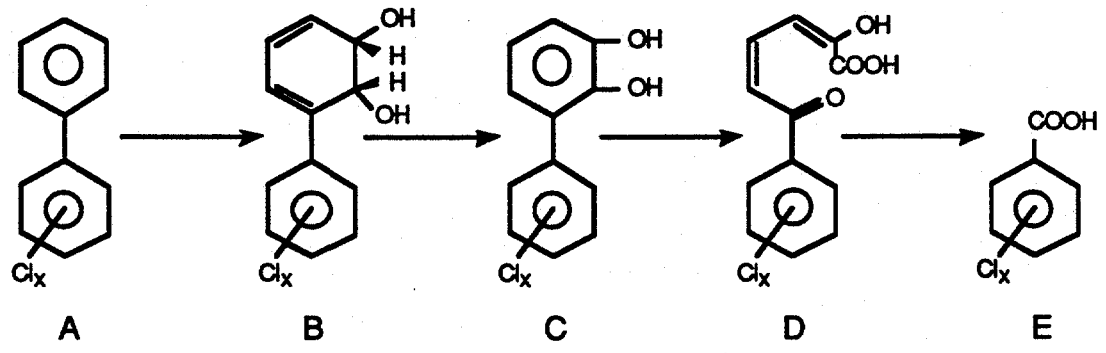


Figure 1-1. Degradation of chlorobiphenyls by the 2,3-dioxygenase pathway. A. polychlorinated biphenyl (PCB); B. chlorinated dihydrodiol; C. chlorinated 2,3-dihydroxybiphenyl; D. chlorinated 2-hydroxy-6-oxo-6-phenylhexa-2,3-dienoic acid; E. chlorinated benzoic acid. The chlorobenzoates (E) are further biodegraded to CO_2, H_2O, Cl^- and biomass by a variety of indigenous aerobic microorganisms present in Hudson River sediments.

A selection of these isolates and their PCB degrading capabilities are shown in Table 1-1 [Bedard et al., 1986]. The aerobic microorganism used for inoculation in this experiment was *Alcaligenes eutrophus* strain H850. This organism is a natural isolate from the Hudson River region and one of the most competent PCB degraders identified to date.

A significant amount of information had been gathered regarding the oxidative degradation of PCBs by aerobic bacteria in laboratory experiments [Abramowicz, 1990; Bedard, 1990]. However, very little was known about this process in the field. In 1987, GE conducted a field study on a PCB-contaminated soil in South Glens Falls, New York. By repeatedly inoculating the soil with large doses of a PCB-degrading strain (*Pseudomonas sp.* LB400), the PCB concentration was reduced by approximately 25% in the surface layer of soil over the course of the summer-long experiment. More details regarding this study are described elsewhere [McDermott et al., 1989].

To our knowledge, the 1991 *in situ* Hudson River Research Study is the first PCB bioremediation study in a river sediment environment. The objectives of this study were to: 1) demonstrate that aerobic PCB biodegradation could occur under actual field conditions, as indicated by prior laboratory investigations, 2) identify the key variables that influence the rate and extent of PCB biodegradation in Hudson River sediments, and 3) investigate the potential for natural aerobic biodegradation to occur in these sediments via indigenous PCB-degrading microorganisms.

CHAPTER 2
SITE CHARACTERIZATION AND FACILITY DESIGN

The Hudson River Research Station (HRRS) was located immediately offshore from the west bank of the Hudson River in the Town of Moreau in Saratoga County, New York approximately 2 miles downstream of Fort Edward (see Figures 2-1 and 2-2). The area, which has been designated Hot Spot #5 by the New York State Department of Environmental Conservation, will be called H7 in this report (GE designation). The site was located 193.5 river miles upstream from the Battery in New York City. The USGS Topographic Maps (7.5 minute series) show the HRRS site on the southern border of the Hudson Falls Quadrangle and the northern border of the Fort Miller Quadrangle, at approximately 43° 15' 0" latitude and 73° 35' 40" longitude. Site access was provided from West River Road.

This site was selected for evaluation based upon earlier laboratory work with this sediment. Samples taken from this location over the past several years have shown evidence of extensive anaerobic dechlorination activity. Also, PCB-degrading aerobic bacteria, including *A. eutrophus* H850 which was used in this study, had been isolated from the banks of the Hudson River near this site.

1990 Harza Engineering Survey

During the summer of 1990, Harza Engineering conducted a survey of the project area at the request of GE. The 230' x 25' area was divided into a rectangular grid with 12 foot spacings between the sampling points (Figure 2-3). Samples were collected by hand-driving a coring tube into the sediment to the point of refusal. The core samples were then sectioned horizontally and analyzed for PCB content to determine the variation in PCB concentration as a function of depth.

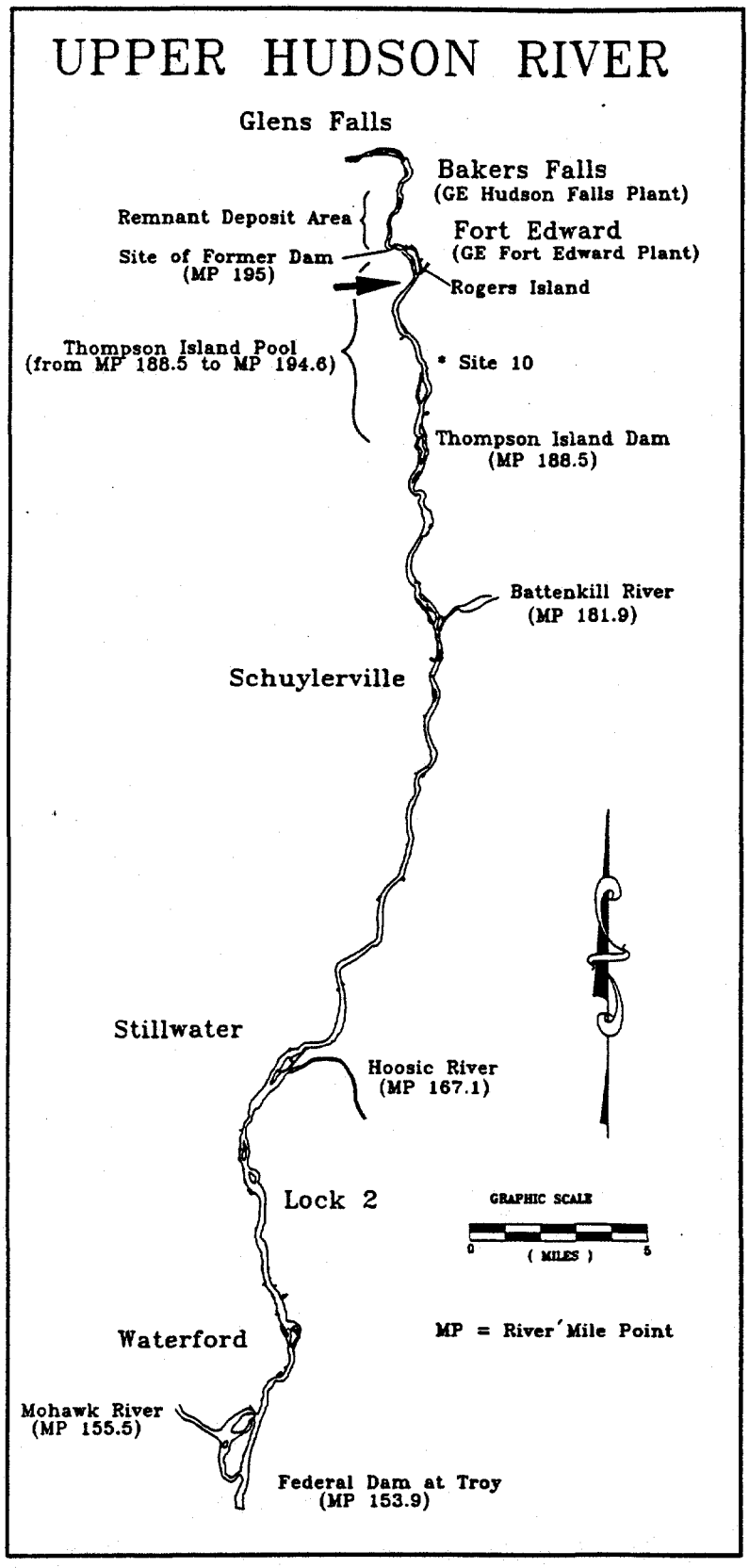


Figure 2-1. Map of Upper Hudson River.

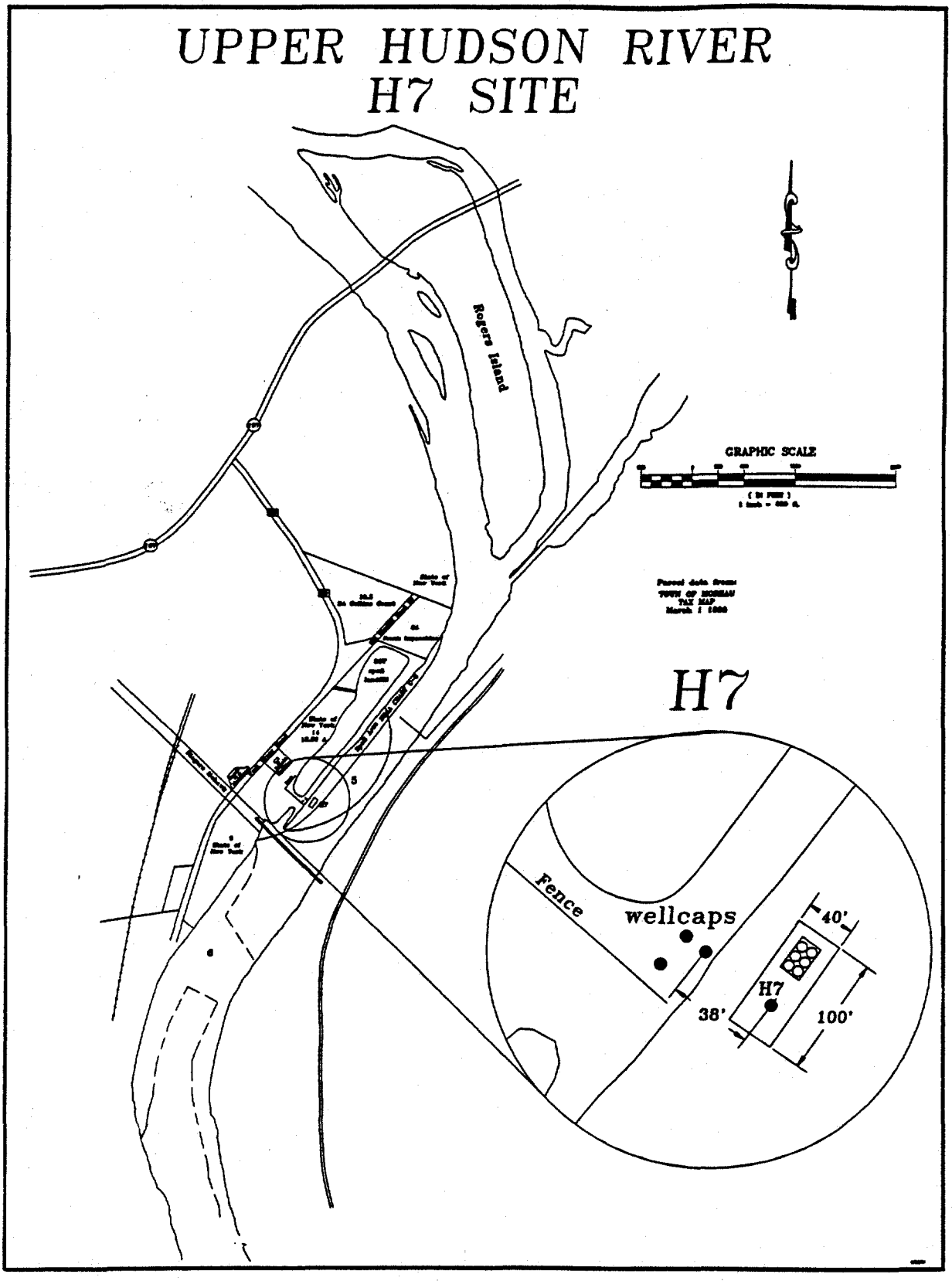


Figure 2-2. Area map of Hudson River Research Station, H7 site.

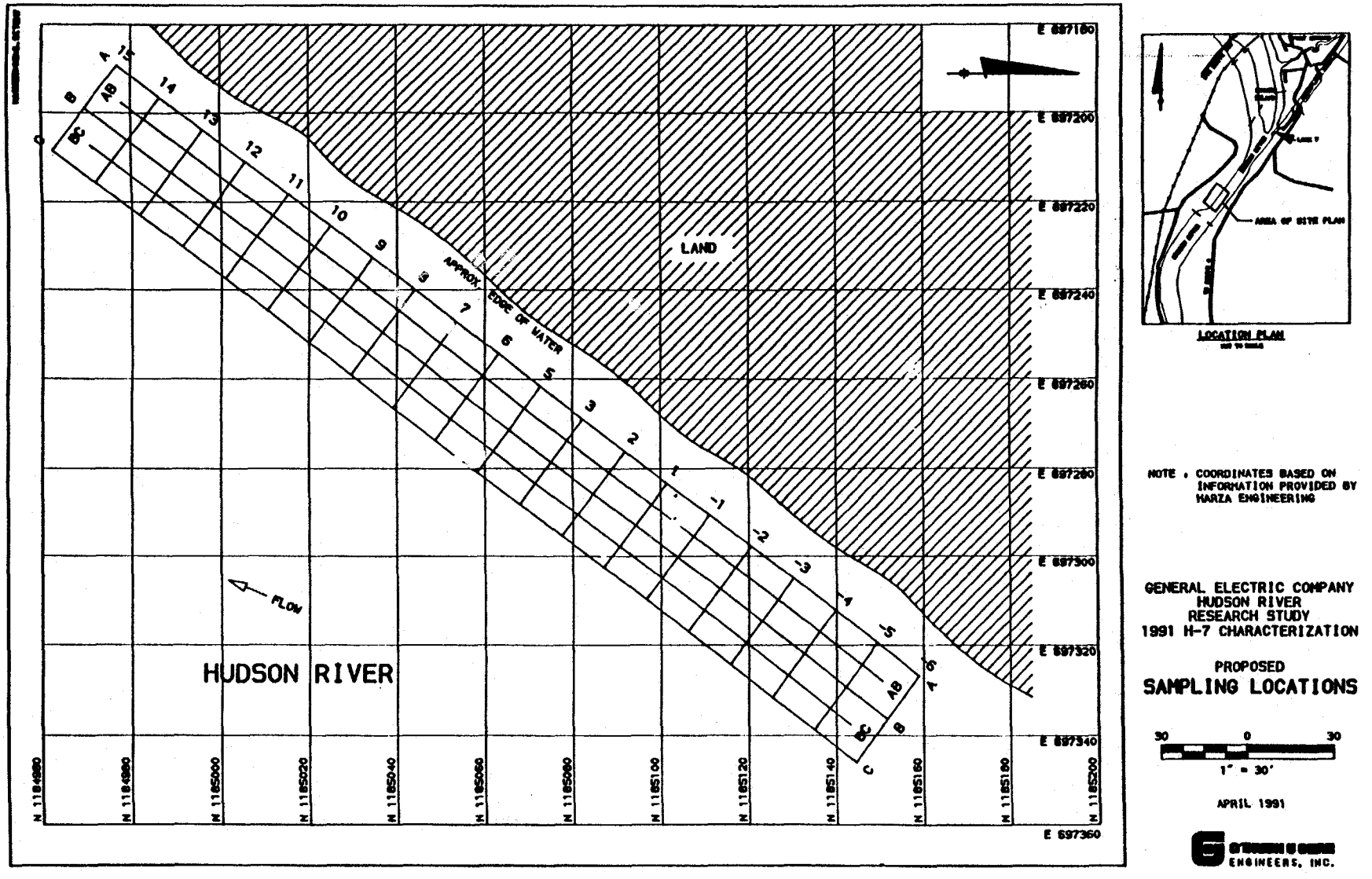


Figure 2-3. Sampling grid for 1990 and 1991 site surveys.

The results of the PCB analyses are contained in Figure 2-4. The height of the bar indicates the depth of the core and the individual layers denote the subsections at various depths. The length scale is indicated in the upper left corner. The PCB distribution at the site was highly variable. The average PCB concentration in the survey was 39.4 ppm (N=150), with a range in PCB values from 0.02 to 730 ppm and a standard deviation of 102 ppm. From the depth profiles, it was evident that most of the PCBs in the area resided in the top 4 to 8 inches of the sediment. Chromatographic analysis indicated the PCB in the area had undergone extensive and widespread dechlorination to mainly mono- and dichlorobiphenyls. A representative chromatogram appears in Figure 2-5. The shallow contamination typical of the upper Hudson River and the extent of dechlorination made this site an appropriate place for this biodegradation experiment.

1991 O'Brien & Gere Engineers Survey

In April of 1991, another PCB survey of the general site area was conducted by O'Brien & Gere Engineers to determine the exact placement of the HRRS platform within the previously investigated area. This survey was referenced to the same sampling grid as the Harza survey, with two notable differences. The sample spacing was closer than in the Harza survey, 6 feet between samples as opposed to 12 feet, and only those areas with greater than 10 ppm PCB in the Harza survey were re-sampled.

Based upon the earlier Harza survey, which found the PCB contamination to be shallow (Figure 2-4), the decision was made to focus on the top six inches of sediment for the biodegradation experiment. Sediments were collected with a 2-5/8 inch diameter core sampling tube. The cores were sectioned and the top six inches of sediment were analyzed by EPA Method 8080 and quantified by Webb and McCall [1973] methodologies. In locations where the core sampler could not collect sufficient material, a Ponar Dredge was used to collect surficial samples. The results of this survey are detailed in Figure 2-6.

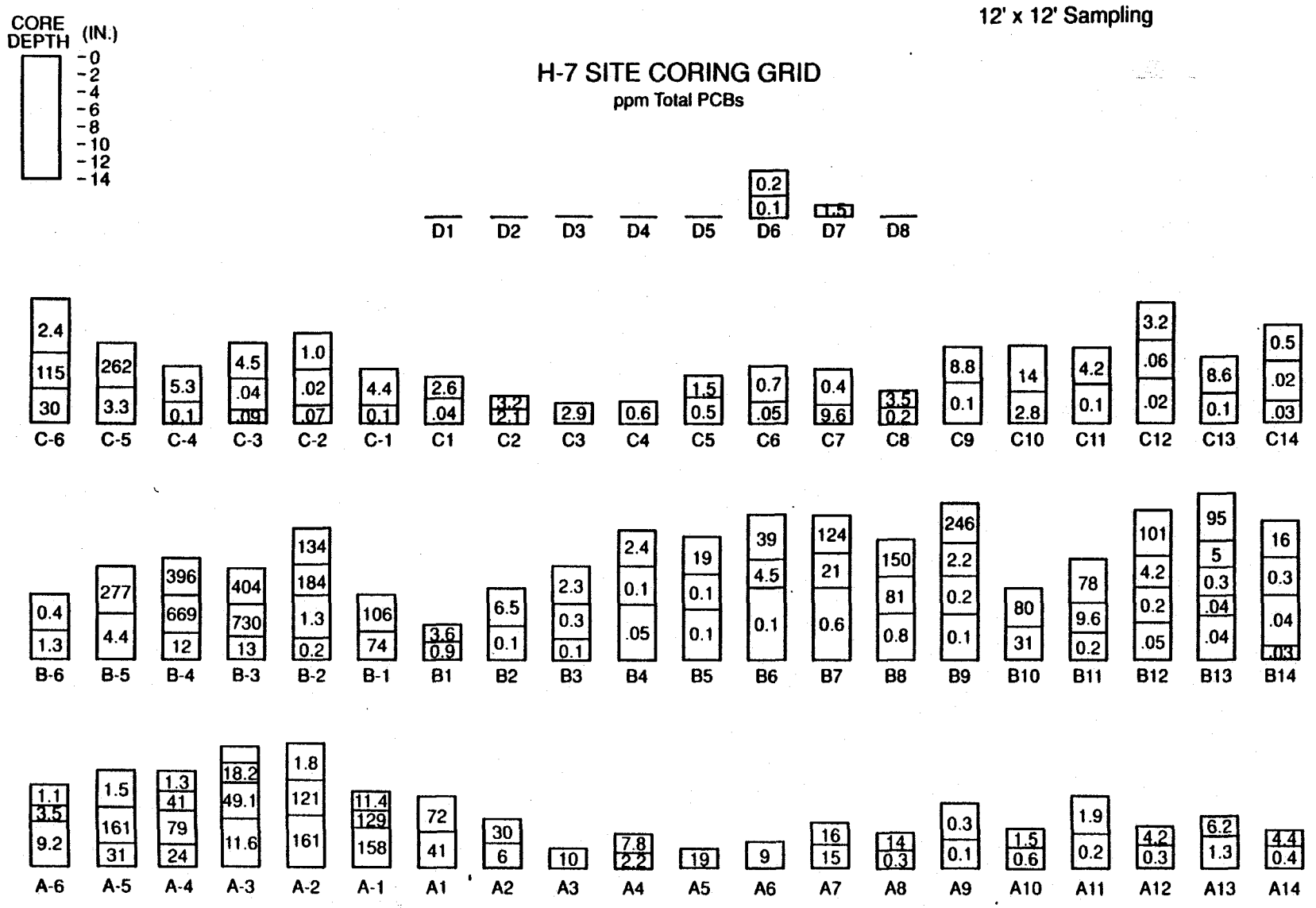


Figure 2-4. Harza Engineering Survey results. PCB concentration is shown as a function of location and depth.

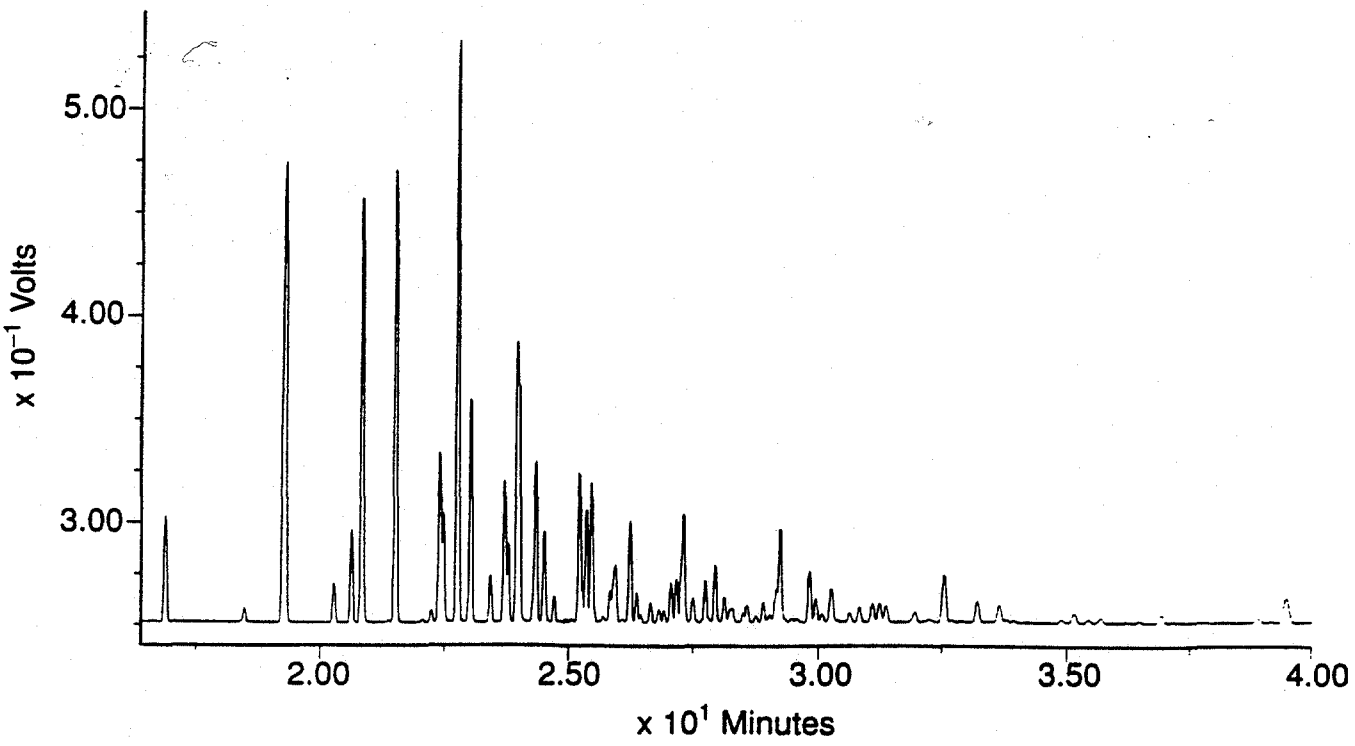
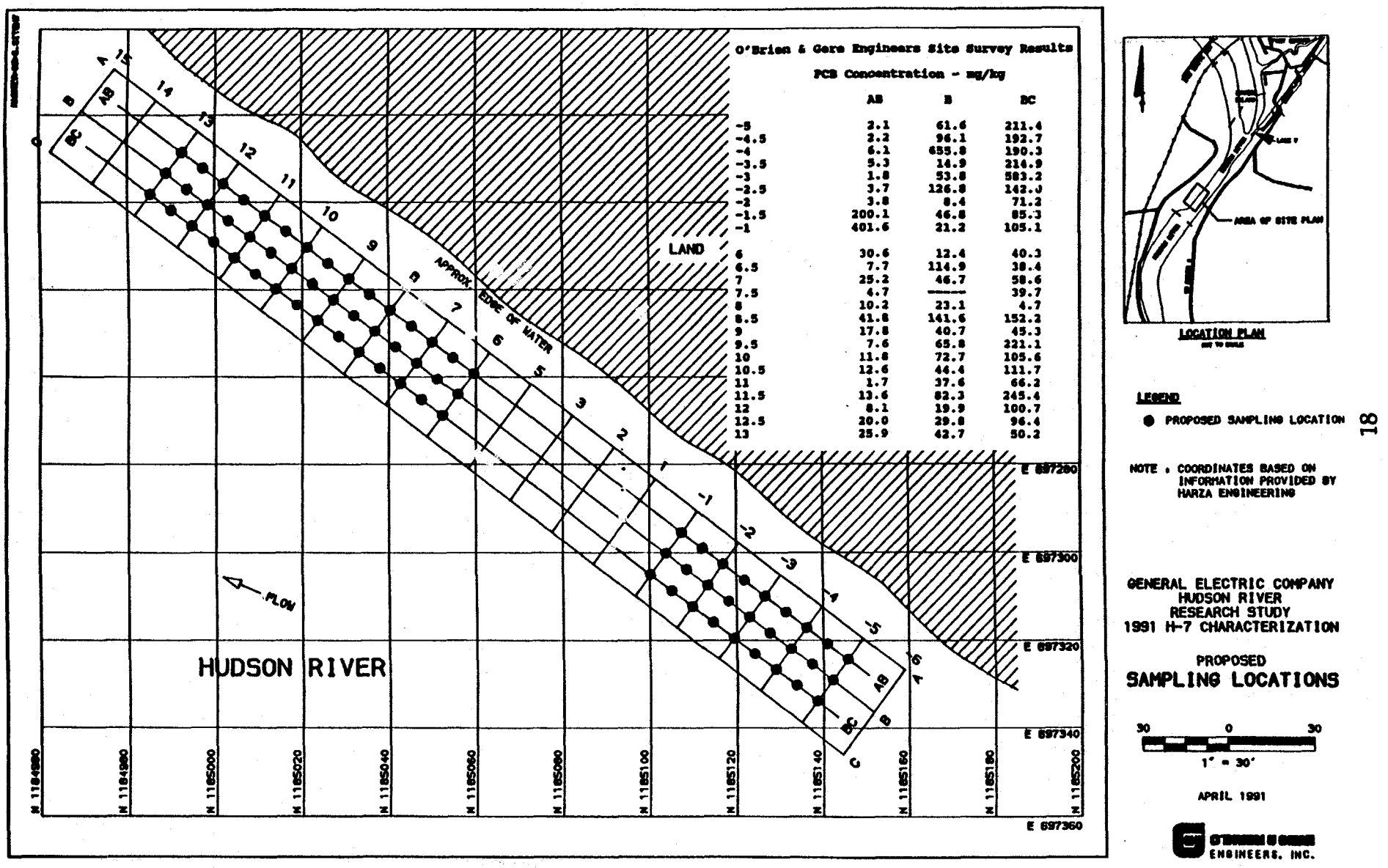


Figure 2-5. Typical gas chromatogram of PCBs from H7 site. Increased proportions of mono- and dichlorobiphenyls reflect extensive *in situ* dechlorination activity.



LEGEND

● PROPOSED SAMPLING LOCATION

NOTE : COORDINATES BASED ON INFORMATION PROVIDED BY HARZA ENGINEERING

GENERAL ELECTRIC COMPANY
HUDSON RIVER
RESEARCH STUDY
1991 H-7 CHARACTERIZATION

PROPOSED
SAMPLING LOCATIONS

30 0 30
1" = 30'

APRIL 1991

ENGINEERS, INC.

Hudson River Research Study

Figure 2-6. O'Brien & Gere Engineers Survey results. PCB concentration for top six inches of sediment or less.

The footprint of the 6 caisson reactors on the river bottom was approximately 25' x 15.5'. On the sampling grid, this footprint covered 10 sample points arranged in a 2 x 5 matrix. The criteria for placement of the reactors were: 1) average PCB concentration in excess of 50 ppm over the 2 x 5 matrix and 2) moderate variation in PCB concentration from point-to-point. Two areas met the first criterion, an upriver area spanning (B,-5) to (BC,-2.5) and a downriver area spanning (B, 8.5) to (BC, 10.5). The upriver area had greater variability, with a high concentration of 655.8 ppm and an adjacent low concentration of 14.9 ppm. In addition, the construction of the platform at the upriver position would have been much more difficult because of limited shore access. In light of these considerations, the downriver position was selected for the platform.

The results of the O'Brien & Gere survey displayed a high degree of variation in PCB concentration throughout the survey area. The difference between adjacent points was often large, despite the fact that samples were taken only 6 feet apart. This is a common feature of heterogeneous environmental samples that complicates any field experiment or operation. This spatial variability would manifest itself when the survey results were compared to the PCB concentrations measured in the caissons at the beginning of the experiment.

Physical Plant Description

The HRRS comprised a research platform constructed immediately off the west bank of the river plus several trailers and a tent on the adjacent land. The river platform supported the six caissons plus the gas handling system and local monitoring equipment. The majority of the support functions, such as hydrogen peroxide and nitrogen supply, set point control, data acquisition, and motor control were land-based.

The detailed design, construction, and installation of the HRRS was subcontracted by GE to OHM Corporation (Findlay, OH).

Land-Based Operations

On the land adjacent to the HRRS platform were a Motor Control trailer, a decontamination trailer, a hydrogen peroxide storage and delivery system, a storage shed, an open screen tent, and a waste storage area. These were all situated in the gravel parking lot located approximately 50 yards from the river bank. Access to the river platform was along a gravel path and suspended metal walkway.

The Motor Control trailer contained the variable frequency drives for the AC motors on the caisson agitators. Although the agitator speeds were adjustable, the settings were not changed once the desired level of agitation was determined during the startup phase prior to the experiment.

The controllers and monitors for the process variables were also housed in the Motor Control trailer. The variables continuously monitored included the following:

1) Temperature
2) pH
3) Dissolved oxygen (DO)
4) Water level
5) Motor torque
6) Gas Flows (N_2 inflow, reactor outgas and suction)
7) Hydrogen peroxide flow

The gas flows and hydrogen peroxide flows were also integrated with totalizers. The process variable information was displayed in the trailer with LED readouts, and recorded for later analysis with strip chart recorders and a personal computer with data acquisition software. The process information was also monitored on a personal computer at CRD in Schenectady via modem.

The remainder of the Motor Control trailer was office space and a small laboratory area for simple chemical analyses.

The decontamination trailer contained lockers and showers for the researchers. This trailer was primarily used for the storage of personal effects and personal protection equipment.

The hydrogen peroxide used as an oxygen source in the experiment was obtained as a 10% solution in water from Degussa Corporation (Richfield, New Jersey). The hydrogen peroxide was delivered in an ISO container mounted on a trailer along with a feed pump system. The feed pump system had a gear pump and a small 40 gallon holding tank. When the smaller tank was nearly empty, it was refilled from the main storage tank automatically. This prevented any back contamination of the peroxide in the storage tank.

A small open screen tent was erected in the parking lot for additional work space. The tent provided protection from the weather and ventilation necessary when small amounts of organic solvent were used for decontamination during sample preparation.

The waste storage area consisted of a lined area surrounded by a berm and a chain-link fence. The area contained a number of 55 gallon drums for disposal of sediment, water, decontamination liquids, and contaminated personal protection equipment.

HRRS Platform

The HRRS platform was a 25' x 37.5' rectangular steel structure erected approximately 10 feet offshore. The longer side (37.5') ran parallel to the shoreline. The structure was supported by 8 steel piles driven an average of 70 feet into the river bottom. This length of pile was necessary because the sediment at the study site was soft and had poor load bearing capability. An aerial view of the platform is depicted in Figure 2-7.

Construction of the platform was done by a barge mounted crane. The decision to construct the platform from the river side was based upon the limited crane access on the shore. The large steel members of the platform were barged downriver from Fort Edward. The piles were driven into the

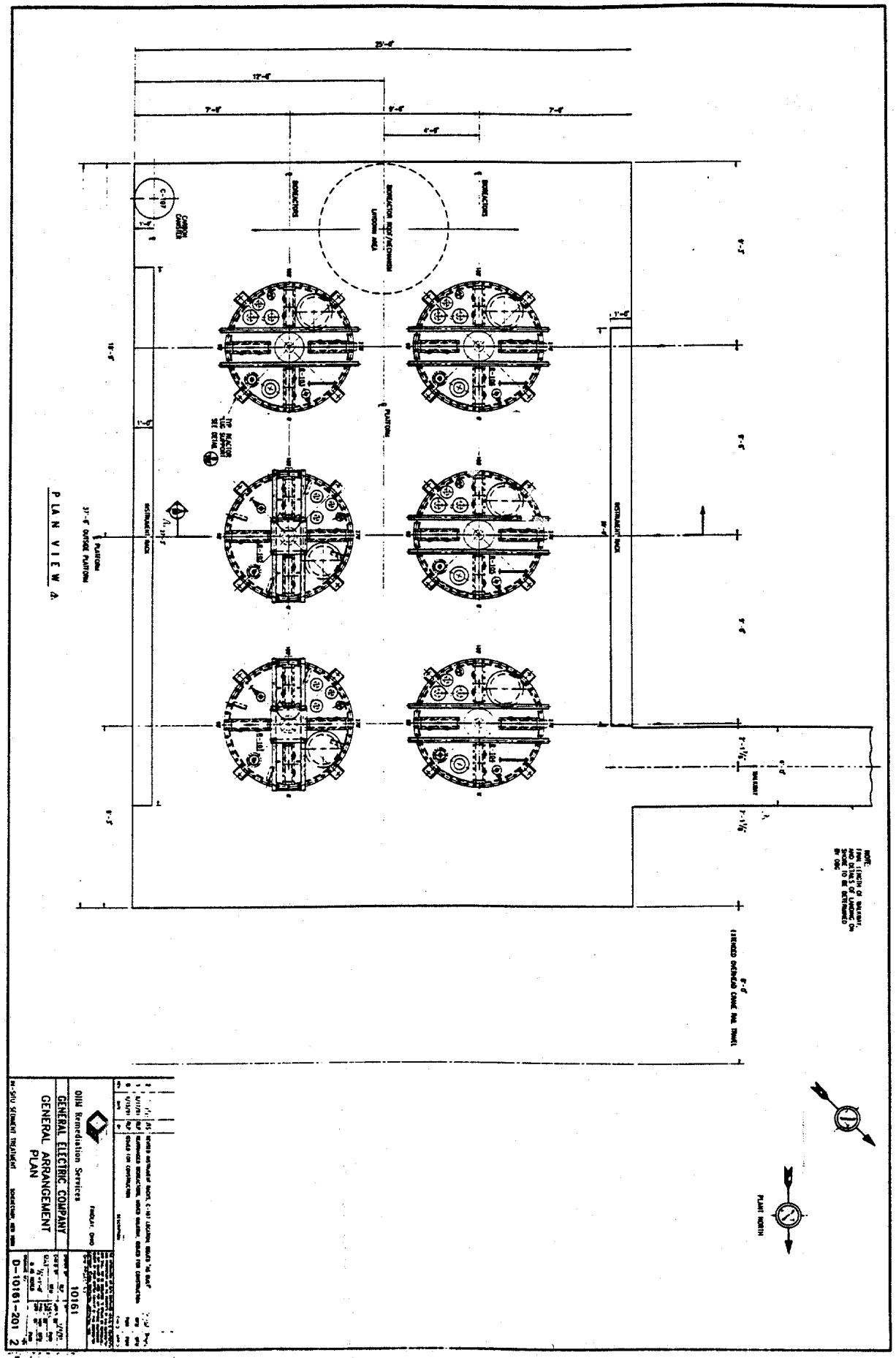


Figure 2-7. Aerial plan view of Hudson River Research Station platform.

sediment with a vibratory hammer on the crane. A silt curtain was installed around the platform to contain any silt disturbed during this operation.

Once the piles were driven and the major cross members of the platform installed, the six foot diameter caissons were driven with the vibratory hammer an average of 8 feet into the sediment. The overall caisson length was 16 feet. This caisson depth, coupled with the very low sediment permeability, provided a virtual seal at the reactor bottom. The water depth in the caissons ranged from 2.5 to 4 feet. Figure 2-8 shows an end view of the platform with the caissons in place. An open metal grating was then installed on the platform to provide a work surface for access to all sides of the caissons.

Caisson Details

The caisson walls were constructed of 6 foot ID spiral welded carbon steel pipe. Wall thickness was 1/2 inch. The caisson tops were 3/8 inch thick flat steel plates bolted onto a flange welded to the top of the caisson walls. The gasket material sealing the caisson top was 1/2 inch Gore-tex™.

Access to the caisson internals was through the caisson top. Caisson lid details are shown in Figure 2-9. Four rectangular sampling ports located 90° apart allowed sediment samples to be taken at four angular positions and at varying radial positions. These sampling ports were 22" x 4" with hinged lids. The lids were bolted to a welded flange and sealed with a 1/8" viton gasket. A 18" manhole provided additional access. All monitoring probes entered through ports on the caisson lid. All seals were maintained with 1/8" viton gaskets.

All caissons were pressure tested to 1 psi prior to installation. During the experiment small leaks were detected in some of the caissons when under pressure. These leaks likely resulted from the frequent opening and closing of the ports which disrupted the seals. When a leak was identified, measures were taken to restore the seal.

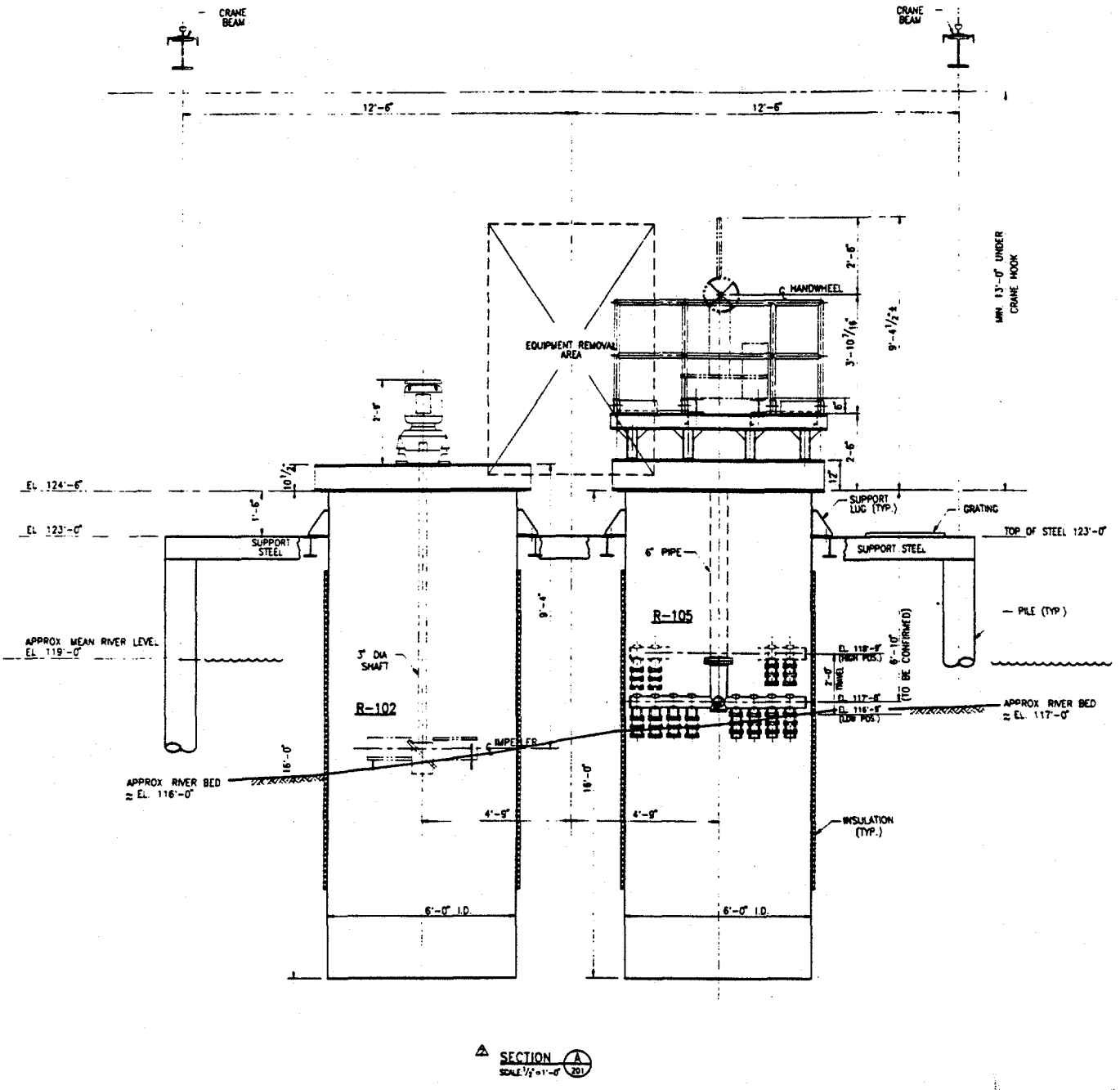


Figure 2-8. End view of Hudson River Research Station (courtesy of OHM Corporation).

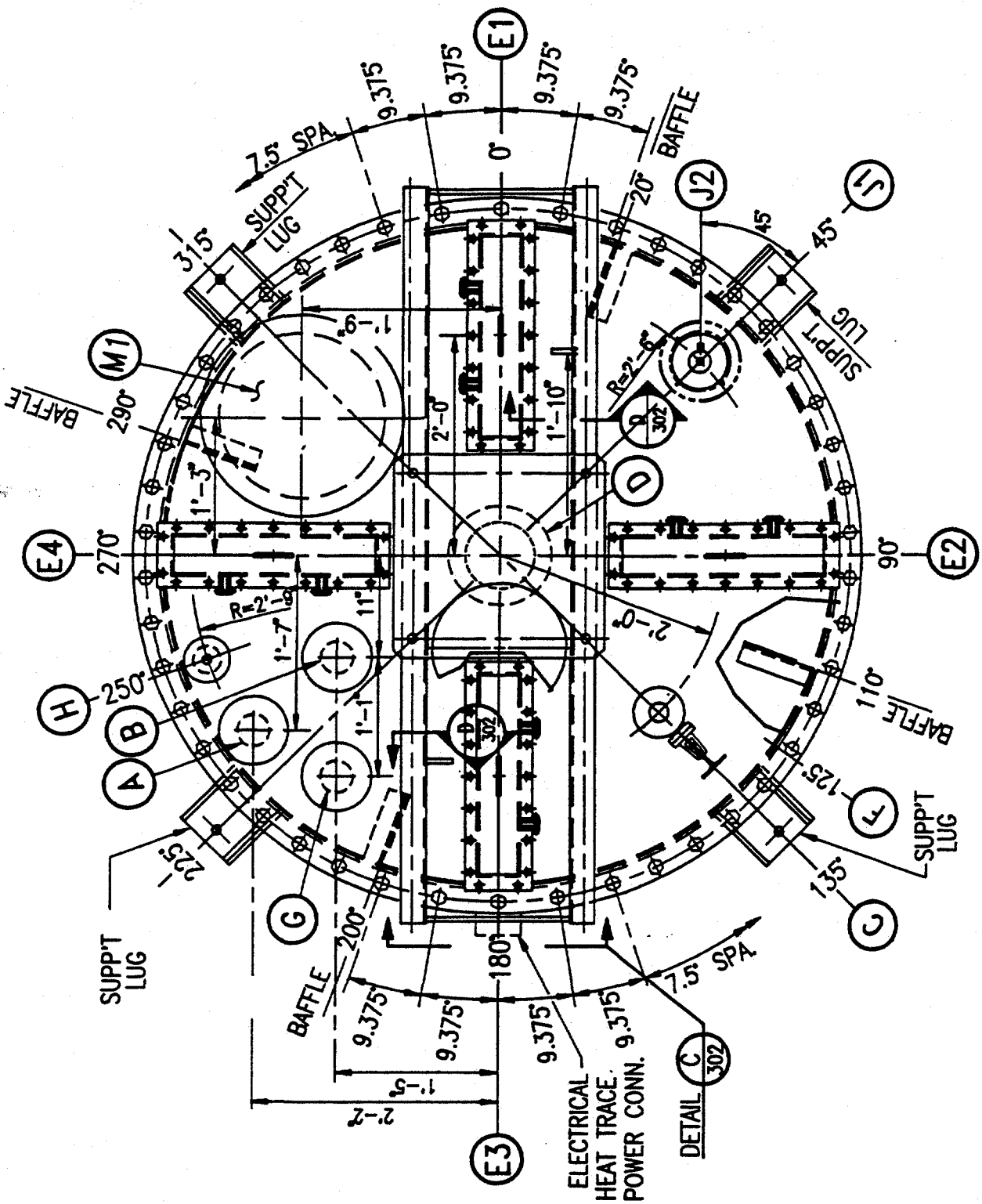


Figure 2-9. Caisson lid details showing four rectangular sampling ports (E_1, E_2, E_3, E_4), manhole entry (M_1) and ports for monitoring probes (A, B, G) (courtesy of OHM Corporation).

The caisson walls were wrapped with heat tracing tape along 5 feet of length. This was then covered with 1 1/2" of fiberglass insulation and a protective aluminum sheath that covered 10 feet of wall length. This provided the research team with the capability to heat the caissons if the ambient water temperature became too low. This situation did not arise during the course of the study so the heaters were never turned on. The entire experiment was conducted at ambient river temperatures.

It was the intent of the research team to assess the impact of agitation on the rate and extent of PCB bioremediation. Therefore two caisson configurations were employed for the study, each utilizing a different mode of mixing the sediment. A high-mix mode of agitation was created using a turbine impeller. The intent was to suspend the lighter fractions of the sediment in the aqueous phase in the caisson. Caissons with this agitator had 4 six inch baffles installed on the inside of the reactor walls with a spacing of two inches between the wall and the baffle edge. Two caissons were configured in this manner. A low-mix mode of agitation was created using a rake mechanism designed to slowly rake or plow through the sediment to provide surface renewal for the exchange of oxygen and chemicals between the sediment and the water column. The intent was to gently mix the sediment but not suspend large amounts of material in the water column. Caissons with this rake agitator also had small water column agitators to induce slow circulation and to eliminate any gradients in the water column.

Agitator Details

The large agitators were installed on the caisson lids with the shafts passing through water seals into the caisson. The small water column agitators had mechanical seals.

The turbine agitators were manufactured by Lightnin (Rochester, New York) with 5 hp variable speed AC motors (0 to 80 rpm). The four-bladed turbine impeller had a 3 foot diameter. This large diameter turbine afforded large pumping volumes (22,250 gpm at maximum rpm) at relatively low tip speeds. The result was very good sediment suspension and circulation at low

rpm with very little vortex formation. This configuration also kept the sediment surface relatively flat. A smaller turbine at high speeds would have scoured the bottom into a conical shape.

The rake agitators were manufactured by EIMCO (Salt Lake City, Utah). The assembly was a drive mechanism for a 12 foot diameter thickener or clarifier. Since the intent of the rake was to turn the sediment over and not sweep it to the center as would normally be done in a thickener, a custom rake had to be designed. The rake design consisted of four arms at 90°, each with four plows. The plows were one foot long and had three angled plates welded onto a 3" x 3" right angle (see Figure 2-10). The plows were arranged in a staggered configuration so that the plow faces would not ride one behind the other. This prevented the formation of mounds of unmixed sediment in between the plow furrows. The rpm range was 0 to 4. The rake was driven with a 5 hp motor.

The depth that the rakes cut into the sediment was adjustable. The drive mechanism allowed the rake arms to be raised and lowered 24 inches. Once the rake assemblies were installed, soundings were taken in the caissons to determine the exact depth of the sediment surface. The rakes were then set to plow the top 6-8 inches of sediment.

The small water column agitators that accompanied the rake mechanisms were manufactured by Lightnin with 6 inch diameter, three-bladed turbines. Due to a specification error the agitators were not functional at the start of the experiment. The agitators were subsequently modified and the turbines set 6 inches below the liquid surface. These small agitators provided gentle circulation in the water column.

Hydrogen Peroxide Delivery System

The hydrogen peroxide storage and pump system was located in the parking lot next to the Motor Control trailer. The peroxide was pumped to the platform through a recirculation loop. This prevented the peroxide from lying stagnant in a feed line when there was no peroxide demand in the

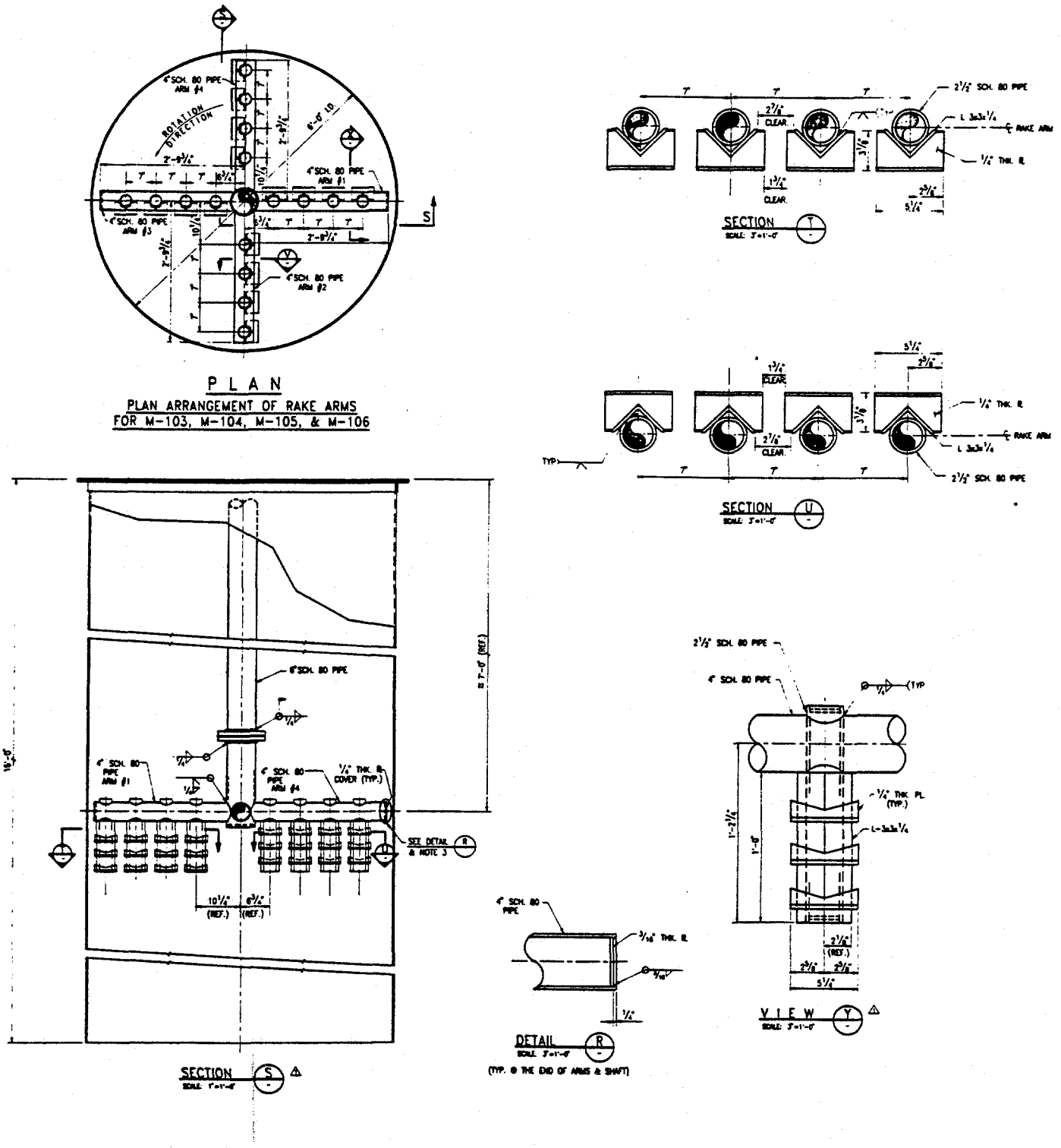


Figure 2-10. Plow arrangement and details for rake mechanisms (courtesy of OHM Corporation).

caissons. The flow rate through this loop was many times the normal flow rate of peroxide to all caissons combined. The supply lines to the caissons were individually connected to this recirculation loop. Each supply line had a control valve and flow meter. The control valve responded to the dissolved oxygen (DO) set point established at the controller in the Motor Control trailer. When the DO fell below the set point, the valve opened allowing hydrogen peroxide to enter the caisson. This quickly decomposed yielding oxygen which was detected by the DO probe.

The dissolved oxygen probes and monitoring system (5340D and 671D, respectively, Great Lakes Instr. Co.) provided a continuous readout of DO in ppm and a system output of 4-20 mA to a loop controller. The controller (MYCRO 352, Moore Prod. Co.) used to meter the peroxide flow to the caissons was a microprocessor-based single loop digital system with integral orifice flowmeters (ABB Kent Taylor, Inc.). Flow rate was measured via differential pressure which was converted to a 4-20 mA signal.

Only the four experimental reactors received hydrogen peroxide. The two control caissons had nitrogen purge lines instead of the peroxide addition lines. The nitrogen purge lines were installed to purge oxygen from the control caissons to keep them anaerobic. However, this system was not used during the experiment because the caissons leaked under the pressure created by the gas purge. Instead, liquid nitrogen was added to the caissons after sampling and prior to restarting the mixer in those cases when it was necessary to purge the headspace of oxygen.

Gas Handling System

Each caisson was connected to the gas handling system through a 3/8" vent line. The gas passed through a mass flow meter and then to a series of beds packed with adsorbent capable of trapping PCBs, XAD-2 resin. There were three resin beds in series on each caisson outlet, with the exception of R101. The outlet to R101, which was a control caisson, was changed from three beds in series to one bed with a smaller aspect ratio containing the same

amount of adsorbent. This was done to decrease the pressure drop across the bed. These adsorbent beds were changed periodically and analyzed for PCB so that volatilization losses could be quantified. The three beds in series allowed the researchers to determine that PCBs did not break through the beds and leave the system. All the outlets ultimately passed through a 55 gallon drum of activated carbon as an additional precaution against emissions.

The gas handling system measured the amount of outgassing and any gas flow that might have entered the caissons when they cooled at night or during cold weather. This was done with check valves and mass flow meters to measure flow in and out of the caissons.

Data Acquisition

A data acquisition system was used, in addition to strip chart recorders, to monitor and record process variables for all 6 caissons. The system was comprised of two IBM compatible PCs each interfaced to a 15-channel acquisition unit (ACRO-400, Analogic Corp.). In addition to monitoring and recording the pH, temperature, water level and gas flows (in and out of each caisson), the dissolved oxygen level and peroxide flow to each caisson were monitored on a real-time history graph. A modem was used to remotely monitor the DO level and peroxide flow history graphs from GE CRD. The data was formatted and loaded into Lotus 1-2-3 spreadsheets for plotting.

Probe and Mass Flow Controller Calibrations

On-line monitoring equipment, such as dissolved oxygen, pH, and temperature probes were checked weekly for response and recalibrated if necessary. This was done in place in the caissons by comparing the on-line readings to those obtained using portable, hand-held instruments calibrated prior to the check. A full recalibration of the instrument was performed if the two readings differed by more than ten percent.

The calibrations of the hydrogen peroxide mass flow controllers (MFCs) were checked manually at the beginning and near the end of the experiment. Table 2-1 shows that the actual flows measured for the four peroxide caissons were slightly higher than the meter readouts. Instantaneous peroxide rates and totalizer amounts were corrected for this difference. The corresponding controller settings given by Table 2-1 show readings only up to 25% open. This upper limit setting was to prevent peroxide addition rates from exceeding 25 ml/min, which could cause accidental sterilization in the caissons. No drift was observed in any of the MFCs over the course of the study.

Table 2-1
Calibrated peroxide flow rates

Reactor	Controller (% open)	Meter Flow (ml/min)	Actual Flow (ml/min)
R102	10	5.0	6.5
	15	10.0	12.0
	20	15.0	17.5
	25	20.0	23.1
R103	10	5.0	7.2
	15	10.0	13.3
	20	15.0	19.3
	25	20.0	25.3
R105	10	5.0	5.6
	15	10.0	10.7
	20	15.0	15.9
	25	20.0	21.0
R106	10	5.0	6.0
	15	10.0	11.7
	20	15.0	17.5
	25	20.0	23.2

Waste Handling, Demobilization and Decommissioning

On October 15, 1991 sixteen drums of waste (10 drums of PCB sediment and debris and 6 drums of wash water) were transported to a TSCA permitted storage facility in Philadelphia, Pennsylvania (PPM, Inc., a subsidiary of USPCI) until the material could be transported to the Rollin's TSCA incinerator in Deer Park, Texas (there is a waiting period of up to 3 months). We are currently awaiting certificates of destruction.

On October 28, 1991 the experimental phase of the HRRS ended. The HRRS was a temporary installation. The entire platform and land-based operations were removed by the end of November. Prior to the disassembly of the HRRS, both the water and the top foot of sediment were pumped out of the caissons. Any equipment that came into contact with sediment was decontaminated upon removal, first with high pressure water, then with acetone. The water from the caissons and all decontamination liquids were placed into a tanker, sampled, and solidified. The sediment from the caissons was sampled and placed in drums. The sediment, associated contaminated personal protection equipment, and solidified liquids generated are scheduled to be transported to the TSCA permitted landfill (Model City, New York) for burial by Chemical Waste Management. The results of the sample analysis done on this material is included in Table C2-4 in Appendix C-2.

CHAPTER 3
EXPERIMENTAL DESIGN AND PROTOCOLS

Experimental Design

The Hudson River Research Study was performed to evaluate the parameters that affect the biological degradation of PCBs in sediments under field conditions. The variables addressed in this study include the addition of oxygen and nutrients and the effect of mixing mode and bacterial inoculation on *in situ* aerobic PCB biodegradation. Mixing is necessary to enhance the dispersal of oxygen and nutrients into the sediment to stimulate aerobic activity, but it was not known how the natural system would respond. A high-mix condition was created using an axial-flow turbine impeller which rotated at 40 rpm. The intent was to suspend only the lighter fractions of the sediment into the water column. This is similar in design to many laboratory mixing systems. A low-mix condition was created using a plow-like rake which rotated at 3 rpm in the sediment. The intent was to simply turn the sediment over periodically without resuspending large amounts of dense material. In both cases only the top 6-8" of sediment, where the PCB levels were highest, were mixed.

The bacterial strain H850 was isolated in 1983 from PCB-containing dredge spoils several hundred yards upstream from the study site [Bedard, et al., 1984]. It has been identified by the American Type Culture Collection (Rockville, Maryland) as an *Alcaligenes eutrophus*. The degradative competence of this organism has been characterized using a variety of PCB congeners in the laboratory (Table 1-1). *A. eutrophus* H850 is able to degrade an unusually broad spectrum of PCB congeners, including some tetra-, penta-, and hexachlorobiphenyls [Bedard et al., 1987]. Due to extensive dechlorination, H7 sediments contain primarily mono- to trichlorobiphenyls (at least 80% of the total PCB). During this study H850 was added to two of the caissons to establish if PCB biodegradation would be enhanced in a competitive natural environment.

The experimental design for the river study is summarized in Table 3-1 and Figure 3-1. Two of the caissons, R101 and R104, are controls for the high- and low-mix agitation systems, respectively. No nutrients, oxygen, or organisms were added to these caissons, in order to limit aerobic biological activity. It was impractical to exclude all oxygen from these systems, thus each caisson was aerobic to some degree. This was not a significant problem for the low-mix caisson R104, but R101 did exhibit elevated oxygen levels in the water column from rapid oxygen equilibration with the headspace. This was particularly pronounced after the caisson was opened for sampling. This phenomenon was minimized by pouring several liters of liquid nitrogen into the caisson to purge the headspace of oxygen prior to closing the caisson and restarting the mixers.

R102, R103, R105, and R106 were experimental caissons. Each received the same additions of nitrogen, phosphate, and biphenyl at the start and at intervals throughout the study. Nitrogen and phosphate were added in the form of reagent grade diammonium phosphate, ammonium sulfate, and potassium phosphate to maintain predetermined minimum nutrient levels in the caissons. Nitrogen and phosphate analyses were performed on a bi-weekly basis to monitor these levels. Biphenyl was added on a fixed schedule at ten- and twenty-day intervals throughout the experiment. The actual addition schedule appears in Table 5-2 of this report.

Oxygen was delivered to the experimental caissons in the form of a 10% hydrogen peroxide solution. This was done on demand via controllers linked to the dissolved oxygen probes. Total peroxide usage was tracked for each experimental caisson. No attempt was made to control the pH or temperature in any caisson, although these variables were continuously monitored.

The experimental caissons differed from each other by agitation mode and bacterial inoculation with H850. R102 contained the high-mix, turbine agitation system, while the other experimental caissons contained the low-mix, rake agitators. R102 and R103 were inoculated with H850 at various points during the study. R105 and R106 were duplicate caissons in which

Table 3-1. Experimental Protocol

Cell 101 : Physical Control for the Turbine Agitated (High-Mix) System

* Turbine agitated system
* Physical mixing only
* Anaerobic environment
* No nutrients, oxygen, or organisms added
* No pH control

Cell 104 :Physical Control for the Rake Agitated (Low-Mix) System

* Rake agitated system
* Physical mixing only
* Anaerobic environment
* No nutrients, oxygen, or organisms added
* No pH control

Cell 102 : Effect of Added Organisms Under High-Mix Conditions

* Turbine agitated system
* Nitrogen, phosphate, and biphenyl added
* Oxygen on demand via hydrogen peroxide
* No pH control
* H850 addition

Cell 103 : Effect of Added Organisms Under Low-Mix Conditions

* Rake agitated system
* Nitrogen, phosphate, and biphenyl added
* Oxygen on demand via hydrogen peroxide
* No pH control
* H850 addition

Cell 105 : Stimulation of the Indigenous Population Under Low-Mix Conditions

* Rake agitated system
* Nitrogen, phosphate, and biphenyl added
* Oxygen on demand via hydrogen peroxide
* No pH control
* No addition of organisms

Cell 106 : Stimulation of the Indigenous Population Under Low-Mix Conditions

* Same as cell 105

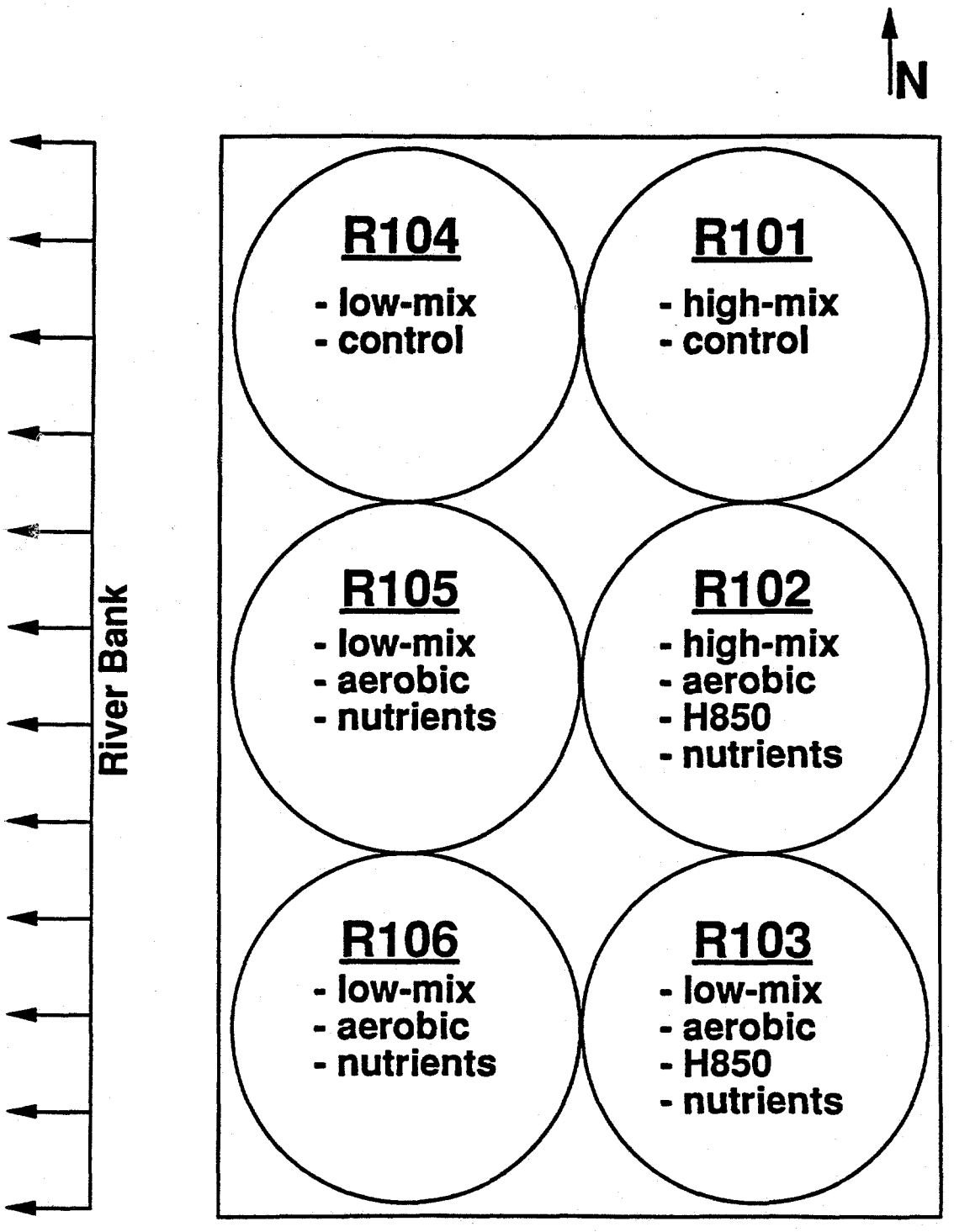


Figure 3-1. HRRS caisson orientation.

only the indigenous bacterial populations were stimulated. No H850 was added. The duplication was used to determine the inherent variability in the experiment. The effect of mixing may be assessed by a direct comparison of caissons R102 and R103. The impact of H850 inoculation may be determined by comparing R103 with R105 and R106.

Sediment cores for PCB analysis were taken from the caissons at the beginning and end of the experiment, and at one week intervals throughout the study. Twelve cores were taken from each caisson at the initial and final time points. This large number of samples was deemed necessary to achieve representative averages, given the anticipated variability of the PCB distributions in the sediment. Four cores were taken from each caisson at the intermediate time points. While conclusions should not be drawn based on data from any single intermediate time point due to the small sample size, these values gave an indication of the rate at which PCB concentrations changed over time.

All the caissons were vented to the atmosphere through vapor traps containing XAD-2 resin to retain any PCBs which may have volatilized from these systems. The vapor traps were removed and the resin analyzed for PCBs after the first week and at three week intervals throughout the duration of the study.

In addition to samples for PCB analysis, samples for bacterial counts, chlorobenzoates, and biphenyl were also taken throughout the study. A minimum of one aqueous sample and two sediment samples per caisson per week were obtained to track both aerobic heterotrophic and PCB-degrading bacterial populations. Additional samples were taken during the first several weeks of the experiment and after each H850 addition to the caissons. The caissons were sampled for chlorobenzoates daily during the first week of the experiment, three times per week from the second through the fourth weeks, and weekly thereafter. In general, one aqueous sample and two sediment samples were collected from each caisson on each sampling day. On sampling days when sediment cores were taken for PCB analysis, four rather than two sediment samples were collected from each caisson. Sediment and aqueous samples were analyzed for biphenyl concentration on a weekly basis.

Aqueous and sediment samples for a battery of standard environmental analyses were taken at the beginning and end of the study from each caisson, along with sediment samples for toxicity testing. In addition, subsamples of sediment from cores taken for PCB analysis at T_o and T_f were analyzed for solid phase total organic carbon content.

Chemicals

All chemicals used in the river study were of reagent grade quality. Dibasic potassium phosphate was purchased from Aldrich Chemical Company in Milwaukee, Wisconsin. Dibasic ammonium phosphate, aluminum potassium sulfate (alum), and acetone were purchased from J.T. Baker Chemical Company in Phillipsburg, New Jersey. Ammonium sulfate was purchased from EM Scientific in Cherry Hill, New Jersey. Biphenyl was purchased from Sigma Chemical Co. in St. Louis, Missouri. Ethanol was purchased from Mallinckrodt Chemical Co. in Paris, Kentucky.

Growth of *Alcaligenes eutrophus* strain H850

The *Alcaligenes eutrophus* strain H850 used in this study for inoculation of caissons R102 and R103 was grown aerobically at 30°C in a phosphate-buffered mineral salts medium (PAS) containing biphenyl as a carbon source and supplemented with 0.005% yeast extract [Bedard et al., 1984]. The bacteria were grown in 15 L and 300 L fermenters with vigorous mixing and aeration. Large-scale cultures were inoculated to an optical density (OD_{615}) of 0.1 with stored frozen H850 cells (e.g. 20 g H850 wet weight per 300 L run). Growth occurred over a 24 hour period to a final OD_{615} of 2.5. Cells were harvested by concentration with a Millipore tangential flow filtration apparatus (40 ft^2 of 0.22 micron Duropore membrane), followed by high speed centrifugation. The cell paste was transferred to plastic bags, quickly frozen as a thin film in liquid nitrogen and then stored at -80°C until needed.

To inoculate a caisson, the frozen H850 was diluted at the study site using phosphate buffer to resuspend the paste. In general the caissons were inoculated with about 0.2 kg of H850 to give an OD_{615} of 0.1 (approximately 1.0 x 10^8 cfu/ml).

PCB-degradative competence of the cells was assessed at various times during the growth phase of the culture and after harvesting. This was done by performing a resting-cell assay using a defined PCB congener mix [Bedard et al., 1986]. Typical results from one culture are shown in Table 3-2. No significant change in the PCB-degradative competence was observed before or after cell harvesting. The viability of the cells, after harvesting, was determined through plate counts on PAS/biphenyl plates.

Table 3-2

Degradation of PCB mixture 1B by *A. eutrophus* H850 during growth phase[a]

PCB Congener	Percent Degradation at: 14 h[b]	24 h	After Harvest	After Freezing
2-4	100	100	100	100
4-4	48	55	55	54
24-4	77	79	84	82
25-25	100	100	100	100
23-25	100	100	100	100
246-24 IS[c]	0	0	0	0
23-23	97	97	100	100
24-34	32	34	38	33
245-23	55	53	56	54
34-34	2	2	2	1
245-245	28	26	26	26

[a] 24 h resting-cell assays using 1.0 OD_{615} cells
[b] hours of growth
[c] IS, Internal Standard.

Sample Collection Procedures

Sediment samples from within the caissons were taken through 4 sampling ports using a 2" diameter sediment corer made by Wildco (model #2420-G55), equipped with a 12' extension handle. The sediment was collected in plastic core liners which could be capped at each end to insure the sediment core remained intact after sampling. A new liner was used for each core taken.

Prior to the initial sediment sampling (T_0) the caissons were allowed to mix for 24 hours. This was done in an attempt to eliminate the most severe gradients in PCB concentration within the caissons prior to sampling. The contents of the caissons were then allowed another 24 hours to settle before sampling. The initial sampling took two days. Cores were taken from the north and south sampling ports of all caissons on the first day of sampling and from the east and west ports during the second day. The caissons were not mixed between sampling events. Cores were taken from the inner, middle, and outer position of each port in an attempt to achieve a radial distribution of samples. A similar protocol was followed for the final caisson sampling (T_f), without the initial 24 hour premix step.

For the weekly sampling time points, the mixers were shut down the morning of the sampling day, allowing the sediment 2-4 hours to settle. One sediment core was taken from each sampling port of each caisson, usually from the middle radial position, for a total of 4 samples per caisson per time point. The order in which the caissons were sampled was always the same and established so that the H850-inoculated caissons were sampled last to minimize the potential for cross-contamination. In addition, the core sampling equipment was decontaminated and sterilized with ethanol between caissons.

After sampling, the core liners were capped and placed on ice. The mixing did suspend a large fraction of the dark, silty, organic-rich material in the sediment, particularly in the rake caissons. One gram of aluminum potassium sulfate (alum) dissolved in 10 ml of deionized water was added to each sample to initiate flocculation and enhance settling of this fraction. The

cores were then allowed to stand on ice overnight to settle. The following day the clear aqueous phase was removed from each of the cores and combined for biphenyl and chlorobenzoate analysis. The sediment heights, measured as colloidal and consolidated sediment layers, were noted and the sediment transferred into labeled glass jars with teflon-lined lids. During this process a few grams of the top 1-2" of sediment were removed from selected cores for sediment phase biphenyl and chlorobenzoate analysis.

In an attempt to improve sample uniformity, only 3-6" of the consolidated sediment layer was transferred into the sample jars. This was necessary because the coring device did not always penetrate the sediment to a uniform depth and sometimes incorporated substantial amounts of underlying, unmixed sediment into the core sample. Including this unmixed sediment layer would have diluted the sample with material of low PCB concentration and skewed the analysis. Samples which contained large consolidated sections were divided in two. In some instances, the lower portion of the core was saved for analysis. These samples were designated with an A suffix and are referenced in Table 5-7. Samples with less than 3" of consolidated material were rejected at this point and had to be retaken. Acceptable samples were placed on ice for transport to the analytical laboratory.

Three vapor traps (each trap: glass construction, one inch diameter and nine inches length) were installed in series on the gas outlet line of each caisson. 130 grams of XAD-2 (Rohm & Haas) polymeric adsorbent was used in each vapor trap to capture PCBs from the vapor phase. The XAD-2 resin was pretreated by washing successively in water, methanol, acetone, and hexane, followed by additional washes with acetone and methanol. The XAD-2 resin was then dried in a vacuum oven at 60°C overnight with a slight nitrogen purge. The treated and dried XAD-2 resin was stored in sealed glass jars until needed. Vapor traps filled with clean XAD-2 resin in the lab were exchanged every three weeks with vapor traps from the Hudson River site. The used resin was transferred to sample jars and sent to the analytical laboratory for PCB analysis.

All samples for biological analysis were obtained by subsampling sediment cores taken directly from the caissons. Aqueous phase samples were obtained by pouring water from the top of the core tubes into a sterile 50 ml sample tube. The sediment samples for biological analysis were taken by removing 3-5 grams of material from the top 1-2" of sediment remaining in the core. No alum was added to these samples. All samples were stored on ice during transfer to CRD for analysis.

Aqueous samples for chlorobenzoate analysis were obtained either by lowering a dip bucket through a caisson sample port, or by collecting the aqueous supernatant from sediment cores taken for PCB analysis. In either case, 350-400 ml aqueous samples were collected in glass jars and capped with teflon-lined lids. Sodium hydroxide (10 ml of 0.75 N NaOH) was added to each of the aqueous samples, which were then transported to CRD on ice and stored at 4°C. The combination of pH adjustment and cold storage inhibited further microbiological activity in the samples. Laboratory tests showed that the addition of alum to settle suspended particulates from the aqueous supernatant of the sediment cores did not affect the concentration of detectable chlorobenzoates in the aqueous phase (data not shown).

Sediment samples for chlorobenzoate and biphenyl analysis were obtained by removing 3-5 g of sediment from the top 1-2" of cores taken for PCB analysis or from cores taken specifically for this analysis. The samples were placed in small glass vials, transported to CRD on ice, and stored at 4°C.

Standard chain-of-custody protocols were maintained for all samples.

PCB Analysis

PCB analysis for this study was done by Northeast Analytical Laboratory, an independent, commercial environmental laboratory in Schenectady, NY. At Northeast Analytical the sediment samples were inspected and logged into their tracking system. Samples containing large amounts of liquid were centrifuged in their containers to separate the solid and liquid phases. In some cases a second addition of alum was made to

facilitate this process. The clear aqueous phase was removed, and the liquid samples were combined for each caisson and extracted with methylene chloride.

The sediment samples were partially air-dried, homogenized, weighed, and subsampled for PCB analysis. The subsampled material was completely air-dried in a low flow fume hood at room temperature. No substantial loss of lower-chlorinated PCB congeners was observed during drying (see Table C2-2). The dried sediment was then ground into a fine powder. Foreign objects and unusually large stones which would artificially skew the analysis were removed at this point. The sediment was reweighed into a tared soxhlet thimble and extracted with hexane/acetone for 16 hours.

Total sample dry weights were obtained as a means to estimate the total PCB content of each core (PCB concentration x sample dry weight = total PCB content). This was done in two ways. Samples taken at the beginning and end of the experiment were air-dried in their entirety. Intermediate time point sample wet weights were obtained and the sediment subsampled and dried to determine the moisture content. Sample dry weights were then calculated from these numbers.

XAD-2 resin samples from the vapor traps were weighed into tared soxhlet thimbles and extracted in bulk with hexane/acetone for 16 hours.

The extract cleanup procedure for all samples included the use of elemental mercury to remove sulfur, sulfuric acid to remove hydrocarbons and colored biogenic compounds, and florisil to remove polar acidic compounds from the sediment extract. This procedure eliminates compounds which interfere with PCB analysis on the gas chromatograph (GC). PCB analyses were performed on two Varian 3400 GCs, using DB-1 (J&W Scientific), bonded polydimethylsilicone, fused silica capillary columns (30 m x 0.25 mm inner diameter) and electron capture detectors. 118 PCB peaks are resolved using this column system. A mixture of Aroclors 1242 and 1260, spiked with known amounts of mono- and dichlorobiphenyls, was used as the primary calibration standard.

Several quality control checks were built into the measurement system. Method blanks, solvent blanks, matrix spikes, and sample duplicates were incorporated into each sample set run on the GCs. These protocols are defined in the project Quality Assurance/Quality Control (QA/QC) Plan submitted as part of the permitting process. A summary of all QA/QC data appears in Appendix C-2 of this report. For more details on the sample cleanup, analysis, and related QA/QC procedures please refer to Northeast Analytical's lab method NEA-PCBHRGC.

Chlorobenzoate and Biphenyl Analysis

All analyses for chlorobenzoate and biphenyl were performed at GE CRD in Schenectady, NY. Aqueous and sediment samples were analyzed for chlorobenzoates using a congener-specific protocol developed by May et al. [in preparation]. The procedures are briefly described below. A complete description of the method is provided in Appendix B-1.

Portions of each aqueous sample were centrifuged to remove suspended particulates. Aliquots of the aqueous supernatants were treated with hydrochloric acid to protonate the polar organic compounds. The polar organic fraction was then extracted into anhydrous ethyl ether containing 4-fluorobenzoic acid (4-FBA) as an internal standard. The extracts were derivatized with pentafluorobenzyl bromide [Kawahara, 1968], and analyzed for chlorobenzoates via gas chromatography-mass spectrometry (GC-MS).

Two serial extractions were performed on each sediment sample. The nonpolar organic fraction containing biphenyl and PCB was removed first, followed by an extraction of the polar organic fraction containing chlorobenzoates and other organic acids. Sediment subsamples were treated with sodium metasilicate as a dispersing agent. The sodium metasilicate also acted as a strong base to deprotonate and therefore contain the polar organic compounds in the sediment during the initial extraction of the nonpolar compounds. Hexane/acetone (90:10) containing 4-fluorobiphenyl as the internal standard was used as the extraction solvent. The extract containing the nonpolar organic fraction was removed and analyzed for biphenyl via gas

chromatography-flame ionization detection. A complete description of the separation and analysis conditions is provided in Appendix B-1.

The sediment subsamples were further treated to extract the polar organic fraction. This procedure was identical to the aqueous phase extraction described above. Hydrochloric acid was added to each subsample along with anhydrous ethyl ether containing 4-FBA as an internal standard. The ether extracts were derivatized with pentafluorobenzyl bromide and analyzed for chlorobenzoates via GC-MS.

The derivatized extracts containing the polar organic fraction were analyzed for chlorobenzoates using GC-MS. A Hewlett-Packard model 5890 Series II gas chromatograph equipped with a DB-1 column (J & W Scientific, Folsom, CA) and a fused silica precolumn (J & W Scientific) was connected to a Hewlett-Packard model 5971A quadrupole mass selective detector operated at an electron energy of 70 eV. Selective ion monitoring was used to quantify the concentrations of the mono- and dichlorobenzoate congeners in each sample extract [May et al., in preparation].

Bacterial Plate Counts

Plate counts to characterize bacterial populations were performed at GE CRD in Schenectady, NY. Within 24 hours of sampling, sediment and aqueous samples were diluted and plated on two types of agar medium using standard plating techniques [*Standard Methods*, Section 9215, 1989]. PAS plates with biphenyl crystals in the cover of the petri dish were used to estimate the concentration of bacteria capable of utilizing biphenyl as the sole source of carbon for growth (biphenyl-metabolizing bacteria). For estimation of the total number of heterotrophic bacteria, each of the diluted samples was spread onto R2A agar [*Standard Methods*, Section 9215, 1989].

Dilutions of sediment samples were made as follows: one gram of sediment was removed from each sample and placed in a 15 ml sterile,

capped polypropylene round bottom tube. Four milliliters of sterile dilution buffer (pH 7.5) was added to each gram of sediment, followed by mixing for 1 minute at high speed on a Vortex-Genie mixer (Fisher Scientific). One milliliter was removed immediately and added to 9 ml of dilution buffer to generate a 10-fold dilution. Subsequent serial dilutions were achieved by mixing the tube at high speed for 15 seconds before 1 ml aliquots were removed and added to 9 ml of sterile dilution buffer. One-tenth milliliter of each of the appropriate dilutions was spread on each of two PAS-biphenyl plates and two R2A agar plates. In most cases, serial dilutions spanning 3 orders of magnitude were plated on both types of plates. The plates were inverted and incubated at 28°C. R2A plates were incubated for 5 days and the PAS-biphenyl plates for 4 to 10 days, depending on the growth rate of the colonies observed.

The number of colonies on each of the plates was determined manually with the aid of a colony counter. Typically, plates containing between 30 and 300 colonies were used to calculate colony-forming units (cfu). In cases where the plates failed to produce a minimum of 30 colonies, the numbers less than 30 were used to approximate the colony-forming units per ml in the four milliliters of slurried sediment. Due to the relatively high number of *A. eutrophus* H850 cells added to caissons R102 and R103, plate counts of biphenyl degraders from these caissons initially reflected the H850 population. Subsequent platings were designed to measure both the number of *A. eutrophus* H850 cells and other biphenyl-metabolizing bacteria.

The water content of the sediment was determined by removing one gram of wet sediment from each of the 12 samples taken on day 40, placing them in a glass vial, and drying for 24 hours at 60° C. The water content of the sediment was calculated from the difference between the two weights.

To estimate the number of viable bacteria in aqueous samples, a 4 ml aliquot was removed from each of the 50 ml aqueous samples, serially diluted, plated , and scored according to the procedure described above for the sediment samples.

Other Analyses

Levels of ammonia nitrogen and orthophosphate in the caissons were quantified colorimetrically using Method 4500-NH_3 C for ammonia and Method 4500-P C for orthophosphate [*Standard Methods*, 1989]. These assays were done on-site and performed using test kits from Hack Company (Loveland, CO).

Standard environmental analyses were performed by Adirondack Environmental Services, Inc. in Albany, NY. Aqueous samples were measured for total phosphate-P by EPA Method 365.3, for ammonia-N by EPA Method 350.1, for nitrate-N by EPA Method 353.1, for nitrite-N by EPA Method 354.1, for sulfate-S by EPA Method 375.4, and for alkalinity as $CaCO_3$ by EPA Method 310.1. Sediment samples were measured for total Kjeldahl nitrogen by EPA Method 9060, for total phosphate-P by EPA Method 365.2, for 5-day biochemical oxygen demand by EPA Method 405.1, for chemical oxygen demand by EPA Method 410.4, for soluble total organic carbon by EPA Method 9060, and for oil and grease by EPA Method 9071.

Sediment cores taken at T_0 and T_f were dried and subsampled for solid phase total organic carbon (TOC). The analysis was done by Hudson Environmental Services Inc. of Queensbury, NY. The analysis followed procedures outlined in EPA Method SW846-9060 and the EPA Lloyd Kahn Method for total organic carbon in solid/sediment samples. All samples were analyzed in duplicate with the mean and standard deviation reported.

Toxicity testing was performed at Battelle Great Lakes Environmental Center in Traverse City, MI. The tests performed were whole sediment acute toxicity tests using *Ceriodaphnia dubia*. This species is identified as an approved toxicity indicator organism by the EPA. These tests were carried out by exposing the test species to whole sediment and monitoring the mortality of these organisms over a 48 hour period. These tests were performed in accordance with Battelle's in-house Standard Operating Procedures, which are based on procedures developed by U.S. EPA (Peltier and Weber) *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (EPA/600/4-85/013), ASTM *Guide for Conducting Sediment*

Toxicity Tests with Freshwater Invertebrates and ASTM *Guide for Collection, Storage, Characterization and Manipulation of Sediment for Toxicological Testing*. The details of the testing procedure are found in Appendix B-2.

CHAPTER 4
PRELIMINARY LABORATORY STUDIES

Aerobic biodegradation of PCBs has been observed and reported by many investigators since the early 1970's [e.g. Ahmed and Focht, 1973; Furukawa et al., 1978]. GE has studied the aerobic biodegradation of PCBs at its Corporate Research and Development Center (CRD) in Schenectady, New York for over ten years. The pathway for oxidative PCB biodegradation is generally well understood [Ahmed and Focht, 1973; Nadim et al., 1987]. A number of microbes that display a wide range of PCB-degrading capabilities have been isolated from the environment (Table 1-1). The best of these organisms can oxidatively degrade most of the congeners in the commercial Aroclor 1242, and several of the higher chlorinated congeners in Aroclor 1254 [Bedard et al., 1987]. The field study conducted by GE in 1987 on PCB-contaminated soil in South Glens Falls was the first attempt to apply this information in the field [McDermott et al., 1989]. The Hudson River Research Study represents a second effort to demonstrate that laboratory results can be reproduced in the field.

Prior to the start of the Hudson River Research Study, laboratory work was initiated to apply GE's understanding of aerobic PCB biodegradation to a specific environmental situation, the endogenous PCBs in Hudson River sediment. Efforts were made to understand those factors which contribute to optimum PCB biodegradation in sediment while modeling experimental conditions as they were anticipated to be encountered in the field. A synopsis of the most significant of these experiments and their results follow.

Shake Flask Treatability Studies

A series of treatability studies were conducted in shake flasks at CRD to assess the potential to biodegrade the PCBs found in Hudson River sediment under conditions similar to those encountered in the field. A simple factorial experimental design was employed so that several variables could be

evaluated simultaneously in each study. In all cases PCB-contaminated Hudson River sediment obtained from the H7 site was diluted with river water and supplemented with simple nitrogen and phosphorus compounds. Results from two such experiments are reported below.

The conditions and design of the first experiment are outlined in Table 4-1. The addition of bacteria (H850), carbon source (biphenyl and sodium succinate), and pH control were the variables examined for their impact on PCB biodegradation. The experiment was run for 21 days, with all flasks run in duplicate. The sediment in one set of flasks was sampled weekly for PCB analysis throughout the course of the experiment. A second set of flasks was not sampled until the end of the experiment so that the total contents of the flasks could be removed and analyzed for PCB content. Sediment was prepared for PCB analysis by removing the standing water by centrifugation, followed by air-drying and soxhlet extraction with hexane/acetone overnight. Negligible amounts of PCBs were found in the water phase after liquid-liquid extraction with hexane-ether. Urethane foam plug stoppers were used in all flasks during the experiment. These were extracted with hexane/acetone and analyzed for PCB content so that losses due to volatilization could be monitored.

Sediment extract cleanup procedures included the use of elemental mercury to remove sulfur compounds, sulfuric acid to remove hydrocarbons and colored biogenic compounds, and florisil to remove polar acidic compounds. PCB analysis was done using a Varian Model 3700 gas chromatograph equipped with a DB-1 (J&W Scientific), bonded polydimethylsilicone, 30 meter fused silica capillary column and an electron capture detector.

Dissolved oxygen and pH levels in the flasks were monitored daily with portable DO and pH meters. pH adjustments were made on a daily basis in those flasks receiving pH control whenever the pH dropped below 6. Adjustments were made by dropwise addition of a 0.5 N NaOH solution until the pH reached 7.2. Nitrogen and phosphate levels were monitored on a weekly basis using test kits from Hack Company (Loveland, Colorado). Populations of biphenyl-metabolizing bacteria were monitored weekly in all

Table 4-1
Treatability Experiment 1 - Experimental Conditions and Design

flask size - 250 ml (Bellco, baffled)
sediment mass - 30 g (wet weight)
liquid volume - 45 ml Hudson River water
temperature - 24°
shaking speed - 175 rpm
duration - 21 days

Flask	H850	BP/Na-Suc	pH control
A	-	-	-
B	-	-	+
C	-	+	-
D	-	+	+
E	+	-	-
F	+	-	+
G	+	+	-
H	+	+	+
I*	-	-	-

* Mercuric chloride killed control (0.15 g $HgCl_2$)

H850 inoculation - 2.5×10^8/ml
Ammonium sulfate (all flasks except killed control) - 0.1 g
Potassium phosphate (") - 0.05 g
Biphenyl - 0.02 g
Sodium succinate - 0.02 g
pH control via NaOH addition (daily) - 0.5 N

flasks using spread plate techniques on PAS medium agar plates with biphenyl crystals on the lids as the sole carbon source [Mondello, 1989].

Total recovery of PCBs from the whole flask extractions is shown in Figure 4-1, normalized to the average dry weight of sediment in the experimental flasks. Three T_o sediment samples were extracted to establish the starting PCB concentration. These were in good agreement and show reproducibility within 5% for the analytical method. PCB recovery in the killed control was generally within experimental variability, at 108% of the

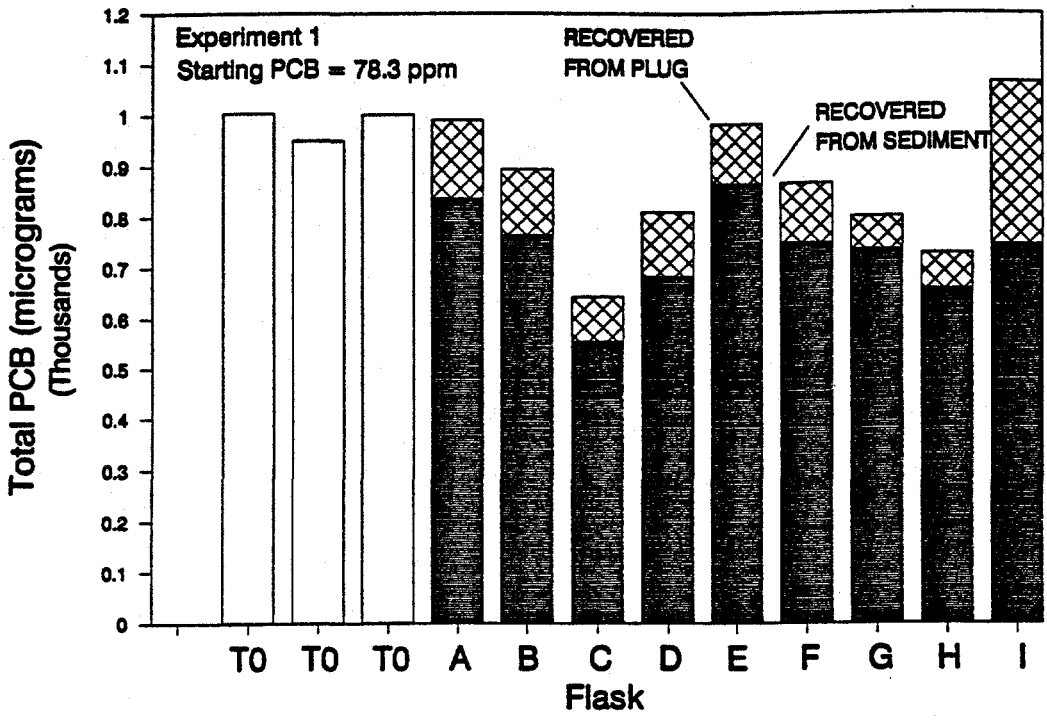


Figure 4-1. Total PCB recovery from shake flasks in experiment 1. Sediment, flasks, and foam plugs were extracted and analyzed for PCB content. PCB totals were normalized to average sediment dry weight. See Table 4-1 for experimental conditions and a description of individual shake flasks.

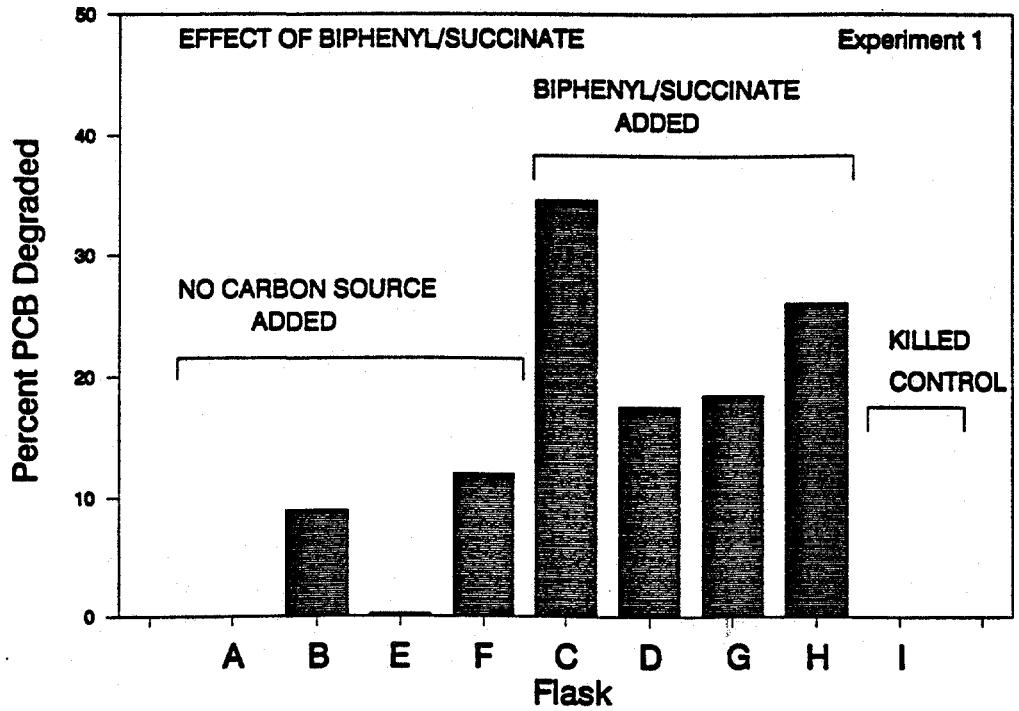


Figure 4-2. Effect of carbon addition on PCB degradation in experiment 1. The percent of PCB degraded in each flask (A through I) is shown. See Table 4-1 for experimental conditions and a description of individual shake flasks.

starting concentration. Approximately 30% of the PCBs were retrieved from the foam plug, indicating that significant volatilization occurred under sterile control conditions. Predictably, most of this loss was of the mono- and dichlorinated biphenyls (data not shown). Volatilization losses from the other flasks were somewhat lower than in the killed control, reflecting the impact of biodegradation on the PCB congeners susceptible to volatilization.

Breakdown of the data into variable grouping showed the presence of carbon source had the greatest impact on PCB biodegradation (Figure 4-2). In those flasks where biphenyl and sodium succinate had been added, percent biodegradation (as calculated by unrecovered PCB divided by starting concentration) averaged 25% versus 5% for those flasks where no carbon was added. Similar groupings of variables showed the addition of H850 to have no effect on the overall extent of PCB biodegradation, although time course analysis did indicate faster biodegradation at early time points with H850 present. pH control appeared to be beneficial when no carbon source was added, but overall its impact was inconclusive. pH control was particularly difficult to maintain in this system because of large pH drops between caustic adjustments.

The population of biphenyl-metabolizing bacteria in flasks without H850 increased from 10^2 to 10^8 cfu/ml in 14 days with biphenyl and sodium succinate present. No substantial increase in the number of biphenyl-metabolizing bacteria was seen in the absence of these substrates (data not shown). In flasks where H850 was added, the number of these organisms declined from 10^8 to 10^6 cfu/ml over the 21 days of the experiment. H850 counts were slightly elevated where biphenyl and sodium succinate were present (data not shown).

Use of biphenyl as a growth substrate is known to be important in maintaining the maximum degradative competence of PCB-degrading bacterial strains over time in the laboratory. Growth of these organisms on other substrates generally led to some loss of PCB-degradative ability [Mondello and Yates, 1989 GE Report]. The presence of biphenyl appears to induce production of the enzymes involved in the PCB-degradative pathway. The combination of biphenyl and sodium succinate was used in preliminary

PCB biodegradation studies by Paul Flathman at OHM Corporation [private communication], although the role of the latter substrate was unclear.

The experimental conditions and design of the second experiment are outlined in Table 4-2. The addition of H850, the addition of sodium succinate versus biphenyl alone, and pH control were further examined in this study for their impact on PCB biodegradation. The experiment was run in the fashion previously described, with the following exceptions: the experiment ran for 28 days; shake flask liquid and solid loading was increased and the shaker speed was decreased to reduce the amount of volatilization from the system; the level of H850 inoculation was 5-fold lower; biphenyl was added to all the experimental flasks, while only half the flasks received sodium succinate; calcium carbonate (0.5 g) was added to those flasks designated for pH control in an attempt to buffer some of the large pH fluctuations encountered in the first experiment; the aerobic heterotrophic bacterial populations were estimated using spread plate techniques with R2A agar, Difco 1826 (Difco Laboratories; Detroit, Michigan).

Total PCB recovery data is shown in Figure 4-3, again normalized to the average dry weight of sediment. Triplicate analyses for T_0 PCB concentrations were reproducible and PCB recovery in the killed control was complete. All flasks showed reduced PCB losses due to volatilization, as evidenced by reduced amounts of material found in the foam plugs. Due to the presence of biphenyl in all but the control flask, degradative losses were considerably higher than in the previous experiment, ranging from 23-37%. Only pH control had a significant effect on biodegradative loss (Figure 4-4). Flasks <u>without</u> pH control out-performed pH adjusted flasks on average by 24% to 32%. Once again there was no difference in PCB reduction between those flasks inoculated with H850 and those that received no inoculation. There also appeared to be no added benefit of sodium succinate addition over that of biphenyl alone.

The measured pH in the uncontrolled shake flasks dropped considerably over the course of the experiment, as illustrated in Figure 4-5. After an initial rapid drop in the first few days the pH in these flasks gradually declined over time, until all the flasks were near pH 5 by the 28th day. The

Table 4-2
Treatability Experiment 2 - Experimental Conditions and Design

flask size - 250 ml (Bellco, baffled)
sediment mass - 40 g (wet weight)
liquid volume - 60 ml Hudson River water
temperature - 24°C
shaking speed - 150 rpm
duration - 28 days

Flask	H850	Na-Suc	pH control
A	-	+	+
B	-	-	+
C	-	+	-
D	-	-	-
E	+	+	+
F	+	-	+
G	+	+	-
H	+	-	-
I*	-	-	-

* Mercuric chloride killed control (0.15 g $HgCl_2$)
H850 inoculation - 0.5×10^7/ml
Ammonium sulfate (all flasks except killed control) - 0.13 g
Potassium phosphate (") - 0.07 g
Biphenyl (") - 0.02 g
Sodium succinate - 0.02 g
pH control via NaOH addition (daily) - 0.5 N
with 0.5 g $CaCO_3$ added to enhance buffering capability

pH in the NaOH adjusted flasks was reasonably stable in the 6-6.5 range (data not shown).

In another experiment the rate of PCB degradation was investigated over a pH range of 4-8 using H850 as the PCB-degrading organism. H850 cells were washed and resuspended to 1.0 OD_{615} in buffer, and a defined PCB congener mix (mix 1B, 11 congeners, 1 ppm/congener) added [Bedard et al., 1986]. These resting-cell assays were quenched at various time points and analyzed for PCB-depletion. Degradation rates were determined from pH 4-7

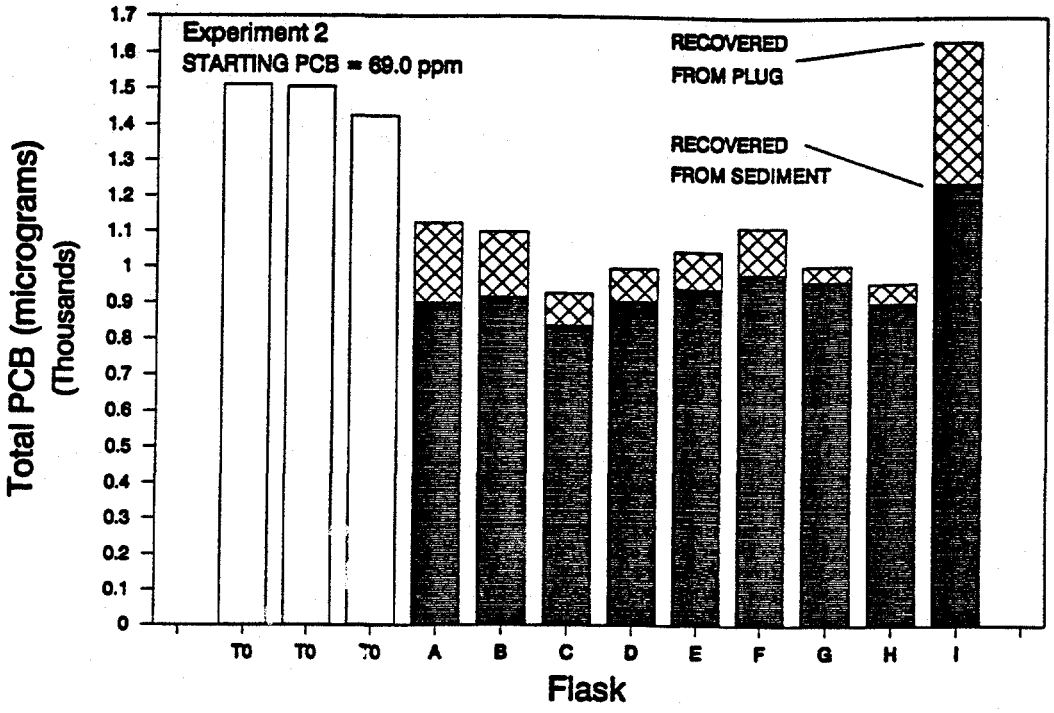


Figure 4-3. Total PCB recovery from shake flasks in experiment 2. Sediment, flasks, and foam plugs were extracted and analyzed for PCB content. PCB totals were normalized to average sediment dry weight. See Table 4-2 for experimental conditions and a description of individual shake flasks.

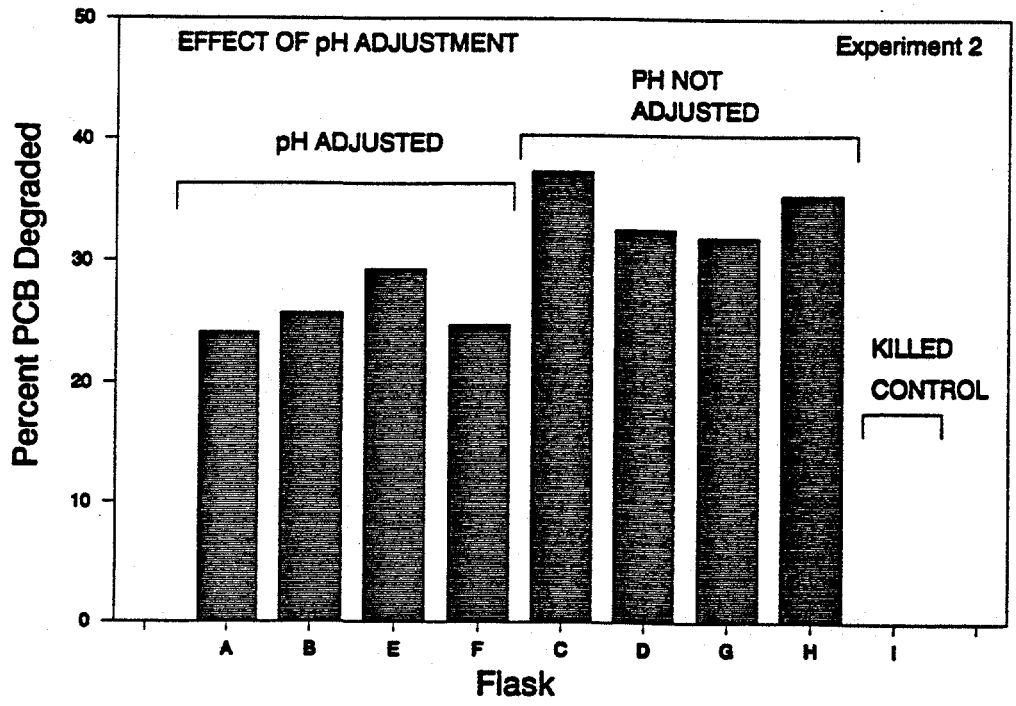


Figure 4-4. Effect of pH adjustment on PCB degradation in experiment 2. The percent of PCB degraded in each flask (A through I) is shown. See Table 4-2 for experimental conditions and a description of individual shake flasks.

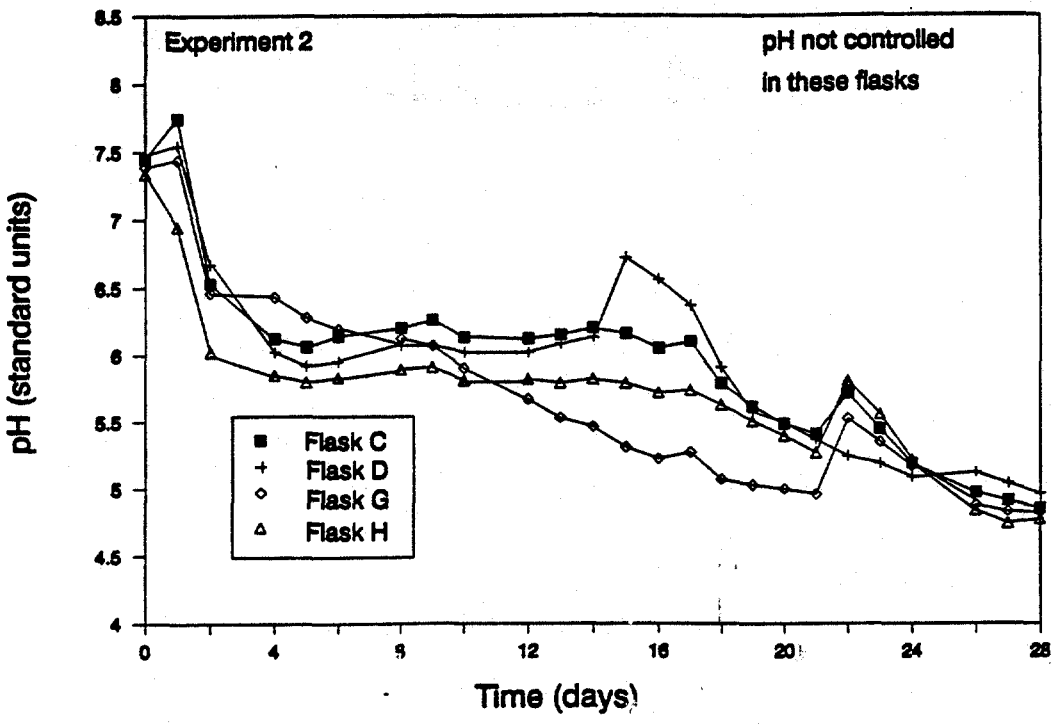


Figure 4-5. pH changes over time in shake flasks in experiment 2. pH was not adjusted in these flasks. See Table 4-2 for experimental conditions and a description of individual shake flasks.

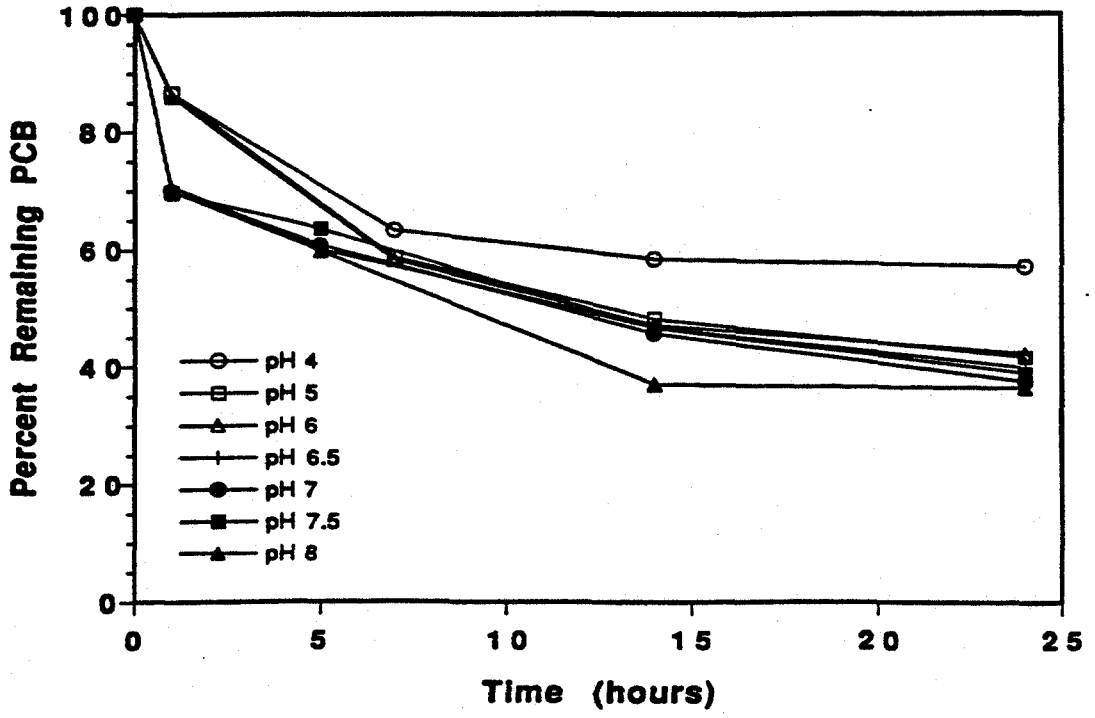


Figure 4-6. The effect of pH on rate of PCB degradation by H850. PCB degradation rates in resting cell assays at various pHs with PCB congener mix 1B are shown.

in 50 mM phosphate-citrate buffer and from pH 6-8 in 50 mM phosphate buffer. The results appear in Figure 4-6. After 8 hours, there was little difference in the rate or extent of PCB degradation in assays between pH 4-8. However, the total degradation observed after 24 hours at pH 4 was 33% less than at pH 5-8. Based upon these experiments it does not appear that pH in the range of 5-8 has a strong effect on the rate or extent of PCB biodegradation.

Growth of the background heterotrophic bacterial population is shown in Figure 4-7 for those flasks without H850 addition. Population counts in flasks where pH was not controlled rose from 10^6 to 10^9 cfu/ml in the first week of the experiment and then leveled off. Population counts climbed to a higher level at day 13 in the pH controlled flasks, before decreasing slightly over the last two weeks of the experiment. The trend was similar with H850 present (data not shown). Indigenous biphenyl-metabolizing bacterial populations were not affected in the same way (Figure 4-8). The population of these organisms increased from 10^3 to 10^8 cfu/ml in 14 days, followed by an equally rapid decline. The decline appeared to be more rapid in those flasks in which the pH was controlled (A and B). No differences between flasks were noted upon addition of H850 (data not shown). In general, H850 populations declined steadily from 10^8 to 10^7 cfu/ml over the course of the experiment.

Attempts were also made to isolate individual PCB-metabolizing bacterial cultures from the H7 sediment used in these experiments. Each enrichment culture contained 100 ml of PAS medium, 5 grams of wet H7 sediment, and a carbon source. Carbon sources including biphenyl, Aroclors 1221 and 1248, succinate, and glucose were used alone or in combination. All cultures were enriched in 250 ml foam-stoppered flasks and incubated at 30°C for 17 days. Several enrichments were subcultured under the same conditions before the different bacterial isolates were obtained.

Culture isolates were grown on biphenyl for 24 to 48 hours, then washed and resuspended in 50 mM sodium phosphate buffer (pH 7.5) to an OD_{600}. Several defined PCB congener mixtures (mix 1B, 2B, and H7) were added to each of the cultures at concentrations of 10 to 50 ppm total PCB. All cultures were incubated at 30°C on a rotary shaker incubator for 24 hours.

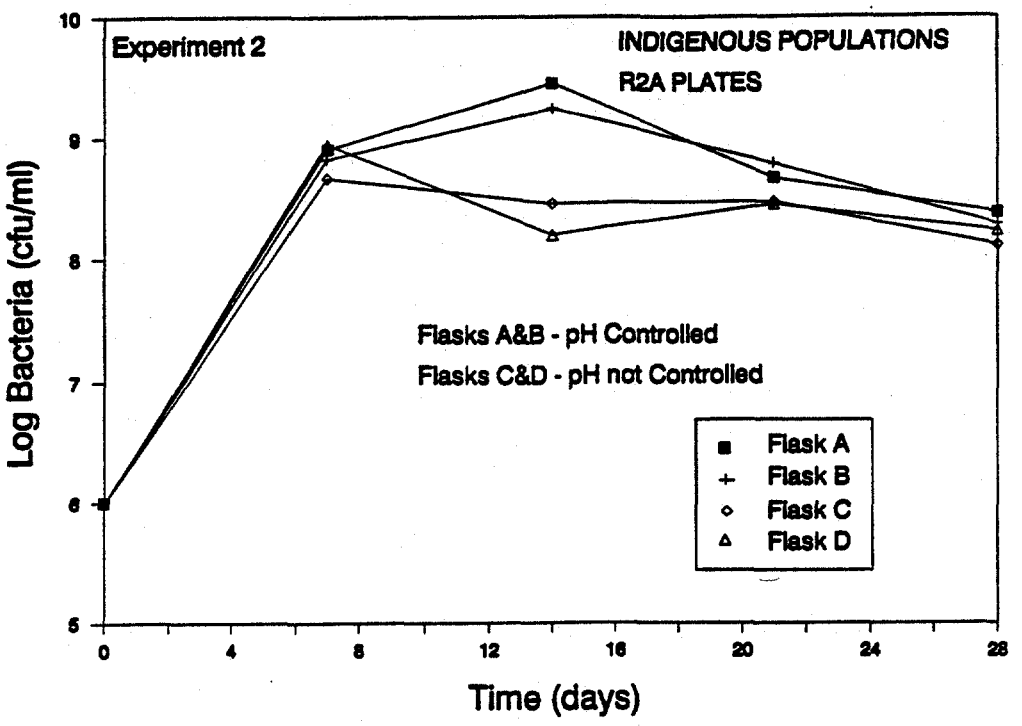


Figure 4-7. Population changes of indigenous heterotrophic bacteria over time in shake flasks in experiment 2. Cfu's shown were counted on R2A agar plates.

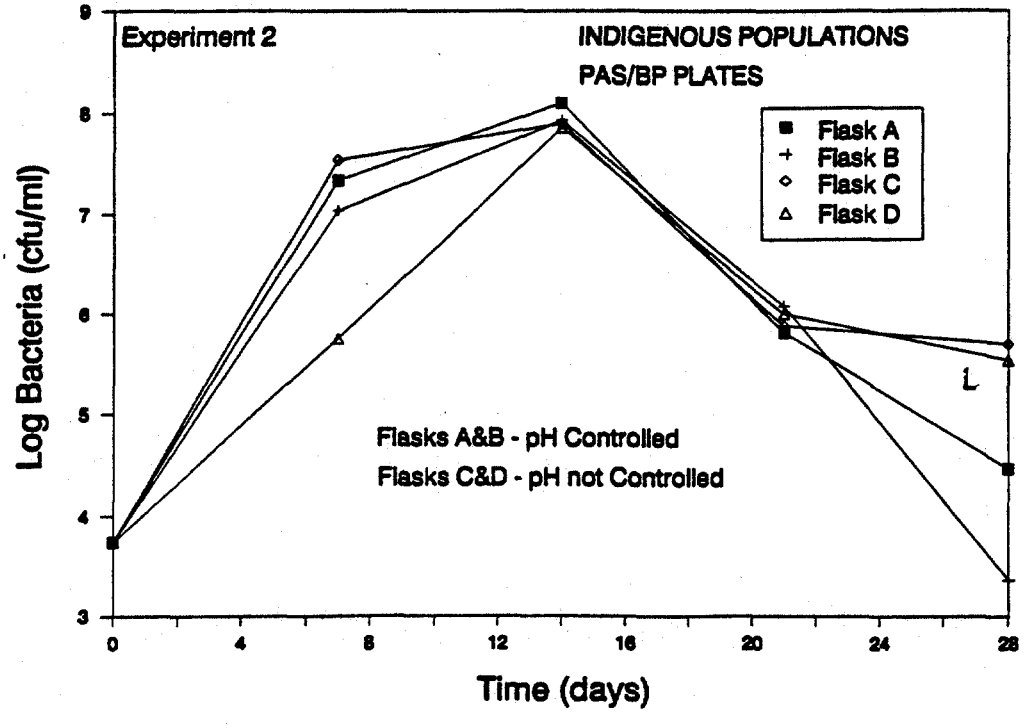


Figure 4-8. Population changes of indigenous biphenyl-metabolizing bacteria over time in shake flasks in experiment 2. Cfu's shown were counted on PAS/biphenyl plates.

The results of these resting-cell assays are shown in Table 4-3. Also included are three previously isolated cultures (LB400, H850, MB1) known to have excellent PCB-degrading ability. The isolates demonstrate a range of PCB-degradative competence. Some are very limited in the number of congeners they will attack, while others exhibit the ability to degrade a range of congeners comparable to that of the best PCB-degrading strains previously isolated. In either case it is clear there are a number of indigenous organisms present in the H7 sediment capable of producing the PCB degradation observed in the shake flask experiments.

One-Liter Reactor Studies

A series of laboratory studies which approximated the conditions selected for the Hudson River study were also performed in glass reactors (1 liter) at 20°C using live H7 sediment and river water (100 gm of sediment, solid/liquid = 1:5). These experiments are summarized in Table 4-4. The experiments were run for 24-28 days. Reactors A and B were maintained in an aerobic state (3 ppm dissolved oxygen) using hydrogen peroxide as an oxygen surrogate while the control reactors D-G were sparged with nitrogen, tightly sealed, and maintained in an anaerobic state for the duration of the experiment. This was verified by nondetectable dissolved oxygen readings on the DO probes inserted into each of the reactors. Reactor C did not have hydrogen peroxide added, but was not tightly sealed and therefore was not totally void of oxygen (i.e. low oxygen/not quantified). All reactors were stirred with a simple, single-bladed glass impeller. Reactors C-G were stirred at 5 rpm. Reactors A and B were stirred at 100 rpm to assure immediate decomposition of the peroxide to oxygen. Urethane foam plugs were present in reactors A and B to capture PCBs removed via volatilization.

Where indicated in Table 4-4, 1.0 gram of biphenyl and/or 0.87 grams of diammonium phosphate were added initially to the 100 grams of sediment in some of the reactors. Reactor B received only 0.1 grams of biphenyl and 0.087 grams of phosphate initially, followed by five more additions when reduced DO cycling suggested reduced microbial activity due to biphenyl limitations.

Note that some reactors (E and F) received only one of the nutrients and one reactor (D) did not receive any nutrient amendment.

About 60% of the PCBs were biodegraded in the aerobic reactors (A and B), while the anaerobic reactors (D through G) lost less than 5% (the error of PCB analysis alone is about 5%). The individual PCB congener distribution, before and after biodegradation, for the peroxide reactor A and the control reactor D are given in Figures 4-9A and 4-9B. The change in the initial 3 peaks (i.e. 2- , 4- and 2-2-/26-chlorobiphenyls) constitute most of the PCB removal in the peroxide reactor. This was expected since these congeners are the most susceptible to aerobic attack. No significant congener-specific losses were observed in any of the anaerobic controls. The amount of PCBs captured in foam plugs in the peroxide reactors during the run was below 0.1% of the PCB initially measured (Table 4-4), indicating that PCB volatilization was not significant when hydrogen peroxide was used as the oxygen source. PCB loss in reactor C was 40%, a value between that representative of peroxide-fed and anaerobic reactors. This suggests that substantial PCB biodegradation can occur under conditions with only low levels of oxygen present (i.e. as in reactor C). Such a case was also observed in the actual field study in control caisson R101.

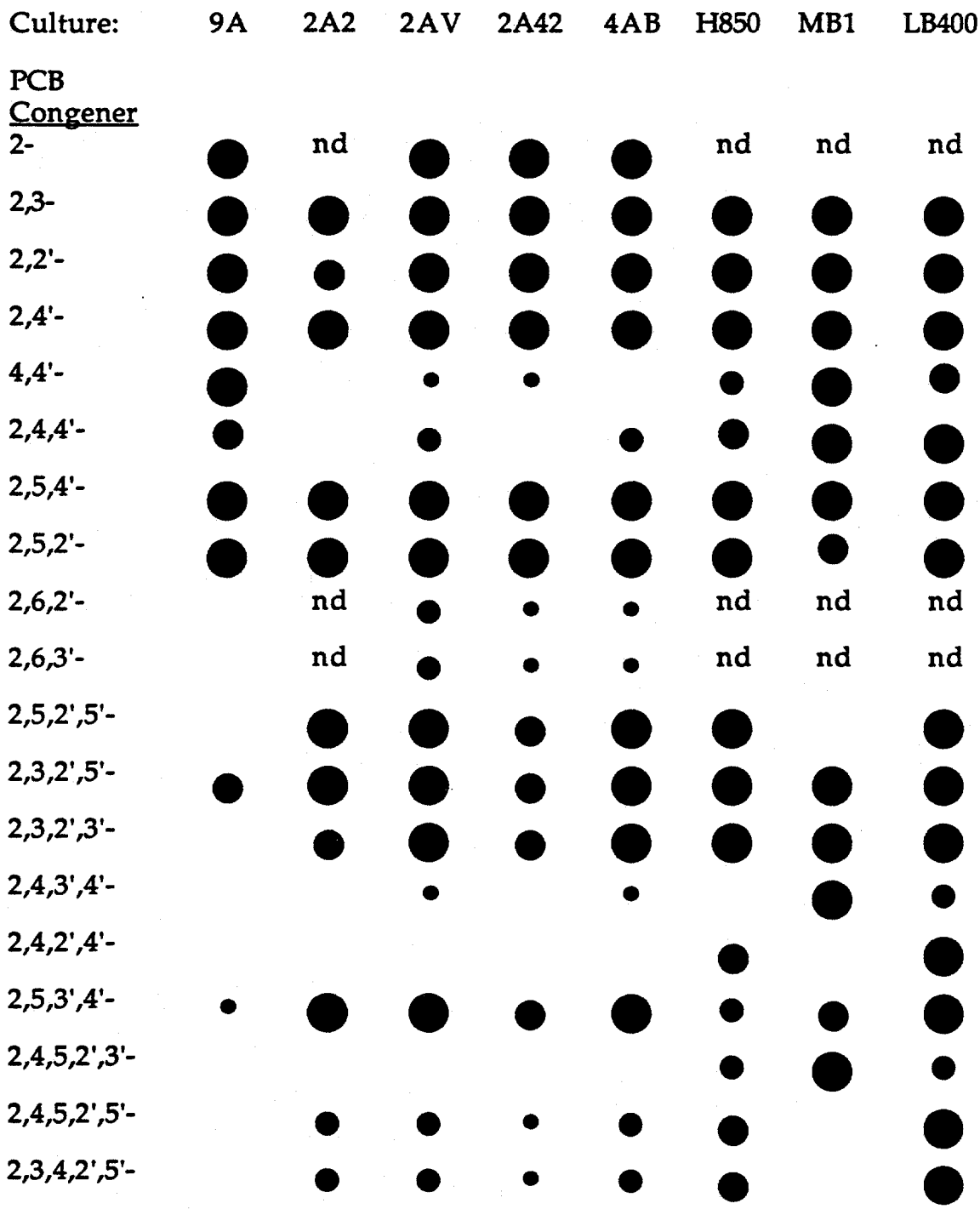
Adding carbon/nutrients in small periodic additions rather than in one large amount may be beneficial, as indicated by the slightly higher PCB loss of 63% for reactor B. Congener-specific PCB removal and the percentage of each PCB congener peak remaining after the aerobic treatment of reactor B are given in Figures 4-10A and 4-10B, respectively. In general, a greater percentage of the more lightly chlorinated congeners are degraded, presumably a function of their greater bioavailability and their greater susceptibility to enzymatic attack. There was evidence of a biocatalytic specificity in the congeners attacked, as evidenced by the recalcitrance to biodegradation of certain congeners, notably 26-2- and 23-2-/26-4-chlorobiphenyl (peaks 6 and 10). Such congener specificity is commonly observed in PCB biodegradation experiments in resting-cell assays, but is not observed where more general abiotic processes are involved.

Table 4-3

PCB Degradative Competence of Bacterial Cultures Isolated from Laboratory Enrichments of Hudson River H7-Site Sediment.

Culture:	1C	1R	2L	2ZS	4Z	4S1	7Z	8A	8M
PCB Congener									
2-	nd	nd	nd	nd	nd	nd	nd	nd	nd
2,3-	●	●	●	●	●	●	●	●	●
2,2'-		●					●		
2,4'-	●	●	●	●	●	●	●	●	●
4,4'-	●	●	●	●	●	●	●	●	●
2,4,4'-	●	●	●	●	●	●	●	●	●
2,5,4'-		●	●	●	●	●		●	●
2,5,2'-									
2,6,2'-	nd	nd	nd	nd	nd	nd	nd	nd	nd
2,6,3'-	nd	nd	nd	nd	nd	nd	nd	nd	nd
2,5,2',5'-									
2,3,2',5'-		●							
2,3,2',3'-	●		●		●		●		
2,4,3',4'-		●						●	●
2,4,2',4'-									
2,5,3',4'-									
2,4,5,2',3'-									
2,4,5,2',5'-									
2,3,4,2',5'-									

Table 4-3 (cont.)



PERCENT DEGRADATION: KEY:

● = 80 - 100 % ● = 60 - 79 % ● = 40 - 59 %
● = 20 - 39 % = < 20 % nd = not determined

Reactor	A Peroxide Amm/Phos Biphenyl- 1 addt'n 24 days	B Peroxide Amm/Phos Biphenyl- 6 addt'n 24 days	C Low O2 Amm/Phos Biphenyl- 1 addt'n 24 days	D No O2 28 days	E No O2 Biphenyl- 1 addt'n 28 days	F No O2 Amm/Phos 28 days	G No O2 Amm/Phos Biphenyl- 1 addt'n 28 days
To (ppm)	106	109	112	106	100	104	106
Tf (ppm)	46	40	67	107	96	100	105
% Loss	57	63	40	0	4	4	1
% Captured	<0.1	<0.1	-	-	-	-	-

Table 4-4. Laboratory results for peroxide (high oxygen level) and anaerobic bioreactors.

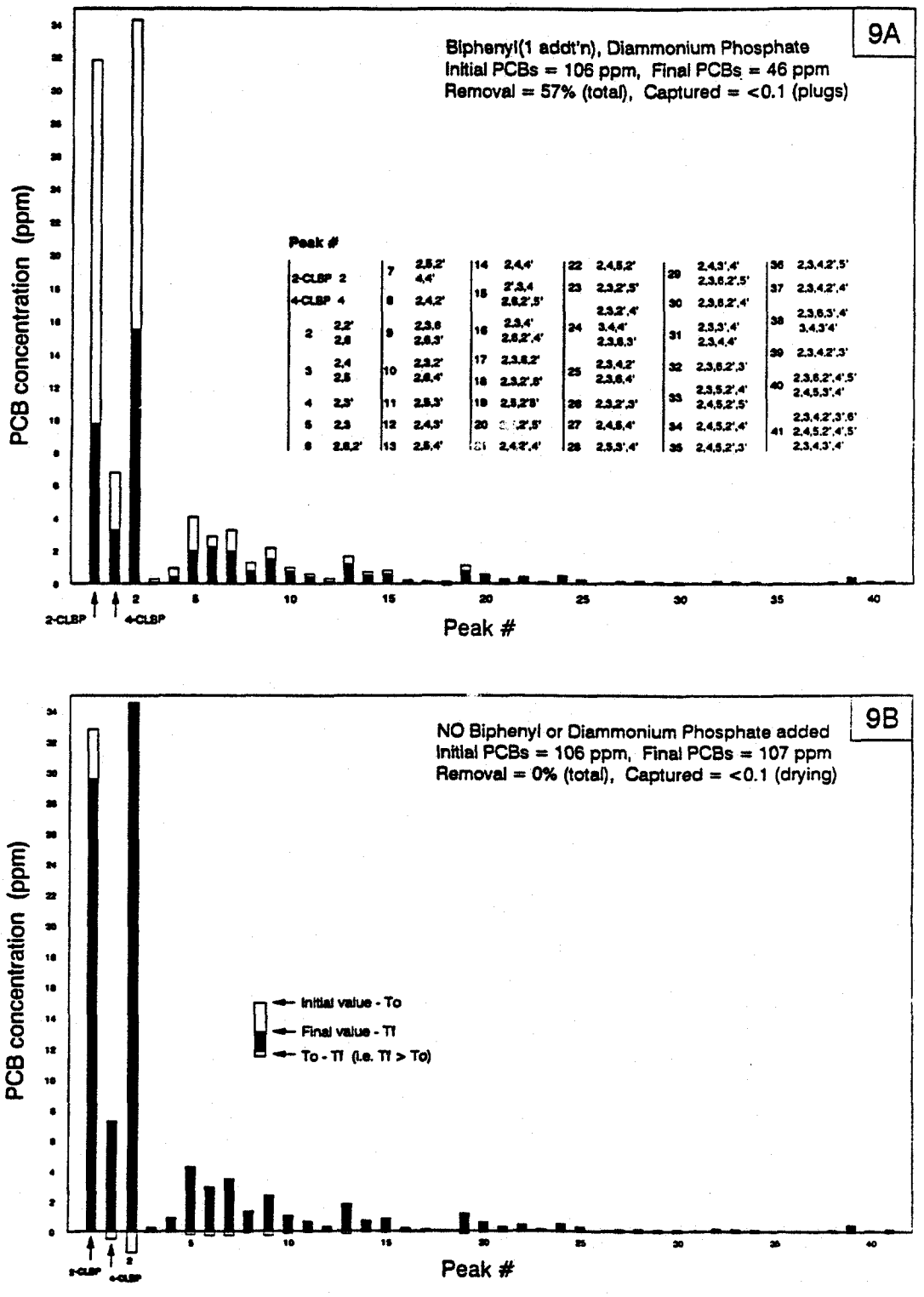


Figure 4-9 PCB recovery in one liter reactor biodegradation studies with H7 sediment and hydrogen peroxide as oxygen source. The initial and final PCB concentrations are displayed in the histograms by congener peak for (A) reactor A (hydrogen peroxide, diammonium phosphate, single addition of biphenyl) and (B) reactor D (N_2 sparged, tightly sealed anaerobic control).

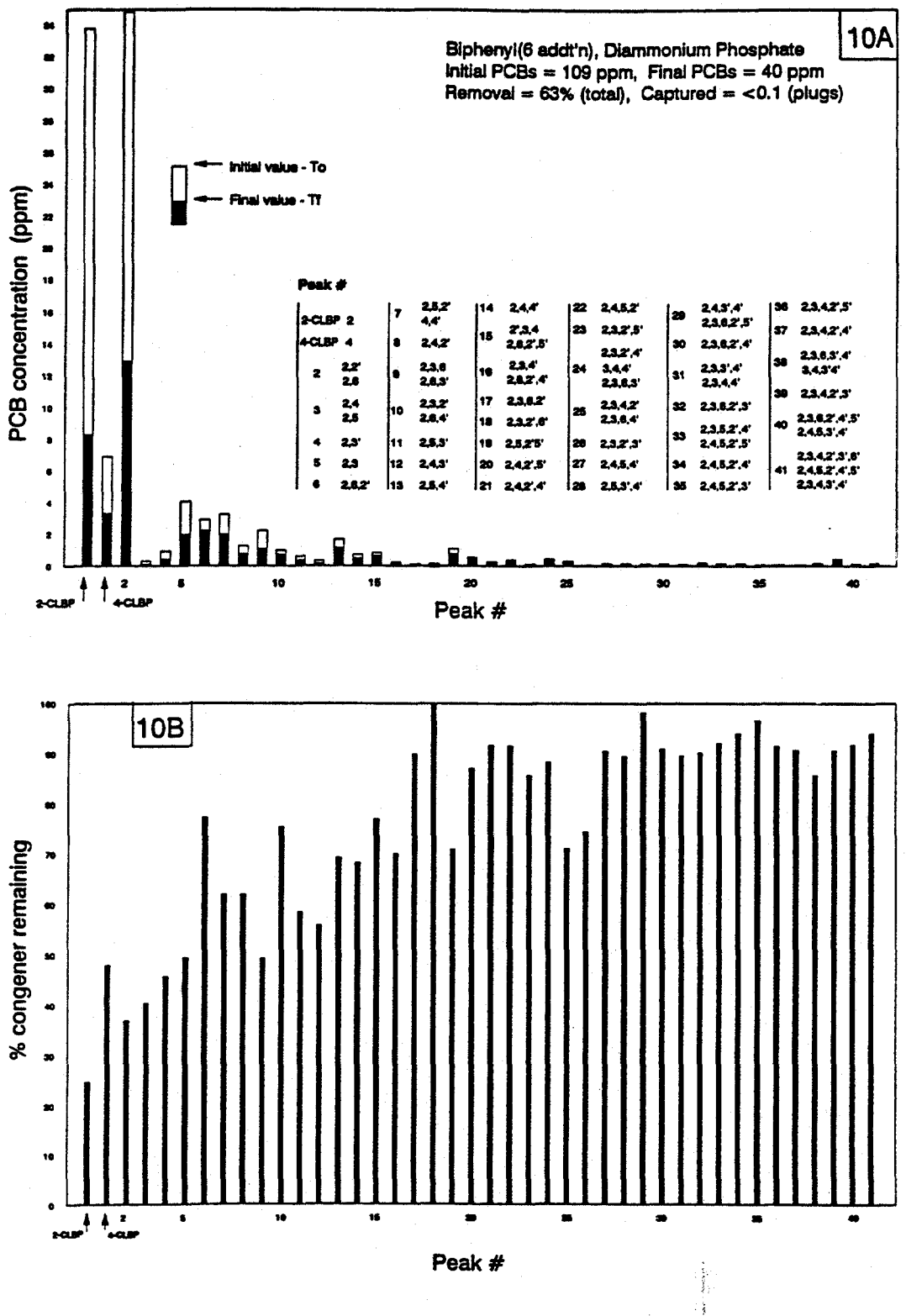


Figure 4-10 PCB recovery after biodegradation in reactor B (hydrogen peroxide, diammonium phosphate, multiple additions of biphenyl). (A) The initial and final PCB concentrations are displayed in the histogram by congener peak and (B) the percent of each congener peak remaining after biodegradation.

A second series of experiments was performed to assess how quickly biodegradation can occur in stirred reactors under optimal conditions. H850 (5×10^8 cfu/ml) and nutrients (ammonium sulfate, 0.65 g; potassium phosphate, 0.35 g) were added to 100 grams of H7 sediment in a 1 liter reactor (1:5 sediment/river water). The reactor was stirred at 80-100 rpm and open to the atmosphere through a foam plug. No peroxide was added. The experiment was stopped and the results analyzed for PCB losses after one week. The results are shown in Figure 4-11A. 50-60% biodegradation of PCBs was observed in this time frame.

A second reactor was set up with nutrients and biphenyl present (ammonia and phosphate at the levels above; biphenyl initially at 1.0 gram), but without an addition of H850. A second addition of biphenyl was made after one week. This experiment was assayed for PCB-biodegradative losses after one and two weeks (Figure 4-11B). After one week, degradative activity had begun (16% of the PCBs were degraded), but by the end of the second week PCB losses were 51%, and congener-specific losses were comparable to those seen in reactors with added H850. With appropriate nutrient addition the indigenous organisms required only two weeks to perform as well as H850 added at 0.5 OD. Control reactors were also run for two weeks without the addition of nutrients of any kind. One of the two received mercuric chloride in an attempt to track abiotic losses. The PCB losses from these two reactors were both less than 4%.

It should be noted that all these laboratory experiments were run at or near room temperature. In order to assess the impact of temperature on the PCB degradation rate, resting-cell assays were performed with H850 cells (1.0 OD_{615}) in 50 mM potassium phosphate buffer (pH 7.5) containing a PCB mix of 8 congeners (100 ppm total), which constitute 80 mole % of the PCB in the sediment. The assays were incubated at 10^o, 20^o, or 25^o C, quenched at various times, and analyzed for total PCB loss. The results appear in Figure 4-12. As expected, temperature has a significant influence on the the initial rate of PCB degradation. However, PCB degradation is evident at the lower temperatures and continues at a slower rate over the time course of the experiment. Thus, when temperatures fall significantly below 20^oC in the

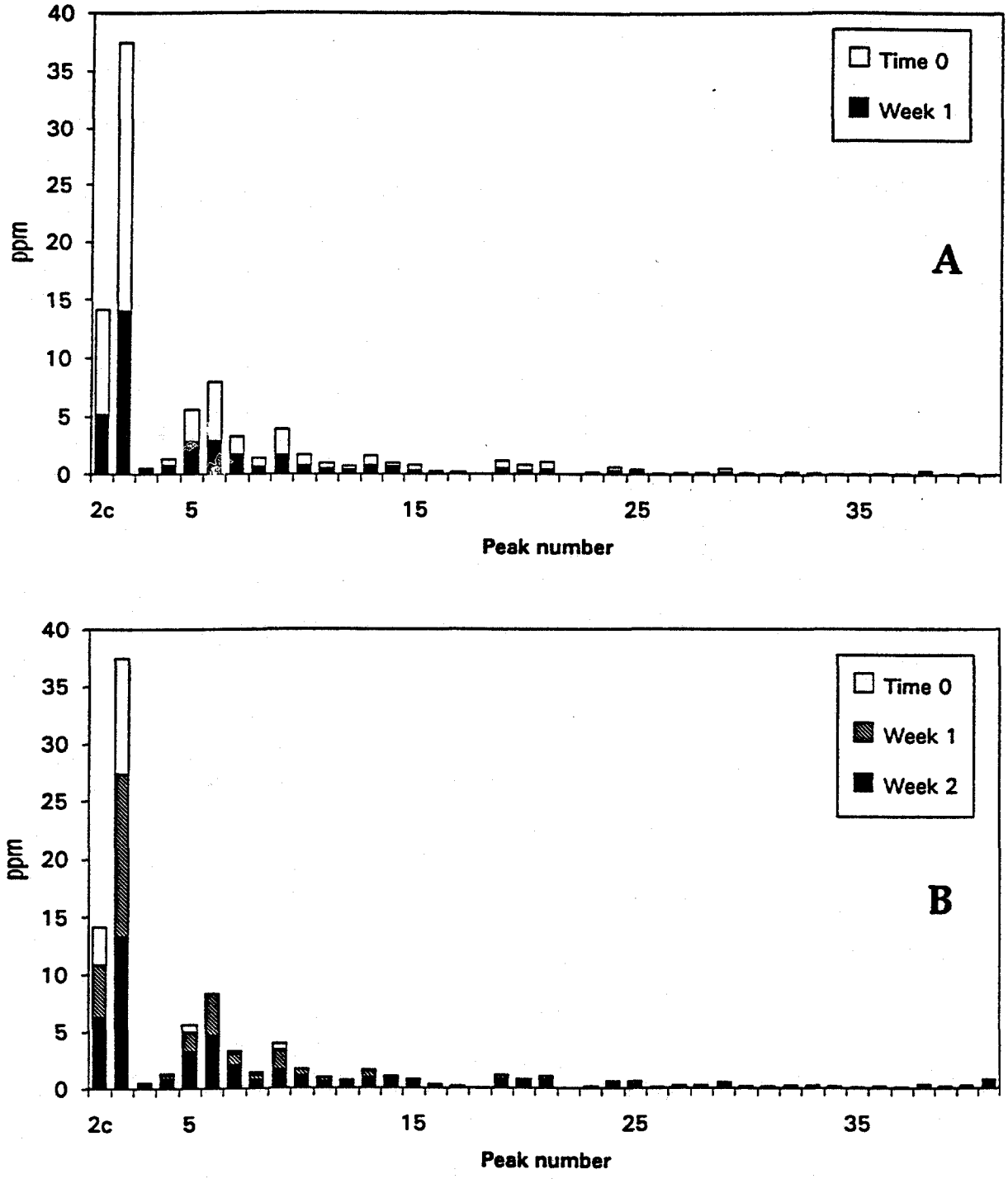


Figure 4-11 PCB recovery in one liter reactor biodegradation studies with H7 sediment : effect of *A. eutrophus* H850 on PCB degradation rate. The initial and final PCB concentrations are displayed in the histograms by congener peak. Aerobic PCB biodegradation is compared (A) with *A. eutrophus* H850 added at 0.5 OD_{615} and (B) with stimulation (diammonium phosphate, biphenyl added) of the indigenous population of PCB-degraders without H850.

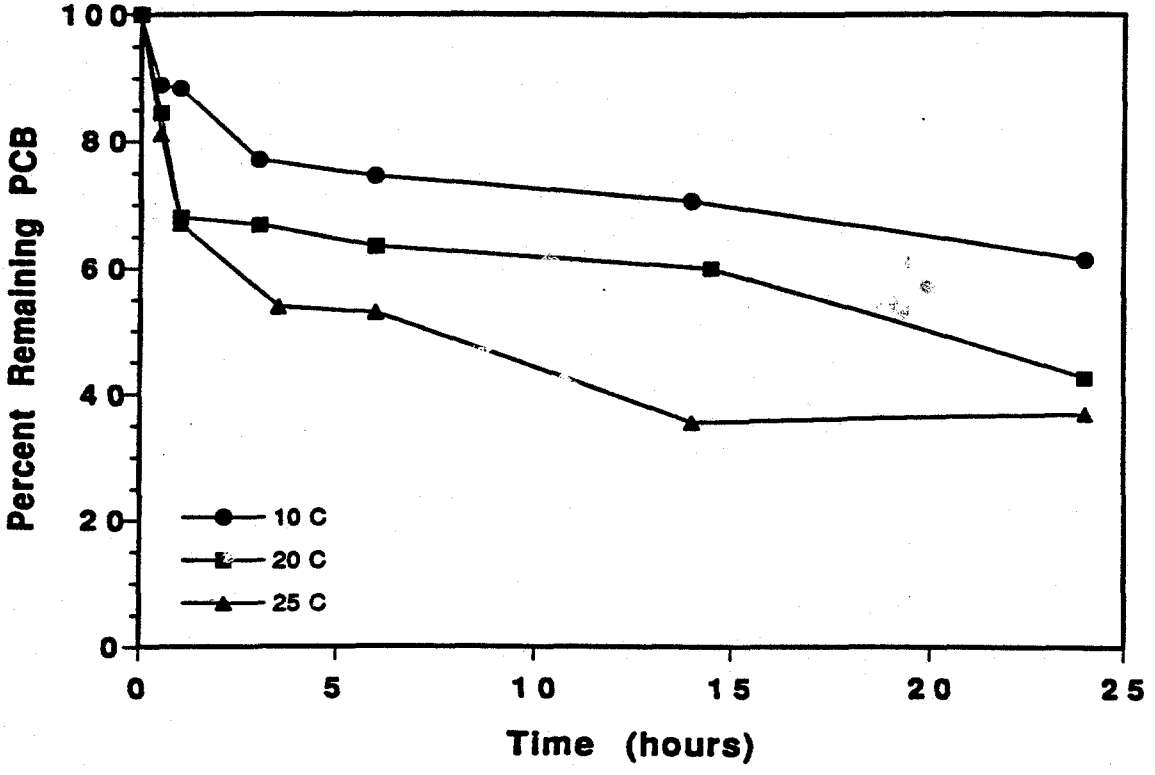


Figure 4-12. Effect of temperature on the rate of PCB degradation by *A. eutrophus* H850. PCB degradation rates in resting cell assays at varying temperatures with PCB congener mix H7 are shown.

field the biodegradation of the PCBs is expected to take longer than in the laboratory.

Bioavailability Measurements

PCB biodegradation did not exceed 50-60% of the starting concentration in any of the laboratory studies dealing with aged, endogenous PCBs in H7 sediments. However, an examination of the PCB congener distribution in this sediment indicates that significantly higher percentages (>90%) of this material ought to be biodegradable, based upon past laboratory experience with these same congeners in the absence of sediment.

It is hypothesized that the primary limitation affecting the rate and extent of PCB biodegradation in H7 sediment is not biochemical, but physical. Desorption of many nonionic organic compounds from sediments displays bimodal kinetics: a "labile fraction" of the organic compound desorbs readily, while a "resistant fraction" desorbs orders of magnitude slower [Karickhoff and Morris, 1985]. Similar desorption kinetics have been studied with both PCB-spiked and environmentally-contaminated sediments [Coates and Elzerman, 1986; Dunnivant, 1988], using a purge and trap technique to monitor contaminant desorption [Mackay et al., 1979]. Recently, a desorption technique using Tenax GC resin as a sink for the desorbed chemical was described [Pignatello, 1990].

The desorption kinetics of PCBs from environmentally-contaminated Hudson River sediment were examined under various conditions using XAD-4 (polystyrene bead resin) as a PCB "sink". The labile fraction measured in these experiments presumably determines the available fraction of PCB for immediate biodegradation.

PCB-contaminated sediment was obtained as a slurry from the Hudson River H7 site. The sediment was air-dried and stored in a glass jar until needed. Sea sand (Aldrich) was washed with water, acetone and then hexane. The sand was spiked with Aroclor 1242 from a dilute hexane solution by careful drying with a rotary evaporator and then stored in a glass jar. XAD-4

resin (Rohm and Haas) was cleaned by washing successively with water, methanol, acetone/hexane, methanol and water. The wet resin (50% water) was stored in a polyethylene jar to inhibit dehydration.

One gram of PCB-contaminated sediment or PCB-spiked sand was contacted with one gram of XAD-4 resin and 5 ml of water in 2 dram glass vials sealed with Teflon-lined caps. Mixing was achieved by placing the vials on a reciprocating shaker during the experiment. Time points were obtained by sacrificing whole vials. The sediment/sand and the XAD-4 phases were separated by salt addition and centrifugation, and three successive extractions with ethyl acetate were used to remove the PCBs from the solid phases. The combined extracts were treated with mercury to remove sulfides and analyzed by GC. PCB mass balances were +/- 10%.

It is well established that non-ionic organic contaminants partition primarily to the polymeric matrix of the sediment [Karickhoff, 1980]. In order to separate the effects of the polymeric organic phases of the sediment on PCB desorption from that of the inorganic phases (largely silica), the desorption of PCBs from spiked sea sand (inorganic phase, no polymeric phase) was compared with that of Hudson River sediment (a mixture of polymeric and inorganic phases).

The result of 170 hours of PCB desorption from H7 sediment and sea sand is shown in Figure 4-13. The PCB levels on the y-axis are normalized to the starting PCB levels of the sand and sediment before desorption (13 ppm and 25 ppm, respectively). PCBs from spiked sea sand desorbed readily (85% desorption in 8 hours). In contrast, only half of the PCBs from the H7 sediment desorbed within 24 hours (labile fraction), while the remaining half did not desorb over the 170 hour time period (resistant fraction). It is likely that the resistant fraction of PCB is not bioavailable in its sorbed state and therefore would not be biodegradable in the time period of the experiment.

It is believed that this resistant fraction is made up of PCBs which are dissolved in the polymeric matrix of the sediment and therefore must diffuse through the polymer before desorption to a "sink" [Pignatello, 1990]. Long desorption times are expected, as diffusion coefficients of PCBs in polymers

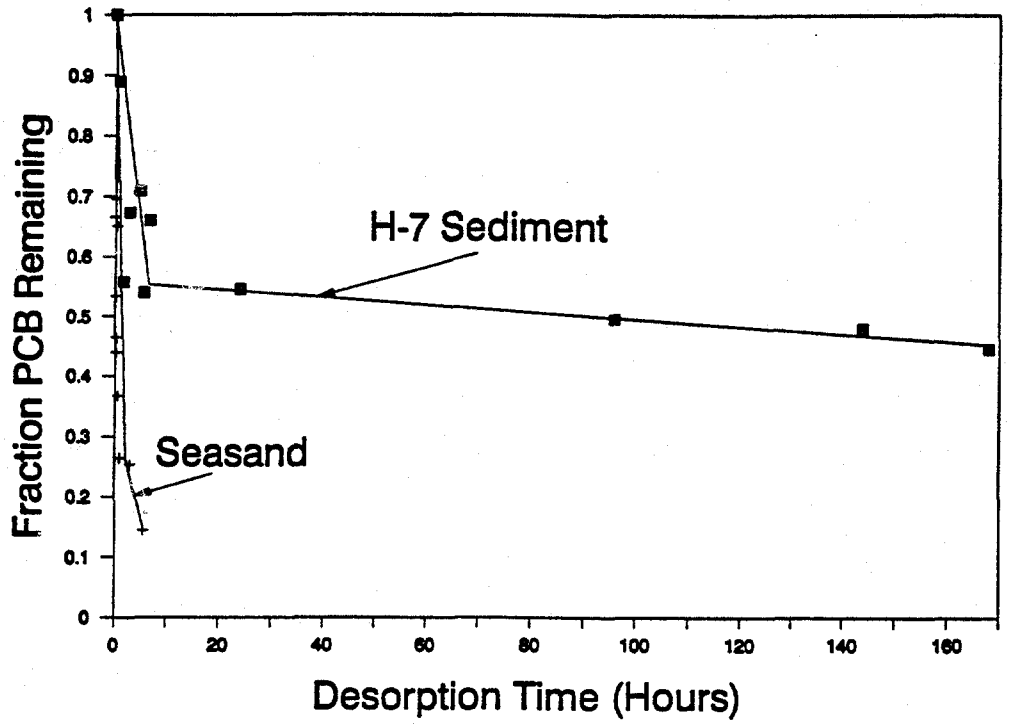


Figure 4-13 Desorption of PCBs from H7 sediment versus spiked sea sand.

are very small. For example, the diffusion coefficient of 4-chlorobiphenyl in polystyrene is 10^{-17} cm^2/sec [Van Hoof and Andren, 1990], while the diffusion coefficient of PCB in water is of the order of 10^{-6} cm^2/sec.

In the previous experiment, half of the PCB made up a resistant fraction which essentially did not desorb over a period of a week. Therefore, the experiment was continued for six months to see the effect of longer desorption times. The results of this experiment are shown in Figure 4-14. Over a period of six months, some of the PCB making up the resistant fraction slowly desorbed from the polymeric matrix of the sediment with uptake by the XAD-4 resin. A half life of 4 months was found for this PCB fraction. Therefore, given enough time, PCBs in the resistant fraction should become available for biodegradation.

The previous experiments describe desorption studies with sediments having relatively low levels of PCB contamination (25 ppm). To assess the impact of PCB loading in the H7 sediment on desorption kinetics and the amount of the resistant fraction, standard one-week desorption experiments were performed with H7 sediment containing 205 ppm of endogenous PCBs. The results of this analysis appear in Figure 4-15. In this case the resistant fraction for this more highly contaminated sediment was only 25%, instead of 50% as was found with sediment containing 25 ppm PCB. It therefore appears that in Hudson River sediments with higher PCB concentrations, a greater percentage of the PCBs are available for biodegradation.

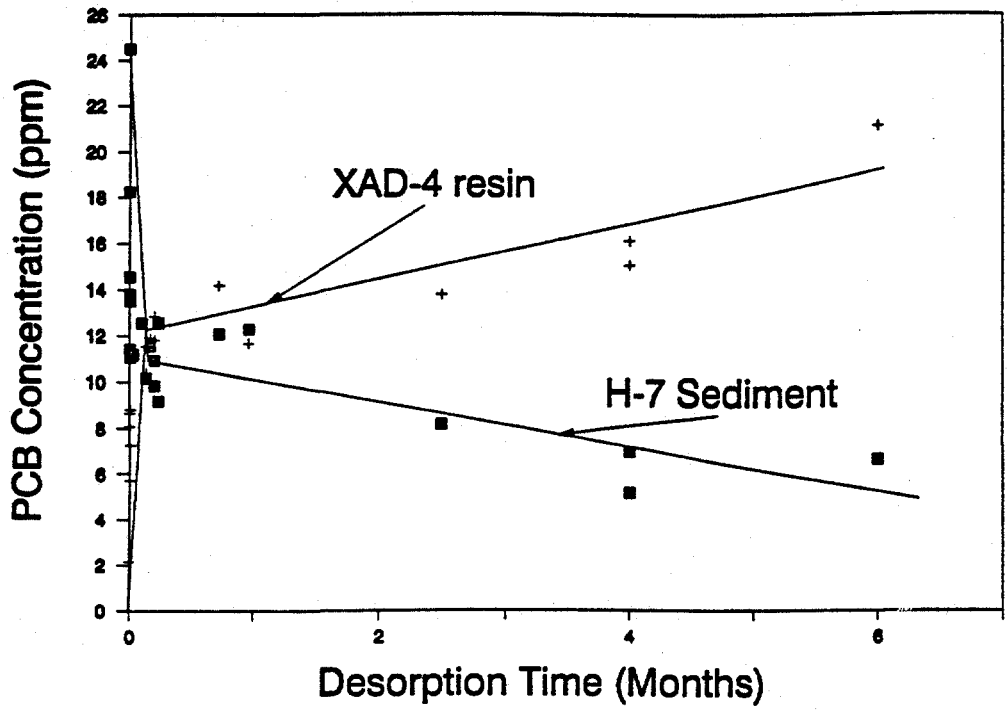


Figure 4-14 The effect of long contact times on PCB desorption from H7 sediment.

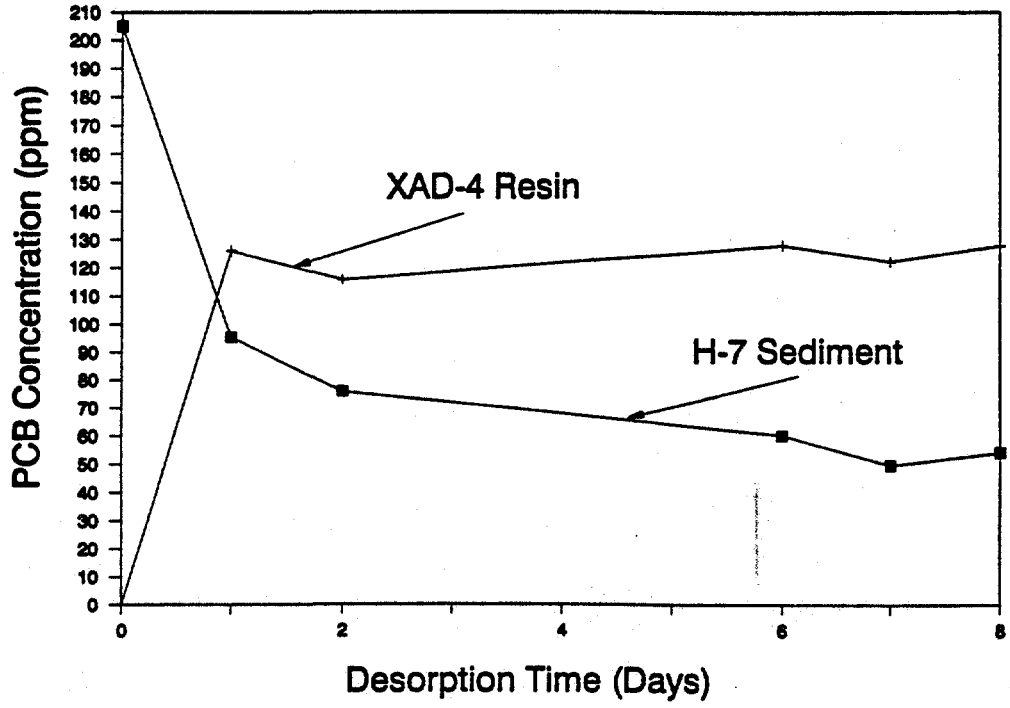


Figure 4-15 The effect of PCB loading on PCB desorption from H7 sediment.

CHAPTER 5
RIVER STUDY RESULTS

The Hudson River Research Study began on August 9, 1991 with the addition of nutrients and bacterial strain H850 to the experimental caissons. Prior to that time the caissons had been mixed for 24 hours, then shut down to allow the sediment to resettle. All initial samples were taken on August 6 and 7. The study ran for 10.5 weeks, until October 21, and the final sampling took place on October 22-24. Caisson R103 was re-dosed with strain H850 and restarted on October 24 for an additional 3 days in a subsequent short experiment.

General Environmental Analyses

The results of the general environmental analyses on aqueous and sediment samples taken from the caissons at the beginning and end of the experiment appear in Table 5-1. The initial sampling was done after the 24 hour pre-mix period. The only difference between caissons at that time was mixing mode. Therefore only two rake and one turbine caisson were sampled for some analyses. Based upon these analyses, some biological activity was detected in the turbine caissons during the premix period, as indicated by decreased ammonia and phosphate levels and elevated nitrate levels in the aqueous phase of R101. This was not unreasonable since these caissons were aerobic during that period due to the introduction of oxygen into the water column from the headspace at the high rate of mixing. It is difficult to make any observations based upon the sediment analyses, since these appear to be inherently more variable. The 5-day sediment biological oxygen demand (BOD_5) was measured as 400-500 mg/kg on average.

Analysis of the samples taken after 10-1/2 weeks (T_f) reflected the impact of both nutrient addition and enhanced biological activity in the experimental caissons. Increased levels of sulfate and ammonia-N in the aqueous phase of the experimental caissons was a direct result of nutrient

Table 5-1
General Environmental Analyses - Initial Sampling

	R101	R102	R103	R104	R105	R106
Water Samples (mg/l)						
Sulfate - S	63	-	-	-	15	12
Phosphate - P	0.02	-	-	-	2.1	4.5
Ammonia - N	0.9	-	-	-	2.2	3.3
Nitrate - N	0.59	-	-	-	0.06	0.06
Nitrite - N	0.02	-	-	-	<0.02	<0.02
Alkalinity (as $CaCO_3$)	26	-	-	-	85	80
Sediment Samples (µg/g)						
Total Kjeldahl N	519	-	1480	1080	-	-
Phosphate, Tot - P	353	-	771	573	-	-
TOC	335	173	254	746	667	308
COD	10,200	2,290	11,000	51,000	13,000	17,700
BOD_5	335	111	335	541	835	478
Oil & Grease	<1	<1	<1	<1	<1	<1

Final Sampling

	R101	R102	R103	R104	R105	R106
Water Samples (mg/l)						
Sulfate - S	82	468	950	580	925	585
Phosphate - P	<0.02	0.03	1.7	0.37	0.06	0.78
Ammonia - N	<0.1	117	158	0.4	268	257
Nitrate - N	0.06	63	5.9	0.65	4.6	3.0
Nitrite - N	<0.02	9.2	6.6	0.17	1.1	2.5
Alkalinity (as $CaCO_3$)	28	33	230	140	260	360
Sediment Samples (µg/g)						
Total Kjeldahl N	350	630	331	518	392	280
Phosphate, Tot - P	26	335	219	209	53	222
TOC	530	845	900	690	1040	930
COD	11,000	16,000	9,400	13,000	9,650	7,330
BOD_5	119	412	83	208	142	76
Oil & Grease	<1	<1	<1	<1	<1	<1

addition, although the elevated sulfate level in R104 is anomalous. The increase in nitrate and nitrite in these caissons resulted from the activity of aerobic nitrifying bacteria. This was especially pronounced in R102. Available phosphate levels remained low in all the caissons, despite repeated phosphate amendments to the experimental reactors. An increase in alkalinity was evident in the low-mix caissons.

Sediment analyses indicated general declines in total Kjeldahl nitrogen, total phosphate, and BOD_5 in both experimental and control caissons. Total soluble organic carbon increased slightly in the experimental caissons, while the chemical oxygen demands were unchanged. Oil and grease levels remained below detectable limits.

The absence of phosphate in the final analyses of the experimental caissons is somewhat unexpected, given the fact that a significant quantity (0.6 kg P/caisson) was added over the course of the study. It is probable that only a small percentage of this material was incorporated into biomass. The majority was probably absorbed by clays or by the Fe or Al oxides/hydroxides in the sediment [Bolt and Bruggenwert, 1978]. In either case the phosphate is expected to remain in a colloidal form and would not be measured in the aqueous (because the colloidal fraction was filtered out) or sediment analyses (because the colloidal fraction did not settle readily).

There were elevated ammonia levels in the aqueous phase of the experimental caissons. These levels were primarily the result of over-estimating the nitrogen requirements necessary to maintain the microbial populations in the caissons. This situation could be avoided in the future by the addition of less nitrogen. A well-balanced, slow-release source of nitrogen and phosphate would be particularly beneficial.

No whole-sediment acute toxicity was evident in any of the sediment samples taken from the caissons before or after the study. Control non-PCB-contaminated river sediments were also shown to exhibit no toxicity. This indicated that the aerobic biodegradation of PCBs and the addition of nutrients used in this study posed no acute toxicity hazard to a sensitive test organism, *Ceriodaphnia dubia*.

Operational Data

The addition schedule for the study is presented in Table 5-2. Initial additions of 1.0 kg diammonium phosphate, 2.0 kg ammonium sulfate, and 1.0 kg biphenyl were made to all experimental caissons. In addition, R102 and R103 were inoculated with 0.19 kg of strain H850. Subsequent additions of diammonium phosphate were made at the end of week 2 and week 3 of the experiment in response to diminished phosphate levels in the aqueous phase. These levels are shown in Figure 5-1. The phosphate source was switched to potassium phosphate in the fifth week of the study to avoid adding additional ammonia to the caissons. It appears that the phosphate was strongly adsorbed to the sediment rather than remaining in the aqueous phase. The availability of the absorbed nutrient to the microorganisms is unclear.

Subsequent additions of 0.25 kg of biphenyl were made at days 20, 30, and 40. When it appeared this was not sufficient to sustain a high indigenous biphenyl-metabolizing population, the addition level was increased to 1.0 kg at day 50. Re-inoculations of R103 and R102 with approximately 0.20 kg of H850 were made at days 12 and 15 respectively, and again to R103 on day 50. An additional 2.0 kg of H850 were added to R103 in an additional experiment subsequent to the final sampling in that caisson.

Operational data for temperature, pH, and dissolved oxygen are given for each of the caissons in Figures 5-2 through 5-4. Temperatures in the caissons ranged from 12-32°C during the 11 weeks of the experiment (Figure 5-2). In general, the caisson temperatures tracked the ambient air temperature (data not shown). Temperatures in the high-mix caissons averaged 5-6°C higher than in the low-mix caissons, a result of the mechanical energy created by the turbines and dissipated as heat. Smaller differences in temperature between individual caissons may be due to water depth or their orientation with respect to the river current. The moderate temperatures throughout the course of the study made it unnecessary to activate the heaters.

Table 5-2
HRRS - Nutrient and Organism Addition Log

DATE	REACTOR	$(NH_4)_2PO_4$	$(NH_4)_2SO_4$	KH_2PO_4	BIPHENYL	H850
8-9-91	R102	1.0	2.0	-	1.0	0.19
8-9-91	R103	1.0	2.0	-	1.0	0.19
8-9-91	R105	1.0	2.0	-	1.0	-
8-9-91	R106	1.0	2.0	-	1.0	-
8-22-91	R102	0.5	-	-	-	-
8-22-91	R103	0.5	-	-	-	0.19
8-22-91	R105	0.5	-	-	-	-
8-22-91	R106	0.5	-	-	-	-
8-27-91	R102	-	-	-	-	0.21
8-30-91	R102	0.5	-	-	0.25	-
8-30-91	R103	0.5	-	-	0.25	-
8-30-91	R105	0.5	-	-	0.25	-
8-30-91	R106	0.5	-	-	0.25	-
9-9-91	R102	-	-	-	0.25	-
9-9-91	R103	-	-	-	0.25	-
9-9-91	R105	-	-	-	0.25	-
9-9-91	R106	-	-	-	0.25	-
9-12-91	R102	-	-	0.5	-	-
9-12-91	R103	-	-	0.5	-	-
9-12-91	R105	-	-	0.5	-	-
9-12-91	R106	-	-	0.5	-	-
9-20-91	R102	-	-	-	0.25	-
9-20-91	R103	-	-	-	0.25	-
9-20-91	R105	-	-	-	0.25	-
9-20-91	R106	-	-	-	0.25	-
9-30-91	R102	-	-	-	1.00	-
9-30-91	R103	-	-	-	1.00	0.21
9-30-91	R105	-	-	-	1.00	-
9-30-91	R106	-	-	-	1.00	-
10-24-91	R103	-	-	-	-	2.10

All notations in kg.

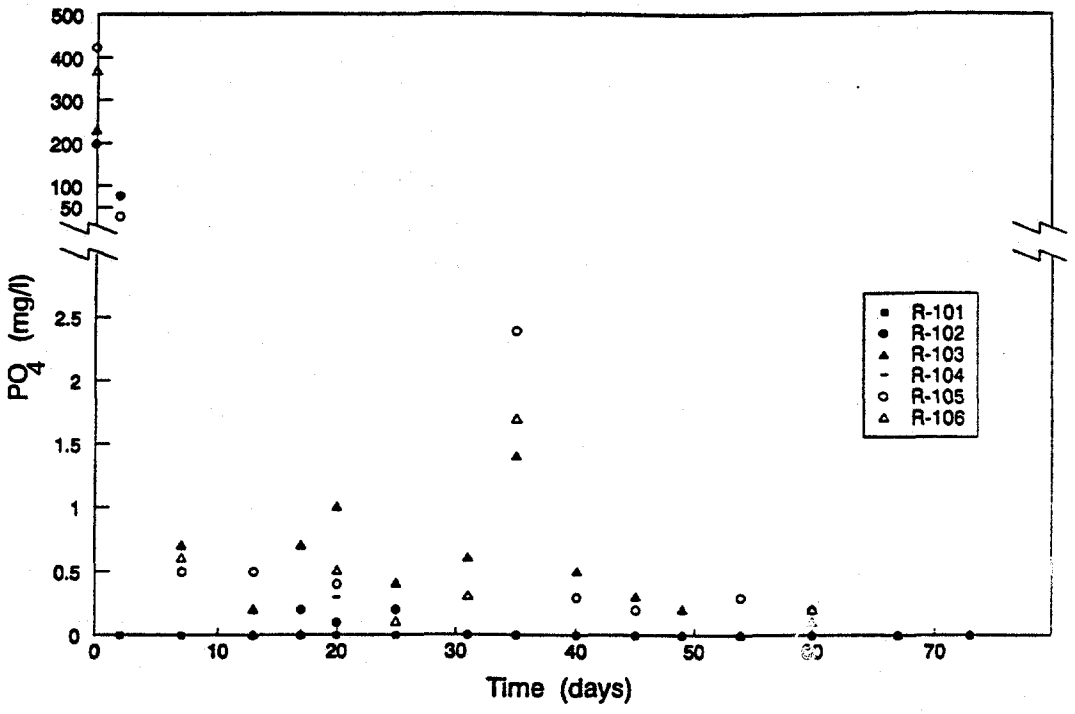


Figure 5-1. Phosphate concentrations in the caissons over time.

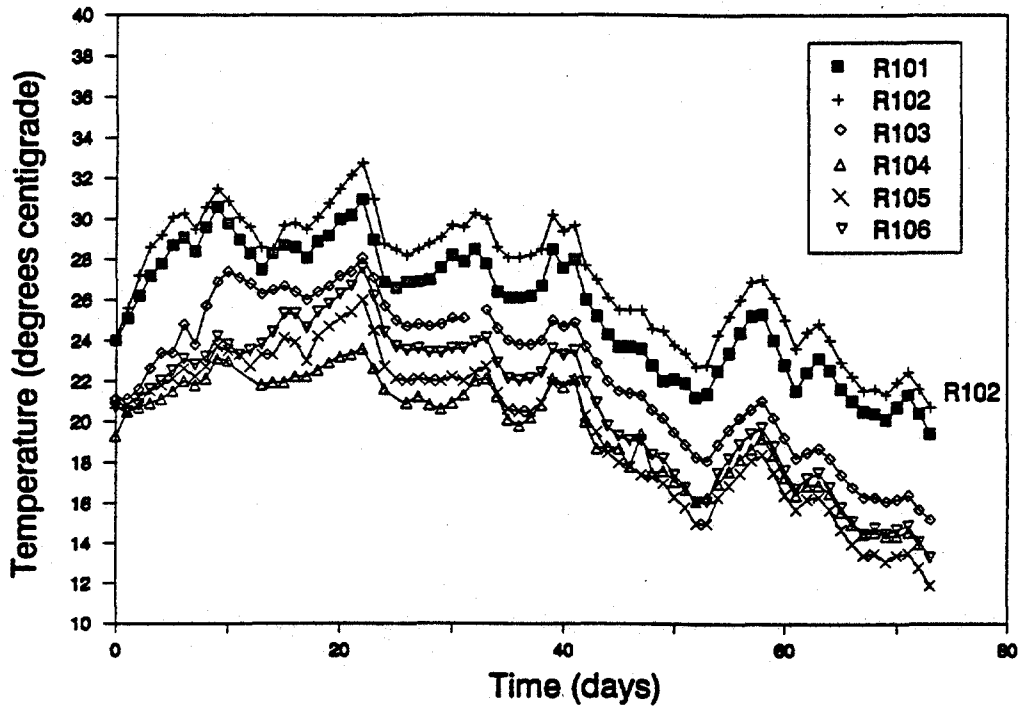


Figure 5-2. Temperature readings in the caissons over time.

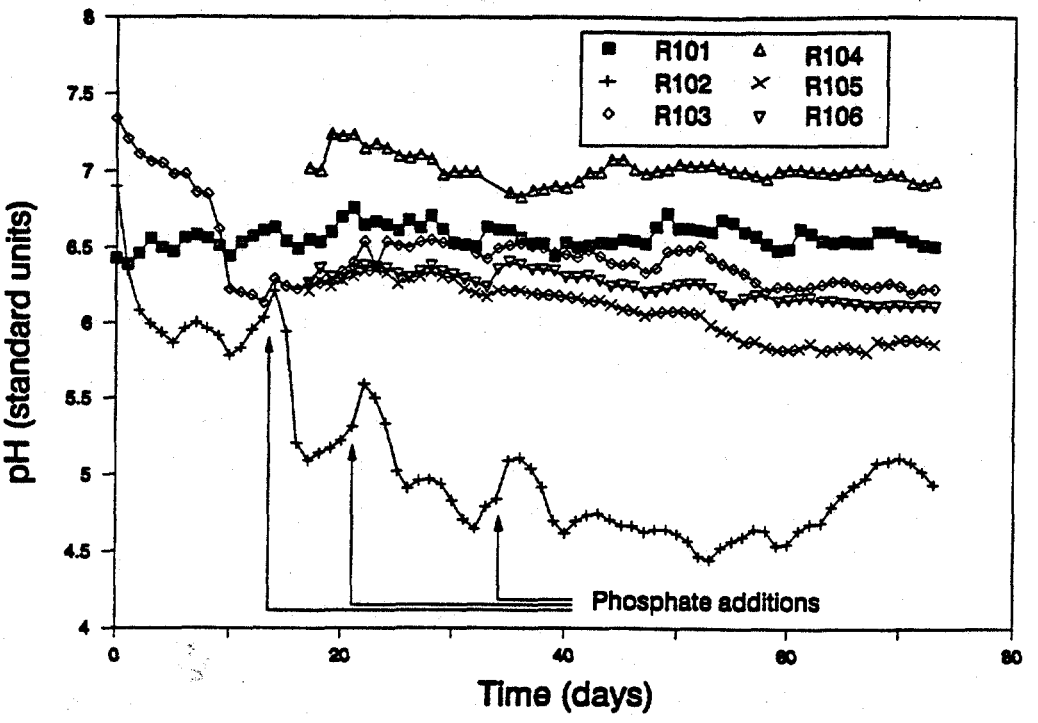


Figure 5-3. pH readings in the caissons over time. Phosphate additions are indicated by arrows. Technical problems prevented accurate pH readings in caissons R104-R106 during the first 2 weeks of the study.

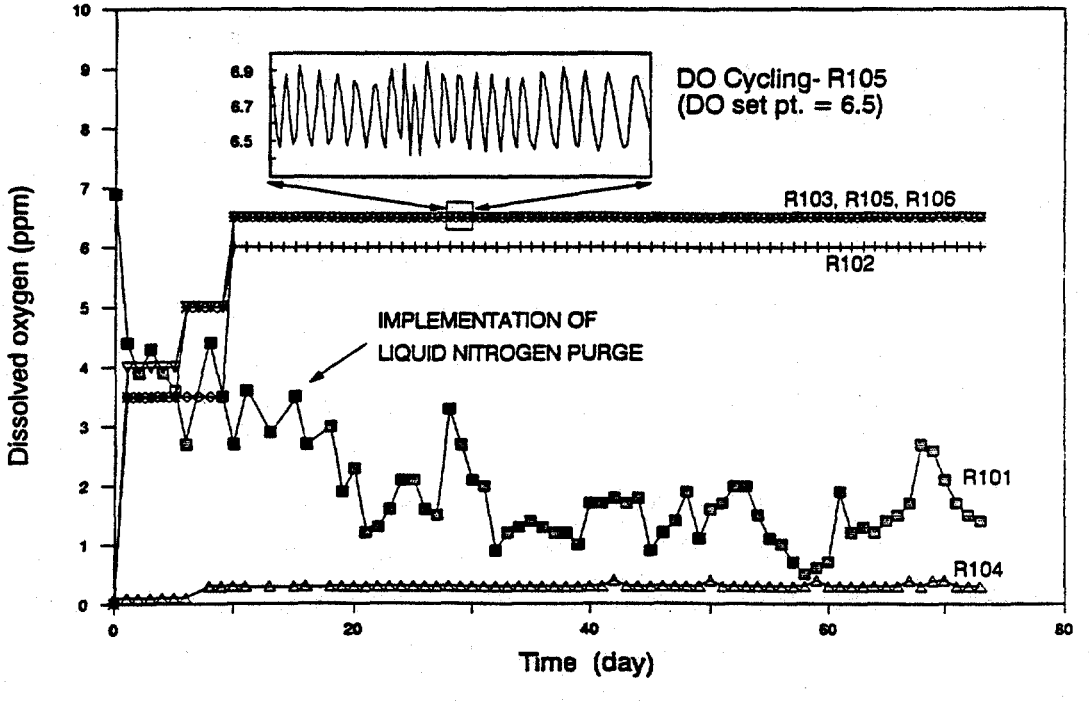


Figure 5-4. Daily dissolved oxygen measurements in individual caissons. DO setpoint values are given for caissons R102, R103, R105, and R106. Representative cycling of dissolved oxygen about the set point in these caissons is illustrated in the inset.

Daily pH measurements appear in Figure 5-3. The pH of the control caissons was stable throughout the experiment at 6.5 and 7.0 for R101 and R104 respectively. The pH in the experimental rake caissons declined slowly from 7.0 to 6.0 or less by week 11. pH data for the first two weeks in caissons R104-R106 is not shown in Figure 5-3 because electrical interferences disrupted pH measurements in those caissons during that period. The pH in R102 showed the most movement, falling rapidly and remaining below 5.0 for most of the experiment. This drop in pH was the result of the strong nitrifying activity in that caisson, and was not unprecedented, having also been observed in laboratory experiments. The addition of phosphate did cause a short term increase in pH in the caissons, most evident in R102. However, after several days the effect was diminished.

Daily dissolved oxygen measurements appear in Figure 5-4. Control setpoint values are plotted for the experimental caissons, while actual readings are given for the control caissons. The DO control setpoints were increased gradually during the first week of the experiment while the peroxide delivery system was monitored. Dissolved oxygen levels were established at less than saturation (8.5 ppm at 23°C) to minimize oxygen losses to the headspace so that an oxygen balance could be established. The setpoint in R102 was slightly lower than the others (6.0 versus 6.5) because that caisson ran at a higher temperature and hence had a lower oxygen solubility at saturation. The actual DO cycling around the control setpoint is illustrated in the inset of Figure 5-4. The liquid nitrogen purge protocol was implemented in caisson R101 on day 14 of the experiment. It was generally successful in reducing the DO level in that caisson to below 2 ppm. DO levels below 2 ppm could not be reliably measured with the probes used in this study.

There was a problem with oxygen delivery to the rake caissons in the first week of the experiment. Small mixers designed to create recirculation in the water column and enhance oxygen delivery to the sediment were installed in all the rake caissons. However, due to a specification error the shaft lengths were not long enough to actually reach the water and the mixers were not initially turned on. Dissolved oxygen measurements using the portable DO meter made during the first week of the study revealed that strong gradients in oxygen concentration were present in the water column in

the absence of the action provided by the auxiliary mixers. When this was noted the shafts were lengthened and the mixers made operational in all the rake caissons, with the exception of control caisson R104. This occurred on experiment day 7 in R103 and on day 10 in R105 and R106. This problem did not affect the operation of the rake mixers during this period.

Biphenyl analysis was performed on selected aqueous samples from caissons R102, R103, R105, and R106 to monitor biphenyl concentrations throughout the experiment. The analytical methods used are described in Appendix B-1. The results of these analyses appear in Figures 5-5A through 5-5D. All 4 caissons receiving biphenyl additions contained little biphenyl in the aqueous-phase after the first 20 days of the experiment. This presumably indicates that the biphenyl in solution was rapidly degraded by the biphenyl-metabolizing microbes in the aqueous-phase. In the low-mix caissons this activity was preceded by a lag period of 7-10 days. This period coincides with the growth of the indigenous biphenyl-degrading bacterial populations in those caissons. It should be noted that these reactors may have received insufficient aeration during the first several days of the experiment, which may have contributed to the length of this period. Such a lag was not observed in high-mix caisson R102.

Less biphenyl was measured in R102 and R103 than in R105 and R106, which may be attributed to inoculation of those caissons with *Alcaligenes eutrophus* H850. The extremely low biphenyl levels observed in R102 may also be related to better oxygenation and mixing in that caisson.

Sediment biphenyl levels were occasionally monitored, although the results were highly variable. The biphenyl evidently did not distribute evenly in the sediment phase, which made it difficult to obtain representative samples for analysis.

The total volume of hydrogen peroxide added to each of the experimental caissons is shown in Figure 5-6. The total peroxide demand in each of the low-mix caissons was comparable, ranging from 182 to 197 liters of peroxide by the end of the experiment. R102 is again the exception, showing a peroxide demand 50% higher than the other caissons. In all cases, a surge in

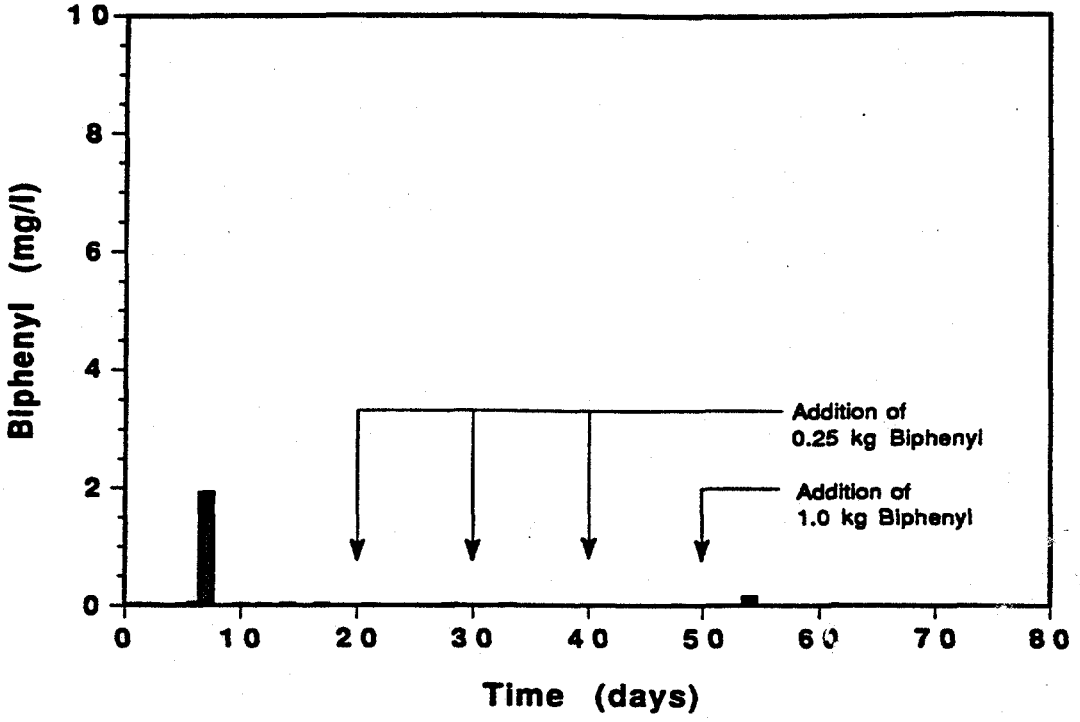


Figure 5-5A. Aqueous-phase biphenyl concentrations in caisson R102. Additions of biphenyl are noted.

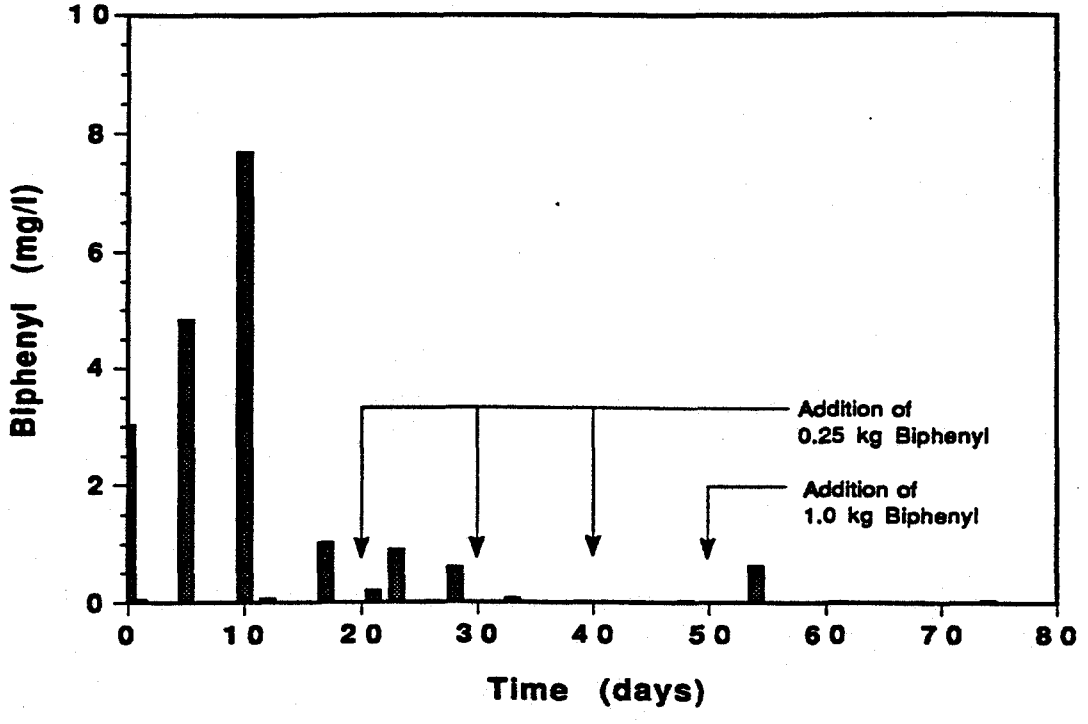


Figure 5-5B. Aqueous-phase biphenyl concentrations in caisson R103. Additions of biphenyl are noted.

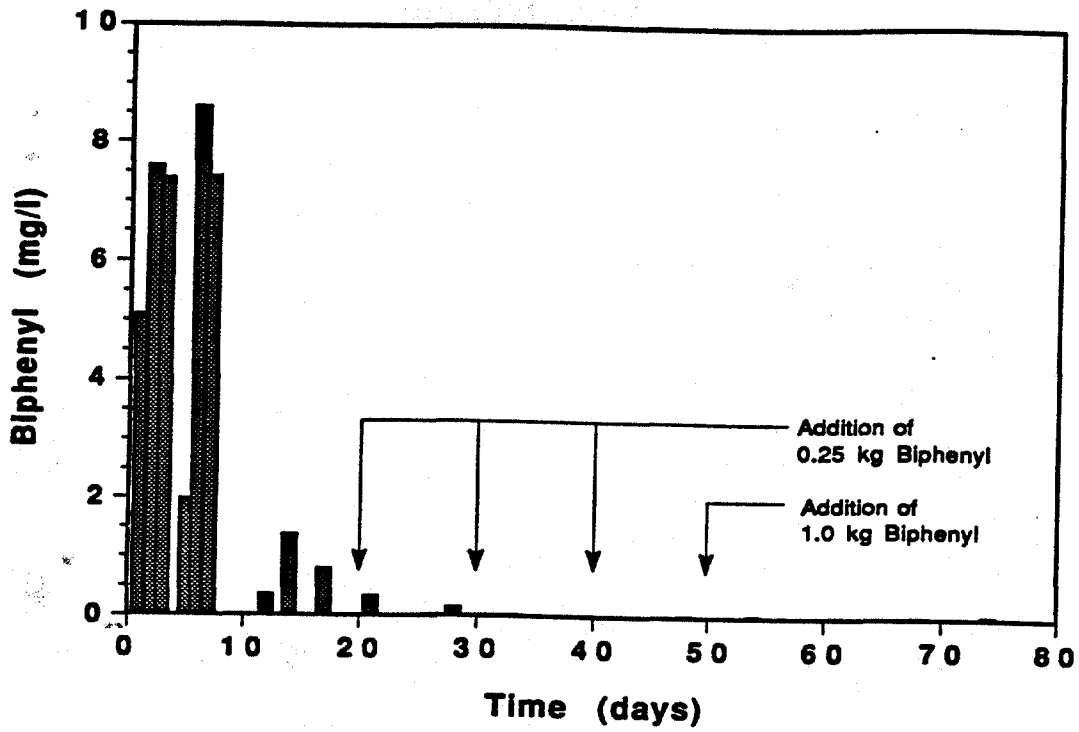


Figure 5-5C. Aqueous-phase biphenyl concentrations in caisson R105. Additions of biphenyl are noted.

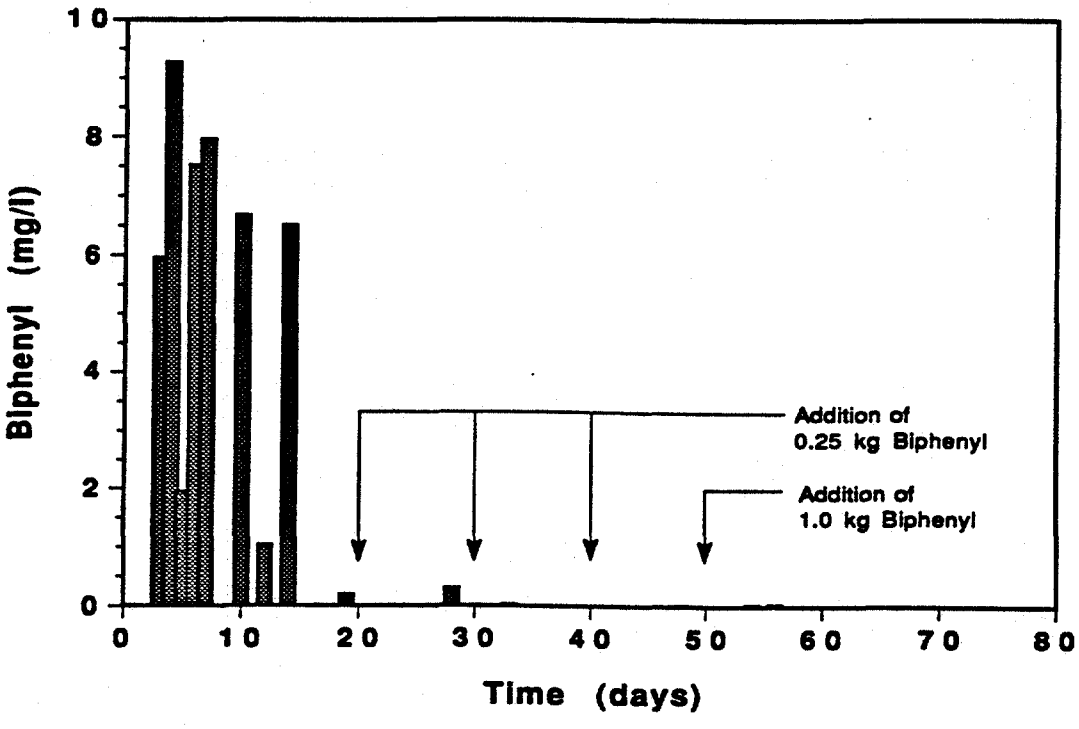


Figure 5-5D. Aqueous-phase biphenyl concentrations in caisson R106. Additions of biphenyl are noted.

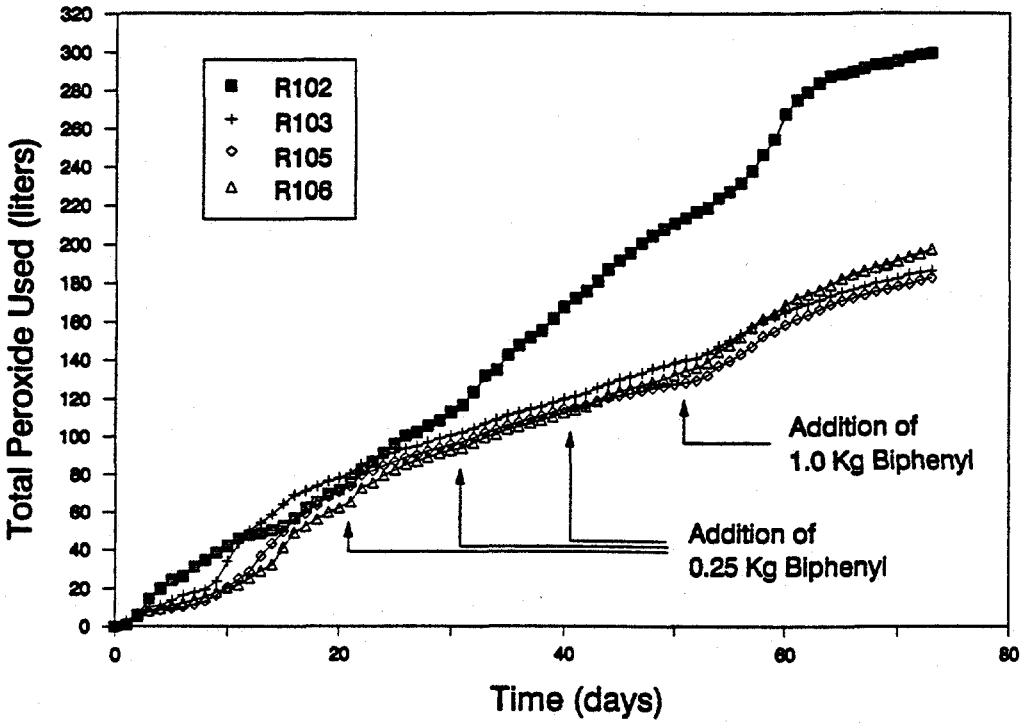


Figure 5-6. Cumulative hydrogen peroxide demand in the caissons over time. Additions of biphenyl to the caissons are indicated by the arrows.

peroxide demand followed the addition of biphenyl to the system. The theoretical ultimate biological oxygen demand in any caisson, assuming an initial sediment BOD_5 of 500 mg/kg and including the 2.75 kg of carbon that was added in the form of biphenyl is 9 kg of O_2 (or 200 liters of hydrogen peroxide). Based upon this analysis, it appears the low-mix caissons were close to achieving the system's ultimate BOD demand. The increased peroxide demand in R102 most likely reflects the additional oxygen demand associated with the biological nitrifying activity, which produced 63 mg/L nitrate and 9.2 mg/L nitrite in that caisson.

Bacterial Plate Counts

The bacterial plate counts done as part of this experiment were designed to track the relative numbers of biphenyl-metabolizing and total aerobic heterotrophic bacteria present in the caissons. The colony-forming units (cfu) reported do not represent the total number of bacteria present in the sediment samples, since exhaustive efforts were not employed to remove all the bacteria from the sediment particles, and all bacteria do not grow on the same agar medium. The cfu in the aqueous samples are probably more representative of the total numbers present in that phase, but these samples also contained significant quantities of lighter sediment particles which had not settled after several hours.

The colony-forming units of indigenous biphenyl-metabolizing bacteria were measured by direct plate counts with biphenyl as the sole source of carbon and energy. Supplemental additions of nutrients such as yeast extract could not be used in the PAS-biphenyl plates without obscuring the analysis due to increased background growth of non-biphenyl-degrading microbes. Some biphenyl-degrading organisms previously isolated from the Hudson River sediment grew very poorly without the addition of trace amounts of yeast extract [Lobos, personal communication]. Therefore, the numbers counted in this study are conservative estimates of the total number of biphenyl-degrading organisms in the caissons.

The sediment samples used for the bacterial plate count had an average water content of 22 percent. Two sediment samples from each of the six caissons were analyzed for water content. The samples ranged from 19 to 27 percent water.

The proliferation of the indigenous biphenyl-metabolizing and total aerobic heterotrophic bacteria which were detected in the sediment from the two high-mix caissons, R101 and R102, is shown in Figure 5-7A. A rapid increase in the population of indigenous biphenyl-metabolizing bacteria occurred in R102 during the first two weeks of the study, followed by a more gradual increase which peaked at about 10^7 cfu/ml after 60 days. Very few biphenyl-metabolizing bacteria were detected in the control caisson R101. The population of total heterotrophs remained relatively constant throughout the course of the study in both of the high-mix caissons. The cfu/ml of total heterotrophs in R102 were about 10-100 times greater than those in R101. The plate counts from the aqueous samples were similar to those in the sediment samples for both R101 and R102, except that tne cfu/ml were consistently 5-10 times higher in the aqueous samples. These appear in Figure 5-7B.

Plate counts of biphenyl-metabolizing and total heterotrophic bacteria in the sediment and aqueous samples from R104, R105, and R106 are shown in Figures 5-8A and 5-8B. A rapid increase in the population of biphenyl-metabolizing bacteria occurred during the first two weeks of stimulation in R105 and R106. The cfu/ml reached a maximum of almost 10^7 in the sediment samples and 10^8 in the aqueous samples after 2-3 weeks. This increase was followed by a gradual decline, which leveled off after 40 days at about 10^4 and 10^5 cfu/ml in the sediment and aqueous samples, respectively. The increase in the total heterotrophic bacteria in R105 and R106 was coincident with that of the biphenyl-metabolizing organisms. The cfu/ml of heterotrophic bacteria increased about 100-fold in the sediment and 1000-fold in the aqueous samples. Maxima of 2×10^7 cfu/ml in sediment and 10^9 cfu/ml in aqueous samples resulted after three weeks. However, in contrast to the biphenyl-metabolizing organisms, there was no significant decline in the total heterotrophic bacteria thereafter. This suggests the decline in biphenyl-metabolizing bacteria observed was not due to predation by protozoa, since total bacterial counts remained high. No significant increase

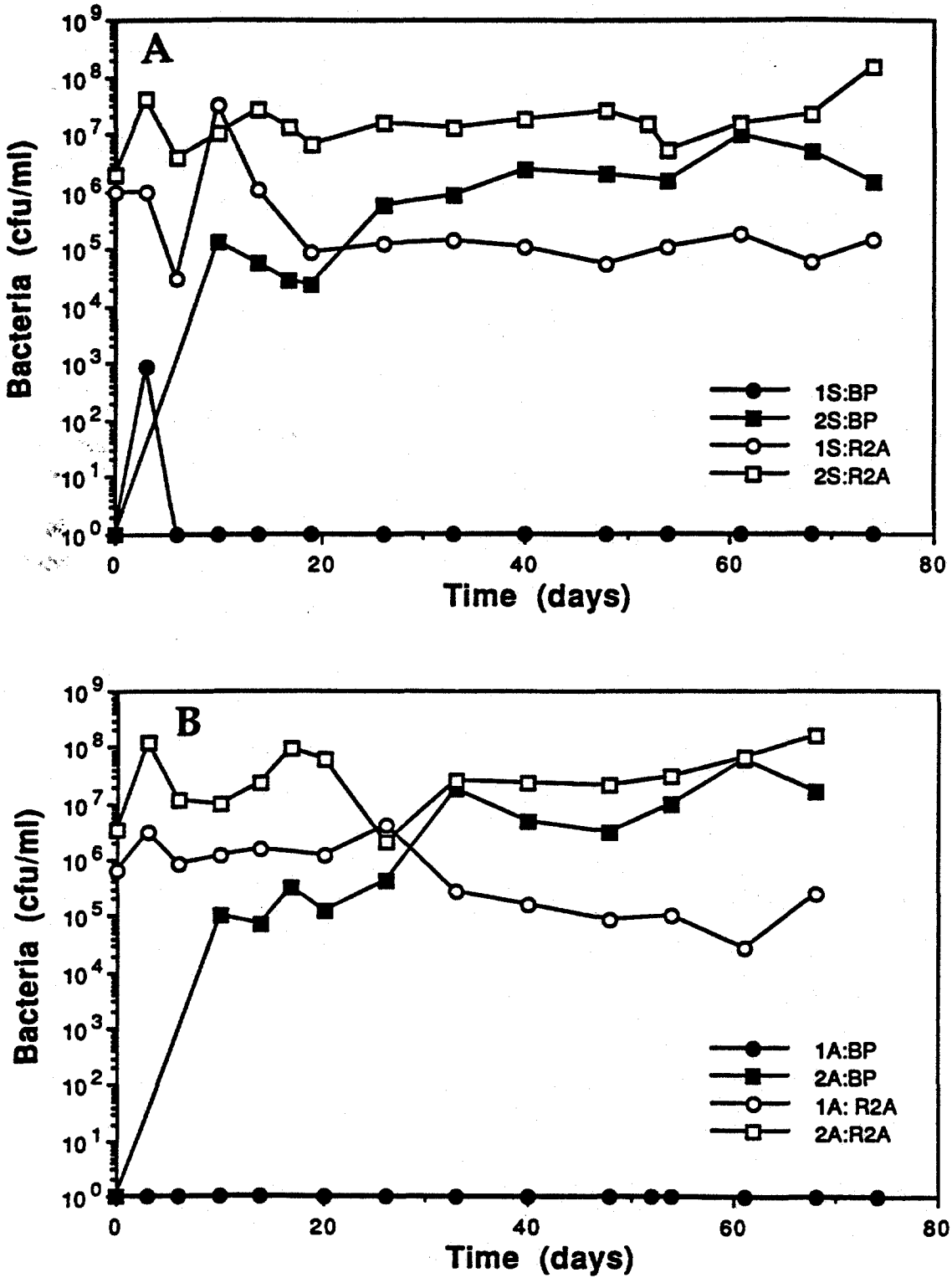


Figure 5.7. Colony forming units (cfu) of indigenous bacteria in sediment (A) and aqueous samples (B) from caissons R101 and R102 versus time. Line labels designate: Caisson #, (1 or 2); Sample type, (S)ediment or (A)queous; and Culture medium, PAS-Biphenyl (BP) or R2A (R2A) plates.

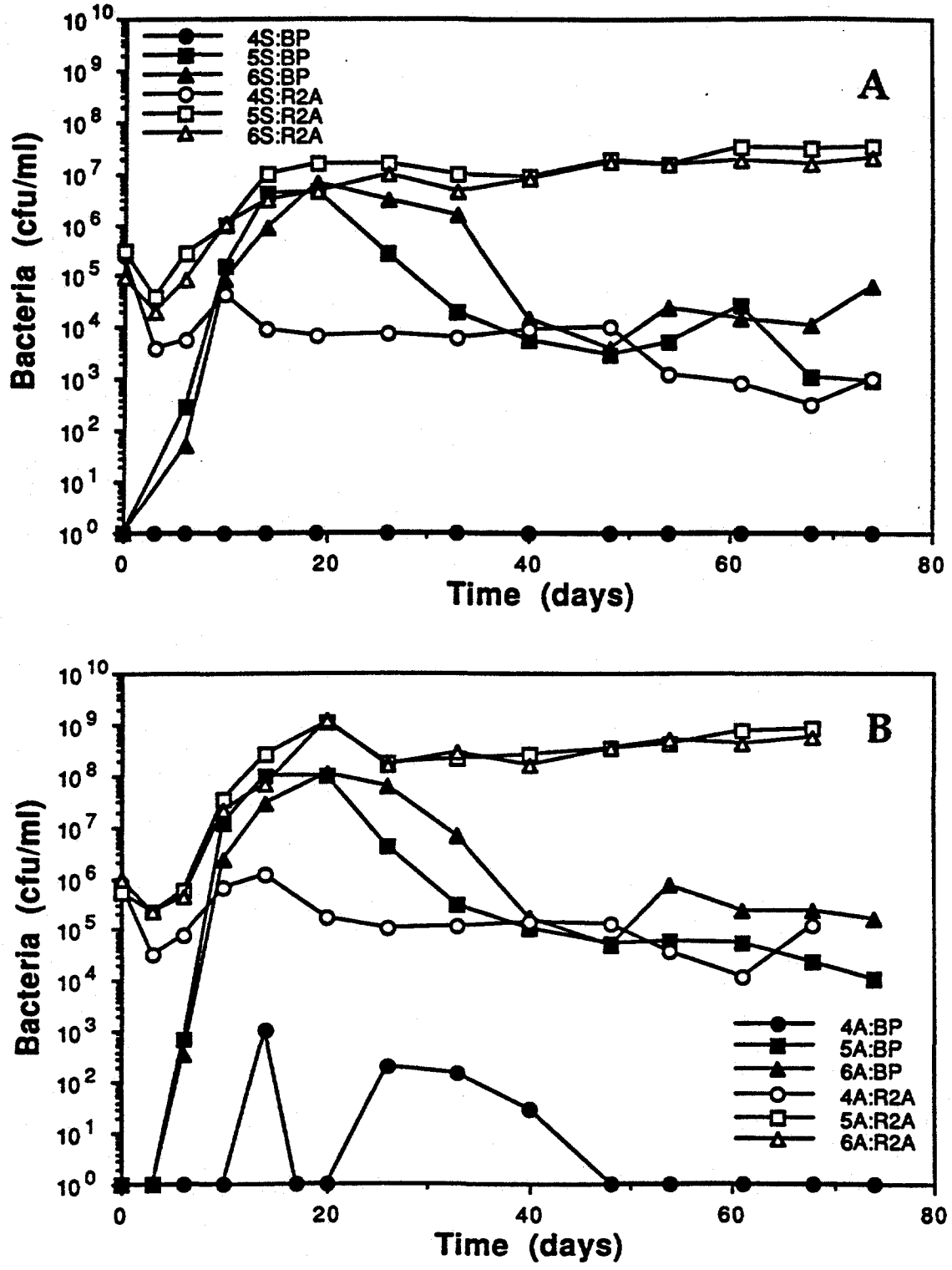


Figure 5-8. Colony forming units (cfu) of indigenous bacteria in sediment (A) and aqueous samples (B) from caissons R104, R105, and R106 versus time. Line labels designate: Caisson #, (4, 5, or 6); Sample type, (S)ediment or (A)queous; and Culture medium, PAS-Biphenyl (BP) or R2A (R2A) plates.

in the number of biphenyl-metabolizing or total heterotrophic bacteria was observed in the control reactor, R104, over the course of the study.

Caissons R102 and R103 were dosed several times with amounts of *A. eutrophus* H850 that were calculated to yield a concentration of about 10^8 cells/ml. The survival of *A. eutrophus* H850 in the two reactors after each dosing is shown in Figure 5-9. Plate counts of both sediment and aqueous phase samples are shown together. After the initial dose, the concentration of *A. eutrophus* H850 dropped below 10^5 cfu/ml after ten days in both caissons. A second dose to R103 resulted in a similar decline in the *A. eutrophus* H850 population after six days, decreasing from 2×10^7 to 10^4 cfu/ml. A third dose of H850 to R103 at day 54 appeared to survive slightly better than the previous two, but still declined below 10^5 cfu/ml after two weeks. The colony-forming units of *A. eutrophus* H850 in the aqueous samples taken from R103 within 24 hours of dosing were reasonably close to the calculated value of 10^8 cells/ml.

The second dose of *A. eutrophus* H850 to R102 resulted in less than 10^5 cfu/ml one day after addition and no further doses were attempted. It should be noted that the pH in R102 was about 5 at this time.

The effect of mixing mode on the indigenous biphenyl-metabolizing and total heterotrophic bacteria is shown in Figure 5-10, which compares colony counts in R102 and R103. Identical additions were made to both caissons to stimulate the growth and activity of biphenyl-metabolizing bacteria, and both were dosed with *A. eutrophus* H850. The large number of H850 cells added at T_0 to R102 and R103 made it difficult to follow the increase in the population of indigenous biphenyl-metabolizing bacteria at early time points. The rapid decrease in H850 colony counts and their distinct colonial morphology made it possible to distinguish this organism from the indigenous biphenyl-metabolizing bacteria after 10 days.

The cfu/ml of indigenous biphenyl-metabolizing bacteria in the low-mix caisson R103 reached a maximum concentration in less than 2 weeks, which was followed by a gradual decline. This was similar to the trend observed in the other low-mix caissons. In contrast, the high-mix caisson

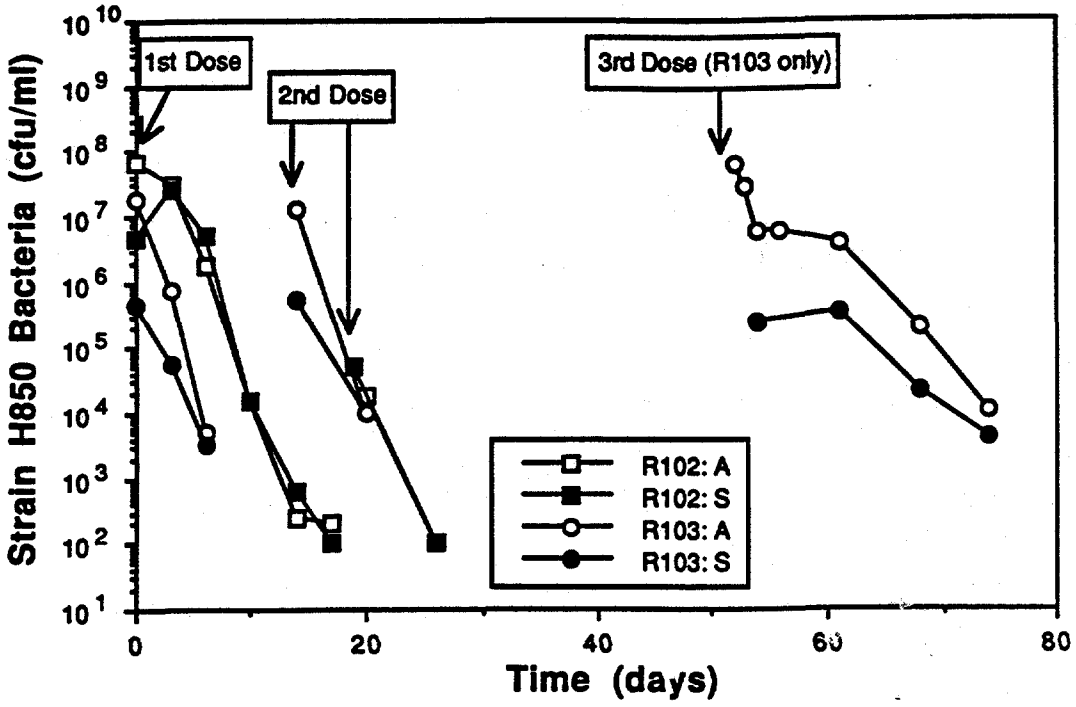


Figure 5-9. Colony-forming units (cfu) of *A. eutrophus* H850 versus time in caissons R102 and R103. Line labels designate: Caisson #, (2 or 3); and sample type, (S)ediment or (A)queous. Culture medium was PAS-Biphenyl agar plates.

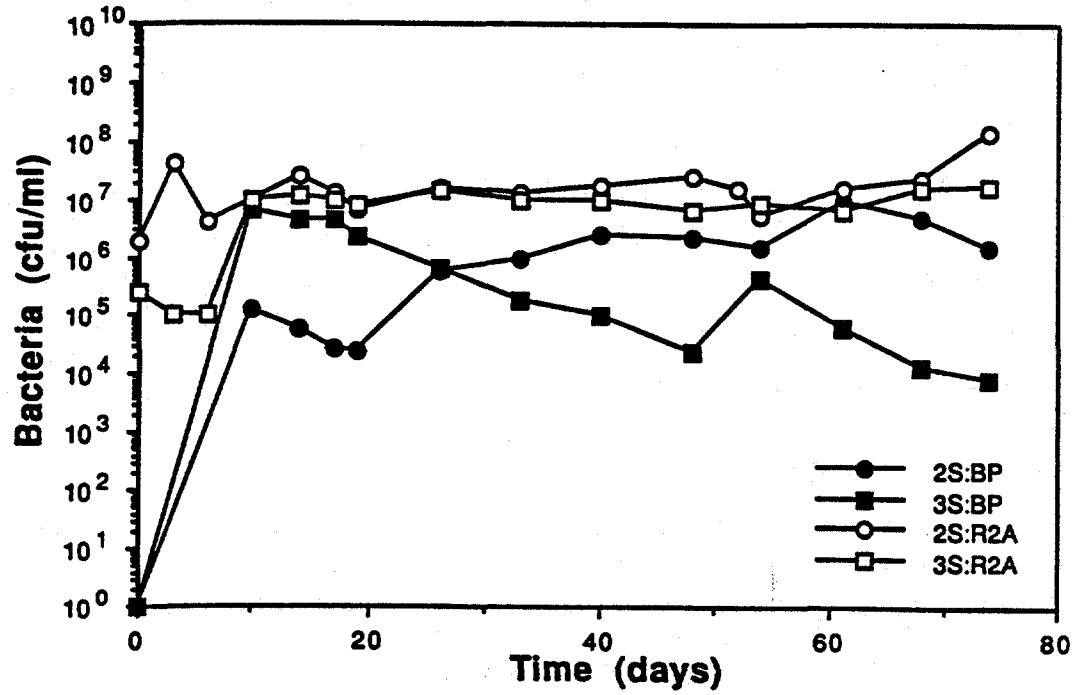


Figure 5-10. Comparison of colony forming units (cfu) of indigenous bacteria in sediment samples from high mix (R102) and low mix (R103) caissons versus time. Line labels designate: Caisson #, (2 or 3); Sediment sample (S); and Culture medium, PAS-Biphenyl (BP) or R2A (R2A) plates.

R102 had a much slower increase in the cfu/ml of indigenous biphenyl-metabolizing bacteria for most of the first 60 days without any significant decline. Both caissons reached maximums of about 10^7 cfu/ml for indigenous biphenyl-metabolizing bacteria in sediment samples. The total heterotrophic bacteria population increased from 10^6 to 10^8 cfu/ml in these samples. Analogous behavior was observed in aqueous samples, except the cfu/ml were 5 to 10 times higher than in corresponding sediment samples (data not shown).

A summary comparison of the number of indigenous biphenyl-metabolizing bacteria present in the aqueous and sediment samples removed from each of the six caissons is shown in Figure 5-11A and 5-11B. The profiles for the growth and decline of this population are very similar in the three low-mix experimental caissons for both aqueous and sediment samples. The indigenous biphenyl-metabolizing bacteria reached maxima of 10^7 and 10^8 cfu/ml in three weeks in sediment and aqueous samples, respectively, followed by a period of decline which leveled off at about 10^4 and 10^5 cfu/ml. The slight increase observed at about experiment day 54 may have been in response to the 1.0 kg of biphenyl that was added to each of the four experimental caissons on day 50 (0.25 kg was added at 10 day intervals from day 20 to day 40). Very few indigenous biphenyl-metabolizing bacteria were detected in the samples from the controls, R101 and R104.

The decline in the biphenyl-metabolizing population observed in the intermediate weeks of the study may have been due to substrate limitation once the initial addition of biphenyl was depleted after the first two weeks. This resulted in a gradual decline in the number of biphenyl-degraders to a concentration (10^4 to 10^5 cfu/ml) which could be maintained by the amount of available biphenyl. The slight increase in the biphenyl-metabolizing population from days 54 - 60 after the 1.0 kg addition supports this contention, as does the absence of biphenyl in the aqueous phase over the period of decline (see Figures 5-5A through 5-5B). This suggests the biphenyl-metabolizing population will not persist long at high levels in the environment once biphenyl is no longer available.

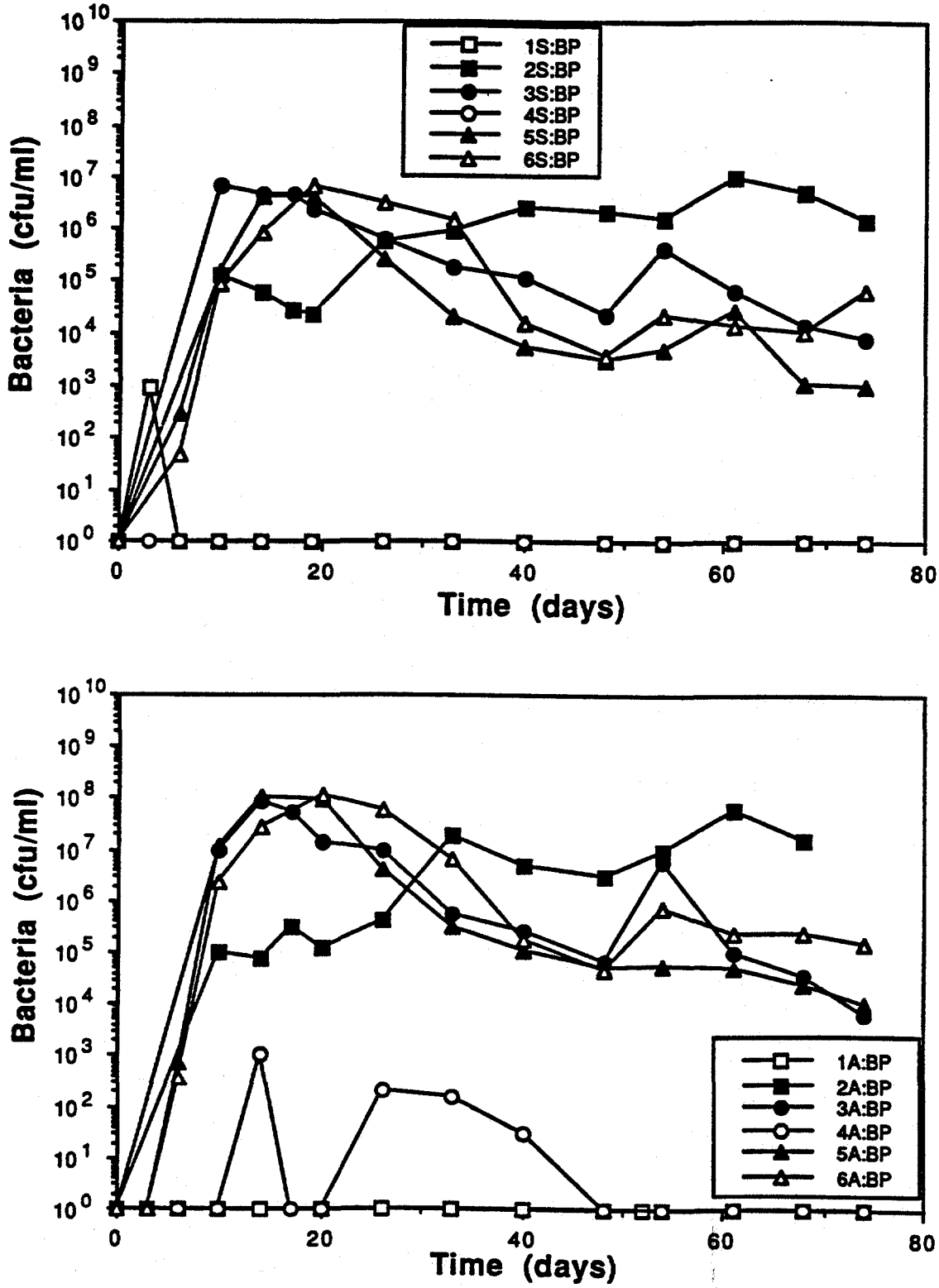


Figure 5-11. Colony forming units (cfu) of indigenous biphenyl metabolizing bacteria in sediment samples versus time. Line labels designate: Caisson #, (1-6); Sample type, (S)ediment; and Culture medium, PAS-Biphenyl (BP) plates.

The fact that the total heterotrophic bacteria survived better than the indigenous biphenyl-metabolizing population may be due to the increased diversity of microorganisms and the wide variety of "natural" carbon sources utilized by the various populations, which may include the dead and decaying biphenyl-metabolizing bacteria.

The indigenous biphenyl-metabolizing population in high-mix caisson R102 reached concentrations of about 10^5 cfu/ml after three weeks. In contrast to the low-mix reactors, the indigenous biphenyl-metabolizing population continued to increase over the next 40 days before reaching a maximum of about 10^7 and 10^8 cfu/ml in the sediment and aqueous samples, respectively. Although there was not a significant decline in the total cfu/ml of the biphenyl-metabolizing bacteria in R102, there were shifts to different populations of biphenyl-metabolizing bacteria.

A variety of colony morphologies were observed for the biphenyl-metabolizing bacteria taken from the four experimental caissons. The colony morphologies of indigenous biphenyl-metabolizing strains were similar in the three low-mix caissons. Most of the colony morphologies for the biphenyl-metabolizing bacteria in the high-mix caisson R102 were very different from those isolated from the low-mix caissons. They were also much slower growing than those in the low-mix reactors.

In addition to mixing there was at least one other significant difference which may have affected the types and growth rate of the biphenyl-metabolizing bacteria in the high-mix reactor R102. The pH in R102 decreased to below 5 after 3 weeks and remained there for the duration of the study. The pH in the other caissons generally remained between 6 and 7 for the entire period. The difference in colony morphologies of biphenyl-metabolizing bacteria observed in R102 may indicate that different populations had been enriched at the lower pH.

Total heterotrophic bacterial counts were important indicators of the overall microbial health of the caissons. Since oxygen was supplied to the reactors in the form of H_2O_2 (which is converted to $O_2 + H_2O$ by the enzyme catalase in aerobic bacteria and by metals present in the sediment), there was

concern that the concentration of H_2O_2 might reach toxic levels if there were insufficient aerobic bacteria present in the caissons. This, however, does not appear to be the case. The total heterotrophic bacterial cfu/ml did not indicate any detrimental effects due to peroxide toxicity.

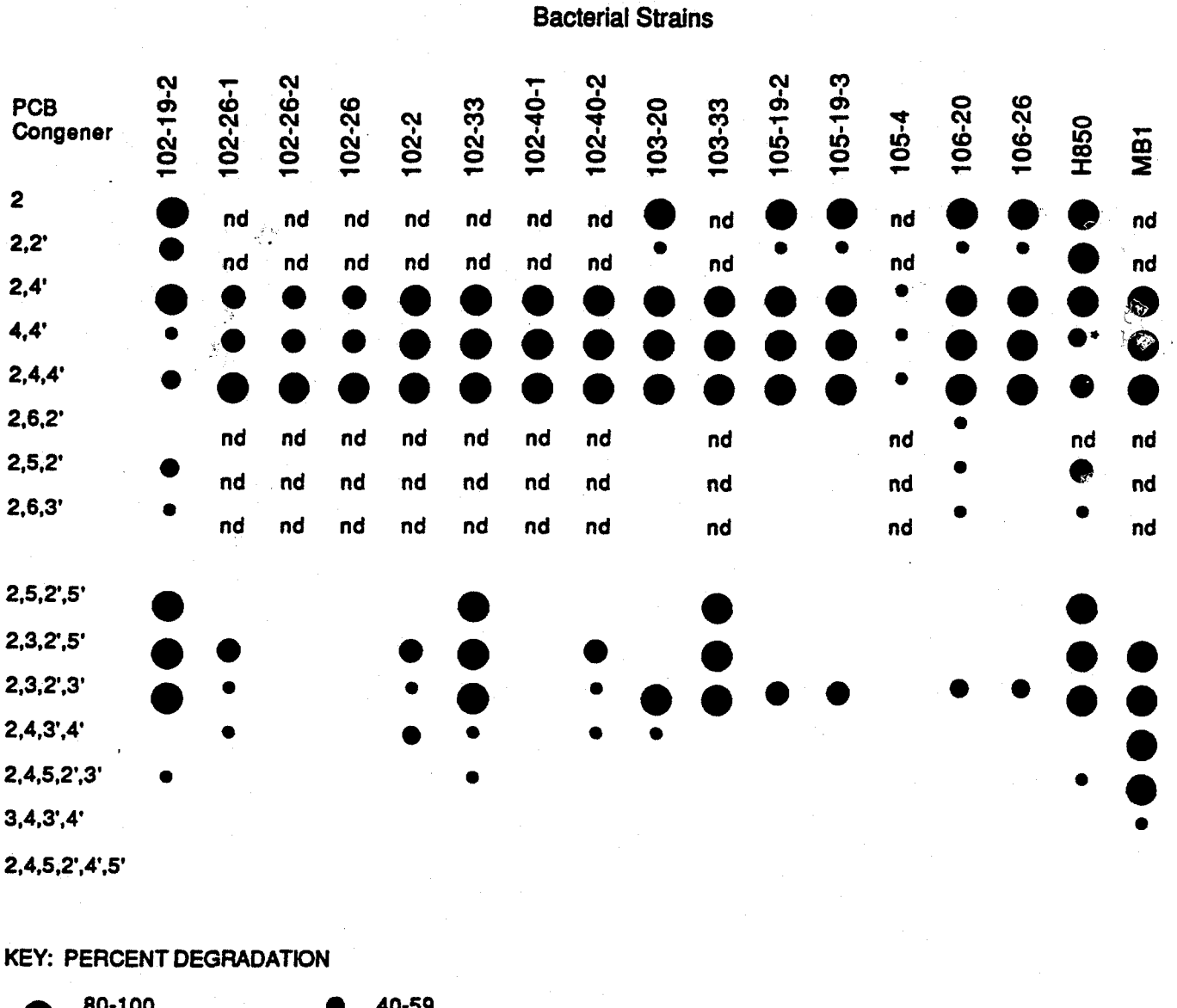
Resting-Cell Assay Results

In the course of enumerating colonies and studying the colonial characteristics of *A. eutrophus* H850 and the indigenous biphenyl-metabolizing strain, it became apparent that there were several different biphenyl-degrading populations present in the caissons. These populations grew at different rates, and some of these outnumbered H850 in the plate counts. In some cases, it became difficult to differentiate between strain H850 and the indigenous population on the biphenyl (BP)/PAS plates. Several of these colonies were picked in order to compare their PCB-degradative competence with that of H850.

This was not an exhaustive survey of the bacteria growing in the experimental caissons. The environment in these caissons was a dynamic one. Some of the colonies were picked when they were the predominant population in the caisson, while others were randomly selected. Colonies were picked and assayed as early as two weeks into the study and as late as six weeks.

Resting-cell assays [Bedard et al., 1986] of these biphenyl-metabolizing isolates were performed using defined PCB congener mixes 1B and congener mixtures consisting of the lower chlorinated PCB congeners that are predominant at the H7 site and throughout the upper Hudson River. The comparative results of PCB congener degradation by bacteria isolated from the caissons are shown in Table 5-3. Caissons R102 and R103 were dosed several times with H850. Isolate 102-19-2 was probably H850, while 102-33 resembled H850 very closely, but had better competence with double *para*-chlorinated biphenyls (4-4-, 24-4-, and 24-34-chlorobiphenyl). Caisson R103 contained isolate 103-33, which also closely resembled H850, but again had better

Table 5-3.
PCB degradative competence of bacterial isolates from Hudson River research station caissons.



competence with double *para*-chlorinated biphenyls (4-4- and 24-4-chlorobiphenyl).

The other isolates from caissons R102, R103, R105 and R106 displayed similar activity, with demonstrated competence mostly for the double *para*-chlorinated congeners. It would appear from these results that most of the indigenous biphenyl-metabolizing bacteria from these four caissons were not superior PCB-degraders as measured by resting-cell assays. They were capable of degrading 2-, 2-2-, 2-4-, and some 4-4-chlorobiphenyl, which comprised most of the PCB at the site of the study.

By the fifth week of the study, biphenyl was not detected in the aqueous phase of the caissons. In addition, the pH of caisson R102 was below pH 5.0, while the other caissons remained at ~pH 6.5. In order to determine whether PCB degradation was adversely affected by the low pH in caisson R102 relative to the other caissons, and whether the lack of biphenyl might be affecting PCB degradation, an H7 PCB congener mix assay was conducted using aqueous samples from the caissons. Aqueous samples taken from all six caissons at day 40 were mixed well, followed by removal of a 1 ml sample for the PCB-degradation assay. The mixture was incubated at 30°C for 48 hours.

From the results shown in Table 5-4 it is clear that there was no detectable PCB-degradative activity in the aqueous phase samples from the control caissons. There was limited activity present in the four experimental caissons. The PCB-degradative competence of microorganisms in caissons R102/R103, which were dosed with H850, did not differ appreciably from caissons R105/R106, which contained only indigenous bacteria. This may be due to the fact that many H850 cells were not viable or had lost most of their competence. In that case the observed PCB-degradative activity could be attributed to the indigenous populations in all four of the experimental caissons. The PCB-degradative competence of the microorganisms in caisson R102 did not appear to have been adversely affected by the low pH. The PCB-

Table 5-4.
Percent degradation of mix H7 PCB congeners in aqueous phase from Hudson River caissons.
Percent Degradation[a]

	Caisson					
PCB Congener	101[b]	102	103	104[b]	105	106
2	0	100	100	0	97	70
2-2	0	0	0	0	0	0
2-4	0	96	0	0	15	0
26-2	0	0	0	0	0	0
25-2	0	0	0	0	0	0
4-4	0	0	0	0	0	0
26-3	0	0	0	0	0	0

[a] 48 hour assay with PCB mix H7
[b] control

degradative performance seen here is not surprising, since it reflected the actual number of PCB-degrading organisms in the caissons, which was approximately 100-1000 fold smaller than the number of cells used in typical resting-cell assays (Tables 1-1 and 5-3). It is significant that any activity was seen under such conditions.

PCB Analysis

Details of the statistical analysis cited in this section appear in Appendix C-1. Data on air-drying of sediments, water analyses, analyses of waste streams, and a summary of QA/QC data generated by Northeast Analytical Laboratory during the course of their PCB analysis appears in Appendix C-2. Representative chromatograms of the PCB distributions

within each caisson at the beginning and end of the study appear in Appendix C-3. Individual results of the analysis of sediment cores, including PCB values, sample dry weights, and total organic carbon values, are presented in Appendix C-4.

Average PCB concentrations for the 12 core samples taken from each of the caissons at T_0 and at week 11 (T_f) are summarized in Table 5-5. The data is represented as averages in PCB concentration, core weight, and total PCB per core, with standard deviations included. Despite efforts to situate the research platform over an area of uniformly high PCB content, the starting PCB concentrations were not uniform among the 6 caissons. This result was not unexpected, given the heterogeneous manner in which PCBs are distributed in river sediments (see Figures 2-4 and 2-6). The average PCB concentration in caisson R101, the high-mix control, was particularly low at 6 ppm. Caisson R102 started at 20 ppm. Average PCB concentrations in the low-mix caissons were higher, ranging from 30 to 50 ppm. High standard deviations in the PCB values at T_0 indicated significant heterogeneity still existed in some of the caissons, even after the 24 hour pre-mix period.

The changes in average PCB concentration observed in each caisson are presented graphically in Figure 5-12. The bars in the figure are 95% confidence limits, indicating the probability that the actual mean of the distribution is within the limits shown. Statistically significant changes in average PCB concentrations were observed in all caissons, with the exception of high-mix control R101. An average PCB reduction of 41% was found in the high-mix experimental caisson, R102. The highest PCB losses were observed in the low-mix caissons R105 and R106. However, PCB losses in R104, the low-mix control, are problematic and complicate the interpretation of these results. This is particularly true in regard to R103, which shows a smaller PCB decrease than the control. This result is indicative of the difficulty in using direct concentration measurements to assess changes in complex heterogeneous environmental samples. These issues will be addressed using two normalization techniques [Atlas, 1981; Pritchard, 1991].

There were substantial differences in sediment morphology produced by the turbine and rake mixing systems. Following the initial 24-hour pre-

Table 5-5
Summary of Analyses of Core Samples
(results are represented as averages +/- one standard deviation)

Caisson	N	PCB (ug/g)	Core Wt (g)	Total PCB (ug)
R101-T_o	12	5.99 +/- 1.88	300 +/- 53	1750 +/- 400
R101 - Tf	12	6.51 +/- 2.53	287 +/- 25	1840 +/- 610
R102 - T_o	12	20.0 +/- 11.0	291 +/- 62	5220 +/- 1360
R102 - Tf	12	11.8 +/- 7.3	271 +/- 63	3130 +/- 1870
R103 - T_o	12	30.2 +/- 10.6	232 +/- 74	6680 +/- 2200
R103 - Tf	12	19.1 +/- 5.0	316 +/- 71	5920 +/- 1540
R103 - 10/28	12	19.9 +/- 4.1	288 /- 48	5600 +/- 970
R104 - T_o	12	39.9 +/- 15.6	265 +/- 58	9990 +/- 2590
R104 - Tf	12	23.1 +/- 9.9	341 +/- 72	7900 +/- 4050
R104-11/4	6	48.9 +/- 4.8	256 +/- 29	12500 +/- 2020
R105 - T_o	12	49.7 +/- 27.8	262 +/- 77	11300 +/- 3770
R105 - Tf	12	13.6 +/- 5.6	447 +/- 61	5980 +/- 2220
R105 - 11/4	6	24.8 +/- 4.8	338 +/- 59	8240 +/- 2860
R106 - T_o	12	39.1 +/- 17.5	237 +/- 65	8640 +/- 3450
R106 - Tf	12	12.3 +/- 4.1	408 +/- 44	4910 +/- 1470

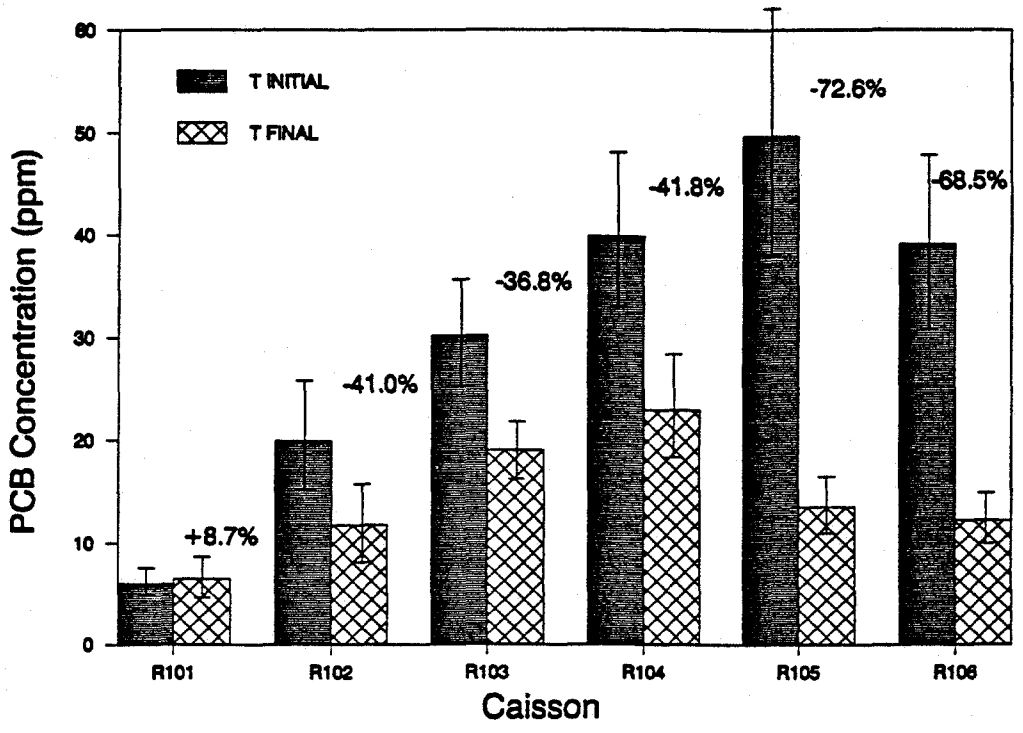


Figure 5-12. Average PCB concentration in the caissons at T_o and T_f. Percent change is indicated. Bars delimit 95% confidence intervals (95% confidence that actual mean is within limits shown).

mix period the sediment in the high-mix caissons had a reddish brown color and sandy texture. This color and morphology did not change appreciably over the course of the experiment. The sandy sediment settled readily and was penetrated easily by the sediment corer, making uniform core samples relatively straightforward to obtain. After the pre-mix period the sediment in all of the low-mix caissons was darker in color and contained both a sandy fraction and a highly organic, colloidal fraction. This latter fraction did not settle rapidly and increased over time as the experiment progressed. It is likely that this fraction was generated by the action of the rakes slowly grinding through the sediment.

There were also natural compositional variations between caissons. The sediment at the south end of the platform (R103, R105, and R106) contained more gravel than that at the north end of the platform, making it more difficult to obtain samples from those caissons. In addition, as the experiment progressed, a hard compacted zone was formed in the sediment below the rakes at the bottom of all the low-mix caissons. It was often necessary to break through this zone in order to take a sediment sample that would remain intact upon withdrawal from the caisson. These difficulties, combined with the fact that the colloidal fraction did not settle readily, created significant challenges in sampling the low-mix caissons and reduces the usefulness of the PCB measurements described in Figure 5-12.

These sampling differences manifested themselves in changes in sample core weights in some of the caissons during the study. Average core weight values (dry weight basis) for all the caissons at T_o and T_f are presented in Figure 5-13. There was little change in average core weights for the high-mix caissons, R101 and R102, reflecting the ease with which those caissons were sampled. On the other hand, the average core weights in the low-mix caissons were significantly increased in each instance at T_f in spite of efforts to reduce sample weight variability at the sample preparation step (see Chapter 3 - Sample Collection Procedures).

A conservative approach to adjust for this variable sample weight is to calculate the total PCB content for each core by multiplying its PCB concentration by its core weight. These results, expressed as average total PCB

in micrograms per sediment core, appear in Figure 5-14. Statistically significant changes in total PCB content were observed in caissons R102, R105, and R106. As expected, the PCB biodegradation results for the high-mix caissons were essentially unchanged by this analysis method. PCB reductions for the other caissons were all smaller as a result of their higher average sample weights at T_f. R105 and R106 show PCB changes of 47.1% and 43.1%, respectively, under this analytical method. However, interpretation of PCB changes in R104 and R103 continues to be problematic.

Bar charts showing the average PCB congener distributions of all 12 sediment samples at T_o and T_f for all of the caissons are shown in Figures 5-15B through 5-15G. Several of the important PCB congener peaks are identified for reference in Figure 5-15A. These results are normalized using a PCB peak recalcitrant to biodegradation as an internal standard so that congener-specific changes will stand out (normalization will be discussed in the following section). Representative chromatograms of these analyses appear in Appendix C-3. Aerobic biodegradation of PCBs is congener specific, meaning some congeners will be degraded more readily and rapidly than others [reviewed in Abramowicz, 1990; Bedard, 1990]. This is particularly true of the mono- and dichlorobiphenyls, which constitute 62-73% of the PCBs in this sediment. Review of the chromatograms in Figure 5-15E indicates very little congener-specific change had taken place in R104, the low-mix control caisson. This indicates that very little biodegradation, or any other congener-specific loss of PCBs, had occurred in that caisson. There appears to be some small loss of PCBs from control caisson R101, particularly in the largest congener peaks (2-, 2-2-/26- and 23-/2-4-chlorobiphenyl).

On the other hand, significant congener-specific changes were evident in all of the experimental caissons. Significant reductions are evident in 2-, 2-2-/26-, 23-/2-4-, and 236-/26-3-chlorobiphenyls (peaks 2, 5, 8, 16) for example, while congeners like 26-2-chlorobiphenyl (peak 10), and some of the tri- and tetrachlorobiphenyls (peak 30 and above) changed much less. This preference for the aerobic attack of lightly chlorinated PCB is well documented [Abramowicz, 1990; Bedard, 1990] and is consistent with laboratory results using these sediments. Some caisson-specific differences in degradation patterns could be discerned from these chromatograms and will be discussed

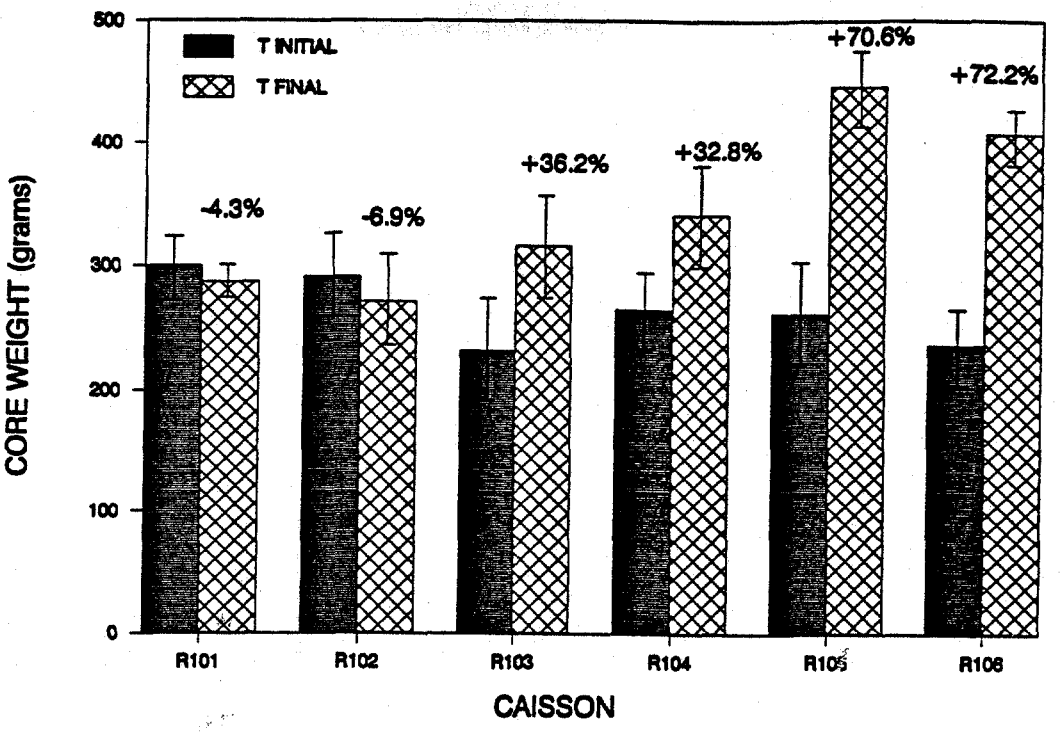


Figure 5-13. Average core dry weight taken from the caissons at T_o and T_f. Percent change is indicated. Bars delimit 95% confidence intervals (95% confidence that actual mean is within limits shown).

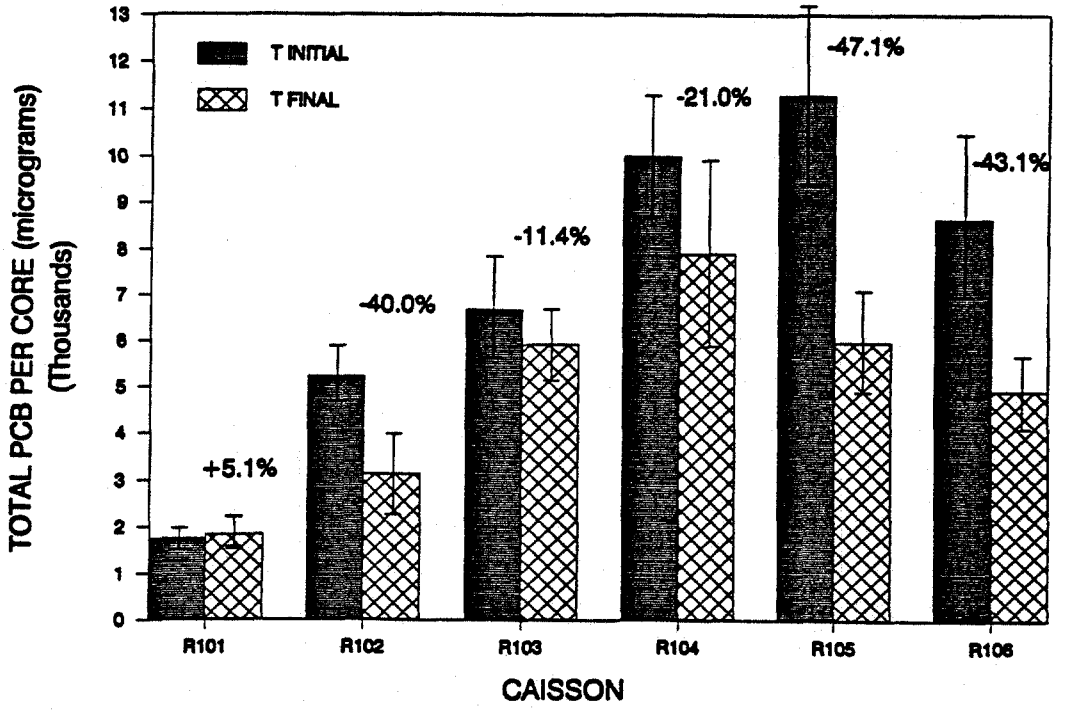
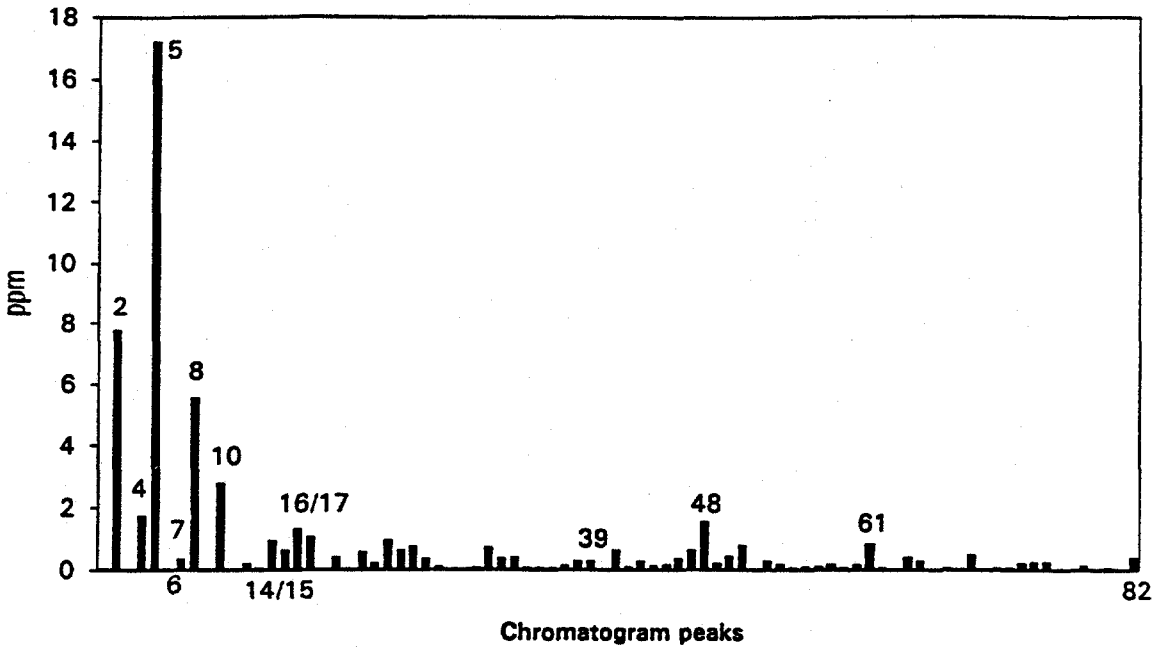


Figure 5-14. Average total PCB per core taken from the caissons at T_o and T_f. Percent change is indicated. Bars delimit 95% confidence intervals (95% confidence that actual mean is within limits shown).



PCB Homolog	Peak	PCB Congener
Mono-chloro	2	2–
	4	4–
Di-chloro	5	2, 2'–/2, 6–
	6	2, 4–/2, 5–
	7	2, 3'–
	8	2, 3–/2, 4'–
Tri-chloro	10	2, 6, 2'–
	14	2, 5, 2'–/4, 4'–
	15	2, 4, 2'–
	16	2, 3, 6–/2, 6, 3'–
	17	2, 3, 2'–/2, 6, 4'–
Normalization References	39	2, 3, 6, 4'–/2, 6, 3', 4'–
	48	2, 3, 5, 6, 2'–/2, 3, 6, 2', 5'–
	61	3, 4, 3', 4'–/2, 3, 6, 3', 4'–
	82	2, 3, 4, 2', 4', 5'–/2, 3, 5, 6, 3', 4'–

Figure 5-15A. Example histogram with selected congener peaks and reference peaks identified.

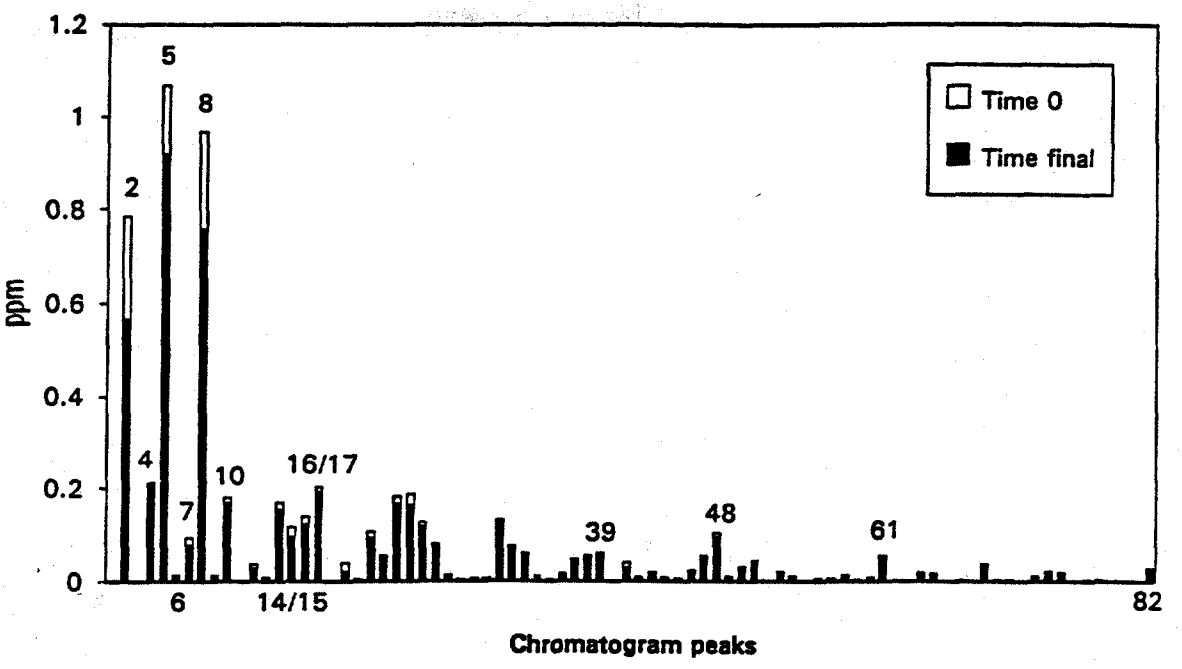


Figure 5-15B. Histogram showing peak-specific PCB concentrations in core samples from caisson R101 at T_0 and T_f. Histograms represent averages of all the cores taken at that time point, normalized to peak 61 to emphasize congener-specific changes. Some selective congener depletion is evident.

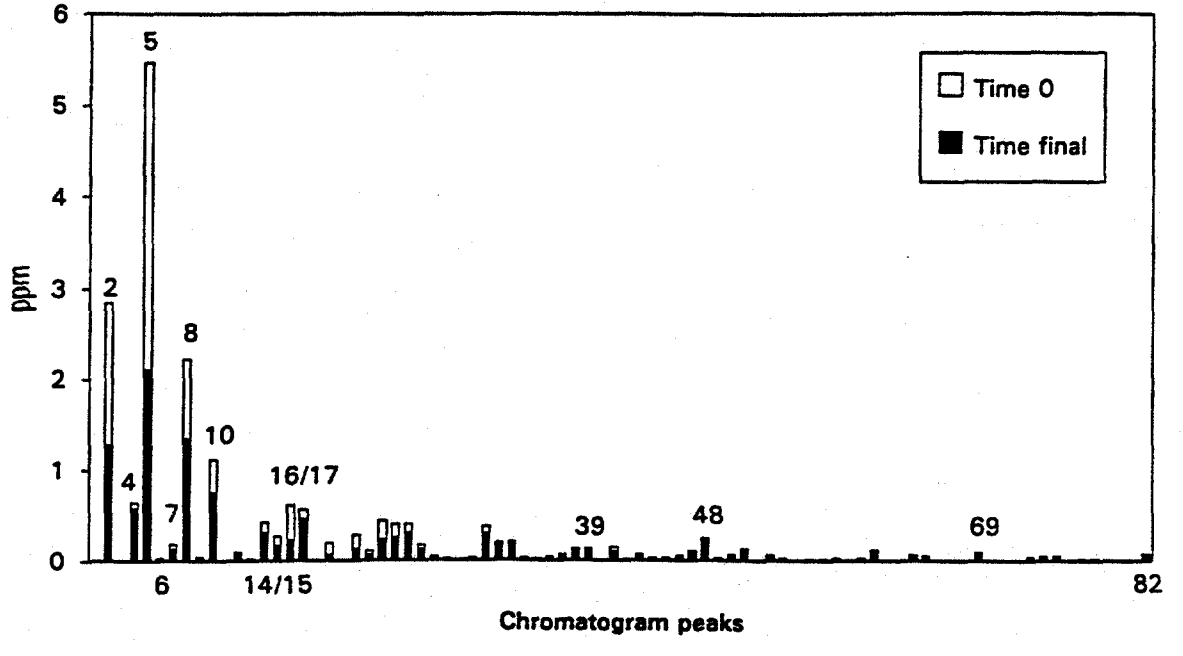


Figure 5-15C. Histogram showing peak-specific PCB concentrations in core samples from caisson R102 at T_0 and T_f. Histograms represent averages of all the cores taken at that time point, normalized to peak 61 to emphasize congener-specific changes. Selective congener depletion is evident.

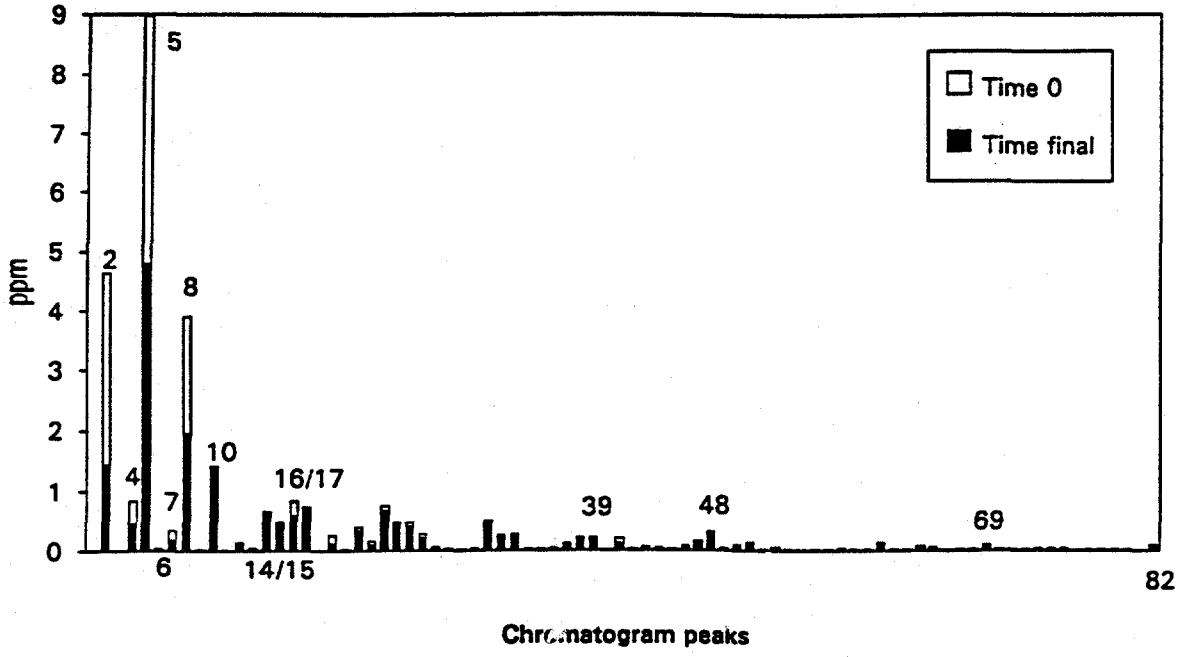


Figure 5-15D. Histogram showing peak-specific PCB concentrations in core samples from caisson R103 at T_0 and T_f. Histograms represent averages of all the cores taken at that time point, normalized to peak 61 to emphasize congener-specific changes. Selective congener depletion is evident.

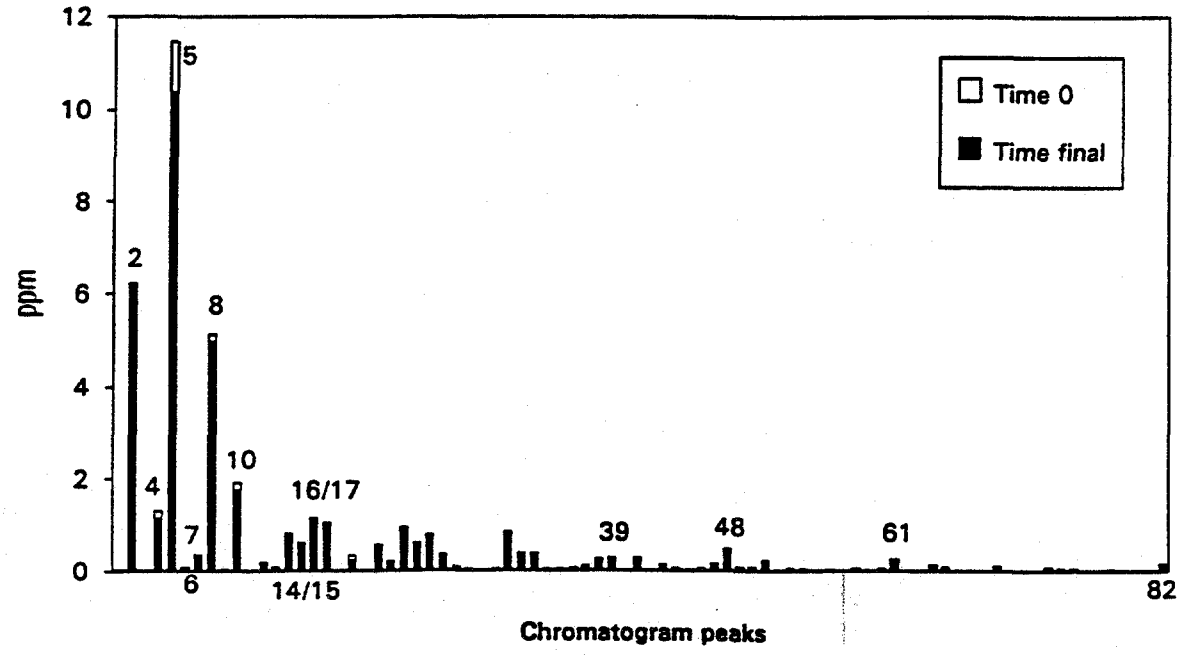


Figure 5-15E. Histogram showing peak-specific PCB concentrations in core samples from caisson R104 at T_0 and T_f. Histograms represent averages of all the cores taken at that time point, normalized to peak 61 to emphasize congener-specific changes. Little selective congener depletion is evident.

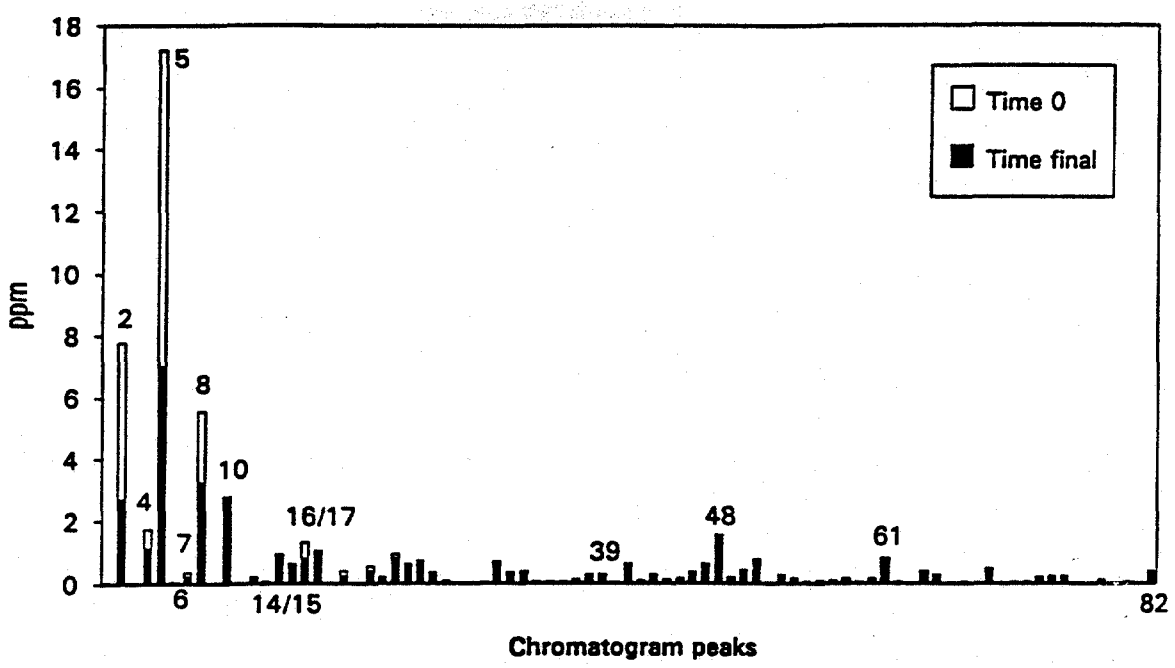


Figure 5-15F. Histogram showing peak-specific PCB concentrations in core samples from caisson R105 at T_o and T_f. Histograms represent averages of all the cores taken at that time point, normalized to peak 61 to emphasize congener-specific changes. Selective congener depletion is evident.

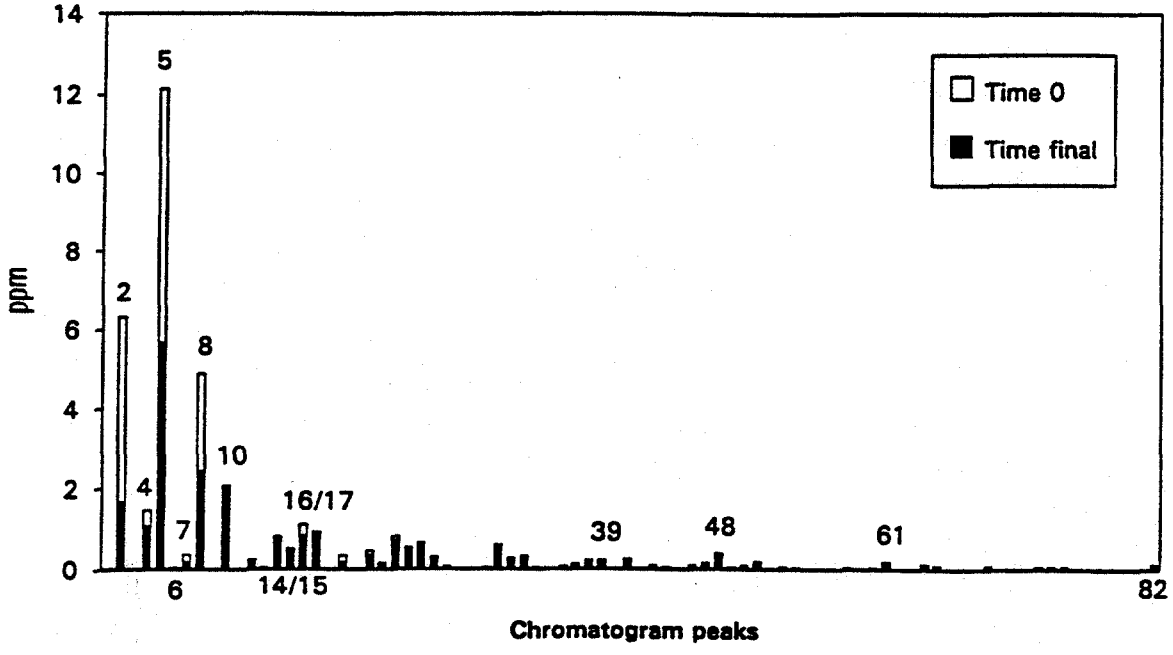


Figure 5-15G. Histogram showing peak-specific PCB concentrations in core samples from caisson R106 at T_o and T_f. Histograms represent averages of all the cores taken at that time point, normalized to peak 61 to emphasize congener-specific changes. Selective congener depletion is evident.

later in this chapter. For R104, changes in total PCB concentration without concomitant changes in congener distribution implies PCB redistribution, rather than biodegradation, occurred in that caisson.

The apparent loss of PCBs from R104 cannot be accounted for by volatilization, since these losses would have been highly congener specific, unlike the PCB distribution observed. In addition, PCB recovery from the XAD vapor traps from all the caissons was minimal relative to the PCB loading in the caissons. These results appear in Table 5-6. Surface wipes of the insides of the caissons after the experiment found inconsequential amounts of PCB (see Table C2-3 in Appendix C-2). In addition, PCBs also did not penetrate below the well-mixed zone of the caissons. Several samples of the sediment below that region were analyzed at both the beginning and end of the study. The results, which appear in Table 5-7, indicate that PCB concentrations in the lower zone generally did not increase in the rake caissons over the course of the experiment. The only elevations of these values occurred in the high-mix caissons, perhaps due to deeper or more complete mixing in those caissons.

It is likely that some of the PCBs in R104 remained associated with the suspended colloidal material in the caisson. This colloidal material represented a significant fraction of the mixed sediment in the low-mix caissons by the end of the experiment, and may not have been adequately sampled at T_f. Two of the caissons, R104 and R105, were re-sampled two weeks after the completion of the experiment. Samples were taken after the tops and mixers had been removed from the caissons and all material adhering to the rake mechanisms had been washed back into the caisson with water. Six samples were taken from each caisson at this time. Under these conditions more of the colloidal material had settled and was available for sampling.

The results of this subsequent analysis are included in Table 5-5. In caissons R104 and R105 the average PCB concentration measured was higher under these conditions than at the T_f sampling. The average PCB concentration and total PCB per core of samples obtained at this later sampling time from R104 were comparable to those values obtained at T_o in

Table 5-6
All Non-zero PCB Vapor Trap Analyses

Time	Trap #	ppm PCB	Trap #	ppm PCB
1 week	R101 T	0		
	R101 M	0.10		
	R101 B	0.06		
4 weeks	R101 T	0.15	R104 T	0
	R101 B	0.21	R104 M	0
			R104 B	1.12
7 weeks	R101 T	0	R106T	0
	R101 M	0.02	R106 M	0
			R106 B	0.33
10 weeks	R102 T	0.02		
	R102 M	0.24		
	R102 B	0		

Key: T = Top trap (furthest downstream)
M = Middle trap
B = Bottom trap (nearest to caisson)

Table 5-7
PCB Analysis of Sediment below the Well-Mixed Zone
(results are represented as averages +/- one standard deviation)

Caisson	N	To	N	Tf
R101	5	0.35 +/- 0.19	3	2.04 +/- 0.81
R102	2	1.72 +/- 1.42	4	6.62 +/- 2.68
R103	0	—	3	3.18 +/- 1.99
R104	6	12.4 +/- 2.8	6	11.9 +/- 12.0
R105	1	21.1	3	4.27 +/- 0.70
R106	2	75.8 +/- 46.5	5	2.86 +/- 1.77

that caisson, indicating significant amounts of PCB had settled from the water column or off the rakes and were now present in the sediment. Some variability is expected in these numbers, since the caisson was not remixed prior to sampling. However, this data suggests the mass balance in R104 could be closed, given adequate time for all the colloidal material in the caisson to settle. The average PCB concentration and core totals obtained in this later sampling were lower in R105 than at T_o, reflecting the effect of biodegradation in that caisson.

It is difficult to overstate the importance of the colloidal sediment fraction in the PCB analysis of these caissons. This material contained a large percentage of the clay and silt fractions of the sediment, both generally high in organic content. It is likely that a large percentage of the PCBs in the sediment resided there as well, since these molecules are known to be strongly associated with these fractions in natural systems [Means et al., 1980; Chiou et al., 1983].

In order to test this premise several core samples were removed from each caisson during the T_f sampling and used to generate a PCB profile as a function of depth and sediment fraction within each core. These samples were removed from the caissons and capped, with great care taken not to disturb the sediment layers during sampling and transport from the site. The samples were then allowed to stand undisturbed for several days to allow the colloidal (floc) layer to settle. Once settling had occurred, the clear water layer was discarded and the floc layer collected. Since a clean separation was not possible between the floc and the sand, an "interface" layer was also taken at this point. The remaining sandy fraction in the core was separated into 6 - 10 cm sections and saved as individual samples. The individual subsections were then air-dried at room temperature, weighed, homogenized, soxhlet extracted, and analyzed for PCB content. Subsamples of the extracted sediment were also dried and heated to 550°C in an oven to combust the organic matter so that an approximate value for the total organic carbon (TOC) could be obtained.

PCB concentrations, sample dry weights, and TOC content for each subsection are summarized in Table 5-8. The PCB concentrations are represented

Table 5-8
Core Subsection Analyses

Caisson		Floc	Interface	Top-sand	Mid-sand	Bottom-sand
R101	PCB (ppm)	16.5	10.0	0.7	2.6	0.4
n=2	Section wt (g)	35.4	59.6	175.3	130.0	119.4
	TOC (%)	3.82	2.59	0.68	1.16	2.67
R102	PCB (ppm)	23.5	23.7	2.1	2.6	-
n=2	Section wt (g)	65.3	75.5	137.4	110.5	-
	TOC (%)	4.95	2.71	1.21	4.90	-
R103	PCB (ppm)	35.7	16.3	8.6	7.1	0.2
n=3	Section wt (g)	126.4	178.9	184.7	209.5	209.3
	TOC (%)	4.27	1.93	2.90	1.50	1.36
R104	PCB (ppm)	95.4	12.0	9.1	1.0	0.9
n=3	Section wt (g)	115.7	104.2	303.4	265.0	215.8
	TOC (%)	4.24	0.55	0.89	2.22	0.94
R105	PCB (ppm)	43.1	20.8	5.6	5.3	-
n=3	Section wt (g)	104.9	82.5	164.7	258.5	-
	TOC (%)	4.24	1.77	0.45	0.47	-
R106	PCB (ppm)	34.0	5.2	2.4	5.2	-
n=2	Section wt (g)	147.9	96.7	228.2	205.6	-
	TOC (%)	3.34	0.59	0.45	0.36	-

Note - % TOC = 0.5 (% weight loss via combustion)

graphically in Figure 5-16. Multiple analyses from the same caisson have been averaged in these summaries. The amount of colloidal material formed was substantially greater in the rake caissons than in the turbine-mixed systems (124 grams on average versus 50.4 grams). The lower amount of floc in caissons R101 and R102 was probably due to the less abrasive nature of the axial-flow turbine mixers. As expected, the largest amounts of total organic carbon and the greatest concentrations of PCBs were generally associated with the floc layer. The amount of PCB generally decreased rapidly with increasing depth into the sand. Caisson R104, the rake control, showed a significantly higher level of PCBs in the colloidal layer than did the other rake caissons. Given that caissons R104, R105, and R106 began the experiment with comparable PCB concentrations, Figure 5-16 suggests that PCB biodegradation in the colloidal phase alone may have been as high as 55-65% in the experimental caissons.

Subsequent to this analysis all T_0 and T_f sediment samples were homogenized, subsampled, and sent out to an external analytical laboratory for solid phase total organic carbon (TOC) analysis. This was done in an effort to obtain a more reliable basis to compare the PCB data. Given that PCBs reside in the organic carbon phase of the sediment and that this phase is more strongly associated with the colloidal fraction, expressing PCB concentration on a total organic carbon basis (mg PCB/g TOC) should be less prone to sampling bias and a better representation of the actual PCB biodegradation in the system.

These results are summarized in Table 5-9. Sediment TOC averages at T_0 correlate with average PCB concentrations in the caissons. R101 and R102 were lowest in TOC, followed by R103. The other caissons are grouped at a higher TOC level. Not surprisingly, at T_f the average TOC content in caissons R104, R105, and R106 dropped substantially, indicating that the colloidal fraction was not adequately sampled at this stage due to the long settling time of this material. The average TOC content in caissons R101, R102 and R103 appeared to increase over the course of the experiment. The most significant increase was in R101. This increase appeared to result from the incorporation of additional organic material from under the well-mixed zone into these samples. This material could be seen visually upon drying and

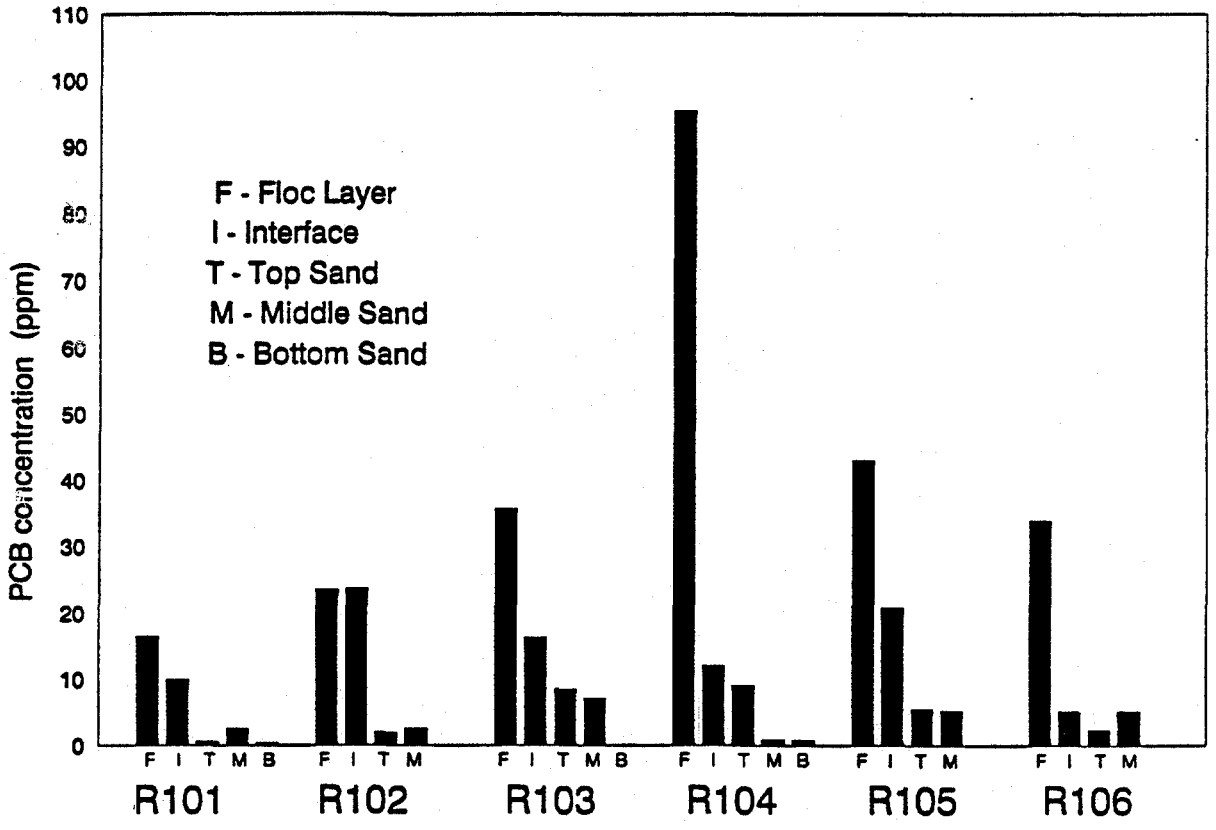


Figure 5-16. PCB concentration as a function of sediment fraction and depth in sediment cores from caissons R101-R106.

Table 5-9
PCB Analysis on a Total Organic Carbon Basis
(results are represented as averages +/- one standard deviation)

Caisson	N	TOC (ppm)	PCB/TOC (mg/g)	% Change
R101 - T_o	12	12,000 +/- 4600	0.56 +/- 0.24	
R101 - T_f	12	23,600 +/- 12,500	0.39 +/- 0.29	-30.7% *
R102 - T_o	12	14,300 +/- 4400	1.35 +/- 0.39	
R102 - T_f	12	16,500 +/- 8000	0.75 +/- 0.36	-44.7%
R103 - T_o	12	18,700 +/- 7100	1.[illegible] +/- 0.65	
R103 - T_f	12	24,600 +/- 5400	0.79 +/- 0.19	- 55.5%
R104 - T_o	12	24,200 +/- 7000	1.6[illegible] +/- 0.40	
R104 - T_f	12	14,300 +/- 2800	1.80 +/- 1.14	+ 8.4% *
R105 - T_o	11	22,300 +/- 9100	2.10 +/- 0.58	
R105 - T_f	10	13,700 +/- 5500	0.98 +/- 0.20	- 53.1%
R106 -T_o	12	21,000 +/- 4500	1.83 +/- 0.72	
R106 -T_f	12	12,300 +/- 2700	0.99 +/- 0.22	-46.0%

* These changes are not statistically significant at a 95% confidence limit.

homogenizing the samples. It was relatively low in PCB concentration. It is not clear what caused the increase in TOC content in R103.

TOC-based PCB values at T_o were lower for R101 than for the other caissons, consistent with the lower average PCB concentrations measured at T_o in those caissons (see Table 5-5 for a comparison). Significant reductions in TOC-based PCB averages at T_f occurred in all the experimental caissons, with losses ranging from 45-55%. Changes in TOC-based PCB values in control caissons R101 and R104 were not statistically significant at a 95% confidence level. Changes in R101 were attributable primarily to the increase in average TOC in that caisson. These results appear to more accurately reflect the

changes in congener distribution evident from viewing the chromatograms and aid substantially in attempting to quantify those changes.

Congener-Specific Analysis

The difficulties in establishing the extent of *in situ* biodegradation in field studies by establishing mass balance and separating biotic from abiotic loss processes are widely recognized. Much work is being done to develop criteria by which evidence of *in situ* biodegradation can be generated [Madsen, 1991]. One approach to address this issue is to use the ratio of easily degraded compounds to recalcitrant compounds in a contaminant mixture as an indication of the extent of biological losses that have occurred [Atlas, 1981]. Changes in this ratio can be used to determine losses that are directly attributable to biological activity. This approach was used most recently in work on the Valdez oil spill, where ratios of degraded C17 and C18 alkanes to more recalcitrant pristane and phytane were used to obtain evidence of *in situ* biodegradation of oil [Pritchard, 1991].

This same concept can be used to build a normalization procedure to study congener-specific losses in PCB mixtures. A congener peak in the PCB mixture known to be recalcitrant from previous laboratory studies was selected as a reference by which the losses in other congeners from biotic and abiotic processes were measured. The general criteria for such a reference peak is that it should be large and distinct enough to be easily quantitated, its component congeners should be similar to the other components of the mixture in physical/chemical characteristics, and it should not be degraded. Since almost all congeners in the 1242 commercial mix are subject to some aerobic biodegradative attack [Bedard, 1987], the resulting loss calculations based on changes in this ratio will be a conservative estimate of the actual extent of biological degradation in the system.

Several congeners believed to be relatively resistant to aerobic attack were chosen as candidates for the normalization peak. These included 236-4- (peak 39), 24-34- (peak 48), 34-34-/236-34- (peak 61) and 234-245-/2356-34- chlorobiphenyls (peak 82). The normalization was carried out for the PCB

distribution in each caisson by calculating the average PCB concentration of the selected reference peak at T_0 and assuming this concentration did not change over the course of the experiment. If this concentration changed in any sample during the experiment, a multiplier was generated to bring this peak to its T_0 concentration. All the other PCB peak concentrations in the sample were then multiplied by the same factor and summed to generate a normalized total PCB concentration for that sample.

This procedure was applied to the T_f data, with the results appearing in Table 5-10 and Figure 5-17. Peak 61 is the largest of the group of reference peaks identified and was used for most of our analyses. All the changes shown in Table 5-10 using this reference peak were statistically significant at the 95% confidence level, with the exception of those changes in R104. A 4% loss of PCBs was observed in this caisson. Losses in the experimental low-mix caissons R103, R105, and R106 all converge to about 40% in this analysis. R102 showed the highest PCB losses of any of the experimental caissons (42%). A higher PCB loss was observed in R101 than in R104 using this method of analysis. This difference was statistically significant and suggests that some aerobic PCB-degradative activity may have occurred in R101, since strict anaerobic conditions were not maintained in that caisson during the experiment.

Table 5-10
Change in PCB using Various Normalization Reference Peaks
(Losses expressed on a weight % basis)

	absolute	pk 10	pk 61	pk 82	pk 39	pk 48
R101	+9.3	-6.9	-14.4	-5.2	-12.5	-6.9
R102	-41.2	-14.6	-42.4	-38.7	-33.8	-32.3
R103	-36.7	-35.5	-37.8	-33.3	-36.5	-32.9
R104	-42.1	+4.4	-4.3	+2.5	-2.7	+0.0
R105	-72.3	-42.4	-40.5	-36.7	-39.0	-36.7
R106	-68.5	-45.5	-38.7	-33.2	-34.7	-33.3

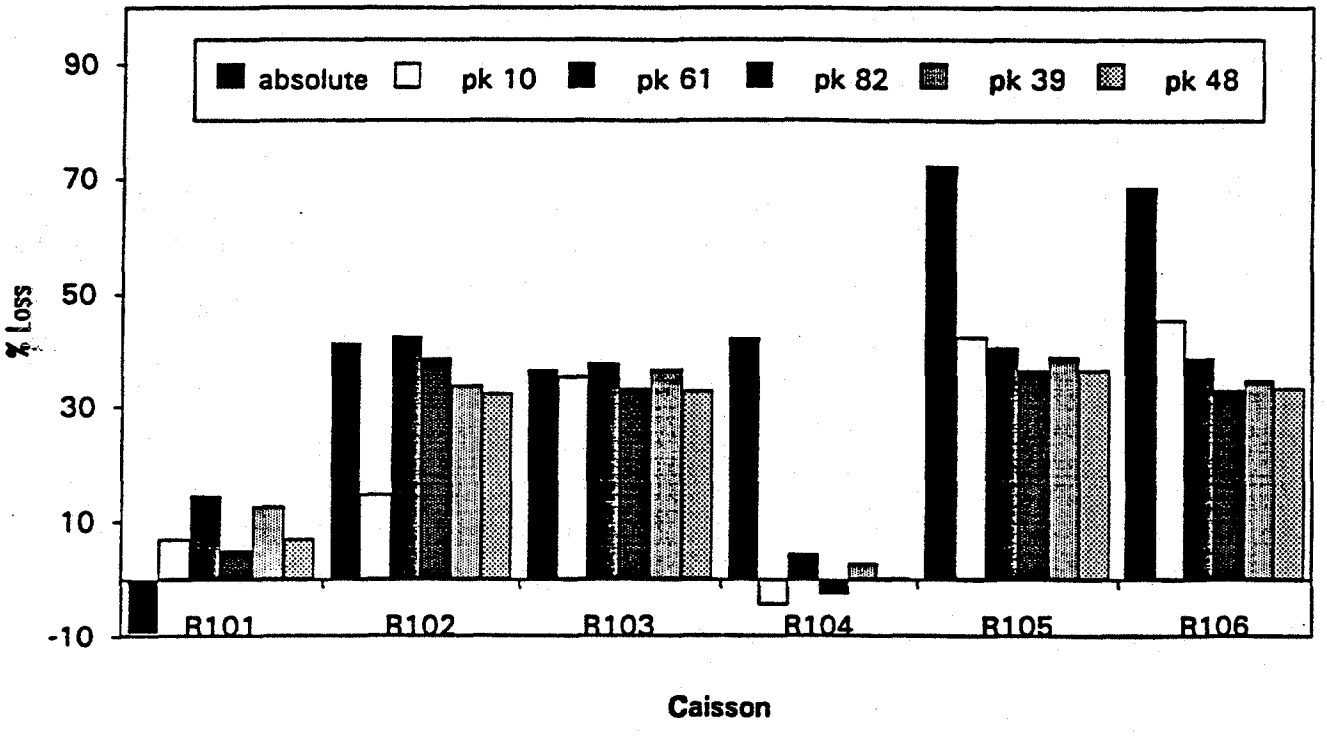


Figure 5-17. The effect of reference peak selection on the % loss of PCB for normalized data. Peaks were selected as normalization reference peaks based on their recalcitrance to degradation in laboratory studies. Only caisson R102 seems significantly effected by the choice of peak 10.

Other peaks selected *a priori* for this procedure (peaks 39, 48, and 82) gave very similar results, as shown in Table 5-10 and Figure 5-17. The similar results observed with all three internal standard peaks validate this approach. The effect of selecting a more degradable internal standard peak can be demonstrated using peak 10 (26-2-chlorobiphenyl). In R102 PCB loss was calculated at only 14% relative to peak 10, a significantly lower loss than generated when any of the other preselected peaks were used for normalization. This difference emerges because 26-2-chlorobiphenyl was degraded to a significant degree in R102, causing the normalized losses to be underestimated in that caisson. This was not true in the other experimental caissons, where peak 10 normalization gave comparable results to other peak selections. This is representative of the wider ranging PCB degradation pattern observed in R102, in which a significant number of the trichlorobiphenyl congeners were attacked (see Figure 5-15C). While this wider degradative competence may have been due to the presence of H850 in R102, it was also possible that the different environment created in that caisson due to better mixing and lower pH stimulated an indigenous PCB-degrading population with broader congener specificities.

It should be noted that the initial PCB distributions in the six caissons were similar, but not identical. The chromatogram of R101 shows that its sediments had undergone less dechlorination prior to the experiment than the sediment in the other caissons. This was true to a lesser extent in R102. This was not unexpected, since it had been observed previously that sediments with lower PCB concentrations typically show less *in situ* dechlorination than when the contamination is at a much higher level. The fact that dechlorination was less advanced in R102 at the outset of the experiment implies that the potential for aerobic biodegradation was lower relative to the other caissons, which started out with a higher percentage of more easily degradable mono- and dichlorobiphenyls. The extent of biodegradation might have been higher than observed had more readily degradable PCB congeners been present in larger quantities.

Rate Data

The rate at which PCBs were degraded in the caissons during the river study can be calculated in several ways. If the absolute PCB concentrations from the caissons are used directly to make this determination, the rates calculated will include loss of PCB due to redistribution in the caissons as well as from biological degradation. A more robust means of calculating the rate of PCB loss is to use the normalized data, which is not affected by the redistribution problem. It must be noted, however, that since normalization yields a conservative estimate of PCB losses, rates generated from this data will also be conservative.

For each time point in a caisson, the normalized PCB levels were averaged and these averages were fitted to a simple linear equation relating PCB concentration to time. Rates of PCB loss were approximated by the slope of the lines.

The normalized data and fitted curves using the linear approximation are presented in Figures 5-18A through 5-18F. The rates derived from these approximations are shown in Table 5-11. The PCB losses from caissons R101-R104 are adequately described by a single linear equation. In R105 and R106 there are two loss regimes and two linear equations are required to fit the data. In R105 PCB was lost at a rate of 0.48 ppm/day for the first six weeks of the study, after which the rate dropped to 0.044 ppm/day. A similar result was obtained in R106, where losses went from 0.28 ppm/day to 0.017 ppm/day at about seven weeks into the experiment. In both cases the change in rate came after 35-40% of the PCB in these caissons had been degraded.

The change in rate may correspond to a change in the bioavailability of the PCBs remaining in the sediment, as had been observed in laboratory desorption experiments (see Figure 4-13). The first rate equation observed in R105 and R106 presumably represents biodegradation of the labile PCB fraction, whereas the second equation represents biodegradation of the resistant fraction. In the laboratory, the break between labile and resistant fraction occurred when about 50% of the PCBs had been removed from H7

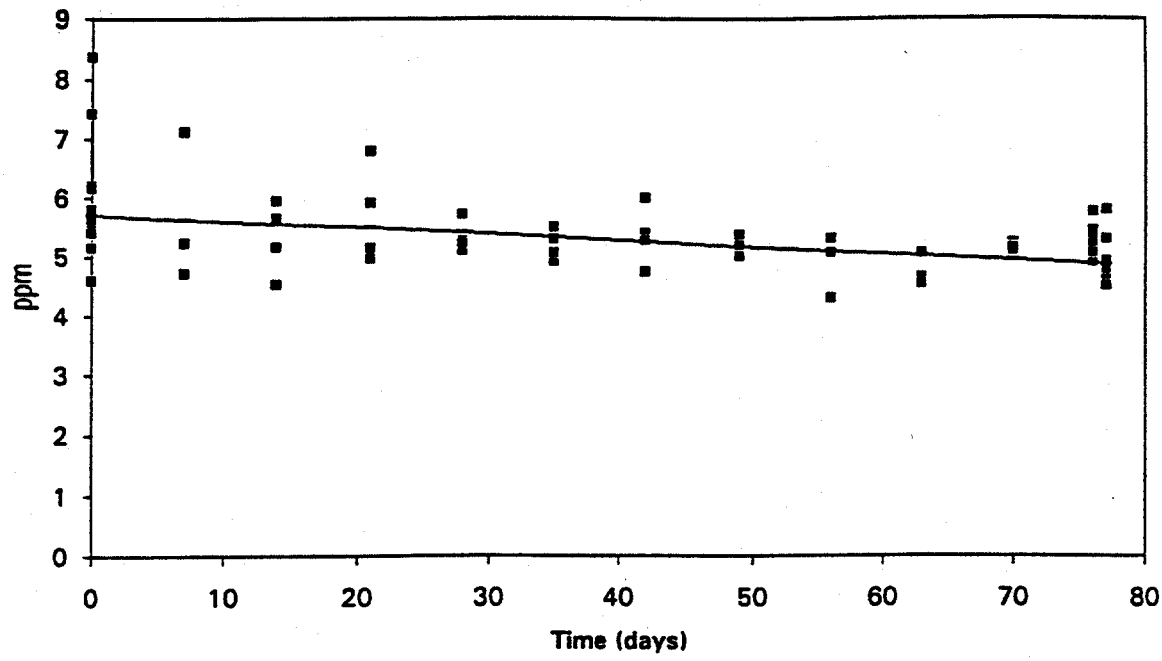


Figure 5-18A. PCB loss versus time in caisson R101. The rate of loss was fit by linear regression through the average data normalized to peak 61.

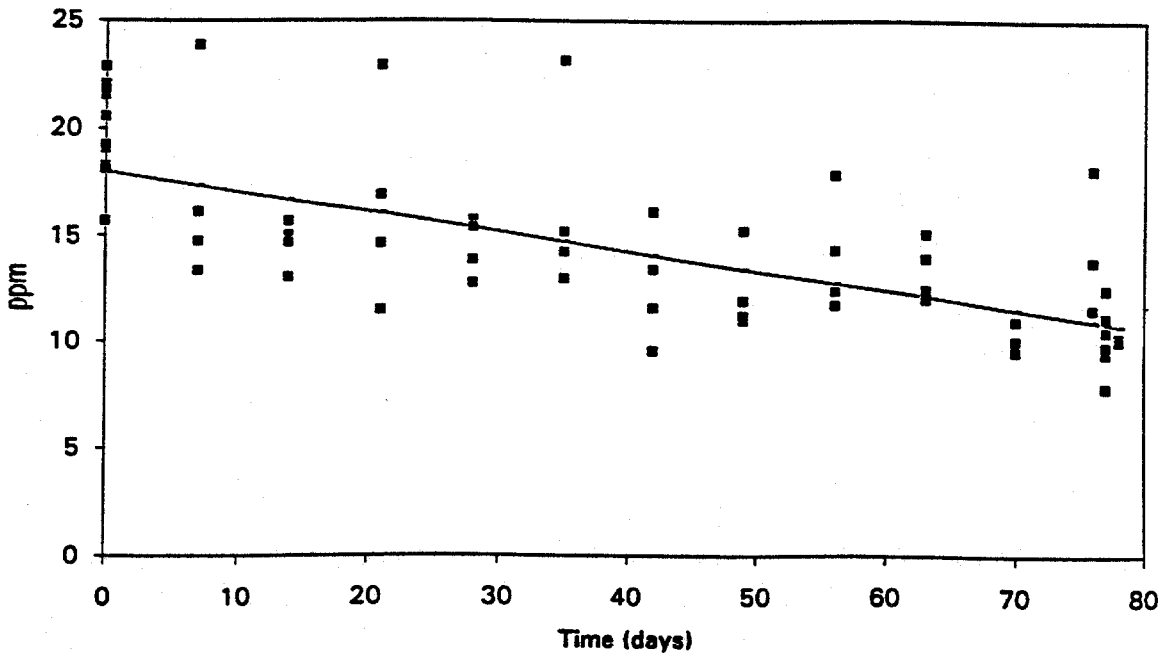


Figure 5-18B. PCB loss versus time in caisson R102. The rate of loss was fit by linear regression through the average data normalized to peak 61.

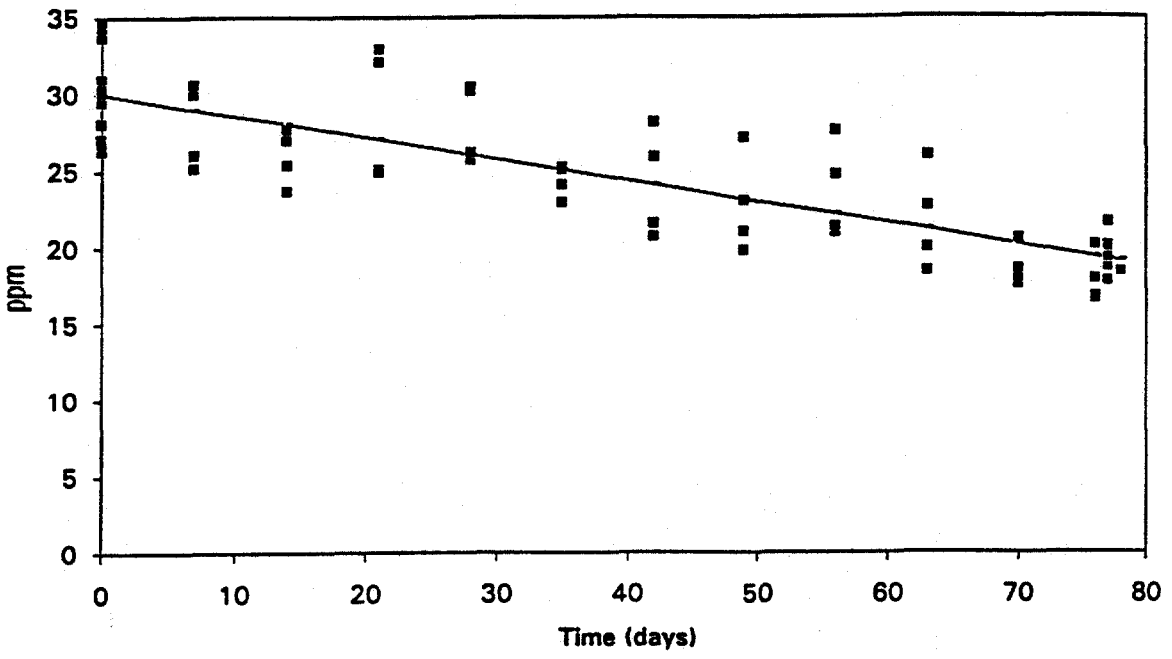


Figure 5-18C. PCB loss versus time in caisson R103. The rate of loss was fit by linear regression through the average data normalized to peak 61.

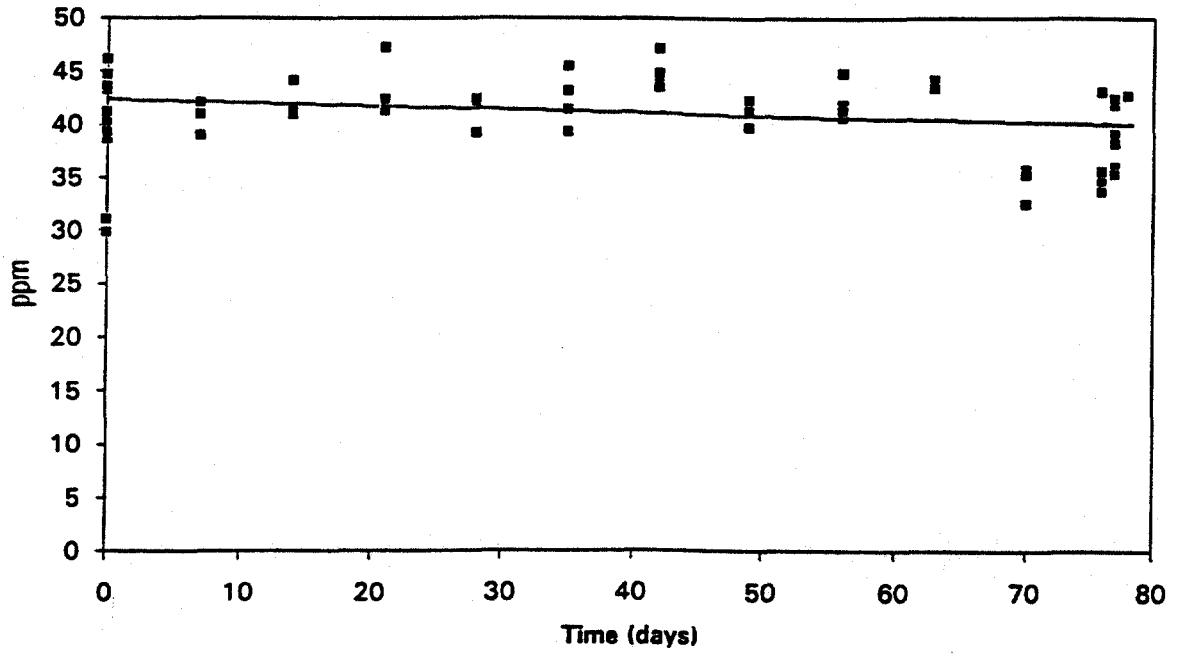


Figure 5-18D. PCB loss versus time in caisson R104. The rate of loss was fit by linear regression through the average data normalized to peak 61.

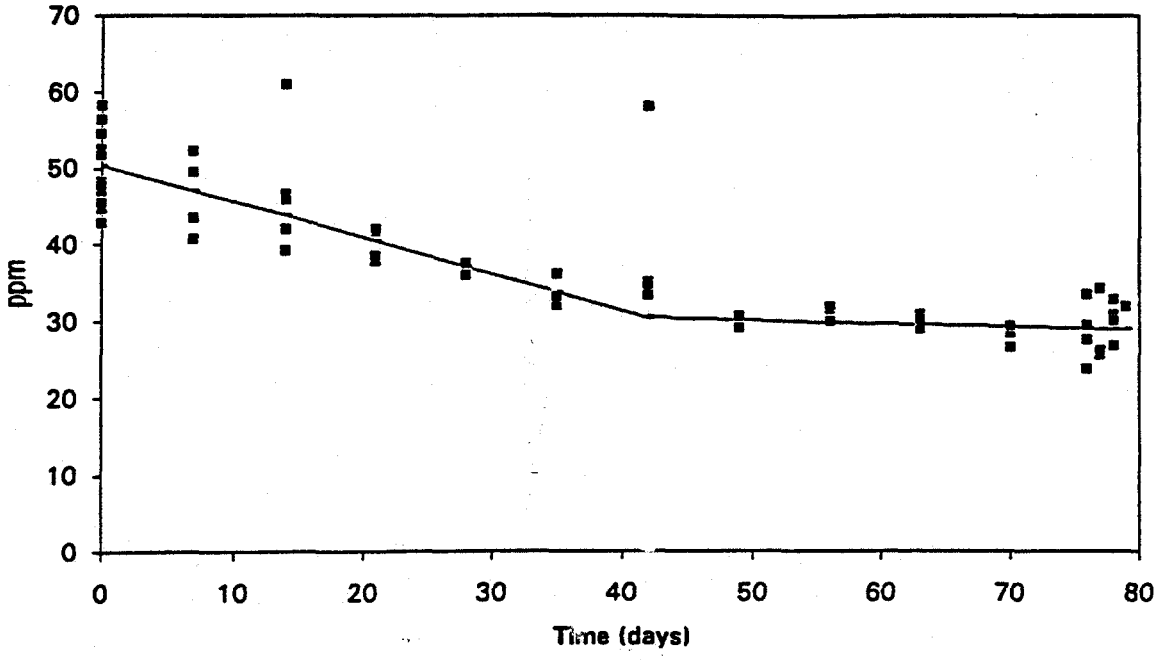


Figure 5-18E. PCB loss versus time in caisson R105. The rate of loss was fit by linear regression through the average data normalized to peak 61. R105 entered the second loss regime at about 6 weeks, most likely due to decreased bioavailability of the remaining PCBs.

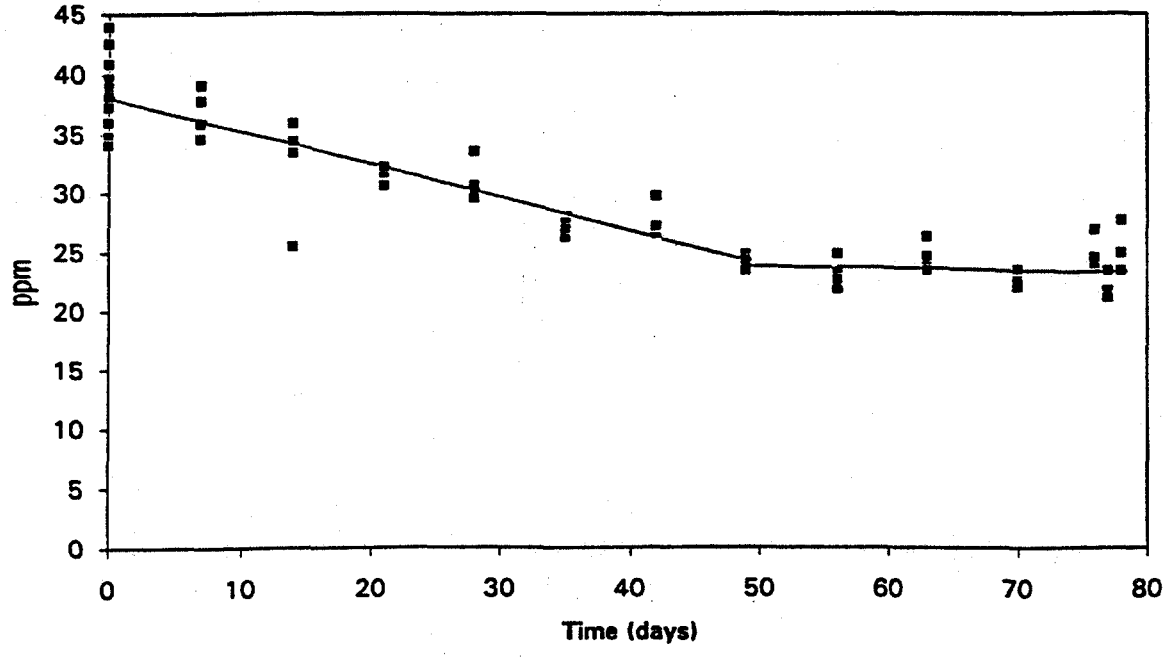


Figure 5-18F. PCB loss versus time in caisson R106. The rate of loss was fit by linear regression through the average data normalized to peak 61. R106 entered a second loss regime at about 7 weeks, most likely due to decreased bioavailability of the remaining PCBs.

Table 5-11
PCB Loss Rates

Caisson		Absolute Rate (ppm/day)	Relative Rate (day^{-1})
R101		-0.011	-0.0019
R102		-0.093	-0.0051
R103		-0.140	-0.0047
R104		-0.031	-0.0007
R105	initial	-0.478	-0.0095
	final	-0.044	-0.0014
R106	initial	-0.282	-0.0074
	final	-0.017	-0.0007

sediment with a comparable PCB content, in reasonable agreement with the caisson data.

The absolute rates of PCB loss observed in R102 and R103 were initially lower than in R105 and R106. This is partially a function of their lower initial PCB concentrations. In order to compare the rates of degradation in each caisson independent of starting concentration, relative rates of PCB loss were calculated by dividing the absolute rate loss by the starting PCB concentration in each loss regime. The results of these calculations appear in Table 5-11. The initial relative loss rates in R105 and R106 were 1.5-2.0 times faster than those in R102 and R103 by this analysis, although losses from all experimental caissons were substantially faster than the controls. Overall PCB losses from the caissons did not reflect these differences in initial rate because the rate of PCB loss in R105 and R106 changed during the experiment. PCB loss rates of the resistant PCB fraction in these caissons were much slower than for the labile fraction, and caused the average overall loss rates for the experiment to be reduced in these caissons. It appears that the transition point between labile and resistant PCB fractions may not have been reached in R102 and R103, although it is likely these caissons were approaching that point and had

the experiment gone long enough, would have shown a change in rate as well.

In order to address this issue a large inoculation of H850 was prepared and added to caisson R103 on October 24th, immediately following the completion of T_f sampling. 2.1 kg of H850 paste was added to R103 to bring the H850 population to 10^9 cfu/ml in the caisson. The mixing and peroxide systems were reactivated for an additional 3 days before the caisson was shut down again on October 27th. Sampling (an additional 12 core samples) was completed on October 28th after 24 hours of settling. The results are included in Table 5-5. There was no significant change in the PCB concentration within the caisson as a result of this large addition of H850, although a spike in chlorobenzoate was noted in the aqueous phase of the caisson following inoculation. This suggests the degradation rate was not primarily limited by biochemical factors at that time, but by bioavailability considerations..

Overall, all the PCB loss rates observed in the field study were 2-3 times slower than those observed in the laboratory. This applies to both the labile and resistant PCB fractions. These differences in loss rates may be attributable to differences in mixing between the field and the laboratory. Reaction vessels in the laboratory were smaller and mixing more complete than in the field, creating smaller length scales over which diffusion and other mass transport mechanisms acted. These impact both the delivery of oxygen and nutrients to the sediment and the desorption of PCB from it. Other variables, such as changes in temperature and PCB-degrading populations may also have contributed to the slower rates observed in the field. The lower temperatures observed in the caissons during the last quarter of the study undoubtedly slowed both physical transport and biochemical degradation rates. The decline in biphenyl-metabolizing organisms observed over the last several weeks of the field study may also have had an effect on biodegradation rates, although the degradation process was probably limited by bioavailability at that time.

Both control caissons did show small PCB losses during the experiment. The absolute rate of loss was higher in R104 than in R101 (0.0307 ppm/day versus 0.0113 ppm/day). However, on a relative basis the rate of

PCB loss in R101 was almost three times faster than in R104 (0.0019 day^{-1} versus 0.0007 day^{-1}). This is another indication that there was some aerobic biological activity in caisson R101.

The normalized data above can also be broken down into mono- and dichlorobiphenyl groups so that the rate of disappearance of these individual homolog groups can be monitored. Plots of this breakdown for all the caissons are shown in Figures 5-19A through 5-19F. In these figures average values of the the ratio of mono-, di-, and trichlorinated homologs to reference peak 82 are used to demonstrate selective attack. In R103, R105, and R106 there appears to be sequential attack of the mono- and then the dichlorinated PCB congeners. This degradation order is consistent with the higher solubility and lower sediment partitioning coefficients of the monochlorobiphenyls and was typically observed in laboratory studies. The ratio of trichlorinated congeners to peak 82 did not change substantially in these caissons. In R102 there was also a general reduction in mono- and dichlorobiphenyls. However, these losses were accompanied by a loss of trichlorinated congeners, particularly in the latter stages of the study. This data once again indicates that the biphenyl-metabolizing organisms present in R102 possessed a broader congener specificity than those in the other caissons.

Homolog specific changes in R101 and R104 were small and are generally obscured by the variability in the data. However, some loss of mono- and dichlorobiphenyls appears to be evident in R101.

Chlorobenzoate Analysis

Aerobic biodegradation of PCBs has been shown to proceed through a series of degradation intermediates, as shown in Figure 5-20 [reviewed in Abramowicz, 1990; Bedard, 1990]. The detection of one or more of these metabolic compounds during *in situ* biodegradation would establish a clear link between the observed loss of PCB and the induced microbiological activity. An analytical method was therefore developed to detect low concentrations of chlorinated benzoates [May et al., in preparation and Appendix B-1], which are metabolic intermediates in the PCB biodegradation

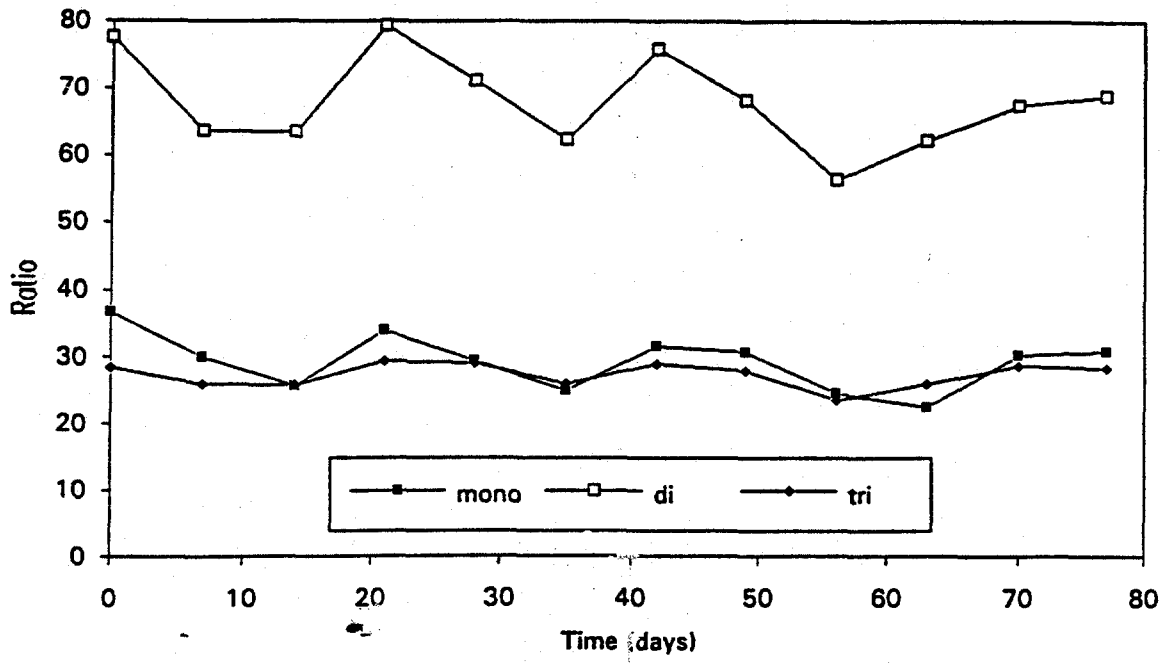


Figure 5-19A. Ratio of mono-, di-, and trichlorinated compounds referenced to peak 82 in R101. Little homolog-specific loss is apparent.

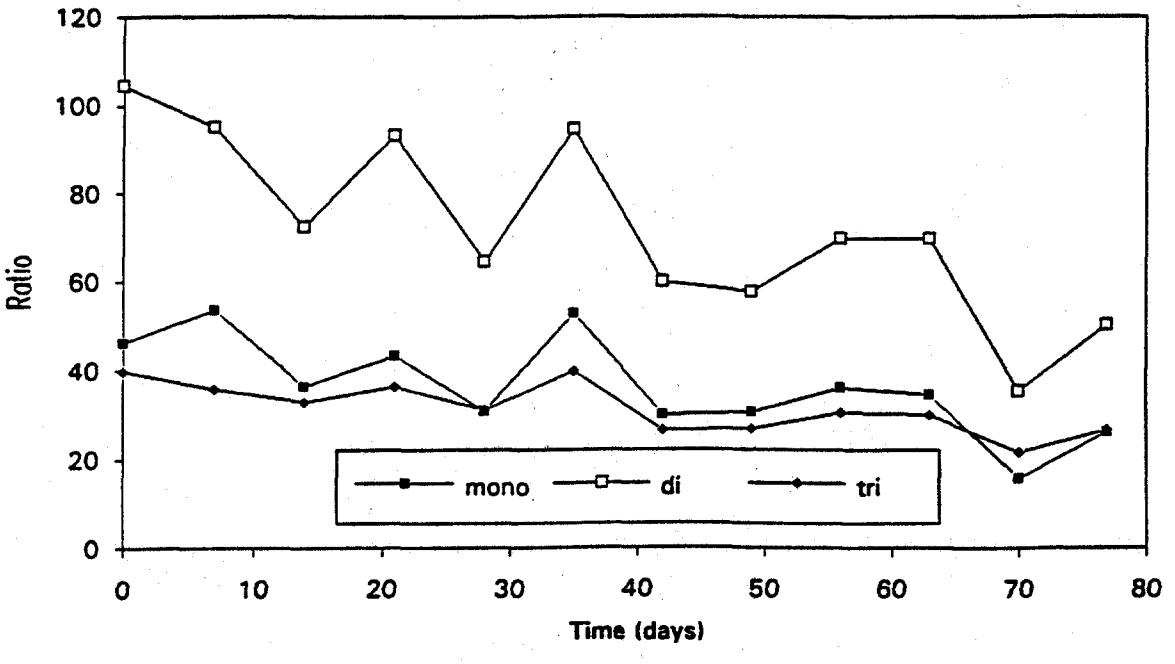


Figure 5-19B. Ratio of mono-, di-, and trichlorinated compounds referenced to peak 82 in R102. Significant loss of trichlorinated compounds is apparent only in R102.

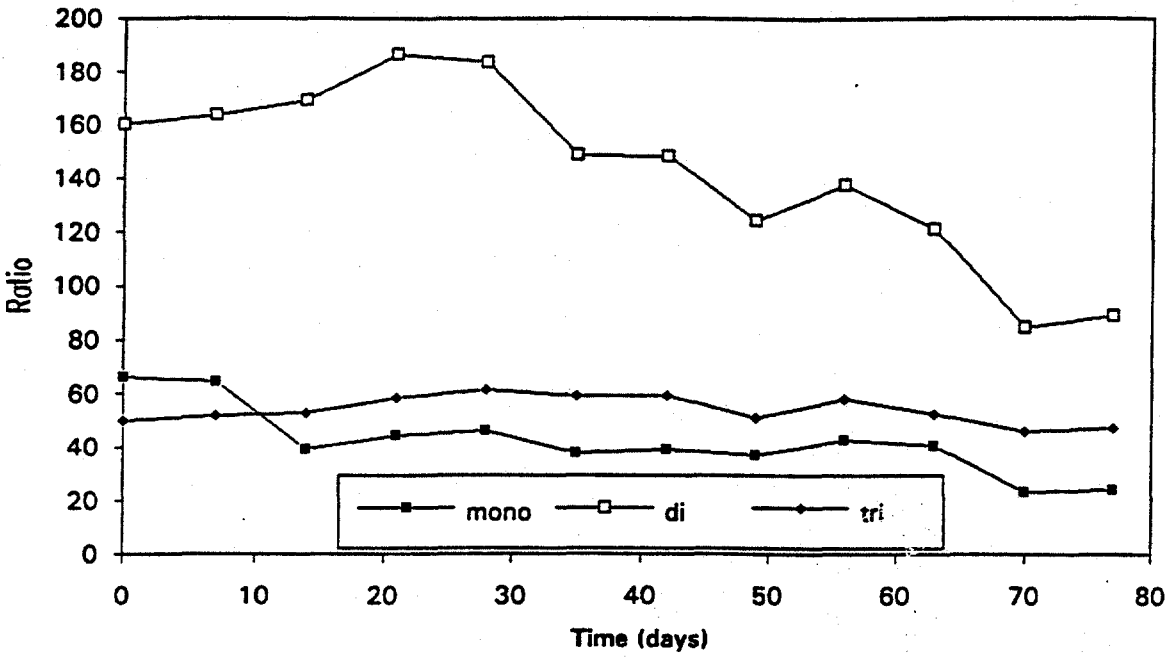


Figure 5-19C. Ratio of mono-, di-, and trichlorinated compounds referenced to peak 82 in R103. Sequential attack first on mono- followed by attack on dichlorinated congeners is apparent.

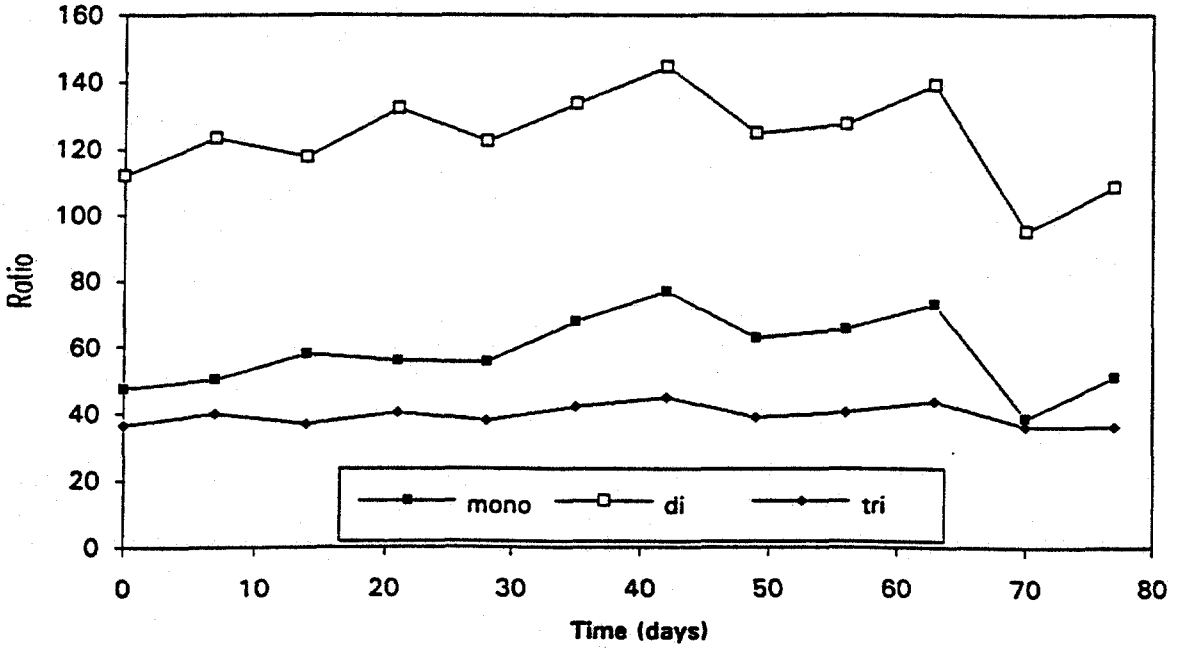


Figure 5-19D. Ratio of mono-, di-, and trichlorinated compounds referenced to peak 82 in R104. Little homolog-specific loss is apparent.

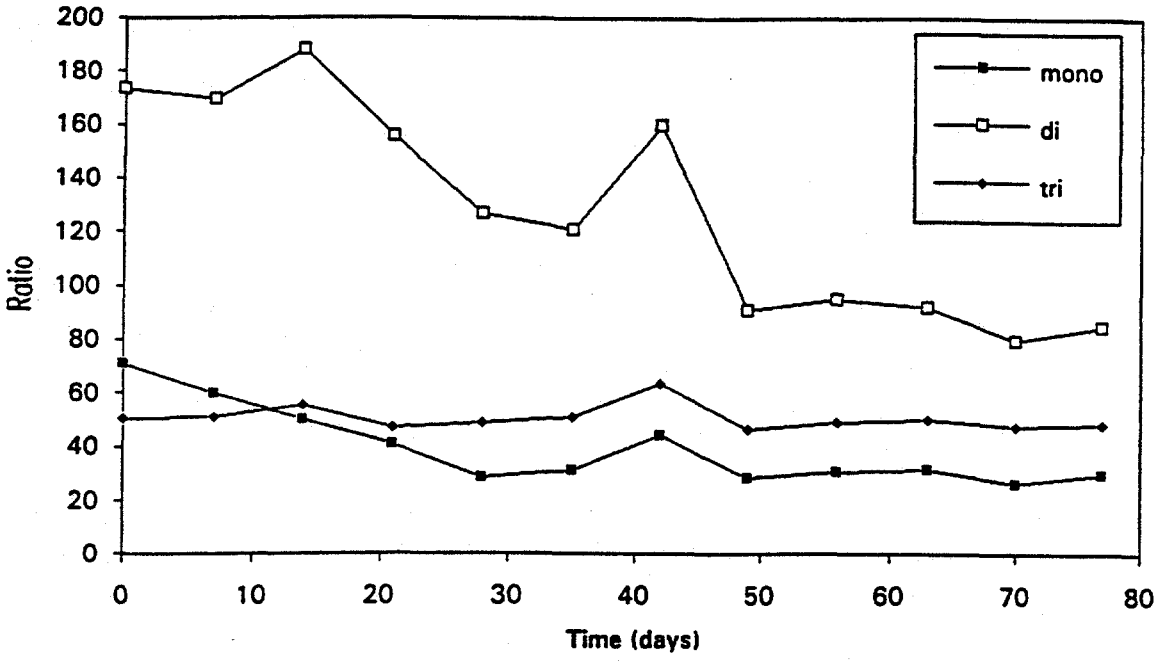


Figure 5-19E. Ratio of mono-, di-, and trichlorinated compounds referenced to peak 82 in R105. Sequential attack on mono- followed by attack on dichlorinated congeners is apparent.

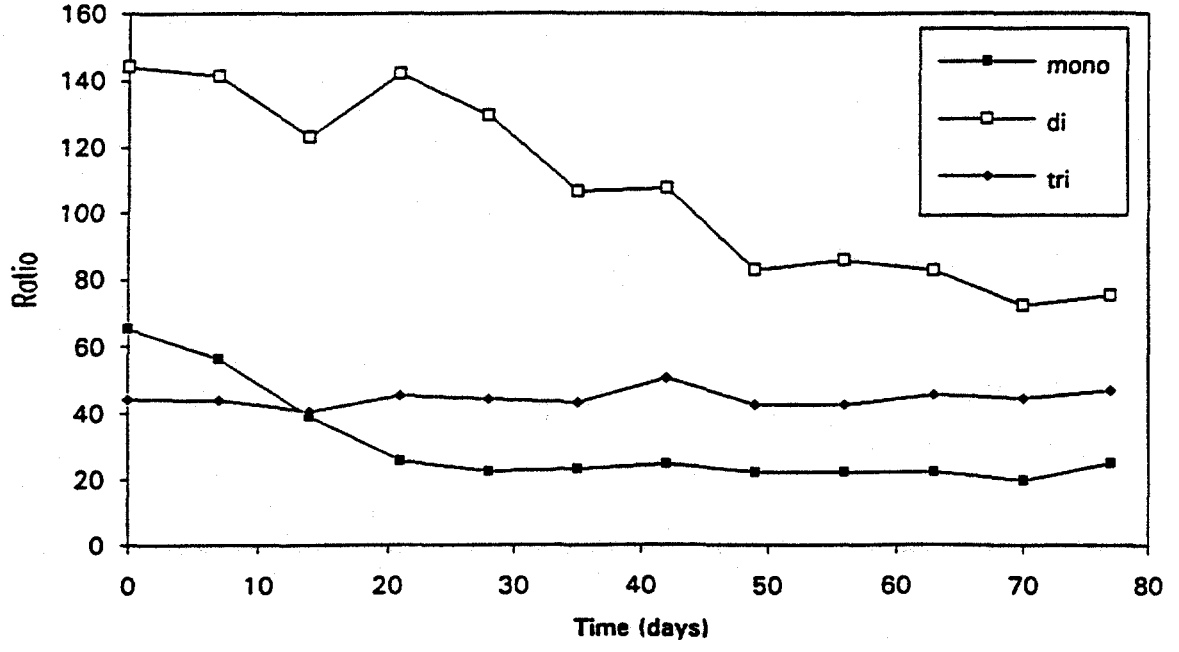


Figure 5-19F. Ratio of mono-, di-, and trichlorinated compounds referenced to peak 82 in R106. Sequential attack on mono-followed by attack on dichlorinated congeners is apparent.

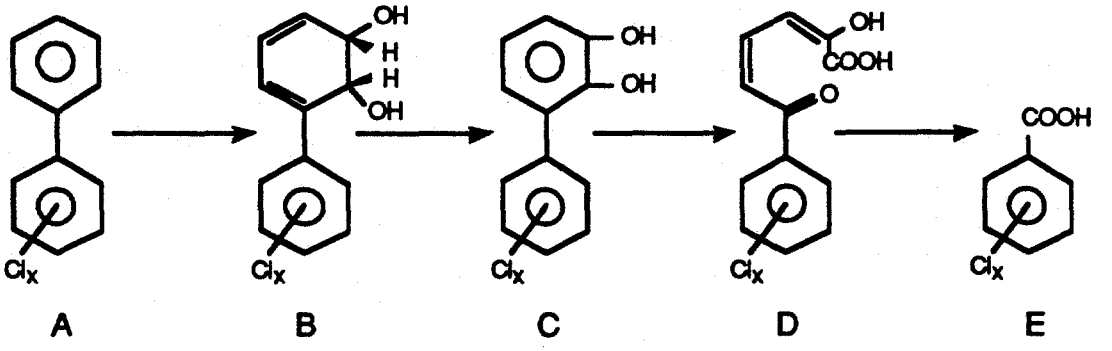


Figure 5-20. Degradation of chlorobiphenyls by the 2,3-dioxygenase pathway. A. polychlorinated biphenyl (PCB); B. chlorinated dihydrodiol; C. chlorinated 2,3-dihydroxybiphenyl; D. chlorinated 2-hydroxy-6-oxo-6-phenylhexa-2,3-dienoic acid; E. chlorinated benzoic acid. The chlorobenzoates (E) are further biodegraded to CO_2, H_2O, Cl^- and biomass by a variety of indigenous aerobic microorganisms present in Hudson River sediments.

pathway. Aqueous and sediment samples were collected from each caisson throughout the experiment and analyzed for chlorobenzoates. Attention focused primarily on the detection of mono- and dichlorobenzoates, since most of the PCBs in the H7 region were mono- and dichlorobiphenyls.

During the experiment, chlorinated benzoates were detected as transient intermediates in the aqueous phase of all of the experimental low-mix caissons R103, R105, and R106 (see Figures 5-21 through 5-23). Increased chlorobenzoate levels were not detected in the corresponding low-mix control, R104. The onset of the elevated chlorobenzoate levels coincided with the activation of water column mixers in each of the experimental caissons (day 7 in R103; day 10 in both R105 and R106). Earlier appearance of these intermediates may have been inhibited by insufficient aeration prior to this event. This observation was also coincident with increasing populations of indigenous biphenyl-metabolizing bacteria and depletion of biphenyl and monochlorinated biphenyls in those caissons during this time period. These

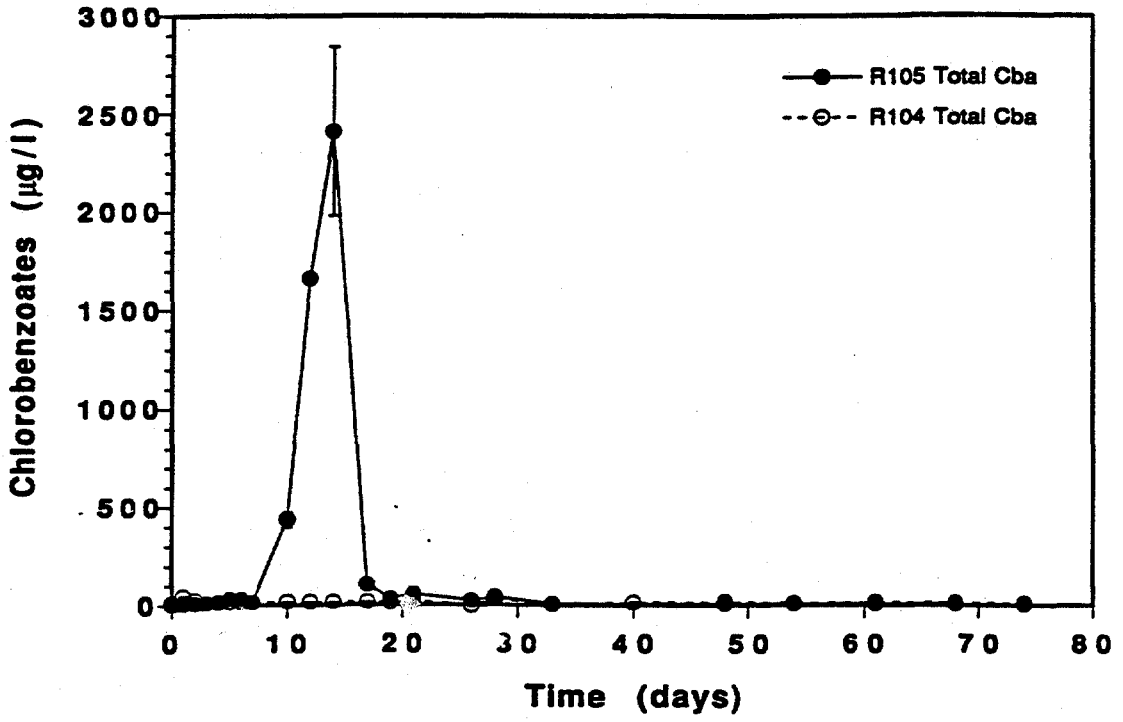


Figure 5-21. Total aqueous-phase chlorobenzoates in R105 compared to R104. Points represent average values for two analyses with ranges shown.

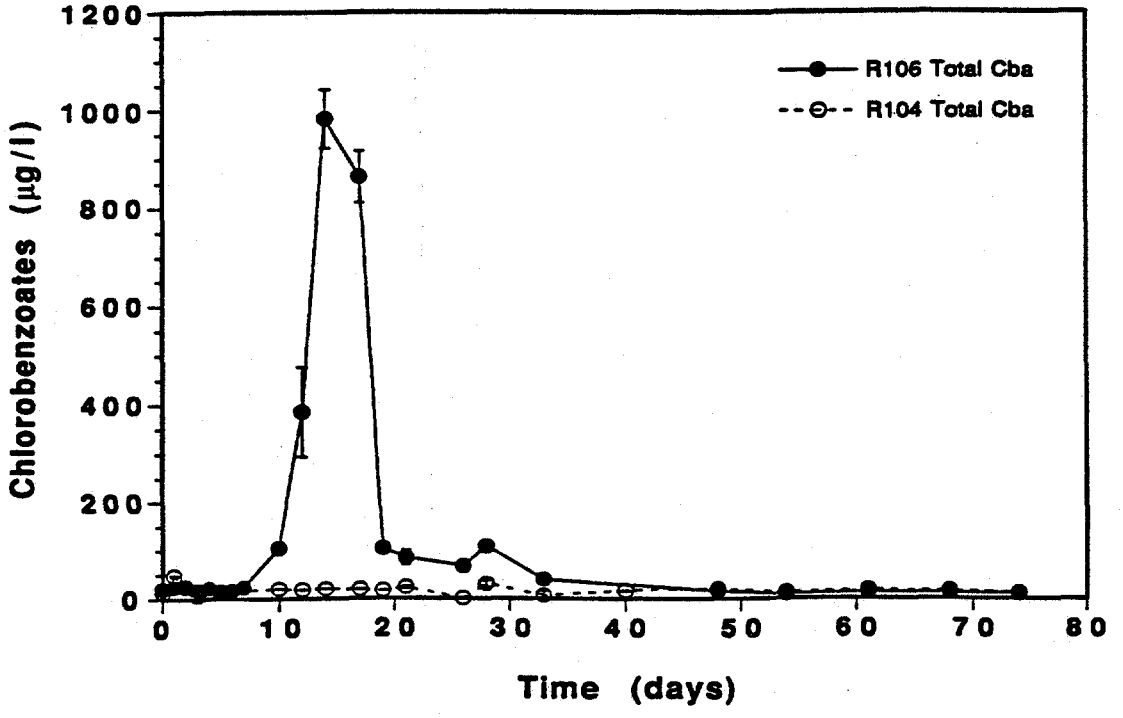


Figure 5-22. Total aqueous-phase chlorobenzoates in R106 compared to R104. Points represent average values for two analyses with ranges shown.

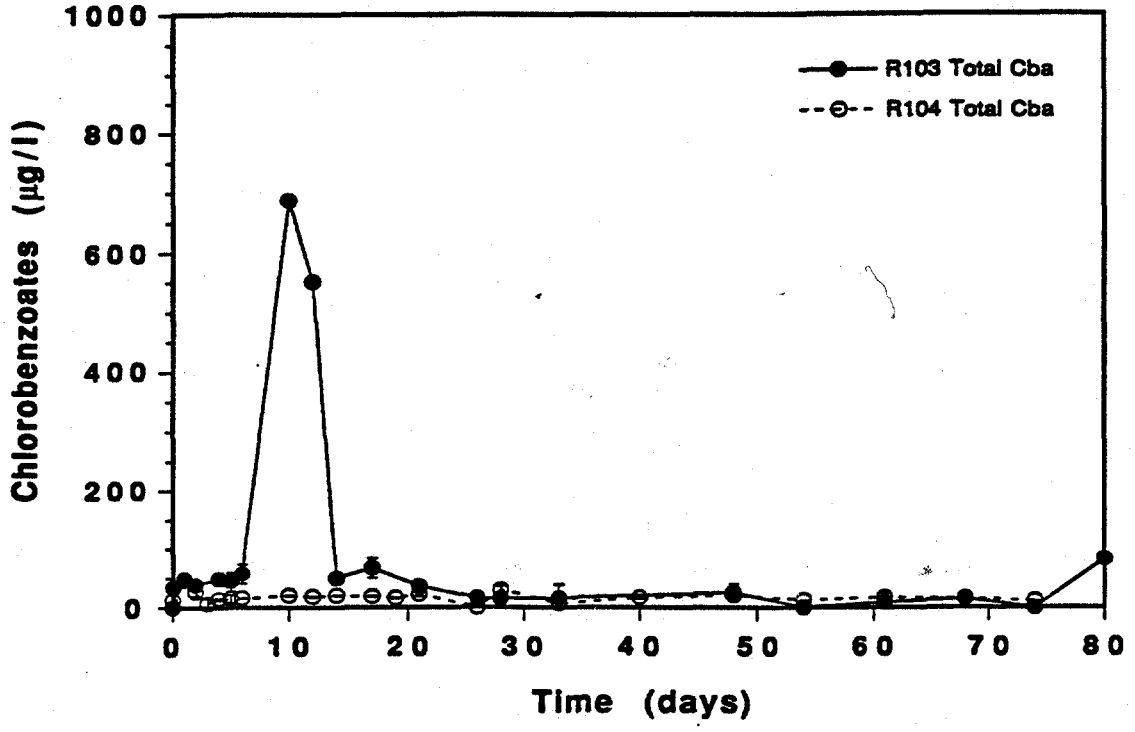


Figure 5-23. Total aqueous-phase chlorobenzoates in R103 compared to R104. Points represent average values for two analyses with ranges shown.

results provide strong qualitative evidence linking the loss of PCBs in these experimental caissons with the stimulated aerobic microbial biodegradation.

The transient nature of the detected chlorobenzoates was expected, since these intermediate compounds are further biodegraded to CO_2, H_2O, Cl^- and biomass by many naturally-occurring microorganisms [Hartmann et al., 1979; van den Tweel et al., 1987; Focht and Hickey, 1990; Hernandez et al., 1991]. Such aerobic chlorobenzoate-degrading microorganisms are present in Hudson River sediments as well. Laboratory studies had predicted the transient nature of the chlorobenzoate levels, particularly in the aqueous phase (data not shown). As a result, the caissons were sampled more extensively during the early stages of the *in situ* experiment in order to observe this transient phenomenon. It is suspected that elevated benzoate and chlorobenzoate levels stimulated growth of the chlorobenzoate-degrading microbial population, or induced increased rates of chlorobenzoate cometabolism by these microbes. The subsequent decreases in chlorobenzoate levels are thought to reflect a stimulation of chlorobenzoate biodegradation rather than a cessation of PCB biodegradation.

To demonstrate that chlorobenzoate-degrading organisms were present in Hudson River sediment and were able to degrade the chlorobenzoates produced from PCB biodegradation, a 1% inoculum of Hudson River sediment collected just north of the H7 site was examined for its ability to degrade benzoate, 3 monochlorinated benzoates, and 4 dichlorobenzoates. In a competition experiment, each substrate was included in PAS medium to a final concentration of 0.1 mM. A profile of chlorobenzoate concentration and chloride concentration vs. time is presented in Figure 5-24A. Both 3-chloro- and 4-chlorobenzoate are plotted as superimposed concentrations since they co-elute by the HPLC method of analysis. Clearly, the consortium of microorganisms selectively degraded these molecules, attacking benzoate first, then the monochlorinated benzoates, and finally the dichlorobenzoates. The chlorobenzoates were metabolized by the microbial consortia, with stoichiometric release of Cl^- (Figure 5-24B). Autoclaved controls did not degrade benzoate or chlorobenzoates.

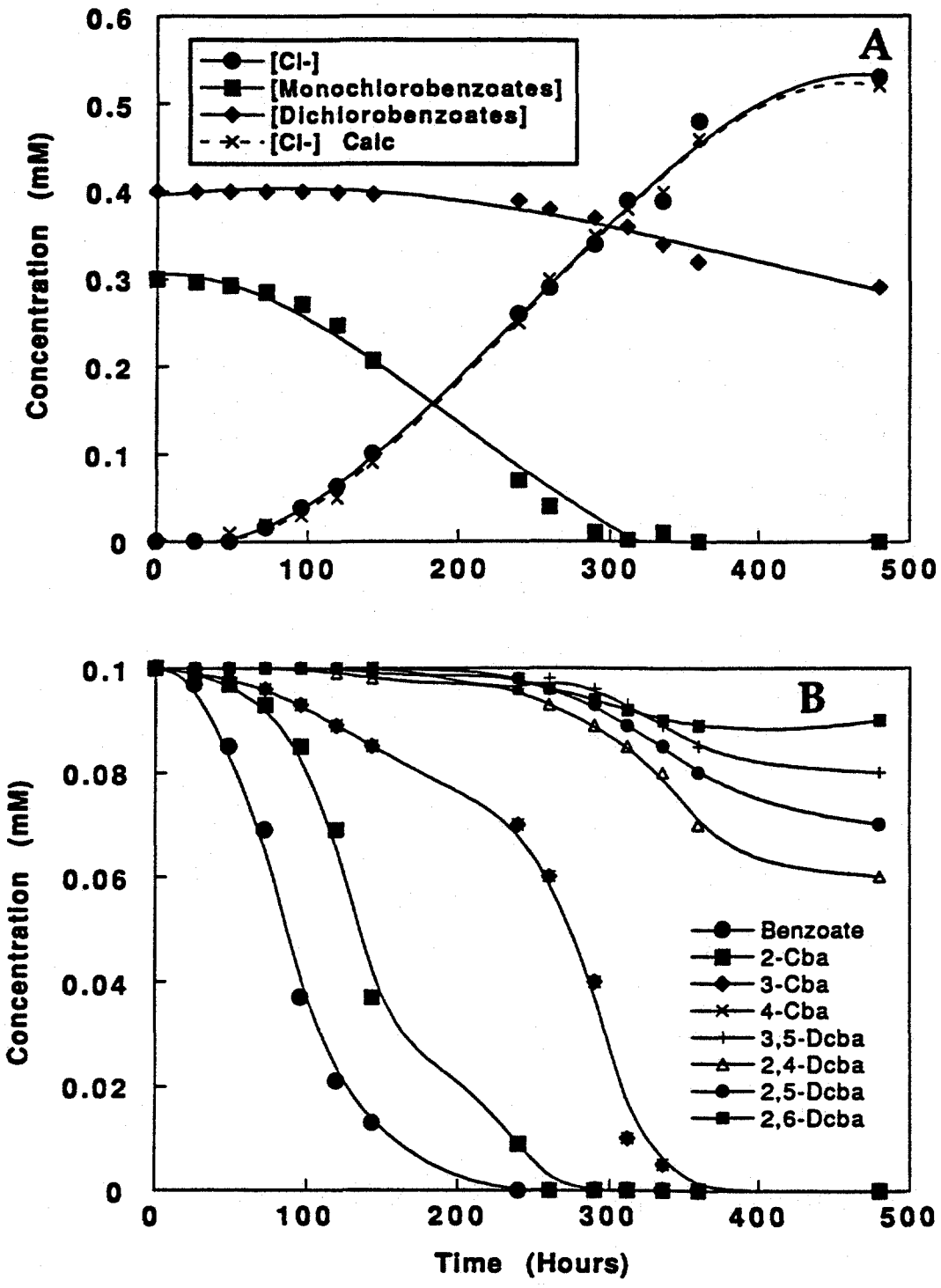


Figure 5-24. The biodegradation of added benzoate and chlorobenzoates in Hudson River Core Sediment WPF-S1 (1-2" segment) (A) shows loss of total monochlorinated benzoates and dichlorobenzoates with concomitant release of chloride. (B) shows loss of individual benzoate and chlorobenzoates as a function of incubation time.

After 5 serial transfers from this culture to fresh media containing 5 mM 2-chlorobenzoate, an "enriched culture" was obtained (KMF2). This culture was able to grow on and metabolize 2-chlorobenzoate. Autoclaved controls were unchanged over the time course of the experiment. Growth of KMF2 on carbon sources other than 2-chlorobenzoate dramatically decreased the competence on this substrate. The kinetic profiles of 2-chlorobenzoate biodegradation and chloride release are shown in Figure 5-25A. This culture did not degrade any of the other mono- or dichlorobenzoates shown in Figure 5-24B (3-, 4-, 35-, 24-, 25-, and 26-chlorobenzoate). Taken together, these data show that microorganisms residing in Hudson River sediment are capable of degrading a wide range of chlorobenzoates. Furthermore, it is clear that individual species may have a high specificity for chlorobenzoate "congeners" (specific chlorinated benzoates), as observed directly with KMF2.

In R105 and R106, which contained only indigenous microflora, the monochlorobenzoates appeared earlier (10-20 days) than the dichlorobenzoates (15-35 days), presumably resulting from a more rapid biodegradation of monochlorinated biphenyls. This is illustrated in Figures 5-26 and 5-27. The formation sequence of these metabolites was consistent with the selective microbial attack on the individual PCB congeners (see Figures 5-19A through 5-19F). Note that monochlorobenzoates can be produced by the biodegradation of both mono- (e.g. 2-chlorobiphenyl) and some dichlorinated (e.g. 2-2-dichlorobiphenyl) congeners, accounting for the large percentage of monochlorobenzoates observed. 2- and 2-2- substituted PCBs represented the largest proportion of PCB congeners in H7 sediment.

The sequential appearance of mono- then dichlorobenzoates was less apparent in caisson R103 (Figure 5-28). The inoculation of this caisson with *Alcaligenes eutrophus* H850 could be expected to affect the complex microbial population dynamics of the indigenous PCB- and chlorobenzoate-degrading bacteria, thereby affecting the order of appearance and disappearance of specific PCB and chlorobenzoate congeners. A noticeable increase in chlorobenzoate level was detected in caisson R103 at day 80 (Figure 5-28). This increase closely followed a large inoculation with *A. eutrophus* H850 at day 77, indicating some further stimulation of aerobic PCB biodegradation activity.

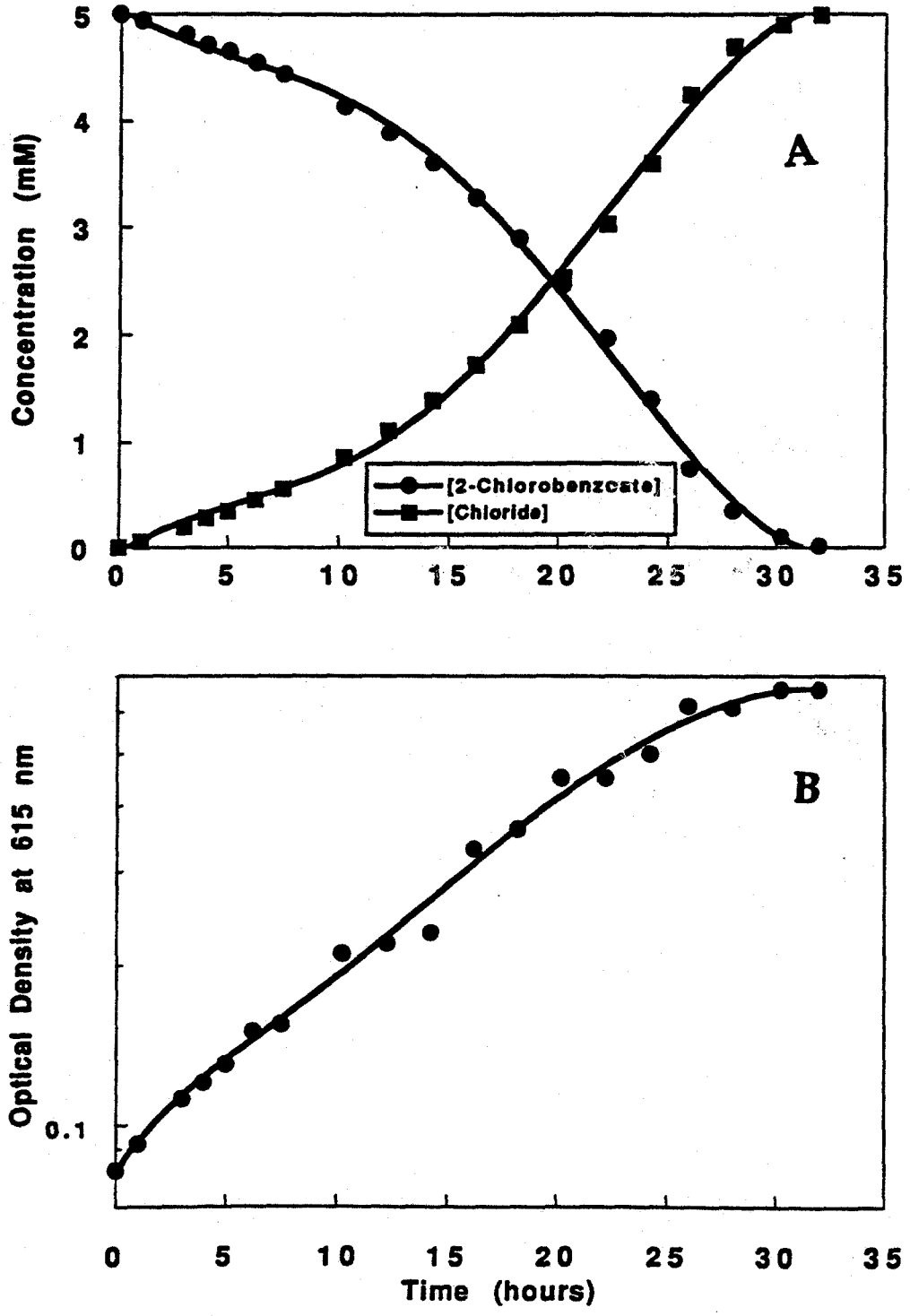


Figure 5-25. The biodegradation of added 2-chlorobenzoate by the enriched culture KMF2. (A) The loss of 2-chlorobenzoate and release of chloride as a function of time in the assay. (B) The growth of KMF2 (as measured by increasing OD_{615}) over time. These plots demonstrate that KMF2 was able to metabolize and grow on 2-chlorobenzoate.

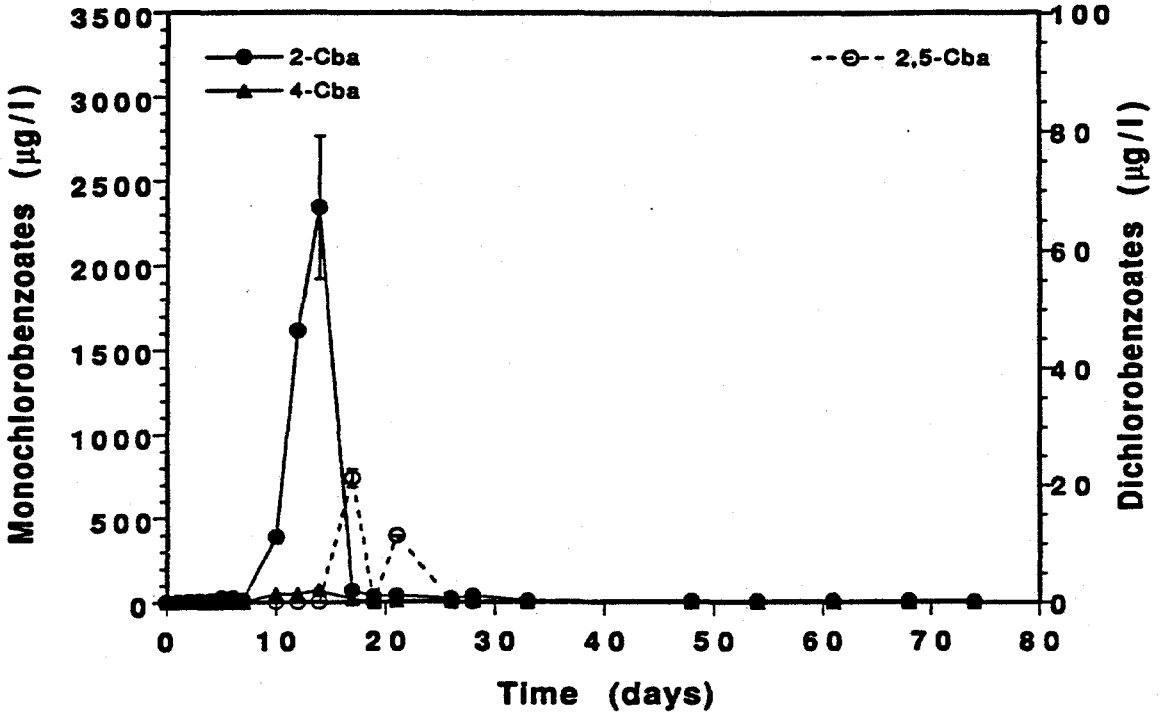


Figure 5-26. Congener-specific aqueous-phase mono- and dichlorobenzoates in R105. Monochlorobenzoates appear earlier than dichlorobenzoates, consistent with the selective attack on PCB congeners observed in this caisson (Figure 5-19F). Points represent average values for two analyses with ranges shown.

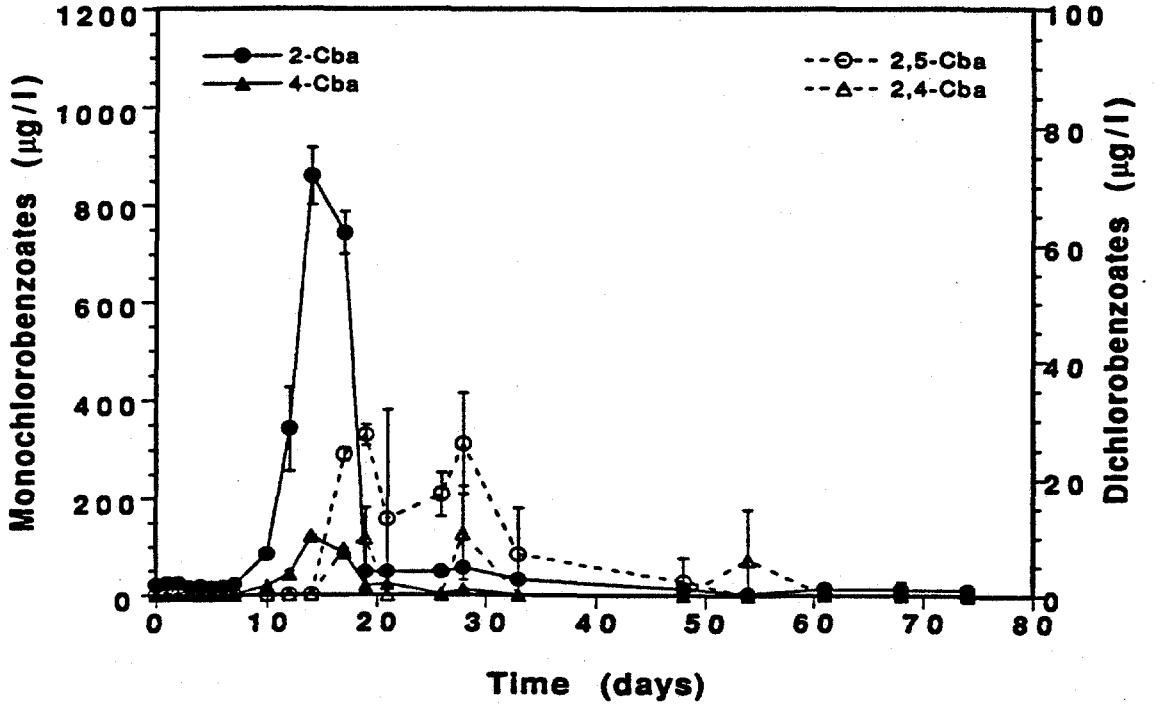


Figure 5-27. Congener-specific aqueous-phase mono- and dichlorobenzoates in R106. Monochlorobenzoates appear earlier than dichlorobenzoates, consistent with the selective attack on PCB congeners observed in this caisson (Figure 5-19G). Points represent average values for two analyses with ranges shown.

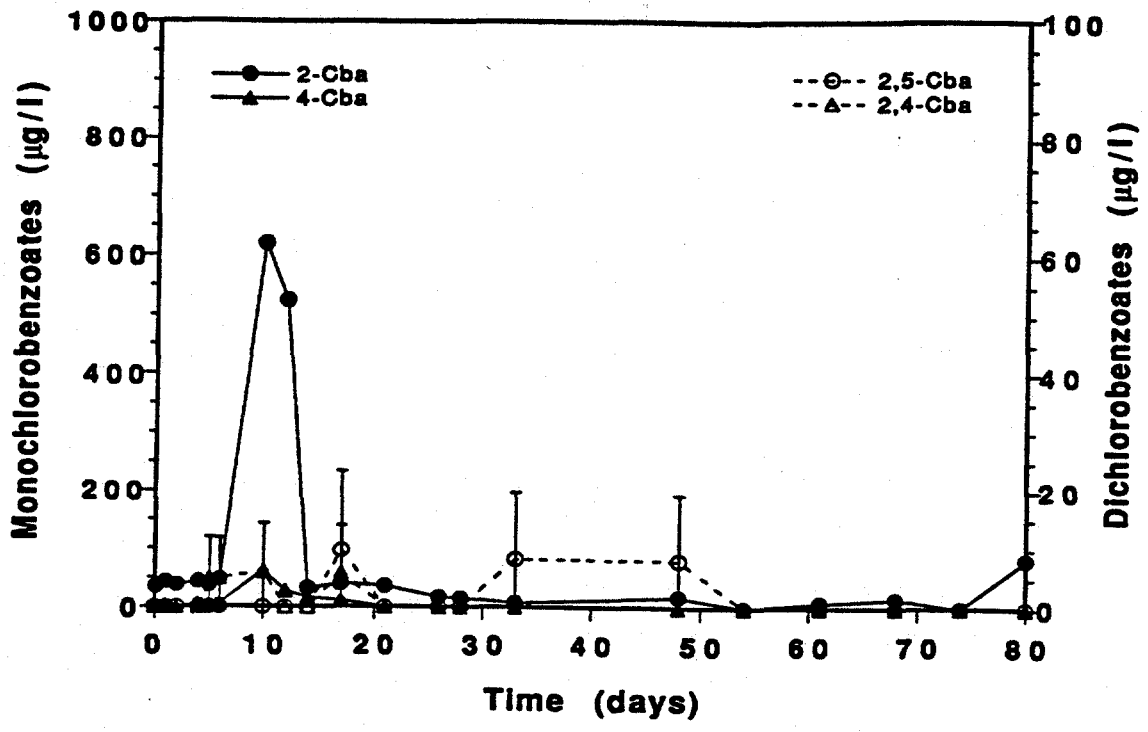


Figure 5-28. Congener-specific aqueous-phase mono- and dichlorobenzoates in R103. Monochlorobenzoates appear earlier than dichlorobenzoates, consistent with the selective attack on PCB congeners observed in this caisson (Figure 5-19D). Points represent average values for two analyses with ranges shown.

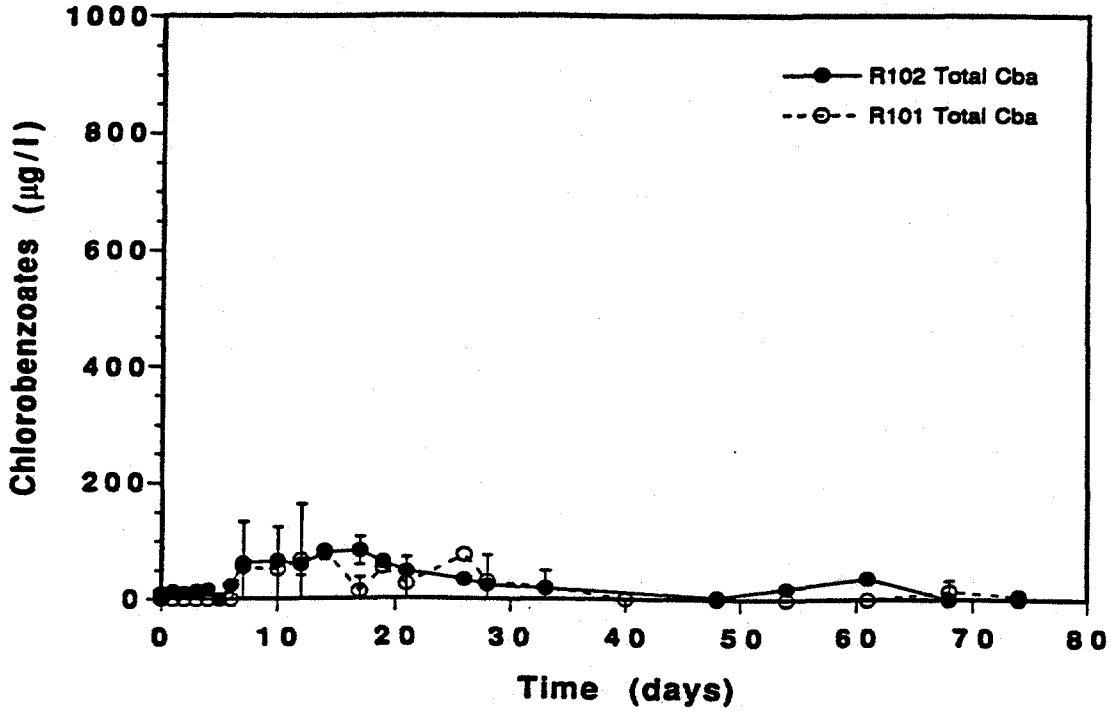


Figure 5-29. Total aqueous-phase chlorobenzoates in R102 compared to R101. Points represent average values for two analyses with ranges shown.

Elevated chlorobenzoate levels were not detected in the aqueous phase of experimental caisson R102 compared to high-mix control R101 (Figure 5-29), even though substantial PCB losses were observed in this caisson. It is possible, however, that the rapid mixing may have created favorable conditions for early stimulation of the indigenous chlorobenzoate-degrading microbial population such that these transient intermediates were not detectable.

Sediment samples were also analyzed for chlorobenzoates. All the experimental caissons consistently exhibited increased levels of chlorobenzoates in the sediment phase compared to the control caissons (data not shown). However, chlorobenzoate concentrations in the sediment phase were less conclusive than those obtained from the aqueous phase due to larger sample heterogeneity. The distribution properties of chlorobenzoates in sediment were not well characterized in this study.

Larger than expected chlorobenzoate levels were detected in sediment samples collected from all the caissons at T_0 (Figure 5-30). Since the caissons underwent a 24-hour pre-mix prior to T_0 sampling, it is interesting to note that the high-mix turbine caissons (R101 and R102) contained statistically significant (95% confidence) elevations in chlorobenzoate levels relative to the low-mix rake caissons (R103, R104, R105, R106). This may indicate a stimulation of some aerobic degradative activity due to mixing/aeration alone, without the addition of any nutrients or microorganisms.

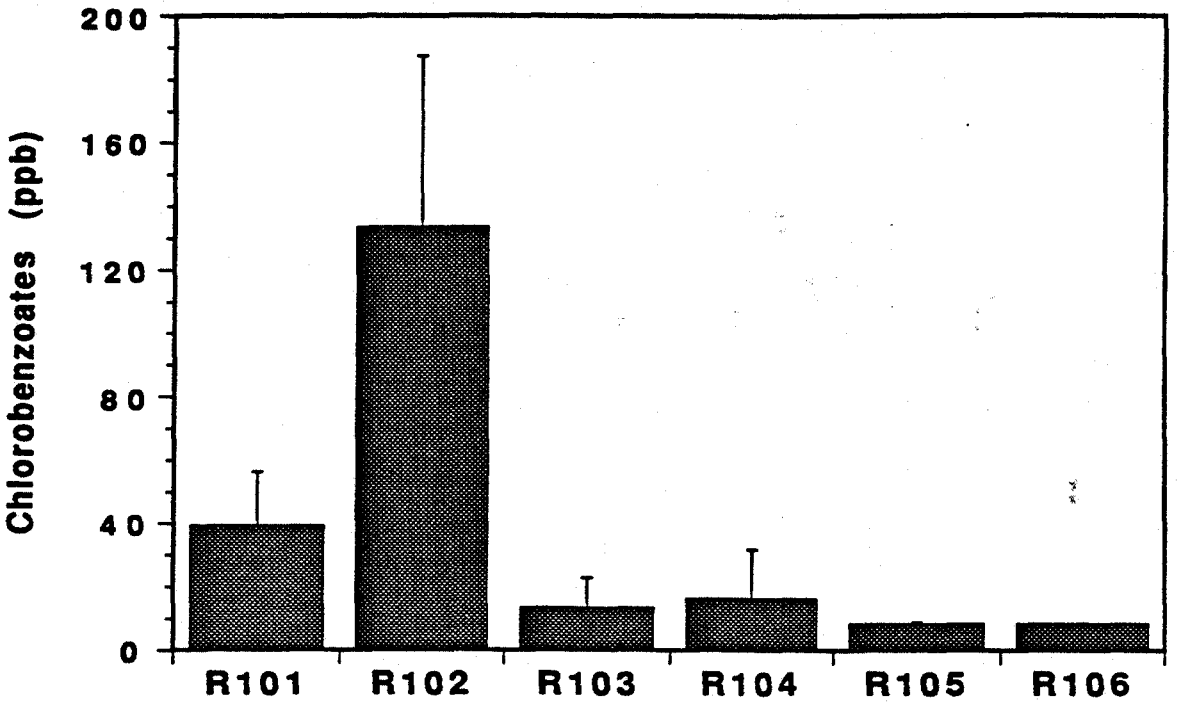


Figure 5-30. Sediment chlorobenzoate concentrations at T_0 after 24 hour pre-mix period. Each point represents the average of three or four analyses with +/- one standard deviation shown.

CHAPTER 6
DISCUSSION

Conclusive demonstrations of biodegradation in the laboratory are aided by the ability to work with homogeneous samples, to utilize sterile controls, and to perform complete mass balances. Such methods are unfortunately difficult to apply when performing *in situ* biodegradation experiments in the environment. Historically, biodegradation was deduced by simply demonstrating reductions in contaminant concentrations during the experiment, although real world difficulties (sampling problems, heterogeneous contaminant concentrations, limited mixing, and abiotic losses) seriously reduced the value of such studies. It is no longer acceptable to infer that biodegradation has occurred or that microorganisms are the causative agents with such limited evidence. Several convergent lines of independent evidence dramatically strengthen such claims. A framework for demonstrating *in situ* biodegradation has recently been published [Madsen, 1991]. This framework is helpful as a basis to evaluate whether successful *in situ* biodegradation occurred in the current field study.

There are at least seven criteria which may be used to establish that *in situ* biodegradation has occurred [Madsen, 1991]. These criteria, as applied to *in situ* biodegradation of PCBs in Hudson River sediments, include: 1) distinguishing biotic from abiotic processes; 2) the detection of metabolic intermediates (chlorobenzoates); 3) the selective disappearance of microbially labile isomers (PCB congeners); 4) the loss of co-reactants that participate in the biodegradation process (oxygen, nutrients); 5) metabolic adaptation; 6) manipulating the site to elicit unique biotic responses (adding nutrients or other metabolic stimulants); and 7) gathering microbiological evidence confirming *in situ* biodegradation (laboratory data, enumerating microorganisms). All of these conditions have been successfully met in the experiment, and are discussed below. In addition, direct PCB losses from the caissons and preliminary evidence for natural aerobic PCB biodegradation in the environment will also be discussed.

1) Control caissons were used in order to distinguish abiotic from biological PCB loss processes. Although these caissons were physically mixed in similar fashion to their experimental counterparts, they received no nutrient or microbial amendments and were blanketed with nitrogen when required to reduce oxygen levels and limit aerobic PCB biodegradation. Limited oxygen levels prevented PCB biodegradation in caisson R104 (low-mix control) (see Table 5-10), although PCB decreases of approximately 10% were observed in control caisson R101 (high-mix control). Since this caisson was not perfectly sealed and small but measurable levels of oxygen were present in the water column, this loss may be due to biodegradation. Other steps to assess the impact of abiotic processes included monitoring vapor traps to measure volatilization, swiping the sides of the caissons to evaluate adherence of PCBs to the vessel walls, and investigating any contribution to PCB losses from Fenton chemistry (see Appendix A-3 for a detailed analysis). No significant PCB losses occurred via these abiotic routes.

2) The detection of metabolic intermediates was accomplished by the development of sensitive GC-MS analytical methods to detect transient chlorobenzoates. It is well known that the primary pathway for aerobic PCB biodegradation metabolizes PCBs to chlorobenzoic acids (CBAs) [reviewed in Abramowicz, 1990; Bedard, 1990]. It is also known that distinct microbial populations can completely mineralize these intermediates [Hartmann et al., 1979; van den Tweel et al., 1987; Focht and Hickey, 1990; Hernandez et al., 1991]. In addition, our laboratory results have shown that Hudson River sediment from the study site contains microorganisms capable of degrading mono- and dichlorobenzoates (see Figure 5-24). Moreover, such transient detection of these metabolites was also observed in comparable laboratory PCB biodegradation experiments (data not shown).

Elevated CBA levels were detected in three of the experimental caissons, although only briefly, as microbial populations capable of degrading these transitory metabolites quickly removed them. The sequence of CBA production (mono- followed by di-CBAs, see Figures 5-26 and 5-27) correlated with the observed progression of PCB biodegradation in the caissons (Figure 5-19). The large proportion of 2-CBA observed is consistent with the known fate of 2-chlorobiphenyl, 2-2-chlorobiphenyl, 2-3-chlorobiphenyl, and 2-4-

chlorobiphenyl all proceeding through the 2-CBA intermediate. Only 4-chlorobiphenyl would proceed through the 4-CBA intermediate, again congruent with the low initial level of 4-chlorobiphenyl. The low levels of di-CBAs observed (25- and 24-CBA) are consistent with the low initial levels of PCB congeners that proceed through these intermediates (25-, 25-2, 24-, 24-2-chlorobiphenyl). The detection of these known PCB biodegradation intermediates and the correlation between PCB congeners degraded and CBA congeners detected offers the strongest evidence that *in situ* biodegradation is responsible for the observed PCB losses.

3) The selective disappearance of microbially labile PCB congeners has been demonstrated several ways. First, the data displayed a selective sequence of PCB biodegradation, from mono- to dichlorobiphenyl (see Figure 5-19). Only small changes of congeners containing three or more chlorines were observed, except for significant losses of 26-3-chlorobiphenyl in all the experimental caissons, and of 26-2-chlorobiphenyl in caisson R102. The observed loss of 26-2-chlorobiphenyl in caisson R102 could result from the addition of PCB-degrading strain H850 to this caisson, or to the unique conditions within this caisson (high-mix, low pH) supporting a different population of microflora. Second, congener-specific metabolites (CBAs) were observed, as described above. Moreover, the sequence of PCB degradation and CBA accumulation is highly correlated. Finally, congener-specific changes were observed within a group of closely related isomers (peak 10, 26-2-chlorobiphenyl; peak 16, 26-3-chlorobiphenyl; peak 17, 26-4-chlorobiphenyl). Only the 26-3-chlorobiphenyl peak was degraded in caissons R103, R105, and R106 (both control caissons R101 and R104 displayed no change in the relative amounts of these trichlorobiphenyls). Note that these three trichlorobiphenyl congeners display similar chemical and physical properties, eliminating the involvement of abiotic mechanisms in the selective disappearance of 26-3-chlorobiphenyl. In R102, significant degradation of both 26-2- and 26-3-chlorobiphenyl also occurred, most likely due to the different microbial populations within this caisson.

4) The loss of co-reactants that participate in the biodegradation process was demonstrated with a variety of nutrients. Losses of nitrogen and phosphorus were difficult to determine, as large excesses were added to the

caissons. Decreases in biphenyl levels were obvious (Figure 5-5) and expected, as PCBs are cometabolized in the presence of biphenyl. Oxygen was clearly utilized in the caissons, as shown by the integrated peroxide usage curves in Figure 5-6. Continuous oxygen demand, reflected in the dissolved oxygen cycling described in Figure 5-4, demonstrated rapid oxygen utilization in the experimental caissons.

5) Metabolic adaptation was demonstrated by increased numbers of PCB-degrading microorganisms in the sediment and by directly measuring the PCB-degradative competence of organisms in the aqueous phase of the caissons. Figure 5-11A clearly demonstrates that increases in the numbers of PCB-degrading microorganisms occurred only in the experimental caissons, coincident with PCB losses. Samples taken directly from each caisson revealed that at least three microbial adaptations had occurred. First, active PCB-degrading microorganisms were present in large numbers only in the experimental caissons, indicating that oxygen, nutrient, and biphenyl additions directly stimulated this microbial population. Second, this population was enriched for organisms capable of degrading the lightly chlorinated PCB congeners (e.g. 2- and 2-4-chlorobiphenyl, Table 5-4). Third, colony morphologies of the biphenyl-metabolizing bacteria changed throughout the course of the experiment, reflecting population shifts responding to altered conditions within the caissons. These data again support the thesis that *in situ* PCB biodegradation occurred in the experiment.

6) Manipulation of the site to elicit unique biological responses involved the addition of oxygen and other nutrients. The reduced levels of PCB biodegradation in the control caissons in the absence of these amendments demonstrated the importance of aerobic metabolism in PCB biodegradation. Oxygen and nutrient additions correlated with increased levels of PCB biodegradation and increased numbers of PCB-degrading bacteria in the experimental caissons. Temporal responses also demonstrated the association of PCB losses and biological activity, as the onset of PCB biodegradation (measured by the sharp rise in chlorobenzoates) correlated with activation of the water column mixers on the low-mix caissons to distribute oxygen in the aqueous phase (day 7 in R103, day 10 in R105 and R106).

7) Efforts to gather additional microbial evidence to confirm *in situ* PCB biodegradation include several independent research areas. First, laboratory biodegradation studies performed prior to the field study demonstrated that approximately 30-63% of the PCB in sediments from the study site could be biodegraded by indigenous microorganisms under various conditions (see Figure 4-3 and 4-11B). Corresponding levels of PCB biodegradation were observed in the field study (38-55%). In addition, enumeration of microorganisms from the caissons demonstrated that numbers of PCB-degrading microorganisms increased by 5-7 orders of magnitude in the caissons where significant PCB biodegradation occurred (see Figure 5-11). The total heterotrophic aerobic population also increased, as shown in Figure 5-7. Furthermore, assays on the PCB-degradative competence of the microbial populations within the caissons during the experiment confirmed that organisms capable of PCB biodegradation were active (see Table 5-4).

A compilation of results of the PCB analyses from the caissons are shown in Table 6-1. These results offer additional support for *in situ* biodegradation. As discussed previously, analysis of results based on measurements of PCB concentrations or total PCB per core in the low-mix caissons are complicated by PCB redistribution within these caissons. This analysis demonstrates the limitations of direct measurements of contaminant losses due to heterogeneities in environmental samples.

Two independent normalization methods were used to correct for these difficulties. Both methods demonstrated that losses of 38-55% occurred in the experimental caissons, while small or insignificant losses occurred in the control caissons. In the first method, PCB values were expressed on a total organic carbon (TOC) basis for each core. As previously discussed, hydrophobic contaminants such as PCB typically reside in the organic fraction of the sediment. This was demonstrated for sediments from the caissons by directly subsectioning samples (see Table 5-8) and correlating TOC and PCB concentrations in caisson R104. It was also demonstrated that the mass balance could be closed in R104 if sampling occurred after the PCB-rich colloidal fraction in that caisson had settled (see Table 5-5).

Table 6-1
Comparison of PCB Analytical Methods
(values expressed as % losses)

Caisson	Average PCB Conc.	Average PCB/core	Average PCB/TOC	Peak 61 PCB Normal
R101	+ 8.7%*	+ 5.1%*	- 30.7%*	- 14.4%
R102	-41.0%	-40.0%	-44.7%	-42.4%
R103	-36.8%	11.4%*	-55.5%	-37.8%
R104	-41.8%	-21.0%*	+ 8.4%*	- 4.3%*
R105	-72.6%	-47.1%	-53.1%	-40.5%
R106	-68.5%	-43.1%	-46.0%	-38.7%

* Indicates changes were not statistically significant at a 95% confidence limit.

The results of expressing the initial and final PCB concentrations on a TOC basis showed that PCB losses ranged from 44.7-55.5% in the experimental caissons, while statistically significant decreases were not observed in either control caisson. These results are consistent with observations from laboratory efforts and with the pattern of losses observed in PCB chromatograms. This technique appears to offer a more valid representation of actual *in situ* PCB biodegradation levels in the field study.

A second method for quantifying PCB biodegradation levels involved normalization of the data using a non-degradable PCB peak as an internal standard. This method is limited by the fact that no PCB congeners present at the site have been demonstrated to be completely non-degradable by aerobic PCB-degrading microorganisms (see Table 5-3). Therefore this normalization technique will represent a conservative measure of actual losses, although this bias can be minimized by selecting a less biodegradable congener as the normalization standard. Several congener peaks were selected as potential internal standard references (peaks 39, 48, 61, and 82). Comparable results were achieved with any of these peaks serving as internal standard,

validating the technique. PCB losses with peak 61 as an internal standard ranged from 37.8-42.4% in the experimental caissons. Again, only small changes were observed in the control caissons. These conservative estimates are consistent with the slightly larger losses of 44.7-55.5% determined using the TOC technique.

Use of internal standard normalization significantly reduced the variability in the PCB analysis. Figure 6-1 displays the losses determined for each of the final samples normalized to peak 61. In general, the PCB biodegradation was quite uniform within the caissons, in contrast to the highly variable results obtained from more direct measurements. An exception included one sample from caisson R102 that has a slight negative effect on estimates of biodegradation within the caisson. Control caisson R101 (low oxygen levels) demonstrated a slight but significant decrease in PCB levels via this analysis.

It should be noted that in caissons R101 and particularly R102, where no significant sampling problems were encountered, all four analytical techniques, including direct concentration measurements, are in excellent agreement. This observation lends strong support to the validity of the two normalization techniques.

The results of this field study demonstrated no significant difference in the extent of PCB biodegradation in the high-mix caisson (R102) compared to the low-mix caissons (R103, R105, R106). Also, the extent of PCB biodegradation was not significantly affected by inoculation with PCB-degrading strain H850. In general, PCB-biodegradation rates in the field were 2-3 fold slower than results with much smaller scale laboratory experiments. Typical laboratory experiments were performed with ≤ 0.1 kg of sediment compared to the 500 kg of PCB-contaminated sediments contained within each caisson. The observed difference in PCB-degradation rate is likely due to the effect of increased length scale on mass transfer and mixing, along with lower experimental temperatures encountered in the field.

The extent of PCB biodegradation in both laboratory and field experiments was limited by the characteristic bioavailability of the PCBs in the

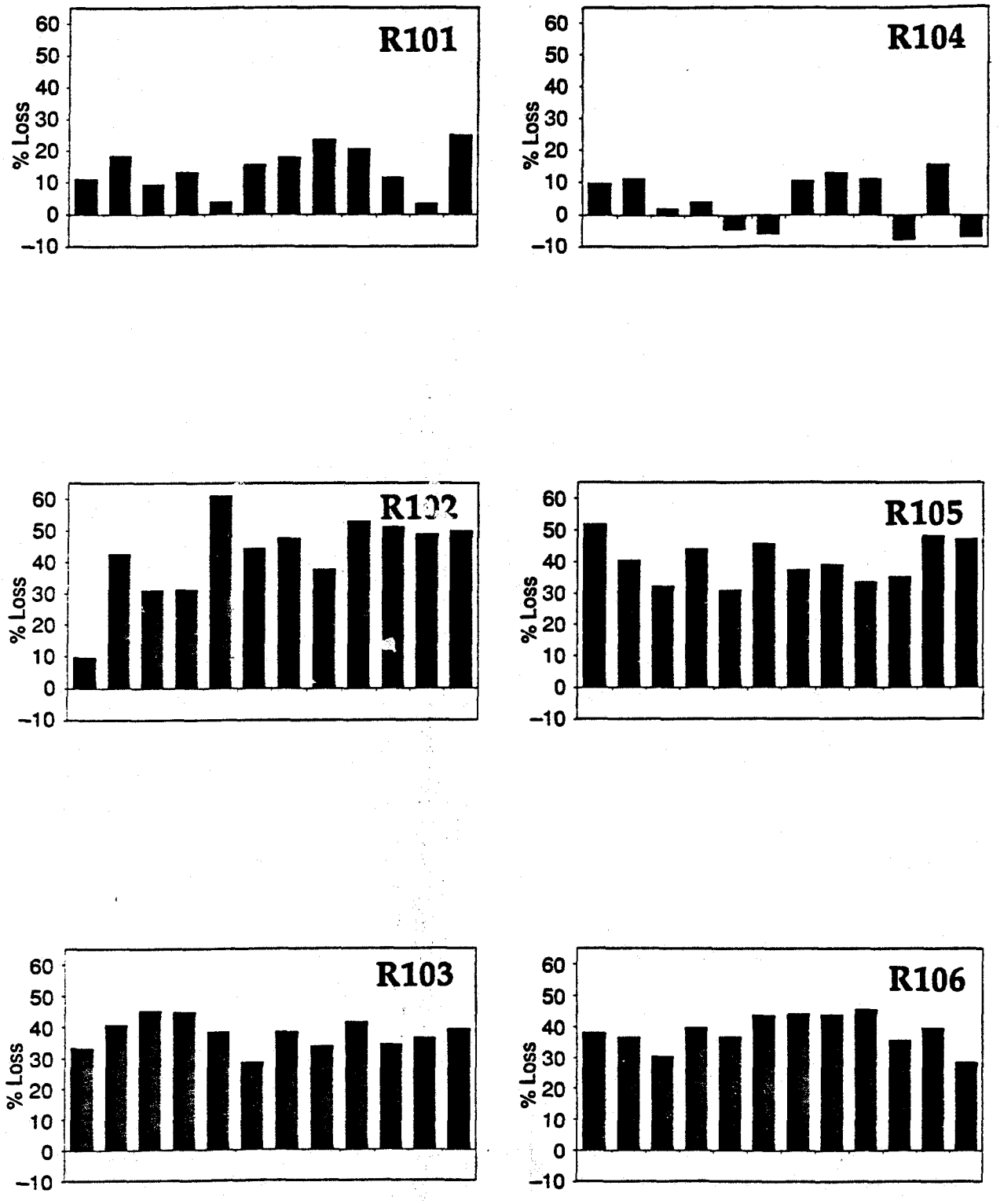


Figure 6-1. Normalized PCB losses for each of the sediment cores at T_f. Data is normalized to peak 61. All changes were statistically significant at a 95% confidence level, with the exception of those observed in R104.

sediments. This phenomenon is commonly observed to varying degrees whenever the biodegradation of hydrophobic contaminants like oil and PCB is carried out in the presence of soils or sediments. At PCB concentrations typical for this Hudson River hotspot (20-40 ppm), only about 50% of the target compounds were shown to be readily available for the bacteria to degrade over the time course of the experiment. This PCB fraction was generally degraded in the experiment. The remaining resistant fraction was not available to the PCB-degrading bacteria and is unlikely to be available to other organisms, as well [Larsson et al., 1992]. This may have important implications for establishing risk estimates. The resistant PCB fraction is expected to become available over long periods of time (months-years), although physical, chemical, and natural aerobic biodegradative processes should mitigate the effects of this slowly desorbing material.

It is important to note that the bioavailability issue is more pronounced at low PCB concentrations. At higher concentrations (e.g. approximately 200 ppm) as much as 75% of the contamination should be available for biodegradation, with a greater percentage available at even higher concentrations. But at the low concentrations typically found in Hudson River sediments, significantly less total PCB is available for accelerated aerobic biodegradation or bioaccumulation through the food chain.

Although the rate of PCB biodegradation was affected by oxygen levels, limited biodegradation appeared to occur in control caisson R101 (no added nutrients or biphenyl). Low levels of oxygen (<2 ppm) were detected in that caisson, presumably resulting from equilibration of oxygen into the water column from the headspace of the caisson. Larger than expected chlorobenzoate levels were detected in sediment samples collected from all the caissons even at T_0 after the 24 hour pre-mix period, with elevated levels apparent in the high-mix caissons in particular (Figure 5-30). Taken together, these results suggest that *in situ* PCB biodegradation occurred even under limited conditions (minimal mixing, no microbial inoculation, low oxygen levels, and no added nutrients or biphenyl) in this field study. Therefore, such aerobic PCB biodegradation may be occurring naturally in the

environment, a hypothesis further supported by the detection of chlorobenzoic acids in undisturbed Hudson River sediments (see below).

Chlorobenzoate Detection in Undisturbed Hudson River Sediments

The presence of dechlorinating and PCB-degrading bacteria in the environment strongly suggests that these processes are ongoing at PCB-contaminated sites. Further evidence is required before a conclusive link between the laboratory observations and the *in situ* degradation mechanisms can be established. The existence of ongoing anaerobic dechlorination in nature was discovered through the analysis of PCB congener-distribution patterns which revealed evidence of environmental modifications [Brown et al., 1987]. During the course of this study, preliminary evidence has been discovered that suggests that PCBs are being aerobically biodegraded in undisturbed Hudson River sediments. This is based on the detection in these sediments of chlorobenzoic acids, which are metabolic intermediates of aerobic PCB biodegradation (Figure 5-20). This result provides qualitative evidence that aerobic PCB biodegradation is occurring in the natural environment.

A core (#WPF-S1) was collected from an undisturbed region of the H7 site ~60 feet upstream of the experimental platform. The core was frozen and sliced into 1" sections, which were then thawed, homogenized, and analyzed for PCBs and chlorobenzoates (CBAs) via GC-MS (see Appendix B-1).

The PCB profile of core #WPF-S1 is shown in Figure 6-2. It contained a high concentration of PCBs (~500 ppm) which were located primarily in the top 5" of sediment. The chlorobenzoate (CBA) concentration was 19 ppb (ng/g dry weight) in the top inch, and decreased linearly with depth (Figure 6-3). Duplicate analyses demonstrated excellent reproducibility, given the low level of analyte. The majority of the detected CBAs were monochlorinated benzoates (Figure 6-4). Traces of dichlorinated benzoates (25- and 24-) were observed at low, non-quantifiable levels (data not shown). CBAs were not detected in sediments collected from an "uncontaminated" section of the Hudson River (Spier Falls).

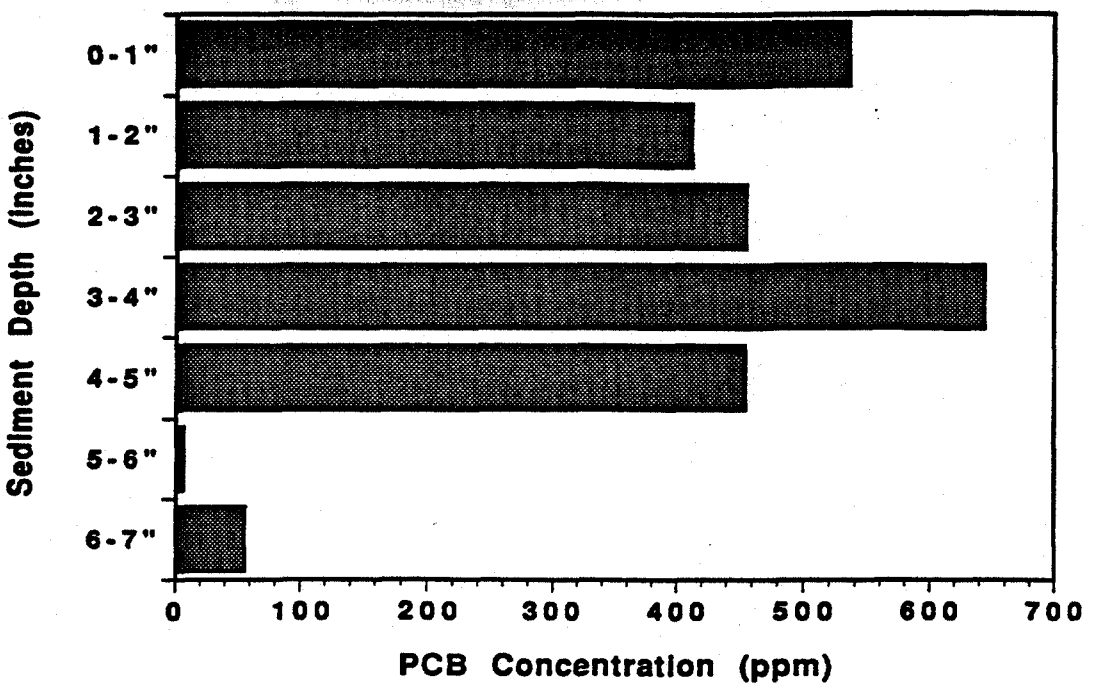


Figure 6-2. PCB concentrations in core #WPF-S1. This sediment core was collected from an undisturbed region of the H7 site, frozen, and sectioned into 1" lengths for PCB analysis.

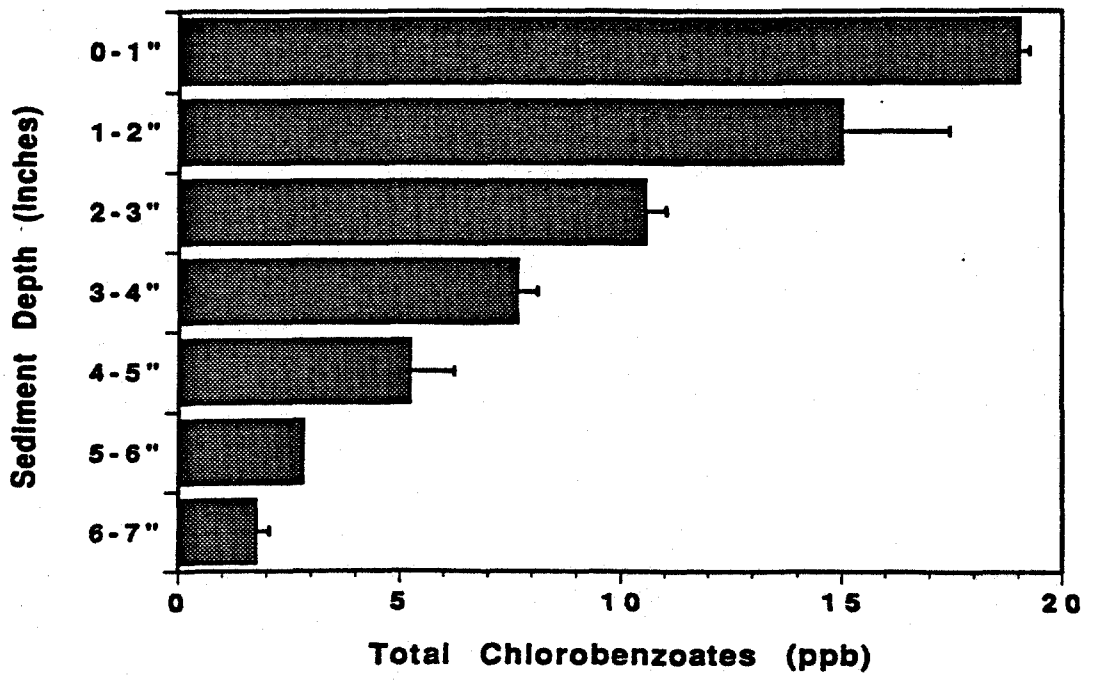


Figure 6-3. Total chlorobenzoate concentrations in core #WPF-S1. Each datum represents the average of two analyses with ranges shown.

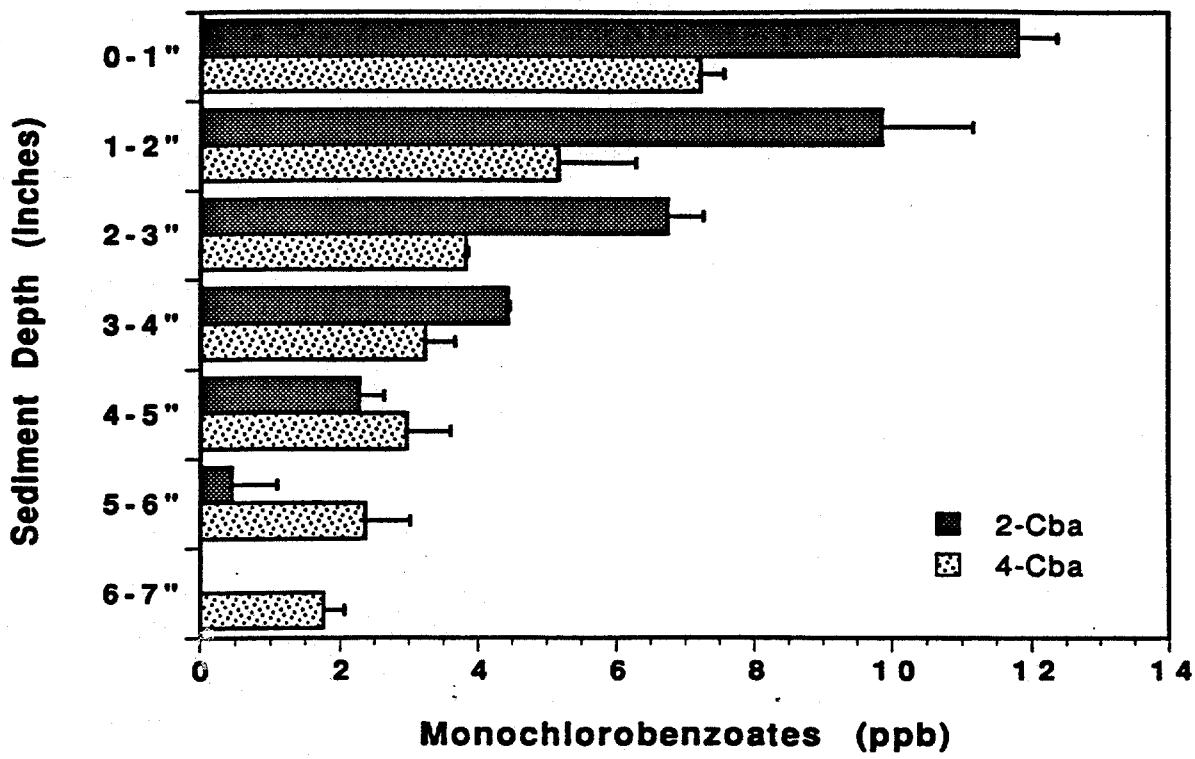


Figure 6-4. Monochlorobenzoate concentrations in core #WPF-S1. Each datum represents the average of two analyses with ranges shown.

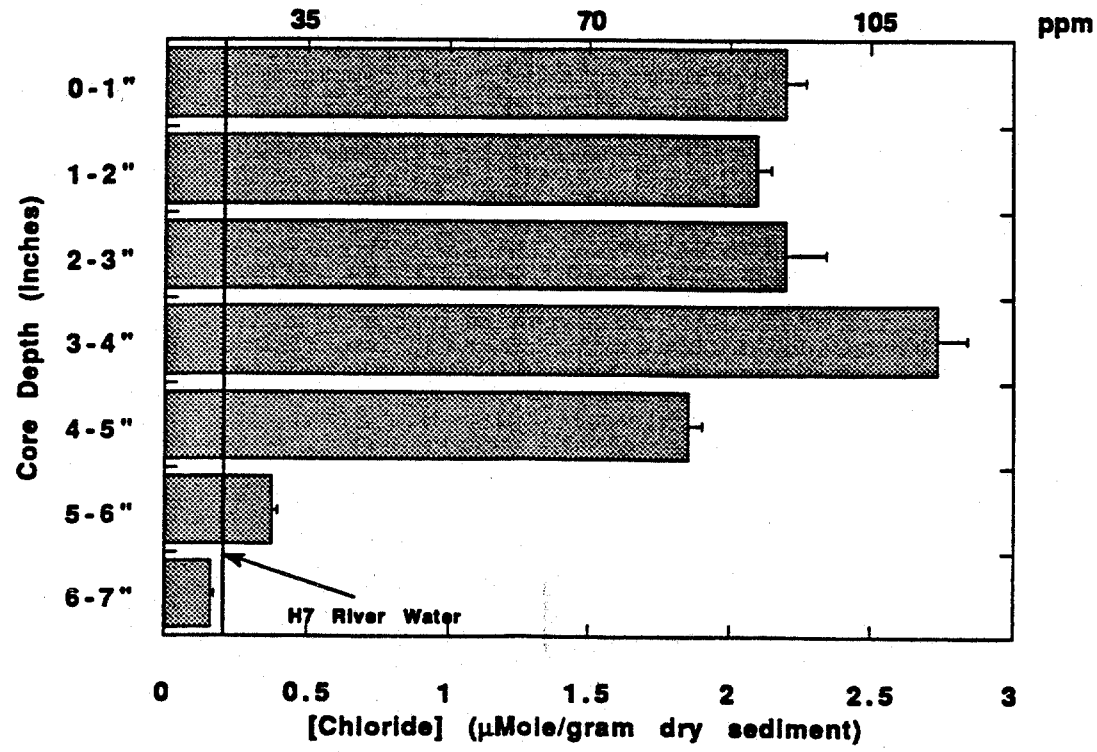


Figure 6-5. Chloride ion concentration in core #WPF-S1. The vertical line represents the chloride ion concentration in the water column. Each datum represents the average of two analyses with ranges shown.

Chloride ion analysis was also performed on sediment from core #WPF-S1 by ion chromatography using a Waters IC-Pak A ion chromatograph column. The final chloride ion concentrations were calculated based on the dry weight of each sample. The results appear in Figure 6-5. Core #WPF-S1 contained elevated levels of chloride ion in the top 5" of the sediment, which correlated well with the PCB contamination [Figure 6-2]. This may be an indicator of aerobic PCB mineralization, anaerobic PCB dechlorination, or some other chloride source. Nevertheless, the correlation between chloride ion and PCBs may provide further evidence for the ongoing loss of PCB in the Hudson River.

Efforts are currently underway to further substantiate these results [Flanagan et al., in preparation]. Sediment cores have been collected from a variety of locations within the Thompson Island Pool area of the Hudson River. In addition, cores will be collected from non-PCB-contaminated areas of the river as well. The cores will be analyzed for chlorobenzoates, other PCB-biodegradation metabolites, and free chloride ion. The goal of this program will be to further investigate the existence of *in situ* aerobic PCB biodegradation, in order to fully understand this important loss mechanism.

CHAPTER 7
CONCLUSIONS

Results from this field study in Hudson River sediments demonstrate that aerobic PCB biodegradation can occur *in situ*. This conclusion is supported by several independent lines of evidence, as well as by a comprehensive set of PCB analyses, including PCB data normalized to total organic carbon levels and to an "internal standard" reference peak.

Six major conclusions can be drawn from these field studies.

1) Indigenous PCB-degrading microorganisms are present in these sediments and can biodegrade the lightly chlorinated PCBs typically present in Upper Hudson River sediments.

2) The observed PCB biodegradation in the field correlated well with previous laboratory research.

3) Aerobic PCB biodegradation can be accelerated in Hudson River sediments *in situ* by the addition of nutrients and supplemental oxygen.

4) Inoculation of extensively dechlorinated sediments with large numbers of a purified PCB-degrading organism resulted in little benefit over the degradation achieved via stimulating the growth of the indigenous microflora alone.

5) Rapid biodegradation of chlorobenzoates indicates that PCBs can be fully metabolized through these intermediates by indigenous microflora present in Hudson River sediments.

6) Evidence from this field study, together with chlorobenzoate analyses in undisturbed river sediments, suggests that aerobic PCB destruction is occurring naturally in the Hudson River. This aerobic biodegradation of lightly chlorinated PCBs complements the extensive anaerobic dechlorination previously documented.

CHAPTER 8
REFERENCES

Abramowicz, D.A. 1990. In: CRC Critical Reviews in Biotechnology, G.G. Steward and I. Russell,(eds.), CRC Press, Inc., Vol. 10 3:241.

Ahmed, M. and D.D. Focht. 1973. Can. J. Microbiol., 19:47.

Atkins, P.W. 1978. Physical chemistry. W.H. Freeman and Company, San Francisco.

Atlas, R.M. 1981. Microbiol. Rev., 45:180.

Bedard, D.L. 1990. In: Biotechnology and Biodegradation, D. Kamely, A. Chakrabarty and G.S. Omenn (eds.), Adv. Appl. Biotechnol. Series, Vol. 4, Portfolio Publishing Co., the Woodlands, TX pp. 369.

Bedard, D.L., Wagner, R.E., Brennan, M.J., Haberl, M.L., Brown, J.R., Jr. 1987. Appl. Environ. Microbiol. 53:1094-1102.

Bedard, D.L., R. Unterman, L.H. Bopp, M.J. Brennan, M.L. Haberl, and C. Johnson. 1986. Appl. Environ. Microbiol. 51:761.

Bedard, D.L., M.J. Brennan, and R. Unterman. 1984. in G. Addis and R. Komai (ed.), Proceedings of the 1983 PCB Seminar, Electrical Power Research Institute, Palo Alto, Calif.

Bolt, G.H. and Bruggenwert, M.G.M., (eds.), 1978. Soil Chemistry A. Basic Elements. Elsevier Science Publishers B.V., the Netherlands.

Buckley, E.H. 1982. Science, 216, 520.

Chiou, C.T., P.E. Porter and D.W. Schmedding. 1983. Environ. Sci. Technol. 17:227.

Clifton, M.J. and A. Savall. 1986. J. App. Electrochem. 16:812.

Coates, J.T. and A.W. Elzerman. 1986. J. Contam. Hydro., 1:191.

Dunnivant, F.M. 1988. Ph.D. Dissertation. Environmental Systems Engineering Department, Clemson University.

Flanagan, W.P., R.J. May, and K.M. Fish. Chlorobenzoate formation as evidence for *in situ* aerobic PCB biodegradation. GE Corporate Research and Development Class 1 Technical Report, in preparation.

Focht, D.D. and W.J. Hickey. 1990. Appl. Environ. Microbiol. 56:3842.

Furukawa, K. 1982. In: Biodegradation and Detoxification of Environmental Pollutants, A.M. Chakrabarty (ed.), CRC Press, Inc., pp. 33.

Furukawa, K., F. Matsmura, and K. Tonomura. 1978. Agric. Biol. Chem., 42:543.

General Electric Company Research and Development Program for the Destruction of PCBs, First Progress Report. 1991. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Second Progress Report. 1990. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Third Progress Report. 1989. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Fourth Progress Report. 1988. General Electric Corporate Research and Development, Schenectady, NY.

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

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

General Electric Company Research and Development Program for the Destruction of PCBs, Seventh Progress Report. 1985. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Eighth Progress Report. 1984. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Ninth Progress Report. 1983. General Electric Corporate Research and Development, Schenectady, NY.

General Electric Company Research and Development Program for the Destruction of PCBs, Tenth Progress Report. 1982. General Electric Corporate Research and Development, Schenectady, NY.

Hartmann, J., W. Reineke and H.-J. Knackmuss. 1979. Appl. Environ. Microbiol., 37:421.

Hatzikonstantinou, H. and S.B. Brown. 1978. Biochem. J. 174:893.

Hernandez, B. S., F.K. Higson, R. Kondrat, D.D. Focht. 1991. Appl. Environ. Microbiol., 57:3361.

Hutzinger, O. and W. Veerkamp. 1981. In: Microbial Degradation of Xenobiotics and Recalcitrant Compounds, Leisinger, T., Hutter, R., Cook, A.M., and Nuesch, J. eds., Academic Press, New York.

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

Jensen, S. 1966. New Sci., 32, 612.

Karickhoff, S.W. and K.W. Morris. 1985. Environ. Tox. Chem., 4:469.

Karickhoff, S.W. 1980. In: Contaminants and Sediments. Baker, R.A., ed., Science/Butterworth, Ann Arbor, MI.

Kawahara, F.K. 1968. Anal. Chem. 40:2073.

Larsson, P., L. Colivin, L. Okla, and G. Meyer. 1992. Environ. Sci. Technol., 26:346.

Mackay, D., W.Y. Shiu, and R.P. Sutherland. 1979. Environ. Sci. Technol., 13:333.

Madsen, E.L. 1991. Environ. Sci. Technol., 25:1663.

May, R.J., W.P. Flanagan, and K.M. Fish. A congener-specific protocol for the quantitative analysis of chlorobenzoates in river sediments. GE Corporate Research and Development Class 1 Technical Report, in preparation.

McDermott, J.B., R. Unterman, M.J. Brennan, R.E. Brooks, D.P. Mobley, D.K. Dietrich and C.C. Schwartz. 1989. Environ. Prog. 8:46.

Means, J.C., S.G. Wood, J.J. Hassett and W.L. Barnwart. 1980. Environ. Sci. Technol. 14:1524.

Mondello, F.J. and J.R. Yates. 1989. in General Electric Company Research and Development Program for the Destruction of PCBs, Eighth Progress Report. General Electric Corporate Research and Development, Schenectady, NY.

Mondello, F.J. 1989. J. Bacteriol. 171, 1725.

Nadim, L., M.J. Schocken, F.J. Higson, D.T. Gibson, D.L. Bedard, L.H. Bopp, and F.J. Mondello. 1987. In: Proceedings of the 13th Annual Research Symposium on Land Disposal, Remedial Action, Incineration, and Treatment of Hazardous Waste. EPA/600/9-87/015. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Northeast Analytical Laboratory Method NEA-PCBHRGC, revision 1, 1990.

Pignatello, J.J. 1990. Environ. Tox. and Chem., 9:1107.

Pourbaix, M. 1974. Atlas of Electrochemical Equilibria in Aqueous solutions. National Association of Corrosion Engineers, Houston.

Pritchard, P.H., C.F. Costa. 1991. Environ. Sci. Technol., 25:372.

Quality Assurance Plan for Research and Development Studies on Biological Method of Elimination of Polychlorinated Biphenyls (PCBs) from Sediments in the Hudson River, dated June, 1991.

Quensen, J.F., III, J.M. Tiedje and S.A. Boyd. 1988. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science 242:752.

Quensen, J.F., III, S.A. Boyd and J.M. Tiedje. 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microb. 56:2360.

Rochkind, M.L., J.W. Blackburn, and G.S Sayler. 1986. In: Microbial decomposition of chlorinated aromatic compounds. EPA/600/2-86/090. U.S. Environmental Protection Agency.

Safe, S. 1980. in Halogenated Biphenyls, Naphthalenes, Dibenzodioxins, and Related Products, Kimbrough, R., (ed.), Elsevier/North Holland Publishers, 77.

Sediak, D.L. and A.W. Andren. 1991. Aqueous-Phase Oxidation of Polychlorinated Biphenyls by Hydroxy Radicals. Environ. Sci. Tech. 25(8):1419.

Standard Methods for the Examination of Water and Wastewater, 1989, 17th edition, American Public Health Association, Washington, DC.

Sundstrom, G., and O. Hutzinger. 1976. Chemosphere 5:267.

Tanabe, S., Hidaka, H., and Tatsukawa, R. 1983, Chemosphere, 12, 277.

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. 1988. In: Reducing Risks from Environmental Chemicals Through Biotechnology, G.S. Omenn et al., (eds.), Plenum Press, New York.

Van Hoof, P.L. and A.W. Andren. 1991. in Organic Substances and Sediments in Water, R.A. Baker (ed.), Vol. 1, Lewis Publishers, Chelsea, MI.

van den Tweel, W.J.J., Kok, J.B. de Bont, J. A.M. Appl. Environ., Microbiol. 1987, 53: 810.

Webb, R.G. and A.C. McCall. 1973. J. Chromat. Sci., 11:366.

APPENDIX A-1
REQUIRED PERMITS

To allow the construction, operation, and eventual removal of the facility, as well as to approve the experimental design, a number of federal, state, and local permits were required.

FEDERAL

1) TSCA Research and Development Permit was obtained from the US EPA (Washington). In addition to information on the design of the experimental protocol, the application included a quality assurance document and a description of the laboratory PCB analytical method. This permit to perform research on the *in situ* biodegradation of PCB in Hudson River sediments was granted on May 14, 1991 for one year.

2) No permit was required from the Army Corps of Engineers, as the project fell within an existing Nationwide Permit (written verification was obtained from the Corps).

STATE

3) New York State Department of Environmental Conservation (NYSDEC) Permit DEC #5-4144-00049/00001-1 was obtained pertaining to Article 15, Title 5 of the Environmental Conservation Law for the protection of water. The permit allowed the construction of a 25 by 37.5 foot platform in the Hudson River supported by pile driven steel supports as well as up to 130 linear feet of driven steel sheet pile retaining wall on the riverbank (4/1/91 to 12/31/91).

4) A New York State Department of Transportation (NYSDOT) Canal Work Permit #T1-91-070 was obtained for surveying and related work on parcel 515 in preparation for construction of the facility (short term permit terminated 4/19/91).

5) NYSDOT Canal Work Permit #T1-90-55C was obtained for construction and operation of the research project in the Hudson River (Champlain Canal) in the vicinity of green buoy #219 (4/15/91 to 12/1/91).

6) NYSDOT Canal Work Permit #T1-90-53C was obtained to take on-shore borings as part of the geotechnical investigation to determine bearing capacities and other structural properties (2/12/91 to 6/30/91).

7) NYSDOT Canal Work Permit #T1-91-04C was obtained to place three acoustic transducers on the sediment bed of the Hudson River near Billings Island (3/5/91 to 7/31/91).

8) Office of General Services Division of Land Utilization Permit was obtained to use state-owned property for three transducers and support structures placed on the river bed (3/1/91 to 10/1/91).

9) Office of General Services Division of Land Utilization Permit was obtained to use state-owned property for placing six caissons on submerged lands in the Hudson River (4/1/91 to 4/1/92).

LOCAL (Town of Moreau)

10) Floodplain Development Permit (4/10/91, no expiration date).

11) Fence Permit #19 (2/20/91 to 2/20/92).

12) Garage and Sheds Accessory Building (Temporary) Permit #20 (2/20/91 to 2/20/92), Trailer Authorization (1/30/91, placement for up to one year).

APPENDIX A-2
ANAEROBIC DECHLORINATION IN HUDSON RIVER SEDIMENTS

Daniel A. Abramowicz, John F. Brown, and
Michael K. O'Donnell

General Electric Company Research and Development Program
For the Destruction of PCBs
Tenth Progress Report
August 1991

INTRODUCTION

The reductive dechlorination of PCBs has been observed in the laboratory and the environment [Brown *et al.*, 1984; 1987a; 1987b; Brown and Wagner, 1990; Quensen *et al.*, 1988; reviewed in Abramowicz, 1990 and Bedard, 1990]. Its occurrence in the environment has been detected via the altered distribution of residual PCB congeners in aquatic sediments from several locations. In general, this microbial reductive dechlorination effects the preferential removal of *meta* and *para* chlorines, resulting in a depletion of highly chlorinated PCB congeners with corresponding increases in lower chlorinated, *ortho*-substituted PCB congeners. This report will focus on recent findings that demonstrate widespread and progressive PCB dechlorination in the upper Hudson River (mile point 195 to 156).

This ubiquitous environmental transformation directly results in gradual losses of all the PCB congeners that are readily bio-accumulated in higher animals, and more rapid losses of the potentially toxic PCB congeners [Safe *et al.* 1985a; 1985b] (e.g. non-*ortho* and mono-*ortho* congeners containing at least four *meta* and *para* chlorines). Therefore, these widespread microbial dechlorination processes have resulted in significant reductions of the theoretical health risks associated with the PCB residues in the upper Hudson River.

RESULTS AND DISCUSSION

1976-1989 Temporal Trends in Upper Hudson PCB Levels

The U.S. Geological Survey has monitored total water PCB concentrations (i.e., the sum of dissolved and suspended particle-bound PCBs) at several stations in the upper Hudson River since 1976. The river survey extends from mile point 194 (location of the GE plant) to mile point 160 (Lock No. 1). The time trend displayed by this data is shown in Figure 2-1. Note that under both low flow (summer average) and high flow conditions, the PCB concentration measured in the water column has decreased exponentially. Although some year-to-year variations exist, the overall trend indicates a half-life of only three years. These consistently large reductions in water column PCB concentrations demonstrate that significant improvements have occurred naturally in the upper Hudson River system.

Two extensive surveys have been performed by the New York State Department of Environmental Conservation (NYSDEC) on this section of the upper Hudson River. The PCB mass estimates for the bottom sediments in the Thompson Island pool determined from these surveys were 61,000 kg in 1979 and 23,000 kg in 1984 [Brown *et al.*, 1988; Sanders, 1989]. The nearly three-fold reduction in these estimates in five years suggests that the total PCB mass is declining. Although the errors associated with these estimates reduce the power of such comparisons, large reductions in upper Hudson fish PCB levels have been directly observed in the same area during this same time period [EPA, 1991 Pumpkinseed from milepoint 175, 1979, 1060 ug/gm-lipid; 1984, 300 ug/gm-lipid]. Sequential anaerobic PCB dechlorination and aerobic PCB biodegradation [Bedard *et al.*, 1987a; GE report, 1990; Abramowicz and Brennan, 1991] may play an important role in the observed environmental changes.

Dechlorination Status of Upper Hudson PCBs in 1984

The first report of anaerobic PCB dechlorination was made by observing unusual PCB congener distributions in Hudson River sediments [Brown *et al.*, 1984]. These initial observations were based upon the limited number of sediment samples available at the time. Confirmation of these

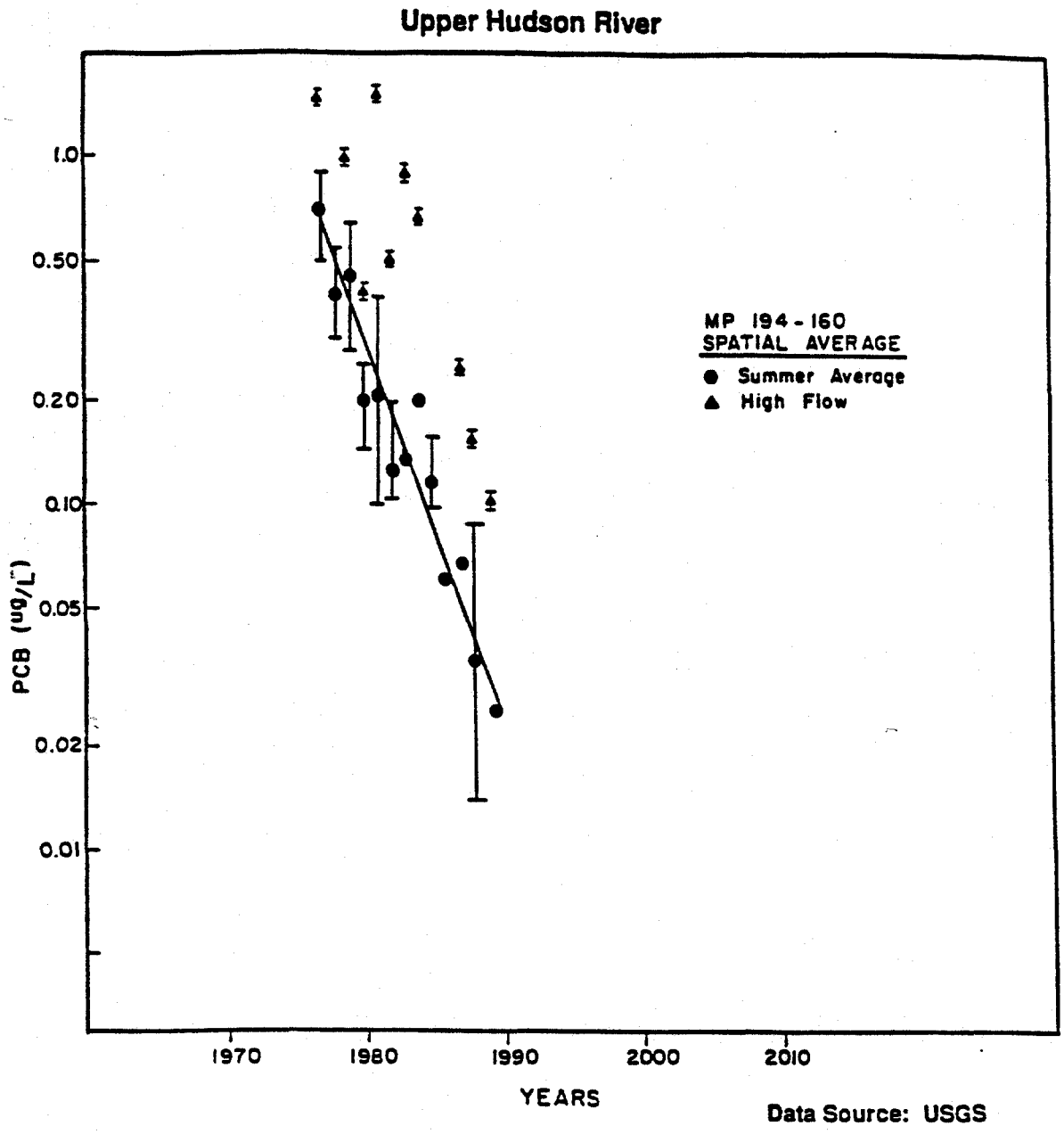


Figure 2-1. Time trend of PCB concentration measured in the water column of the upper Hudson River from 1976 to 1989. The data is presented as the spatial averages of measurements from monitoring stations located between mile point 194 to 160. Data obtained from the U.S. Geological Survey.

environmental changes was obtained by Bopp *et al.* [1984], who noted that "in every core from the upper Hudson examined thus far, a significant shift toward relative higher abundances of peaks with retention times corresponding to lower chlorinated PCB congeners has been observed. Further evidence of anaerobic dechlorination of PCB congeners in sediments of natural systems should be sought."

An extensive survey of the Thompson Island Pool, a six mile stretch of the upper Hudson, was performed by the NYSDEC in 1983-1984 [Brown *et al.*, 1988]. This survey resulted in 2073 packed column PCB analyses of sediments collected from ~1000 sampling locations (mile point 194.5 to 188.5). The stored data base contains the peak areas from the original packed GC analyses in addition to total PCB concentrations. The authors used ratios of these peak areas to estimate the extent and breadth of PCB dechlorination in the sediments. In Figure 2-2, the source locations of samples containing at least 10 ppm PCB (545 of the 2073 samples) are mapped. Note that most of the PCB accumulations lay along the edges of the River, in sedimentation zones. One measure of dechlorination can be obtained by determining the ratio of mono-*ortho* tetrachlorinated PCBs (peak 70) to mono-*ortho* trichlorinated PCBs (peak 47). In pure Aroclor 1242, the principal material used in the GE plants, this ratio is ~1.8. Any sample with a ratio ≤ 1 has been considered "significantly dechlorinated" and is circled in Figure 2-2. Quantitative determination of the average chlorine level is not possible since the response factors for the individual peaks were not determined in the original analysis. Over 70% of the 1984 samples displayed significant dechlorination, with peak 70/peak 47 ratios ≤ 1. In fact, the average peak ratio was < 0.5 at all PCB concentrations surveyed, as shown in Table 2-1. Therefore the peak ratio had decreased nearly four-fold on average for all samples containing > 5 ppm PCB, and decreased five-fold on average for samples at > 100 ppm PCB (see Table 2-1). The number of samples within the different PCB concentration ranges showing significant dechlorination are given in Table 2-2. This shows that the prevalence of significant dechlorination increased from 63% of the samples in the lowest PCB concentration range (5-10 ppm) to 93% in the highest range (> 100 ppm). Both the Table 2-1 and Table 2-2 results indicate that environmental PCB dechlorination may proceed faster and more

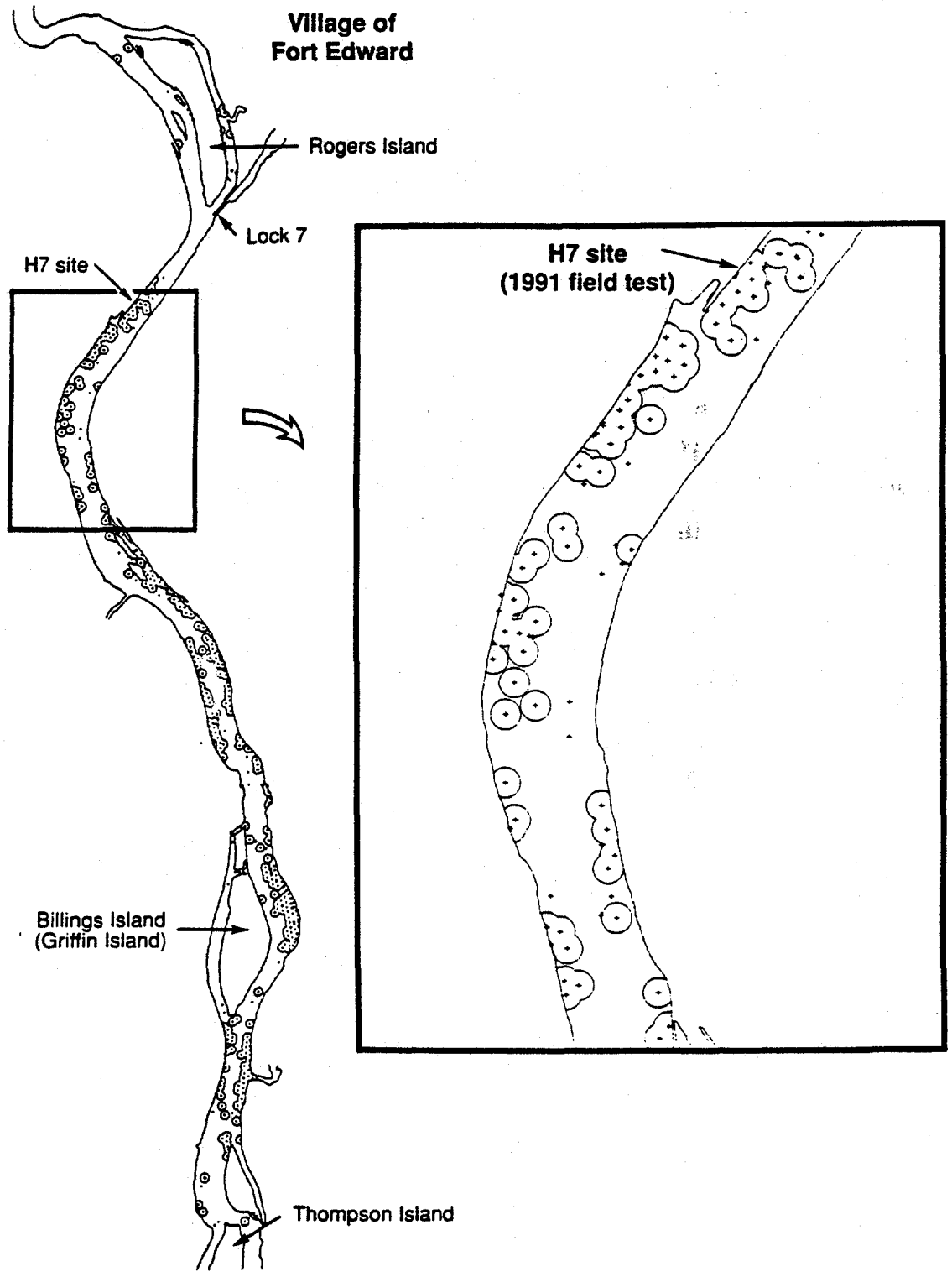


Figure 2-2. Locations of sediment PCB accumulations in the upper Hudson River, based upon re-analysis of the 1984 survey of the Thompson Island Pool performed by the NYSDEC. + Indicates samples containing ≤ 10 ppm PCB. Circles indicate samples containing a ratio of peak 70/peak 47 ≤ 1.

TABLE 2-1. Effect of total PCB concentration on a measure of tetrachlorobiphenyl dechlorination in upper Hudson River sediments in 1984.

Dechlorination in Hudson River (1984 DEC data)

[PCB] ppm	#Samples $0 < \frac{\text{Peak 70}}{\text{Peak 47}} \leq 1$	Avg [PCB] ppm	$\frac{\text{Peak 70}}{\text{Peak 47}}$ (Avg)
≥ 5	447	83	0.49
≥ 10	384	95	0.48
≥ 20	314	114	0.44
≥ 50	190	167	0.40
≥ 100	111	232	0.36

TABLE 2-2. Effect of total PCB concentration on the proportion of upper Hudson River sediments showing significant dechlorination of tetrachlorobiphenyl in 1984.

Dechlorination in Hudson River (1984 DEC data)

PCB Range	# Samples	# Samples $0 < \frac{\text{Peak 70}}{\text{Peak 47}} \leq 1$	%
5-10 ppm	100	63	63%
10-20 ppm	108	70	65%
20-50 ppm	179	124	69%
50-100 ppm	92	79	86%
> 100 ppm	119	111	93%

extensively in sediments containing higher concentrations, in agreement with previous laboratory studies [Quensen, 1988].

The peak ratio discussed above (peak 70/peak 47) is an indicator of overall dechlorination from packed column PCB analyses. Similar results supporting widespread PCB dechlorination in the upper Hudson were obtained with other peak ratios. Examples include peak 37/peak 21 (indicator of pattern B and B' dechlorination), peak 21/peak 16 (indicator of pattern C dechlorination), and peak (37+40)/peak 11 (indicator of mono-CB formation). Pattern designations are defined in Brown *et al.*, 1987a.

These results indicate that microbial PCB dechlorination was already widespread throughout upper Hudson River sediments in 1984. Extensive changes had occurred in sediments exhibiting a broad range of PCB concentrations, even as low as 5 ppm. More quantitative comparisons would require high resolution PCB analysis (capillary GC), as shown in the following section.

Dechlorination Status of Upper Hudson PCBs in 1990

Extensive high resolution PCB analysis of upper Hudson River sediments has been performed at the H7 site (mile point 193.5). PCB chromatograms displaying typical PCB distributions from 1982-1990 are shown in Figure 2-3. Note that even the earliest sample (Figure 2-3a) displays a considerable level of *ortho*-substituted products (2- and 2-2/26-CB, see arrows), compared to Aroclor 1242, indicating that dechlorination was already well advanced. However, a significant amount of the more highly chlorinated PCB congeners still remained at this early time point. Over the next eight years (Figure 2-3b and 2-3c), dechlorination continued until over 80% of the total PCBs in the sediment samples consisted of 2-CB and 2-2/26-CB. The chromatogram displayed in Figure 2-3c represents an extensively dechlorinated environmental sample, and is similar to dechlorination Pattern C previously described [Brown *et al.*, 1987a]. The average chlorine level has been reduced from originally 3.6 to ~2.0 in this sample, indicating the removal of most of the *meta* and *para* chlorine substitution. It is not possible to determine accurate environmental dechlorination rates from

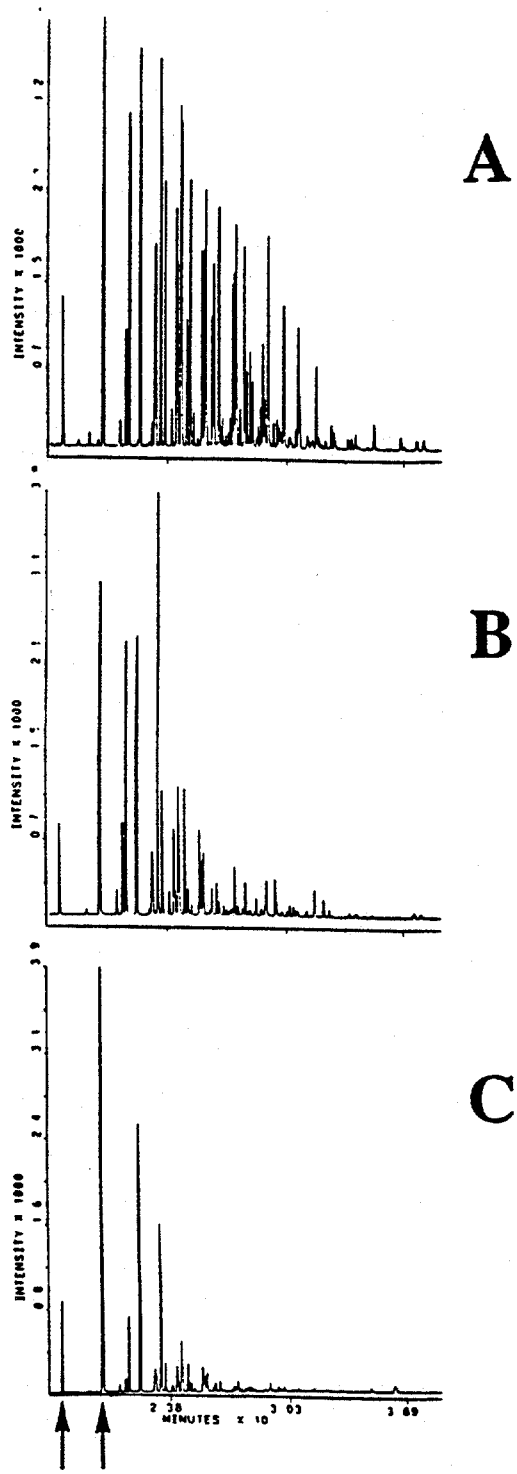


Figure 2-3. DB-1 Capillary gas chromatograms showing the progression of anaerobic dechlorination with time in Hudson River sediments at the H7 site. Panel A, 1982; Panel B, 1985; Panel C, 1990. Arrows indicate 2-CB and 2-2-CB.

these few samples, as significant spatial variations in dechlorination activity may exist at the different sample sites.

In order to determine the spatial variation in this dechlorination activity, a dense grid of core samples was obtained from the H7 site in the summer of 1990 (68 sampling sites on 12 foot centers with 151 high resolution PCB analyses). The results of the capillary PCB analyses are shown in Figure 2-4. Note the extensive variations in PCB concentrations even in adjacent samples and core sections. The corresponding dechlorination levels, expressed as average chlorine content per biphenyl, are shown in Figure 2-5. Sediments obtained before the onset of dechlorination contained ~3.6 Cl/BP. The mean of these average Cl/BP ratios from the H7 site is 2.3 ($n = 62$, $\sigma = 0.3$), similar to the distribution represented in Figure 2-3a. Extensive anaerobic dechlorination had occurred uniformly throughout the entire site, at both low and high PCB concentrations.

In hopes of identifying minimally dechlorinated sites that could be used for future field studies of techniques for accelerating PCB dechlorination rates, additional sampling was performed in 1990. Eighteen locations (ranging between mile points 163 and 195) were selected as the **least** dechlorinated areas based upon the 1984 NYSDEC survey results and other sampling. The results of the 1990 survey and high resolution capillary PCB analyses are shown in Table 2-3. Site 11 (mile point ~169, 3.4 Cl/BP) appeared to be the least active, but further sampling revealed that significant dechlorination had occurred even at this site (elevated levels of 2- and 2-2/26-CB). The high average chlorine level was found to originate from additional contamination from a more highly chlorinated PCB mixture as observed from high resolution analysis. This was most probably the result of a small, localized spill of Aroclor 1254, since subsequent sampling nearby yielded only 2.7 and 2.6 Cl/BP (dechlorinated Aroclor 1242 only). Likewise, additional sampling at site 18 yielded 2.3 and 2.3 Cl/BP. The mean of these average Cl/BP ratios from the survey was 2.5 ($n = 32$, $\sigma = 0.3$), when samples not contaminated with Aroclor 1254 from sites 11 and 18 were used. Therefore, even in sites selected for minimal dechlorination in 1984, significant changes from the original 3.6 Cl/BP had occurred by 1990. The chromatographic changes observed in the environmental samples indicated the selective loss

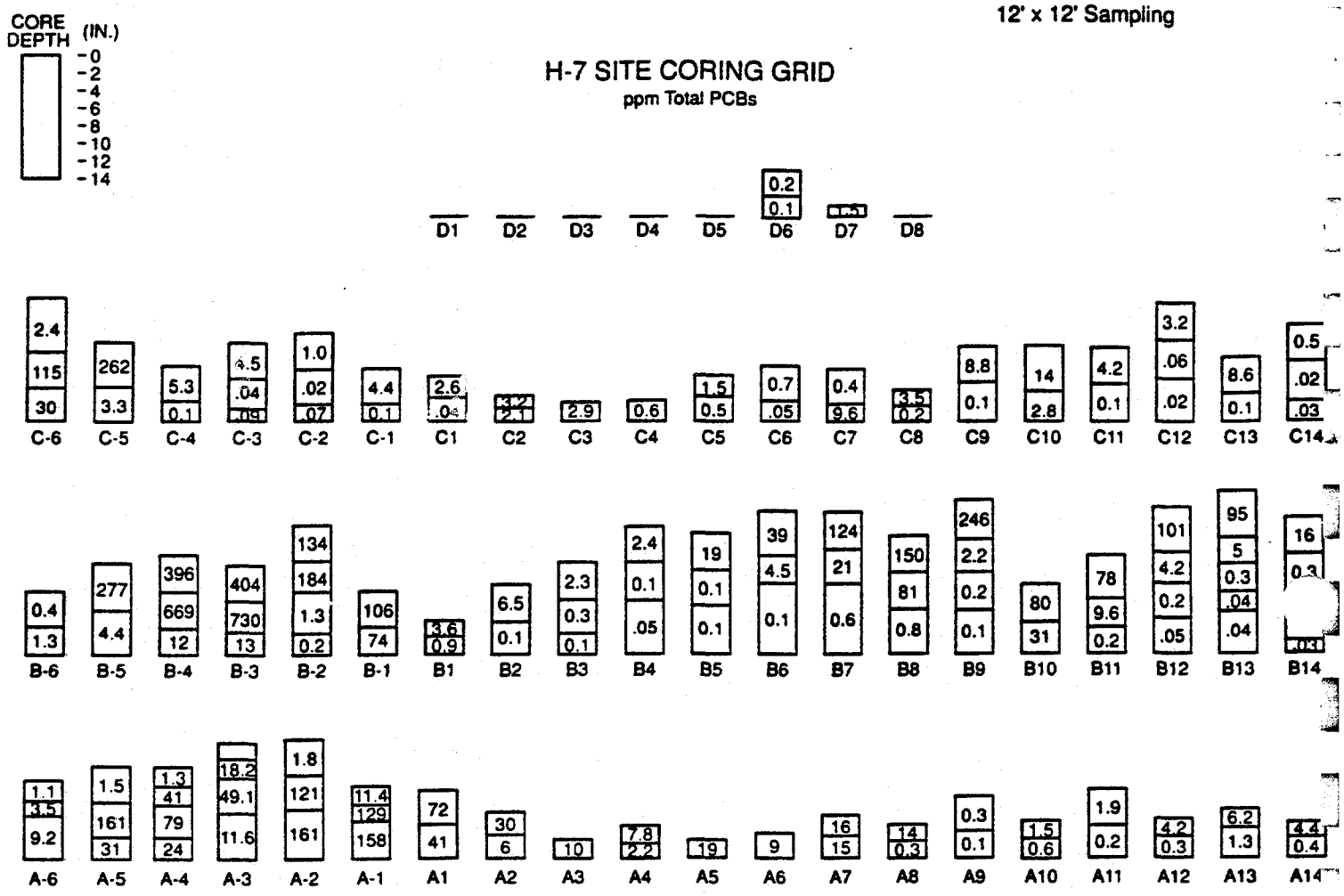


Figure 2-4. Local distribution of sediment PCB accumulations at the upper Hudson River H7 site (mile point 193.5). Cores were taken on 12 foot centers and subdivided at visible stratigraphic boundaries. PCB concentrations are shown within the boxes in ppm (dry weight). The core depth is indicated by the height of the box at each sampling location.

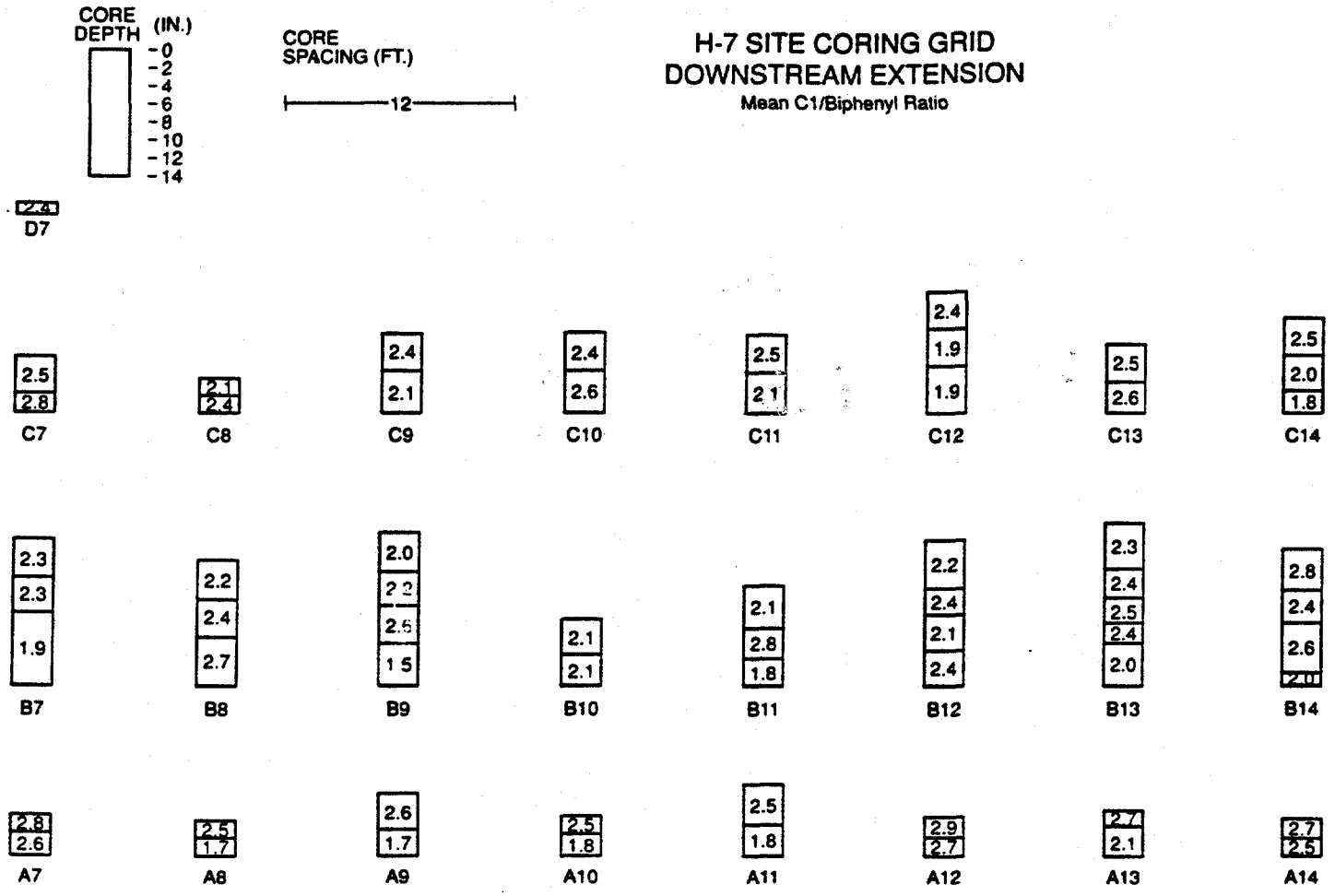


Figure 2-5. Local distribution of sediment PCB chlorine contents at the upper Hudson River H7 site (mile point 193.5). Cores were taken on 12 foot centers and subdivided at visible stratigraphic boundaries. Average Cl/BP ratios are shown within the boxes. The core depth is indicated by the height of the box at each sampling location.

TABLE 2-3. Average chlorine/biphenyl ratios of upper Hudson River (mile point 163-195) sediment PCBs in samples collected in 1990 from sites showing relatively little dechlorination in 1984.

Average Cl/biphenyl (1990 Survey)

Site #	Location	Sample 1	Sample 2	Sample 3
1	Bakers Falls	--	--	--
2	Near Remnant 1	--	--	--
3	Near Remnant 4	--	--	--
4	Hotspot 6	2.3	2.4	2.4
5	Hotspot 14	2.2	2.2	2.3
6	Hotspot 16	2.1	2.3	2.3
7	Hotspot 18	2.4	2.3	2.4
8	Hotspot 19	2.4	2.3	--
9	Hotspot 28	2.8	3.1	2.9
10	Hotspot 31	3.0	3.1	3.2
11	Hotspot 36	3.3	3.5	3.3
12	Hotspot 39	2.3	2.7	3.2
13	Hotspot 40	2.7	2.8	2.8
14	Rogers Island	2.8	--	--
15	Hotspot 5	--	--	--
16	Hotspot 8	--	--	--
17	Hotspot 9	2.4	--	--
18	Griffin Island	2.8	--	--

of *meta* and *para* chlorines, which is characteristic of natural microbial dechlorination.

SUMMARY AND CONCLUSIONS

Several different indicators (decreasing PCB levels in sediments, fish, and the water column) have established that PCB levels in the upper Hudson River are declining. It is now also known from 1984 and 1990 sediment survey data that anaerobic PCB dechlorination has occurred on a wide scale throughout the upper Hudson. It is also known that aerobic microorganisms capable of degrading the lightly-chlorinated, *ortho*-substituted products of anaerobic activity are widespread and common in upper Hudson sediments (J.H. Lobos, personal communication). Therefore sequential anaerobic dechlorination / aerobic biodegradation may be playing a significant role in removing PCBs from the upper Hudson River.

In addition, the pervasive dechlorination process already completed has resulted in reduced sediment PCB concentrations of highly chlorinated PCB congeners. These include those congeners that can bio-accumulate in fish and those that are potentially toxic. Therefore microbial dechlorination alone has already reduced any theoretical health risks associated with the residual sediment PCBs in the upper Hudson River.

This report presents the first detailed mappings of widespread PCB dechlorination in the environment. However, anaerobic biodegradation is not limited to the Hudson River. PCB-containing sediments from Escambia Bay, FL; Hoosic River, MA; Hudson River, NY; Kalamazoo, MI; Massena, NY; New Bedford Harbor, MA; Sheboygan River, WI; Silver Lake, MA; Waukegan Harbor, IL; and Woods Pond, MA all display environmental PCB dechlorination [Brown *et al.*, 1987b; Abramowicz, 1990]. Recent reports on New Bedford Harbor [Brown and Wagner, 1990; Lake *et al.*, 1991] indicate that the observed activity at that site is also not localized. Even uncontaminated sediments (from an Adirondack marsh near Stony Creek, NY; Center Pond, MA; Red Cedar River, MI; Saline River, MI; and the Hudson River at Spier Falls, NY) contain microorganisms capable of catalyzing the reductive dechlorination of PCBs [GE Report, 1990]. This evidence suggests that the

metabolic capability utilized in this process is common and widespread among many different anaerobic microorganisms.

REFERENCES

Abramowicz, D.A. and M.J. Brennan. 1991. In: Biological remediation of contaminated sediments with special emphasis on the Great Lakes. Aerobic and anaerobic biodegradation of endogenous PCBs. C.T. Jafvert and J.E. Rodgers (eds), EPA/600/9-91/001, pp. 78-86.

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., 3:179-183.

Brown, J.F., Jr, and R.E. Wagner. 1990. PCB movement, dechlorination, and detoxication in the Acushnet estuary. Environ. Toxicol. Chem., 9:1215 -1233.

Brown, M.P., M.B. Werner, C.R. Carusone, and M. Klein. 1988. Distributions of PCBs in the Thompson Island pool of the Hudson River. Albany, NY, NYSDEC. Final report of EPA grant C361167-01, Hudson River PCB reclamation demonstration project sediment survey.

General Electric Company Research and Development Program for the Destruction of PCBs, Ninth Progress Report. 1990. General Electric Corporate Research and Development, Schenectady, NY.

Lake, J.L., R.J. Pruell, and F.A. Osterman. 1991. In: Organic substances and sediments in water, Lewis publishers. Dechlorinations of PCBs in sediments of New Bedford harbor, in press.

Sanders, J.E. 1989. The PCB pollution problem in the upper Hudson River: from environmental disaster to environmental gridlock. Northeast. Environ. Sci., 8:1-86.

APPENDIX A-3
FENTON CHEMISTRY

A recent paper by Sediak and Andren [1991] described the hydroxylation of PCBs by Fenton's reagent. The authors reacted 0.01 mM 2-chlorobiphenyl with 0.8 mM Fe^{2+} and 0.2 mM H_2O_2 over a pH range of 1 to 7. The extent of reaction was a strong function of pH with approximately 90% of the 2-chlorobiphenyl being hydroxylated in the pH range of 2.3 to 4.3. At pH 7, the extent of reaction dropped to a value of 20%. All 7 isomers of hydroxy-2-chlorobiphenyl were detected and were of the same order of magnitude in concentration. This suggested a relatively non-selective attack by the hydroxy radical at each of the nonchlorinated ring positions.

Sediak and Andren also subjected a commercial mix of PCB congeners, Aroclor 1242, to Fenton's reagent. The relative rates of reaction for the individual congeners were proportional to the number of nonhalogenated sites. While the attack of the hydroxyl radical on the biphenyl ring was relatively non-selective, the authors reported slightly higher reactivity at the *meta* and *para* positions. The authors speculated that this may have resulted from higher steric strain for the *ortho*-hydroxylated biphenyls.

Hydrogen peroxide was added to the HRRS reactors to maintain a set level of dissolved oxygen (DO). There was concern that the reaction of hydrogen peroxide with naturally occurring ferrous ions in the reactors could have resulted in the loss of PCBs via Fenton chemistry. The following addresses that concern.

Fenton's reagent is a mixture of hydrogen peroxide and ferrous ion that forms hydroxy radicals in the following reaction:

$$H_2O_2 + Fe^{2+} = OH\bullet + Fe^{3+} + OH^-$$

The hydroxy radicals produced can rapidly oxidize organic compounds. The second-order rate constant for radical formation is 76 l mol^{-1} s^{-1} [Clifton and

Savall, 1986]. The decomposition of hydrogen peroxide to water and oxygen is catalyzed by ferric ion (Fe^{3+}) with an apparent second-order rate constant (first-order in both Fe^{3+} and H_2O_2) of 4×10^2 l mol^{-1} s^{-1} at neutral pH.

The DO of the reactors was maintained at 3.5 ppm or greater throughout the course of the experiment. The initial set points were conservatively low to avoid accidental sterilization of the reactors if a malfunction occurred in the control system. With this level of dissolved oxygen and near neutral pH, the water in the reactors was calculated to be mildly oxidizing with an electrochemical potential of 750 mV relative to a standard hydrogen electrode [Atkins, 1978]. At lower pH or higher DO the electrochemical potential would be higher. At this potential, ferrous ion is not thermodynamically stable [Pourbaix, 1974] and the equilibrium concentration of ferric ion, as $Fe(OH)_2^+$, would be 6 to 7 orders of magnitude greater than ferrous concentration.

In addition to the ferric ion catalyzed decomposition of hydrogen peroxide, other catalytic decomposition routes of hydrogen peroxide were operative in the reactors; the most notable involved catalase. The decomposition of hydrogen peroxide to water and oxygen catalyzed by bacterial catalase is very rapid, having an apparent second-order rate constant of 7×10^7 l mol^{-1} s^{-1} [Hatzikonstantinou and Brown, 1978]. With the increased bacterial population during the experiment, one would expect instantaneous DO response to the addition of hydrogen peroxide. This rapid response was observed at the HRRS and in the laboratory.

Considering the low ferrous ion content relative to ferric ion and the decomposition of hydrogen peroxide by elevated bacterial catalase levels, the production of hydroxy radicals in the reactors should have been extremely low. The small amount of hydroxy radicals that may have been produced would have rapidly reacted with dissolved organic matter. The biphenyl that was added to the reactors as a carbon source was itself fifty times more abundant than the PCBs. If hydroxylation of aromatic organic compounds was occurring in the reactors via Fenton chemistry, one would expect to find significantly more hydroxybiphenyl than hydroxychlorobiphenyl.

Water samples collected over the first ten days of the experiment were tested for the presence of mono- and dihydroxybiphenyls. The highest concentration of ferrous ion would have occurred shortly after the experiment started, before the sediment had been well mixed and was still anaerobic. Biphenyl was also abundant at this time. These conditions would favor the hydroxylation of biphenyl via Fenton chemistry. The water analyses were performed by GC-MS with a sample preparation similar to that used for the analysis of chlorobenzoates (Appendix B-1). The results are contained in Table A3-1.

The results show the presence of small (<0.5 ppm) amounts of monohydroxybiphenyl in the T_o samples and the later samples, including control reactor R-104. There was no dihydroxybiphenyl detected. There was some increase in the amount of 2-hydroxybiphenyl in the later samples and the appearance of 4-hydroxybiphenyl which was not present in the T_o samples. Very notable was the lack of any detectable 3-hydroxybiphenyl. If the increase in hydroxybiphenyl was the result of Fenton chemistry, one would expect to find 3-hydroxybiphenyl in levels comparable to 2-hydroxybiphenyl and 4-hydroxybiphneyl [Sediak and Andren, 1991]. The selective hydroxylation in the *ortho* and *para* positions and the presence of 2-hydroxybiphenyl prior to the addition of hydrogen peroxide strongly suggest an alternative oxygenase pathway, possibly the result of fungal activity.

With all the considerations presented above, if Fenton chemistry was operative at all during the HRRS experiment, its contribution to the reduction of PCB concentration was insignificant compared to the biologically mediated PCB degradation.

Table A3-1.
GC-MS analysis of reactor water samples for hydroxybiphenyls.

(-), none detected; (+), detected; (++), detected at increased level.
Duplicate samples collected from the reactors are denoted "a".

		Hydroxybiphenyl		
Reactor	Sample	*Ortho*	*Meta*	*Para*
R-102	t=0	+	-	-
	t=2 days	++	-	-
R-103	t=0	-	-	-
	t=2 days	+	-	-
R-104	t=0	+	-	-
	t=0, a	+	-	-
	t=2 days	+	-	-
	t=2 days, a	+	-	-
R-105	t=0	-	-	-
	t=0, a	+	-	-
	t=1 day	+	-	+
	t=1 day, a	++	-	+
	t=2 days	++	-	+
	t=2 days, a	+	-	+
R106	t=0	+	-	-
	t=0,a	+	-	-
	t=2 days	+	-	+
	t=2 days, a	+	-	+
	t=4 days	++	-	++
	t=10 days	++	-	+

References

Atkins, P.W. 1978. Physical Chemistry. W.H. Freeman and Company, San Francisco.

Clifton, M.J. and A. Savall. 1986. Numerical Models for Reactions Catalysed by Homogeneous Mediators: the Case of Fenton's Reagent. J. App. Electrochem. 16:812-818.

Hatzikonstantinou, H. and S.B. Brown. 1978. Catalase Model Systems. Biochem. J. 174:893-900.

Pourbaix, M. 1974. Atlas of Electrochemical Equilibria in Aqueous Solutions. National Association of Corrosion Engineers, Houston.

Sediak, D.L. and A.W. Andren. 1991. Aqueous-Phase Oxidation of Polychlorinated Biphenyls by Hydroxy Radicals. Environ. Sci. Tech. 25(8):1419-1427.

APPENDIX B-1
CHLOROBENZOATE AND BIPHENYL ANALYTICAL METHODOLOGY

Chlorobenzoate (CBA) concentrations were monitored throughout this study using a congener-specific method developed by May et al. [in preparation]. Biphenyl and benzoic acid concentrations were also determined. Both methods are described below.

Extract Preparation for Aqueous Phase Chlorobenzoate Analysis

A 6 ml aliquot of each aqueous sample was centrifuged at 1600 rpm for 30 minutes to remove suspended particulates. A 3 ml aliquot of the supernatant was transferred to a small glass vial and treated with 1 ml of 6 N hydrochloric acid (HCl) to protonate the polar organic compounds. The polar organic fraction was extracted by adding 2.00 ± 0.01 ml of anhydrous ethyl ether containing 0.537 ppm 4-fluorobenzoic acid (4-FBA) as internal standard (TABLE 3; Ether B). The vials were tightly capped with teflon-lined lids and placed on a reciprocating shaker overnight. The vials were then centrifuged at 1600 rpm for 30 minutes. The organic phase containing the polar organic compounds was derivatized as described below, and analyzed for chlorobenzoates via gas chromatography-mass spectrometry (GC-MS).

Extract Preparation for Sediment Phase Chlorobenzoate and Biphenyl Analysis

Two serial extractions were performed on each sediment sample. The nonpolar organic fraction containing biphenyl and PCB was removed first, followed by an extraction of the polar organic fraction containing chlorobenzoates. The initial nonpolar organic extraction was intended as a cleanup/matrix reduction step, and also as a means of performing in-house PCB analysis for rapid feedback.

Sediment subsamples (2-3 g) were transferred to preweighed glass vials and treated with sodium metasilicate as a dispersing agent (1 ml of a 3% solution). The sodium metasilicate also acted as a strong base (pH 12.2) to deprotonate the polar organic compounds and therefore retain them in the sediment phase during the initial extraction of the nonpolar compounds. The nonpolar organic fraction was extracted by adding 3.00 ± 0.01 ml of a hexane/acetone (90:10) extraction solvent containing 0.446 ppm 4-fluorobiphenyl (4-FBP) as internal standard (Table B1-1; SES). The vials were tightly capped with teflon-lined lids, placed on a reciprocating shaker overnight, and then centrifuged at 1600 rpm for 30 minutes. The organic phase containing the nonpolar organic compounds was transferred to a clean glass vial. The sample vial and contents were washed three times with 1 ml aliquots of anhydrous ethyl ether. The rinsates were added to the vials containing the extracted nonpolar organic fraction. The extracts were concentrated to 3 ml under a nitrogen stream and then stored at 4°C.

The sediment subsamples were further treated to extract the polar organic fraction. This procedure was identical to the aqueous phase extraction described above. Hydrochloric acid (1 ml of 6 N HCl) was added to each subsample along with an anhydrous ethyl ether extraction solvent containing 4-FBA as internal standard (Table B1-1; Ether B). The vials were tightly capped, placed on a reciprocating shaker overnight, and centrifuged at 1600 rpm for 30 minutes. The organic phase containing the polar organic fraction was derivatized with pentafluorobenzyl bromide as described below and analyzed for chlorobenzoates via GC-MS.

Following the two serial extractions, the remaining aqueous acid phase was carefully removed from each sediment vial with a clean Pasteur pipet. The sediment vials were air-dried for 18-24 hours, and then placed in an 80°C heating block for 18-24 hours. A dry sediment pellet weight was obtained by subtracting the weight of the preweighed glass vial from the weight of the vial containing the dried sediment.

Table B1-1.
Extraction solvent recipes.

Sediment Extraction Solution (SES)
90% hexane 10% acetone 0.446 ppm 4-fluorobiphenyl (4-FBP)
Ether B
100% anhydrous ethyl ether 0.537 ppm 4-fluorobenzoate (4-FBA)

Extract Derivatization for Chlorobenzoate Analysis

Sample extracts of the polar organic fraction were derivatized with pentafluorobenzyl bromide prior to analysis for chlorobenzoates [Kawahara, 1968]. Round-bottom flasks (50 ml) were washed, rinsed in acetone and allowed to dry. The sample extracts were placed in the flasks along with 10 ml of spectrophotometric grade acetone, 20 ml pentafluorobenzyl bromide (Pierce, Rockford, IL), and ~0.2 g potassium carbonate (K_2CO_3) added as a catalyst and drying agent. The flasks were refluxed overnight and then evaporated to 2 ml under a nitrogen stream. The derivatized extracts containing the polar organic fraction were analyzed for chlorobenzoates via GC-MS.

Gas Chromatography-Mass Spectrometry for Chlorobenzoate Analysis

A Hewlett-Packard model 5890 Series II gas chromatograph equipped with a 30 m x 0.25 mm (I.D.) column of 0.25 mm DB-1 phase (J & W Scientific, Folsom, CA) and a 1 meter uncoated fused silica pre-column (0.53 mm I.D.) was connected to a Hewlett-Packard model 5971A quadrupole mass selective detector operated at an electron energy of 70 eV. A splitless injection was used, with a 1 minute delay before purge. The injection volume was 1 µl. Helium at a linear gas velocity of 25 cm/sec was the carrier gas. The injector

and transfer line were maintained at 270°C. The ion source pressure was maintained at 2.8×10^{-5} +/- 1.0×10^{-5} Torr. Selected ion monitoring (SIM) was used to quantify the concentrations of the mono- and dichlorobenzoate congeners in each derivatized sample extract. For each congener, both the molecular ion and an ion fragment were monitored. The SIM parameters and GC temperature program for the chlorobenzoate analysis are given in Table B1-2. A typical chromatogram of the calibration standard is shown in Figure B1-1.

Gas Chromatography-Mass Spectrometry for Biphenyl and Benzoic Acid Analysis

Total ion chromatographs (TIC) were used to quantify the concentrations of the biphenyl and benzoic acid in each derivatized sample extract. The GC-MS system described above was used without modification. The GC temperature program for the biphenyl and benzoic acid analysis is given in Table B1-3.

Quality Assurance/Quality Control

The Hewlett-Packard model 5971A mass spectrometer was tuned and mass calibrated once for each analysis set using a standard autotune routine. Initial determination of the relative response factor and instrumentation concentration range for each analyte was determined by multilevel calibration (Figure B1-2). A calibration standard containing all of the chlorobenzoate congeners of interest plus the internal standard 4-FBA (Table B1-4) was injected before each chlorobenzoate analysis set, and once for every 5 sample injections. Retention times and response factors were updated based on the calibration standard injections. An internal standard (4-FBA) was included in the extraction solvent to normalize against variations in sample handling during the extraction procedure. Clean glass beads (~2 g) were periodically extracted as a method blank using the same procedures and solvents as for the samples.

Table B1-2
GC-MS method for chlorobenzoate analysis

Column:	30 m DB-1 0.25 mm I.D. 0.25 mm film thickness
Pre-column:	1 m uncoated fused silica 0.53 mm I.D.
Injection:	Splitless; 1 minute delay before purge
Injector Temperature:	270°C
Transfer Line Temperature:	270°C
Temperature Program:	90°C for 2 min. 20°C/min. to 150°C 6°C/min. to 270°C Hold at 270°C for 5 min.
Solvent Delay:	7 min.
SIM Parameters:	

Time (min)	Ions
7.00 to 11.10	123, 181, 320
11.10 to 13.30	105, 181, 302
13.30 to 14.00	139, 181, 336
14.50 to 16.90	173, 181, 370
16.90 to 30.00	181, 207, 404

Dwell Time:	300 msec per channel

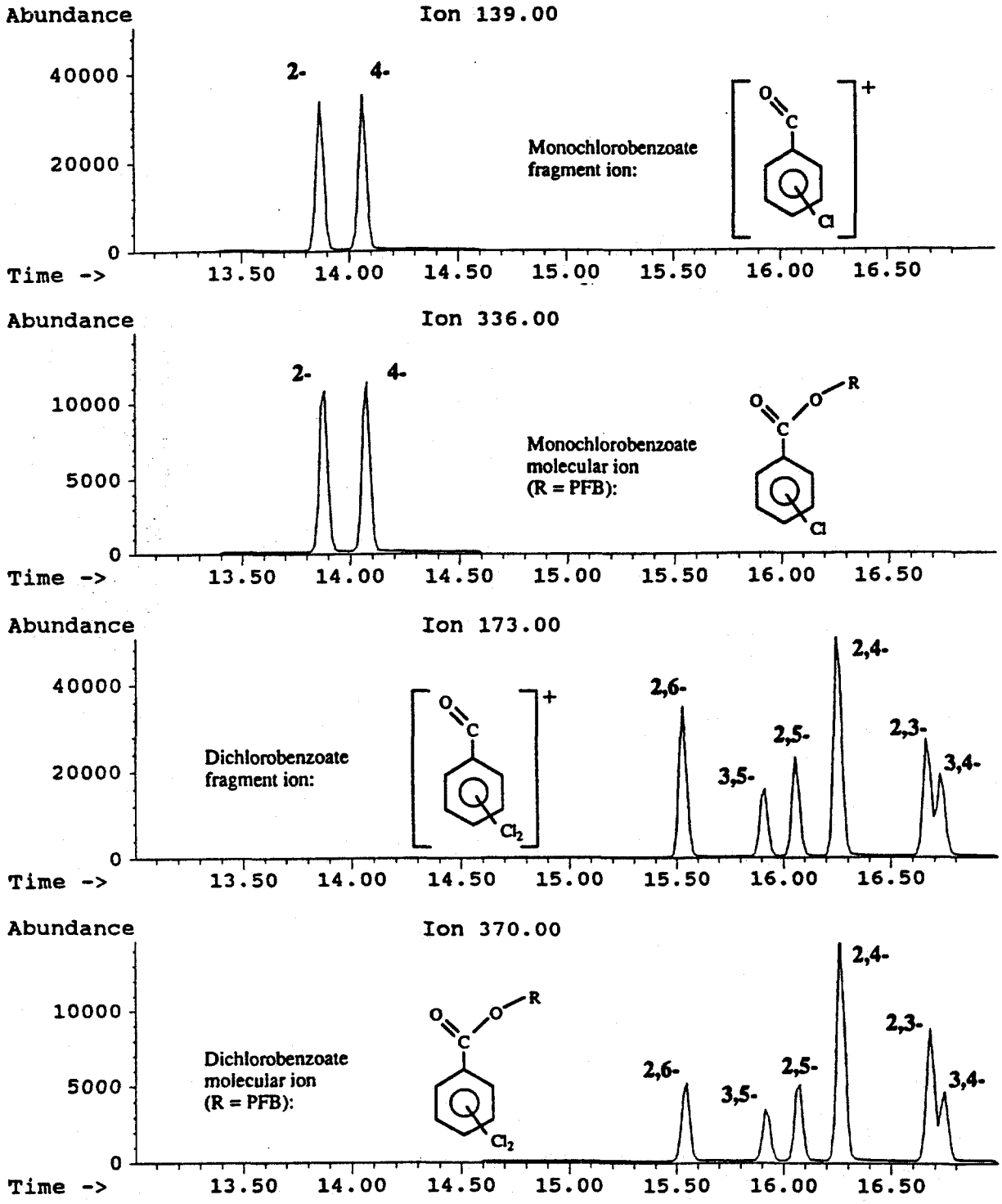


Figure B1-1. Typical GC-MS SIM chromatogram of a calibration standard containing mono- and dichlorobenzoates derivatized with pentafluorobenzyl bromide (PFB-Br).

Table B1-3.
GC-MS method for biphenyl and benzoate analysis.

Column:	30 m DB-1 0.25 mm I.D. 0.25 mm film thickness
Pre-column:	1 m uncoated fused silica 0.53 mm I.D.
Injection:	Splitless; 1 minute delay before purge
Injector Temperature:	270°C
Transfer Line Temperature:	270°C
Temperature Program:	90°C for 2 min. 30°C/min. to 150°C 20°C/min. to 270°C Hold at 270°C for 4 min.
Solvent Delay:	7 min.
SIM Parameters:	

Time (min)	Ions
6.00 to 7.00	153, 154
7.00 to 8.01	188, 190
8.01 to 8.15	123, 336
8.15 to 8.40	105, 302
8.40 to 14.00	105, 181

Dwell Time:	300 msec per channel

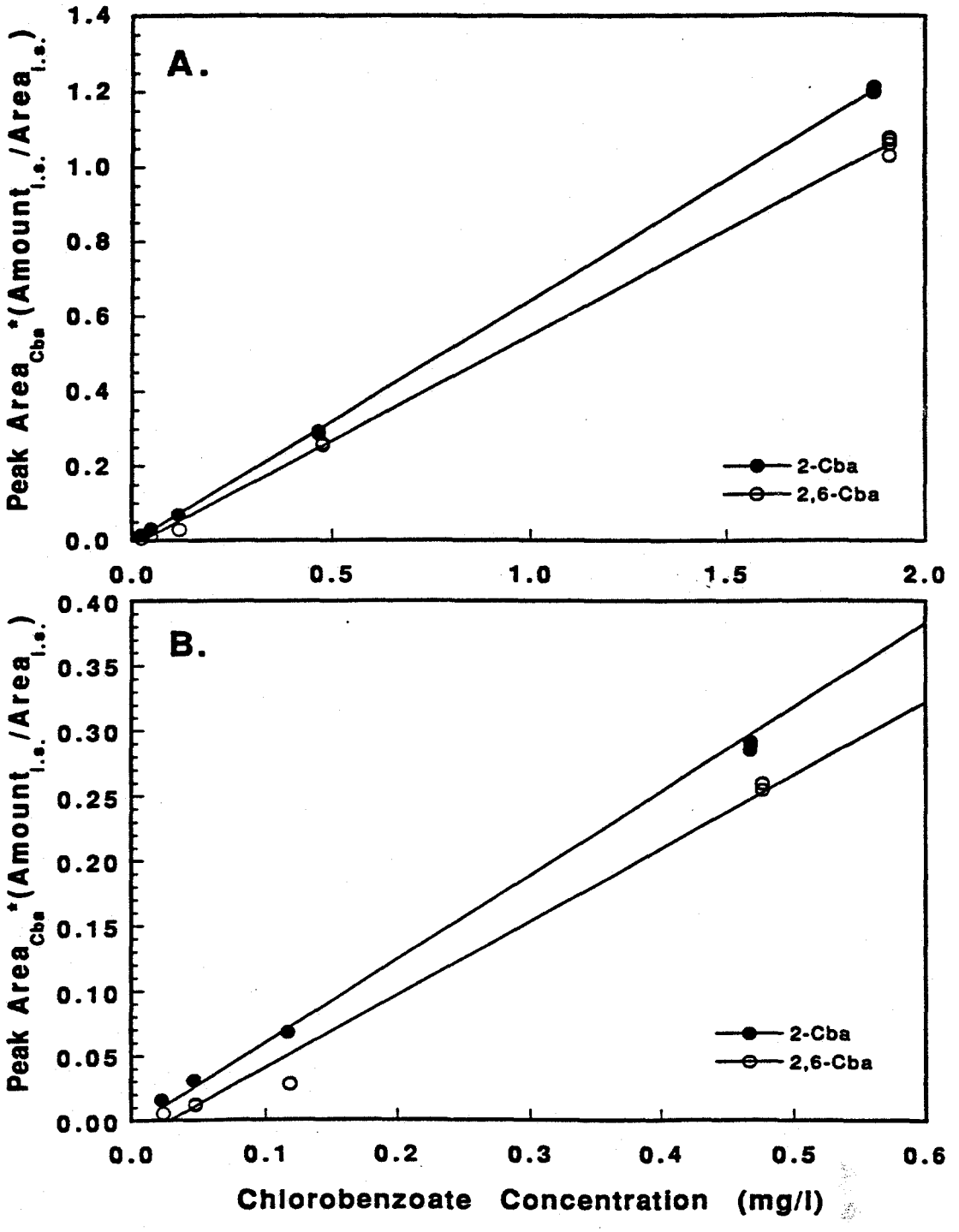


Figure B1-2. Typical multipoint GC-MS calibration for a selected mono- (2-) and dichlorobenzoate (2,6-) analyte. $r^2_{(2\text{-Cba})} = 0.9994$; $r^2_{(2,6\text{-Cba})} = 0.9992$. Plot B is an expanded version of plot A.

Table B1-4.
Chlorobenzoate (CBA) calibration standard

Compound	Concentration (ppm)
4-FBA	0.537
Benzoate	0.537
2-CBA	0.581
4-CBA	0.595
2,6-CBA	0.591
2,5-CBA	0.680
2,4-CBA	1.18
2,3-CBA	0.766
3,4-CBA	0.584
3,5-CBA	0.595

APPENDIX B-2
TOXICITY TESTING PROTOCOL

Standard Operating Procedure (SOP) of Battelle Laboratories Static Acute Whole Sediment Toxicity Tests with *Ceriodaphnia dubia*

The *C. dubia* toxicity tests were conducted in accordance with Battelle's Standard Operating Procedures, which are based on procedures developed by U.S. EPA (Peltier and Weber) *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (EPA/600/4-85/013), ASTM *Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates* and ASTM *Guide for Collection, Storage, Characterization and Manipulation of Sediment for Toxicological Testing.*

Introduction

This SOP describes the methodology for conducting a static acute whole sediment toxicity test with *Ceriodaphnia dubia.* During this test, *Ceriodaphnia dubia* are continuously exposed for 48 hours to whole sediment laboratory water over-lying the sediment. *Ceriodaphnia dubia* survival is recorded daily for the duration of the test period. The survival data are used to estimate the lethality of the test sediment.

Test System

Test organisms. Young *Ceriodaphnia dubia* used for testing must come from laboratory reared animals and must be less than 24 hours old at test initiation.

Feeding. *Ceriodaphnia dubia* are not fed during the test period.

Methods. Place young *Ceriodaphnia dubia* (<24 hours-old at test initiation) in test chambers and subject to test conditions for 48 hours. *Ceriodaphnia dubia*

must be transferred with a large-bore pipette into test chambers, which then must be covered with a pane of glass or watch glasses to minimize evaporation.

Test Chambers/Test Volume. 30 ml borosilicate glass beakers containing 10 ml of test sediment and 15 ml of dilution water are used as test chambers.

Test Materials. Test materials (sediments) are stored at 4°C in the dark (unless otherwise specified), but allowed to gradually come to 20°C before test initiation. Portions of the test sediment are homogenized in volumetric flasks and then aliquots of the sediment are measured and placed into the test beakers.

Over-lying Water. Over-lying water will be laboratory water (see SOP on Preparation of Reconstituted Water, EEF D-07 or EEF D-20).

Controls. Controls must be set up and treated identically with regard to experimental conditions as test chambers, except that a reference sediment (Spier Falls) is used in place of the test material.

Test Concentrations. The concentration tested will be 100 per cent whole sediment.

Randomization. *Ceriodaphnia dubia* are assigned completely at random from the stock to the test beakers. A two-stage transfer procedure is needed. *Ceriodaphnia dubia* from the culture stock are randomly transferred into beakers containing laboratory water which corresponds to each test group. The order of assignment is determined from a table of random numbers or another method of random allocation. A second transfer is then made into beakers then randomly placed in the environmental chamber (see SOP on randomization, EEF B-06/07).

Replication. Four test chambers, each containing five *Ceriodaphnia dubia* (a total of 20 animals per concentration), are required for each experimental condition.

Aeration. Aeration of test solutions is not performed during testing.

Light and Photoperiod. Fluorescent light bulbs (ambient laboratory illumination) with a 16-hour light: 8-hour dark photoperiod were automatically controlled. Acceptable ambient laboratory levels of light intensity range from 323 to 1076 lux.

Temperature. Tests are conducted in a controlled environment chamber which maintains the test temperature at 20 ± 2°C. A continuously operating recording thermometer provides a permanent record of the air temperature and is checked daily during the test period.

Water Quality Measurements. Because *Ceriodaphnia dubia* are extremely sensitive to mechanical stress, such as the introduction of analytical probes into the test chambers, dissolved oxygen, pH, and temperature measurements are made in test chambers at the beginning of the test (before the animals are introduced) and at the end of the test (after survival observations). Temperature is also measured in at least one control chamber at 24 hours.

pH. The pH of the test material is not adjusted.

Endpoints. The number of live and dead (or immobilized) *Ceriodaphnia dubia* are counted at 24 hours and 48 hours. Immobilized test organisms are those unable to sustain their position in the water column or those that do not react to gentle prodding.

General Acute Test Procedures

1. Transfer parental generation to new culture beakers containing food 24 hours before the start of the test to ensure that only <24-hour-old *Ceriodaphnia dubia* will be available for testing (note time of change on acclimation form).

2. Label all test beakers.

3. Prepare test material by adjusting the temperature to 20°C, homogenize the sediment and then add the over-lying water to each test chamber containing sediment.

4. Allow test solutions to settle overnight to allow the overlying water to clear.

5. Measure and record on data form the dissolved oxygen, pH, and temperature in all test chambers and the controls at the beginning of the test (0 hours) before addition of test animals.

6. Randomly add <24-hour-old *Ceriodaphnia dubia* until each beaker contains five individuals.

7. Randomize control and test concentrations into rows and then cover with glass (EEF B06/07).

8. At the end of 24 hours and 48 hours, count and record on the data form the number of dead or immobilized *Ceriodaphnia dubia* per beaker.

9. At 24 hours, measure and record on the data form the water temperature in at least one control chamber.

10. At the end of the test (48 hours), measure and record on the data form dissolved oxygen, pH and temperature in all test chambers for every test concentration (with surviving animals) and the controls.

APPENDIX C-1
STATISTICAL DESIGN, SAMPLING, AND DATA ANALYSIS TASKS

C.B. Morgan
Management Science and Statistics Program

Introduction

This report will describe how statistical design, sampling, and data analysis methods were used to quantitatively assess whether there had been a change in the concentration of PCB within each of the six caisson reactors during the eleven week experimental study. Although many analyses were performed on the sediment samples to identify soil properties, hydrology, percent of PCB congeners present, etc., this section specifically focuses on the assessment of the PCB changes within each caisson using core sediment samples.

Sampling Plan

The sampling plan was developed on the basis of the quantitative assessment to be made, the characteristics of the study site, and the restrictions on sampling within each caisson. Core sediment samples were taken initially at week T_o, prior to starting the experimental program, and at week T_f, the end of the experimental program. The top six to eight inches of sediment within each caisson was mixed using either a rapid stirring or slow raking process for about twenty-four hours prior to obtaining the initial core samples. These core measurements would be used to establish the baseline PCB concentration level for each caisson. Core sediment samples were also taken on a weekly basis within each caisson. The number of cores taken in any time period was restricted to avoid depleting the volume of sediment within the caisson. In addition, the cost of collecting, processing, and analyzing each soil sample was incorporated into determining the sample sizes.

A grid pattern was employed to define sampling regions within each caisson. Each caisson area had four geographical subdivisions or strata called ports (North, South, East and West). The ports were the primary means of access to the sediment area of each caisson. Each port also had three geographical subdivisions called sections (Inner, Outer and Middle). A systematic sampling procedure was employed within each caisson. Twelve sediment samples - one per section within each port - were taken from each caisson at week T_0 and T_f. The number of samples taken was appropriate for the size of the sample area. Four sediment samples (one from each port) were also taken from each caisson on a weekly basis.

This sampling plan was developed to provide access, at least in the initial and final sampling periods, to as much of the spatial extent of the sampling area as possible. The sample collection and handling procedures are outlined in Chapter 3 of this report. Each physical core sample was delivered to Northeast Analytical Laboratories (NEA) where it was mixed and homogenized. A random subsample of the homogenized core sample was selected for chemical extraction and analyzed to measure the PCB concentration in the sample. In addition, total core dry weights were measured so that the total PCB content of each core could be determined.

The Data

Samples were identified according to the number of the caisson from which the sample was obtained, the date the sample was collected, and codes specifying the port (N,S,E,W) and section of the port (I,M,O) from which the sample was taken. The analytical findings included the measured PCB concentration (µg /g), the core sample dry weight (g), and the measured total PCB level (µg). A complete listing of the data is provided in Appendix C-4.

Distribution of PCB Concentration for each Caisson

Each caisson area was small enough that with stirring or raking it would be possible to establish a fairly homogeneous sediment layer during the experimental program. The initial PCB concentrations within each caisson were not identical. Thus, the final comparisons would be based on the change of concentration within each caisson area, not between the caissons.

Figure C1-1 provides a boxplot of the measured PCB concentration levels for each sediment sample within each caisson at the initial time, week T_o. Figure C1-2 provides a similar boxplot for the measurements obtained at the final week, T_f. The boxplot or schematic plot allows an easy comparison of the distribution of concentration measurements for the six caissons. The bottom and top edges of the box are located at the sample 25th and 75th percentiles, respectively. (The distance between the 25th and 75th sample percentiles is the interquartile range.) The horizontal line in the interior of each box is located at the sample median (50th percentile). The central vertical lines or "whiskers" extend from the box as far as the data extend to a distance of about 1.5 interquartile ranges. Extreme observations which are beyond 3 interquartile ranges are noted with a small dot. Figures C1-3 and C1-4 provide similar boxplots for the measured total PCB levels for the weeks T_o and T_f, respectively.

The spread and presence of outliers in some of this data is not unexpected, particularly given the nonhomogeneous nature of the site, the short period of the initial mixing processes, and the difficulties encountered in obtaining core samples from some caissons. Such variability was inherent in the experimental process. For that reason it was decided not to reject any samples from the data set solely on a statistical basis. Hence the PCB results presented here and in Chapter 5 reflect all the data collected in the experiment.

Before defining a statistical test to determine whether a change in the PCB concentration within a caisson had occurred, the distribution of the PCB concentrations within each caisson should be established. This distribution is

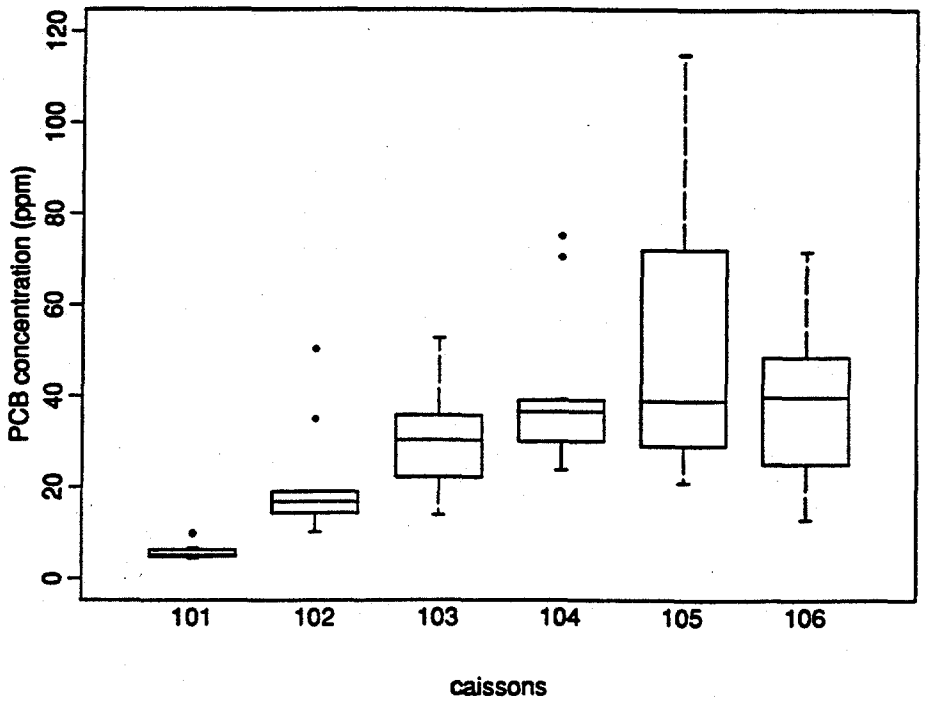


Figure C1-1. Boxplot of PCB concentration in caissons at T_o.

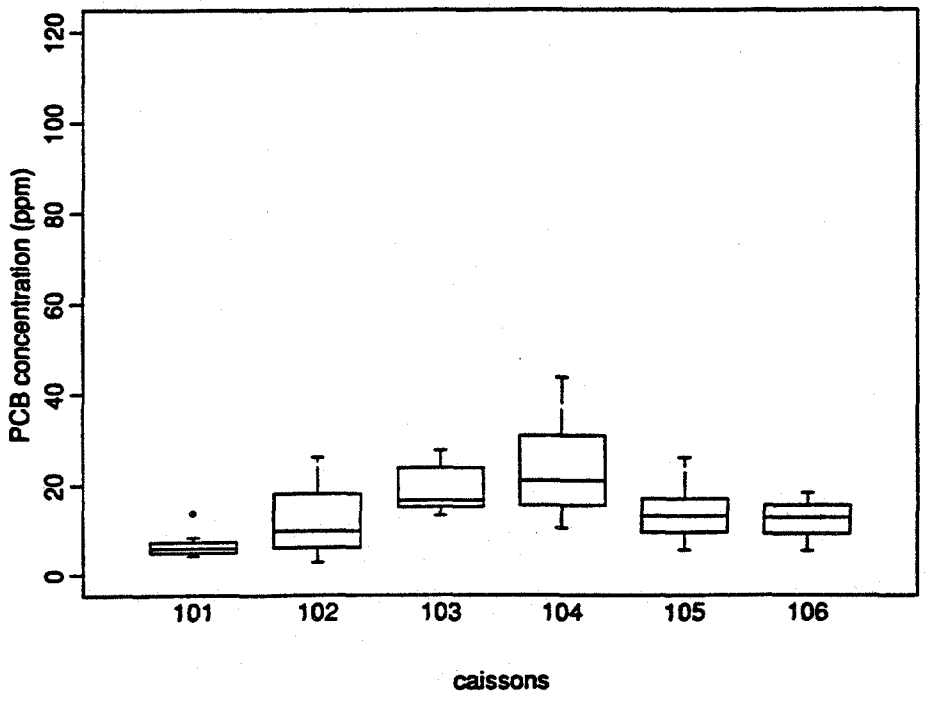


Figure C1-2. Boxplot of PCB concentration in caissons at T_f.

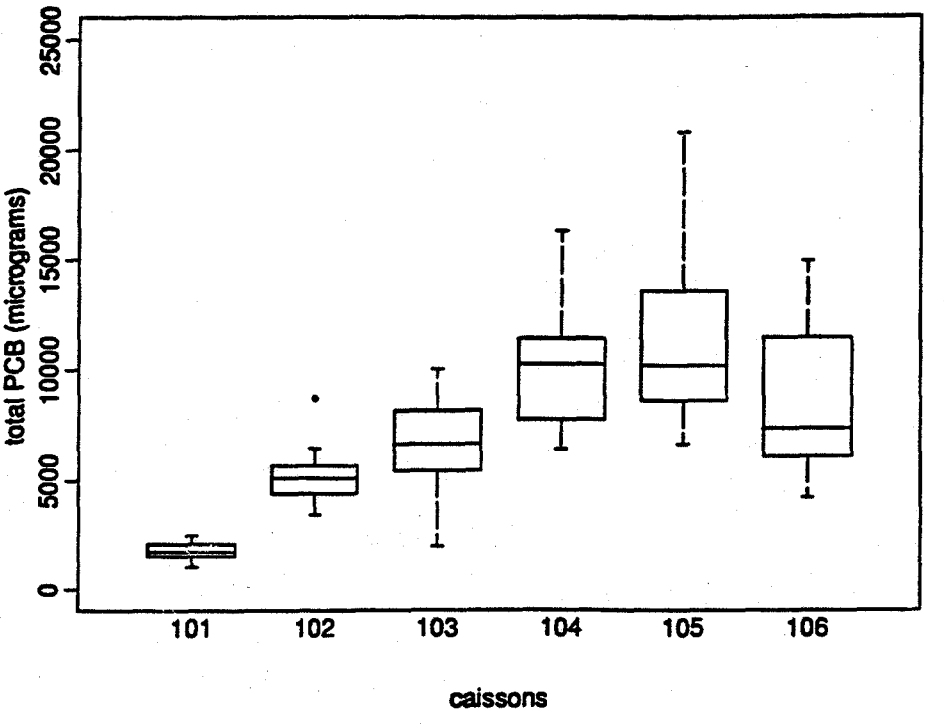


Figure C1-3. Boxplot of total PCBs in caissons at T_0.

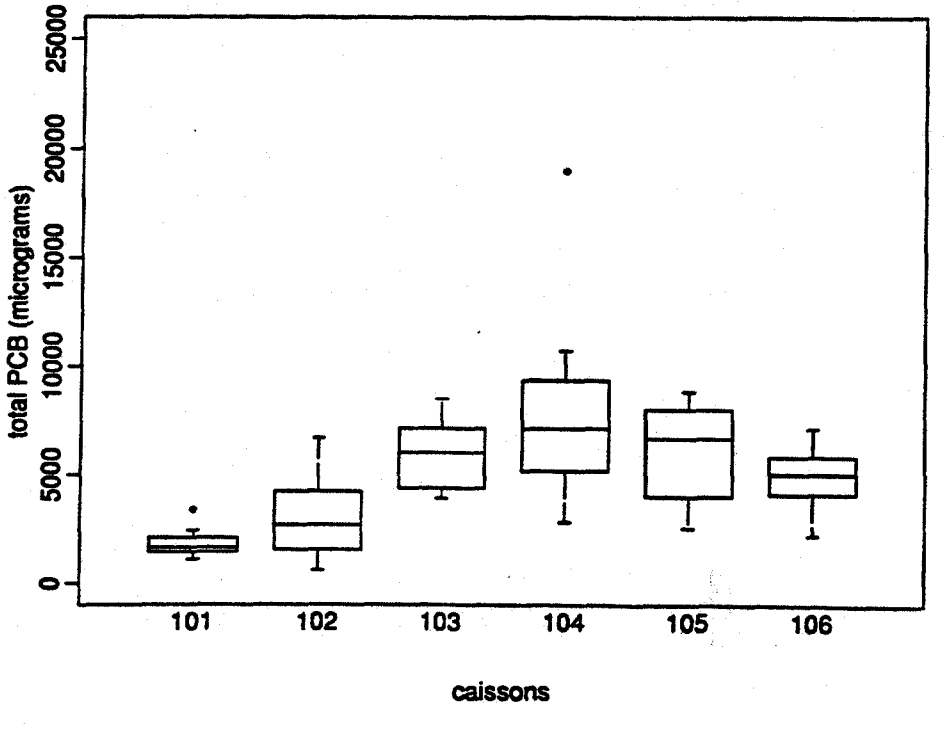


Figure C1-4. Boxplot of total PCBs in caissons at T_f.

expected to vary depending on the site location, sediment composition, and methods of collecting and analyzing the soil samples. Since the chemical concentrations cannot be less than zero, the distribution of concentrations is not likely to be symmetric but skewed to the right (i.e., have a long tail to the right). However by taking the natural logarithm of the observations a normal distribution of values can often be obtained. Figures C1-5 - C1-8 show boxplots for the natural logarithms of the data obtained at T_o and T_f.

Table C1-1 shows the sample size (N), minimum, maximum, mean, standard error about the mean, and the coefficient of variation (cv) for the PCB concentration (CPCB) and total core PCB measurements (PCBTOT) with each caisson as well as the same summary statistics for the natural logarithms of these measurements. (The coefficient of variation is the ratio of the standard deviation and the mean and expresses the spread of the data in relative terms.) The code N in the margin denotes if the sample values are from a normal distribution. This determination was made using the Shapiro-Wilk test for normality on the sample data.

In many cases the natural logarithm transformation did yield a normal distribution for the sample data. This suggests the use of the mean and natural logarithms in the statistical tests. For comparative purposes, the statistical tests were conducted using both the original and transformed data. Often the results were similar using either form of the data. Furthermore, the use of the mean should not present any problems for the analyses since none of the measurements were recorded as below the equipment detection limit.

Statistical Findings

The t statistic for testing the equality of means x_1 and x_2 from two independent samples with n_1 and n_2 observations is

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{S_p \sqrt{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

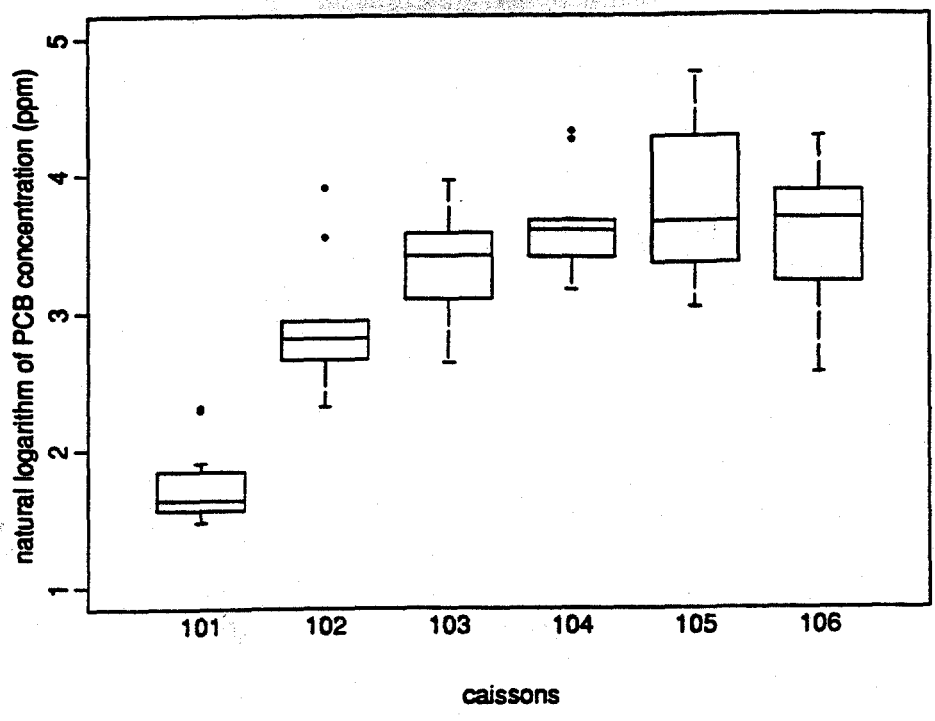


Figure C1-5. Boxplot of the natural logarithm of PCB concentration in caissons at T_0.

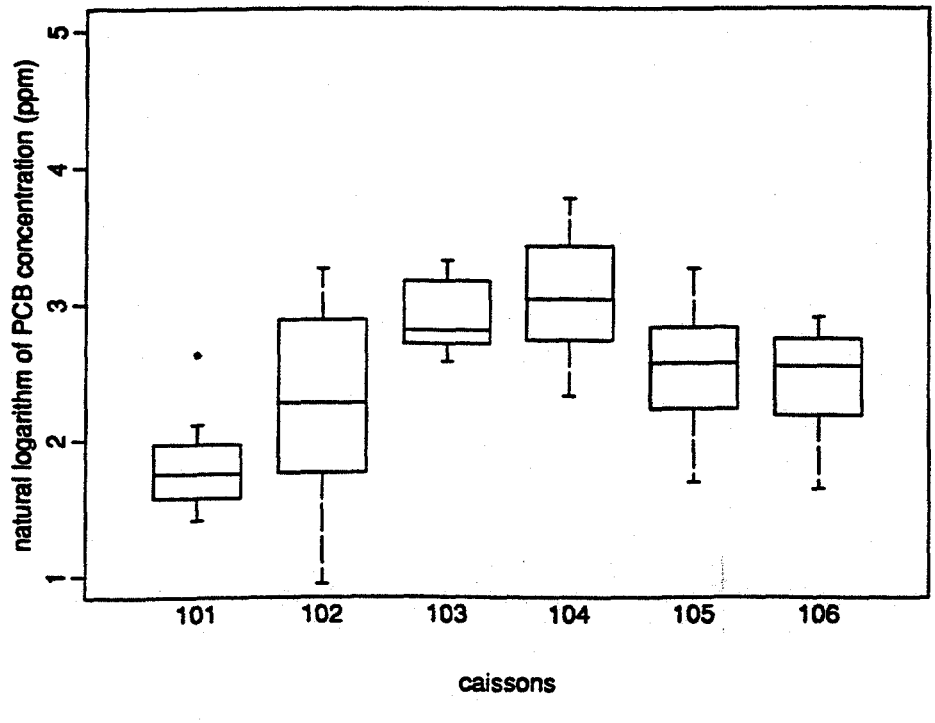


Figure C1-6. Boxplot of the natural logarithm of PCB concentration in caissons at T_f.

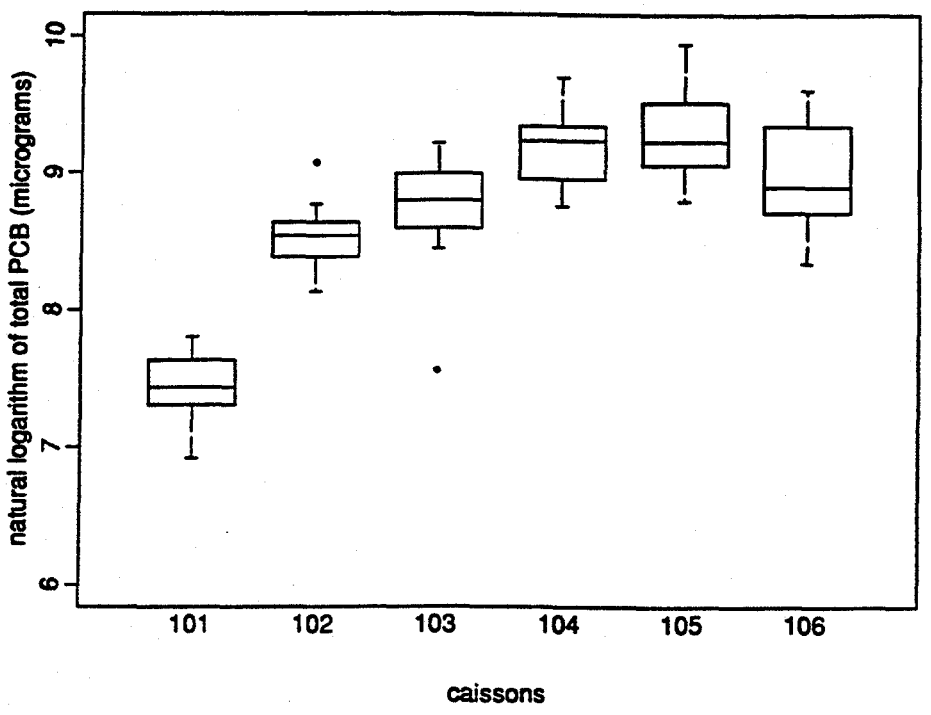


Figure C1-7. Boxplot of natural logarithm of total PCB in caissons at T_o.

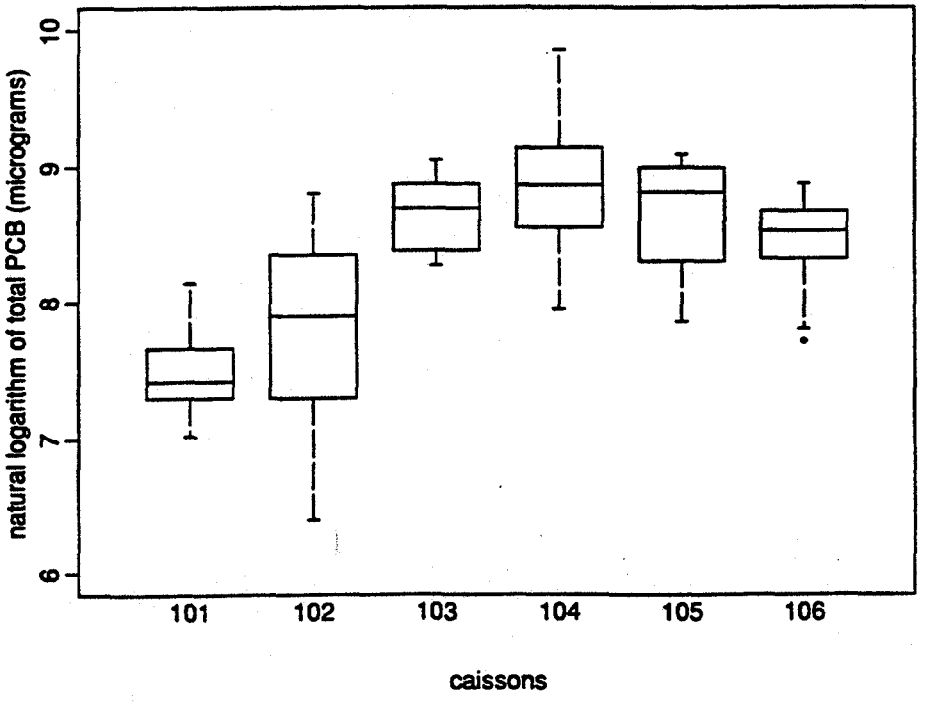


Figure C1-8. Boxplot of natural logarithm of total PCB in caissons at T_f.

TABLE C1-1. Summary Statistics for PCB Concentration Data Initial Week

Variable	N	Minimum	Maximum	Mean	Std Error	CV	
CAISSON=101							
CPCB	12	4.3600	10.0800	5.9908	0.5674	32.8064	
LCPCB	12	1.4725	2.3106	1.7499	0.0815	16.1261	
PCBTOT	12	1010.5	2444.4	1747.2	121.4	24.0797	N
LPCBTOT	12	6.9182	7.8016	7.4377	0.0727	3.3838	N
DRYWT	12	202.5	380.5	300.2	16.0419	18.5108	N
CAISSON=102							
CPCB	12	10.1100	50.3600	19.9808	3.3171	57.5090	
LCPCB	12	2.3135	3.9192	2.8852	0.1313	15.7630	
PCBTOT	12	3405.1	8741.5	5220.4	411.1	27.2794	N
LPCBTOT	12	8.1330	9.0758	8.5290	0.0744	3.0203	N
DRYWT	12	128.1	365.6	291.3	18.5454	22.0533	N
CAISSON=103							
CPCB	12	13.9300	52.6900	30.1650	3.2046	36.8008	N
LCPCB	12	2.6340	3.9644	3.3435	0.1087	11.2616	N
PCBTOT	12	1933.3	10037.4	6677.8	662.3	34.3587	N
LPCBTOT	12	7.5670	9.2141	8.7340	0.1266	5.0228	
DRYWT	12	104.5	374.5	231.8	22.2770	33.2915	N
CAISSON=104							
CPCB	12	23.6500	75.2700	39.9158	4.6927	40.7256	
LCPCB	12	3.1634	4.3211	3.6252	0.1007	9.6220	N
PCBTOT	12	6373.7	16312.9	9989.3	781.5	27.1020	N
LPCBTOT	12	8.7599	9.6997	9.1768	0.0767	2.8948	N
DRYWT	12	151.7	336.9	264.9	17.4238	22.7866	N
CAISSON=105							
CPCB	12	20.8200	114.9	49.7292	8.3770	58.3537	
LCPCB	12	3.0359	4.7444	3.7666	0.1561	14.3560	N
PCBTOT	12	6603.1	20845.7	11332.5	1135.3	34.7042	N
LPCBTOT	12	8.7953	9.9449	9.2862	0.0926	3.4561	N
DRYWT	12	120.0	395.2	262.0	23.2293	30.7190	N
CAISSON=106							
CPCB	13	12.8700	71.6200	39.1646	5.0496	46.4874	N
LCPCB	13	2.5549	4.2714	3.5560	0.1419	14.3865	N
PCBTOT	13	4194.3	14934.6	8641.9	996.7	41.5828	N
LPCBTOT	13	8.3415	9.6114	8.9854	0.1147	4.6042	N
DRYWT	13	134.6	339.5	237.2	18.7321	28.4792	N

N denotes Shapiro-Wilk test for normality was not rejected

TABLE C1-1. Summary Statistics for PCB Concentration Data
Final Week

Variable	N	Minimum	Maximum	Mean	Std Error	CV	
CAISSON=101							
CPCB	12	4.0900	13.8800	6.5100	0.7619	40.5443	
LCPCB	12	1.4085	2.6304	1.8161	0.0961	18.3300	N
PCBTOT	12	1115.3	3440.9	1843.7	183.7	34.5194	N
LPCBTOT	12	7.0169	8.1435	7.4723	0.0901	4.1769	N
DRYWT	12	246.2	331.4	287.2	7.5573	9.1166	N
CAISSON=102							
CPCB	12	2.5600	26.0400	11.7508	2.2066	65.0494	N
LCPCB	12	0.9400	3.2596	2.2490	0.2064	31.7872	N
PCBTOT	12	598.3	6675.8	3129.0	562.4	62.2586	N
LPCBTOT	12	6.3941	8.8062	7.8193	0.2226	9.8637	N
DRYWT	12	122.6	373.6	271.4	18.8615	24.0723	N
CAISSON=103							
CPCB	12	13.1600	27.5800	19.1100	1.5109	27.3875	N
LCPCB	12	2.5772	3.3171	2.9167	0.0776	9.2124	N
PCBTOT	12	3917.7	8525.0	5914.3	465.6	27.2702	N
LPCBTOT	12	8.2733	9.0508	8.6502	0.0802	3.2102	N
DRYWT	12	240.7	501.7	315.7	21.3004	23.3699	
CAISSON=104							
CPCB	12	10.1800	43.5100	23.0908	2.9834	44.7573	N
LCPCB	12	2.3204	3.7730	3.0507	0.1267	14.3891	N
PCBTOT	12	2837.2	19035.6	7897.1	1219.6	53.5006	
LPCBTOT	12	7.9506	9.8541	8.8611	0.1417	5.5400	N
DRYWT	12	265.7	478.7	341.0	21.7111	22.0577	N
CAISSON=105							
CPCB	12	5.4200	25.9600	13.5925	1.6846	42.9319	N
LCPCB	12	1.6901	3.2566	2.5186	0.1327	18.2491	N
PCBTOT	12	2583.2	8865.3	6017.1	678.5	39.0606	N
LPCBTOT	12	7.8568	9.0899	8.6173	0.1307	5.2532	N
DRYWT	12	341.5	534.8	449.0	17.0101	13.1230	N
CAISSON=106							
CPCB	12	5.1700	18.2400	12.3133	1.2423	34.9485	N
LCPCB	12	1.6429	2.9036	2.4402	0.1204	17.0910	N
PCBTOT	12	2249.7	7162.8	4906.1	441.9	31.2016	N
LPCBTOT	12	7.7186	8.8767	8.4434	0.1058	4.3422	N
DRYWT	12	355.2	495.	407.0	13.1430	11.1873	N

N denotes Shapiro-Wilk test for normality was not rejected

where S_p^2 is the pooled variance and

$$S_p^2 = \frac{[(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2]}{(n_1 + n_2 - 2)}$$

where S_1^2 and S_2^2 are the sample variances of the two groups.

The use of this t statistic requires the assumption that the population variances of the two groups are equal, i.e. $\sigma_1^2 = \sigma_2^2$. Under the assumption of unequal variances, an approximate t statistic is computed as

$$t' = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)}}$$

Additional details on the calculation of the t statistic and approximate t statistic are given in Dixon and Massey, 1969.

Table C1-2 shows the results of the t statistic test for the original and the natural logarithm of the data. Using the original data values, the test of the null hypothesis that the average initial PCB concentration equals the average final PCB concentration was rejected at the 5% significance level for all caissons except caisson R101. The results were identical when the natural logarithm of the data was used instead. In the cases where the null hypothesis was rejected, the initial average logarithm PCB concentration was greater than the final average logarithm PCB concentration. As shown in Table C1-2, a similar analysis was conducted using the total PCB content values. The total PCB content is obtained by multiplying the measured PCB concentration of the core by the dry weight of the core. In this case the null hypothesis states that average initial PCB content equals the average final PCB content. This hypothesis was rejected at the 5% significance level for caissons R102, R105 and R106. The differences in these results compared with PCB concentrations reflect the effect of increasing core weights in the low-mix caissons over time.

Table C1-2
PCB and Total PCB Two Sample t Test Results

Comparison of sample means at T_o and T_f

NULL HYPOTHESIS H_0: $\mu_1 = \mu_2$

ALTERNATIVE HYPOTHESIS H_A: $\mu_1 \neq \mu_2$

CAISSON -->	101	102	103	104	105	106
VARIABLE						
PCB CONC.	A	R	R	R	R	R
LN PCB CONCENTRATION	A	R	R	R	R	R
TOTAL PCB	A	R	A	A	R	R
LN TOTAL PCB	A	R	A	A	R	R

R means reject the null hypothesis at the 5% significance level
A means cannot reject the null hypothesis at the 5% significance level

PCB Total Organic Carbon Data Analysis

Total organic carbon (TOC) was measured for each core sample taken during the T_o and T_f samplings of the caissons. Average PCB concentrations in those sediment cores could then be calculated on a TOC basis. Figures C1-9 and C1-10 provides boxplots of the measured PCB TOC concentration levels for each sediment sample within each caisson at week T_o and T_f, respectively. Figures C1-11 and C1-12 show similar plots for the natural logarithms of the data. Table C1-3 shows the sample size (N), minimum, maximum, mean, standard error about the mean, and the coefficient of variation (CV) for the measured PCB levels (variable PCBTOC) within each caisson as well as the summary statistics for the natural logarithms of the

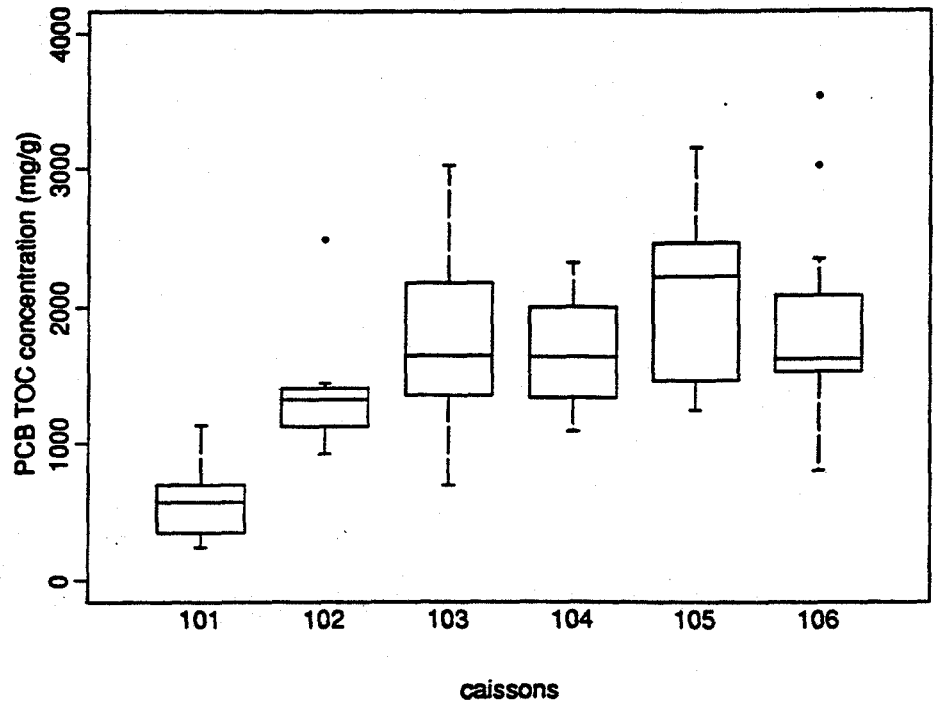


Figure C1-9. Boxplot of PCB TOC concentration in caissons at T_0.

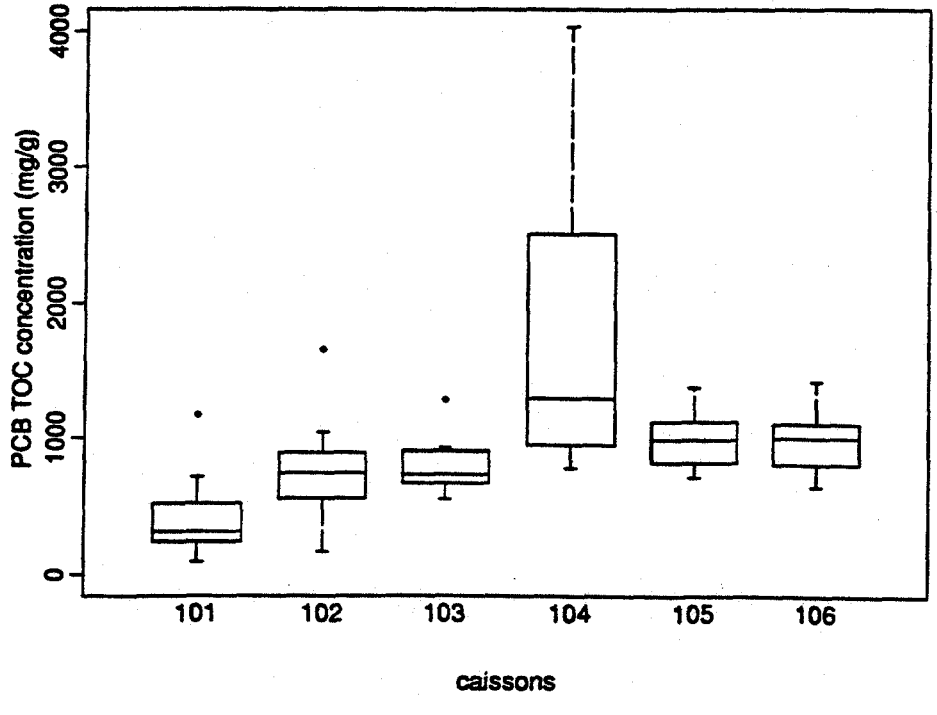


Figure C1-10. Boxplot of PCB TOC concentration in caissons at T_f.

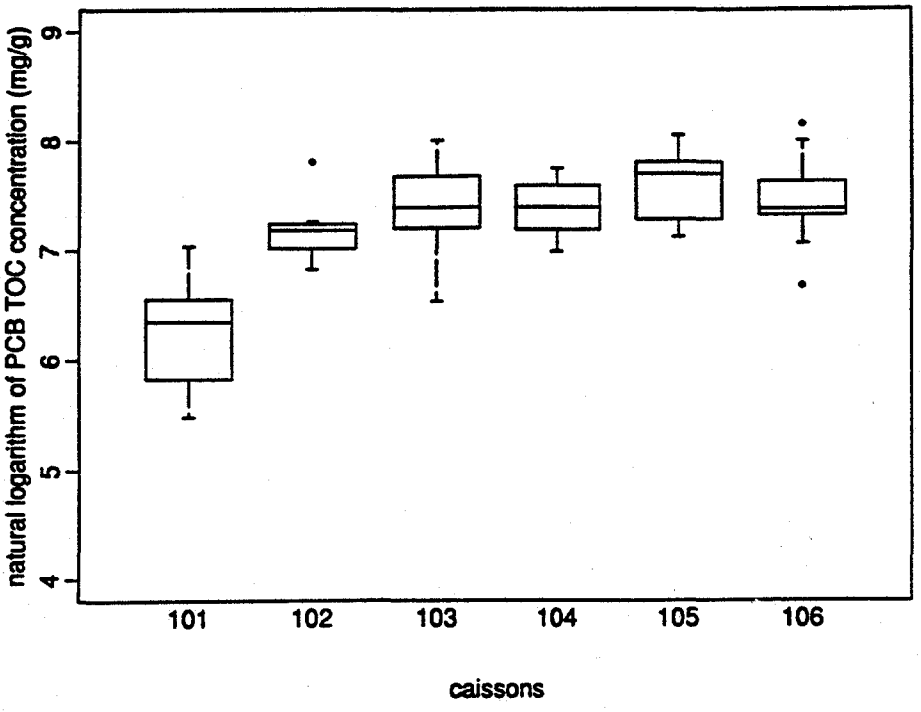


Figure C1-11. Boxplot of natural logarithm of PCB TOC concentration in caissons at T_o.

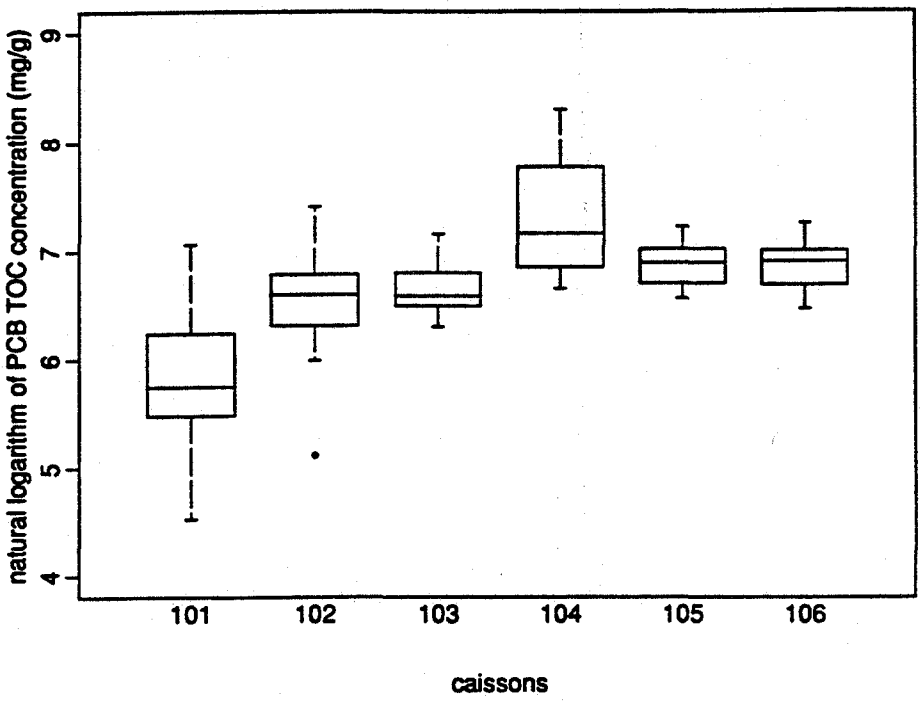


Figure C1-12. Boxplot of natural logarithm of PCB TOC concentration in caissons at T_f.

TABLE C1-3. Summary Statistics for Total Organic Carbon Data Initial Week

Variable	N	Minimum	Maximum	Mean	Std Error	CV	
CAISSON=101							
PCBTOC	12	241.6	1138.6	563.0	73.9128	45.4767	N
LPCBTOC	12	5.4873	7.0376	6.2336	0.1383	7.6863	N
CAISSON=102							
PCBTOC	12	925.0	2499.3	1353.7	116.2	29.7328	
LPCBTOC	12	6.8298	7.8238	7.1781	0.0734	3.5425	
CAISSON=103							
PCBTOC	12	692.1	3029.9	1774.2	196.9	38.4427	N
LPCBTOC	12	6.5397	8.0163	7.4084	0.1186	5.5451	N
CAISSON=104							
PCBTOC	12	1084.7	2323.1	1662.9	119.4	24.8809	N
LPCBTOC	12	6.9891	7.7507	7.3876	0.0727	3.4072	N
CAISSON=105							
PCBTOC	11	1243.2	3156.0	2097.3	183.2	28.9658	N
LPCBTOC	11	7.1254	8.0571	7.6070	0.0932	4.0624	N
CAISSON=106							
PCBTOC	13	800.4	3542.0	1834.7	209.2	41.1126	N
LPCBTOC	13	6.6851	8.1724	7.4430	0.1084	5.2492	N

N denotes Shapiro-Wilk test for normality was not rejected

TABLE C1-3. Summary Statistics for Total Organic Carbon Data Final Week

Variable	N	Minimum	Maximum	Mean	Std Error	CV	
CAISSON=101							
PCBTOC	12	92.7000	1175.2	412.7	84.5302	70.9483	
LPCBTOC	12	4.5294	7.0692	5.8253	0.1893	11.2560	N
CAISSON=102							
PCBTOC	12	167.9	1664.4	746.9	107.5	49.8465	N
LPCBTOC	12	5.1234	7.4172	6.4902	0.1625	8.6729	N
CAISSON=103							
PCBTOC	12	545.4	1289.1	788.7	57.7614	25.3706	N
LPCBTOC	12	6.3015	7.1617	6.6440	0.0676	3.5256	N
CAISSON=104							
PCBTOC	12	775.9	4032.4	1801.2	344.9	66.3330	
LPCBTOC	12	6.6540	8.3021	7.3234	0.1700	8.0416	N
CAISSON=105							
PCBTOC	10	708.7	1379.1	984.0	66.1193	21.2481	N
LPCBTOC	10	6.5634	7.2292	6.8717	0.0663	3.0510	N
CAISSON=106							
PCBTOC	12	642.4	1427.7	987.5	66.1323	23.1999	N
LPCBTOC	12	6.4652	7.2638	6.8705	0.0670	3.3804	N

N denotes Shapiro-Wilk test for normality was not rejected

these measurements (variable LPCBTOC) for each sampling point. The code N in the right margin denotes the Shapiro-Wilk test for normality was not rejected for the sample values at a 5% significance level. Data for R102 at T_o and R101 and R104 at T_f failed the normality test using the untransformed values. In all but one case (data for R102 for T_o) the natural logarithm transformation did yield a normal distribution for the sample data. This suggests the use of the natural logarithms of the data in the statistical tests.

Table C1-4 shows the results of the two-sample t test using both the measured PCB values and the natural logarithms (ln) of the data. The tests assume the two samples are random samples from independent normal distributions and have equal variances. The test of the null hypothesis that the mean of initial PCB distribution in a caisson is less than or equal to the mean of the final PCB distribution in that caisson was rejected at the 5% significance level for all caissons except R101 and R104. Similar results were obtained using the logarithmic values.

Table C1-4
PCB/TOC Two Sample t Test Results

Comparison of sample means at T_o and T_f

NULL HYPOTHESIS H_0: $\mu_1 \leq \mu_2$

ALTERNATIVE HYPOTHESIS H_A: $\mu_1 > \mu_2$

CAISSON	101	102	103	104	105	106
VARIABLE						
LN PCB TOC	A	R	R	A	R	R
PCB TOC	A	R	R	A	R	R

R means reject null hypothesis at 5% significance level
A means cannot reject null hypothesis at 5% significance level

Table C1-5 provides approximate 95% confidence intervals on the difference between the average initial and final values for the variables discussed to this point. The intervals provide some quantitative bounds on the magnitude of the difference and give an indication of the uncertainty in the estimates. Note that in several cases the intervals are approximate when the assumptions of equality of the variances and normality are not valid. In addition, some of the confidence intervals enclose zero. Additional discussion of confidence intervals is given in Hahn and Meeker [1991].

Normalized PCB Concentration Data

The normalized PCB concentration data were also collected during the study and used to model PCB concentration as a function of time. These measurements were used to provide a conservative estimate of the rate of PCB loss, as discussed in Chapter 5. Table C1-6 shows the summary statistics on the initial and final week data for the normalized data values (variable NORMAL) and the natural logarithm of the normalized data (variable LNORMAL). the Shapiro-Wilk test of the hypothesis that the data are from a normal distribution was rejected at the 5% significance level in only one instance, the initial week logarithmic data values for caisson R104. Boxplots of the data values are given in Figures C1-13 through C1-16. Table C1-7 shows the results of the two-sample t test using the initial and final week normalized data measurements. The analyses were conducted using both the normalized data and the natural logarithm of the normalized data. The null hypothesis that the mean values for the initial and final weeks are equal was rejected for all caissons except caisson R104 (5% significance level). Table C1-8 provides approximate 95% confidence intervals on the difference between the average initial and final values for the normalized data.

TABLE C1-5. 95% Confidence Intervals on Differences Between Initial and Final Means

CAISSON	TEST	INITIAL - FINAL MEAN	95% CONFIDENCE LIMITS LOWER	UPPER
101	CPCB	-0.52	-2.49	1.45
101	LCPCB	-0.07	-0.33	0.20
101	PCBTOT	-96.57	-553.34	360.21
101	LPCBTOT	-0.03	-0.27	0.21
101	PCBTOC	150.29	-82.59	383.18
101	LPCBTOC	0.41	-0.08	0.89
102	CPCB	8.23	0.03	16.49
102	LCPCB	0.64	0.13	1.14
102	PCBTOT	2091.41	646.68	3536.14
102	LPCBTOT*	0.71	0.22	1.20
102	PCBTOC	606.77	278.52	935.03
102	LPCBTOC*	0.69	0.32	1.06
103	CPCB*	11.06	3.71	18.40
103	LCPCB	0.43	0.15	0.70
103	PCBTOT	763.58	-915.54	2442.71
103	LPCBTOT	0.08	-0.23	0.39
103	PCBTOC*	985.53	559.97	1411.10
103	LPCBTOC	0.76	0.48	1.05
104	CPCB	16.82	5.29	28.36
104	LCPCB	0.57	0.24	0.91
104	PCBTOT	2092.21	-912.11	5096.53
104	LPCBTOT	0.32	-0.02	0.65
104	PCBTOC*	-138.36	-895.38	618.67
104	LPCBTOC*	0.06	-0.32	0.45
105	CPCB*	36.14	18.41	53.86
105	LCPCB	1.25	0.82	1.67
105	PCBTOT	5315.37	2572.29	8058.46
105	LPCBTOT	0.67	0.34	1.00
105	PCBTOC*	1113.30	689.31	1537.29
105	LPCBTOC	0.74	0.49	0.98
106	CPCB*	26.85	15.68	38.02
106	LCPCB	1.12	0.73	1.50
106	PCBTOT*	3735.83	1414.00	6057.66
106	LPCBTOT	0.54	0.22	0.87
106	PCBTOC*	847.26	377.30	1317.23
106	LPCBTOC	0.57	0.30	0.84

* indicates test for equality of sample variances or normality assumption rejected hence limits are "approximate"

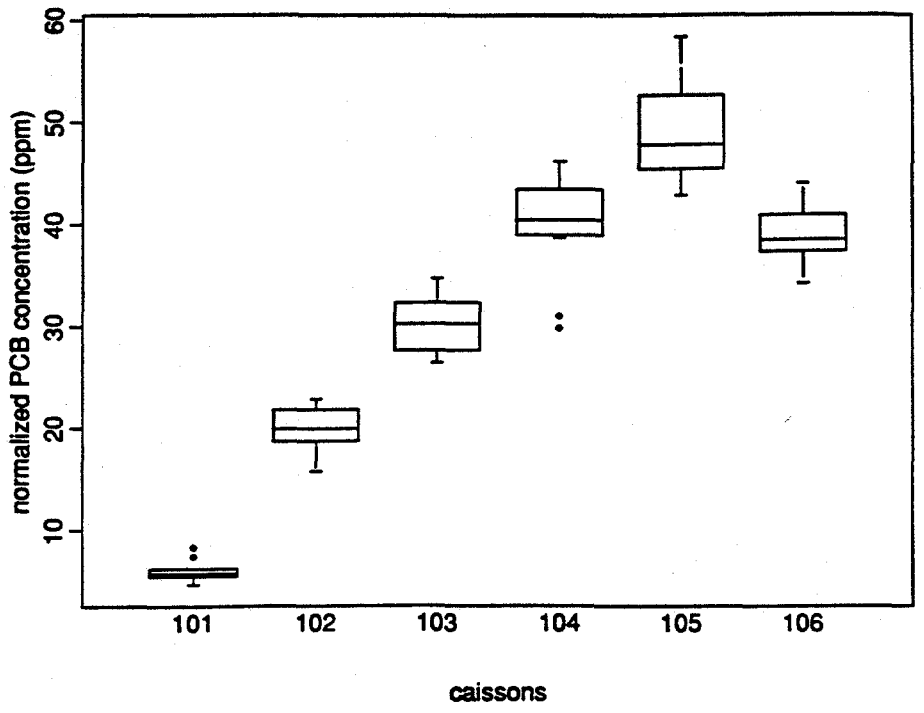


Figure C1-13. Boxplot of normalized PCB concentration in caissons at T_0.

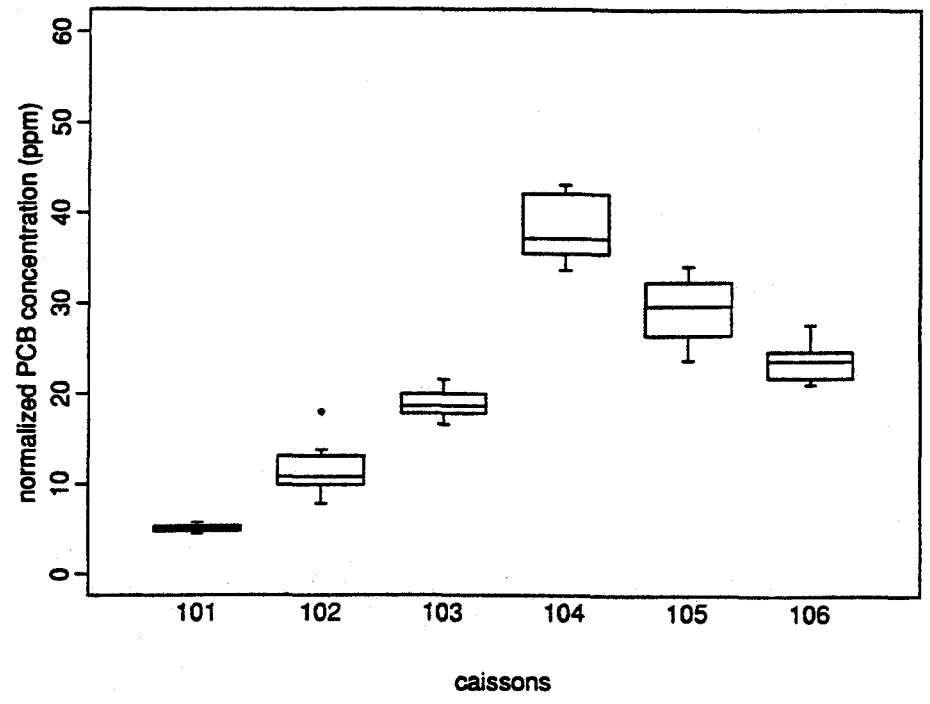


Figure C1-14. Boxplot of normalized PCB concentration in caissons at T_f.

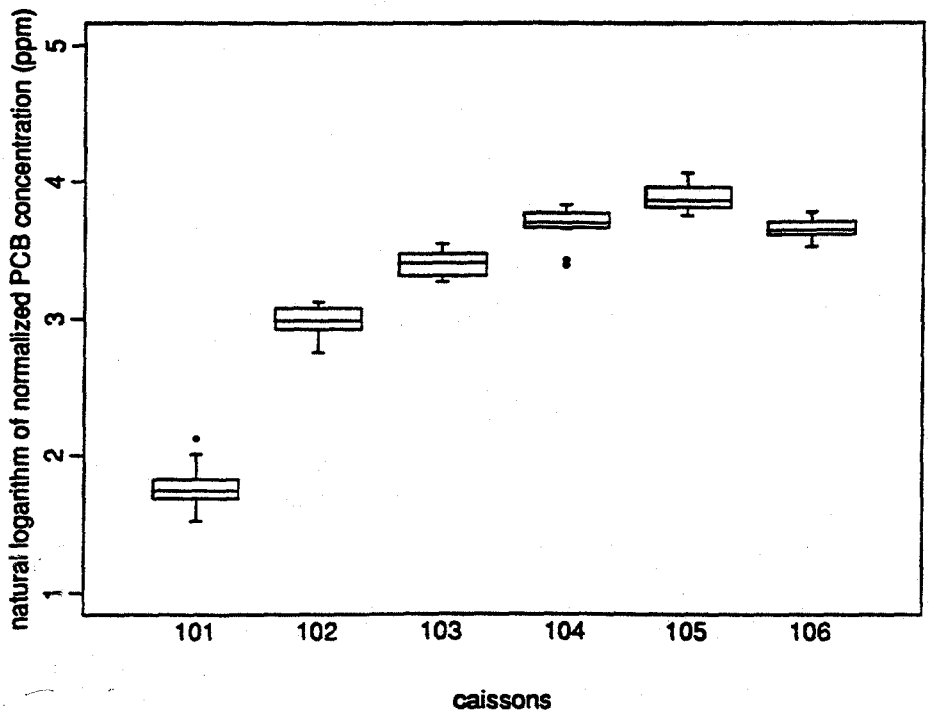


Figure C1-15. Boxplot of natural logarithm of normalized PCB concentration in caissons at T_O.

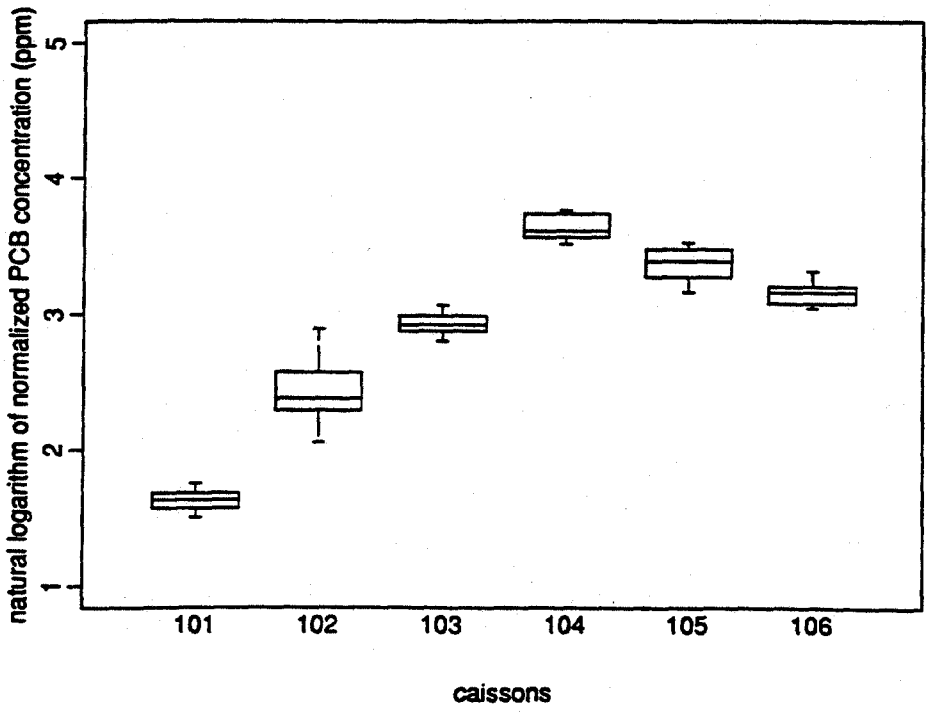


Figure C1-16. Boxplot of natural logarithm of normalized PCB concentration in caissons at T_f.

TABLE C1-6. Summary Statistics for Normalized PCB Concentration Data Final Week

Variable	N	Minimum	Maximum	Mean	Std Error	CV	
CAISSON=101							
NORMAL	12	4.4985	5.7820	5.1256	0.1202	8.1221	N
LNORMAL	12	1.5037	1.7547	1.6312	0.0234	4.9779	N
CAISSON=102							
NORMAL	12	7.7929	18.0508	11.5157	0.7805	23.4774	N
LNORMAL	12	2.0532	2.8932	2.4207	0.0635	9.0839	N
CAISSON=103							
NORMAL	12	16.5324	21.5587	18.7624	0.4261	7.8670	N
LNORMAL	12	2.8053	3.0708	2.9290	0.0227	2.6834	N
CAISSON=104							
NORMAL	12	33.6857	43.0671	38.1946	1.0059	9.1228	N
LNORMAL	12	3.5171	3.7628	3.6389	0.0261	2.4852	N
CAISSON=105							
NORMAL	12	23.7271	34.1247	29.3683	0.9809	11.5703	N
LNORMAL	12	3.1666	3.5300	3.3737	0.0339	3.4846	N
CAISSON=106							
NORMAL	12	21.0419	27.6555	23.7548	0.6018	8.7754	N
LNORMAL	12	3.0465	3.3198	3.1643	0.0250	2.7344	N

N denotes Shapiro-Wilk test for normality was not rejected

TABLE C1-6. Summary Statistics for Normalized PCB Concentration Data Initial Week

Variable	N	Minimum	Maximum	Mean	Std Error	CV	
CAISSON=101							
NORMAL	12	4.5907	8.3630	5.9906	0.2952	17.0720	N
LNORMAL	12	1.5240	2.1238	1.7779	0.0464	9.0379	N
CAISSON=102							
NORMAL	12	15.6897	22.8347	19.9806	0.6073	10.5296	N
LNORMAL	12	2.7530	3.1283	2.9894	0.0315	3.6445	N
CAISSON=103							
NORMAL	12	26.3219	34.6740	30.1644	0.8323	9.5579	N
LNORMAL	12	3.2704	3.5460	3.4025	0.0274	2.7883	N
CAISSON=104							
NORMAL	12	29.8337	46.1103	39.9154	1.4345	12.4491	N
LNORMAL	12	3.3956	3.8310	3.6789	0.0388	3.6517	
CAISSON=105							
NORMAL	13	42.8240	58.2971	49.3959	1.3505	9.8576	N
LNORMAL	13	3.7571	4.0656	3.8955	0.0268	2.4840	N
CAISSON=106							
NORMAL	13	34.1103	43.9225	38.7365	0.7954	7.4036	N
LNORMAL	13	3.5296	3.7824	3.6543	0.0206	2.0276	N

N denotes Shapiro-Wilk test for normality was not rejected

Table C1-7
Normalized PCB Two Sample t Test Results

Comparison of sample means at T_o and T_f

NULL HYPOTHESIS H_0: $\mu_1 \leq \mu_2$

ALTERNATIVE HYPOTHESIS H_A: $\mu_1 > \mu_2$

CAISSON	101	102	103	104	105	106
VARIABLE						
NORMALIZED PCB	R	R	R	A	R	R
LN NORMALIZED PCB	R	R	R	A	R	R

R means reject null hypothesis at 5% significance level
A means cannot reject null hypothesis at 5% significance level

Samples with Low Dry Weight Values

Several core samples had sample dry weight values which were less than 150 grams. These samples were believed to be "outliers" and potentially could be omitted from the analyses. Several detection of outlier tests were performed on the data. Method One (Wadsworth, 1990) involved creating a z statistics defined as the

$$z_i = \left[\frac{(x_i - \text{median})}{\text{MAD}}\right]$$

where MAD is the median of the absolute deviation of the x_i from the sample median.

This approach uses fairly robust estimators - the median and MAD - for the central location and scale (dispersion) parameters of the distribution. However, this test indicated that basically all the low weight units and several

TABLE C1-8. 95% Confidence Intervals on Differences Between Initial and Final Means of Normalized PCB Values

CAISSON	TEST	INITIAL - FINAL MEAN	95% CONFIDENCE LIMITS LOWER	UPPER
101	NORMAL*	0.8650	0.2039	1.5261
101	LNORMAL*	0.1467	0.0389	0.2545
102	NORMAL	8.4649	6.4139	10.5159
102	LNORMAL*	0.5688	0.4219	0.7157
103	NORMAL*	11.4019	9.4627	13.3411
103	LNORMAL	0.4735	0.3997	0.5473
104	NORMAL	1.7208	-1.9127	5.3543
104	LNORMAL	0.0400	-0.0570	0.1369
105	NORMAL	20.0277	16.5243	23.5311
105	LNORMAL	0.5218	0.4330	0.6106
106	NORMAL	14.9817	12.8914	17.0721
106	LNORMAL	0.4899	0.4234	0.5564

* indicates test for equality of sample variances or normality assumption rejected hence limits are "approximate"

additional units would be considered outliers. Given the number of samples identified it was deemed inappropriate to omit all these cases from the analysis or to use their "nearest-neighbor" values instead. When only the low dry weight samples (samples with weights less than 150 g) were omitted from the analysis and the t statistic test repeated, the test results did not change.

A second method of outlier detection (Dixon and Massey, 1969) identified only two sample units as outliers. Both observations were the low dry weight units from caisson R102. Performing the analysis with these two observations omitted did not change the t statistic results for caisson R102.

Other Statistical Analyses of the Data

This section has summarized the two-sample t test results of the data using initial and final sampling measurements of PCB concentration, total core PCB levels, and PCB TOC concentrations. In many cases a statistically significant difference was observed for the variables measured. However, that statistical analysis of the data was not limited to the t test procedures. A Wilcoxon rank sum test, a nonparametric counterpart to the two sample t test, was also performed on the data. This test is valid under less restrictive assumptions and only requires that the samples come from continuous populations. The results of the nonparametric test closely duplicated the t test results for each of the caissons at the 5% significance level using both the original and transformed values of the variables.

References

Dixon, W.J. and F.J. Massey. Introduction to Statistical Analysis. McGraw-Hill, New York, 1969.

Wadsworth, H.M. Handbook of Statistical Methods for Engineers and Scientists. McGraw-Hill, New York, 1990.

Hahn, G.J. and W.Q. Meeker. Statistical Intervals: A Guide for Practitioners. John Wiley and Sons, Inc., New York. 1991.

APPENDIX C-2
Additional PCB and QA/QC Data

Water Phase Analyses

PCB samples received by Northeast Analytical Laboratory (NEA) sometimes contained significant quantities of water. Air-drying such samples would have taken several days or even weeks and compromised pre-extraction hold times for these samples. Excess water was removed from these sediments by centrifuging the samples in their original containers. Sometimes small (1 gram per core) additions of alum were made to enhance settling during this process. The clear water phase was removed and combined by caisson for subsequent PCB analysis. PCB from the water column was removed by liquid-liquid extraction using methylene chloride as the extraction solvent. PCB analysis was performed according to standard procedures by GC.

The results of a series of these analyses appear in Table C2-1. Data on the water phase obtained at week 0 (T_o), week 4, and week 11 (T_f) are shown. All values are in the low ppb range, three orders of magnitude lower than PCB concentrations in the associated sediment. The combined volume of water removed from the samples from any caisson was generally less than one liter. This phase contributes negligibly to the PCB content of the sediment and was therefore not considered in the analysis.

Effect of Air Drying on PCB Analysis

Some concern has been expressed in the past about the effect air-drying of sediment samples may have on subsequent PCB analyses. Of particular concern is the potential for loss of some of the more lightly chlorinated congeners to volatilization during the drying process. An experiment was conducted by Northeast Analytical to generate sufficient data to address this concern. Two methods of soil drying, air drying (current NEA method) and

Table C2-1
PCB Concentrations in Separated Water
Northeast Analytical Preparation

	PCB Concentration (ppb)		
Caisson	Week 0	Week 4	Week 11
R101	1.25	0.28	0.64
R102	4.64	0.71	1.91
R103	2.65	1.40	3.98
R104	2.77	3.74	9.68
R105	2.61	3.79	4.11
R106	8.37	5.59	3.76

sodium sulfate drying (US EPA SW846, Method 3540), were compared in a simple test so that the potential for volatilization losses due to air drying could be evaluated.

Prior to the experiment a sediment sample from the study (R104-EI-0814-01) was homogenized and six subsamples were removed. Three of the subsamples were air-dried in a low-flow hood at room temperature overnight. The other three samples were dried with the addition of sodium sulfate per EPA SW846. The chemical reagent was added 1:1 by weight based upon the wet weight of the sediment sample and blended with the sediment until the mixture was dry. The dry weight of the chemically dried samples was calculated using the average moisture content of the air-dried samples (38%). Once dry, both sets of samples underwent identical soxhlet extraction and cleanup procedures as outlined in Northeast Analytical Laboratory Method NEA-PCBHRGC. Extracts were analyzed by NEA using 118 peak capillary analysis. PCB concentrations in both cases were calculated on a dry weight sediment basis.

The results of the analyses appear in Table C2-2. The average PCB concentrations differ by about 10%, with the air-dried samples being higher (23.4 ppm versus 21.3 ppm). More importantly, there is essentially no

Table C2-2
Comparison of Sediment Drying Methods
(values are represented as average +/- one standard deviation)

	Air-Dried	Chemically-Dried
Number Samples	3	3
PCB Average (ppm)	23.4 +/- 0.5	21.3 +/- 0.6
Homolog Dist. (Mole %)		
Mono	26.7 +/- 0.2	26.4 +/- 0.8
Di	44.6 +/- 0.2	44.2 +/- 0.2
Tri	16.8 +/- 0.2	17.2 +/- 0.3
Tetra	7.5 +/- 0.1	7.7 +/- 0.2
Penta	2.7 +/- 0.1	2.7 +/- 0.1
Hexa	1.0 +/- 0.0	1.0 +/- 0.0
Hepta	0.4 +/- 0.0	0.4 +/- 0.1
Octa	0.2 +/- 0.0	0.2 +/- 0.0
Nona	0.1 +/- 0.0	0.1 +/- 0.0

difference in the average homolog distribution between the sample sets. If volatilization was significant, we would expect to see a depletion of at least the monochlorobiphenyls in the air-dried samples. This is clearly not the case.

Equipment Sampling Prior to Decontamination

In an effort to check for PCB mass balance a series of extractions and wipes were made at the conclusion of the experiment to insure significant PCB sinks did not exist in or on the equipment exposed to the sediment or headspace inside the caissons. This was done prior to the final decontamination operation. Two foot sections of the gasketing material under the caisson lids were removed and soxhlet extracted with hexane/acetone. Two foot sections of vent lines were removed and rinsed

with acetone. Swipe tests with acetone were done of 100 cm^2 areas of the caissons directly above and below the water line and on the surfaces of a turbine and rake mixer.

The results appear in Table C2-3. PCB content in the gaskets and vent lines was minimal (less than 2 micrograms total). Caisson and turbine surfaces were lightly contaminated, probably primarily due to residual sediment. Only the rake surface was significantly contaminated. A large buildup of the black colloidal material was noted on the horizontal surfaces of these mixers when they were pulled from the caissons during demobilization. Even after this material was washed from the rakes with water, a black organic film remained on the surface. Apparently this film was high in PCB content and the primary contributor to the PCB levels measured in the wipe. Although the PCB content is high, the horizontal surface area of the rake mechanism is not substantial enough to contribute significantly to the PCB loading within the caissons.

PCB Analysis of Waste Streams

After completion of the experiment the contents of the caissons were pumped out into small tank trucks. The liquid was removed first and transferred to three compartments in a tanker. Each compartment was sampled separately (Table C2-4, aqueous samples 01-03). The top 10-12 inches of sediment was then removed from the caissons and pumped into drums. This material was subsampled periodically during that operation (Table C2-4, sediment samples 01 - 04). The 55 gallon carbon filter was also sampled during this period.

The results of these analyses appear in Table C2-4. Analyses were run on packed column GC and quantitated using both the Dye Color Manufacturers Association (DCMA) 10 congener standardization method and standardization based on Aroclor mixtures amended with mono- and dichlorobiphenyls. The DCMA analyses typically yielded values at least three times higher than those by amended Aroclor standards. It appears the

Table C2-3
Caisson Equipment Analysis - Prior to Decontamination

Gasket Material - Gore-tex™ (2 foot sections - soxhlet extracted)

R102-GAS-1104-01	0.30 micrograms
R104-GAS-1104-01	1.05 micrograms
R105-GAS-1104-01	0.08 micrograms

Vent Lines - stainless steel (1 foot section - rinsed with acetone)

R101-EXT-1105-01	0.00 micrograms
R102-EXT-1105-01	0.00 micrograms
R104-EXT-1105-01	0.00 micrograms

Caisson Surface - above water line (wipe sample)

R102-BD-AWL	7.11 ug/100 cm^2
R105-BD-AWL	4.27 ug/100 cm^2

Caisson Surface - below water line (wipe sample)

R102-BD-BWL	0.72 ug/100 cm^2
R105-BE-BWL	7.15 ug/100 cm^2

Mixer Surface - before decontamination (wipe sample)

R102-BD-PROP	1.16 ug/100 cm^2
R105-BD-RAKE	48.55 ug/100 cm^2

TABLE C2-4
PCB Content of Waste Streams
(All values in ppm)

Sample	DCMA	Amended Aroclor
COMP-AQ-1104-01	3.4	1.1
COMP-AQ-1104-02	3.8	1.2
COMP-AQ-1104-03	0.03	0.01
COMP-CARBON-1104-01	0.19	0.01
COMP-SED-1105-01	49.0	16.7
COMP-SED-1105-02	40.7	12.9
COMP-SED-1105-03	43.1	13.5
COMP-SED-1105-04	11.4	4.1

Note - all PCB values except for activated carbon are on a wet weight basis.

conservative assignment of response factors and the presence of non-PCB contaminant peaks in the extracts causes this method to significantly overpredict the PCB levels of these samples.

Summary of PCB QA/QC Data

The QA/QC data pertaining to the PCB analysis performed by Northeast Analytical Laboratory as specified in the GE Quality Assurance Plan submitted to EPA in June of 1991 is summarized in this section. Northeast Analytical used two GCs for this analysis. In general samples were run in groups of approximately twenty. Each group of twenty included on average one method blank, one solvent blank, one matrix spike, one duplicate

sample, and two calibration standards. In general one equipment wipe sample was taken during each day of sampling.

Results of the method blank analysis appear in Figure C2-1. Method blanks were obtained by soxhlet extracting clean sand and carrying the extract through the cleanup procedure in parallel with the sediment extracts. These were used to detect laboratory PCB contamination. No significant PCB is detected, with the exception of low level contamination very early in the analysis sequence. It is suspected that this arose from contaminated glassware. Several of the sediment samples run during that period were subsequently reanalyzed, with no systematic change in PCB concentration noted. It does not appear this contamination significantly affected the samples run during that period. Solvent blanks showed no PCB contamination throughout the analysis period (data not shown).

Results of matrix spike analysis appear in Table C2-5. Matrix spikes were performed by spiking a sediment sample with a known amount of PCB standard dissolved in hexane, subjecting the sample to the soxhlet and cleanup procedures, and comparing the measured PCB level with an unspiked replicate to evaluate method recovery. Spike recoveries were excellent, averaging 95% for the analysis period.

Results of duplicate analysis appear in Table C2-6 Duplicate analyses were performed by subsampling the homogenized sediment core twice and subjecting each sample to parallel drying, extraction, and cleanup procedures in order to evaluate sample representativeness. In this case duplicate analyses were very consistent, generally within 5%.

Data from routine calibration checks appear in Figure C2-2. Average values for the standards run on both the GCs were slightly higher than the nominal standard concentration overall (1.27 versus 1.22). This could be the result of some solvent evaporation during sample preparation, storage, or injection. There was virtually no difference between machines in terms of averages and standard deviations. There seemed to be some minor cycling in values over time, appearing on both machines concurrently.

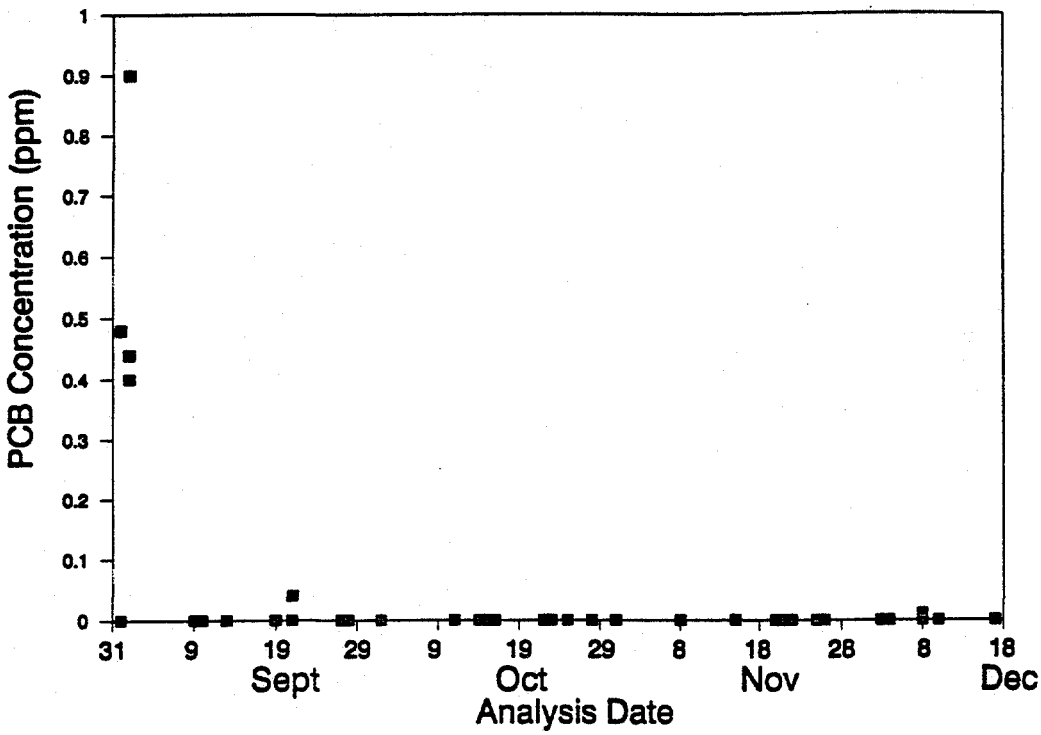


Figure C2-1. Summary of NEA method blank analysis over time.

Table C2-5
Spike Recovery Results

Date	Sample ID	Percent Recovery
08 Sept 91	R101-WI-0807-01A S	81.7%
10 Sept 91	R101-EM-087-01 S	87.9%
10 Sept 91	R101-EO-0807-01 S	85.1%
10 Sept 91	R102-EM-0807-01 S	86.6%
10 Sept 91	R102-EO-0807-01 S	105.1%
13 Sept 91	R106-SO-0806-01 S	98.1%
18 Sept 91	R103-NM-0807-02 S	110.0%
19 Sept 91	R107-SF-0808-02 S	100.6%
19 Sept 91	R101-EI-0807-01 S	102.9%
21 Sept 91	R105-SM-0828-01 S	103.2%
28 Sept 91	R-102-EM-0814-01 S	88.7%
28 Sept 91	R103-EI-0814-01 S	84.7%
29 Sept 91	R102-EM-0821-01A S	90.0%
03 Oct 91	R101-SM-0821-01 S	95.6%
03 Oct 91	R101-EM-0821-01 S	94.1%
03 Oct 91	R101-NM-0904-01 S	90.0%
03 Oct 91	R101-SM-0904-01 S	90.8%
16 Oct 91	R106-WM-0904-01 S	101.2%
16 Oct 91	R101-NM-0911-01 S	115.8%
22 Oct 91	R104-NI-0911-01 S	128.2%
24 Oct 91	R103-NI-0918-01 S	94.0%
26 Oct 91	R106-XM-3 S	96.7%
26 Oct 91	R105-WI-0918-01 S	83.6%
29 Oct 91	R104-EO-0926-01 S	82.0%
31 Oct 91	R101-SM-1002-01 S	85.6%
01 Nov 91	R104-NM-1002-01 S	100.0%
08 Nov 91	R102-SM-1009-01 S	83.0%
09 Nov 91	R105-SM-1009-01 S	95.4%
15 Nov 91	R101-WM-1016-01 S	95.8%
16 Nov 91	R104-S0-1016-01 S	86.7%
16 Nov 91	R105-NM-1016-01 S	86.9%
16 Nov 91	R101-NI-1022-01 S	105.2%
21 Nov 91	R104-NM-1022-01 S	76.1%
22 Nov 91	R104-NM-1022-01A S	96.8%
26 Nov 91	R101-WM-1023-01 S	90.1%
26 Nov 91	R101-WI-1023-01 S	83.6%
04 Dec 91	R105-NO-1024-03 S	85.8%
05 Dec 91	R106-SI-1024-02 S	91.5%
08 Dec 91	R101-XB-4 S	89.4%
08 Dec 91	R103-SO-1028-01 S	108.6%
09 Dec 91	R103-EM-1028-01 S	112.4%
11 Dec 91	R104-NEO-1104-01 S	111.6%
11 Dec 91	R105-SWI-1104-01 S	99.9%
17 Dec 91	R103-EM-0807-01 R S	93.0%

Table C2-6
Results of Duplicate Analyses

Sample ID	PCB	% Difference
R102-NM-0806-01	15.09	+ 4.6%
R102-NM-0806-D	15.79	
R103-S0-0806-01	21.06	+ 6.5%
R103-S0-0806-01 D	22.43	
R105-NI-0806-01A	21.14	- 6.9%
R105-NI-0806-01A D	19.69	
R104-NI-0814-01	38.50	- 7.2%
R104-NI-0814-01 D	35.71	
R101-NM-0821-01	2.38	+ 11.8%
R101-NM-0821-01 D	2.66	
R101-NM-0828-01	3.77	+ 3.1%
R101-NM-0828-01 D	3.89	
R102-WM-0828-01	13.43	- 0.7%
R102-WM-0828-01 D	13.34	
R102-SM-0904-01	22.31	+ 1.5%
R102-SM-0904-01 D	22.64	
R104-WI-0904-01A	8.29	- 2.8%
R104-WI-0904-01A D	8.06	
R104-WI-0911-01	24.50	- 9.2%
R104-WI-0911-01 D	22.25	
R103-SI-0918-01	21.30	- 4.4%
R103-SI-0918-01 D	20.37	
R102-EM-0926-01	4.72	+ 6.8%
R102-EM-0926-01 D	5.04	
R102-EM-1002-01	17.94	+ 2.3%
R102-EM-1002-01 D	18.35	
R106-SM-1009-01	2.21	+ 3.2%
R106-SM-1009-01 D	2.28	
R104-WM-1016-01	15.41	+ 3.3%
R104-WM-1016-01 D	15.92	

Table C2-6
Results of Duplicate Analyses cont.

Sample ID	PCB	% Difference
R106-EM-1016-01	6.54	- 2.0%
R106-EM-1016-01 D	6.41	
R105-NM-1022-01	10.25	- 7.2%
R105-NM-1022-01 D	9.51	
R104-NI-1022-01	14.13	+ 2.3%
R104-NI-1022-01 D	14.46	
R105-SI-1022-01A	4.54	+ 0.4%
R105-SI-1022-01A D	4.56	
R103-SM-1023-02	14.60	+ 4.7%
R103-SM-1023-02 D	15.29	
R105-WI-1023-01	13.19	+ 7.7%
R105-WI-1023-01 D	14.21	
R101-EI-1023-01	4.09	+ 0.2%
R101-EI-1023-01 D	4.10	
R106-WO-1023-01	13.25	+ 0.5%
R106-WO-1023-01 D	13.32	
R103-SI-1024-03A	0.80	- 5.1%
R103-SI-1024-03A D	0.76	
R103-SM-1028-01	22.34	+3.2%
R103-SM-1028-01 D	23.06	
R106-RK-1102-01	34.35	+ 4.2%
R106-RK-1102-01 D	35.81	
R102-SI-0814-01-R	10.07	+ 0.4%
R102-SI-0814-01-R D	10.11	

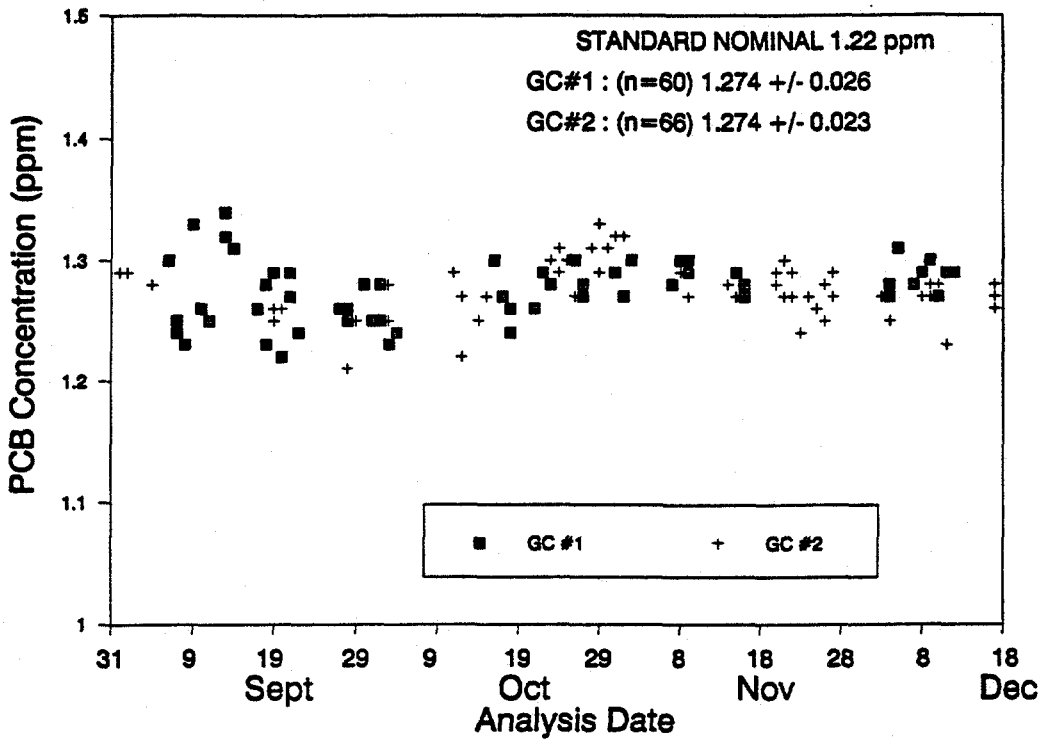


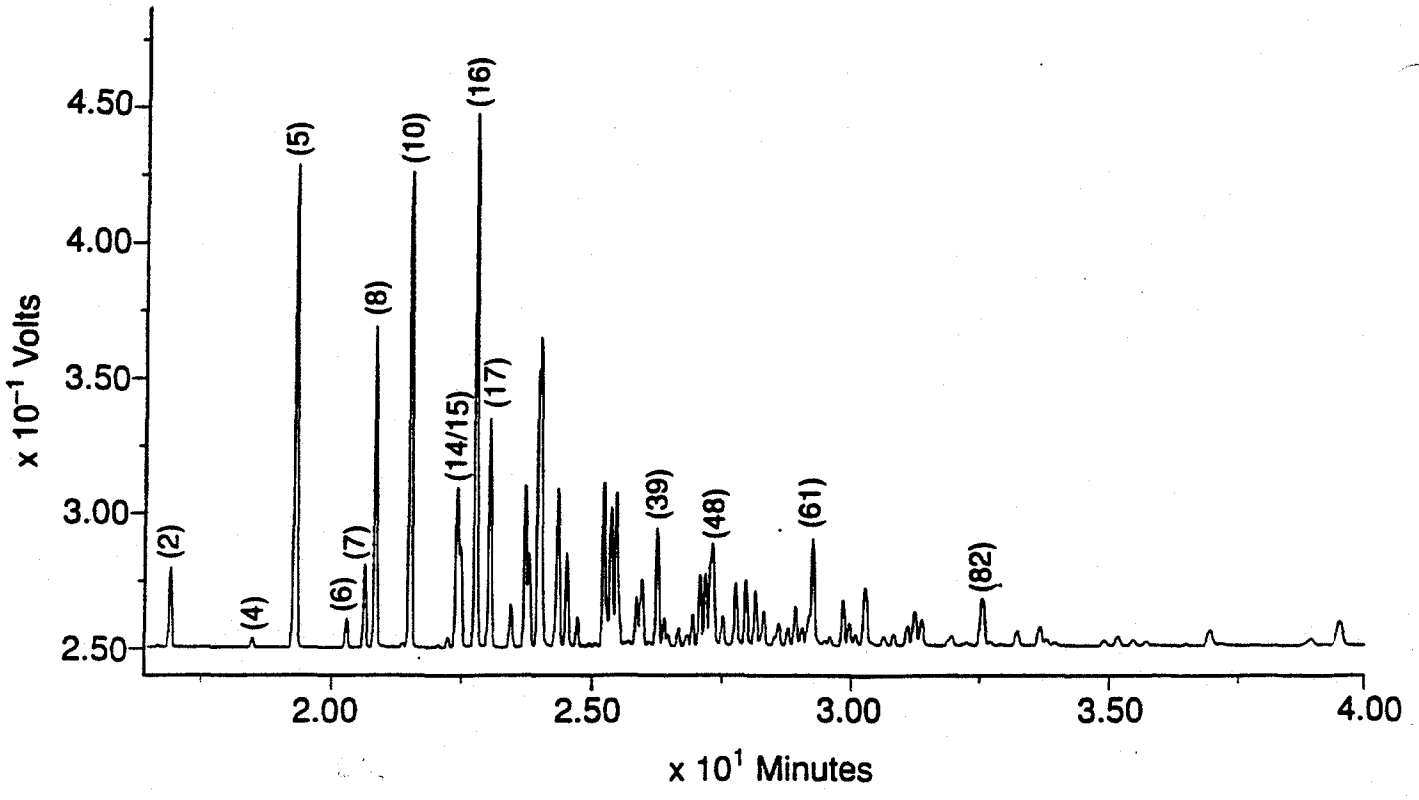
Figure C2-2. Summary of NEA calibration checks over time.

Table C2 -7
Results of Equipment Blank Analysis

Date Taken	Total PCB (micrograms)
06 Aug 91	1.57
07 Aug 91	1.26
21 Aug 91	0.83
04 Sept 91	0.86
11 Sept 91	1.03
18 Sept 91	0.21
26 Sept 91	0.51
02 Oct 91	0.46
16 Oct 91	0.66
22 Oct 91	0.59

Results of equipment blanks appear in Table C2-7. Equipment blanks were obtained by wiping down the corer with a laboratory wipe saturated in ethanol after a complete decontamination sequence was performed on the equipment in order to assess the effectiveness of the field decontamination procedures. Contamination after decontamination was very low, all below 2 micrograms per wipe.

APPENDIX C-3
PCB CHROMATOGRAMS



PCB Homolog	Peak	PCB Congener
Mono-chloro	2	2–
	4	4–
Di-chloro	5	2, 2'–/2, 6–
	6	2, 4–/2, 5–
	7	2, 3'–
	8	2, 3–/2, 4'–
Tri-chloro	10	2, 6, 2'–
	14	2, 5, 2'–/4, 4'–
	15	2, 4, 2'–
	16	2, 3, 6–/2, 6, 3'–
	17	2, 3, 2'–/2, 6, 4'–
Normalization References	39	2, 3, 6, 4'–/2, 6, 3', 4'–
	48	2, 3, 5, 6, 2'–/2, 3, 6, 2', 5'–
	61	3, 4, 3', 4'–/2, 3, 6, 3', 4'–
	82	2, 3, 4, 2', 4', 5'–/2, 3, 5, 6, 3', 4'–

Figure C3-1A. Example chromatogram with selected congener peaks and reference peaks identified.

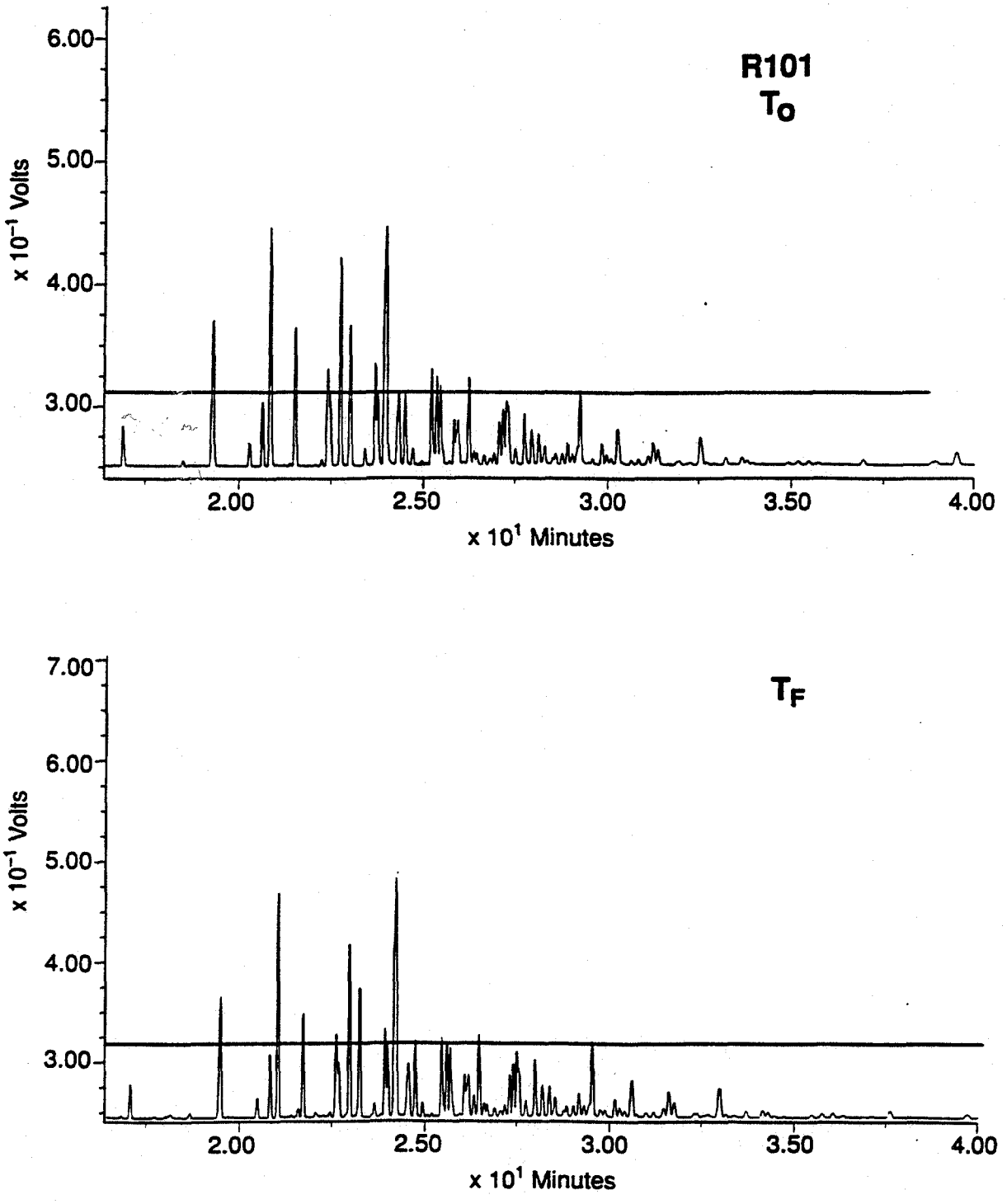


Figure C3-1B. Typical chromatograms from caisson R101 at T_o and T_f showing small amounts of selective congener attack.

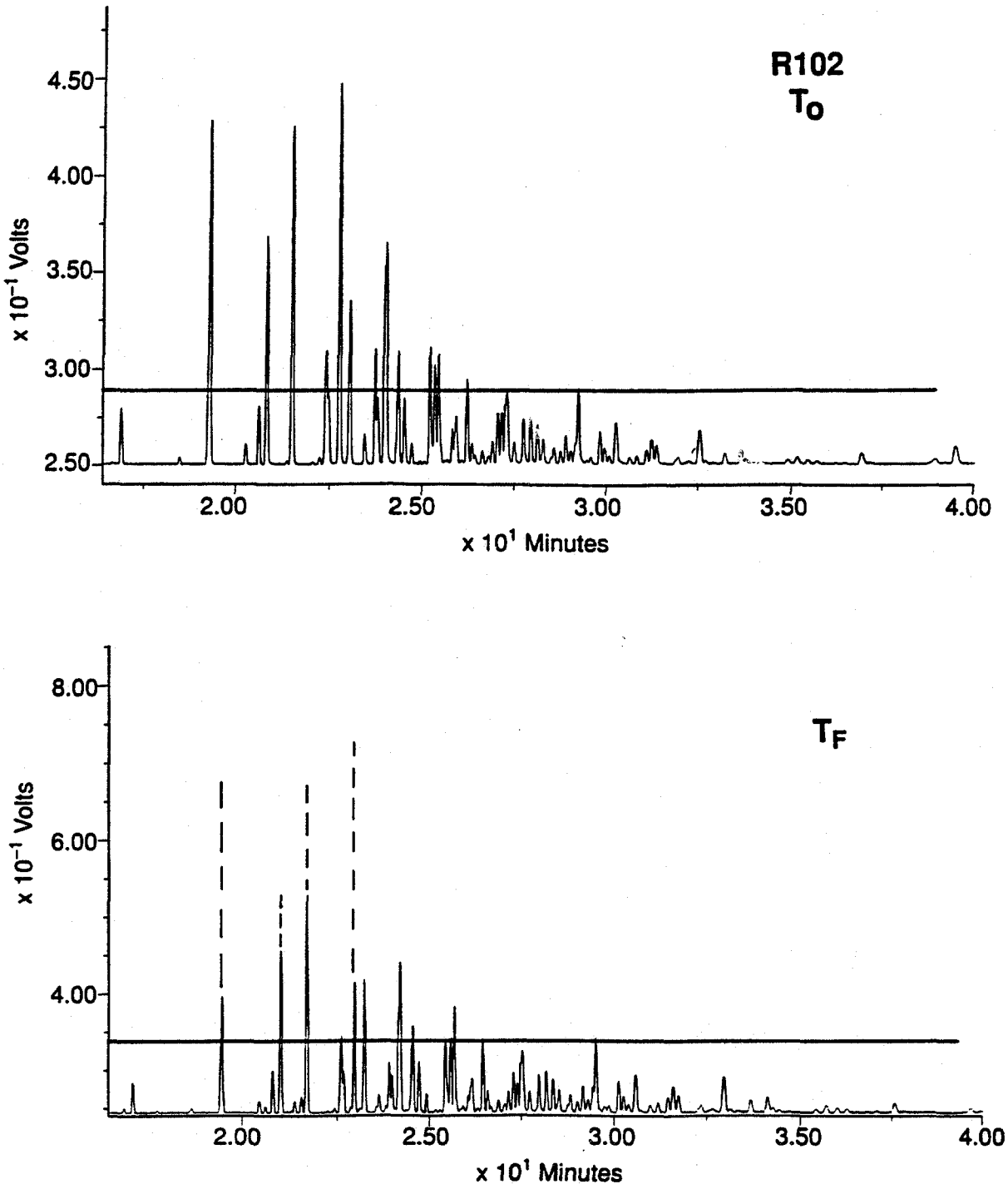


Figure C3-1C. Typical chromatograms from caisson R102 at T_o and T_f showing selective congener attack.

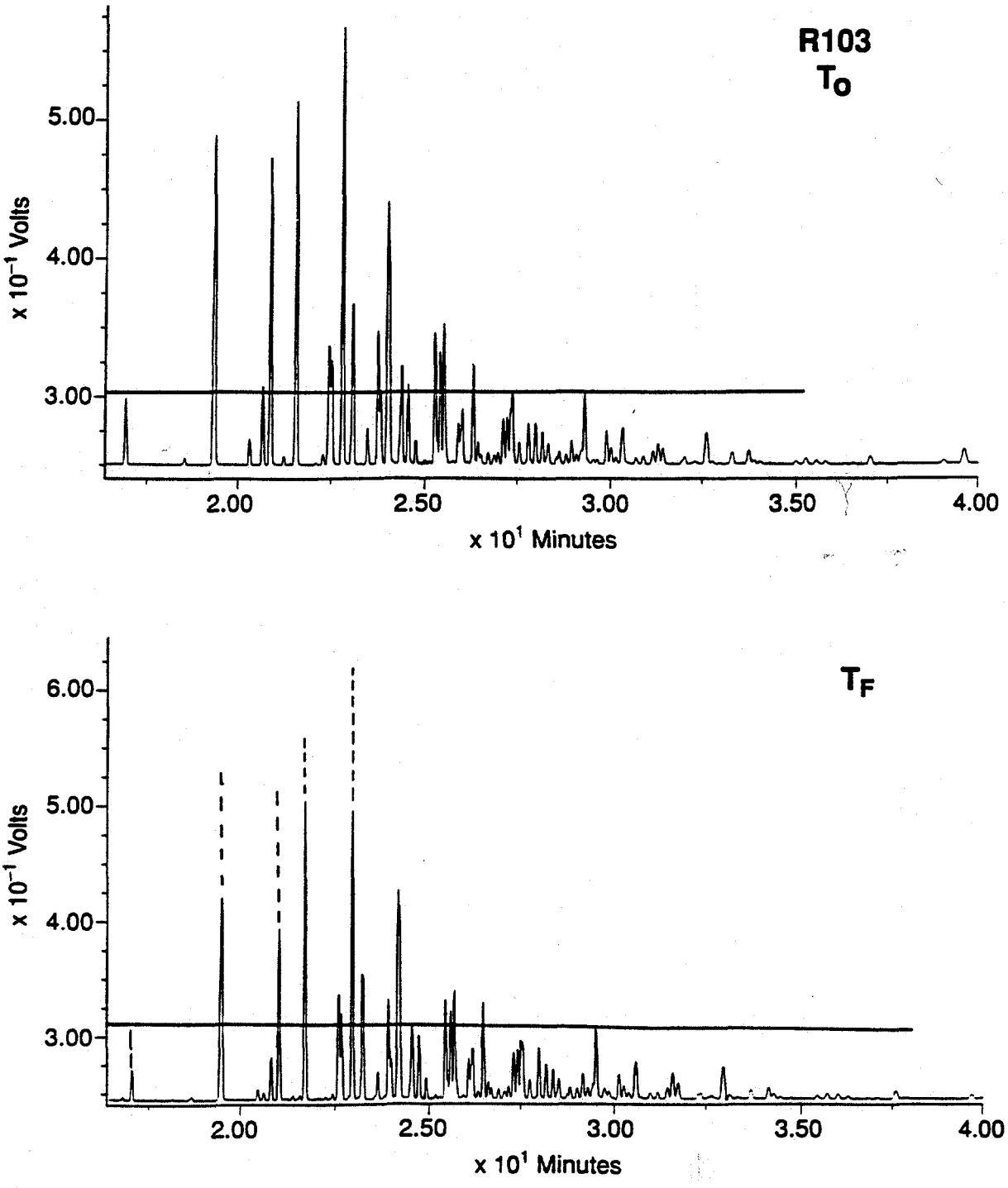


Figure C3-1D. Typical chromatograms from caisson R103 at T_o and T_f showing selective congener attack.

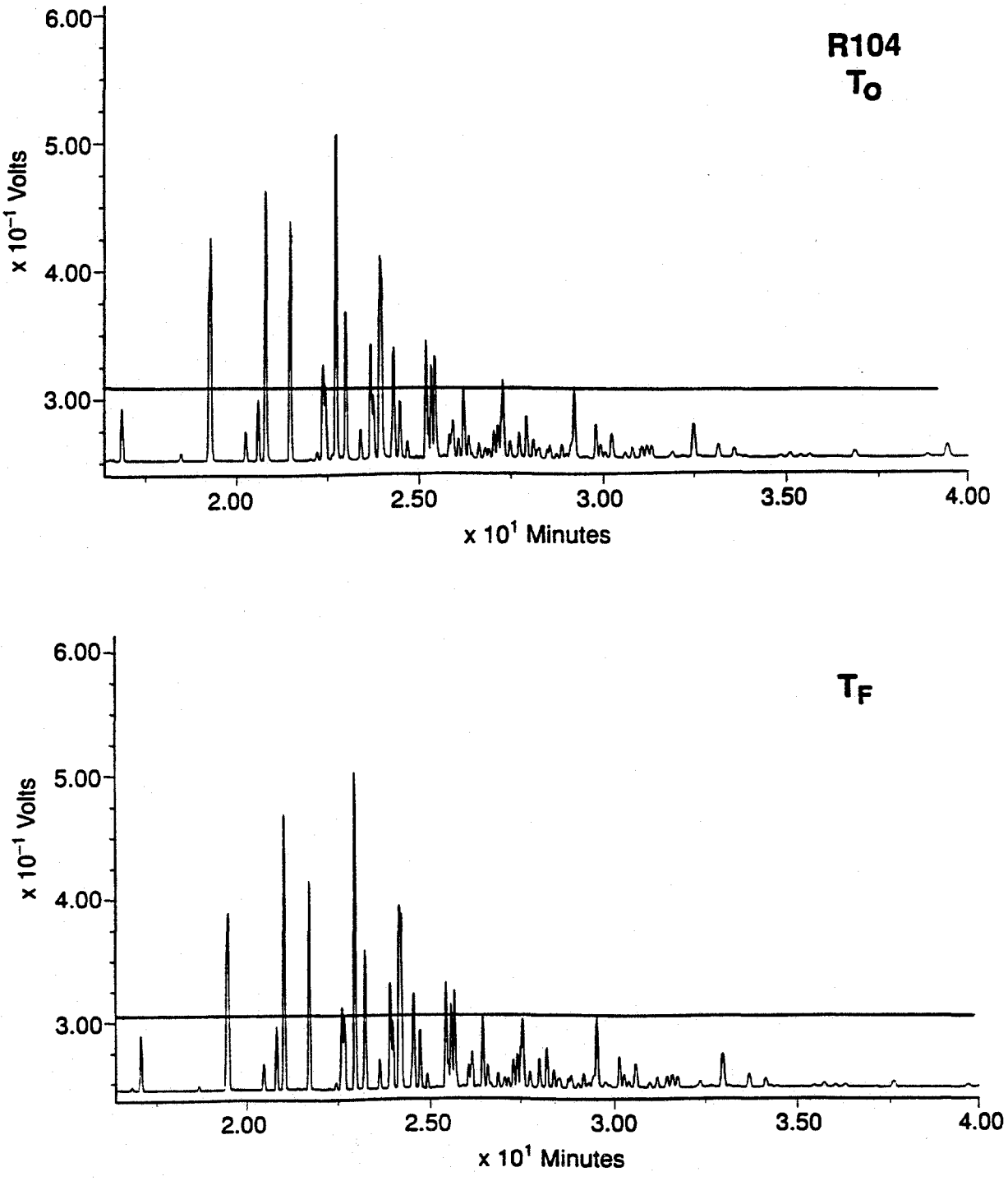


Figure C3-1E. Typical chromatograms from caisson R104 at T_o and T_f showing little selective congener attack.

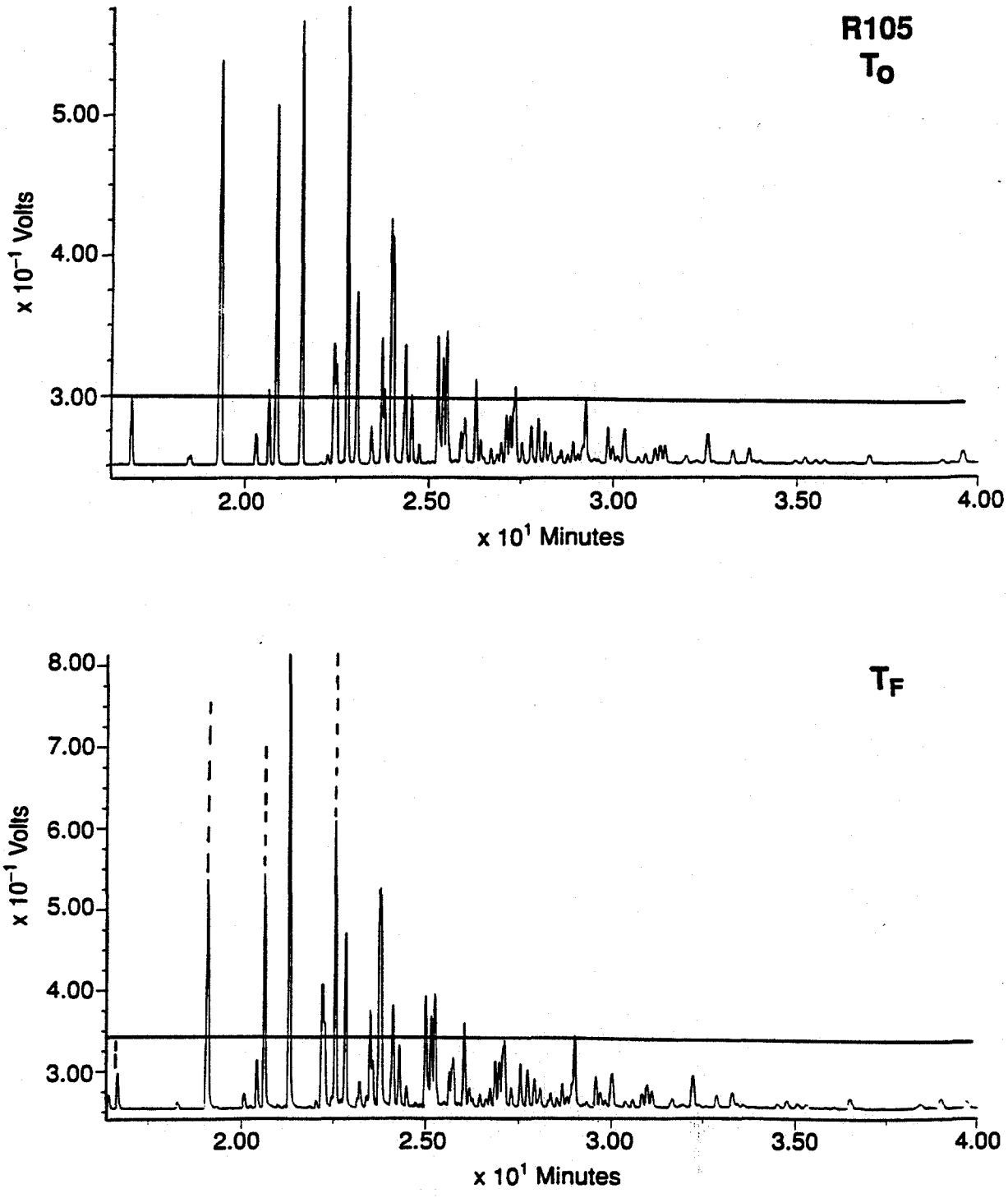


Figure C3-1F. Typical chromatograms from caisson R105 at T_o and T_f showing selective congener attack.

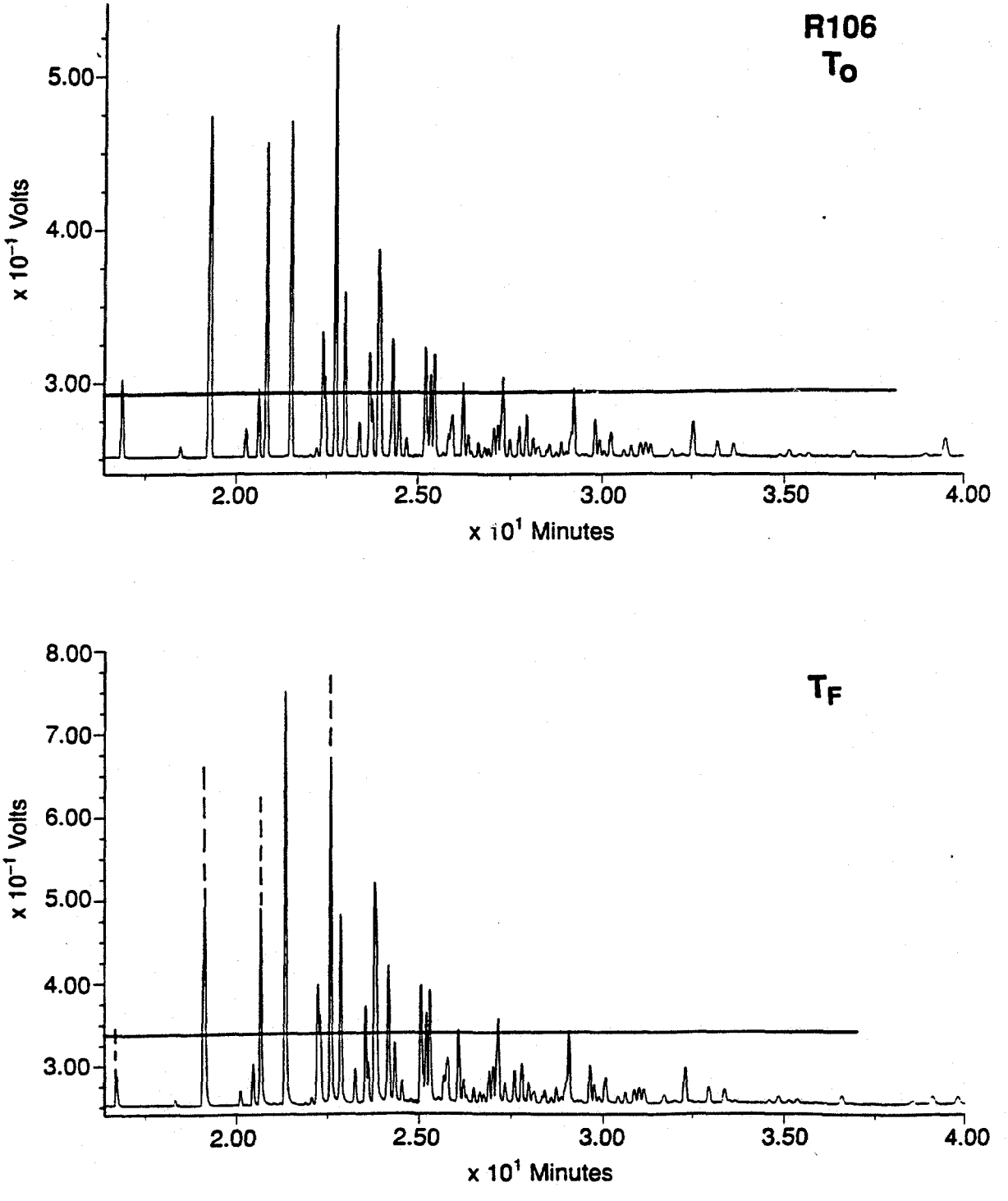


Figure C3-1G. Typical chromatograms from caisson R106 at T_o and T_f showing selective congener attack.

APPENDIX C-4
Summary of PCB Data

T0 PCB DATA

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R101-NI-0806-01	9.86	235.7	2324.0	0.00866	1138.6
R101-NM-0806-01	5.61	380.5	2134.6	0.01022	548.9
R101-NO-0806-01	4.61	327.5	1509.8	0.00952	484.2
R101-SI-0806-01	4.74	308.7	1463.2	0.00795	596.2
R101-SM-0806-01	10.08	242.5	2444.4	0.01301	774.8
R101-SO-0806-01	5.25	379.5	1992.4	0.00736	713.3
R101-EI-0807-01	4.99	202.5	1010.5	0.0115	433.9
R101-EM-0807-01	6.7	268	1795.6	0.00975	687.2
R101-EO-0807-01	4.98	330.6	1646.4	0.0082	607.3
R101-WI-0807-01	4.79	337.1	1614.7	0.01824	262.6
R101-WM-0807-01	4.36	296.1	1291.0	0.01805	241.6
R101-WO-0807-01	5.92	293.8	1739.3	0.02212	267.6
R101-NM-0806-01A	0.17	65.7	11.2		
R101-EM-0807-01A	0.66	247	163.0		
R101-EO-0807-01A	0.48	192.2	92.3		
R101-WI-0807-01A	0.17	143.4	24.4		
R101-WM-0807-01A	0.28	148.3	41.5		
R102-NI-0806-01	14.81	365.6	5414.5	0.01064	1391.9
R102-NM-0806-01	15.09	286.2	4318.8	0.01156	1305.4
R102-NO-0806-01	50.36	128.1	6451.1	0.02015	2499.3
R102-SI-0806-01	15.05	326.1	4907.8	0.01205	1249.0
R102-SM-0806-01	18.42	290.3	5347.3	0.0129	1427.9
R102-SO-0807-02	18.98	235.3	4466.0	0.01405	1350.9
R102-EI-0807-01	10.82	314.7	3405.1	0.00783	1381.9
R102-EM-0807-01	13.73	348.5	4784.9	0.01376	997.8
R102-EO-0807-01	18.99	296	5621.0	0.01893	1003.2
R102-WI-0807-01	10.11	345.3	3491.0	0.01093	925.0
R102-WM-0807-01	18.36	310.2	5695.3	0.01447	1268.8
R102-WO-0807-01	35.05	249.4	8741.5	0.02429	1443.0
R102-NM-0806-01 D	15.79	286.2	4519.1		
R102-SM-0806-01A	0.3	120.2	36.1		
R102-EM-0806-01A	3.13	155.2	485.8		

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R103-NI-0807-02	29.47	231.4	6819.4	0.02248	1310.9
R103-NM-0807-02	52.69	190.5	10037.4	0.01739	3029.9
R103-NO-0806-01	44.53	216.6	9645.2	0.01607	2771.0
R103-SI-0807-02	18.5	104.5	1933.3	0.02673	692.1
R103-SM-0806-01	23.25	288.3	6703.0	0.01019	2281.6
R103-SO-0806-01	21.06	313.5	6602.3	0.01105	1905.9
R103-EI-0807-01	38.58	174.3	6724.5	0.02695	1431.5
R103-EM-0807-02	31.26	181.2	5664.3	0.02231	1401.2
R103-EO-0807-01	31.14	150	4671.0	0.02066	1507.3
R103-WI-0807-01	32.92	289.2	9520.5	0.03011	1093.3
R103-WM-0807-01	24.65	267.6	6596.3	0.01382	1783.6
R103-WO-0807-01	13.93	374.5	5216.8	0.00669	2082.2
R103-SO-0806-01 D	22.43	313.5	7031.8		
R104-NI-0806-01	75.27	151.7	11418.5	0.0324	2323.1
R104-NM-0807-02	70.68	230.8	16312.9	0.03813	1853.7
R104-NO-0806-01	37.57	283.4	10647.3	0.01695	2216.5
R104-SI-0806-01	29.35	328.8	9650.3	0.01709	1717.4
R104-SM-0806-01	37.91	278.6	10561.7	0.02139	1772.3
R104-SO-0806-01	23.65	269.5	6373.7	0.01628	1452.7
R104-EI-0807-01	33.92	336.9	11427.6	0.02682	1264.7
R104-EM-0807-01	38.91	173.4	6747.0	0.01814	2145.0
R104-EO-0807-01	26.52	300.3	7964.0	0.02256	1175.5
R104-WI-0807-01	39.28	289.3	11363.7	0.02807	1399.4
R104-WM-0807-01	35.34	213	7527.4	0.03258	1084.7
R104-WO-0807-01	30.59	322.9	9877.5	0.01974	1549.6
R104-NO-0806-01A	17.54	61.4	1077.0		
R104-NM-0807-02A	11.53	128.4	1480.5		
R104-SM-0806-01A	14.87	149.5	2223.1		
R104-EI-0807-01A	10.55	118.7	1252.3		
R104-EO-0807-01A	10.62	185.2	1966.8		
R104-WO-0807-01A	9.54	129.2	1232.6		
R105-NI-0806-01	114.94	120	13792.8	0.04154	2767.0
R105-NM-0806-01	67.62	222.6	15052.2		
R105-NO-0806-01	39.72	285.6	11344.0	0.01613	2462.5
R105-SI-0806-01	81.86	162.1	13269.5	0.03616	2263.8
R105-SM-0806-01	41.72	245.4	10238.1	0.02264	1842.8
R105-SO-0806-01	32.41	305	9885.1	0.0146	2219.9
R105-EI-0807-01	76.47	272.6	20845.7	0.02423	3156.0
R105-EM-0807-01	33.59	272.1	9139.8	0.02702	1243.2
R105-EO-0807-01	38.04	203.1	7725.9	0.01698	2240.3

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R105-WI-0807-01	25.47	395.2	10065.7	0.02037	1250.4
R105-WM-0807-01	20.82	385.6	8028.2	0.00961	2166.5
R105-WO-0807-01	24.09	274.1	6603.1	0.01652	1458.2
R105-NI-0806-01A	21.14	—			
R105-NI-0806-01A D	19.69	—			
R106-NI-0806-01	71.62	189.5	13572.0	0.02022	3542.0
R106-NM-0806-01	58.34	195.9	11428.8	0.01928	3025.9
R106-NO-0806-01	48.48	150.9	7315.6	0.02329	2081.6
R106-NO-0807-02	39.67	184.8	7331.0	0.02279	1740.7
R106-SI-0806-01	24.99	221.9	5545.3	0.01933	1292.8
R106-SM-0806-01	18.48	258.5	4777.1	0.01573	1174.8
R106-SO-0806-01	12.87	325.9	4194.3	0.01608	800.4
R106-EI-0807-01	43.99	339.5	14934.6	0.02885	1524.8
R106-EM-0807-01	25.97	292.2	7588.4	0.01705	1523.2
R106-EO-0807-01	24.26	266.5	6465.3	0.0155	1565.2
R106-WI-0807-01	64.27	206.8	13291.0	0.02737	2348.2
R106-WM-0807-01	31.12	316	9833.9	0.01927	1614.9
R106-WO-0807-01	45.08	134.6	6067.8	0.02788	1616.9
R106-NI-0806-01A	122.2	45.3	5536.6		
R106-NM-0806-01A	29.28	139.1	4072.8		
R107-SF-0808-01	0.09	237.6	21.4		
R107-SF-0808-02	0.05	397.9	19.9		

T1 PCB DATA

R101-NI-0814-01	5.55	245.5	1362.5		
R101-SI-0814-01	4.33	279.4	1209.8		
R101-EI-0814-01	5.06	253.6	1283.2		
R101-WI-0814-01 R	3.9	226.1	881.8		
R101-WI-0814-01	11.54	226.1	2609.2		
R102-NI-0814-01	7.43	258.2	1918.4		
R102-SI-0814-01 R	10.07	261.3	2631.3		
R102-EI-0814-01	19.03	209.1	3979.2		
R102-WI-0814-01	7.95	278.9	2217.3		
R102-SI-0814-01	4.41	261.3	1152.3		

Sample ID	PCB Conc	Dry Wt	PCB Tot
R103-NI-0814-01	57.77	199	11496.2
R103-SI-0814-01	65.08	259.7	16901.3
R103-EI-0814-01	27.2	351.3	9555.4
R103-WI-0814-01	24.37	209.8	5112.8
R104-NI-0814-01	38.5	259.1	9975.4
R104-SI-0814-01	41.21	223.8	9222.8
R104-EI-0814-01	21.49	380.2	8170.5
R104-WI-0814-01	58.11	217.9	12662.2
R104-NI-0814-01 D	35.71	259.1	9252.5
R105-NI-0814-01	50.43	249.8	12597.4
R105-SI-0814-01	49.73	323.7	16097.6
R105-EI-0814-01	66.78	228.1	15232.5
R105-WI-0814-01	45.09	229	10325.6
R106-NI-0814-01	35.27	386.4	13628.3
R106-SI-0814-01	37.63	276.8	10416.0
R106-EI-0814-01	76.46	251.8	19252.6
R106-WI-0814-01	66.71	261.2	17424.7
T2 PCB DATA			
R101-NM-0821-01	2.38	296.1	704.7
R101-SM-0821-01	4.82	341.4	1645.5
R101-EM-0821-01	6.68	309.6	2068.1
R101-WM-0821-01	5.2	286.4	1489.3
R101-NM-0821-01 D	2.66	296.1	787.6
R102-NM-0821-01	7.87	271.9	2139.9
R102-SM-0821-01	13.94	334.3	4660.1
R102-EM-0821-01	18.6	232.9	4331.9
R102-WM-0821-01	12.52	292.8	3665.9
R102-EM-0821-01A	10.94	272.7	2983.3
R103-NM-0821-01	34.64	402.2	13932.2
R103-SM-0821-01	44.92	265.2	11912.8
R103-EM-0821-01	30.37	378.5	11495.0
R103-WM-0821-01	39.08	262.1	10242.9

Sample ID	PCB Conc	Dry Wt	PCB Tot
R104-NM-0821-01	20.34	521.7	10611.4
R104-SI-0821-01	45.29	314.6	14248.2
R104-EM-0821-01	23.12	420.9	9731.2
R104-WM-0821-01	22.24	418.9	9316.3
R104-EM-0821-01A	3.03	122.7	371.8
R105-NM-0821-01	38.27	376.8	14420.1
R105-SM-0821-01	30.17	304.8	9195.8
R105-WM-0821-02	25.31	366.8	9283.7
R105-WM-0821-01	46.53	399.9	18607.3
R105-WM-0821-02A	5.55	244.5	1357.0
R106-NM-0821-01	52.2	264.3	13796.5
R106-SM-0821-01	27.98	349.6	9781.8
R106-EM-0821-01	43.36	215.3	9335.4
R106-NM-0821-02	32.85	361.2	11865.4
R102-WM-0821-01	51.4	355	18247.0
T3 PCB DATA			
R101-NM-0828-01	3.77	377.6	1423.6
R101-SM-0828-01	3.67	319	1170.7
R101-EM-0828-01	2.7	338.5	914.0
R101-WM-0828-01	4.18	334.9	1399.9
R101-NM-0828-01 D	3.89	377.6	1468.9
R101-EM-0828-01A	1.83	137.7	252.0
R102-WM-0828-01	13.43	345.2	4636.0
R102-SM-0828-01	10.36	341.7	3540.0
R102-EM-0828-01	26.64	300.3	8000.0
R102-WM-0828-01	8.92	288.7	2575.2
R102-WM-0828-01 D	13.34	345.2	4605.0
R102-EM-0828-01A	18.49	149	2755.0
R103-NI-0828-01	23.89	264.1	6309.3
R103-SI-0828-01	24.45	346.4	8469.5
R103-EM-0828-01	26.86	350.1	9403.7
R103-WI-0828-01	30.23	225.2	6807.8

Sample ID	PCB Conc	Dry Wt	PCB Tot
R103-EM-0828-01A	4.35	196.9	856.5
R104-NI-0828-01	20.46	369.3	7555.9
R104-SI-0828-01	30.85	413.3	12750.3
R104-EI-0828-01	34.11	391.3	13347.2
R104-WI-0828-01	25.02	462.1	11561.7
R105-NI-0828-01	23.13	287.2	6642.9
R105-SM-0828-01	20.25	471.5	9547.9
R105-EM-0828-01	31.02	294.8	9144.7
R105-WM-0828-01	27.21	401.4	10922.1
R106-NI-0828-01	20.08	457.5	9186.6
R106-SI-0828-01	24.96	374.5	9347.5
R106-EI-0828-01	17.27	494.2	8534.8
R106-NI-0828-01	28.33	447.7	12683.3
T4 PCB DATA			
R101-NM-0904-01	6.42	304	1951.7
R101-SM-0904-01	4.91	362.6	1780.4
R101-EM-0904-01	5.37	273.3	1467.6
R101-WM-0904-01	5.96	302.1	1800.5
R101-NM-0904-01A	1.52	170.3	258.9
R102-NM-0904-01	7.07	349.9	2473.8
R102-SM-0904-01	22.31	215.5	4807.8
R102-EM-0904-01	18.76	337.7	6335.3
R102-WM-0904-01	13.04	320.1	4174.1
R102-SM-0904-01 D	22.64	215.5	4878.9
R102-WM-0904-01A	0.78	139.4	108.7
R103-NI-0904-01	44.49	214.5	9543.1
R103-SI-0904-01	31.32	209.3	6555.3
R103-EI-0904-01	25.1	331	8308.1
R103-WI-0904-01	28.71	250.9	7203.3
R103-NI-0904-01A	5.84	271.5	1585.6

Sample ID	PCB Conc	Dry Wt	PCB Tot
R104-NI-0904-01	25.43	300.1	7631.5
R104-SI-0904-01	24.25	336.1	8150.4
R104-EI-0904-01	27.51	312.6	8599.6
R104-WI-0904-01	29.04	315.5	9162.1
R104-NI-0904-01A	9.44	413.1	3899.7
R104-WI-0904-01A	8.29	440.5	3651.7
R104-WI-0904-01A D	8.06	440.5	3550.4
R105-NI-0904-01	20.8	300.9	6258.7
R105-SI-0904-01	23.19	309.9	7186.6
R105-EI-0904-01	15.9	265	4213.5
R105-WM-0904-01	19.27	324.7	6257.0
R105-SI-0904-01A	3.72	269.3	1001.8
R105-WM-0904-01A	4.24	189.4	803.1
R106-NI-0904-01	11.46	342.1	3920.5
R106-SI-0904-01	14.28	297.6	4249.7
R106-EM-0904-01	17.07	313.5	5351.4
R106-WM-0904-01	10.93	373.5	4082.4
R106-NI-0904-01A	8.17	278.5	2275.3
R106-WM-0904-01A	3.11	395.9	1231.2
R106-NI-0904-01A R	2.85	278.5	793.7

T5 PCB DATA

R101-NM-0911-01	7.1	341.1	2421.8
R101-SM-0911-01	7.98	429	3423.4
R101-EM-0911-01	5.17	352.2	1820.9
R101-WM-0911-01	6.76	342.1	2312.6
R102-NM-0911-01	10.73	329.1	3531.2
R102-SM-0911-01	17.3	373.4	6459.8
R102-EM-0911-01	12.9	478.4	6171.4
R102-WM-0911-01	20.17	353.1	7122.0
R103-NI-0911-01	23.32	309.3	7212.9
R103-SI-0911-01	7.44	365.1	2716.3
R103-EM-0911-01	13.84	302.6	4188.0
R103-WM-0911-01	18.03	366.8	6613.4

Sample ID	PCB Conc	Dry Wt	PCB Tot
R104-NI-0911-01	27.51	342.1	9411.2
R104-SI-0911-01	28.84	311.2	8975.0
R104-EI-0911-01	26.29	326.2	8575.8
R104-WI-0911-01	24.5	347.5	8513.8
R104-WI-0911-01 D	22.25	347.5	7731.9
R105-NO-0911-01	15.19	301.9	4585.9
R105-SI-0911-01	4.22	441.8	1864.4
R105-EI-0911-01	6.53	419.8	2741.3
R105-WM-0911-01	15.02	301.2	4524.0
R106-NI-0911-01	9.11	297.6	2711.1
R106-SI-0911-01	11.69	369.7	4321.8
R106-EI-0911-01	2.1	370.9	4487.9
R106-WM-0911-01	11.23	369.5	4149.5
T6 PCB DATA			
R101-NO-0918-01	4.24	336	1424.6
R101-SO-0918-01	3.87	413.3	1599.5
R101-EO-0918-01	7.53	334.1	2515.8
R101-WO-0918-01	4.57	356.8	1630.6
R102-NI-0918-01	12.92	360.8	4661.5
R102-SI-0918-01	10.1	283	2858.3
R102-EO-0918-01	7.69	373.7	2873.8
R102-WM-0918-01	12.6	343	4321.8
R103-NI-0918-01	18.93	351	6644.4
R103-SI-0918-01	21.3	314	6688.2
R103-EM-0911-01	17.55	465.6	8171.3
R103-WI-0918-01	9.92	323.4	3208.1
R103-SI-0918-01 D	20.37	314	6396.2
R104-NI-0918-01	19.53	318.2	6214.4
R104-SI-0918-01	13.81	416.2	5747.7
R104-EI-0918-01	18.78	358.9	6740.1
R104-WI-0918-01	22.87	302.1	6909.0

Sample ID	PCB Conc	Dry Wt	PCB Tot
R105-NM-0918-01	14.64	401.3	5875.0
R105-SM-0918-01	13.47	398.7	5370.5
R105-EI-0918-01	14.16	387	5479.9
R105-WI-0918-01	11.54	415.6	4796.0
R106-NI-0918-01	8.06	341.6	2753.3
R106-SI-0918-01	11	380	4180.0
R106-EI-0918-01	8.85	391.1	3461.2
R106-WI-0918-01	13.97	371.9	5195.4
T7 PCB DATA			
R101-NO-0926-01	6.03	291.8	1759.6
R101-SM-0926-01	9.34	340.7	3182.1
R101-EM-0926-01	6.38	351.6	2243.2
R101-WO-0926-01	4.99	256.6	1280.4
R102-NO-0926-01	5.85	299.2	1750.3
R102-SM-0926-01	5.25	358.6	1882.7
R102-EM-0926-01	4.72	409.2	1931.4
R102-WM-0926-01	35.91	254.5	9139.1
R102-EM-0926-01 D	5.04	409.2	2062.4
R103-NO-0926-01	10.83	323.4	3502.4
R103-SO-0926-01	14.54	277.2	4030.5
R103-EO-0926-01	8.79	319	2804.0
R103-WM-0926-01	11.84	209.4	2479.3
R104-NO-0926-01	17.13	284.3	4870.1
R104-SM-0926-01	22.3	334.3	7454.9
R104-EO-0926-01	15.08	338	5097.0
R104-WO-0926-01	16.11	354.7	5714.2
R105-NM-0926-01	11.8	371.8	4387.2
R105-SM-0926-01	15.02	353.5	5309.6
R105-EM-0926-01	14.31	340.3	4869.7
R105-WM-0926-01	12.18	347	4226.5
R106-NM-0926-01	11.6	368.4	4273.4
R106-SM-0926-01	8.11	347.1	2815.0
R106-EM-0926-01	9.72	358.3	3482.7
R106-WM-0926-01	6.61	398.1	2631.4

Sample ID	PCB Conc	Dry Wt	PCB Tot
T8 PCB DATA			
R101-NO-1002-01	4.63	307.6	1424.2
R101-SM-1002-01	4.96	367.6	1823.3
R101-EM-1002-01	3.74	384.3	1437.3
R101-WM-1002-01	2.45	245.4	601.2
R102-NM-1002-01	5.29	388.2	2053.6
R102-SM-1002-01	14.26	374.3	5337.5
R102-WM-1002-01	17.38	228.3	3967.9
R102-EM-1002-01	17.94	311.2	5582.9
R102-EM-1002-01 D	18.35	311.2	5710.5
R103-NM-1002-01	15.64	272.3	4258.8
R103-SM-1002-01	22.22	251	5577.2
R103-WO-1002-01	12.37	386.4	4779.8
R103-EM-1002-01	10.81	354.6	3833.2
R104-NM-1002-01	15.34	292.4	4485.4
R104-SM-1002-01	21.95	280.3	6152.6
R104-WM-1002-01	21.05	260.5	5483.5
R104-EO-1002-01	47.16	177.8	8385.0
R105-NM-1002-01	24.9	195.9	4877.9
R105-SO-1002-01	3.91	405.1	1583.9
R105-WM-1002-01	8.52	359.9	3066.3
R105-EM-1002-01	20.73	201	4166.7
R106-NM-1002-01	10.26	341.2	3500.7
R106-SM-1002-01	12.24	378.9	4637.7
R106-WI-1002-01	10.04	358.8	3602.4
R106-EM-1002-01	10.4	357.2	3714.9
T9 PCB DATA			
R101-NM-1009-01	4.71	305.7	1439.8
R101-SM-1009-01	4.44	413.2	1834.6
R101-WM-1009-01	2.34	249.2	583.1
R101-EM-1009-01	4.89	363.6	1778.0
R102-NM-1009-01	3.96	363.6	1439.9
R102-SM-1009-01	12.88	315.9	4068.8
R102-EM-1009-01	10.22	357.7	3655.7

Sample ID	PCB Conc	Dry Wt	PCB Tot
R102-WM-1009-01	21.19	420.2	8904.0
R103-NM-1009-01	10.92	278.6	3042.3
R103-SO-1009-01	11.92	299.7	3572.4
R103-WO-1009-01	12.01	297.7	3575.4
R103-EM-1009-01	19.58	207.3	4058.9
R104-NM-1009-01	27.04	322.5	8720.4
R104-SM-1009-01	32.27	252.5	8148.2
R104-WM-1002-01	25.94	324.6	8420.1
R104-EM-1009-01	22.1	363.3	8028.9
R105-NM-1009-01	10.33	354.4	3661.0
R105-SM-1009-01	8.33	385.2	3208.7
R105-WM-1009-01	14.5	314.7	4563.2
R105-EM-1009-01	13.59	286.9	3899.0
R106-NO-1009-01	13.56	287.3	3895.8
R106-SM-1009-01	2.21	500.4	1105.9
R106-WM-1009-01	9.79	277.5	2716.7
R106-EM-1009-01	10.59	360.4	3816.6
R106-SM-1009-01 D	2.28	500.4	1140.9

T10 PCB DATA

R101-NM-1016-01	4.88	338	1649.4
R101-SM-1016-01	5.68	357.4	2030.0
R101-EM-1016-01	6.25	413.3	2583.1
R101-EM-1016-01	6.19	401.4	2484.7
R102-NM-1016-01	5.27	319	1681.1
R102-SM-1016-01	4.66	317.1	1477.7
R102-EM-1016-01	8.39	369.2	3097.6
R102-WM-1016-01	10.83	237.3	2570.0
R103-NM-1016-01	11.13	311.1	3462.5
R103-SM-1016-01	9.89	319.7	3161.8
R103-EM-1016-01	10.7	341	3648.7
R103-WM-1016-01	13.88	284.1	3943.3
R104-NM-1016-01	13.89	321.4	4464.2
R104-SO-1016-01	14.91	338.9	5053.0
R104-EM-1016-01	18.53	314.6	5829.5

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R104-WM-1016-01	15.41	315.3	4858.8		
R104-WM-1016-01 D	15.92	315.3	5019.6		
R105-NM-1016-01	8.25	328.5	2710.1		
R105-SO-1016-01	7.6	319.5	2428.2		
R105-EO-1016-01	10.42	356	3709.5		
R105-WM-1016-01	11.74	394	4625.6		
R106-NM-1016-01	11.16	337.1	3762.0		
R106-SM-1016-01	9.03	346	3124.4		
R106-EM-1016-01	6.54	382.1	2498.9		
R106-WO-1016-01	12.15	313.4	3807.8		
R106-EM-1016-01 D	6.41	382.1	2449.3		
T11 PCB DATA					
R101-NI-1022-01R	8.25	297.3	2452.7	0.00702	1175.2
R101-NM-1022-01R	6.1	286.3	1746.4	0.0177	344.6
R101-NO-1022-01	13.88	247.9	3440.9	0.0281	494.0
R101-SI-1022-01	7.42	279.2	2071.7	0.01041	712.8
R101-SM-1022-01	4.82	331.4	1597.3	0.02435	197.9
R101-SO-1022-01	4.82	316	1523.1	0.01948	247.4
R101-EI-1023-01	4.09	296.7	1213.5	0.0115	355.7
R101-EM-1023-01	5.45	273.9	1492.8	0.02342	232.7
R101-EO-1023-01	4.53	246.2	1115.3	0.04886	92.7
R101-WI-1023-01	5.27	277.3	1461.4	0.00985	535.0
R101-WM-1023-01	6.87	317	2177.8	0.02462	279.0
R101-WO-1023-01	6.62	276.7	1831.8	0.02317	285.7
R101-NM-1022-01	6.54	286.3	1872.4		
R101-EI-1023-01 D	4.1	296.7	1216.5		
R101-EI-1023-01A	2.38	116.6	277.5		
R101-WI-1023-01A	0.91	155.6	141.6		
R101-WO-1023-01A	2.82	104.5	294.7		
R102-NI-1022-01	22.12	301.8	6675.8	0.01329	1664.4
R102-NM-1022-01	6.91	303.7	2098.6	0.01332	518.8
R102-N0-1024-02	26.04	231.7	6033.5	0.02859	910.8
R102-SI-1022-01	9.38	281	2635.8	0.00904	1037.6
R102-SM-1022-01	11.56	330.5	3820.6	0.0159	727.0
R102-SO-1024-02	20.4	212.9	4343.2	0.02723	749.2

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R102-EI-1023-01	2.56	327.2	837.6	0.00433	591.2
R102-EM-1023-01	7.36	373.6	2749.7	0.00852	863.8
R102-EO-1023-01	9.99	260	2597.4	0.01701	587.3
R102-WI-1023-01	4.15	248.7	1032.1	0.01038	399.8
R102-WM-1023-01	15.66	263.4	4124.8	0.02102	745.0
R102-WO-1023-01	4.88	122.6	598.3	0.02906	167.9
R102-SI-1022-01A	11.03	111.4	1228.7		
R102-SO-1024-02A	5.28	260.4	1374.9		
R102-EO-1023-01A	6.25	220.9	1380.6		
R102-WM-1023-01A	3.9	138.6	540.5		
R103-NI-1022-01	27.58	279.5	7708.6	0.0298	925.5
R103-NM-1022-01	22.77	295.7	6733.1	0.02478	918.9
R103-NO-1022-01	24.66	345.7	8525.0	0.01913	1289.1
R103-SI-1024-03	15.99	271.1	4334.9	0.02705	591.1
R103-SM-1023-02	14.6	501.7	7324.8	0.02042	715.0
R103-SO-1022-01	17.14	405.2	6945.1	0.01952	878.1
R103-EI-1023-01	22.12	240.7	5324.3	0.03034	729.1
R103-EM-1023-01	13.16	297.7	3917.7	0.02413	545.4
R103-EO-1023-01	13.5	339.5	4583.3	0.0164	823.2
R103-WI-1023-01	16	276.8	4428.8	0.02304	694.4
R103-WM-1023-01	26.35	263.9	6953.8	0.03646	722.7
R103-WO-1023-01	15.45	271.3	4191.6	0.02446	631.6
R103-SM-1023-02 D	15.29	501.7	7671.0		
R103-SI-1024-03A D	0.76	443.5	337.1		
R103-SI-1024-03A	0.8	435.2	348.2		
R103-EM-1023-01A	3.07	119.7	367.5		
R103-WI-1023-01A	5.67	209.5	1187.9		
R104-NI-1022-01	14.13	413.3	5839.9	0.01554	909.3
R104-NM-1022-01	15.81	314.3	4969.1	0.01604	985.7
R104-NO-1024-02	22.37	478.7	10708.5	0.01277	1751.8
R104-SI-1022-01	20.37	265.7	5412.3	0.01565	1301.6
R104-SM-1022-01	10.18	278.7	2837.2	0.01312	775.9
R104-SO-1022-01	14.89	303.9	4525.1	0.01692	880.0
R104-EI-1023-01	43.51	437.5	19035.6	0.01079	4032.4
R104-EM-1023-01	29.12	290.6	8462.3	0.01835	1586.9
R104-EO-1023-01	21.16	382.1	8085.2	0.01646	1285.5
R104-WI-1023-01	15.86	389.6	6179.1	0.01547	1025.2
R104-WM-1023-01	32.19	270.1	8694.5	0.00844	3814.0
R104-WO-1023-01	37.5	267.1	10016.3	0.01148	3266.6

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R104-NI-1022-01 D	14.46	413.3	5976.3		
R104-NI-1022-01A	6.59	138.8	914.7		
R104-NM-1022-01A	6.8	419.2	2850.6		
R104-NO-1024-02A	38.62	217.9	8415.3		
R104-SO-1022-01A	8.23	510.2	4198.9		
R104-EM-1023-01A	6.13	112.8	691.5		
R104-WO-1023-01A	4.96	169.9	842.7		
R105-NI-1024-02	19.14	422.1	8079.0		
R105-NM-1022-01	10.25	403.6	4136.9		
R105-NO-1024-03	12.81	534.8	6850.8	0.01263	1014.3
R105-SI-1022-01	8.36	469.1	3921.7	0.01027	814.0
R105-SM-1022-01	5.42	476.6	2583.2	0.0064	846.9
R105-SO-1022-01	16.77	515.3	8641.6	0.01749	958.8
R105-EI-1024-02	16.18	474	7669.3	0.01351	1197.6
R105-EM-1024-02	6.39	420.7	2688.3	0.0057	1121.1
R105-EO-1023-02	11.58	366.1	4239.4	0.01634	708.7
R105-WI-1023-01	13.19	496.8	6552.8	0.01713	770.0
R105-WM-1025-02	25.96	341.5	8865.3	0.02521	1029.8
R105-WO-1023-01	17.06	467.6	7977.3	0.01237	1379.1
R105-NM-1022-01 D	9.51	403.6	3838.2		
R105-WI-1023-01 D	14.21	496.8	7059.5		
R105-SI-1022-01A D	4.56	306	1395.4		
R105-NM-1022-01A	3.31	192.5	637.2		
R105-NO-1024-03A	4.95	215.9	1068.7		
R105-SI-1022-01A	4.54	306	1389.2		
R106-NI-1022-01 R	5.98	376.2	2249.7	0.00743	804.8
R106-NM-1022-01	10.67	437.8	4671.3	0.01249	854.3
R106-NO-1022-01	5.17	473.5	2448.0	0.00684	755.8
R106-SI-1024-02	7.31	495.5	3622.1	0.01138	642.4
R106-SM-1024-02	12.17	399.1	4857.0	0.01509	806.5
R106-SO-1022-01 R	12.1	434.7	5259.9	0.01167	1036.8
R106-EI-1023-01	15.89	355.2	5644.1	0.01113	1427.7
R106-EM-1024-02	15.15	400.1	6061.5	0.01427	1061.7
R106-EO-1023-01	18.24	392.7	7162.8	0.01418	1286.3
R106-WI-1023-01	16.91	403.5	6823.2	0.0156	1084.0
R106-WM-1023-01	14.92	356.8	5323.5	0.01323	1127.7
R106-WO-1023-01	13.25	358.5	4750.1	0.01378	961.5

Sample ID	PCB Conc	Dry Wt	PCB Tot	TOC	PCB/TOC
R106-NI-1022-01	7.01	376.2	2637.2		
R106-WO-1023-01 D	3.32	358.5	4775.2		
R106-NM-1022-01A	6.23	—			
R106-SI-1024-02A	1.7	374.7	637.0		
R106-SO-1022-01A	3.1	—			
R106-EI-1023-01A	1.62	70.4	114.0		
R106-WO-1023-01A	1.67	206.1	344.2		
R103-NI-1028-01	17.44	317.1	5530.2	0.01827	954.6
R103-NM-1028-01	20.9	277.6	5801.8	0.02605	802.3
R103-NO-1028-01	27.03	300.9	8133.3	0.0273	990.1
R103-SI-1028-01	13.76	392.8	5404.9	0.01854	742.2
R103-SM-1028-01	22.34	294.1	6570.2	0.01668	1339.3
R103-SO-1028-01	13.05	319.5	4169.5	0.01737	751.3
R103-EI-1028-01	21.22	249	5283.8	0.0308	689.0
R103-EM-1028-01	20.56	212.5	4369.0	0.02086	985.6
R103-EO-1028-01	25.9	224	5801.6	0.01731	1496.2
R103-WI-1028-01	21.55	248.8	5361.6	0.02159	998.1
R103-WM-1028-01	19.13	287.4	5498.0	0.02435	785.6
R103-WO-1028-01	15.93	334.4	5327.0	0.01687	944.3
R103-SM-1028-01 D	23.06	282.2	6507.5		
R104-NEI-1104-01	46.1	201.6	9293.8	0.02503	1841.8
R104-NEM-1104-01	54.27	254	13784.6	0.02476	2191.8
R104-NEO-1104-01	46.91	244.4	11464.8	0.01598	2935.5
R104-SWI-1104-01	41.63	290.6	12097.7	0.02047	2033.7
R104-SWM-1104-01	55.78	284.2	15852.7	0.02204	2530.9
R104-SWO-1104-01	48.82	258.6	12624.9	0.01842	2650.4
R105-NEI-1104-01	30.38	349.3	10611.7	0.02318	1310.6
R105-NEM-1104-01	28.09	328.8	9236.0	0.01733	1620.9
R105-NEO-1104-01	26.88	273	7338.2	0.02245	1197.3
R105-SWI-1104-01	8.81	409.8	3610.3	0.01099	801.6
R105-SWM-1104-01	24.33	260.4	6335.5	0.0293	830.4
R105-SWO-1104-01	30.18	408.3	12322.5	0.022	1371.8