TASK I.A.1

POTENTIAL DETOXIFICATION OF SHEBOYGAN HARBOR PCB'S

TD
427
.P65
P68
199x

UNCLASSIFICATED
INFORMATION
GPO

WISCONSIN COASTAL MANAGEMENT PROGRAM

TD427.P65P68 1997

WISCONSIN DEPARTMENT
OF ADMINISTRATION
P.O. BOX 7868
MADISON, WI 53707
608-266-8234

POTENTIAL DETOXIFICATION OF SHEBOYGAN HARBOR PCB'S

COASTAL ZONE
INFORMATION CENTER

This study was funded in part with financial assistance provided by the State of Wisconsin, Division of Energy and Intergovernmental Relations, Coastal Management Program through a grant under the Coastal Zone Management Improvement Act of 1980, as amended, administered by the Office of Ocean and Coastal Resource Management, National Oceanic and Atmospheric Administration.

The development of this project was under the supervision of William C. Sonzogni, University of Wisconsin, Madison, Wisconsin.

US Department of Commerce
NOAA Coastal Services Center Library
2234 South Hobson Avenue
Charleston, SC 29405-2413

EXECUTIVE SUMMARY

POTENTIAL DETOXIFICATION OF SHEBOYGAN HARBOR PCBs

William C. Sonzogni
Principal Investigator

Over the last two years research has been conducted on Wisconsin's Sheboygan River, a tributary to Lake Michigan located north of the City of Milwaukee. The Sheboygan River was studied because it is contaminated by polychlorinated biphenyls (PCBs), so much that it has been declared a Superfund Site by the U.S. Environmental Protection Agency and a Great Lakes Area of Concern by the U.S./Canadian International Joint Commission. The river is also a source of PCBs to Lake Michigan. Further, the PCBs, at least some of more toxic PCB compounds, are a potential health risk to inhabitants of the river or users of the river. Thus, this research was designed to provide important information on a major environmental problem affecting the coastal zone of the Great Lakes.

The focus of the research was to determine whether PCB dechlorination is occurring in the river's sediments. High resolution gas chromatographic analyses were made on sediment, water, and fish collected from the river and harbor. In addition, several soil samples from a confined disposal facility holding dredged sediment were also analyzed. Laboratory experiments were also conducted to try and replicate some of the processes that occur in the natural environment. Information from other investigators and the scientific literature was used when possible to supplement findings.

Overall, the research was successful. All of the objectives were met. Major findings of the research are summarized below.

1. Data collected on PCBs in sediments indicate the distribution of PCB compounds (congeners) has changed relative to the distribution of PCBs that originally polluted the river.

2. It appears, based on all the available evidence, that the changes in the PCB distribution are the result of dechlorination of the PCBs by anaerobic microorganisms in the sediment.

3. Dechlorination was observed mainly in those section of the river with accumulation of muck (sedimentation areas) and where total PCB concentrations were high.

4. The fact that many of the higher chlorinated PCB compounds (congeners) are being dechlorinated indicates that some natural detoxification is occurring, as the more highly chlorinated PCB compounds are generally considered to be more toxic.

5. The results suggest that it may be possible to modify natural processes to speed up the dechlorination as a bioremediation measure. Complete degradation of the PCBs may even be possible.

6. PCB concentrations in the harbor (about 20 km downstream from the source) were much lower than the source area and the distribution of PCB compounds did not show evidence of degradation.

7. PCB concentrations in the water were much lower than in sediment, but concentrations increased downstream from the source. The distribution of PCB compounds varied between the

2. One thesis was published as a result of this research, and two research papers are written so far and will be submitted to refereed scientific journals.

3. Several presentations have been made on the project at scientific meetings, including an international workshop on biological remediation of contaminated sediments sponsored by the U.S. Environmental Protection Agency. A summary of the presentation is to be published in the workshop proceedings.

4. The multidimensional gas chromatograph, purchased for the Wisconsin Laboratory of Hygiene partly through this project, will be a continuing resource for future PCB and other complex organic analysis. As a result of demonstrating the ability of this machine to measure "toxic" PCB compounds in this project, Fort Howard Paper Company has decided to purchase one of these machines to help them in their efforts to free the lower Fox River and Green Bay from PCB pollution.

5. The Wisconsin Department of Natural Resources has become increasingly concerned about the presence of certain PCB compounds (congeners), and methods to analyze these compounds refined during this project will be used in the future by the State Laboratory of Hygiene in doing analyses for the Department.

6. Information on PCBs developed in this project will be useful to the Green Bay Mass Balance Study now ongoing on the Fox River and Green Bay.

7. Results of this study will be useful to the current Superfund investigation and remedial action plan. In fact,

dissolved and particulate PCBs.

8. The types of PCBs found in fish from the Sheboygan River were different from the PCB pattern found in contaminated sediments.

9. While experiments to induce anaerobic dechlorination of PCBs using bacteria extracted from Sheboygan River sediments were negative, further experiments would likely produce positive results. Based on recent laboratory work by others (with different river sediments), it is clear dechlorination can be demonstrated in the laboratory although results are highly variable. Key factors controlling dechlorination are not fully understood.

10. The multidimensional (heart cutting) gas chromatograph is an effective tool for resolving PCB compounds (congeners) that otherwise can not be measured.

11. Some of the "toxic" PCBs (having dioxin-like effects), that can't be resolved by conventional high resolution techniques, were quantified using the multidimensional gas chromatograph. Concentrations were always very low and there were indications that dechlorination of several of these compounds was occurring.

This research has led to a number of benefits and products other than the research findings. The most important of these are listed below.

1. Training was provided to students and technicians. A Master of Science degree in Water Chemistry was received by one student working on the project.

this study probably had an influence or helped stimulate current efforts to bioremediate dredged Sheboygan sediment through microbial processes.

8. The results of this study will be useful to other coastal sites with in-place pollution due to PCBs. Natural dechlorination may be a useful remedial strategy in some situations.

9. The Assessment and Remediation of Contaminated Sediments (ARCS) Program, set up under the 1987 amendments to the U.S. Clean Water Act, has set the Sheboygan River as one of its pilot study areas. Data from this study will be essential for this Program, and information has already been given to program officials.

10. Last but not least, findings from this study will benefit the living near or using the Sheboygan River. Knowing that some of the more highly chlorinated, and thus potentially most toxic, can be dechlorinated provides hope that the PCB problem can eventually be solved.

The hard work, support and cooperation of many individuals made this study possible. Co-principal investigators included Professors David Armstrong and Anders Andren, and Dr. John Konrad of the Wisconsin Department of Natural Resources' Bureau of Research. Laura Maack Brondyk served as Assistant Researcher throughout most of the project, and played a major role in initiating the project. Margaret David served as the student researcher for the project, and the core findings of the project are described in her Master of Science dissertation. Robert

Lawrence served as an Assistant researcher during the second half of the study. Many staff at the Wisconsin Laboratory of Hygiene contributed their expertise, particularly to the analytical chemistry part of the work. Notable contributors were David Degenhardt, Thomas Gibson, Carol Buelow William Krick and Thomas Dunnick. Professor Ronald Schell and Terry Kurzynski provided bacteriological expertise.

Finally, the generous financial support of the Wisconsin Coastal Management Program and Wisconsin Sea Grant Program are gratefully acknowledged. In addition to the fiscal support, the administrative support and general encouragement given by the staffs of these organizations was really appreciated. The Wisconsin State Laboratory of Hygiene and the Water Chemistry Program of the University of Wisconsin, as well as the Wisconsin Department of Natural Resources also provided support for this project.

ATTACHMENTS

1. PCB Congener Distribution in Sheboygan River Sediment Fish and Water (M.S. Thesis by Margaret David).

2. Concentrations of Toxic Polychlorinated Biphenyl Congeners in Sheboygan River (USA) Sediments (paper submitted to the Bulletin of Environmental Contamination and Toxicology).

3. PCB Dechlorination in the Sheboygan River, Wisconsin (summary to be published in proceedings of a workshop on biological remediation of contaminated sediments).

4. Summary of Laboratory Experiments to Dechlorinate PCBs using Bacteria Extracted from Sheboygan River Sediments.

5. An Evaluation of the Potential for PCB Dechlorination in Confined Disposal Facilities.

PCB CONGENER DISTRIBUTION IN SHEBOYGAN RIVER SEDIMENT, FISH AND WATER

Margaret Meigs David

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
(Water Chemistry Program,
Department of Civil and Environmental Engineering)

March 7, 1990

Acknowledgements

I would like to thank my parents for their undying support and encouragement. They have been great parents and fantastic friends. Without my friend David, this project would be an abysmal skeleton of what it now is. David's ideas and criticisms were excellent and his drive to truly understand science, inspiring. I am very glad that my younger brother and I have been in graduate school at the same time. Andrew has been a source of humor and hope, as well as an amazingly fluid mechanics and differential equations tutor. Insightful comments have come from Peter who is omniscient and of course, my older brother. He constantly piques my curiosity about the world and desire to learn more.

I would especially like to extend my gratitude to all of those in the State Laboratory of Hygiene who worked with me. Carol Buelow and Tom Gibson have been extraordinarily helpful. Carol has saved me many a time from complete and total frustration and has been a mentor to me. Dave Degenhardt has made my life much simpler and for him I hope that National Dave Degenhardt day will become a state holiday with big band music and ice cream.

I would like to thank Bill Sonzogni for even considering me as a research assistant and then for getting involved in the dirty work of the science--sampling oozing, cold, wet

river sediment. Laura Maack and Debbie DeLuca were great colleagues with whom one could discuss detection limits and decaffeinated coffee. I would also like to thank Dr. Armstrong who inspite of hectic schedules has always made time to discuss this project and provide very thoughtful criticism. And lastly, I would like to thank Dr. Andren whose spunky interesting lectures got me involved in water chemistry in the first place.

TABLE OF CONTENTS

Chapter 1 Introduction.....1

Chapter 2 Objectives.....4

Chapter 3 Literature Review of Aerobic and Anaerobic Bacteria.....6

3.1. Aerobic Degradation of PCBs.....6
3.1.1. Introduction.....6
3.1.2. Enzyme Systems.....7
3.1.3. Aerobic Microbial Degradation Studies of PCBs.....10

3.2. Anaerobic Degradation of PCBs.....15
3.2.1. Introduction.....15
3.2.2. Anaerobic Degradation of Structurally Similar Compounds.....17
3.2.3. Anaerobic Microbial Degradation Studies of PCBs.....21

Chapter 4 Site Description and Historical Use of PCBs.....30

Chapter 5 Sample Collection, Materials and Methods.....38

5.1. Sample Collection.....38
5.1.1. Sediment.....38
5.1.2. Fish.....41
5.1.3. Water.....42

5.2. Materials.....43
5.2.1. Solvents.....43
5.2.2. Chromatographic Adsorbents.....43
5.2.3. Standards.....43
5.2.4. Other Chemicals and Materials.....44
5.2.5. Equipment.....45
5.2.6. Glassware.....45

5.3. Extraction and Cleanup Methods.....45
5.3.1. Sediment.....45
5.3.2. Fish.....49
5.3.3. Water.....52

5.4. Gas Chromatography.....57

5.5. Grain Size Analysis of Sediment.....59

Chapter 6 Results.....64

6.1. Grain Size.....64

6.2. Sediment Samples from the Sheboygan River.....65
6.2.1. Total Concentrations of PCBs in Sediment..65
6.2.2. Homolog Distribution Patterns in Sediment.71
6.2.3. Most Prominent Congeners in Sediment.....76
6.2.4. Accuracy, Precision and Confirmatory Analysis for Sediment Samples.....81

6.3. Sediment Samples from the Harbor.....83
6.3.1. Total PCB Concentrations in Harbor Sediments.....83
6.3.2. Homolog Distribution in Harbor Sediment...83
6.3.3. Most Prominent Congeners in Harbor Sediment.....86
6.3.4. Precision and Accuracy for Harbor Samples.88

6.4. Water Samples from the Sheboygan River.....88
6.4.1. Total PCB Concentrations in Water.....88
6.4.2. PCB Homolog Distribution in Water.....90
6.4.3. Most Prominent Congeners in Water.....92
6.4.4. Accuracy, Precision, and Confirmatory Analysis for Water.....93

6.5 Fish.....95
6.5.1. Total PCB Concentrations in Fish.....95
6.5.2. Homolog PCB Distribution in Fish.....95
6.5.3. Most Prominent Congeners in Fish.....99

Chapter 7 Discussion of Results.....101

7.1. Discussion of Sediment Results.....101
7.1.1. Grain Size Correlations with PCB Concentration.....101
7.1.2. Total PCB Concentrations in Sediment.....102

7.2. Discussion of Possible Mechanisms causing the Congener Distribution in Sediment.....105
7.2.1. Physical Processes Affecting the Distribution of PCBs in Sediment.....106
7.2.2. Biological Processes Affecting the Distribution of PCBs in the Sediment.....112

7.3. Discussion of Water Results.....123
7.3.1. Total PCB Concentrations in Water.....123
7.3.2. Homolog and Congener Distribution in Water.....125

7.4. Discussion of Fish Results.....126
7.4.1. Total PCB Concentrations in Fish.....126
7.4.2. Homolog Patterns in Fish.....131
7.4.3. Most Prominent Congeners in Fish.....132

Chapter 8 Conclusions.....140

8.1. Sediment.....140
8.2. Water.....142
8.3. Fish.....144
8.4. Concluding Remarks.....145

Bibliography.....147

Appendices
Appendix A Limits of Detection
Appendix B PCB Congener Results

List of Tables

Chapter 3

Table 3-1 Homolog Distribution for Pattern AB and B.....25
Table 3-2 Energy Derived from the Oxidation of Glucose to Carbon Dioxide and Water using Various Oxidants.....26

Chapter 4

Table 4-1 PCB Mixtures Used at Tecumseh Products Site....36

Chapter 5

Table 5-1 Operating Conditions for the Gas Chromatograph.....58

Chapter 6

Table 6-1 Results of Sieved Sediment Samples.....66
Table 6-2 Total PCB Concentrations in River Sediment.....69
Table 6-3 Most Prominent Congeners in Sediment.....78
Table 6-4 Percent Recovery from Spiked Sediment.....81
Table 6-5 Duplicate Sediment Samples.....82
Table 6-6 Total PCB Concentrations in Harbor Sediment....84
Table 6-7 Most Prominent Congeners found in Harbor Samples.....86
Table 6-8 Total PCB Concentrations in Water.....89
Table 6-9 Most Prominent Congeners in Water.....93
Table 6-10 Total PCB Concentrations in Fish.....96
Table 6-11 Most Prominent Congeners in Fish.....99

Chapter 7

Table 7-1 PCB Concentrations in Sheboygan River Sediment.....103
Table 7-2 Changes in Sheboygan River Sediment Relative to Aroclors (Sediment Samples with concentrations greater than 50 ppm).....119
Table 7-3 Comparison of Congeners Predicted by Brown et al. based on Anaerobic Degradation to Congeners Observed in Study.....120
Table 7-4 Total PCB Concentrations in Water.....124
Table 7-5 Total PCB Concentrations in Fish from the Sheboygan River.....127
Table 7-6 A Comparison of Most Prominent Congeners in Fish.....133
Table 7-7 Clearance Rates for Specific Congeners.....136
Table 7-8 BCF Values Reported in the Literature.....140

List of Figures

Chapter 3

Figure 3-1 Major Aerobic Degradation Pathway for PCBs.....9
Figure 3-2 Anaerobic Degradation of Chlorobenzoates.....19

Chapter 4

Figure 4-1 The Sheboygan River and Tributaries.....31

Chapter 5

Figure 5-1 Map of Sample Sites.....39
Figure 5-2 Analysis of PCBs in Sediment.....47
Figure 5-3 Analysis of PCBs in Fish.....51
Figure 5-4 Analysis of PCBs in Water.....53

Chapter 6

Figure 6-1 Map of Sheboygan River illustrating Average Sediment Core.....67
Figure 6-2 Homolog Distribution in Sediment (Concentration >50 ppm Sites B, C, D, E).....73
Figure 6-3 Homolog Distribution in Sediment (Concentration >50 ppm Site T).....74
Figure 6-4 Homolog Distribution in Sediment (Concentration <50 ppm, Sites B,C,D,E).....75
Figure 6-5 Homolog Distribution in Sediment (Concentration <50 ppm, Site T).....77
Figure 6-6 Most Prominent Congeners in Sediment (Sites B,C,D,E).....79
Figure 6-7 Most Prominent Congeners in Sediment (Site T).....80
Figure 6-8 Homolog Distribution in Harbor Sediment.....85
Figure 6-9 Most Prominent Congeners in Harbor Sediment...87
Figure 6-10 Homolog Distribution in Water.....91
Figure 6-11 Most Prominent Congeners in Water.....94
Figure 6-12 Homolog Distribution in Fish.....97
Figure 6-13 Homolog Distribution in Fish and Sediments...98
Figure 6-14 Most Prominent Congeners in Fish.....100

Chapter 7

Figure 7-1 Ortho vs Meta/Para Congeners in Sediment.....115
Figure 7-2 Ortho vs Meta/Para Congeners in Spiked Sediment.....117
Figure 7-3 Correlation between Fish Fat and PCB Concentration.....129

Figure 7-4 Correlation between Packed
and Capillary Column Results.....130
Figure 7-5 Predicted BCF from Mackay
versus Calculated BCF from Field data.....138

Chapter 1 Introduction

Polychlorinated biphenyls are a class of 209 compounds comprised of two benzene rings with one to ten chlorines. The physical and chemical properties of the 209 compounds, referred to as congeners, vary dramatically. Some congeners are oily liquids at room temperature, while others are white powdery solids. Vapor pressures vary orders of magnitudes among the congeners; boiling points span almost 200 degrees (Erickson, 1986).

Because of their wide range of physical and chemical properties, thermal stability, and chemical resistance to acids and bases, PCBs have been used in many industries for a wide variety of purposes. PCBs were commonly used in transformer oils, dielectric fluids, hydraulic fluids, plasticizers, and carbonless copy paper (Erickson, 1986; National Acaedemy of Science, 1979).

PCBs were first manufactured in the late 1920s by direct chlorination of biphenyl with chlorine gas. Monsanto Chemical Corporation, one of the largest producers of PCBs, marketed PCB mixtures under the tradename of Aroclor. Each Aroclor mix was sold as a weight percent of chlorine. The last two digits of the Aroclor name signified the weight percent. Aroclor 1242 contains 42 percent chlorine by weight and consists predominantly of the trichlorinated congeners (Furukawa,

1982).

In the early 1930s, PCBs were found to have delitrious health effects and workplace threshold limit values were set. It was not until 1977, however, that PCBs were banned from commercial use because of possible health effects (Erickson, 1986). The scientific community is uncertain about the exact risks posed by PCBs. When humans are exposed to PCBs in large doses, they are thought to elicit one or more of the following responses: wasting syndrome, skin disorders such as chloroacne, thymic and splenic atrophy, liver damage, endocrine and reproductive dysfunction, tetratogenesis and carcinogenesis (hepatocarcinoma)(Parkinson and Safe, 1987). Several congeners are thought to be more toxic than others. These "toxic congeners" have a planar configuration similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin, one of the most toxic environmental contaminants known to man. Like dioxin, the toxic congeners are thought to bind to the direboxynucleic acid (DNA) at sites which are coded for enzyme synthesis (Chantry, 1989).

Because PCBs were used extensively in a variety of industries before their toxicological hazards were identified and because they are relatively inert, their presence in the environment is ubiquitous. PCBs have been measured in air, water, sediment, soil and biota around the world. Many sites

in the United States contain high concentrations of PCBs (above 50 ppm). Under federal regulation, these sites are required to be cleaned up to background or limits set by the Environmental Protection Agency (EPA). The cost of cleaning up such sites is tremendous and the success of cleaning up such sites is often marginal.

The fate of PCBs in the environment is not completely understood, in part because it has been difficult to quantify all the physical and chemical properties associated with the different congeners. Aerobic microbial degradation of the lower chlorinated PCBs has been shown to occur under certain conditions. Recently, there has been evidence that anaerobic degradation of PCBs occurs as well (Brown et al., 1987b; Chen et al., 1988; Rhee et al., 1989; Quensen et al., 1988). This finding is significant because PCBs and PCB-contaminated waste is often stored in anaerobic environments such as landfills, and sediments. Determining if anaerobic degradation occurs and how it occurs has substantial implications for the treatment and disposal of PCBs. Significant economic gains could be achieved if sediments could be treated in situ. In addition, the risks of exposure would be considerably diminished if PCBs and PCB-contaminated material were treated in place.

Chapter 2 Objectives

The overall purpose of this study is to examine PCB-contaminated sediment from the Sheboygan River to determine if anaerobic dechlorination is occuring. The observed congener distribution will be compared to the predicted distributing based on existing knowledge of partitioning relationships, diffusion equations, Henry's Law constants, and observed biodegradation patterns.

The study objectives are fourfold. The first objective is to determine the distribution of PCB congeners in the Sheboygan River sediments and to determine the variation with depth and distance. The second objective is to determine if there is a significant difference between the congener distribution found in the river sediment versus the congener distribution found in the Aroclors originally used at the industrial site.

The third objective is to evaluate processes such as partitioning and biodegradation that could account for the change in the distribution of the congeners. The work of Burkhard et al.(1985a, 1985b) will be used to evaluate the effects of partitioning and volatilization on the congener distribution. The work of Brown et al. (1987), Rhee et al. (1989) and Quensen et al.(1988) will be used to evaluate the effects of anaerobic biodegradation on the congener

distribution. The last objective was to examine the congener distribution in other matrixes such as fish and water and to compare these distributions to that in the sediment.

Chapter 3 Literature Review of Aerobic and Anaerobic Bacteria

One of the goals of this research was to determine whether sediment from the Sheboygan River had PCB congener patterns characteristic of anaerobic dechlorination as described by Brown et al. (1984), Quensen et al. (1988), Chen et al. (1988) and Rhee et al. (1989). This chapter presents a synopsis of existing knowledge of aerobic microbial degradation as well as anaerobic microbial degradation.

An understanding of aerobic processes is important since rivers are dynamic systems that vary temporally and spatially, scouring and depositing sediments in a somewhat random fashion. As a result of these processes, it is likely that sediment that was anaerobic becomes aerobic and vice versa. Therefore, sediment samples may have been exposed to both types of environments.

This chapter contains a brief overview of the following subjects; aerobic microbial degradation, aerobic bacteria, PCB-degrading enzymes and the environment necessary for PCB-degradation. A similar overview of anaerobic degradation follows the discussion of aerobic microbial degradation.

3.1. Aerobic Degradation of PCBs

3.1.1. Introduction

Since the first discovery that aerobic bacteria degrade

PCBs (Ahmed and Focht, 1973), many aerobic, PCB-degrading bacteria have been identified. These bacteria are apparently widely distributed in the environment. For example, Chantry (1989) cites sixteen PCB-metabolizing genera in a review of the literature. Several of these genera have been found in the environment, notably Pseudomonas, Vibrio, Aeromonas, Microccus, Acinetobacter, Bacillus, and Streptomyces (Furukawa, 1982). In sediments from the Hudson River, which contain PCBs, twenty isolates from five PCB-degrading genera have been identified: Acetobacter, Acinetobacter, Alcaligenes, Klebsiella and Pseudomonas (Furukawa, 1982).

3.1.2. Enzyme Systems

Two major aerobic enzyme systems are thought to enable bacteria to degrade PCBs. All PCB-degrading bacteria employ a dioxygenase enzyme, in which both atoms of the oxygen molecule are added to the product (Stryer, 1981). In contrast, mammals use a monooxygenase system for PCB metabolism. A monooxygenase system, also referred to as a mixed function oxygenase system, is one in which one atom of oxygen is added to the product and one atom goes to form water (Stryer, 1981).

The most common enzyme system used by aerobic PCB-degrading bacteria is the 2,3 dioxygenase enzyme. Serving as

an electron acceptor, molecular oxygen is attached to an vacant 2,3 site (or 5,6 site) on the aromatic ring to produce a cyclic peroxide intermediate and then a cis-dihydrodriol (see figure 3-1). The cis-dihydrodriol then forms a 2,3 dihydroxy compound which undergoes oxidative meta ring cleavage between the first and second carbon atom to form a benzoic acid (Safe, 1984). Although the chlorobenzoates can be mineralized by bacteria, there are no known bacteria which can both degrade PCBs and mineralize the chlorobenzoates.

A second pathway for the degradation of PCBs, a 3,4 dioxygenase, has been proposed by Bedard and coworkers (Bedard et al., 1987a; Bedard et al., 1987b) based on experiments with the bacteria, Alcaligenes, and Aroclor 1242. Three of Bedard and coworker's experimental results suggest an alternative enzyme to the 2,3 dioxygenase. First, PCBs that were sterically hindered for the 2,3 dioxygenase enzyme (i.e chlorinated at the 2,3 sites) were readily metabolized by Alcaligenes H850. Second, Alcaligenes was not able to metabolize para chlorinated biphenyls; however, it easily metabolized ortho chlorinated biphenyls. These results are in striking contrast to the conclusions drawn from previous experiments which indicated that ortho chlorinated congeners are extremely resistant to degradation by bacteria using the 2,3 dioxygenase system (Furukawa, 1982; Furukawa et al.,

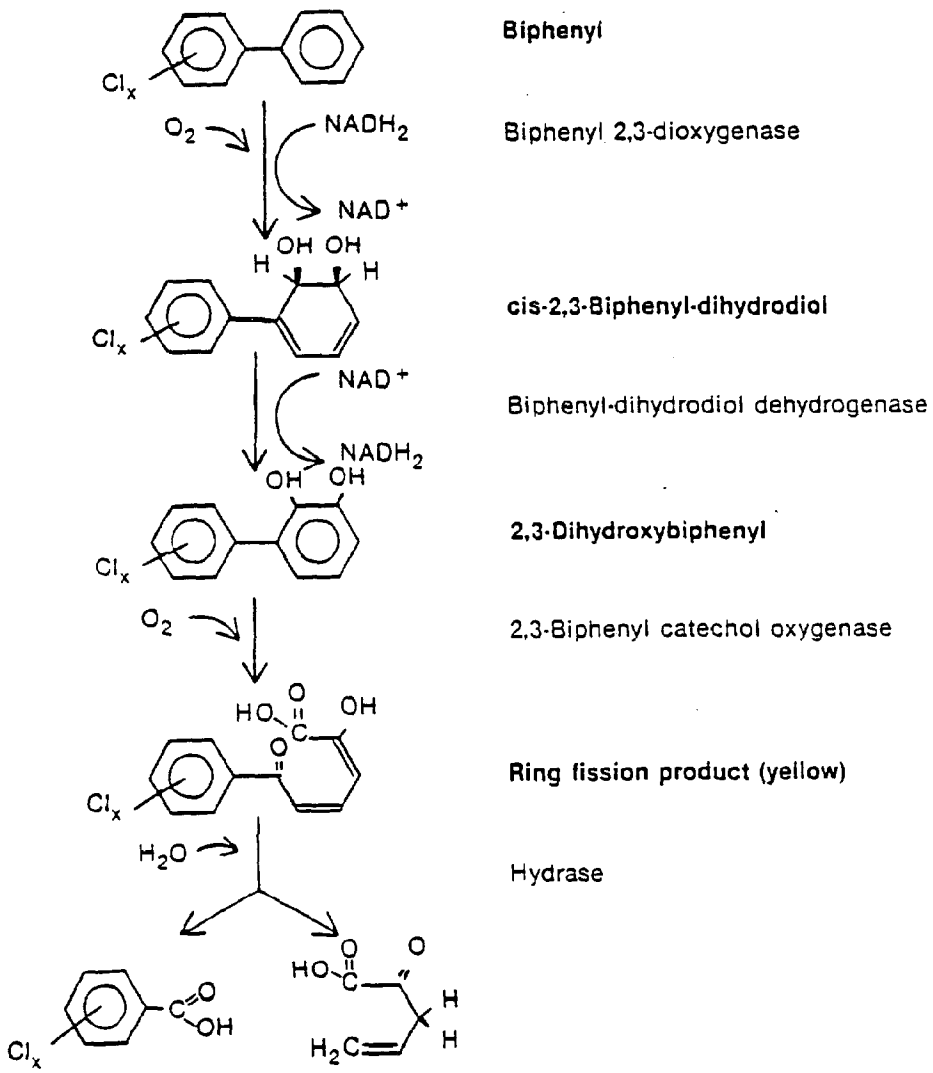


Figure 3-1 Major Aerobic Degradation Pathway For PCBs

(Source: General Electric Research and Development, 1987)

1978). Lastly, there is some evidence that the expected metabolites from a 3,4 dioxygenase pathway are formed (Bedard et al., 1987b).

3.1.3. Aerobic Microbial Degradation Studies of PCBs

Many different bacteria have been found to oxidize PCBs. Although they use similar enzyme systems, the extent and the rate of degradation among bacterial strains differ depending on the number and the position of chlorines on the biphenyl molecule. Three experimental results are important. First, as the chlorine content of the PCB increases, the ability of bacteria to metabolize PCB aerobically decreases. Second, mixed cultures of bacteria appear to increase the biodegradation rates of PCBs. This finding is important since natural environments often contain mixed cultures of bacteria. Thirdly, many bacteria appear to require an additional substrate for growth, a process referred to as cometabolism.

Tucker and coworkers (1972) examined the ability of microorganisms in activated sewage sludge to degrade Aroclor mixtures at concentrations of 2.5 and 5.0 ppm. Most of the degradation of the mono and di chlorobiphenyls in Aroclor 1221 took place in less than a day. There was a 26 percent decrease in Aroclor 1242 in two days and no degradation of the

more highly chlorinated Aroclor 1254. From these results, Tucker and coworkers concluded that as the degree of chlorination increased, the degree of biodegradation decreased.

Experiments performed by Wong and Kaiser (1975) also support this conclusion. Microorganisms (Achromobacter and Pseudomonas) from lake water were placed in 0.05 percent solution of Aroclor 1221, 1242, and 1254. The bacteria metabolized Aroclor 1221 and 1242 but not Aroclor 1254. Aroclor 1221 was completely degraded in one month. Rates for Aroclor 1242 and 1254 were not established.

Furukawa, Tomizuka and Kamibayashi (1983) also found that as the degree of chlorination increased, the ability of the microorganisms to degrade PCBs decreased. Using PCB contaminated soil (50 ppm) and the bacteria, Acinetobacter, these researchers found that Kaneclor 200 (predominantly dichlorobiphenyls) was rapidly degraded in four hours. Kaneclor 300 (primarily trichlorobiphenyls) and Kaneclor 400 (primarily tetrachlorobiphenyls) were also susceptible to biodegradation but to a lesser degree. Kaneclor 500 (primarily pentachlorobiphenyls) was recalcitrant. Similarly, microbes from activated sewage sludge were unable to degrade Kaneclor 500 at concentrations of 0.1, 5, and 10 ppb (Kaneko et al., 1976).

Mixed bacterial cultures may be better able to degrade PCBs than pure strains. A mixed strain was found to degrade Aroclor 1242 in five days, a rate considerably faster than values previously reported in the literature (Clark et al., 1979). The increase in the rate may be because only readily water soluble PCBs were used (a solution was saturated with Aroclor 1242). This may have biased the experiment because the lower chlorinated congeners are more soluble and also more easily degraded. In addition, the PCBs may have been more available to bacteria since they were in solution and not adsorbed to particulates. Again the lower chlorinated congeners were most easily degraded.

Furukawa and coworkers (1983) extensively studied two bacterial strains, Alcaligenes and Acinetobacter, both of which degrade PCBs using a 2,3 dioxygenase enzyme. The following generalizations about aerobic PCBs degradation result from their work and summarize much of the aerobic research to date.

1) As the number of chlorines increased on the biphenyl molecule, the ability of the bacteria to degrade the compound decreased. This decrease may be due to increased steric hinderance as additional chlorines are added to the molecule. Congeners with greater than four chlorines were recalcitrant.

2) PCBs containing chlorines in the ortho position of the

ring were also resistant to degradation. These congeners may be sterically hindered; the 2,3 dioxygenase system requires vacant sites at either 2,3 or 5,6 position. If both ortho positions are filled on the opposite ring to the ring that the enzyme is attacking, the attack by the enzyme will be hindered.

3) PCBs containing all the chlorine atoms on a single ring were generally degraded faster than isomers with the same total number of chlorines distributed on both rings.

4) Ring cleavage occured more often with congeners that had fewer chlorines.

5) PCBs having adjacent unchlorinated sites, particularly at the 2,3 position, were more readily degraded than those congeners chlorinated at the 2,3 position.

In addition to these factors, it appears that a substrate for growth may be important since PCB-degrading bacteria are unable to completely mineralize PCBs.

Cometabolism, as defined by Horvath (1972), is "any oxidation of substances without the utilization of the energy derived from the oxidation to support microbial growth." There is evidence that the higher chlorinated congeners cannot be degraded without additional carbon sources. "Although complete mineralization of monochlorobiphenyls has been reported, no culture with the ability to completely mineralize

more highly chlorinated biphenyls or to grow on any congener that has one or more chlorine on each ring has unequivocally been described" (Kohler et al., 1988). A variety of substrates have been used. Biphenyl is by far the most common; however, acetate has also been used (Clark et al., 1979).

Brunner and coworkers (1985) found the most important factor affecting PCB degradation in soil was enrichment of the soil with biphenyl. After incubating soil for 210 days, Brunner and coworkers noted that little if any degradation took place in soil that was not inoculated with a growth medium (such as biphenyl) or with bacteria (such as Acinetobacter). In contrast, when the soil was inoculated with a growth medium such as biphenyl, degradation increased tenfold. Inoculation with bacteria increased biodegradation, but not as dramatically. With the exception of 4-chlorobiphenyl, Acinetobacter cannot grow upon any of the PCBs because it is unable to dehalogenate the ring fission products (Brunner et al., 1985).

Kohler also noted that the addition of biphenyl substantially increased the ability of bacteria to metabolize PCBs. When bacteria, Acinetobacter and Arthobacter, were grown in the presence of biphenyl, degradation rates of Aroclor 1254 increased substantially (Kohler et al, 1988).

Interestingly it has been found that adding Aroclor 1221 increased the ability of the Pseudomonas to degrade Aroclor 1254 (Lui, 1980). This phenomenon is suggestive of cometabolism. Aroclor 1254 (300 ppm) was degraded in 18 days to 0.59 ppm. In contrast, when Aroclor 1221 was added, the bacteria degraded the same concentration of Aroclor 1254 in 8 days. Sodium ligninsulfonate was also added to these cultures resulting in the formation of an emulsion. It is hypothesized that the emulsion increased the surface area of PCBs available to the bacteria and therefore expedited the biodegradation of these compounds (Lui, 1980).

Another example of cometabolism is a study of four congeners by Parson and Sijm (1988). Grown on biphenyl and 4-chlorobiphenyl in a continuous culture system, Pseudomonas could metabolize 4-chlorobiphenyl, 2,2'3,3' (congener 40), 2,2'5,5' (congener 52), 2,3,'4'5 (congener 70) and 3,3'4,4' (congener 77). If, however, the Pseudomonas were cultured on nutrient broth or benzoates they eventually lost the ability to metabolize these four congeners (IUPAC # 40, 52, 70, 77).

3.2. Anaerobic Degradation of PCBs

3.2.1. Introduction

Although the fate of PCBs in aerobic sediments has been

well studied, very little is known about the fate of PCBs in anaerobic sediments such as landfills, and confined disposal facilities. Since anaerobic environments such as river bottoms and lake bottoms represent the final sink for PCBs (National Academy of Science, 1979), it is important to determine what, if any, processes PCBs undergo in anaerobic environments.

In contrast to the plethora of information that exists about aerobic microbial degradation, there is surprisingly little information about anaerobic microbial degradation. Anaerobic microbial metabolic pathways are not well understood and anaerobic bacteria which can degrade PCBs have not been isolated.

The first studies presented little if any experimental evidence of anaerobic degradation. Work by Kaneko and coworkers (1976) showed that anaerobic incubation of Kaneclor 500 (equivalent to Aroclor 1254) in the presence of sewage sludge for twenty days resulted in no degradation. Similarly, when Aroclor 1242 was incubated anaerobically in soil enriched with biphenyl and Acinetobacter, no evidence for degradation was seen (Brunner et al., 1985).

Although these studies were useful, they do not conclusively demonstrate that these compounds do not degrade in an anaerobic environment. Alternatively, these

experiments may indicate that the environment present in the experiment was not suitable for anaerobic degradation. A variety of environmental conditions can affect the type of microbial degradation and the organisms present. Some of the more common variables are pH, temperature, nutrients (such as yeast extract), reduction potential, substrate, and enrichment techniques.

3.2.2. Anaerobic Degradation of Structurally Similar Compounds

Although initial studies of anaerobic degradation of PCBs presented little evidence of anaerobic degradation, structurally similar compounds such as the chlorophenols (Woods et al., 1989; Mikesell and Boyd, 1988; Krumme and Boyd, 1988), 4-chlororesorcinol (Fathepure et al., 1987) and chlorobenzoates (Horowitz et al., 1983; Suflita et al., 1982, Palmer et al., 1989) have been shown to be degraded by anaerobic organisms. Chlorobenzoates are perhaps the most relevant of the three compounds mentioned above because they are intermediate products in the microbial metabolism of PCBs.

Mono, di, and trihalobenzoates have been found to be completely mineralized to carbon dioxide and methane using bacteria from lake sediments and sewage sludge as well as enriched cultures grown on 3-chlorobenzoate (Suflita et al., 1982; Horowitz et al., 1983). Dechlorination was always

accompanied by methane production. The proposed reductive dechlorination pathway is shown in figure 3-2. In contrast to aerobic degradation, removal of the chlorine was necessary before the chlorobenzoate could be mineralized to carbon dioxide and methane.

The dechlorination was thought to be a biologically mediated reaction for two reasons. First, the reaction was inhibited if oxygen was present or if the sediments had been sterilized (e.g., autoclaved, radiated, or poisoned with formaldehyde). Obligate anaerobes, for obvious reasons, do not function in the presence of oxygen. Autoclaving terminates biological degradation because most enzymes are denatured above 39 degrees C (Keeton, 1980). Zeikus and Winfrey (1976) also found similar temperature limitations in their study of methanogenic organisms in aquatic environments.

Second, a lag period was observed that lasted 1 week to 52 weeks depending on the position and number of chlorines on the benzoate. Once the microbes were acclimated, no lag period was seen. A lag period is commonly found in biologically mediated degradation. Time is needed to select for a population with the necessary enzymes for metabolizing the chemical. There was a preferential loss of the chlorines in the meta position indicating that position of the chlorine was important.

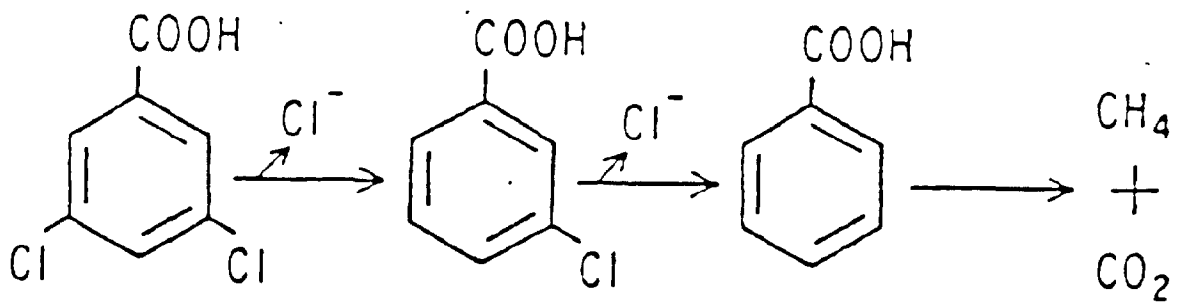


Figure 3-2 Anaerobic Degradation of Chlorobenzoates

(Source: Suflita et al., 1982)

The lag period was found to be correlated to the amount of substrate present. For example, 4-amino 3,5 dichlorobenzoate at low concentrations (13 to 26 μM) was not degraded after 21 weeks whereas higher concentrations (820 μM) were degraded within four weeks (Horowitz et al., 1983).

In experiments with chlorophenol, the position and the number of chlorines also influenced the degree of degradation. Ortho chlorinated congeners had the shortest lag period (7-21 days). Meta chlorinated isomers followed with a lag period of 31-49 days and para chlorinated congeners were not degraded (Woods et al., 1989). No dechlorination of the monochlorophenols or chlorines from the position para to the hydroxyl group (a metabolite) was seen in the entire 7 months of the experiment.

In studies with 4-chlororescorinol, a product of dye and pharmaceutical industries, it was found that the lag period could be decreased from three weeks to two days by adding yeast extract and trypticase. In addition, the rate of dechlorination increased (Fathepure et al., 1987).

These studies suggest three generalizations. First, position and number of chlorines affect the ability of microorganism to degrade the compound. Second, the

degradation pathway may be a function of the concentration of the compound. High concentration may induce degradation to occur whereas low concentrations may not. Third, additional substrates such as yeast extract may be necessary for dechlorination to occur.

3.2.3 Anaerobic Microbial Degradation Studies of PCBs

Few articles in the existing literature describe anaerobic degradation of PCBs. Much of the current knowledge of anaerobic degradation has come from the General Electric Research and Development Center in New York. This section describes their study of anaerobic dechlorination in sediments from the Hudson River and two other research studies of anaerobic dechlorination.

Between 1952-1973, an estimated 362,000 kg of Aroclor 1242 was released into the Hudson River in New York (Brown et al., 1984) contaminating over 134 metric tons of sediment (Brown et al., 1987b). Hudson River sediments were analyzed by the General Electric Research Group to determine the total concentration of PCBs in the sediment and the distribution of congeners. The concentrations ranged from 1 ppm to 2700 ppm (Brown et al., 1984). When the chromatograms from the sediment samples were compared to a standard chromatogram of the original source material, Aroclor 1242, a loss of the

higher chlorinated congeners was seen. A corresponding gain in the lower chlorinated congeners was observed. In particular, there was a relative decrease in the levels of tri, tetra and penta chlorobiphenyls and increase in the levels of mono and di chlorobiphenyls. These results were attributed to anaerobic microbial degradation.

This finding is particularly significant for at least three reasons. First, all prior aerobic studies had suggested that the more highly chlorinated congeners (notably those containing more than four chlorines) were very resistant to degradation.

Second, this degradation process appears to dechlorinate the more highly chlorinated congeners such as the "toxic congeners" and thus reduce the risk that these congeners present. The "toxic congeners" have a 4,4' chlorine substitution with at least two chlorines in the meta position and no more than one ortho chlorine which is adjacent to the meta chlorine. Although the overall concentration of PCBs in the sample remains the same, the reduction in the number of chlorines increases the potential for aerobic degradation to occur and decreases potential risk to health and biota. The lower chlorinated congeners are generally thought to be less toxic than higher chlorinated congeners.

Lastly, typical PCB residues in the sediment today are

the more highly chlorinated biphenyls, even though they represent only about 35 percent of the total PCBs manufactured (Furukawa, 1982). It is important to identify bacteria cr processes which can degrade the more highly chlorinated congeners since they are a significant proportion of the PCBs in the environment.

Brown and coworkers (1984) have identified several chromatographic patterns which they attribute to different bacterial strains. Within the Hudson River, five patterns A, B, C, E, and X (Brown et al., 1984) occur. They are outlined briefly below and discussed in more detail in chapter seven. Each of the patterns shows a loss of meta and para congeners.

The congener distribution of pattern A is similar to Aroclor 1242. Relative to Aroclor 1242, concentrations of mono and di chlorinated congeners and tri and tetra chlorinated congeners are reduced (e.g., congeners 18, 17, 28, 20/21/33, 22, 44, 37, 70/76, 66 and 56/60). Relative concentrations of the penta and hexa chlorinated congeners are slightly enhanced. This pattern was typically seen in sediment samples from surface-water interfaces.

Pattern B was found in many of the Hudson River samples. Again in comparison to Aroclor 1242, pattern B is characterized by a decrease in the concentrations of congeners eluting after congener 52/49/47 as well as a decrease in

congeners 28/31 and 22. In general, there was an increase in the mono, di and tri chlorinated congeners. Within the dichlorinated congeners, congener 6 and congener 8 increase. Within the trichlorinated congeners, congeners 17, 16, 32 and 27 are substantially increased. Pattern B' was the same as B with the exception that congener 6 was not present. Table 3-1 shows the homolog weight percents for typical A and A/B patterns.

In comparison to Pattern B, pattern C showed less removal of the higher chlorinated congeners with the exception of congeners 52/49/47, which were readily removed. The di and tri chlorinated groups decreased, most notably congeners 6, 5/8, 17 16/32 and 27. Congeners 1, 3, and 4/10 correspondingly increased.

Pattern E shows a selective loss of the higher chlorinated congeners and in general was found in combinations with pattern B and C. Pattern X was similar to pattern B; however, levels of congeners 1, 6, and 5/8 were below pattern B; levels of 19, 24, and 28 were above pattern B.

Brown and coworkers found a significant decrease in the meta and para chlorines in all of the above patterns. Thermodynamical analysis shows that the meta and para dechlorination is favored over ortho dechlorination. The standard reduction potentials (E) of congeners with chlorines

in the ortho position, are more negative than isomers

Table 3-1 Homolog Distributions for Pattern AB and B

Pattern	Conc (ppm)	(# of Samples)	Wt % of Homolog Group (Number of Chlorines)				
			1	2	3	4	5
Pattern A+B	162.1	(8)	6.6	26.6	39.6	18.1	8.1
Pattern B	659	(13)	13.0	41.6	30	13.7	4.0
Aroclor 1242			0.7	13.8	46.2	30.5	8.5

(Source: Brown et al., 1984)

containing the same number of chlorines (Rusling and Miaw, 1989). For example, the toxic congener 3, 3', 4, 4' does not contain any ortho chlorines and it has a more positive standard potential. It is also one of the more easily reduced compounds. A more positive standard potential indicates a greater tendency for the reaction to occur as indicated by the Nernst equation: delta G = -n F (delta E).

Bacteria which are able to anaerobically degrade PCBs may have a thermodynamic advantage over those that do not (Brown et al., 1987b). Since the PCB nucleus is not destroyed when reductive dechlorination occurs, it is possible that the

microbes use the PCBs as electron acceptors rather than a carbon source (Brown et al., 1987b). Brown and coworkers compared the calculated values of Gibbs free energies for the oxidation of glucose for a variety of reactants (table 3-2). The second highest energy level of any acceptor is hexachlorobenzene, which is similar in structure to PCBs. Note the energy value is four times higher than

Table 3-2 Energy Derived from the Oxidation of Glucose to Carbon Dioxide and Water using Various Oxidants

Oxidant	Reduced Product	Gibbs Free Energy (kcal/mole)
Oxygen	Water	-676.1
Hexachlorobenzene	Benzene	-410.16
Monochlorobenzene	Benzene	-369.5
Sulfate	Sulfur	-131.78
Carbon Dioxide	Methane	- 95.63

(Source: Brown et al., 1987)

carbon dioxide.

Two other research groups have examined the ability of anaerobes to degrade PCBs. Both groups concluded that dechlorination occurs. Their experiments were carried out under different conditions and subsequently their conclusions are different although not necessarily contradictory.

Quensen et al. (1988) extracted anaerobes from the Hudson River at a site which ranged in concentration of PCBs from 60 to 562 ppm. After introducing the anaerobic culture into a

series of sterilized, "clean" Hudson River sediments, PCBs were added at concentrations of 14 ppm, 140 ppm and 700 ppm to different reaction vessels. Controls were established using sterilized samples and no inoculation.

Reductive dechlorination occured most rapidly in the more highly contaminated sediments (Quensen et al., 1988). In the 700 ppm sample, 53 percent of the chlorine was removed in four months with an increase in the mono and di chlorinated biphenyls of 71 percent. No dechlorination was observed in the least concentrated sample (14 ppm). The sterilized control sediments and sediment containing microorganisms from a PCB-free site in the Hudson River showed no evidence of dechlorination.

Dechlorination appeared to occur predominantly from meta and para positions. This finding supports the thermodynamic advantage of the meta and para positions postulated by Brown et al. (1987) and Rusling and Miaw (1989). It is interesting to note that Quensen and coworkers observed a stepwise decrease in the chlorine composition of the 700 ppm sample with time.

The Wadsworth Center for Laboratory and Research, Department of Health, in New York also preformed several anaerobic experiments. An enriched bacterial culture from the Hudson River was introduced into a medium containing 20 ppm

of Aroclor 1221 (a lower chlorinated Aroclor consisting mainly of mono and di chlorinated biphenyls) and a 90.6 percent decrease was observed in the monochlorinated biphenyls and a 65.5 percent decrease in the dichlorinated biphenyls (Chen et al., 1988).

When bacteria were incubated anaerobically with pure monochlorinated biphenyls (0.75 ppm) for 80 days, complete degradation of the chlorinated biphenyls was seen. In contrast, when a higher chlorinated congener, 2,4,2',4' tetrachlorobiphenyl was used, no anaerobic degradation was seen.

Absence of dechlorination in the higher chlorinated congener does not conclusively demonstrate the inability of bacteria to anaerobically degrade the higher chlorinated congeners. Insufficient incubation period or insufficient quantities of substrate could inhibit degradation. The anaerobic bacteria were incubated for 40 days with the higher chlorinated congeners and 80 days with the lower congeners. In contrast, Quensen and coworkers used an incubation period of 112 days. It is possible that a lag period may occur before significant dehalogenation takes place. A lag was observed in Quensen's work where very little dechlorination occured in the first month. In addition, Quensen and coworkers found dechlorination to occur only in the more

highly concentrated samples (above 700 ppm) and not in the less concentrated samples (14 ppm). The concentration of tetrachlorobiphenyl in the experiment by Wadsworth Center was considerably lower (0.25 ppm (Chen et al., 1988)). This may be an insufficient quantity of substrate to support microbial growth.

Recently, this same research group found significant anaerobic degradation to occur in untreated sediments, biphenyl-amended sediment, and biphenyl-amended and bacteria-inoculated sediment from the Hudson River and Moreau sediment (Rhee et al., 1989). The concentrations of PCBs were much higher than previous experiments, 707 ppm and 936 ppm respectively. These sediments were under a nitrogen atmosphere. No degradation was observed under an anaerobic carbon dioxide/hydrogen gas environment with similarly treated sediments. It is unclear why. Quensen and coworkers (1988) found bacterial degradation to occur in an environment which was 80 % nitrogen and 20 % carbon dioxide.

The biphenyl-amended sediments showed a significant decrease in the higher chlorinated congeners. This suggests that biphenyl may be needed for the degradation of the higher chlorinated congeners, a finding which has also been suggested for the aerobic cometabolism of the higher chlorinated congeners.

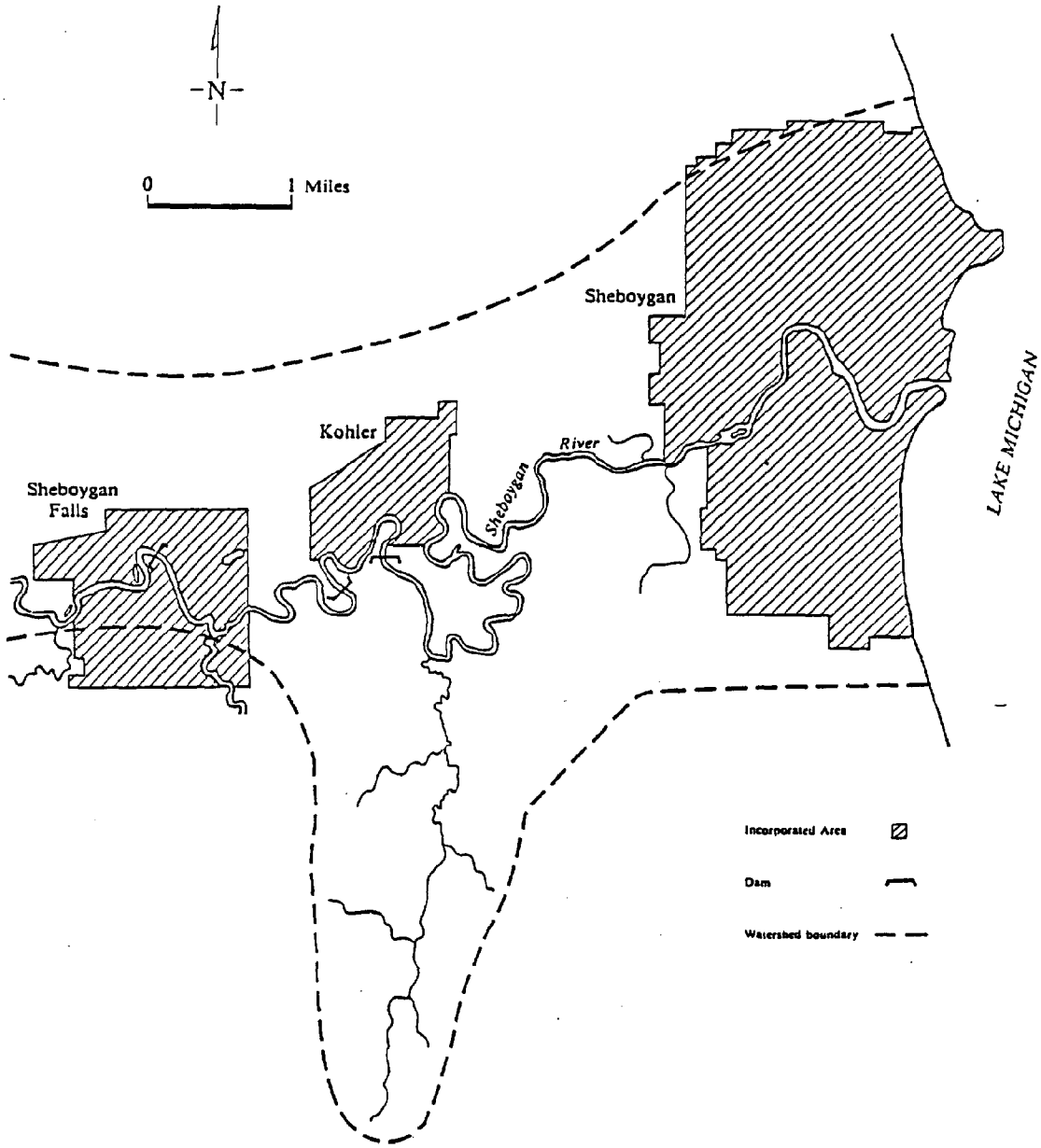
Chapter 4 Site Description and Historical Use of PCBs

This chapter describes the hydrology and geology of the study area. The history of PCB usage at the site is presented as well as a brief summary of the regulatory precautions that were enacted to protect human health and the environment.

The Sheboygan River flows westward and drains into Lake Michigan at the city of Sheboygan. Sheboygan is approximately 89 km directly north of Milwaukee. The river is a total of 220 stream km long and has a drainage basin of approximately 272 square km (Wisconsin Department of Natural Resources, 1989). Figure 4-1 shows a map of the Sheboygan River and drainage basin as well as its two tributaries, the Onion and the Mullet rivers. Beginning at the Sheboygan Falls dam and ending at the harbor in Sheboygan, the 22.4 km river segment studied is a small portion of the total length. This portion of the river drains approximately 57 square km (Wisconsin Department of Natural Resources, 1989). The river is slowed by two small dams located near the village of Kohler.

The Sheboygan River has a mean annual discharge of approximately 7.3 cubic meters per second (258 cfs) based on data collected from 1942 to 1986. The lowest flow for this century 0.0283 cubic meters per second (1 cfs) was

Figure 4-1 The Sheboygan River and Tributaries



(Source: Wisconsin Department of Natural Resources, 1989)

recorded in August, 1922. The highest flow was 217 cubic meters per second (7,680 cfs) in March, 1975. Average annual rainfallduring 1978 to 1986 was 762 cm per year (Wisconsin Department of Natural Resources, 1989). Although, the Wisconsin Department of Natural Resources (WDNR) concluded that the river bottom from Sheboygan Falls to Sheboygan was generally scoured in 1980, major areas of deposition were noted, above both of the dams, near Kiwanis Park in Sheboygan and near the Eight street island in Sheboygan. The average rate of deposition was calculated to be 10 cm/yr (Wisconsin Department of Natural Resources, 1989). The river deposits approximately 22,923 cubic meters (30,000 cubic yards) of sediment a year into the harbor (Wisconsin Department of Natural Resources, 1989).

The Sheboygan River lies in the geologic province of the Eastern ridges and lowlands. The dominant feature is a plain interspersed with north-south ridges, paralleling Lake Michigan. The sedimentary bedrock is comprised alternatingly of weather resistant dolomites which form the ridges, and erodible sandstones and shales which form the lowlands (Paull and Paull, 1976). Glacial features such as kames, eskers, drumlins and kettle holes are abundant. Within the river, the sediment is predominantly fine-grained

sand interspersed with silt and occasionally clay.

The Sheboygan River and harbor have been extensively studied since the area was first identified as an "Area of Concern" by the International Joint Commission and as a "Superfund site" by the Environmental Protection Agency (EPA). An "Area of Concern" is defined as a locality where water quality objectives, standards, criteria or guidelines are not being met and remedial actions are necessary to restore the water quality. A "Superfund site" is an area which, according to EPA's national ranking system, poses a substantial risk to human health and the environment. Once an area is designated as a Superfund site, parties responsible for cleaning up the site are identified, where possible, and remediation efforts are initiated under the supervision of government agencies. If no responsible party can be identified, money from the national hazardous waste trust fund can be used to clean up the site. A lengthy study of the extent of contamination and other relevant information such as groundwater flow, potential routes of exposure, and possible treatment alternatives is necessary before any remediation can begin.

Contamination of the river sediments by PCBs was first identified in 1978, when the WDNR found sediment containing 190 ppm of PCBs downstream from Tecumseh Products, a die

casting plant located on the river at Sheboygan Falls. Further sampling at the Tecumseh Products site revealed soil samples with concentration of PCBs as high as 120,000 ppm. The samples were found to contain oil-soaked soil as well as oil soaked rags, pressure hoses and other refuse (Wisconsin Department of Natural Resources, 1989).

When the Sheboygan River and harbor was identified as a Superfund site, Tecumseh Products was named as potentially responsible party. Tecumseh Products and the previous company at the industrial site, the Die Cast Corporation, used hydraulic fluids in the manufacture of outdoor motors. Use of hydraulic fluids containing PCBs commenced after a large fire occurred at the Die Cast plant. The cause of the fire was attributed to the combustion of hydraulic fluids. Subsequently the Die Cast Corporation switched to a hydraulic fluid which was fire resistant.

Monsanto Company began to manufacture fire resistant hydraulic fluids containing PCBs in the early 1950's. The first formulation that they marketed was Pydrol F9, the formulation believed to be used at Tecumseh Products and the Die Cast Corporation. Table 4-1 contains the chronology of PCB use at these two companies. The major formulations used by Tecumseh contained Aroclor 1248 and 1254. Tecumseh Products acquired the Die Cast Corporation in 1966 and used

the same equipment as the Die Cast Corporation. Based on interviews and available records, it is thought that the Die Cast Corporation also used Pydrol F9.

Tecumseh Products ceased using hydraulic fluids containing PCBs in 1971 because the major producer of PCBs restricted sales to closed systems such as transformers. The government did not prohibit the manufacture of PCBs and use of PCBs until July 1, 1977 (Federal Register 42 part 6531 and Federal Register 44 part 315141). Before PCBs were regulated, Tecumseh Products used material from the plant and soil from around the plant to construct a low dike at the river's edge. Since the dike sloped at 45 degree angle into the river, it was relatively easy for sediment to be introduced into the river during high water events (Wisconsin Department of Natural Resources, 1989).

In 1979, Tecumseh Products voluntarily removed 2,046 cubic meters (72,300 cubic feet) of PCB-contaminated material from the dike and surrounding area. This material was sent to a federally-licensed disposal area outside of Cincinnati, Ohio.

Since that time the area has been designated an "Area of Concern" by the IJC as well as "Superfund site". The EPA has extensively sampled the river and some of the harbor. The U.S. Army Corps of Engineers has completed

Table 4-1 PCB Mixtures used at the Tecumseh Products Site

Company	Date	Formulation used
Die Cast Corp	1959-1966	Thought to have used Pydrol F9 (52.5% 1248)
Tecumseh Products	1966-1969	Pydrol F9 (52.5% 1248 [a])
	1970-1971	Chemtrend HF30 (65% 1254, 4.6% 1248 [a])

[a] -- The remaining percentage is a non-PCB product

(Source: Wisconsin Department of Natural Resources, 1989; Mark Thimke, Lawyer for Tecumseh Products, Lauderin and Associates, Milwaukee, WI, personal communication; Paul Micheal, Analytical Chemist, Monsanto, Chicago, IL, personal communication.)

several studies of the harbor to assess the nature and extent of contamination. Contaminated sediments in the harbor have prevented dredging of the harbor since 1969. This has resulted in a substantial loss to the shipping industry because barges must travel with less than a full cargo to get in and out of the harbor.

A fish consumption advisory has been in effect since 1978 when it was found that fish from the Sheboygan area exceeded the Food and Drug Administration limit of 5 ppm,

which was subsequently lowered to 2 ppm in 1984 (Federal Register 38 part 18096, Federal Register 49, part 21514). A waterfowl consumption advisory was issued in 1987 when the waterfowl were found to exceed the 3 ppm fat based limit set by the Food and Drug Administration (Wisconsin Department of Natural Resources, 1989).

Most recently, the EPA has begun the removal this fall of 1,910 cubic meters (2,500 cubic yards) of sediment from the river in the vicinity of Tecumseh Products and Rochester Park. These highly contaminated sediments will be placed in a confined disposal facility and monitored to determine if anaerobic and aerobic biodegradation is occurring. In addition, 1,854 square meters (20,000 square feet) of sediment will be amoured in place by covering the sediments with a fabric liner, followed by sand and gravel. The sediments will remain amoured until final treatment decisions are made in 1991 (United States Environmental Protection Agency, 1989).

Chapter 5 Sample Collection, Materials and Methods

This chapter delineates how sediment, fish and water samples were collected and prepared for high resolution gas chromatography analysis. A summary of operating conditions of the gas chromatographs is included as well as a discussion of the quality control measures used. Lastly, the procedure used for analyzing grain size is explained.

5.1. Sample Collection

5.1.1. Sediment

Two sediment sampling trips were made to the Sheboygan River (December 2, 1988 and April 28, 1989). On the first trip, five sites were sampled: above the Sheboygan Falls dam (Sheboygan Falls, site A), at Rochester Park (Sheboygan Falls, site B), above the upper Kohler dam (Kohler, site C), above the lower Kohler dam (Kohler, site D) and near Kiwanis Park (Sheboygan, site E). Figure 5-1 shows the exact locations. On the second trip, samples were only taken at Rochester Park (Site T).

Based on information from previous studies, these five sampling sites were selected because it was thought they would represent concentrations varying from high (Rochester Park) to low (above Sheboygan Falls dam). At each site, an effort was made to sample areas of deposition, since there

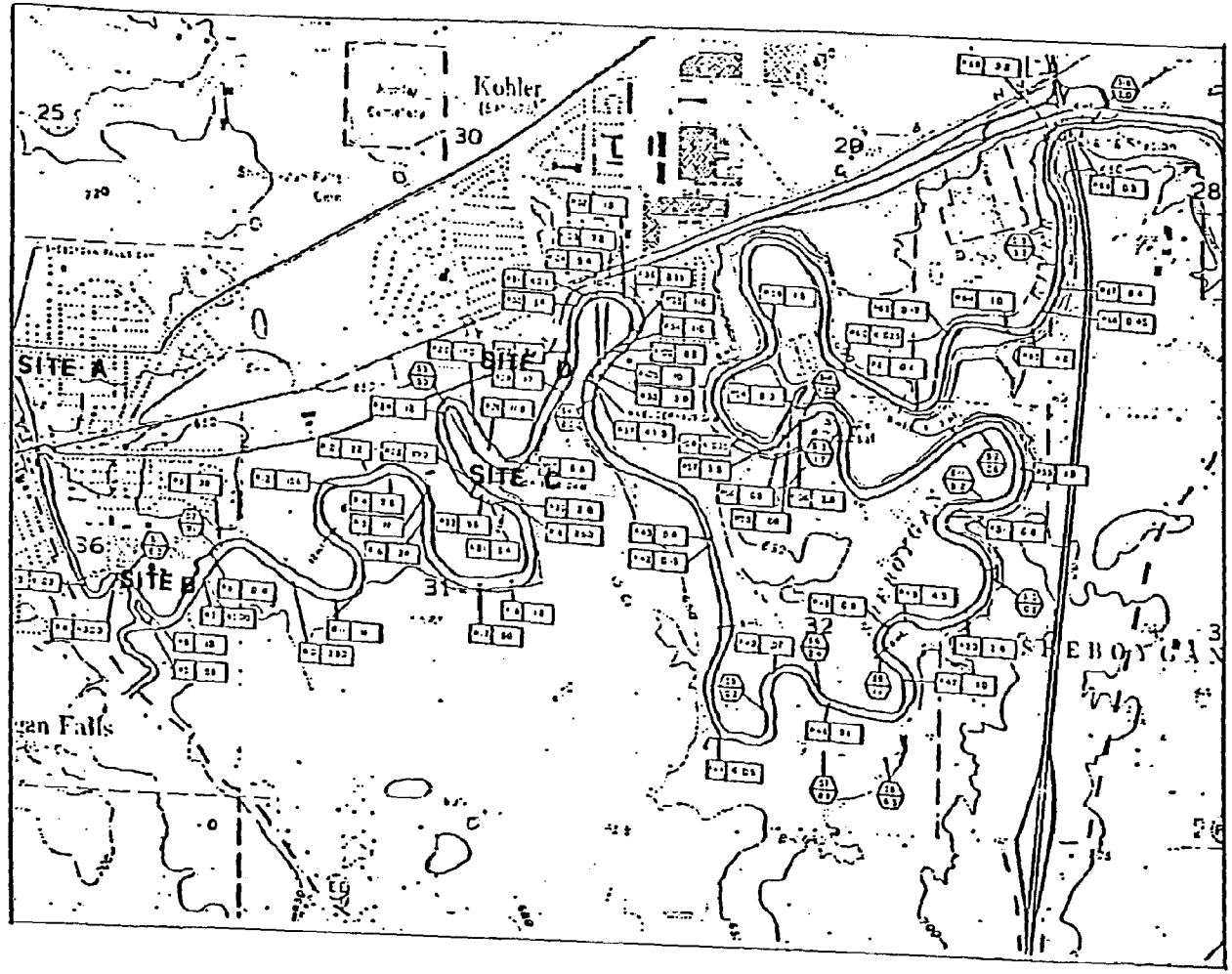


Figure 5-1 Map of Field Site

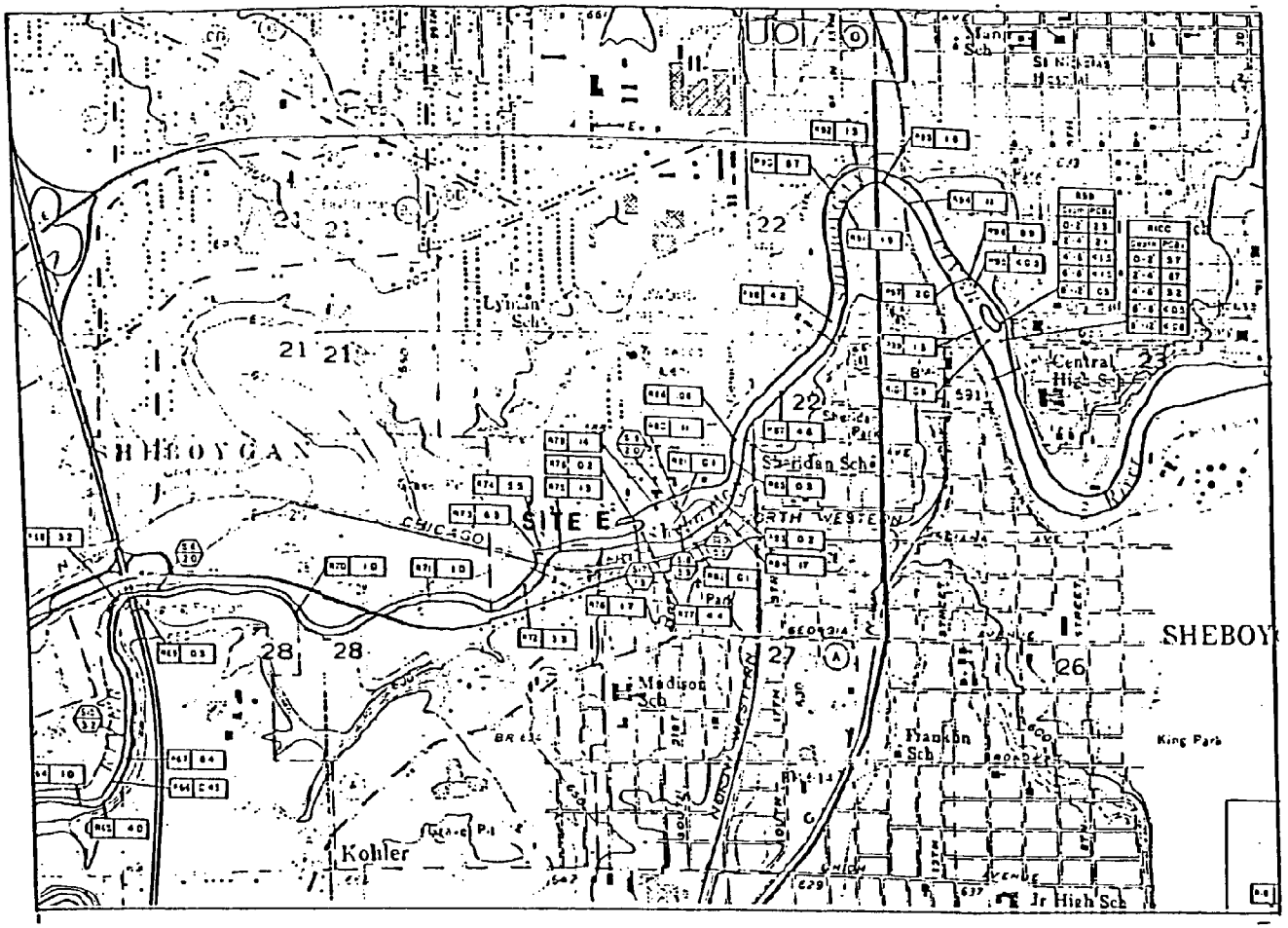


Figure 5-1 (cont.)

is evidence that all things equal that finer grained material contains higher concentrations of PCBs than coarser grained material (Karickhoff, 1979).

Sediment cores were taken with a hand-held cylindrical coring device which was plunged into the soft sediment. The coring device was then slowly removed and a cap placed on the bottom of the cylinder while it was still under water. Since the cylinders, approximately 8 cm in diameter and 1.5 meters in length, were made out of clear plexiglass, it was relatively easy to examine the core. In all cores , the sediment-water interface remained undisturbed and intact with distinct boundaries between different sediment compositions. The cores were extruded out of the cylinders using a hydraulic pump. Each core was divided into 15 cm segments which were put into glass mason jars with teflon caps and stored at 4 degrees C until time of analysis.

5.1.2. Fish

Fish samples were collected by the Wisconsin Department of Natural Resources in the summer of 1988. Ten of the fish were collected in the Kiwanis Park area; the remaining eleven fish were caught in the harbor area. The fish tissue was frozen until time of analysis.

5.1.3 Water

Water samples were collected from the Sheboygan River at five locations corresponding to the five locations of the sediment cores. Two sampling trips were made. One on August 3 to collect water from Kiwanis park, above Sheboygan Falls and Rochester Park; and a second on August 17 to collect water from Kiwanis park, above the upper Kohler dam, and below the lower Kohler dam.

At each site, samples were taken from a boat in the middle of the river. A narrow-neck one liter bottle enclosed in a steel cage was dropped gently over the edge of the boat and lowered to the river bottom, usually no more than one to two meters. The bottle was then pulled at a rate such that the bottle was just filled when it was a few centimeters from the water's surface. This method, referred to as the depth integrated profile method, is thought to give a more representative sample than a single point sample (personal communication, Leo Haus, United States Geologic Survey, 1989). The one-liter bottle was cleaned according to State Laboratory of Hygiene's procedure for water sampling bottles (1989) and was rinsed with river water before sampling. A clean bottle was used at each site.

The narrow-neck bottle was emptied into a nineteen-liter beverage canister. The bottle was lowered several

more times until the canister was filled. The canisters were rinsed with hexane three times before leaving the lab. The canisters were brought back to the lab and filtered within three days.

5.2. Materials

5.2.1. Solvents

Solvents: Acetone, Cyclohexane, Ethyl Ether, Hexane, Iso-octane, Methylene Chloride. High purity (Baxter, Burdick and Jackson).

5.2.2. Chromatographic Adsorbents

Florisil: Pesticide residue grade, 60/100 mesh, activated at 130 degrees C for 12 hours and stored until use in an air tight container at room temperature (Floridin Co.).

Silica gel: 100-200 mesh, grade 923, activated at 130 degrees and stored until use at 130 degrees C until use. Deactivated with 5 % distilled water at least one hour prior to use (Davison Co.).

5.2.3. Standards

Aroclor standards 1232, 1242, 1254, 1260 in methanol were obtained from the EPA-EMSL Quality Assurance Branch in Cincinnati, OH and diluted to appropriate levels. Internal standards, congener 30 and 204, were ordered from Ultra-

Scientific.

5.2.4. Other Chemicals and Materials

Sodium Sulfate: granular, heated at 130 degrees overnight to activate (Mallinckrodt).

Copper: granular, 20-30 mesh (J.T. Baker Inc.)

Glass wool: soxhlet-extracted in 50/50 acetone hexane for four hours (Corning glassworks).

Boiling Chips: soxhlet-extracted in 50/50 acetone/hexane for four hours (Cargille Scientific Co).

Glass Filters: 29.3 cm diameter GC 50 grade, wrapped in aluminum foil and ashed for ten hours at 400 degrees C and stored in foil packets until used (Micro Filtration Systems).

XAD-2 Resin: (Sigma Chemical Co.) Resin was extracted for 24 hours each in methanol, acetone, hexane, methylene chloride and then extracted four hours in acetone, hexane and acetone. The resin was then rinsed with distilled water and stored in glass bottles filled with distilled water until needed.

ASTM Soil Sieves for grain size analysis

19-liter stainless steel canisters (Beverage Industry).

Bio-beads Gel Resin (SX-3): for the Gel Permeation Column (Biorad).

5.2.5. Equipment

Hewlett Packard 5880 Gas Chromatograph with DB-5 column and ECD detector.

Hewlett Packard 5890 Gas Chromatograph with DB-1 column and ECD detector.

Hewlett Packard 5790 Gas Chromatograph with a Packed Column.

Low Pressure Gel Permeation Column Autoprep 1001 Chromatograph (ABC Labs)

Rotovap and air blowdown apparatus

Filtration apparatus: 293 mm millipore filtration head

5.2.6. Glassware

Cleaning protocol: Glassware was washed with laboratory detergent, rinsed with hot municipal tap water and two rinses of distilled water, and dried at 180 C. For water analysis, all glassware was rinsed three times with hexane.

General glassware as well as chromatographic columns (2 cm id and 1 cm id), resin columns (3 cm by 17 cm), 500 ml separatory funnels, glass soxhlets and flasks were needed.

5.3. Extraction and Cleanup Methods

5.3.1. Sediment

The method used to cleanup sediments was based on the State Laboratory of Hygiene procedure for congener specific

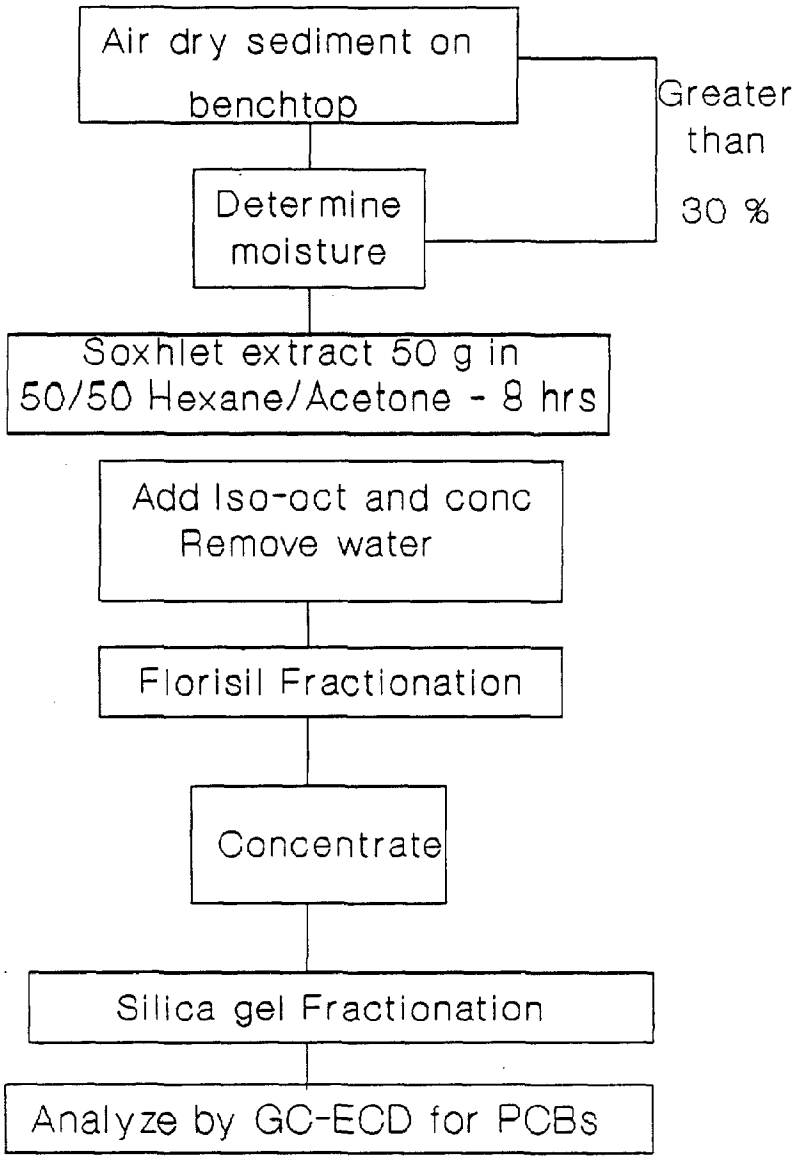
PCB analysis (State Laboratory of Hygiene, 1989). A schematic of this process is shown in figure 5-2 and a summary of the method used is given below.

The sample was removed from the mason jars, placed on aluminum foil and allowed to air dry until the sample had less than 30 percent moisture but at least 10 percent moisture. To insure that a homogenized representative sample was used, the sediment was sieved through a number 10 sieve and the residuals, predominantly twigs and shell fragments, discarded. Ten grams of the sediment was dried overnight at 120 degree C to determine the moisture content of the soil.

Fifty grams of the sediment was placed in an acetone-washed filter thimble which was placed into an acetone-washed soxhlet. Three hundred ml of a 50/50 hexane/acetone solution was poured into a soxhlet flask. Before the soxhlet, flask, and distillation column were assembled, granular copper was added to the soxhlet flask to remove any sulfur interference and to insure uniform boiling. The sediment was soxhlet-extracted for 8 hours. The temperature was set so that the solvent would cycle about five to eight times an hour.

Once the extraction apparatus had cooled, the sediment was discarded and the acetone-hexane solution containing the

Figure 5-2 Analysis of PCBs in Sediment



PCBs was placed in a warm water bath and concentrated to approximately two ml under a gentle stream of filtered air. The sample was diluted with approximately ten ml of iso-octane and again concentrated to remove any remaining acetone. Enough sodium sulfate was added to the sample to adsorb any water remaining in the beaker after the blowdown step.

Florisil columns were prepared by pouring hexane into a twenty ml column until it reached the base of the bulb. Next the column was filled with two grams of sodium sulfate, followed by 22 grams of Florisil and then another two grams of sodium sulfate. The hexane was drained to the top of the first sodium sulfate layer and the eluant discarded. The sample was poured into the column and eluted at a rate of five ml/minute. When the sample reached the top of the sodium sulfate layer, the beaker that had contained the sample was rinsed with a small amount of hexane and added to the column. After the first rinse reached the sodium sulfate layer, a second rinse was added to the column. The sample was eluted with 200 ml of a 96/4 percent hexane/ethyl ether solution.

When all of the solution was eluted, the sample was concentrated in a warm water bath under a stream of air to approximately five ml before silica gel fractionation. The

silica gel columns were prepared by filling a column (10 mm) to the base of the bulb with hexane, adding two grams of sodium sulfate, five grams of deactivated silica gel and then another two grams of sodium sulfate. Best results were found when the silica gel was deactivated with five percent distilled water and allowed to equilibrate for an hour in a sealed Erlenmeyer flask.

The sample was eluted by the same procedure used for the florisil fractionation with the following two exceptions: the flow rate was 1-2 ml/min and the sample was eluted with 50 ml of hexane. When all of the hexane had eluted through the column, the sample was concentrated and the solvent switched from hexane to iso-octane. Next the sample was diluted to 50 ml and screened on the packed column to determine if further dilution (or concentration) of the sample was necessary before performing high resolution gas chromatography.

5.3.2. Fish

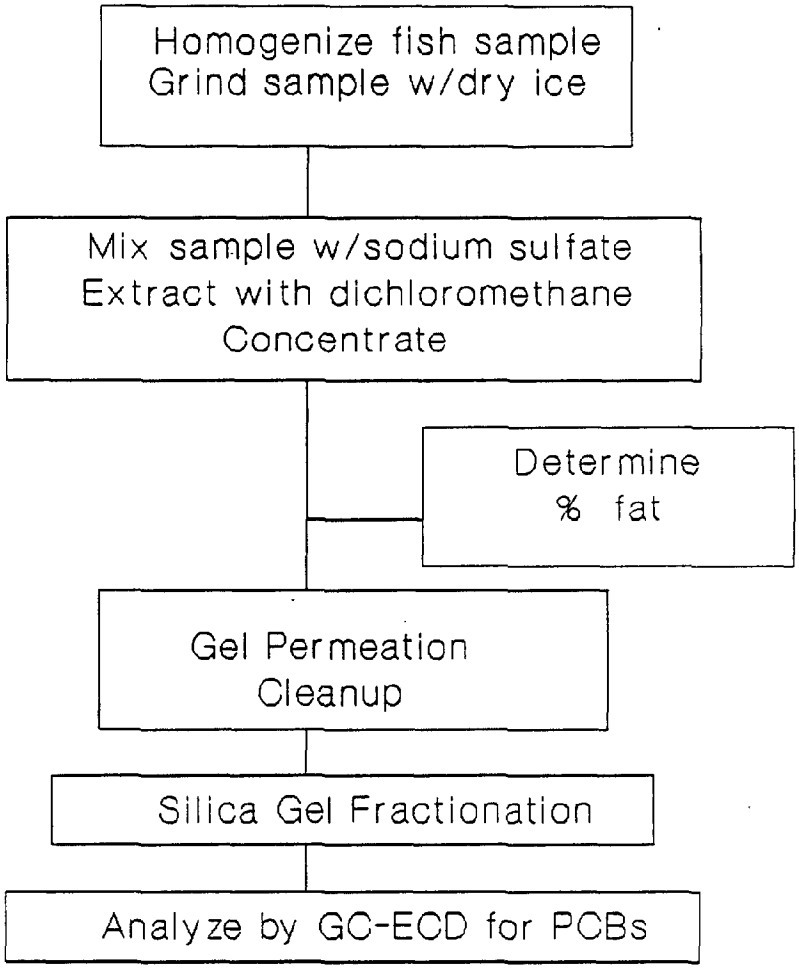
The procedure for extracting PCBs from fish was similar to that used for sediment and for water with the exception that a gel permeation column was necessary to remove fat which would interfere with high resolution gas chromatography. The procedure used was that of the State

Laboratory of Hygiene (State Laboratory of Hygiene, 1989) and is shown schematically in figure 5-3.

The entire sample, weighing approximately 200 grams was ground up in a high speed blender with dry ice to homogenize the sample. The dry ice was allowed to sublime overnight. Ten grams of the sample was mixed with 60 grams of sodium sulfate to remove any moisture in the sample and then the sample was poured into a 20 mm i.d. chromatographic column containing two centimeters of sodium sulfate. Dichloromethane (230 ml) was used to elute the sample at a rate of 5 ml/min. Once the dichloromethane was through the column, the sample was concentrated to five ml in a warm water bath under a gentle stream of filtered air. Cyclohexane was added to the sample until a final volume of ten ml was reached. A two ml aliquot was taken from the ten ml sample and was weighed. The solvent was evaporated from the sample and reweighed to determine the percent fat in the sample.

To remove the fat from the sample, a gel permeation column (GPC) was used. GPC is based on the principle of size exclusion. Larger molecules, such as fats move relatively quickly through the column; however, smaller molecules such as PCBs, DDE and toxaphene, move more slowly because they permeate the multitude of smaller channels in

Figure 5-3 Analysis of PCBs in Fish



the gel which are not available to the larger fat molecules. Five ml of each sample was injected onto the GPC. The first 100 ml of eluate to come off the column was discarded because it contained the larger fat molecules. The second 160 ml was collected as this fraction contained the PCBs and was concentrated to five ml.

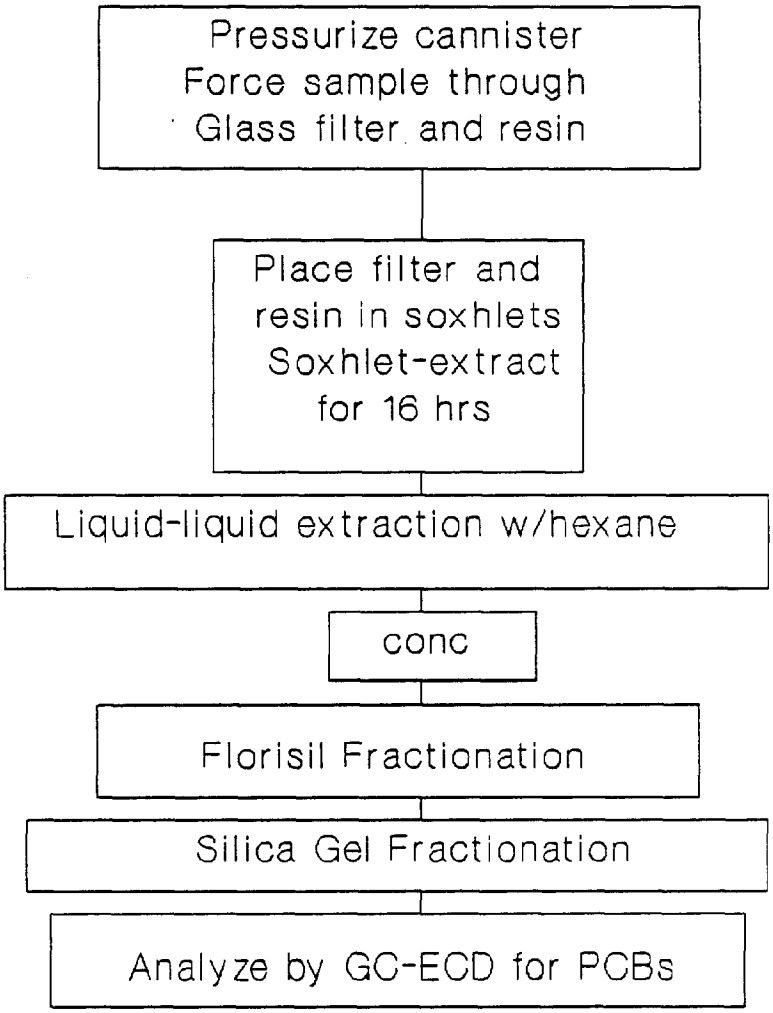
Silica gel fractionation was performed using the same procedure as that for sediment: five grams of silica gel (5% deactivated with water) and 50 ml of hexane for the eluant. After silica gel fractionation, the sample was concentrated to five ml in iso-octane and diluted if necessary before analyzing on the gas chromatograph.

5.3.3. Water

PCBs were extracted from the water using the filterhead-resin bed method. Although a brief summary of this procedure is given below, a more detailed discussion can be found by Marti (1984), Crane (1986) and the State Laboratory of Hygiene (1989). Figure 5-4 shows a schematic of the procedure used.

After the samples were brought back to the lab, the 19-liter beverage canister containing the water sample was connected to a tank of ultra pure nitrogen gas by a teflon hose and also connected to the filterhead by copper tubing.

Figure 5-4 Analysis of PCBs in Water



An ashed glass filter was placed on the filterhead and wetted with distilled water before the filterhead was fastened securely in place. An XAD resin column was attached to the bottom of the filterhead. The canister was pressurized with approximately 5 psi of gas; causing the water to flow from the canister through the filterhead, the resin and into a carboy located below the filterhead. To maintain a flow rate of 200 ml/minute, it was necessary to have the gas pressure at 1 to 2 psi.

The resin columns were prepared by fitting a short bit of tubing with a clamp over the end of a glass column (3 cm by 17 cm). The tubing was attached to a water aspirator to help pack the column. The column was then filled with distilled water. A small portion of glass wool was pushed into the column and any air bubbles driven out by tapping the wool with a glass stirring rod. Enough XAD-resin was poured into the column so that the column had a resin bed depth of 14 cm. A small portion of glass wool was placed on the top. The packed resin remained in distilled water until just after the canister was pressurized whereupon the tubing and clamp at the end of the column were removed and sample water began to flow through the resin.

Once all of the water had been filtered, the volume of the filtrate in the carboy was measured and the filtrate

discarded. Next the resin column was uncoupled from the filterhead, and inverted over a hexane-rinsed soxhlet. Acetone was added until the resin would flow into the soxhlet. Both glass wool plugs were added to the soxhlet. The column was then rinsed with 60 ml of acetone and 40 ml of hexane. Enough of the 60/40 acetone/hexane solution was added to insure a final volume in the soxhlet and flask of 350 ml. After boiling chips were dropped into the flask, the flask and soxhlet were placed on a hot plate and soxhlet-extracted for 16 hours.

The filter was peeled off the filterhead, wrapped tightly and placed into a soxhlet. Then the beverage canister was wiped with a small bit of glass wool to remove any particles. The canister was rinsed three times with 50 ml of a 60/40 acetone/hexane solution. The filterhead was also wiped with glass wool to remove particulates and both pieces of glass wool were placed in the soxhlet containing the filter. Three consecutive rinses of 50 ml of hexane/acetone were siphoned from the canister, through the filterhead and caught in a beaker. These rinses were added to the other rinses in the soxhlet containing the filter. Again enough 60/40 acetone/hexane solution was added to the soxhlet and flask until a final volume of 350 ml was reached. Boiling chips were added and the filter was

soxhlet-extracted for 16 hours.

Since the filter, resin and glass wool contained water, all of the samples needed to be liquid-liquid extracted after soxhlet-extraction. The sample was poured into a 500 ml separatory funnel and extracted three times with 75 ml of hexane. Next the sample was placed on to a rotovap to concentrate the sample to approximately 30 ml whereupon it was transferred to a water bath and concentrated under a gentle stream of air to ten milliliters. About 20 ml of iso-octane was added and the sample was concentrated again. This step was repeated for all of the samples.

Once the samples were concentrated to approximately five ml, they were ready for florisil fractionation and silica gel fractionation. The general procedure for preparing and eluting the compounds was the same as that for sediments; however, for florisil fractionation, only eight grams of florisil were needed for the column and only 50 ml of 94/6 hexane-ethyl ether was needed for the eluate. The same procedure was used for silica gel fractionation.

All samples were concentrated to five ml after fractionation and screened on the packed column to determine if further concentration of the sample was necessary before analyzing the sample on the high resolution gas chromatograph.

5.4. Gas Chromatography

High resolution gas chromatography was used for the analysis of PCBs in all matrixes. Table 5-1 contains the operating conditions for the three machines used. All of the samples were first screened on the packed column, and then run on the Hewlett Packard (HP) 5880. Confirmatory analysis of congeners was done on the HP 5890.

The HP 5880 was calibrated before each run using a 0.61 μg/g standard containing Aroclor 1232, 1248, 1262 at 0.25 mg/l, 0.18 mg/l, 0.18 mg/l respectively; the HP 5890 was calibrated with a 1.22 μg/g standard of similar composition. No more than ten samples were run in a single batch and periodically a standard was run at the end of the batch to assure that later samples were correctly identified. Each sample was spiked with internal standards 30 (12.5 ng) and 204 (13.8 ng) to correct for variability in injection sample size and to correct for drift in the rention times of the congeners. Ten percent of the samples were run in duplicate to assess the precision of the analysis. A matrix spike for each batch of samples was run (a single batch typically contained 10 samples) to assess the accuracy of the analysis.

Table 5-1
Operating conditions for the Gas Chromatographs

Hewlett-Packard 5880 Gas Chromatograph
- 60 m DB5 column, 0.2 mm ID
- Hydrogen carrier gas, Nitrogen makeup gas
- Electron Capture Detector at 320 degrees C
- Oven temperature program
  - 90 C -- 0.5 min
  - 90 - 150 C at 10 C/min;
  - 150 - 220 C at 1 C/min;
  - 220 - 270 C at 3 C/min, hold for 2 min
- Injection size of 3μl

Hewlett-Packard 5890 gas chromatograph
- 60 m DB1 column, 0.2 mm ID
- Hydrogen carrier gas, Nitrogen makeup gas .
- Electron Capture Detector at 320 degrees C
- Oven temperature program, same as HP 5880
- Injection size of 2μl

Hewlett-Packard 5790 gas chromatograph
- 6 ft x 4 mm column
- Packed with 4% SE 30/ 6% OV 210
- Oven temperature of 235 degrees C
- Injection size 2μl

Confirmatory analysis was done for approximately ten percent of the samples using the HP 5890. Additional confirmatory analysis was done by State Lab of Hygiene personnel on a mass spectrometer for specific congeners.

Limits of dectection were available for each method from the State Laboratory of Hygiene (State Laboratory of Hygiene, 1989). Limit of dectection is defined as the response that is three times the standard deviation of the

background noise. Congener values which fell below the limit of dectection were not reported. Limits of dectection for each method are included in Appendix A. In addition, congeners which deviated from the standard retention time by more than a tenth of a minute were not reported. For congeners which coelute, this value was relaxed to a twentieth of a minute.

5.5. Grain Size Analysis of Sediment

Grain size analysis determines the percent of sand, silt and clay in a sediment sample. The larger particles such as pebbles and sand can be physically separated and classified using sieves having different sized apertures; however, the smaller particles such as clay and silt cannot be separated using sieves and therefore must be separated by gravitational methods (such as a sedimentation test).

For sedimentation tests, most often a slurry containing fine particles and distilled water is made up and a hydrometer which measures the density of a solution is placed in the slurry. At given time intervals, readings are taken. As the time of the analysis increases, the heavier particles settle out and the solution becomes less dense.

Sedimentation tests are based on Stoke's Law which describes the velocity of spherical particles of a known

density in a solution of known density and viscosity

$$v = \frac{\gamma_s - \gamma_w}{18\eta} D^2$$

where

v - velocity (cm/sec)

γ_s - the specific weight of a solid (g/cm^3)

γ_w - the specific weight of water (g/cm^3)

η - the viscosity (g·sec/cm2)

D - the diameter of the particle

The equation can be rewritten to solve for the diameter at any time since all other variables are known. (The velocity can be calculated indirectly by knowing the depth of the hydrometer and the time.)

Grain size analysis was preformed on 18 of the sediment samples. Because a large amount of fine material was recommended for the sedimentation test (Das, 1986 and Head, 1980), a small subset of the total samples (ten samples) was used for the sedimentation test because other samples contained insufficient quantities of sediment to do the test.

The method used for sieving was based on the American Society for Testing and Materials (ASTM), Standard Method for Particle Size Analysis, Method D422-63 (American Society of Testing and Materials, 1987). Slight modifications to this procedure were suggested by Norm Severson (personal

communication, Department of Civil Engineering, Madison, WI 1989).

A summary of the method follows (for further detail, see ASTM D422). A sediment sample weighing between 100 to 200 grams was placed in a No. 200 mesh ASTM sieve over a large basin. None of the sample was pretreated with hydrogen peroxide or hydrochloric acid as it was desired to determine the particle size distribution in an unaltered environmental sample. The hydrogen peroxide removes organic matter and the hydrochloric acid removes carbonate cement between particles.

The sample was rinsed with a steady stream of tap water (referred to as "wet-washing") until the water running out from the sieve was clear. Wet-washing is necessary because there is a tendency for the finer clay and silt particles to adhere to the larger particles when a sample is dry sieved. The turbid, murky water from wet-washing was collected in the basin and allowed to settle overnight. The water was siphoned off and the sample was dried, crushed to break up any aggregation of particles, and weighed.

Fifty grams of the fine sediment was used for the sedimentation test. A 150 ml of a deflocculating agent was added to the sediment to disperse large flocs of aggregated particles which may settle out in the absence of a

deflocculating agent. The deflocculating agent replaces the divalent and trivalent cation with sodium and causes the clay micelles to repel (Hillel, 1982). The dispersing agent was 35.7 grams of sodium hexametaphosphate (commercially known as Calgon) and 7.94 grams of sodium carbonate in one liter of distilled water.

The sample was then mixed using a high speed mechanical stirrer for two minutes. The slurry was transferred into a one-liter sedimentation cylinder and diluted with distilled water to the one-liter mark. After a rubber stopper was placed in the end of the cylinder, the cylinder was inverted sixty times in one minute. Immediately after the last inversion, a hydrometer (ASTM 152 H) was placed in the slurry and readings were taken at the following intervals: 0 seconds, 15 seconds, 30 seconds, 1 minute, 2 minutes, 4 minutes, 8 minutes, 15 minutes, 30 minutes, 60 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours. Corrections for temperature, meniscus level and density of the deflocculating agent were made to the hydrometer readings (see Das, 1986 for further detail).

The sample remaining on the sieve after being wet-washed was dried in an oven (120 degrees C) and sieved through a number 10 sieve and a number 40 sieve. The former separates the large sand grains from the medium sand

grains while the latter separates the medium sand grains from the fine sand grains (American Society for Testing and Materials, 1987). A number 10 sieve is routinely used in preparation of sediment samples for gas chromatography analysis. Unfortunately, sediment samples were prepared for gas chromatography analysis before it was decided that the samples would be analyzed for grain size and subsequently the material that remained on the number 10 sieve was discarded before it was weighed. Since the sediment is a fine grained fluvial deposit, almost all of the sediment passed through a number 10 sieve. In addition, most of the matter not passing was organic matter such as twigs, material which would not ordinarily be used in a grain size test.

Chapter 6 Results

This chapter contains the results of grain size analyses as well as the concentration and distribution of the PCB congeners in sediment, water and fish samples collected from the Sheboygan River.

6.1. Grain Size

In general, the sediment is comprised of fine-grained sand and silt interspersed with clay and organic debris. The grain size test results determining the sand, silt, and clay fractions are also given in table 6-1.

The sediment samples are composed predominantly of fine sand, although a few samples are comprised of almost equal percentages of fine sand, and silt and clay. In general, the proportions of fine sand and fines (silt and clay) remain the same for different locations, reflecting the relative homogenity of the fluvial deposits.

The medium sand fraction is probably overestimated since this fraction was in part composed of organics such as leaf fragments, and grass. Organic matter can be removed using hydrogen peroxide. Hydrogen peroxide was not used in the pretreatment of samples since it was desired to measure the grain size in an unaltered state representative of field conditions.

In cases where there was sufficient sample, sedimentation tests were performed to separate silt from clay. These results are summarized in table 6-1. As can be seen from the table, the silt fraction is higher than the clay fraction in all but one of the samples and in many of the samples the silt fraction is almost twice the clay fraction. Again the variation of the percentages of silt and clay with location is small.

6.2. Sediment Samples from the Sheboygan River

6.2.1. Total Concentrations of PCBs in Sediment

The total PCB concentrations ranged from a high of 1590 ppm found above the Upper Kohler dam to a low of 0.04 ppm found above the Sheboygan Falls dam (background concentrations). Although there is considerable variation among the samples, in general total PCB concentrations decreased as one moves downstream away from the source. The average value for each core is shown on a map in figure 6-1. Values for each individual 15 cm section of core are given in table 6-2.

As seen from the samples (A samples) collected above Sheboygan Falls Dam, the background concentrations are very low (an average of 0.05 ppm). For the remaining cores, the top 15 cm of the core, containing the sediment-water

Table 6-1 Results of Sieved Sediment Samples
Based on ASTM Definitions[a]

Sample	PCB Conc (ppm, dry wt)	Sand Fraction Medium (2.0mm-425μm)	Sand Fraction Fine (425μm-75μm)	Fine Fraction Silt (74μm-5μm)	Fine Fraction Clay (<5μm)
A1-2	0.04	4.9	62.3	20.4	12.6
A2-2	0.02	8.2	66.9	16.2	8.7
B1-1	8.5	6.4	64.8	18.9	9.9
B1-2	345.9	8.0	53.9	23.9	14.2
B1-3	334.2	8.8	59.0	19.3	13.1
C1-3	9.8	5.3	47.3	28.4	18.4
D2-3	300.3	15.9	43.8	41.1*	
D2-4	4.2	6.4	61.6	31.7*	
E3-1	7.7	8.0	62.9	29.3*	
T1A	50.3	8.9	69.4	19.8	9.5
T2B	76.9	5.4	76.4	18.4*	
T2C	97.8	4.6	62.3	14.8	18.4
T2D	3.5	5.2	65.4	21.0	8.7
T3B	60.9	5.5	63.8	15.7	15.0
T3C	0.5	2.4	90.5	6.8*	
T3D	0.4	3.4	80.7	15.9*	
T4B	12.5	5.4	85.0	9.6*	

* -- Indicates combined silt and clay fractions
[a]American Society of Testing and Materials, 1987

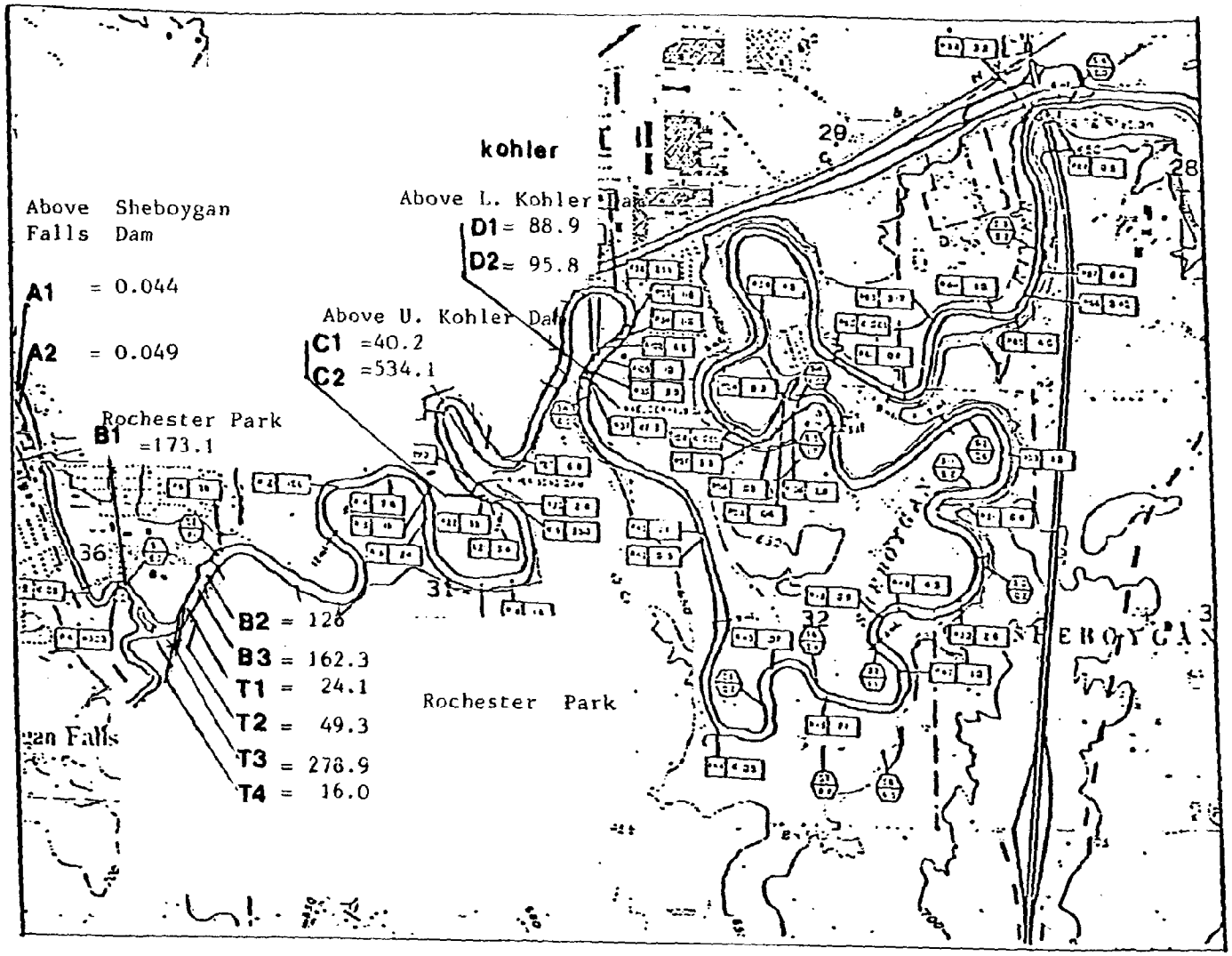


Figure 6-1 Map of Sheboygan River illustrating Average Sediment Core Concentrations

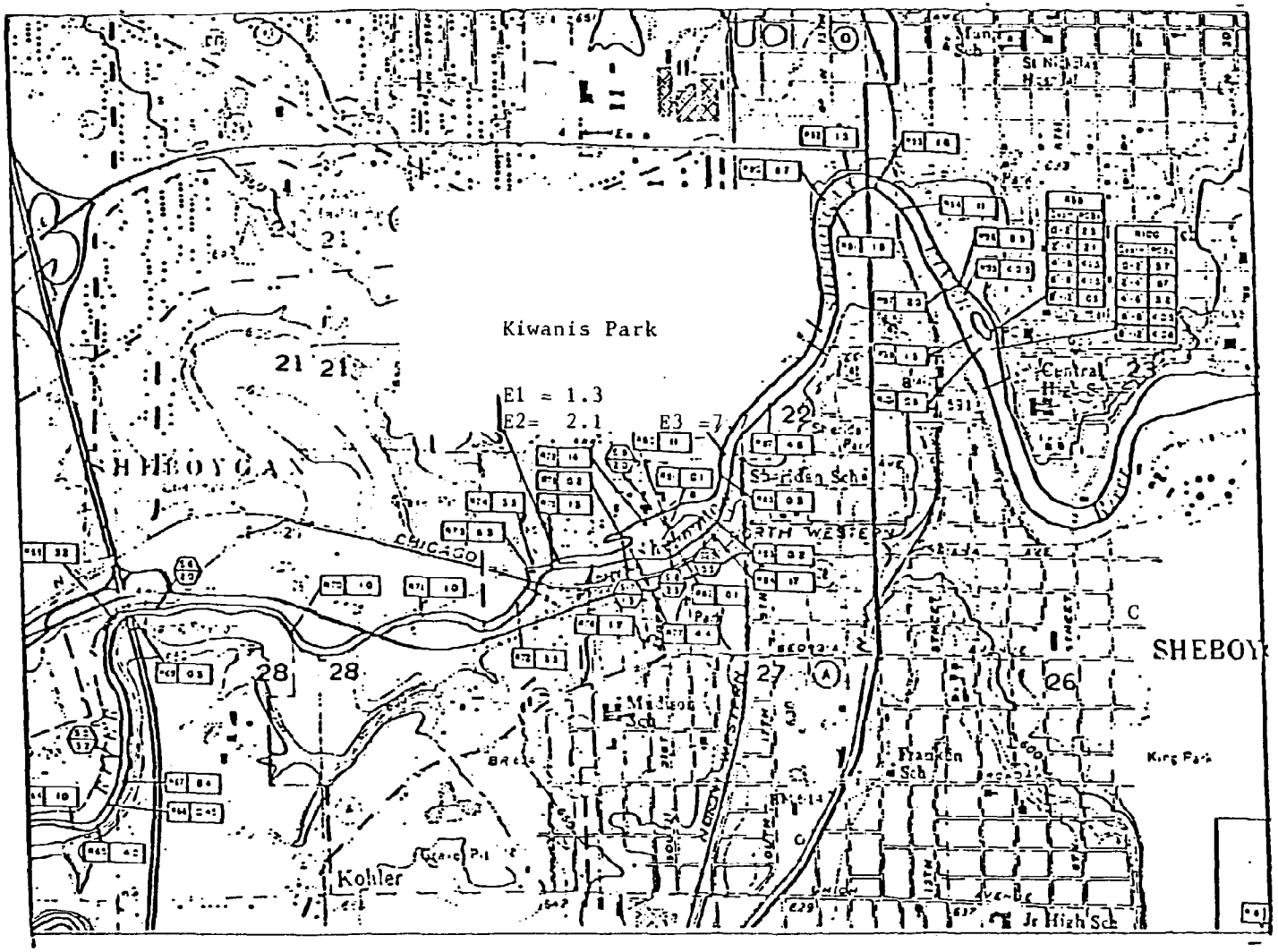


Figure 6-1 Map of Sheboygan River (Continued)

Table 6-2 Total PCB Concentrations in River Sediment
(ppm, dry wt)

Sample	Concentration	
Above Sheboygan Falls Dam		
A1-1	0.0354	
A1-2	0.0176	
A1-3	0.0381	
A1-4	0.0866	
A1-5	0.0916	
		Ave. 0.044
A2-1	0.033	
A2-2	0.0235	
A2-3	0.0663	
A2-4	0.1611	
A2-5	0.0581	
		Ave. 0.049
Rochester Park		
B1-1	8.5	
B1-2	345.9	
B1-3	334.2	
B1-4	3.6	
		Ave. 173.1
B2-1	113.9	
B2-2	143.1	
B3 (composite)	162.3	
		Ave. 139.8
Above Upper Kohler Dam		
C1-1	28.7	
C1-2	8.6	
C1-3	9.8	
C1-4	113.6	
		Ave. 40.2
C2-1	3.9	
C2-2	12.4	
C2-3	1585.8	
		Ave. 534.0
Above Lower Kohler Dam		
D1-1	4.4	
D1-2	117.9	
D1-3	144.5	
		Ave. 88.9

Table 6-2 Total Concentration of PCBs in River Sediment (cont.)

Sample	Concentration	
Above Lower Kohler Dam (cont.)		
D2-1	8.2	
D2-2	70.5	
D2-3	300.3	
D2-4	4.2	
		Ave. 95.8
Kiwanis Park		
E1-1	1.9	
E1-2	0.7	
E2-1	2.1	
E3-1	7.7	
Rochester Park (Second Sampling Trip)		
T1A	50.3	
T1B	21.3	
T1C	0.8	
		Ave. 24.1
T2A	18.8	
T2B	76.9	
T2C	97.8	
T2D	3.5	
		Ave. 49.3
T3A	1053.7	
T3B	60.9	
T3C	0.5	
T3D	0.4	
		Ave. 278.9
T4A	8.6	
T4B	12.5	
T4C	26.8	
		Ave. 16.0

interface, had relatively low concentrations of PCBs (an average of 21.4 ppm excluding sample T3A). The next two core segments (15-30 cm and 30-45 cm) typically had the highest concentrations (an average of 174.4 ppm), while the bottom core segment (45-60 cm) had on average the lowest concentrations (an average of 2.4 ppm excluding sample C1-4).

6.2.2. Homolog Distribution Patterns in Sediment

Samples from the first sampling trip (in which all sites were sampled) could be divided into two distinct groups based on their homolog patterns. One pattern had an enrichment in the lighter chlorinated congeners relative to the original Aroclor patterns while the other pattern had a distribution similar to the original Aroclor patterns. This distinction appeared to be a function of concentration. Samples containing PCB concentrations greater than 50 ppm appeared to be enriched with the lighter chlorinated congeners, whereas those with less than 50 ppm PCBs were not.

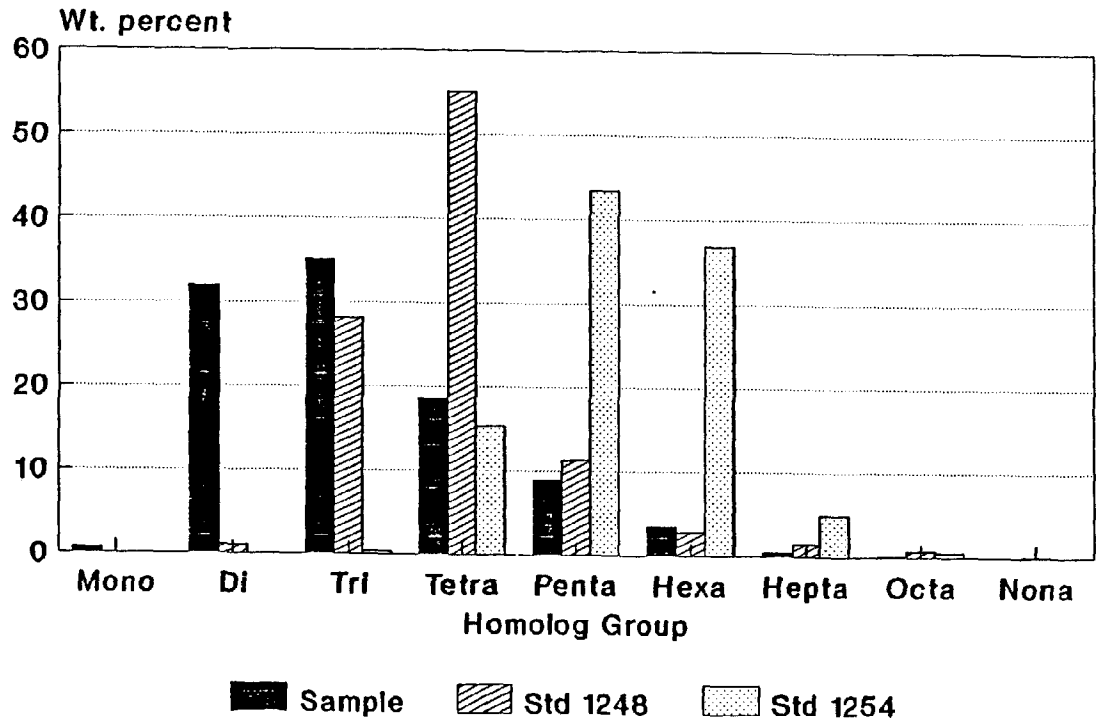
Samples containing 50 ppm or more PCBs had significantly higher concentrations of mono and dichlorinated congeners compared to the original Aroclors introduced into the river. Using a multivariate t test, these samples were found to be statistically different in composition from Aroclor 1248, Aroclor 1254, and a 50/50 mixture of Aroclor 1248 and 1254

All p values were less than 0.05. A p value smaller than 0.05 means that the samples are significantly different at the ninety fifth percentile confidence interval. There is less than a five percent chance that the sample came from a distribution that contained the Aroclor mixes. Figure 6-2 and 6-3 show the homolog distribution of sediment samples with PCB concentrations above 50 ppm from the first and second sampling trips. Although the two sets of data are from different sampling trips, there is no statistical difference between these samples (p=0.8870, using a test of two means).

In samples containing PCBs concentrations less than 50 ppm from the first sampling trip (sites B,C,D,E), the homolog patterns slightly resembled the original Aroclors introduced into the river; however, they were statistically different from Aroclor 1248, Aroclor 1254 and a 50/50 mix of Aroclor 1248 and 1254 (using a multivariate t test p values were found to be less than 0.05 for all comparisons). This is illustrated graphically in figure 6-4.

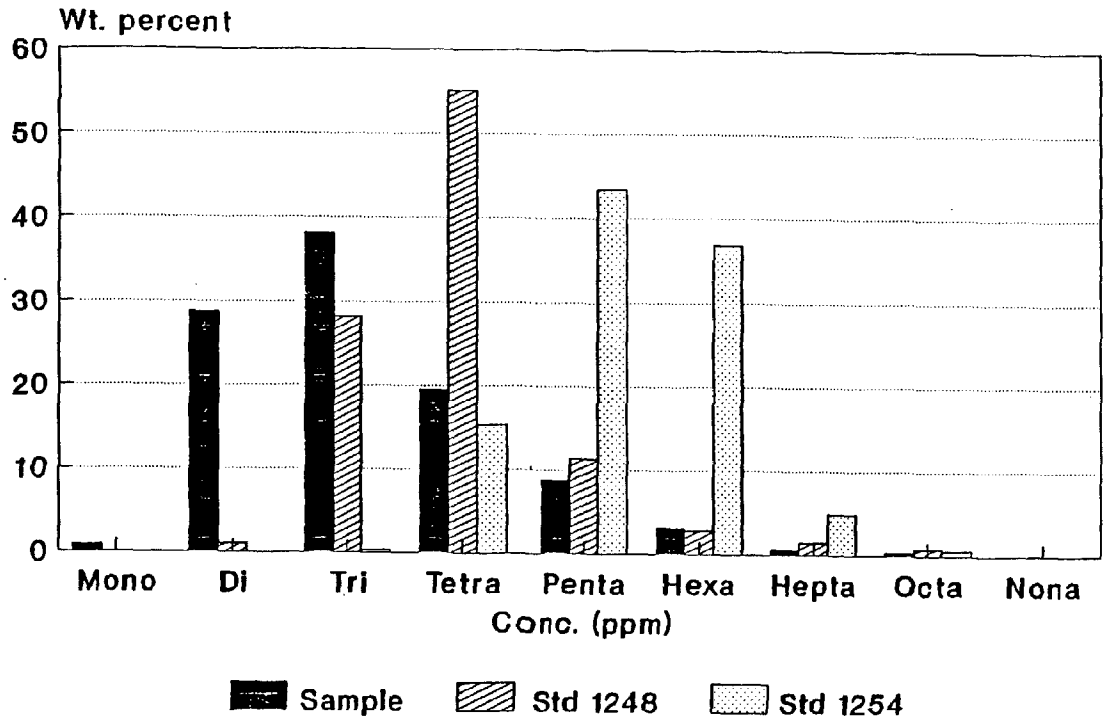
There were too few samples with concentrations less than 50 ppm from the second sampling trip (Rochester Park site, T samples) to use a multivariate t test. These samples were found to be statistically different from the di, tri, tetra, hepta and octa homolog groups of the original Aroclors using a univariate t test ($p < 0.05$). This is illustrated in

Figure 6-2 Homolog Distribution in Sediment (Conc.> 50 ppm, Sites B,C,D,E)



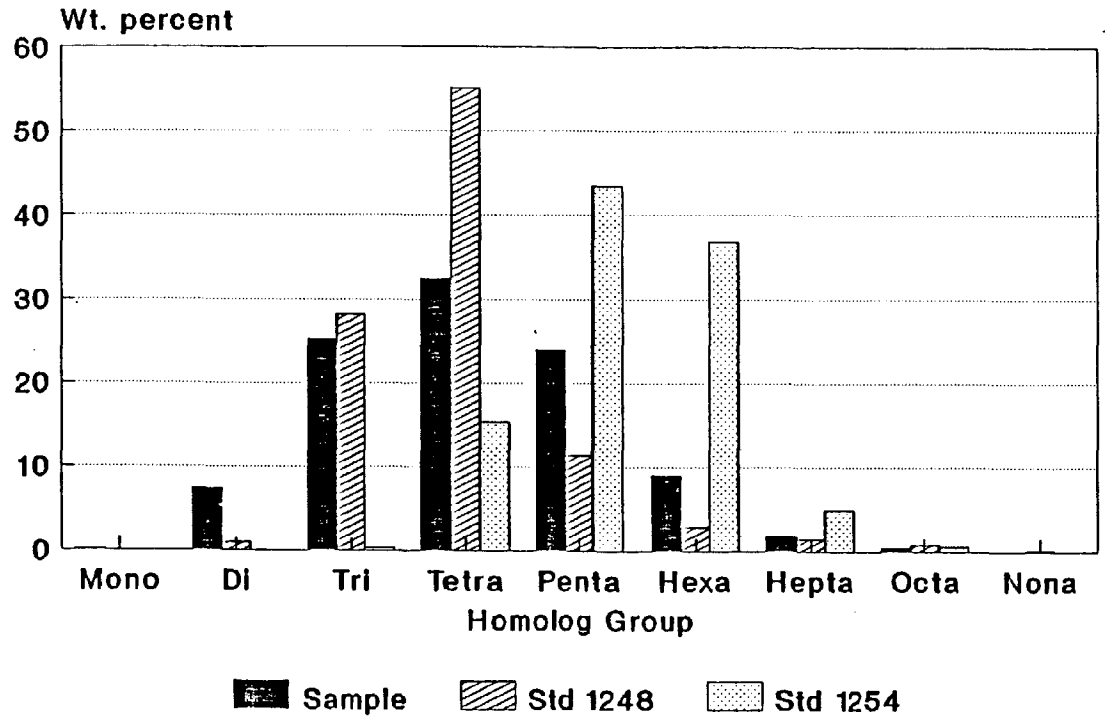
(Ave. of 11 samples)

Figure 6-3 Homolog Distribution in Sediment (Conc. > 50 ppm, Site T)



(Ave. of 5 samples)

Figure 6-4 Homolog Distribution in Sediment (Conc.< 50 ppm, Sites B,C,D,E)



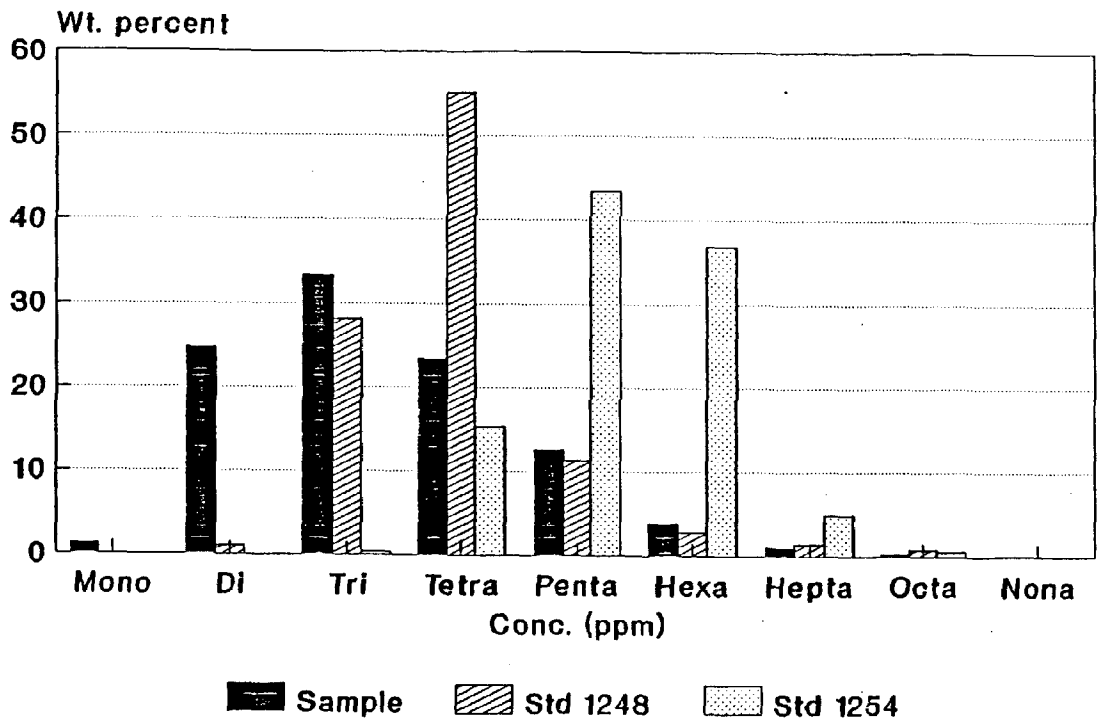
(Ave. of 14 samples)

figure 6-5. Interestingly, the samples from the second sampling trip containing less than 50 ppm (Rochester Park, T samples) were very similar to the distribution of the more highly concentrated samples from the same area (p= 0.8875).

6.2.3. Most Prominent Congeners in Sediment

The most prominent congeners are listed in table 6-3. These results are also presented graphically in figure 6-6 and figure 6-7. Relative to the original Aroclors, particularly high concentrations of congeners 5/8 (congeners 5 and 8 coelute) were seen. The highest concentrations were found in samples containing more than 50 ppm PCBs, 29.2 percent on average. In contrast, samples containing less than 50 ppm of PCBs, contained only 11.4 percent on average. Areas of high concentrations of PCBs, such as Rochester Park, had high concentrations of congener 5/8 throughout the core, independent of concentration in each individual segment of core. Samples from Rochester Park had on average 24 percent congener 5/8 whereas all other sites had on average 11.5 percent. Congeners 4/10, 16/32, 17, 24/27, 47/48 and 26 were also abundant in comparison to the original percentages in the Aroclors. Although 28/31, 52, 66/95, and 77/110 were prominent congeners, they were present in diminished quantities in the sample relative to the original quantities

Figure 6-5 Homolog Distribution in Sediment (Conc. < 50 ppm, Site T)



(Ave. of 9 samples)

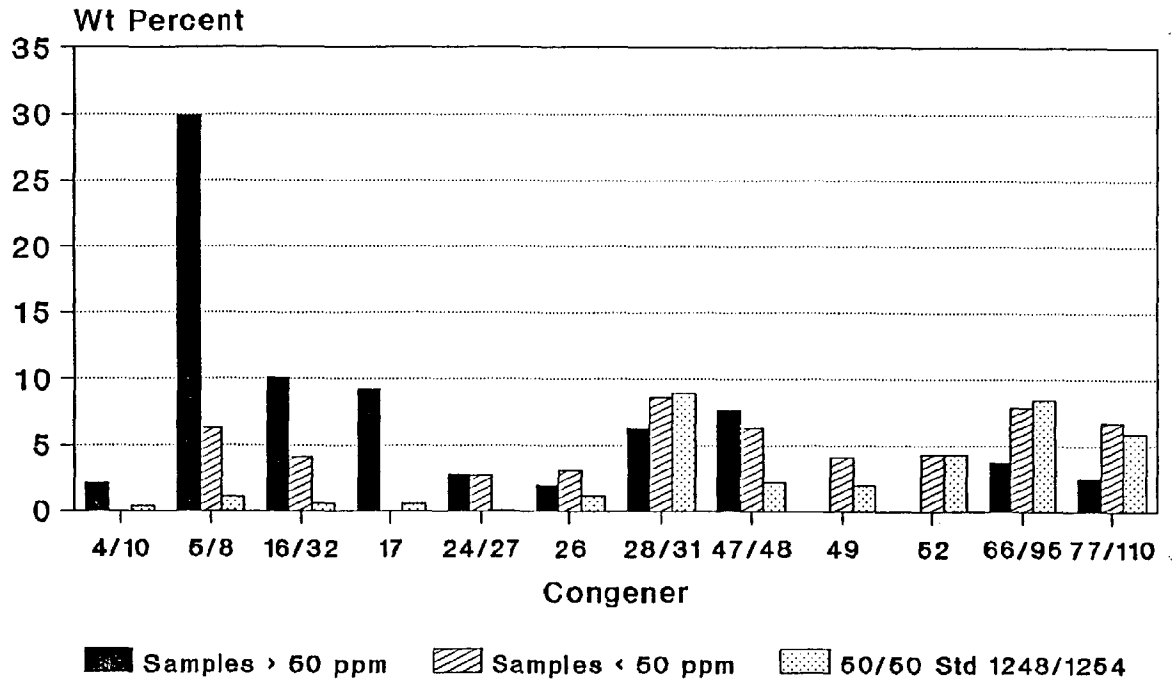
Table 6-3 Most Prominent Congeners in Sediment
(Weight Percent of Total)

Congener	1st trip samples >50 ppm	2nd trip (R.Park only) samples >50 ppm	Aroclor 1248[a]	Aroclor 1254[a]
4/10	2.1		1.6	0.03
5/8	29.9	27.4	2.1	0.06
17	9.2	10	1.2	<0.1
16/32	10.1	9.8	1.9	<0.1
47/48	7.7	7.2	4.0	0.4
24/27	2.8	3.4	0.18	<0.1
28/31	6.3	6.6	17.7	0.6
66/95	3.7	4.1	9.0	7.6
77/110	2.5		2.5	9.2
26	2.0	2.9	0.4	<0.1
52		2.4	4.4	4.2
49		2.0	3.0	1.0

Congener	1st trip samples <50 ppm	2nd trip (R. Park only) samples <50 ppm	Aroclor 1248[a]	Aroclor 1254[a]
5/8	6.3	17.3	2.1	0.06
17		10.3	1.2	<0.1
16/32	4.1	11.3	1.9	<0.1
47/48	6.3	8.3	4.0	0.4
28/31	8.6	9.9	17.7	0.6
66/95	7.9	4.0	9.0	7.6
77/110	4.1	3.0	2.5	9.2
26	3.1		0.4	<0.1
52	4.3	1.8	4.4	4.2
49	4.1	2.2	3.0	1.0
41/64/71		2.9	0.7	<0.1

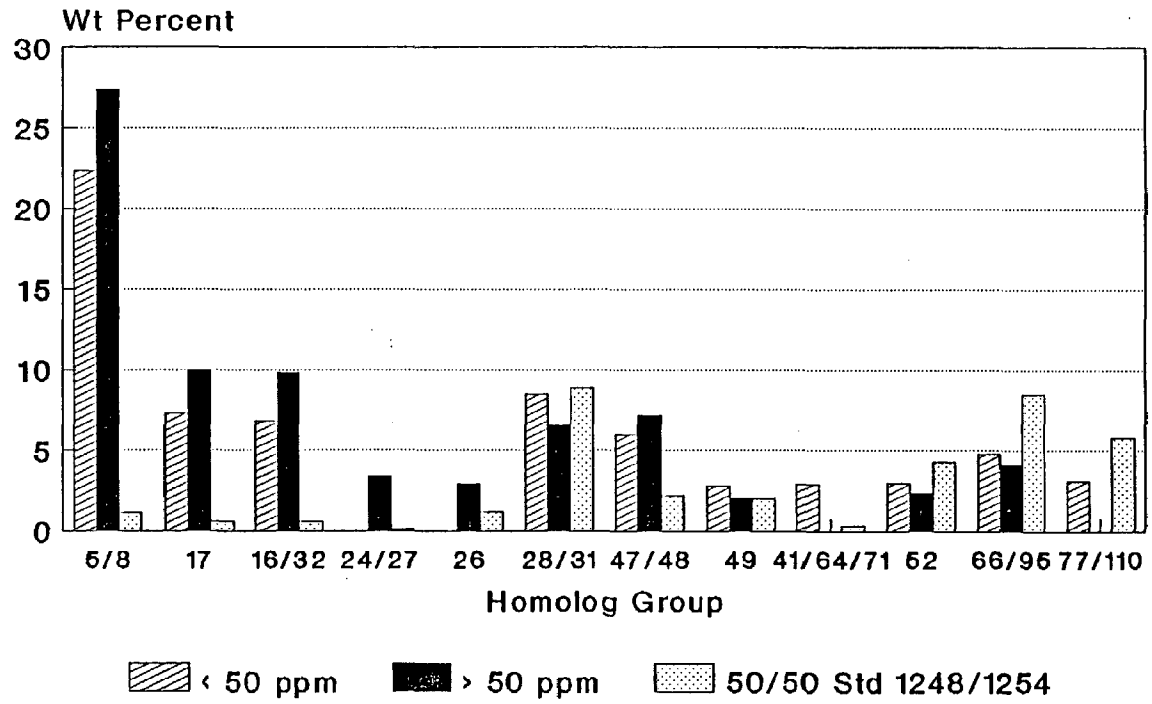
[a] Manchester, 1988

Figure 6-6 Most Prominent Congeners in Sediment (Sites B,C,D,E)



(Ave of 11 Samples, <50 ppm
Ave of 14 Samples, <50 ppm)

Figure 6-7 Most Prominent Congeners in Sediment (Site T)



(Ave of 5 samples >50 ppm
Ave of 9 samples <50 ppm)

in the Aroclor mixtures.

6.2.4. Accuracy, Precision and Confirmatory Analysis for Sediment Samples

A duplicate sample and a spiked sample were included in every batch of samples run. Sediment was spiked with an Aroclor mixture of Aroclor 1232, 1248 and 1262 at a level of 0.61 ppm. The recoveries for the spikes are listed in the table below and are calcluated by summing the individual congener concentration. The recoveries varied from a high of 108.1 percent to a low of 81.8 percent with an average recovery of 92.5 percent.

Table 6-4 Percent PCB Recovery from Spiked Sediment

Run 1	81.8
Run 2	108.3
Run 3	81.9
Run 4	95.4
Run 5	94.4
Run 6	94.5
Run 7	89.2
Run 8	94.4

Values for duplicate samples are given in table 6-5. Confirmatory analysis was performed on approximately 25 percent of the samples (15 samples out of a total of 59 samples) using a DB-1 column. Good correlation was found between the two data sets (correlation coefficient of 0.983).

Since many of the samples had very high concentrations of congener 5/8, additional confirmatory analysis of this particular congener was sought. Six of the samples, containing high concentrations of PCBs (342.8 ppm on average) and high concentrations of congener 5/8 (43.7 percent on average), were analyzed using an Electron Impact Gas Chromatography Mass Spectrometer. All of the spectra

Table 6-5 Duplicate Sediment Samples

Duplicate	Results	Mean	Percent Agreement
1	345.9, 414.8	380.4	90.9
3	4.6, 5.2	4.9	93.9
2	7.0, 8.2	7.6	92.1
7	76.9, 69.3	73.1	94.8
6	0.53, 0.49	0.51	92.2
4	97.8, 134.8	116.3	84.1
5	4.4, 5.1	4.75	92.6
8	20.74, 20.92	20.83	99.6

confirmed that the previously identified congener 5/8 peak was indeed a dichlorinated biphenyl. The sample spectra were compared to a library of known spectra compiled by the National Bureau of Standards. A computer algorithm designed to match the most significant peaks determined the agreement between the sample and known standard. Good agreement was found for all spectra as indicated by the percent match which

varied between 83.2 and 96.6 percent with an average match of 91.8 percent.

6.3. Sediment Samples from the Harbor

Three cores from the Sheboygan Harbor were obtained from the EPA and analyzed to determine the PCB congener distribution. The cores were located near the mouth of the harbor. Two cores were taken at a sediment depth of approximately 30.5 cm (1 ft). The remaining core ranged from the surface-water interface to a depth of 50.8 cm (20 inches).

6.3.1. Total PCB Concentrations in Harbor Sediments

Table 6-6 contains boring depths of the core and the concentration of PCBs found in each segment of the three cores.

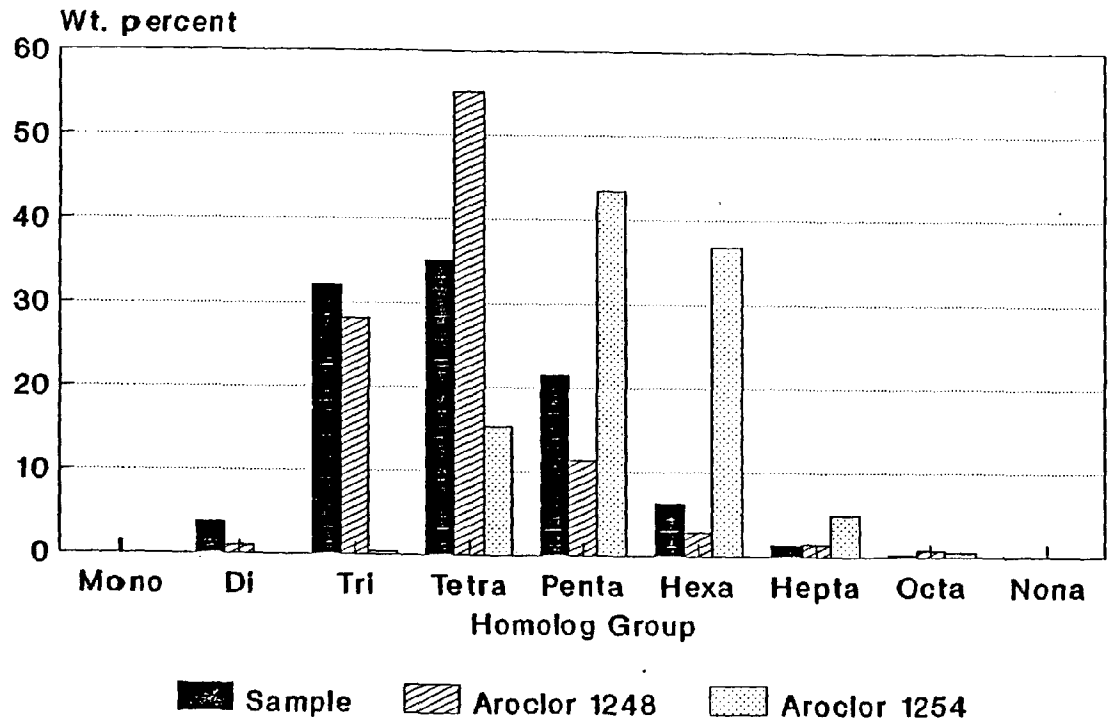
6.3.2. Homolog Distribution in Harbor Sediment

The homolog distribution are similar in distribution to the original Aroclor mixtures (see figure 6-8). They are however statistically different ($p < 0.05$). In addition, the patterns appear to be similar to the homolog patterns seen for sediment samples containing less than 50 ppm PCBs.

Table 6-6 Total PCB Concentrations in Harbor Sediment

Core	PCB Conc. (ppm, dry wt.)
H18	
0-1.3 cm	36.3
1.3-5 cm	15.7
5-10 cm	83.6
10-15 cm	30.7
15-20 cm	3.5
20-31 cm	1.4
31-41 cm	0.3
41-51 cm	0.9
H11	
31-41 cm	3.5
41-51 cm	27.7
H15	
20-31 cm	91.2

Figure 6-8 Homolog Distribution in Harbor Sediment



(Ave. of 11 samples)

6.3.3. Most Prominent Congeners in Harbor Sediment

The most prominent congeners are listed in table 6-7. Most of these congeners are also found in high concentrations in the original Aroclor mixtures 1248 and 1254 as noted in the table below. The most prominent congeners are also presented graphically in figure 6-9.

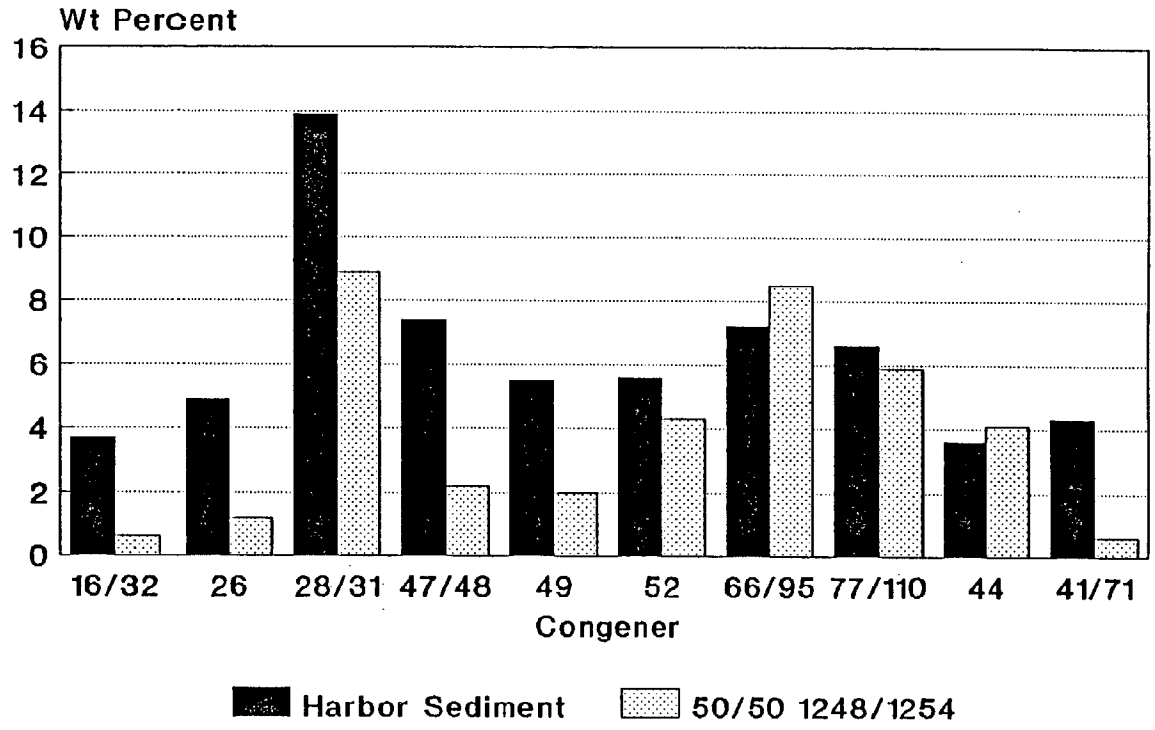
Table 6-7 Most Prominent Congeners found in Harbor Samples (Weight percents)

Congener	Sample	Aroclor 1248[a]	Aroclor 1254[a]
28/31	13.9	17.7	0.6
47/48	7.4	4.0	0.4
66/95	7.2	9.0	7.6
77/110	6.6	2.5	9.2
49	5.5	3.0	1.0
52	5.6	4.4	4.2
26	4.9	0.4	<.1
41/64/71	4.3	0.7	0.4
44	3.6	5.2	3.0
16/32	3.7	1.9	<.1

[a] Manchester, 1988

Congener 28/31 is the most abundant congener in the harbor samples. It is also the most abundant congener in Aroclor 1248. Congeners 47/48, 49, 52, 26, 41/64/71, and 16/32 are enriched in the harbor sediment relative to their concentrations in the Aroclors. The only congener found in diminished quantities relative to the Aroclor mixtures was congener 66/95.

Figure 6-9 Most Prominent Congeners in Harbor Sediment



(Ave of 11 Samples)

6.3.4. Precision and Accuracy for Harbor Samples

The recovery from spiked sediment was 101.6 percent and was calculated by summing the individual congeners. The results of the duplicate analysis preformed on the samples were 1.4 ppm and 1.5 ppm.

6.4. Water Samples from the Sheboygan River

6.4.1. Total PCB Concentrations in Water

The water samples are divided into particulate and dissolved concentrations based on an operational definition. The portion of the sample that was able to pass through the 0.7 micron filter was defined as the dissolved fraction and the portion that remained on the filter was the particulate fraction. The particulate and dissolved concentrations are given in table 6-8.

The background concentrations were low, 0.007 and 0.003 ppb for the particulate and dissolved fraction, respectively. The particulate concentrations in the non-background samples varied from an average low of 0.162 to an average high of 0.502 ppb. The lowest particulate concentration in the water occured at Rochester Park, the site of the highest average sediment PCB concentrations, 173.1 and 139.8 ppm. This would be expected since the river water has been in relatively short contact with the contaminated sediment. The highest

Table 6-8 Total PCB Concentrations in Water (ppb)

Site	Concentration
Above Sheboygan Falls Dam (Background)	
Dissolved	
Water sample 1	0.003
Particulate	
Water sample 1	0.007
Rochester Park	
Dissolved	
Water sample 1	0.028
Water sample 2	0.047
Particulate	
Water sample 1	0.162
Water sample 2	0.301
Above Upper Kohler Dam	
Dissolved	
Water sample 1	0.190
Water sample 2	0.204
Particulate	
Water sample 1	0.464
Water sample 2	0.432
Above Lower Kohler Dam	
Dissolved	
Water sample 1	0.152
Water sample 2	0.179
Particulate	
Water sample 1	0.508
Water sample 2	0.452
Kiwanis Park	
Dissolved	
Water sample 1	0.071
Water sample 2	0.069
Particulate	
Water sample 1	0.275
Water sample 2	0.327

particulate concentration in the water was above the Lower Kohler dam which had average sediment core concentrations of 88.9 and 95.8 ppm.

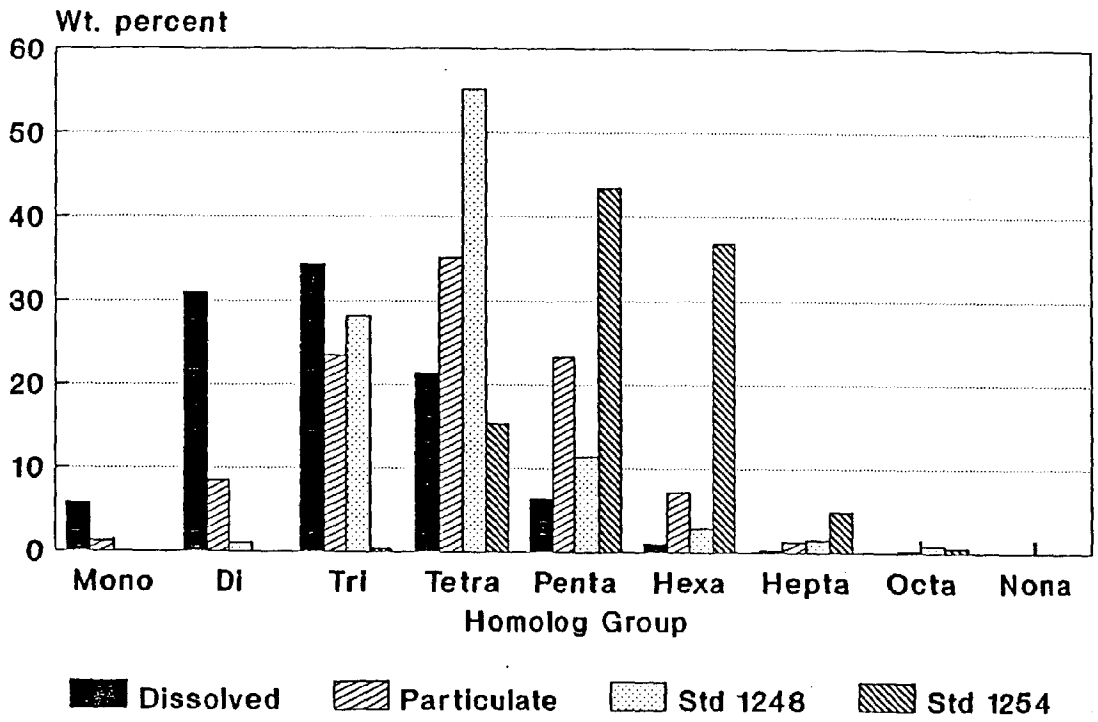
The dissolved concentrations varied between 0.028 and 0.204 ppb. The lowest concentrations were again found at Rochester Park, the site of highest PCB sediment concentrations. The highest dissolved concentrations were found above the Upper Kohler dam which had sediment concentrations of 40.2 and 534 ppm (the average of 534 ppm was disceptively high because the highest single 15 cm sediment concentration was found in this core, the remainder of the core had low concentrations of PCBs).

6.4.2. PCB Homolog Distribution in Water

The homolog groups for the dissolved and particulate fractions are illustrated in figure 6-10. In the dissolved fraction, there is a dramatic increase in the lighter chlorinated congeners relative to the original Aroclor mixture, particularly the mono, di and trichlorinated congeners. Correspondingly, there is a decrease of the heavier chlorinated congeners, notably the penta, hexa, hepta and octachlorinated homolog groups.

In comparison to the dissolved fraction, the particulate fraction contains decreased quantities of the mono, di and

Figure 6-10 Homolog Distribution in Water



(Ave. of 8 samples)

trichlorinated congeners; however, there is still an enrichment of these lower chlorinated congeners relative to the original Aroclors. In general, the distribution is much more similar to the original Aroclors than the dissolved fraction.

Not surprisingly, the homolog distribution for the dissolved fraction was not found to be statistically similar to the original Aroclors or a 50/50 mix of the original Aroclors ($p < 0.05$ for univariate t tests of each homolog group). The particulate fraction was also not statistically similar to the original Aroclors with the exception of the monochlorinated congeners and the nanochlorinated congeners which are in very small concentrations in both the sample and in the Aroclors ($p < 0.05$ for univariate t tests of each homolog group).

6.4.3. Most Prominent Congeners in Water

The most prominent congeners found in water are listed in table 6-9 and shown graphically in figure 6-11. Congeners 1, 4/10, 5/8, 17, 16/32, and 41/64/71 were in higher concentrations in the water samples than in the original Aroclors. Congener 70/76 was in lower concentrations than the Aroclors but still remained one of the most prominent congeners in the sample. The remaining

Table 6-9 Most Prominent Congeners in Water (Dissolved and Particulate Fraction)

Congener	Diss.	Part.	Aroclor 1248[a]	Aroclor 1254[a]
1	3.4		<0.1	<0.1
4/10	2.9		1.59	0.03
5/8	26.0	7.6	2.1	0.06
17	7.9	3.6	1.2	<0.1
16/32	3.6		1.9	<0.1
28/31	10.7	9.7	17.74	0.6
47/48	3.4	6.1	4.0	0.4
41/64/71	2.8	4.7	0.74	0.4
52	4.0	3.5	4.4	4.2
66/95	3.3	7.8	9.0	7.6
70/76		4.2	7.1	4.4
77/110		5.9	2.5	9.2
44		3.1	5.2	3.0

[a] Manchester, 1988

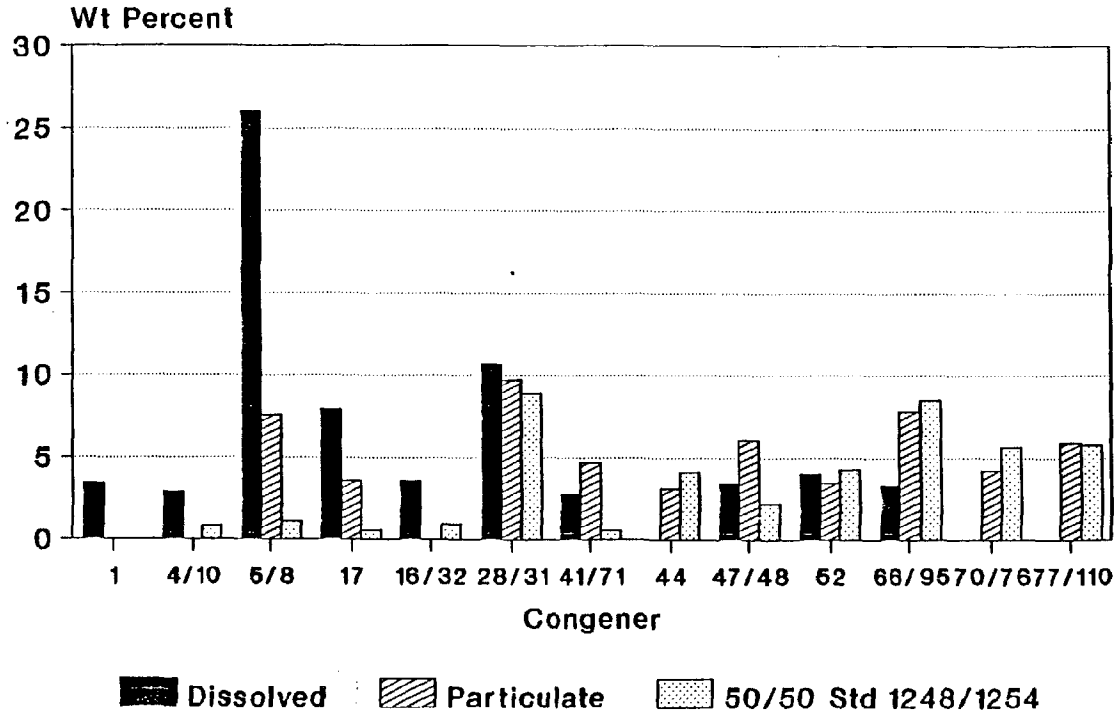
congeners were of intermediate value.

6.4.4. Accuracy, Precision and Confirmatory Analysis for Water

The recoveries from spiking water with an Aroclor mixture of 1232/1248/1262 in acetone were 83.1 and 91.7. All samples with the exception of the background sample were run in duplicate and are shown in table 6-8.

Confirmatory analysis was performed on approximately thirty percent of the samples (six samples) using a DB-1 column. The correlation coefficient for the two data sets indicate good agreement (Correlation coefficient = 0.977).

Figure 6-11 Most Prominent Congeners in Water



(Ave of 8 samples)

6.5. Fish

6.5.1. Total PCB Concentrations in Fish

The fish concentrations varied from a low of 1.3 ppm to a high of 22.0 ppm. These results are summarized in table 6-10. The highest values were found in carp and channel catfish; both of which are bottom feeding fish and have high concentrations of fat.

6.5.2. Homolog PCB Distribution Patterns in Fish

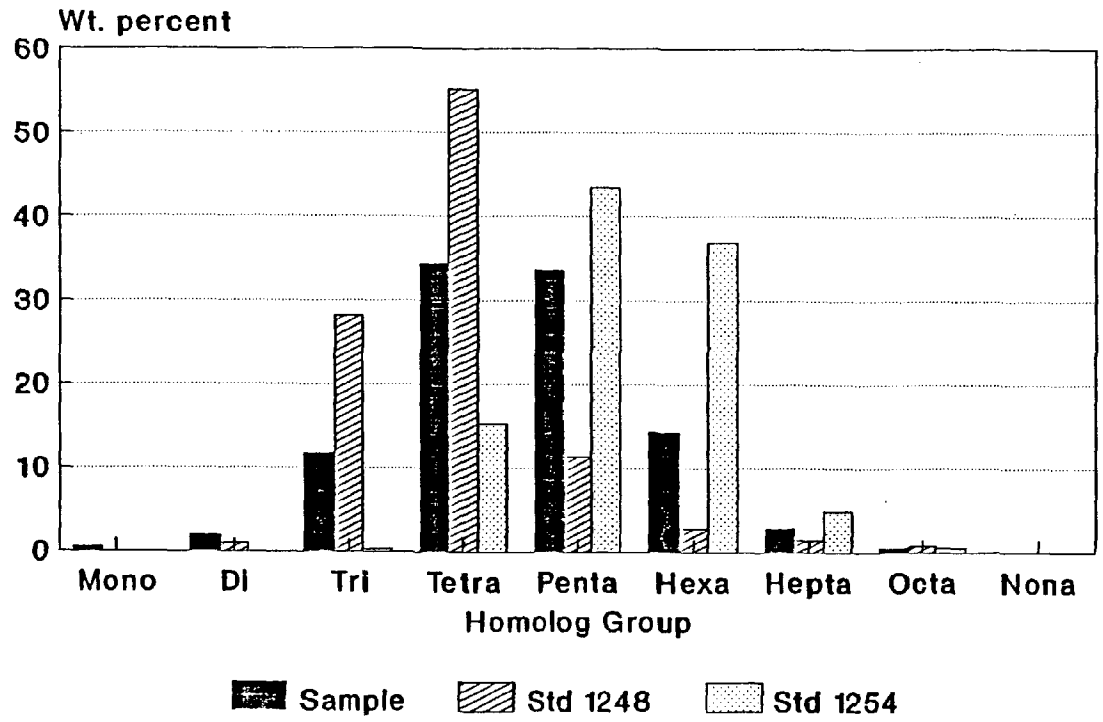
The average homolog distribution pattern is shown in figure 6-12 with the Aroclor 1248 and 1254 and in figure 6-13 with the homolog distribution for the river sediment and the harbor sediment. Relative to the Aroclors, there was a slight increase in the mono and dichlorinated congeners in the fish samples, probably due to the significant concentrations of the mono and di chlorinated congeners in the water and sediment.

Although the fish samples were enriched in the higher chlorinated congeners and appeared to be more similar to the Aroclors than the water or sediment samples, the samples were not found to be statistically similar to Aroclor 1248, Aroclor 1254 or a 50/50 mix of Aroclor 1248 and 1254 using a multivariate t test ($p < 0.0001$).

Table 6-10 Total PCB Concentrations in Fish

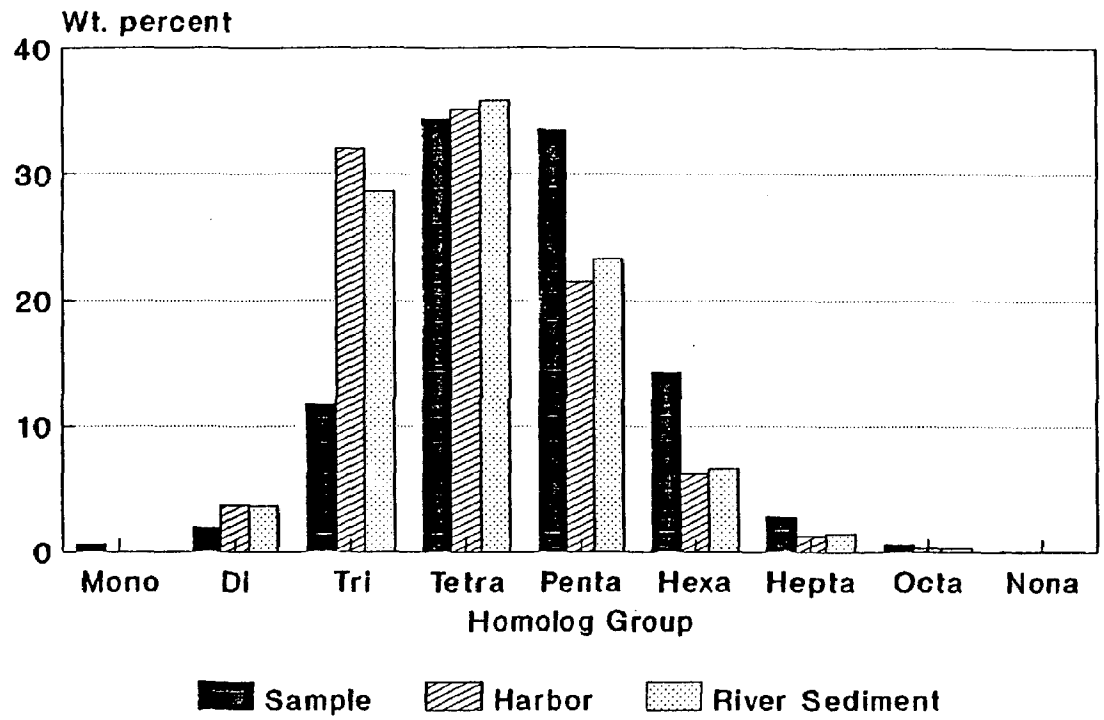
Species	Capillary Column	Packed Column	% Fat
Small Mouth Bass	3.4	3.7	0.6
"	4.3	4.2	0.7
"	3.9	3.5	0.4
"	4.3	4.2	0.8
"	5.8	4.4	1.2
"	6.8	9.0	3.0
"	2.3	2.6	0.8
"	2.0	2.3	0.5
Bluegill	2.7	2.2	1.4
Walleye	10.1	6.7	1.8
Northern Pike	3.6	6.6	0.5
"	4.6	3.9	1.0
"	6.9	6.2	1.0
"	6.4	3.8	1.6
Rock Bass	5.1	6.0	2.1
"	1.3	1.4	0.4
Channel Catfish	9.5	13.0	5.1
"	22.0	32.0	3.4
"	13.9	26.0	6.5
Carp	22.0	21.0	4.1
"	18.9	15.0	13.0

Figure 6-12 Homolog Distribution in Fish



(Ave. of 21 samples)

Figure 6-13 Homolog Distribution in Fish and Sediments



(Ave. of 21 samples)

6.5.3. Most Prominent Congeners in Fish

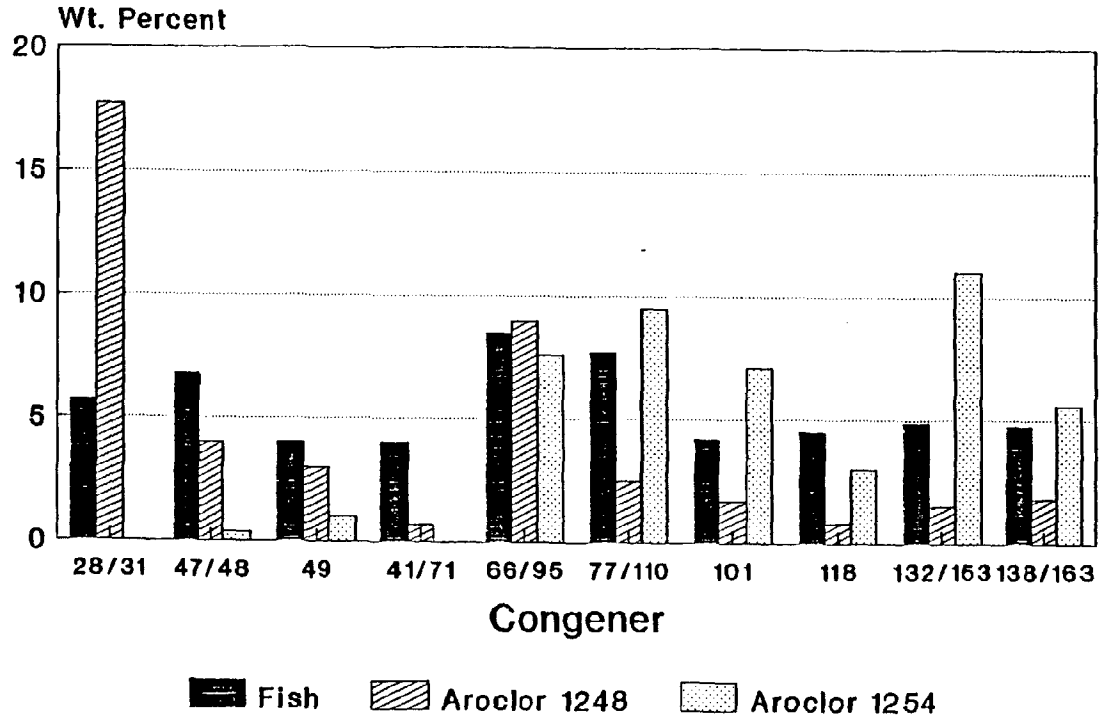
The most prominent congeners are listed in table 6-11 and also presented in figure 6-14.

Table 6-11 Most Prominent Congeners in Fish

Congener	Sample	Aroclor 1248[a]	Aroclor 1254[a]
28/31	5.7	17.7	<0.1
47/48	6.8	4.0	0.4
49	4.0	3.0	1.0
41/64/71	4.0	0.7	<0.1
66/95	8.5	9.0	7.6
77/110	7.7	2.5	9.5
101	4.2	1.7	7.1
118	4.5	0.8	3.0
132/153/105	4.9	1.5	11.0
138/163	4.8	1.8	5.6

[a] Manchester, 1988

Figure 6-14 Most Prominent Congener in Fish



Chapter 7 Discussion of Results

In this section results from the sediment, water and fish analyses are compared to results from the literature. Explanations for the congener patterns are evaluated by comparing predicted patterns to observed patterns.

7.1. Discussion of Sediment Results

7.1.1. Grain Size Correlations with PCB Concentration

Since sediment samples composed of smaller particles have greater surface area for adsorption relative to samples of the same volume consisting of larger particles, it is predicted that smaller particles will have higher concentrations of PCBs relative to larger particles (Simmons et al., 1980). This tendency is enhanced by the fact that smaller particles are often comprised of organic carbon which has been positively correlated with PCB content (Elzerman and Coates, 1987). In this study, to examine the effect of grain size on PCB concentration, the two variables were correlated.

The correlation of grain size and concentration of PCBs is confounded by spatial effects such as distance from the source and the depth of the core. To control for spatial variation, samples from only one site (Rochester Park, samples B and T) were correlated with the grain size. Other samples were not correlated because there were too few samples at each

site to give a meaningful correlation. PCB concentration depends slightly on grain size; as the grain size decreased, the concentration of PCBs increased (R^2 value of 0.52 for a log log plot). The outlying points were samples from either the surface of the core or from the bottom of the core. One possible explanation for these outlying points is that sediment which is in the bottom of the core is older sediment and may not have been exposed to significant concentrations of PCBs. Conversely, sediment at the top of the core may be newer and cleaner sediment. Both of these samples would therefore be likely to have low concentrations of PCBs because they were not exposed to the same amount of PCBs as the middle section of core.

7.1.2. Total PCB concentrations in Sediment

Total sediment concentrations of PCBs ranged from 0.016 ppm to 1586 ppm as seen in table 6-2. Although higher concentrations were found by the Superfund remedial investigation on the Sheboygan River, the range of concentrations are comparable (table 7-1). The sediment results show a decreasing trend with distance from the source, similar to the results found in this study.

Although reported concentrations from other highly contaminated sites is comparable to Sheboygan River

Table 7-1 PCB Concentrations in Sheboygan River Sediment[a]

This Study[b]		Remedial Investigation[c]	
Core	Concentration (ppm)	Core	Concentration (ppm)
B1	173.1	R4	4300
		R5	59
B2/B3	139.8	R6	15
T1	24.1	R7	4500
T2	49.3	R8	0.4
T3	278.9	R9	28.0
T4	16		
C1	40.2	R19	250
C2	534	R20	2.8
		R21	5.4
		R22	93
D1	88.9	R34	110
		R103	72
		R104	5.4
D2	95.8	R37	<1.5
		R35	2.0
E1	0.8	R85	0.3
E2	2.1	R86	4.2
E3	7.7	R87	4.6

[a] The two sets of cores are grouped according to general proximity
[b] Samples are averages of cores which were 30 to 45 cm deep
[c] Remedial investigation (EPA Superfund study) samples are composite core samples which were 30 to 45 cm deep (Blasland and Bouck Engineers, 1988)

sediments, the PCB concentrations in Sheboygan River sediments are considerably higher than other sites in the Great Lakes region. Brown and coworkers found concentrations as high as 2600 ppm in Hudson River sediments with an average concentration in the hot spot areas of 75 ppm (Brown et al., 1984). In the tidal Hudson, sediment PCB concentrations dropped to an average of 10 ppm (Bopp et al., 1981). PCB concentrations in the Great Lakes are much lower. In Lake Ontario, sediment values were found in the range of 200-1200 ppb (Oliver et al., 1989).

Within the cores, as mentioned in Chapter 6, the top section of the core generally contained low concentrations of PCBs. The middle section of core usually contained the highest concentrations of PCBs and the bottom section of core typically had the lowest concentrations of PCBs. One possible explanation for this distribution is that the bottom section of sediment was deposited before significant contamination occured whereas the top section of core was deposited after significant contamination took place and contains the newer cleaner sediment.

There was one exception to this general trend. In sample T3A the highest concentration was in the top segment of core. T cores were collected four months after all other cores. During these four months, federal and state remedial

investigations of the site continued and it is possible that the sediments were disturbed by other investigators.

7.2 Discussion of Possible Mechanisms Causing the Congener Distribution in Sediment

The results from the sediment analysis clearly indicate that the sediment PCB patterns are significantly different from the Aroclor originally introduced into the river. The mechanisms which can alter the congener distribution in the sediment can be divided into three processes-- chemical, physical-chemical and biological.

Abiotic chemical reactions in the sediment affecting the PCB congener distribution are unlikely. Photolysis and oxidation reactions are unlikely to occur in the subsurface anaerobic environment present in most of the sediments. Although chlorinated methanes and ethanes have been found to undergo reductive dechlorination (Wade et al., 1969; Klecka and Gonsoir, 1984), reductive dechlorination of PCBs is thought not to occur in the conditions present in anaerobic sediments (Quensen et al., 1988; Brown et al., 1987b; Hutzinger et al., 1974). For example, reductive dechlorination of PCBs has been demonstrated to occur by Boyer and colleagues (1985) at 50-90 degrees C using sodium hypophosphite. These temperatures are well above the

temperatures found in the environment. Furthermore, the reduction of chlorinated ethanes and methanes by iron is not strictly abiotic because iron is present as iron porphyrin which is a biological enzyme present in hemeglobin, cytochromes and chlorophyll (Fessenden and Fessenden, 1982). In general, there are very few abiotic pathways which completely mineralize organic compounds (Alexander, 1981).

7.2.1. Physical-Chemical Processes Affecting the Distribution of PCBs in Sediment

Four physical-chemical processes can affect the distribution of PCB congeners in sediment: sediment-water partitioning, diffusion, air-water partitioning, and colloidal partitioning. A discussion of each follows with an evaluation of the predicted congener distribution based on the mechanism compared to the observed distribution.

Sediment-Water Partitioning. Probably, the most significant partitioning process affecting the distribution of congeners in the sediment is the partitioning between sediment and water (Bopp et al., 1981, Burkhard, 1985a). The partitioning of hydrophobic compounds between sediment and water can be described by the following relationship (Karickhoff, 1981):

$$Kp=S^e/P^e$$

where Kp is the equilibrium partition coefficient,

S^e is the equilibrium sorbed pollutant concentration,

P^e is the equilibrium aqueous phase concentration.

The partition coefficient is corrected for the organic carbon present in the sediment. The corrected partition coefficient is expressed as the organic carbon based partition coefficient, K_{oc}, and is related to the partition coefficient by the following equation:

$$K_{oc}=K_p*oc$$

where K_{oc} is the organic carbon based partition coefficient,

K_p is the partition coefficient,

oc is the fraction of organic carbon.

The octanol-water partition coefficient (K_{ow}) can be related to the organic carbon based partition coefficient (K_{oc}) by the following relationship (Karickhoff et al., 1979):

$$\text{Log } K_{oc}=1.0 \text{ Log } K_{ow} -0.21 \qquad (R^2= 1.00).$$

Using this relationship, the K_{oc} can be estimated from the K_{ow}. Since the octanol-water partition coefficient tends to increase with increasing chlorination (Shiu and Mackay, 1986), the partition coefficient for PCBs should also increase with increasing chlorination.

The above relationships predict that the higher chlorinated congeners should remain in the sediment while the lower chlorinated congeners should partition into the water.

Field studies at other sites show an enrichment of the higher chlorinated congeners in sediment which has been attributed to partitioning (Furukawa, 1982; Hansen, 1987). For example, sediment from the Puget Sound in Washington shows an enrichment of the higher chlorinated congeners and loss of lower chlorinated congeners to the water (Pavlou and Dexter, 1979).

Using the relationships of Karickhoff (1979) and predictions of Henry's Law constants and octanol-water partition coefficients, Burkhard (1984) and Burkhard et al. (1985) modelled the partitioning of Aroclor mixtures in a three phase system containing air, water and suspended particulate matter. Beginning with an equimolar distribution of congeners in all phases, Burkhard and colleagues examined the effects of partitioning into different phases on the congener distribution in each phase.

The air phase was found to be depleted in the higher chlorinated congeners above IUPAC 160. In the water phase there was some enrichment of the lower chlorinated congeners below IUPAC 40 and some depletion of congeners above IUPAC 120. The most dramatic change was found in the distribution of the congeners in the suspended particulate matter phase. Congeners below IUPAC 80 were significantly depleted while congeners above IUPAC 80 were significantly enriched. The

results of Burkhard (1984) and Burkhard et al. (1985) illustrate that the distribution of congeners in sediment is dramatically affected by the sediment-water partition coefficients. Equilibrium modelling using sediment-water partition coefficients predicts increases in higher chlorinated congeners and decreases in lower chlorinated congeners in the sediment.

Diffusion. Diffusion of congeners from the sediment back to the water is slow relative to sedimentation rates and is inversely related to the partition coefficient (Fisher et al., 1983; DiToro et al., 1985). The diffusion rate of PCBs in the presence of sediment (D^*_s) is function of the inverse of the partition coefficient and is described by the following relationship (DiToro et al., 1985):

$$D^*_s = D_s/(1+m*K_p/\phi)$$

where D^*_s is the diffusion rate in the presence of sediment (cm^2/day),

D_s is the aqueous diffusion rate (no sediment present) (cm^2/day),

m is the sediment solids concentration,

K_p is the partition coefficient,

ϕ is the porosity of the sediment.

microns such as humic and fulvic acids, microemulsions and mineral precipitates (McCarthy and Zachara, 1989). The overall effect of colloids in the partitioning of organic contaminants is to increase the "apparent" solubility of a compound in water and decrease the "apparent" partition coefficient.

The effect of colloidal partitioning on the sediments may be limited since PCBs were probably introduced into the Sheboygan River adsorbed to sediment (Wisconsin Department of Natural Resources, 1989). Hydraulic fluid containing PCBs was spilled on to dirt and debris which were later used to build a flood dike. Since the PCBs were already adsorbed to dirt that later became river sediment, colloidal partitioning could only occur after the PCBs were desorbed from the sediment into the water and eventually adsorbed onto the colloids. The first step of this process is governed by sediment-water partition coefficients which, as explained before, gives a distribution that is enriched in the higher chlorinated congeners and depleted in the lower chlorinated congeners. Therefore, it appears unlikely that colloidal partitioning has a major influence on the distribution of congeners in the Sheboygan River sediments.

None of the processes described above adequately explain the distribution seen in the Sheboygan River sediments. Since

This relationship illustrates that higher chlorinated congeners are more likely to remain in the sediment than the lower chlorinated congeners because the higher chlorinated congeners have higher partition coefficients and subsequently lower diffusion rates.

Air-Water Partitioning. The distribution of congeners between water and air phase can be important because it can affect the distribution between the water and sediment. The effect of air-water partitioning of congeners on the overall distribution of PCB congeners in sediment is likely to be small in comparison to sediment-water partitioning because the sediments are not in direct contact with the air. The vapor partitioning of PCBs from sediment in the Hudson River was calculated to have a small effect relative to sediment-water partitioning (Bopp et al., 1981). Even if one were to assume that the transport of PCBs from the sediment to the water and then to air were significant, the congener patterns seen in the Sheboygan sediments still would not be predicted. Henry's Law constants, which describe the partitioning of PCBs between the air and water, show no particular trend with regard to molecular weight (Burkhard et al., 1985b).

Colloidal Partitioning. Currently, there is considerable debate about the effect colloids have on partitioning. Colloids are defined as particles with diameters less than 10

sediment-water partition coefficients increase with molecular weight, an enrichment of the higher chlorinated congeners and a depletion of the lighter chlorinated congeners is predicted based on the partition coefficients. Equilibrium partitioning models such as Burkhard's do not predict the specific enrichment of a select small group of congeners such as 5/8, 17, and 24/27 which were found in this study. Instead, these models predict a gradual continuum of increasing concentrations of the higher chlorinated congeners in the sediments.

Henry's Law constants show no particular trend with molecular weight. The most prominent congeners seen in the sediment do not exhibit particularly low Henry's Law constants and therefore their presence cannot be explained in terms of these constants.

In summary, neither physical-chemical processes nor abiotic chemical reactions readily explain the distribution of congeners in Sheboygan River sediments. Consequently, one or more biological processes may better explain the measured distribution. Possible biological processes are discussed next.

7.2.2. Biological Processes affecting the Distribution of PCBs in the Sediment

Aerobic and anaerobic microbial degradation are probably the most likely biological processes that could affect the PCB distribution in sediment. Aerobic degradation, if it occurs at all, occurs in the top few centimeters of sediment where oxygen is present. Because most of the samples analyzed were slices of the core located 45 to 60 cm below the sediment surface, very little of the sediment was thought to be aerobic. Aerobic PCB-degrading microbes metabolize the lower chlorinated congeners, those with less than four chlorines, but generally do not metabolize the higher chlorinated congeners (Furukawa, 1982).

The pattern seen in the sediments is more typical of anaerobic degradation in which the higher chlorinated congeners are metabolized to produce lower chlorinated congeners. Several results in this study suggest anaerobic microbial degradation. First, there is a shift in the congener pattern from the higher chlorinated congeners to the lower chlorinated congeners. This cannot be accounted for by simple physical-chemical partitioning. Second, there appears to be some selectivity as to which congeners were lost that is characteristic of enzymatic microbial degradation. Lastly, the congener patterns appear to be a function of the concentration of PCBs in the sediment that is typical of biological degradation.

A shift in congener distribution from the higher chlorinated congeners to the lower chlorinated congeners was seen by Quensen and coworkers (1988) in their laboratory study of dechlorination by anaerobic bacteria. Over a period of four months, Quensen and coworkers saw a stepwise dechlorination of the higher chlorinated congeners to produce the lower chlorinated congeners. Brown and coworkers (1987a and 1987b) postulated such a stepwise dechlorination process based on their results from Hudson River sediment cores.

With regard to congener selectivity, there is a loss of meta and para chlorinated congeners compared to ortho chlorinated congeners. Figure 7-1 contains the average number of meta and para chlorines and ortho chlorines calculated for each sediment sample along with averages calculated for the Aroclor mixtures (General Electric Research and Development, 1987). When Aroclor mixture averages are plotted, a linear relationship can be seen, indicating that any combination of Aroclors will also fall on this line. Therefore, any deviation from this linearity may be attributed to a physical or biological process and not a simple mixing of different Aroclors (General Electric Research and Development, 1987). Brown and coworkers maintain that an upward deviation indicates evaporation, elution and oxidative biodegradation whereas a downward deviation indicates

Figure 7-1 Ortho vs Meta/Para Congener in Sediment Samples

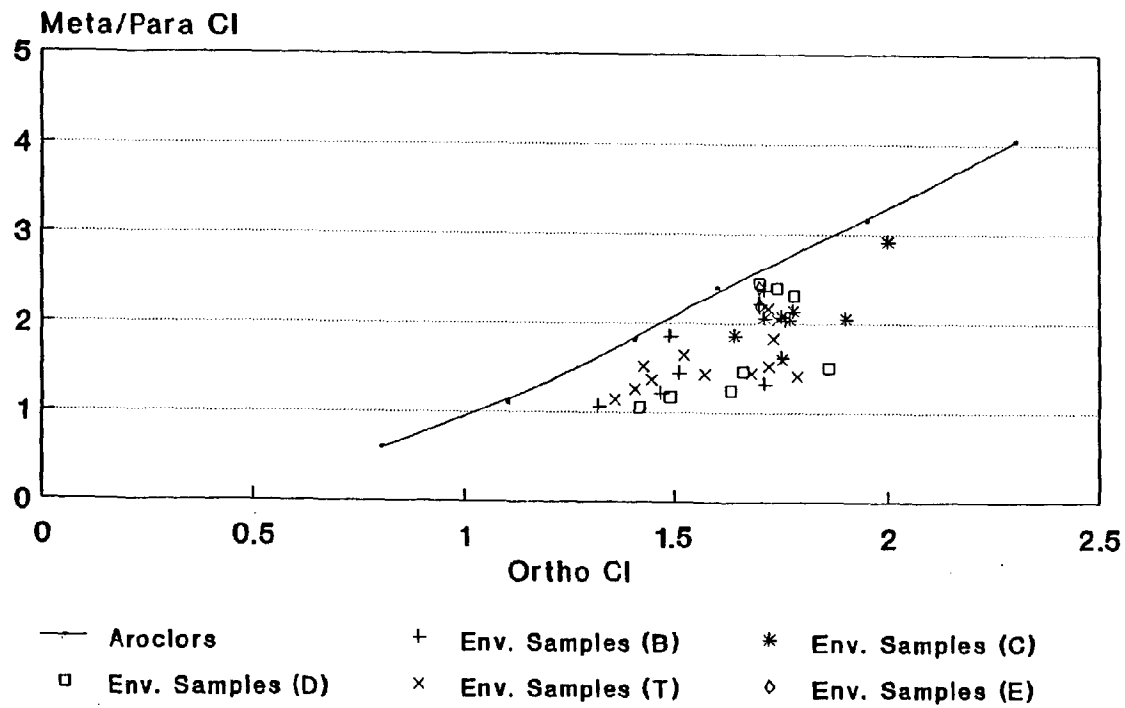


Fig 7-1

anaerobic dechlorination (General Electric Research and Development, 1987).

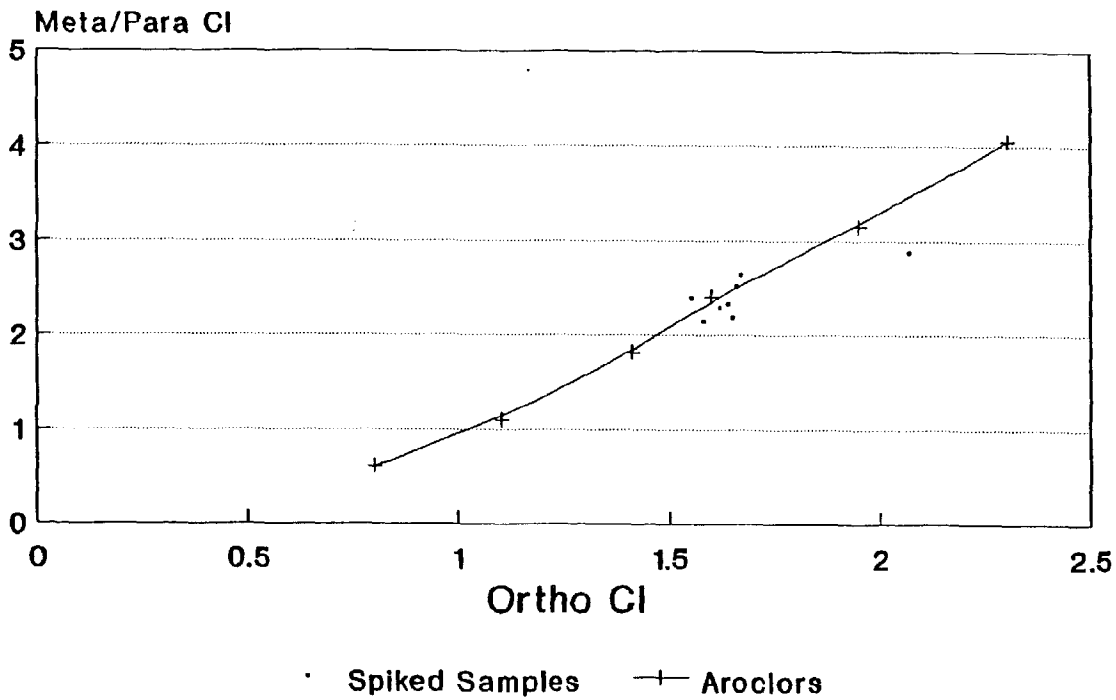
Most of the sediment samples from the Sheboygan River fall below the Aroclor mixture line indicating a depletion in the meta/para chlorines relative to the ortho chlorines. For comparison, the average meta and para and ortho chlorines for the spiked sediment samples ("clean" sediments spiked with Aroclors) were calculated. The spiked sediments could be thought of as a control since they do not undergo microbial degradation. These are shown in figure 7-2. Note that they all lie along the line as predicted (General Electric Research and Development, 1987). In a laboratory study of anaerobic dechlorination a marked loss in average number of meta/para chlorines per biphenyl molecule was also seen (Quensen et al., 1988). Brown and coworkers found similar results in their field study of Hudson River sediments (1987a).

Exactly why bacteria selectively dechlorinate meta and para chlorines is unclear. Rusling and Miaw (1989) have shown that reductive dechlorination of meta and para chlorines gives a greater Gibbs free energy value than reductive dechlorination of ortho chlorines. It is possible that bacteria which selectively dechlorinate meta and para chlorines have a biological advantage over those that do not.

With regards to specific congeners, several congeners are

Fig 7-2

Figure 7-2 Ortho vs Meta/Para Congeners in Spiked Sediments



found in abundance which would not be expected based simply on physical partitioning or on the original weight percents present in the Aroclor mixtures. Table 7-2 contains the average weight percent of each congener in the samples containing over 50 ppm PCBs as well as the weight percents of congeners in Aroclor 1248, Aroclor 1254 and a 50/50 combination of Aroclors 1248 and 1254.

In the sediment samples, there is a general trend of enrichment of the lighter chlorinated congeners and depletion of the heavier chlorinated congeners. In particular, several congeners were significantly enriched in comparison to the 50/50 combination of Aroclor 1248 and Aroclor 1254. Congeners with a greater than two percentage point increase, relative to the 50/50 mixture, were congeners 5/8, 19, 17, 24/27, 16/32, 26 and 47/48. Congeners with a greater than two percentage point decrease were 18, 28/31, 52, 44, 70/76, 66/95, 56/60, 101, 77/110, 132/153/105, and 138/163.

It is interesting to note that these results are comparable to the results of Brown and coworkers (1987b). Table 7-3 shows the congeners Brown and coworkers found to be significantly depleted and enriched in the sediments. With the exception of congener 16 which coelutes with congener 32 in this study, and congeners 47 and 49, all of the congeners

Table 7-2 Changes in Congeners in Sheboygan River Sediment Relative to Aroclors (Sediment Samples with conc. greater than 50 ppm)

Congener	Concentration (Wt. Percent)			
	Sample Average	Aroclor[a] 1248	Aroclor[a] 1254	50/50 Mixture
1	0.6	<0.1	<0.1	
3	b.d.	<0.1	<0.1	
4/10	2.1	0.8	<0.1	
7	0.1	0.1	<0.1	
6	0.8	0.1	<0.1	
5/8	29.1	2.1	0.1	++
19	2.5	0.5	0.1	++
18	0.1	5.0	0.1	--
17	9.4	1.2	<0.1	++
24/27	3.0	0.2	<0.1	++
16/32	10.0	2.0	<0.1	++
26	2.3	0.4	<0.1	++
28/31	6.4	17.7	0.6	--
33	0.1	2.9	<0.1	
22	0.5	0.6	<0.1	
45	0.4	0.9	<0.1	
46	0.1	0.5	<0.1	
52	2.1	4.4	4.2	--
49	2.4	3.0	1.0	
47/48	7.5	4.0	0.4	++
44	1.1	5.2	3.0	--
37/42	1.4	5.2	0.1	
41/64/71	2.0	2.5	0.4	
40	0.1	1.1	0.1	
74	0.4	2.6	0.9	
70/76	0.3	7.1	4.4	--
66/95	3.8	9.0	7.7	--
91	0.9	<0.1	0.9	
56/60	0.13	5.9	0.9	--
84/92	1.4	1.4	4.0	
101	0.8	1.7	7.1	--
99	0.5	0.9	2.5	
97	0.2	0.9	2.2	
87	0.1	0.9	3.1	
85	0.1	0.9	1.5	
136	0.1	0.1	1.0	
77/110	2.4	2.5	9.2	--
82	b.d.	<0.1	0.9	
151	0.2	0.1	0.8	

Table 7-2 Continued

Congener	Sample Average	Aroclor 1248	Aroclor 1254	
135/144	0.2	0.1	1.4	
149	0.8	0.4	5.0	
118	0.4	0.8	3.0	
146	0.3	<0.1	2.2	
132/153/105	1.1	1.5	11.0	--
141	b.d.	<0.1	0.5	
137/176	b.d.	<0.1	0.5	
138/163	0.6	0.3	5.6	--
182/187	0.2	0.1	0.2	
183	0.1	<0.1	0.3	
185	b.d.	<0.1	0.1	
174	b.d.	0.1	0.4	
177	0.1	<0.1	0.1	
171/202	b.d.	<0.1	0.1	
172/197	b.d.	<0.1	0.1	
180	0.1	0.8	1.0	
170/190	0.2	<0.1	1.0	
201	0.1	0.1	<0.1	
196/203	b.d.	0.1	<0.1	
195/208	b.d.	<0.1	<0.1	
194	b.d.	<0.1	<0.1	
206	b.d.	<0.1	<0.1	

[a] -- data from Manchester, 1988
b.d. -- indicates below detection

-- indicates a decrease by at least two percentage points from 50/50 ratio

++ indicates an increase by at least two percentage points from 50/50 ratio

Table 7-3 Comparison of Congeners Predicted by Brown et al.(1987b) based on Anaerobic Degradation to Congeners Observed in this Study

Diminished Congeners	This Study[a]	Enriched Congeners	This Study[a]
33	-	2	+
22	-	6	+
74	-	8	++
70	--	4	+
66	--	10	+
56	--	17	++
60	--	27	++
118	-	32	++
105	--	19	++
18	--	53	n.m.
16	++		
44	--		
42	n.m.		
87	-		
85	-		
82	-		
101	--		
99	-		
97	-		
153	--		
138	--		
141	-		
52	--		
49	+		
47	++		

[a] indicates samples with concentration above 50 ppm
- indicates present in diminished quantities relative to 50/50 ratio
-- indicates a decrease by at least two percentage points from 50/50 ratio
+ indicates present in increased quantities relative to 50/50 ratio
++ indicates an increase by at least two percentage points from 50/50 ratio
n.m. not measured in this study

which were found to be depleted in Brown's study were also found to be depleted in this study.

The concentration of PCBs in the sediment appeared to be a significant variable. As mentioned before in areas of low PCB concentrations, the sediment chromatograms appeared to be similar to the original Aroclors. Chromatograms from areas of high concentration had much higher weight percents of the lower chlorinated congeners relative to the original Aroclors. Other researchers have noted that anaerobic dechlorination appears to occur only when there are high concentrations of PCBs present (Brown et al., 1984, Brown et al., 1987b, Quensen et al., 1988, Rhee et al., 1989).

Microbial degradation is often restricted to areas of high substrate concentrations. For example, toluene, xylene and naphthalene are metabolized by bacteria at high concentrations but not at low concentrations (Alexander, 1985). The minimum concentration of a chemical which is needed to support growth of a microbial population is referred to as the threshold concentration (Alexander, 1985). Above the threshold concentration organisms are able to obtain sufficient energy for both maintenance and growth. As the concentration of a chemical compound decreases, the amount of the compound available to the organism by diffusion decreases until insufficient quantities are available for both growth and

maintenance (Alexander, 1985). Below this threshold concentration, additional energy sources must be found to support growth of the population, since the organism is no longer able to completely mineralize the substrate.

Cometabolism occurs below the threshold concentration to provide additional sources of nutrients for the organism. Anaerobic dechlorination of higher chlorinated congeners in some cases has been shown to occur by cometabolism with the addition of biphenyl (Rhee et al., 1989). Based on the different chromatographic patterns seen for high and low concentrations of PCBs, it is quite possible that some threshold concentration exists for the anaerobic dechlorination of PCBs.

7.3. Discussion of Water Results

7.3.1. Total PCB Concentrations in Water

The total PCB concentrations in water are similar to other values which have been reported for the Sheboygan River (see table 7-4). In a survey of Wisconsin Rivers, Marti (1984) found the Sheboygan River contained 0.103 (s.d. 0.036) ppb total PCB in the river. The values reported in this study are considerably higher than the concentrations found by Crane (Crane, 1990) in the Fox River which ranged from 1.43 to 34.4 ppt for the dissolved fraction and 2.11 to 103 ppt for the

particulate fraction or by Swackhammer (1985) in Lake Michigan which ranged from 0.3 to 3 ppt total PCBs. Mudroch and coworkers (1989) found the Wheately Harbor in Lake Erie to have concentrations ranging from 0.086 ppb to 0.291 ppb total PCBs. In Hamilton Harbor in Lake Ontario, concentrations ranged from 0.086 ppb to 0.213 ppb total PCBs (Mudroch et al., 1989). However, the concentrations in sediment from the Great Lakes are lower than those from the Sheboygan River.

Table 7-4 Total PCB Concentrations in Water

Site	Concentration (ppb)		
	WDNR 1978	Remedial Investigation 1987	This Study
Above Sheboygan Falls Dam	<0.3	<0.05 P <0.05 D	0.007 P 0.003 D
Rochester Park	<0.4		0.213 P 0.038 D
Above Upper Kohler Dam	<0.2	0.267 P 0.118 D	0.448 P 0.197 D
Above Lower Kohler Dam		0.150 P 0.078 D	0.480 P 0.166 D
Sheboygan	0.3	0.159 P 0.059 D	0.301 P 0.070 D

P - Particulate Fraction
D - Dissolved Fraction

Source: Wisconsin Department of Natural Resources, 1989

7.3.2. Homolog and Congener Distribution in Water

The weight percents of each of the homolog groups did not vary greatly among the samples. Higher concentrations of the lower chlorinated congeners were found in the dissolved phase than in the particulate phase. This would be expected since the lower chlorinated congeners are in general more soluble and less likely to partition onto the particulate fraction.

Congeners 1, 4/10, 5/8, 17, 16/32, 28/31, 41/64/71, 47/48, 52 and 66/95 were the most prominent congeners in the dissolved fraction. Congeners 5/8, 17, 28/31, 47/48, 41/64/71, 52, 66/95, 70/76, 44 and 77/110 were the most prominent congeners in the particulate fraction. Since the solubility of PCB congeners generally decreases with increasing chlorination and since the most prominent congeners listed in table 6-9 do not show particularly high solubilities (Shiu and MacKay, 1986), it is likely that their abundance in the water column is a function of their high concentrations in the sediment.

The distribution and relative percentages of the most prominent congeners in the water increasingly reflect the congener distribution of the sediments in the lower reaches. For example, at Rochester Park congener 5/8 constitutes 2.9 percent of the particulate fraction of PCBs. In contrast, at

the other sites, congener 5/8 is 9.2 percent of the particulate fraction. The weight percents of congeners 47/48 and congener 17 also increase at the other sites. In the dissolved phase, this trend is more pronounced. At Rochester Park, congener 5/8 constitutes 14.6 percent of the dissolved PCB concentration. The average for the other sites for congener 5/8 is 30 percent. The weight percents of congeners 17, 16/32 and 47/48 in the dissolved phase also increase. Most likely, these increases in specific congeners, which are seen in abundance in the sediment, are due to the scouring of the river bottom.

7.4. Discussion of Fish Results

7.4.1 Total PCB concentrations in Fish

The total PCB concentrations found in the fish that were analyzed are similar to other reports in the literature. The Wisconsin Department of Natural Resources routinely has fish analyzed from the Sheboygan River. Recent results are listed in table 7-5. In the Kiwanis Park area, the Wisconsin Department of Natural Resources found the fish to contain an average total PCB concentration of 8.4 ppm which is comparable to 7.6 ppm found in this study (Wisconsin Department of Natural Resources, 1989). In the Harbor area, the average totals of 42.7 ppm are considerably higher than

those found in this study.

Ten of the fish in this study were from the Kiwanis Park area and eleven were from the harbor. In a survey of fish in Wisconsin rivers (including the Sheboygan River) values ranged from 0.07 to 7.0 ppm (Maack and Sonzogni, 1988). Carp and lake trout from the Wisconsin side of Lake Michigan were found to have PCB concentrations similar to those reported in carp and other fish in this study (16.3 ppm for carp and a range of 2.9- 33.8 ppm for trout (Kleinert, 1976 in Swain, 1983)).

Table 7-5 Total PCB Concentration in Fish from the Sheboygan River (ppm)

Site	Year	# of Fish	Ave Conc.(ppm)	Species
Above Sheb. Falls	1987	6	<0.30	Varied
Below Sheb. Falls	1987	7	24.9	Carp, Sm Bass
Upper Kohler Dam	1987	3	7.67	Sm Bass
Lower Kohler Dam	1985	9	9.65	Varied
Kiwanis Park	1986	9	8.4	Varied
Sheboygan	1983	17	42.7	Varied

(Source: Wisconsin Department of Natural Resources, 1989)

In the more highly contaminated Hudson River, fish were found to have total PCB concentrations ranging from below detection to 7.07 ppm (Califano et al., 1982).

The highest total PCB concentrations were found in fish that had high concentrations of fat to body weight. This finding suggests a positive correlation between percent fat and the level of PCBs in fish. To test this hypothesis, the percent fat was regressed against the total PCB concentration. The results are plotted in figure 7-3. The regression equation for this line follows:

Log PCB = 1.061 log fat - 0.644 R^2 value of 0.71.

The correlation between percentage of fat in the fish and the PCB contamination is significant.

The fish samples were analyzed using both a capillary column and a packed column. The two methods were compared to see how well they agreed. Twenty one samples were analyzed with both types of columns. The measurements are compared by regression:

Log Cap Col = 1.05 log Packed Col + 0.0005 R^2 value 0.88.

These results are plotted in figure 7-4. This result is important since it implies that packed column measurements made over the last twenty years (by comparing chromatographic patterns of a sample with that of pure Aroclors (Erickson,1986)) produce results similar to that

Figure 7-3 Correlation between Fish Fat and PCB Concentration

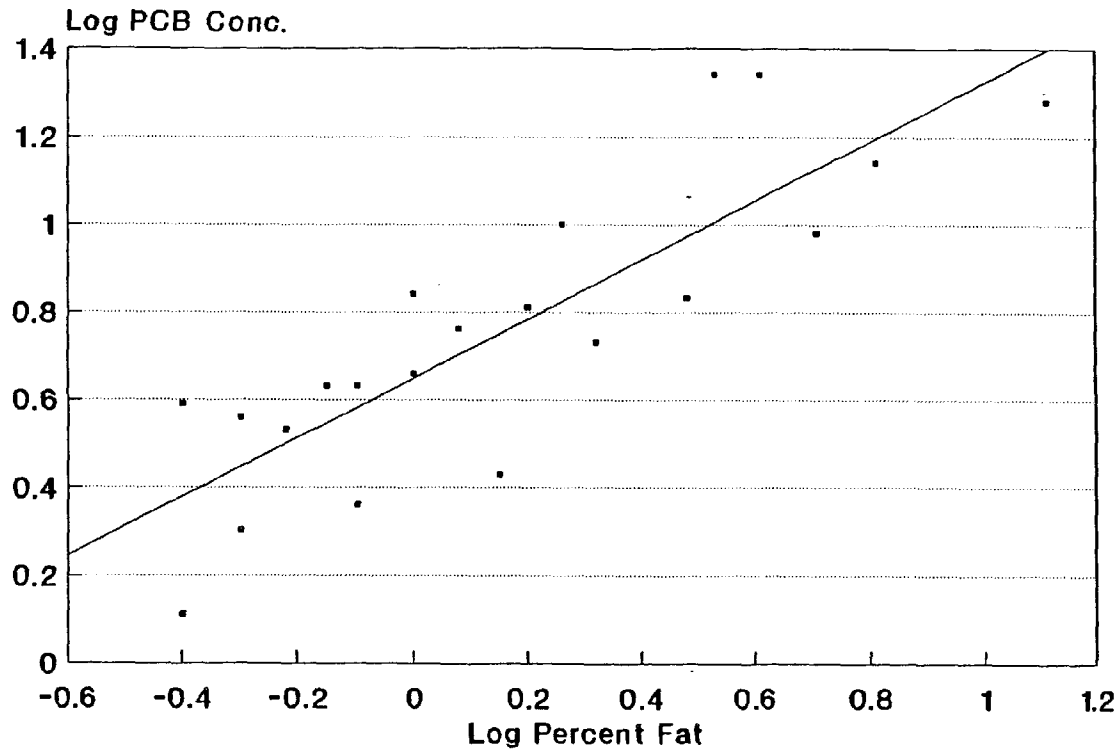
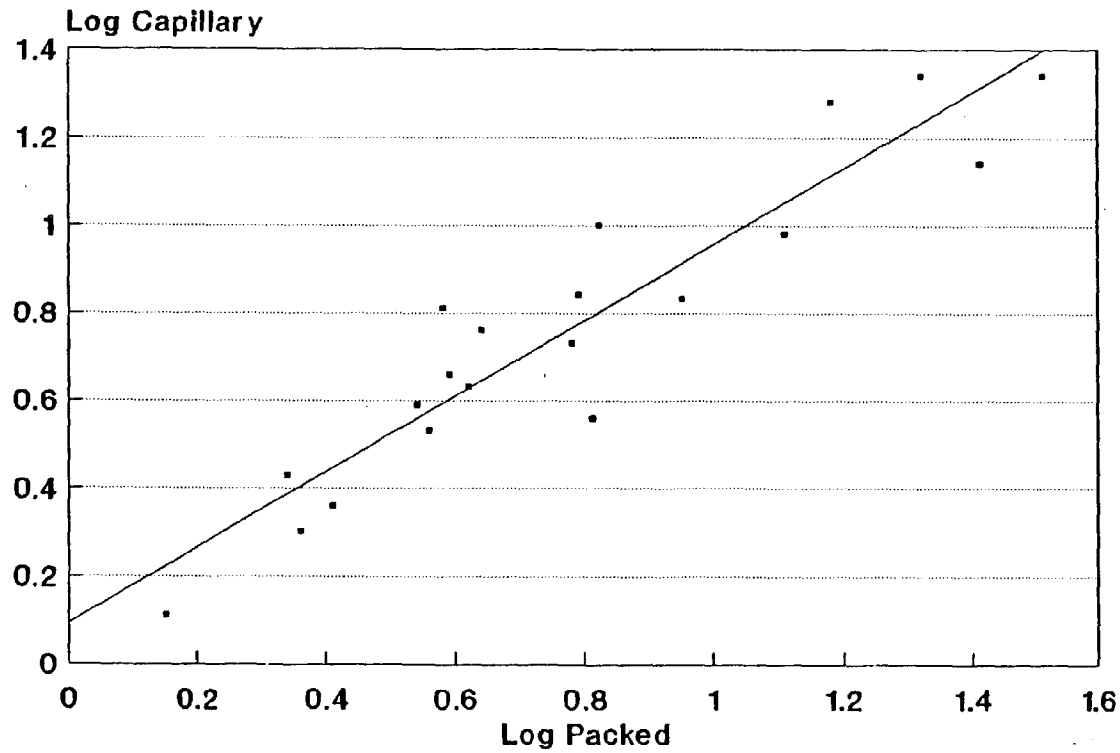


Figure 7-4 Correlation between Packed and Capillary Column Results



obtained by summing individual congeners. Maack and Sonzogni (1988) also found good agreement for total PCBs measured by packed and capillary column chromatography.

7.4.2. Homolog Patterns in Fish

The enrichment of the higher chlorinated congeners and depletions of the lower chlorinated congeners seen in the fish homolog patterns (figure 6-12) has been observed by others. In the heavily PCB contaminated Hudson River, Califano and coworkers (1982) found that fish congener patterns were more typical of Aroclor 1254 than of the material introduced into the river, Aroclor 1242. Because fish generally are unable to metabolize the higher chlorinated congeners (Niimi and Oliver, 1989; Dekock and Lord, 1988; Bruggeman et al., 1981; Lech and Peterson, 1983), it is not surprising to see an enrichment in the higher chlorinated congeners. Work by Peterson and Lech (1983) suggests that fish are only able to metabolize mono, di and tri chlorinated biphenyls. It is interesting to note that fish tend to metabolize PCBs much more slowly than mammals (Lech and Peterson, 1983).

The higher chlorinated congeners tend to bioaccumulate not only within the fish but also within the entire food chain. Since many of the fish in this study are carnivorous and are

therefore higher on the food chain, it would be expected that higher total concentrations of PCBs should be found in the fish in comparison to other biological compartments (van der Oost et al., 1988; Oliver and Niimi, 1988). In particular, higher concentrations of the higher chlorinated congeners is expected (Oliver and Niimi, 1988). The data collected in this study support these findings as the fish are significantly enriched in the higher chlorinated congeners relative to the water and overall are enriched in total PCB concentration relative to the water.

7.4.3 Most Prominent Congeners in Fish

The most abundant congeners found by Maack and Sonzogni (1988), and Niimi and Oliver (1988) are listed in table 7-6. The standard deviations for the most abundant congeners found in this study were fairly small indicating that the percentages of each congener amongst the samples was relatively constant. This occured despite the variation in species, PCB content and weight of the fish.

As mentioned before several of the congeners seen in high concentrations in the fish in this study are prominent in commercial Aroclors. For example, in Aroclor 1248 the top three congeners are 28/31, 70/76, and 66 in the following percentages, 17.7, 7.1 and 7.1 (Manchester, 1988).

Table 7-6 A Comparison of Most Prominent Congeners
(Weight Percent)

Congener	This Study Species Varied[c]	Niimi and Oliver[a] Br. Trout	Niimi and Oliver[a] Lk. Trout	Niimi and Oliver[a] Rainbow Trout	Maack and Sonzogni[b] Species Varied
	Percent				
28/31	5.7 (1.4)				7.9
47/48	6.8 (0.9)				
49	4.0 (0.47)				
41/64/71	4.0 (0.66)				
66/95	8.5 (0.78)				7.4
70/76					4.5
101	4.2 (0.3)	6.3	6.1	7.2	6.0
118	4.5 (0.6)	5.5	6.3	5.6	2.7
77/110	7.7 (0.9)	5.6	4.8	5.8	6.8
132/153 /105	4.9 (0.85)	7.3	10.8	9.1	11.0 2.6[d]
138/163[e]	4.8 (0.81)	5.4	5.9	5.7	8.1
146					3.4
149		5.1	3.4	4.8	4.3
180		4.2	4.6	4.5	3.7

[a] Oliver and Niimi, 1989
[b] Maack and Sonzogni, 1988
[c] Parentheses indicate standard deviation
[d] Congener 105 was reported separately from congeners 132/153
[e] Congener 138/163 was reported together in this study; however the other researchers reported congener 138 alone.

In Aroclor 1254, the top three congeners 153/132/105, 110 and 95 constitute the following percentages, 11.0, 9.2, and 7.6 (Manchester, 1988). Congeners 47/48, 49, 41/64/71 were prominent congeners in the sediment which may account for their presence in the fish.

Oliver and Niimi (1988) found that twelve congeners, 153, 101, 84, 138, 110, 180, 87/97, 149, 187/182 and 105, contributed to over half the total percentage of PCBs in fish. Maack and Sonzogni (1988) found the ten most prominent congeners contributed over half of the total percentage (66 percent). In this study, it was also found that the ten most abundant congeners contributed over half the total percentage of PCBs in fish (55 percent).

Several congeners have been identified as being significantly more toxic than other congeners (Safe et al., 1985). They are characterized as containing at most one ortho chlorine and two para and at least two meta chlorines. Congeners containing no ortho chlorines are considered the most toxic; their structure is similar to TCDD and elicits similar toxic responses (Safe et al., 1985). Of the nonortho chlorines, only congener 77/110 was detected using the methods described in this study (see table 6-11). Although congeners 77/110 were detected, it is unlikely that the contribution of congener 77 is significant (Hansen, 1987; Maack and Sonzogni,

1988). Of the mono ortho chlorinated congeners, 105 and 118 were detected (see table 6-11). The precise quantities of congener 105 are not known since this congener coelutes with 153 and 132; however, Maack and Sonzogni (1988) found congener 105 to contribute approximately twenty percent of the combined total of the three congeners in their study of Wisconsin fish. Congener 118 was found in all fish (see table 6-11).

Maack and Sonzogni (1988) found only two of the monochlorinated biphenyls, congener 118 and 105, in their study. They were unable to determine the presence or absence of congener 77 since it coeluted with congener 110 which was present in large concentrations in the sample. Congener 77 has been found in fish from the Waukegan Harbor in Lake Michigan in quantities slightly diminished from the original Aroclor introduced, Aroclor 1254 (Huckins et al., 1988). Congener 105 was also found in fish from the Waukegan Harbor in approximately the same quantities as that in Aroclor 1254.

The retention of the various PCB congeners is thought to be a function of selective elimination of congeners rather than selective uptake (Bruggeman et al., 1981). Uptake efficiencies have been found to be independent of their partition coefficients (Oppenhuizen and Schrap, 1988; van der Oost et al., 1988). However, the elimination rates are found to be a function of hydrophobicity. For the higher

chlorinated congeners, elimination rates are very low (van der Oost et al., 1988; Bruggeman et al., 1981). Conversely, the lower chlorinated congeners have been found to have high rates of clearance. In one study of dichlorobiphenyl, the compound was found to be completely excreted 14 days after the fish had been switched from a contaminated environment to a clean one (Bruggeman et al., 1984). Table 7-7 gives a few of the clearance rates for PCBs as reported in the literature. Note that the clearance rates increase with increasing molecular weight.

Table 7-7 Clearance Rates for Specific Congeners

Congener	Halflife (days)	Source
2,5	10	Bruggeman et al., 1981
2,3',4',5	60	"
2,2',5	14.4	"
3,3,4,4	34.6	Huckins et al., 1988
2,3,5	2.5	"
2,2',3,3'5,5'	139*	Oppenhuizen and Schrap, 1988
2,2',3,3',4,4',6,6'	139*	"

* -- Based on elimination rates given in their paper

It is well known that PCBs can be bioaccumulated to rather high levels in aquatic organisms even though the ambient concentrations in the water may be considerably lower (Oliver

and Niimi, 1988; Gooch and Hamdy, 1983). The ability of an organism to accumulate a chemical from water is often characterized by the bioconcentration factor, i.e., the concentration of the chemical in the organism divided by the concentration in the surrounding water (MacKay, 1982).

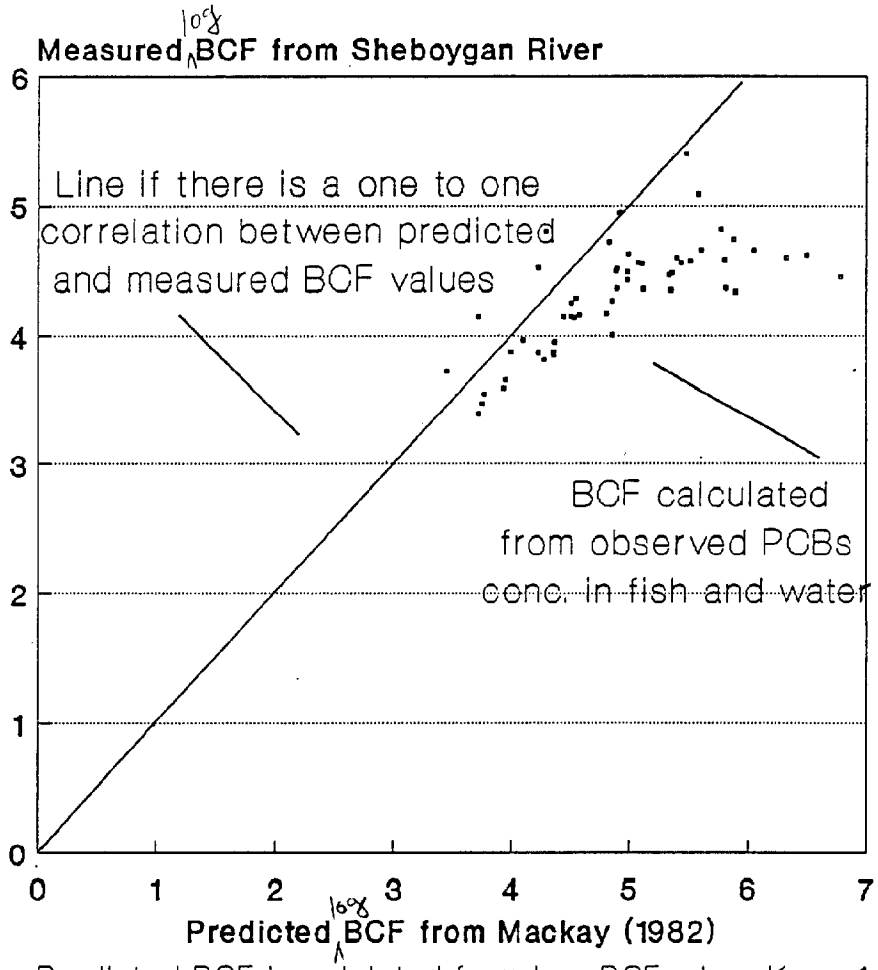
Mackay predicted a relationship between the bioconcentration factor and the octanol-water partition coefficient based on thermodynamics. Incorporating laboratory data from a Veith et al. (1979) into the thermodynamic prediction, Mackay developed the following equation:

$$\text{Log BCF} = \text{Log } K_{ow} - 1.32 \qquad (R^2 = 0.95).$$

Figure 7-5 is a comparison between the BCF values calculated from this study and Mackay's predicted relationship. The bioconcentration factors found in this study were obtained by dividing the average fish concentration of a particular congener by the concentration in water of that particular congener. Then the octanol-water coefficient for the particular congener was found and a second BCF value calculated using the relationship of Mackay.

With the exception of the first two congeners and two other congeners, the BCF values calculated in this field study were lower than those predicted by Mackay (1982). This is surprising because the BCF calculated from laboratory studies usually underestimates the amount actually accumulated by fish

Figure 7-5 Predicted BCF from Mackay versus Calculated BCF from Field data



Predicted BCF is calulated from Log BCF = Log Kow -1.32

• **BCF (from data)**

BCF (from data) is (conc. PCB in fish)/(conc. PCB in water)

fig 7-5

in the environment, in part because it ignores diet as a contribution of PCBs. It has been shown that the diet is a major source of PCBs to fish particularly with regards to the higher chlorinated PCBs (Niimi and Oliver, 1988; Rubenstein et al., 1984; Bruggeman et al., 1984). Although the BCF values in this study were slightly lower than the values suggested by Mackay, these values are within the range of values reported by Mackay (1982) in a survey of the literature (see table 7-8).

Above a log octanol-water partition coefficient of about six, the relationship between the octanol-water partition coefficient and bioconcentration factor is no longer a simple linear relationship. This deviation can be seen in figure 7-5 in which Mackay's predicted line continues upward whereas the BCF values based on field data start to decrease at a log BCF value of about five, corresponding to a log K_{ow} of about six. It is therefore difficult to predict the bioconcentration factor for chemicals having log octanol-water partition coefficients above six. Other researchers also have reported that the relationship between bioconcentration factor and the octanol-water partition coefficient is no longer linear after there are six or more chlorines on the biphenyl (Bruggeman et al., 1984).

Table 7-8 BCF Values Reported in the Literature
(from Mackay, 1982)

Relationship	Cite
BCF = 0.907log Kow -0.361	Baughman et al., 1984
BCF = 0.837log Kow -0.770	"
BCF = 1.160log Kow -0.75	Metcalf et al. 1975
BCF = 0.85log Kow -0.7	Veith et al. 1979
BCF = 0.542log Kow +0.124	Neeley et al. 1974
Log BCF =1.15 log Kow -0.4	This study

Several reasons have been suggested for this decline. The more highly chlorinated compounds may not be as readily available to the fish relative to the lower chlorinated congeners due to their low solubility in water. In addition, the higher chlorinated congeners are more likely to form colloids that can not permeate fish membranes and therefore will not be adsorbed (Bruggeman et al., 1984). It is also thought that compounds with molecular weight (greater than 600) or large volumes may not be adsorbed because they are too large to permeate membranes (Oliver and Niimi, 1986).

Chapter 8 Conclusions

8.1. Sediment

The overall finding from this study is that the PCB congener distributions from highly contaminated sediments (greater than 50 ppm) are considerably different from the PCB congener distributions of the Aroclors originally used at the industrial site. The highly contaminated sediments are enriched in mono, di, and tri chlorinated congeners relative to the original Aroclors. In sediments containing concentrations of PCBs less than 50 ppm, the congener distributions were similar to the original Aroclors.

The enrichment in the highly contaminated sediments with lower chlorinated congeners is not easily explained by existing physical-chemical partitioning relationships or known abiotic chemical reactions. This suggests that a biotic process ~~is~~ may be responsible for the enrichment. Anaerobic dechlorination by bacteria has been shown to occur in other highly contaminated river sediments. The similarity of the PCB congener distribution in highly contaminated areas of the Sheboygan River to the results from other anaerobic microbial degradation studies at highly contaminated sites suggests that the congener distribution is the result of microbial degradation. Microbial degradation is also suggested by the concentration dependence of the patterns seen in the

sediments. Other studies of anaerobic microbial degradation of organic compounds have found a similar concentration dependency.

Specific findings are listed below:

1) The river sediment is largely composed of fine grain sand (40 to 90 percent of the sample). Grain size correlated with PCB content. Smaller particles were found to contain higher concentrations of PCBs relative to larger particles of the same volume.

2) Total PCB concentrations varied spatially in the river sediment. With depth, PCB concentrations in sediment cores were the lowest in surface sediments (top 15 cm of the sediment core) and the bottom sediments (greater than 45 cm from the sediment-water interface). Highest concentrations were found in the center of the core (15-45 cm down from the sediment-water interface).

3) The total concentration of PCBs diminished with distance from the source, presumably due to the transport of PCBs downstream.

4) In sediment samples with greater than 50 ppm PCBs

concentrations of congeners 4/10, 5/8, 17, 24/27, 16/32, 47/48, and 26 were found to be enriched in the sediment compared to the original Aroclors. Congeners 5/8 were in the greatest abundance (29.2 percent on average). In six of the samples, congeners 5/8 were found to comprise a large percentage of the sample (43.7 percent on average).

5) Congeners with a greater than two percent increase relative to a 50/50 mix of Aroclor 1248 and Aroclor 1254 were 5/8, 19, 17, 24/27, 16/32, 26 and 47/48. Congeners 5/8, 17, 24/27 and 16/32 were also prominent congeners in the Hudson River where anaerobic dechlorination has been reported to occur.

6) Congeners with a greater than two percent decrease relative to a 50/50 mixture of Aroclor 1248 and 1254 were 18, 28/31, 52, 44, 70/76, 66/95, 56/60, 101, 77/110, 132/153/105, and 138/163. In the Hudson River, congeners 44, 70/76, 66/95, 132/153/105, and 138 were also found in diminished quantities.

7) Harbor sediment samples had congener patterns similar to the original Aroclors and to the river sediments containing less than 50 ppm.

8.2. Water

The total PCB concentrations in water were highest several miles downstream from the source. The water samples were less contaminated directly at the source and in the harbor. The variation of total PCBs in water is most likely due to the transport of PCBs downstream. The fact that the highest concentrations were found several miles downstream from the source is not surprising. Water concentrations at the source would be lower because of constant inflow of "clean water" at Rochester Park and subsequent dilution of the contaminated river water.

Other findings are summarized below:

1) With distance from the source, the water developed a congener profile that was similar to the sediments. Presumably, this is due to the entrainment of sediment particles in the water column as a result of turbulent scouring.

2) Higher concentrations of the lighter chlorinated congeners were found in the dissolved phase compared to the particulate phase. These results would be expected based on the partition coefficients and solubility constants for these congeners.

3) Congeners 4/10, 5/8, 17, 16/32, 41/64/71 were found to be

in high concentrations in the water relative to the original Aroclors used at the source. These congeners were found to be prominent congeners in the sediment.

8.3. Fish

The congener distribution in fish bore little resemblance to the sediment or water distribution. From a modelling and regulatory perspective, this finding is significant because some researchers have advocated using sediment as a predictor of congener distributions in fish. Clearly, for rivers such as the Sheboygan River or Hudson River, use of these models is inappropriate since the fish are enriched in higher chlorinated congeners while the sediment is not.

Specific findings are listed below:

1) In contrast to the water distribution, the fish do not contain the lighter chlorinated congeners. This is consistent with previous studies of fish that show the lighter chlorinated congeners are metabolized and the heavier chlorinated congeners are bioaccumulated.

2) There was a positive correlation between the weight percent fat in fish and PCB content.

3) Fish samples were analyzed for total PCBs using both

capillary column and packed column techniques. Total PCBs measured by both techniques were similar.

4) Congeners 28/31, 47/48, 49, 41/64/71, 66/95, 101, 118, 77/110, 132/153/105 and 138/163 were prominent in fish. Congeners 28/31, 70/76, 66, 153/132/105, 110, 95 are the top three congeners in Aroclor 1248 and Aroclor 1254 respectively. Congeners 47/48, 49, 41/64/71 were found to be in abundance in the sediment. Congeners 77, 105, and 118, which are considered to be among the most toxic PCBs, coelute so their relative importance (in terms of concentration in fish) is not clear.

8.4. Concluding Remarks

Further studies of anaerobic dechlorination of PCBs is warranted to determine if environmental conditions can be enhanced to encourage reductive dechlorination of PCBs. Specifically, investigations are needed to identify the microorganisms responsible for dechlorination, to determine the necessity of additional substrates (cometabolism), and to determine the type of anaerobic environment necessary (nitrogen atmosphere versus carbon dioxide atmosphere).

In addition, further research is warranted to determine the feasibility of using anaerobic microbial degradation as

a large-scale treatment option for PCBs. Currently, the Environmental Protection Agency (EPA) is conducting a pilot project on anaerobic microbial degradation of PCBs using highly contaminated Sheboygan River sediments. At present however, much remains to be learned before microbial anaerobic degradation can be thought of as a efficient and cost-effective, large-scale treatment option.

Bibliography

Ahmed, M., and D.D. Focht. 1973. Degradation of Polychlorinated Biphenyls by two species of Achromobacter. Can. J. Microbiol., Vol. 19.

Alexander, M. 1981. Biodegradation of Chemicals of Environmental Concern. Science, Vol. 211. pp. 132-138.

Alexander, M. 1985. Biodegradation of Organic Chemicals. Environ. Sci. Technol., Vol. 18. No. 2. pp. 106-111.

American Society of Testing and Materials (ASTM). 1987. Vol. 4.08 Soil and Rock; Building Stones ASTM, Philadelphia, PA.

Baker, J.E., Capel, P.D. and S.J. Eisenreich. 1986. Influence of Colloids on Sediment-Water Partition Coefficients of Polychlorobiphenyls Congeners in Natural Waters. Environ. Sci. Technol. Vol. 20. No. 11. pp.1136-1143.

Bedard, D.L., Haberl, M.L., May, R.J., and M.J. Brennan. 1987a. Evidence for Novel Mechanism of PCB Metabolism in Alcaligenes eutrophus H850. Appl. Environ. Microbiol., Vol.53. No. 5. pp. 1103-1112.

Bedard, D.L., Wagner, R.E., Brennan, M.J., Haberl, M.L. and J.F. Brown. 1987b. Extensive Degradation of Aroclors and Environmentally transformed Polychlorinated Biphenyls by Alcaligenes eutrophus H850. Appl. Environ. Microbiol. Vol. 53., No. 5. pp. 1094-1102.

Blasland and Bouck Engineers. 1988. Preliminary Report on Results of Sheboygan River and Harbor Remedial Investigations. Syracuse. New York.

Bopp, R.F., Simpson, H.J., Olsen, C.R. and N. Kostyk. 1981. Polychlorinated Biphenyls in Sediments of the Tidal Hudson River, New York. Environ. Sci. Technol., Vol. 15. No. 2. pp. 210-216.

Boyer, S.K., McKenna, J., Karliner, J. and M. Nirsberger. 1985. A Mild and Efficient Process for Detoxifying Polychlorinated Biphenyls. Tetrahedron Letters. Vol. 26. No. 31. pp. 3677-3680.

Brown, J.F., Wagner, R.E., Bedard, D.L., Brennan, M.J., Carnahan, J.C., May, R.J. and T.J. Tofflemire. 1984. PCB Transformations in Upper Hudson Sediments. Northeastern Environmental Science, Vol. 3. No. 3/4. pp. 166-178.

Brown, J.F., Bedard, D.L., Brennan, M.J., Carnahan, J.C., Feng, H. and R.E. Wagner. 1987a. Polychlorinated Biphenyl Dechlorination in Aquatic Sediments. Science, Vol. 236. pp. 709-712.

Brown, J.F., Wagner R.E., Feng, H., Bedard, D.L., Brennan, M.J., Carnahan, J.C. and R.J. May. 1987b. Environmental Dechlorination of Polychlorinated Biphenyls. Environmental Toxicology and Chemistry, Vol. 6. pp. 579-593.

Bruggeman, W.A., Marton, L.B.J.M., Kooiman, D. and O. Hutzinger. 1981. Accumulation and Elimination Kinetics of Di, Tri, and Tetra chlorobiphenyls by Goldfish after Dietary and Aqueous Exposure. Chemosphere. Vol. 10. No. 8. pp. 911-832.

Bruggeman, W.A., Oppenhuizen, A., Wijbenga, A. and O. Hutzinger. 1984. Bioaccumulation of Super Lipophilic Chemicals in Fish. Toxicology and Environmental Chemistry. Vol. 7. pp. 173-189.

Brunner, W., Sutherland, F.H., and D.D. Focht. 1985. Enhanced Biodegradation of Polychlorinated Biphenyls in Soil by Analog Enrichment and Bacterial Inoculation. J. Environ. Qual. Vol. 14. No. 3. pp. 324-328.

Burkhard, L.P. 1984. Physical-Chemical Properties of Polychlorinated Biphenyls: Measurement, Estimation, and Application to Environmental Systems. PhD Thesis. Water Chemistry Program, University of Wisconsin, Madison, Wisconsin.

Burkhard, L.P., Armstrong, D.E., and A.W. Andren. 1985a. Partitioning Behavior of Polychlorinated Biphenyls. Chemosphere. Vol. 14. No. 11/12. pp.1703-1716.

Burkhard, L.P., Armstrong, D.E. and A.W. Andren. 1985b. Henry's Law Constants for Polychlorinated Biphenyls. Environ. Sci. Technol. Vol. 19. No. 7. pp.590-596.

Califano, R.J., O'Connor, J.M. and J.A. Hernadez. 1982. Polychlorinated Biphenyl Dynamics in Hudson River. Striped Bass. I Accumulation in Early Life History Stages. Aquatic Toxicology. Vol. 2. pp.187-204.

Chantry, W. 1989. Biodegradation of PCBs Sorbed to Sewage Sludge Lagoon Sediments in An Aerobic Digester. PhD Thesis. Department of Civil and Environmental Engineering, University of Wisconsin, Madison, Wisconsin.

Chen, M., Hong, C.S., Bush, B. and G.Y. Rhee. 1988. Anaerobic Biodegradation of PCBs by Bacteria from Hudson River Sediments. Ecotoxicology and Environmental Safety, Vol. 16. pp. 95-105.

Clark, R.R., Chian, E.S.K. and R.A. Griffin. 1979. Degradation of Polychlorinated Biphenyl by Mixed Microbial Cultures. Appl. Environ. Microbiol. Vol. 37. No. 6. pp. 680-685.

Connor, M.S. 1984. Fish/Sediment Concentration Ratios for Organic

Compounds. Environ. Sci. Technol. Vol. 18. No. 1. pp. 31-35.

Crane, J. 1986. Preliminary Report for PhD examination. University of Wisconsin, Madison, Wisconsin.

Crane, J. 1990. The Influence of Water/Particle Partitioning on the Transport of Water Column PCB congeners in a Contaminanted Lake System. PhD Thesis, Water Chemistry Program, University of Wisconsin, Madison.

Das, B.M. 1986. Soil Mechanics Laboratory Manual. Engineering Press, San Jose, CA.

Dekock, A.C. and D.A. Lord. 1988. Kinetics of Uptake and Elimination of Polychlorinated Biphenyls by an Estuarine Fish Species (Rhabdosargus Holubi) after Aqueous Exposure. Chemosphere. Vol. 17. No. 12. pp.2381-2390.

DiToro, D.M., Jeris, J.M. and D. Clarcia. 1985. Diffusion and Partitioning of Hexachlorobiphenyl in Sediments. Environ. Sci. Technol. Vol. 19. No. 12. pp.1169-1172.

Elzerman, A.W. and J.T. Coates. 1987. Hydrophobic Organic Compounds on Sediments: Equilibria and Kinetics of Sorption in Sources and Fates of Aquatic Pollutants R. Hites and S.J. Eisenreich (Eds.) American Chemical Society, Washington D.C.

Erickson, M. 1986. Analytical Chemistry of PCBs. Butterworth Publishers. Boston.

Fessenden, R.J. and J.S. Fessenden. 1982. Organic Chemistry. Willard Grant Press. Boston, MA.

Fathepure, B.Z., Tiedje, J.M. and S.A. Boyd. 1987. Reductive Dechlorination of 4-Chlororesorcinol by Anaerobic Microorganisms. Environ. Tox. and Chem. Vol. 16. pp. 929-934.

Fisher, J.B., Petty, R.L. and W. Lick. 1983. Release of Polychlorinated Biphenyls from Contaminated Lake Sediments: Flux and Apparent Diffusivities of Four Individual PCBs. Environ. Pollut. Series. B. Vol. 5. pp 121-132.

Furukawa, K., Tonomura, K. and A. Kamibayashi. 1978. Effect of Chlorine Substitution on the Biodegradability of PCBs. Appl. and Environ. Microbiol. Vol. 35. No. 2. pp 223-227.

Furukawa, K. 1982. Microbial Degradation of PCBs in Biodegradation and Detoxification of Environmental Pollutants A.M. Chakrabarty (Ed.). CRC Press, Boca Raton, FL.

Furukawa, K., Tomizuka, N. and A. Kamibayashi. 1983. Metabolic Breakdown of Kaneclors (PCBs) and their Products by Acinetobacter sp. Appl. Environ. Microbiol. Vol. 46.

General Electric Research and Development Center. 1987. Research and Development Program for Destruction of PCBs. Seventh Progress Report. Schenectady, New York.

General Electric Research and Development Center. 1988. Research and Development Program for Destruction of PCBs. Seventh Progress Report. Schenectady, New York.

Gooch, J.A. and M.K. Hamdy. 1983. Uptake and Concentration Factor of Aroclor 1254 in Aquatic Organisms. Bull. Environ. Contam. Toxicol. Vol. 31. pp. 445-452.

Hansen, L.G. 1987. Environmental Toxicology of PCBs in PCBs: Mammalian and Environmental Toxicology S. Safe (Ed.). Springer Verlag, Berlin.

Hawker, D.W. and D.W. Connell. 1988. Octanol-Water Partition Coefficients of Polychlorinated Biphenyl Congeners. Environ. Sci. Technol. Vol. 22. pp. 382-387.

Head, K.H. 1980. Manual of Soil Laboratory Testing. Pentech Press, London.

Hillel, D. 1982. Introduction to Soil Physics. Academic Press, New York.

Horowitz, A., Suflita, J.M. and J.M. Tiedje. 1983. Reductive Dehalogenations of Halobenzoates by Anaerobic Lake Sediment Microorganisms. Appl. Environ. Microbiol. Vol. 45. No. 5. pp. 1459-1465.

Horvath, R. 1972. Microbial Co-Metabolism and the Degradation of Organic Compounds in Nature. Bacteriological Reviews, Vol. 36. No. 2.

Horzempa, L.M. and D.M. DiToro. 1983. The Extent of Reversibility of Polychlorinated Biphenyl Adsorption. Water Res. Vol. 17. No. 8. pp.851-859.

Huckins, J.N., Schwartz, T.R., Petty, J. and C.M. Smith. 1988. Determination, Fate and Potential Significance of PCBs in Fish and Sediment Samples with Emphasis on Selected AHH-inducing Congeners. Chemosphere. Vol. 17. No. 10. pp.1995-2016.

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

Kaneko, M., Morimoto, K. and S. Nambu. 1976. The Response of Activated Sludge to a Polychlorinated Biphenyl. Water Res.

Vol. 10. pp. 157-163.

Karickhoff, S.W. 1979. Sorption of Hydrophobic Pollutants on Natural Sediments. Water Res. Vol. 13. pp. 241-248.

Karickhoff, S.W. 1981. Semi-empirical Estimation of Sorption of Hydrophobic Pollutants on Natural Sediments and Soils. Chemosphere. Vol. 10. No. 8. pp. 833-846.

Keeton, W.T. 1980. Biological Science. W.W. Norton and Co. New York.

Klecka, G.M. and S.J. Gonsoir. 1984. Reductive Dechlorination of Chlorinated Methanes and Ethanes by Reduced Iron (II) Porphyrins. Chemosphere. Vol. 13. No. 3. pp. 391-402.

Kohler, H.P.E., Kohler-Staub, and D.D. Focht. 1988. Cometabolism of PCB: Enhanced Transformation of Aroclor 1254 by Growing Bacterial Cells. Appl. Environ. Microbiol. Vol. 54. No. 8. pp. 1940-1945.

Krumme, M.L. and S.A. Boyd. 1988. Reductive Dechlorination of Chlorinated Phenols in Anaerobic Upflow Bioreactors. Water Res. Vol. 22. No. 2. pp. 171-177.

Lech, J. and R. Peterson. 1983. Biotransformation and Persistence of Polychlorinated Biphenyls in Fish in PCBs: Human and Environmental Hazards, D'ltri, F.M. and M. Karin (Eds.). Butterworth Publishers, Boston.

Lui, D. 1980. Enhancement of PCB Biodegradation by Sodium Ligninsulfonate. Water Res. Vol. 14. pp. 1467-1475.

Maack, L. and W.C. Sonzogni. 1988. Analysis of Polychlorobiphenyl Congeners in Wisconsin Fish. Arch. Environ. Contam. Toxicol. Vol. 17. pp. 711-719.

Mackay, D. 1982. Correlation of Bioconcentration Factors. Environ. Sci. Technol. Vol. 16. pp. 274-278.

Mackay, D. and A.I. Hughes. 1984. Three Parameter Equation describing the Uptake of Organic Compounds by Fish. Environ. Sci. Technol. Vol. 18. No. 6. pp. 439-444

Manchester, J. 1988. Seasonal Variation in the Amount and Distribution of Atmospheric PCB Congeners. Masters Thesis. Water Chemistry Program, Dept. of Civil and Environmental Engineering. University of Wisconsin, Madison, Wisconsin.

Marti, E.A. 1984. PCBs in Sixteen Lake Michigan Tributaries. Master's Thesis, Water Chemistry, University of Wisconsin-Madison.

McCarthy, J.F. and J.M. Zachara. 1989. Subsurface Transport of

Contaminants. Environ. Sci. Technol. Vol. 23. No. 5. pp.

Mikesell, M.D. and S.A. Boyd. 1988. Enhancement of Pentachlorophenol Degradation in Soil through Induced Anaerobiosis and Bioaugmentation with Anaerobic Sewage Sludge. Environ. Sci. Technol. Vol. 22. No. 12. pp. 1411-1414.

Mudroch, A., Onuska, F.I., and L, Kalas. 1989. Distribution of Polychlorinated Biphenyls in Water, Sediment and Biota of Two Harbours. Chemosphere. Vol. 18. No. 11/12. pp. 2141-2154.

National Academy of Science. 1979. Polychlorinated Biphenyls. National Academy of Science. Washington, D.C.

Niimi, A.J. and B.G. Oliver. 1988. Influence of Molecular Weight Molecular Volume on Dietary Absorption. Can. J. Fish. Aquatic Sci. Vol. 145. pp. 222-227

Niimi, A.J. and B.G. Oliver. 1989. Distribution of Polychlorinated Biphenyl Congeners and other Halocarbons in Whole Fish and Muscle among Lake Ontario Salmonids. Environ. Sci. Technol. Vol. 23. No. 1. pp.83-88.

Oliver, B.G., Charlton, M.N. and R.W. Durham. 1989. Distribution, Redistribution and Geochronology of PCB congeners and other Chlorinated Hydrocarbons in Lake Ontario Sediments. Environ. Sci. Technol. Vol. 23. pp. 200-208.

Oliver, B.G. and A.J. Niimi. 1988. Trophodynamic Analysis of Polychlorinated Biphenyl Congeners and Other Chlorinated Hydrocarbons in Lake Ontario Ecosystem. Environ. Sci. Technol. Vol. 22. pp. 388-397.

Opperhuizen, A., Velde, E.W., Gobas, F.A.P.C., Liem, D.A.K. and J.M.D. Steen. 1985. Relationship between Bioconcentration in Fish and Steric Factors of Hydrophobic Chemicals. Chemosphere. Vol. 14. No. 11/12. pp.1871-1896.

Opperhuizen, A. and S.M. Schrap. 1988. Uptake Efficiencies of Two Polychloro Biphenyls in Fish after Dietary Exposure to Five Different Concentrations. Chemosphere. Vol. 17. No. 2.

Palmer, D.T., Linkfield, T.G., Robinson, J.B., Genthner, B.R.S. and G.E. Pierce. 1989. Determination and Enhancement of Anaerobic Dehalogenation: Degradation of Chlorinated Organics in Aqueous Systems. U.S. Environmental Protection Agency Research and Development, Risk Reduction Engineering Lab, Cincinnati, OH. EPA/600/S2-88/054.

Parkinson, A. and S. Safe. 1987. Mammalian Biologic and Toxic Effects of PCBs in Polychlorinated Biphenyls: Mammalian and

Environmental Toxicology. Springer-Verlag. New York.

Parsons, J., Veerkamp, W. and O. Hutzinger. 1983. Microbial Metabolism of Chlorobiphenyls. Toxicological and Environmental Chemistry. Vol. 6. pp. 327-350.

Parsons, J.R. and D.T.H.M. Sijm. 1988. Biodegradation Kinetics of Polychlorinated Biphenyls in Continuous Cultures of a Pseudomonas Strain. Chemosphere. Vol.17. No.9. pp.1755-1766.

Paull, R.K. and R.K. Paull. 1976. Geology of Wisconsin and Upper Michigan. Kendall/Hunt Publishing. Dubuque, Iowa.

Pavlou, S.P. and R.N. Dexter. 1979. Distribution of Polychlorinated Biphenyls (PCBs) in Estuarine Ecosystems. Testing the Concept of Equilibrium Partitioning in the Marine Environment. Environ. Sci. Technol. Vol. 13. No. 1.

Quensen, J.F., Tiedje, J.M. and S.A. Boyd. 1988. Reductive Dechlorination of Polychlorinated Biphenyls by Anaerobic Microorganisms from Sediments. Science. Vol. 242. pp. 752-754.

Rhee, G.Y., Bush, B., Brown, M.P., Kane, M. and L. Shane. 1989. Anaerobic biodegradation of Polychlorinated Biphenyls in Hudson River Sediments and Dredged Sediments in Clay Encapsulation. Water Res. Vol. 23. No. 8. pp. 957-964.

Rubenstein, N.I. Lores, E. and N.R. Gregory. 1983. Accumulation of PCBs, Mercury and Cadmium by Nereis Virens, Mercenaria Mercenaria and Palaemonetes Pugio from Contaminated Harbor Sediments. Aquatic Toxicology. Vol. 3. pp.249-260.

Rubenstein, N.I., Gilliam, W.T. and N.R. Gregory. 1984. Dietary Accumulation of PCBs from Contaminated Sediment Source by a Demersal Fish (Leiostomus Xanthurus). Aquatic Toxicology. Vol. 5. pp. 331-342.

Rusling, J.F. and C.L. Miaw. 1989. Kinetic Estimation of Standard Reduction Potentials of Polyhalogenated Biphenyls. Environ. Sci. Technol. Vol. 23. No. 4. pp. 476-479.

Safe, S. 1984. Microbial Degradation of PCBs in Microbial Degradation of Organic Compounds D.T. Gibson (Ed.). Marcel Dekker. New York.

Safe, S., Bandiera, S., Sawyer, T., Zmudzka, B, Mason, G., Romkes, M., Denomme, M.A., Sparling, S., Okey, A.B., and T. Fujita. 1985. Binding to the 2,3,7,8 -TCDD Receptor Protein and AHH Induction--Halogenated Biphenyls. Environ. Health. Persp. Vol. 61. pp.21-33.

Safe, S. 1987. Determination of 2,3,7,8-TCdd Toxic Equivalent Factors (TEFs): Support for the Use of the in Vitro AHH induction Assay. Chemosphere. Vol. 16. No. 4. pp. 791-802.

Shaw, G.R. and D.W. Connell. 1984. Physicochemical Properties Controlling Polychlorinated Biphenyl Concentrations in Aquatic Organisms. Environ. Sci. Technol. Vol. 18. pp. 18-23.

Shiu, W.Y. and D. Mackay. 1986. A Critical Review of Aqueous Solubilities, Vapor Pressures, Henry's Law Constants and Octanol-Water Partition Coefficients of the Polychlorinated Biphenyls. J. Phys. Chem. Ref. Data. Vol.15. No. 2. pp.911-929.

Simmons, M.S., Bialosky, D.I. and R. Rossman. 1980. PCB Contamination in Surficial Sediments of NE Lake Michigan. J. Great Lakes Res. Vol. 6. No. 2. pp. 167-171.

State Laboratory of Hygiene. 1989. Methods and Quality Control Manual. Organic Chemistry Unit. Wisconsin State Laboratory of Hygiene. Madison, Wisconsin.

Steen, W.C., Paris, D.F., and G.L. Baughman. 1978. Partitioning of Selected Polychlorinated Biphenyls to Natural Sediments. Wat. Res. Vol. 12. pp. 655-657.

Stryer, L. 1981. Biochemistry. W.H. Freeman and Co. San Francisco.

Suflita, J.M., Horowitz, A., Shelton, D.R. and J.M. Tiedje. 1982. Dehalogenation: A Novel Pathway for the Anaerobic Biodegradation of Haloaromatic Compounds. Science. Vol. 218. pp. 1115-1117.

Swackhammer, D.L. 1985. The Role of Water-Particle Partitioning and Sedimentation in Controlling the Fate and Transport of PCBs in Lakes. PhD Thesis. Oceanography and Limnology. University of Wisconsin. Madison, Wisconsin.

Swain, W. 1983. An Overview of the Scientific Basis for Concern with Polychlorinated Biphenyls in the Great Lakes in PCBs: Human and Environmental Hazards. F. D'Ltri and M.A. Kamrin (Ed.). Butterworth Publishers, Boston, MA.

Tucker, E.S., Saeger, V.W. and O. Hicks. 1975. Activated Sludge Primary Biodegradation of Polychlorinated Biphenyls. Bull. Environ. Contam. Toxicol. Vol. 14. No. 6.

United States Environmental Protection Agency. 1989. Fact sheet Sheboygan River and Harbor Update. September 1985. Region 5. Office of Public Affairs, Chicago, IL.

van der Oost, R. Heida, H. and A. Opperhuizen. 1988.

Polychlorinated Biphenyl Congeners in Sediment, Plankton, Molluscs, Crustaceans and Eel in a Freshwater Lake. Implications of Using Reference Chemicals and Indicator Organisms in Bioaccumulation Studies. Arch. Environ. Contam. Toxicol. Vol. 17. pp. 721-729.

Wade, R.S., Harlin, R. and C.E. Castro. 1969. Note: Organic Matter and Reduced Iron Porphyrins. Journal of the American Chemical Society. Vol.91. No. 26. pp. 7530.

Wisconsin Department of Natural Resources. 1989. Sheboygan River Remedial Action Plan. Wisconsin Department of Natural Resources, Madison, Wisconsin.

Wong, P.T.S. and K.L.E. Kaiser. 1975. Bacterial Degradation of PCBs II Rate Studies. Bull. Environ. Contam. Toxicol. Vol. 13. No. 2.

Woods, S.L., Ferguson, J.F. and M.M. Benjamin. 1989. Characterization of Chlorophenol and Chloromethoxybenzene Biodegradation during Anaerobic Treatment. Environ. Sci. Technol. Vol. 23. pp. 62-68.

Wu, S. and D.M. Gshwend. 1986. Sorption Kinetics of Hydrophobic Organic Compounds to Natural Sediments and Soils. Environ. Sci. Technol. Vol. 20. No. 7. pp.717-725.

Zeikus, J.G. and M.R. Winfrey. 1976. Temperature Limitations of Methanogenesis in Aquatic Sediments. Appl. Environ. Microbiol. Vol. 31. pp99-107.

Concentrations of Toxic Polychlorinated Biphenyl Congeners in Sheboygan River (USA) Sediments

by W. Sonzogni

L. Maack

T. Gibson

J. Lawrence

Laboratory of Hygiene
and Water Chemistry Program
University of Wisconsin
Madison, WI 53706

In the U. S. polychlorinated biphenyls (PCBs) were produced in mixtures (Aroclors) of the 209 different PCB congeners. The Aroclors differ not only in the congeners contained in the mixture, but also in the weight percent of the congeners. Until recently almost all environmental and biological samples were analyzed for PCBs by matching the chromatographic pattern to the pattern of pure Aroclors (Webb and McCall 1973; Erickson 1986), and results were reported as an Aroclor or mixture of Aroclors. However, with the refinement of high resolution capillary gas chromatographic techniques, it is now possible to identify and quantify individual PCB congeners in environmental samples (Mullin et al. 1984; Maack and Sonzogni 1988). Note that in this paper congeners will be referred to by their International Union of Pure Applied Chemistry (IUPAC) numbers and by an abbreviation of their structure. For example, 3, 3', 4, 4' tetrachlorinated biphenyl is referred to as congener 77 (34-34).

Interest in individual congener concentrations has also increased because recent toxicological data indicate the potency of congeners varies widely (Safe 1987; Tanabe et al. 1987). Those congeners presently thought to be most toxic are the non-ortho chlorinated PCBs, since these co-planer compounds structurally resemble the highly potent 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD). Congeners 77 (34-34), 126(345-34) and 169 (345-345) are non-ortho chlorinated and most resemble dioxin. They are believed to be the most toxic congeners, at least in terms of dioxin-like properties (Safe et al. 1985; Tanabe et al. 1987). Congener 81 (345-4) also has no ortho chlorines; however, it is structurally different enough from TCDD that it is not considered to be as toxic as the compounds above. The mono-ortho congeners, such as 105

Send reprint requests to W. Sonzogni at the above address.

(234-34), 118 (245-34), 123 (345-24), 114 (2345-4) and 167 (245-345) are also thought to be toxic (have toxic properties related to dioxin), although they are less potent (Safe et al. 1985; Safe 1987).

Unfortunately, most of the congeners mentioned above are difficult to analyze, even by high resolution gas chromatography. These compounds tend to co-elute with other congeners. Special separation techniques are required to identify them. To date, the only separation techniques reported in the literature are (1) carbon column absorption (Stalling et al. 1983) and multidimensional gas chromatography (Duinker et al. 1988). The purpose here is to present results of analyses of river sediments for these toxic PCB congeners using a multidimensional chromatography technique.

MATERIALS AND METHODS

Sediment samples were collected from the Sheboygan River, a Wisconsin tributary to Lake Michigan. The river is polluted with PCBs from the mouth to about 22.6 km (14 miles) upstream. Waste hydraulic fluids containing Aroclor 1248 and Aroclor 1254, were the source of the contamination (David 1990). The polluted section of the river is a U.S. federal "Superfund" site as well as one of the Great Lakes "Areas of Concern" as defined by the U.S./Canadian International Joint Commission.

Sediment cores were collected in December of 1988 and April of 1989 using a metal corer 90 cm (3 ft) long and 7.6 cm (3 in.) in diameter. All samples were collected near the original source of PCBs at Rochester Park (about 22.6 km upstream from the mouth). A hydraulic extruder was used to remove the sediment from the corer. The cores were segmented into 15 cm sections and kept cool (4°C) until analysis.

Sediment was air dried and sieved to form a homogeneous sample. PCBs were extracted from 50 g sediment samples by soxhleting for eight hours with acetone and hexane (1:1). Granular copper was added to the soxhlet flask to insure uniform boiling and to remove sulfur interferences. After concentrating the sample, anhydrous sodium sulfate was added to remove water from the extract. The hexane-acetone solution was then exchanged for 2,2,4-trimethyl pentane (iso-octane). PCBs were separated from other contaminants using Florisil and silica gel. Hexane and 6% ethyl ether were the eluting solvents used for the Florisil fractionation and hexane for the silica gel fractionation. The first silica gel fraction was subsequently used for PCB quantitation. Hexane was exchanged with isooctane prior to gas chromatographic analysis. Samples were concentrated to

10 or 50 mL prior to injection of an aliquot into the gas chromatograph.

PCB congeners were quantitated using a Siemens Sichromat 2-8 multidimensional gas chromatograph. The instrument was equipped with two 30 m capillary columns (DB-5 and DB-1; J and W Scientific). Each column was connected to an individual ^{63}Ni electron capture detector. The columns were also contained in separate ovens. Eluate from the DB-5 column passed through the column's detector producing a chromatogram similar to that produced in conventional high resolution gas chromatography. However, when a "T-piece" connecting the DB-5 column to the DB-1 column was activated, eluate was diverted to the DB-1 column. Thus, a selected part of the eluate (e.g., the eluate eluting between a specific time interval) was cut to the DB-1 column. The cut is accomplished on a real time basis according to pneumatic (gas flow) differences between the two columns, the timing of which is controlled by the analyst.

The temperature of the oven containing the DB-5 column was ramped from 90°C to 160°C at a rate of 20°C per minute, then from 160°C to 260°C at a rate of 4°C per minute. The final temperature was held for one minute. The temperature of the column containing the DB-1 oven was held at 160°C for 20 minutes and then increased at a rate of 4°C per minute to 240°C. The final temperature was held for eight minutes. The injector temperature was 250°C; the detectors were heated to 300°C. The carrier gas and make-up gas were hydrogen and nitrogen, respectively.

By cutting a portion of the eluate after it passed through the DB-5 column to and through the less polar DB-1 column, increased separation of the compounds in the cut was obtained. The chromatogram produced from the second (DB-1) column and its associated detector represented the response of the cut components, while the chromatogram of the first column (DB-5) represented the rest of the compounds in the injected mixture (the chromatogram for the first column is interrupted by the cut). For a further discussion of the instrument, see Duinker et al. (1988).

For the eight congeners studied, pure standards were used for identification (based on retention times) and quantification. A detector limit of 1 ng/g, based on a signal to noise ratio of approximately 3, was made. Although replicate analyses were made to check results, no alternate method was available to confirm the identification of the congeners in the unknown.

RESULTS AND DISCUSSION

Results of the analyses of Sheboygan River sediment samples for total PCBs and eight "toxic" congeners are summarized in Table 1. Each of these congeners was identified and quantified in at least one of the samples. Note that all sediment core samples analyzed had total PCB concentrations greater than 50 μg/g.

Congeners 118 (245-34), 105 (234-34), and 77 (34-34) were detected in all of the samples reported here. Concentrations ranged from about 5 to 1500 ng/g. The remaining toxic congeners, 81 (245-4), 114 (2345-4), 167 (245-345), 126 (345-34), and 169 (345-345), were detected less frequently in the analyzed samples. Concentrations of these congeners ranged from non-detectable to slightly over 100 ng/g.

Very few measurements of toxic congeners in environmental samples have as yet been reported in the literature. Tanabe et al. (1987) reported various levels of congeners 77 (34-34), 126(345-34), and 169 (345-345) in fish, marine mammals, and terrestrial animals in Japan. Duinker et al. (1988) reported seal blubber from the Dutch Wadden Sea contained 100, 800 and 5200 ng/g of congeners 77 (34-34), 118 (245-34), and 105 (234-34), respectively. Congeners 126 (345-34), 81 (345-4), 167 (245-345), and 114 (23 45-4) were all found in seal blubber at concentrations less than 10 ng/g. Note that for the Sheboygan sediments reported here, congeners 77 (34-34), 118 (245-34), and 105 (234-34) were also found at concentrations higher than the rest of the congeners. To the best of the authors' knowledge, no other data on concentrations of these congeners in aquatic sediment have been published.

Table 2 presents the weight percents of the congeners in the samples. Also included are the weight percents of congeners in several Aroclors as determined by Duinker et al. (1988). The weight percents of the toxic congeners in the sediments were generally lower than those found in Aroclors 1248 and 1254 (the primary PCB mixtures discharged to the river) and in the other Aroclor mixtures listed in Table 2. The weight percents of the most prominent toxic congeners (77, 118, and 105) were about one order of magnitude lower than the weight percents of these congeners in Aroclor 1248. Some weight percents for congener 81 (345-4) were higher than reported for the Aroclors, although the congener was detected only in four of the eight samples reported.

Anaerobic reductive dechlorination of PCBs, similar to that reported by Brown et al. (1987), Quensen et al. (1988), and Rhee et al. (1989), has been reported to

occur in Sheboygan River sediments (David 1990). Dechlorination of non-ortho chlorinated congeners has been found to occur more readily than dechlorination of ortho chlorinated PCBS (Brown and Wagner 1990). Congeners 77 (34-34), 118 (245-34), and 105 (234-23) are all non-ortho chlorinated PCBs, suggesting that dechlorination might play a role in the depletion.

Of the toxic congeners, congener 118 (245-34) was found in Sheboygan River sediments in the highest weight percent. Congener 118 is also found in all the Aroclors in Table 2 in the highest weight percent of the toxic congeners. It also appears to be the most common toxic congener in environmental samples. It was found in highest concentrations (relative to other toxic congeners) in seal blubber (Duinker et al. 1988), as well as in the sediments collected for this study. Congener 118, unlike the other toxic congeners, can often be resolved and quantified without multidimensional gas chromatography or other special techniques. It has thus been reported in a variety of matrices, such as fish and the blood of sport fish eaters (Maack and Sonzogni 1988; Fiore et al. 1989 and Sonzogni et al. 1991).

The eight toxic PCBs studied here appear to be present in the sediment, but in relatively low concentrations compared to total PCBs or other more abundant congeners. The concentrations of the eight congeners are low relative to the congeners with which they co-elute as well. For example, toxic congener 77 (34-34) co-elutes with congener 110 (236-34), but 77, in the sediments studied here, made up less than 4 percent of the total of the co-eluting pair. It should again be emphasized that the toxic congeners have been so labeled because of their dioxin-like structure and the potential health effects of dioxin. More research needs to be conducted on the toxicological effects of the PCB congeners. The effects of exposure to environmental levels needs to be considered as well as toxicological endpoints other than that for dioxin. For example, the neurotoxicological impacts of specific congeners or combinations of congeners needs to be studied. Perhaps new analytical tools, such as multidimensional gas chromatography, will help make such research more feasible.

Acknowledgements: The authors gratefully acknowledge the sampling assistance of William Wawrzyn and Thomas Aartila of the Wisconsin Department of Natural Resources. This research was supported under a grant from the Wisconsin Coastal Management Program.

Table 1. Concentration of various toxic PCB congeners in Sheboygan River sediment samples of different total PCB concentrations.

Total PCB in Sample (μg/g)	Congener (IUPAC Number) 77	118	105	167 (ng/g)	114	126	169	81
50.3	31	140	80	44	ND[a]	ND	ND	ND
1050	360	1480	490	80	110	10	19	90
76.9	24	129	27	ND	ND	9	9	ND
97.8	21	318	41	ND	ND	ND	ND	ND
256	46	395	15	ND	ND	ND	ND	75
73.4	5	103	11	ND	ND	ND	ND	ND
97.5	11	162	6	ND	ND	ND	11	ND
124	16	86	11	ND	ND	ND	ND	50

[a]ND = not detected (concentration less than 1 ng/g)

Table 2. Weight Percent of Various Toxic PCB Congeners in Sheboygan River Sediment Samples and Weight Percent of Congeners in Several Aroclors[a].

Total PCB in Sample (μg/g)	Congener (IUPAC Number) 77	118	105	167	114	126	169	81
	(weight %)							
50.3	0.06	0.27	0.15	0.09	- -[b]	- -	- -	- -
1050	0.03	0.14	0.05	0.01	0.01	<0.01	<0.01	0.01
76.9	0.03	0.16	0.04	- -	- -	0.01	0.01	- -
97.8	0.02	0.33	0.04	- -	- -	- -	- -	- -
256	0.02	0.15	0.01	- -	- -	- -	- -	0.03
73.4	0.01	0.14	0.01	- -	- -	- -	- -	- -
97.5	0.01	0.17	0.01	- -	- -	- -	0.01	- -
124	0.01	0.07	0.01	- -	- -	- -	- -	0.04
Aroclor 1242	0.50	1.80	0.33	<0.01	<0.01	<0.01	<0.01	<0.01
Aroclor 1248	0.30	3.35	0.55	<0.01	<0.01	<0.01	<0.01	<0.01
Aroclor 1254	<0.01	8.45	2.03	0.05	<0.01	0.08	0.08	<0.01
Aroclor 1260	<0.01	1.15	0.08	0.15	<0.01	0.05	0.05	<0.01

[a]Aroclor weight percents from Duinker et al. 1988
[b]Congeners not detected in samples (weight percents less than 0.002%).

References

Brown JF, Bedard DL, Brennan MJ, Carnahan JC, Feng H, Wagner RE (1987) Polychlorinated biphenyl dechlorination in aquatic sediments. Science 236:709-712

Brown JF, Wagner RE (1990) PCB movement, dechlorination, and detoxication in the Acushnet Estuary Environ Toxicol Chem 9:1215-1233

David M (1990) PCB congener distribution in Sheboygan River sediment, fish and water. MS Thesis, Water Chemistry Program, Univ of WI, Madison 159p

Duinker JC, Schultz DE, Petrick G (1988) Multidimensional gas chromatography with electron capture detection for the determination of toxic congeners polychlorinated biphenyl mixtures. Anal Chem 60:478-482

Erickson MD (1986) Analytical Chemistry of PCBs. Butterworth Publishers, Stoneham, MA

Fiore BJ, Anderson HA, Hanrahan LP, Olson LJ, Sonzogni WC (1989) Sport fish consumption and body burden levels of chlorinated hydrocarbons; A study of Wisconsin anglers. Archives of Environ Health 44:82-88

Maack L, Sonzogni WC (1988) Analysis of polychlorobiphenyl congeners in Wisconsin fish. Arch Environ Contam Toxicol 17:711-719

Mullin MD, Pochini CM, McCrindle S, Romkes M, Safe SH, Safe LM (1984) High resolution PCB analysis: Synthesis and chromatographic properties of all 209 PCB congeners. Environ Sci Technol 18:468-476

Safe S, Safe L, Mullin M (1985) Polychlorinated biphenyls: Congener-specificanalysis of commercial mixture and a human milk extract. J Agric Food Chem 33:24-29

Safe S (1987) Determination of 2,3,7,8-TCDD toxic equivalent factors (TEFs); Support for the use of the in vitro AHH induction assay. Chemosphere 16:791-802

Sonzogni W, Maack L, Gibson T, Degenhardt D, Anderson H, Fiore B (1991) Polychlorinated biphenyls congeners in blood of Wisconsin sport fish consumers. Arch Environ Contam Toxicol, in press

Stalling DL, Smith LM, Petty JD, Hogan JW, Johnson JL, Rappe C, Buser HR (1983) Residues of polychlorinated debenzo-p-dioxins and debenzofurans in Laurentean Great Lakes fish. In: Tucker RE, Young AL, Gray AP (ed) Human and environmental risks of chlorinated dioxins and related compounds, Plenum

Tanabe S, Kannan N, Subramanian A, Watanabe S, Tatsukawa R (1987) Highly toxic coplanar PCBs: Occurrence, source, persistency, and toxic implications to wildlife and humans. Environ Pollut 47:147-163

Webb RG, McCall AC (1972) Identities of polychlorinated biphenyl isomers in aroclors. J Assoc Offic Anal Chem 55: 746-752

PCB Dechlorination in the Sheboygan River, Wisconsin

Extended Abstract to be Published in Proceedings of a Workshop on Biological Remediation of Contaminated Sediments

by William C. Sonzogni and Margaret M. David
Laboratory of Hygiene and Water Chemistry Program
University of Wisconsin
Madison, WI 53706

The Sheboygan River in Wisconsin flows into Lake Michigan at the city of Sheboygan, located 90 km north of Milwaukee. Due to the high concentrations of PCBs in the river sediment, the Sheboygan River and Harbor area has received national attention. The main source of contamination was from a die casting plant located in the Village of Sheboygan Falls. The contamination source area is about 22 km upstream from the mouth of the river.

Hydraulic fluids containing PCBs were used by the die casting plant from 1959 to 1971 (11). Apparently, a large fire occurred at the plant prior to 1959 that was caused by combustion of the hydraulic fluids then in use. Fluids containing PCBs were subsequently put in use because of their fire resistance. Based on interviews and available records, a product called Pydrol F9 was used between 1959 and 1969 and a product called Chemtrend HF30 was used between 1970 and 1971. Pydrol F9 contains Aroclor 1248, while Chemtrend HF30 contains mostly Aroclor 1254 with a small percentage of Aroclor 1248. In 1971 the use of hydraulic fluids containing PCBs ceased.

Material from the plant (oil soaked rags, hoses and other refuse) and soil from around the plant was used to construct a low dike at the edge of the Sheboygan River. The dike sloped at

a 45 degree angle to the river, so erosion of the diked material into the river occurred relatively easily (11). Concentrations of PCBs in the soil samples were as high as 120,000 μg/g. The die casting plant is the only known major source of PCBs to the river, therefore, the congeners deposited in the sediments were most likely the components of Aroclor 1248 and 1254.

In an article in Science it was reported that biological reductive dechlorination of PCBs was occurring in Hudson River sediment. There was evidence that anaerobic dechlorination was also occurring in other aquatic sediments, including Sheboygan River sediments (4). The Sheboygan evidence was based on observations of chromatograms obtained from the U.S. Army Corps of Engineers.

As result of the published reports that dechlorination could occur and because of new analytical capabilities to do congener specific PCB analysis, research was begun to examine the distribution of PCB congeners in the Sheboygan River sediment and to determine whether anaerobic dechlorination may be occurring. The intent was to determine the congener distribution in Sheboygan River sediment and assess whether some transformations had occurred. Results of congener distributions in Sheboygan River sediment relative to distributions in Aroclors will be summarized below as well as information on the occurrence of "toxic" congeners. Finally, a summary of evidence so far to degrade PCBs in the laboratory using bacteria from Sheboygan River sediments will be made.

Total PCB concentrations ranged from 1586 μg/g found

downstream from the source to 0.04 μg/g above the source (considered to represent background levels). Although there is considerable variation in the sediment PCB concentrations, in general, the values were found in areas of sediment deposition in the river. In the individual cores, the top segment of core (0-15 cm) and the bottom segment of core (45-60) cm had relatively low concentrations of PCBs. The highest concentrations were found in the 15-45 cm segments.

Sediment samples were also analyzed for PCB congeners using high resolution gas chromatography. Samples containing total PCB concentrations greater than 50 μg/g appeared to be enriched with the lower chlorinated congeners whereas those with less than 50 μg/g PCBs were not. Samples containing 50 μg/g or more PCBs and significantly higher concentrations of mono- and di- chlorinated congeners when compared to aroclors 1248 and 1254 which were originally introduced into the river. Using a multivariate ANOVA statistical test, samples containing PCB concentrations less than 50 μg/g, were found to be statistically different from Aroclor 1248, Aroclor 1254 and an equal parts mixture of Aroclors 1248 and 1254 (p values were <0.05). In sediment samples containing PCB concentrations less than 50 μg/g, the homolog patterns were more similar to the patterns of the Aroclor 1248 and 1254 than the more contaminated sediments.

The most prominent congeners in the sediments with total PCB concentrations greater than 50 μg/g were (IUPAC #) 5/8, 17, 16/32, 47/48, and 28/31. Relative to the original Aroclors, particularly high concentrations of congeners 5 and 8 were seen

(congeners 5 and 8 coelute). To confirm the presence of congeners 5/8 six of the samples containing high concentrations of PCBs (342.8 μg/g on average) and high concentrations of congener 5/8 (43.7 percent on average) were analyzed using an electron impact gas chromatography mass spectrometer. All samples contained high concentrations of dechlorinated congeners. In samples containing less than 50 μg/g of PCBs, the most prominent congeners were similar, but their weight percents were generally reduced.

The result from the sediment analyses indicate that the PCB congeners and their respective weight percentages in sediments with high PCB concentrations are significantly different from the Aroclors originally introduced into the river. Although physical-chemical processes such a sediment-water partitioning are important in determining the distribution of congeners in sediments, it is unlikely that it is the dominant process influencing the distribution of congeners. Sediment-water partition coefficients generally increase with molecular weight and thus an enrichment of the higher chlorinated congeners in the sediments not lower chlorinated congeners as observed would be predicted.

Diffusion of congeners out of the sediment and into the water is slow relative to sedimentation rates and is inversely related to partition coefficients (7,8). Therefore, a distribution enriched in the higher chlorinated PCBs would be predicted (opposite of what was observed in this study).

Another possibility to account for the change in congener

patterns in abiotic chemical reactions. PCBs have been shown to undergo abiotic reductive dechlorination in the laboratory; however, the conditions in the laboratory (high temperatures, excess base, and the presence of a catalyst) are considerably different from those in the environment (3). In general, it is thought that there are very few abiotic pathways which completely mineralize organic contaminants (1).

It is possible, however, that the Aroclors undergo biological dechlorination. Recent work by Quensen et al. (9), Chen et al. (6), Rhee et al. (10), and Brown et al. (4,5) suggest that PCBs can undergo anaerobic microbial degradation. Several results in this study suggest such a process.

First, there is a shift in the congener pattern from the higher chlorinated congeners to the lower chlorinated congeners as observed by Quensen et al. (9) in a laboratory experiment and as noted by Brown et al. (4,5) in a field study of hudson River sediments. This enrichment in lower chlorinated congeners cannot be accounted for by physical-chemical partitioning relationships or diffusion processes.

Second, there appears to be a structural selectivity as to which congeners are depleted in the sediment. Congeners containing chlorines in the ortho position are enriched, whereas congeners containing chlorines in the meta or para position are depleted. This is consistent with the results obtained by Quensen et al. (9) and Brown et al. (4) in their anaerobic microbial dechlorination work.

Third, several congeners are found in abundance that would

not be expected based on physical-chemical partitioning relationships or on the original weight percentages present in the Aroclor mixtures. In sediment samples with concentrations of PCBs above 50 μg/g, congeners that were significantly enriched are 5/8, 19, 17, 24/27, 16/32, 26, and 47/48. Congeners that were significantly depleted are 18, 28/31, 52, 44, 70/76, 66/95, 56/60, 101, 77/110, 132/153/105, and 138/163. These changes are comparable to Brown et al.'s (5) findings.

Fourth, the concentration of PCBs in the sediment appears to be important. Microbial degradation is often restricted to areas of high substrate concentrations. For example, toluene, xylene, and naphthalene are metabolized by bacteria at high concentrations but not at low concentrations (2). Threshold concentrations exist for many contaminants and are the minimum concentration of a chemical which is needed to support growth of a microbial population (2). Below the threshold concentration, additional energy sources must be found to support growth of the population, since the organism is no longer able to completely mineralize the substrate. Based on the different chromatographic patterns seen for high and low concentrations of PCBs in the Sheboygan River, it may be that a threshold concentration exists for the anaerobic dechlorination of PCBs.

To confirm that microbial processes are actually responsible for degrading PCBs, laboratory experiments have been conducted similar to those reported by Quensen et al. (9) and Rhee et al. (10). Using bacteria extracted from Sheboygan sediments, degradation was attempted using growth medium and anaerobic

conditions suitable for microbial dechlorination of PCBs. However, to date no dechlorination has been observed in the experiments. The reason for the lack of dechlorination activity is not clear, but it is suspected that the conditions that favor degradation are very complicated (e.g., may involve very precise Eh conditions and may involve several different species or stains or organisms) and may be difficult to consistently reproduce in the laboratory.

Finally, Sheboygan sediments have been analyzed for the presence of non-ortho or coplanar PCBs. These congeners are believed to be the most toxic (at least in terms of dioxin like toxic properties), but generally coelute with other congeners using capillary column gas chromatography. A multidimensional ("heart cutting") gas chromatograph that uses two high resolution columns in series was used to separate coeluting congeners. Results to date indicate that several congeners of toxicological interest are found in sediment samples, albeit at low concentrations. Congeners 118, 105 and 77 were detected in 83 percent of the samples analyzed, at average concentrations of about 0.25, 0.06, and 0.04 $\mu g/g$ respectively. The average composition of these congeners was 0.13, 0.03, and 0.02 percent, respectively. Congeners 81, 114, 167, 126, and 169 were also detected in some of the samples all at concentrations less than 0.03 $\mu g/g$. While the concentrations of these congeners are low relative to total concentrations of PCBs or to the congener they coelute with, the fact that they are present may be important toxicologically. Research is ongoing in this area.

This work was supported by grants from the Wisconsin Coastal Management Program and the Wisconsin Sea Grant Program.

References

1. Alexander, M. (1981). Biodegradation of chemicals of environmental concern. Science, 211: 132-138.

2. Alexander, M (1985) Biodegradation of organic chemicals. Environ. Sci. Technol., 18:106-111.

3. Boyer, S.K., J McKenna, J. Karliner, and M. Nirsberger (1985). A mind and efficient process for detoxifying polychlorinated biphenyls. Tetrahedron Letters, 26; 3677-3680.

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

5. Brown, J.F., R.E. Wagner, H. Fend, D.L. Bedard, M.J. Brennan, J.C. Carnahan, and R.J. May (1987b). Environmental dechlorination of polychlorinated biphenyls. Environ. Toxicology and Chemistry, 6: 579-593.

6. Chen, M., C.S. Hong, B Bush, and G.Y. Rhee (1988). Anaerobic biodegradation of PCBs by bacteria from Hudson

River sediments. Ecotoxicology and Environ. Safety, 16: 95-105.

7. DiToro, D.M., J.M. Jeris, and D. Clarcia (1985). Diffusion and partitioning of hexachlorobiphenyl in sediments. Environ. Sci. Technol., 19: 1169-1172.

8. Fisher, J.B., R.L. Petty, and W. Lick (1983) Release of polychlorinated biphenyls from contaminated lake sediments: flux and apparent diffusivities of four individual PCBs. Environ. Pollut. Series B., 5: 121-132.

9. Quensen, J.F., J.M. Tiedje, and S.A. Boyd (1988). Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms form sediments. Science, 242" 752-754.

10. Rhee, C.Y., B. Bush, M.P. Brown, M. Kane, and L. Shane (1989). anaerobic biodegradation of polychlorinated biphenyls in Hudson River sediments and dredged sediments in encapsulation. Water Research, 23: 957-964.

11. Wisconsin Department of Natural Resources (1989). Sheboygan River remedial action plan. Environmental Quality Division, Madison, Wisconsin.

SUMMARY OF LABORATORY EXPERIMENTS TO DECHLORINATE PCBs USING BACTERIA EXTRACTED FROM SHEBOYGAN RIVER SEDIMENTS

In May of 1989 the first experiments were run to induce dechlorination of PCBs in the laboratory. Anaerobic bacteria were extracted from three sediment cores using the procedure that will be described subsequently with the exception that a chopped mean anaerobe media was used. PCBs were added to nine vials to produce concentrations of 60 ppm, and to nine vials to produce concentrations of 600 ppms (to give 18 incubating vials, excluding controls).

After initial PCB analysis, this run was abandoned for the following reasons. First, the sediment from which the anaerobes were extracted had low PCB concentrations (18 ppm) so that dechlorinating anaerobes may not have been established in the sample. Second, the media used (a standard chopped meat carbohydrate based anaerobic media) was different than used by Quenson et al. (1988) in their original work. This media presented sampling and extraction problems for PCB analysis. Thus, a new experiment was started.

In the second experiment medium was prepared after Quensen (1988 and personal communication, Michigan State University, 1989).

The composition of the medium (in mg/L) is listed below:

KH_2PO_4	270
K_2HPO_4	350
NH_4Cl	530
$CaCl_2$	75
$MgCl_2$	100
$MnCl_2$	0.5
$ZnCl_2$	0.05
$CuCl_2 \cdot 2H_2O$	0.038
$CoCl_2 \cdot 6H_2O$	0.05
$FeCl_2 \cdot 4H_2O$	20
H_3BO_3	0.05
$NaMnO_4 \cdot 2H_2O$	0.01
$NiCl_2 \cdot 6H_2O$	0.05
$Na_2SeO_3 \cdot 5H_2O$	0.05

The medium was not buffered with $NaHCO_3$ nor reduced with sulfide, as these additions were noted in Quensen's procedures as optional. After autoclaving for about thirty minutes, the pH of the mineral medium was adjusted to 7.0 with dilute sodium hydroxide.

A sediment sample was obtained from an area of known high PCB contamination with the expectation that it would contain bacteria capable of dechlorinating PCBs. An additional sample was obtained from a site, upriver from the contamination source, to serve as an uncontaminated sediment source. The samples with high PCB contamination, which tested anaerobic to methylene blue, were placed in anaerobe jars with 10 mL of sterile water.

Twenty grams of uncontaminated sediment and 35 mL mineral medium were added to each of eight serum bottles in a CO_2/H_2 glove box. The bottles were left open for 24 hours in the glove box, injected with 50 μL ethanol, sealed, incubated at 37°C until methane production was observed, and, after two weeks, were autoclaved at 121°C for 1 hr.

Anaerobic microbes were obtained from the PCB-contaminated sediment by first transferring the sediment from the anaerobe jar to a glove box. In the glove box (CO_2/H_2 atmosphere) a sample of several hundred grams of PCB-contaminated sediment was shaken vigorously with 500 mL of the mineral medium. After 15 min settling, the liquid was decanted and saved. Aliquots (35 mL) of the decanted liquid were added to each of the eight autoclaved serum bottles of uncontaminated sediment.

Aroclors 1248 and 1254 (1:1 mixture dissolved in dimethylsulfoxide) were added to serum bottles (containing sediment and inoculated medium) in two concentrations. The resulting concentrations of PCBs in the vials were 55 ppm and 436 ppm. Duplicate vials were prepared at each concentration. The remaining four bottles, used as controls, were inoculated with anaerobic medium, but were then twice autoclaved for 15 minutes before addition of the Aroclor standards. All eight sealed serum bottles were stored in the dark at room temperature (23-26°C).

Part way through the experiment it was feared that the sediment sample from which the bacteria were extracted may not have had enough dechlorinating bacteria. Therefore, an additional sediment sample was obtained to maximize the

probability of the existence of dechlorinating anaerobes. The sample obtained was subsequently analyzed for PCBs and it contained more than 100 ppm of PCBs. Therefore, this sample should have contained dechlorinating bacteria. This sediment was extracted and the extract added to the media as described above. However, PCBs were spiked to give a resulting concentration of 436 ppm. Two vials were set up this way (no additional controls were prepared).

Samples of slurry, 2 mL each, were withdrawn from the vials for PCB analysis on an approximately monthly schedule. The samples were withdrawn into centrifuge tubes with 2 mL acetone and 10 mL hexane, agitated on a mechanical shaker for 10 min, decanted, agitated with an additional 10 mL hexane for 10 min, decanted and the liquid added to the previous extracts. The liquid was then blown down to near dryness and taken into about 3 mL iso-octane. The iso-octane extracts were then each eluted through a column of 8g florisil and 2g Na_2So_4 with 200 mL 6% ether/hexane. An aliquot of the sample was diluted in iso-octane until packed column GC screening indicated a suitable concentration for congener analysis with capillary GC. The samples were then analyzed for PCB congeners using a Hewlett Packard 5880 gas chromatograph equipped with a 60 m DB-5 coated capillary column.

The PCB concentrations measured in the monthly analyses were only about 30 percent of the expected concentration. Apparently, the procedure used to extract PCBs from the media was incomplete. However, the distribution of PCB congeners that were extracted

were nearly identical to the PCB congener distribution of the original Aroclors added. Cross streak plate tests of the samples after three months did reveal anaerobic activity (Clostridium and Bacillus organisms were tentatively identified). Consequently, despite anaerobic conditions, anaerobic dechlorination apparently did not occur (at least not measurably) in this experiment.

Part way through the experiment, the two vials that were set up using extracts from the sediments with PCBs greater than 100 ppm were opened in the glove box and cysteine sulfide was added along with 40 mL of ethanol. The cysteine sulfide was added in an amount equivalent to the amount of sodium sulfide specified by Quenson (Michigan State University, East Lansing, MI, 1989; personal communication) as an optional addition. These chemicals ensure that fully reducing conditions are occurring in the sample. It was thought that this addition would remove any uncertainty posed by not originally adding the sulfide.

After seven months the incubation was ended. All analyses up to this point indicated that no dechlorination was occurring, although the amount of PCBs extracted from subsamples was always less than expected. Either the vials were subsampled in such a way that the subsample obtained was unrepresentative (unlikely), or the extraction procedure was not fully removing the PCBs from the sediment/media mixture.

For a final analysis for PCBs after the seven month incubation, special procedures were taken to ensure better extraction of the sediment/media mixture. The vials (2) containing bacteria extracted from sediment with high PCBs and to

which the sulfide was added were analyzed by this special procedure. These vials were most likely to show dechlorination.

PCBs were first extracted from the decant of the vial with 50 mL of hexane. The extraction was repeated four times and the extract was saved. The residue left in the vial was then vigorously shaken with 35 mL of acetone in order to suspend all the material. The vial was then sonicated for three minutes to break up the particles as finely as possible to ensure good contact with the solvent. After the contents settled, the acetone was poured off and saved with the hexane extracts. This acetone extraction procedure was repeated three more times, so that the sonicated sediment/media mixture was extracted a total of four times with acetone.

The sediment/media mixture was further extracted with 35 mL of hexane. The mixture was sonicated for three minutes during each of the four successive extractions. After settling, the decant from each extraction was saved.

For PCB analysis, all the decants following extraction were mixed together. The resulting mixture was concentrated to 10 mL by rotoevaporation. The concentrated solution was cleaned up on a column containing 22.0 g of florisil and two 1 cm layers of Na_2SO_4 (to remove water and other interferences). The column was eluted with 220 mL of 6% either/hexane. The solvent leaving the column was evaporated by blow-down. Iso-octane was added to adjust the volume to 250 mL. Finally, a 1.0 mL aliquot was diluted to 50 mL with iso-octane.

Following screening by packed column chromatography, the

iso-octane extracts were analyzed by capillary column gas chromatography. For the two vials analyzed by this procedure, 88% and 89% of the PCBs were recovered. Thus, repeated extractions of sonicated samples was effective at extracting PCBs from the sediment/media mixture.

Despite the good recoveries, no evidence was found of dechlorination. Congener patterns closely resembled the distributions of congeners originally spiked into the vials. Consequently, none of the laboratory experiments produced evidence that bacteria extracted from Sheboygan River sediments produce dechlorination.

Despite the negative results of the experiments conducted, it is believed that more work would produce laboratory evidence of dechlorination. Several other laboratories around the country have now reported successful laboratory PCB dechlorination experiments. Dr. G-Yull Rhee of the New York Department of Health has now duplicated Quenson et al.'s experiments with bacteria extracted from the Hudson River (Rhee et al. 1989). However, Dr. Rhee (New York State Department of Health and School of Public Health, SUNY at Albany, Albany, NY, 1990; personal communication) has indicated that getting the bacteria to grow properly is very tricky. He indicated that in his work it would not be unusual to have only one of four replicates show dechlorination. Both Dr. Rhee and Dr. Quenson agree that the factors that may effect dechlorination in the laboratory are not clear. For example, it is not clear whether the addition of sulfide and ethanol are necessary or what electrode potential

should be obtained for the organisms to grow. Both believe that a consortium or organisms, rather that an individual species, is responsible for the dechlorination activity. Thus, while these studies laboratory experiments were not positive, it is believed that additional work, including a larger number of replicate samples, would produce evidence of dechlorination. The laboratory experiments of this study do show, however, that the conditions under which dechlorination occurs are not clear and that more needs to be learned if anaerobic dechlorination is to be used as a bioremediation tool.

References

1. Quensen, J.F., J.M. Tiedje, and S.A.Boyd (1988). Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms form sediments. Science, 242: 752-754.

2. Rhee, C.Y., B. Bush, M.P. Brown, M. Kane, and L. Shane (1989). Anaerobic biodegradation of polychlorinated biphenyls in Hudson River sediments and dredged sediments in encapsulation. Water Research, 23: 957-964.

An Evaluation of the Potential for PCB Dechlorination in Confined Disposal Facilities

Wisconsin State Laboratory of Hygiene
University of Wisconsin
Madison, WI 53706

EXISTING CONFINED DISPOSAL FACILITIES

Dredging of Great Lakes harbors to maintain navigation dates back to the early 1800's. Natural siltation from tributary rivers prompted periodic dredging, and dredging has been generally accepted as necessary and normal harbor maintenance. Typical disposal methods were to side cast the dredgings to adjacent areas or, more commonly, to deposit the material into deeper waters.

Until the mid 1960's immediate economic concerns governed the focus of this disposal. However, a new awareness of lake eutrophication problems led to a questioning of open water disposal techniques. The U.S. Army Corps of Engineers (1969) in a study titled "Dredging and Water Quality Problems in the Great Lakes" concluded that "in-lake disposal of heavily polluted dredging must be considered presumptively undesirable." In 1970, Congress generated PL 91-611 to create the Dike Disposal Program for the Great Lakes (Anonymous, 1989). The intent was to hold, in a confined facility, a ten year supply of dredgings, and to initiate a Dredged Material Research Program (DMRP). The U. S. Army Corps of Engineers (COE) conducted the DMRP at their Vicksburg (Mississippi) Waterways Experimental Station (WES).

The DMRP study was a comprehensive project, involving universities, private research laboratories, and other federal agencies. The main conclusions of the DMRP study, as indicated in U.S. Environmental Protection Agency (1987), were fourfold:

1. No single disposal alternative is suitable for a region, nor should any single alternative be dismissed from consideration.
2. Environmental considerations are stronger than other considerations to necessitate long range regional planning as a lasting, effective solution to disposal problems.
3. Fears concerning short term release of contaminants to site waters are unfounded especially if the geochemical environment is not changed.
4. To be environmentally effective, a confined site must retain a high percentage of finer particles, since this is where contaminants are carried.

Since 1978 there has been some additional dredged material research. However, the research focus has shifted from eutrophication to toxic substances. During the late 1970's and 1980's many harbor sediments were found to contain high concentrations of heavy metals and organic pollutants, such as PCBs. The research has not, however, provided a clear understanding of the impact that toxic substances in a CDF may be having on the Great Lakes or what happens to toxic chemicals over time when placed in a CDF.

Existing confined disposal facilities (CDF'S) in the Great Lakes basin are listed in Table 1. Other than the Superior/ Duluth facility (constructed in 1978), Wisconsin's CDF'S are all sited in or along Lake Michigan. In addition to the six Wisconsin facilities listed in Table 1, three additional sites

have been proposed (Menominee, Sturgeon Bay, and Sheboygan) for construction. The U. S. COE is responsible for dredge and fill operations, including the operation of CDF'S. Guidelines and criteria for dredging and filling are, however, set by the U.S. Environmental Protection Agency (EPA).

The Sheboygan Harbor was dredged by the COE in the mid 1950's and annually up to 1969 when dredging stopped due to controversy over the presence of heavy metals. Some contaminated harbor sediments were removed in 1981 and 1984. As stricter disposal regulations, particularly for PCBs have been put into effect, dredging has been halted due to the lack of a means of disposal.

GENERAL DESIGN OF CONFINED DISPOSAL FACILITIES

Each CDF is unique to the needs of the individual site. Upland CDF'S generally have earthen dikes, whereas in-lake CDF'S generally are encased by stone dikes to withstand wave action. The overall design goals are to hold within the facility as high a percent of the sediment particles dredged as possible.

The design of CDF'S has evolved since the first facility was constructed in the early 1960's to a point where there are now certain design similarities (U.S. Army Corps of Engineers, 1986). Designs now allow limited permeability so precipitation can drain into the lake. As environmental requirements became more stringent, steel sheet pile was incorporated into the design. This pile was used in the three Wisconsin CDF'S, thus further ensuring the dredged material is retained in the dike.

Recently constructed dikes have utilized a core of limestone which "cements" over time. A layer of locally available lake sand is now typically incorporated by layering the sand on the disposal side of the dike. This sand is uniform, has low permeability, and will not change or deteriorate in time. Some designs incorporate a weir with a skimmer to retain possible sediment overflow as the permeability of the CDF approaches zero.

PCB'S IN CDF'S

Very little data are available on PCB concentrations within CDF'S. Published data exists for only a few sites. The concentrations of PCB'S would be expected to vary from one CDF to another depending on the concentration of PCB's in the sediment which was dredged. Since PCB's in sediment can vary widely even within a small area (sediments are not homogeneous), concentrations within CDF's also undoubtedly vary widely.

The few data that are available on PCB's measured from samples collected from CDF's indicate relatively low total PCB levels. Clark (1988) reported a value of 1.1 $\mu g/g$. from sediments in the Calumet harbor CDF. Rathburn (1988), in a study of the Saginaw Bay diked facility, reported concentrations of PCB's congeners in water collected from inside the facility (indike), immediately outside the facility in Saginaw Bay (outdike), and at an adjacent site in the Saginaw River channel. An example of these data are shown in Table 2. The data show PCB concentrations are higher in water inside the facility than outside the facility. However, concentrations in water outside the facility are generally higher than PCBs in the reference

station (Saginaw River channel).

The specific congeners found in highest concentration in the Saginaw Bay study (Table 2) are similar to those found in highest concentration in the Sheboygan River. For example, co-eluting congeners 28/31 are the most prevalent in the Saginaw Bay study samples (Table 2), as they were in most Sheboygan samples.

Whether or not PCB's leak from a CDF continues to be a major uncertainty. The Saginaw Bay study was initiated to find the extent of leakage (as well biological uptake of the PCB's). The general conclusion of the study was that no appreciable leakage from the CDF was occurring.

If polychlorinated biphenyls (PCBs) can be dechlorinated in river sediments, a logical question to ask is whether PCBs contained in confined disposal facilities (CDFs) also undergo bacterial dechlorination. If dechlorination does occur in confined disposal facilities, these facilities would be an attractive way of detoxifying dredged PCB contaminated sediment. In the case of the Sheboygan River, these is interest in building a marina in Lake Michigan just north of the harbor. If such a facility could be designed in concert with the concept of a confined disposal facility, it would be especially attractive if it also promoted dechlorination of any PCBs in the sediment.

In this study the likelihood of dechlorination occurring in confined disposal facilities along the Wisconsin shore of Lake Michigan was considered. Attempts were made to gather information on PCBs in confined disposal facilities to see if any evidence for dechlorination was available. Evidence for

dechlorination in similar facilities, notably sewage sludge lagoons, was also sought. Finally, some samples were taken from the confined sludge facility off of Milwaukee and analyzed for PCBs.

Two samples were collected from the Milwaukee CDF. The CDF might be expected to contain relative high amounts of PCBs because of the industry in the area. However, specific data on the PCB content of the dredged material originally placed in the facility was not found. Nor was any data found from past measurements of PCBs made on samples taken from the facility.

Two "soil" samples were taken from the Milwaukee facility with the permission of the Corps of Engineers. Because the soil was hard, crusted and contained surface vegetation, sampling was difficult. It was also unclear what would be a representative sample, or where to sample to get high PCB concentrations.

The two samples analyzed had low PCB concentrations. No evidence of dechlorination was discernable from the PCB pattern. It was hoped that samples with higher PCB concentrations could be obtained, as such samples might show a greater tendency for dechlorination (analogous to Sheboygan River sediments). As it was, there was no evidence to suggest that dechlorination may be occurring, or at least occurring at a rate that would be noticeable.

A question that comes up is whether sediments below the top of the facility are anaerobic. Deeper core samples would be desirable in this regard, as dredged material several feet below the surface might be anaerobic while near the surface the dredged

material might be aerobic. Based on what was available in the literature, such measurements have not been made.

One indication that dechlorination might not occur (at least not at a meaningful rate), is that a sewage lagoon with PCB contamination has been extensively studied and there is no evidence of any PCB dechlorination occurring there. The Madison Metropolitan Sewerage District has several lagoons that have been filled for many years and now pose a disposal problem because of the high concentration of PCBs (about 50 ppm) in the sludge. The lagoons, which do go anaerobic, have been extensively monitored over the years. However, monitoring results indicate that the PCBs have been very stable over the years (Nemke, J., Director, Madison Metropolitan Sewerage District, Madison, WI, 1990; personal communication). While there may be special conditions in these specific lagoons that prevent dechlorination, one might expect the conditions in an anaerobic lagoon to be conducive to dechlorination.

Based on experiments attempting to achieve bacterial dechlorination in the laboratory, it is clear that the dechlorination process is highly sensitive to a variety of factors (some of which are not known or understood). Whether the right conditions are in place in CDFs or lagoons is not known. It is also clear that for dechlorination to occur in a measurable fashion or at a fast enough rate, PCB concentrations must be relatively high. Unless "hot spots" or areas of high concentration occur in CDFs, concentrations may be too low to see measurable PCB dechlorination.

Thus, an analysis of the available information suggest that dechlorination probably does <u>not</u> occur to any important extent in CDFs. More research should probably be conducted to confirm this hypothesis, including more rigorous sampling to assay PCBs in CDFs. Alternately, research might be conducted on how to promote or accelerate PCB dechlorination. Current research conducted on sediments from the Sheboygan River in pilot confined treatment facilities (in conjunction with Superfund cleanup of the river) may uncover some ways of promoting dechlorination that could be applied to CDFs.

b:\cdf1.jan

Table 1. Great Lakes Confined Disposal Facilities

Location	Type[a]	Size (acres)	Capacity (cu. yd.)	Year
Calumet Harbor, Chicago, IL	L	42	1,300,000	1984
Michigan City, IN	U	3.3	25,000	1978
Bolles Harbor, MI	L	24.6	335,000	1977
Monroe County, MI	I	685	18,640,000	1978/ 1981
Frankfort, MI	U	--	107,000	1982
Detroit, MI	I	80	1,900,000	1960
Dickinson Island, MI	U	174	2,000,000	1976
Grand Haven, MI	U	36	310,000	1974
Holland, MI	U	27.7	370,000	1977
Inland Route, MI	U	8.6	19,500	1982
Monroe, MI	L	89	4,200,000	1985
Saginaw Bay, Village of Sebewaing, MI	U	180	84,000	1979
Erie Pier, MN	L	82	1,000,000	1978
Buffalo, NY	L	33	1,500,000	1968
Buffalo, NY	L	45	1,500,000	1972
Buffalo, NY	L	40	6,900,000	1977
Cleveland, OH	L	56	2,760,000	1974
Cleveland, OH	L	88	6,130,000	1979
Huron, OH	I	63	2,150,000	1975
Lorain, OH	L	58	1,850,000	1977
Toledo, OH	L	150	5,000,000	1977
Toledo, OH	L	242	10,000,000	1976
Erie, PA	L	23	1,600,000	1979
Green Bay, WI	I	60	1,200,000	1979
Green Bay, WI	U	400	300,000	1965
Kenosha, WI	L	25	750,000	1975
Kewaunee, WI	L	28	500,000	1982
Manitowoc, WI		24	800,000	1975
Milwaukee, WI	I	454	1,600,000	1975

[a] L = In-lake adjacent to land
I = In-lake CDF island
U = Upland

Table 2. PCB Congener Concentrations (ng/L) in Saginaw Bay CDF Water Samples (From Rathburn et al. 1988)

Congener Number	Indike	Outdike	Saginaw River Channel
004 + 010	0.27	0.04	0.043
006	1.4	0.055	0.023
007	0.061	0	0.013
012	0	0.032	0.043
013	0.08	0	-----
016	1.2	0.1	0.04
017	3.4	0.19	0.1
018	3.6	0.31	0.092
019	0.3	0.032	0
022	2	0.13	0.019
024	0.05	0.017	0
025	----	----	0.073
027	0.39	0.036	0.017
029	0.035	0.021	0
031 + 028	13	0.96	0.4
032	3.5	0.43	0.16
033	2.1	0.027	0.052
037	---	-----	0.064
040	1.7	0.1	0.029
041 + 071	3.1	0.23	0.13
042	2.4	0.19	0.083
043	0.5	0	0
044	5.2	0.51	0.28
045	1.2	0.093	0.03
046	0.58	0.051	0.027
047 + 048	1.6	0.15	0.17
049		0.43	0.23
051	0.36	0.013	----
052		0.57	0.17
053	1.2	0.1	0.049
056 + 060	2	0.25	0.13
063	0.37	0.031	0.0075
064	2.2	0.019	0.092
066	3.4	0.4	0.23
070 + 076	3.4	0.41	0.19
074	1.3	0.13	0.068
077	0.65	0.068	0.034
081	0.17	0.024	0.0083
082	0.51	0.057	0.021
083	0.29	0.027	0
085	0.78	0.097	0.052
087	0.98	0.13	0.072
089	0.12	0.011	0
091	0.79	0.073	0.041
092 + 084	2.6	0.25	0.14

100	0.21	0	0
101	2.1	0.28	0.092
105 + 132 + 153	2	0.35	0.16
107	0.18	0.027	0.013
110	3.5	0.4	0.2
118	1.4	0.19	0.1
119	0.2	0.017	0.015
128	0.29	0.044	0.092
129	0	0	0.0083
130	0.14	0.023	0.028
134 + 114	0.3	0.04	0.028
135 + 144	0.16	0.047	0.0092
136	0.12	0.017	0
137 + 176	0	0	0
141	0.2	0.036	0.028
146	0.27	0.044	0.021
149	0.87	0.12	0.083
151	0.23	0.033	0.021
156	0.19	0.025	0.011
157 + 200	0	0.027	0.019
158	0.021	0.033	0.036
163 + 138	1.4	0.25	0.15
167	----	----	0.0075
170 + 190	0.48	0.083	0.051
172 + 197	0.16	0.021	0.0092
173	0	0	-----
174	0.23	0.047	0.03
175	0.23	0.036	0
177	0.16	0.031	0.02
178	0.098	0.039	0.0083
180	0.67	0.13	0.049
183	0.22	0.033	0.019
185	0	0	0.0045
187 + 182	0.29	0.059	0.035
189	0.089	0	0
191	0.4	0.016	----
193	0.044	0.0093	0.011
194	0.14	0.031	0.016
196	0.14	0.032	0.016
198	0.084	0.019	0.006
199	0	0	0.0047
201	0.23	0.052	0.033
202 + 171	0.12	0.019	0.0042
203	0.072	0.027	0.018
205	0	0	0.0033
206	0.11	0.037	0.013
207	0.044	0.0093	0.0033
208 + 195	0.42	0.072	0.026
209	0.005	0.001	0.0008
Total PCB'S	91.4	10.2	5.2

Blanks = rejected congeners; zeros = concentrations lower than analytical detection limit.

REFERENCES:

1. Anonymous (1989) Diked Disposal Program - Resume (P.L. 91-611), Q/A section, U.S. Army Corps of Engineers, Buffalo, NY.

2. Clark, J.U., McFarland, V.A., and Dorkin, J. (1988) Evaluating Bioavailability of Neutral Organic Chemicals in Sediments: A confined Disposal Facility Case Study. Water Quality 88 Seminar Proceedings., Feb. 23-24, Charleston, SC, U.S. Army Corps of Engineers, Vicksburg, MS.

3. Rathburn, J., Kreis, R., Lancaster, E., Mac, M., and Zabik, M. (1988) Pilot Confided Disposal Facility Biomonitoring Study: Channel/Shelter Island Dike Facility, Saginaw Bay, Bay City, Michigan, Report of the Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, MN

4. U.S. Army Corps of Engineers (1969) Dredging and Water Quality Problems in the Great Lakes, Buffalo District, Buffalo, NY.

5. U.S. Army Corps of Engineers (1986) Appendix B, Dredged Material Disposal Facilities on the Great Lakes, Draft Environmental Impact Statement, Indiana Harbor Confined Disposal Facility, North Central District, Chicago, IL.

6. U.S. Environmental Protection Agency (1987) Report on Great Lakes Confined Disposal Facilities, draft, Great Lakes Program Office, Region V, Chicago, IL.

NOAA COASTAL SERVICES CENTER LIBRARY
3 6668 14104 8233