

Chapter 2

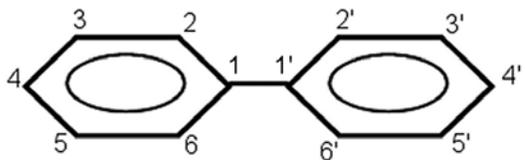
PCB/trans-Nonachlor Study Overview

2.1 PCB Introduction

2.1.1 Physical/Chemical Properties

Polychlorinated biphenyls (PCBs) are a class of synthetic organic chemicals characterized by two six-membered aromatic carbon rings joined by a single carbon-carbon bond that constitute the parent biphenyl. One or more chlorine atoms are attached to carbon atoms in the two ring structures. There are 209 possible arrangements of chlorine atoms, and each of the arrangements is referred to as a PCB “congener.” The carbon atoms on both of the aromatic rings are numbered 1 through 6, to indicate their position around each ring. The superscript prime (') is used to distinguish similar positions on the two rings. The two carbon atoms linking the rings are numbered 1 and 1'. Each of the remaining 10 carbon atoms (numbered 2 to 6 and 2' to 6') can bond with one hydrogen or chlorine atom. The formal chemical name of each of the 209 congeners identifies the specific positions and the total number of chlorine atoms in the congener, e.g., 2,2',4,5'-tetrachlorobiphenyl.

Figure 2-1. Generalized 2-D Structure of Biphenyl



There are 3 possible PCB congeners that contain a single chlorine atom. The two ring structures are symmetrical, such that the structures 2-monochlorobiphenyl and 2'-monochlorobiphenyl are identical. In addition, the two rings can rotate along the carbon bond that joins them, such that the 2 and 6 positions on each ring are equivalent, as are the 3 and 5 positions. By convention, the equivalent position with the lower number is used to describe the compound. Thus, a monochlorobiphenyl with the chlorine attached in the 2 or 2', or 6 or 6' position is named 2-monochlorobiphenyl. The monochlorobiphenyl with the chlorine attached in the 3 or 3', 5 or 5' position is named 3-monochlorobiphenyl. The monochlorobiphenyl with the chlorine attached in the 4 or 4' position is named 4-monochlorobiphenyl.

Table 2-1. Numbers of Congeners in Each Level of Chlorination

No. of Chlorine Atoms	Level of Chlorination	No. of Congeners
1	monochlorobiphenyl	3
2	dichlorobiphenyl	12
3	trichlorobiphenyl	24
4	tetrachlorobiphenyl	42
5	pentachlorobiphenyl	46
6	hexachlorobiphenyl	42
7	heptachlorobiphenyl	24
8	octachlorobiphenyl	12
9	nonachlorobiphenyl	3
10	decachlorobiphenyl	1

PCBs are often described in terms of the “level of chlorination,” which refers to the number of chlorine atoms attached to the biphenyl ring (e.g., monochlorobiphenyls, dichlorobiphenyls, and trichlorobiphenyls). The numbers of PCB congeners in each level of chlorination are shown in Table 2-1. The physical and chemical properties of PCBs are dependent on the number of chlorine atoms and their respective positions in the two ring structures, thus, they vary with the congener. In general, PCBs with the same number of chlorine atoms have similar physical and chemical properties.

GLNPO adopted the numbering system developed by Ballschmiter and Zell (1980) that simplifies the identification of each congener by assigning each possible congener a number 1 through 209. That notation is used throughout the remainder of this discussion.

There is a subset of PCB congeners in which the pattern of chlorine substitution causes "steric hindrance" that limits the rotation of the two aromatic rings around their common carbon-carbon bond such that the two rings lie in the same plane. These congeners are called "coplanar" PCBs and as a result of their flat configuration, they have a greater ability to penetrate the walls of living cells and generally exhibit greater toxicological effects (see Section 2.3).

In general, PCBs are chemically inert, nonflammable, and do not transmit electrical current. These properties, combined with high melting and boiling points made PCBs useful in a wide variety of industrial applications, particularly as dielectric fluids in electrical transformers and capacitors. Flash points for PCBs are in the range of 170 to 380°C (H. Fiedler, 2001)

PCBs are readily soluble in organic solvents, but have low solubility in water. Water solubility decreases dramatically as the number of chlorine atoms attached to the parent biphenyl structure increases. For example, the water solubility of unsubstituted biphenyl has been reported to be within the range of 5.9 to 7.5 mg/L, while the water solubility of PCB 209, containing 10 chlorine atoms, is estimated at 4×10^{-6} mg/L, or 4 ng/L (Shiu and Mackay, 1986). The vapor pressures of PCB congeners decrease in a similarly dramatic fashion with increased chlorination. Dunnivant and Elzerman (1988) report vapor pressures for several dichlorobiphenyl congeners on the order of 2×10^{-6} atmospheres, while the vapor pressures for some hexachlorobiphenyls are on the order of 3×10^{-10} or 3×10^{-11} atmospheres. In the LMMB Study, PCBs served as a model for conservative organic pollutants.

2.1.2 History of PCB Production

In the United States, the Monsanto Company produced commercial mixtures of PCBs by chlorinating biphenyl and sold the mixtures under the trade name Aroclor. There were nine Aroclor mixtures produced in the U.S. and they were differentiated by a series of four-digit numbers (e.g., Aroclor 1242). The names of eight of the nine mixtures begin with the number "12," representing the 12 carbon atoms in the parent biphenyl, and end in a two-digit number that represents the percentage of chlorine (by weight) in the mixture. Aroclor 1016 was the only mixture that violated this naming scheme, because its number began with "10" instead of "12," and it contained more than 16% chlorine. The nine Aroclor mixtures are shown in Table 2-2, along with their percent chlorine (by weight), and the approximate percentage of domestic production that they comprised for the period from 1957 to 1977.

Table 2-2. U.S. Domestic Production of Commercial Aroclor Mixtures from 1957 to 1977*

Mixture	Percent Chlorine by Weight	Percent of U.S. Production
Aroclor 1016	41	13
Aroclor 1221	21	1
Aroclor 1232	32	<1
Aroclor 1242	42	52
Aroclor 1248	48	7
Aroclor 1254	54	16
Aroclor 1260	60	11
Aroclor 1262	62	1
Aroclor 1268	68	<1

*Adapted from Brown, 1994. Total production percent is greater than 100%, due to rounding.

Table 2-3 contains the typical compositions of the five Aroclors with the greatest U.S. production, in terms of the percentages of various levels of chlorination.

PCB production facilities were built in Austria, Germany, France, Great Britain, Italy, Japan, Spain, the USSR, and the U.S. PCB mixtures were produced in Germany under the trade name Clophens, in Japan under the names Kanechlors and Sanotherm, and in France as Phenoclor and Pyralene. World-wide production of Aroclors is estimated to have been 1.5 million metric tonnes (3.3 billion pounds) (Rantanen, 1992; Ivanov and Sandell, 1992).

Table 2-3. Typical Composition (%) of the Five Aroclor Mixtures with Greatest U.S. Production*

Biphenyls by Level of Chlorination	Aroclor				
	1016	1242	1248	1254	1260
Monochlorobiphenyls	2	1	<1%	<1%	<1%
Dichlorobiphenyls	19	13	1	<1%	<1%
Trichlorobiphenyls	57	45	21	1	<1%
Tetrachlorobiphenyls	22	31	49	15	<1%
Pentachlorobiphenyls	<1%	10	27	53	12
Hexachlorobiphenyls	<1%	<1%	2	26	42
Heptachlorobiphenyls	<1%	<1%	<1%	4	38
Octachlorobiphenyls	<1%	<1%	<1%	<1%	7
Nonachlorobiphenyls	<1%	<1%	<1%	<1%	1
Decachlorobiphenyl	<1%	<1%	<1%	<1%	<1%

*Adapted from PCBs: Cancer Dose-Response Assessment and Application to Environmental Mixtures, EPA/600/P-96/001F, September 1996.

2.1.3 Regulatory Background

PCB production and export in the U.S. was halted in October 1977 under the auspices of the Toxic Substances Control Act (TSCA). Use and import of PCBs were banned in Japan in 1972. In addition to the production ban instituted under TSCA, EPA regulates PCBs, as Aroclors, under a wide range of environmental statutes. For example, Aroclors are regulated in effluent guidelines developed under the Clean Water Act and administered through the National Pollutant Discharge Elimination System (NPDES). The Office of Water has established water quality criteria (WQC) for freshwater and marine systems. The freshwater chronic WQC is 0.014 µg/L of total Aroclor. The marine chronic WQC is 0.030 µg/L. The WQC for human health is 4.5×10^{-5} µg/L. Under the Safe Drinking Water Act, EPA has established a maximum contaminant limit (MCL) of 0.50 µg/L for total Aroclor. Under the auspices of the Resource Conservation and Recovery Act (RCRA) EPA has placed Aroclors on Appendix VIII (hazardous substances) and Appendix IX (groundwater monitoring), and has established a Universal Treatment Standard (UTS) of 1 mg/kg of Aroclors in non-wastewaters and 0.10 mg/L in wastewaters. PCBs are included in the Toxics Release Inventory (TRI) developed under the Emergency Planning and Community Right to Know Act (EPCRA).

2.1.4 Fate and Effects

The fate and effects of PCBs in the environment are driven by the physical properties of the individual PCB congeners. In general, PCBs are hydrophobic and lipophilic. Therefore, they are more likely to be found in soils, sediments, and tissues, than dissolved in water. PCBs found in water are likely to be associated with the particulate phase, with some of the lower chlorinated congeners present in the dissolved phase.

Baker and Eisenreich (1990) examined the behavior of PCBs in Lake Superior, finding that PCBs volatilize when river inputs to the lake are relatively high in PCBs. They calculated volatilization rates for PCBs that are approximately equal to the rate of atmospheric deposition into the lake. These findings support a model of PCB cycling proposed by Mackay and Patterson (1986) in which PCBs dissolved in rain or sorbed onto air particulates are transported to surface water. This input results in a fugacity

gradient that favors volatilization from the water into the atmosphere, where the PCBs are dissolved in rain water or sorbed onto particulates again, thus repeating the cycle.

2.1.5 Biological Transformations

PCBs can be degraded by microorganisms by several mechanisms, including both aerobic and anaerobic processes. Although PCBs are generally resistant to aerobic breakdown, Rochkind *et al.* (1986) found that microorganisms of the genera *Acetobacter*, *Alcaligenes*, and *Pseudomonas* are capable of degrading PCBs under aerobic conditions. However, the rates of degradation are greatly influenced by the chlorine substitution pattern in each congener. Rochkind *et al.* found that:

- higher chlorinated PCBs degrade more slowly under aerobic conditions than those with fewer chlorine atoms;
- PCBs with chlorines on only one ring are metabolized more rapidly than PCBs in the same level of chlorination, but with the chlorine atoms distributed on both rings;
- the ring with fewer chlorines will be hydroxylated first, and;
- chlorine atoms attached at the *ortho* position (2 or 6 on the ring) significantly inhibit degradation.

In anaerobic environments, PCBs will undergo reductive dechlorination (loss of chlorine), with the degradation rate related to the number of chlorine atoms on the PCB. As a result, the more highly chlorinated congeners are more readily dechlorinated under anaerobic conditions. Fiedler *et al.* (1994) found that the chlorines in the *meta* and *para* positions (3, 4, and 5 on the ring) were more readily lost by reductive dechlorination, resulting in an apparent increase in the prevalence of the *ortho*-substituted PCBs in the environment.

PCBs are lipophilic, concentrating in fatty tissues of organisms. As a result, PCBs are not readily excreted from organisms as intact chlorinated biphenyls. The metabolic transformation of PCBs requires that the molecule undergo hydroxylation to make it more polar, and thus more water soluble. In higher organisms, PCBs are hydroxylated through a metabolic pathway involving the hepatic monooxygenase system mediated by the enzyme Cytochrome P-450. As with other transformations of PCBs, the rates are related to the level of chlorination and the substitution patterns.

As a result of both their lipophilic nature and their low rates of biodegradation, PCBs accumulate, or bioconcentrate, to higher concentrations in each subsequent trophic level of an ecosystem. Fiedler *et al.* (1994) has shown that both the total PCB concentrations and the concentrations of the "toxic" or "dioxin-like" PCBs (see Section 2.1.6) increase in typical aquatic systems consisting of phytoplankton, zooplankton, plankton-eating fish, piscivorous fish, and piscivorous birds.

2.1.6 Toxicity

EPA has classified PCBs as carcinogens. Other human health effects include disruption of endocrine systems. Effects of short-term exposures in humans include chloracne, changes in skin pigmentation, numbness of the limb and general weakness. Long-term exposures have been associated with changes in liver function, irritations of the nose, throat and intestinal tract, fertility problems, birth defects, premature births, and neurological and developmental problems in newborns.

Many of these health effects of PCBs are similar to those related to exposures to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and are believed to result from the actions of a subset of the 209 possible PCB congeners known as the "coplanar" PCBs. The coplanar PCBs have structures that resemble the 2,3,7,8-substituted PCDDs/PCDFs, in which the two aromatic rings lie in one plane and are believed to be more readily transported across cell membranes. The World

Health Organization (WHO) has classified 12 PCB congeners as the “toxic” PCBs, or the “dioxin-like” PCBs, based on structure-activity relationships (see Table 2-4 and Van den Berg *et al.*, 1998). However, for reasons discussed in Section 2.6, the toxic PCBs were not the primary focus of the LMMB Study.

Table 2-4. World Health Organization Toxic PCB Congeners

Congener Number	PCB Congener	Structural Group
77	3,3',4,4'-Tetrachlorobiphenyl	Non- <i>ortho</i> substituted PCB
81	3,4,4',5-Tetrachlorobiphenyl	Non- <i>ortho</i> substituted PCB
126	3,3',4,4',5-Pentachlorobiphenyl	Non- <i>ortho</i> substituted PCB
169	3,3',4,4',5,5'-Hexachlorobiphenyl	Non- <i>ortho</i> substituted PCB
105	2,3,3',4,4'-Pentachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
114	2,3,4,4',5-Pentachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
118	2,3',4,4',5-Pentachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
123	2',3,4,4',5-Pentachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
156	2,3,3',4,4',5-Hexachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
157	2,3,3',4,4',5'-Hexachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
167	2,3',4,4',5,5'-Hexachlorobiphenyl	Mono- <i>ortho</i> substituted PCB
189	2,3,3',4,4',5,5'-Heptachlorobiphenyl	Mono- <i>ortho</i> substituted PCB

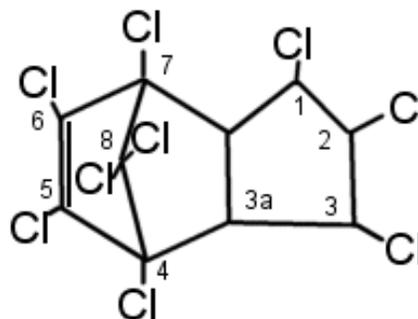
The term “*ortho*” in Table 2-4 refers to the position of the chlorines attached to the phenyl ring structure, relative to the carbon-carbon bond between the two rings. Chlorines attached at the 2, 2', 6, or 6' positions of the biphenyl structure (see Figure 2-1) are in the “*ortho*” position. A “non-*ortho*” congener does not have any chlorines in the 2, 2', 6, or 6' positions, while a “mono-*ortho*” congener has a chlorine attached at one of those positions.

2.2 *trans*-Nonachlor Introduction

2.2.1 Physical/Chemical Properties

trans-Nonachlor is the common name for 1,2,3,4,5,6,7,8,8-nona-chloro-3a,4,7,7a-tetrahydro-4,7-methanoindan, a member of the class of cyclodiene pesticides. *trans*-Nonachlor is a component of the pesticide formulation “technical chlordane,” comprising about 1.1% of that mixture of at least 140 related compounds. *trans*-Nonachlor differs from the two structural isomers of the compound chlordane (*cis*- and *trans*-chlordane) by the addition of one additional chlorine atom, in the number 3 position of the molecule (see Figures 2-2 and 2-3).

Figure 2-2. Generalized 2-D Structure of *trans*-Nonachlor



Compared to the two isomers of chlordane, there is very little published physical-chemical data for *trans*-nonachlor. For example, Mackay *et al.* (1992) do not list *trans*-nonachlor at all. The general properties of *trans*-nonachlor can be inferred from those of chlordane. The addition of one more chlorine atom to the chlordane structure increases the melting and boiling points for *trans*-nonachlor, decreases the water solubility, and decreases the vapor pressure. *trans*-Nonachlor has low solubility in water, but is readily soluble in organic solvents. It has a melting point above 100°C and a boiling point above 175°C.

In the LMMB Study, *trans*-nonachlor serves as a model for the cyclodiene pesticides.

2.2.2 *trans*-Nonachlor Production

trans-Nonachlor was not produced as a pure compound, but was one of the major components of technical chlordane mixtures. Chlordane is produced through the chlorination of cyclopentadiene to form hexachloropentadiene. This intermediate product is condensed to form chlordene, which undergoes additional chlorination to produce *cis*- and *trans*-chlordane, plus *trans*-nonachlor, heptachlor, and several other major components. Chlordane mixtures were first produced in the U.S. in 1948 and various formulations of chlordane were widely used as pesticides on food crops and lawns, and for termite control from 1948 to 1988. In April 1988, all commercial uses in the U.S. were banned (USEPA, 1988).

Since 1988, the only U.S. domestic manufacturer of chlordane has been the Velsicol Chemical Company of Memphis, TN. Production data for chlordane are difficult to obtain, but EPA estimated that 3.5 to 4 million pounds of chlordane were distributed in 1986. Data from the Toxics Release Inventory for 1990 indicate that 100,000 to 1 million pounds of chlordane were produced that year.

EPA estimated that more than 7.5 million pounds of chlordane were used for home, lawn, and garden purposes in the U.S. in 1974 (USEPA, 1975). Other sources estimate total production in 1974 was on the order of 21 million pounds, suggesting that over 13 million pounds of chlordane were exported that year (WHO, 1988).

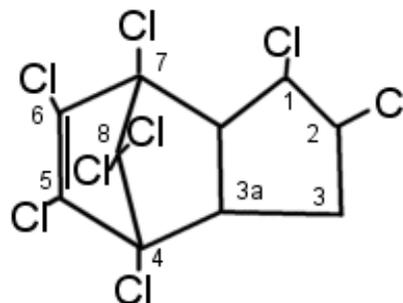
2.2.3 Regulatory Background

trans-Nonachlor has been regulated in the U.S. as a component of chlordane. Beginning in 1975, EPA ordered a halt to the use of chlordane and the related pesticide heptachlor for most household and agricultural uses, citing an imminent human cancer hazard. The 1975 action limited the use of chlordane to underground injection for termite control and treatment of the roots and tops of non-food plants. From July 1983 to April 1988, EPA further restricted the use of chlordane to underground injection for termite control. In April 1988, EPA canceled all commercial uses of chlordane in the U.S.

Regulation of *trans*-nonachlor has typically been accomplished by analogy to chlordane. Thus, the maximum contaminant limit (MCL) established under the Safe Drinking Water Act is 2 µg/L for *trans*-nonachlor, the same MCL used for chlordane. Similarly, the water quality criteria developed under the Clean Water Act for *trans*-nonachlor have the same values as were developed for chlordane.

Chlordane has been banned in 47 countries, including the U.S., and 14 additional countries have severely restricted its use.

Figure 2-3. Generalized 2-D Structure of *trans*-Chlordane



2.2.4 Fate and Effects

All of the components of chlordane have been found to bioaccumulate, with *trans*-nonachlor the most bioaccumulative of the components. In general, *trans*-nonachlor is hydrophobic and lipophilic. Therefore, it is more likely to be found in soils, sediments, and tissues than dissolved in water. When found in water, *trans*-nonachlor is likely to be associated with the particulate phase, rather than actually dissolved in water.

2.2.5 Toxicity

As with other aspects of *trans*-nonachlor, the majority of the data on toxicity has been determined for either technical chlordane mixtures or the predominant chlordane isomers. To date, the data from human studies have not provided sufficient evidence to conclude that either chlordane or *trans*-nonachlor is a human carcinogen. However, mice fed low levels of chlordane in food developed liver cancer. Therefore, *trans*-nonachlor is considered to be a probable human carcinogen. Other human health effects include neurological effects, blood dyscrasia, hepatotoxicity, immunotoxicity, and endocrine system disruption.

2.3 Study Design

2.3.1 Description

PCBs and *trans*-nonachlor were chosen for analysis in the LMMB Study as representatives of the persistent, bioaccumulative chlorinated compounds. PCB congeners and *trans*-nonachlor were measured in vapor, precipitation, particulates, atmospheric dry deposition, water in the open lake, tributaries, sediment, lower pelagic food web organisms, and fish. The data generated from this study were used to estimate an overall mass balance of PCBs and *trans*-nonachlor in Lake Michigan (see Section 1.4).

2.3.2 Scope

To develop a mass balance of PCBs and *trans*-nonachlor in Lake Michigan, all significant sources and stores of PCBs and *trans*-nonachlor in the environment were measured. Significant sources and stores included tributary inputs, atmospheric inputs from the vapor phase, particulate phase, and precipitation, sediment, lower pelagic food web organisms, and fish. The specific components that were studied are shown in Table 2-5.

Field sampling was conducted from February 1994 through October 1995, with an additional sampling cruise in May 1996 to retrieve sediment traps and collect samples at Stations LM94-11, LM94-17, LM94-18, LM94-21S and LM94-32.

2.3.3 Organization/Management

The responsibility for collecting and analyzing PCBs and *trans*-nonachlor samples from the various components was divided among multiple principal investigators (Table 2-5). Each principal investigator developed a Quality Assurance Project Plan (QAPP) that was submitted to EPA's Great Lakes National Program Office. The QAPPs detailed the project management, study design, and sampling and analysis procedures that would be used in the study and the quality control elements that would be implemented to protect the integrity of the data. The LMMB Quality assurance program is further discussed in Section 2.6, and detailed information on the quality assurance activities and data quality assessment specific to each ecosystem component are discussed in Chapters 3-5.

Table 2-5. Components Sampled by Principal Investigators

Ecosystem Compartment	Component	Principal Investigator
Atmosphere	Vapor Particulate Precipitation	Clyde Sweet , Illinois State Water Survey (Sleeping Bear Dunes from 4/94 to 7/94 and all other sites)
	Vapor Particulate Precipitation	Ron Hites and Ilora Basu , Indiana University (Sleeping Bear Dunes <i>only</i> , from 8/94 to 10/95)
	Dry deposition	Steve Eisenreich , University of Minnesota Gray Freshwater Biological Institute and Rutgers University
Tributary	Dissolved Total	William Sonzogni , University of Wisconsin, Wisconsin State Lab of Hygiene
Open Lake	Dissolved Total	Eric Crecelius , Battelle Sequim
Sediment	Surficial Resuspended	Patricia Van Hoof and Brian Eadie , Great Lakes Environmental Research Laboratory, National Oceanic and Atmospheric Administration
Lower Pelagic Food Web Organisms	Mysis Diporeia Zooplankton Phytoplankton	Deborah Swackhamer , University of Minnesota
Fish	Lake Trout Slimy sculpin Deepwater sculpin Coho salmon Alewife Bloater chub Smelt	Robert Hesselberg and James Hickey , United States Geological Survey, Biological Resources Discipline (formerly National Biological Service)

2.4 Sampling Locations

2.4.1 Atmospheric Components

Atmospheric samples were collected at eight shoreline sampling stations and 16 open-lake sampling stations within Lake Michigan and two open-lake sampling stations in Green Bay. In addition, three out-of-basin land-based sampling stations were established as regional background sites to represent air coming over Lake Michigan during periods of southwest or northwest prevailing winds. The sampling locations and sampling frequencies for the LMMB Project were selected through discussions with experts in the field during several workshops, including the Great Lakes Mass Balance Planning Workshop in April 1992 and the LMMB Planning Meeting in September 1993. Site-selection criteria considered predominant annual wind directions, source areas, and episodic summer events. In general, sites were selected to be regionally representative of land-use categories and to represent the different potential sources of pollutants in this study (e.g., releases associated with population centers versus applications of agricultural activities). The sampling frequencies were designed to capture the expected variability at the sites (e.g., more frequent sampling in urban areas versus less frequent sampling in remote areas and more frequent sampling in spring summer, and fall months).

The shoreline atmospheric sampling stations include those specific to the LMMB Study as well as several that are part of the Integrated Atmospheric Deposition Network (IADN). Samples were collected from

the land-based IADN stations at the Brule River, Eagle Harbor, and Bondville from April 1994 through October 1995. Sampling at these IADN stations was governed by study design and quality assurance programs specific to IADN, but generally similar to those in the LMMB Study, so the data have been incorporated into the LMMB database. The locations of the shoreline atmospheric PCB sampling stations within the Lake Michigan basin are shown in Figure 2-4. The site classifications, planned sampling frequencies, and types of vapor-phase and particulate-phase samples are shown in Table 2-6. These frequencies were generally followed as sampling schedules permitted and except in cases of sampler malfunction, lack of precipitation, or when circumstances prevented retrieval of a sample.

Table 2-6. Site Classifications, Planned Frequencies, and Types of Vapor-phase and Particulate-phase Samples Collected at Shoreline and Out-of-basin Stations

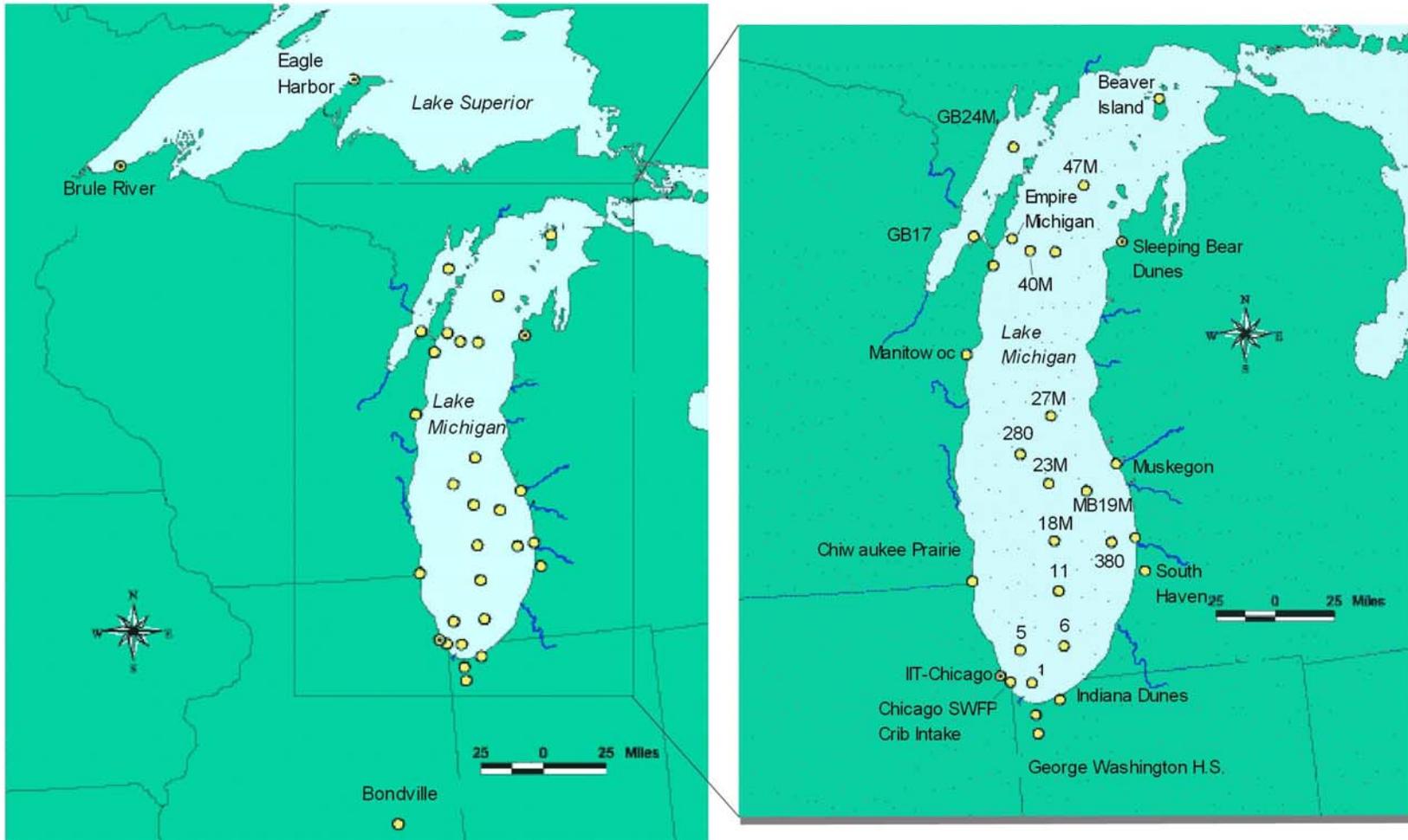
Site Classification	Planned Frequency	Site Names	Sample Type
Urban - Major urban sources within 1 km	One 24-h composite collected every 3 days	IIT Chicago	Multiple 24-h samples composited to represent 1 sample per month
Urban-influenced - Major urban sources within 10 km	One 24-h composite collected every 3 days	Chiwaukee Prairie	Multiple 24-h samples composited to represent 1 sample per month
		Indiana Dunes	
		Manitowoc	
		Muskegon	
Rural - Urban sources generally 10 to 50 km away, but agricultural sources within 1 km	One 24-h composite collected every 6 days	Bondville	Multiple 24-h samples composited to represent 1 sample per month
		South Haven	
Remote - No urban areas or major sources of air pollutants within 50 km	One 24-h composite collected every 12 days	Beaver Island	Multiple 24-h samples composited to represent 1 sample per month
		Brule River	
		Eagle Harbor	
		Sleeping Bear Dunes	Individual 24-h samples analyzed and mathematically composited to represent 1 sample per month

PCBs and *trans*-nonachlor were measured in vapor, particulates, and precipitation samples collected at 16 locations in Lake Michigan during seven cruises of the *Research Vessel Lake Guardian* between April 1994 and October 1995 and at two stations in Green Bay. Because these open-lake samples were collected on board the ship, they are single-day samples, and were not composited by month.

Monthly integrated dry deposition samples were collected from the following stations: 68th Street Crib, Sleeping Bear Dunes, Harrison Crib, IIT Chicago and South Haven. In addition, two 4-day composite dry deposition samples were collected on the ship in July 1994.

Figure 2-4. Atmospheric Sampling Stations

Atmospheric Stations ● IADN Stations ⊙



2.4.2 Tributaries

Tributary samples were collected from 11 rivers that flow into Lake Michigan (Figure 2-5). These tributaries included the Menominee, Fox, Sheboygan, and Milwaukee Rivers in Wisconsin; the Grand Calumet River in Indiana; and the St. Joseph, Kalamazoo, Grand, Muskegon, Pere Marquette, and Manistique Rivers in Michigan. With the exception of the Pere Marquette River, these tributaries were selected for the LMMB Study because of elevated concentrations of contaminants in resident fish. The Pere Marquette River was selected because it has a fairly large and pristine watershed. Samples collected from the Pere Marquette River can be used to estimate loads from the small portion of the Lake Michigan watershed that was not monitored in this study. The 11 monitored tributaries represent greater than 90% of the total river flow into Lake Michigan and an even higher percentage of the total tributary load of pollutants into Lake Michigan.

Table 2-7 describes specific watershed characteristics and impairment information for each of the monitored tributaries. Of the 11 tributaries, 6 (the Kalamazoo, Manistique, Menominee, Fox, Sheboygan, and Grand Calumet Rivers) are classified as Great Lakes areas of concern (AOCs). Areas of concern are severely degraded geographic areas within the Great Lakes Basin. They are defined by the US-Canada Great Lakes Water Quality Agreement (Annex 2 of the 1987 Protocol) as “geographic areas that fail to meet the general or specific objectives of the agreement where such failure has caused or is likely to cause impairment of beneficial use or the area’s ability to support aquatic life.” Most of the eleven tributaries are also listed on the Clean Water Act Section 303(d) list of impaired water bodies due to contamination from mercury, PCBs, and other pollutants.

Figure 2-5. Tributary Sampling Stations



Table 2-7. Watershed Characteristics for Tributaries Monitored in the LMMB Study

Tributary	Watershed area (mi ²)	Total river miles in watershed	Riparian Habitat		IWI Score ^a	Impaired for ^b	Area of Concern
			Forested	Agricultural/Urban			
St. Joseph	4685	3743	25-50%	>50%	3- less serious problems, low vulnerability	<i>E. coli</i> , mercury, PCBs, pathogens, macro-invertebrate community	
Kalamazoo	2047	1560	25-50%	>50%	3- less serious problems, low vulnerability	Mercury, PCBs	X
Grand (lower)	2003	2014	25-50%	>50%	5- more serious problems, low vulnerability	PCBs, pathogens	
Muskegon	2686	1886	25-50%	>50%	5- more serious problems, low vulnerability		
Pere Marquette	2644	1356	25-50%	>50%	3- less serious problems, low vulnerability	Mercury, PCBs	
Manistique	1464	1061	>75%	20-50%	1- better quality, low vulnerability	Mercury, PCBs, pathogens	X
Menominee	2306	1660	>75%	20-50%	1- better quality, low vulnerability	Dioxin, PCBs, mercury, pathogens	X
Fox (lower)	442	700	25-50%	>50%	6- more serious problems, high vulnerability	PCBs, organic enrichment, dissolved oxygen	X
Sheboygan	2201	1699	25-50%	>50%	5- more serious problems, low vulnerability	PCBs, mercury	X
Milwaukee	864	802	25-50%	>50%	5- more serious problems, low vulnerability	PCBs	
Grand Calumet	1039	760	25-50%	>50%	5- more serious problems, low vulnerability	PCBs, pesticides, lead, mercury, dissolved oxygen, cyanide, chlorides, impaired biotic community, oil and grease, copper	X

^aEPA's Index of Watershed Indicators Score for assessing the health of aquatic resources.

^bBased on 1998 listing of Clean Water Act Section 303(d) impaired waters.

2.4.3 Open Lake

Open-lake water column samples were collected from 38 sampling locations on Lake Michigan, 2 sampling locations in Green Bay, and 1 sampling location on Lake Huron (Figure 2-6). Open-lake samples were collected during eight cruises of the *R/V Lake Guardian* between April 1994 and September 1995. Due to field conditions and other considerations, samples were not collected at all 41 stations during each cruise. The dates of the eight cruises and the total number of stations occupied are shown in Table 2-8.

The stations used in the LMMB Study and shown in Figure 2-6 include many historical stations used by GLNPO and other programs. Stations established specifically for the LMMB Study are shown in Figure 2-6 with identifiers that begin with "MB." The identifier for the station in Lake Huron begins with "LH," as seen in the upper right corner of Figure 2-6. The identifiers for the two stations in Green Bay begin with "GB," as seen in the upper left portion of Figure 2-6. Some of the stations are those specified by GLNPO as "Master" stations that are used for various purposes beyond the LMMB Study. The identifiers for those master stations end in "M."

The first survey occurred in the early spring just after "ice out" in April 1994. The second survey was in early summer (June 1994) after the onset of stratification and following the spring runoff period of agricultural chemicals from crop land. The third survey was in late summer (August 1994) during later stages of stratification. The fourth and fifth surveys, conducted in October 1994 and January 1995, sampled only a few of the Lake Michigan sites. The sixth survey occurred in March 1995 just after ice out. The seventh and eighth surveys occurred in August and September 1995, during stratification.

Figure 2-6. Open-Lake Water Column Sampling Stations

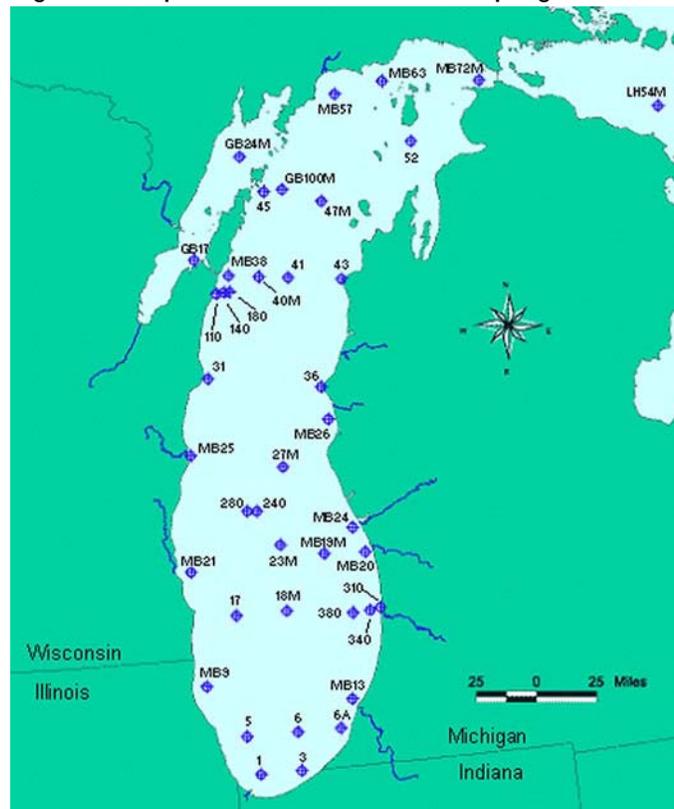


Table 2-8. Open-lake Cruise Dates and Number of Stations Occupied

Cruise Date	Number of Stations Occupied
April 1994	39
June 1994	14
August 1994	41
October 1994	37
January 1995	4
March 1995	40
August 1995	19
September 1995	41

2.4.4 Sediment

In 1994 and 1995, 117 sediment samples were collected from Lake Michigan and 6 samples were collected from Green Bay, by box coring, Ponar dredge, and gravity coring. The sediment sampling locations were selected to help define the three depositional zones (depositional, transitional, and non-depositional). The locations and the sampling device used at each location are shown in Figure 2-7.

In addition, sediment traps were deployed at eight locations in Lake Michigan (see Figure 2-8). However, samples could not be retrieved from the traps at two of those locations. Sample retrieval was successful at trap locations 1, 2, 5, 6, 7, and 8.

Figure 2-7. Sediment Sampling Stations

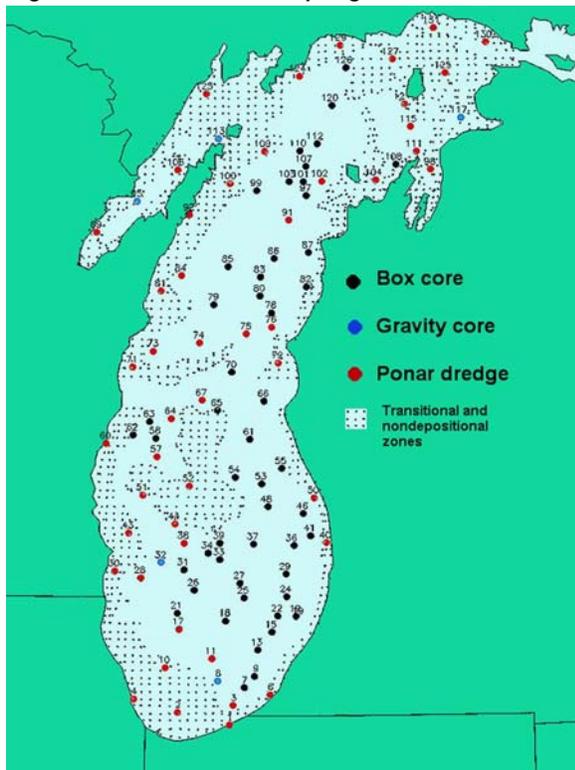


Figure 2-8. Sediment Trap Locations



2.4.5 Lower Pelagic Food Web Organisms

Plankton sampling locations were selected by GLNPO and the PIs in advance of sampling. The sites included eight stations in three biological sampling areas or “biota boxes” (Stations 110, 140, 180, 240, 280, 310, 340, and 380), three master stations (18M, 27M, and 47M), and a fourth biota box centered around Station 5, near Chicago. The four biota boxes are outlined in red in Figure 2-9. Samples were collected on seven occasions, from June 1994 to September 1995. In addition, zooplankton were collected from Station 19M in January 1995 and phytoplankton were collected from Stations 23M and 41 in June 1994. A total of 72 zooplankton and 71 phytoplankton samples were collected during the study.

In addition to the plankton samples, samples of *Mysis* and *Diporeia* were to be collected at the stations where lake trout were collected (near biota boxes 1-3) and at one site NE of Chicago. *Mysis* and *Diporeia* samples were collected from stations 140, 180, 240, 280, 340, 380, 47M and 5. Samples of *Mysis* also were collected at stations 27M and 18M. A total of 53 *Mysis* samples and 39 *Diporeia* samples were

collected. *Mysis* feed on both phytoplankton and zooplankton, while *Diporeia* feed to detritus and phytoplankton (see Figure 7-5 in Chapter 7).

2.4.6 Fish

Specimens of lake trout, coho salmon, bloater chub, alewife, smelt, deepwater sculpin, and slimy sculpin were collected using various means. Two subsets of alewife and bloater chub were differentiated, based on total length (see Table 2-9). Coho salmon were differentiated into three subsets by age (hatchery, yearling, and adult). In their adult stages, the lake trout and Coho salmon are piscivorous fish, while the other fish species collected are forage fish that generally feed on plankton and detritus (see Figure 7-5 in Chapter 7).

Where possible, five fish were composited for analysis. Fish were collected during the spring, summer, and fall of 1994, and the spring and fall of 1995, from three of the four biota boxes in Figure 2-9 (fish were *not* collected from the biota box at Station 5, near Chicago).

Additional fish were collected from locations throughout the lake by means ranging from gill nets to hook-and-line angling. Table 2-9 contains a summary of the numbers of individual fish collected, the total number of locations from which they were collected, and the total number of samples submitted for analysis, by species. Table 2-10 contains a summary of the sample collection techniques used, by species.

Figure 2-9. Sampling Stations for Lower Pelagic Food Web Organisms and Fish

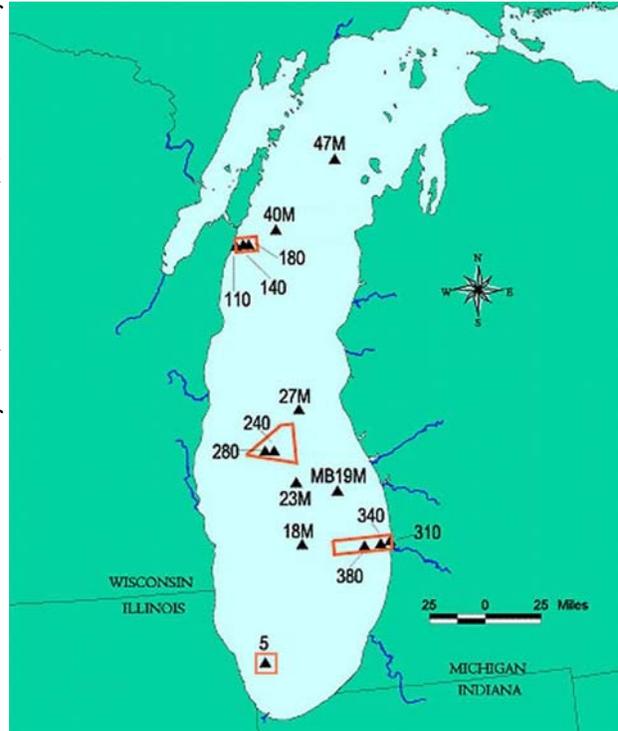


Table 2-9. Number of Fish Collected by Species and Location

Species	Total Number of Individual Fish Collected	Number of Locations	Number of Composite Samples Created
Lake Trout	1087	3	246
Slimy sculpin	315	3	69
Deepwater sculpin	325	3	74
Smelt	365	3	73
Coho salmon - adult	238	79	54
Coho salmon - yearling	38	22	8
Coho salmon - hatchery	25	1	5
Alewife >120 mm	347	3	70
Alewife <120 mm	298	3	60
Bloater chub >160 mm	334	3	67
Bloater chub <160 mm	348	3	70

Table 2-10. Number of Fish Collected by Technique

Species	Number of Fish Collected by Technique				
	Hook and Line	Gill Net	Bottom Trawl	Harvest Weir	Dip Net
Lake Trout	-	1029	58	-	-
Slimy sculpin	-	25	290	-	-
Deepwater sculpin	-	-	325	-	-
Smelt	-	25	340	-	-
Coho salmon - adult	235	3	-	-	-
Coho salmon - yearling	29	-	-	9	-
Coho salmon - hatchery	-	-	-	-	25
Alewife >120 mm	-	-	347	-	-
Alewife < 120 mm	-	-	298	-	-
Bloater chub >160 mm	-	-	334	-	-
Bloater chub <160 mm	-	-	348	-	-

2.5 Sampling Methods

Full details of the sampling methods used in the LMMB Study have been published by EPA in a Methods Compendium (USEPA, 1997d and USEPA, 1997e). A brief summary is provided below.

2.5.1 Atmospheric Components

Each shoreline site had a 10-meter meteorological tower and a number of meteorological instruments including wind speed and wind direction sensors at a height of 10 m (Met-One, Grants Pass, OR), a solar radiation sensor (LI-Cor, model LI 200S, Lincoln, NE), temperature and relative humidity sensors (Campbell Scientific, Logan, UT), and a standard Belfort rain gauge (Belfort Instrument, Baltimore, MD) with a Nipher wind shield. All of the meteorological sensors were automatically recorded every 6 seconds using a datalogger (Campbell Scientific, model 21X, Logan, UT).

2.5.1.1 Vapor Fraction

Airborne semivolatile organic contaminants, which include PCBs and *trans*-nonachlor, were collected using high-volume (Hi-Vol) samplers for organics, modified to include an aluminum tube behind the filter holder that accommodated a vapor trap consisting of a stainless steel cartridge of XAD-2[®] resin. The samplers were operated for a 24-hour period at a flow rate of 34 m³ per hour. The frequencies of sampling depended on the nature of the site (e.g., urban versus remote), as described in Section 2.4.1. The samplers were checked each week for proper functioning, to collect a sample, or to set up for the next collection. Samples from shoreline sites, except those collected at Sleeping Bear Dunes, were physically composited to yield one sample per site representing the entire month. Samples from Sleeping Bear Dunes were not composited, but analyzed separately. The results for each sample at Sleeping Bear Dunes were mathematically composited to yield one result for the entire month.

The samples collected aboard the *R/V Lake Guardian* were not composited, but were analyzed individually, because there was at most one sample per station in any given month and not all stations were sampled on every cruise. In addition, the results for a small number of samples could not be tied to

specific locations or master stations (e.g., the samples were collected while the *R/V Lake Guardian* steamed between stations). The locations of these samples are listed as “unrecorded.”

2.5.1.2 Particulate Fraction

Airborne particulate organic contaminants were collected on 20.3 x 25.4 cm pre-fired quartz fiber filters using high-volume samplers for organics. The samplers were operated for a 24-hour period at a flow rate of 34 m³ per hour. The frequencies of sampling depended on the nature of the site (e.g., urban versus remote), as described in Section 2.4.1. The samplers were checked each week for proper functioning, to collect a sample, or to set up for the next collection. Samples from shoreline sites, except those collected at Sleeping Bear Dunes, were physically composited to yield one sample per site representing the entire month. Samples from Sleeping Bear Dunes were not composited, but analyzed separately. The results for each sample at Sleeping Bear Dunes were mathematically composited to yield one result for the entire month.

The samples collected aboard the *R/V Lake Guardian* were not composited, but were analyzed individually, because there was at most one sample per station in any given month and not all stations were sampled on every cruise. In addition, the results for a small number of samples could not be tied to specific locations or master stations (e.g., the samples were collected while the *R/V Lake Guardian* steamed between stations). The locations of these samples are listed as “unrecorded.”

2.5.1.3 Precipitation Fraction

A MIC sampler (Meteorological Instruments of Canada) with a 0.212 m² stainless steel catch basin was used for collecting precipitation samples for analysis of PCBs and *trans*-nonachlor. The sampler was modified for all-weather operation by enclosing and insulating the space underneath the sampler. The temperature in the enclosure was maintained at 10 to 15°C during the winter using a small space heater. The collector also was fitted with a precipitation sensor and a retractable cover. The catch basin remained covered to prevent evaporation until precipitation was detected by the sensor.

During sample collection, precipitation passed through a column containing XAD-2[®] resin that adsorbed PCBs and *trans*-nonachlor in the precipitation sample. Glass wool plugs inserted on either side of the XAD-2[®] resin trapped any particles in the sample. The sampler was in operation continuously for four-week periods in order to collect the required 5 L of precipitation, and was checked each week to ensure proper functioning and sample collection. The XAD columns were sealed with PTFE caps, transported to the testing laboratory, and stored in air-tight containers at -18°C until analysis. In the laboratory, the precipitation collection funnel was rinsed with water and wiped with a piece of clean quartz fiber filter paper to remove adhering particles. The filter paper and rinsings were included as part of the sample.

2.5.1.4 Dry Deposition

Dry particle deposition was measured using deposition plates. Strips of Mylar[®] (approximately 5.7 cm by 1.8 cm) were coated with a layer of Apeizon[®] L grease about 5 µm thick to keep particles from bouncing off the surface of the strip. This approach was designed to address concerns that traditional sampling methods such as high volume samplers may underestimate the deposition of large particles (>10 µm).

The strips were attached to plates made from polyvinyl chloride (PVC). The leading edge of each PVC plate was tapered at about a 10° angle to provide a laminar flow of air over the plate and reduce turbulence. The deposition plates were mounted in an Eagle II automatic dry deposition collector at each site. Each collector contained two deposition plates. A wind vane on the collector points the leading

edge of the plates into the wind and a moisture sensor activates a motorized cover that protects the plates from rain or snow. A timer monitored the total time that the plates were exposed.

The plates were exposed to dry deposition for periods ranging from 4 to 97 days. The total length of time over which samples were collected represented 20 to 30% of the duration of the LMMB Study. The longer exposures (e.g., 97 days) were required in rural areas where particle deposition rates were low.

Completed sample strips were weighed in the field, transferred to clean wide-mouth jars, frozen, and shipped to the laboratory for analysis.

2.5.2 Tributaries

The number and timing of sampling events were dependent upon the stability of the tributaries and the timing of increased flow events. Tributaries with greater stability (i.e., those that are less responsive to precipitation events) were sampled less frequently than those that were more variable. Sampling was timed to collect approximately one-third of the samples during base flow conditions (i.e., when flows were below the 20th percentile of the historic flow regime) and approximately two-thirds of the samples when flows were above the 20th percentile.

Tributary samples were collected as near to river mouths as possible without being subject to flow reversals that are common near river mouths in Lake Michigan. Composite samples were obtained using the USGS quarter-point sampling procedure. In this procedure, the stream is visually divided into three equal flow areas. At the center of each flow area, samples were collected from 0.2 and 0.8 times the depth. All six samples were then composited and pumped (using a peristaltic pump) through a 0.7- μ m glass fiber filter. Filters were removed, folded in quarters, and wrapped in aluminum foil for particulate PCB analysis. The filtrate was then passed through a 250-g XAD-2[®] resin column at a flow rate of 500-1000 mL per minute to trap dissolved organics. Samples were chilled and delivered to the testing laboratory for analysis.

2.5.3 Open Lake

Open-lake samples were collected from various depths depending upon the stratification conditions. During stratification, open-lake stations were sampled at the mid-epilimnion and mid-hypolimnion. During non-stratified periods, samples were collected at mid-water column depth and two meters below the surface. At master stations, during times of non-stratification, were sampled at mid water column, one meter below the surface, and two meters off the bottom. During times of stratification, master stations were sampled at one meter below the surface, mid-epilimnion, mid-hypolimnion, and two meters off the bottom. In addition, Stations 18M and 41 were sampled at the thermocline and 2 meters below the surface during stratification.

Water samples were collected using an onboard sampling and filtration system on the *R/V Lake Guardian*. Samples were drawn with a peristaltic pump through a hose held overboard at a specific depth. A volume of water ranging from 100 to 1000 L was pumped through a "Pentaplate" filtration apparatus holding up to five glass-fiber filters in parallel. The filtered water was discharged into glass carboys and subsequently pumped through a column containing 250 g of XAD-2[®] resin at a flow rate of 500-1000 mL per minute to trap dissolved organics. The total volume of water passed through the filtration and XAD-2[®] systems was measured in the glass carboys and recorded.

After sampling was complete, the filters and the XAD-2[®] resin column were removed from the sampling apparatus. The filters were frozen onboard the ship, and transferred to the laboratory for analysis while still frozen. The resin columns were cooled to 4°C and transferred to the laboratory for analysis.

2.5.4 Sediment

Sediment samples were collected using three types of equipment. A modified Soutar box corer was used to retrieve cores approximately 60 cm in length, with well-preserved sediment-water interfaces. Samples also were collected using a gravity corer and a Ponar dredge, depending on the nature of the lake bottom at the collection point. Surficial sediment samples were collected from the cores by sectioning the cores into intervals ranging from 0.5 to 1.5 cm thick. The sections were transferred to glass containers, frozen onboard the ship, and transferred to the laboratory while still frozen.

Sediment trap samples were collected and split while still wet. The 60-mL trap bottles were allowed to settle for 25 hours, under refrigeration. The overlying water (~25 mL) was poured off into a beaker, and the remaining trap sample was poured into the splitter reservoir through a 700- μm screen. The sample was split into four subsamples and washed through the splitter using the overlying water in the beaker and distilled water. The subsamples were transferred to glass containers, frozen onboard the ship, and transferred to the laboratory while still frozen.

2.5.5 Lower Pelagic Food Web Organisms

Phytoplankton were collected using a device called a phytovibe. This device was specially designed and constructed for GLNPO for collecting large volumes of plankton for analysis of chemical contaminants such as mercury and PCBs. The phytovibe consists of a pair of inverted pyramids constructed of stainless steel mesh lined with 10- μm Nitex netting. Water is pumped by a submersible pump through nylon tubing into the top of the device, which has an opening that is 1 m². The end of the nylon tubing is covered with 100- μm netting to remove zooplankton. In order to prevent plugging of the netting with plankton, the phytovibe is shaken by a motor. The samples were washed down into a detachable sampling cup with lake water and collected for processing. Sampling times ranged from 6 to 14 hours, depending on plankton concentration in the water and sample size needed for a particular analysis.

The depth of collection was chosen based on interpretations of the temperature, fluorescence, and bathymetric profiles from the ship, with the objective of choosing a depth that maximized the occurrence of phytoplankton that were being grazed. This generally corresponded to the epilimnion or the subthermocline chlorophyll maximum in stratified conditions.

Zooplankton were collected in nested Nitex nets of two different mesh sizes (102- μm and 500- μm) during standard vertical tows, from near the bottom to the surface. The 500- μm nets were used to exclude larger organisms, including small fish, from the zooplankton samples. The number of tows performed was dependent on the mass of sample collected per tow. *Diporeia* spp. were collected in benthic tows, and *Mysis* spp. were collected in vertical and benthic tows.

Plankton samples were transferred to glass containers, frozen onboard the ship, and transferred to the laboratory while still frozen.

2.5.6 Fish

Whole fish were collected intact, with all body fluids and no incisions, except lake trout, which had their stomachs removed. Fish were wrapped in aluminum foil, placed in polyethylene bags, tagged, and frozen onboard the vessel. The fish were aged by checking for coded wire tags on the head and for fin clips. Fish were then composited by age, location, species, and size range. Samples were homogenized at the laboratory using a 40-qt vertical cutter mixer for large fish, 12-qt Stephan Machinery vertical cutter for medium-sized fish, or a high-speed 2-qt Robot Coupe for small fish. Additional information on the numbers of fish collected and the composite samples that were created can be found in Section 2.4.6.

2.6 Analytical Methods

Full details of the analytical methods used in the LMMB Study have been published by EPA in a methods compendium (USEPA, 1997d and USEPA 1997e). The general considerations in the choice of methods are discussed in Section 2.6.1. The convention for the data presented in this report is discussed in Section 2.6.2, and brief summaries of the specifics of the analyses for each lake component are provided in Sections 2.6.3 to 2.6.7.

2.6.1 General Analytical Considerations

Three significant, and interrelated aspects of the analytical methods for the PCBs and *trans*-nonachlor used in this study were the selection of analytical instrumentation, the selection of specific PCB congeners of interest, and the provision of a common set of analytical standards to all of the investigators. The vast majority of the commonly used analytical techniques for PCB analysis employ gas chromatography as a means of separating the PCB congeners. Traditionally, methods for the analysis of Aroclor mixtures have employed an electron capture detector (ECD) and produce chromatographic data that require that the analyst look for patterns of chromatographic peaks that resemble those in an authentic standard of each Aroclor. However, as a result of the changes that can occur in environmental samples (e.g., “weathering”), the pattern of peaks present in an environmental sample often changes enough to make it difficult or impossible to identify a pattern attributable to a specific Aroclor mixture.

Given these difficulties with Aroclor analysis, the most common alternative is to perform analyses for the individual PCB congeners. At present, there are no gas chromatography columns that can completely separate all 209 individual PCBs. Many of the congeners pass through the gas chromatograph at the same time, a phenomenon called “coelution.” Using an electron capture detector, it is not possible to distinguish between two PCB congeners that coelute. Using a mass spectrometer as a detector permits the analyst to distinguish some coeluting congeners because the mass spectrometer can differentiate the masses of the individual compounds. Therefore, if two congeners coelute but have different molecular weights (e.g., a tetrachlorobiphenyl and a pentachlorobiphenyl), the mass spectrometer can distinguish between them. However, even the combination of gas chromatography and mass spectrometry (GC/MS) cannot separate all 209 congeners. The costs of GC/MS analyses are substantially higher than those for GC/ECD. For this reason and others described below, GC/ECD was chosen as the primary analytical technique for the PCBs. GC/ECD also is applicable to the analysis of *trans*-nonachlor, and the *trans*-nonachlor results often were determined from the same analyses as the PCB congeners. The combination of gas chromatography and negative ion chemical ionization mass spectrometry was used for the analysis of PCB congeners in fish tissues.

A critical aspect of any analytical procedure is the availability of authentic standards of the analytes of interest. PCB analyses present a significant challenge in this regard because Aroclors were produced on an industrial scale as mixtures of PCB congeners, with some degree of variation between production lots over time. The commercial production of those Aroclors was outlawed over 20 years ago, but EPA has issued a small number of permits for the manufacture of Aroclors for use as analytical standards. Although the manufacturers of the standards go to great lengths to ensure that their standards resemble the original commercial mixtures, some differences are expected, and those differences can affect the end results of the analyses.

In order to address the concerns about methods and standards for the PCBs, EPA developed an approach to selecting appropriate PCB congeners for analysis and for providing common analytical standards to all of the investigators in the LMMB. Michael Mullin of EPA developed a mixture of specific proportions of Aroclors 1232, 1248, and 1262 that was designed to contain all of the Aroclor-derived congeners that are

likely to be found in environmental samples. That mixture is known as the “Mullin mix” and was provided to all of the investigators in the LMMB for use as a common reference standard.

In addition, information on the specific PCB congeners contained in the Mullin mix was used to focus the LMMB PCB analyses on those congeners that represent a significant portion of mass of Aroclors that may have been released into the environment. This is a critical aspect of the LMMB Study. Although the toxic effects of PCBs are certainly of interest in Lake Michigan, the purpose of the LMMB Study is to develop a mass balance for the anthropogenic pollutants described in Chapter 1. Therefore, the investigators focused on PCB congeners that occur in relatively high concentrations in the Aroclors that may have been released into the environment.

The majority of the toxic effects of PCB exposure are associated with the 12 WHO toxic congeners. However, not all of these congeners can be readily separated by GC methods and most of the toxic congeners are present in environmental samples at concentrations that require very sensitive analytical methods (e.g. high resolution mass spectrometry). Data on the composition of congeners in the Mullin mix were used to select those congeners that represent a significant portion of the mass of Aroclors 1232, 1248, and 1262 and that the investigators were able to detect reliably. Table 2-11 contains a list of the 45 PCB congeners that constitute at least 1% of the mass of the Mullin mix of the three Aroclors.

Collectively, these 45 congeners contribute 77.31% of the mass of the Mullin mix. There are 77 additional congeners that have been identified in the Mullin mix, each of which contributes 0.01% to 0.99% of the total mass. Collectively, these 77 congeners contribute 22.69% of the mass of the Mullin mix.

Data for the Mullin mix indicate that 10 of the 12 WHO toxic congeners are present in the mix at concentrations that could be measured. Seven of those 10 toxic congeners can be separated from all the other congeners and represent 1.17% of the mass of the Mullin mix. PCB 118 is the toxic congener with the highest concentration, representing 0.6% of the mass in the Mullin mix. Collectively, the 10 toxic congeners that are present and the other 5 coeluting congeners represent 5.50% of the mass.

The data in Table 2-11 demonstrate why looking for *just* the toxic PCB congeners would make it difficult to develop a robust mass balance for Lake Michigan, since such an approach would ignore 95 to 99% of the mass of PCBs present in Aroclors.

The use of the Mullin mix as a common analytical standard across all of the laboratories provides an important control mechanism in the analysis of the PCBs and provided a mechanism with which to select a basic set of PCB congeners of interest for the LMMB Study. Differences in the study requirements and specific instruments used in each laboratory, matrix-specific sample preparation and extraction techniques, and other factors enabled each of the laboratories to report results for additional PCB congeners. However, different numbers of additional congeners or coeluting groups of congeners being reported in each of the laboratories. The maximum number of congeners reported for each laboratory is shown in Table 2-12.

The “total” PCB concentrations also were determined for each sample in the study. The totals are simply the sum of all of the PCB congeners that were found in the sample. However, each PI determined the total PCB concentrations based on the specific congeners determined in that laboratory and some adjustments were made when interferences from non-PCB analytes were apparent.

Table 2-11. PCB Congeners that Contribute at Least 1% of the Mass of the Mullin Mix

PCB Congener	Concentration in Mullin Mix (ng/mL)	% of Total Mass in Mullin Mix
PCB-008+005	15	7.71
PCB-001	14	7.20
PCB-031+028	12	6.17
PCB-003	8.5	4.37
PCB-066	7.1	3.65
PCB-180	6.6	3.39
PCB-044	5.0	2.57
PCB-052	5.0	2.57
PCB-132+153+105	4.8	2.47
PCB-203+196	4.6	2.36
PCB-015+017	4.5	2.31
PCB-018	4.4	2.26
PCB-056+060	4.4	2.26
PCB-201	4.4	2.26
PCB-033	4.2	2.16
PCB-070+076	3.9	2.00
PCB-022	3.6	1.85
PCB-187+182	3.6	1.85
PCB-163+138	3.5	1.80
PCB-004+010	3.4	1.75
PCB-123+149	3.3	1.70
PCB-174	3.2	1.64
PCB-041+071	2.8	1.44
PCB-049	2.8	1.44
PCB-074	2.6	1.34
PCB-032	2.5	1.29
PCB-016	2.4	1.23
PCB-064	2.2	1.13
PCB-194	2.1	1.08
PCB-095	2.0	1.03
PCB-170+190	2.0	1.03

+ indicates congeners that typically coelute

Table 2-12. Maximum Number of PCB Congeners Reported, by Laboratory

Laboratory	Maximum Number of PCB Congeners or Groups of Coeluting Congeners
Battelle, Sequim	105
Indiana University	108
University of Wisconsin, Wisconsin State Lab of Hygiene	65 (78)*
University of Minnesota	110
National Oceanic and Atmospheric Administration	105
Rutgers University	86
Illinois State Water Survey	107
United States Geological Survey, Biological Resources Discipline	80

*The figure in parentheses represents the number of congeners reported in analyses conducted after a change in the laboratory's standard operating procedure that was instituted in November 1994.

2.6.2 Data Presented in this Report

As noted in Section 2.1, there are 209 possible PCB congeners, and the investigators in this study reported results for 65 to 110 of these congeners, depending on the capabilities of each laboratory (Table 2-12). Given the hundreds of samples that were collected for some media, it is impractical to present *all* of the PCB congener results for any of the media in this report, let alone the entire LMMB Study. This report seeks to strike a balance between the depth of the data presentation and a desire to limit the report to a manageable size. Therefore, except as noted below, throughout the remainder of this report, summaries are presented for the results for the following analytes:

- PCB congener 33 (2',3,4-trichlorobiphenyl)
- PCB congener 118 (2,3',4,4',5-pentachlorobiphenyl)
- PCB congener 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl)
- Total PCBs
- *trans*-nonachlor

The three PCB congeners were selected for presentation for the following reasons:

- These congeners do not coelute with any other congeners that were abundant in the Aroclors in any of the LMMB media (thus allowing presentation and data interpretation across all media in the LMMB report),
- They were reported by all but one of the investigators in the LMMB, thus enabling direct comparisons across all media (except dry deposition),
- They represent different levels of chlorination of the parent biphenyl structure and thus should represent a variety of environmental fates for PCBs,
- None were used as surrogates in the analysis,
- All three congeners are present in the "Mullin mix" standard of Aroclors used for the LMMB Study, and
- PCB 118 represents one of the coplanar congeners currently deemed "toxic" because of its biological activity, while PCBs 33 and 180 currently are *not* classified in the "toxic" group.

The only exception to this approach occurs in Chapter 6, on the sediment analyses, where the principal investigator found that PCB 33 coeluted with the organochlorine pesticide heptachlor. As a result, the presence of heptachlor in the sediment samples lead to high recoveries of PCB 33 in matrix spike

samples. Therefore, the principal investigator provided detailed graphics for the coeluting PCB congener pair 28+31 and not PCB 33. The coelution with heptachlor was not evident in the matrix spike recoveries from samples of other matrices, and other matrices would not be expected to retain heptachlor from historical sources to the extent that may occur in sediments.

2.6.3 Atmospheric Components

The preparation and analysis of three of the four fractions of the atmospheric samples followed similar procedures. Soxhlet extraction was used to extract the PCBs and *trans*-nonachlor from:

- XAD-2[®] resin used to collect the vapor phase samples,
- Glass fiber filters used to collect the particulate samples, and
- XAD-2[®] resin used to collect the precipitation samples.

Hexane:acetone (50:50) was used for all three Soxhlet extractions and the extracts were concentrated by rotary evaporation.

For all fractions, interfering compounds were removed and analytes were fractionated with silica gel. The hexane fraction contained all of the PCBs and the second fraction (40% dichloromethane, 60% hexane) contained *trans*-nonachlor. PCBs and *trans*-nonachlor were determined by capillary gas chromatography with electron-capture detection.

The dry deposition samples were prepared by extracting the Mylar[®] strips with dichloromethane and hexane in an ultrasonic bath. The extracts were subjected to a cleanup procedure that employed deactivated silica gel and deactivated alumina which removed most of the Apeizon[®] L grease from the extracts. PCBs and *trans*-nonachlor were determined by capillary gas chromatography with electron-capture detection. Because some grease remained in the concentrated extracts, a 1-m glass capillary pre-column was attached to the front end of the GC column and the remaining grease was deposited on the pre-column. Half of the 1-m pre-column was cut off after 3 to 5 sample injections and the pre-column was replaced after 6 to 10 injections. Combined with frequent maintenance and replacement of the glass injector liners in the GC, the pre-column prevented the residual grease from compromising the resolution of the GC column.

To minimize the potential effects of sorption of PCBs and *trans*-nonachlor from the gas phase onto the grease strips, the deposition of PCBs onto the field blank associated with each sample was used to correct the sample results, based on the mass of PCBs normalized to the surface area of the sample.

2.6.4 Tributaries and Open Lake

The XAD-2[®] resins and glass fiber filters used to collect tributary samples were extracted separately with a sequence of Soxhlet extractions, followed by volume reductions and rinses. Florisil and silica gel column chromatography were used to cleanup tributary sample extracts prior to analysis, allowing fractionation of the PCBs from *trans*-nonachlor and the majority of the other organochlorine pesticide compounds in the samples. Following this fractionation, PCB congeners and *trans*-nonachlor were then determined by the analyses of separate extracts using capillary gas chromatography with electron-capture detection.

The XAD-2[®] resins and glass fiber filters used to collect open-lake samples were extracted separately with a sequence of Soxhlet extractions, followed by volume reductions and rinses, in a fashion similar to that for the tributary samples. For the open-lake samples, cleanup procedures included the use of acidified silica gel and alumina to remove polar interferences from the sample extracts. Subsequent to the

start of the study, the laboratory performing the analyses of the open-lake samples adjusted their extract cleanup procedures and included a second acidified silica gel cleanup, in an attempt to overcome potential interferences that were present on the XAD-2[®] resin used to collect water samples. As a result of those changes, the laboratory did not obtain separate cleanup fractions containing the PCBs and *trans*-nonachlor, but analyzed the sample extracts on two dissimilar GC columns (DB-5 and DB-1701). While the DB-1701 column provided clear chromatographic separation of any *trans*-nonachlor in the sample, this analyte coeluted with PCB 99 on the DB-5 column. As a result of the potential coelution, the reported concentrations of PCB 99 in open-lake samples are probably biased by any *trans*-nonachlor present in the samples.

2.6.5 Sediment

Approximately 15-30 g of thawed wet sediment was extracted with dichloromethane in an ultrasonic bath held at 30°C. The extracts were dried over sodium sulfate and passed through a cleanup column. PCBs were separated from pesticides and polyaromatic hydrocarbons by column chromatography fractionation. PCBs and *trans*-nonachlor were determined by capillary gas chromatography with electron-capture detection.

2.6.6 Lower Pelagic Food Web Organisms

Lower pelagic food web organism samples were thawed, rinsed with methanol, then extracted by a Soxhlet procedure (four hours with methanol; then 16-24 hours with dichloromethane). The methanol fraction was back-extracted with hexane. The hexane fraction containing the PCBs was combined with the dichloromethane fraction and concentrated by rotary evaporation. Lipids were removed from the extracts, and the extracts were cleaned up and eventually reduced to 200-300 µL prior to analysis. PCBs were determined by capillary gas chromatography with electron-capture detection. *trans*-Nonachlor was analyzed by electron capture negative ionization (ECNI) gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM), using methane as the reagent gas.

2.6.7 Fish

Homogenized fish tissue samples were thawed and then extracted with a 90/10 mixture of petroleum ether/ethyl acetate. Extracts were concentrated and the lipid content was determined from an aliquot of the concentrated extract. The extracts were then prepared for analysis by separation and removal of lipids using a gel permeation chromatography system. PCBs and *trans*-nonachlor were determined by electron capture negative ionization (ECNI) gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM), using methane as the reagent gas.

2.7 Quality Implementation and Assessment

As described in Section 1.5.5, the LMMB quality assurance (QA) program prescribed minimum standards to which all organizations collecting data were required to adhere. The goal of the QA program was to ensure that all data gathered during the LMMB Study met defined standards of quality with specified levels of confidence. Data quality was defined, controlled, and assessed through activities that included development of study QAPPs, use of SOPs, and data verification. These activities are described in detail in *The Lake Michigan Mass Balance Study Quality Assurance Report* (USEPA, 2001b).

Specific quality control elements implemented in the sampling and analysis of PCB and *trans*-nonachlor included:

- use of standard operating procedures and trained personnel for field sampling and laboratory analysis;
- determination of method sensitivity through calculation of method detection limits;
- preparation and analysis of a variety of blanks to characterize contamination associated with specific sample handling, storage, and analysis processes including field blanks, lab reagent blanks, bottle blanks, trip blanks, and lab solvent blanks;
- collection and analysis of field or laboratory duplicate samples;
- preparation and analysis of a variety of quality control samples including standard reference samples and performance standards;
- use of internal and surrogate standards for all field samples;
- use of a standardized data reporting format; and
- preparation and analysis of matrix spike samples to characterize the applicability of the analytical method to the study sample matrices.

In addition, each researcher's laboratory was audited during an on-site visit at least once during the time LMMB samples were being analyzed. The auditors reported positive assessments and did not identify issues that adversely affected the quality of the data. Prior to data submission, each researcher submitted electronic test files containing field and analytical data according to the LMMB data reporting standard. GLNPO reviewed these test data sets for compliance with the data reporting standard and provided technical assistance to the researchers.

An intercomparison study for the PCB analyses was conducted among all PIs and the EPA ORD laboratory in Duluth, Minnesota. A detailed discussion of the intercomparison study is provided in *The Lake Michigan Mass Balance Study Quality Assurance Report* (USEPA, 2001b).

Because all of the PIs were analyzing different types of samples, such as vapor, fish, and sediment, and employing sample-specific preparation procedures, the intercomparison study focused on the instrumental analysis portion of the study. Each of the PIs was provided with two solutions of PCB congeners prepared by Ultra Scientific in consultation with Dr. Mike Mullin of EPA ORD. The solutions included PCB congeners with retention times and response factors covering the ranges observed in environmental samples as well as congeners that are known to be difficult to resolve.

The results submitted by each PI were evaluated by comparison to: 1) the gravimetric true value of the solution as prepared and 2) the 95% confidence intervals for specific congeners based on the full set of results from the study. A goal of 30 percent bias from the true gravimetric mean was established for the intercomparison study.

This goal was not always met. Comparison of the results from each PI to the gravimetric true mean indicated a high bias for some congeners and low bias for others. With few exceptions, results that exhibited more than 30% bias from the true value were associated with coeluting congeners. The results from all of the PIs were within the 95% confidence intervals calculated for the PCB congeners, indicating that none of the results were likely to be outliers. However, the relatively low number of degrees of freedom (i.e., eight laboratory participants) limit the statistical power of this evaluation.

The variability of the analytical results among PIs also was evaluated by calculating the relative standard deviation (RSD) among results for each congener or group of coeluting congeners. Although the RSD values for the majority of the congeners were less than 30%, the results for congeners 37, 77, 77+110, 81, and 123+149 in one of the two solutions in the intercomparison study exceeded 30% RSD. Coelution is a problem for all of these six congeners. However, these congeners represent only a small portion of the

total mass of PCBs. Only the coeluting pair of 123+149 is present in the Mullin mix at greater than 1%, and PCB-77 and PCB-81 represent only 0.15% and 0.08% of the Mullin mix, respectively. Therefore, the RSD results greater than 30% for these congeners in the intercomparison study are not likely to have a large influence on the study goal of developing a mass balance of PCBs for Lake Michigan.

Overall, the results of the intercomparison study suggest the PIs were successfully employing analytical procedures consistent with what is expected for determination of PCB congeners in environmental samples and did not indicate any major analytical difficulties associated with any of the labs in the study.

Prior to sample collection, Quality Assurance Project Plans (QAPPs) were developed by the PIs and submitted to GLNPO for review. In the QAPPs, the PIs defined MQOs in terms of six attributes: sensitivity, precision, accuracy, representativeness, completeness, and comparability. The MQOs were designed to control various phases of the measurement process and to ensure that the total measurement uncertainty was within the ranges prescribed by the DQOs. The MQOs for PCBs and *trans*-nonachlor are listed in Section 7 of *The Lake Michigan Mass Balance Study Quality Assurance Report* (USEPA, 2001b).

The PI-defined MQOs also were used in the data verification process. GLNPO conducted data verification through the LMMB QA Workgroup. The workgroup was chaired by GLNPO's Quality Assurance Manager and consisted of quality control coordinators that were responsible for verifying the quality of specific data sets. Data verification was performed by comparing all field and QC sample results produced by each PI with their MQOs and with overall LMMB Study objectives. If the results failed to meet MQOs and corrective actions were not feasible, the results were flagged to inform data users of the failure. These flags were not intended to suggest that data were not useable; rather they were intended to caution the user about an aspect of the data that did not meet the predefined criteria. In addition, a wide variety of flags were applied to the data to provide detailed information to data users. For example, the flag LAC (laboratory accident, no result reported) was applied to sample results to document that a sample was collected, but no result was reported due to a laboratory accident. The frequencies of flags applied to PCB and *trans*-nonachlor study data are provided in the Quality Implementation Sections of each of the following chapters. The flag summaries include the flags that directly relate to evaluation of the MQOs to illustrate some aspects of data quality, but do not include all flags applied to the data to document sampling and analytical information (such as LAC). In order to provide detailed quality information to data users, the study data are maintained in the GLENDA database with all applied flags. Detailed definitions of the flags can be found in the *Allowable Codes Table* on GLNPO's web site at www.epa.gov/glnpo under *Result Remark, List of QC flags* (lab_rmrk).

Comparability of the data from the various PIs was enhanced by the use of standardized reporting units for samples from similar matrices. In addition, the analyses of PCBs incorporated the use of surrogate compounds that were added to the sample before extraction. These surrogates provide an estimate of the efficiency of the entire sample preparation, extraction, cleanup, and analysis process, as applied to each sample. The recoveries of the surrogates were used by most of the investigators to correct the results of the target PCBs for any apparent losses or gains of the surrogates. The dry deposition PCB data were *not* corrected for surrogate recoveries, but were corrected for the potential contribution of PCBs absorbed into the Apeizon[®] L grease directly from the vapor phase, using field blank results.

For *trans*-nonachlor analyses, the PIs had difficulty identifying a surrogate compound that worked well for those matrices analyzed by gas chromatography with electron-capture detection (GC/ECD). The analyses of lower pelagic food web organisms and fish were performed by GC/MS techniques, which permitted the use of a stable ¹³C-labeled form of *trans*-chlordane as a surrogate. Therefore, the lower pelagic food web and fish results were corrected for surrogate recoveries.

Of the GC/ECD analyses for *trans*-nonachlor, *only* the data from the atmospheric samples collected at Sleeping Bear Dunes were corrected for surrogate recoveries. As with the PCB results, the dry deposition *trans*-nonachlor data were *not* corrected for surrogate recoveries, but were corrected for the potential contribution of *trans*-nonachlor absorbed into the Apeizon[®] L grease directly from the vapor phase, using field blank results.

The PIs participating in the study also conducted real-time data verification. PIs applied best professional judgement during sampling, analysis, and data generation, based on their experience monitoring PCB and *trans*-nonachlor in the environment. In most cases, when sample results were questionable, the PI reanalyzed the sample or clearly documented the data quality issues in the database through the application of data quality flags or by including comments in the database field, "Exception to Method, Analytical." Because the flags and comments are maintained in the database for each sample result, data users are fully informed of data quality and can evaluate quality issues based on their intended use of the data. The level of documentation that GLNPO is maintaining in the study database is unprecedented for a database of this size and will serve as a model for future efforts.

GLNPO also conducted data quality assessments in terms of four of the six attributes used as the basis for the MQOs, specifically sensitivity, precision, and bias. For example, system precision was estimated as the mean relative percent difference (RPD) between results for field duplicate pairs. Similarly, analytical precision was estimated as the mean RPD between results for laboratory duplicate pairs. Bias was estimated using the mean recovery of spiked field samples or other samples of known concentration such as laboratory performance standards. A summary of data quality assessments is provided for the PCB and *trans*-nonachlor study data in the Quality Implementation Section of each of the following chapters.