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BIOLOGICAL AND CHEMICAL ASSESSMENT OF CONTAMINATED GREAT LAKES SEDIMENT

BIOLOGICAL AND CHEMICAL ASSESSMENT OF CONTAMINATED GREAT LAKES SEDIMENT

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ABSTRACT

Section 118 (c)(3) of the Clean Water Act (CWA), as amended by the Water Quality Act of 1987, authorized the USEPA Great Lakes National Program Office (GLNPO) to carry out a 5-year project dealing with the Assessment and Remediation of Contaminated Sediments (ARCS) at selected Great Lakes Areas of Concern (AOC). Sediment assessment procedures performed by the National Fisheries Contaminant Research Center (NFCRC) on samples from Indiana Harbor, Buffalo River, and Saginaw River AOCs included: Elutriate Toxicity Tests (Chapters 2 and 3), Whole Sediment Toxicity Tests (Chapter 4), Benthic Community Structure (Chapter 5), and Mutagenicity (Chapter 6) and Genotoxicity (Chapter 7) Assays.

Sediment samples collected from Indiana Harbor were severely contaminated compared to either Buffalo or Saginaw River based on sediment toxicity and chemistry. While sediment samples from Saginaw River were generally less contaminated compared to Buffalo River, considerable spatial variability in contamination was evident at all three AOC. About 41% of the elutriates samples prepared from these sediments were designated as toxic using either *Daphnia magna* or Microtox assays. Interpretation of toxicity using *Selenastrum capricornutum* was complicated by variable nutrient and inorganic carbon concentrations in the elutriate samples.

About 68% of the whole sediment samples were toxic to *Hyalella azteca*, *Chironomus riparius*, or *Chironomus tentans* in 10-d to 28-d exposures. Fourteen- and 28-d exposures with *Hyalella azteca* which monitored effects on survival, body length, and sexual maturation, identified a higher proportion of toxic samples compared to either 14-d exposures with *Chironomus riparius* or 10-d exposures with *Chironomus tentans*.

Oligochaetes and chironomids were the dominant taxa at each AOC indicating impacted benthic communities. Average number of midges with mouth part deformities ranged from 45 to over 77% in samples from the AOCs. Artificial substrate samplers which were colonized *in situ* sampled a more diverse taxa compared to benthic grab samplers.

Over 90% of organic extracts from sediment samples were classified as mutagenic or genotoxic using Ames and Mutatox assays. Toxicity of the organic extracts complicated interpretations in the Ames assay, but was not a problem in the Mutatox assay.

Each sediment sample contained a complex mixture of inorganic and organic contaminants. Additional studies are required to determine specific contaminants that may be causing adverse effects (e.g., sediment spiking and Toxicity Identification Evaluations).

Data from these studies are to be evaluated with the Sediment Quality Triad which will integrate data from laboratory exposures, benthic community structure, and chemical analyses to provide complementary evidence for the degree of pollution-induced degradation in aquatic communities at each AOC. Results of the Triad evaluations will be discussed in a later report.

This report was submitted in fulfillment of USFWS Contract Number DW14933874-1 by the National Fisheries Contaminant Research Center, U.S. Fish and Wildlife Service and Battelle Contract Number 89934235-0 by Battelle Northwest Laboratories under partial sponsorship of the U.S. Environmental Protection Agency. This report covers a period from 03/01/89 to 11/10/92, and work was completed as of 11/10/92.

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CHAPTER 2: ELUTRIATE TOXICITY TESTS: *DAPHNIA MAGNA* AND MICROTOX

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CHAPTER 3: ELUTRIATE TOXICITY TESTS: *SELENASTRUM CAPRICORNUTUM*

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CHAPTER 4: WHOLE SEDIMENT TOXICITY TESTS

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CHAPTER 5: BENTHIC COMMUNITY STRUCTURE EVALUATIONS

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CHAPTER 7: MUTATOX GENOTOXICITY ASSAY

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INTRODUCTION

Section 118 (c)(3) of the Clean Water Act (CWA), as amended by the Water Quality Act of 1987, authorized the USEPA Great Lakes National Program Office (GLNPO) to carry out a 5-year project dealing with the Assessment and Remediation of Contaminated Sediments (ARCS) in the Great Lakes. Annex 14 of the Great Lakes Water Quality Agreement of 1978, as amended, between the United States and Canada stipulated the cooperating parties identify the nature and extent of sediment pollution in the Great Lakes, develop methods to assess effects, and evaluate the technological capability of programs to remedy such pollution. Information from these activities is to be used to guide the development of Remedial Action Plans (RAPs) for the identified Great Lakes Areas of Concern (AOC) as well as Lake-wide Management Plans (Ross et al. 1992).

Areas of Concern include major municipal and industrial centers on Great Lakes rivers, harbors, and connecting channels where beneficial uses are impaired (International Joint Commission 1987, 1988a, 1988b). Toxic contamination of bottom sediments is often a major problem in these areas. Additionally, areas exist where impairment of beneficial uses (e.g., navigation, swimming, fishing) of water or biota have been documented. There are currently 42 AOC: 25 in U.S. waters, 12 in Canadian waters, and 5 international connecting channels. The CWA designates five AOC for priority consideration: Saginaw River, MI; Sheboygan Harbor, WI; Indiana Harbor, IN; Ashtabula River, OH; and Buffalo River, NY. This report describes research designed to evaluate the toxicity in AOCs where sediments do not have to be removed to maintain navigational channels, but present a hazard to the ecosystem if left in place.

In order to implement the ARCS program, a management framework was established by GLNPO, which included an Activities and Integration Committee and four technical Work Groups: (1) Toxicity-Chemistry, (2) Risk Assessment-Modeling, (3) Engineering-Technology, and (4) Communications-Liaison. The goal of the Toxicity-Chemistry Work Group was to assess the nature and extent of bottom sediment contamination at selected Great Lakes AOC. The Risk Assessment-Modeling Work Group was charged with evaluating the ecological impacts resulting from contaminated sediments and developing techniques for evaluating various remedial alternatives. The Engineering-Technology Work Group evaluated procedures for removal and remediation of contaminated sediments and the Communications-Liaison Work Group facilitated flow of information between Work Groups and to the public. The Activities Integration Committee had oversight over the entire ARCS program and coordinated Quality Control and Quality Assurance (Ross et al. 1992).

The sediment assessment procedures conducted by the National Fisheries Contaminant Research Center (NFCRC) on samples from Indiana Harbor, Buffalo River, and Saginaw River included: Elutriate Toxicity Tests (Chapters 2 and 3), Whole Sediment Toxicity Tests (Chapter 4), Benthic Community Structure (Chapter 5), and Mutagenicity (Chapter 6) and Genotoxicity (Chapter 7) Assays. A separate project by Wright State University (WSU) provided additional toxicity information on splits of select sediment samples from each AOC (Burton et al. 1992). The NFCRC and WSU projects were designed to evaluate sediment evaluation procedures suggested by both the International Joint Commission (International Joint Commission 1988a, 1989) and ASTM (ASTM E 1383-92).

REFERENCES CITED IN THE INTRODUCTION

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CONCLUSIONS AND RECOMMENDATIONS

GENERAL

- Sediment samples collected from Indiana Harbor were severely contaminated compared to either Buffalo or Saginaw River based on sediment toxicity and chemistry. While sediment samples from Saginaw River were generally less contaminated compared to Buffalo River, considerable spatial variability in contamination was evident at all three AOCs.

- Each sediment sample contained a complex mixture of inorganic and organic contaminants. Additional studies are required to determine specific contaminants that may be causing adverse effects (e.g., sediment spiking and Toxicity Identification Evaluations).

- Multiple assessment procedures are needed to thoroughly evaluate sediment contamination. Data from these studies are to be evaluated with the Sediment Quality Triad which will integrate data from laboratory exposures, benthic community structure, and chemical analyses to provide complementary evidence for the degree of pollution-induced degradation in aquatic communities at each AOC. Results of the Triad analyses will be discussed in a later report.

- Additional research is needed dealing with factors that control bioavailability of sediment-associated contaminants (e.g., organic carbon, particle size, acid volatile sulfides). Further investigations are needed on factors that control the desorption rates of sediment-sorbed contaminants.

CHAPTER 2: ELUTRIATE TOXICITY TESTS: *DAPHNIA MAGNA* AND MICROTOX

- Elutriate toxicity tests were conducted with *Daphnia magna* (48-h exposures) and Microtox (5- and 15-min exposures). About 33% of the 30 elutriate samples tested with daphnids were identified as toxic (i.e., EC50 less than the full-strength elutriate). Forty-one percent of the 39 samples tested with Microtox were identified as toxic. These results indicate that the Microtox test was a more sensitive tool for evaluating the toxicity of sediment elutriate samples compared to the *Daphnia magna* test.

- Toxicity and chemical analyses of elutriates prepared from Buffalo and Saginaw River sediment samples indicated that sediment contamination at these AOCs may not be as extensive relative to Indiana Harbor.

- Sediment resuspension should be kept to a minimum if contaminated sediments are removed during remediation based on the toxicity and contamination of the elutriate samples.

- Microtox and Daphnia magna toxicity tests with elutriates are useful tools for assessing the potential hazards of contaminated sediments. While the elutriate test can be used to evaluate potential effects of open-water disposal of dredged material, the test may not be appropriate for evaluating in situ effects of contaminated sediments on aquatic organisms.

CHAPTER 3: ELUTRIATE TOXICITY TESTS: SELENASTRUM CAPRICORNUTUM

- Elutriate toxicity tests were conducted using a 24-h ^{14}carbon assimilation algal assay. Interpretation of toxicity using Selenastrum capricornutum was complicated by variable nutrient and inorganic carbon concentrations in the elutriate samples. All of the elutriate samples tested stimulated carbon assimilation of Selenastrum capricornutum in one or more of the dilutions.

- Attempts to modify the algal medium to provide unlimited nutrients were not successful. An algal medium which supports greater growth potential should be developed in order to evaluate the toxicity of environmental samples with high concentrations of nutrients to algae.

- To evaluate the effect of storage on toxicity, additional elutriates were prepared from select whole sediment samples stored for 12 to 16 months after collection. Storage of whole sediments for over a year did not dramatically change toxicity of elutriate samples in the Selenastrum capricornutum or in the Microtox tests.

- An approach for evaluating toxicity in samples that may be stimulatory is presented. This approach needs to be evaluated using reference toxicants.

CHAPTER 4: WHOLE SEDIMENT TOXICITY TESTS

- Whole sediment toxicity tests were conducted with amphipod Hyalella azteca, and the midges Chironomus riparius and Chironomus tentans. About 68% of the sediment samples were toxic to Hyalella azteca, Chironomus riparius, or Chironomus tentans in 10-d to 28-d exposures.

- Fourteen- and 28-d exposures with Hyalella azteca which monitored effects on survival, body length, and sexual maturation, identified a higher proportion of toxic samples compared to either 14-d exposures with Chironomus riparius or 10-d exposures with Chironomus tentans.

- Chronic sediment toxicity tests need further development to better determine effects of complex chemical mixtures. These tests should incorporate sublethal responses using representative benthic organisms.

- Toxic responses observed in the whole sediment tests need to be compared to benthic community effects and sediment chemistry to determine if the test are predictive.

CHAPTER 5: BENTHIC COMMUNITY STRUCTURE EVALUATIONS

- Oligochaetes and chironomids were the dominant taxa at each AOC indicating impacted benthic communities.

- Average number of midges with mouth part deformities ranged from 45 to over 77% in samples from the AOCs.

- Results of variance partitioning estimates indicates station and replicates accounted for most of the explained variability.

- Artificial substrate samplers which were colonized in situ sampled a more diverse taxa compared to benthic grab samplers.

- Since most of the variance in benthic community estimates was between stations and replicates, future studies should sample more stations and replicates using a smaller grab sampler.

- Frequency of deformities in chironomids exposed in laboratory toxicity tests (Chapter 4) should be measured and compared to the frequency of deformities in the chironomids collected from the field.

- Assessments of benthic community should use both artificial substrate and grab samplers.

- Additional research is needed evaluating specific contaminant, abiotic, and biotic factors controlling invertebrate distributions in sediments.

CHAPTER 6: AMES MUTAGENICITY ASSAY

- In order to assess the mutagenicity of Great Lakes sediments, unfractionated organic extracts of sediments were screened for mutagenicity using the Ames Salmonella/microsome assay.

- Extracts of sediment samples from every station were both toxic and mutagenic to the bacteria. Toxicity was controlled by varying rat hepatic S9 concentrations and extract dilution.

- Of the four bacterial strains tested, only strain TA98 detected mutagenicity at every station. Although extracts were not fractionated, chemical analysis indicated that as a class, PAHs comprise the greatest percentage by weight of total identified organics. Sediment mutagenicity is likely attributable to PAH compounds.

- Fractionation of samples before testing may allow a greater degree of discrimination and aid in identifying compounds causing the observed mutagenicity.

- Methods should be developed to test elutriate and porewater samples to better evaluate bioavailability of sediment associated contaminants. Studies utilizing passive membrane bag samplers may be useful for this purpose.

CHAPTER 7: MUTATOX GENOTOXICITY ASSAY

- Organic extracts of sediment samples were evaluated with the new activated Mutatox Genotoxicity Assay using rat hepatic S9 for exogenous metabolic activation and a dark mutant strain of the luminescent bacterium Photobacterium phosphoreum for detection of environmental DNA-damaging substances (genotoxins).

- A genotoxic response was indicated when the test chemical restored the luminescent state in bacteria. The degree of light increase indicated the relative genotoxicity of the sample.

- Toxicity of the organic extracts complicated interpretations in the Ames assay, but was not a problem in the Mutatox assay.

- Samples from 27 of 28 stations exhibited evidence of genotoxins, 23 of 28 stations (82%) were designated genotoxic, four were suspect (14%), and one was negative (3%).

- The activated Mutatox Assay was a sensitive, specific, predictive, and rapid test for detecting the presence of genotoxins in complex environmental samples.

CHAPTER 1: OVERVIEW

Ingersoll, C.G., D.R. Buckler, E.A. Crecelius, and T.W. La Point

Determining the effects of contaminants in sediment is a challenging new area in environmental toxicology. Mounting evidence exists of environmental degradation in areas where water quality criteria are not exceeded, yet organisms in or near sediments are adversely affected (Chapman 1986). Historically, evaluations of contaminant effects have emphasized surface waters, not sediments. Most assessments of water quality focus on water-soluble compounds and sediment is considered to be a safe repository of sorbed contaminants (Maki et al. 1984). This approach emphasizes testing organisms in the water column without considering the fate of chemicals in sediment.

The assessment of sediment quality is often limited to chemical characterizations. However, quantifying contaminant concentration alone cannot provide enough information to adequately evaluate potential adverse effects, interactions among chemicals, or the time-dependent availability of contaminants to aquatic organisms (Dillon and Gibson 1986). Because of poorly understood relationships between concentrations of contaminants in sediment and their bioavailability, determining effects of contaminated sediments on aquatic organisms requires use of benthic community surveys and controlled toxicity and bioaccumulation tests (Seelye and Mac 1984).

The goal of the National Fisheries Contaminant Research Center (NFCRC) project was to assess the nature and extent of bottom sediment contamination at selected Great Lakes Areas of Concern (AOC) in the United States (Ross et al. 1992). Up to 12 stations were sampled from each of three AOCs (Saginaw River, MI; Buffalo River, NY; and Indiana Harbor, IN; Table 2.1). Stations were selected by the GLNPO ARCS Toxicity-Chemistry Work Group (Table 1.1). Personnel from USEPA Grosse Ile Large Lakes Research Station (LLRS), Grosse Ile, MI collected and shipped sediment samples to NFCRC for the sediment assessments. Personnel from Battelle Marine Science Laboratories, Sequim, WA performed physical and chemical analyses on the sediment samples. Personnel from the USFWS National Fisheries Center-Great Lakes, Ann Arbor, MI conducted fish sediment bioaccumulation studies and fish tumor surveys (Mac et al. 1992). NFCRC personnel assessed sediment contamination in each sample using elutriate and whole sediment toxicity tests, benthic community surveys, and mutagenicity and genotoxicity assays. The NFCRC project was conducted in cooperation with a Wright State University project that provided additional toxicity information for select stations at each AOC (Burton et al. 1992).

Data from these studies are to be evaluated with both the Sediment Quality Triad (Triad; Chapman 1986) and the Apparent Effects Threshold (AET; Barrick et al. 1988) approaches in order to assess the biological hazard of contaminants associated with sediments in Great Lakes AOC. Triad and AET analyses will be discussed in a later report. The Triad will integrate data from: (1) laboratory exposures (e.g., sediment toxicity); (2) benthic community structure (e.g., species abundance); and (3) chemical and physical analyses (e.g., organic and inorganic contaminants, grain size) and will provide strong, complementary evidence for the degree of pollution-induced degradation in aquatic communities (Chapman 1986). The data obtained from the studies will also be used to generate Apparent Effects Threshold (AET) values. An AET is defined as the sediment concentration of a given chemical above which statistically significant biological effects (e.g., sediment toxicity)

are always expected. If any chemical exceeds its AET for a particular biological indicator, an adverse biological effect is predicted for that indicator. If all chemical concentrations are below their AET for a particular biological indicator, then no adverse effect is predicted. The AET has been applied to contaminated sediment in marine environments (e.g., the Puget Sound, Barrick et al. 1988), however this approach has yet to be applied to freshwater ecosystems.

DESCRIPTION OF AREAS OF CONCERN AND STATION SELECTION

Indiana Harbor

The Indiana Harbor and the Grand Calumet River (Figure 1.1) receive heavy industrial and municipal inputs creating severe water and sediment quality problems. There are 39 permitted outfalls on the Grand Calumet River and Indiana Harbor Canal. Contaminant problems are aggravated by the lack of industrial pretreatment and the small size of the upstream drainage basin (Brannon et al. 1989). Toxic substances are of particular concern in this AOC because most of the flow in this system enters Lake Michigan. Although source control during the past decade has improved water quality, sediments and ground water in the area continue to be a repository for many contaminants including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dioxins and furans, chlorinated pesticides, heavy metals, and many other pollutants (International Joint Commission 1987). Surface sediments are contaminated as are sediments buried by deposition.

A total of 50 USEPA Superfund sites have been identified and listed in the Indiana Harbor-Grand Calumet River AOC (Simmers et al. 1989). Benthic invertebrates collected from the area are indicative of pollution tolerant organisms (e.g., oligochaetes). Carp (*Cyprinus carpio*) collected from the Grand Calumet River had deformities including eroded fins, swollen eyes, deformed lower jaws, and concentrations of PCBs and chlordane exceeding U.S. Food and Drug Administration action levels (Simmers et al. 1989). Channelization and historic dredging have also reduced diversity of habitat and diversity of aquatic organisms at the AOC. No dredging activities have occurred since 1972 because of concerns regarding severely contaminated sediment. For additional detail on the Indiana Harbor-Grand Calumet River AOC see International Joint Commission (1987), Brannon et al. (1989), and Simmers et al. (1989).

Buffalo River

The Buffalo River (Figure 1.2) has had documented pollution problems (e.g., bacteria, oil, phosphorus, chlorine, phenol, mercury, and organic dyes) since the 1940s (International Joint Commission 1987). Updated municipal wastewater treatment facilities and controls on industrial discharges have reduced concentrations of many waterborne pollutants, however the major problem in the Buffalo River continues to be contaminated sediments. Sediments are contaminated with heavy metals, industrial organic chemicals, PCBs, and PAHs (International Joint Commission 1987). Surface sediments are contaminated as are sediments buried by deposition. Fisheries resources and populations of benthic invertebrates are severely impaired and fish consumption advisories limit public use of the river. An increased frequency

of fish tumors and other deformities has also been reported. River sediments at some locations are contaminated with cyanide and metals to a level that prohibits open lake disposal of dredge materials. Channelization and dredging of the Buffalo River have also reduced diversity of habitat and the diversity of aquatic organisms. For additional details on the Buffalo River AOC see International Joint Commission (1987) and Lee et al. (1989).

Saginaw River

The Saginaw River (Figure 1.3) receives industrial and municipal wastewater discharges from four major urban areas (Flint, Saginaw, Bay City, and Midland). There are 127 wastewater treatment facilities and 87 industries that discharge directly into the Saginaw River and Saginaw Bay (Brandon et al. 1989). Eutrophication (e.g., phosphorus) and toxic materials have been identified as causing degraded conditions in Saginaw River and Saginaw Bay. Contaminants in sediment include heavy metals, PCBs, polybrominated biphenyls, and DDT. Surface sediments are contaminated as are the sediments buried by deposition. Sediments are most severely contaminated around Saginaw and Bay City. Over 100 hazardous waste sites and 13 USEPA Superfund sites are located in the Saginaw River basin (International Joint Commission 1987). Pollutants of concern in fish collected from the river include hexachlorobenzene, furans and dioxins, diphenyl ethers, styrenes, and terphenyls. Fish consumption advisories have been issued due to elevated levels of PCBs in tissues. Benthic communities have been effected by both toxic materials and eutrophication. Toxic materials may also be affecting reproductive success of fish-eating birds in Saginaw Bay. For additional details on the Saginaw River AOC see International Joint Commission (1987) and Brandon et al. (1989).

Station Selection

Stations were selected by the GLNPO ARCS Toxicity-Chemistry Work Group (Tables 1.1 and 2.1). Attempts were made to select stations representing a gradient in sediment contamination. Additionally, an effort was made to provide some degree of geographical coverage of the AOCs. Chemical, biological, and geophysical characterizations (water, sediment, biota) for each AOC had been conducted on a limited basis by state and federal agencies, universities, and International Joint Commission studies. These data were available through reports, journal articles, and computer database systems (International Joint Commission 1987, Brannon et al. 1989, Simmers et al. 1989, Lee et al. 1989, Brandon et al. 1989).

GENERAL METHODS

Sediment Collection, Handling, and Storage

Contaminants in field-collected sediment may include carcinogens, mutagens, and other potentially toxic compounds. Testing of sediments was started before chemical analyses were completed so contact with sediment was minimized by using gloves, laboratory coats, safety glasses, and respirators. Samples were manipulated and tested under negative pressure.

Sediments were collected and shipped to participating laboratories between August 1989 and July 1990 by personnel from the USEPA Large Lakes

Research Station, Grosse Ile MI (Table 1.1). Station location was determined with a global positioning system. A stainless steel Ponar grab (23- x 23-cm) was used to collect benthic invertebrates at each station (Chapter 5). All benthos samples were collected before samples for toxicity testing were collected to minimize disturbance of the sediments and associated invertebrates. Sediments from the first surveys of Indiana Harbor, Buffalo River, and Saginaw River were collected with the Ponar grab. Sediments from the third survey of Saginaw River were collected with a Van Veen (41- x 51-cm) grab sampler. The grab samplers collected material from the upper 10 to 20 cm of the sediment. Multiple casts of the grab samplers were made and an attempt was made to avoid sampling the same location twice. Sediment was placed in 5-L plastic buckets with plastic liners for transport to shore. Total volume collected ranged from 15 to over 100 L/station. On shore, the sediment was poured into a cement mixer, a plastic cover was placed over the mouth of the mixer, and the sediments were homogenized for 15 min. The cement mixer was washed with soap and site or city water between samples. After homogenization, sub-samples of the sediment were placed in high density polyethylene (HDPE) containers. Sediment samples were shipped on ice to the laboratories via over-night courier. Upon arrival, the sediments were inventoried and held in the dark at 4°C.

Core samples were collected from select stations during the third survey of the Saginaw River (Table 2.1) using a portable electro-mechanical vibrator (Model M3000, Wacker Corporation, Milwaukee, WI). A flexible shaft was attached to a custom-made stainless steel core head via a Model H45 vibrator head. The core tubes were 7.6 cm diameter and penetrated to a depth of up to 1.8 m. See Chapter 3 for additional details on coring procedures.

Sediment samples were generally stored for no more than three weeks before the start of testing. Elutriate toxicity tests with Microtox were conducted upon receipt of the sediments and again at the start of the whole sediment toxicity tests to document potential changes in sediment toxicity with storage (Chapters 2, 3, and 4). The control sediment was a fine silt- and clay-particle size soil obtained from an undisturbed agricultural area. This control sediment has been used previously to evaluate contaminated sediments (Ingersoll and Nelson 1990, ASTM E 1383-92).

Physical and Chemical Characterization of Sediments

Sediment physical characterization included organic carbon, percentage water, and particle size (Chapter 4). Sediment chemical characterization included total metals (Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn), organometals (butyltins and methyl mercury), acid volatile sulfide (AVS) and simultaneously extracted metals (SEM), chlorinated pesticides, total polychlorinated biphenyls (PCBs), polychlorinated dioxins and furans, and polycyclic aromatic hydrocarbons (Chapter 4). Metal concentrations in elutriate and porewater samples were also measured for select samples (Chapter 2).

Quality Control and Quality Assurance

All studies were conducted in basic accordance with Good Laboratory Practice outlined in the Federal Register (160.120; 40 CFR Ch. 1; 7-1-85 edition; subpart G - "Protocol for and Conduct of a Study") and were in basic

accordance with NFCRC guidelines for the provisions of good care and humane handling of test animals during culturing and experimentation. Data are permanently archived by NFCRC and summary data have been transmitted to the GLNPO Project Officer. Data verification reports can be obtained from Dr. Brian Shumacher (USEPA, P.O. Box 93478, Las Vegas, Nevada 89193-3478, 702/798-2100).

Precision is a term that describes the degree to which data generated from replicate measurements differ. Accuracy is the difference between the value of the measured data and the true value. The acceptability of tests was determined by estimating precision (e.g., replication) and by the response of organisms to control sediment or dilution water (e.g., survival). Determining the accuracy of tests with field samples is not possible since the true values are not known. No procedures have been developed to directly measure the accuracy of sediment tests. However, accuracy was indirectly estimated by evaluating the sensitivity of organisms with reference chemicals. Performance audits were conducted by the NFCRC Quality Assurance Officer and GLNPO personnel. Specific reference toxicants, analytical standards, calibration checks, and quality check frequency are described in Chapters 2 to 7.

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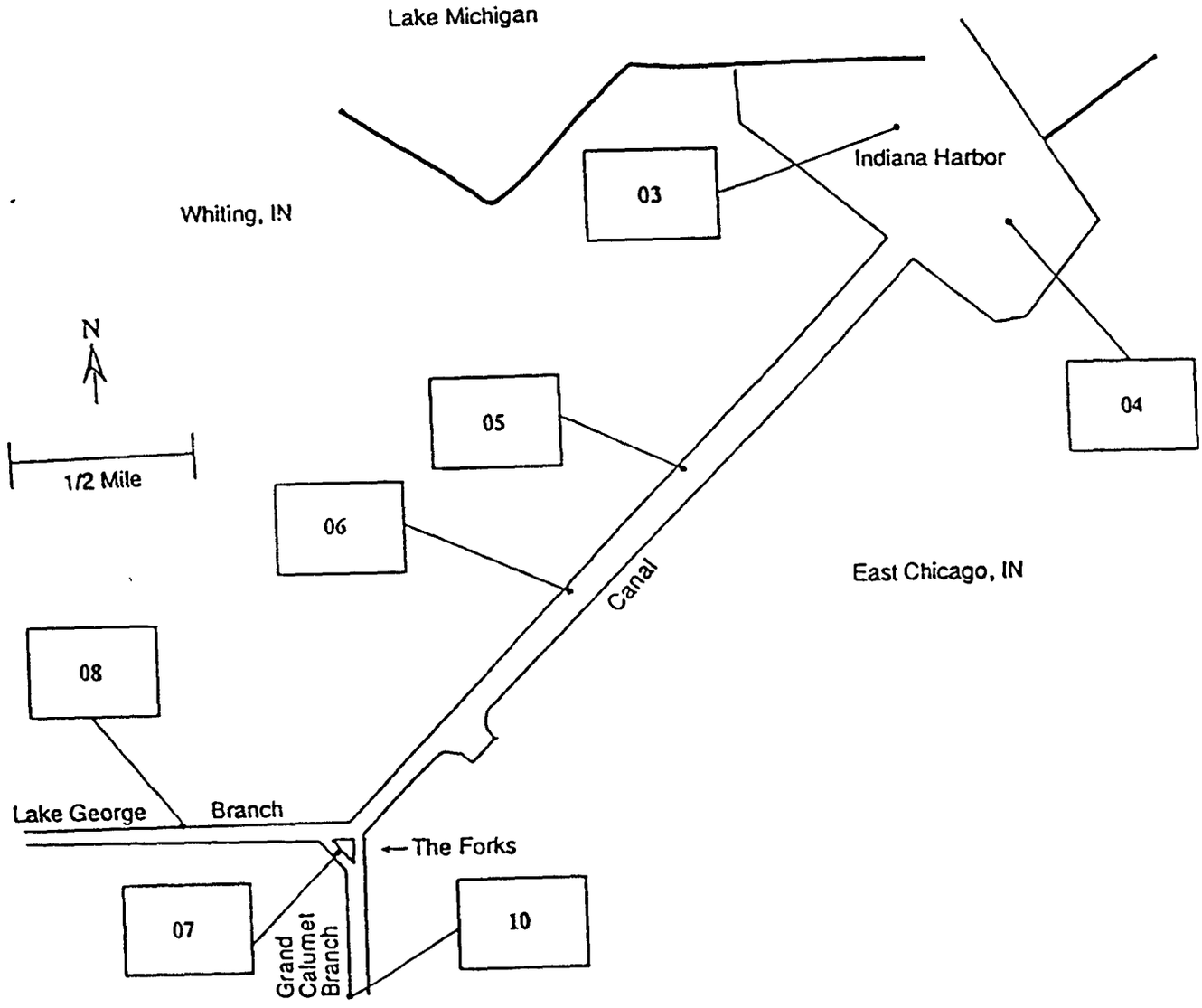

Figure 1.1 Indiana Harbor sediment sampling stations. See Table 2.1 for a complete description of sampling numbers.

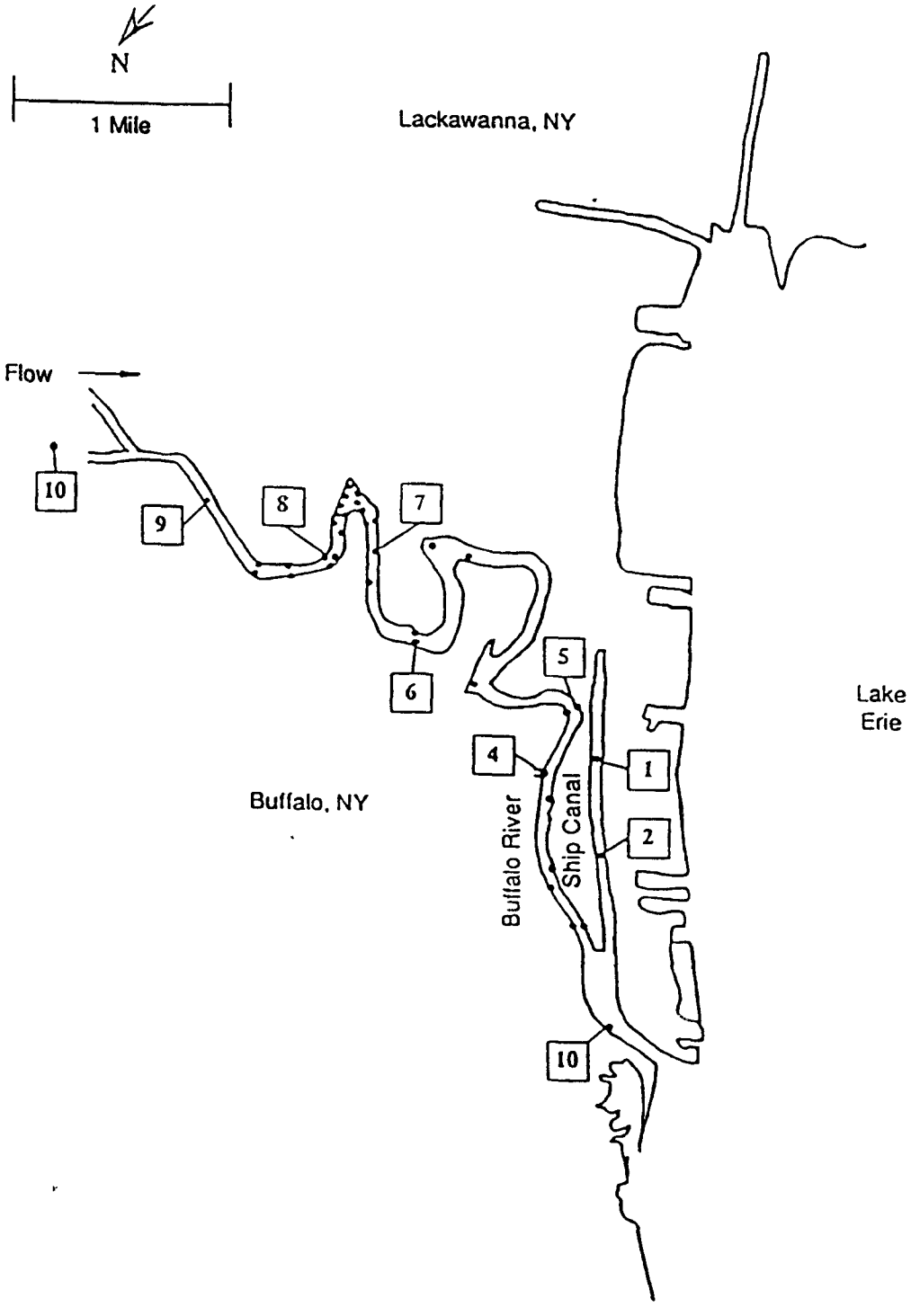

Figure 1.2 Buffalo River sediment sampling stations. See Table 2.1 for a complete description of the sampling numbers.

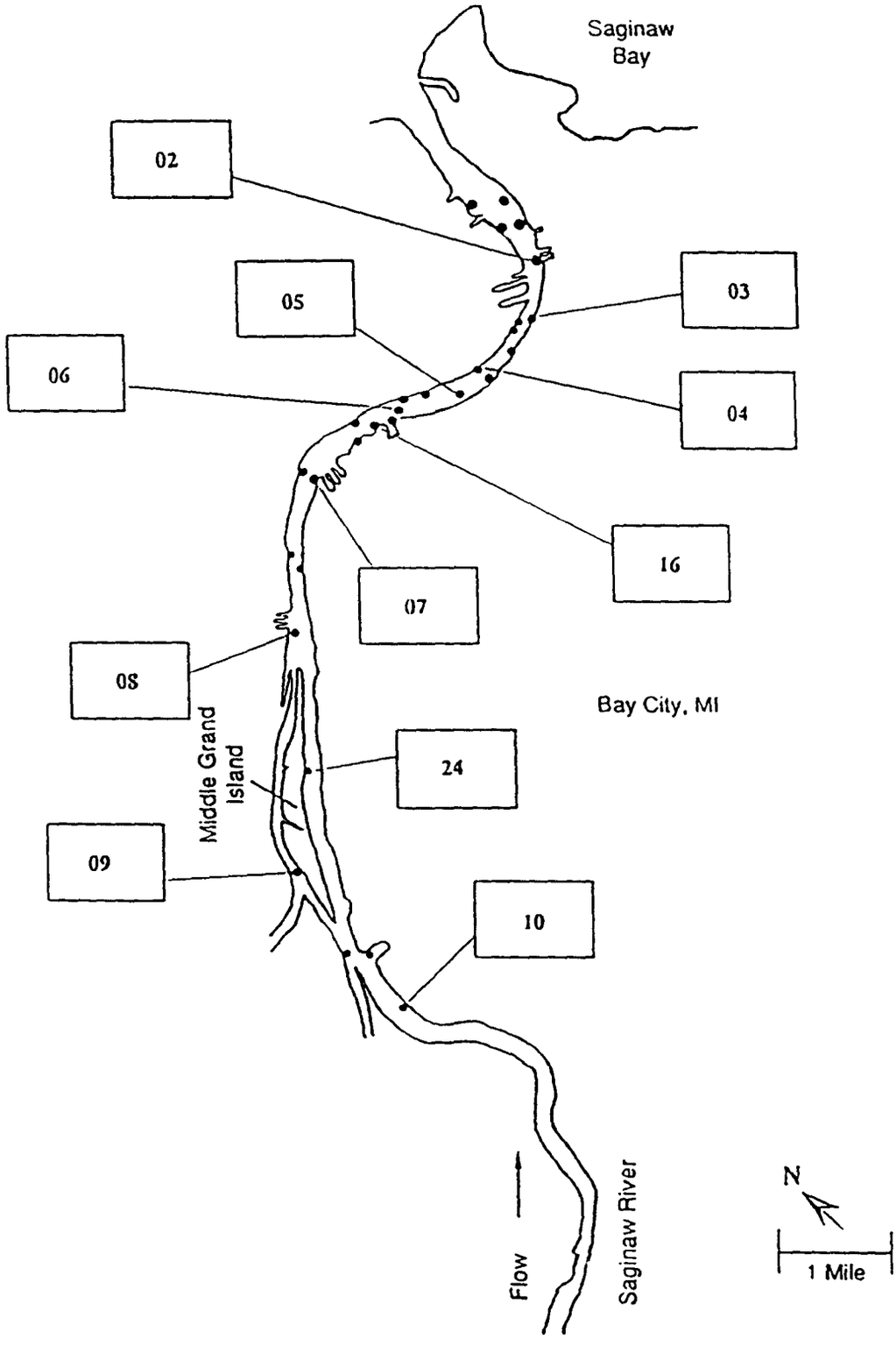

Figure 1.3 Saginaw River sediment sampling stations. See Table 2.1 for a complete description of the sampling numbers.

Table 1.1. USEPA GLNPO ARCS Toxicity-Chemistry Work Group.

Name	Affiliation
P.E. Ross	The Citadel; Charleston, SC
G.A. Burton, Jr.	Wright State University; Dayton, OH
D.C. Cowgill	USEPA-GLNPO; Chicago, IL
E.A. Crecelius	Battelle; Sequim, WA
J.C. Filkins	USEPA-LLRS, Grosse Ile, MI
R.G. Fox	USEPA-GLNPO; Chicago, IL
J.P. Giesy, Jr.	Michigan State University; East Lansing, MI
C.G. Ingersoll	USFWS; Columbia, MO
P.F. Landrum	NOAA; Ann Arbor, MI
M.J. Mac	USFWS; Ann Arbor, MI
T.J. Murphy	DePaul University; Chicago, IL
J.E. Rathbun	AScI, Grosse Ile; MI
V.E. Smith	AScI, Grosse Ile; MI
H.E. Tatem	USCOE; Vicksburg, MS

CHAPTER 2: ELUTRIATE TOXICITY TESTS; DAPHNIA MAGNA AND MICROTOX[R]

Coyle, J.J., E.A. Crecelius, and C.G. Ingersoll

INTRODUCTION

Contaminated sediments are a widespread problem in the maintenance of acceptable water quality in freshwater, coastal, and offshore marine environments (Gannon and Beeton 1969, EPA/COE 1977, Shuba et al. 1978, NRC 1989). The increased concern of resource managers and the scientific community over the ecological role and significance of contaminated sediments, and the lack of established correlations between concentrations of sediment-associated contaminants and biological effects has lead to the development of sediment bioassessment procedures (EPA/COE 1977, Prater and Anderson 1977). Tests that measure bioconcentration and biological effects are used in these sediment assessment procedures rather than chemical analyses to determine the presence or absence of certain pollutants. Typically, these procedures involve evaluating the liquid, suspended particulate, and solid phases of sediment samples for potential biological effects and bioconcentration (Shuba et al. 1978). Thus, organisms that inhabit the water column are exposed to the liquid and suspended particulate phases, and benthic and epibenthic organisms are exposed to the solid phase.

The elutriate test was recommended for use by the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency as part of a process developed to evaluate proposals to discharge dredged materials into ocean waters, and to evaluate the potential of the dredged materials to impact ocean ecology (EPA/COE 1977). The Corp of Engineers (1978) evaluated and recommended the elutriate test as a means of predicting effects of open-water disposal of dredged materials and suggested that the elutriate test may be useful in testing other solid waste. In elutriate tests, usually the aqueous phase of a 4:1 water to sediment mixture is separated and aquatic organisms are exposed to various dilutions of the aqueous phase for a specified period.

Results of elutriate tests depend on temperature, composition of dilution water, extent of sediment contamination, condition of the test organisms and other factors (Shuba et al. 1978). Elutriate tests provide information on the potential toxicity of specific constituents that may be released from contaminated sediments to the water column under various field conditions, e.g., the open water disposal of dredged materials (Shuba et al. 1978, Palermo 1986), but they do not necessarily reflect the toxicity of the in-place sediments (Seelye and Mac 1984). Thus, results of elutriate tests can be used to support inferences about the potential toxicity of the contaminated sediments from which the elutriates are prepared and to identify the biologically active constituents of the contaminated sediment. In addition to assessing the potential hazard of contaminated sediments to aquatic organisms, the results of elutriate tests can be used to compare the relative toxicity of contaminated sediments from different locations (Shuba et al. 1978) and to study the biological availability of contaminants associated with sediments (Seelye and Mac 1984).

In the present study, elutriate tests were used to assess the toxicity of sediment samples collected from three Great Lakes Areas of Concern (AOC). The AOCs include major municipal and industrial centers on Great Lakes rivers, harbors and connecting channels where beneficial uses (e.g., drinking,

swimming, fishing, navigation) are impaired, or where environmental quality criteria are exceeded to the point that such impairment is likely (IJC 1988). There are currently 42 AOCs -- 25 in U.S. waters, 12 in Canadian waters, and five in international connecting channels (IJC 1988). Five AOCs have been specifically designated for "priority consideration" (FWPCA 1987)-- Saginaw Bay (MI), Sheboygan Harbor (WI), Indiana Harbor (IN), Ashtabula River (OH) and Buffalo River (NY). Toxicity tests were conducted with elutriates prepared from sediments collected from three of these five AOCs -- Indiana Harbor, Saginaw River, and Buffalo River. The tests were conducted with *Daphnia magna* and *Photobacterium phosphoreum* (Microtox^R). *Selenastrum capricornutum* were also exposed to these elutriates in acute toxicity tests (Chapter 3). Results of these studies will be used to determine the hazard of contaminated sediments to Great Lakes aquatic resources beyond the context of navigational dredging and dredged material disposal. Eventually, data generated from the studies will be used in two assessment techniques for evaluating the biological hazard of sediment-associated contaminants in the Great Lakes AOCs -- the Sediment Quality Triad method (Chapman and Long 1983, Long and Chapman 1985) and the Apparent Effects Threshold method (Beller et al. 1986, Barrick et al. 1988).

MATERIALS AND METHODS

SEDIMENT COLLECTION, STORAGE AND HANDLING

Summary of Samples Tested

The sediment samples tested were collected within each AOC from Stations selected by the Great Lakes National Program Office (GLNPO) Toxicity and Chemistry Work Group (see Table 1.1 in Chapter 1). The samples were collected by personnel of the U.S. Environmental Protection Agency's (USEPA) Large Lakes Research Station, Grosse Ile, MI. About 10 L of bulk sediment grab samples or four L of bulk core samples were collected from seven Stations in Indiana Harbor, IN in August 1989; from 10 Stations in Buffalo River, NY in October 1989; and from 12 Stations in Saginaw River, MI in October 1989 and June 1990 (Table 2.1).

Sampling Methods

A contaminant-free silt-clay sized particle soil collected from Florissant, Missouri was used as a control sediment. This soil has been previously used in sediment toxicity tests as a control sediment (Adams et al. 1985, Ingersoll and Nelson 1990).

A 23- X 23-cm standard Ponar sampler was used to collect the grab samples. To insure that true surficial sediments were collected, care was taken to avoid sampling from the same spot when successive Ponar casts were required to obtain a desired sample volume. The core samples were collected with a portable light-weight (8.1 kg) device assembled by Large Lake Research Station personnel. The coring device was constructed of a Wacker Model M3000, three horse-power electro-mechanical vibrator (Wacker Corporation, Milwaukee, WI) and a flexible shaft attached to a custom-made stainless steel core head via a Model H45 vibrator head. The core head accepted 7.6 cm diameter core tubes. This coring device did not produce sufficient power to collect cores more than 1.5 to 1.8 m long. To collect a core sample, (1) the sampling vessel was three-way-anchored and its position determined, (2) a core tube was slid into the core head and secured, (3) the coring apparatus was lowered into the water and the vibrator head was turned on, (4) the apparatus was allowed to penetrate the sediment until it stopped or until all of the core tube had entered the sediment, (5) the apparatus was raised onto the deck of the vessel, and (6) the core tube was removed from the vibrator head and the core sample was removed from the core tube. The core samples were transported to shore and separated into different fractions according to depth and identified with X2, X3, 01, or 02 extensions to the sample number (Table 2.1). The samples were placed in 10-L high density polyethylene screw-topped containers, and manually homogenized by stirring for 2 to 3 min. The samples were then packed in synthetic ice, and shipped to the National Fisheries Contaminant Research Center (NFCRC) via over-night courier.

Sample Receipt

Upon receipt, the samples were inventoried. Sediment temperatures upon receipt ranged from 8 to 21°C. The samples were then placed into 18.9 L plastic secondary containers equipped with water-tight sealing lids, labelled,

and stored (≤ 5 d) in the dark in a walk-in cooler at 4°C until the elutriates were prepared (generally less than two weeks).

Sediment Homogenization

All sediment samples were mixed at room temperature of about 20°C with a modified 30.5 cm bench-top drill press (Dayton Model 3Z993). The drill-press was modified by (1) extending the motor-support column 35 cm, and (2) installing an adjustable speed, reversible, 1/2 HP, direct current motor (General Electric Model 5BPB56KAA100) regulated with a SCR direct current motor controller (Dayton Model 2M171C). This motor controlled the speed of the auger between 0 and 500 RPM and direction of rotation. A limit-switch-controlled, AC motor (Dayton, Model 4Z063A) raised and lowered the drill press platform. Sediments were mixed with a stainless steel auger (7.6 cm diameter, 38 cm overall length, 25.4 cm bit length) obtained from Augers Unlimited, Exton, PA. Teflon[R] spatulas and a 40 x 2 x 0.6 cm stainless steel bar was used to remove sediment that adhered to the auger and sample containers.

The samples were mixed in the original shipping containers. A container was placed on the platform of the sediment homogenizer directly under the auger. The platform was then slowly raised to immerse the auger, which was turning at minimum speed, into the sample. The platform was raised to a point where the auger was about 2 cm from the bottom of the sample container. Then, the auger speed was gradually increased to about 350 RPM. The sample was allowed to mix for 10 min while the container was manually rotated on the platform. The auger speed was reduced, the homogenizer platform was lowered, and the stainless steel bar was used to probe the sides and bottom of the sample container to loosen unmixed sediment. The unmixed sediment was moved to the center of the sample container with the stainless steel bar, the platform was raised, and the sample was mixed for an additional 10 min. Between samples, the auger and other tools that were in contact with the sample were (1) were rinsed with well water, (2) washed with soap and water, and (3), sequentially rinsed with acetone, well water, 10% HCl, well water, and deionized water (three rinses).

Elutriate and Pore Water Preparation

Elutriates were prepared immediately after homogenization by mixing one part of sediment with four parts of reconstituted dilution water. The dilution water (hardness 134 mg/L as $CaCO_3$; alkalinity 60-65 mg/L as $CaCO_3$; pH 7.8 to 8.0; conductivity 300 µmhos/cm; sulfate 72 mg/L) was reconstituted according to procedures of Ingersoll et al. (1990). To prepare the elutriates, a 200 ±0.05 g sub-sample of each homogenized sediment was removed from the containers with a disposable polypropylene syringe (60 cc). The syringe was modified by drilling a 0.9 cm hole in the tip of the syringe barrel. The sub-sample was placed into a tared 1000-mL polypropylene centrifuge bottle and weighed on a calibrated electronic pan balance (A&D Engineering Inc. Model FX-3000). Then 800 g of the dilution water was added to the tared centrifuge bottle, cap, and sediment sample.

The contents of the centrifuge bottle were then mixed end over end for 30 min in an extraction apparatus. This apparatus consisted of a 31- x 23- x 18-cm stainless steel box with a removable top and six 10- x 10- x 18-cm compartments. One centrifuge bottle was placed into each compartment. The

box was attached with a flexible bushing to the motor drive of a 1/10 HP, 14 RPM, shaded pole gear motor (Dayton Model 3M136A) supported by a metal frame constructed of 2.54 cm^2 tubing. The motor rotated the box and samples (end over end) at 12 RPM on two lubricated pillow-block bearing assemblies.

After the 30-min mixing, the samples were centrifuged at 5,200 RPM (7,000 x G) with a large capacity, refrigerated centrifuge (International Equipment Co. Model PR-7000) equipped with a Model 966 rotor. The overlying water was decanted through a U.S. Standard # 50 stainless steel sieve into a 3-L glass bottle. The elutriate samples were not filtered. Sub-samples of each elutriate sample were removed from the 3-L bottles and stored ($\leq$ 5 d) in one-L amber bottles equipped with Teflon^R-lined screw-on tops in the dark at 4°C until they were tested (generally less than one week).

Porewater samples were prepared by Battelle's Marine Sciences Laboratory, Sequim Washington from about 40 L of sediment samples. Aliquots of the 40-L samples were extracted in acid-cleaned 500-mL Teflon jars by centrifugation in a modified clothing extractor at 2000 RPM for 15 min. The pore water was decanted into clean 150-mL glass centrifuge tubes and then centrifuged again at 2000 RPM for one h. Then the pore water was pipetted without filtration into 500-mL acid-cleaned Teflon bottles, acidified to pH 2 with HNO_3, and stored at room temperature for metal analyses.

TEST PROCEDURES

Daphnia magna Tests

Daphnia magna were exposed in static acute toxicity tests for 48 h to the full-strength (100%) elutriates and 50%, 25%, and 12.5% volumetric dilutions of the 100% elutriates; an elutriate prepared from the control sediment; and to a negative control of dilution water. The treatments were not replicated. Graduated cylinders and pipettes were used to prepare dilutions of each elutriate. The 100% elutriates and the dilution water were aerated before dilutions were prepared to ensure that initial oxygen concentrations were adequate among treatments at the beginning of each test.

Daphnids were cultured according to procedures described by the USEPA (1984) as modified by Ingersoll et al. (1990). Adult daphnids were isolated from laboratory cultures on the day before a test was started. On the day the test was started (Day 0), daphnids (<24 h old) were removed from the isolated culture and placed into 250 mL beakers containing 200 mL of culture water. Before a test was started groups of the young daphnids were acclimated to the dilution water over a 4-h period. The daphnids were placed first into a solution containing 50% culture water and 50% dilution water for 2 h and then into a solution containing 75% dilution water and 25% culture water for 2 h. Smooth large-bore glass tubes were used to transfer ten of the daphnids, in groups of five each, from the 75:25% acclimation solution directly into each 250-mL test beaker containing 200-mL of test solution. The treatments were stocked in the following order: dilution water control, control sediment elutriate (if tested), and 12.5, 25, 50, and 100% elutriate. Test temperature was maintained at 20°C with a ventilated temperature-controlled water bath. A 16:8 (light:dark) photoperiod with a light intensity of about 25 to 30 footcandles was maintained with fluorescent and incandescent light bulbs suspended above the water baths. Mortality was recorded in all treatments at 24 and 48 h of the test. Lack of mobility in response to prodding with a

blunt probe during a 5-s observation period was the criterion used to determine death.

Microtox^R Tests

All acute toxicity tests with the luminescent bacteria, (Photobacterium phosphoreum) were performed with a Model 500 Microtox^R instrument at 15°C (Microbics 1988). The instrument measured the light output of the luminescent bacteria before and after exposure to dilutions of osmotically-adjusted elutriate samples. Metabolic inhibition in the luminescent organisms occurs if a sample is toxic, and the subsequent reductions in light output are used to derive a dose-response curve from which the effective concentration (i.e., EC50) of the sample is determined.

The Microtox^R tests were simplified through partial automation. A RS232 interface was used to transmit measured light intensities from the Microtox^R instrument to a Compaq^R III (Model 40) personal computer. A manufacturer supplied computer program (written in BASIC) was used to determine the Median Effective Concentrations (EC50) of the sample. The computer program estimated the EC50 value from linear regression equations obtained by plotting log concentration vs. log gamma (normalized ratio of light lost during test to light remaining at time of measurement). No light normalization was performed for the 100% percent test (described below).

Elutriates samples were tested using either "100%" or "Standard" test methods. Less toxic samples were tested undiluted in the 100% Test by substituting sodium chloride for the Microbics^R Osmotic Adjusting Solution (MOAS) to osmotically adjust the sample. Elutriate dilutions up to 45% were evaluated in the Standard Tests. Color corrections were not necessary for any of the elutriate samples tested. For sediment samples evaluated with the 100% method, the full-strength (100%) elutriate and 45, 22.5, 11.3, 5.6% dilutions were tested. Occasionally, primary dilutions of the 100% elutriate were prepared for samples showing high toxicity. In tests were a primary dilution was required, 11.0, 5.5, 2.7, and 1.3% dilutions of the full strength elutriates were evaluated. Acute toxicity values for each sample were estimated as 5-and 15-min EC50s, (the percent dilution of the elutriates that produced a 50% reduction in light output of the luminescent bacteria within 5 or 15 min). In all toxicity tests, duration of exposure is an important factor that influences effects. Generally, Photobacterium phosphoreum in the Microtox^R test system react rapidly to organic compounds and toxicity is frequently elicited within 5 min; however, metals take longer to elicit toxicity in the Microtox^R system and require at least 15 min to stabilize (Microtox^R, 1988). The 5-min EC50s were estimated as intermediate values to provide information on the rate at which toxicity developed. Because the sediment samples contained a mixture of organic and inorganic compounds, these 5-min EC50s should not be used to evaluate their toxicity in the Microtox^R test.

Standard elutriate tests evaluated 45, 22.5, 11.3, and 5.6% dilutions of the full-strength samples. Standard tests were conducted in the following manner:

1) clean cuvettes were placed in the Reagent Well and in incubator block Wells A1 through A5 and B1 through B5 of the Microtox^R instrument,
2) one mL of deionized water was placed in the cuvette in the Reagent Well to reconstitute the bacteria samples,

3) 500 μl of diluent (2% sodium chloride solution) was added to the cuvettes in Wells B1 through B5 with a micropipet,
4) one mL of the diluent was added to the cuvettes in Wells A1 through A4,
5) 250 μl of MOAS (22% sodium chloride to osmotically adjust the sample) and 2.5 mL of elutriate or phenol standard were pipetted into the cuvette in Well A5 and mixed by aspiration with a micropipet,
6) one mL of this osmotically adjusted elutriate or phenol standard was used to prepare 50% serial dilutions in the cuvettes in Well A4, A3, A2, and,
7) the dilutions were mixed by aspiration with a micropipet and allowed 5 min to reach test temperature of 15°C.

Vials of Microtox[R] Reagent (Photobacterium phosphoreum) were stored frozen at -20°C until they were reconstituted on the day of the test. The reagent was added to the 1 mL of cooled reconstitution solution in the Reagent Well cuvette, swirled, and returned to the Well, where it was aspirated 20 times with a 500 μl micropipette. Ten (10) μl of this reagent solution was pipetted into the cuvettes in Wells B1 through B5 and thoroughly mixed by repeated aspiration with a 250 μl micropipette. The diluted reagent solutions were held for 15 min to allow the temperature to stabilize to 15°C.

To begin a test, the reagent sample from Well B1 was placed in the turret Well to calibrate the instrument. Illumination of the "Ready" light on the display panel indicated that the instrument was properly calibrated. The reagent samples in Wells B2, B3, and B4 were then placed in the turret, and the initial light levels (I_0) were recorded by the interfaced personal computer. Then 500 μl of the samples in Wells A1 (Blank), A2, A3, A4, and A5 were transferred, respectively, to the B1, B2, B3, B4, and B5 Reagent Wells. The samples and reagents were mixed by aspiration with the 500 μl micropipette at least twice. Then light outputs of test cuvettes B1 through B5 were recorded at 5 and 15 min. Dilutions lower than 5.6% were tested in the same manner except that the serial dilutions were prepared from a 50% solution of the full-strength elutriate sample.

The "100%" test methods used for undiluted elutriate samples differed from the "Standard" test methods. The manufacturer-supplied computer program could not be used in the 100% test, so light intensity values were recorded manually and the EC50s were calculated by manually entering the data into the computer program. Further, the 100% test differed from the Standard test in that initial I_0 values were not recorded for each dilution. Thus, to calculate the EC50s, the initial I_0 value obtained for sample A1 was assumed to be the same as the I_0 values for samples A2 through A5. This assumption may have diminished the accuracy of the test because the sample dilutions may not have had the same initial luminescence. However, the potential loss of accuracy associated with the 100% test was accepted in order to evaluate greater than 45% dilutions of elutriate samples for toxicity.

The 100% test was performed in the following manner:
1) the lyophilized bacteria were reconstituted and placed in the Reagent Well as previously described,
2) one mL of diluent was added to cuvettes A1, A2, A3, and A4,
3) analytical reagent grade sodium chloride (50 mg) and 2.5 mL of the elutriate were added to cuvette A5 and mixed by aspiration to dissolve the sodium chloride,
4) 500 μl of the sample was removed from cuvette A5 and discarded, and another 1.0 mL was removed and mixed with the diluent in cuvette A4,

5) one mL of the sample in A4 was used to prepare 50% serial dilutions in Wells A3 and A2, and

6) then 1.0 mL of the sample in A2 was withdrawn and discarded.

The temperature of the samples were allowed to stabilized for 5 min to 15°C. Ten μl of reconstituted reagent was mixed with the samples in Wells A1, A2, A3, A4, and A5. After five min the instrument was calibrated by placing cuvette A1 (reagent blank) in the turret. Calibration light intensities were recorded after 5 and 15 min for the samples in cuvettes A1 through A5.

Microtox[R] tests were conducted to determine if the toxicity of elutriates prepared from stored sediment samples changed over time. Elutriates were prepared from mixed sediment samples each time stored sediment samples were homogenized. The elutriates were tested with the same methods described above with one exception. While original elutriates were stored for about 1 week at 4°C in the dark before they were tested, subsequent elutriates were tested within 24 h after they were prepared.

Water Quality Characterization

Immediately after preparation, water quality characteristics of the dilution water and 100% elutriate samples were determined (APHA et al., 1975). Dissolved oxygen (mg/L) was measured with a YSI Model 54-A oxygen meter. Conductivity (μmhos/cm, corrected to 25°C) was measured with a YSI Model 33 S-C-T conductivity meter. The pH and alkalinity (mg/L as $CaCO_3$) was measured with an Orion 940E ionalyzer and a 81-72BN pH probe. Total hardness (mg/L as $CaCO_3$) was determined by burette titration. Ammonia (mg/L) was measured with an Orion 940E ionalyzer and a 95-12 ammonia electrode. Turbidity (NTU) was measured with a Cole-Palmer Model 8391-35 turbidity meter. Unionized ammonia was determined by converting the total ammonia measured in the samples to unionized ammonia, and then correcting for pH and temperature (Thurston et al. 1974). After preparation of the dilution water and 100% elutriates, samples for chloride (mg/L) were placed in 250 mL I-CHEM bottles, labeled, and stored at 4 ± 3°C until analysis with an Orion 940E ionalyzer and a 94-17B electrode. The pH, dissolved oxygen, and conductivity were measured at the beginning and end of each daphnid test in the 100 and 25% treatments, and in the dilution water control.

About 500 mL of each 100% elutriate sample were placed in Teflon[R] bottles, acidified to pH 2 with redistilled hydrochloric acid, and shipped via over-night courier to Battelle Marine Sciences Laboratory, Sequim, Washington for metal analyses.

Elutriate and porewater samples were analyzed for Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, Se, and Zn. With the exception of Hg and Zn in elutriates, all porewater and elutriate samples were analyzed for metals by direct injection graphite furnace atomic absorption without sample preparation. The Zn in elutriates was quantified by flame atomic absorption. The Hg in elutriates were analyzed by cold vapor atomic fluorescence with sub-ng/L detection limits. Organics prevalent in many of the samples were broken down before Hg analysis by use of a bromine monochloride/UV oxidation procedure (Bloom and Crecelius 1983).

Quality Control and Quality Assurance

The precision of a test describes the degree to which data obtained from replicate measurements differ. The accuracy of data is the difference between measured values and true values. The accuracy of data obtained from toxicity tests with field samples cannot be determined since the true values are not known. Currently, no methods exist to directly measure the accuracy of sediment toxicity tests. However, indirect estimates of the accuracy of data from the elutriate toxicity tests were obtained by measuring the sensitivity of test organisms to waterborne reference toxicants. Test acceptability was determined by using methods described for waterborne acute and chronic toxicity tests (USEPA 1985a).

Elutriates prepared from the AOC sediments, control sediment elutriates and dilution water controls were tested. The control sediment was a fine soil containing silt-clay sized particles which has previously been used in sediment toxicity testing (Adams et al. 1985, Ingersoll and Nelson 1990). To determine the acceptability of the toxicity tests, responses (e.g., survival) of test organisms to elutriates prepared from sediments collected at the AOCs were compared to their responses to the control sediment elutriate and the dilution water control.

The EC50s in the daphnid tests were calculated with either probit, binomial, or moving average methods as appropriate (Stephen 1977). A reference toxicant test with Instant Ocean^R was conducted to evaluate the sensitivity of daphnids used in the elutriate toxicity tests. The daphnids were exposed in a 48-h static acute toxicity test to concentrations (1.5 to 33.0 g/L) of Instant Ocean^R dissolved in well water. Results of this reference toxicant test are shown in Table 2.2.

All Microtox^R tests were conducted with equipment, supplies, and reagents that were recommended by, or obtained from the manufacturer. A 90 mg/L solution of phenol prepared in deionized water was used to conduct reference toxicant tests at the beginning of each days test run. The EC50s for the reference toxicant tests averaged 19.7% and ranged from 17.8% to 22.5% for the full-strength phenol solution, which was within the manufacturer's established acceptable range of 14 to 29%. These reference toxicant tests were conducted concurrently with the first AOC sample elutriates (E-1) Microtox^R tests.

RESULTS

INTER-AOC ELUTRIATE WATER QUALITY SUMMARY

Generally, a wide range in elutriate water quality characteristics was measured across the AOCs. Ranges in elutriate water quality measurements were: pH, 6.6 to 8.0; alkalinity, 80 to 400 mg/L as $CaCO_3$; hardness, 115 to 283 mg/L as $CaCO_3$; dissolved oxygen, 1.8 to 7.1 mg/L; conductivity 335 to 951 µmhos/cm; unionized ammonia, 0.01 to 0.59 mg/L; chloride 8.9 to 26.2 mg/L; and turbidity, 14.3 to 198.0 NTU (Table 2.3). Ranges of water quality measurements for elutriates prepared from control sediment were: hardness, 118 to 120 mg/L as $CaCO_3$; oxygen, 7.9 to 9.4 mg/L; conductivity, 238 to 259 µmhos/cm; chloride, 9 to 11 mg/L; turbidity, 29 to 54 NTU; pH, 7.0; alkalinity, 44 mg/L as $CaCO_3$; and unionized ammonia, 0.01 mg/L (Table 2.3). Mean pH, alkalinity, hardness, conductivity, and chloride measurements were relatively similar in elutriates across the AOCs (Table 2.3). Mean dissolved oxygen was lowest in Indiana Harbor elutriates; was intermediate in elutriates from Buffalo River and Saginaw River (third survey); and was highest in elutriates from the first survey of Saginaw River (Table 2.3). Mean unionized ammonia and turbidity in elutriates from Indiana Harbor and the first survey of Saginaw River were similar, and were slightly higher than unionized ammonia and turbidity in elutriates from Buffalo River and the third survey of Saginaw River (Table 2.3).

INDIANA HARBOR

Elutriate Water Quality Characterization

Generally, measured water quality parameters were similar in the control elutriate and Indiana Harbor elutriates. Exceptions were that alkalinity in the Indiana Harbor elutriates was three to nine times higher than in the elutriate prepared from control sediment; unionized ammonia ranged from 0.1 to 0.6 mg/L in the Indiana Harbor elutriates and was much lower (0.01 mg/L) in the control elutriate; and turbidity was about two to four times higher in Indiana Harbor elutriates than in the control elutriate (Table 2.3). Measured water quality was also relatively similar among the Indiana Harbor elutriates treatments except that alkalinity was about 4 times higher in elutriate sample IH-01-10 compared to other elutriate samples (Table 2.3). During the daphnid test with Indiana Harbor elutriates the pH, dissolved oxygen and conductivity were similar across treatments (Table 2.4).

Elutriate samples from Indiana Harbor contained elevated total metal concentrations compared to the control elutriate (Table 2.5). Generally, the concentrations of metals in samples IH-01-04 and IH-01-10 were similar and lowest; metal concentrations in samples IH-01-03, IH-01-05, IH-01-06, and IH-01-08 were intermediate; and metal concentrations in sample IH-01-07 were the highest (Table 2.5).

Total metal concentrations were also elevated in porewater samples prepared from Indiana Harbor sediments. Pore water from sample IH-01-06 contained the lowest metal concentrations and samples IH-01-03 and IH-01-04 contained metal concentrations that were intermediate between concentrations in samples IH-01-06 and IH-01-07 (Table 2.6).

Toxicity

Daphnia magna Tests --

All the Indiana Harbor elutriates, except the elutriate from sample IH-01-06, were toxic to *Daphnia magna*. Elutriates from samples IH-01-03, IH-01-05, IH-01-07, and IH-01-08 were the most toxic, and 48-h EC50s for daphnids (as percent dilutions of the 100% elutriates) ranged from 0.7 to 25.0%; elutriates from samples IH-01-04 and IH-01-10 elicited intermediate toxicity to daphnids and 48-h EC50s ranged from 39.4 to 70.7%; and the undiluted (100%) elutriate from sample IH-01-06 was not acutely toxic to daphnids (Table 2.7).

Microtox^R Tests--

All of the Indiana Harbor elutriates were toxic to *Photobacterium phosphoreum* in the Microtox^R test. Elutriates from samples IH-01-06, IH-01-08, and IH-01-10 were the most toxic with 15-min EC50s ranging from 4.3 to 6.8% of the 100% elutriates (Table 2.7). Sample IH-01-03 elicited intermediate toxicity with an EC50 of 20.3%, and samples IH-01-05, IH-01-04, and IH-01-07 were the least toxic in the Microtox^R test with EC50s that ranged from 27.3 to 34.4% dilutions. With the exception of sample IH-01-07, Indiana Harbor elutriates elicited a slow response in the Microtox^R tests, and 5-min EC50s were about half the 15-min EC50s (Table 2.7).

BUFFALO RIVER

Elutriate Water Quality Characterization

The pH, alkalinity, hardness, dissolved oxygen, and conductivity of elutriates prepared from Buffalo River sediments were generally similar. The exceptions among the sample elutriates were that alkalinity was slightly lower in samples BR-01-02 and BR-01-03; hardness was slightly lower in samples BR-01-09; chloride was slightly higher in samples BR-01-01 and BR-01-02; and turbidity was slightly lower in samples BR-01-01, BR-01-02, and BR-01-10 (Table 2.3). Unionized ammonia in elutriates from sample BR-01-01 was 68 to 137 times higher compared to elutriates from the other Buffalo River samples which contained relatively low (0.01 to 0.02 mg/L) unionized ammonia concentration (Table 2.3). During the daphnid test with Buffalo River elutriates the pH, dissolved oxygen and conductivity were similar across treatments (Table 2.4).

A wide range of metal concentrations was observed in Buffalo River elutriates (Table 2.8). Zinc and Pb concentrations were higher in Buffalo River elutriates compared to the other metals, and concentrations of Cr, Cu, Ni, Pb, and Zn were highest in sample BR-01-06 (Table 2.8).

Buffalo River sediment pore waters contained low μg/L concentrations of metals, and concentrations of As, Cr, and Cu were higher compared to the other metals (Table 2.9).

Toxicity

Daphnia magna Tests--

Elutriates prepared from Buffalo River sediments were generally less toxic to daphnids than Indiana Harbor elutriates. Exceptions were the elutriates from sample BR-01-03 for which the 48-h EC50 was 76.2%, and the elutriate from sample BR-01-06 for which the 48-h EC50s was 0.11% of the full-strength elutriate (Table 2.7).

Microtox^R Tests--

Microtox^R indicated that the elutriate from sample BR-01-06 was the most toxic with a 15-min EC50 of 6.7% of the full-strength elutriate (Table 2.7). Elutriates from samples BR-01-01, BR-01-03, and BR-01-05 elicited intermediate toxicity in the Microtox^R test and 15-m EC50s ranged from 40.5 to 65.8% dilutions of the full-strength elutriates (Table 2.7). The remaining elutriates from samples BR-01-02, BR-01-04, BR-01-07, BR-01-08, BR-01-09, and BR-01-10 were not toxic in the Microtox^R test (Table 2.7). Buffalo River elutriates elicited a rapid response in the Microtox^R tests, and 5- and 15-min EC50s were similar with the exception of sample BR-01-06 for which the 15-min EC50 was about three times lower than the 5-min EC50 (Table 2.7).

SAGINAW RIVER (FIRST SURVEY)

Elutriate Water Quality Characterization

Measured water quality was generally similar in elutriates prepared from sediments collected during the first survey of Saginaw River (Table 2.3). Compared to the elutriate prepared from control sediment, alkalinity was two to five times higher; ammonia was 6 to 283 times higher; chloride was 2 to 4 times higher; and turbidity was 3 to 8 times higher in the Saginaw River elutriates (Table 2.3). During the daphnid test with Saginaw River (first survey) elutriates the pH, dissolved oxygen and conductivity were similar across treatments (Table 2.4). Elutriates prepared from Saginaw River sediment samples collected during the first survey contained low μg/L metal concentrations, and concentrations of As, Cu, Pb, and Zn were higher compared to the other metals (Table 2.10). Elutriate sample SR-01-06 contained the highest concentrations of Cd, Cr, Cu, Ni, Pb, and Zn (Table 2.10). Pore water from samples SR-01-03 and SR-01-10 contained low ($\leq$ 4.0 μg/L) metal concentrations and metal concentrations in pore water from sample SR-01-06 were higher and ranged from < 1.0 to 36.0 μg/L (Table 2.11).

Toxicity

Daphnids magna Tests--

Generally, Saginaw River elutriates were not toxic to daphnids. Exceptions were elutriates from SR-01-06 and SR-01-07 which elicited 48-h EC50s for daphnids of 12.5 to 25% dilutions of the full-strength elutriates (Table 2.7).

Microtox^R Tests--

The Microtox^R test indicated that the elutriate from sample SR-01-06 collected during the first survey of Saginaw River was toxic with an EC50 of 13.0% of the full-strength elutriate (Table 2.7). The remaining elutriates from first survey samples SR-01-02, SR-01-03, SR-01-04, SR-01-07, SR-01-09, and SR-01-10 were not toxic (EC50 > 100%). Essentially no differences were observed between 5- and 15-min EC50 values for the Saginaw River (first survey) elutriates.

SAGINAW RIVER (THIRD SURVEY)

Elutriate Water Quality Characterization

The water quality of elutriates from sediment samples collected during the third survey of Saginaw River was generally similar (Table 2.3). Elutriates prepared from core samples contained higher unionized ammonia concentrations (0.1 to 0.3 mg/L) compared to the elutriates prepared from grab samples (Table 2.3). During the daphnid test with Saginaw River elutriates (third survey) the pH, dissolved oxygen and conductivity were similar across treatments (Table 2.4). With the exception of Zn concentrations that ranged from <7 to 148 μg/L, metals concentrations in elutriates prepared from sediment grab samples collected during the third survey of Saginaw River were $\leq$ 10.5 μg/L (Table 2.12). With the exception of Hg, concentrations of metals in elutriates prepared from sediment core samples were generally higher than in those prepared from grab samples (Table 2.12). Metal concentrations in pore water from sediment samples collected during the third survey of Saginaw River ranged from 0.002 μg/L for Hg to 10.7 μg/L for Zn (Table 2.13).

Toxicity

Daphnia magna Tests--

None of the elutriates prepared from grab samples collected during the third survey of Saginaw River were toxic to daphnids (Table 2.7). Elutriates prepared from core samples were not test with daphnids.

Microtox^R Tests--

Only one of the elutriates (SR-03-16) prepared from grab samples collected during the third survey of Saginaw River were toxic in the Microtox^R test (Table 2.7). However, the Microtox^R tests conducted with elutriates prepared from the core samples indicated that samples SR-03-02-X2 and SR-03-06-X2 were the most toxic with EC50s that ranged from 31.0 to 34.0% dilutions of the full-strength elutriates; the elutriate from core sample SR-03-06-01 elicited intermediate toxicity (EC50 of 54%); and the remaining elutriates from third survey Saginaw River core samples elicited Microtox^R EC50s $\geq$ 77% (Table 2.7). Essentially no differences were observed between 5- and 15-min EC50 values for the Saginaw River (third survey) elutriates prepared from all grab samples and most of the core samples.

MICROTOX[R] SEDIMENT STORAGE EVALUATIONS

With few exceptions, the Microtox[R] test indicated that the toxicity of whole sediment samples did not change during storage up to 26 d in the laboratory at 4°C. Indiana Harbor samples were stored the longest (26 d). The remaining samples were stored between 21 and 26 d. Of the 19 samples tested, only 2 elicited a different toxicity over time (Table 2.14). After 14 d of storage, elutriates prepared from sample IH-01-07 (Indiana Harbor) elicited increased toxicity compared to the toxicity observed in initial testing, and elutriates prepared from sample BR-01-03 (Buffalo River) did not elicit toxicity but had been toxic in initial testing. All other samples exhibited no changes in toxicity.

DISCUSSION

Photobacterium phosphoreum (Microtox^R) and Daphnia magna have been previously used to measure sediment toxicity (Burton et al. 1989, Giesy et al. 1990, Giesy and Hoke 1989, Ankley et al. 1989). In the present study, these two organisms were used in elutriate toxicity tests to screen sediment samples from each AOC for acute toxicity. The toxic effects observed in the Daphnia magna and Microtox^R acute toxicity tests represent integrated responses to the interaction (i.e., additive, synergistic or antagonistic) of multiple contaminants that were present in the elutriates. Without applying Toxicity Identification Evaluation (TIE) procedures (Mount and Anderson-Carnahan 1988, Mount and Anderson-Carnahan 1989, Mount 1989) the toxicity observed in these tests cannot be attributed to any particular chemical constituent of the elutriates.

The rotary procedure used to prepare the elutriates for this study was similar to elutriate preparation procedures that have been shown to produce reasonable estimates of bioavailable contaminants in sediment samples (Daniels et al. 1989). The elutriates from each AOC were analyzed for metals but not for organic contaminants, and some of the elutriates contained elevated concentrations of heavy metals. Albeit no analyses for organic contaminants were performed on the elutriates, organic compounds were present in the whole sediment samples from which the elutriates were prepared. Undoubtedly, some fraction of these organic contaminants desorbed into the elutriate sample during preparation. Ionic contaminants may desorb from sediment particles agitated in water, and the concentration of these contaminants in the water phase is dependent on chemical desorption processes which are classified as either fast (taking place in minutes to hours) or slow (days to weeks) (Karichoff 1980). In sediment systems, chemical desorption rates are dependent on partition coefficients, organic carbon content of the sediment, ionic strength of the aqueous phase, and contact time between the chemical and the sediment. Thus, the elutriates tested in these studies probably contained complex mixtures of both inorganic and organic compounds which singly or in combination contributed to the observed toxicity.

INDIANA HARBOR

Daphnia magna

With the exception of elutriate sample IH-01-06 which was not toxic at full-strength (100%), all the elutriates prepared from Indiana Harbor sediments were highly toxic to daphnids. The elutriate test used in the present study is an efficient indicator of soluble constituents that may be released from disturbed sediments and become available to organisms in the water column. Contaminant-induced biological effects in aquatic organisms are a function of biologically available contaminant concentrations and exposure time. Daphnid EC50s (as percent dilutions of the full-strength elutriates) for the elutriates that were toxic ranged from 0.7 to 70.7% indicating that the elutriates contained increasing concentrations of toxic components, or toxic components that were differentially available to the organisms. These results were confirmed by the presence of elevated heavy metal concentrations in the elutriate samples. Furthermore, elutriate sample IH-01-07 which contained the highest concentrations of metals also elicited the highest

toxicity to daphnids (EC50 of 0.7%). Apparently contaminant availability was lower in elutriate sample IH-01-06 compared to the other elutriates, since this sample elicited no toxicity in daphnids, but it contained concentrations of metals similar to some of the other samples which were toxic. The Indiana Harbor elutriates contained elevated unionized ammonia concentrations that probably also contributed to the observed responses; however, concentrations of unionized ammonia in most of the elutriates were below concentrations (0.5 to 2.8 mg/L NH_3) reported to be acutely toxic to daphnids (USEPA 1985b).

All of the elutriates probably contained toxic components other than metals, since a wide array of contaminants such as organometals, polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenols (PCBs), dioxins, and pesticides were measured in the whole sediment samples from which the elutriates were prepared. Refer to Chapter 4 in this report for information on concentrations of contaminants in the whole sediment samples from Indiana Harbor.

Microtox^R

Indiana Harbor elutriates elicited substantial toxicity in the Microtox^R tests which was consistent with the elevated concentrations of metals in the elutriate samples. The toxicity observed in the Microtox^R test with Indiana Harbor elutriates indicated that spatial differences in contaminant loads exist within the AOC. Elutriates prepared from sample IH-01-08 collected from the Lake George Branch, sample IH-01-10 collected from Grand Calumet Branch, and sample IH-01-07 collected near the "Forks" (See Figure 1.1 in Chapter 1) area elicited the highest toxicity. Generally, samples collected closer to Indiana Harbor exhibited less toxicity. This distribution of toxicity may be related to contaminant discharge patterns, the dredging and removal of contaminated sediments from the main channel of the harbor, or differences in contaminant cycling and distribution processes among the sampling stations

The presence of ammonia in the samples may not have influenced the Microtox^R tests because the sensitivity of *Photobacterium phosphoreum* to ammonia is low. In porewater toxicity identification evaluations, Microtox^R was unresponsive to samples whose toxicity to *C. dubia* and fathead minnows was associated primarily with ammonia (Ankley 1990). .

Subsequent testing of two elutriates prepared from sample IH-01-07 indicated that the sample was more toxic than it had been shown to be in the initial elutriate test. This discrepancy cannot be explained, so the Microtox^R results for elutriate sample IH-01-07 must be cautiously interpreted.

BUFFALO RIVER

Daphnia magna

Compared to Indiana Harbor, sediments in Buffalo River probably contain lower concentrations of toxic components. Only Buffalo River elutriate samples BR-01-03 (EC50 76.2%) and BR-01-06 (EC50 0.11%) were toxic to daphnids, and the other elutriate samples were not toxic at full-strength. These conclusions were supported in part by the concentrations of metals that were measured in the elutriate samples. Whereas measured metal concentrations were generally low and similar among the elutriate samples, concentrations of

Pb, for example, in samples BR-01-03 and BR-01-06 were much higher, and were 1.0 to 3.0 times higher than the National Acute Water Quality Criteria (EPA 1986) for Pb (82.0 μg/L, normalized to 140 mg/L hardness).

The Pb concentration in elutriate sample BR-01-05 was two times higher than the National Water Quality Criteria, but the full-strength elutriate was not toxic to daphnids. Apparently, there were differences in contaminant availability to the daphnids or antagonistic interactions among the Buffalo River elutriate samples tested. Differences in contaminant availability or contaminant interactions may have resulted in the pattern of mortality that prevented the calculation of an EC50 for daphnids exposed to dilutions of elutriate BR-01-07. Despite having unionized ammonia concentrations exceeding those reported to be acutely toxic to daphnids (0.5 to 2.8 mg/L NH_3, USEPA 1985b), the elutriate prepared from BR-01-01 sediment was not toxic to Daphnia magna. The physical and chemical characteristics of sample BR-01-01 may have reduced ammonia toxicity.

Daphnids in the Buffalo River elutriate tests were probably also exposed to other contaminants in addition to the metals, such as organometals, PAHs, PCBs, dioxins, and pesticides because they were measured in the whole sediment samples. Refer to Chapter 4 in this report for information on concentrations of contaminants in the whole sediment samples from Buffalo River.

Microtox

Elutriate BR-01-06 exhibited the highest Microtox^R toxicity (15-min EC50, 6.7%), followed by BR-01-01 (15-min EC50,40.5%), BR-01-03 (15-min EC50, 44.2%) and BR-01-05 (15-min EC50, 65.8%). Compared to Station BR-01-06, elutriates prepared from sediments collected upstream (BR-01-05) and downstream (BR-01-07) were considerably less toxic. This spatial distribution of toxicity implies the presence of a localized source of pollution near Station BR-01-06.

The toxic effects observed in the Microtox^R tests appeared to be related to the concentrations of select metals in the elutriates. With the exception of Station BR-01-01, elutriates that exhibited Microtox^R toxicity (BR-01-03, BR-01-05 and BR-01-06) contained concentrations of Cu, Cd, and Pb equal to or greater than the Chronic National Water Quality Criteria for these metals. The concentrations of Cu and Cd measured in BR-01-01 approached the Chronic Water Quality Criteria for these metals and may have been sufficient to elicit the observed Microtox^R toxicity. The increasing toxicity observed in BR-01-06 between 5- and 15-min readings suggest that the primary toxic agent is one or more metals (Elnabarawy et al. 1988). However, as previously noted, the toxicity measured in these elutriates cannot be attributed to individual constituents of the elutriates (e.g., metals). Reported Microtox^R 15-min EC50s for single metals are much higher for Hg, (29 μg/L), Cu (250 μg/L), Cd (17000 μg/L) than those measured in the Buffalo River elutriates tested (Elnabarawy et al. 1988).

SAGINAW RIVER (FIRST AND THIRD SURVEYS)

Daphnia magna

Sediment samples from Saginaw River contain lower concentrations of contaminants compared to Indiana Harbor or Buffalo River. Only elutriate

samples SR-01-06 (EC50 >12%) and SR-01-07 (EC50 >12) prepared from Saginaw River (first survey) sediments were toxic to daphnids. All other elutriates from the first and third survey of Saginaw River were not toxic to daphnids at full-strength. These conclusions were supported by the generally higher concentrations of metals and probably other toxic components (see Chapter 4) not measured in elutriate SR-01-06 and SR-01-07 prepared from Saginaw River (first survey) sediment samples.

Microtox[R]

All samples collected from Saginaw River during the first and third surveys were evaluated with the 100% Microtox[R] assay because historical information indicated that Saginaw River sediments contained low concentrations of contaminants. The toxicity of effluents have been determined within an order of magnitude using either the 100% or Standard Microtox[R] tests and the 100% Microtox[R] test has been recommended for use with fairly non-toxic samples (Tarkepea and Hansson 1989).

The low Microtox[R] toxicity observed in the Saginaw River elutriates were consistent with concentrations of metals measured in the samples. The elutriate prepared from grab sample SR-01-06 was the only sample from either the first or third survey that exhibited toxicity. This sample contained concentrations of Cu and Cd higher than the Acute and Chronic Water Quality Criteria for these metals. Further, the concentration of Pb was nearly 10 times higher than the Chronic Water Quality Criterion value. However, the concentrations of Cu and Cd in SR-01-06 were lower than the EC50s determined for the individual metals (Elnabarawy et al. 1988) indicating that interactive toxicity between these metals, or toxicity from other contaminants not measured probably occurred. The presence of a contaminant source near Station SR-01-06 is suggested since it was the only Saginaw River sample that exhibited Microtox[R] toxicity.

Grab samples were collected from Station 06 in the Saginaw River during both the first and third surveys, but measured metal concentrations and Microtox[R] toxicities differed for the two surveys. Metal concentrations in elutriate sample SR-01-06 were 2 to 20 times higher than in SR-03-06 and Microtox[R] toxicity observed in these two samples was consistent with their metal concentrations. These results indicate that considerable differences in contaminant loading may exist in Saginaw River.

The results of Microtox[R] tests on elutriates from core samples indicate that historical patterns of contaminant distribution exist in the Saginaw River. For example, while core sample SR-03-02-X2 (20 to 50 cm deep) was more toxic than sample SR-03-02-X3 (50 to 88 cm deep), core samples SR-03-05-01 and SR-03-05-02 did not exhibit increasing toxicity with depth. Further, at Station 06, where four core samples were taken, differences in toxicity in relation to core depth were observed. While core SR-03-06-01 (0 to 61 cm deep) was more toxic than sample SR-03-06-02 (61 to 122 cm deep), sample SR-03-06-X2 (an oily core sample taken between 13 to 33 cm deep) was the most toxic core tested. The sample SR-03-06-X3, taken just below the oily core between 33 to 81 cm deep elicited only slight toxicity. The heterogenous distribution of sediment toxicity has been reported previously (Stemmer et al. 1990, Burton 1991). In addition to historical contamination, local disturbances and re-suspension related to shipping and dredging activities in

the Saginaw River may have contributed to the observed variation in toxicity with core depth.

The Microtox^R test was the only test conducted with elutriates prepared from stored sediment samples. Microtox^R elutriate test results demonstrated (with few exceptions) that the toxicity of the stored samples did not change appreciably during storage for up to 26 d, although sediment storage times no longer than 2 weeks are recommended (ASTM E 1383-92 1991). Apparently, changes in toxicity of the stored sediment samples as indicated by the Microtox^R test were minimized by maintaining the samples at 4°C in their original, screw-topped shipping containers.

INTER-AOC TOXICITY COMPARISONS

Elutriates prepared from whole sediment samples are efficient indicators of sediment-associated contaminants that may be released to the water column (Shuba et al. 1977). The elutriates and pore waters evaluated in the present study were centrifuged rather than filtered before they were tested. The samples were centrifuged to minimize the possibility of reducing toxicity and subsequently underestimating the potential toxicity of the sediments samples from each AOC.

The chemical constituents of sediments are associated with the interstitial water or surfaces of the solid or particulate phase (Engler 1980). These constituents are available at varying degrees for release to the water column, ranging from essentially no release of insoluble components associated with mineral lattices to the highly available components of the interstitial water. The chemical constituents present in elutriates generated in the present study probably represent interstitial water components and loosely-bound, easily-exchangeable components of the solid phase sediment.

Apparently, the elutriate preparation process was highly efficient in extracting many metals from field collected sediment samples. The elutriate preparation methods used in the present study required mixing a 1:4 ratio of sediment to water for 30 min. Mixing the sediments with oxygenated water may have been sufficient to oxidize metal-AVS complexes. Once released from the AVS-complexes, the metals were maintained in solution by the addition of HCl to the samples. Assuming that pore water comprises 20 to 50% of field collected sediments samples, elutriate preparation resulted in a 9- to 21-fold dilution of the pore water present in the original samples. Despite this dilution, elutriate samples contained higher metal concentrations (except Hg) than full-strength porewater samples.

In contrast to other metals, Hg concentrations were frequently higher in porewater samples compared to those in elutriates (IH-01-04, BR-01-01, SR-01-06, and all samples from the third survey of Saginaw River). Hg has a strong affinity for organic substances in soils and natural waters (Lindqvist et al. 1991). Whereas the elutriate preparation process was highly efficient in extracting cationic metals from the sediment, apparently the process was less effective in extracting Hg from the sediment. The lower Hg concentrations measured in the elutriates probably represent a dilution of Hg which was present in sediment pore water.

Elutriate toxicity tests have been recommended as a means of evaluating the potential ecological effects of open-water disposal of dredged materials (EPA/COE 1977). However, conclusions derived from elutriate toxicity tests in relation to potential ecological effects at open-water sites where dredged

materials are disposed may not be relevant for in-place contaminated sediments in inland or coastal waters. For example, potential ecological effects at open water disposal sites are believed to be minimal because of settling and dilution that prevent contaminants released from the sediment from reaching toxic levels; however, ecological effects may develop in inland or coastal waters where dilution may not be sufficient and contaminants may be continually released from sediments due to wave action, activity of organisms, and human disturbances.

Responses observed in the Microtox^R and daphnid elutriate tests indicate that sediments in Indiana Harbor are more toxic than those in Buffalo River which are more toxic than sediments in Saginaw River. Although the responses observed cannot be related to any particular component in the elutriates tested due to interactive toxicity that can occur in complex contaminant mixtures, there were trends in the relation between concentrations of individual metals and the responses observed. For example, Cu concentrations in all the Indiana Harbor elutriates exceeded both the Acute and Chronic National Water Quality Criteria; all the elutriates from Indiana Harbor contained Pb concentrations that exceeded the National Acute Water Quality Criteria; and concentrations of Hg and Cd (except sample IH-01-04) in the Indiana Harbor elutriates exceeded the National Chronic Water Quality Criteria (Figure 2.1). Also, unionized ammonia concentrations in all the Indiana Harbor elutriates far exceeded the National Acute Water Quality Criteria (Figure 2.2). The elutriates from Buffalo River were less toxic than those from Indiana Harbor, and only three of the Buffalo River elutriates contained concentrations of Cu and Pb that exceeded the National Acute Water Quality Criteria and none contained concentrations of Cd and Hg that exceeded the Acute Criteria (Figure 2.3). Moreover, unionized ammonia in all the Buffalo River elutriates (except sample BR-01-01) were below the National Acute Criteria (Figure 2.2). The Saginaw River elutriates were less toxic than elutriates from Indiana Harbor and Buffalo River with only one sample containing Cu and Cd concentrations that exceeded National Acute and Chronic Criteria, although some of the samples exceeded the Chronic Criteria for Pb and Hg (Figure 2.4). Unionized ammonia in the Saginaw River elutriates were elevated but were lower than those in the Indiana Harbor elutriates (Figure 2.2) that were more toxic.

Thirty-three percent of the 30 elutriate samples tested with daphnids were conservatively identified as toxic, i.e., EC50s of less than the full-strength elutriate were determined. Fifty-three percent of the 39 samples tested with Microtox^R were conservatively identified as toxic. These results indicate that the Microtox^R test is more sensitive than Daphnia magna tests for sediment bioassessments. The Microtox^R test has been previously reported to be more sensitive than the D. magna test for assessing sediment toxicity (Giesy and Hoke 1989). However, because bacteria and protozoans did not exhibit a clear, interpretable response in elutriate tests, their use in sediment testing was discouraged (Shuba et al. 1977). Results of the present study indicate that the daphnid and Microtox^R elutriate tests are useful tools for assessing sediment toxicity.

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Table 1. Sample numbers and sample station locations for sediments collected from three Great Lakes Areas of Concern (AOC) and tested at the National Fisheries Contaminant Research Center (NFCRC).

AOC and sample number[1]	Water depth (m)	Core depth (m)	Location of sampling station Latitude	Longitude
Indiana Harbor, Indiana				
IH-01-03	ND[2]	NA[3]	41° 40.42′	87° 26.35′
IH-01-04	ND	NA	41° 40.07′	87° 26.17′
IH-01-05	ND	NA	41° 39.65′	87° 27.09′
IH-01-06	ND	NA	41° 39.30′	87° 27.58′
IH-01-07	ND	NA	41° 38.75′	87° 28.34′
IH-01-08	ND	NA	41° 38.81′	87° 28.84′
IH-01-10	ND	NA	41° 38.38′	87° 28.28′
Buffalo River, New York				
BR-01-01	5.5	NA	42° 51.42′	78° 52.04′
BR-01-02	4.6	NA	42° 52.18′	78° 52.28′
BR-01-03	6.1	NA	42° 52.66′	78° 53.06′
BR-01-04	ND	NA	42° 51.97′	78° 52.14′
BR-01-05	6.7	NA	42° 51.71′	78° 52.04′
BR-01-06	6.1	NA	42° 51.79′	78° 51.25′
BR-01-07	6.1	NA	42° 51.63′	78° 50.93′
BR-01-08	ND	NA	42° 51.69′	78° 50.73′
BR-01-09	0.9	NA	42° 51.68′	78° 50.02′
BR-01-10	ND	NA	42° 51.94′	78° 49.05′
Saginaw River, Michigan (first survey)				
SR-01-02	ND	NA	43° 37.42′	83° 50.53′
SR-01-03	0.9	NA	43° 37.25′	83° 50.58′
SR-01-04	ND	NA	43° 36.84′	83° 51.40′
SR-01-06	0.6	NA	43° 36.74′	83° 52.16′
SR-01-07	ND	NA	43° 36.46′	83° 53.17′
SR-01-09	2.7	NA	43° 33.76′	83° 54.57′
SR-01-10	ND	NA	43° 32.66′	83° 53.05′

Table 1 continued

AOC and sample number[1]	Water depth (m)	Core depth (m)	Location of sampling station Latitude	Longitude
Saginaw River, Michigan (third survey)				
SR-03-01	6.4	NA	43° 45.30′	83° 47.48′
SR-03-02	2.0	NA	43° 37.42′	83° 50.53′
SR-03-05	1.8	NA	43° 36.72′	83° 51.67′
SR-03-06	0.7	NA	43° 36.74′	83° 52.16′
SR-03-08	0.9	NA	43° 35.33′	83° 53.88′
SR-03-16	6.7	NA	43° 36.70′	83° 51.80′
SR-03-24	0.8	NA	43° 34.55′	83° 54.16′
SR-03-02-X2	2.0	0.2-0.5	43° 37.42′	83° 50.53′
SR-03-02-X3	2.0	0.5-0.9	43° 37.42′	83° 50.53′
SR-03-05-01	1.8	0.0-0.6	43° 36.72′	83° 51.67′
SR-03-05-02	1.8	0.6-1.2	43° 36.72′	83° 51.67′
SR-03-06-01	0.7	0.0-0.6	43° 36.74′	83° 52.16′
SR-03-06-02	0.7	0.6-1.2	43° 36.74′	83° 52.16′
SR-03-06-X2	0.7	0.1-0.3	43° 36.74′	83° 52.16′
SR-03-06-X3	0.7	0.3-0.8	43° 36.74′	83° 52.16′

[1] The Large Lakes Research Station Number includes the site code, survey number, transect number, and the sample fraction number (core samples only).

[2] ND = not Determined

[3] NA = Not applicable

Table 2. Dissolved Oxygen (DO), pH, and survival of *Daphnia magna* during a 48-h reference toxicant test with Instant Ocean[R] salinity. The 48-h EC50 was 25.3 with 95% confidence limits of 21.6 - 13.0 0/00 salinity for the survival data shown.

Instant ocean[R] salinity (0/00)	DO (mg/L)		pH		Survival (%)	
	0-h	48-h	0-h	48-h	24-h	48-h
Control (well water)	7.9	8.2	8.4	8.3	100	100
33.0	7.1	8.0	8.0	8.1	0.0	
19.8	--	--	--	--	0.0	
11.8	--	--	--	--	0.0	
7.1	7.5	8.1	8.2	8.2	100.0	80.0
4.2	--	--	--	--	100.0	100.0
2.5	--	--	--	--	100.0	100.0
1.5	7.9	8.0	8.3	8.3	100.0	100.0

Table 3. Measured elutriate water qualtiy for each area of concern (AOC) and the mean with standard error (in parentheses across stations for each AOC.

AOC and sample number	pH	Alkalinity, (mg/L as $CaCO_3$)	Hardness, (mg/L as $CaCO_3$)	D.O., (mg/L)	Conductivity, (μmhos/cm)	Unionized ammonia, (mg/L)	Chloride, (mg/L)	Turbidity, (NTU)
Indiana Harbor								
Control	6.98	44.00	118.00	7.90	259.46	--	10.58	53.50
IH-01-03	7.64	120.00	155.00	3.50	335.14	0.12	26.21	143.00
IH-01-04	7.78	160.00	185.00	4.00	432.43	0.09	10.58	101.00
IH-01-05	7.89	112.00	150.00	4.00	362.16	0.24	8.25	96.40
IH-01-06	7.26	160.00	168.00	3.6	486.49	0.27	24.16	78.50
IH-01-07	7.51	146.00	115.00	3.30	383.78	0.37	10.58	95.00
IH-01-08	7.16	160.00	160.00	1.80	443.24	0.09	11.88	198.00
IH-01-10	7.42	400.00	183.00	3.20	951.35	0.59	18.88	139.80
Means	7.5	163	154	3.91	456.76	0.25	15.14	113.15
	(0.11)	(36.62)	(9.32)	(0.62)	(74.91)	(0.07)	(2.46)	(15.99)
Buffalo River								
BR-01-01	9.17	108.00	160.00	4.40	464.86	1.37	43.80	72.20
BR-01-02	7.98	80.00	160.00	8.20	400.00	0.03	33.10	14.302
BR-01-03	7.08	82.00	150.00	4.90	347.03	0.02	14.30	112.00
BR-01-04	7.01	116.00	160.00	3.40	367.57	0.02	29.20	115.00
BR-01-05	7.38	102.00	160.00	4.00	335.14	0.02	15.40	91.00
BR-01-06	7.24	112.00	150.00	3.20	367.57	0.02	19.70	156.00
BR-01-07	7.20	116.00	150.00	3.00	378.38	0.01	14.90	121.60
BR-01-08	7.19	146.00	180.00	3.00	410.81	0.02	18.20	154.00
BR-01-09	7.05	134.00	160.00	3.00	421.62	0.02	15.60	160.00
BR-01-10	7.21	106.00	160.00	3.20	378.38	0.01	15.40	39.80
Means	7.45	110.20	159.00	4.03	387.14	0.15	21.96	103..59
	(0.21)	(6.43)	(2.77)	(0.51)	(12.10)	(0.14)	(3.18)	(15.70)
Saginaw River (First Survey)								
Control	7.02	44.00	120.00	9.40	237.84	0.01	8.90	29.00
SR-01-02	7.43	168.00	208.00	3.10	507.03	0.05	34.60	57.00
SR-01-03	7.48	210.00	256.00	2.90	498.38	0.07	20.20	63.00
SR-01-04	7.57	154.00	200.00	3.20	432.43	0.08	18.90	69.00
SR-01-06	7.55	118.00	120.00	3.80	356.76	1.75	16.70	64.20

Table 3 continued..

AOC and sample number	pH	Alkalinity, (mg/L as $CaCO_3$)	Hardness, (mg/L as $CaCO_3$)	D.O., (mg/L)	Conductivity, (µmhos/cm)	Unionized ammonia, (mg/L)	Chloride, (mg/L)	Turbidity, (NTU)
Saginaw River (first survey)								
SR-01-07	7.52	164.00	196.00	3.30	430.27	0.09	20.40	66.90
SR-01-09	7.54	146.00	192.00	3.10	410.81	0.08	21.30	36.00
SR-01-10	7.71	106.00	176.00	4.80	340.54	0.04	17.30	27.70
Mean	7.48	138.75	183.50	4.20	401.76	0.27	19.79	51.60
	(0.07)	(17.59)	(16.08)	(0.77)	(31.31)	(0.21)	(2.53)	(6.24)
Saginaw River (third survey)								
Grab samples								
SR-03-01	6.91	104.00	150.00	3.40	335.14	0.01	9.50	36.20
SR-03-02	6.65	128.00	180.00	3.50	400.00	0.01	15.60	17.60
SR-03-05	6.96	110.00	170.00	4.00	378.38	0.01	15.60	27.50
SR-03-06	7.09	92.00	160.00	7.10	345.95	0.01	14.50	14.20
SR-03-08	7.14	126.00	170.00	3.20	400.00	0.02	14.10	40.90
SR-03-16	7.16	130.00	180.00	3.00	400.00	0.03	15.90	66.20
SR-03-24	7.36	162.00	190.00	2.50	486.49	0.09	27.50	75.50
Core samples								
SR-03-02-X2	7.52	144.00	200.00	4.30	513.51	0.20	17.50	100.00
SR-03-02-X3	7.74	136.00	190.00	5.70	486.49	0.25	18.10	38.30
SR-03-05-01	7.74	132.00	172.00	4.20	410.81	0.16	16.60	72.50
SR-03-05-02	7.47	168.00	192.00	4.50	518.92	0.16	22.10	75.00
SR-03-06-01	7.81	140.00	160.00	4.40	410.81	0.28	19.60	75.00
SR-03-06-02	8.00	116.00	160.00	4.30	421.62	0.32	20.50	23.00
SR-03-06-X2	7.49	148.00	192.00	2.70	513.51	0.17	24.60	159.00
SR-03-06-X3	7.81	108.00	172.00	3.70	454.05	0.21	19.20	25.00
Mean	7.39	129.60	200	4.03	431.71	0.13	18.06	56.39
	(0.10)	(5.50)	(3.82)	(0.30)	(15.58)	(0.03)	(1.15)	(10.01)

Table 4. Mean and standard error of the mean (in parentheses) water qualtiy during 48-hour acute toxicity tests with Daphnia magna exposed to elutriates from each Area of Concern (AOC).

AOC and sample number	pH	D.O., (mg/L)	Conductivity, (µmhos/cm)
Indiana Harbor			
Control	8.18 (0.05)	8.45 (0.03)	276.75 (13.25)
IH-01-03	8.26 (0.01)	8.20 (0.19)	330.81 (13.26)
IH-01-04	8.31 (0.06)	8.35 (0.09)	369.19 (29.52)
IH-01-05	8.31 (0.01)	8.13 (0.19)	332.16 (14.56)
IH-01-06	8.33 (0.05)	8.18 (0.13)	374.87 (29.40)
IH-01-07	8.11 (0.08)	8.05 (0.41)	363.52 (18.31)
IH-01-08	8.30 (0.02)	8.50 (0.07)	391.08 (23.71)
IH-01-10	8.26 (0.03)	8.45 (0.17)	567.57 (113.96)
Buffalo River			
BR-01-01	8.64 (0.25)	9.33 (0.12)	356.76 (36.66)
BR-01-02	8.39 (0.0)	9.85 (0.25)	313.51 (34.47)
BR-01-03	8.24 (0.17)	9.90 (0.37)	329.73 (41.05)
BR-01-04	8.31 (0.12)	9.70 (0.24)	352.70 (31.78)
BR-01-05	8.32 (0.13)	9.70 (0.17)	332.43 (31.94)
BR-01-06	8.14 (0.14)	9.10 (0.11)	353.01 (23.82)
BR-01-07	8.23 (0.12)	9.63 (0.22)	324.32 (5.58)
BR-01-08	8.29 (0.10)	9.60 (0.23)	338.74 (17.30)
BR-01-09	8.17 (0.16)	9.43 (0.17)	334.23 (16.61)
BR-01-10	8.25 (0.13)	9.50 (0.19)	320.00 (7.23)
Saginaw River (first survey)			
Control	7.98 (0.25)	9.05 (0.40)	294.87 (19.02)
SR-01-02	8.27 (0.22)	9.08 (0.19)	403.51 (33.23)
SR-01-03	8.36 (0.24)	9.10 (0.28)	481.62 (76.94)
SR-01-04	8.34 (0.19)	9.17 (0.35)	400.27 (45.39)
SR-01-06	7.91 (0.22)	9.05 (0.29)	339.19 (20.39)
SR-01-07	8.24 (0.17)	9.43 (0.49)	356.21 (62.33)
SR-01-09	8.17 (0.23)	9.30 (0.45)	372.16 (29.76)
SR-01-10	8.15 (0.21)	9.48 (0.51)	334.05 (11.09)

Table 4 continued.

AOC and sample number	pH	D.O., (mg/L)	Conductivity, (μmhos/cm)
Saginaw River (third survey)			
Grab samples			
SR-03-01	8.11 (0.09)	8.22 (0.55)	321.30 (9.16)
SR-03-02	8.08 (0.11)	8.22 (0.52)	355.68 (22.31)
SR-03-05	8.08 (0.12)	8.14 (0.57)	352.43 (20.33)
SR-03-06	8.11 (0.11)	8.10 (0.48)	327.57 (13.63)
SR-03-08	8.08 (0.14)	8.24 (0.62)	343.79 (20.41)
SR-03-16	8.06 (0.14)	8.16 (0.61)	352.43 (22.09)
SR-03-24	8.06 (0.15)	8.26 (0.59)	386.60 (35.91)
Core samples			
SR-03-02-X2	8.00 (0.11)	7.95 (1.02)	420.54 (60.35)
SR-03-02-X3	8.14 (0.08)	9.33 (0.87)	377.57 (35.63)
SR-03-05-01	8.11 (0.11)	8.90 (0.91)	340.54 (38.27)
SR-03-05-02	8.08 (0.14)	8.58 (0.72)	409.46 (45.78)
SR-03-06-01	8.01 (0.07)	8.44 (0.86)	341.19 (31.00)
SR-03-06-02	8.16 (0.07)	9.08 (1.04)	380.54 (31.34)
SR-03-06-X2	7.87 (0.16)	7.30 (0.96)	450.00 (57.39)
SR-03-06-X3	8.11 (0.07)	8.83 (0.99)	400.54 (28.06)

Table 5. Concentrations of metals in elutriates prepared from Indiana Harbor sediment samples.

	Metal concentration (μg/L)								
Sample number	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
FLOR-01-01	<0.03	<1.8	0.05	1.1	2.6	0.0011	2.5	12	6.9
IH-01-03	<0.03	15	3.3	61	65	0.0383	23	489	517
IH-01-04	<0.03	5.5	1	37	28	0.0233	8.8	293.9	183
IH-01-05	0.21	9.8	2.9	73	42	0.0345	18	391.9	535
IH-01-06	0.26	10.2	2.1	86	36	0.0772	15	343.9	348
IH-01-07	1.3	152	69	4700	1329	0.6670	650	11756	10870
IH-01-08	<0.03	24.8	5.8	196	70	0.1203	46	2449	814
IH-01-10	0.45	13.0	2.6	73	23	0.0046	8.8	637	248

TABLE-2.5

Table 6. Concentration of metals in Indiana Harbor sediment porewater samples. The ranges for replicates are shown in parentheses.

	Metal concentration (µg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
IH-01-03	<0.05	2.3	1.1	13.2	21.4	0.02	10.4	96.8	60.8
IH-01-04	<0.05	3.7 (2.9-4.4)	1.0	28.6 (27.3-29.8)	21.3 (19.1-23.4)	0.03 (0.02-.03)	7.5 (5.8-9.2)	92.8 (89.5-96.0)	50.3 (49.6-50.9)
IH-01-06	<0.05	<1.0	0.19	2.46	5.3	0.004	5.9	5.3	6.3
IH-01-07	<0.05	3.2	14.3	350.0	126.3	0.06	171.4	1284.0	1081.0

Table 7. Acute toxicity of Indiana Harbor, Buffalo River, and Saginaw River elutriates to *Daphnia magna* and *Photobacteria phosphorum* (Microtox[R]). The 48-h EC50's for daphnids and 5 and 15 min EC50's for MicrotoxR (95% confidence intervals in parentheses) are percent dilutions of full-strength elutriate samples. Survival of daphnids in dilution water controls ranged from 80 to 100%.

Sample number	EC50 and 95% confidence interval		
	Daphnia magna (48 h)	Microtox[R] (5 min)	Microtox[R] (15 min)
Indiana Harbor			
Control	>100	>100	>100
IH-01-03	1.0 (0.008 - 9.6)	33.6 (29.0 - 39.0)	20.3 (18.7 - 21.9)
IH-01-04	70.7 (25 - 100)	58.7 (27.6 - 124.8)	31.5 (27.1 -36.6)
IH-01-05	25.0 (18 - 100)	48.4 (38.2 - 61.2)	27.3 (25.4 - 29.3)
IH-01-06	>100 (ND[a])	9.0 (8.3 - 9.8)	4.3 (3.2 - 5.8)
IH-01-07	0.7 (0.001 - 3.1)	37.2 (27.3 - 50.8)	34.4 (23.6 - 50.2)
IH-01-08	5.4 (2.5 - 11.0)	12.1 (11.5 - 12.7)	6.0 (5.4 - 6.7)
IH-01-10	39.4 (30.2 - 53.0)	12.9 (12.2 - 13.7)	6.8 (5.8 - 7.9)
Buffalo River			
BR-01-01	>100 (ND)	33.4 (23.6 - 47.4)	40.5 (25.4 - 64.4)
BR-01-02	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
BR-01-03	76.2 (50 - 100)	40.8 (38.5 - 43.3)	44.2 (32.8 - 59.5)
BR-01-04	>100 (ND)	>100 (ND)[b]	>45 (ND)
BR-01-05	>100 (ND)	63.5 (37.3 - 108.3)	65.8 (52.5 - 82.4)
BR-01-06	0.11 (0.001 - 12.5)	19.3 (17.1 - 21.8)	6.7 (6.0 - 7.5)
BR-01-07	ND	55.5 (41.7 - 73.8)	>45 (ND)
BR-01-08	<100 (ND)	>100 (ND)[b]	>100 (ND)[b]
BR-01-09	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
BR-01-10	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
Saginaw River, first survey			
SR-01-02	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-01-03	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-01-04	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-01-06	>12.5 - 100	14.1 (12.0 - 16.6)	13.0 (9.5 -17.7)
SR-01-07	>12.5 - 100	>100 (ND)[b]	>100 (ND)[b]
SR-01-09	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-01-10	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]

Table 7 continued

Sample number	EC50 and 95% confidence interval		
	Daphnia (48 h)	Microtox[R] (5 min)	Microtox[R] (15 min)
Saginaw River, third survey			
Grab samples			
SR-03-01	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-03-02	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-03-05	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-03-06	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-03-08	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
SR-03-16	>100 (ND)	>100 (ND)[b]	95.0 (37.2 -242.9)[b]
SR-03-24	>100 (ND)	>100 (ND)[b]	>100 (ND)[b]
Core samples			
SR-03-02-X2	ND	43.1 (35.0 - 53.0)[b]	34.0 (26.7 - 43.4)[b]
SR-03-02-X3	ND	ND	96.8 (0.26 - >100)[b]
SR-03-05-01	ND	80.5 (53.1 - 122.1)[b]	77.3 (56.3 - 105.9)[b]
SR-03-05-02	ND	88.1 (55.6 - 139.5)[b]	ND
SR-03-06-01	ND	36.1 (31.1 - 41.8)[b]	53.7 (42.3 - 68.3)[b]
SR-03-06-02	ND	76.5 (44.7 - 131.0)[b]	>100 (ND)[b]
SR-03-06-X2	ND	30.5 (19.8 - 47.0)[b]	31.1 (22.4 - 43.1)[b]
SR-03-06-X3	ND	71.3 (54.3 - 93.5)[b]	95.7 (64.0 - 143.1)[b]

[a] Not determined
[b] 100 % elutriates testesd

Table 8. Concentrations of metals in elutriates prepared from Buffalo River sediment samples.

	Metal concentration (μg/L)									
Sample Number	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn
BR-01-01	<0.03	27	1.5	22	14	0.005	6.6	45	<0.25	122
BR-01-02	<0.03	0.7	<0.15	1.4	3.2	0.004	<5	8.5	<0.25	22
BR-01-03	0.33	15	2.2	44	34	0.01	5.5	115	<0.25	150
BR-01-04	<0.03	8	0.83	10	16	0.004	<5	40	<0.25	60
BR-01-05	0.12	8.4	2.9	24	30	0.009	7.7	156	<0.25	161
BR-01-06	0.04	17	2	31	60	0.10	11	238	<0.25	306
BR-01-07	0.04	12	1.2	10	17	0.007	9.9	62	<0.25	67
BR-01-08	<0.03	12	0.61	2.3	11	0.006	<5	25	<0.25	28
BR-01-09	<0.03	9.8	0.44	1.3	7.4	0.003	<5	23	<0.25	28
BR-01-10	<0.03	2.6	<0.15	0.9	4.8	0.002	<5	13	<0.25	17

Table 9. Concentration of metals in Buffalo River sediment porewater samples. The ranges for replicates are shown in parentheses.

	Metal concentration (µg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
BR-01-01	<0.05	14.5	0.1	2.5	3.3	*0.1 (±0.003)	<4.0	5.7	0.9
BR-01-03	<0.05	1.8 (1.2-2.3)	<0.07	0.7	1.9 (1.3-2.6)	0.009 (.0098-.0096)	<4.0	1.8 (1.6-2)	<0.93
BR-01-07	<0.052	1.7	<0.07	0.3	1.65	0.004	<4.0	0.8	<0.93
BR-01-08	<0.05	<1.0	<0.07	0.2	1.65	0.002	<4.0	<0.7	<0.93
BR-01-09	<0.052	1.7	<0.07	0.27	2.3	0.002	<4.0	<0.7	<0.93

Table 10. Concentrations of metals in elutriates from sediment samples collected during the first Survey of Saginaw River.

	Metal concentration (μg/L)								
Sample number	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SR-01-02	<0.09	5.9	0.17	3.1	7.6	0.0057	<5.0	11.2	28.0
SR-01-03	<0.09	8.6	0.11	3.0	5.6	1.00	<5.0	11.8	28.0
SR-01-04	<0.09	5.6	0.35	4.8	10.4	0.003	<5.0	15.1	50.0
SR-01-06	<0.09	4.6	7.4	253.0	98.0	0.005	124.0	39.2	139.0
SR-01-07	<0.09	3.4	0.24	2.6	7.2	0.002	<5.0	11.2	33.0
SR-01-09	<0.09	5.3	0.11	1.2	3.6	0.002	<5.0	5.0	28.0
SR-01-10	<0.09	2.2	<0.06	1.2	<1.2	0.008	<5.0	3.4	17.0

Table 11. Concentration of metals in porewater from sediments collected during the first survey of Saginaw River.

	Metal concentration (µg/L)								
Sample number	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SR-01-03	<0.05	<1.0	<0.07	0.8	<0.57	0.0034	<4.0	<0.7	<0.93
SR-01-06	<0.052	<1.0	0.84	24.4	19.8	0.006	97.8	36.2	19.0
SR-01-10	<0.052	1.7	<0.066	0.33	2.6	0.002	<4.0	0.81	<0.93

Table 12. Concentrations of metals in elutriates prepared from sediments collected during the third survey of Saginaw River. The ranges for replicates are shown in parentheses.

Sample number	Metal concentration (µg/L)								
	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Grab samples									
SR-03-01	<0.085	1.7	<0.18	2.85	4.5	0.0004	<4.3	9.7	9.4
SR-03-02	<0.085	2.6	<0.18	1.22	3.00	0.0004	4.9	5.5	<7.2
SR-03-05	<0.085	2.6	0.29	3.52	4.51	0.001	<4.3	9.7	31.0
SR-03-06	0.096	2.8 (2.6-2.9)	0.34	2.98	4.88	0.001	5.7 (4.9-7.4)	8.2 (7.6-8.9)	31.0
SR-03-08	<0.085	2.3	0.52	4.34	6.01	0.0009	<4.3	18.6	53.0
SR-03-16	0.12	3.2	0.52	6.37	9.39	0.002	6.1	25.4	127.0
SR-03-24	0.12	5.2	0.43	3.80	10.5	0.001	4.9	32.2	148.0
Core samples									
SR-03-02-X2	1.09	66.2	8.53	334.0	122.0	0.01	16.0	166.0	148.0
SR-03-02-X3	0.19 (.15-.24)	9.0	0.16 (.15-.19)	9.36 (9.2-9.5)	9.56 (9.2-10)	0.002 (.0021-.0024)	<4.3	12.3	9.4
SR-03-05-01	0.54	42.1	4.51	83.0	35.0	0.003	13.5	52.9	75.0
SR-03-05-02	0.29	63.3	1.01	34.0	16.0	0.005	6.1	35.5	39.0
SR-03-06-01	0.73	7.6	6.11	334.0	61.0	0.005	95.8	49.9	16.0
SR-03-06-02	0.07	5.8	0.069	3.1	12.0	0.003	<4.3	11.4	9.4
SR-03-06-X2	1.36	4.1	14.1	597.0	107.0	0.006	209.0	133.0	302.0
SR-03-06-X3	0.13	6.7	0.44	14.6	17.0	0.005	8.6	17.3	24.0

Table 13. Concentration of metals in porewater from sediment samples collected during the third survey of Saginaw River. The ranges for replicates are shown in parentheses.

	Metal concentration (µg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SR-03-01	<0.46	1.42	<0.17	0.71	2.7	0.002	3.15	3.5	1.35
SR-03-02	<0.46	0.84	<0.17	0.88	1.1	0.0031	3.15	3.5	2.44
SR-03-06	<0.46	1.60	<0.17	1.9	3.0	0.003	4.90	3.5	10.7
SR-03-08	<0.46	3.23 (2.3-3.9)	<0.17	2.66 (2.5-2.8)	3.56 (3.0-4.3)	0.0045 (0.003-0.006)	4.90	4.86 (4.1-6.4)	2.88 (2.3-3.3)

Table 14. Acute toxicity of elutriates prepared from stored sediments from Indiana Harbor, Buffalo River, and Saginaw Riv to Photobacterium phosphoreum in Microtox[R] tests. The 5 and 15 min EC50's (95% confidence intervals in parentheses) are percent dilutions of full-strength elutriate samples. The E1 elutriates were prepared from sediment samples that were stored up to 5 d; E2's from samples stored 14-19 d; and E3's from samples stored 17-26 d.

Sample number	Elutriate number	Days of storage	Percent sample dilution tested	Microtox[R] EC50's and 95% confidence intervals 5 min	15 min
Indiana Harbor					
IH-01-03	E1	4	45.0	33.6 (29.0 - 39.0)	20.3 (18.7 - 21.9)
IH-01-03	E2	19	45.0	31.0 (24.5 - 39.1)	14.3 (13.7 - 15.0)
IH-01-03	E3	26	45.0	32.7 (28.0 - 38.4)	18.8 (17.8 - 20.0)
IH-01-04	E1	4	45.0	58.7 (27.6 - 124.8)	31.5 (27.1 - 36.6)
IH-01-04	E2	19	45.0	61.8 (22.8 - 167.9)	34.1 (28.0 - 41.6)
IH-01-04	E3	26	45.0	51.5 (10.4 - 254.7)	33.5 (25.0 _ 45.0)
IH-01-06	E1	5	45.0	9.0 (8.3 - 9.8)	4.3 (3.2 - 5.8)
IH-01-06	E2	19	45.0	10.1 (9.4 -10.7)	5.0 (4.3 - 5.6)
IH-01-06	E3	26	45.0	9.0 (8.7 - 9.2)	4.7 (3.7 - 6.0)
IH-01-07	E1	5	45.0	37.2 (27.3 - 50.8)	34.4 (23.6 - 50.2)
IH-01-07	E2	19	45.0	1.3 (0.7 - 2.5)	0.6 (0.2 - 1.5)
IH-01-07	E3	26	11.2	3.8 (3.5 - 4.3)	3.3 (2.8 - 3.8)
Buffalo River					
BR-01-01	E1	1	45.0	33.4 (23.6 - 47.4)	40.5 (25.4 - 64.4)
BR-01-01	E2	15	45.0	42.0 (32.6 - 54.0)	49.6 (44.0 - 55.8)
BR-01-03	E1	1	45.0	40.8 (38.5 - 43.3)	44.2 (32.8 - 59.5)
BR-01-03	E2	15	100.0	>100 (NC)	>100 (NC)
BR-01-07	E1	2	45.0	55.5 (41.7 - 73.8)	>45 (NC)
BR-01-07	E2	15	100.0	70.7 (48.7 - 102.5)	91.8 (28.3 - 298.0)
BR-01-08	E1	3	100.0	>100 (NC)	>100 (NC)
BR-01-08	E2	15	100.0	>100 (NC)	>100 (NC)
BR-01-09	E1	3	100.0	>100 (ND)	>100 (ND)
BR-01-09	E2	15	100.0	>100 (ND)	>100 (ND)
Saginaw River (first survey)					
SR-01-03	E1	1[1]	100.0	>100 (ND)	>100 (ND)
SR-01-03	E2	14[2]	100.0	>100 (ND)	>100 (ND)
SR-01-03	E3	22[3]	100.0	>100 (ND)	>100 (ND)
SR-01-06	E1	3	45.0	14.1 (12.0 - 16.6)	13.0 (9.5 -17.7)

Sample number	Elutriate number	Days of storage[1]	Initial test concentrations[4]	Microtox[R] EC50's and 95% confidence intervals 5 min	15 min
SR-01-06	E3	22	45.0	20.7 (20.0 - 21.4)	12.0 (6.0 - 24.0)
SR-01-10	E1	2	100.0	>100 (ND)	>100 (ND)
SR-01-10	E2	14	100.0	100 (ND)	>100 (ND)
SR-01-10	E3	22	100.0	>100 (ND)	>100 (ND)
Saginaw River, (third survey)					
SR-03-01	E1	1	100.0	>100 (NC)	>100 (NC)
SR-03-03	E2	14	100.0	92.2 (61. - 14.)	>100 (NC)
SR-03-03	E3	17	100.0	>100 (ND)	>100 (NC)
SR-03-02	E1	1	100.0	>100 (ND)	>100 (ND)
SR-03-02	E2	14	100.0	>100 (ND)	>100 (ND)
SR-03-02	E3	17	100.0	>100 (ND)	>100 (ND)
SR-03-05	E1	1	100.0	>100 (ND)	>100 (ND)
SR-03-05	E2	14	100.0	>100 (ND)	>100 (ND)
SR-03-05	E3	17	100.0	>100 (ND)	>100 (ND)
SR-03-06	E1	2	100.0	>100 (ND)	>100 (ND)
SR-03-06	E2	14	100.0	>100 (ND)	>100 (ND)
SR-03-06	E3	17	100.0	>100 (ND)	>100 (ND)
SR-03-08	E1	2	100.0	>100 (ND)	>100 (ND)
SR-03-08	E2	14	100.0	>100 (ND)	>100 (ND)
SR-03-08	E3	21	100.0	>100 (ND)	>100 (ND)
SR-03-16	E1	2	100.0	>100 (ND)	95.0 (37.2 - 242.9)
SR-03-16	E2	14	100.0	>100 (ND)	92.3 (63.1 - 135.1)
SR-03-16	E3	21	100.0	>100 (ND)	>100 (ND)
SR-03-24	E1	2	100.0	>100 (ND)	>100 (ND)
SR-03-24	E2	14	100.0	>100 (ND)	>100 (ND)
SR-03-24	E3	21	100.0	>100 (ND)	>100 (ND)

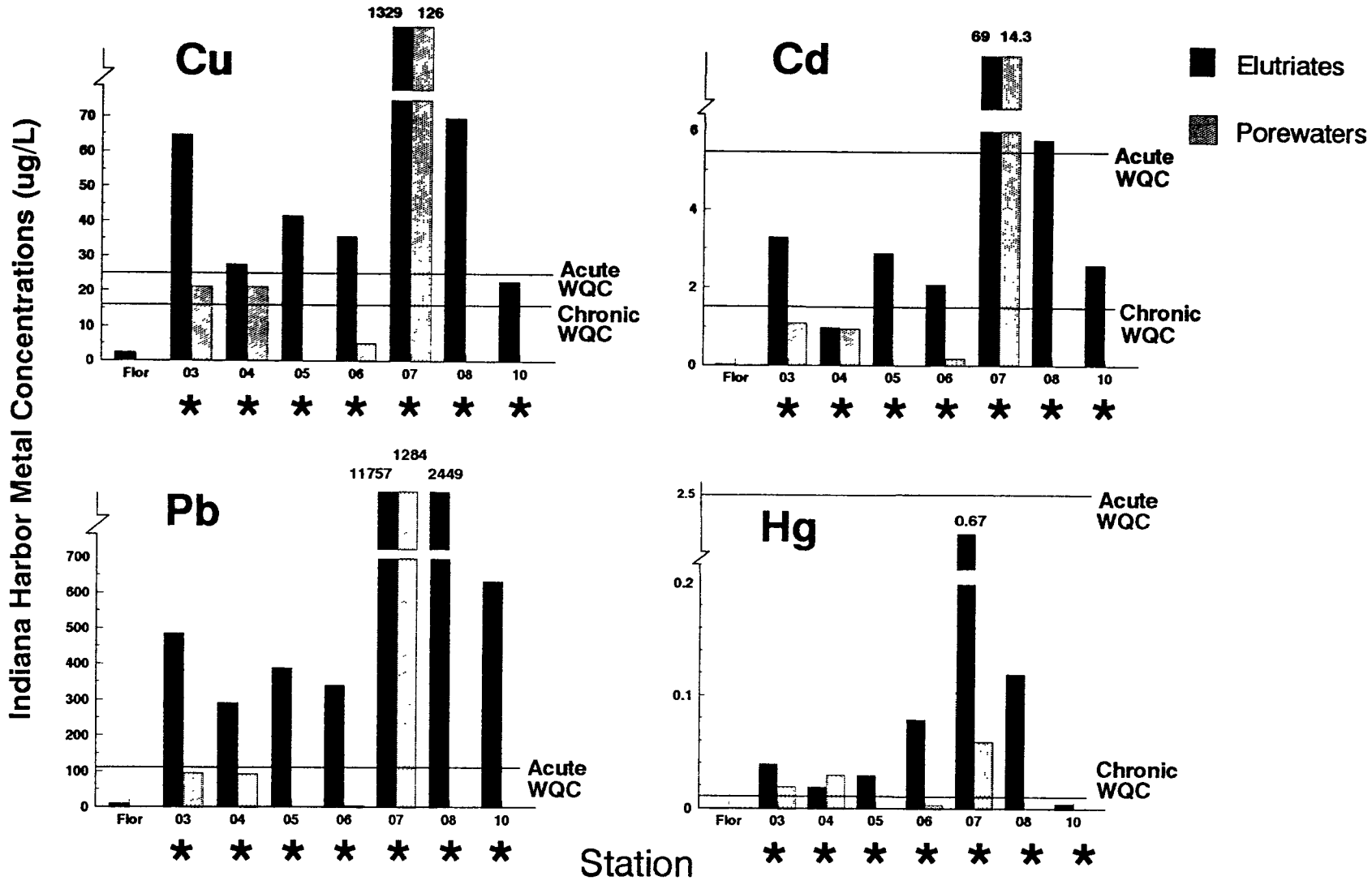

Figure 2.1 Concentrations of select metals (total) in elutriates and pore waters prepared from Indiana Harbor whole sediment samples. Asterisks indicate samples that elicited effects in the daphnid or Microtox[R] test and the horizontal lines show the National Acute or Chronic Water Quality Criteria, adjusted to 140 mg/L hardness for Cu, Cd, and Pb (EPA 1986). Toxicity tests were not conducted with porewater samples.

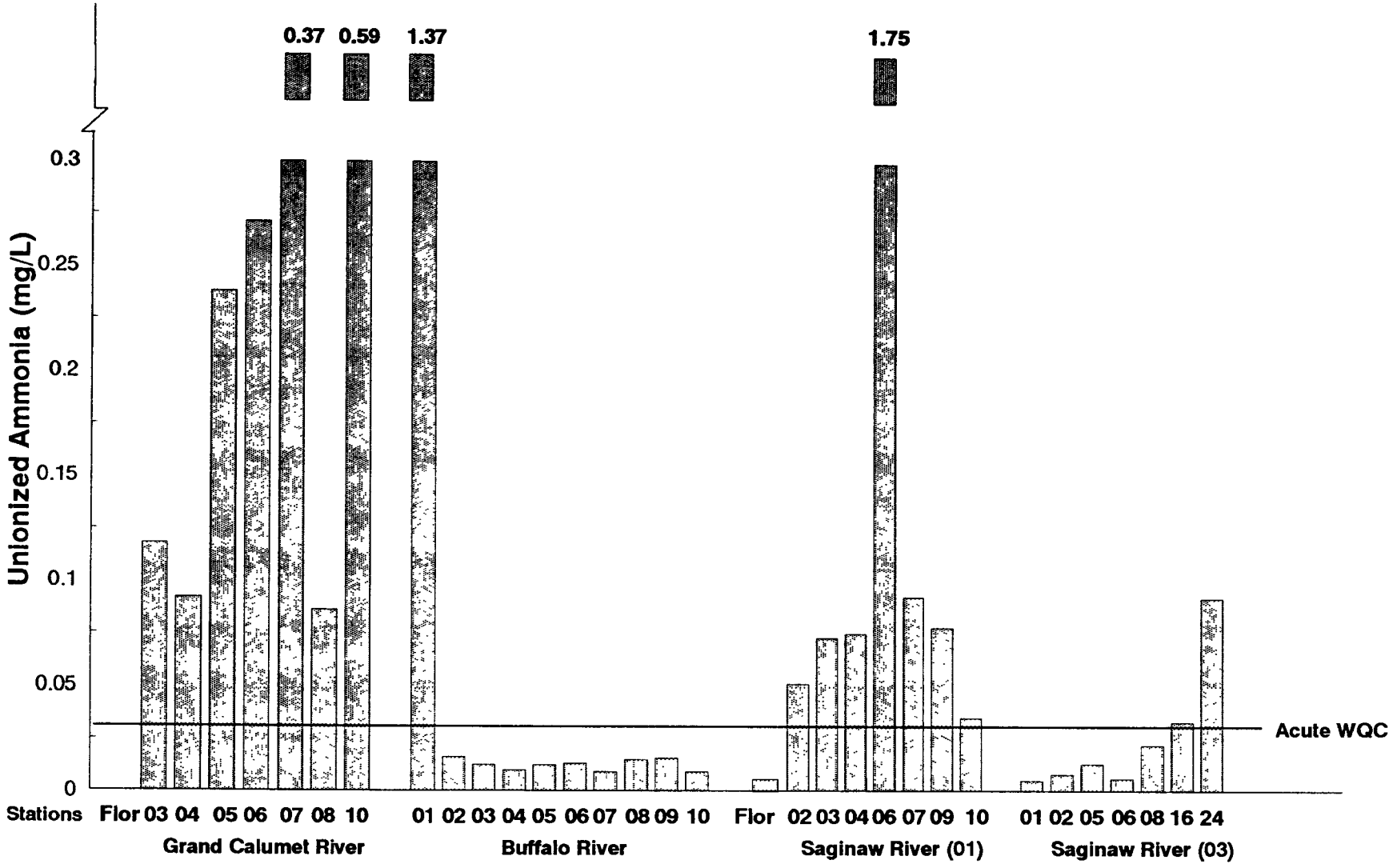

Figure 2.2 Concentrations of unionized ammonia in elutriates prepared from Indiana Harbor, Buffalo River, and Saginaw River whole sediment samples. The horizontal line shows the National Acute Water Quality Criteria for unionized ammonia (EPA 1986).

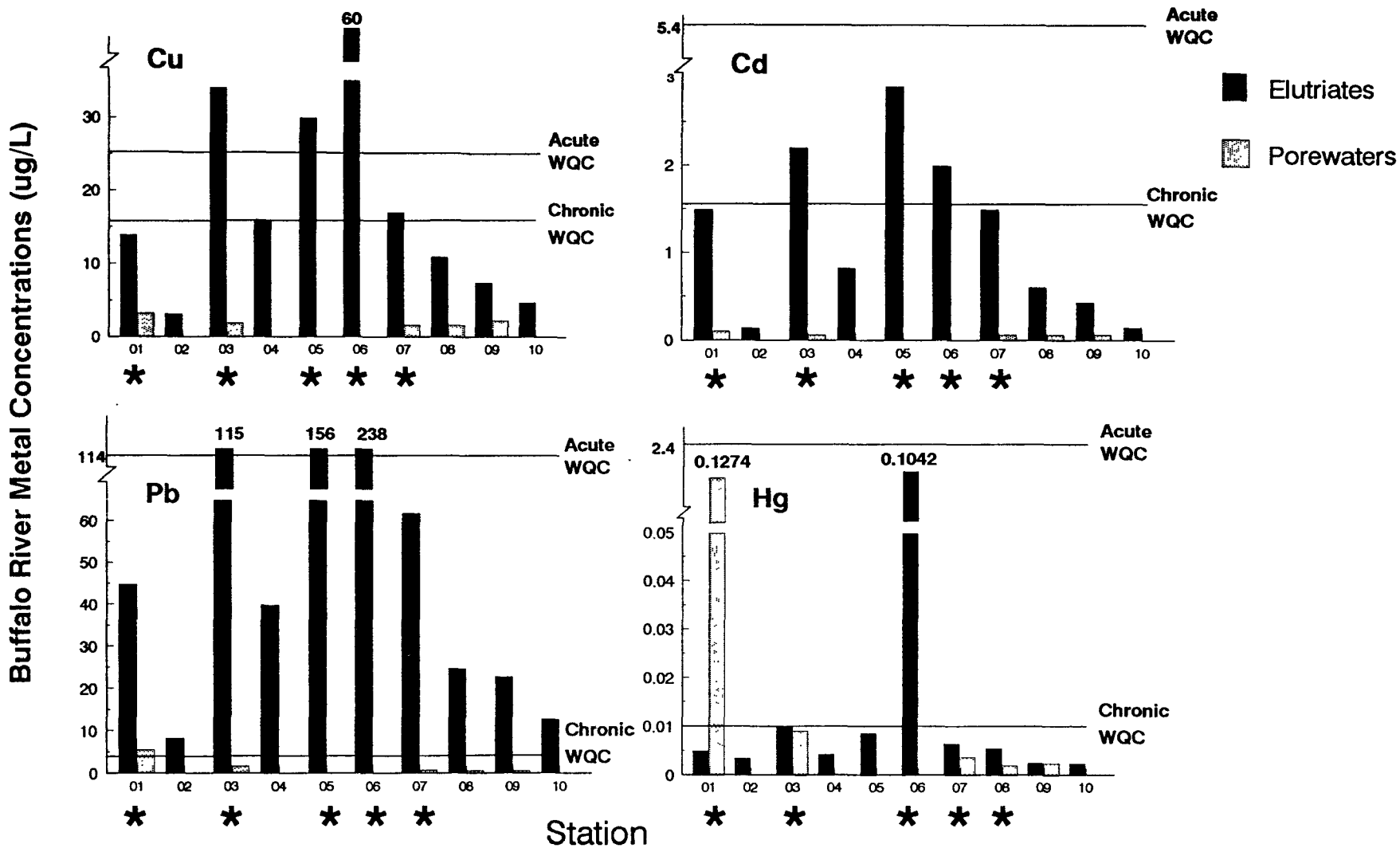

Figure 2.3 Concentrations of select metals (total) in elutriates and pore waters prepared from Buffalo River whole sediment samples. Asterisks indicate samples that elicited effects in the daphnid or Microtox[R] test and the horizontal lines show the National Acute or Chronic Water Quality Criteria, adjusted to 140 mg/L hardness for Cu, Cd, and Pb (EPA 1986). Toxicity tests were not conducted with porewater samples.

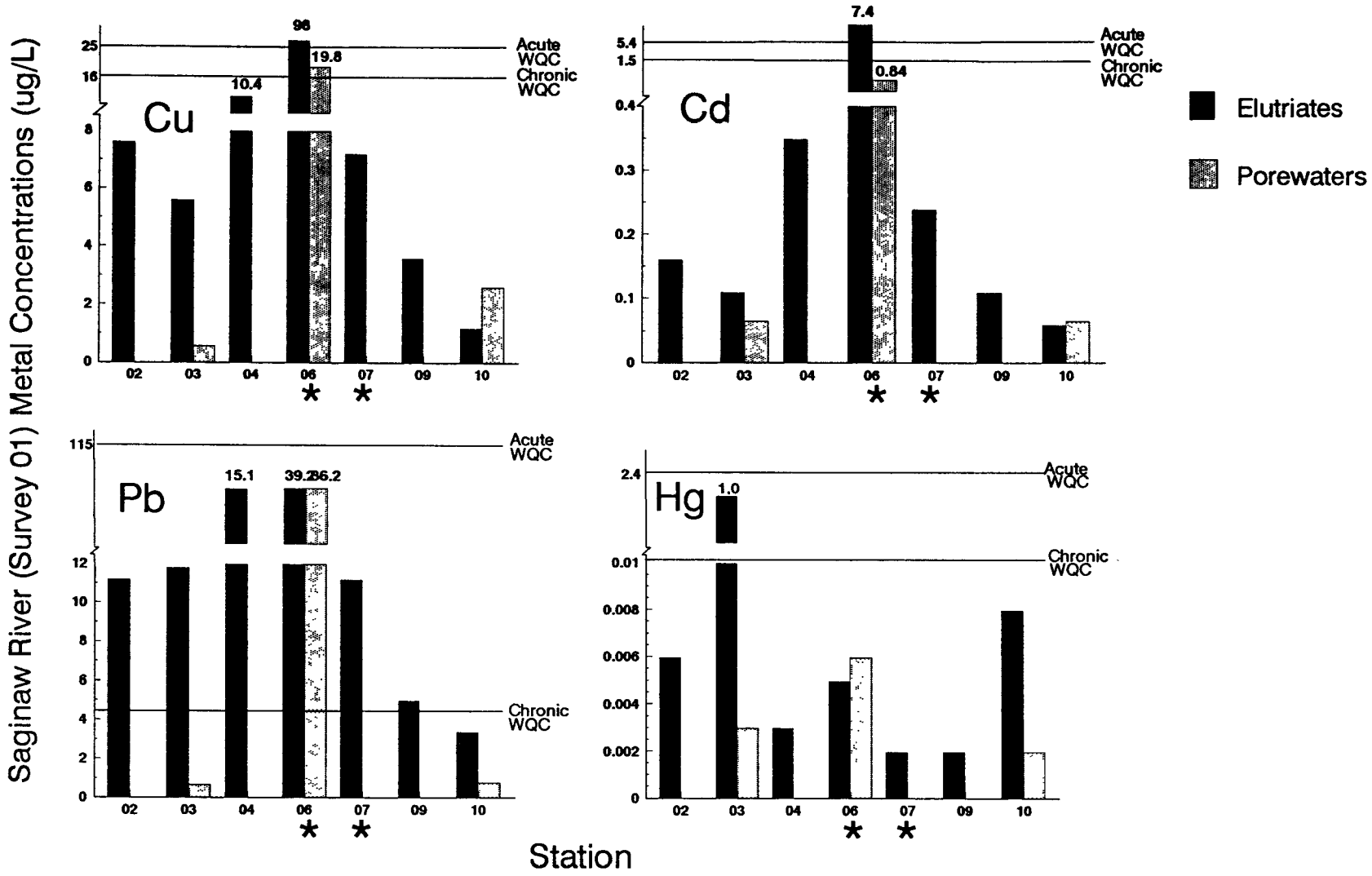

Figure 2.4 Concentrations of select metals (total) in elutriates and pore waters prepared from Saginaw River (first survey) whole sediment samples. Asterisks indicate samples that elicited effects in the daphnid or Microtox^{R} test and the horizontal lines show the National Acute or Chronic Water Quality Criteria, adjusted to 140 mg/L hardness for Cu, Cd, and Pb (EPA 1986). Toxicity tests were not conducted with porewater samples.

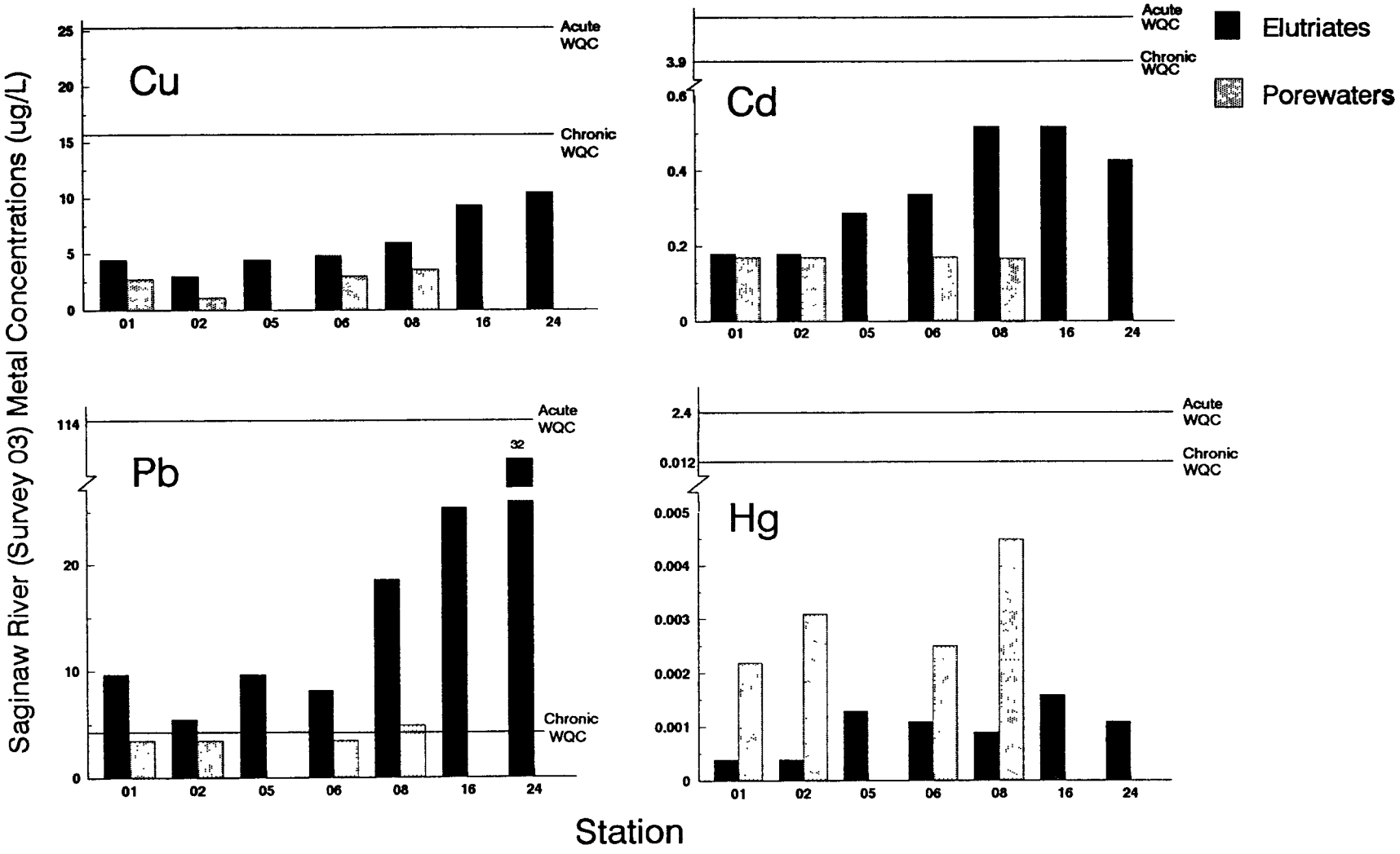

Figure 2.5 Concentrations of select metals (total) in elutriates and pore waters prepared from Saginaw River (third survey) whole sediment samples. Asterisks indicate samples that elicited effects in the daphnid or Microtox[R] test and the horizontal lines show the National Acute or Chronic Water Quality Criteria, adjusted to 140 mg/L hardness for Cu, Cd, and Pb (EPA 1986). Toxicity tests were not conducted with porewater samples.

Table 2.1 Sample numbers and sample station locations for sediments collected from three Great Lakes Areas of Concern (AOC) and tested at the National Fisheries Contaminant Research Center (NFCRC).

AOC and sample number[1]	Water depth (m)	Core depth (m)	Location of sampling station Latitude	Longitude
Indiana Harbor, IN				
IH-01-03*	ND[2]	NA[3]	41° 40.42′	87° 26.35′
IH-01-04*	ND	NA	41° 40.07′	87° 26.17′
IH-01-05	ND	NA	41° 39.65′	87° 27.09′
IH-01-06*	ND	NA	41° 39.30′	87° 27.58′
IH-01-07*	ND	NA	41° 38.75′	87° 28.34′
IH-01-08	ND	NA	41° 38.81′	87° 28.84′
IH-01-10	ND	NA	41° 38.38′	87° 28.28′
Buffalo River, NY				
BR-01-01*	5.5	NA	42° 51.42′	78° 52.04′
BR-01-02	4.6	NA	42° 52.18′	78° 52.28′
BR-01-03*	6.1	NA	42° 52.66′	78° 53.06′
BR-01-04	ND	NA	42° 51.97′	78° 52.14′
BR-01-05	6.7	NA	42° 51.71′	78° 52.04′
BR-01-06	6.1	NA	42° 51.79′	78° 51.25′
BR-01-07*	6.1	NA	42° 51.63′	78° 50.93′
BR-01-08*	ND	NA	42° 51.69′	78° 50.73′
BR-01-09*	0.9	NA	42° 51.68′	78° 50.02′
BR-01-10	ND	NA	42° 51.94′	78° 49.05′
Saginaw River, MI (first survey)				
SR-01-02	ND	NA	43° 37.42′	83° 50.53′
SR-01-03*	0.9	NA	43° 37.25′	83° 50.58′
SR-01-04	ND	NA	43° 36.84′	83° 51.40′
SR-01-06*	0.6	NA	43° 36.74′	83° 52.16′
SR-01-07	ND	NA	43° 36.46′	83° 53.17′
SR-01-09	2.7	NA	43° 33.76′	83° 54.57′
SR-01-10*	ND	NA	43° 32.66′	83° 53.05′

Table 2.1 continued

AOC and sample number[1]	Water depth (m)	Core depth (m)	Location of sampling station Latitude	Longitude
Saginaw River, MI (third survey)				
Grab samples				
SR-03-01*	6.4	NA	43° 45.30′	83° 47.48′
SR-03-02*	2.0	NA	43° 37.42′	83° 50.53′
SR-03-05*	1.8	NA	43° 36.72′	83° 51.67′
SR-03-06*	0.7	NA	43° 36.74′	83° 52.16′
SR-03-08*	0.9	NA	43° 35.33′	83° 53.88′
SR-03-16*	6.7	NA	43° 36.70′	83° 51.80′
SR-03-24*	0.8	NA	43° 34.55′	83° 54.16′
Core samples				
SR-03-02-X2	2.0	0.2-0.5	43° 37.42′	83° 50.53′
SR-03-02-X3	2.0	0.5-0.9	43° 37.42′	83° 50.53′
SR-03-05-01	1.8	0.0-0.6	43° 36.72′	83° 51.67′
SR-03-05-02	1.8	0.6-1.2	43° 36.72′	83° 51.67′
SR-03-06-01	0.7	0.0-0.6	43° 36.74′	83° 52.16′
SR-03-06-02	0.7	0.6-1.2	43° 36.74′	83° 52.16′
SR-03-06-X2	0.7	0.1-0.3	43° 36.74′	83° 52.16′
SR-03-06-X3	0.7	0.3-0.8	43° 36.74′	83° 52.16′

[1] The Large Lakes Research Station Number includes the site code, survey number, transect number, and the sample fraction number (core samples only).

[2] ND = Not determined

[3] NA = Not applicable

* Indicate samples tested in whole sediment tests

Table 2.2 Dissolved Oxygen (DO), pH, and survival of *Daphnia magna* during a 48-h reference toxicant test with Instant Ocean[R] salinity. The 48-h EC50 was 25.3 with 95% confidence limits of 21.6 to 13.0 0/00 salinity for the survival data shown.

Instant ocean[R] salinity (0/00)	DO (mg/L)		pH		Survival (%)	
	0-h	48-h	0-h	48-h	24-h	48-h
Control (well water)	7.9	8.2	8.4	8.3	100	100
33.0	7.1	8.0	8.0	8.1	0.0	
19.8	ND	ND	ND	ND	0.0	
11.8	ND	ND	ND	ND	0.0	
7.1	7.5	8.1	8.2	8.2	100.0	80.0
4.2	ND	ND	ND	ND	100.0	100.0
2.5	ND	ND	ND	ND	100.0	100.0
1.5	7.9	8.0	8.3	8.3	100.0	100.0

ND = Not determined

Table 2.3 Measured water qualtiy for elutriate samples prepared from sediments collected from each area of concern (AOC) and the mean with standard error (in parentheses) across stations for each AOC.

AOC and sample	pH	Alkalinity, (mg/L as $CaCO_3$)	Hardness, (mg/L as $CaCO_3$)	D.O., (mg/L)	Conductivity, (μmhos/cm)	Unionized ammonia, (mg/L)	Chloride, (mg/L)	Turbidity, (NTU)
Indiana Harbor								
Control	6.98	44.00	118.00	7.90	259.46	ND	10.58	53.50
IH-01-03	7.64	120.00	155.00	3.50	335.14	0.12	26.21	143.00
IH-01-04	7.78	160.00	185.00	4.00	432.43	0.09	10.58	101.00
IH-01-05	7.89	112.00	150.00	4.00	362.16	0.24	8.25	96.40
IH-01-06	7.26	160.00	168.00	3.6	486.49	0.27	24.16	78.50
IH-01-07	7.51	146.00	115.00	3.30	383.78	0.37	10.58	95.00
IH-01-08	7.16	160.00	160.00	1.80	443.24	0.09	11.88	198.00
IH-01-10	7.42	400.00	183.00	3.20	951.35	0.59	18.88	139.80
Means	7.5	163	154	3.91	456.76	0.25	15.14	113.15
	(0.11)	(36.62)	(9.32)	(0.62)	(74.91)	(0.07)	(2.46)	(15.99)
Buffalo River								
BR-01-01	9.17	108.00	160.00	4.40	464.86	1.37	43.80	72.20
BR-01-02	7.98	80.00	160.00	8.20	400.00	0.03	33.10	14.302
BR-01-03	7.08	82.00	150.00	4.90	347.03	0.02	14.30	112.00
BR-01-04	7.01	116.00	160.00	3.40	367.57	0.02	29.20	115.00
BR-01-05	7.38	102.00	160.00	4.00	335.14	0.02	15.40	91.00
BR-01-06	7.24	112.00	150.00	3.20	367.57	0.02	19.70	156.00
BR-01-07	7.20	116.00	150.00	3.00	378.38	0.01	14.90	121.60
BR-01-08	7.19	146.00	180.00	3.00	410.81	0.02	18.20	154.00
BR-01-09	7.05	134.00	160.00	3.00	421.62	0.02	15.60	160.00
BR-01-10	7.21	106.00	160.00	3.20	378.38	0.01	15.40	39.80
Means	7.45	110.20	159.00	4.03	387.14	0.15	21.96	103..59
	(0.21)	(6.43)	(2.77)	(0.51)	(12.10)	(0.14)	(3.18)	(15.70)
Saginaw River (first survey)								
Control	7.02	44.00	120.00	9.40	237.84	0.01	8.90	29.00
SR-01-02	7.43	168.00	208.00	3.10	507.03	0.05	34.60	57.00
SR-01-03	7.48	210.00	256.00	2.90	498.38	0.07	20.20	63.00
SR-01-04	7.57	154.00	200.00	3.20	432.43	0.08	18.90	69.00
SR-01-06	7.55	118.00	120.00	3.80	356.76	1.75	16.70	64.20

Table 2.3 continued.

AOC and sample	pH	Alkalinity, (mg/L as $CaCO_3$)	Hardness, (mg/L as $CaCO_3$)	D.O., (mg/L)	Conductivity, (µmhos/cm)	Unionized ammonia, (mg/L)	Chloride, (mg/L)	Turbidity, (NTU)
Saginaw River (first survey)								
SR-01-07	7.52	164.00	196.00	3.30	430.27	0.09	20.40	66.90
SR-01-09	7.54	146.00	192.00	3.10	410.81	0.08	21.30	36.00
SR-01-10	7.71	106.00	176.00	4.80	340.54	0.04	17.30	27.70
Mean	7.48	138.75	183.50	4.20	401.76	0.27	19.79	51.60
	(0.07)	(17.59)	(16.08)	(0.77)	(31.31)	(0.21)	(2.53)	(6.24)
Saginaw River (third survey)								
Grab samples								
SR-03-01	6.91	104.00	150.00	3.40	335.14	0.01	9.50	36.20
SR-03-02	6.65	128.00	180.00	3.50	400.00	0.01	15.60	17.60
SR-03-05	6.96	110.00	170.00	4.00	378.38	0.01	15.60	27.50
SR-03-06	7.09	92.00	160.00	7.10	345.95	0.01	14.50	14.20
SR-03-08	7.14	126.00	170.00	3.20	400.00	0.02	14.10	40.90
SR-03-16	7.16	130.00	180.00	3.00	400.00	0.03	15.90	66.20
SR-03-24	7.36	162.00	190.00	2.50	486.49	0.09	27.50	75.50
Core samples								
SR-03-02-X2	7.52	144.00	200.00	4.30	513.51	0.20	17.50	100.00
SR-03-02-X3	7.74	136.00	190.00	5.70	486.49	0.25	18.10	38.30
SR-03-05-01	7.74	132.00	172.00	4.20	410.81	0.16	16.60	72.50
SR-03-05-02	7.47	168.00	192.00	4.50	518.92	0.16	22.10	75.00
SR-03-06-01	7.81	140.00	160.00	4.40	410.81	0.28	19.60	75.00
SR-03-06-02	8.00	116.00	160.00	4.30	421.62	0.32	20.50	23.00
SR-03-06-X2	7.49	148.00	192.00	2.70	513.51	0.17	24.60	159.00
SR-03-06-X3	7.81	108.00	172.00	3.70	454.05	0.21	19.20	25.00
Mean	7.39	129.60	200	4.03	431.71	0.13	18.06	56.39
	(0.10)	(5.50)	(3.82)	(0.30)	(15.58)	(0.03)	(1.15)	(10.01)

ND = Not determined

Table 2.4 Mean and standard error of the mean (in parentheses) water qualtiy during 48-h acute toxicity tests with Daphnia magna exposed to elutriates prepared from sediments collected from each Area of Concern (AOC).

AOC and sample	pH	D.O., (mg/L)	Conductivity, (μmhos/cm)
Indiana Harbor			
Control	8.18 (0.05)	8.45 (0.03)	276.75 (13.25)
IH-01-03	8.26 (0.01)	8.20 (0.19)	330.81 (13.26)
IH-01-04	8.31 (0.06)	8.35 (0.09)	369.19 (29.52)
IH-01-05	8.31 (0.01)	8.13 (0.19)	332.16 (14.56)
IH-01-06	8.33 (0.05)	8.18 (0.13)	374.87 (29.40)
IH-01-07	8.11 (0.08)	8.05 (0.41)	363.52 (18.31)
IH-01-08	8.30 (0.02)	8.50 (0.07)	391.08 (23.71)
IH-01-10	8.26 (0.03)	8.45 (0.17)	567.57 (113.96)
Buffalo River			
BR-01-01	8.64 (0.25)	9.33 (0.12)	356.76 (36.66)
BR-01-02	8.39 (0.0)	9.85 (0.25)	313.51 (34.47)
BR-01-03	8.24 (0.17)	9.90 (0.37)	329.73 (41.05)
BR-01-04	8.31 (0.12)	9.70 (0.24)	352.70 (31.78)
BR-01-05	8.32 (0.13)	9.70 (0.17)	332.43 (31.94)
BR-01-06	8.14 (0.14)	9.10 (0.11)	353.01 (23.82)
BR-01-07	8.23 (0.12)	9.63 (0.22)	324.32 (5.58)
BR-01-08	8.29 (0.10)	9.60 (0.23)	338.74 (17.30)
BR-01-09	8.17 (0.16)	9.43 (0.17)	334.23 (16.61)
BR-01-10	8.25 (0.13)	9.50 (0.19)	320.00 (7.23)
Saginaw River (first survey)			
Control	7.98 (0.25)	9.05 (0.40)	294.87 (19.02)
SR-01-02	8.27 (0.22)	9.08 (0.19)	403.51 (33.23)
SR-01-03	8.36 (0.24)	9.10 (0.28)	481.62 (76.94)
SR-01-04	8.34 (0.19)	9.17 (0.35)	400.27 (45.39)
SR-01-06	7.91 (0.22)	9.05 (0.29)	339.19 (20.39)
SR-01-07	8.24 (0.17)	9.43 (0.49)	356.21 (62.33)
SR-01-09	8.17 (0.23)	9.30 (0.45)	372.16 (29.76)
SR-01-10	8.15 (0.21)	9.48 (0.51)	334.05 (11.09)

Table 2.4 continued.

AOC and sample	pH	D.O., (mg/L)	Conductivity, (μmhos/cm)
Saginaw River (third survey)			
Grab samples			
SR-03-01	8.11 (0.09)	8.22 (0.55)	321.30 (9.16)
SR-03-02	8.08 (0.11)	8.22 (0.52)	355.68 (22.31)
SR-03-05	8.08 (0.12)	8.14 (0.57)	352.43 (20.33)
SR-03-06	8.11 (0.11)	8.10 (0.48)	327.57 (13.63)
SR-03-08	8.08 (0.14)	8.24 (0.62)	343.79 (20.41)
SR-03-16	8.06 (0.14)	8.16 (0.61)	352.43 (22.09)
SR-03-24	8.06 (0.15)	8.26 (0.59)	386.60 (35.91)
Core samples			
SR-03-02-X2	8.00 (0.11)	7.95 (1.02)	420.54 (60.35)
SR-03-02-X3	8.14 (0.08)	9.33 (0.87)	377.57 (35.63)
SR-03-05-01	8.11 (0.11)	8.90 (0.91)	340.54 (38.27)
SR-03-05-02	8.08 (0.14)	8.58 (0.72)	409.46 (45.78)
SR-03-06-01	8.01 (0.07)	8.44 (0.86)	341.19 (31.00)
SR-03-06-02	8.16 (0.07)	9.08 (1.04)	380.54 (31.34)
SR-03-06-X2	7.87 (0.16)	7.30 (0.96)	450.00 (57.39)
SR-03-06-X3	8.11 (0.07)	8.83 (0.99)	400.54 (28.06)

Table 2.5. Concentrations of total metals in elutriates prepared from Indiana Harbor sediment samples.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Control	<0.03	<1.8	0.05	1.1	2.6	0.0011	2.5	12	6.9
IH-01-03	<0.03	15	3.3	61	65	0.0383	23	489	517
IH-01-04	<0.03	5.5	1	37	28	0.0233	8.8	293.9	183
IH-01-05	0.21	9.8	2.9	73	42	0.0345	18	391.9	535
IH-01-06	0.26	10.2	2.1	86	36	0.0772	15	343.9	348
IH-01-07	1.3	152	69	4700	1329	0.6670	650	11756	10870
IH-01-08	<0.03	24.8	5.8	196	70	0.1203	46	2449	814
IH-01-10	0.45	13.0	2.6	73	23	0.0046	8.8	637	248

Table 2.6. Concentration of totals metals in Indiana Harbor sediment porewater samples. The ranges for duplicate samples are in parentheses.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
IH-01-03	<0.05	2.3	1.1	13.2	21.4	0.02	10.4	96.8	60.8
IH-01-04	<0.05	(2.9-4.4)	1.0	(27.3-29.8)	(19.1-23.4)	(0.02-0.03)	(5.8-9.2)	(89.5-96.0)	(49.6-50.9)
IH-01-06	<0.05	<1.0	0.19	2.46	5.3	0.004	5.9	5.3	6.3
IH-01-07	<0.05	3.2	14.3	350.0	126.3	0.06	171.4	1284.0	1081.0

Table 2.7 Acute toxicity of Indiana Harbor, Buffalo River, and Saginaw River elutriates to *Daphnia magna* and *Photobacterium phosphoreum* (Microtox^R). The 48-h EC50's for daphnids and 5 and 15 min EC50's for MicrotoxR (95% confidence intervals in parentheses) are percent dilutions of full-strength elutriate samples. Survival of daphnids in dilution water controls ranged from 80 to 100%.

	EC50 and 95% confidence interval		
Sample	*Daphnia magna* (48-h)	Microtox^R (5-min)	Microtox^R (15-min)
Indiana Harbor			
Control	>100	>100	>100
IH-01-03	1.0 (0.008 - 9.6)	33.6 (29.0 - 39.0)	20.3 (18.7 - 21.9)
IH-01-04	70.7 (25 - 100)	58.7 (27.6 - 124.8)	31.5 (27.1 -36.6)
IH-01-05	25.0 (18 - 100)	48.4 (38.2 - 61.2)	27.3 (25.4 - 29.3)
IH-01-06	>100	9.0 (8.3 - 9.8)	4.3 (3.2 - 5.8)
IH-01-07	0.7 (0.001 - 3.1)	37.2 (27.3 - 50.8)	34.4 (23.6 - 50.2)
IH-01-08	5.4 (2.5 - 11.0)	12.1 (11.5 - 12.7)	6.0 (5.4 - 6.7)
IH-01-10	39.4 (30.2 - 53.0)	12.9 (12.2 - 13.7)	6.8 (5.8 - 7.9)
Buffalo River			
BR-01-01	>100	33.4 (23.6 - 47.4)	40.5 (25.4 - 64.4)
BR-01-02	>100	>100[a]	>100[a]
BR-01-03	76.2 (50 - 100)	40.8 (38.5 - 43.3)	44.2 (32.8 - 59.5)
BR-01-04	>100	>100[a]	>100[a]
BR-01-05	>100	63.5 (37.3 - 108.3)	65.8 (52.5 - 82.4)
BR-01-06	0.11 (0.001 - 12.5)	19.3 (17.1 - 21.8)	6.7 (6.0 - 7.5)
BR-01-07	ND	55.5 (41.7 - 73.8)	46 -100
BR-01-08	<100	>100[a]	>100[a]
BR-01-09	>100	>100[a]	>100[a]
BR-01-10	>100	>100[a]	>100[a]
Saginaw River (first survey)			
SR-01-02	>100	>100[a]	>100[a]
SR-01-03	>100	>100[a]	>100[a]
SR-01-04	>100	>100[a]	>100[a]
SR-01-06	>12.5 - 100	14.1 (12.0 - 16.6)	13.0 (9.5 -17.7)
SR-01-07	>12.5 - 100	>100[a]	>100[a]
SR-01-09	>100	>100[a]	>100[a]
SR-01-10	>100	>100[a]	>100[a]

Table 2.7 continued

Sample	EC50 and 95% confidence interval		
	Daphnia (48-h)	Microtox[R] (5-min)	Microtox[R] (15-min)
Saginaw River (third survey)			
Grab samples			
SR-03-01	>100	>100[a]	>100[a]
SR-03-02	>100	>100[a]	>100[a]
SR-03-05	>100	>100[a]	>100[a]
SR-03-06	>100	>100[a]	>100[a]
SR-03-08	>100	>100[a]	>100[a]
SR-03-16	>100	>100[a]	>100[a]
SR-03-24	>100	>100[a]	>100[a]
Core samples			
SR-03-02-X2		43.1 (35.0 - 53.0)[a]	34.0 (26.7 - 43.4)[a]
SR-03-02-X3		>100[a]	>100[a]
SR-03-05-01		>100[a]	>100[a]
SR-03-05 02		88.1 (55.6 - 139.5)[a]	>100[a]
SR-03-06-01		>100[a]	53.7 (42.3 - 68.3)[a]
SR-03-06-02		76.5 (44.7 - 131.0)[a]	>100[a]
SR-03-06-X2		30.5 (19.8 - 47.0)[a]	31.1 (22.4 - 43.1)[a]
SR-03-06-X3		71.3 (54.3 - 93.5)[a]	95.7 (64.0 - 143.1)[a]

ND = Not determined
[a] 100% elutriates testesd

Table 2.8 Concentrations of total metals in elutriates prepared from Buffalo River sediment samples.

	Metal concentration (μg/L)									
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Se	Zn
BR-01-01	<0.03	27	1.5	22	14	0.005	6.6	45	<0.25	122
BR-01-02	<0.03	0.7	<0.15	1.4	3.2	0.004	<5	8.5	<0.25	22
BR-01-03	0.33	15	2.2	44	34	0.01	5.5	115	<0.25	150
BR-01-04	<0.03	8	0.83	10	16	0.004	<5	40	<0.25	60
BR-01-05	0.12	8.4	2.9	24	30	0.009	7.7	156	<0.25	161
BR-01-06	0.04	17	2	31	60	0.10	11	238	<0.25	306
BR-01-07	0.04	12	1.2	10	17	0.007	9.9	62	<0.25	67
BR-01-08	<0.03	12	0.61	2.3	11	0.006	<5	25	<0.25	28
BR-01-09	<0.03	9.8	0.44	1.3	7.4	0.003	<5	23	<0.25	28
BR-01-10	<0.03	2.6	<0.15	0.9	4.8	0.002	<5	13	<0.25	17

Table 2.9 Concentrations of total metals in Buffalo River sediment porewater samples. The ranges for duplicate samples are in parentheses.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
BR-01-01	<0.05	14.5	0.1	2.5	3.3	0.1 (±0.003)	<4.0	5.7	0.9
BR-01-03	<0.05	(1.2-2.3)	<0.07	0.7	(1.3-2.6)	(0.0098-0.0096)	<4.0	(1.6-2.0)	<0.93
BR-01-07	<0.052	1.7	<0.07	0.3	1.65	0.004	<4.0	0.8	<0.93
BR-01-08	<0.05	<1.0	<0.07	0.2	1.65	0.002	<4.0	<0.7	<0.93
BR-01-09	<0.052	1.7	<0.07	0.27	2.3	0.002	<4.0	<0.7	<0.93

Table 2.10. Concentrations of total metals in elutriates from sediment samples collected during the first survey of Saginaw River.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SR-01-02	<0.09	5.9	0.17	3.1	7.6	0.0057	<5.0	11.2	28.0
SR-01-03	<0.09	8.6	0.11	3.0	5.6	1.00	<5.0	11.8	28.0
SR-01-04	<0.09	5.6	0.35	4.8	10.4	0.003	<5.0	15.1	50.0
SR-01-06	<0.09	4.6	7.4	253.0	98.0	0.005	124.0	39.2	139.0
SR-01-07	<0.09	3.4	0.24	2.6	7.2	0.002	<5.0	11.2	33.0
SR-01-09	<0.09	5.3	0.11	1.2	3.6	0.002	<5.0	5.0	28.0
SR-01-10	<0.09	2.2	<0.06	1.2	<1.2	0.008	<5.0	3.4	17.0

Table 2.11. Concentrations of total metals in porewater from sediments collected during the first survey of Saginaw River.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SR-01-03	<0.05	<1.0	<0.07	0.8	<0.57	0.0034	<4.0	<0.7	<0.93
SR-01-06	<0.05	<1.0	0.84	24.4	19.8	0.006	97.8	36.2	19.0
SR-01-10	<0.05	1.7	<0.066	0.33	2.6	0.002	<4.0	0.81	<0.93

Table 2.12 Concentrations of total metals in elutriates prepared from sediments collected during the third survey of Saginaw River. The ranges for duplicates are in parentheses.

Sample	Metal concentration (μg/L)								
	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
Grab samples									
SR-03-01	<0.085	1.7	<0.18	2.85	4.5	0.0004	<4.3	9.7	9.4
SR-03-02	<0.085	2.6	<0.18	1.22	3.00	0.0004	4.9	5.5	<7.2
SR-03-05	<0.085	2.6	0.29	3.52	4.51	0.001	<4.3	9.7	31.0
SR-03-06	0.096	(2.6-2.9)	0.34	2.98	4.88	0.001	(4.9-7.4)	(7.6-8.9)	31.0
SR-03-08	<0.085	2.3	0.52	4.34	6.01	0.0009	<4.3	18.6	53.0
SR-03-16	0.12	3.2	0.52	6.37	9.39	0.002	6.1	25.4	127.0
SR-03-24	0.12	5.2	0.43	3.80	10.5	0.001	4.9	32.2	148.0
Core samples									
SR-03-02-X2	1.09	66.2	8.53	334.0	122.0	0.01	16.0	166.0	148.0
SR-03-02-X3	(0.15-0.24)	9.0	(0.16-(0.19)	(9.2-9.5)	(9.2-10.0)	(0.0021-0.0024)	<4.3	12.3	9.4
SR-03-05-01	0.54	42.1	4.51	83.0	35.0	0.003	13.5	52.9	75.0
SR-03-05-02	0.29	63.3	1.01	34.0	16.0	0.005	6.1	35.5	39.0
SR-03-06-01	0.73	7.6	6.11	334.0	61.0	0.005	95.8	49.9	16.0
SR-03-06-02	0.07	5.8	0.069	3.1	12.0	0.003	<4.3	11.4	9.4
SR-03-06-X2	1.36	4.1	14.1	597.0	107.0	0.006	209.0	133.0	302.0
SR-03-06-X3	0.13	6.7	0.44	14.6	17.0	0.005	8.6	17.3	24.0

Table 2.13 Concentrations of total metals in porewater from sediment samples collected during the third survey of Saginaw River. The ranges for duplicate samples are in parentheses.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
SR-03-01	<0.5	1.4	<0.2	0.7	2.7	0.002	3.15	3.5	1.3
SR-03-02	<0.5	0.8	<0.2	0.9	1.1	0.0031	3.15	3.5	2.4
SR-03-06	<0.5	1.6	<0.2	1.9	3.0	0.003	4.90	3.5	10.7
SR-03-08	<0.5	(2.3-3.9)	<0.2	(2.5-2.8)	(3.0-4.3)	(0.003-0.006)	4.90	(4.1-6.4)	(2.3-3.3)

Table 2.14 Acute toxicity of elutriates prepared from stored sediments from Indiana Harbor, Buffalo River, and Saginaw River to Photobacterium phosphoreum in Microtox[R] tests. The 5- and 15-min EC50's (95% confidence intervals in parentheses) are percent dilutions of full-strength elutriate samples. The E1 elutriates were prepared from whole sediment samples that were stored up to 7 d; E2's from whole sediment samples stored 18 to 21 d; and E3's from whole sediment samples stored 21 to 28 d. "Days Storage" is the number of days between first collection at AOC and day of Microtox[R] test.

Sample	Elutriate number	Days of storage	Percent sample dilution tested	Microtox[R] EC50's and 95% confidence intervals 5-min	15-min
Indiana Harbor					
IH-01-03	E1	6	45.0	33.6 (29.0 - 39.0)	20.3 (18.7 - 21.9)
IH-01-03	E2	21	45.0	31.0 (24.5 - 39.1)	14.3 (13.7 - 15.0)
IH-01-03	E3	28	45.0	32.7 (28.0 - 38.4)	18.8 (17.8 - 20.0)
IH-01-04	E1	6	45.0	58.7 (27.6 - 124.8)	31.5 (27.1 - 36.6)
IH-01-04	E2	21	45.0	61.8 (22.8 - 167.9)	34.1 (28.0 - 41.6)
IH-01-04	E3	28	45.0	51.5 (10.4 - 254.7)	33.5 (25.0 _ 45.0)
IH-01-06	E1	7	45.0	9.0 (8.3 - 9.8)	4.3 (3.2 - 5.8)
IH-01-06	E2	21	45.0	10.1 (9.4 -10.7)	5.0 (4.3 - 5.6)
IH-01-06	E3	28	45.0	9.0 (8.7 - 9.2)	4.7 (3.7 - 6.0)
IH-01-07	E1	7	45.0	37.2 (27.3 - 50.8)	34.4 (23.6 - 50.2)
IH-01-07	E2	21	45.0	1.3 (0.7 - 2.5)	0.6 (0.2 - 1.5)
IH-01-07	E3	28	11.2	3.8 (3.5 - 4.3)	3.3 (2.8 - 3.8)
Buffalo River					
BR-01-01	E1	5	45.0	33.4 (23.6 - 47.4)	40.5 (25.4 - 64.4)
BR-01-01	E2	20	45.0	42.0 (32.6 - 54.0)	49.6 (44.0 - 55.8)
BR-01-03	E1	5	45.0	40.8 (38.5 - 43.3)	44.2 (32.8 - 59.5)
BR-01-03	E2	20	100.0	>100 (NC)	>100 (NC)
BR-01-07	E1	7	45.0	55.5 (41.7 - 73.8)	>45 (NC)
BR-01-07	E2	20	100.0	70.7 (48.7 - 102.5)	91.8 (28.3 - 298.0)

Table 2.14 continued

Sample	Elutriate number	Days of storage	Percent sample dilution tested	Microtox[R] EC50's and 95% confidence intervals 5-min	15-min
Buffalo River					
BR-01-08	E1	8	100.0	>100	>100
BR-01-08	E2	20	100.0	>100	>100
BR-01-09	E1	8	100.0	>100	>100
BR-01-09	E2	20	100.0	>100	>100
Saginaw River (first survey)					
SR-01-03	E1	5	100.0	>100	>100
SR-01-03	E2	18	100.0	>100	>100
SR-01-03	E3	26	100.0	>100	>100
SR-01-06	E1	7	45.0	14.1 (12.0 - 16.6)	13.0 (9.5 -17.7)
SR-01-06	E2	18	45.0	17.8 (16.5 - 19.2)	14.8 (12.9 - 17.0)
SR-01-06	E3	26	45.0	20.7 (20.0 - 21.4)	12.0 (6.0 - 24.0)
SR-01-10	E1	6	100.0	>100	>100
SR-01-10	E2	18	100.0	100	>100
SR-01-10	E3	26	100.0	>100	>100
Saginaw River, (third survey)					
SR-03-01	E1	5	100.0	>100	>100
SR-03-03	E2	18	100.0	92.2 (61. - 14.)	>100
SR-03-03	E3	21	100.0	11.9 (34.6 - 355.1)	>100)
SR-03-02	E1	5	100.0	>100	>100
SR-03-02	E2	18	100.0	>100	>100
SR-03-02	E3	21	100.0	>100	>100
SR-03-05	E1	5	100.0	>100	>100
SR-03-05	E2	18	100.0	>100	>100
SR-03-05	E3	21	100.0	>100	>100
SR-03-06	E1	6	100.0	>100	>100
SR-03-06	E2	18	100.0	>100	>100
SR-03-06	E3	21	100.0	>100	>100

Table 2.14 continued.

Sample	Elutriate number	Days of storage	Percent sample dilution tested	Microtox[R] EC50's and 95% confidence intervals 5-min	15-min
Saginaw River, (third survey)					
SR-03-08	E1	6	100.0	>100	>100
SR-03-08	E2	18	100.0	>100	>100
SR-03-08	E3	25	100.0	>100	>100
SR-03-16	E1	6	100.0	>100	>100
SR-03-16	E2	18	100.0	>100	92.3 (63.1 - 135.1)
SR-03-16	E3	25	100.0	>100	>100
SR-03-24	E1	6	100.0	>100	>100
SR-03-24	E2	18	100.0	>100	>100
SR-03-24	E3	25	100.0	>100	>100

CHAPTER 3: ELUTRIATE TOXICITY TESTS: SELENASTRUM CAPRICORNUTUM

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INTRODUCTION

Algae serve a critical functional role in aquatic ecosystems. They convert solar energy and carbon dioxide to organic matter or algal biomass. Algal biomass serves as food for herbivorous and omnivorous animals, from Daphnia to paddlefish. Thus, algae are a primary base for the transfer of energy to consumer levels of trophic food webs. Algae also serve numerous other critical ecological functions. They cycle chemical elements through the ecosystem by incorporating and transferring dissolved nutrients, vitamins, metals, and other elements to other ecosystem components. If not consumed, algae die and are decomposed by bacteria, thereby transforming, solubilizing and recycling chemicals to be used again as nutrients by algae and other organisms. The flow of energy and materials through the ecosystem is affected by phytoplankton population dynamics, which are in turn influenced by temperature, solar radiation, nutrient concentration and animal grazing. Anthropogenic disturbance, such as the introduction of toxicants, can alter the structure and function of the phytoplankton community (Amblard et al. 1990, Bokn 1990, Havens and Heath 1990, Molander et al. 1990, Rai et al. 1990). Such changes can ultimately affect the entire aquatic ecosystem.

Algae were used in the present study to evaluate the toxicity of the Great Lakes sediments, through testing of elutriates of sediment samples. Elutriates and their use in sediment testing are discussed in Chapter 2. Currently, there is no method for exposing algae directly to whole sediments, an elutriate most closely simulates the most likely exposure conditions for natural algal populations. A single-species laboratory toxicity test was selected that was rapid and had small volume requirements. The species selected for testing was Selenastrum capricornutum, recommended in standard methods (EPA 1985, IJC 1988, Clesceri et al. 1989, ASTM 1990). The method is derived from the U.S. Environmental Protection Agency's standard 96-h algal assay procedure (AAP) (Miller et al. 1978). The AAP was originally designed to measure eutrophication. However, it has been recommended for use in testing the toxicity of complex effluents (Porcella 1983) and has been widely used to test single chemicals (Elnabarawy and Welter 1984, Whitton 1984, Greene et al. 1988). The AAP has a 96-h duration, cell-counts are the test endpoint and flasks are the test vessels; thus labor, time, space and equipment requirements may be prohibitive for screening large numbers of samples. The AAP has been converted into a 24-h photosynthesis test with smaller sample volume requirements (Ross et al. 1988, Sloterdijk et al. 1989). Algal cells are exposed to an elutriate supplemented with nutrients and radioactive inorganic carbon. Toxicity is measured in terms of relative radioactive carbon uptake compared to controls. Our tests are modifications of their 24-h radioactive carbon assimilation (photosynthesis) tests.

The objective of this chapter is to identify AOC samples from the Great Lakes toxic to algae. Toxicity is defined for the algal assay as negatively affecting carbon assimilation of algal test subpopulations. The short generational time and meager volume requirements of a microscopic algae allows testing of subcultures comprised of thousands of individuals and production of one or more generations within a 24-h period. Algal test responses are population responses incorporating effects upon individual cells' metabolism, growth, mortality and reproduction. It is assumed that if elutriate has a toxic effect on assay algal populations, natural algal communities in contact with sediment at the sampling site may also experience a negative effect. Effects may range from slower growth rates to lethality. Either may have profound effects on the ability of a species to compete for resources and could result in changes in algal communities, balance with bacterial competitors, phytoplankton consumers, and creating a ripple of effect throughout the ecosystem.

MATERIALS AND METHODS

METHOD DEVELOPMENT

The original objective of our study was to evaluate the toxicity of elutriate samples prepared from all of the sediment samples listed in table 2.1. Initially, elutriates from all of the sediments except the third survey of Saginaw River samples were tested using a method similar to that described by Ross et al. (1988).

Elutriates were filtered through 0.7 μm Whatman GF-F glass fiber filters to remove indigenous algae. Exposure volume was 11.2 mL, placed within a 16-mL capped borosilicate glass tube. Nutrients were added in 1 mL of medium concentrate to produce the same nutrient concentration as the culture medium for the control treatment (Table 3.1). Algal exposure occurred at a density of 190,000 cells/mL, for 24 h at 21 ± 2° C with 400 to 500 ftc (approximately 40 to 50 $\mu E/m^2$-sec) of light. At 20 h, sodium ^{14}C-bicarbonate was added to produce a concentration of 1 to 1.15 μCi/mL. At 24 h, algal ^{14}carbon assimilation was halted by adding HCl and air bubbling for 5 min to remove unassimilated ^{14}carbon. The amount of radioactive carbon assimilated was measured in a beta liquid scintillation counter.

Results from these exposures indicated the test required modification to determine elutriate toxicity. The high nutrient concentrations likely to occur in the elutriates violated two assumptions of the algal test design: equal concentrations among treatments of (1) non-radioactive inorganic carbon (Kusk and Nyholm 1991) and (2) nutrients limiting growth.

Non-radioactive inorganic carbon concentrations of elutriates (estimated from pH and alkalinity), were about 10 times higher than the concentration in the algal medium. Therefore radioactive inorganic carbon was diluted with non-radioactive inorganic carbon e.g., radioactive carbon assimilation decreased with increased elutriate concentration. The method was revised to include measurement of non-radioactive inorganic carbon in test solutions when radioactive carbon was added, at 20 h. A carbon dioxide ion selective electrode (Fisher model 13-620-506/507) was used to determine total inorganic non-radioactive carbon upon an acidified 20-mL sub-sample from each test replicate. Test volume was increased to 24.4 mL and 25-mL capped borosilicate glass tubes used as test chambers. The 4-mL portion remaining after sub-sampling received radioactive carbon. Algal response was measured as total inorganic carbon assimilated, calculated from measurements of radioactive carbon assimilated and total inorganic non-radioactive carbon available, rather than simply radioactive carbon assimilated.

The second problem became apparent after the variable non-radioactive inorganic carbon concentration problem was resolved. Elutriates generally stimulated algal carbon assimilation. Reducing algal cell density, increasing medium concentration, or adding sodium nitrate, potassium phosphate dibasic or sodium EDTA, singly, or in combination, did not increase algal carbon assimilation in the control. Addition of sodium bicarbonate (60 mg/L) did enhance growth of algae in the control, but elutriates were still stimulatory. The nutrient concentration problem was not completely resolved. Only the presence or absence of toxicity could be determined.

Based on the previous findings, the original method was revised. The revised method will be described fully under the heading Toxicity Testing.

EFFECTS OF SAMPLE STORAGE

Whole sediments were stored for 12 to 16 months at 4° C before we could re-test samples with the revised method. Because toxicity may change with storage (Burton 1991), only a few samples were re-tested.

Storage effects were determined by preparing and testing elutriates from stored whole sediments using the original algal method of initial studies. Storage effects tests using the original algal method were conducted concurrent with toxicity re-testing using the revised method. Although the

toxicity of elutriates using the initial algal method could not be interpreted, any change in the response pattern for a particular treatment over time could indicate a change in toxicity if carbon concentrations were unchanged. If changes in inorganic non-radioactive (^{12}carbon) concentrations occurred during storage, the ratio of ^{14}carbon/^{12}carbon would also change upon testing and affect ^{14}carbon assimilation. Therefore Microtox was also performed as a second method to detect changes in toxicity with storage. The Microtox test was selected for detection of long-term storage effects for the same reasons it was used to evaluate weekly changes in sediment toxicity described in Chapters 2 and 4. Methods for Microtox testing are described in Chapter 2.

Both algal and Microtox methods indicated that storage of samples for approximately a year would not affect the classification of elutriates as toxic or non-toxic with the revised algal method. Toxicity to algae of these elutriates of stored samples, using the revised algal method, is reported in the remainder of this chapter.

SAMPLES TESTED USING THE REVISED METHOD

Elutriates prepared from sediment samples from three Great Lakes Areas Of Concern (AOC) were re-tested: Indiana Harbor, IN (IH-01-07); Buffalo River, NY (BR-01-03 and BR-01-08); Saginaw River and Bay, MI (SR-01-06 and SR-01-10).

In addition to testing sediments from the AOCs, we tested a soil elutriate (Missouri River sediment) from Florrissant, MO for comparison as a control sediment from an uncontaminated site (Ingersoll and Nelson 1990).

Sediment collection, handling, and storage as well as elutriate preparation procedures are described in Chapter 2.

ALGAL CULTURE

Selenastrum capricornutum Printz was cultured before tests under axenic conditions in borosilicate glass flasks, with a 1:5 (v/v) medium:air ratio. The culture medium was AAP medium but with EDTA reduced by two-thirds (Table 3.1). Culture flasks were continuously agitated at 50 to 100 rpm on a rotary shaker table under constant light (300-350 ftc, approximately 30-35 $\mu E/m^2$-sec).

An inoculum was prepared for each study from fresh culture stocks during the exponential growth phase. We quantified stock culture cell density, concentrated cells by centrifugation, rinsed cells with fresh medium, and then diluted the algae in culture medium to an inoculum density of 10.5×10^6 cells/mL.

TOXICITY TESTING

Algal tests were conducted over a 24-h exposure period. Radiolabeled carbon was added after 20 h of exposure. At 24 h (4 h incubation), the test was ended. Photosynthesis was estimated as the amount of total carbon assimilated by S. capricornutum. Nutrients (Table 3.1) were supplied equally to all treatments.

The tests were conducted under sterile conditions. Bacteria, as well as spurious algae, were removed from elutriates samples with 0.45 μm Gelman Metricel™ GN-6 cellulose acetate filters and glass fiber prefilters. All dilution water and glassware were autoclaved. Minimization of bacteria prevented continual addition of an unknown and varying amount of carbon dioxide from bacterial decomposition of organic material.

Five serial dilutions were tested: 1%, 10%, 25%, 50%, and 94% elutriate. Deionized water (<1 megohms/cm) was used as dilution water and as the negative control (0% elutriate) treatment. Exposure chambers were 25-mL capped borosilicate glass tubes. Exposure volume was 24.4 mL.

Six percent of each treatment was composed of medium and algal

additions. Nutrients were added in 1 mL of concentrated medium to all control and treatment tubes before addition of algae. Dilution of the medium concentrate in the control treatment produced the same nutrient concentration, except for sodium bicarbonate, as the culture medium, and at least the culture medium concentration for elutriates with intrinsic nutrients. Sodium bicarbonate concentration was increased so that dilution of the medium concentrate in treatments produced a four-fold increase in carbon from the culture medium. Algal inoculum was added to produce an exposure density of 190,000 cells/mL.

Total exposure time was 24 h at 21 $\pm$ 2° C and 400 to 500 ftc (approximately 40 to 50 $\mu E/m^2$-sec) of light. After 20 h of exposure, total inorganic carbon was determined before treatments were spiked and incubated with ^{14}carbon. Total dissolved carbon dioxide (equivalent to total inorganic carbon) of acidified sub-samples removed before spiking, was measured with a carbon dioxide ion selective electrode (Fisher model 13-620-506/507). After sub-sampling, all treatments were spiked with 1 to 1.15 μCi of sodium ^{14}C-bicarbonate per mL of test volume. Incubation period was 4 h. At the end of the incubation, unmetabolized ^{14}carbon was removed by acidifying treatments with concentrated HCl and bubbling for 5 min with air. Scintillation cocktail (Safety Solve®) was added and radioactive carbon assimilation was measured in a Beckman LS 3801 Beta Liquid Scintillation Counter. Treatment responses were total inorganic carbon assimilation values, calculated using Vollenweider's (1974) equation:

Carbon assimilated = carbon available * ^{14}carbon assimilated / ^{14}carbon available.

To compare test results of different batches of algae (different dates), responses were standardized as "percent of control" = (Treatment response / average control response) * 100%. (A treatment with a response of 100% would indicate no difference from controls.)

Blanks (additional control and 94% dilution treatment tubes which received no algal inoculum) were included to quantify exposure fluorescence due to factors other than S. capricornutum ^{14}carbon assimilation.

WATER QUALITY CHARACTERIZATION

Alkalinity, pH, unionized ammonia, total chloride, turbidity, dissolved oxygen, hardness and conductivity were measured on 0.45μm filtered elutriates and control (dilution) water using the same methods described in Chapter 2. Additionally, inorganic carbon was calculated from pH and alkalinity data (Stumm and Morgan 1981).

PHYSICAL AND CHEMICAL CHARACTERIZATION OF SAMPLES

No chemical analysis was performed on the samples tested with the revised algal method, filtered elutriates prepared from stored sediments. Unfiltered elutriates, prepared shortly after sediment arrival, were analyzed for inorganic contaminants (Chapter 2) and whole sediments were analyzed for organic and inorganic contaminants (Chapter 4).

QUALITY CONTROL AND QUALITY ASSURANCE

Measures of quality control are reported in Table 3.2. The algal toxicity test was performed with a reference toxicant, copper sulfate. Negative (0% elutriate) control assimilation was determined by comparing control assimilation to blank treatments which did not receive S. capricornutum. Control assimilation was considered acceptable if it was greater than blank assimilation: The actual amount of assimilation was not expected to be consistent between tests because it is a function of several factors besides culture health including age of the algal cells, the specific activity of the ^{14}carbon, and the length of time between reading and preparation of scintillation samples. Unassimilated ^{14}carbon removal

efficiency was determined as:

(the average ^{14}carbon spike (dpm) - ^{14}carbon remaining in blanks after bubbling (dpm)) / the average ^{14}carbon spike (dpm).

Removal efficiency, while a function primarily of sample acidification and bubbling, is also influenced by sample chemistry and the presence of bacteria or other microorganisms. The carbon dioxide ion selective electrode was calibrated to dilutions of a commercially prepared sodium bicarbonate standard.

RESULTS

WATER QUALITY CHARACTERIZATION

Water quality results for the 0.45 μm filtered elutriates prepared from stored sediments are presented in Table 3.4. Alkalinity, inorganic carbon, hardness, conductivity, unionized ammonia, total chloride, and turbidity varied most between AOC elutriate samples and control water (deionized water with nutrients added). The control sediment was similar to the control water for unionized ammonia, alkalinity, inorganic carbon and total chloride, but more similar to AOC elutriate samples in terms of hardness, conductivity and turbidity.

EXPOSURES

All of the elutriates of the stored samples were stimulatory i.e., produced greater algal carbon assimilation than the negative control (0% elutriate), at one or more dilution concentrations (Figure 3.1). A positive relationship between carbon assimilation and elutriate concentration was evident for the control sediment and SR-01-10. Inhibition, i.e. mean response less than the mean negative control response, occurred only in the 1% elutriate concentrations of BR-01-03 and SR-01-06, but IH-01-07 and BR-01-08 did exhibit a decrease in stimulation at some of the higher concentrations which may indicate toxicity.

CHEMICAL ANALYSIS

Results of whole sediment chemical analyses for selected organic and inorganic compounds and metal chemistry for elutriates are reported in chapters 4 and 2, respectively. Elutriate metal chemistry from chapter 2 is presented in Table 3.3 for the subset of sites re-tested with the algal assay; no chemical analyses were performed on the filtered elutriates prepared from stored sediments.

Aging of the sediment and filtration of the elutriate propably altered metal concentrations in the elutriates tested with the algal assay from the elutriates described in chapter 2. The chemistry data is best used as an indicator of relative site potential for toxicity. Assuming any contaminant loss to be proportional to concentration regardless of the sample, the elutriate prepared from IH-01-07 was the most contaminated, and the elutriates prepared from the control sediment and SR-01-10 sediment were least contaminated with metals. Organic toxicants were probably also present, but relative concentrations in algal elutriates are difficult to estimate from only whole sediment data.

DISCUSSION

It was possible to classify sediments as toxic or non-toxic with the algal assay, by comparing responses within an elutriate dilution series. A toxic sediment is defined to be a sediment, that if suspended, is likely to have adverse effects on natural algal populations. Effects may be sublethal, such as reduced carbon assimilation rates, or lethal, to individual algal cells, but either may ultimately result in algal population declines and / or changes in algal community structure.

The algal stimulation produced by all the elutriates necessitated the different approach to data interpretation from the planned comparison to a negative control. Sediment elutriates may have contained both growth promoting and inhibiting elements relative to the AAP medium. For a given treatment, the response observed may have been the difference between stimulation and inhibition relative to the AAP medium.

Stimulation also made it impossible to determine effective concentrations (Nyholm and Kallqvist 1989) for elutriates. Thus, toxic sediments could not be ranked or categorized by severity of toxicity as in Chapter 2.

Test materials of a complex chemical nature may produce algal stimulation relative to a negative control of AAP medium. Thomas et al. (1986) reported stimulation from more than half the elutriate samples tested, but none from identified toxicants tested as single chemicals. Stimulation is reported in response to resuspended sediment (Ahlf et al. 1989), industrial wastes (Walsh et al. 1982), textile mill effluents (Walsh et al. 1980) as well as elutriates (Thomas et al. 1986). The phenomenon is not specific to photosynthetic carbon assimilation, but is reported from growth (cell enumeration) toxicity tests (Walsh et al. 1980, Walsh et al. 1982, Thomas et al. 1986) and a test measuring chlorophyll a (Ahlf et al. 1989). Also, algal stimulation does not appear to be a initial compensatory response to elutriate toxicity in our test, for the following reasons: (1) the control sediment was stimulatory and (2) the magnitude (about 200 to 300% of control) of stimulation from the samples.

Elutriates and other complex mixtures, through perhaps either greater concentrations and variety of nutrients or in creating more growth-conducive chemical environments with pH, pH buffering, redox potential, etc., encourage greater algal growth than the AAP medium alone can support. Water quality measurements (Table 3.4) illustrate the disparities between the AAP medium and the elutriates, especially between alkalinity, inorganic carbon concentration, hardness, and conductivity. The elutriates have the greater ion concentrations, and perhaps nutritive concentrations as well.

In the absence of toxicants, growth increases with elutriate concentration. Increasing elutriate concentration of the control sediment increased stimulation (Figure 3.1). A resuspended sediment produced chlorophyll a stimulation of Ankistrodesmus bibraianus in an assay using a chamber device technique, while an elutriate, a more dilute preparation of the same sediment, was not stimulatory in a growth assay (Ahlf et al. 1989).

If potential growth was equally limited in all treatments, i.e., the normal test conditions for single chemical testing, observed when we tested copper sulfate, treatment responses would be less than or equal to the negative control. The null response curve for the sample dilution series would be a slopeless line equivalent to the negative control response ("no stimulation, no toxicity", Figure 3.2). Toxicity would be indicated by decreased carbon assimilation relative to the negative control as in "no stimulation, constant toxicity" and "no stimulation, increasing toxicity", Figure 3.2. Results could be expressed as the effective concentration which reduced growth (assimilation) potential by 50% (i.e., EC50).

Great Lakes samples were labeled toxic or non-toxic using the following procedure: Results were interpreted without relation to negative controls, because each treatment has a different unknown potential for growth in the absence of toxicants (dependent upon the concentration of elutriate and its concentration of nutritive elements). The AAP medium was intended to function

as a negative control, but instead served only as a means of standardizing algal responses for different test dates.

Without a negative control, the null response for a sample dilution series was an unknown. Sample toxicity can be indicated by deviation of the dilution-series response curve ("stimulation, increasing toxicity", Figure 3.2) from the shape of a hypothetical non-toxic sample's response curve ("stimulation, no toxicity", Figure 3.2), however. This null response curve would be a function of the increasing concentrations of limiting sample nutrients. It would have a positive slope, although a plateau might be reached, and the 0% elutriate control response would be the lowest value. An actual negative control curve could not be constructed for a given sample dilution series because points are functions of unknown nutrient concentrations.

Constant toxicity across dilutions would have a response curve indistinguishable from the null (no toxicity) response curve, and therefore may not be recognized ("stimulation, constant toxicity", Figure 3.2). However, increasing toxicity would be indicated by a decrease in stimulation ("stimulation, increasing toxicity", Figure 3.2). Thus, samples could be classified as toxic, or with less certainty, non-toxic.

SEDIMENT TOXICITY

The control sediment and SR-01-10 produced increasing stimulation with increasing concentration, as expected for a non-toxic sample (Figure 3.1). There was no evidence of toxicity for either sample at any dilution.

The IH-01-07 and BR-01-08 elutriates were classified as toxic (Figure 3.1). After exhibiting increasing stimulation, a decline occurred at the upper dilutions.

The BR-01-03 elutriate may have been toxic. Algal response was similar to the control sediment and SR-01-10, but the response was lower than the negative control at the lowest dilution (Figure 3.1).

The SR-01-06 elutriate was classified as toxic but with less certainty than the IH-01-07 and BR-01-08 samples. The response curve vacillated between dilutions unlike other elutriates, and although stimulation tended to increase with increasing concentration, carbon assimilation was less than the negative control at the lowest dilution.

CHEMISTRY

The elutriate with the highest metal concentrations (IH-01-07) was also most certainly classified as toxic with the previous approach, although algae could be responding to contaminants other than those analyzed.

Chemical analyses and algal toxicity tests were performed upon samples from the same sediments, but they were not performed upon the same samples. The processes of storage, elutrification and filtration affect the concentrations of toxicants in the algal test samples. Storage may decrease or increase sediment toxicity as some toxicants volatilize, and others degrade chemically or microbially to lesser or more toxic forms. Elutrification, discussed in chapter 2, may extract water soluble toxicants from the sediment, while toxicants organically bound to particulate matter may be lost when the elutriate is poured off. Filtration may further reduce contaminant levels by removing more particulate matter (Burton 1991). It is impossible to say what chemical concentrations were present in the elutriates tested in the algal assay, and therefore to attempt to correlate chemistry and algal toxicity.

SUMMARY AND RECOMMENDATIONS

Elutriates prepared from selected stored sediment samples were tested with an algal ^{14}carbon assimilation method developed for use with materials of varying carbon concentrations. IH-01-07, BR-01-08, BR-01-03, and SR-01-06 were classified as toxic to algae. The control sediment and SR-01-10 were non-toxic. It was not possible to correlate sample chemistry and algal toxicity, but sites identified as toxic did have higher concentrations of suspected toxicants than the sites designated non-toxic. These results suggest sites to target for clean up exist at all 3 AOC, but that SR-01-10 may serve as a non-toxic reference site for algae.

All elutriates were stimulatory relative to the negative control, AAP medium. Stimulation complicated interpretation of results and prevented calculation of effective concentrations. The AAP medium did not suffice as a negative control and nutrient supplement for elutriates. It did not provide unlimited algal nutrients or other unknown growth-limiting factors in all treatments of the toxicity test. Samples were classified as toxic or non-toxic based upon dilution series response curves, but effect concentrations (i.e., EC50s) could not be calculated.

The sensitivity demonstrated by S. capricornutum to sediment contaminants warrants the development of an algal medium that can be used to evaluate toxicity, without the complication of stimulation. With the right medium, this algal test could be a consistent, sensitive, rapid, reproducible, small-volume, means of screening and ranking environmental samples for toxicity.

There are several approaches that could be taken to find a medium appropriate to toxicity testing of environmental samples. Further experimentation could be done on appropriate algal density, medium concentration, and concentrations of medium nutrients individually or in combination; effects of aerating chambers or modifying the AAP medium by adding a pH buffer or a soil extract or other organic materials (Berman et al. 1991); or another published medium could be substituted. Bold's and OECD media produce significantly greater algal growth than the AAP medium (Millington et al. 1988).

The ideal medium would have an excess of nutrients such that nutrients inherent to test materials would not stimulate growth. However, the ideal medium would not have nutrient concentrations in excess of what is necessary to provide unlimited growth. Algal nutrients may affect the toxicity of other materials (Hornstrum 1990) or become toxicants with increasing concentrations (Malone et al. 1975). Finally, an ideal medium would be defined, i.e., one in which all materials and their concentrations are identified, for comparability of results between laboratories.

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LIST OF FIGURES IN CHAPTER 3

Figure 3.1 S. capricornutum responses to elutriate sample doses (Mean and standard deviation).

Figure 3.2 Hypothetical S. capricornutum dose-response curves for stimulatory and nonstimulatory samples.

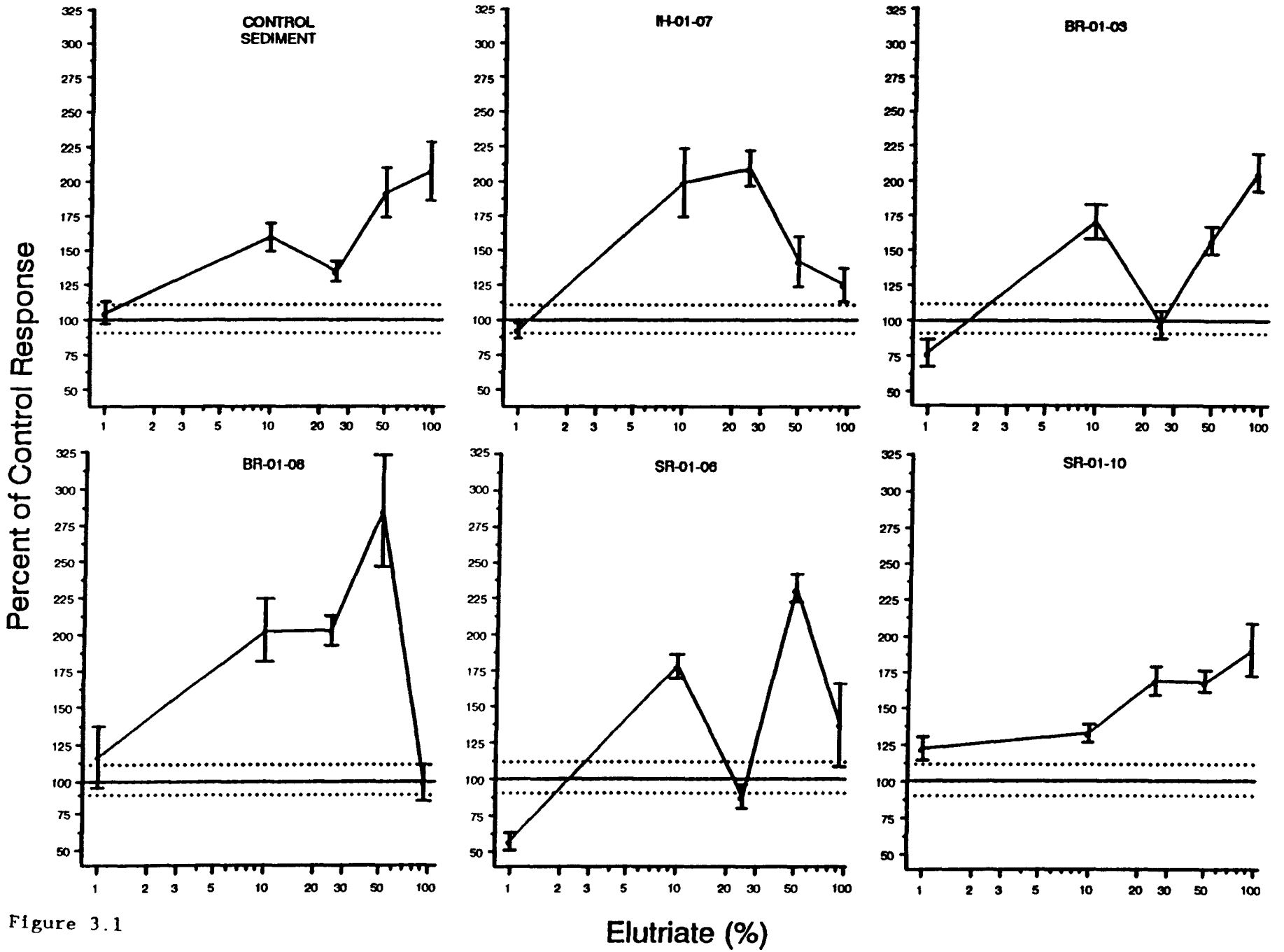
CONTROL SEDIMENT
H-01-07
BR-01-03
BR-01-08
SR-01-06
SR-01-10
Percent of Control Response
Elutriate (%)
325
300
275
250
225
200
175
150
125
100
75
50
1
2
3
5
10
20
30
50
100

Figure 3.1

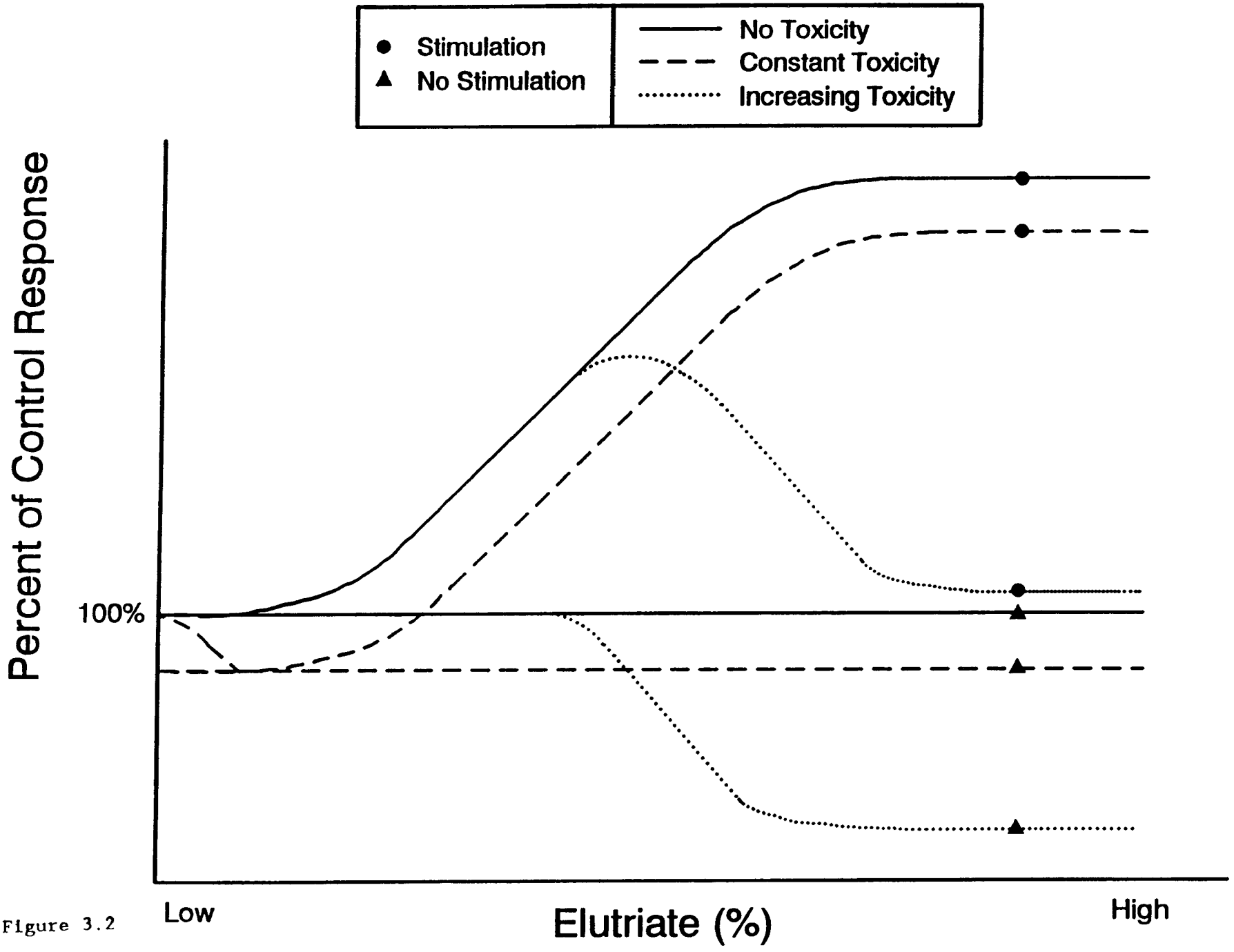
Stimulation
No Stimulation
No Toxicity
Constant Toxicity
Increasing Toxicity
Percent of Control Response
100%
Low
Elutriate (%)
High

Figure 3.2

Table 3.1. Algal culture medium (after Miller et al. 1978).

Compound	Concentration
$NaNO_3$	25.500 mg/L
$MgCl_2 \cdot 6H_2O$	12.160
$CaCl_2 \cdot 2H_2O$	4.410
$MgSO_4 \cdot 7H_2O$	14.70
K_2HPO_4	1.044
$NaHCO_3$	15.00[1]
H_3BO_3	185.52 μg/L
$MnCl_2 \cdot 4H_2O$	415.38
$ZnCl_2$	3.27
$CoCl_2 \cdot 6H_2O$	1.428
$CuCl_2 \cdot 2H_2O$	0.012
$Na_2MoO_4 \cdot 2H_2O$	7.26
$FeCl_3 \cdot 6H_2O$	160.00
$Na_2EDTA \cdot 2H_2O$	100.0[2]
pH	7.5[1]
C : N : P	30 : 50 : 1 mol.[1]

[1] Parameter altered for the revised algal test: $NaHCO_3$ = 60.000 mg/L, pH = 8.1, C : N: P = 119 : 50 : 1 mol.
[2] Concentration reduced from the Algal Assay Procedure medium concentration of 300.0 μg/L.

Table 3.2. Measures of quality assurance for algal assays.

Assay	Cu^{2+} EC50 (μg/L)	Control Response[1] (Control / Blank ^{14}C Assimilation)	$^{14}CO_{2(free)}$ Removal Efficiency (%)
IH-01-07, CONTROL[2], BR-01-08, SR-01-10	NM	104	99.97-99.98
BR-01-03, SR-01-06	10 (3-18)[3]	58	99.94-99.97
Couture et al. 1981	4 < EC50 < 8	NA	NA

[1]Algal carbon assimilation in the 0% elutriate control, prepared by adding nutrient medium to deionized water.
[2]Elutriate prepared Dec. 1990, from stored, dried soil of control sediment.
[3]Mean and 95% confidence interval.
NM Not measured.
NA Information not available.

Table 3.3. Concentrations of metals in non-filtered elutriates prepared within 2 weeks of sample receipt. Note, algal assays were performed on filtered elutriates prepared from the same samples after ≥ 1 year storage.

	Metal concentration (μg/L)								
Sample	Ag	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
CONTROL SEDIMENT	<0.03	<1.8	0.05	1.1	2.6	0.0011	2.5	12	6.9
IH-01-07	1.3	152	69	4700	1329	0.6670	650	11756	10870
BR-01-03	0.33	15	2.2	44	34	0.01	5.5	115	150
BR-01-08	<0.03	12	0.61	2.3	11	0.006	<5	25	28
SR-01-06	<0.09	4.6	7.4	253.0	98.0	0.005	124.0	39.2	139.0
SR-01-10	<0.09	2.2	<0.06	1.2	<1.2	0.008	<5.0	3.4	17.0

Table 3.4. Water quality of elutriates tested with the algal assay.

Sample	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	D.O. (mg/L)	Conductivity (μmhos/cm)	Unionized Ammonia (mg/L)	Total Chloride (mg/L)	Turbidity (NTU)	Inorganic Carbon[1] (μmol/L)
CONTROL SEDIMENT[2]	7.5	40	116	9.7	238	0.002	6.9	2.6	850
IH-01-07	8.2	124	168	10.8	378	0.869	11.3	8.1	2500
BR-01-03	8.0	88	132	9.2	300	0.205	10.1	0.4	1800
BR-01-08	8.1	152	188	10.5	378	0.463	11.2	1.3	3100
SR-01-06	8.1	NM[3]	172	8.6	372	0.169	15.3	2.4	NM[3]
SR-01-10	8.0	124	180	9.8	322	0.100	15.4	1.6	2500
0% ELUTRIATE CONTROL	8.1	36	16	9.5	81	0.005[4]	7.3	0.2	720

[1]Inorganic carbon is estimated from pH and alkalinity data (Stumm and Morgan 1981).
[2]Elutriate prepared from stored dried soil 1990.
[3]Values not measured.
[4]Values below detectable limits.

CHAPTER 4: WHOLE SEDIMENT TOXICITY TESTS

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INTRODUCTION

Contaminated sediments are a persistent source of toxic materials to aquatic resources because of their ability to accumulate contaminants in concentrations frequently greater than that of the overlying water (Medine and McCutcheon 1989), and as a result, can have significant effects on biota and the overlying water quality. During re-suspension and redistribution, and accumulation in depositional areas, contaminated sediment may cause deleterious effects to the benthos and food chain organisms. Because indigenous benthic organisms are continuously exposed to complex mixtures of contaminants associated with sediment, they may exhibit lethal and sublethal effects such as, mortality, diminished growth, delayed maturation, and reproductive impairment.

The interaction of complex mixtures of sediment-associated contaminants can be integrated by using whole sediment toxicity tests (Swartz et al., 1979). Whole sediment laboratory toxicity tests can measure the effect of all bioavailable contaminants, where bioavailable is defined as the fraction of the total contaminant in the interstitial water and on the sediment particles that is available for biological processes (Landrum and Robbins 1990). If the test organism's critical life stages are used, ecotoxicologically relevant information can be obtained (Samoiloff, 1989). Toxicity assessments using aquatic invertebrates provide valuable information on the relative degree of aquatic resource degradation and a direct measure of contaminant bioavailability (Swartz et al. 1982, Nebeker et al. 1984, Breteler et al. 1989, Scott and Redmond 1989, Ingersoll and Nelson 1990). Test organisms exposed to field-collected sediments integrate interactions among complex mixtures of contaminants that may be present at the sediment collection site.

TOXICITY TESTS

The objective of the whole sediment laboratory exposures was to evaluate the biological effects of sediment contamination to benthic macroinvertebrates. The laboratory experimental design was based on the assumption that the behavior and effects of the sediment-associated contaminants is similar to that in *in situ* sediments. Relative comparisons between species sensitivities were made. Sediment contaminant concentrations, and the toxicity responses observed, were compared to recommended threshold concentrations used to identify sediment toxicity (Barrick et al. 1988; Long and Morgan 1990; SMS 1991).

The present studies evaluated the potential toxicity of whole sediments collected from three Areas of Concern (AOCs) within the Great Lakes: Indiana Harbor, IN; the Buffalo River, NY; and the Saginaw River, MI. The studies were conducted for the Assessment and Remediation of Contaminated Sediments (ARCS) with the U.S. Environmental Protection Agency's (USEPA) Great Lakes National Program Office (GLNPO). Three test species were exposed in flow-through or static exposures, in partial life-cycle (10-d, 14-d and 28-d) toxicity tests. The test organisms included: *Hyalella azteca* (Amphipoda) 14

and 28-d flow-through exposures; Chironomus riparius (Diptera) 14-d flow-through exposure; and Chironomus tentans (Diptera) 10-d static exposure. The toxicological endpoints measured were survival and growth (body length) for all test species, antennal segment number and sexual maturation for H. azteca. These species have been recommended by the International Joint Commission (1987) and ASTM (E 1383-90) for whole sediment toxicity evaluations.

Hyalella azteca (Amphipoda)

The amphipod, H. azteca, is a common and widely distributed Talitrid amphipod inhabiting permanent lakes, ponds, and streams, throughout most of the American continent and the Caribbean (Bousfield 1958, DeMarch 1981, Pennak 1989). Its life cycle is divided into three stages: (1) immature, including the first five instars; (2) juvenile, including instars 6 and 7; and (3) adult, including the 8th instar and older (Cooper 1965, Pennak 1989). Adult males are larger than females and have an enlarged second gnathopod propodus (Giesler 1944). As a toxicity test organism, H. azteca has several desirable characteristics: (1) it has a short generation time, (2) is easily collected from natural sources or laboratory cultured, (3) is ecologically relevant, and (4) is sensitive to contamination (Maciorowski 1975, Borgmann et al. 1989). For assessment and management of contaminated sediments, H. azteca has been used in sediment toxicity testing, both under laboratory and field conditions (Landrum and Scavia 1983, Cairns et al. 1984, Nebeker et al. 1984, 1986, Nebeker and Miller 1988, Burton et al. 1989a, Borgmann and Munawar 1989, Ingersoll and Nelson 1990).

Chironomus riparius and Chironomus tentans (Diptera)

Chironomus tentans and C. riparius (midge larvae) live most of their life cycle as burrowing aquatic insects. They are frequently found in large numbers in North American lakes and ponds (ASTM E 1383-90). Owing to their frequently high density, chironomids constitute a biomass of considerable ecological importance as nutrient processors and as food organisms for higher trophic levels. The life cycles of C. riparius and C. tentans can be divided into three stages: (1) a larval stage (first four instars); (2) a pupal stage; and (3) an adult stage. Under optimal conditions C. riparius larvae will pupate in 15 to 21 days at 20°C and C. tentans larvae will pupate after 24 to 28 days at 20°C. Because midge larvae are in direct contact with the sediment and consume a wide variety of organic substances, they are sound biological indicators of habitat quality and represent an important route of entry for contaminants into food webs. Due to their short life span and ease of laboratory culture, midges have been used frequently for sediment toxicity testing (Adams 1985, Giesy et al. 1988, Pittinger et al. 1989, Ingersoll and Nelson 1990).

MATERIALS AND METHODS

SUMMARY OF SEDIMENT SAMPLES TESTED

The GLNPO ARCS Toxicity-Chemistry Work Group (Table 1.1) selected stations for whole sediment toxicity evaluations. Stations were chosen based on the expected presence of contaminants which would elicit both acute and chronic effects. Of the 29 stations sampled between 1988 and 1990, from the three AOC, 19 were chosen for whole sediment toxicity evaluation. The following Great Lakes sediment samples were evaluated:

Indiana Harbor, IN, IH-01-03, IH-01-04, IH-01-06 and IH-01-07;
Buffalo River, NY, BR-01-01, BR-01-03, BR-01-07, BR-01-08 and BR- 01-09;
Saginaw River, MI (First survey), SR-01-03, SR-01-06 and SR-01-10[1];
Saginaw River, MI (Third survey), SR-03-01, SR-03-02, SR-03-05, SR-03-06, SR-03-08, SR-03-16 and SR-03-24)[2].

For additional information regarding locations, water depths, and sample types, see Table 2.1. For descriptions of collection methods see Chapter 1 (Overview) this report.

Sediment Storage

Sediment sampling at all AOCs required more than one day. Reported storage days are the number of days between the first date of sediment collection at an AOC, and the date when sediments were put into test chambers.

Indiana Harbor--

Sediment samples were collected from Indiana Harbor between 8/9/89 and 8/10/89. Sediment from Stations IH-01-03, IH-01-04 and IH-01-06 were collected on 8/9/89, and from Station IH-01-07 on 8/10/89. All samples were received at the National Fisheries Contaminant Research Center (NFCRC) on 8/11/89.

Elutriates were prepared on 8/15/89, 8/30/89 and 9/6/89 (see Chapter 2). Sediments were homogenized and put into test chambers on the days indicated: 14 d *H. azteca*, 8/30/89 (21 days storage); *C. riparius* and *C. tentans*, 9/6/89 (28 days storage). The time between collection and placing sediment in test chambers ranged from 21 to 28 days.

Buffalo River--

Sediment samples were collected from the Buffalo River between 10/6/89 and 10/8/89. Sediment was collected from the following stations on the days indicated: BR-01-03, BR-01-04 -- 10/6/89; BR-01-06, BR-01-07, BR-01-09 -- 10/7/89; and BR-01-01, BR-01-08 -- 10/8/89. Samples arrived at NFCRC on 10/10/89 at 8°C. Elutriates were prepared on 10/11/89 and 10/25/89 (see

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[1] Referred to as SR-01 when discussing stations, or Saginaw River (1) when discussing AOCs.

[2] Referred to as SR-03 when discussing stations, or Saginaw River (3) when discussing AOCs.

Chapter 2). Sediments were homogenized and put into test chambers on the days indicated: C. riparius and 28-d H. azteca 10/23/89 (17 days storage); 14 d H. azteca and C. tentans 10/25/89 (19 days storage). The time between collection and placing sediment into test chambers ranged from 17 to 19 days.

Saginaw River (1)--

Sediment samples were collected between 12/01/89 and 12/02/89 from Saginaw River (1). Sediment was collected from the following stations on the days indicated: SR-01-10 -- 12/01/89; and SR-01-03, SR-01-06 -- 12/02/89. Samples arrived at NFCRC on 12/5/89. Elutriates were prepared on 12/6/89, 12/19/89, and 12/27/89 (see Chapter 2). Sediment was homogenized and put into test chambers on the days indicated: 28 d H. azteca, 12/19/89 (18 storage days); C. riparius, 12/20/89 (19 storage days); 14 d H. azteca 12/27/89 (26 storage days). The time between collection and placing sediment into test chambers ranged from 18 to 26 days.

Saginaw River (3)--

Sediment samples were collected between 6/21/90 and 6/23/90 from Saginaw River (3). Sediment was collected at the stations on the following days: SR-03-02, SR-03-05, SR-03-06 -- 6/21/90; SR-03-08, SR-03-16, SR-03-24 -- 6/22/90; and SR-03-01 -- 6/23/90. Samples arrived at NFCRC on 6/26/90 at temperatures between 20°C to 21°C. Elutriates were prepared on 6/26/90, 07/09/90, 07/12/90 and 07/16/90. Sediment was homogenized and put into test chambers on the days indicated: 28 d H. azteca 07/09/90 (18 days storage), C. riparius 7/12/90 (21 days storage), 14 d H. azteca, 7/16/90 (25 days storage), and 7/18/90 C. tentans (27 days storage). The time between collection and placing sediment into test chambers ranged from 18 to 27 days.

EXPOSURE PROCEDURES

Whole sediment tests conducted include the flow-through tests for amphipods in 14- and 28-d exposures and C. riparius in a 14-d exposure following procedures described in Ingersoll and Nelson (1990). The midge, C. tentans, was tested in 10-d static exposures following procedures described in Giesy et al. (1988). The effects of contaminants on survival and body length were measured for all test species. In addition, effects on sexual maturation, antennal segment number and increase in body length were measured for amphipods. A fine silt- and clay-particle size soil was used for the control sediment in all studies (Adams et al. 1985, Ingersoll and Nelson 1990).

Test animals were cultured at 20°C (~2°C) with a photoperiod of 16:8 h light:darkness, and a light intensity of about 25 to 50 fc. Amphipods were mass cultured in 80-L glass aquaria containing 50 L of NFCRC water (pH 7.8, hardness 286 mg/L as $CaCO_3$, alkalinity 258 mg/L as $CaCO_3$, chloride 22 mg/L, and conductivity 600 umhos/cm) with about three water additions daily, and gentle aeration with air stones. Amphipods were cultured on hard maple leaves (Acer spp.) that had been soaked in NFCRC water for about 30 d and then rinsed with well water to remove any tannic acid. Also, ground Tetra^R Standard Mix Fish Food was fed weekly ad libitum (Ingersoll and Nelson 1990). Amphipods were collected for testing by placing a part of the leaf substrate in a U.S.

Standard stainless steel sieve stack (#25, #40, and #60) and rinsing with culture water. Amphipods, about second instar, stopped by the #60 sieve ($\leq$2 mm), were used for testing.

Both of the midges were mass cultured in polyethylene chambers (30 x 30 x 30 cm) that were covered with nylon screen and contained 3 L of well water. Chironomus riparius larvae were fed a combination of Cerophyl^R, green algae Selenastrum capricornutum, and Hartz^R dog treats (Ingersoll and Nelson 1990). The C. tentans chambers contained a shredded paper towel substrate, and larvae were fed ground Tetrafin^R in a well water suspension (Giesy et al. 1988). Chironomus riparius were isolated for testing by placing fresh egg cases in individual 200-mL crystallization dishes containing about 100 mL of culture water at 20°C. Midge larvae began hatching after two to three days and were collected for testing within 24 h of hatching. Chironomus tentans were isolated for testing by placing fresh egg cases in glass baking dishes containing culture water at 20°C. Larvae used for testing hatched after two to three days and were maintained for 10 to 12 days (second instar), with gentle aeration, and a diet of S. capricornutum and ground Tetrafin^R.

The reconstituted water used in the sediment tests is described in Chapter 2 (pH 8.0, hardness 134 mg/L as $CaCO_3$, alkalinity 60 to 65 mg/L as $CaCO_3$, sulfate 72 mg/L). Test animals were acclimated to the overlying water before tests started on Day 0, by placing them at 2-h intervals into 50:50 and 25:75 mixtures of culture water to overlying water (Ingersoll and Nelson, 1990). At the start of each test (Day 0) a sub-sample of about 20 animals were preserved with a sugar-formalin mixture (Ingersoll and Nelson 1990) and archived. Test animals were also preserved and archived at the end of each study. Animals were tested at 16:8 h light:darkness photoperiod and light intensity of 25-50 fc (Ingersoll and Nelson, 1990, ASTM E 1383-90).

Flow-through Tests

The day before tests started (Day -1) sediment was homogenized, then sediment and overlying water were added to each of four replicate test chambers (Ingersoll and Nelson, 1990). Test chambers for amphipods and C. riparius exposures contained 200 mL homogenized whole sediment and 800 mL reconstituted overlying water (Ingersoll and Nelson 1990). Gentle aeration of the overlying water was started, cover glasses placed on the test chambers, and test systems equilibrated overnight in a 20°C water bath. Tests were started on Day 0, using methods described in Ingersoll and Nelson (1990), with the addition of 20 amphipods or 50 C. riparius larvae into each test chamber. Larvae of C. riparius and amphipods were acclimated to the overlying water, then counted into 30 mL beakers containing overlying water. Animals were released into the test chambers and any animals on the surface were gently submersed or replaced. Modified Mount and Brungs proportional flow-through diluters delivered overlying water (Mount and Brungs 1967). To allow the larvae to settle into the sediment (Pittinger et al. 1989), water delivery (1.25 volume additions test chamber^{-1}*day^{-1}) was started on Day 1 of the C. riparius test, and on Day 0 in the amphipod tests (Ingersoll and Nelson 1990). Cover glasses and airlines were removed from the test chambers before the water flow started. Test chambers were observed daily for the presence of animals in the water column or on the sediment surface. Water condition (e.g., surface film, cloudiness) was recorded, and sediment surface was

observed for the presence of mold, unconsumed food particles, or signs of burrowing behavior.

Amphipods were fed Purina[R] Rabbit Pellets in a water suspension (Ingersoll and Nelson 1990) three times weekly at 6 mg for the first 7 d, then 12 mg to test end. Chironomus riparius larvae were fed a combination of Cerophyl[R] (10 mg daily for the first 6 d), S. capricornutum (6 X 10^7 cells daily), and Hartz[R] dog treats (10 mg daily) (Ingersoll and Nelson 1990). When excessive mold was present on the sediment surface of any replicate, food was withheld for the entire treatment.

At test end, sediments were sieved to retrieve surviving test organisms using methods outlined in Ingersoll and Nelson (1990). Remaining debris and sediment from the collecting pans was preserved and later inspected under a low power binocular microscope to recover any small animals (particularly amphipods) which were not seen at test end.

Preserved amphipods were measured using a Zeiss[R] Interactive Digital Analysis System in combination with a Zeiss SV8 stereomicroscope at a magnification of 25 X (Ingersoll and Nelson 1990). Two measurements of amphipod body length (~0.1 mm), along the curve of the dorsal surface from the base of the first antenna to the tip of the third uropod, were recorded, and antennal segments were counted. To determine sexual maturation, amphipods were sexed by the appearance of an enlarged propodus on the second gnathopod for males, and the presence of eggs in the brood pouch or lack of an enlarged propodus for females (Nelson and Brunson 1992). Archived amphipods from Day 0 were measured for comparisons to amphipods at the end of the 14- and 28-d exposures. Chironomus riparius larvae were photographed on a 1 cm gridded background and C. riparius lengths were digitized from enlarged prints (20 x 30 cm) with a Houston Instrument True-grid[R] 1017 digitizing board. Up to 20 midge per replicate were measured from the anterior of the labrum to the posterior of the last abdominal segment (Smock, 1980).

Static Tests

Sediments collected from Indiana Harbor, Buffalo River and Saginaw River (3) were tested with C. tentans using static, 10-d whole sediment toxicity test methods, described by Giesy et al. (1988), with some modifications. Due to diminished reproduction in C. tentans cultures, test organisms were not available to conduct whole sediment tests on sediment samples collected from Saginaw River (1). Additionally, only 10 individuals per station were tested in Saginaw River (3) due to a larger number of samples for this survey and limited space in the water bath.

Two days before the start of the test, 7.5 g of homogenized sediment from each station was placed in 15 50 mL polypropylene conical centrifuge tubes, or 10 tubes for Saginaw River (3) exposures. Forty mL of overlying water was added to each test chamber. Sediment and water were mixed by capping chambers and inverting five times in accordance with Giesy et al. (1988). Test chambers containing sediment from the same station were inserted in a styrofoam holder and placed in a vented, temperature controlled (23~1°C) waterbath. One day before introduction of test organisms, caps were removed and gentle aeration (about 3 bubbles*s^{-1}) were delivered to the chambers. Tests were started by placing one second instar C. tentans larva in each test chamber.

Biological observations (e.g., organism behavior) and observations of sediment and water conditions (e.g., presence of mold, water clarity) were performed daily on each test chamber. TetraFin^R (6 mg/day) was added to each chamber in an aqueous suspension (Giesy et al. 1988). If mold was observed on the sediment surface or if the overlying water appeared cloudy, feeding was withheld until conditions cleared or the test ended. Water lost to evaporation was replaced as needed with temperature acclimated deionized water (Giesy et al. 1988).

At the end of the 10-d exposure, sediment was passed through a U.S. Standard #50 sieve to recover larvae. Surviving C. tentans larvae from test chambers for each station were counted and combined into a single glass vial, preserved and archived. Growth (dry weight) was determined gravimetrically for midge exposed to Buffalo River sediments. Larvae were blotted on absorbent paper to remove excess water, then individuals were placed on pre-weighed pans and dried at 80°C. Chironomus tentans exposed to Indiana Harbor and Saginaw River (3) sediments were measured from photographs using methods described above for C. riparius.

To determine if the Microtox^R test response to elutriates prepared from stored sediment samples had changed over time, Microtox^R tests were conducted for all sediments used in whole sediment tests. Before each whole sediment test, stored sediment samples were homogenized, elutriates prepared, then tested for Microtox^R toxicity. Elutriates prepared from the stored sediments were tested with the same methods described in Chapter 2 with one exception: elutriates prepared for the Microtox^R and Daphnia magna tests were stored for about 1 week at 4°C, in the dark, whereas elutriates prepared from stored sediments were tested within 24 h.

WATER QUALITY CHARACTERIZATION

The reconstituted overlying water described in Chapter 2 was used as the overlying water in the whole sediment exposures (pH 8.0, hardness 134 mg/L as $CaCO_3$, alkalinity 60 to 65 mg/L as $CaCO_3$, sulfate 72 mg/L). The methods used for measuring water quality characteristics were the same as described in Chapter 2.

Whole sediment exposures began on separate days because of the time required to set up individual tests. Because of the staggered test set-up, water quality assessment efforts were combined. Before starting water flow on Day 0 of the first flow-through exposure, 50 mL of overlying water was removed from each of the four replicates per treatment and pooled. The pH, dissolved oxygen, conductivity, water hardness, alkalinity, turbidity, ammonia, and chloride were measured and the values were used as Day 0 water quality determinations for the remaining whole sediment tests within an AOC.

In the 14-d exposures with amphipods and C. riparius, the pH, dissolved oxygen, and conductivity were determined on Day 7. On Day 13, pH, dissolved oxygen, conductivity, total water hardness, alkalinity, turbidity, ammonia, and chloride were measured. In the amphipod 28-d exposures, pH, dissolved oxygen, and conductivity were measured on Days 7, 14, and 21; pH, dissolved oxygen, conductivity, total water hardness, alkalinity, turbidity, ammonia, and chloride were measured on Day 27. In the C. tentans exposure, pH, dissolved oxygen, conductivity, total water hardness, alkalinity, turbidity, ammonia, and chloride were measured on Day 10; no Day 0 water quality determinations were conducted. For the Day 10 water quality determinations,

about 40 mL of exposure water per chamber (within each treatment) was collected and pooled.

STATISTICAL ANALYSIS

Survival (except C. tentans), body length (H. azteca, C. riparius, C. tentans), body weight (C. tentans), increase in length, antennal segment number and sexual maturation (H. azteca) were tested for differences among means. Before testing for differences, survival, increase in length, and sexual maturation data were arcsine square root transformed. Variances were compared using the general linear model (GLM) one way analysis of variance (ANOVA) least squares means Type III test at alpha = 0.05 (SAS 1988). Homogeneity of variance was tested using an *F* test, at alpha = 0.05, in the GLM and ANOVA procedures. Survival data for C. tentans was analyzed using a categorical model procedure (SAS 1988) that performed a statistical analysis of multivariate categorical data described by Koch et al. (1977). Significant differences for sediment treatments are presented as differences compared to control, and not differences between stations or AOCs. Sediment samples were considered toxic if any test organism response was significantly different from the control treatment.

SEDIMENT PHYSICAL AND CHEMICAL CHARACTERIZATIONS

Sediment physical characterization included (1) percentage solids, and (2) particle size. Sediment chemical characterization included (1) metals, (2) organometals, (3) acid volatile sulfide (AVS) and simultaneously extracted metals (SEM), (4) chlorinated pesticides, (5) polychlorinated biphenyls (PCBs), (6) polychlorinated dibenzo-dioxins (PCDDs) and -furans (PCDFs), (7) select polynuclear aromatic hydrocarbons (PAHs), and (8) total organic carbon (TOC). All chemicals analyzed are listed in Table 4.1.

All chemical analyses on sediment samples were provided by Battelle Laboratory, Sequim, WA. The samples for chemistry were collected by personnel of the Large Lakes Research Station, Grosse Isle, MI, and shipped by overnight courier to Battelle. In most cases, one 2-L bottle of sample from each station was collected and shipped for sediment chemical analyses. The samples were then placed in an automated, continuously monitored cold storage room until they were analyzed. For analyses, the samples were sub-sampled as follows:

1. 50 g for metals, percentage solids, and TOC analyses,
2. 250 g for analysis of PAH,
3. 50 g for tributyltin analysis,
4. 20 g for AVS and 20 g for methylmercury analyses, and
5. 100 g for Ames (Chapter 6) and Mutatox (Chapter 7) assays.

The percentage solids in each sediment sample was estimated by freeze drying the sample and then comparing wet and dry weights. Freeze drying provided a fine, powdery sample that could be more uniformly homogenized. The TOC in samples was determined with a Leco Model WR-12 carbon determinator. Samples were pre-treated with concentrated HCl to remove inorganic carbon. Then the samples were burned at 800°C in an oxygen atmosphere connected to a boat inlet which transferred the evolved CO_2 directly into an organic carbon analyzer. Particle size was determined with a Gilson Model WV-2 wet siever,

using U.S. Standard #18 (1 mm), 60 (250 μm), 230 (63 μm) and 400 (38 μm) sieves.

Acid volatile sulfides were determined with the method of Cutter and Oattes (1987). In this method, hydrogen sulfide is selectively generated, cryogenically trapped, separated by gas chromatography, and detected by photoionization. The SEM are determined by analyzing the 1NHCl solution produced during the AVS determination. The 1NHCl is filtered through a 0.3μm pore filter and analyzed by graphite furnace atomic absorption. Because sediments samples were homogenized in the field and exposed to air, they may not be representative of ambient field conditions, but are representative of the sediment samples used in the toxicity tests which were homogenized and stored in a similar manner. Sub-samples for AVS analyses should be isolated before open air homogenization.

Simultaneously extracted metal concentrations were not determined for Saginaw River (3), but were estimated using the vigorously extracted metal concentrations (μg/dry g). Saginaw River (3) vigorously extracted, individual metal concentrations were converted to molar concentrations (μM/dry g). To obtain the estimates of the SEM concentration, the Saginaw River (3) metal concentrations (μM/dry g) were multiplied by the average Saginaw River (1) SEM concentration for each metal.

The sediment samples were analyzed for total metals concentrations by use of the USEPA method 200.4 (USEPA 1990). These techniques are not intended to measure biologically significant metals. The samples were completely dissolved by digestion with nitric, perchloric and hydrofluoric acids in Teflon[R] pressure vessels and then analyzed by use of cold vapor atomic absorption, or graphite furnace atomic absorption. For crustal elements that are difficult to dissolve with strong acids a portion of the freeze-dried samples was ball-milled to about 120 mesh, pelletized, and analyzed with x-ray fluorescence (Nielson and Sanders 1983).

In methylmercury analyses, the homogenized samples were digested in 10 mL of a 25 percentage solution of potassium hydroxide in methanol at 60°C for two to four hours. Samples were allowed to cool for 24 h and an additional 10 mL of methanol was added and mixed well by shaking. Before analysis undissolved solids were allowed to completely settle. The samples were analyzed with a cold vapor atomic fluorescence technique (Bloom 1989). The technique is based on the emission of 254 nm radiation by exiting mercury atoms in an inert gas stream. An ethylating agent, sodium tetraethylborate, was added to the sample digestate to form a volatile methylethylmercury derivative. The derivative was then purged onto graphite carbon traps for pre-concentration and removal of interferences. Then the sample was subjected to cryogenic chromatography and pyrolytic degradation to elemental mercury, which was quantified with a cold vapor atomic fluorescence detector.

In analyses for organotins, samples were extracted with 0.2% tropolone in methylene chloride, then filtered through glass wool. The filtrates were derivitized with 1 mL hexyl magnesium bromide, a Grignard's reagent, and cleaned-up with a Florisil column. Organotin concentrations were measured with a Hewlett Packard Model 5890 gas chromatograph equipped with a flame photometric detector.

Three groups of organic chemicals were measured for each sediment sample: (1) PAH, (2) PCB and chlorinated pesticides, and (3) PCDDs and PCDFs. The analytical procedure for each chemical group included solvent extraction, extract purification with column chromatography, and chemical quantification

with capillary column gas chromatography. In the analyses for pesticides and PCBs, Aldrin, B-BHC, Gamma Chlordane, 4,4,-DDD, 4,4,-DDE, 4,4,-DDT, Dieldrin, Endosulfan I, Endosulfan II, Endrin, Endrin aldehyde, Endrin ketone, Heptachlor epoxide, Aroclor 1242 and 1254 were detected in some samples, but either a less than 25% difference between two gas chromatography columns for detected concentrations was observed, or the analyses were conducted at secondary sample dilution factors.

Polynuclear aromatic hydrocarbons in sediment samples were determined according to the USEPA method 3550 (USEPA 1986a). Before extraction, three isotopically labelled surrogate PAH compounds (D10-fluorene, D10-anthracene, D10-pyrene) were added to the samples. Then the samples were extracted with methylene chloride in a Soxhlet extractor. Potential interferences by pigments, lipids and other macromolecules were removed by use of the USEPA gel permeation chromatography (GPC) method 3540 (USEPA 1986a). Then the extracts were exchanged into hexane and analyzed with the USEPA Gas Chromatography/Mass Spectrometry (GC/MS) method 8270 (USEPA 1986a).

Aroclors quantified were 1016, 1221, 1232, 1242, 1248, 1254 and 1260 for sediments from Indiana Harbor, Buffalo River and Saginaw River (1); and 1242, 1254 and 1260 for Saginaw River (3). Aroclors were extracted from the sediment samples according to the USEPA method 3550 (USEPA 1986a). The GC surrogate compound dibutyl chlorendate (DBC) was added to the samples, and the samples were subsequently extracted with methylene chloride using sonication. Potential interferences by oily-type materials from highly contaminated sediments, lipids, and other macromolecules were eliminated by use of GPC or alumina column chromatography (USEPA 1986a, methods 3540 and 3610). Aroclors were quantified by USEPA method 8080 (1986a) using a DB-5 fused silica capillary column (0.25 mm diameter x 30 m) and a Hewlett-Packard 5890 gas chromatography equipped with an electron capture detector (GC/ECD) and a computer for data acquisition. A dual column analysis was always performed simultaneously and results from both columns were accepted if they showed no more than 50% variation.

The USEPA isotope dilution method 8290 (USEPA 1986a) was used to extract and clean-up the sediment samples for analysis of PCDDs and PCDFs. Isotopically labelled PCDDs and PCDFs were added to the samples before extraction. The samples were extracted with benzene in a Soxhlet extractor for 18 h. Then a three-step column chromatography procedure with acidified silica gel, alumina, and AX-21 activated carbon on silica gel was used to enrich the samples and remove interferences. Isotopically labelled 2,3,7,8,-TCDD was added to the samples before the enrichment to determine the efficiency of the method. Two internal standards were added to the samples after sample enrichment to determine percentage recoveries. The PCDDs and PCDFs were quantified with capillary column gas chromatography and high resolution mass spectrometry. Concentrations were determined by selected-ion-monitoring chromatography of groups of ion masses described in the USEPA method 8290 (USEPA 1986a).

QUALITY CONTROL AND QUALITY ASSURANCE

The precision of the whole sediment toxicity tests were evaluated using four replicate treatments, except for C. tentans tests, in which one midge larva per replicate was used, for 10 to 15 replicates. The acceptability of the toxicity tests was assessed by response of the test organisms to the

control sediment for survival. Survival in the control sediment was within acceptable ranges for all of the H. azteca tests, greater than 80% as recommended by American Society for Testing and Materials (ASTM) (E 1383-90). Midge control survival for ASTM test acceptability is 70% (E 1383-90), and was met for all tests except the C. riparius Buffalo River sediment exposure (60.5% survival, Table 4.18), and the C. tentans Saginaw River (3) sediment exposure (60% survival, Table 4.35). The results from these two midge studies should be interpreted with caution.

Test organism sensitivity was evaluated by exposing the organisms to saline water in acute toxicity tests. These reference toxicant tests are routinely conducted by NFCRC to monitor the sensitivity and condition of laboratory test organisms. Reference toxicant tests were conducted with NFCRC water and Instant Ocean^R sea salts (pH 8.0, salinity 33 g/L). The 48-h LC50 for C. riparius was 9 g/L salinity and for C. tentans was 10 g/L salinity. The 72-h LC50 for H. azteca was 17 g/L salinity.

Accuracy and precision of the chemical analyses were determined by analysis of one blank, one matrix spike, one certified reference material, and one sample in duplicate or triplicate for each set of 20 samples. Acceptable recovery values ranged from 85 to 115% of the spike concentration for metals, and from 70 to 130% of the spike concentration for organics and organometals. Analytical values for reference materials were acceptable if they were within 20% of the certified ranges. The acceptable coefficient of variation for duplicate or triplicate sample analyses was $\leq$20%.

During chemical analyses, three to five standards containing concentrations that bracketed the expected range of concentrations in the samples were used for daily instrument calibrations. In analyses of samples for metals by atomic absorption spectrophotometry, these standards were analyzed as matrix spikes, and the slopes from linear regression analyses were used to estimate sample concentrations. The minimum acceptable r^2 in the regression analyses was 0.97. The standards for each sample set were analyzed at the beginning and end of each analytical run. The analytical results were accepted if the values for standards were within 90 to 110% of their certified values. For some samples analyzed by atomic absorption, average response factors, rather than linear regression, were used for instrument calibration. The accuracy of this calibration method was checked by dividing each response factor by the average response value. The calibration values were accepted if they were within 5% of the average response value.

During chemical analyses, the method's detection limits (MDL) was estimated according to procedures in the USEPA Federal Register (1984). The MDLs were calculated as:

$$3\sigma_n$$

where σ_n is the standard deviation of the response factors of 3 or 4 calibration spikes or blanks (if applicable). Additional MDLs were calculated as:

$$\text{Student } t_{0.01,\alpha-1} \times {}^{o}{}_{n}$$

where ${}^{o}{}_{n}$ is the standard deviation of the measured concentration of a standard value within an order of magnitude of the absolute detection limit of the instrument. If standard values were not available, 15 replicates of certified reference materials were used to obtain ${}^{o}{}_{n}$, and to calculate non-matrix-specific MDLs.

RESULTS

EVALUATION OF SEDIMENT STORAGE USING MICROTOX TESTS

With few exceptions, the Microtox[R] test indicated that the toxicity of sediment samples did not change during laboratory storage, for up to 26 d at 4°C. Indiana Harbor sediments were stored the longest over other AOCs, for 26 d. The remaining samples were stored between 15 and 22 days before the last whole sediment tests were started. Only two of the 19 samples tested exhibited different toxicity over time, see Table 2.14 in Chapter 2. After 14 days of storage, elutriates prepared from sample IH-01-07 (Indiana Harbor) elicited increased toxicity, and elutriates prepared from sample BR-01-03 (Buffalo River) exhibited decreased toxicity. All other samples exhibited no appreciable changes in toxicity as measured by Microtox[R] toxicity.

INDIANA HARBOR

Physical and Chemical Characterization

Concentrations of TOC, total solids, and organometals in Indiana Harbor sediment samples are listed in Table 4.2. Total organic carbon ranged from 8.8 to 12.2% in Stations IH-01-05, IH-01-06, IH-01-07, IH-01-08 and IH-01-10 and in Stations IH-01-03 and IH-01-04 from 5.6 to 7.8%. Solids ranged from 19.6% in sediment from Station IH-01-10 to 50.2% in sediment from Station IH-01-05. Organometals in Indiana Harbor sediment samples ranged from less than 0.1 ng/dry g for methylmercury to 1500 ng/dry g for tributyltin.

Metals in Indiana Harbor sediments ranged from 0.7 μg/dry g for Hg to 7960 μg/dry g for Zn (Table 4.3). Concentrations of Cr, Cu, Fe, Mn, Pb, and Zn were the highest, and the other metals ranged from less than 1.0 to 100 μg/dry g (Table 4.3).

Acid volatile sulfides and eight SEM for Indiana Harbor stations were analyzed (Table 4.4). Among the Indiana Harbor stations, Station IH-01-07 was the highest for extractable Cd, Cr, Fe, Mn, Ni, Pb and Zn (Table 4.4). The AVS ranged from 16 μM/dry g in Station IH-01-04 to 71.4 μM/dry g in Station IH-01-07, and all SEM/AVS ratios were less than one (Table 4.4).

The Indiana Harbor sediments consisted primarily of particles that were less than 38 μm in size (36.8% to 77.7%) with the remainder of the particles ranging from .063 mm to 1.0 mm in size (Table 4.5). Particles greater than 1.0 mm in size comprised only a small portion (0.2% to 1.0%) of the sediments from Indiana Harbor.

Sediments from Indiana Harbor contained PAH and semivolatile compound concentrations that ranged from less than 25 ng/dry g for dimethylphthalate to 290,000 ng/dry g for 2-methylnaphthalene (Table 4.6). Station IH-01-07 contained fluorene, phenanthrene, anthracene, fluoranthene and pyrene (Table 4.6). Polychlorinated dibenzo-dioxins and -furans in the Indiana Harbor samples ranged from 3.8 pg/dry g for 1,2,3,7,8-pentachlorodibenzofuran to 43,000 pg/dry g for octachlorodibenzodioxin (Table 4.7).

Concentrations of PCBs in the sediment from Indiana Harbor for Aroclor 1242 ranged from 3,000 (IH-01-03) to 43,000 ng/dry g (IH-01-07), and in addition, Aroclor 1254 was detected in sediment from Station IH-01-04 (1,000 ng/dry g) (Table 4.8). Pesticides in the Indiana Harbor sediments were below analytical detection limits (Table 4.9).

Hyalella azteca Exposure

Overlying Water Quality Characterization--

In the Indiana Harbor whole sediment amphipod exposure, mean overlying water dissolved oxygen, pH, and hardness were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.137 mg/L in all of the treatments, with the highest unionized ammonia level (0.320 mg/L) in overlying water from Station IH-01-06 (Table 4.10); unionized ammonia in the overlying water of control was 0.057 mg/L (Table 4.10). Alkalinity in all of the treatments, including control, were about 1.5 times greater than the in-flowing reconstituted water (Table 4.10). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride was elevated 1.5 to two times in all treatments (except control) relative to the reconstituted water (Table 4.10). Turbidity in all treatments was about five to six times greater than control (Table 4.10).

Toxicity--

Amphipod survival was significantly reduced in 14-d exposures to whole sediment samples from Stations IH-01-03 and IH-01-06, and no amphipods survived in Stations IH-01-04 and IH-01-07 (Table 4.11). The sublethal endpoints, number of antennal segments and percent sexually mature (identifiable secondary sex characteristics) of surviving amphipods exposed to Indiana Harbor sediments, were not significantly different after 14 days (Table 4.11). Because Indiana Harbor sediments were lethal in 14-d exposures, amphipod 28-d exposures were not conducted.

By Day 1, amphipods were observed floating on the water surface in all Indiana Harbor stations (except the control) within the first 24 h of the 14-d exposure, most notably in Station IH-01-07. An oily film was present on the overlying water surface in all stations, especially heavy in IH-01-07. Two feedings were withheld from Station IH-01-07 because of sediment surface mold. Indigenous species seen during amphipod recovery at test end included oligochaetes and ostracods.

Chironomus riparius Exposure

Overlying Water Quality Characterization--

During the Indiana Harbor C. riparius exposure, overlying water pH, dissolved oxygen, and hardness were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.140 mg/L in all of the treatments, with the highest unionized ammonia level, (0.320 mg/L) in overlying water from Station IH-01-06 (Table 4.10). Unionized ammonia in the control overlying water was 0.055 mg/L (Table 4.10). Alkalinity in all of the treatments, including control, were about 1.5 times greater than the in-flowing reconstituted water (Table 4.10). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride was 1.5 to two times greater in all of the treatments (except control) relative to the

reconstituted water (Table 4.10). Turbidity in the treatments was about two times greater than control (Table 4.10).

Toxicity--

All of the Indiana Harbor sediments were toxic to C. riparius (Table 4.11). Whereas 75% of the larvae survived in the control, survival in sediment from Indiana Harbor was 0 to 2.0% (Table 4.11). Body length was not measured for the limited number of survivors.

An oily film developed on the overlying water surface of all treatments. Controls were fed algae and Hartz[R] dog treats Cerophyl[R] throughout the study. All test chambers containing sediment from Indiana Harbor stations were fed only algae between Day 5 and the end of the test, because of surface sediment mold. Oligochaetes, fingernail clams, and snails were found in the Indiana Harbor sediments at the end of the study.

Chironomus tentans Exposure

Overlying Water Quality Characterization--

In the C. tentans whole sediment exposure, unionized ammonia concentrations were greater than 0.292 mg/L in all of the treatments, with the highest unionized ammonia level, (1.110 mg/L) in overlying water from Station IH-01-07 (Table 4.10). Alkalinity in all of the treatments, including control, was about 1.5 to two times greater than the reconstituted water (Table 4.10). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride was 1.5 to two times greater in all of the treatments (including control) relative to the reconstituted water (Table 4.10). Turbidity in all treatments was about two to four times greater than control (Table 4.10).

Toxicity--

All C. tentans exposed to sediment from Stations IH-01-06 and IH-01-07 died within 10 days. Survival and growth (body length) of C. tentans larvae exposed to sediment from Stations IH-01-03 and IH-01-04 were significantly reduced compared to the control sediment (Table 4.11).

Black sediment surface mold required withholding food for two days in the control sediment, one day in Stations IH-01-03 and IH-01-04, and three days in Stations IH-01-06 and IH-01-07. An oil sheen was observed on the overlying water surface of the exposure chambers containing sediment from Stations IH-01-03, IH-01-04 and IH-01-07. Indigenous species were not observed at the end of the C. tentans exposure.

BUFFALO RIVER

Physical and Chemical Characterization

Concentrations of TOC, total solids, and organometals in Buffalo River sediments are listed in Table 4.12. With the exception of Station BR-01-01 that contained about 9.0% TOC, concentrations of TOC in the Buffalo River sediments were similar ($\leq$ 2.0%). Solids ranged from 30% in sediment from

Station BR-01-01 to 74% in sediment from Station BR-01-02. Organometal concentrations in the Buffalo River samples ranged from less than 0.05 ng/dry g for methylmercury to 36 ng/dry g for tributyltin.

Metals in sediment from Station BR-01-01 were elevated over sediments from all other Buffalo River sediment (Table 4.13). The Buffalo River samples contained a broad range of metal concentrations (Table 4.13): Cr, Cu, Mn, Pb, and Zn ranged from less than 1.0 μg/dry g to 1386 μg/dry g; all other metals were ≤ 57 μg/dry g. Concentrations of AVS and eight SEM for Buffalo River stations were analyzed (Table 4.4). The AVS in sediments from Buffalo River stations ranged from 1.0 μM/dry g (BR-01-02) to 161 μM/dry g (BR-01-01). All SEM/AVS ratios were less than one (Table 4.4).

The distribution of sizes for sediment particles in the Buffalo River sediments are shown in Table 4.14. With the exception of Station BR-01-02, the Buffalo River sediments consisted primarily of particles that were less 0.038 mm in size (43.7% to 92.0%); Station BR-01-02 consisted mainly of particles that ranged from 0.063 to 0.25 mm (58.5%) and from 0.25 to 1.0 mm (38.5%) (Table 4.14). Particles greater than 1.0 mm in size comprised only a small portion (0.1% to 14.0%) of the Buffalo River sediments (Table 4.14).

Concentrations of PAHs in Buffalo River sediments ranged from less than 18 ng/dry g for dichlorobenzene to 59000 ng/dry g for bis-(2-ethylhexyl) phthalate (Table 4.15). Polychlorinated dibenzo-dioxins and -furans in the Buffalo River samples ranged from less than the analytical detection limit up to 12,000 pg/dry g for octachlorodibenzodioxin (Table 4.16). Aroclors and pesticides in all samples from Buffalo River were below analytical detection limits (Tables 4.8 and 4.9).

Station BR-01-01, located nearest to the end of the shipping canal, had the most distinctive physical and chemical characteristics compared to the other sediments collected at the Buffalo River study area. Sediment from Station BR-01-01 had the lowest dry weight and a TOC value more than four times higher than other stations. In addition, Station BR-01-01 had the highest measured values for AVS (Table 4.4), methylmercury, tri-, di-, and monobutyltin (Table 4.12), nine out of 12 metals (Table 4.14); 13 out of 20 semi-volatile organic compounds (Table 4.15); and 22 out of 25 PCDDs and PCDFs (Table 4.16). Sediments from Station BR-01-07 and BR-01-08 had the lowest TOC values of any Buffalo River sediments tested, and also had intermediate to lower levels of AVS, methylmercury, and tri-, di- and monobutyltin, compared to other sediments tested.

Hyalella azteca Exposures

Overlying Water Quality Characterization--

In the Buffalo River amphipod 14- and 28-d whole sediment exposures, overlying water dissolved oxygen and pH were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.052 mg/L in all of the treatments, with the highest unionized ammonia level, (0.117 mg/L) in overlying water from Station BR-01-09 (28-d exposure) (Table 4.17). Unionized ammonia in control overlying water was 0.004 mg/L (Table 4.17). Alkalinity, in all of the treatments, including control, was about 1.5 times greater (mean alkalinity for Station BR-01-09 was three times greater) than the in-flowing reconstituted water (Table 4.17). Overlying water hardness was elevated about 1.5 times for sediment from

Station BR-01-09 over the reconstituted water (Table 4.17). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride was 1.5 to two times greater in all of the treatments (including control), relative to the reconstituted water (Table 4.17). Turbidity in all treatments (except BR-01-07) of the 14-d Hyalella azteca exposure was elevated about four to eight times relative to control; Station BR-01-07 in the 14-d exposure was elevated about 21 times; and Station BR-01-07 of the 28-d exposure, was elevated about four times (Table 4.17).

Toxicity--

Amphipod 14-d survival in sediments from Station BR-01-03 was significantly reduced. Additionally, amphipod 28-d survival was significantly reduced in sediments from Stations BR-01-01, BR-01-03, BR-01-07 and BR-01-09 (Table 4.18). Amphipods exposed to sediments from Station BR-01-03 exhibited the lowest survival for both 14-d (63.8%) and 28-d (75.0%) exposures. Body length for surviving 14-d H. azteca was significantly reduced in exposures from all stations, but body length was significantly reduced in 28 days only in sediments from Stations BR-01-03 and BR-01-07 (Table 4.18). Similarly, amphipods exposed to sediments from BR-01-03 and BR-01-07 in the 28-d exposure exhibited significant reduction for increase in length (Figure 4.1). The number of antennal segments in the 14-d control amphipods was significantly reduced for all stations, except Station BR-01-09, but was the same between Stations BR-01-01 and BR-01-08, and Stations BR-01-03 and BR-01-07. Amphipods in the 28-d sediment exposure exhibited no significant differences for number of antennal segments and had similar number of segments to the 14-d amphipods (Table 4.18). Sexually mature amphipods for the 14-d exposure was significantly different (6.1%) in sediments from all stations except BR-01-03 (4.2%), however, no significant reduction in percent sexually mature was evident at the end of the 28-d exposure (Table 4.18).

Except for the control sediment, a film was present on the overlying water surface in all Buffalo River sediments, in both the 14- and 28-d exposures. In the 28-d exposure, at least one feeding was withheld from all stations, except for BR-01-08, because of sediment surface mold. No amphipods were observed during the 14- or 28-d exposures. Indigenous species observed at the end of the 14- and 28-d tests included oligochaetes, ostracods, and one leach in station BR1-07 of the 14-d test.

Chironomus riparius Exposure

Overlying Water Quality Characterization--

In the Buffalo River C. riparius whole sediment exposure, overlying water dissolved oxygen and pH were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.051 mg/L in all treatments, with the highest unionized ammonia level (0.118 mg/L) in overlying water from Station BR-01-09 (Table 4.17). Unionized ammonia in control overlying water was 0.004 mg/L (Table 4.17). Alkalinity, in all treatments, including control, were about 1.5 times greater (about three times greater in Station BR-01-09) than the reconstituted water (Table 4.17). Water hardness was elevated about 1.5 times in sediment from Station BR-01-09 over

the reconstituted water (Table 4.17). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride was 1.5 to two times greater in all of the treatments relative to the reconstituted water (Table 4.17). Turbidity in all treatments was elevated about seven to 15 times relative to control (Table 4.17).

Toxicity--

Control survival (61%) was not within the limits of test acceptability (70%) (ASTM E 1383-90) so C. riparius Buffalo River data should be interpreted with caution. Survival of C. riparius was significantly reduced in sediments from Stations BR-01-07 or BR-01-08 (Table 4.18). Survival did not differ significantly in sediments from Stations BR-01-01, BR-01-03 and BR-01-09 (Table 4.18). Only C. riparius larvae exposed to Station BR-01-03 sediment were significantly smaller than larvae exposed to the control and the other sediments, except Station BR-01-08. Body length did not differ significantly for C. riparius larvae exposed to the control or sediments from Stations BR-01-01, BR-01-07, BR-01-08, and BR-01-09 (Table 4.18).

By Day 7 all test sediments exhibited surface mold which temporarily required withholding Hartz^R dog treats. Beginning at Day 8, and continuing until the end of the exposures, the amount of Hartz^R dog treats added to the test chambers was decreased by 50%, to minimize development of sediment surface mold. At the end of the exposures, snails, oligochaetes and several unidentified midge larvae were found in the Buffalo River sediments.

Chironomus tentans Exposure

Overlying Water Quality Characterization--

In the Buffalo River C. tentans whole sediment exposure, overlying water dissolved oxygen, and pH were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.066 mg/L in all treatments, with the highest unionized ammonia level, (0.559 mg/L) in overlying water from Station BR-01-01 (Table 4.17). Unionized ammonia in the control overlying water was 0.547 mg/L (Table 4.17). Alkalinity, in all of the treatments, including control, were about 1.5 times greater (about three times greater in sediment from Station BR-01-09) relative to the reconstituted water (Table 4.17). Overlying water hardness was elevated about 1.5 times for all treatments, except sediments from Station BR-01-03 over the reconstituted water (Table 4.17). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride was three to seven times greater in all of the treatments relative to the reconstituted water (Table 4.17).

Toxicity--

Survival of C. tentans was not significantly reduced in the Buffalo River test (Table 4.18). While larval dry weight was reduced in sediments from Stations BR-01-07 and BR-01-08, no significant effect was observed in sediments from Stations BR-01-01, BR-01-03 or BR-01-09 (Table 4.18).

Sediment surface mold required withholding food for five days for the control sediments and sediment from Station BR-01-01; six days for BR-01-03; seven days for sediments from Stations BR-01-08 and BR-01-09; and eight days for sediment from Station BR-01-07. Indigenous species were not observed when test organisms were recovered at the end of the C. tentans exposure.

SAGINAW RIVER (1)

Physical and Chemical Characterization

Concentrations of TOC, total solids, and organometals in sediments from the Saginaw River (1) are listed in Table 4.19. With the exception of sediments from Station SR-01-10 that contained less than 1.0% TOC, TOC in Saginaw River were similar and ranged from 2.0 to 3.8%. Solids ranged from 33.0% in sediment from Station SR-01-07 to 64.0% in sediment from Station SR-01-10. Sediments contained low (ng/dry g) concentrations of organometals.

Saginaw River (1) sediments contained a broad range of metal concentrations: Cr, Cu, Mn, Pb, and Zn ranged from 16.0 μg/dry g for Cu in Station SR-01-10 to 819 μg/dry g for Mn in Station SR-01-03 (Table 4.20). With exception of Fe, that ranged from 1.2 to 3.2% in Saginaw River (1) sediments, all other metals were ≤ 157 μg/dry g (Table 4.20).

Concentrations of AVS and eight SEM for Saginaw River (1) sediments were analyzed (Table 4.21). The AVS in Saginaw River (1) sediments ranged from 1.5 μM/dry g in Station SR-01-10 to 15.5 μM/dry g in Station SR-01-06. All SEM/AVS ratios were less than one (Table 4.21).

Sediment samples from Saginaw River (1) consisted primarily of particles that were less than 0.038 mm in size (19.4% to 79.3%), and particles that ranged from 0.063 mm to 0.25 mm (10.4% to 45.9%). Particles greater than 1.0 mm in size comprised only 0.1% to 5.4% of the samples (Table 4.22).

Concentrations of PAHs and other semivolatile compounds in Saginaw River (1) sediments ranged from less than the analytical detection limit of 4.0 ng/dry g for dichlorobenzene up to 13000 ng/dry g for bis(2-ethylhexyl) phthalate (Table 4.23). Saginaw River (1) sediments contained PCDDs and PCDFs that ranged from 1.7 pg/dry g for 1,2,3,4,7,8-hexachlorodibenzodioxin in Station SR-01-10 to 22,000 pg/dry g for total tetrachlorodibenzofuran (TCDD) in Station SR-01-04 (Table 4.24). Levels of PCBs measured in sediment ranged from 300 ng/dry g (Arochlor 1254) to 60,000 ng/dry g (Aroclor 1232) in sediment from Station SR-01-06 (Table 4.8). Pesticides in sediments from Saginaw River (1) were below analytical detection limits (Table 4.9).

Hyalella azteca Exposures

Overlying Water Quality Characterization--

Amphipod 14- and 28-d exposures to Saginaw River (1) sediments, overlying water dissolved oxygen, hardness, and pH were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.021 mg/L in all of the treatments, with the highest unionized ammonia level, (0.064 mg/L) in overlying water from Station SR-01-06 for both the 14- and 28-d exposures (Table 4.25). Unionized ammonia in the control overlying water were 0.003 mg/L (14-d exposure) and 0.004 mg/L (28-d exposure) (Table 4.25). Alkalinity, in all of the treatments except control, was

elevated about two times compared to the reconstituted water (Table 4.25). Alkalinity for the control overlying water was 48 mg/L in the 14-d exposure, and 66 mg/L in the 28-d exposure (Table 4.25). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride, in all of the treatments, was elevated two times compared to the reconstituted water (Table 4.25). Turbidity in all treatments was elevated about four to 10 times relative to control (Table 4.25).

Toxicity--

Amphipod 14-d and 28-d survival in sediments from Station SR-01-06 was significantly reduced. Survival of amphipods ranged from 92.5% in sediments from Station SR-01-03 to 10.0% in sediments from Station SR-01-06 (Table 4.26). No significant differences were detected in the 14-d exposure sublethal responses (body length, number of antennal segments and percent mature), but was significantly reduced in sediment exposures from Station SR-01-06 and Station SR-01-03 in the 28-d exposure (Table 4.26). Increase in 28-d amphipod body length was significantly reduced in exposures from Stations SR-01-03 and SR-01-06 sediments (Figure 4.1).

In the 28-d exposure, gravid females or amplexed pairs were observed in the control and in Station SR-01-10, however no young were isolated at the end of the test. By Day 1, amphipods were observed floating on the water surface in Station SR-01-06 of the 28-d exposure. In the 14-d exposure, at least one feeding was withheld from the control and Station SR-01-06 because of mold, and a build-up of food on the sediment surface. Indigenous species observed at the end of the 14- and 28-d tests included oligochaetes, ostracods, and cyclops. In sediment from Station SR-01-06, only one oligochaete was observed.

Chironomus riparius Exposure

Overlying Water Quality Characterization--

In the Saginaw River (1) C. riparius exposure, overlying water dissolved oxygen, hardness, and pH were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.021 mg/L in all of the treatments, with the highest unionized ammonia level (0.063 mg/L) in overlying water from Station SR-01-06 (Table 4.25). The unionized ammonia concentration for the control overlying water was 0.003 mg/L (Table 4.25). Alkalinity, in all of the treatments except control, were elevated about two times compared to the in-flowing reconstituted water (Table 4.25). Alkalinity for the control overlying water was 34 mg/L about 0.5 times less than the reconstituted water (Table 4.25). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride, in all of the treatments, was elevated two times compared to the reconstituted water (Table 4.25). Turbidity in all treatments was elevated about three to six times compared to control (Table 4.25).

Toxicity--

Both survival and body length were significantly reduced among larvae exposed to sediment from Station SR-01-06 (Table 4.26). Survival and body length of C. riparius in sediment exposures from Stations SR-01-03 and SR-01-10 did not differ significantly (Table 4.26).

An oily surface water film was observed in all test chambers beginning at Day 5. By Day 7, mold on the sediment surface required withholding Cerophyl[R] in all treatments. Surface sediment conditions in test chambers containing Station SR-01-06 and control sediment were generally clearer than sediments from Stations SR-01-03 and SR-01-10. Additions of Hartz[R] dog treats were withheld from Stations SR-01-03 and SR-01-10 during the last four days of the exposure because of sediment surface mold. At the end of the exposures, oligochaetes were observed in the sediments from Saginaw River (1).

Chironomus tentans Exposure

No exposures with Chironomus tentans were conducted with samples collected from the Saginaw River (1) because of problems with the cultures.

SAGINAW RIVER (3)

Physical and Chemical Characterization

Concentrations of TOC, total solids, and organometals in sediments from Saginaw River (3) are listed in Table 4.27. With the exception of the sediment grab sample from Station SR-03-06 that contained 0.2% TOC, TOC in the Saginaw River (3) grab samples ranged from 1.0% to 4.0%. Total organic carbon in core samples from Saginaw River (3) ranged from 0.6% in Station SR-03-06-02 to 5.0% in Station SR-03-02-X2. Solids ranged from 47.3% to 76.4% in Saginaw River (3) sediment grab samples and from 49.2% to 76.3% in sediment core samples. The Saginaw River (3) grab samples contained low concentrations of organometals that ranged from less than 0.1 ng/dry g for methylmercury to 10.0 ng/dry g for tributyltin.

Generally, the sediments from Saginaw River (3) contained a broad range of low (μg/dry g) metal concentrations. In grab samples, Cr, Cu, Mn, Pb, and Zn ranged from 16.9 μg/dry g for Pb in Station SR-03-06 to 549 μg/dry g for Mn in Station SR-03-24; all other metals in the grab samples were ≤ 92.9 μg/dry g (Table 4.28).

AVS in Saginaw River (3) grab samples ranged from 1.2 μM/dry g in Station SR-03-06 to 8.9 μM/dry g in Station SR-03-24 (Table 4.21). The estimated SEM/AVS ratios were all less than one (Table 4.21).

Sediments from Saginaw River (3) consisted primarily of particles that were less 0.038 mm in size (2.3% to 54.9%) and particles that ranged from 0.063 mm to 0.25 mm (22.7% to 77.7%) (Table 4.29). Particles greater than 1.0 mm in size comprised only 0.1% to 1.4% of the samples (Table 4.29).

Generally, PAHs in the sediments from Saginaw River (3) were below analytical detection limits, but concentrations of some PAHs were detected (Table 4.30). For example, pyrene ranged from less than the detection limit up to 1800 ng/dry g in grab samples and up to 2700 ng/dry g in core samples, and phenanthrene ranged from less than detection limits up to 1000 ng/dry g in grab samples (Table 4.30).

The samples from Saginaw River (3) contained PCDDs that ranged from less than detection limits up to 6400 pg/dry g of octachlorodibenzodioxin in grab sample SR-03-08, and up to 74000 pg/dry g of heptachlorodibenzofuran in core sample SR-03-02-X2 (Table 4.31). Total dioxins ranged from 5.7 pg/dry g in sediment from Station SR-03-06 to 230 pg/dry g in sediment from Station SR-03-08; and total furans 230 pg/dry g in sediments from Station SR-03-01 (first replicate) to 3100 pg/dry g in sediments from Station SR-03-06 (Table 4.31).

With the exception of Endrin ketone, which ranged from 2.0 to 380 ng/dry g, pesticides in sediments from the Saginaw River (3) were below analytical detection limits or present at low ng/dry g concentrations (Table 4.32). In Saginaw River (3) sediment, Aroclor 1260 was generally below detection limits; Aroclor 1254 ranged from less than detection limits up to 95 ng/dry g in grab samples and up to 8100 ng/dry g in core samples; and Aroclor 1242 ranged from less than detection limits up to 470 ng/dry g in grab samples and up to 79,000 ng/dry g in core samples (Table 4.33).

Hyalella azteca Exposures

Overlying Water Quality Characterization--

In the Saginaw River (3) amphipod 14- and 28-d whole sediment exposures, overlying water dissolved oxygen, hardness, alkalinity, pH, and chloride were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.019 mg/L in all treatments, with the highest unionized ammonia level (0.118 mg/L, 14-d exposure; and 0.101 mg/L; 28-d exposure) in overlying water from Station SR-03-24 (Table 4.34). Unionized ammonia for the control overlying water was 0.005 mg/L (14-d exposure) and 0.004 mg/L (28-d exposure) (Table 4.34). Dissolved oxygen was at acceptable levels (greater than 40 percent of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Turbidity in all treatments, except Station SR-03-01, was elevated about three to eight times compared to control, but turbidity in SR-03-01 was similar to control (Table 4.34).

Toxicity--

Amphipod survival, body length and number of antennal segments were not significantly reduced in 14-d or 28-d exposures, for all Saginaw River (3) stations (Table 4.35). However, in the 28-d exposure, increase in amphipod body length was reduced significantly in sediment exposures from Stations SR-03-06 and SR-03-08 (Figure 4.1). Amphipods exposed to sediment from Station SR-03-05 exhibited delayed sexual maturation in the 14-d exposure.

In both the 14- and 28-d exposures, mold was present on the sediment surface in all treatments, except Station SR-03-01. A film was present on the water surface of all treatments in the 28-d exposure, and in all treatments, except the control and Station SR-03-01, of the 14-d exposure. One feeding was withheld from the control treatment in the 28-d exposure due food build-up on the sediment surface. Gravid females were observed in sediments from Stations SR-03-16 and SR-03-24 at the end of the 14-d exposure, however, no young were isolated. Indigenous species observed at the end of the 14- and 28-d tests were oligochaetes, most notably in Station SR-03-01.

Chironomus riparius Exposure

Overlying Water Quality Characterization--

In the Saginaw River (3) whole sediment C. riparius exposure, overlying water dissolved oxygen, hardness, alkalinity, pH, and chloride were similar to in-flowing reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.015 mg/L in all of the treatments, with the highest unionized ammonia level (0.101 mg/L) in overlying water from Station SR-03-24 (Table 4.34). Unionized ammonia in the control overlying water was 0.004 mg/L (Table 4.34). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Turbidity in all treatments, except Station SR-03-01, was elevated about three to five times compared to control, but turbidity in the sediment exposure from Station SR-03-01 was similar to control.

Toxicity--

Survival of C. riparius larvae was not significantly different in sediments for all stations from Saginaw River (3) (Table 4.35). Larval body length exposed to sediments from all Saginaw River (3) stations was significantly greater than that of C. riparius larvae exposed to the control sediment. For C. riparius, body length ranged from 10.1 mm for the control to 12.5 mm for Stations SR-03-02 and SR-03-16 (Table 4.35).

All treatments were fed Cerophyl^R, Hartz^R dog treats and algae for the first six days of the test. Beginning Day 7, additions of Hartz^R dog treats were withheld from four of the seven Saginaw River (3) sediments. All feeding was withheld on Day 10 and aeration started in all treatments, due to a combination of low dissolved oxygen and the presence of black mold on the surface of the sediments. Aeration was continued until the end of the test. Surface mold disappeared after aeration started. Oligochaetes and snails were found in the Saginaw River (3) sediments at the end of the exposures.

Chironomus tentans Exposure

Overlying Water Quality Characterization--

In the Saginaw River (3) C. tentans exposure, overlying water dissolved oxygen, hardness, and pH, were similar to reconstituted water described earlier. Unionized ammonia concentrations were greater than 0.074 mg/L in all treatments, with the highest unionized ammonia level (0.886 mg/L) in overlying water from Station SR-03-01 (Table 4.34). Unionized ammonia in the control overlying water was 0.376 mg/L (Table 4.34). Dissolved oxygen was at acceptable levels (greater than 40% of saturation, ASTM E 1383-90) in all treatments throughout the exposure. Chloride, in all treatments except Station SR-03-01, were elevated about two times compared to the reconstituted water (Table 4.34). Turbidity in all treatments (except Station SR-03-01) was elevated about three times compared to control, but turbidity in the sediment exposure from Station SR-03-01 was similar to control.

Toxicity--

Survival of C. riparius in the control (60%) was less than the recommended limit for test acceptability (70%) (ASTM E 1383-90), therefore, Saginaw River (3) whole sediment exposure results should be interpreted with caution. Survival of C. tentans exposed to Saginaw River (3) stations ranged from 0 to 80% (Table 4.35). Samples were grouped into two general classes, those exhibiting survival greater than the control (SR-03-05, SR-03-08 and SR-03-24) and those with survival less than the control (SR-03-01, SR-03-02, and SR-03-06, and SR-03-16). No C. tentans survived in Station SR-03-06 and was the only treatment significantly different from control (Table 4.36). Due to difficulties controlling the air flow during these exposures, several chambers in the study had increased overlying water evaporation. In three treatments: control, SR-03-01 and SR-03-08, overlying water evaporated resulting in the loss of test organisms.

Except in the control, mold developed by Day 5 on the surface of all Saginaw River (3) sediments. Sediment surface mold required withholding food for the last five test days in all Saginaw River (3) sediments. Indigenous species were not observed at the end of the test.

DISCUSSION

INDIANA HARBOR

Overlying Water Quality Characteristics

Overlying water quality for the amphipod and midge exposures differed from the in-flowing reconstituted water in unionized ammonia, chloride, alkalinity and turbidity. Chloride levels in the overlying water were 1.5 and two times greater in all treatments compared to control. Culture water chloride levels are about 20 mg/L (Kemble et al. 1992) indicating that the two-fold chloride increase in the Indiana Harbor exposures would probably not be directly toxic to amphipods or midges.

Unionized ammonia concentrations in the overlying water were $\geq$ 0.137 mg/L which is above the 4-d unionized ammonia criterion of 0.035 mg/L at pH 8.0 and 20°C (USEPA 1986b). However, unionized ammonia concentrations of about 1 mg/L were not toxic to either amphipods or C. tentans in 10-d water only exposures (G. Ankley, USEPA, Duluth, MN, personal communication). Unionized ammonia, in treatment IH-01-07, of the 10-d C. tentans exposure, was 1.110 mg/L, high enough that it may have contributed to the observed toxicity. However, amphipod and C. riparius exposures had complete mortality in the IH-01-07 sediment exposure, despite having lower unionized ammonia. Unionized ammonia levels in the overlying water of the C. tentans static exposure were higher than unionized ammonia levels in the amphipod and C. riparius treatments. This is most likely because the amphipods and C. riparius were exposed in a flow-through system while the C. tentans exposure was in a static system. In addition, higher test temperature in the C. tentans exposure (C. tentans 23°C; H. azteca or C. riparius 20°C) may also have caused the increase in unionized ammonia levels (Thurston et al. 1974).

Toxicity Tests

The reductions in survival of amphipods, C. riparius or C. tentans, exposed to whole sediments from Indiana Harbor, indicate that the quality of sediments is not adequate to sustain populations of the test organisms or organisms of equal or greater sensitivity. Higher trophic level organisms which exploit midge larvae or amphipods as food items would likely be reduced within the reaches of the Indiana Harbor sediments sampled for these exposures, due to the probable absence of these benthic organisms.

Because all of the sediments from Indiana Harbor elicited severe effects on test organism survival, spatial differences in sediment contamination were difficult to identify. Observed effects on survival could not be attributed to any specific contaminant measured in the Indiana Harbor sediments (Figures 4.2 and 4.3). The presence of concentrations of a broad range of contaminants in the sediments provides additional evidence that poor sediment quality exists in Indiana Harbor. However, because the sediments had a complex mixture of contaminants, interactive toxicity (i.e., additivity, synergism, or antagonism) (Marking 1985) may have occurred during the exposures.

Given the extreme spatial differences in sediment toxicity reported even in small study areas (Stemmer et al. 1990), combined with the limited number stations tested in the present study, it is difficult to determine if a gradient of toxicity exists at Indiana Harbor. However, C. tentans test

results suggest a trend. Samples collected from Indiana Harbor (Stations IH-01-03 and IH-01-04) exhibited less toxicity compared to samples collected from the main channel (Station IH-01-06) or at the junction of the Lake George and Grand Calumet Branch (Station IH-01-07). Sediments from the two up-channel locations had elevated metals and organic compound concentrations compared to the other Indiana Harbor stations.

Sediment from Station IH-01-07, despite having an intermediate TOC level, compared to other Indiana Harbor sediments, generally contained the highest concentration of organic contaminants (Table 4.37). Station IH-01-07 also had the highest measured metal concentrations (Tables 4.3 and 4.4). This chemical profile suggests that Station IH-01-07, located at the convergence of the Grand Calumet Branch and the Lake George Branch, may be accumulating contaminants from both systems.

Together, Stations IH-01-06 and IH-01-07 had the highest concentrations of 16 out of 20 measured PAH. Of the 25 PCDDs and PCDFs measured, Stations IH-01-06 and IH-01-07 accounted for 23 of the highest measurements. Interestingly, Station IH-01-03 (collected from Indiana Harbor proper) which had the lowest toxicity to C. tentans, also contained the highest concentrations of the toxic 2378 TCDD isomer (Helder 1981, Walker and Peterson 1991). These findings suggest that either 2378 TCDD is not toxic to C. tentans larvae at these concentrations, or that it was not bioavailable under the conditions tested.

BUFFALO RIVER

Overlying Water Quality Characteristics

Overlying water quality for the amphipod and midge Buffalo River whole sediment exposures were similar to the in-flowing reconstituted water (except unionized ammonia, and chloride). Culture water chloride levels are about 20 mg/L (Kemble et al. 1992) indicating that the two-fold chloride increase in the Indiana Harbor exposures would probably not be directly toxic to amphipods or midges. Survival of C. tentans was not adversely affected even with a four-fold increase in chloride levels. Unionized ammonia concentrations in the overlying water in all treatments, except control, were $\geq$ 0.051 mg/L which were above the 4-d unionized ammonia criterion of 0.035 mg/L at pH 8.0 and 20°C (USEPA 1986b). However, unionized ammonia concentrations of about 1 mg/L were not toxic to either amphipods or C. tentans in 10-d water only exposures (G. Ankley, USEPA, Duluth, MN, personal communication). Unionized ammonia levels in the C. tentans exposure were higher than in the amphipod and C. riparius treatments. This is most likely because the amphipods and C. riparius were exposed in a flow-through system while the C. tentans exposure was in a static system. However, higher test temperature in the C. tentans exposure (C. tentans 23°C; H. azteca or C. riparius 20°C) may also have caused the increase in unionized ammonia levels (Thurston et al. 1974).

Toxicity Tests

Buffalo River sediment exposures were not consistently toxic to amphipods or C. riparius. Amphipods identified toxicity (i.e., reduced survival) in four of the five stations, whereas C. riparius identified two of the five stations (Table 4.18). Amphipods were more sensitive than the midge

in identifying toxicity in sediment from Buffalo River stations. In addition, the results imply that differences in the spatial distribution of contaminant loads existing in the Buffalo River stations may adversely influence survival of H. azteca or C. riparius populations, or organisms of equal or greater sensitivity. This implication is supported by the broad range in concentrations of heavy metals, PAHs, and dioxins that were measured in Buffalo River sediments (Tables 4.4, 4.12, 4.15 and 4.16). Differences in the spatial distribution of contaminants can result from differences in contaminant loading patterns, differences in contaminant cycling processes, or contaminant transport (Burton 1991).

Of the five sediment samples tested, metal concentrations where higher in stations closer the mouth of the river (Stations BR-01-01 and BR-01-02) compared to stations sampled further up-stream (Stations BR-01-07, BR-01-08, BR-01-09). Organic contaminants measured in the sediments did not follow the trend of progressively higher concentrations near the mouth of the river as seen with metals. While organic contaminants increased between Station BR-01-09 and BR-01-07, in many cases concentrations of organic contaminants were lower in Station BR-01-08 compared to Station BR-01-09. Growth effects were not seen in sediments from Station BR-01-01 for midge or amphipods (28-d), despite the higher levels of metal and organic contaminants found compared to Station BR-01-08, in which amphipods (14-d) and C. tentans larvae showed significant growth effects (Table 4.18). The high TOC found in sediment from Station BR-01-01 may have minimized the availability of the PAHs, dioxins, and furans and reduced their toxicity (Table 4.37).

The adverse effects of Buffalo River sediment on amphipod (14- and 28-d) suggest that contaminants were bioavailable, but that a longer exposure (28-d) was necessary to demonstrate effects on survival. In contrast though, the 28-d exposure produced significant differences in growth (body length) at two stations, while growth effects (body length) were observed at all stations in the 14-d exposure (Table 4.18), which constituted a 50% reduction in number of stations producing effects in 28 days. Percentage sexual maturation was significantly different at four stations at 14-d, but by 28 days all amphipods were sexually mature and not significantly different.

The amphipod Buffalo River whole sediment exposures demonstrate the need to conduct both short- and long-term exposures to measure both lethal and sublethal effects, as the information obtained is not always predictable from one exposure or one endpoint. Disproportionate survival from the 14- to 28-d exposure can be expected if the sediment-sorbed contaminants are slow to accumulate in organisms (Landrum 1988), produce delayed effects at the site of action (Landrum et al. 1985), or exhibit slow desorption kinetics (i.e., increased equilibration time between the contaminant and sediment) (Kenega and Goring 1980, Karickhoff 1981, Landrum and Robbins 1990). For a better understanding of the desorption kinetics of non-labile contaminants and its relationship to bioavailability, rate limiting factors of sediment-sorbed contaminants needs further investigation. But, if volatile contaminants are lost from the test system or toxic compounds degrade, increased survival may be expected in the 28-d exposures, in which case further investigation is needed to assess the fate of the labile and degradable compounds in such sediment test systems.

Effects of Buffalo River whole sediment on the survival and body length of C. riparius were less severe than the effects elicited by Indiana Harbor sediments; however, the Buffalo River results must be cautiously interpreted

because survival of C. riparius (60.5%) in the control was less than the 70% recommended by ASTM E1383-90 (1990). These effects of Buffalo River sediments on C. riparius were consistent with overall lower measured concentrations of heavy metals, PAHs, and dioxins in the Buffalo River sediments compared to Indiana Harbor.

The Buffalo River sediments did not elicit uniform toxicity to C. riparius. Survival of C. riparius from Stations BR-01-01, BR-01-03 and BR-01-09, were similar to control. Sediments from Stations BR-01-07 and BR-01-08 elicited effects on survival similar to sediments from Stations BR-01-01 and BR-01-03, but were significantly different from the control and the sediment from Station BR-01-09 (Table 4.18). The only significant effect elicited by exposure of C. tentans to Buffalo River sediments was the decreased weight of organisms exposed to sediments from Station BR-01-07 and BR-01-08. Otherwise, Buffalo River contaminated sediments were not toxic to C. tentans.

Exposure of C. tentans to sediment collected from Station BR-01-01 did not result in significant decreases in survival or length. Two hypotheses may be postulated to explain the lack of effects noted during the 10 day C. tentans exposure to sediments from Station BR-01-01. The high TOC found in sediment from Station BR-01-01 may have minimized the availability of the PAHs, dioxins, and furans and reduced their toxicity (Table 4.37). The other possibility is that the duration of the test (10-d) was insufficient for contaminants present to produce toxic effects.

Withholding food to prevent additional growth of mold on the sediment surface during the C. tentans exposures may have contributed to the decrease in organism length in sediments from Stations BR-01-07 and BR-01-08. During the 10 day exposures, food was withheld from chambers containing sediments from Station BR-01-07 a total of eight days, and larvae exposed to sediments from Station BR-01-08 were not fed a total of seven days. In comparison, organisms exposed to sediment collected from Stations BR-01-01, BR-01-03 and the control sediment were not fed on four to five days during the exposure period. However, results of the C. tentans test conducted with sediment collected from Station BR-01-09 with similar physical and chemical characteristics contradicts the possibility of a feeding effect. Similar to tests with sediment from Stations BR-01-07 and BR-01-08, the larvae exposed to sediment from Station BR-01-09 were not fed for seven days but showed no decrease in length by Day 10.

SAGINAW RIVER (1) and (3)

Overlying Water Quality Characteristics

Overlying water quality in the amphipod and midge exposures to Saginaw River sediment were similar to the in-flowing reconstituted water (except unionized ammonia for all exposures). Unionized ammonia concentrations for H. azteca and C. riparius in the whole sediment overlying water were $\geq$ 0.040 mg/L in all treatments (except treatments SR-03-01, SR-03-06, and control), which is above the 4-d unionized ammonia criterion of 0.035 mg/L at pH 8.0, and 20°C (USEPA 1986b). Unionized ammonia concentrations in the 10-d C. tentans exposures were $\geq$ 0.074 mg/L, which is above the 4-d unionized ammonia concentration of 0.035 mg/L at pH 8.0 and 20°C (USEPA 1986b). Unionized ammonia levels in the C. tentans exposures were higher than unionized ammonia levels in the amphipod and C. riparius exposures. This is most likely because

the amphipod and C. riparius exposures were in a flow-through system while the C. tentans exposure was in a static system. In addition, higher temperature in the C. tentans exposure (C. tentans 23°C; H. azteca or C. riparius 20°C) may also have caused the increase in unionized ammonia levels (Thurston et al. 1974). However, unionized ammonia concentrations of about 1 mg/L were not toxic to either amphipods and C. tentans in 10-d water only exposures (G. Ankley, USEPA, Duluth, MN, personal communication).

Although dissolved oxygen in the 10-d C. tentans exposure was in the acceptable range (greater than 40% saturation, ASTM E 1383-90), problems with aeration were common (either no air flow or to much air flow to a tube). This may have caused dissolved oxygen concentrations to drop below 40% saturation during the exposure or caused supersaturation in some of the tubes. Rapid air flow in one case (treatment SR-03-08) bubbled one of the exposure tubes dry.

Toxicity Tests

Test results with whole sediment exposures from Saginaw River indicate that sediment quality in Saginaw River may be more suitable for sustaining H. azteca, C. riparius and C. tentans populations than the sediment quality in Indiana Harbor and Buffalo River. Only the sediment from Station SR-01-06 in Saginaw River (1) elicited significant reductions in the survival and growth for both amphipods and C. riparius. One sediment exposure from Saginaw River (3) adversely effected growth (C. tentans, Station SR-03-06), and one sediment effected amphipod sexual maturation (Tables 4.26 and 4.35). These results were consistent with the generally higher measured concentrations of heavy metals, methylmercury, PAHs, and dioxins in the sediment from Station SR-01-06 compared to the other sediments from Saginaw River. Also, these results suggest a difference in the spatiotemporal distribution of toxicity and contamination in Saginaw River sediments that may influence the distribution and abundance of test organism populations as well as indigenous organisms. Sediment collected from Station SR-03-06, which resulted in complete mortality of exposed C. tentans, had elevated concentrations of PCBs and some tetra- and penta-furans, compared to other stations.

Amphipod survival in Saginaw River (1) exposures was reduced significantly in sediment from Station SR-01-06 in both 14- and 28-d tests. Additionally, effects on body length, antennal segment number and sexual maturation were observed at 28 days (Table 4.26). Body length at 28 days was the most sensitive amphipod sublethal endpoint, since it detected toxicity for all Saginaw River (1) sediments, whereas number of antennal segments and sexual maturation could be predicted from reduced survival (Table 4.26). Sediments from Saginaw River (3) were not lethal to amphipods at 14- or 28-d exposures, and none of the sublethal endpoints added further sensitivity, with the exception of one station (SR-03-05) at 14-d for percentage sexual maturation (Table 4.35).

Samples collected from Saginaw River (1) were not evaluated using C. tentans whole sediment tests. Larvae were not available at the time tests were started.

The validity of the biological response of C. tentans exposed to sediments collected from the Saginaw River (3) is uncertain. Control survival was less than the test acceptability criteria at 70% (ASTM E 1383-90). Low control survival may indicate that the cohort of test organisms used in the exposures may have been unhealthy or subjected to some environmental or

handling stress which decreased their vitality. In addition to low control survival, technical problems with regulating air flow to the chambers resulted in excessive evaporation of three chambers and the need for unusually high water replacement in others. Lastly, due to an attempt to test all grab samples, the number of replicates was reduced from 15 to 10. Exposing fewer individuals enhanced the importance of mortalities in the controls.

COMPARISONS BETWEEN AREAS OF CONCERN AND SPECIES SENSITIVITIES

As mentioned earlier, a broad range of contaminant concentrations were measured in the sediment samples from each AOC. But because of the potential for these contaminated sediments to elicit toxicity as a result of contaminant interactions, the responses observed in the whole sediment tests cannot be attributed to any specific sediment-associated contaminant or component (i.e., particle size, TOC, ammonia). The survival of each species in the whole sediment tests was inversely related to the sum of metal and PAH concentrations in sediments from all stations across AOCs (Figures 4.2 and 4.3). The total metals, associated with high mortality, was less than 5000 μg/dry g for all test species. The total PAHs, associated with high mortality, was about 50 μg/dry g for H. azteca and C. riparius, and about 200 μg/dry g for C. tentans (Figures 4.2 and 4.3).

Understanding the complex interactions of factors that control the bioavailability of sediment-associated organic contaminants is fundamental to evaluating the hazards of contaminated sediments with any degree of certainty. Factors affecting the bioavailability of sediment-sorbed organic compounds, and subsequently, the toxicity that these compounds may elicit include: (1) the chemical characteristics of the contaminants, for example, hydrophobicity, (2) sediment composition and characteristics, for example, total organic carbon and particle size (Förstner 1990, Landrum and Robbins 1990, DiToro et al. 1991), and (3) the behavior and physiological characteristics of the test organisms, for example, burrowing activity, feeding, waste elimination (Riedel et al. 1987, Landrum and Robbins 1990). In the present studies, many of the organic contaminants were hydrophobic, and as such, their bioavailability may be regulated by sediment characteristics, such as organic carbon or fine grain sediment particles. To evaluate organic carbon interactions, concentrations of low and high molecular weight PAHs and total PCBs, were normalized with percentage organic carbon (Table 4.37), then compared with recommended threshold concentrations (Table 4.38). Concentrations of low and high molecular weight PAHs and total PCBs, on a dry weight basis, were also compared to recommended threshold concentrations (Tables 4.37 and 4.38).

Concentrations of total PAHs (ng/dry g) and total PCBs (ng/dry g) exceeded the Effects Range-Median (ER-M), estimates of the concentrations at or above which effects were often detected at the 50th percentile (Long and Morgan 1990), in all Indiana Harbor sediments (Table 4.38), whereas all Buffalo River and Saginaw River (1) sediments only exceeded the PCB threshold, and one sediment (Station BR-01-01) exceeded the total PAH threshold (Table 4.38). The reported total PAH Effects Range-Low (ER-L), an approximation of the concentration at which adverse effects were first detected at the 10th percentile, and ER-M, in Long and Morgan (1990), has a low subjective degree of confidence, and has a moderate degree of confidence for total PCBs. Compared to the Long and Morgan (1990) ER-M reported concentrations, the

recommended threshold values reported by Barrick et al. (1988), based on amphipod Apparent Effects Threshold (AET) concentrations (Table 4.38), identified fewer sediments (Stations IH-01-06 and -07) as elevated for both low and high molecular weight PAHs; and only one sediment (IH-01-03) was identified as elevated for the high molecular weight PAHs (Tables 4.37 and 4.38). The amphipod AET (Barrick et al. 1988) identified all Indiana Harbor, Buffalo River and Saginaw River (1) sediments as elevated for total PCBs, similar to Long and Morgan (1990) (Tables 4.37 and 4.38).

Recommended threshold values from Puget Sound (SMS 1991), established by AET and equilibrium partitioning methods, were contrasted with sediment concentrations that were normalized for TOC (Table 4.38). Similar to amphipod AET thresholds (Barrick et al. 1988) in Tables 4.37 and 4.38, the SMS (1991) thresholds for PAHs, identified three Indiana Harbor sediments as elevated (IH-01-03, -06, and -07). Similar to the AET thresholds reported in Barrick et al. (1988), concentrations of total PCBs exceeded the SMS (1991) recommended threshold concentrations in all Indiana Harbor, Buffalo River and Saginaw River (1) sediments, but the SMS (1991) also identified Saginaw River (3) sediments (SR-03-05, -06, and -08) as elevated for PCBs (Tables 4.37 and 4.38).

Single-compound tests with different TOC concentrations are providing evidence that TOC and contaminant bioavailability are related. Measurement of contaminant concentrations in the sediment pore water (DiToro et al. 1991), rather than in the bulk sediment as in the present study, may better reflect contaminant concentrations that the benthic test organisms encounter. This does not imply that pore water or bulk sediment contaminant concentrations normalized to TOC, are the only, or primary routes of exposure. All exposure pathways are equally capable of contributing to the chemical activity of a compound in an equilibrium analysis (DiToro et al. 1991). Further investigations are needed to evaluate the influence of TOC on organic contaminant bioavailability in sediments containing complex mixtures of contaminants. Further, the relation between recommended threshold concentrations for organic contaminants normalized to TOC or those evaluated on a dry weight basis needs to be established.

Metal concentrations (μg/dry g) measured in the sediments tested in the present study (Tables 4.3, 4.13, 4.20 and 4.28) were compared to recommended threshold concentrations (Table 4.39). Metal concentrations (μg/dry g) that exceeded recommended threshold concentrations (not AVS normalized), reported for Puget Sound (SMS 1991), also exceeded the other reported thresholds, with the exception of the ER-M value (Long and Morgan 1990) for As and Cd (Table 4.39). All the Indiana Harbor sediments contained at least three metals at concentrations that exceeded the SMS (1991) and the Long and Morgan (1990) thresholds, and sediments from Stations BR-01-01, SR-01-06 and SR-03-16 contained two metals that exceeded (Table 4.39). Although metal concentrations (Tables 4.3, 4.13, 4.20 and 4.28) exceeded recommended thresholds (Table 4.39), no relation between AVS and SEM were apparent. All SEM/AVS ratios were less than one (Tables 4.4 and 4.21), indicating that the divalent SEM may not have been bioavailable (DiToro et al. 1990, Carlson et al. 1991). Measuring metals in sediments that are simultaneously released during the AVS extraction is a more reasonable extraction procedure rather than the strong acid total metal extraction procedure, because it provides a better estimate of bioavailable metal concentrations (DiToro et al. 1990). Additionally, the metal concentrations in the sediment can be normalized to

AVS (DiToro et al. 1990). However, additional studies are needed with other metals and complex mixtures of metals to further delineate the influence of SEM/AVS on metal bioavailability in sediments (Carlson et al. 1991).

Sediment contaminants that exceed recommended threshold concentrations are predicted to have an adverse effect on aquatic resources. Comparisons of recommended threshold concentrations and test organism responses (i.e., significant reductions in survival or growth, Tables 4.11, 4.18, 4.26 and 4.35) are listed in Table 4.40. Three situations were observed in comparing the recommended threshold concentrations to observed responses of the test organisms. The first situation was designated as a positive hit (+) where sediment concentrations exceeded recommended thresholds and toxicity occurred, or sediment concentrations did not exceed thresholds and no toxicity occurred (true positive and negative). The second situation was designated as a negative hit (-) where sediment concentrations did not exceed thresholds and toxicity occurred (false negative). The third situation was designated as a zero hit (0) where sediment concentrations did exceed thresholds and no toxicity occurred (false positive). The reported recommended thresholds were ranked according to increasing ability to predict, or not to predict, the test organism responses in the present study.

For positive hits (+) (thresholds were predictive):

Metals -- SMS (1991) > Long and Morgan (1990);

PAHs -- Long and Morgan (1990) > SMS (1991) = Barrick et al. (1988);

PCBs -- Barrick et al. (1988) > SMS (1991) > Long and Morgan (1990).

For negative hits (-) (thresholds not predictive, false negatives):

Metals -- Long and Morgan (1990) > SMS (1991);

PAHs -- SMS (1991) = Barrick et al. (1988) > Long and Morgan (1990);

PCBs -- Long and Morgan (1990) = Barrick et al. (1988) > SMS (1991).

For zero hits (0) (thresholds not predictive, false positives):

Metals -- SMS (1991) = Long and Morgan (1990);

PAHs -- SMS (1991) = Long and Morgan (1990) = Barrick et al. (1988);

PCBs -- SMS (1991) = Long and Morgan (1990) > Barrick et al. (1988).

The rankings should be interpreted with caution. For example, SMS (1991) predicted toxicity at Station SR-03-06 in total PCB concentration, but did not predict toxicity for metals or PAHs. Although a negative hit indicated that a threshold concentration for metals or PAHs was not exceeded, the total PCB threshold concentration predicted the toxicity. To evaluate the negative hits (false negatives) of the recommended threshold concentrations all chemical classes need to be assessed.

In comparing recommended thresholds and observed toxicity, the occurrence of false positives (sediments that exceeded contaminant recommended thresholds but toxicity was not observed) is important for assessing the predictive value of the threshold concentrations. Four stations in Saginaw River (3), 57% of the stations, are characterized by this situation (Stations SR-03-05, SR-03-08, SR-03-16 and SR-03-24) (Table 4.40). The SMS (1991) and Long and Morgan (1990) recommended threshold concentrations for metals and PCBs were not predictive. It appears that problems are associated with defining the toxicity of individual contaminants from data on complex mixtures of contaminants as observed in the above comparison of recommended thresholds. Similar findings have been reported by Jenkins and Gillam (1991). The occurrence of false positives indicates the need for further development of the threshold concentration approach in sediment hazard assessments, as the current thresholds have varying degrees of uncertainty associated with them.

Although the sediments contained complex mixtures of contaminants that may have been complexed by either TOC (e.g., hydrophobic organics) or AVS (e.g., SEM), sediment particles may have also played an important role in the sorption process which influences contaminant bioavailability. Normalization to TOC may account for the extent of sorption to sediments, but fine sediment particles (e.g., clays) may also contribute significantly to sorption and reduce contaminant bioavailability (Landrum and Robbins 1990). If a large proportion of the sediment particles are fines (less than 63 μm), normalization to organic carbon alone, to estimate bioavailable organics, may be misleading (Landrum and Robbins 1990). For example, all reduced survival responses compared to control, for at least one species tested in the present study, occurred in sediments (except sediment from Station SR-01-06) containing greater than 90% fine sediment particles (Table 4.37). These results suggest that a relationship may occur between particle size and contaminant availability. However, further evaluation of the organic contaminant data for these studies is needed to define relations between percentage fine sediment particles, or TOC, to test organism responses. Both acute and chronic endpoints should be evaluated in relation to contaminant concentrations.

Sediment tests, and test organisms selected to predict effects on indigenous biota, should be sensitive and able to identify affected areas, such that subsequent remedial actions are directed accurately to the areas in need (Becker et. al 1990). Although the amphipod exposures always had high control survival, greater than or equal to the ASTM test acceptability at 80% (ASTM E 1383-90), no one endpoint (survival, growth, sexual maturation), or duration (14- or 28-day), consistently identified toxicity. Combining all AOCs for amphipod endpoint contrasts between 14- and 28-d exposures, 28-d survival identified 17% more toxic sediments than 14-d exposures, with only an 11% agreement on toxic sediments, and 72% agreement on non-toxic sediments (Figure 4.4). When 14- to 28-d growth (body length) were contrasted they matched closely in ability to identify toxic sediments, with a 1% difference, and had the lowest percentage of non-toxic sediments (55%) to the other amphipod endpoints (Figure 4.4). Although 28-d exposure survival may exceed the 14-d survival (Tables 4.18, 4.26 and 4.35), 28-d exposures will identify more toxic sediments than 14-d exposures, for both survival or growth (Figure 4.4). Although comparisons across AOCs for amphipods, indicated that growth (body length) was a sound indicator of toxicity, growth did not consistently estimate toxicity at all AOCs, when compared to the other sublethal endpoints (increase in length, number of antennal segments, and sexual maturation). Because of the inconsistencies observed in the ability of amphipod sublethal endpoints to identify toxicity, several endpoints should be assessed in the amphipod tests for a thorough toxicity evaluation.

Amphipod antennal segment number and body length were not predictors of sexual maturation. For example, almost 100% of the amphipods exposed to Buffalo River sediments were mature at 28 days and had about 14 antennal segments, yet almost 4% were mature at 14 days which also had 14 antennal segments (Table 4.18). The number of amphipod antennal segments at 28 days is lower for Buffalo River exposures than the other AOCs, and is also lower than values Nelson and Brunson (1992) reported for amphipods at the same age (about 20 segments at six weeks old). In addition, using Saginaw River (1) 28-d amphipod responses, Nelson and Brunson (1992) observed that the relationship between body length and antennal segment number was not a predictor of sexual

maturation, or age, by instar. Nelson and Brunson (1992) also observed that overlapping ranges for body length and antennal segment number occur because of individual variation, density dependent factors (i.e., competitive interactions), and density independent factors (i.e., temperature).

Given the population mean (μ) and the population variance ($\sigma 2$) for endpoints such as antennal segment number or sexual maturation (Tables 4.11, 4.18, 4.26 and 4.35) future studies can be designed using the following formula:

$$16 * \sigma 2/(P * \mu)$$

where: $\sigma 2$ is the population variance, P is the percentage of μ (i.e., expected biological differences), and μ is the population mean. This calculation should be used as a guide to determine whether or not to conduct studies using those endpoints. The formula will estimate the number of treatment observations (n) at $\alpha = 0.05$ with power of the test set at 80% (Snedecor and Cochran 1980).

Relative species sensitivity will frequently vary with the sediment type (i.e., geochemical characteristics) and the types of sediment-associated contaminants being evaluated (Burton et al. 1989a,b, DeWitt et al. 1989, Ingersoll and Nelson 1990). Important factors to consider in assessing the quality of contaminated sediments are: species used, types of tests conducted, and biological endpoints measured. These elements of contaminated sediment assessment are crucial to decisions regarding the ecological and toxicological significance of contaminated sediments. The species, endpoints, and tests used in this study were not equally capable of identifying toxic sediments across AOCs. Although not equal in identifying toxic sediments, the relative species sensitivity (i.e., identifying toxic sediments across AOCs) was apparent.

Comparisons of survival and growth for test species relative sensitivity, expressed as percentage of hits (identified toxicity) from Table 4.41 include:

Survival --

C. riparius 14-d (37%) > H. azteca 28-d (33%) > H. azteca 14-d (32%) > C. tentans 10-d (31%);

Growth --

H. azteca 28-d (33%) > C. tentans 10-d (31%) > H. azteca 14-d (29%) > C. riparius 14-d (13%);

Growth and Survival --

H. azteca 28-d (66%) > C. tentans 10-d (62%) > H. azteca 14-d (61%) > C. riparius 14-d (50%).

The number of stations tested and the number of endpoints measured varied between species. For example, C. tentans percentage hits for survival (31%) was assessed in 84% of the stations, whereas C. riparius percentage hits for survival (37%) was assessed at 100% of the stations (Table 4.41). The proportions of hits and no hits to the number of stations influences the final percentage determination. Caution must be taken with these comparisons as both C. riparius (Buffalo River) and C. tentans (Saginaw River (3)) exposures did not meet ASTM test acceptability criteria as stated earlier. In addition, to fully evaluate species sensitivity exposure procedures should be identical (e.g., temperature).

Amphipod survival in exposures to whole sediments from Waukegan Harbor was more sensitive in identifying toxicity than C. riparius adult emergence (Ingersoll and Nelson 1990), but amphipod 48 h survival to the same Waukegan

sediment tested at another laboratory was not as sensitive as Ceriodaphnia dubia survival (Burton et al. 1989). Two day amphipod exposures were inadequate to identify toxicity with the Waukegan sediments, whereas, the 10- and 29-d amphipod sediment exposures produced lethal and sublethal effects (Ingersoll and Nelson 1990).

Growth of C. tentans was identified as the most sensitive endpoint in studies conducted with Detroit River whole sediments over that of mortality for Daphnia magna or Hexagenia limbata (Giesy et al. 1988). In another Detroit River whole sediment evaluation, C. tentans was less sensitive than D. magna (Giesy et al. 1990). An important distinction between the C. tentans exposures and the whole sediment tests with C. riparius and amphipods is that the former tests were conducted with single test organisms in individual test chambers. This configuration eliminates the potential of density dependent effects, primarily growth, by preventing competition for common resources (i.e., food), within a test container, and makes retrieving the organism somewhat easier at the end of the test.

CONCLUSIONS AND RECOMMENDATIONS

Changes in the physicochemical and biological status of the sediment samples, similar to those described by Patrick et al. (1977) (i.e., sediment redox state, molecular diffusion) and Riedel et al. (1987) (i.e., bioturbation), may have occurred during sampling, transportation, and testing (Burton 1991). Test organism responses from laboratory whole sediment exposures may not reflect in situ responses because of the physicochemical and biological changes that occur during sediment manipulation. But laboratory exposures are useful tools to assess the bioavailability of complex mixtures of contaminants under controlled conditions. Responses of the test organisms exposed to sediments from each AOC, and measured concentrations of contaminants in the sediments tested, provided strong evidence that a gradient in environmental toxicity exists across the AOCs. However, specific contaminants causing the effects could not be identified. Sediments from Indiana Harbor were generally more toxic to the test organisms than sediments from Buffalo River which were generally more toxic than the sediments from Saginaw River.

The contaminant exposure that benthic infaunal organisms encounter in the environment often involves a prolonged exposure with slow contaminant accumulation, that often results in sublethal responses, not acute toxicity (Chapman 1989). Chronic sediment toxicity tests are needed to better define (1) the nature of time-integrated contaminant uptake in complex chemical mixtures; (2) sublethal responses using several sensitive benthic organisms, covering phylogenetically diverse groups; and (3) the ability to estimate toxicity accurately (identifying toxicity without false predictions) and efficiently (consistently identifying toxicity). A better understanding of the importance of sublethal responses is needed. Although interpreting sublethal responses is not as clear as survival data, with continued development and guidance, sublethal endpoints will provide additional toxicity information necessary to make sound sediment quality decisions. Additionally, sublethal responses need to be compared to benthic community effects and sediment chemistry to see if the sublethal endpoints are predictive (Chapman 1989).

A comprehensive approach to evaluating sediment quality using sublethal test methods is to use a tiered testing approach (Burton et al. 1989a,b, Becker et al. 1990, USEPA/USACE 1991, Burton 1991) and several types of toxicity tests, with various species and durations (Burton et al. 1989a,b, Ingersoll and Nelson 1990, Burton 1991), over that of a single species, single duration, and survival as the only toxicity endpoint.

Because of biological, physical, and chemical relationships with sediment associated contaminants, as well as unknown relationships, there is no single whole sediment toxicity test ideal for all evaluations. It is essential to use multiple toxicity tests as demonstrated by the varied responses of the test species exposed to contaminated sediments in the present study. The use of multiple toxicity tests is needed to accurately (discriminating between toxic and non-toxic areas) and efficiently estimate sediment toxicity. It is necessary to better understand what the test responses mean biologically, ecologically and economically. Moreover, of equal importance is the necessity that laboratory sediment toxicity test results be field-verified (i.e., in situ exposures) and comparisons made to benthic community responses, to establish the ability of laboratory exposures to predict biological effects in the field.

The following recommendations are a summary of the future research needs identified as important issues relevant to whole sediment toxicity testing and interpretation of test results. Many of these issues are central to the understanding of contaminant bioavailability and the relevance of sediment studies with field-collected sediments.

- The use of multiple toxicity tests is needed to accurately and efficiently estimate sediment toxicity.
- Chronic sediment toxicity tests need further development to better define the nature of time-integrated contaminant uptake in complex chemical mixtures, and sublethal responses, using sensitive benthic organisms, covering phylogenetically diverse groups.
- Sublethal responses need to be compared to benthic community effects and sediment chemistry to see if the sublethal endpoints are predictive.
- Laboratory sediment toxicity test results need to be field-verified (i.e., in situ exposures) and comparisons made to benthic community responses, to establish the ability of laboratory exposures to predict biological effects in the field.
- Additional studies are needed with other metals and complex mixtures of metals to further determine SEM/AVS relationships and its influence on bioavailability.
- Rate limiting factors of sediment-sorbed contaminants need further investigation for a better understanding of the desorption kinetics of non-labile contaminants, and the relationship to bioavailability.
- Further investigation is needed to evaluate the influence of TOC and organic contaminant bioavailability in complex mixtures, and the relationship to recommended threshold values, at varying TOC levels, or those evaluated on a dry weight basis.
- Further investigation of organic contaminants is needed to define relationships between percentage fines or TOC to test organism responses, including acute and chronic endpoints, in relation to contaminant concentrations.

SUMMARY

Responses of the test organisms exposed to sediments from each AOC, and measured concentrations of contaminants in these sediments, provided strong evidence that a gradient in environmental toxicity exists across the AOCs. However, specific contaminants causing the effects could not be identified. Sediments from Indiana Harbor were generally more toxic to the test organisms than sediments from Buffalo River which were generally more toxic than the sediments from Saginaw River.

Comparisons of survival and growth for test species relative sensitivity expressed as percentage of hits (ability to identify toxicity) include:

Survival --

C. riparius 14-d > *H. azteca* 28-d > *H. azteca* 14-d > *C. tentans* 10-d;

Growth --

H. azteca 28-d > *C. tentans* 10-d > *H. azteca* 14-d > *C. riparius* 14-d;

Growth and Survival --

H. azteca 28-d > *C. tentans* 10-d > *H. azteca* 14-d > *C. riparius* 14-d.

INDIANA HARBOR

Indiana Harbor sediments were toxic to all of the test species. The sediments caused significant reduction in survival or body length, but all test species did not identify toxicity with equal sensitivity for the Indiana Harbor sediment exposures. Survival of *C. tentans* was greater than survival of amphipods or *C. riparius*. The acute toxicity observed, however, did verify extensive chemical contamination. Overall, *C. riparius* and *H. azteca* were the most sensitive species in the Indiana Harbor sediment exposures.

BUFFALO RIVER

Buffalo River sediments significantly reduced amphipod survival in the 28-d exposure at 80% of the stations. Survival of *C. riparius* was significantly reduced in the 14-d exposure in two stations (40%), but survival was not significantly reduced for *C. tentans* for any stations in the 10-d exposure. Amphipod 14-d growth identified 100% of the Buffalo River sediments as toxic, and significant differences from control for antennal segment number or sexual maturation was predicted by amphipod reductions in body length at 80% of the stations. Growth of *C. riparius* identified 20% of the Buffalo River sediments as toxic, and growth of *C. tentans* identified 40% of the stations as toxic. Overall, *H. azteca* was the most sensitive species in the Buffalo River sediment exposures.

SAGINAW RIVER (1)

Saginaw River (1) sediment from Station SR-01-06 was toxic to amphipods and *C. riparius*, with significant reductions in survival and growth. The amphipod 28-d growth response identified 100% of the Saginaw River (1) stations toxic, whereas *C. riparius* growth identified only 33% of the stations. Overall, *H. azteca* was the most sensitive indicator of toxicity in the Saginaw River (1) sediments tested.

SAGINAW RIVER (3)

Saginaw River (3) sediments were not toxic to the species tested in the whole sediment exposures. Survival at any stations, with the exception of C. tentans (Station SR-03-06), was not significantly different from control. Growth for amphipods and C. tentans was not significantly reduced compared to control. Contaminated sediment from Station SR-03-05 exposed to H. azteca delayed sexual maturation in the 14-d exposure, but not in the 28-d exposure. Results from the toxicity tests for all test species indicate an equal sensitivity to Saginaw River (3) sediments, except Station SR-03-06.

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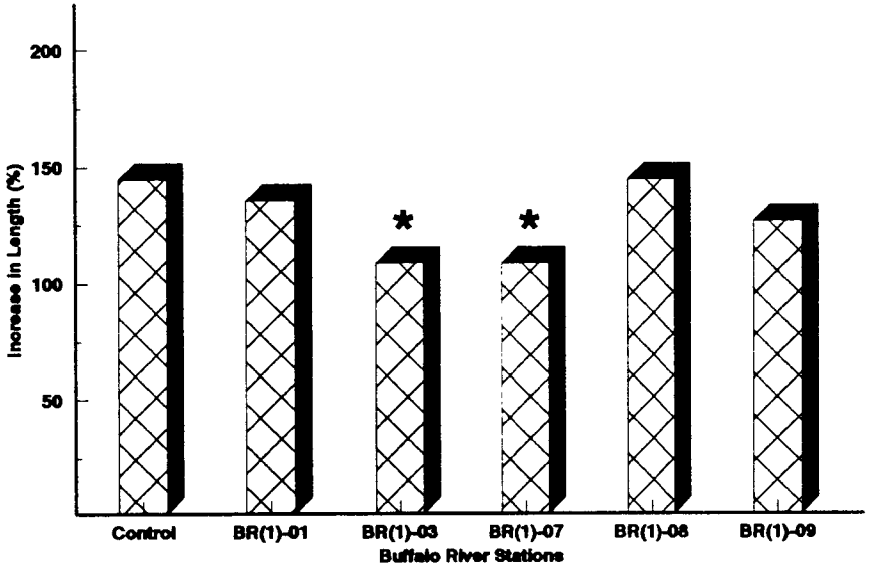
4.1 Amphipod percentage increase in body length for the 28-d exposures, significant differences from control denoted with an *.

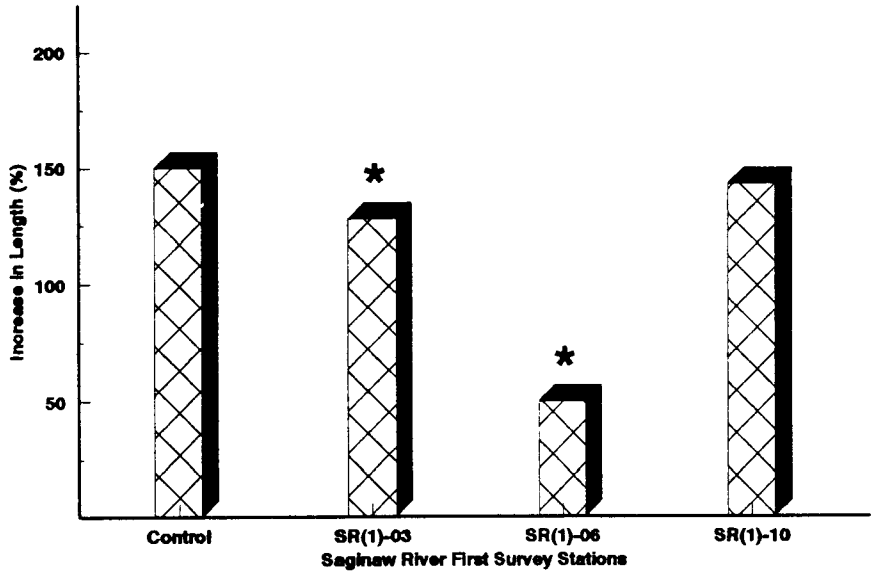
4.2 Relation of summed total metal concentrations to the survival of each species tested in sediments across all Areas of Concern.

4.3 Relation of summed total PAH concentrations to the survival of each species tested, in sediments across all Areas of Concern.

4.4 Comparison of H. azteca test sensitivity. The percentage hits are based on the number of stations that elicited significant reductions in survival, body length or sexual maturation.

Figure 4.1


200
150
100
50
Increase in Length (%)
*
*
Control
BR(1)-01
BR(1)-03
BR(1)-07
BR(1)-08
BR(1)-09
Buffalo River Stations


200
150
100
50
Increase in Length (%)
*
*
Control
SR(1)-03
SR(1)-06
SR(1)-10
Saginaw River First Survey Stations

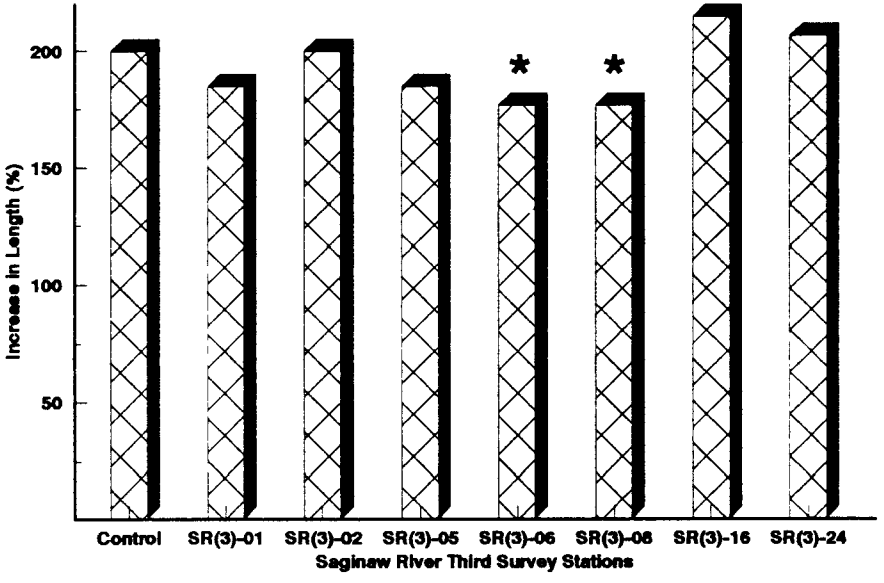

200
150
100
50
Increase in Length (%)
*
*
Control
SR(3)-01
SR(3)-02
SR(3)-05
SR(3)-06
SR(3)-08
SR(3)-16
SR(3)-24
Saginaw River Third Survey Stations

Figure 4.2

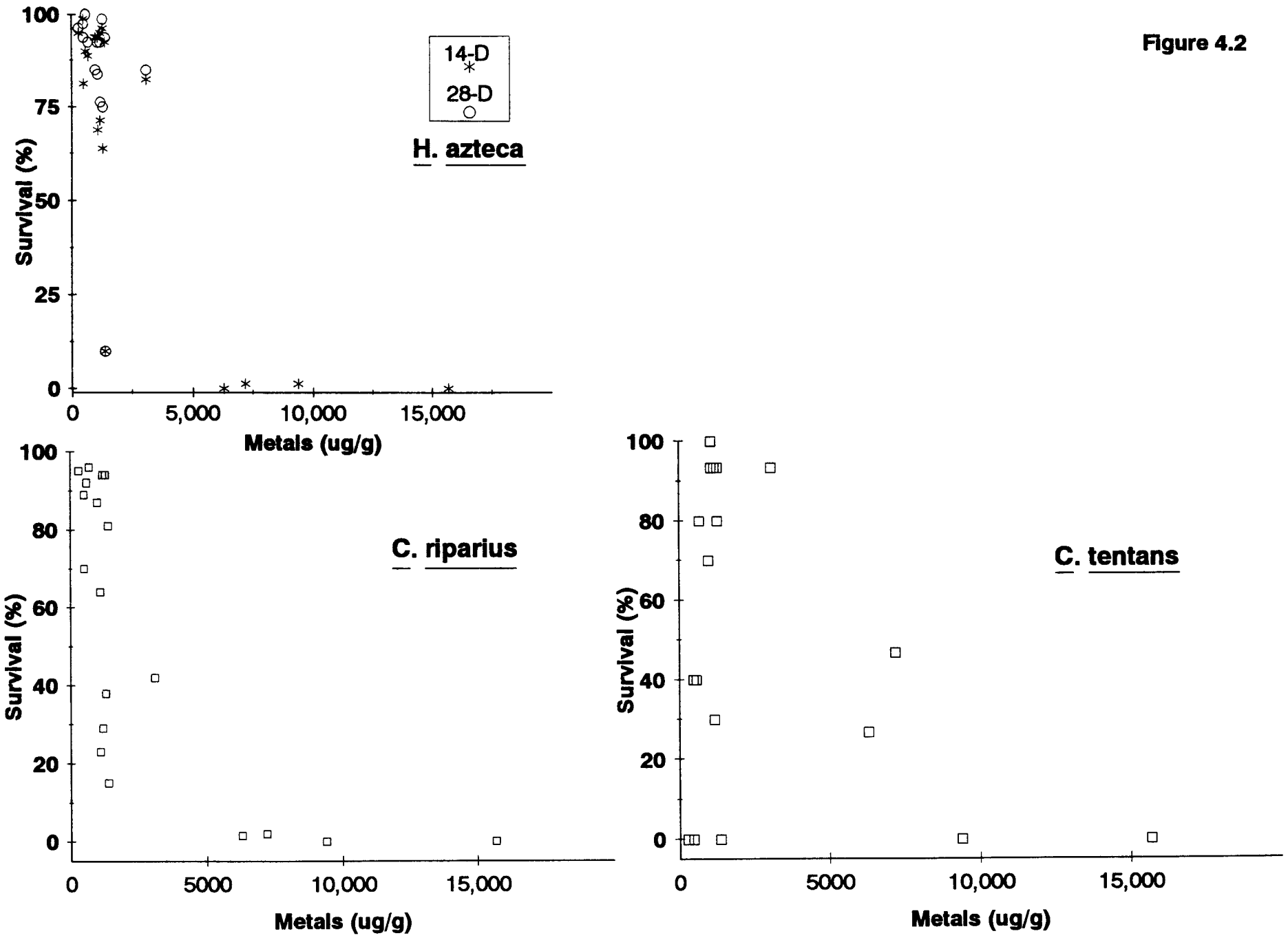
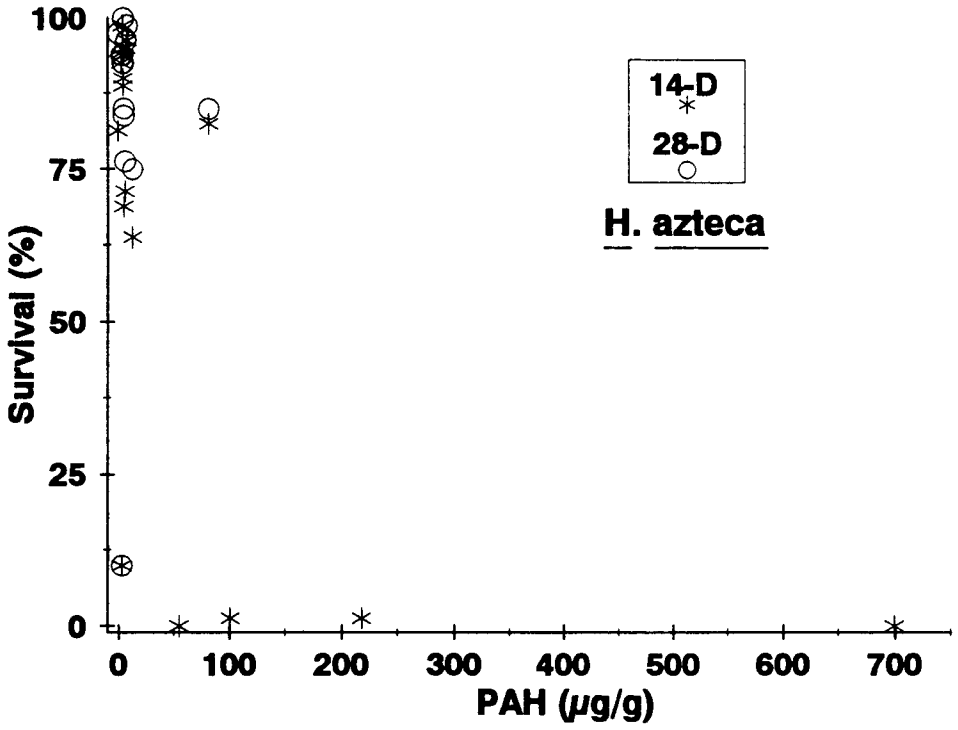
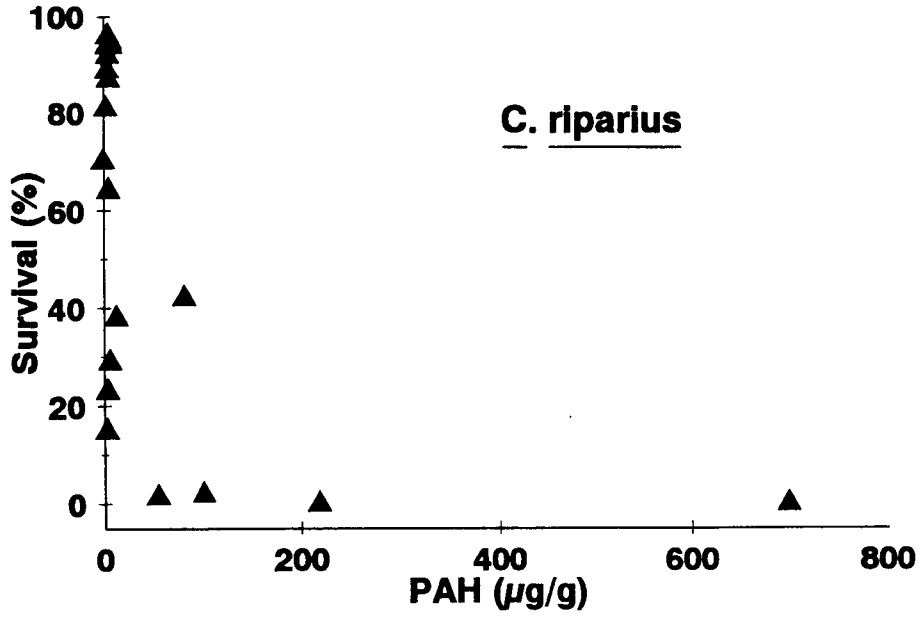

14-D
28-D
H. azteca
Survival (%)
Metals (ug/g)
C. riparius
Survival (%)
Metals (ug/g)
C. tentans
Survival (%)
Metals (ug/g)

Figure 4.3


14-D
28-D
H. azteca
Survival (%)
PAH (µg/g)
0
25
50
75
100
0
100
200
300
400
500
600
700


C. riparius
Survival (%)
PAH (µg/g)
0
20
40
60
80
100
0
200
400
600
800

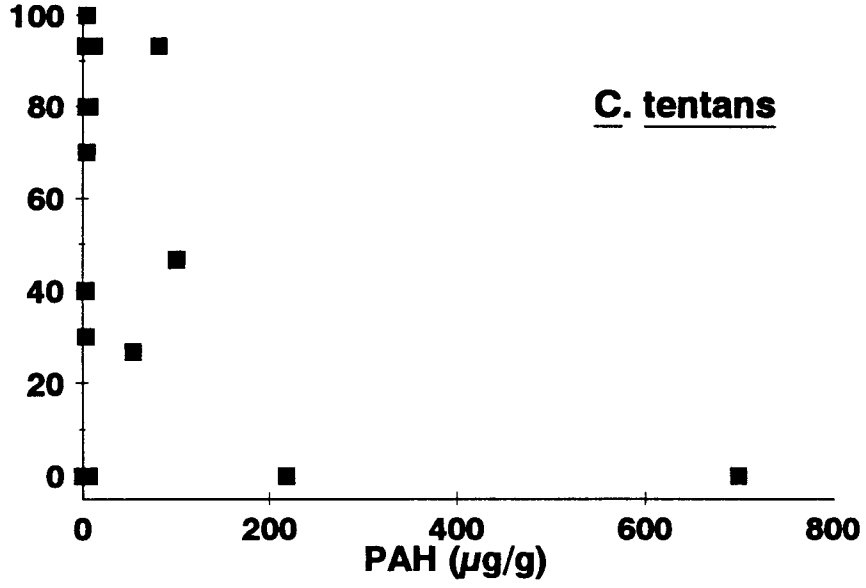
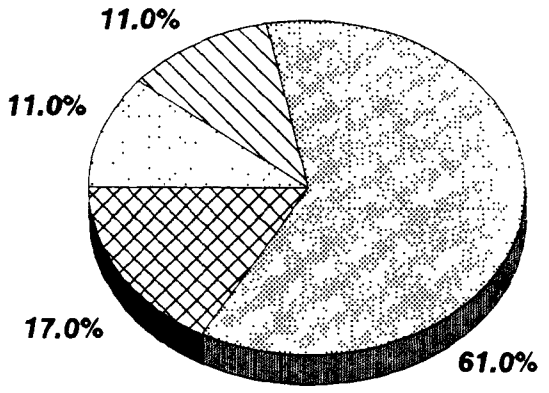
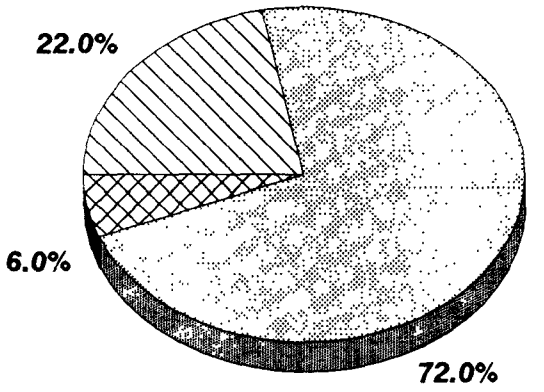

C. tentans
PAH (µg/g)
0
20
40
60
80
100
0
200
400
600
800

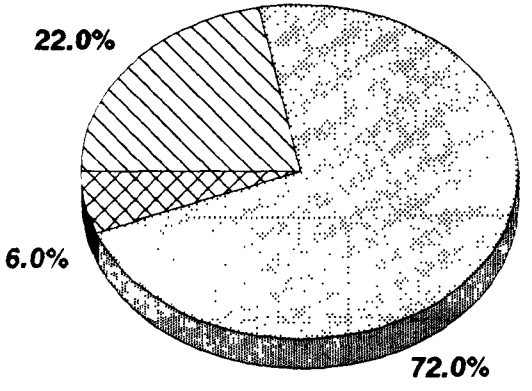
Figure 4.4



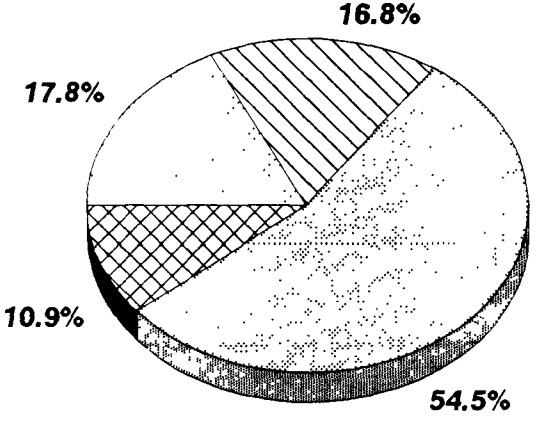
HA-28
Survival / Growth



HA-28
Survival / Sexual Maturation



HA-28
Growth / Sexual Maturation



HA-28 Growth / HA-14 Growth

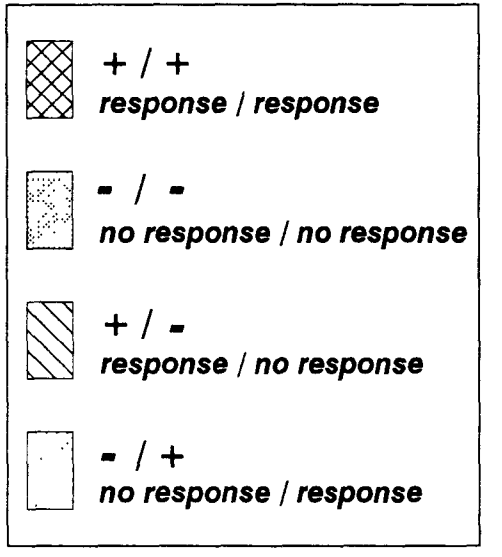


Table 4.1 Chemical names for compounds analyzed in the sediments from stations tested in laboratory whole sediment toxicity tests.

CHEMICAL CODES	CHEMICAL NAME
2378-TCDF	2,3,7,8-Tetrachlorodibenzofuran
Total TCDF	Total Tetrachlorodibenzofuran
2378 TCDD	2,3,7,8-Tetrachlorodibenzodioxin
Total TCDD	Total Tetrachlorodibenzodioxin
12378-PeCDF	1,2,3,7,8-Pentachlorodibenzofuran
23478-PeCDF	2,3,4,7,8-Pentachlorodibenzofuran
Total PeCDF	Total Pentachlorodibenzofuran
12378-PeCDD	1,2,3,7,8-Pentachlorodibenzodioxin
Total PeCDD	Total Pentachlorodibenzodioxin
123478-HxCDF	1,2,3,4,7,8-Hexachlorodibenzofuran
123678-HxCDF	1,2,3,6,7,8-Hexachlorodibenzofuran
123789-HxCDF	1,2,3,7,8,9-Hexachlorodibenzofuran
234678-HxCDF	2,3,4,6,7,8-Hexachlorodibenzofuran
Total HxCDF	Total Hexachlorodibenzofuran
123478-HxCDD	1,2,3,4,7,8-Hexachlorodibenzodioxin
123678-HxCDD	1,2,3,6,7,8-Hexachlorodibenzodioxin
123789-HxCDD	1,2,3,7,8,9-Hexachlorodibenzodioxin
Total HxCDD	Total Hexachlorodibenzodioxin
1234678-HpCDF	1,2,3,4,6,7,8-Heptachlorodibenzofuran
1234789-HpCDF	1,2,3,4,7,8,9-Heptachlorodibenzofuran
Total HpCDF	Total Heptachlorodibenzofuran
1234678-HpCDD	1,2,3,4,6,7,8-Heptachlorodibenzodioxin
Total HpCDD	Total Heptachlorodibenzodioxin
OCDF	Octachlorodibenzofuran
OCDD	Octachlorodibenzodioxin
Ag	Silver
As	Arsenic
Cd	Cadmium
Cr	Chromium
Cu	Copper
Fe	Iron
Hg	Mercury
Mn	Manganese
Ni	Nickel
Pb	Lead
Se	Selenium
Zn	Zinc
TOC	Total Organic Carbon
AVS	Acid Volatile Sulfides
SOLIDS	Total Solids
Methyl-mercury	Methyl-mercury
TBT	Tributylin
DBT	Dibutylin
MBT	Monobutylin
1,4 DCB	1,4-Dichlorobenzene

Table 4.1 (Continued).

Naph	Naphthalene
2-MNaph	2-Methylnaphthalene
Acnaph	Acenapthene
DM PH	Dimethyl Phthalate
DBF	Dibenzofuran
Fluore	Fluorene
Phen	Phenanthrene
Anth	Anthracene
Fluora	Fluoranthene
Pyrene	Pyrene
BBPh	Butyl Benzyl Phthalate
BaAnth	Benzo(a)anthracene
BisPh	Bis(2-Ethylhexyl)Phthalate
Chrys	Chrysene
DnOPh	Di-n-octylphalate
BbFluor	Benzo(b)Fluoranthene
BkFluor	Benzo(k)Fluoranthene
BaPyr	Benzo(a)Pyrene
IndPyr	Indeno (1,2,3-cd)Pyrene
BghiPer	Benzo(g,h,i)perylene
PCB 1016	PCB 1016
PCB 1221	PCB 1221
PCB 1232	PCB 1232
PCB 1242	PCB 1242
PCB 1248	PCB 1248
PCB 1254	PCB 1254
PCB 1260	PCB 1260
Aldrin	Aldrin (pesticide)
A-BHC	Hexachlorocyclohexane-Alpha
B-BHC	Hexachlorocyclohexane-Beta
D-BHC	Hexachlorocyclohexane-Delta
Chlordane	Chlordane
4,4,DDD	
4,4,DDE	
4,4,DDT	
Dieldrin	Dieldrin
Endosulfan I	Alpha Endosulfan
Endosulfan II	Beta Endosulfan
Endosulfan sulfate	Endosulfan sulphate
Endrin	Endrin
Endrin Aldehyde	Endrin Aldehyde
Endrin Ketone	Endrin Ketone
Heptachlor	Heptachlor
Heptachlor epoxide	Heptachlor epoxide
Lindane (G-BHC)	Hexachlorocyclohexane-Gamma
Toxaphene	Toxaphene
Methoxychlor	Methoxychlor

Table 4.2 Concentrations of total organic carbon, percent solids, and organometals in whole sediment samples from Indiana Harbor, IN. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

Sample	TOC (%)	Solids (%)	Methyl-mercury (ng/dry g)	TBT (ng/dry g)	DBT (ng/dry g)	MBT (ng/dry g)
IH-01-03	7.7	40.8	<0.1	240	47	7.4
IH-01-04	5.6	44.8	2.0	110	32	7.2
IH-01-05	11.1	50.2	<0.1	300	58	17.0
IH-01-06	11.6	29.8	<0.1	1500	370	39.0
IH-01-07	8.8	46.7	0.5	(14-19)	(11-19)	12.0 28.0)
IH-01-08	10.4	33.0	1.4	370	110	12.0
IH-01-10	12.3	19.6	<0.1	530	160	26.0

< Indicates that compound was not detected at detection limits shown.

Table 4.3 Concentrations of metals (μg/dry g) in whole sediment samples from Indiana Harbor, IN. See Table 4.1 for an explanation of chemical abbreviations.

	Metal (μg/dry g)											
Sample	Ag	As	Cd	Cr	Cu	Fe(%)	Hg	Mn	Ni	Pb	Se	Zn
IH-01-03	0.2	60	9.1	572	226	19.7	0.9	2420	50	589	2.6	3250
IH-01-04	0.04	32	5.2	407	182	14.4	0.7	1970	50	396	2.3	2250
IH-01-05	0.02	45	10.4	580	219	23.4	0.9	2740	<50	415	2.0	2290
IH-01-06	6.0	52	11.7	1132	379	7.9	1.9	2410	103	878	3.8	4460
IH-01-07	7.1	93	24.2	2610	287	28.8	2.1	3280	<58	1354	3.1	7960
IH-01-08	4.7	56	12.4	780	284	12.1	1.8	1674	95	1223	3.9	3540
IH-01-10	5.2	63	18.4	1412	354	21.4	1.8	2450	88	791	3.3	4080

< Indicates compound not detected at detection limit shown.

Table 4.4 Simultaneously extracted metals (μM/dry g) and the acid volatile sulfides (μm/dry g) in Indiana Harbor, IN, and Buffalo River, NY whole sediment samples. See Table 4.1 for an explanation of chemical abbreviations.

Sample	AVS	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	SUM Cd,Cr,Cu Ni,Pb,Zn	SUM SEM/AVS
					(μM/dry g)						
IH-01-03	33.5	0.013	0.790	0.280	152	3.41	0.083	0.494	1.740	3.40	0.010
IH-01-04	15.7	0.007	0.428	0.247	95	2.74	0.058	0.289	1.008	2.04	0.130
IH-01-05	21.8	0.008	0.796	0.212	96	2.91	0.069	0.345	1.171	2.60	0.119
IH-01-06	52.6	0.012	1.160	0.110	104	2.35	0.114	0.515	1.564	3.47	0.066
IH-01-07	71.4	0.032	4.994	0.267	283	4.61	0.154	1.255	4.552	11.25	0.158
IH-01-08	54.1	0.011	0.784	0.023	78	1.61	0.041	0.431	1.131	2.42	0.045
IH-01-10	31.7	0.008	0.740	0.059	83	1.59	0.106	0.285	0.878	2.08	0.066
BR-01-01	161.0	0.012	0.288	0.062	64	2.40	0.111	0.189	0.419	1.08	0.007
BR-01-02	1.3	0.001	0.007	0.002	1	0.04	0.013	0.011	0.009	0.05	0.038
BR-01-03	5.8	0.003	0.064	0.069	22	1.16	0.030	0.067	0.102	0.33	0.058
BR-01-04	4.2	0.002	0.015	0.054	21	1.56	0.002	0.045	0.068	0.19	0.045
BR-01-05	16.9	0.004	0.043	0.050	21	1.01	0.024	0.074	0.131	0.32	0.019
BR-01-06 REP1	10.7	0.003	0.039	0.079	23	1.20	0.022	0.104	0.164	0.41	0.038
BR-01-06 REP2	10.4	0.004	0.034	0.080	21	1.06	0.020	0.097	0.151	0.38	0.034
BR-01-07	5.7	0.005	0.024	0.047	21	1.42	0.043	0.036	0.053	0.20	0.036
BR-01-08	2.6	0.002	0.013	0.035	13	1.09	0.023	0.026	0.072	0.17	0.067
BR-01-09	5.1	0.005	0.025	0.029	16	1.35	0.044	0.037	0.039	0.18	0.035
BR-01-10	13.5	0.004	0.009	0.032	17	1.04	0.015	0.030	0.045	0.14	0.010

Table 4.5 Particle size distribution (mm), on a dry weight basis, in whole sediment samples from Indiana Harbor, IN.

	Particle Size (mm)				
Sample	>1.0	0.25 - 1.0	0.063 - 0.25	0.038 - 0.063	<0.038
IH-01-03	1.0	2.0	14.4	5.5	72.3
IH-01-04	0.2	2.2	26.1	6.3	63.6
IH-01-05	4.4	10.5	40.6	6.2	36.8
IH-01-06	1.7	2.0	12.3	6.1	77.7
IH-01-07	0.9	3.2	11.5	4.8	75.5
IH-01-08	0.9	2.3	16.4	6.5	70.5
IH-01-10	0.4	1.1	13.1	9.9	72.1

Table 4.6 Concentrations of polynuclear aromatic and other semivolatile compounds (ng/dry g) in whole sediment samples from Indiana Harbor, IN. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Polynuclear Aromatic Hydrocarbon (ng/dry g)									
Sample	1-4 DCB	Naph	2-M Naph	DMPh	DBF	Fluore	Phen	Anth	Fluora	Pyrene
IH-01-03	82	7300	2000	<68	2300	2400	7000	2400	8600	16000
IH-01-04	31	3600	930	<43	920	790	3400	1400	4800	5500
IH-01-05	45	6300	1200	<25	1200	1400	5100	2200	7200	10000
IH-01-06	380	8500	5400	<95	5700	3200	9900	3400	14000	40000
IH-01-07	125 (110-140)	4550 (4100-5000)	21015 (<31-42000)	<52 (<37-<68)	26530 (<61-53000)	30530 (<61-61000)	151500 (33000-270000)	215000 (130000-300000)	80000 (40000-120000)	46500 (38000-55000)
IH-01-08	160	6100	20000	<110	6900	12000	79000	26000	56000	43000
IH-01-10	930	24000	4200	<95	2400	3200	13000	3500	9600	16000

Sample	BBPh	BaAnth	BisPh	Chrys	DnOPh	BbFluor	BkFluor	BaPyr	IndPyr	BghiPer
IH-01-03	<160	7300	10000	8600	1900	7800	10000	10000	7300	9600
IH-01-04	<100	4200	4700	5200	4100	5600	4200	7000	5300	6300
IH-01-05	<58	5800	3800	7200	430	6300	5100	5700	6600	8800
IH-01-06	16000	16000	290000	26000	37000	24000	23000	25000	2000	28000
IH-01-07	<122 (<85-<160)	32000 (25000-39000)	7150 (5900-8400)	31500 (24000-39000)	<130 (<90-<170)	20500 (19000-22000)	13500 (12000-15000)	31000 (21000-41000)	10100 (9200-11000)	17500 (14000 21000)
IH-01-08	<240	30000	18000	33000	2600	26000	21000	29000	19000	31000
IH-01-10	<220	6900	15000	9400	<240	8900	9700	9200	5800	7600

< Indicates that compound was not detected at detection limit shown.

Table 4.7 Concentrations of dioxins and furans (pg/dry g) in whole sediment samples from Indiana Harbor, IN. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)												
Sample	2378-TCDF	Total TCDF	2378-TCDD	Total TCDD	12378-PeCDF	23478-PeCDF	Total PeCDF	12378-PeCDD	Total PeCDD	123-478-HxCDF	123-678-HxCDF	123-789-HxCDF	234-678-HxCDF
IH-01-03	290	860	130	190	27	29	340	<52	ND	41	<66	32	<5.6
IH-01-04	27	400	ND	37	12	21	190	ND	22	16	12	10	ND
IH-01-05	11	170	ND	32	3.8	7.8	76	ND	35	15	6.8	5.2	ND
IH-01-06	600	3700	<59	490	56	120	1300	42	510	130	76	55	13
IH-01-07	610 (480-740)	3450 (2400-4500)	73.5 (<37-<110)	195 (160-230)	104 (28-<180)	106 (82-130)	1350 (1300-1400)	80 (<76-84)	1900	225 (210-240)	98 (86-110)	77.5 (56-<99)	85.5 (<31-<140))
IH-01-08	320	2200	<39	230	27	89	680	29	140	95	<45	32	<13
IH-01-10	310	1700	<18	110	30	68	720	20	66	86	43	30	<18

Table 4.7 (Continued).

Sample	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)											
	Total HxCDF	123-478-HxCDD	123-678-HxCDD	123-789-HxCDD	Total HxCDD	1234-678-HpCDF	1234-789-HpCDF	Total HpCDF	1234-678-HpCDD	Total HpCDD	OCDF	OCDD
IH-01-03	700	53	73	97	950	<38	660	660	1400	3300	1600	6700
IH-01-04	250	13	23	14	350	180	ND	380	410	980	180	2300
IH-01-05	220	17	31	19	420	220	8.8	510	580	1200	250	2900
IH-01-06	1900	130	210	380	2500	1600	81	4200	5100	9300	6900	43000
IH-01-07	3600 (3500-3700)	390 (220-560)	420 (360-480)	660 (520-<800)	6800 (4600-9000)	3150 (3000-3300)	96 (72-120)	7700 (6600-8800)	10750 (6500-15000)	23000 (15000-31000)	17300 (2600-32000)	43500 (41000-46000)
IH-01-08	2100	<47	230	290	2600	340	700	8200	4700	5300	12000	25000
IH-01-10	920	32	99	260	1700	810	36	1500	1600	3100	2500	12000

< Indicates that compound was not detected at detection limit.
ND = Not detected.

Table 4.8 Polychlorinated biphenyls (ng/dry g) in whole sediment samples from Indiana Harbor, IN, and the Buffalo River, NY. See Table 4.1 for an explanation of chemical abbreviations.

Sample	Polychlorinated Biphenyl (ng/dry g)						
	PCB 1016	PCB 1221	PCB 1232	PCB 1242	PCB 1248	PCB 1254	PCB 1260
IH-01-03	<360	<360	<360	10000	<360	<360	<360
IH-01-04	<360	<360	<360	3000	<360	1000	<360
IH-01-06	<500	<500	<500	24000	<500	<500	<500
IH-01-07	<410	<410	<410	43000	<410	<410	<410
BR-01-01	<510	<510	<510	<510	<510	<510	<510
BR-01-03	<300	<300	<300	<300	<300	<3400	<300
BR-01-07	<360	<360	<360	<360	<360	<360	<360
BR-01-08	<340	<340	<340	<340	<340	<340	<340
BR-01-09	<360	<360	<360	<360	<360	<360	<360
SR-01-03	<340	<340	<340	<340	<340	<340	<340
SR-01-06	<300	<300	60000	<300	7900	300	<300
SR-01-10	<260	<260	<260	440	2300	<260	<260

< Indicates that compound was not detected at detection limit shown.

Table 4.9 Pesticide ranges of analytical detection limits (ng/g) measured in whole sediment samples from Indiana Harbor, IN, Buffalo River, NY, and Saginaw River, MI. See Table 4.1 for an explanation of chemical abbreviations.

	Area of Concern			
Pesticide (ng/dry g)	Indiana Harbor	Buffalo River	Saginaw River (1)	Saginaw River (3)
Aldrin	NA	24-51	26-43	2.5-460
A-BHC	34-69	24-51	26-43	2.5-360
B-BHC	34-69	24-39	26-43	2.8-380
C-BHC	34-69	24-51	26-43	2.5-380
Gamma cholordane	≥ 36	24-51	26-43	2.5-360
Alpha Chlordane	34-69	24-51	26-43	2.5-380
4,4,DDD	34-69	24-39	26-43	2.5-380
4,4,DDE	≥ 36	24-51	26-43	2.5-380
4,4,DDT	34-69	24-39	26-43	2.5-380
Deildrin	34-69	24-39	26-43	2.5-380
Endosulfan I	34-69	24-51	26-43	2.5-380
Endosulfan II	34-69	24-51	26-43	2.5-380
Endosulfan sulfate	34-69	24-51	26-43	2.5-380
Endrin	34-69	24-51	26-43	2.5-380
Endrin aldehyde	34-69	24-51	26-43	2.5-380
Endrin ketone	34-69	24-51	26-43	2.5-380
Heptachlor	34-69	24-51	26-43	2.5-380
Heptachlor epoxide	≥ 36	24-51	26-43	2.5-500
Lindane (G-BHC)	34-69	24-51	26-43	2.5-380
Toxaphene	340-500	140-510	260-430	2.5-3800
Methoxychlor	170-340	120-250	130-220	13-1600

NA indicates not applicable.

Table 4.10 Mean (standard error of the mean in parentheses) measured overlying water quality for Indiana Harbor, IN whole sediment toxicity tests.

Sample	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	D.O. (mg/L)	Conductivity (µmhos/cm)	Unionized Ammonia (mg/L)	Chloride (mg/L)	Turbidity (NTU)
Hyalella azteca - 14-day								
Control	8.5(0.49)	82(18.0)	142(14.0)	8.7(0.29)	263(07.2)	0.057(0.05)	8.6(0.03)	7.8(05.55)
IH-01-03	8.1(0.09)	82(06.0)	156(04.0)	7.9(0.48)	299(23.6)	0.137(0.13)	10.4(1.09)	35.8(34.20)
IH-01-04	8.1(0.12)	98(18.0)	146(10.0)	8.0(0.55)	323(24.7)	0.280(0.28)	12.7(0.95)	39.2(37.71)
IH-01-06	8.1(0.09)	90(14.0)	160(08.0)	7.9(0.44)	316(31.2)	0.320(0.31)	14.8(2.82)	44.2(42.80)
IH-01-07	8.0(0.09)	80(08.0)	156(04.0)	7.8(0.40)	317(23.3)	0.297(0.27)	12.1(1.68)	31.6(30.18)
Chironomus riparius - 14-day								
Control	8.3(0.60)	84(16.0)	136(08.0)	8.2(0.53)	270(10.8)	0.055(0.05)	8.6(0.02)	16.7(03.35)
IH-01-03	8.1(0.10)	82(06.0)	150(02.0)	8.2(0.38)	312(17.0)	0.140(0.13)	10.5(1.02)	35.6(34.46)
IH-01-04	8.1(0.12)	96(20.0)	146(10.0)	8.2(0.45)	322(23.0)	0.280(0.28)	12.1(1.59)	39.2(37.75)
IH-01-06	8.0(0.11)	90(14.0)	158(10.0)	8.2(0.33)	323(28.0)	0.325(0.31)	14.3(3.27)	44.1(42.93)
IH-01-07	8.0(0.09)	78(10.0)	152(08.0)	7.9(0.35)	311(17.7)	0.298(0.27)	13.1(0.67)	31.5(30.31)
Chironomus tentans - 10-day								
Control	8.7(0.73)	92(08.0)	130(02.0)	7.6(1.60)	296(59.3)	0.292(0.17)	10.9(2.28)	21.2(07.90)
IH-01-03	8.3(0.04)	116(28.0)	182(30.0)	7.7(1.20)	402(71.7)	0.565(0.24)	13.5(1.94)	40.9(29.10)
IH-01-04	8.3(0.11)	102(14.0)	168(32.0)	8.2(0.95)	380(29.9)	0.364(0.31)	17.8(4.13)	84.2(07.30)
IH-01-06	8.2(0.09)	110(06.0)	174 (06.0)	7.5(1.35)	412(51.6)	0.709(0.07)	20.3(2.75)	74.3(12.75)
IH-01-07	8.2(0.08)	104(16.0)	166 (06.0)	7.9(0.70)	397(67.0)	1.110(0.42)	16.5(2.78)	40.2(21.65)

Table 4.11 Responses of *Hyalella azteca*, *Chironomus riparius*, and *Chironomus tentans* exposed to whole sediment samples from Indiana Harbor, IN. Mean values (standard errors in parentheses) within columns and tests with common letters are not significantly different ($p \leq 0.05$).

Test	Survival (%)	Total Body Length (mm)		Antennal Segment (#)	Mature (%)
		Start	End		
Hyalella azteca 14-day					
Control	92.5(.06)a	2.1	2.5(0.2)	16.3(0.5)	1.2(.06)
IH-01-03	1.3(.06)b		2.4(1.8)	17.0(4.6)	0.0
IH-01-04	0.0		--	--	--
IH-01-06	1.3(.06)b		1.7(1.8)	15.0(4.6)	0.0
IH-01-07	0.0		--	--	--
Chironomus riparius 14-day					
Control	75.0(3.5)a		12.9(0.3)		
IH-01-03	2.0(0.8)b		--		
IH-01-04	1.5(1.0)b		--		
IH-01-06	0.0		--		
IH-01-07	0.0		--		
Chironomus tentans 10-day					
Control	100.0a	6.4	18.7(0.9)a		
IH-01-03	46.7b		10.3(1.4)b		
IH-01-04	26.7b		9.8(1.8)b		
IH-01-06	0.0		--		
IH-01-07	0.0		--		

Table 4.12 Concentrations of total organic carbon, percentage solids, and organometals in whole sediment samples from Buffalo River, New York. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

Sample	TOC (%)	Solids (%)	Methyl-mercury (ng/dry g)	TBT (ng/dry g)	DBT (ng/dry g)	MBT (ng/dry g)
BR-01-01	8.9	30.0	1.6	26.0	36.0	15.0
BR-01-02	0.25	73.9	<0.05	<0.5	0.7	<0.5
BR-01-03	2.0	50.5	<0.05	14.0	4.6	<0.8
BR-01-04	1.8	46.8	<0.05	3.1	2.6	<0.8
BR-01-05	1.7	52.4	<0.05	4.6	2.5	<0.7
BR-01-06	(2.1-2.2)	(47.7-49.1)	(2.1-3.4)	(3.3-3.4)	(2.5-4.6)	(1.1-1.7)
BR-01-07	1.7	44.1	<0.05	2.5	<0.9	<0.9
BR-01-08	1.7	45.9	<0.05	1.7	0.9	<0.8
BR-01-09	2.2	41.7	<0.05	1.3	<0.9	<0.9
BR-01-10	1.9	42.8	<0.05	1.3	<1.0	<1.0

< Indicates that compound was not detected at detection limits shown.

Table 4.13 Concentrations of metals (μg/dry g) in whole sediment samples from Buffalo River, NY. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Metal (μg/dry g)											
Sample	Ag	As	Cd	Cr	Cu	%Fe	Hg	Mn	Ni	Pb	Se	Zn
BR-01-01	0.5	34.0	4.0	312	148.0	5.5	1.9	1386	57.0	286	3.8	900
BR-01-02	<0.03	<1.4	0.04	<13	8.2	0.3	0.02	40	5.2	28	<0.5	32
BR-01-03	0.4	13.0	1.4	113	67.0	4.4	0.6	685	45.0	107	<0.9	286
BR-01-04	0.2	12.0	1.0	77	50.0	4.2	0.2	789	50.0	67	<0.8	224
BR-01-05	0.2	<4.5	1.6	100	60.0	5.4	0.3	673	47.0	314	<1.0	371
BR-01-06	(0.21-0.24)	(12.0-13.0)	1.2	(109-130)	(90.0-93.0)	4.2	(1.6-1.8)	(628-630)	(46.0-(52.0)	(143-(151)	(0.8-0.9)	(387 389)
BR-01-07	0.1	12.0	0.9	92	49.0	4.1	0.2	726	44.0	70	<0.9	195
BR-01-08	0.1	12.0	0.7	70	46.0	3.7	0.1	731	43.0	51	<0.9	166
BR-01-09	0.1	11.0	0.7	56	41.0	3.4	0.1	726	40.0	49	<0.8	159
BR-01-10	0.1	8.2	0.6	46	35.0	3.0	0.1	556	34.0	43	<0.8	142

< Indicates that compound was not detected at detection limit shown.

Table 4.14 Particle size distribution (mm), on a dry weight basis, in whole sediment samples from Buffalo River, NY.

	Particle Size (mm)				
Sample	>1.0	0.25 - 1.0	0.063 - 0.25	0.038 - 0.063	<0.038
BR-01-01	11.7	11.7	30.5	7.6	43.7
BR-01-02	0.3	38.3	58.5	0.2	0.9
BR-01-03	2.8	4.1	30.5	7.0	54.0
BR-01-04	0.2	0.6	6.9	3.7	92.0
BR-01-05	14.3	14.1	14.6	5.2	51.5
BR-01-06	0.5	0.7	4.7	5.4	89.1
BR-01-07	0.1	0.7	6.3	5.1	86.4
BR-01-08	0.3	0.8	9.2	9.5	74.3
BR-01-09	0.5	2.0	18.9	10.0	67.0
BR-01-10	1.6	10.3	23.3	7.9	55.8

Table 4.15 Concentrations of polynuclear aromatic and other semivolatile compounds (ng/dry g) in whole sediment samples from Buffalo River, NY. See Table 4.1 for an explanation of chemical abbreviations.

	Polynuclear Aromatic Hydrocarbons (ng/dry g)									
Sample	1,4 DCB	2-M Naph	Naph	DMPh	DBF	Fluore	Phen	Anth	Fluora	Pyrene
BR-01-01	730	20000	2400	<86	1600	1800	6100	1700	7500	6100
BR-01-02	<18	<29	<29	<34	<30	<30	<36	<34	<55	<68
BR-01-03	810	470	230	<73	120	400	1400	1100	1900	2100
BR-01-04	58	<67	<67	<79	<71	<71	580	170	1200	880
BR-01-05	380	180	190	<63	140	380	2700	640	2700	2500
BR-01-06	590	790	2100	<48	1200	3400	10000	4300	5100	6700
BR-01-07	68	140	83	<45	63	140	680	240	990	1100
BR-01-08	54	59	<47	<56	<50	<50	460	120	760	690
BR-01-09	<26	<42	45	<50	<45	46	540	100	1200	750
BR-01-10	<36	<57	<57	<68	<61	<61	520	99	840	910

Table 4.15 (Continued).

	Polynuclear Aromatic Hydrocarbons (ng/dry g)									
Sample	BBPh	BaAnth	BisPh	Chrys	DnOPh	BbFluor	BkFluor	BaPyr	IndPyr	BghiPer
BR-01-01	15000	3500	39000	4000	38000	7000	9500	5800	3800	3800
BR-01-02	<79	<21	880	<27	<84	<30	<41	<27	<45	<55
BR-01-03	<170	680	7000	1100	<180	1000	910	470	520	620
BR-01-04	<180	460	3100	610	<200	640	650	600	<100	<130
BR-01-05	1500	870	8800	1200	6300	1400	1200	1200	640	460
BR-01-06	6100	1800	41000	2600	24000	1500	1500	1300	990	1100
BR-01-07	2600	470	14000	690	7800	670	600	620	250	260
BR-01-08	<130	260	2400	440	210	550	370	350	78	<91
BR-01-09	210	310	2700	470	560	610	460	440	220	240
BR-01-10	1500	330	59000	480	1300	770	430	460	160	170

< Indicates that compound was not detected at detection limit shown.

Table 4.16 Concentrations of dioxins and furans (pg/dry g) in whole sediment samples from Buffalo River, NY. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)												
Sample	2378-TCDF	Total TCDF	2378-TCDD	Total TCDD	12378-PeCDF	23478-PeCDF	Total PeCDF	12378-PeCDD	Total PeCDD	123-478-HxCDF	123-678-HxCDF	123-789-HxCDF	234-678-HxCDF
BR-01-01	7.6	51.0	<2.0	12.0	1.7	2.8	52.0	<3.3	3.6	3.8	<2.5	4.2	2.8
BR-01-02	<2.2	ND	<2.3	ND	<0.8	<0.6	ND	<0.9	ND	<0.7	<0.6	<0.7	<1.0
BR-01-03	4.4	12.0	<1.0	ND	1.0	<1.1	33.0	<1.3	ND	1.6	<0.8	<2.3	<1.1
BR-01-04	<2.2	1.5	<1.7	ND	<1.3	<2.2	19.0	<0.9	5.1	<7.1	<1.0	<2.5	<1.1
BR-01-05	<3.7	ND	<1.7	ND	<0.7	<1.5	16.0	<1.4	ND	<1.5	<0.8	<2.2	<1.1
BR-01-06	5.2	37.0	<2.1	ND	<0.7	<1.0	83.0	<1.5	ND	<2.4	<1.1	<1.2	10.0
BR-01-07	4.7	10.0	<1.3	ND	2.7	<1.1	36.0	<0.9	ND	11.0	3.6	<1.5	<2.3
BR-01-08	<2.4	ND	<1.3	ND	<0.6	<1.6	9.7	<0.6	ND	<2.7	<1.5	<1.4	<0.9
BR-01-09	<2.0	ND	<1.9	ND	<0.7	<1.5	3.1	<0.7	ND	<1.5	<0.6	<1.1	<1.0
BR-01-10	<2.2	ND	<0.9	ND	<0.7	<1.3	3.5	<3.5	7.4	<1.2	<0.7	<1.6	<0.9

Table 4.16 (Continued).

	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)											
Sample	Total HxCDF	123-478-HxCDD	123-678-HxCDD	123-789-HxCDD	Total HxCDD	1234-678-HpCDF	1234-789-HpCDF	Total HpCDF	1234-678-HpCDD	Total HpCDD	OCDF	OCDD
BR-01-01	67.0	9.2	36.0	19.0	190.0	150.0	9.1	640.0	1200.0	2000.0	780.0	12000.0
BR-01-02	ND	<1.1	<0.9	<1.0	ND	<1.8	<1.1	3.8	6.5	12.0	<3.9	53.0
BR-01-03	24.0	<1.2	<3.2	<2.1	14.0	13.0	<1.9	42.0	64.0	120.0	39.0	560.0
BR-01-04	26.0	<1.7	<2.4	<1.7	ND	11.0	<4.2	32.0	43.0	74.0	26.0	340.0
BR-01-05	12.0	<1.1	<1.8	<1.8	8.0	12.0	<1.7	34.0	52.0	93.0	32.0	400.0
BR-01-06	110.0	<1.4	<3.5	<1.8	14.0	23.0	<3.8	61.0	54.0	100.0	42.0	400.0
BR-01-07	54.0	<2.0	<1.5	<1.4	ND	15.0	<3.1	46.0	39.0	69.0	34.0	290.0
BR-01-08	5.6	<0.8	<1.4	<0.8	ND	7.8	<0.95	22.0	28.0	50.0	21.0	250.0
BR-01-09	8.7	<0.8	<1.1	<0.5	5.0	2.8	<0.7	15.0	33.0	59.0	20.0	250.0
BR-01-10	13.0	<0.9	<1.2	<1.4	4.0	5.2	<0.8	27.0	36.0	61.0	20.0	260.0

< Indicates that compound was not detected at detection limit shown.
ND = Not detected.

Table 4.17 Mean (standard error of the mean in parentheses) measured overlying water quality for Buffalo River, NY whole sediment toxicity tests.

Sample	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	D.O. (mg/L)	Conductivity (µmhos/cm)	Unionized Ammonia (mg/L)	Chloride (mg/L)	Turbidity (NTU)
Hyalella azteca - 14-day								
Control	7.9 (0.09)	90 (6.0)	160 (0.0)	8.4 (0.24)	283 (17.3)	0.004 (0.00)	12.1 (0.25)	1.2 (0.69)
BR-01-01	8.3 (0.06)	87 (1.0)	165 (5.0)	7.5 (0.07)	329 (18.3)	0.063 (0.06)	21.3 (10.05)	13.4 (12.90)
BR-01-03	8.1 (0.06)	87 (1.0)	166 (4.0)	7.7 (0.33)	307 (8.8)	0.052 (0.05)	12.1 (1.10)	10.2 (9.65)
BR-01-07	8.1 (0.07)	93 (7.0)	170 (10.0)	7.6 (0.22)	319 (8.5)	0.074 (0.07)	12.1 (1.85)	42.7 (42.27)
BR-01-08	8.1 (0.05)	113(27.0)	175 (15.0)	7.7 (0.38)	332 (14.3)	0.069 (0.06)	11.9 (2.10)	16.1 (15.45)
BR-01-09	8.1 (0.11)	180(92.0)	220 (60.0)	7.3 (0.35)	408 (93.2)	0.116 (0.11)	19.7 (8.95)	8.0 (7.52)
Hyalella azteca - 28-day								
Control	7.9 (0.06)	82 (2.0)	162 (2.0)	8.5 (0.14)	281 (15.6)	0.004 (0.00)	11.9 (0.45)	10.2 (8.30)
BR-01-01	8.2 (0.09)	82 (6.0)	163 (7.0)	7.8 (0.13)	324 (10.5)	0.065 (0.05)	21.2 (10.15)	17.7 (8.65)
BR-01-03	8.1 (0.06)	88 (0.0)	169 (1.0)	7.9 (0.17)	318 (3.9)	0.052 (0.05)	11.7 (1.55)	10.5 (9.26)
BR-01-07	8.2 (0.07)	94 (6.0)	170 (10.0)	7.9 (0.12)	319 (4.9)	0.075 (0.07)	12.3 (1.60)	43.4 (41.60)
BR-01-08	8.2 (0.08)	116(24.0)	179 (11.0)	7.9 (0.18)	329 (8.0)	0.072 (0.06)	12.6 (1.40)	16.6 (14.90)
BR-01-09	8.2 (0.09)	184(88.0)	228 (52.0)	7.6 (0.16)	382 (53.4)	0.117 (0.11)	19.9 (8.70)	8.6 (6.90)
Chironomus riparius - 14-day								
Control	8.0 (0.14)	82 (2.0)	158 (2.0)	7.9 (0.52)	284 (22.0)	0.004 (0.00)	11.2 (1.10)	2.9 (1.07)
BR-01-01	8.1 (0.14)	85 (3.5)	167 (3.0)	6.8 (0.50)	331 (21.0)	0.065 (0.05)	20.6 (10.70)	21.9 (4.40)
BR-01-03	8.0 (0.03)	86 (2.0)	161 (9.0)	7.7 (0.47)	313 (10.5)	0.051 (0.05)	12.0 (1.20)	12.4 (7.45)
BR-01-07	8.1 (0.05)	92 (8.0)	168 (12.0)	7.5 (0.42)	317 (13.0)	0.074 (0.07)	10.9 (3.00)	46.1 (38.88)
BR-01-08	8.1 (0.05)	112(28.0)	173 (17.0)	7.7 (0.54)	329 (17.0)	0.069 (0.06)	11.2 (2.85)	17.7 (13.80)
BR-01-09	8.0 (0.05)	182(90.0)	216 (64.0)	7.3 (0.55)	408 (93.3)	0.118 (0.11)	18.9 (9.75)	13.7 (1.80)
Chironomus tentans - 10-day								
Control	8.1 (0.32)	156(72.0)	200 (40.0)	7.9 (1.05)	418 (180.4)	0.547 (0.54)	26.3 (13.95)	22.3 (3.80)
BR-01-01	8.4 (0.05)	156(68.0)	237 (67.0)	7.4 (0.20)	581 (233.0)	0.559 (0.42)	53.4 (22.10)	23.2 (3.15)
BR-01-03	8.3 (0.22)	114(26.0)	187 (17.0)	7.9 (0.55)	375 (68.0)	0.066 (0.05)	23.4 (10.20)	23.3 (3.45)
BR-01-07	8.3 (0.09)	130(30.0)	200 (20.0)	7.1 (0.90)	412 (92.8)	0.095 (0.08)	19.8 (5.85)	55.5 (29.50)
BR-01-08	8.3 (0.09)	150(10.0)	205 (15.0)	7.4 (1.10)	424 (80.9)	0.119 (0.04)	25.3 (11.30)	31.5 (0.00)
BR-01-09	8.2 (0.13)	208(64.0)	240 (40.0)	7.1 (0.90)	490 (77.3)	0.232 (0.04)	32.1 (3.50)	45.8 (30.25)

Table 4.18 Responses of *Hyalella azteca*, *Chironomus riparius*, and *Chironomus tentans* exposed to whole sediment samples from Buffalo River, NY. Mean values (standard error in parentheses) within columns and tests with common letters are not significantly different ($p \leq 0.05$).

Test	Survival (%)	Total Body Length(mm) Start	Total Body Length(mm) End	Antennal Segments (#)	Mature (%)
Hyalella azteca 14-day					
Control	82.5(.07)ab	1.3	2.1(.04)a	14.7(.09)c	6.1(.05)a
BR-01-01	82.5(.07)ab		1.8(.04)bc	14.3(.08)a	0.0(.05)b
BR-01-03	63.8(.07)c		1.8(.05)bc	14.1(0.1)b	4.2(.05)ab
BR-01-07	71.3(.07)bc		1.8(.04)bc	13.8(0.1)b	0.0(.05)b
BR-01-08	93.8(.07)a		1.8(.04)b	14.1(.09)a	1.4(.05)b
BR-01-09	68.8(.07)bc		1.9(.04)c	14.4(0.1)c	0.0(.05)b
Hyalella azteca 28-day					
Control	95.0(.05)a	1.1	2.7(.09)b	14.7(0.2)	97.4(.04)a
BR-01-01	85.0(.05)bc		2.6(.09)b	14.2(0.2)	100.0(.04)a
BR-01-03	75.0(.05)c		2.3(0.1)a	14.3(0.2)	98.4(.04)a
BR-01-07	76.3(.05)c		2.3(0.1)a	14.1(0.2)	100.0(.04)a
BR-01-08	92.5(.05)ab		2.7(.09)b	14.0(0.2)	100.0(.04)a
BR-01-09	83.8(.05)bc		2.5(.09)ab	14.5(0.2)	100.0(.05)a
Chironomus riparius 14-day					
Control	60.5(11.0)a		11.7(0.4)a		
BR-01-01	41.5(11.8)ab		12.5(0.5)a		
BR-01-03	38.0(07.0)ab		10.2(0.4)b		
BR-01-07	29.0(06.0)b		11.7(0.5)a		
BR-01-08	23.0(07.3)b		11.4(0.7)ab		
BR-01-09	63.5(05.4)a		12.7(0.4)a		
Chironomus tentans 10-day		Wt.(mg)	Wt.(mg)		
Control	86.7a	0.1	3.6(0.3)b		
BR-01-01	93.3a		3.2(0.3)b		
BR-01-03	93.3a		3.1(0.3)ab		
BR-01-07	93.3a		2.5(0.3)a		
BR-01-08	93.3a		2.6(0.3)a		
BR-01-09	100.0a		3.2(0.2)b		

Table 4.19 Concentrations of total organic carbon, percentage solids, and organometals in whole sediment samples from Saginaw River, MI first survey. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

Sample	TOC (%)	Solids (%)	Methyl-mercury (ng/dry g)	TBT (ng/dry g)	DBT (ng/dry g)	MBT (ng/dry g)
SR-01-02	(3.1-3.2)	(36-37)	0.1	(19.0-20.0)	(11.0-14.0)	<1.1
SR-01-03	3.0	36	<0.1	15.0	21.0	<1.0
SR-01-04	2.6	44	<0.1	14.0	7.4	<0.9
SR-01-06	2.1	56	<0.1	6.9	10.0	1.2
SR-01-07	3.0	33	<0.1	12.0	9.0	<1.1
SR-01-09	3.8	42	<0.1	8.3	5.8	<0.8
SR-01-10	1.0	64	<0.1	2.2	2.3	<0.6

< Indicates that compound was not detected at detection limit shown.

Table 4.20 Concentrations of metals (μg/dry g) in whole sediment samples from Saginaw River, MI first survey. The ranges for samples analyzed in replicate are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Metal (μg/dry g)											
Sample	Ag	As	Cd	Cr	Cu	Fe(%)	Hg	Mn	Ni	Pb	Se	Zn
SR-01-02	0.6	(13.0-14.0)	(0.9-1.0)	(74-84)	(46.0-49.0)	2.8	(0.1-0.2)	(671-672)	(36-38)	(56-58)	(<0.2-0.5)	(386-389)
SR-01-03	0.6	13.1	0.9	90	49.0	3.1	0.2	819	37	55	0.3	352
SR-01-04	0.5	10.9	1.0	73	45.0	2.6	0.1	661	35	511	0.4	326
SR-01-06	1.5	3.6	10.0	319	187.0	1.5	0.3	334	157	86	0.3	381
SR-01-07	0.6	16.0	1.0	84	51.0	3.2	0.2	817	43	58	<0.2	372
SR-01-09	0.2	9.1	0.6	46	31.0	1.8	0.1	374	28	39	0.5	319
SR-01-10	0.1	5.1	0.2	40	16.0	1.2	0.05	305	15	19	0.5	99

< Indicates that compound was not detected at detection limit shown.

Table 4.21 Simultaneously extracted metals (μM/dry g) and the simultaneously extracted metals/acid volatile sulfide ratios for Saginaw River, MI first survey sediments. Also included are the Saginaw River, MI third survey sediment estimated SEM and SEM/AVS ratios. See Table 4.1 for an explanation of chemical abbreviations.

Sample	AVS (μM/dry g)	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Sum SEM Cd,Cr,Cu Ni,Pb,Zn	Sum SEM/AVS
		(μM/dry g)									
SR-01-02 REP1	4.9	0.002	0.033	0.064	19	1.39	0.021	0.036	0.186	0.34	0.069
SR-01-02 REP2	4.4	0.004	0.027	0.061	20	1.44	0.035	0.039	0.192	0.36	0.082
SR-01-03	6.0	0.002	0.035	0.050	26	1.98	0.019	0.044	0.185	0.34	0.056
SR-01-04	5.6	0.002	0.032	0.042	20	1.51	0.016	0.031	0.165	0.29	0.052
SR-01-06	15.5	0.012	0.417	0.018	15	0.51	0.223	0.050	0.195	0.92	0.059
SR-01-07	3.6	0.001	0.014	0.030	14	1.07	0.012	0.024	0.107	0.18	0.051
SR-01-09	5.8	0.002	0.014	0.029	14	0.74	0.017	0.029	0.181	0.27	0.047
SR-01-10	1.5	0.001	0.008	0.014	8	0.52	0.009	0.011	0.055	0.09	0.065
SR-03-02	3.3	0.001	0.011	0.021		0.57	0.010	0.019	0.082	0.14	0.043
SR-03-05	3.7	0.002	0.015	0.029		0.73	0.019	0.021	0.108	0.20	0.054
SR-03-06	1.2	0.001	0.015	0.016		0.19	0.006	0.011	0.028	0.08	0.067
SR-03-08	5.8	0.005	0.042	0.048		0.77	0.026	0.043	0.170	0.33	0.057
SR-03-16	3.2	0.008	0.026	0.046		0.95	0.021	0.036	0.180	0.32	0.100
SR-03-24	8.9	0.003	0.026	0.044		1.06	0.020	0.042	0.266	0.40	0.045
SR-03-02-X2	5.0	0.015	0.130	0.133		1.12	0.027	0.069	0.110	0.48	0.096
SR-03-02-X3	4.9	0.001	0.013	0.015		0.48	0.008	0.011	0.023	0.07	0.015
SR-03-05-01	7.4	0.013	0.068	0.076		0.89	0.036	0.044	0.108	0.35	0.047
SR-03-05-02	10.0	0.006	0.071	0.074		1.10	0.030	0.057	0.097	0.33	0.033
SR-03-06-01	10.9	0.018	0.113	0.126		0.56	0.076	0.047	0.146	0.52	0.048
SR-03-06-02	24.6	0.001	0.016	0.023		0.30	0.008	0.015	0.030	0.10	0.004
SR-03-06-X2	36.8	0.047	0.305	0.331		1.17	0.214	0.105	0.350	1.36	0.037
SR-03-06-X3	8.4	0.003	0.038	0.037		0.37	0.020	0.025	0.055	0.18	0.021

Table 4.22 Particle size distribution (mm), on a dry weight basis, in whole sediment samples from Saginaw River, MI first survey.

	Particle Size (mm)				
Sample	>1.0	0.25 - 1.0	0.063 - 0.25	0.038 - 0.063	<0.038
SR-01-02	0.1	0.9	17.0	10.1	71.4
SR-01-03	0.1	0.9	10.4	9.6	79.3
SR-01-04	0.2	6.0	21.3	9.0	55.6
SR-01-06	5.4	41.0	28.2	6.0	19.8
SR-01-07	0.0	0.3	8.8	9.6	79.3
SR-01-09	0.5	1.6	45.9	9.6	39.0
SR-01-10	5.3	40.2	27.7	5.9	19.4

Table 4.23 Concentrations of polynuclear aromatic hydrocarbons and other semivolatile compounds (ng/dry g) in whole sediment samples from Saginaw River, MI first survey. Ranges for samples analyzed in replicate are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

Sample	Polynuclear Aromatic Hydrocarbon (ng/dry g)									
	1-4 DCB	Naph	2-M Naph	DMPh	DBF	Fluore	Phen	Anth	Fluora	Pyrene
SR-01-02	(33-47.0)	(34-46)	(35.0-41)	(76-80)	(18-22)	(27-34)	(220-310)	(42-70)	(130-190)	(470-670)
SR-01-03	33	35	41	99	<16	38	340	66	190	550
SR-01-04	47	48	37	110	20	<12	270	54	160	460
SR-01-06	130	55	63	68	38	69	390	38	160	570
SR-01-07	52	53	48	77	<18	25	290	30	280	570
SR-01-09	25	27	17	21	<15	<15	99	19	130	190
SR-01-10	<4	<6	<6	16	<7	<7	27	<8	36	44

Sample	BBPh	BaAnth	BisPh	Chrys	DnOPh	BbFluor	BkFluor	BaPyr	IndPyr	BghiPer
SR-01-02	(<34-<51)	(140-180)	(1700-2400)	(290-330)	(<36-54)	(150-400)	(200-220)	(180-240)	(110-120)	(160-190)
SR-01-03	1300	170	13000*	400	2200	220	280	280	220	310
SR-01-04	270	160	1900	300	76	130	300	260	210	270
SR-01-06	<25	300	4200	500	430	310	400	210	160	290
SR-01-07	240	190	3800	390	<49	320	220	310	200	220
SR-01-09	<39	64	840	120	<42	61	61	<13	<22	<27
SR-01-10	<18	15	170	24	<19	<7	<9	<6	<10	<13

* Indicates above linear range.
< Indicates compound not detected at detection limit shown.

Table 4.24 Concentrations of dioxins and furan (pg/dry g) in whole sediment samples from Saginaw River, MI first survey. The ranges for replicates are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)												
Sample	2378-TCDF	Total TCDF	2378-TCDD	Total TCDD	12378-PeCDF	23478-PeCDF	Total PeCDF	12378-PeCDD	Total PeCDD	123-478-HxCDF	123-678-HxCDF	123-789-HxCDF	234-678-HxCDF
SR-01-02	430	1300	(14-17)	140	(170-220)	(140-160)	(790-820)	(13-14)	(74-86)	(160-180)	(37-45)	(19-22)	(5.5-11)
SR-01-03	410	1100	11	100	220	150	790	9.5	60	190	35	17	6
SR-01-04	12000	22000	13	120	1800	2500	8700	15	73	880	200	88	29
SR-01-06	2100	4900	38	230	630	540	2800	22	120	400	76	46	17
SR-01-07	500	1400	12	160	210	200	1000	17	110	270	61	30	6.4
SR-01-09	300	950	9.3	94	130	120	650	7.3	50	110	28	13	<3.6
SR-01-10	110	320	5.9	54	34	29	170	4.2	15	41	7.4	4.1	<2.3

Sample	Total HxCDF	123-478-HxCDD	123-678-HxCDD	123-789-HxCDD	Total HxCDD	1234-678-HpCDF	1234-789-HpCDF	Total HpCDF	1234-678-HpCDD	Total HpCDD	OCDF	OCDD
SR-01-02	(750-820)	(5.6-7.3)	(42-69)	(17-30)	(360-460)	(1100-1300)	(46-57)	(2200-3000)	(700-1300)	(1200-2300)	(1400-2800)	(5700-14000)
SR-01-03	680	10	32	9.5	270	770	40	1600	530	980	1100	4100
SR-01-04	2100	6.9	39	16	340	1200	63	2300	640	1200	1300	4900
SR-01-06	1500	12	100	26	620	1900	69	4000	1100	1900	2700	9300
SR-01-07	950	8.9	48	20	360	1100	85	1800	630	1200	1300	5000
SR-01-09	430	3.3	21	11	200	540	26	980	300	550	610	2200
SR-01-10	210	1.7	11	5.6	98	400	12	770	160	300	400	1300

Table 4.25 Mean (standard error of the mean in parentheses) measured overlying water quality for Saginaw River, MI first survey whole sediment toxicity tests.

Sample	pH	Alkalinity, (mg/L as $CaCO_3$)	Hardness, (mg/L as $CaCO_3$)	D.O. (mg/L)	Conductivity (µmhos/cm)	Unionized Ammonia (mg/L)	Chloride (mg/L)	Turbidity (NTU)
Hyalella azteca - 14-day								
Control	8.0 (0.04)	48 (0.0)	154 (10.0)	8.3 (0.40)	295 (16.0)	0.003 (0.00)	9.0 (0.96)	2.6 (1.10)
SR-01-03	8.0 (0.02)	117 (27.0)	186 (18.0)	7.5 (0.40)	349 (26.2)	0.058 (0.05)	12.2 (4.26)	8.9 (7.15)
SR-01-06	8.0 (0.03)	92 (8.0)	162 (2.0)	7.6 (0.47)	323 (10.3)	0.064 (0.06)	11.0 (2.91)	16.3 (15.70)
SR-01-10	8.0 (0.03)	96 (8.0)	172 (8.0)	7.9 (0.44)	326 (11.7)	0.021 (0.02)	15.3 (7.33)	28.4 (26.60)
Hyalella azteca - 28-day								
Control	7.9 (0.05)	66 (18.0)	152 (8.0)	8.3 (0.23)	298 (9.0)	0.004 (0.00)	10.0 (0.05)	2.9 (0.85)
SR-01-03	8.1 (0.07)	120 (24.0)	188 (16.0)	7.2 (0.24)	341 (15.3)	0.059 (0.05)	12.4 (4.10)	10.6 (5.41)
SR-01-06	7.9 (0.02)	94 (6.0)	160 (0.0)	7.5 (0.20)	320 (8.2)	0.064 (0.06)	11.0 (2.95)	16.4 (15.58)
SR-01-10	8.0 (0.05)	96 (8.0)	172 (8.0)	7.6 (0.25)	323 (9.6)	0.021 (0.02)	15.6 (7.05)	29.9 (25.1
Chironomus riparius - 14-day								
Control	7.8 (0.09)	34 (14.0)	148 (4.0)	7.9 (0.67)	299 (14.8)	0.003 (0.00)	11.6 (1.55)	7.1 (3.40
SR-01-03	7.8 (0.11)	122 (22.0)	186 (18.0)	6.6 (0.77)	357 (21.6)	0.057 (0.05)	12.4 (4.10)	21.0 (5.00
SR-01-06	7.7 (0.13)	90 (10.0)	160 (0.0)	6.9 (0.84)	310 (13.6)	0.063 (0.06)	10.4 (3.50)	17.0 (15.0
SR-01-10	7.8 (0.13)	98 (6.0)	170 (10.0)	7.0 (0.83)	333 (7.5)	0.021 (0.02)	15.5 (7.15)	42.0 (13.0

Table 4.26 Responses of Hyalella azteca and Chironomus riparius exposed to whole sediment samples from Saginaw River, MI first survey. Mean values (standard error in parentheses) within columns and tests with common letters are not significantly different ($p \leq 0.05$).

Test	Survival (%)	Total Body Length(mm) Start	Total Body Length(mm) End	Antennal Segments (#)	Mature (%)
Hyalella azteca 14-day					
Control	80.0(0.1)a	1.4	2.4(0.1)	15.2(0.3)	4.9(.11)
SR-01-03	92.5(0.1)a		2.3(0.9)	15.4(0.3)	11.4(.16)
SR-01-06	10.0(0.1)b		2.1(0.3)	15.0(0.9)	0.0(.16)
SR-01-10	81.3(0.1)a		2.4(0.1)	15.6(0.3)	16.2(.11)
Hyalella azteca 28-day					
Control	97.5(0.06)a	1.4	3.5(.07)a	20.2(0.3)a	98.8(.19)a
SR-01-03	93.8(0.06)a		3.2(.07)c	19.8(0.3)a	100.0(.19)a
SR-01-06	10.0(0.06)b		2.1(0.2)b	16.2(0.9)b	37.5(.19)b
SR-01-10	97.5(0.06)a		3.4(.07)c	20.0(0.3)a	100.0(.19)a
Chironomus riparius 14-day					
Control	77.5(0.5)a		11.3(0.3)a		
SR-01-03	81.0(05.1)a		12.0(0.3)a		
SR-01-06	14.5(03.3)b		5.9(0.5)b		
SR-01-10	70.0(13.0)a		11.5(0.3)a		

Table 4.27 Concentrations of total organic carbon, percent solids, and organometals in whole sediment samples from Saginaw River, MI third survey. The ranges for replications are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

Sample	TOC (%)	Solids (%)	Methylmercury (ng/g)	TBT (ng/g)	DBT (ng/g)	MBT (ng/g)
Grab samples						
SR-03-01	(1.0-1.1)	(55.1-56.8)	(<0.1-<.2)	(<0.4-<1.0)	(<0.4-3.3)	(<0.4-<0.8)
SR-03-02	1.3	55.2	<0.2	6.2	3.0	1.4
SR-03-05	1.4	61.3	<0.1	3.9	2.7	0.8
SR-03-06	0.2	76.4	<0.1	0.8	0.7	0.4
SR-03-08	3.6	47.3	<0.1	6.6	3.7	0.7
SR-03-16	2.9	52.1	<0.2	10.0	6.1	1.8
SR-03-24	4.0	48.7	<0.2	9.2	5.6	0.9
Core samples						
SR-03-02-X2	5.0	53.0	<0.2	<0.6	3.1	2.8
SR-03-02-X3	1.5	67.5	<0.2	<0.4	<0.4	<0.2
SR-03-05-01	3.1	59.0	<0.1	4.0	4.2	1.6
SR-03-05-02	2.9	53.8	<0.2	<0.9	1.9	1.3
SR-03-06-01	1.9	64.4	<0.2	3.9	6.3	3.0
SR-03-06-02	0.6	73.5	<0.2	(<0.4-7.0)	(<0.4-6.6)	(<0.8-<3.1)
SR-03-06-X2	4.1	49.2	<0.2	33.0	25.7	11.6
SR-03-06-X3	0.8	76.3	<0.1	0.7	<0.3	0.8

< Indicated that compound was not detected at detection limit shown.

Table 4.28 Concentrations of metals (μg/dry g) in whole sediment samples from Saginaw River, MI third survey. The ranges for replicates are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Metal (μg/dry g)										
	Ag	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Se	Zn
Grab sample											
SR-03-01	(0.18-0.19)	(6.4-41.1)	(0.7-0.8)	(42.0-67.0)	20.3-24.0)	(0.09-0.1)	(304-340)	(18.5-19.1)	(22.6-26.8)	(<0.8-0.9)	(69.8-81.6)
SR-03-02	0.3	25.0	0.5	24.7	24.0	0.1	293	15.3	29.8	<0.8	166.3
SR-03-05	0.4	16.6	0.9	34.0	33.1	0.1	379	28.5	34.2	<0.8	219.0
SR-03-06	0.2	15.2	0.5	34.8	18.3	0.04	99	8.3	16.9	<0.7	56.3
SR-03-08	0.6	12.0	2.0	95.0	54.8	0.1	397	37.9	68.7	1.2	347.0
SR-03-16	0.5	92.9	2.9	58.0	51.9	0.1	492	30.9	58.0	2.0	367.0
SR-03-24	0.5	14.4	1.1	59.0	49.8	0.2	549	29.3	67.7	1.0	541.0
Core sample											
SR-03-02-X2	2.8	217.0	5.5	292.0	150.7	0.6	578	40.2	110.9	3.0	224.0
SR-03-02-X3	0.1	24.1	0.5	29.6	16.6	0.1	250	11.7	17.4	<0.8	46.1
SR-03-05-01	1.1	60.2	4.9	154.0	86.3	0.3	460	53.3	70.5	<0.8	219.0
SR-03-05-02	0.8	59.5	2.3	161.0	84.2	0.6	569	43.5	91.0	1.7	197.0
SR-03-06-01	1.3	14.5	6.9	255.0	142.8	0.3	292	111.4	75.8	<0.8	298.0
SR-03-06-02	0.2	21.5	0.42	35.3	26.6	0.2	155	11.9	24.0	<0.7	2.0
SR-03-06-X2	3.3	40.6	17.4	687.0	375.0	0.7	604	316.0	168.2	<0.9	714.0
SR-03-06-X3	0.35	70.1	1.2	86.0	42.2	0.3	194	30.2	39.8	<0.76	111.6

< Indicates compound not detected at detection limit shown.

Table 4.29 Particle size distribution (mm), on a dry weight basis, in whole sediment samples from Saginaw River, MI third survey. Ranges for samples analyzed in replicate are shown in parentheses.

	Particle Size (mm)				
Sample	>1.0	0.25 - 1.0	0.063 - 0.25	0.038 - 0.063	<0.038
SR-03-01	0.1	0.9	56.5	13.2	29.2
SR-03-02	0.6	2.7	77.7	6.1	12.7
SR-03-05	(0.3-0.5)	(9.3-9.4)	(60.6-63.1)	(9.9-9.9)	(17.8-15.0)
SR-03-06	0.3	40.0	58.2	1.5	2.3
SR-03-08	0.4	2.7	52.3	13.6	29.9
SR-03-16	0.6	5.5	41.1	12.9	38.8
SR-03-24	1.4	5.3	22.7	14.3	54.9

Table 4.30 Concentrations of polynuclear aromatic and other semivolatile compounds (ng/dry g) in whole sediment samples from Saginaw River, MI third survey. The ranges for replicates are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Polynuclear Aromatic Hydrocarbons (ng/dry g)														
Sample	1,2 DCB	1,3 DCB	1,4, DCB	4-Methph	Naph	2-M Naph	DMPh	Ace-Naph	DBF	Fluore	Phen	Anth	Di-n-B	Fluora	Pyren
Grab samples															
SR-03-24	<290	<290	290	300	<290	<290	<290	<290	<290	<290	1000	<290	290	1200	1800
SR-03-06	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160	<160
SR-03-05	<200	<200	<200	<200	<200	<200	<200	<200	<200	<200	720	<200	<200	630	880
SR-03-02	<250	<250	<250	370	<250	<250	<250	<250	<250	<250	510	<250	270	500	710
SR-03-16	<230	<230	<230	320	<230	<230	<230	<230	<230	<230	460	<230	<230	420	750
SR-03-08	<250	<250	<250	450	<250	<250	<250	<250	<250	<250	580	<250	<250	580	1000
SR-03-01	(<250-270)	(<250-270)	(<250-270)	(410-870)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250-270)
Core samples															
SR-03-05-01	320	<250	450	<250	<250	<250	<250	<250	<250	<250	950	<250	<250	610	2000
SR-03-06-X2	<290	<290	340	1000	<290	<290	<290	<290	<290	<290	1300	<290	<290	1000	2700
SR-03-02-X3	<190	<190	<190	<190	<190	<190	<190	<190	<190	<190	<190	<190	<190	<190	260
SR-03-06-X3	<170	<170	<170	<170	<170	170	<170	<170	<170	<170	440	<170	<170	370	880
SR-03-05-02	<310	<310	<210	<310	<310	<310	<310	<420	<310	<310	1100	<310	<310	490	2100
SR-03-02-X2	680	1100	1300	<290	<290	<410	<290	<290	<290	300	3300	800	<290	1400	6600
SR-03-06-02	<170	<170	<170	<170	<170	<170	<170	<170	<170	<170	350	<170	<170	250	850
SR-03-06-01	<170	<170	<170	<170	<170	<170	<170	<170	<170	<170	1200	210	<170	870	2000

Table 4.30 (Continued).

Sample	Polynuclear Aromatic Hydrocarbons (ng/dry g)									
	BBPh	BaAnth	BisPh	Chrys	Di-n-O	BbFluor	BkFluor	BaPyr	IndPyr	BghiPer
Grab samples										
SR-03-24	<290	690	1000	600	<290	600	<290	440	<290	<290
SR-03-06	<160	<160	310	<160	<160	<160	<160	<160	<160	<160
SR-03-05	370	340	840	330	<200	300	<200	220	<200	<200
SR-03-02	1300	<250	1000	<250	<250	<250	<250	<250	<250	<250
SR-03-16	2800	250	8300	270	2000	<230	<230	<230	<230	<230
SR-03-08	340	340	1000	440	<250	390	<250	<250	<250	<250
SR-03-01	(<250-270)	(<250-270)	(910-2300)	(<250-270)	(<250-1000)	(<250-270)	(<250-270)	(<250-270)	(<250-270)	(<250 270)
Core samples										
SR-03-05-01	<250	720	1900	740	380	310	280	<250	<250	<250
SR-03-06-X2	<290	950	31000	1000	8800	<290	<290	<290	<290	<290
SR-03-02-X3	<190	<190	420	<190	<190	<190	<190	<190	<190	<190
SR-03-06-X3	<170	350	740	320	<170	<170	<170	<170	<170	<170
SR-03-05-02	5200	630	21000	690	6600	<310	<310	<310	<310	<310
SR-03-02-X2	<290	2000	4000	2200	<290	680	620	<290	<290	<290
SR-03-06-02	<170	220	170000	<170	<170	<170	<170	<170	<170	<170
SR-03-06-01	2000	750	22000	790	4500	<170	<170	<170	<170	<170

* Compound not detected at detection limit shown.

Table 4.31 Concentrations of dioxins and furans (pg/dry g) in whole sediment samples from Saginaw River, MI third survey. Ranges for samples analyzed in replicate are noted in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)												
Sample	2378-TCDF	Total TCDF	2378-TCDD	Total TCDD	12378-PeCDF	23478-PeCDF	Total PeCDF	12378-PeCDD	Total PeCDD	123-478-HxCDF	123-678-HxCDF	123-789-HxCDF	234-678-HxCDF
Grab samples													
SR-03-24	290	1100	8.0	64.0	120	110	560	ND	31	200	41	19.0	5.8
SR-03-06	1600	3100	1.3	5.7	240	250	850	1.3	3.8	95	24	8.9	3.8
SR-03-05	710	1600	11.0	50	260	190	940	15	24	230	43	15.0	7.6
SR-03-02	1700	3400	5.2	38	480	690	2500	ND	11	350	60	16.0	4.6
SR-03-16	240	460	6.2	17	110	72	280	ND	ND	120	25	7.0	2.1
SR-03-08	580	1700	32.0	230	250	160	940	20	150	290	82	22.0	111.0
SR-03-01	(99-110)	(230-330)	(4-4.5)	(18-26)	(36-50)	(23-32)	(120-220)	(3.5-4.4)	(20-27)	(52-73)	(24 28)	(6.8-8.2)	(1-1.9)
Core samples													
SR3-05-01	1300	3400	16.0	140	550	400	2700	ND	400	1200	280	73.0	21
SR3-06-X2	6300	15000	110.0	740	1900	1500	7800	ND	310	1200	220	88.0	21
SR3-02-X3	1500	3300	ND	1.2	1500	670	3800	ND	ND	1200	210	46.0	23
SR3-06-X3	490	960	3.6	9.3	180	130	640	ND	26	220	42	14	ND
SR3-05-02	1100	3800	ND	14	520	330	2000	ND	ND	ND	130	36	ND
SR3-02-X2	2700	9600	19	400	850	850	8100	56	990	2700	720	190	98
SR3-06-02	870	1600	ND	2.2	340	210	1100	ND	ND	260	52	11	2.9
SR3-06-01	8600	20000	42	320	2800	2100	11000	28	120	1800	1600	110	35

Table 4.31 (Continued).

Sample	Polychlorinated-dibenzo-dioxins or Polychlorinated-dibenzo-furans (pg/dry g)											
	Total HxCDF	123-478-HxCDD	123-678-HxCDD	123-789-HxCDD	Total HxCDD	1234-678-HpCDF	1234-789-HpCDF	Total HpCDF	1234-678-HpCDD	Total HpCDD	OCDF	OCDD
Grab samples												
SR-03-24	610	4.1	ND	15	190	210	68	770	310	550	850	2600
SR-03-06	200	1.1	3.4	2.6	33	49	4.5	120	40	74	89	340
SR-03-05	490	6.2	ND	15	280	520	44	1700	700	1100	1600	5100
SR-03-02	660	ND	9.3	3.9	87	280	33	570	180	310	530	1800
SR-03-16	340	ND	18	13	160	540	21	1300	430	790	940	4100
SR-03-08	1200	9.5	60	27	460	930	61	2100	700	1300	1800	6400
SR-03-01	(230-370)	(1.9-2.8)	(11-13)	(6.1-6.7)	(65-150)	(440-450)	(11-13)	(770-810)	(210-220)	(390-420)	(540-730)	(2200-2500)
Core samples												
SR3-05-01	5900	30	260	98	1500	9400	450	18000	2500	4400	12000	25000
SR3-06-X2	4600	19	ND	66	1200	4300	240	10000	3800	6400	10000	34000
SR3-02-X3	1900	ND	ND	ND	22	230	64	450	50	86	290	550
SR3-06-X3	510	1.7	18	6.6	120	550	37	1100	200	350	790	1900
SR3-05-02	880	ND	ND	ND	97	980	79	1700	34	34	1200	180
SR3-02-X2	22000	95	ND	210	3300	39000	1100	74000	12000	22000	56000	12000
SR3-06-02	440	ND	ND	ND	3.7	110	15	180	9.5	16	140	89
SR3-06-01	5600	ND	67	22	380	2400	110	5000	1400	2500	4300	14000

ND = Not detected.

Table 4.32 Concentrations of pesticides (μg/g) in the Saginaw River, MI third survey whole sediment samples. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.

	Pesticide											
Sample	Aldrin	A-BHC	B-BHC	C-BHC	Gamma Chlor-dane	Alpha Chlor-dane	4,4,DDD	4,4,DDE	4,4,DDT	Dieldrin	Endo-sulfan I	Endo-sulfan II
SR-03-01	(3.8-4.6)	(<3.3-<3.4)	(<3.3-<3.4)	(<3.3-<3.4)	(<3.3-5.7)	(<3.3-<3.4)	(<3.4-7.6)	(<3.3-3.9)	(<3.4-13.0)	(3.3-<3.4)	(<3.3-<3.4)	(<3.4-9.4)
SR-03-02	8.0	<3.3	6.4	<3.3	5.9	<3.3	8.4	5.6	<3.3	<3.3	<3.3	<3.3
SR-03-05	9.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	<2.8	7.7
SR-03-06	<2.5	<2.5	3.0	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
SR-03-08	<3.8	<3.8	<3.8	<3.8	9.0	<3.8	<3.8	11.0	<3.8	<3.8	12.0	11.0
SR-03-16	<3.2	<3.2	9.0	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2	5.2
SR-03-24	<3.9	<3.9	<3.9	<3.9	7.0	<3.9	13.0	<3.9	<3.9	7.7	6.0	<3.9
SR-03-02-X2	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0
SR-03-02-X3	<2.9	<2.9	7.0	<2.9	5.3	<2.9	<2.9	<2.9	<2.9	<2.9	<2.9	<2.9
SR-03-05-01	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(6.4-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)	(<3.9-<320.0)
SR-03-06	(2.7-460.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)	(<2.5-<270.0)
SR-03-06-X2	1400.0	880.0	<380.0	500.0	<380.0	<380.0	<380.0	<380.0	<380.0	<380.0	<380.0	<380.0
SR-03-06-X3	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0

Table 4.32 (Continued).

Sample	Pesticide								
	Endosulfan Sulfate	Endrin	Endrin Aldehyde	Hepta-Chlor	Heptachlor Epoxide	Lindane (G-BHC)	Toxaphene	Methoxy-chlor	Endrin Ketone
SR-03-01	(<3.3-<3.4)	(<3.3-<3.4)	(<3.4-17.0)	(<3.3-<3.4)	(<3.3-11.0)	(<3.3-<3.4)	(<33.0-<34.0)	(<17.0)	(<3.3-<3.4)
SR-03-02	<3.3	<3.3	<3.3	<3.3	<3.3	<33.0	<33.0	<16.0	<3.3
SR-03-05	<320.0	<320.0	<320.0	<320.0	<320.0	<320.0	<3200.0	<1600.0	<320.0
SR-03-06	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<25.0	12.0
SR-03-08	<3.8	<3.8	<3.8	<3.8	17.0	17.0	<38.0	<19.0	<3.8
SR-03-16	<3.2	<3.2	<3.2	<3.2	40.0	<3.2	<3.2	32.0	16.0
SR-03-24	<3.9	4.1	<3.9	<3.9	15.0	<3.9	39.0	<19.0	<3.9
SR-03-02-X2	<360.0	<360.0	<360.0	<360.0	<360.0	<360.0	<3600.0	<1800.0	<360.0
SR-03-02-X3	<2.9	<2.9	<2.9	<2.9	5.3	<2.9	<29.0	<15.0	<2.9
SR-03-05-01	<320.0	<320.0	<320.0	<320.0	<320.0	<320.0	<3200.0	<1600.0	<320.0
SR-03-05-02	<3.9	4.5	<3.9	<3.9	<3.9	<3.9	<39.0	<19.0	<3.9
SR-03-06-01	<270.0	<270.0	<270.0	<270.0	<270.0	<270.0	<2700.0	<1350.0	<270.0
SR-03-06-02	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<25.0	<13.0	<2.5
SR-03-06-X3	<260.0	<260.0	<260.0	<260.0	<260.0	<260.0	<2600.0	<1300.0	<260.0

< Indicated that compound was not detected at detection limit shown.

Table 4.33 Polychlorinated biphenyl concentrations and analytical detection limits (ng/dry g) for whole sediments from the Saginaw River, MI third survey. See Table 4.1 for an explanation of chemical abbreviations.

	Polychlorinated Biphenyl (ng/dry g)		
	1242	1254	1260
Grab Sample			
SR-03-24	38[1]	38[1]	38[1]
SR-03-06	95	25[1]	25[1]
SR-03-05	370	120	29[1]
SR-03-02	240	95	32[1]
SR-03-16	470	87[1]	32[1]
SR-03-08	21	94	38[1]
SR-03-01	210 (72[1]-260)	34[1] (33[1]-34[1])	34[1] (33[1]-34[1])
Core Sample			
SR-03-05-01	3700	3200[1]	3200[1]
SR-03-06-X2	79000	8100	3800[1]
SR-03-02-X3	29[1]	29[1]	29[1]
SR-03-06-X3	4300	2600[1]	2600[1]
SR-03-05-02	62	100	38[1]
SR-03-02-X2	410	35[1]	36[1]
SR-03-06-02	96	25[1]	25[1]
SR-03-06-01	28000	2700[1]	2700[1]

[1] Indicates that compound was not detected at detection limit shown.

Table 4.34 Mean (standard error of the mean in parentheses) measured overlying water quality for Saginaw River, MI third survey whole sediment toxicity tests.

Sample	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	D.O. (mg/L)	Conductivity (µmhos/cm)	Unionized Ammonia (mg/L)	Chloride (mg/L)	Turbidity (NTU)
Hyalella azteca - 14-day								
Control	7.8 (0.05)	54 (2.0)	131 (1.0)	8.2 (0.09)	283 (9.0)	0.005 (0.00)	7.1 (0.04)	8.9 (8.10)
SR-03-01	8.1 (0.05)	76 (0.0)	149 (3.0)	8.0 (0.20)	293 (6.2)	0.024 (0.01)	6.7 (0.89)	8.4 (7.65)
SR-03-02	8.0 (0.06)	82 (10.0)	149 (5.0)	7.4 (0.13)	315 (16.1)	0.049 (0.03)	8.7 (2.32)	27.6 (26.40)
SR-03-05	8.1 (0.04)	82 (8.0)	148 (4.0)	7.7 (0.17)	305 (7.9)	0.049 (0.04)	8.5 (2.71)	34.7 (33.35)
SR-03-06	8.1 (0.04)	76 (0.0)	151 (3.0)	7.7 (0.06)	315 (15.5)	0.030 (0.02)	8.5 (2.74)	33.0 (32.00)
SR-03-08	8.0 (0.06)	84 (8.0)	152 (4.0)	7.5 (0.19)	319 (15.3)	0.047 (0.04)	8.0 (2.29)	60.5 (59.50)
SR-03-16	8.0 (0.05)	82 (10.0)	150 (6.0)	7.5 (0.24)	323 (14.8)	0.061 (0.05)	8.4 (2.02)	27.0 (26.00)
SR-03-24	8.0 (0.10)	88 (12.0)	156 (8.0)	7.3 (0.47)	341 (26.6)	0.118 (0.11)	10.2 (4.58)	48.1 (46.95)
Hyalella azteca - 28-day								
Control	7.8 (0.07)	62 (6.0)	139 (9.0)	8.2 (0.24)	286 (7.3)	0.004 (0.00)	6.8 (0.33)	8.8 (8.20)
SR-03-01	8.1 (0.04)	76 (0.0)	145 (1.0)	7.7 (0.19)	292 (4.3)	0.019 (0.01)	7.5 (0.12)	8.5 (7.50)
SR-03-02	8.0 (0.05)	84 (8.0)	153 (1.0)	7.6 (0.22)	308 (9.2)	0.047 (0.04)	8.6 (2.42)	27.5 (26.56)
SR-03-05	8.0 (0.06)	81 (9.0)	152 (0.0)	7.6 (0.23)	304 (9.2)	0.049 (0.05)	8.7 (2.52)	34.5 (33.52)
SR-03-06	8.0 (0.04)	74 (2.0)	153 (1.0)	7.9 (0.21)	303 (7.9)	0.026 (0.02)	8.8 (2.44)	32.9 (32.09)
SR-03-08	8.0 (0.05)	83 (9.0)	150 (6.0)	7.6 (0.26)	307 (9.4)	0.040 (0.04)	8.2 (2.10)	60.9 (59.10)
SR-03-16	8.0 (0.06)	84 (8.0)	154 (2.0)	7.4 (0.33)	310 (9.5)	0.062 (0.06)	8.4 (1.96)	27.7 (25.30)
SR-03-24	7.9 (0.06)	86 (14.0)	158 (6.0)	7.3 (0.28)	308 (19.0)	0.101 (0.10)	10.7 (4.11)	48.5 (46.55)
Chironomus riparius - 14-day								
Control	7.9 (0.16)	60 (4.0)	141(11.0)	7.9 (0.50)	287 (11.2)	0.004 (0.00)	7.1 (0.05)	12.1 (4.90)
SR-03-01	8.0 (0.13)	76 (0.0)	149(03.0)	7.6 (0.42)	285 (1.1)	0.015 (0.01)	7.3 (0.32)	11.1 (4.90)
SR-03-02	7.9 (0.22)	77 (15.0)	149(05.0)	7.0 (0.96)	309 (15.9)	0.047 (0.04)	8.8 (2.23)	33.5 (20.50)
SR-03-05	7.9 (0.21)	83 (7.0)	152(00.0)	7.1 (0.89)	306 (14.4)	0.049 (0.05)	8.9 (2.29)	40.0 (28.00)
SR-03-06	8.0 (0.12)	76 (0.0)	153(01.0)	7.5 (0.61)	307 (9.0)	0.026 (0.02)	8.9 (2.27)	37.8 (27.25)
SR-03-08	7.9 (0.19)	86 (6.0)	154(02.0)	7.2 (0.80)	311 (12.0)	0.040 (0.04)	8.5 (1.77)	69.5 (50.50)
SR-03-16	8.0 (0.16)	88 (4.0)	158(02.0)	7.3 (0.62)	310 (15.3)	0.063 (0.06)	8.5 (1.89)	36.0 (17.00)
SR-03-24	7.9 (0.18)	92 (8.0)	160(04.0)	7.2 (0.75)	329 (21.9)	0.101 (0.10)	10.7 (4.14)	57.8 (37.25)

TABLE 4.34 (Continued).

Sample	pH	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)	D.O. (mg/L)	Conductivity (umhos/cm)	Unionized Ammonia (mg/L)	Chloride (mg/L)	Turbidity (NTU)
Chironomus tentans - 10-day								
Control	8.3 (0.17)	112 (56.0)	153(23.0)	6.1 (2.50)	326 (68.0)	0.376 (0.37)	9.3 (2.18)	20.8(3.75)
SR-03-01	8.4 (0.23)	150 (74.0)	169(23.0)	6.6 (1.50)	348 (74.7)	0.886 (0.85)	9.4 (1.86)	14.3 (1.75)
SR-03-02	8.4 (0.25)	114 (22.0)	163(09.0)	7.1 (1.05)	352 (27.3)	0.361 (0.25)	12.3 (1.25)	38.0 (16.00)
SR-03-05	8.6 (0.42)	99 (9.0)	166(14.0)	8.0 (0.15)	351 (30.9)	0.074 (0.04)	12.8 (1.55)	58.5 (9.50)
SR-03-06	8.5 (0.30)	134 (58.0)	185(31.0)	7.8 (0.55)	370 (60.8)	0.846 (0.79)	12.1 (0.90)	36.0 (29.00)
SR-03-08	8.3 (0.23)	134 (42.0)	180(24.0)	7.2 (0.88)	368 (47.9)	0.219 (0.13)	13.7 (3.40)	76.5 (43.50)
SR-03-16	8.5 (0.33)	110 (18.0)	172(16.0)	7.9 (0.35)	374 (49.0)	0.248 (0.10)	14.2 (3.75)	35.5 (17.50)
SR-03-24	8.4 (0.22)	108 (8.0)	178(14.0)	7.6 (0.63)	389 (33.5)	0.188 (0.06)	18.1 (3.30)	64.0 (31.00)

Table 4.35 Responses of Hyalella azteca, Chironomus riparius, and Chironomus tentans exposed to whole sediment samples from Saginaw River, MI third survey. Mean values (standard error in parentheses) within columns and tests with common letters are not significantly different ($p \leq 0.05$).

Test	Survival (%)	Total Body Length (mm) Start	Total Body Length (mm) End	Antennal Segment (#)	Mature (%)
Hyalella azteca - 14 day					
Control	85.0(0.1)	1.2	1.9(.06)ab	15.0(0.1)	12.6(.08)ab
SR-03-01	98.8(0.1)		2.0(.05)b	15.2(0.1)	15.9(.08)ab
SR-03-02	90.0(0.1)		2.0(.06)ab	15.0(0.1)	6.5(.08)bc
SR-03-05	88.8(0.1)		1.8(.06)a	14.9(0.1)	2.8(.08)c
SR-03-06	95.0(0.1)		1.8(.05)a	14.9(0.1)	9.4(.08)abc
SR-03-08	93.8(0.1)		1.9(.05)a	15.0(0.1)	7.8(.08)bc
SR-03-16	95.0(0.1)		2.0(.05)ab	15.3(0.1)	20.7(.08)a
SR-03-24	96.3(0.1)		2.0(.05)b	15.3(0.1)	18.2(.08)ab
Hyalella azteca - 28 day					
Control	96.3(.09)	1.3	3.9(0.1)ab	19.6(0.3)abc	74.9(.04)
SR-03-01	93.8(.09)		3.7(0.1)a	20.0(0.2)ac	83.8(.04)
SR-03-02	100.0(.09)		3.9(0.1)ab	20.2(0.3)ac	75.0(.04)
SR-03-05	92.5(.09)		3.7(0.1)a	19.9(0.2)ac	83.1(.04)
SR-03-06	96.3(.09)		3.6(0.1)a	19.5(0.2)bc	72.1(.04)
SR-03-08	89.5(.09)		3.6(0.1)a	18.9(0.3)b	71.5(.04)
SR-03-16	92.5(.09)		4.1(0.1)b	20.2(0.3)ac	71.6(.04)
SR-03-24	98.8(.09)		4.0(0.1)b	20.2(0.2)a	82.3(.04)
C. riparius - 14 day					
Control	91.5(3.3)		10.1(0.2)e		
SR-03-01	88.5(7.6)		11.0(0.2)d		
SR-03-02	92.0(4.8)		12.5(0.2)ab		
SR-03-05	96.0(2.8)		11.8(0.2)c		
SR-03-06	94.5(3.8)		11.7(0.2)c		
SR-03-08	87.0(3.4)		11.9(0.2)bc		
SR-03-16	94.0(3.5)		12.5(0.2)a		
SR-03-24	93.5(2.2)		11.9(0.2)c		

Table 4.35 (Continued).

Test	Survival (%)	Total Body Length (mm)	
		Start	End
C. tentans - 10 day			
Control	60.0*	5.6	14.6(1.3)
SR-03-01	40.0*		12.6(1.6)
SR-03-02	40.0*		15.4(1.6)
SR-03-05	80.0*		16.2(1.2)
SR-03-06	0.0*		--
SR-03-08	70.0*		16.8(1.2)
SR-03-16	30.0*		16.2(1.9)
SR-03-24	80.0*		15.3(1.2)

* See Table 4.36 for categorical model statistics.

Table 4.36 Categorical comparison matrix of Chironomus tentans survival data for Saginaw River, MI third survey. Values above the diagonal separator are probability values and below the separator are the Chi square values. Probability values with * indicate that significant differences exist in the comparison between sites ($p \leq 0.05$).

	Saginaw River Third Survey Stations							
	01	02	05	06	08	16	24	Control
01	****	1.00	0.079	0.102	0.185	0.640	0.079	0.374
02	0.00	****	0.079	0.102	0.185	0.640	0.079	0.374
05	3.08	3.08	****	**0.008***	0.6075	**0.033***	1.00	0.336
06	2.67	2.67	7.05	****	**0.017***	0.181	**0.008***	**0.032***
08	1.76	1.76	0.26	5.73	****	0.82	0.607	0.640
16	0.22	0.22	4.53	1.79	3.02	****	**0.033***	0.185
24	3.08	3.08	0.00	7.05	0.26	4.53	****	0.336
Control	0.79	0.79	0.92	4.60	0.22	1.76	0.92	****

Table 4.38 Recommended threshold concentrations (ng/dry g) for polynuclear aromatic hydrocarbons and total polychlorinated biphenyls. See Table 4.1 for an explanation of chemical abbreviations.

Compound	Recommended Threshold Concentrations			
	Barrick et al. (1988) (ng/dry g)	USEPA (1988) (ng/dry g)	SMS (1991) (ng/dry g OC)	Long and Morgan (1990)* (ng/dry g)
Low Molecular Weight PAH	24,000	---	370,000	---
Naph	2,400	---	99,000	340-2,100
Acnaph	2,000		16,000	150-650
Fluore	3,600	---	23,000	35-640
Phen	6,900	1,000	100,000	255-1,380
Anth	13,000	---	220,000	85-960
High Molecular Weight PAH	69,000	---	960,000	---
Fluora	30,000	19,000	160,000	600-3,600
Pyrene	16,000	13,000	1,000,000	350-2,200
BaAnth	5,100	13,000	110,000	230-1,600
Chrys	9,200	---	110,000	400-2,800
BbkFlour**	7,800	---	230,000	---
BaPyr	3,000	11000	99,000	400-2,500
IndPyr	1,800	---	33,000	---
BghiPer	1,400	---	31,000	---
Total PAH	---		1,330,000	4,000-35,000
Total PCBs	1,900	420	12,000	50-400

* The values for each compound indicates the range between the Effects Range-Low (ER-L) and Effects Range-Median (ER-M).

** BbFlour and BkFlour are combined and listed as BbkFlour.

Table 4.39 Recommended metals threshold concentrations (μg/dry g) for sediment. See Table 4.1 for an explanation of chemical abbreviations.

Metals	Recommended Threshold Concentrations					
	USEPA/ USACE (1977) (μg/dry g)	MOE (1978) (μg/dry g)	IEPA (1984) (μg/dry g)	IJC (1988) (μg/dry g)	SMS (1991) (μg/dry g OC)	Long and Morgan (1990)* (μg/dry g)
As	3-8	8.0	11.0	1.1	57	33-85
Cd	>6	1.0	1.0	0.6	5.1	5-9
Cr	25-75	25.0	23.0	37.1	260	80-145
Cu	26-50	25.0	60.0	21.0	390	70-390
Hg	1	0.3	0.01	0.03	---	0.15-1.3
Ni	20-50	25.0	---	32.8	---	30-50
Pb	40-60	50.0	38.0	27.5	450	35-110
Zn	90-200	100.0	100.0	120.0	410	120-270

* The values for each metal indicates the range between the Effects Range-Low (ER-L) and Effects Range-Median (ER-M).

Table 4.37 Percentage fine particle size (<63 μm) for sediments from all AOCs tested in whole sediment exposures. Concentrations of low and high molecular weight polynuclear aromatic hydrocarbons (ng/dry g), and total polychlorinated biphenyls (ng/dry g), and concentrations normalized with percentage total organic carbon for low and high molecular weight polynuclear aromatic hydrocarbons (μg/dry g OC), and total polychlorinated biphenyls (μg/dry g OC). See Table 4.1 for an explanation of chemical abbreviations.

Station	Particle Size (%)	TOC (%)	PAHs (ng/dry g)			PAH/TOC (μg/dry g OC)			PCBs	
	Fines (<63 μm)		Molecular Weight			Molecular Weight		Sum of PAH/TOC	Total (ng/dry g)	PCB/TOC (μg/dry g OC)
			Low	High	Sum	Low	High			
IH-01-03*	92.2	7.7	19100	85200	1043000	248.05	1106.49	1354.55	21160∞	274.81
IH-01-04*	96.0	5.6	9190	52800	61990	164.11	942.86	1106.96	17960∞	320.71
IH-01-06*	96.1	11.6	30400	198000	228400	262.07	1706.90	1968.97	27000∞	232.76
IH-01-07*	91.8	8.8	401580	292600	694180	4563.41	3211.36	7774.77	45460∞	516.59
BR-01-01*	81.8	8.9	12000	51000	63000	134.83	573.03	707.87	3570∞	40.11
BR-01-03*	91.5	2.0	3940	9300	13240	197.00	465.00	662.00	5200∞	260.00
BR-01-07*	97.8	1.7	1143	19650	20793	67.24	1155.88	1223.12	2520∞	148.24
BR-01-08	93.0	1.7	580	3498	4078	34.12	205.76	239.88	2380∞	140.00
BR-01-09*	95.9	2.2	731	4700	5431	33.23	213.64	246.86	2520∞	114.55
SR-01-03	99.3	3.0	479	2620	3099	15.97	87.33	103.30	2380∞	79.33
SR-01-06*	54.0	2.1	552	2900	3452	26.29	138.10	164.38	69400∞	3304.76
SR-01-10	53.0	1.0	27	119	146	2.70	11.90	14.60	4040∞	404.00
SR-03-01	98.9	1.0	1040	2250	3290	104.00	225.00	329.00	114☼	11.40
SR-03-02	96.5	1.3	1260	2960	4220	96.92	227.69	324.62	145☼	11.15
SR-03-05	88.2	1.4	1320	3300	4620	94.29	235.71	330.00	519☼	37.07
SR-03-06*	62.0	0.2	640	1400	2040	320.00	700.00	1020.00	367☼	183.50
SR-03-08	95.8	3.6	1330	3750	5080	36.94	104.17	141.11	589☼	16.36
SR-03-16	92.3	2.9	1150	2840	3990	39.66	97.93	137.59	153☼	5.28
SR-03-24	91.9	4.0	1870	6200	8070	46.75	155.00	201.75	278☼	6.95

* Survival for at least one test species was significantly reduced from control. Tests that did not meet ASTM test acceptability criteria were not included (C. riparius 14-d Buffalo River and C. tentans 10-d Saginaw River (3).

∞ PCBs analyzed: Aroclor 1016, 1221, 1232, 1242, 1248, 1254 and 1260.

☼ PCBs analyzed: Aroclor 1242, 1254 and 1260.

Table 4.40 Comparisons of recommended threshold concentrations and test organism responses from whole sediment exposures. A hit was considered positive (+) if threshold concentrations were predictive (sediment concentrations exceeded thresholds and toxicity occurred, or sediment concentrations did not exceed thresholds and no toxicity occurred. A hit was considered negative (-) if sediment concentrations did not exceed thresholds and toxicity occurred. A hit was considered a zero (0) if sediment concentrations did exceed thresholds and no toxicity occurred. Percentages represent proportion of total hits to the number of AOC stations tested.

Station	SMS (1991)			Long and Morgan (1990)☼			Barrick et al. (1988)	
	Metals	PAHs	PCBs	Metals	PAHs	PBCs	PAHs	PCBs
IH-01-03*	+	+	+	+	+	+	+	+
IH-01-04*	+	-	+	-	+	+	-	+
IH-01-06*	+	+	+	+	+	+	+	+
IH-01-07*	+	+	+	+	+	+	+	+
BR-01-01*	+	-	+	+	+	+	-	+
BR-01-03*	-	-	+	-	-	+	-	+
BR-01-07*	-	-	+	-	-	+	-	+
BR-01-08*	-	-	+	-	-	+	-	+
BR-01-09*	-	-	+	-	-	+	-	+
SR-01-03*	-	-	+	-	-	+	-	+
SR-01-06*	+	-	+	+	-	+	-	+
SR-01-10*	-	-	+	-	-	+	-	+
SR-03-01	+	+	+	+	+	+	+	+
SR-03-02	+	+	+	+	+	+	+	+
SR-03-05	+	+	0	+	+	0	+	+
SR-03-06*	-	-	+	-	-	-	-	-
SR-03-08	+	+	0	+	+	0	+	+
SR-03-16	0	+	+	0	+	+	+	+
SR-03-24	0	+	+	0	+	+	+	+
Total + (%)	52.6	47.4	89.5	47.4	57.9	84.2	47.4	94.7
Total - (%)	36.9	52.6	0	42.1	42.1	5.3	52.6	5.3
Total 0 (%)	10.5	0	10.5	10.5	0	10.5	0	0

☼ Indicates concentrations exceeded the Effects Range-Median.
* Survival or growth for at least one test species was significantly reduced from control.

Table 4.41 Comparison of test species sensitivity across Areas of Concern. The percentage hits are based o the number of contaminated sediments that elicited significant reductions in survival or body length compared to control. A positive indicates a hit and a negative indicates no hit.

	Survival				Growth			
Station	HA14*	HA28^	CR14∞	CT10′	HA14*	HA28^	CR14∞	CT10′
IH-01-03	+	NA	+	+	-	NA	NA	+
IH-01-04	+	NA	+	+	NA	NA	NA	+
IH-01-06	+	NA	+	+	-	NA	NA	NA
IH-01-07	+	NA	+	+	NA	NA	NA	NA
BR-01-01	-	+	-	-	+	-	-	-
BR-01-03	+	+	-	-	+	+	+	-
BR-01-07	-	+	+	-	+	+	-	+
BR-01-08	-	-	+	-	+	-	-	+
BR-01-09	-	+	-	-	+	-	-	-
SR-01-03	-	-	-	NA	-	+	-	NA
SR-01-06	+	+	+	NA	-	+	+	NA
SR-01-10	-	-	-	NA	-	+	-	NA
SR-03-01	-	-	-	-	-	-	-	-
SR-03-02	-	-	-	-	-	-	-	-
SR-03-05	-	-	-	-	-	-	-	-
SR-03-06	-	-	-	+	-	-	-	NA
SR-03-08	-	-	-	-	-	-	-	-
SR-03-16	-	-	-	-	-	-	-	-
SR-03-24	-	-	-	-	-	-	-	-
Hits (%)	32	33	37	31	29	33	13	31
Stations (%)	100	79	100	84	89	79	79	68
	HA14*	HA28^	CR14∞	CT10′	HA14*	HA28^	CR14∞	CT10′

* H. azteca 14 day exposure.
^ H. azteca 28 day exposure.
∞ C. riparius 14 day exposure.
′ C. tentans 10 day exposure.
NA Indicates not applicable.

Appendix 4.1 Concentrations of metals (μg/dry g) in control sediment. See Table 4.1 for an explanation of chemical abbreviations.

Metal (μg/dry g)										
Ag	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Se	Zn
0.117	8.9B	0.18	52B	19B	0.022	675B	19.4B	19.6B	<0.16	52.5B

B Indicates that data was not available for procedural blank.
< Indicates compound not detected at detection limit shown.

Appendix 4.2 Concentrations of polynuclear aromatic and other semivolatile compounds (ng/dry g) for control sediment. Ranges for samples analyzed in replicate are shown in parentheses. See Table 4.1 for an explanation of chemical abbreviations.*

Polynuclear Aromatic Hydrocarbon (ng/dry g)

1-4 DCB	Naph	Acenaphl	Acenaph	2-M Naph	DMPh	DBF	Fluore
NA	B	<5.77	<10.26	NA	NA	NA	<7.37

DThiop	Phen	Anth	Fluora	Pyrene	BBPh	BaAnth	BisPh
<5.24	B	<4.77	8.495 (7.25-9.74)	B	NA	<3.76	NA

Chrys	DnOPh	BbFlour	BkFlour	BaPyr	IndPyr	D(a,h)Anth	BghiPer
4.46 (4.19-4.72)	NA	8.52 (8.05-8.99)	<3.23	<4.04	<2.54	<3.22	<2.85

* Other organic chemical analyses (Aroclor 1242, 1248, 1254, and 1260; and pesticides) were conducted for the control sediment. All pesticides and Aroclors were not detected at or above the detection limits used for the contaminated sediments from all AOCs. Total organic carbon was 1.02 percent.

NA Indicates that data is not available.

B Indicates that analyte was detected in the method blank associated with sample.

< Indicates that compound was not detected at detection limit shown.

CHAPTER 5: BENTHIC COMMUNITY STRUCTURE EVALUATIONS

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INTRODUCTION

Contaminated sediments are a major source of pollution in the United States and represent a continual threat to all levels of the aquatic ecosystem (Sorensen et al. 1977, Landrum and Robbins 1990). Sediments act as a repository for a whole array of organic and inorganic contaminants, and can accumulate the contaminants to extremely high concentrations (Shimp et al. 1971, Oschwald 1972, Medine and McCutcheon 1989). Benthic macroinvertebrates are integrally tied to the sediment and therefore are continuously exposed to contaminants in sediments.

The entire aquatic ecosystem is linked together by virtue of interdependent trophic levels. Therefore it is no longer adequate to study single components of the ecosystem when making assessments of sediment toxicity (Burton 1991). Complete ecological assessments of sediment toxicity require the use of resident biota as indicators of sediment quality. For the assessment to be successful, closely integrated biological, chemical, and physical data are required. Sediments integrate historical water quality, hence, the spatial and temporal distribution of resident organisms can reflect the degree to which chemicals in the sediments are toxic. Field surveys of invertebrates provide an essential component of biological assessments of toxicity associated with contaminated sediments and have several advantages: (1) indigenous benthic organisms complete all or most of their life cycles in the aquatic environment and serve as continuous monitors of sediment quality, (2) benthic invertebrates are relatively sedentary and are representative of local conditions, (3) macroinvertebrates are relatively easy to collect and are abundant and ubiquitous across a broad array of sediment types, (4) a field assessment of natural populations can be used to screen potential sediment contamination, (if a spatial gradient of contamination exists, extrapolations of inter-species sensitivity, water quality differences, or chemical interaction [additivity, antagonism, or synergism] are not necessary), and (5) results of an assessment of indigenous populations are biologically interpretable, which should quantify resource damage in a manner more easily understood by managers, regulators and the general public (Cook 1976, Pratt and Coler 1976, Davis and Lathrop 1989).

This chapter describes the results of quantitative invertebrate surveys taken simultaneously with sediment collection from stations within the Areas of Concern (AOC) sampled for the GLNPO ARCS project. The objective of the survey is to describe species distributions and abundances across 3 AOCs: Indiana Harbor, Buffalo River, and Saginaw River. An attempt will be made to relate the observed species abundance and community structure to sediment metal concentrations and other physical and chemical parameters. This information, when analyzed in the context of measures of sediment toxicity will provide a more complete representation of the effects of in-situ contaminants on the benthic invertebrate communities.

MATERIALS AND METHODS

The GLNPO ARCS Toxicity-Chemistry Work Group (Table 1.1) selected stations for whole sediment toxicity and benthic macroinvertebrate assessments based on the expected presence of contaminants which could be deleterious to resident aquatic biota. Three areas of concern (AOC), Buffalo river (BR-01), Indiana Harbor (IH-01), and Saginaw River (SR-01) were sampled once in 1989; Saginaw River (SR-03) was sampled a second time in June 1990 (See Table 2.1 for a complete listing of the stations sampled). Seven stations were sampled in Indian Harbor and Saginaw River (first and third survey) while 10 stations were sampled in Buffalo River. A standard ponar dredge (529 cm^2 area; 4.69 L volume) was used to collect benthic invertebrates and bulk sediments at each station.

Sediments were analyzed for a variety of chemical and physical characteristics. Sediment chemical characteristics included (1) total metals, (2) organometals, (3) simultaneously extracted metals (SEM) and acid volatile sulfide (AVS), (4) chlorinated pesticides, (5) polychlorinated biphenyls (PCB), (6) select polynuclear aromatic hydrocarbons (PAH), and (8) polychlorinated dioxins and furans. Sediment physical characteristics included (1) total organic carbon, (2) inorganic carbon, and (3) sediment particle size. For additional information on chemical and physical characteristics of the sediments, see chapter 4 of this document.

A total of 155 benthic grab samples (about 5 grabs/station) were collected from all AOCs. Artificial substrates were put at 5 stations in Buffalo River, 4 stations at Indiana Harbor and 6 stations at Saginaw River. All 155 benthic samples were collected before sediment samples for chemistry and toxicity testing in an effort to minimize potential disturbance of the sediments and associated invertebrates. The 5 replicate Ponar samples were taken at each station within a 100 m^2 area. Each collected sample was sieved through a 500 μm brass screen using station water for rinsing. Material retained by the sieve was rinsed into 500-mL Ball glass jars and preserved with 10% buffered formalin. All jars were filled to the top with formalin to prevent damage to the benthic invertebrates during transport. Before shipment, jars were placed into a cardboard box with dividers to protect individual samples. Each box was triple bagged with 114 liter plastic sacks and put into a larger box and surrounded by styrofoam packing. Each container was labeled with an 11 digit number describing designation codes for collection site, survey number, transect number, station number, sample type, replicate, and sample fraction. The 11 digit sample number was recorded in the field log book, on the sample container and in the sample container on a piece of paper with a soft lead (#2) pencil.

Samples were received at the National Fisheries Contaminant Research Center (NFCRC) in Columbia, MO, and immediately inspected for shipping damage. Samples broken in shipping were recorded and discarded. Before sorting, samples were rinsed thoroughly with tap water to remove formalin and excess silt or mud. The "clean" samples were drained of excess water, returned to the original jars, filled with 95% ethanol and allowed to soak for at least 24 h to facilitate extraction of any volatiles. After the 24 h extraction period, each sample was rinsed again with tap water to remove the ethanol and volatiles. The samples were placed into a 4-L wide-mouth jar, agitated with tap water to float the invertebrates and lighter detrital material while leaving the snails, clams and heavier material in the bottom of the jar.

Aliquots of the sample were sequentially removed from the jar to sort the benthic invertebrates. This continued until the entire sample had been sorted. Approximate sorting time ranged from 3 to 20 h/sample.

A 4X or greater binocular dissecting microscope was used to sort the samples. Organisms were sorted and enumerated into the following orders or families: Oligochaeta, Chironomidae, Bivalvia, Gastropoda, Ephemeroptera, Odonata, Plecoptera, Hemiptera, Megaloptera, Tricoptera, Coleoptera, Diptera, Hirudinea, and Amphipoda. These samples were used to estimate macroinvertebrate abundance (number/m^2), species composition, and taxa richness. Taxonomic identification was made to the lowest level for each group by NFCRC personnel using published taxonomic keys (Wiederholm 1983; Merritt and Cummins 1984; Pennak 1989; Thorp and Covich 1991). Oligochaeta were identified to genus and species (when possible) by Mark Wetzel at Invertaxon, Urbana, IL. Mollusca were identified to genus and species by Jeff Garner at the Aquatic Resources Center, Franklin, TN. Chironomidae samples were identified to genus by NFCRC personnel and confirmations of identifications were made by Dr. Leonard Ferrington, University of Kansas, Lawrence, KS.

Chironomidae were examined for deformities in mouthpart structures. These deformities consisted of various types of asymmetry, missing teeth, extra teeth, fusion among various teeth, and labial separation described by several investigators (Saether 1970; Hamilton and Saether 1971; Hare and Carter 1976; Warwick et al 1987; Warwick 1989). Individual Chironomidae were mounted on slides and examined for deformities in the mentum and ligula (Tanypodinae). Occurrence of deformities was expressed as a proportion of the total number of Chironomidae at each station.

Artificial substrates were constructed from a 3M™ synthetic mesh and stainless steel wire rotisserie chicken baskets (Stauffer et al. 1976). Each substrate consisted of 5 pieces of mesh (20X20cm) folded in half and placed beside each other in a chicken basket. The rotisserie baskets were 26 cm in length, 17 cm in diameter and 53.34 cm in circumference (Figure 5.1). The baskets were wired shut and three baskets were wired to a cinder block at each sampling station. The baskets were on six foot wires and sat horizontally on the bottom near the cinderblock. A wire was attached to the cinderblock and the other end was connected to a recognizable land mark on shore to facilitate retrieval of the artificial substrates.

Artificial substrates were put at 5 stations in Buffalo River (October 1989), 4 stations in Indiana Harbor (August 1991), and 6 stations at Saginaw River (survey 3, June 1990). The artificial substrates were incubated at stations for 30 d. The substrates were lifted to the surface and then placed into plastic dishpans. The mesh substrate material was removed and placed in 4-L wide-mouth jars and preserved in 4% buffered formalin. Water and mud remaining in the dishpans was poured through a 250 µm mesh sieve and anything retained on the sieve was preserved with the synthetic mesh.

Samples were rinsed in the laboratory through a 250 µm mesh sieve and then each piece of mesh was unraveled under water in a dishpan. Sediment and organisms were retained in the water, sieved (250 µm mesh) and stored in 70% ethanol; the mesh was discarded. The entire preserved sample was placed in a pan for sorting at 12X magnification. A 100 organism sub-sample was removed from the sample; an attempt was made to sub-sample organisms in proportion to their abundance in the sample. At Indiana Harbor Station 3, each sample contained thousands of tiny (<500 um), just-settled zebra mussels (Dreissena

sp.). A 100 organism sub-sample of invertebrates other than zebra mussels was picked to determine other organisms present at Station 3 of Indiana Harbor. At all stations, organisms too rare to be included in the 100 animal sub-samples were qualitatively recorded.

Statistical analysis was performed with the Statistical Analysis System (SAS) computer package for personnel computers (Statistical Analysis System 1988). Relations among the benthic invertebrate abundance within and among areas of concern were analyzed with a nested analysis of variance (ANOVA) (Snedecor and Cochran 1980). Comparisons between benthic invertebrate abundance and physical and chemical data were compared with correlation analysis and multivariate regression statistics. If not reported, statements of statistical significance imply $p \leq 0.05$.

RESULTS

BENTHIC INVERTEBRATE ABUNDANCE AND COMMUNITY STRUCTURE IN GRAB SAMPLES

Indiana Harbor

Samples were collected at Indiana Harbor in August, 1989. Benthic invertebrate abundance (number/m^2) for the entire community (Table 5.1) at each station ranged from 609/m^2 at IH-01-07 to a maximum of 493,917/m^2 at IH-01-10. Except for IH-01-10, total abundance values were less than 7,000/m^2, with abundance values at 4 of the 7 stations less than 4,000/m^2 (Table 5.1). Within the benthic invertebrate community the Oligochaeta were numerically dominant at all stations sampled, bivalves comprised the majority of the remaining community in 3 of the 7 stations. Oligochaeta abundance accounted for 91% to 100% of the community across all stations sampled, with the remainder of the benthic community abundance coming from the Bivalvia, Hirudinea and Chironomidae (Table 5.2).

Oligochaeta Abundance--

Oligochaeta abundance ranged from 552/m^2 at IH-01-07 to 493,887/m^2 at IH-01-10 (Table 5.1). As with total abundance, Oligochaeta abundance values were all less than 6,000/m^2 with the exception of IH-01-10 and abundance was less than 3,000/m^2 at 40% of the stations (Table 5.1). The Oligochaeta community was made up entirely of the family Tubificidae (Table 5.3). The Oligochaeta were comprised of 1 family, 5 genera and 8 species.

Limnodrilus sp. was the most common genera occurring at all 7 stations sampled. *Quistadrilus* sp. was present at 6 stations with *Aulodrilus* sp. and *Ilyodrilus* sp. present at 2 stations each. *Tubifex* sp. was present at only 1 station. The *Limnodrilus* sp. were represented by 3 species (*L. cervix*, *L. hoffmeisteri*, *L. udekemianus*). *Limnodrilus hoffmeisteri* occurred at all 7 stations, with *L. cervix* occurring at 5 stations and *L. udekemianus* occurring at 2 stations. The genera *Aulodrilus* sp. was represented by 2 species, *A. limnobius* and *A. pluriseta*. *Aulodrilus pluriseta* occurred at two stations and *A. limnobius* occurred at 1 station. The remaining genera were represented by 1 species each (*Ilyodrilus templetoni*, *Quistadrilus multisetosus*).

Chironomidae Abundance--

Chironomidae abundance ranged from none in samples from several stations to 8/m^2 at IH-01-10 (Table 5.1). The Chironomidae community was comprised of 1 subfamily (Orthocladinae) and 1 genera (*Cricotopus* sp.) (Table 5.4). Only 2 individuals were found among all the stations sampled at Indiana Harbor.

Mollusca Abundance--

The Mollusca were represented by 1 class (Bivalvia), 1 family, 3 genera, and at least 3 species (Table 5.5). Mean Bivalvia abundance ranged from none in samples from several stations to 8/m^2 at IH-01-03 and IH-01-07 (Table 5.1). *Musculium* sp. and *Pisidium* sp. were the most frequently observed genera each occurring at 2 of 10 stations. *Sphaerium* sp. occurred only at IH-01-07. The abundance of these genera was extremely low when they were present.

Buffalo River

Samples were collected at Buffalo River in October 1989. Benthic invertebrate abundance (number/m^2) for the entire community (Table 5.1) at each station ranged from a low of 2,294/m^2 at BR-01-09 to a high of 19,418/m^2 at BR-01-06. Total abundance values at all stations were less than 20,000/m^2, with abundance values at 7 of the 10 stations less than 10,000/m^2 (Table 5.1). Within the benthic invertebrate community the Oligochaeta were numerically dominant at all stations sampled, Chironomidae comprised the majority of the remaining community in 7 of the 10 stations. Combined Oligochaeta and Chironomidae abundance accounted for 92% of the community at BR-01-04 to 99% of the total benthic invertebrate community at several stations, with the remainder of the benthic community abundance coming from the Gastropoda, Bivalvia and Hirudinea (Table 5.6).

Oligochaeta Abundance--

Oligochaeta abundance ranged from 1,984/m^2 at BR-01-09 to 19,157/m^2 at BR-01-06. As with total abundance, Oligochaeta abundance values were all less than 20,000/m^2 with abundance less than 9,000/m^2 at 70% of the stations (Table 5.1). The Oligochaeta community was dominated by the family Tubificidae to almost entire exclusion of other families (Table 5.7). The Oligochaeta were comprised of 2 families (Naididae, Tubificidae), 5 genera and 7 species.

The Naididae were represented at 1 station BR-01-01 by 1 species (Dero digitata). The Tubificidae were present at all stations. Limnodrilus sp. was the most common genera occurring at all 10 stations followed by Quistadrilus sp. at 6 stations and Dero sp., Aulodrilus sp. and Tubifex sp. occurring at 1 station each. The Limnodrilus sp. were represented by 3 species. Limnodrilus hoffmeisteri was found at 9 stations, L. cervix was found at 5 stations and L. claperedianus at 1 station. The remaining genera were represented by 1 species each (Aulodrilus pigueti, Quistadrilus multisetosus, Tubifex).

Chironomidae Abundance--

Chironomidae abundance ranged from 11/m^2 at BR-01-05 to 2,771/m^2 at BR-01-10 (Table 5.1). Except for BR-01-10, all Chironomidae abundance was less than 500/m^2. The Chironomidae community was comprised of 3 subfamilies (Chironomini, Tanipodinae, Tanytarsini) and 11 genera (Table 5.8).

The Chironomini were represented by 7 genera and were present at all stations except BR-01-05 and BR-01-07 (Table 5.8). Cryptochironomus sp. was the most frequently observed genera and occurred at 8 of 10 stations sampled. Chironomus sp. and Cladopelma sp. were found at 4 stations, Dicrotendipes sp. and Polypedilum sp. occurred at 2 stations, and Microchiromomus sp. and Glyptotendipes sp. each occurred at 1 station.

The Tanypodinae were represented by 3 genera and occurred at all stations sampled. Procladius sp. was found at all stations. Coelotanypus sp. occurred at 3 of 10 stations with Tanypus sp. occurring at 1 station.

The Tanytarsini were found at only 1 station, BR-01-10. The Tanytarsini were represented by the genus Tanytarsus.

Station BR-01-10 had the highest taxa richness with 3 families and 8 genera (Table 5.8). BR-01-01 and BR-01-09 had the next highest taxa richness

with 2 families and 7 genera followed by BR-01-09 with 2 families and 6 genera represented. The taxa richness of the remaining stations was 4 or less.

Mollusca Abundance--

The Mollusca were represented by 2 classes (Gastropoda and Bivalvia), 6 families, 10 genus and a minimum of 11 species (Table 5.9). Mean Gastropoda abundance ranged from 0 at several stations to 378/m^2 at BR-01-04. Mean Bivalvia abundance ranged from 0 at BR-01-10 to 217/m^2 at BR-01-03. Two of the Gastropoda in the Buffalo River samples, Valvata lewisi (Currier, 1968) and Cincinnatia cincinnatiensis (Anthony, 1849) are reported uncommon in New York state (Eileen Jokinen, University of Connecticut, Storrs, CT, personal communication).

The Gastropoda were represented by 4 families, 4 genera and 5 species (Table 5.9). The Gastropoda were present at 6 of 10 stations sampled. Valvata lewisi was the most frequently observed species occurring at 5 of 10 stations. Cincinnatia cincinnatiensis was the next most abundant species occurring at 3 of 10 stations sampled. Bithynia tentaculata and Laevapex fucus occurred at 2 stations each with Valvata tricarinata occurring at only 1 station. The abundance of these three species was low.

Station BR-01-04 had the highest taxa richness with 4 families, 4 genera, and 4 species represented. BR-01-03 had the next highest taxa richness with 3 families, 3 genera, and 4 species present. Samples from stations BR-01-02, BR-01-05, BR-01-08, BR-01-09, and BR-01-10 did not contain Gastropoda.

The Bivalvia were represented by 2 families, 6 genera and at least 7 species (Table 5.9). The Bivalvia were present at 8 of 10 stations sampled. Musculium sp. and Pisidium sp. were the most frequently observed taxa occurring at 7 of 10 stations sampled. Sphaerium sp. was the next most frequently observed species occurring at 6 of 10 stations. The Sphaeridae were the most dominant family at any of the stations.

The Unionidae were represented by 2 genera and 3 species (Table 5.9). The Unionidae were found at 4 stations. The abundance of the Unionidae were relatively small compared to the Sphaeridae.

Saginaw River (first survey)

Samples were collected from Saginaw River (first survey) in December, 1989. Benthic invertebrate abundance (number/m^2) for the entire community (Table 5.1) at each station ranged from 888/m^2 at SR-01-10 to a maximum of 7,129/m^2 at SR-01-03. Total abundance values at all stations were less than 8,000/m^2. Abundance values were evenly distributed across the stations. Within the benthic invertebrate community, the Oligochaeta were numerically dominant at all stations sampled, Chironomidae comprised the majority of the remaining community at 6 of the 7 stations. Combined Oligochaeta and Chironomidae abundance accounted for 99% to 100% of the total benthic invertebrate community at all stations, with the remainder of the benthic community abundance coming from the Bivalvia, Diptera, Coleoptera and Tricoptera (Table 5.10).

Oligochaeta Abundance--

Oligochaeta abundance ranged from 813/m^2 at SR-01-10 to 6,974/m^2 at SR-01-03. Oligochaeta abundance values were all less than 7,000/m^2 (Table 5.1). The Oligochaeta community was dominated by the family Tubificidae to almost entire exclusion of other families (Table 5.11). The Oligochaeta were comprised of 2 families, 5 genera, and 8 species.

The Naididae were represented at 1 station SR-01-09 by 1 species (Dero digitata). The Tubificidae were present at all stations. Limnodrilus sp. was the most common genera occurring at all 7 stations, followed by Ilyodrilus sp. at 5 stations, Quistadrilus sp. at 2 stations and Tubifex sp. at 1 station. The Limnodrilus sp. were represented by 4 species (L. cervix, L. claparedianus, L. hoffmeisteri, L. maumeensis). Limnodrilus hoffmeisteri and L. cervix were found at all 7 stations, with L. maumeensis occurring at 2 stations and L. claparedianus at 1 station. Ilyodrilus templetoni occurred at 5 of 7 stations with Quistadrilus multisetosus at 2 stations and Tubifex tubifex 1 station.

Chironomidae Abundance--

Chironomidae abundance ranged from 11/m^2 at SR-01-06 to 363/m^2 at SR-01-09 (Table 5.1). The Chironomidae community was comprised of 3 subfamilies (Chironominae, Tanipodinae, Orthocladinae) and 6 genera (Table 5.12).

The Chironomini were represented by 4 genera and were present at 5 of 7 stations. Cryptochironomus sp. was the most frequently occurring genera and was present at 3 of 7 stations. Of the remaining genera only the Chironomus sp. occurred at more than 1 station (SR-01-02, SR-01-09).

The Tanypodinae were represented by 1 genera (Procladius sp.) and occurred at all stations except SR-01-06. Procladius sp. was the most abundant genera at all stations it was in occurrence.

The Orthocladinae were represented by the genera Cricotopus sp. and occurred only at SR-01-02. Cricotopus sp. abundance only made up a small proportion of the abundance at SR-01-02.

Station SR-01-02 had the highest taxa richness with 3 families and 4 genera represented (Table 5.12). Station SR-01-09 had the next highest taxa richness with 2 families and 3 genera represented, while SR-01-07 and SR-01-10 had only 2 families and 2 genera represented. The remaining stations had only 1 family and 1 genus present. The abundance of the genera at SR-01-10 was low compared to most stations in Saginaw River.

Mollusca Abundance--

The Mollusca were represented by 1 class (Bivalvia), 1 family, 3 genus and at least 3 species (Table 5.13). Mean Bivalvia abundance ranged from 0 at several stations to 16/m^2 at SR-01-06 (Table 5.1). Sphaerium sp. was the most frequently observed genera each occurring at 2 of 10 stations. Musculium sp. and Pisidium sp. occurred only at SR-01-06. The abundance of all these genera was extremely low.

Saginaw River (third survey)

Samples were collected at Saginaw River (third survey) in June, 1990. Benthic invertebrate abundance (number/m^2) for the entire community at each station ranged from 321/m^2 at SR-03-06 to a maximum of 7,152/m^2 at SR-03-01 (Table 5.1). Total abundance values at all stations were less than 8,000/m^2, with abundance values at 3 of the 7 stations less than 2,000/m^2. Within the benthic invertebrate community the Oligochaeta were numerically dominant at all stations sampled, Chironomidae comprised the majority of the remaining community in all 7 stations. Combined Oligochaeta and Chironomidae abundance accounted for 99% of the community at several stations to 100% of the total benthic invertebrate community at SR-03-01 and SR-03-06, with the remainder of the benthic community abundance coming from the Bivalvia, Diptera and Coleoptera (Table 5.14).

Oligochaeta Abundance--

Oligochaeta abundance ranged from 302/m^2 at SR-03-06 to 4,944/m^2 at SR-03-01. Oligochaeta abundance values were all less than 5,000/m^2 with abundance less than 1,000/m^2 at 40% of the stations (Table 5.1). The Oligochaeta community was dominated by the family Tubificidae to almost entire exclusion of other families (Table 5.15). The Oligochaeta were comprised of 2 families, 5 genera and 7 species.

The Naididae were represented at 2 stations (SR-03-05 and SR-03-16). Station SR-03-05 had 1 species (Dero digitata), while the Naidid at SR-03-16 was unidentifiable. The Tubificidae were present at all stations. Limnodrilus sp. was the most common genera occurring at all 7 stations, followed by Ilyodrilus sp. at 4 stations, Aulodrilus sp. at 2 stations, and Quistadrilus sp. and Tubifex sp. at 1 station. The Limnodrilus sp. were represented by 3 species (L. cervix, L. hoffmeisteri, L. maumeensis) and were found at all 7 stations. Ilyodrilus templetoni occurred at 4 of 7 stations, Aulodrilus pluriseta occurred at 2 of 7 stations with Quistadrilus multisetosus occurring at 1 station.

Chironomidae Abundance--

Chironomidae abundance ranged from 15/m^2 at SR-03-06 to 2,204/m^2 at SR-03-01 (Table 5.1). The Chironomidae community was comprised of 3 subfamilies (Chironominae, Tanipodinae, Tanytarsini) and 7 genera (Table 5.16).

The Chironomini were represented by 3 genera and occurred at all stations (Table 5.16). Chironomus sp. was the most frequently observed genus and occurred at all 7 stations. Cryptochironomus sp. was found at 5 of 7 stations and Microchironomus sp. occurred at 2 stations.

The Tanypodinae were represented by 3 genera and occurred at all stations sampled. Procladius sp. was found at all stations while Tanypus sp. occurred in low abundance at SR-03-08 and SR-03-24 and Coelotanypus sp. occurred only at SR-03-08.

The Tanytarsini were represented by Tanytarsus sp. and occurred only at SR-03-08. Tanytarsus sp. constituted a relatively small proportion (<5%) of the Chironomidae community at SR-03-08.

Station SR-03-08 had the highest taxa richness with 3 families and 7 genera represented. Stations SR-03-16 and SR-03-24 had the next highest taxa richness with 2 families and 4 genera present.

Mollusca Abundance--

The Mollusca were represented by 1 class (Bivalvia), 1 family, 1 genus and 1 species (5.17). Mean Bivalvia abundance ranged from none in samples from several stations to 11/m^2 at SR-03-05. Musculium sp. (Sphaeridae) was the only genus of Bivalvia collected at any of the stations. Musculium sp. was collected at 3 of 7 stations and abundances were extremely low.

COMPARISON AMONG AOCs

Mean total abundance values ranged from 2,783/m^2 in Saginaw River (survey 3) to 9,323/m^2 at Buffalo River (Table 5.18). Median values for total benthic invertebrate abundance ranged from 2003/m^2 in Indiana Harbor (station 10 deleted) to 8,108/m^2 in Buffalo River samples. Indiana Harbor station 10 was deleted from some comparisons because inclusion of IH-01-10 drastically skewed the data from Indiana Harbor and was not comparable to the other AOCs. Deleting station IH-01-10 from certain comparisons was justified because station IH-01-10 was on the upstream side of a low clearance bridge and therefore did not receive the heavy disturbance caused by boat traffic common at all other stations. Total invertebrate abundance was in general lowest at Indiana Harbor and highest at Buffalo River.

Comparisons between the three AOCs show that overall mean Oligochaeta abundance ranged from 2,429/m^2 at Saginaw River (survey 3) to 8,726/m^2 at Buffalo River (Table 5.18). Median values for Oligochaeta abundance ranged from 1,994/m^2 at Indiana Harbor (station 10 deleted) to 7,333/m^2 at Buffalo River again indicating that Oligochaeta abundance was generally lowest at Indiana Harbor and highest at Buffalo River.

The Oligochaeta community across all areas of concern was comprised of 2 families, 6 genera, and 12 species. All AOCs except Indiana Harbor had representatives of the Naididae. Buffalo River, Indiana Harbor, and Saginaw River (survey 1) had representatives of the genus Tubifex sp. present, while Tubifex sp. was entirely absent from the Saginaw River (survey 3) samples. All AOCs had similar numbers of genera and species present in the samples.

Overall mean Chironomidae abundance ranged from none in samples from Indiana Harbor (station 10 deleted) to 465/m^2 in Saginaw River (survey 3). Chironomidae abundance was similar between Buffalo River and Saginaw River (survey 3), but were not found in Indiana Harbor (station 10 deleted) sediment samples. In Saginaw River Chironomidae abundance was higher during survey 3 (June 1990) compared to survey 1 (December 1989) (Table 5.18).

The Chironomidae community across all AOCs was comprised of 4 subfamilies (Chironominae, Tanipodinae, Tanytarsini, Orthocladinae) and 12 genera. Buffalo River had the highest taxa richness with 10 of the eleven genera present. Saginaw River had 7 genera of Chironomidae in the third survey and 6 genera in the first survey. Three genera were similar between the two Saginaw river surveys, with Saginaw River having 4 genera in the third survey which were not present in the first survey. Indiana Harbor had 2 Chironomidae (Cricotopus sp.) present at Station 10. There were no Chironomidae at any of the other Indiana Harbor stations.

The Mollusca were represented by 2 classes (Gastropoda and Bivalvia), 6 families, 9 genera, and at least 11 species. Buffalo River contained the highest taxa richness with all the family, genera and species represented. Indiana Harbor and Saginaw River (survey 1) had a relatively depauperate community with only 1 family (Sphaeridae) and 3 genera found in these areas. Saginaw River (third survey) had only 1 family (Sphaeridae) and 1 genus present.

Comparisons of mean abundance between stations within an AOC demonstrated no consistent trends (Table 5.19). Buffalo River and Indiana Harbor had the most consistent pattern in the Oligochaeta and total abundance. Oligochaeta and total abundance at Buffalo River tended to be highest in the middle numbered stations. Oligochaeta and total abundance at Indiana Harbor (station 10 excluded) tended to be higher in the low numbered stations and lowest in the higher numbered stations. Discrepancies in the pattern occurred with the 2 upper stations and the lowest station at Buffalo River and station 10 at Indiana Harbor. There was no recognizable pattern with the Saginaw River stations. Chironomidae abundance demonstrated no pattern with respect to stations at any of the AOCs.

BENTHOS-CHEMISTRY COMPARISONS IN GRAB SAMPLES

Comparisons between measured concentrations of metals and macroinvertebrate abundance estimates using correlation analysis indicated that no single metal explained a significant amount of the variation associated with macroinvertebrate abundance at $p \leq 0.05$. This result was the same regardless of whether the data was compared across AOCs or across stations within an AOC.

Comparisons between macroinvertebrate mean total abundance and total simultaneously extracted metals (SEM, weak acid extracts of Cd, Cr, Cu, Ni, Pb, and Zn), total polyaromatic Hydrocarbons (PAH, normalized to total organic carbon) and total Polychlorinated Biphenyls (PCB, normalized to total organic carbon) demonstrated a consistent pattern of decreasing abundance with increasing contaminant concentration (Figures 5.2 to 5.4).

Relations between SEM and mean Oligochaeta abundance demonstrated a downward curvilinear plot (Figure 5.5). Below 1 μmol/dry g Oligochaeta abundance seems to be independent of SEM concentration. Between 1 μmol/dry g and 4 μmol/dry g Oligochaeta abundance tended to increase and low Oligochaeta abundance was associated with SEM concentrations above 4 μmol/dry g.

Similar relations are observed between the SEM and mean Chironomidae, mean Bivalvia and mean Gastropoda abundance (Figure 5.6 to 5.8). The threshold was about 1 μmol/dry g.

Comparisons of total PAH's and mean Oligochaeta abundance demonstrated at concentrations below 2,000 μg/dry g Oligochaeta abundance seems to be independent of PAH concentration (Figure 5.9) Above 2,000 μg/dry g PAH concentration was associated with low Oligochaeta abundance. Similar relations were observed between total PAH's and mean Chironomidae and mean Bivalvia abundance (Figure 5.10 and 5.11). Chironomidae abundance tended to be reduced at PAH concentrations around 500 μg/dry g. Bivalvia abundance tended to be lower above 2,000 μg/dry g, similar to the response of the Oligochaeta. There was insufficient data to make any statement concerning the response of Gastropoda abundance to PAH levels (Figure 5.12).

Comparisons of total PCB and mean Oligochaeta abundance demonstrates a downward curvilinear plot with Oligochaeta abundance being independent of PCB concentration below 500 µg/dry g (Figure 5.13). Above 500 µg/dry g PCB, Oligochaeta abundance tended to be lower. Similar relations were observed between total PCB concentration and mean Chironomidae and mean Bivalvia abundance (Figures 5.14 and 5.15). Chironomidae and Bivalvia abundance tended to be more variable at concentrations of PCB's below 250 µg/dry g. Concentrations above 500 µg/dry g were associated with decreased Chironomidae and Bivalvia abundance. There was insufficient data to make any statements about Gastropoda/PCB relations (Figure 5.16).

DEFORMITIES IN CHIRONOMIDAE FROM GRAB SAMPLES

Chironomidae were examined for deformities in the mentum and ligula (Procladius sp.). Deformities in the Chironomidae larvae consisted of various types of asymmetry, missing teeth, extra teeth and fusion among various teeth. Mouth part deformities in the Chironomidae community ranged from a low of 0% at 2 stations BR-01-05 and SR-01-06 to a maximum of 100% at IH-01-10 (Table 5.20). On average the lowest percentage of deformities was from Buffalo River samples (7.0 %), followed by Saginaw River first survey (12.7%), Saginaw River third survey (16.7) and Indiana Harbor (100%). Deformities were present in 19 out of 42 replicate at Buffalo River, 17 out of 29 at Saginaw River survey 1, 23 out of 30 at Saginaw River survey 3 and 2 for 2 at Indiana Harbor.

ARTIFICIAL SUBSTRATES VS. GRAB SAMPLES

The ponar grab samples and the artificial substrates collected different taxa at all the AOCs. The ponar grab samples collected an infaunal community comprised primarily of Oligochaeta and Chironomidae (Table 5.1). Gastropoda, Bivalvia, Hirudinea and rare occurrences of other aquatic insect larvae made up a very small percentage of the grab samples at all the stations.

Artificial substrates collected a much different fauna than the grab samples (Figure 5.17 to 5.19). The artificial substrate samples primarily collected the epifaunal community. Artificial substrates in Indiana Harbor station 3 were almost entirely Dreissena sp. (zebra mussels) (Table 5.17). Along with the Dreissena sp., Station 3 contained largely Turbellaria, Oligochaeta, and Amphipoda. The community at Station 3 also included Cordylophora sp., Hirudinea, Decapoda, and Cyclopoid and Harpacticoid copepods. The number of Dreissena sp. decreased substantially at Indiana Harbor Station 4. Turbellaria and Oligochaeta made up the rest of the community. Throughout the Indiana Harbor samples, occasional Zygoptera nymphs, Hirudinea and Bivalvia were also present. At Station 6 there were relatively few animals, but what was there was predominantly Oligochaeta.

The Buffalo River stations were dominated by Amphipoda and Isopoda with the remainder of the community comprised of Oligochaeta, Chironomidae, and Turbellaria (Table 5.18). Buffalo River station 8 had large numbers of Turbellaria (Flatworms). Oligochaeta abundance collected with the artificial substrates at the Buffalo River stations was always low.

The macroinvertebrate community in Saginaw River was dominated by Amphipoda (Gammarus sp.), Chironomidae and Coelenterata (Hydra sp.) (Table 5.19). Amphipoda were most abundant at Saginaw River Station 2; Chironomidae

at stations 6, 8, and 16; Hydra sp. at Station 5; and Station 24 had relatively even abundances of Amphipoda and Chironomidae.

The density of animals in the artificial substrates was an order of magnitude greater than the density of animals in the grab samples at Saginaw River (Figure 5.20). Similar data was not available to compare the results at Buffalo River and Indiana Harbor.

VARIATION IN BENTHIC INVERTEBRATE SAMPLING USING PONAR GRABS

In this study variation in the estimate of benthic invertebrate abundance in grab samples was partitioned into three separate units: (1) AOC variability defined as the difference in benthic invertebrate estimates between AOCs; (2) station variability, defined as station to station differences in benthic estimates in a particular AOC; and (3) sampling variability defined as differences between benthic invertebrate abundance estimates between individual replicates at each station.

Estimates of variance components (Nested Analysis of Variance; [ANOVA], Snedecor and Cochran 1982) identified that combined station and sampling variability accounted for 74% to 99% of the explained variability in the estimates for all major groups (Table 5.21). Variability associated with AOC ranged from 1% to 26% for all major taxa.

Station variability explained almost 50% of the variability in the estimates of Oligochaeta abundance (Table 5.21). Variability associated with AOC and sampling each accounted for 25% of the remaining variability in the estimates of Oligochaeta abundance.

Sampling variability accounted for 62% of the variability in the estimates of Chironomidae abundance. Variability associated with station to station differences accounted for 37% of the associated variability. Variability associated with AOC was extremely low, accounting for only 1% of the total associated variability in the estimates of Chironomidae abundance.

Variability associated with Bivalvia and Gastropoda estimates demonstrated contrasting results. Station variability accounted for 25% of the variability in the estimates of Bivalvia abundance, but accounted for 59% of the variability in the estimates of Gastropoda abundance (Table 5.21). Sampling variability demonstrated the opposite, accounting for 55% of the variability in estimates off Bivalvia abundance while accounting for only 37% of the variability in the estimates of Gastropoda abundance. Variability associated with AOC was 19% for estimates of Bivalvia abundance and only 4% for estimates of Gastropoda abundance.

DISCUSSION

BENTHIC INVERTEBRATE ABUNDANCE AND COMMUNITY STRUCTURE IN GRAB SAMPLES

The abundance estimates of benthic invertebrates in this study are most likely conservative because of the sampling device used to collect the invertebrates (Resh 1979), and the size of the mesh used to sieve the invertebrates (Brinkhurst 1974, Resh 1979, Heushelle 1982). Ponar samplers are heavy by nature in order to penetrate the sediment surface more evenly and efficiently. Problems arise as the sampler nears the sediment. A shock wave of displaced water impacts the sediment just before the sampler, many times causing small organisms and surface dwellers to be pushed out of the way of the sampler (Flannagan 1970, Howmiller 1971, Howmiller and Beeton 1971, Millbrink and Wiederholm 1973). The effect of this type of disturbance is not easily quantified and was not quantified in this study. However, any bias caused by this type of sampler should be consistent across stations and AOC's.

All samples were sieved through a 500 μm brass screen upon collection in the field. Although this size screen is good at separating the benthic invertebrates from the sediments, many of the smaller organisms such as the Naidids pass through with the sediments (Brinkhurst 1974, Resh 1979, Heushelle 1982). A more appropriate method of sieving may be to sieve samples through a 500 μm mesh screen followed by a 250 μm screen to collect organisms that pass through a 500 μm mesh screen (Burt et al. 1991, Dr. Ralph Brinkhurst, Aquatic Resources Center, Franklin TN, personnel communication).

COMPARISON AMONG AOCs

Benthic Invertebrate Abundance and Species Composition

Comparisons among the three AOCs indicated that Indiana Harbor was the least suitable to the benthic invertebrates and Buffalo River was the most suitable. This was based on overall abundances (BR>SR>IH) and total number of genera and species (BR=33>SR=20>IH=14) present at each AOC. Buffalo River had many genera that were not present or were present in low numbers in Indiana Harbor and Saginaw River samples. Varying degrees of contamination at these AOCs could influence abundance and species composition, but differences in substrate quality, and amount and quality of food sources can not be discounted. There was no relation that could be attributed to any single contaminant or physical variable at any of the stations.

Indiana Harbor

Indiana Harbor was a very depauperate benthic invertebrate community. Except for the 2 Chironomidae collected at IH-01-10, no other aquatic insect orders were present at the stations in Indiana Harbor. The Bivalvia were rare occurrences at three stations (IH-01-03, IH-01-04, IH-01-07). The genera of Bivalvia present have been indicated as being somewhat tolerant of organic contamination (Carr and Hiltunen 1965, Fuller 1974, Bode 1988).

The invertebrate community, dominated by the Oligochaeta family Tubificidae, is indicative of a benthic invertebrate community subjected to heavy organic pollution (Brinkhurst et al. 1972, Brinkhurst and Cook 1974, Cook and Johnson 1974, Burt et al. 1991). All of the Tubificidae genera

present in Indiana Harbor are known to be very tolerant of organic pollutants (Kennedy 1965, Brinkhurst et al 1972). Limnodrilus hoffmeisteri, one of the most pollution tolerant Oligochaeta species, was the most abundant species at all stations sampled.

Oligochaeta abundance was lowest at stations IH-01-07 (junction of Lake George Branch and Grand Calumet Branch) and IH-01-06 (main channel), and highest at station IH-01-10. Sediments from station IH-01-07 generally had the highest concentrations of metals (Tables 4.3 and 4.4) and organic contaminants (Table 4.37) with station IH-01-06 having the next highest concentration of metals and organic contaminants. This would tend to indicate that the combined input of metals and organic contaminants from both the Grand Calumet Branch and the Lake George Branch is having a pronounced effect on the Oligochaeta communities at these stations. Samples from these stations were also among the most toxic in laboratory exposures (see Chapters 2 and 4). High concentrations of metals may reduce Oligochaeta abundance by reducing the bacteria on which they feed.

Oligochaeta abundance was extremely high at station IH-01-10, approaching 1,000,000/m^2 in individual grab samples. Although metals concentrations are the second lowest of any stations sampled, the most pronounced reason for the higher abundances may be the high density of aquatic vegetation present at IH-01-10. Large amounts of vegetation were most likely present because station IH-01-10 was on the upstream side of a low clearance bridge, which tended to minimize the amount of disturbance caused by boat traffic (Joe Rathbun, ASCI Corporation, Grosse Ile, MI, personal communication). This vegetation most likely provided a habitat which collected decaying plant material resulting in a considerable amount of bacterial activity.

Many of the grab samples had large remnants of Gastropoda and Bivalvia shells. The stations sampled were depositional areas and the shells may have washed in from upstream, but the possibility that resident Mollusca communities at these locations have died off can not be excluded with the data in this study.

Buffalo River

Benthic invertebrate communities samples from the Buffalo River exhibited a wide range of abundance across all stations. Although the Oligochaeta were the most abundant taxa, several stations had significant number of representatives from the Chironomidae, Bivalvia, and Gastropoda. On rare occasions, representatives from the orders Ephemeroptera, Odonata, Hemiptera, Tricoptera, Coleoptera, Diptera (other than Chironomidae), Hirudinea, and Amphipoda were present.

The Oligochaeta community was dominated by the family Tubificidae, with species which are generally considered tolerant of organic and metal pollution (Kennedy 1965, Brinkhurst et al 1972, Burt et al. 1991). Limnodrilus hoffmeisteri was the most abundant Oligochaeta at all stations except for BR-01-01 and BR-01-10. The reasons for this are not clear, but it may be a result of food preference or habitat preference not being met at these stations (Verdonschot 1989).

The Chironomidae community at Buffalo River consisted primarily of tolerant genera (Hilsenhoff 1982, 1987, Bode 1988). The exception to this generalization was Tanytarsus sp. at BR-01-10 which reportedly prefers less

organically enriched environments (Krieger 1984). Procladius sp., Chironomus sp. and Cryptochironomus sp. are generally considered to be the most frequently encountered genera in heavily polluted environments (Cook and Johnson 1974, Krieger 1984). These three genera were generally the most frequently sampled genera at Buffalo River.

Several genera and species of Bivalvia and Gastropoda were present in Buffalo River. Two genera, Musculium sp. and Pisidium sp., have been reported from systems receiving organic pollution (Carr and Hiltunen 1965, Fuller 1974). Species of the genus Sphaerium may be somewhat tolerant to organic contaminants by virtue of being in the family Sphaeridae which reportedly is tolerant of organic pollution (Bode 1988, Plafkin et al. 1989).

Representatives from the family Unionidae were collected only at 4 of 10 stations and their abundances were always low. This could be an indication that these organisms are less tolerant of contaminants than some of the Sphaeridae, however members in this family tend to be large so the ponar grabs may not efficiently collect the Unionidae.

Representatives from the genera Valvata sp. and Bithynia sp. have been reported to be somewhat tolerant of organic pollution (Carr and Hiltunen 1965, Krieger 1984). Even so the occurrence of these genera was limited to 6 of 10 stations and abundances of these genera were usually low at most stations. The lack of abundance may be due to toxic effects of metals and organic contaminants, insufficient grazing material, lack of suitable habitat , or a combination of all the above. Interestingly, 2 species of Gastropoda, Valvata lewisi and Cincinnatia cincinnatiensis which are reportedly uncommon in New York State (Eileen Jokinen, University of Connecticut, Storrs, CT, personal communication) seems to be the most abundant Gastropoda species at the Buffalo River stations. Other reported occurrences for these species in New York are in Oneida Lake, Seneca Lake, and a few tributaries in the Oswego drainage basin. All of these areas are subject to high organic enrichment. This may be indicative of a tolerance for organic contaminants which allows these species to out compete other Gastropoda and survive in these polluted areas.

Concentrations of metals and organic contaminants were not consistently related to patterns of invertebrate abundance. Station BR-01-04 and BR-01-10 had the lowest metal concentration and the highest number of taxonomic orders (8) of the Buffalo River stations. Concentrations of contaminants may have had an inconsistent effect on the benthic invertebrate community because of differences in spatial distribution of contaminants or that these contaminants were not readily bioavailable to the organisms (Burton 1991).

Saginaw River (first and third survey)

The benthic invertebrate communities sampled from the Saginaw River exhibited a fairly narrow range of abundance. As with Indiana Harbor and Buffalo River, the Oligochaeta were the most abundant taxa at all stations. The Oligochaeta constituted a higher percentage of the invertebrate community across stations during the first survey sampled in December 1989. Samples from the third survey in June of 1990 had a higher percentage of the family Chironomidae present.

The Oligochaeta were dominated by the pollution-tolerant Tubificidae similar to Indiana Harbor and Buffalo River. Limnodrilus hoffmeisteri was more dominant in the samples from the first survey compared to the third survey where distributions were spread more evenly across several other species.

These differences may be attributed to seasonal variability, spatial or temporal variability, differing food sources, or a combination of all the above.

The Chironomidae community at Saginaw River was predominantly pollution-tolerant genera (Hilsenhoff 1982, 1987, Bode 1988). The exception was Tanytarsus sp. at SR-03-08 which was present but in very low abundance. Procladius sp. and Chironomus sp. were most frequently collected and are reported tolerant of metal and organic pollution (Cook and Johnson 1974, Krieger 1984).

The only Mollusca present at Saginaw River were those in the family Sphaeridae. All three genera, Musculium sp., Pisidium sp. and Sphaerium sp. were present in the first survey, but only Musculium sp. was present in the third survey. The reasons for this are unclear. Perhaps these genera were present in very low abundances during the third survey and were missed when the ponar grabs were taken, or that these genera were not present at any of the stations in the third survey due to loss of habitat or increased contamination.

There were no families of Gastropoda present in Saginaw River. The exact reason for this is not clear from the data, but may be the result of contaminants in the area or the habitat may be insufficient to support this group. Further investigation designed to identify contaminant, abiotic and biotic factors controlling Gastropoda and overall invertebrate distributions need to be conducted before any definitive conclusions can be made.

Benthos-Chemistry Comparisons

Comparisons between concentrations of Simultaneously extracted metals (SEM), total PAHs and total PCBs all demonstrated a consistent pattern of decreasing abundance with increasing contaminant concentration (Figures 5.2 to 5.4). Regardless of the contaminant examined there seemed to be a concentration below which invertebrate abundance was independent of contaminants. There also tended to be a concentration above which invertebrate abundance was consistently reduced. This suggests a threshold concentration of contamination below which invertebrate abundance is controlled by other factors and above which the influence of the contaminant is more pronounced. Even at low concentrations of contaminants the community is still indicative an impacted environment.

The value of this threshold limit, regardless of contaminant examined, seems to be consistently higher for the Oligochaeta community compared to the other invertebrate orders. This is consistent with the findings that identify Oligochaeta as some of the most tolerant aquatic invertebrates (Hilsenhoff 1982, 1987; Bode 1988).

DEFORMITIES IN CHIRONOMIDAE

Different genera of Chironomidae exhibit different levels of susceptibility or tolerance to contaminants (Hamilton and Saether 1971; Hare and Carter 1976; Warwick 1985, 1988; Wiederholm 1984). Some genera are quite intolerant and low levels of contaminants eliminate these genera from Chironomidae communities, while some genera such as Procladius sp., Chironomus sp. and Cryptochironomus sp. are more tolerant and persistent (Warwick 1985, Bode 1988). A relationship between increased contamination and the presence

of Chironomidae deformities has long been documented by many investigators (Hamilton and Saether 1971, Warwick 1980, 1985, Tennessen and Gottfried 1983, Cushman 1984, Wiederholm 1984). Some of the reported deformities include: thickening of the exoskeleton, enlargement and darkening of the head capsule, asymmetry in mouth parts, missing or fused lateral teeth, and antennal deformities.

The mentum and ligula (Tanypodinae) of Chironomidae were examined for deformities at all AOCs. The specimens had various mouthpart deformities including missing lateral and central teeth, asymmetry in the mentum, badly deformed and twisted lateral teeth on the mentum and missing teeth on the ligula (Procladius sp.). None of the specimens examined exhibited antennal deformities. The occurrence of deformities in this study tended to occur in the Procladius sp. and Chironomus sp. genera. Even when other genera were present they did not seem to display frequent mouth part deformities. The reasons for this are not clear but may be due to these genera dying before they can exhibit abnormalities.

The occurrence of deformities in Chironomidae reportedly is less than 1% in non-impacted communities (Wiederholm 1984; Warwick et al. 1987). Several investigators have suggested deformity frequency in the range of 5% to 25% or greater is indicative of moderate to severe contamination (Wiederholm 1984, Warwick et al. 1987). Given this criteria, occurrence of deformities in the Chironomidae community at the three AOCs indicates the areas ranged from moderately to heavily polluted. The stations that had Chironomidae without deformities were also the areas that had few or no Chironomidae. Only 2 Chironomidae were collected from Indiana Harbor (station 10) and both had deformities.

As with the abundance data, the frequency of deformities in Buffalo River indicates less toxic conditions compared to either Indiana Harbor or Saginaw River. It is interesting that the occurrence of deformities is consistently high at the Saginaw River stations, which indicates that contaminants were at a sufficient level impact the Chironomidae community at most stations.

Although it would seem that the occurrence of these deformities may be related to the degree of contamination in the sediments, the limited number of samples made comparisons difficult. Frequency of deformities exhibited by Chironomidae exposed in laboratory sediment toxicity tests (Chapter 4) need to be compared to the frequency of deformities in the field-collected Chironomidae. A more specific study designed to elucidate the relations between particular contaminants and Chironomidae mouthpart deformities and which would encompass a broad range of impacted and non-impacted areas would be beneficial to future studies.

ARTIFICIAL SUBSTRATES VS. GRAB SAMPLES

Several investigators have examined the advantages and disadvantages in using artificial substrates to examine the benthic invertebrate community (Cairns 1982). The artificial substrates used in the present study were modified from Stauffer et al. (1976). These substrates were very easy to work with and the synthetic mesh was easily pulled apart thereby allowing easy access to the invertebrates with a minimum of specimen damage.

The invertebrates from the artificial substrates were processed following the methods outlined in the rapid bioassessment protocols (Plafkin

et al. 1989). Invertebrates were enumerated into major taxonomic groups and no further identification of these taxa was attempted. Therefore a rigorous analysis of the data have yet to be attempted. The results of the data for the artificial substrate samples compared to the ponar grab samples should be used with caution.

The artificial substrates collected a benthic invertebrate community which was considerably more diverse and abundant than the benthic grabs. This indicates there were more invertebrates in the areas sampled than would be predicted by the ponar grabs alone. Perhaps quality substrate is limited in these areas. Invertebrates collected on the artificial substrates may not be indicative of the true abundance of benthic invertebrates in these areas. The artificial substrates may act as a focal point of colonization for surrounding invertebrates inhabiting the sediments.

The differences observed between the ponar grabs and artificial substrates collected presents a problem for benthic community assessments. Estimates of the benthic community composition made from the ponar grabs would indicate a severely impacted community based on the low diversity of taxa. Considerably more invertebrates were at the station based on the results of the artificial substrates samples. This becomes extremely important if potential food chain bioaccumulation estimates are being considered. Since the artificial substrates probably sample the epibenthic community more readily available to vertebrates future studies may require the use of both types of samplers to make estimates the benthic invertebrate community.

VARIATION IN BENTHIC INVERTEBRATE SAMPLING USING PONAR GRABS

In large scale ecological studies it is important to identify sources of variability so that a meaningful interpretation of the data can be made (Collins and Spruleß 1983) and so future studies can be designed to address major sources of variation. The results of the variance partitioning indicates that station and sampling variability accounted for most of the explained variability in the taxa estimates. It is not uncommon for station to station variance to account for a considerable amount of the explained variation between invertebrate abundance estimates (Lewis 1978, Threlkeld 1983). The differences associated between stations may be due to (1) some stations being closer to contaminant effluent outfalls, (2) variation in substrate composition and structure which would preclude colonization by the invertebrates, (3) depth of the station which may prohibit invertebrates due to wave action or shipping traffic, or (4) a combination of all of these factors.

Variance associated with AOC was significant in the estimates of Oligochaeta and Bivalvia abundance while minimal in the estimates of Chironomidae and Gastropoda abundance. The benthic invertebrate abundances between the areas of concern were quite similar. This is not an unexpected considering all three of these areas have received substantial industrial and municipal input and the bulk of the community consists of Oligochaeta. Future studies would be enhanced if a relatively pristine reference station could be identified and sampled.

The remaining variance was associated with sampling technique. Invertebrate estimates between successive grabs could vary by as much as 68% depending on the taxa. Since benthic invertebrate populations exhibit a patchy distribution in the sediment, the result is not unusual (Elliott 1977).

But for most studies with benthic invertebrates you would want this estimate to be as low as possible (eg. additional replicates).

Partitioning of the variance into different components indicates that future studies might provide better data if: (1) smaller size grabs were taken, but with more grabs to try to minimize the influence of a patchy benthic invertebrate distribution; and (2) more stations were sampled in a given AOC in an attempt to obtain stations which would better sample across the gradient of effluents from upstream to downstream. A main reason for limiting the amount of grabs taken from an area of concern is the time and cost it takes to process the samples once they are taken. By taking smaller samples, but still taking the same area of sample, the processing time would be shorter and the estimates between grabs should have a lower variance (Leigh Frederickson, University of Missouri, Columbia, MO, personal communication).

SUMMARY AND RECOMMENDATIONS

Oligochaeta and Chironomidae abundance constituted over 90% of the benthic invertebrate community collected with the ponar grab at all stations and Areas of Concern. This overwhelming dominance by these 2 taxonomic groups is indicative of an impacted benthic invertebrate community. Buffalo River had the largest number of genera and species (n=33) present, followed by Saginaw River (n=20) and Indiana Harbor (n=14). Based on measures of taxa dominance and taxa richness Indiana Harbor was the least suitable Area of Concern to benthic invertebrates and Buffalo River was the most suitable.

Comparisons between concentrations of simultaneously extracted metals (SEM), total PAH, and total PCB with measures of benthic invertebrate abundance demonstrates a consistent pattern of decreasing invertebrate abundance with increasing contamination. The data suggest a threshold concentration below which abundance may be controlled by factors other than contaminant concentration and above which the influence of the contaminant is pronounced. However, direct cause and effect relations between invertebrate abundance and any individual contaminant were not demonstrable with the data at this time.

The occurrence of Chironomidae deformities was pronounced at all Areas of Concern. Buffalo River consistently had the lowest occurrence of deformities across all stations. The occurrence of Chironomidae deformities was more prevalent at Saginaw River than Buffalo River. The 2 Chironomidae collected from Indiana Harbor were both deformed. Overall, the percentages of deformities at all the Areas of Concern would indicate that these area are moderately to severely polluted.

The ponar grabs and artificial substrates collected tremendously different numbers and taxa composition of aquatic invertebrates. While the grab samples were predominantly Oligochaeta and Chironomidae, the artificial substrates collected predominantly Amphipoda, Isopoda, Turbellaria, and Dreissena sp.. Indications are the ponar grabs may be missing a considerable component of the benthic invertebrate community. Incorporation of artificial substrates may enhance the estimation of benthic invertebrate community composition.

Results of variance partitioning indicates station and replicates accounted for most of the explained variability in the estimation of invertebrate abundance collected with the ponar grab. Variance associated with Area of Concern was significant in the estimation of Oligochaeta and Bivalvia abundance, while minimal in the estimation of the remaining taxa abundance.

The following recommendations are concerns and future research identified as important factors relevant to interpreting results of benthic invertebrate surveys.

- Additional research is needed to evaluate specific contaminant, biotic and abiotic factors controlling invertebrate abundance and community structure in sediments.
- Sediment chemistry and physical variables need to be obtained from splits of the samples from which the invertebrates are collected to overcome problems associated with benthic invertebrate patchiness and heterogenous contaminant deposition.

- Assessment of benthic communities should use both artificial substrates and grab samples.
- Frequency of deformities in Chironomidae exposed in laboratory toxicity tests (Chapter 4) should be measured and compared to the frequencies of deformities in the Chironomidae collected from the field.
- With most of the variance in benthic community estimates associated with station and replicates, future studies should sample more stations and take more replicates using smaller grab samples.

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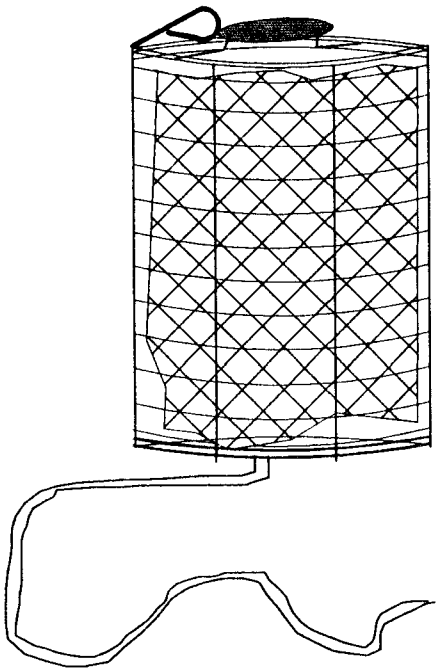
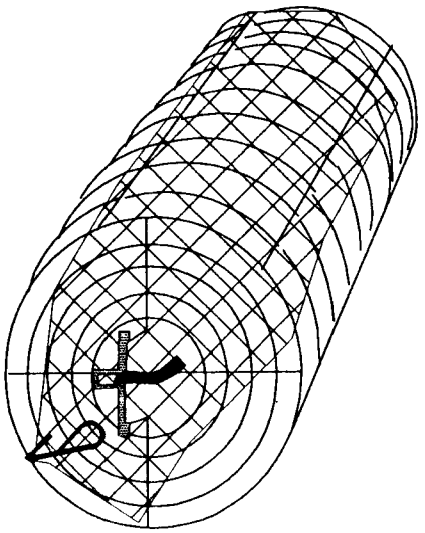
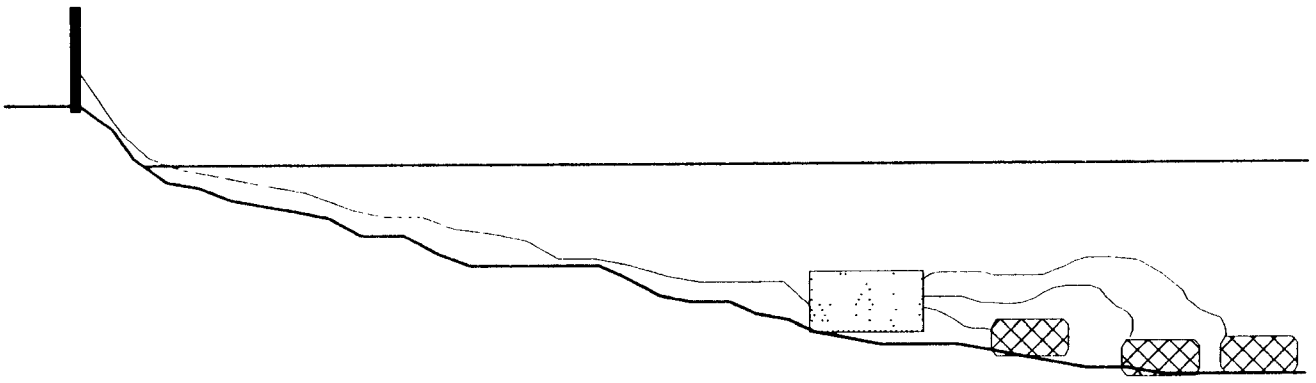
Figure 5.1

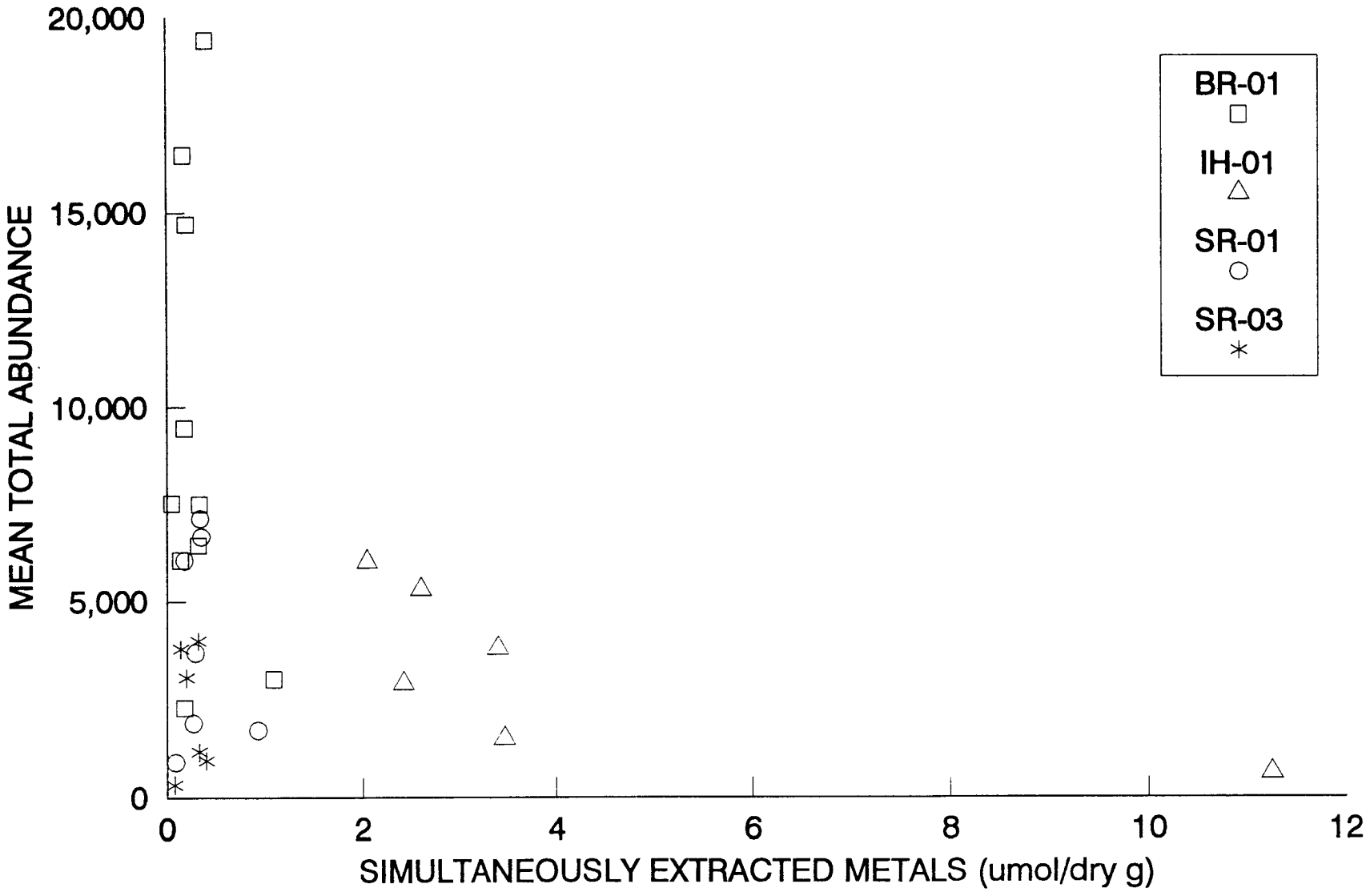






River

Figure 5.2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5,000
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0
2
4
6
8
10
12
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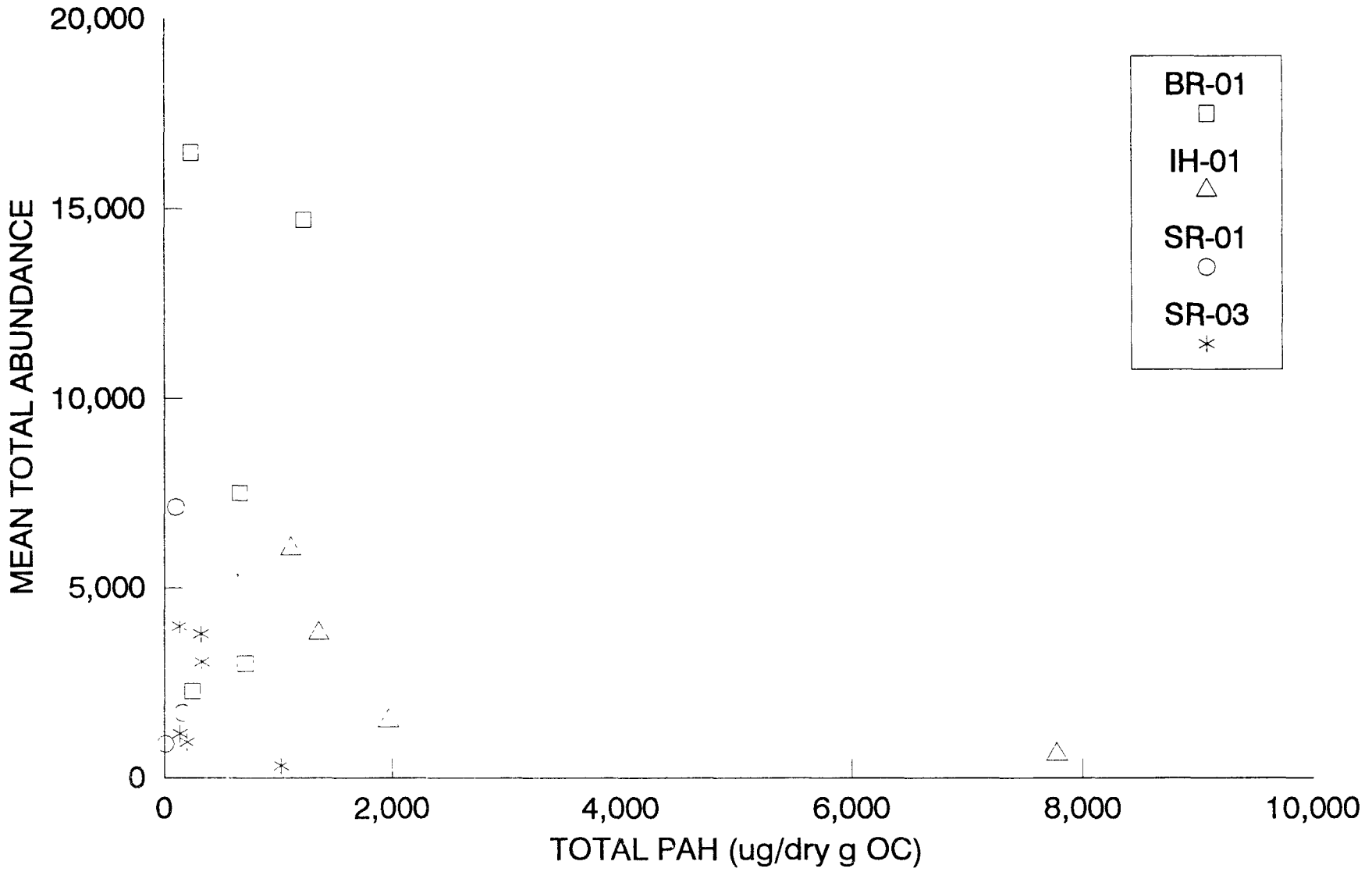
IH-01
SR-01
SR-03

Figure 5.3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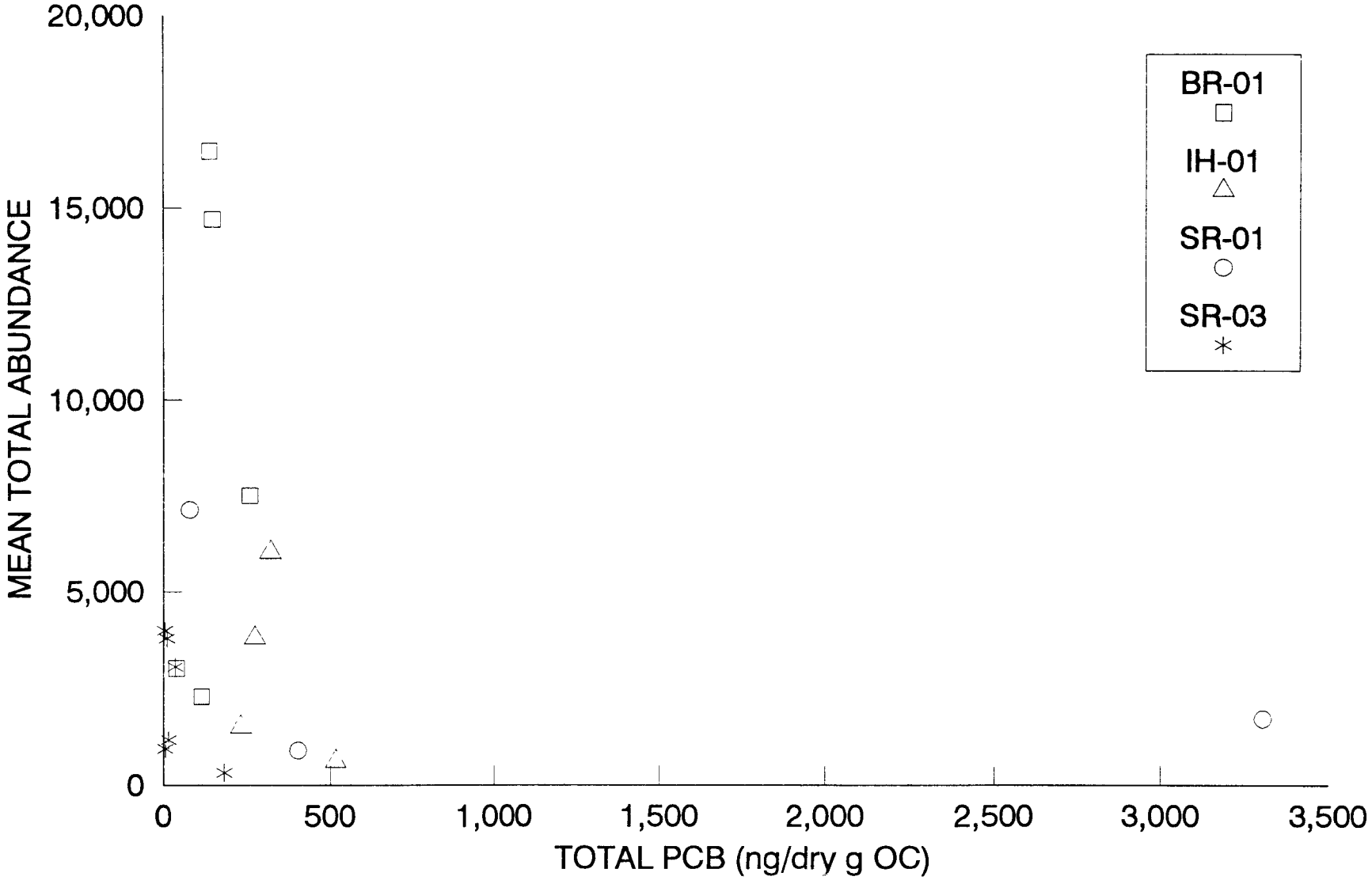
IH-01
SR-01
SR-03

Figure 5.4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3,500
TOTAL PCB (ng/dry g OC)
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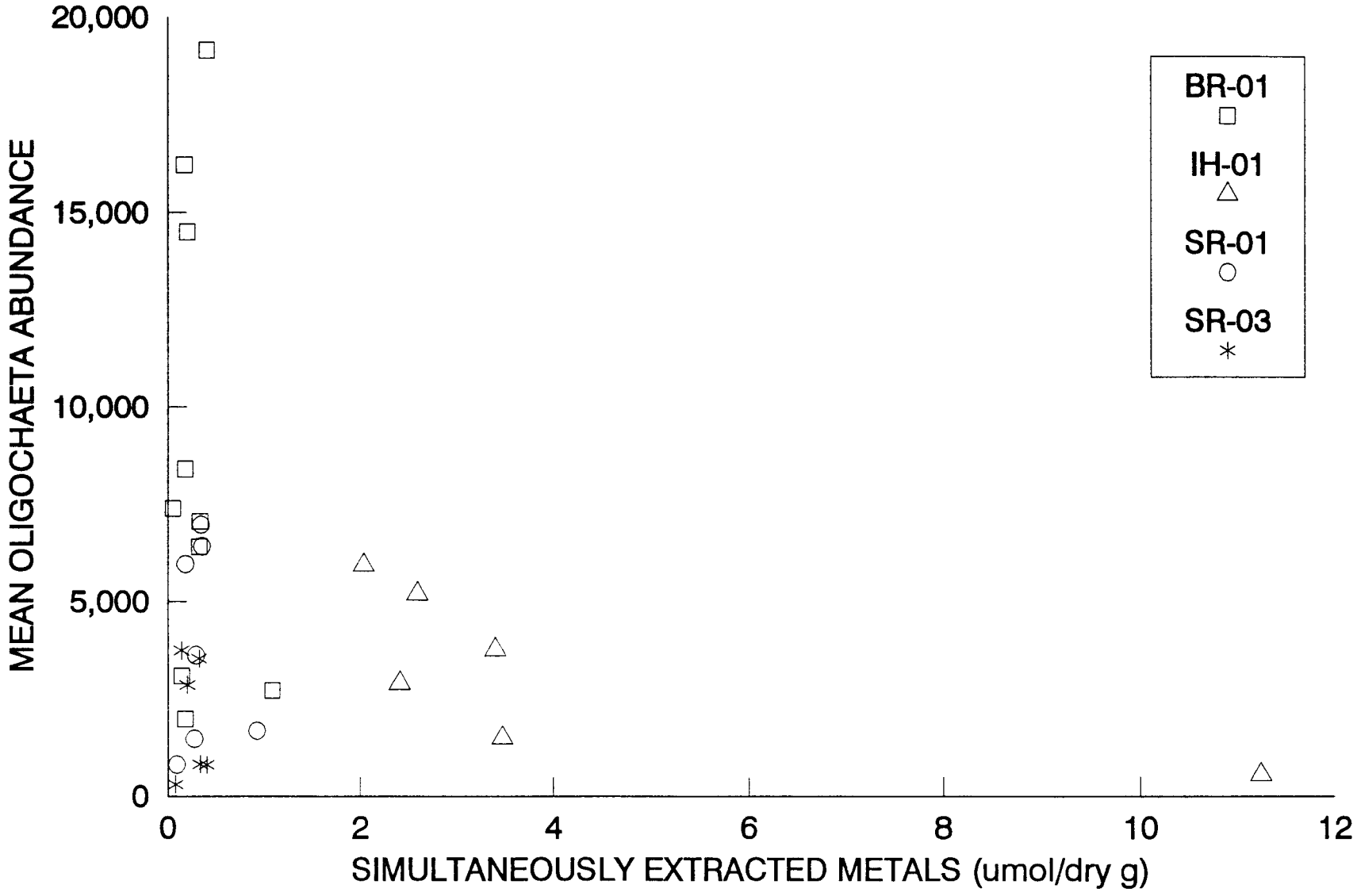
SR-01
SR-03

Figure 5.5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0
0
2
4
6
8
10
12
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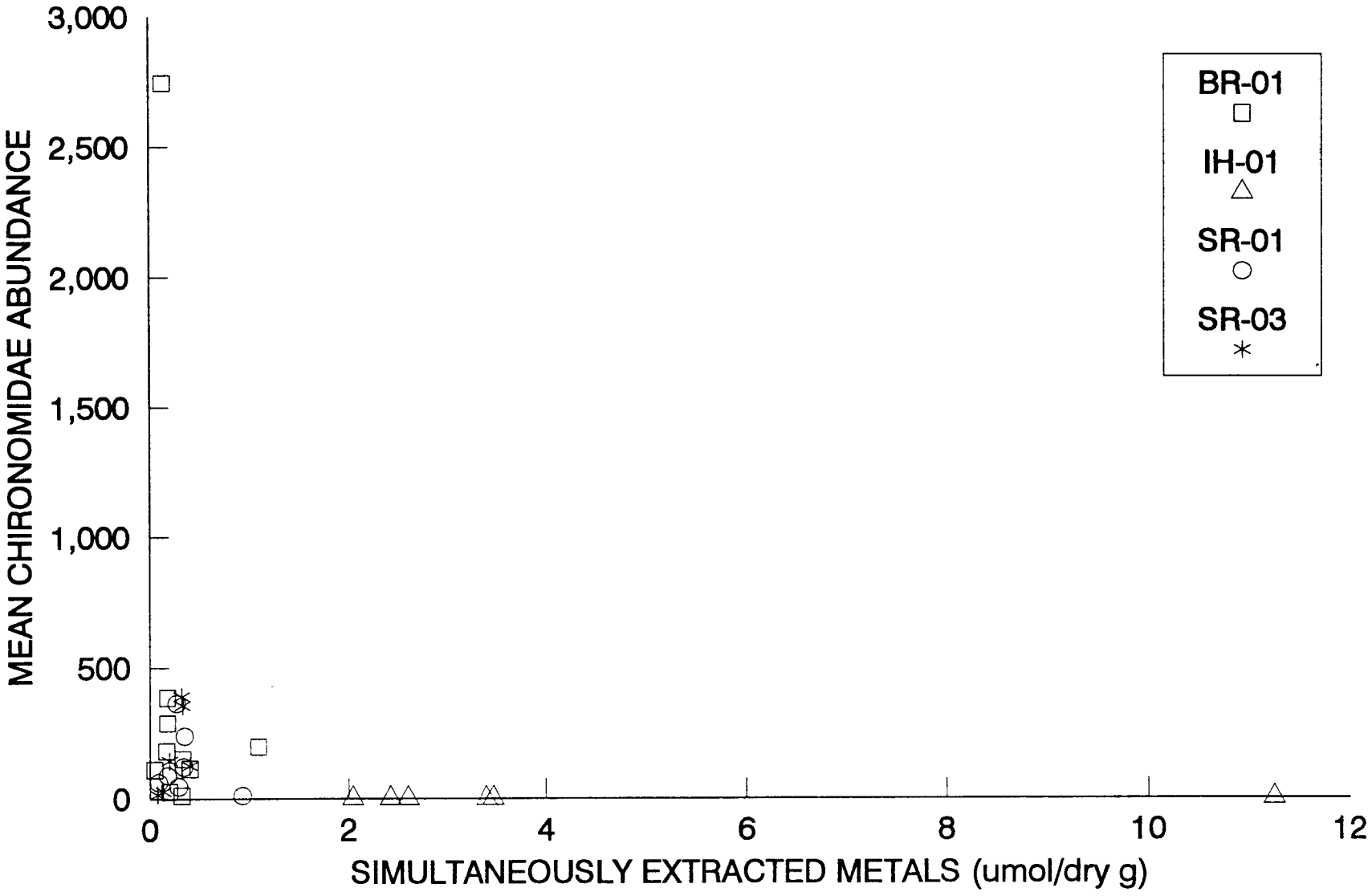
BR-01
IH-01
SR-01
SR-03

Figure 5.6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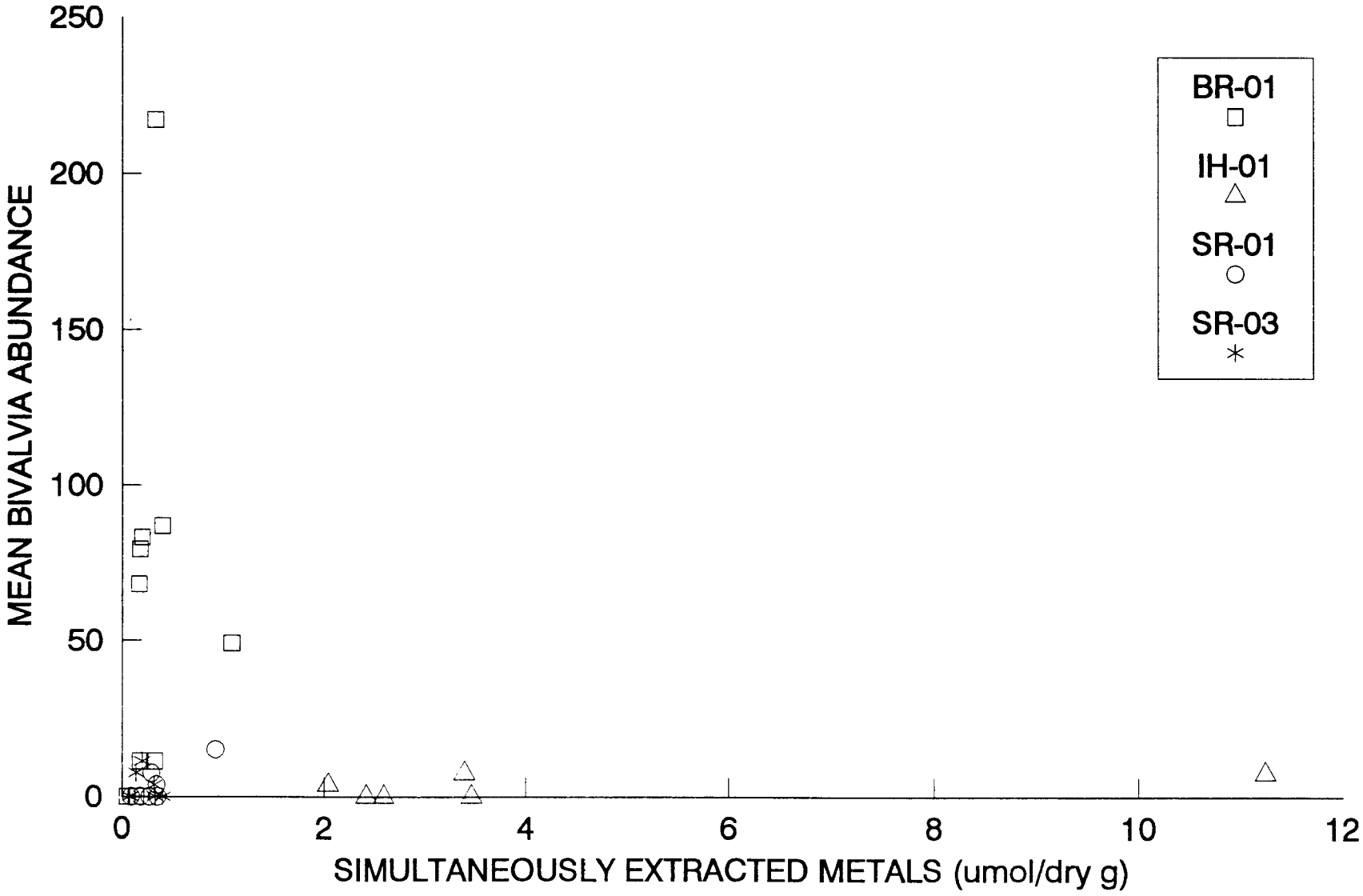
1,500
1,000
500
0
0
2
4
6
8
10
12
SIMULTANEOUSLY EXTRACTED METALS (umol/dry g)
BR-01
IH-01
SR-01
SR-03

Figure 5.7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2
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8
10
12
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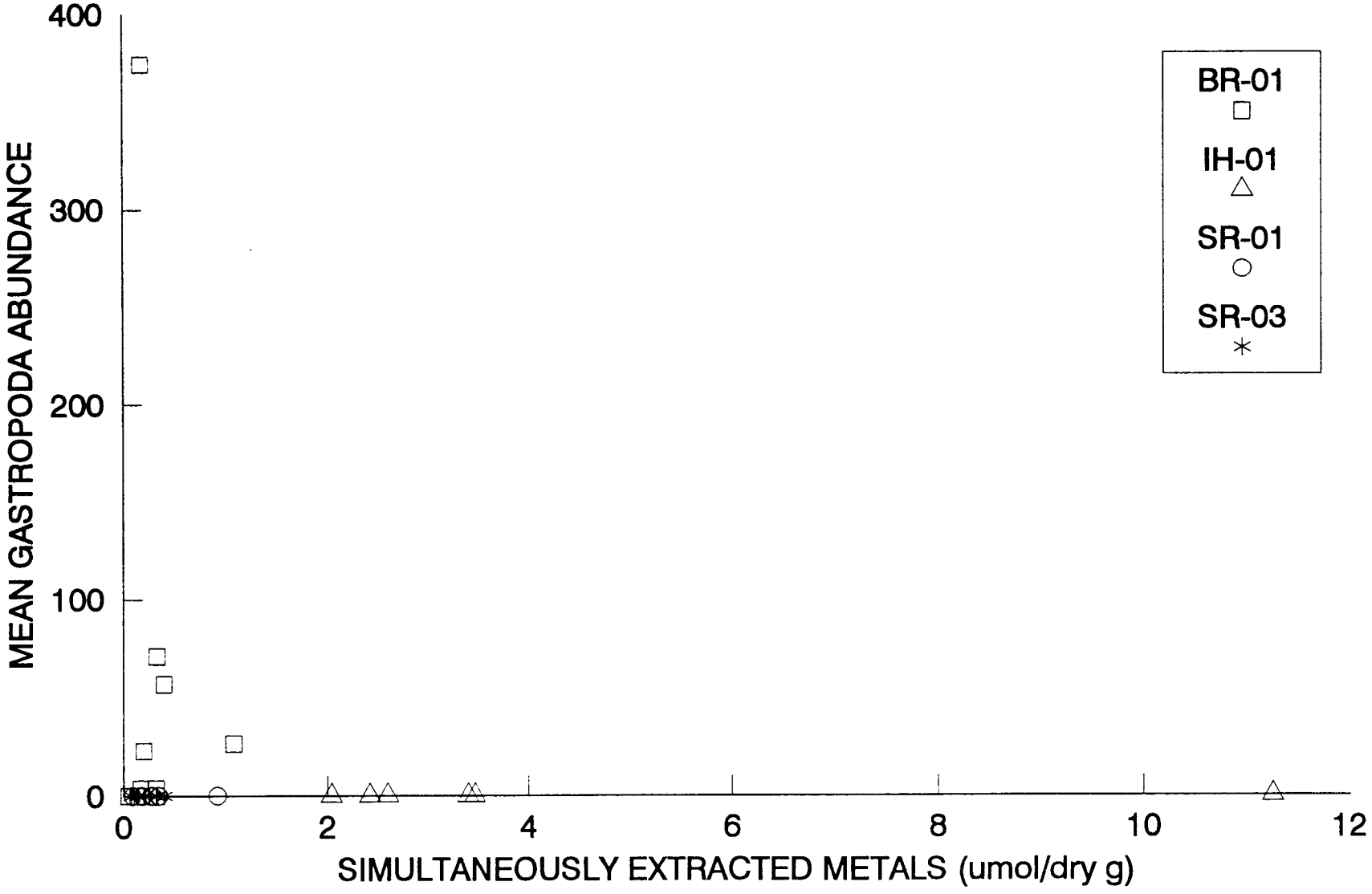
BR-01
IH-01
SR-01
SR-03

Figure 5.8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2
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8
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12
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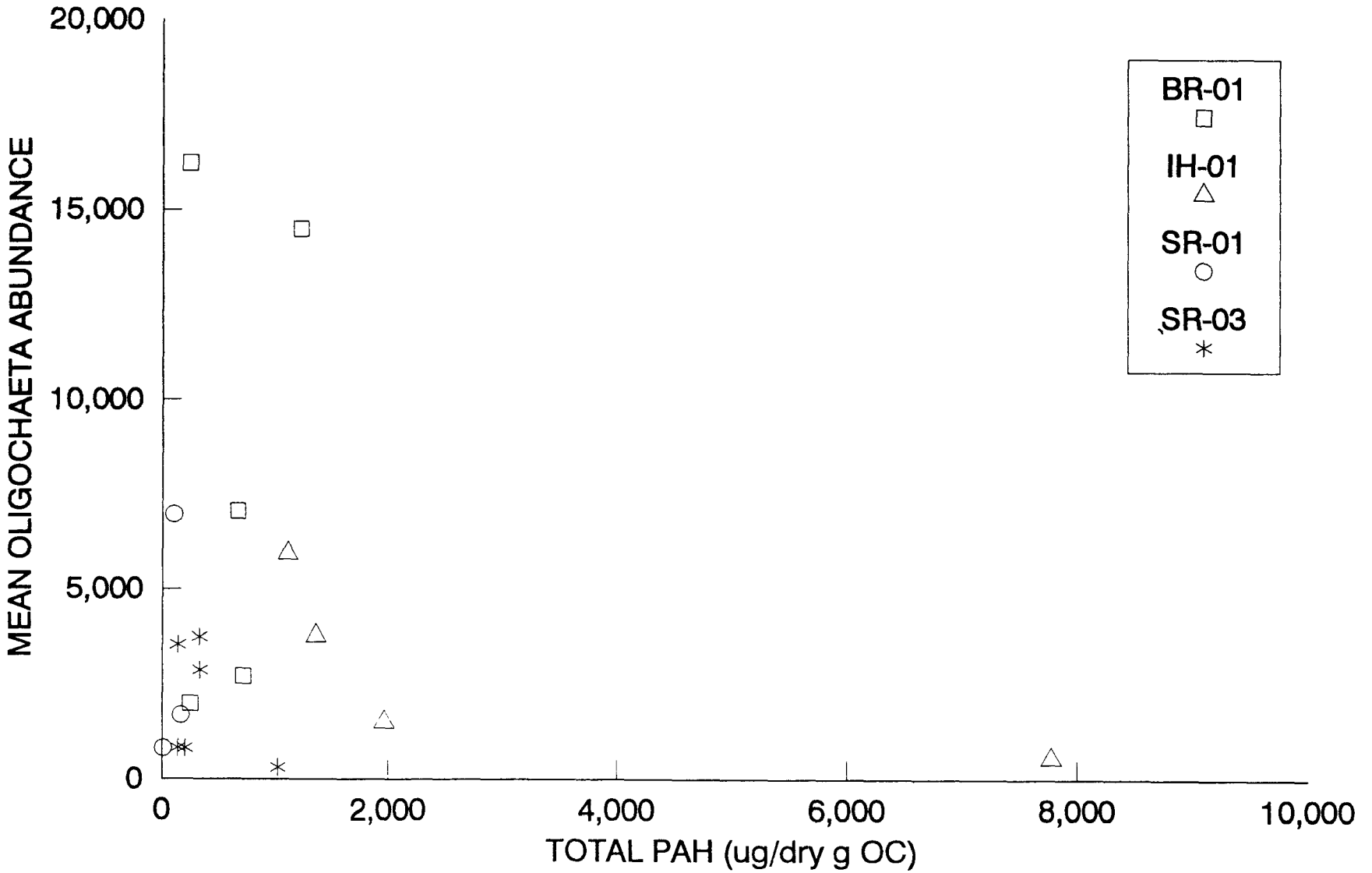
BR-01
IH-01
SR-01
SR-03

Figure 5.9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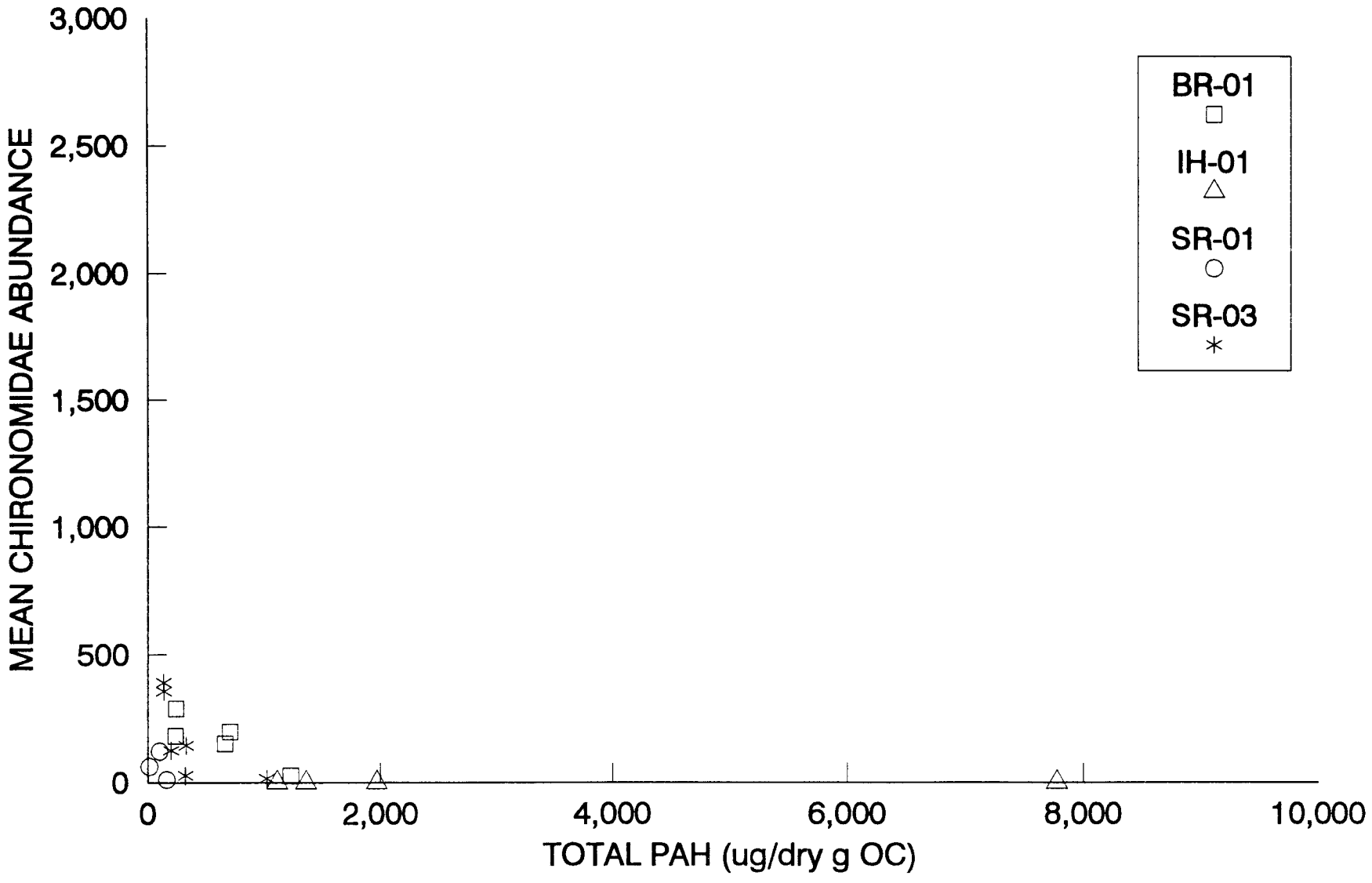
10,000
TOTAL PAH (ug/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.10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1,000
500
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0
2,000
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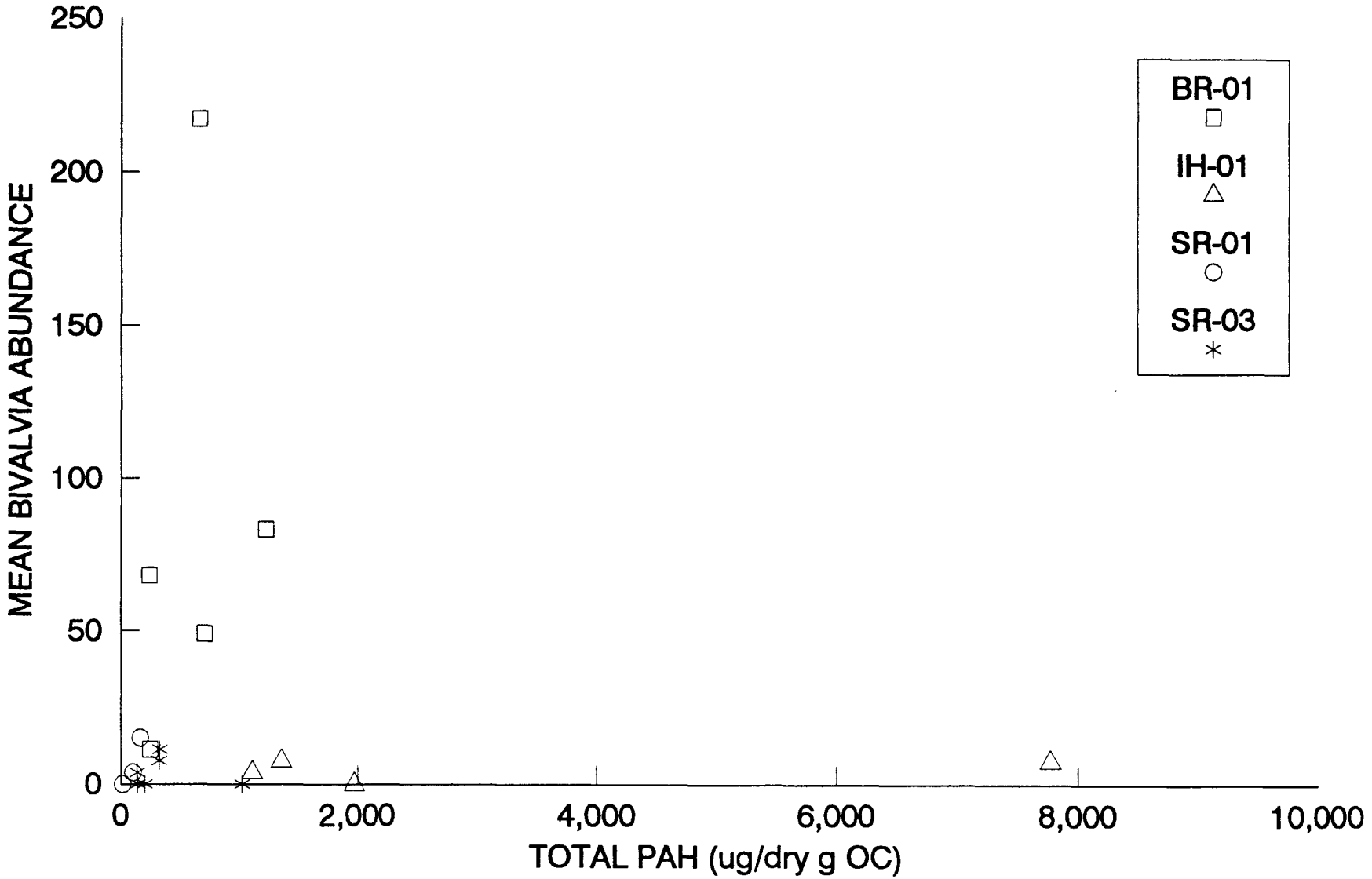
6,000
8,000
10,000
TOTAL PAH (ug/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.11


MEAN BIVALVIA ABUNDANCE
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150
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50
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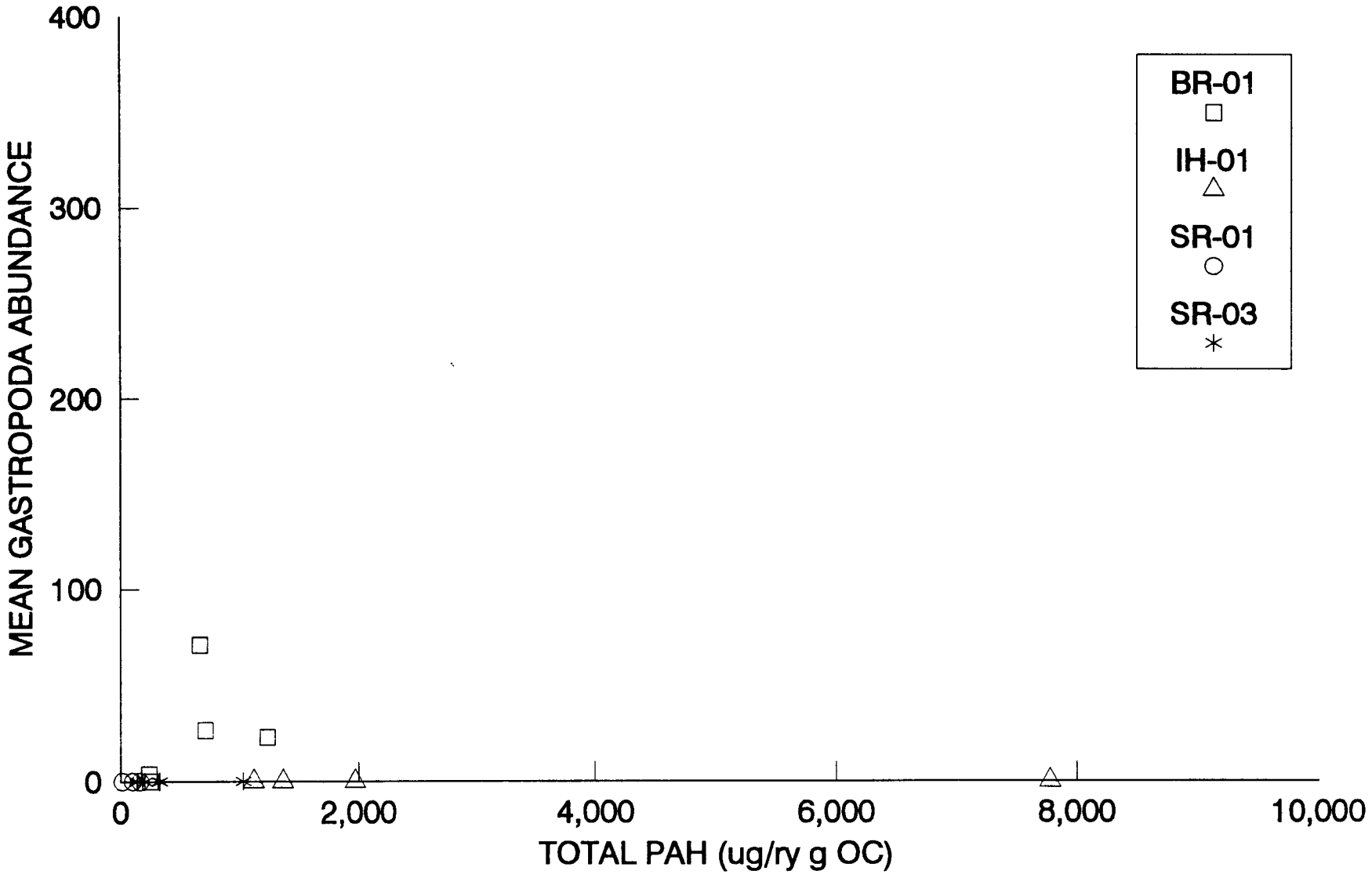
10,000
TOTAL PAH (ug/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.12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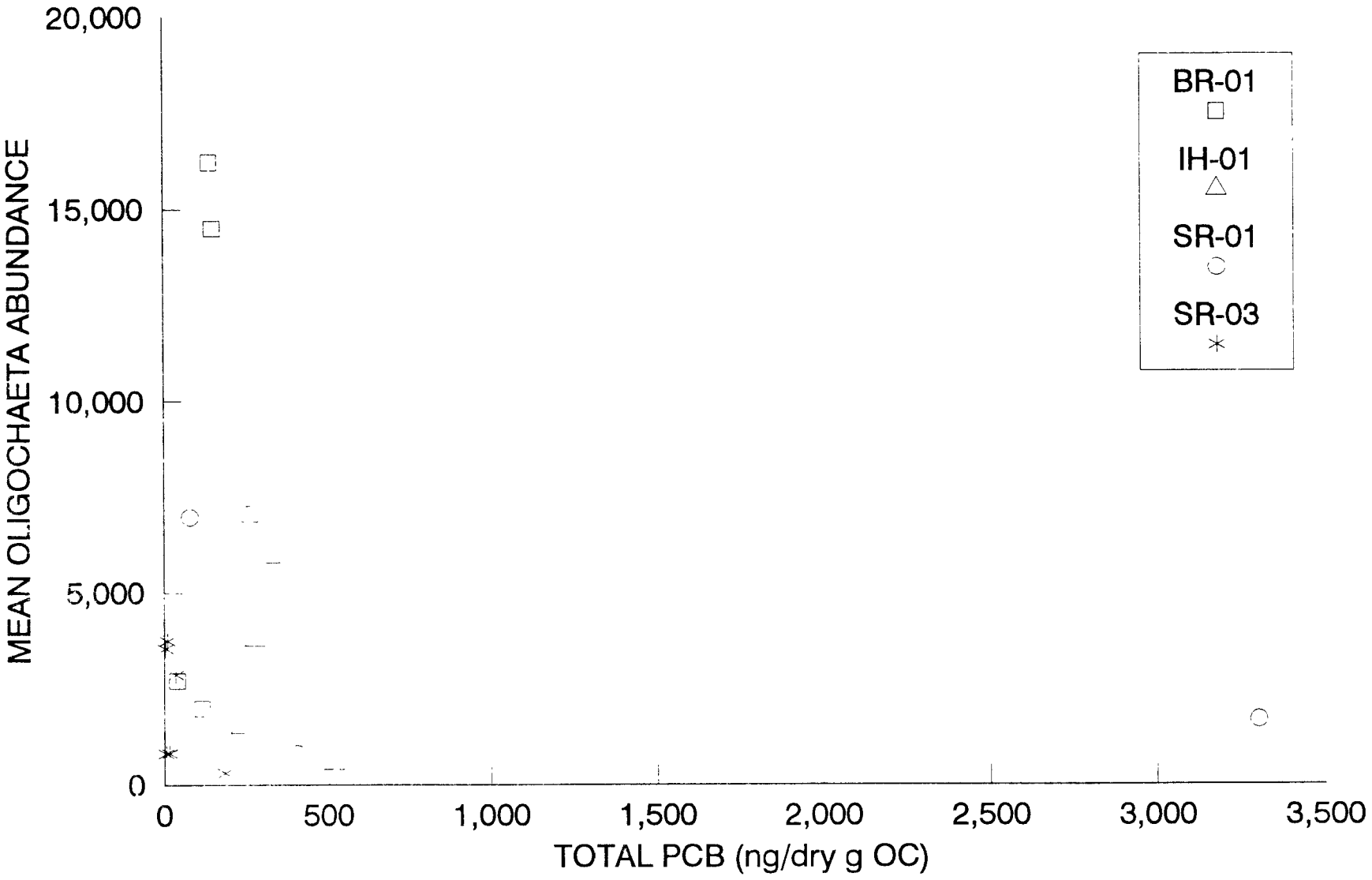
8,000
10,000
TOTAL PAH (ug/ry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.13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1,000
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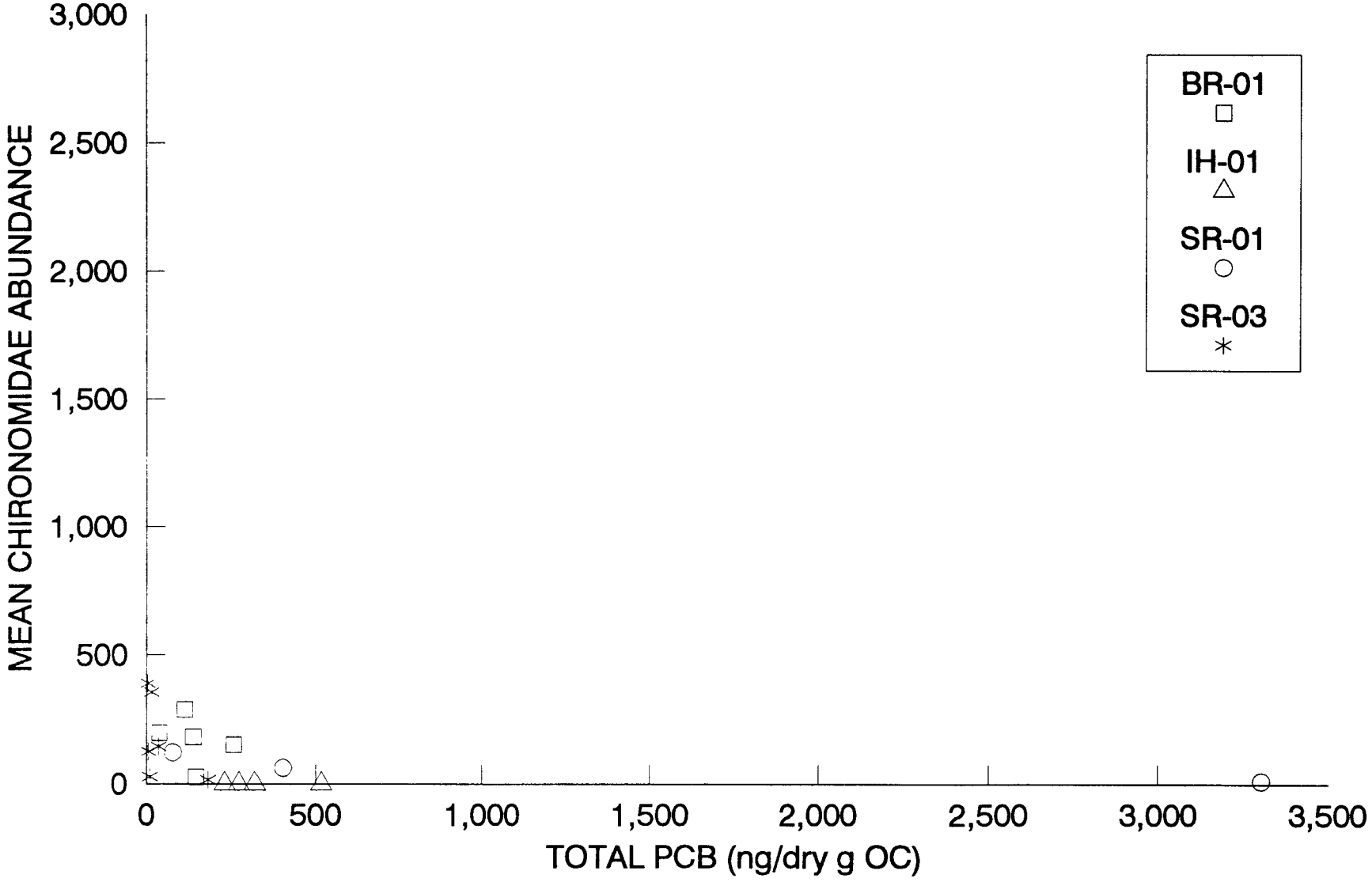
3,000
3,500
TOTAL PCB (ng/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.14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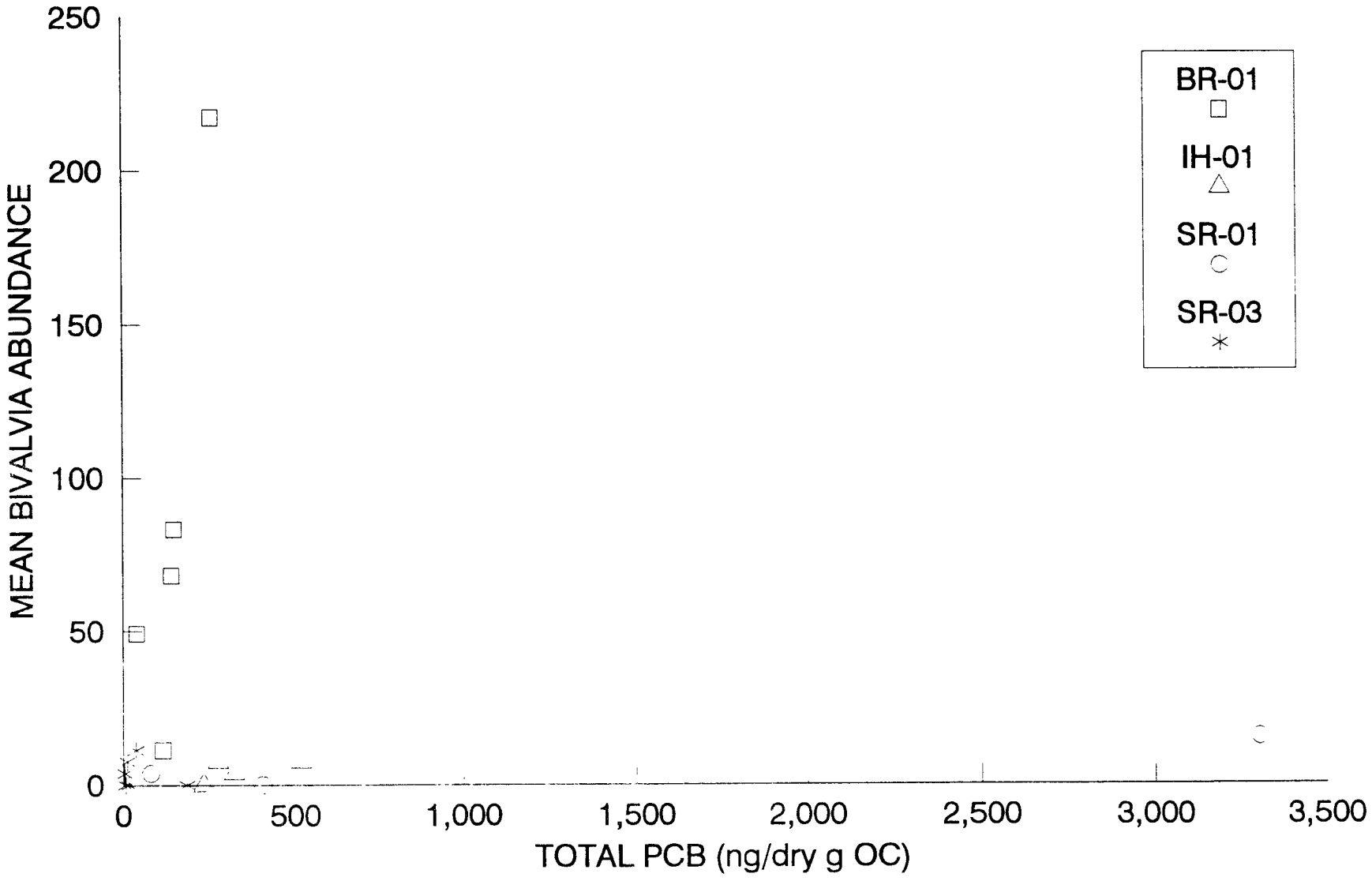
3,000
3,500
TOTAL PCB (ng/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.15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0
500
1,000
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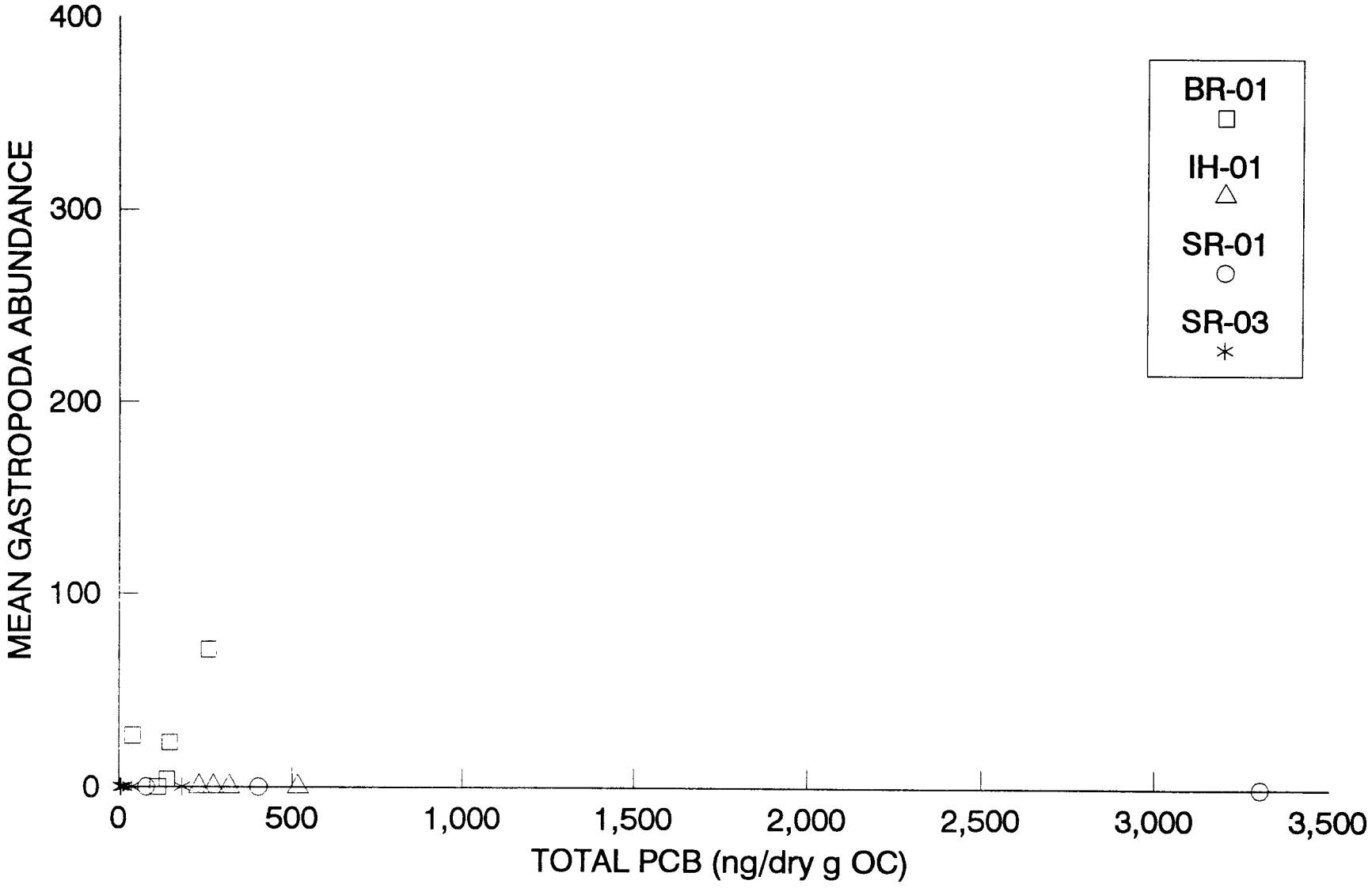
3,000
3,500
TOTAL PCB (ng/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.16


MEAN GASTROPODA ABUNDANCE
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100
0
0
500
1,000
1,500
2,000
2,500
3,000
3,500
TOTAL PCB (ng/dry g OC)
BR-01
IH-01
SR-01
SR-03

Figure 5.17

Indiana Harbor

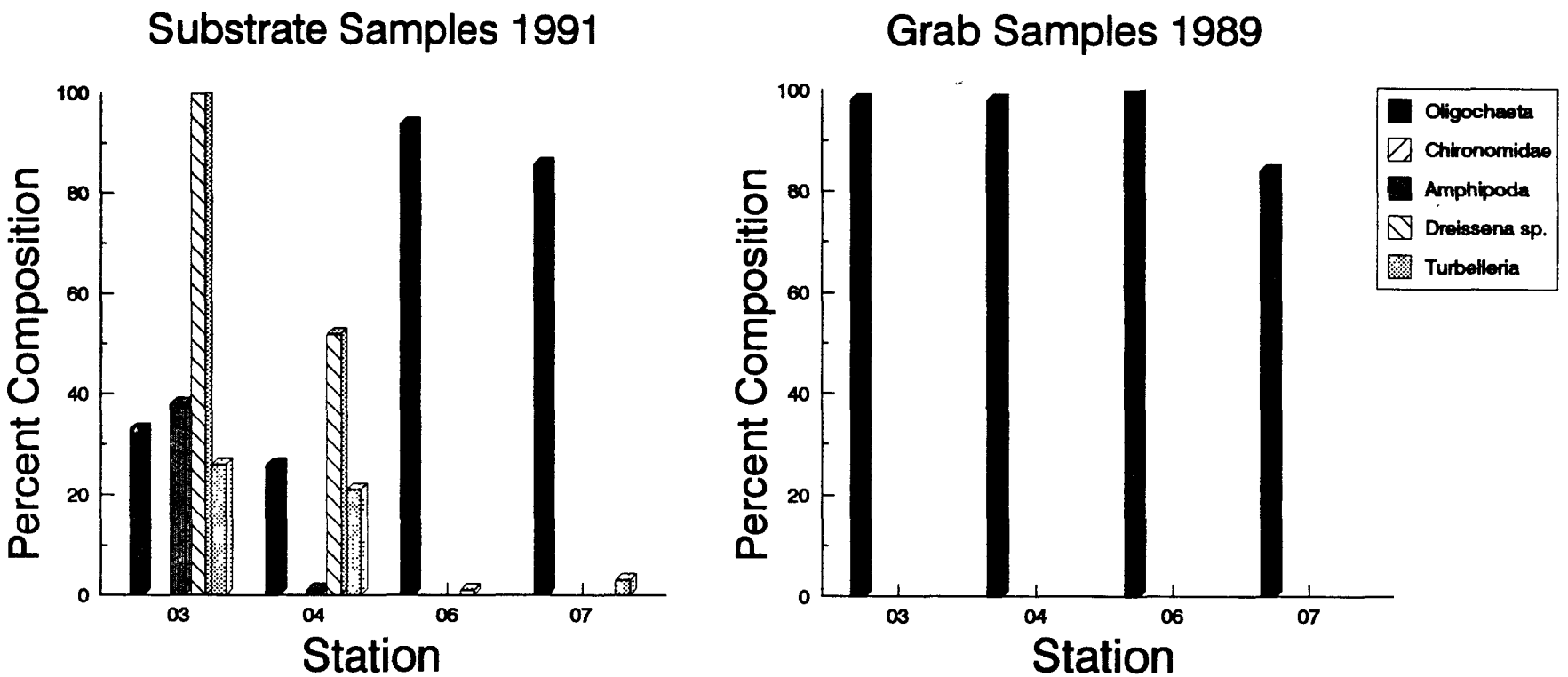


Figure 5.18

Buffalo River

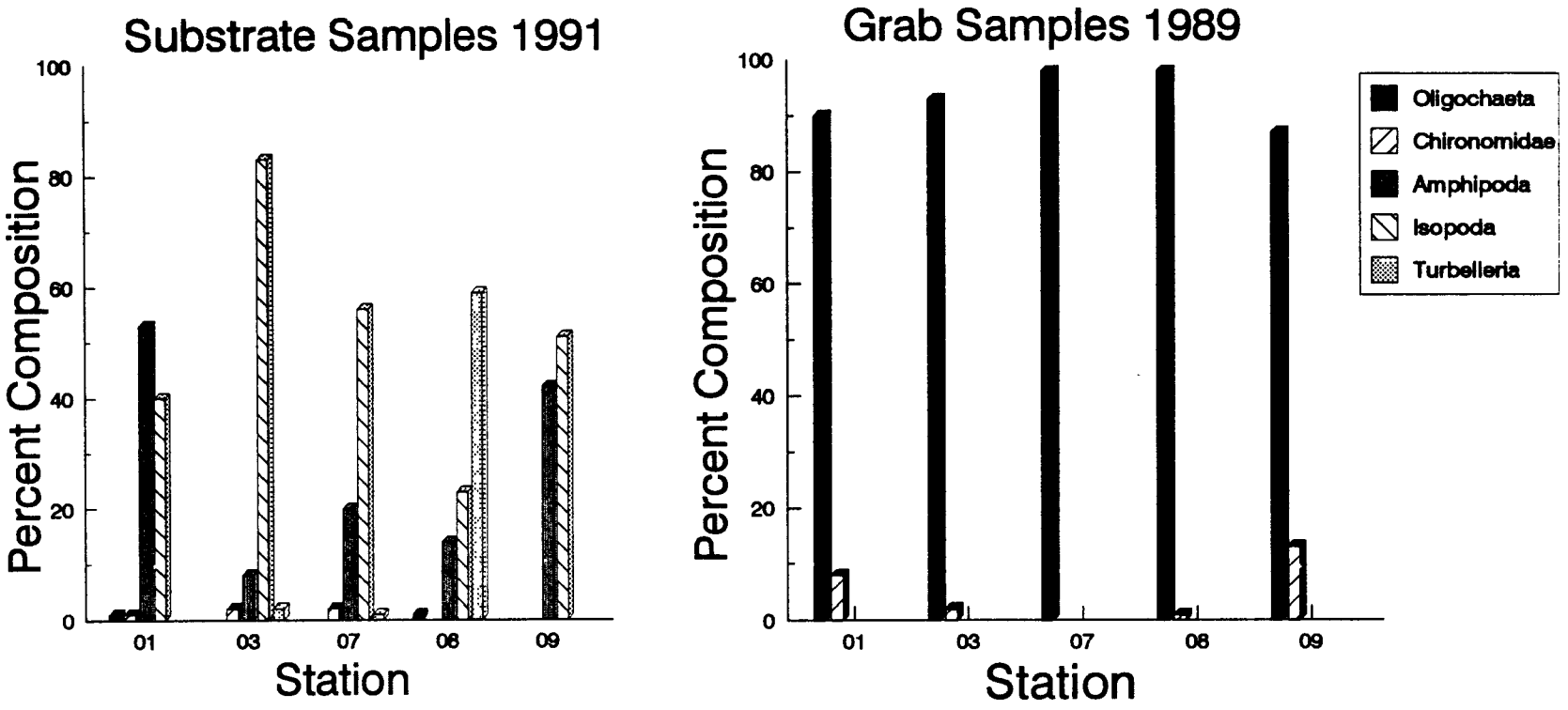
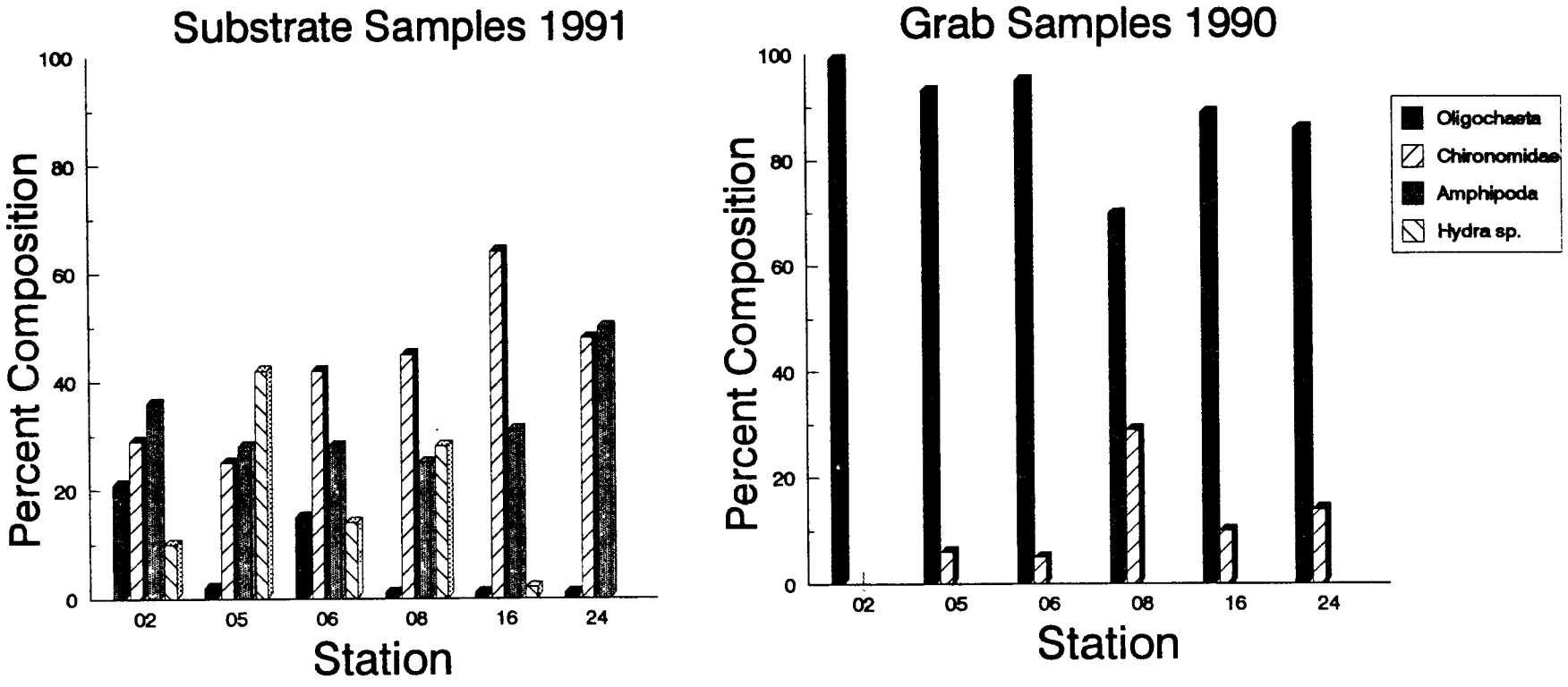


Figure 5.19

Saginaw River



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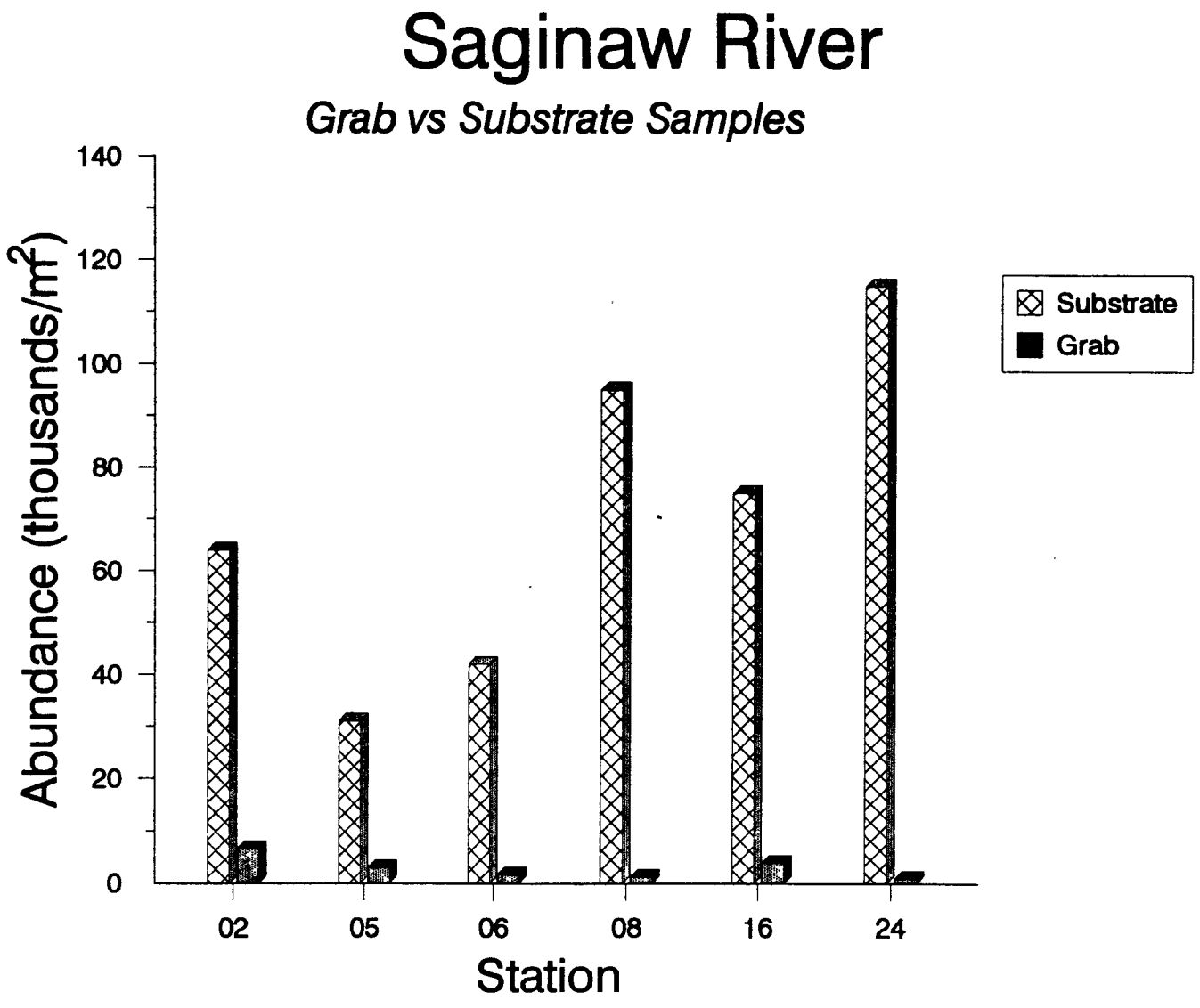
Saginaw River
Grab vs Substrate Samples
Abundance (thousands/m2)
140
120
100
80
60
40
20
0
02
05
06
08
16
24
Station
Substrate
Grab

Table 5.1 Continued

AOC	Oligochaeta	Chironomidae	Bivalvia	Gastropoda	Total
SR-01-02[3]	6418 (761)	238 (77)	0 (0)	0 (0)	6664 (822)
SR-01-03	6974 (242)	121 (22)	4 (4)	0 (0)	7129 (239)
SR-01-04	3621 (515)	45 (18)	8 (5)	0 (0)	3686 (511)
SR-01-06	1682 (432)	11 (5)	16 (7)	0 (0)	1709 (437)
SR-01-07	5954 (365)	87 (14)	0 (0)	0 (0)	6056 (377)
SR-01-09	1478 (204)	363 (79)	0 (0)	0 (0)	1890 (287)
SR-01-10	813 (630)	61 (55)	0 (0)	0 (0)	888 (693)
SR-03-01	4944 (863)	2204 (372)	0 (0)	0 (0)	7152 (1194)
SR-03-02	3735 (648)	23 (9)	8 (5)	0 (0)	3780 (658)
SR-03-05	2861 (244)	144 (35)	11 (8)	0 (0)	3047 (227)
SR-03-06	302 (108)	15 (7)	0 (0)	0 (0)	321 (114)
SR-03-08	820 (240)	360 (105)	0 (0)	0 (0)	1157 (296)
SR-03-16	3538 (253)	389 (45)	4 (4)	0 (0)	3977 (240)
SR-03-24	805 (203)	125 (36)	0 (0)	0 (0)	941 (238)

[1]BR-Buffalo River
[2]IH-Indiana Harbor
[3]SR-Saginaw River

Table 5.1 Mean abundance data (number/m^2, standard error in parentheses) for major benthic invertebrate groups. Values are averages (n=5) of the abundance values obtained for each station within an AOC

Station	Oligochaeta	Chironomidae	Bivalvia	Gastropoda	Total
BR-01-01[1]	2714 (1045)	197 (60)	49 (40)	27 (27)	3013 (1190)
BR-01-02	7394 (1389)	110 (44)	0 (0)	0 (0)	7530 (1419)
BR-01-03	7059 (1712)	151 (46)	217 (103)	72 (14)	7536 (1787)
BR-01-04	8407 (989)	386 (37)	79 (42)	378 (118)	9461 (1210)
BR-01-05	6403 (1920)	11 (8)	12 (8)	4 (4)	6445 (1927)
BR-01-06	19157 (2756)	114 (44)	87 (32)	57 (16)	19418 (2809)
BR-01-07	14496 (1838)	26 (11)	84 (22)	23 (11)	14708 (1839)
BR-01-08	16220 (2254)	181 (19)	68 (13)	4 (4)	16473 (2248)
BR-01-09	1984 (492)	287 (86)	12 (8)	0 (0)	2294 (553)
BR-01-10	3092 (860)	2771 (1633)	0 (0)	0 (0)	6067 (2423)
IH-01-03[2]	3765 (1618)	0 (0)	8 (8)	0 (0)	3791 (1623)
IH-01-04	5935 (2090)	0 (0)	4 (4)	0 (0)	6025 (2081)
IH-01-05	5198 (1742)	0 (0)	0 (0)	0 (0)	5307 (1788)
IH-01-06	1501 (915)	0 (0)	0 (0)	0 (0)	1501 (915)
IH-01-07	552 (272)	0 (0)	8 (8)	0 (0)	609 (255)
IH-01-08	2903 (1430)	0 (0)	0 (0)	0 (0)	2907 (1435)
IH-01-10	493887 (180680)	8 (8)	0 (0)	0 (0)	493917 (180676)

Table 5.2 Percentage contribution of each taxa to the total number of taxa collected from Indiana Harbor, IN, August 1989.

	STATION						
Taxa	03	04	05	06	07	08	10
Oligochaeta	99	98	98	100	91	100	100
Chironomidae	0	0	0	0	0	0	<1
Bivalvia	<1	<1	0	0	1	0	0
Gastropoda	0	0	0	0	0	0	0
Ephemeroptera	0	0	0	0	0	0	0
Odonata	0	0	0	0	0	0	0
Pelcoptera	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	0
Trichoptera	0	0	0	0	0	0	0
Coleoptera	0	0	0	0	0	0	0
Diptera	0	0	0	0	0	0	0
Hirudinea	<1	1	2	0	8	<1	<1
Amphipoda	0	0	0	0	0	0	0

Table 5.3 Mean abundance values (number/m^2, n=5/station) for aquatic Oligochaeta species collected from Indiana Harbor, IN, August 1989.

Taxa	STATION 03	04	05	06	07	08	10
Naididae							
Dero digitata	0	0	0	0	0	0	0
UN[1]	0	0	0	0	0	0	0
Tubificidae							
Aulodrilus pigueti	0	0	0	0	0	0	0
Aulodrilus limnobius	0	77	0	0	0	0	0
Aulodrilus pluriseta	113	0	0	0	0	0	0
Ilyodrilus templetoni	0	0	0	0	15	0	1975
Limnodrilus cervix	128	0	0	0	0	0	0
Limnodrilus cervix variant	72	101	0	0	6	38	0
Limnodrilus claparedianus	0	0	0	0	0	0	0
Limnodrilus clap.-cerv. complex	0	0	0	0	0	0	0
Limnodrilus hoffmeisteri	926	1056	790	102	238	1112	35560
Limnodrilus maumeensis	0	0	0	0	0	0	0
Limnodrilus udekemianus	72	53	0	0	0	0	0
Limnodrilus sp.	166	0	0	21	10	52	0
Quistadrilus multisetosus "l"	15	30	0	0	0	0	0
Quistadrilus multisetosus "m"	0	178	858	102	6	38	0
Tubifex tubifex	0	0	0	0	0	6	0
UIW/OCC[2]	2191	4445	3524	1246	258	1646	419310
UW/CC[3]	30	0	26	30	15	0	37535

[1]unidentifiable naidid
[2]unidentifiable, without capilliform chaetee (mostly Tubificidae)
[3]unidentifiable, with capilliform chaetee (mostly Tubificidae)

Table 5.4 Mean abundance values (number/m^2, n=5/station) for Chironomidae genera collected from Indiana Harbor, IN, August 1989.

	STATION						
Taxa	03	04	05	06	07	08	10
Tanypodinae							
Coelotanypus sp.	-	-	-	-	-	-	-
Procladius sp.	-	-	-	-	-	-	-
Tanypus sp.	-	-	-	-	-	-	-
Tanytarsini							
Tanytarsus	-	-	-	-	-	-	-
Chironomini							
Chironomus sp.	-	-	-	-	-	-	-
Cladopelma sp.	-	-	-	-	-	-	-
Cryptochironomus sp.	-	-	-	-	-	-	-
Dicrotendipes sp.	-	-	-	-	-	-	-
Glyptotendipes sp.	-	-	-	-	-	-	-
Microchironomus sp.	-	-	-	-	-	-	-
Polypedilum sp.	-	-	-	-	-	-	-
Orthocladinae							
Cricotopus sp.	-	-	-	-	-	-	8
Total	0	0	0	0	0	0	8
Taxa Richness	0	0	0	0	0	0	1

Table 5.5 Mean abundance values (numbers/m^2, n=5/station) for Mollusca genera and species collected from Indiana Harbor, IN, August 1989.

	STATION						
Taxa	03	04	05	06	07	08	10
Gastropoda							
Valvatidae							
Valvata lewisi	-	-	-	-	-	-	-
Valvata tricarinata	-	-	-	-	-	-	-
Bithyniidae							
Bithynia tentaculata	-	-	-	-	-	-	-
Ancylidae							
Laevapex fucus	-	-	-	-	-	-	-
Hydrobiidae							
Cincinnatia cincinnatiensis	-	-	-	-	-	-	-
Total	0	0	0	0	0	0	0
Taxa Richness	0	0	0	0	0	0	0
Bivalvia							
Sphaeriidae							
Musculium sp.	4	4	-	-	-	-	-
Pisidium sp.	4	-	-	-	4	-	-
Sphaerium sp.	-	-	-	-	4	-	-
Sp. unidentified	-	-	-	-	-	-	-
Unionidae							
Anodonta imbecillis	-	-	-	-	-	-	-
Anodonta grandis	-	-	-	-	-	-	-
Eliptio complanata	-	-	-	-	-	-	-
Total	8	4	0	0	8	0	0
Taxa Richness	2	1	0	0	2	0	0

Table 5.6 Percentage contribution of each taxa to the total number of taxa collected from Buffalo River, NY, October 1989.

	STATION									
Taxa	01	02	03	04	05	06	07	08	09	10
Oligochaeta	90	98	94	89	99	99	99	98	87	51
Chironomidae	7	2	2	4	<1	<1	<1	1	12	45
Bivalvia	2	0	3	<1	<1	<1	<1	<1	<1	0
Gastropoda	<1	0	<1	4	<1	<1	<1	<1	0	0
Ephemeroptera	0	0	0	0	0	0	0	0	0	<1
Odonata	0	0	0	0	0	0	0	0	0	<1
Pelcoptera	0	0	0	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0	0	0	<1
Megaloptera	0	0	0	0	0	0	0	0	0	0
Trichoptera	0	0	<1	0	0	0	0	0	0	0
Coleoptera	0	0	0	<1	0	0	0	0	0	<1
Diptera	0	0	0	<1	0	<1	<1	<1	0	2
Hirudinea	<1	0	<1	<1	<1	0	<1	0	0	0
Amphipoda	<1	<1	0	<1	0	0	0	0	<1	<1

Table 5.7 Mean abundance values (number/m^2, n=5/station) for aquatic Oligochaeta species collected from Buffalo River, NY, October 1989.

Taxa	STATION 01	02	03	04	05	06	07	08	09	10
Naididae										
Dero digitata	16	0	0	0	0	0	0	0	0	0
UN[1]	0	0	0	0	0	0	0	0	0	0
Tubificidae										
Aulodrilus pigueti	0	30	0	0	0	0	0	0	0	0
Aulodrilus limnobius	0	0	0	0	0	0	0	0	0	0
Aulodrilus pluriseta	0	0	0	0	0	0	0	0	0	0
Ilyodrilus templetoni	0	0	0	0	0	0	0	0	0	0
Limnodrilus cervix	100	0	0	0	0	0	0	0	0	0
Limnodrilus cervix variant	100	0	0	0	0	77	58	0	54	62
Limnodrilus claparedianus	0	0	0	0	0	0	0	0	0	0
Limnodrilus clap.-cerv. complex	30	0	0	0	0	0	0	0	0	0
Limnodrilus hoffmeisteri	0	370	1884	1224	160	1743	2305	1233	125	49
Limnodrilus maumeensis	0	0	0	0	0	0	0	0	0	0
Limnodrilus udekemianus	0	0	0	0	0	0	0	0	0	0
Limnodrilus sp.	43	111	494	588	0	747	449	277	26	37
Quistadrilus multisetosus "l"	100	30	219	261	0	0	0	277	10	12
Quistadrilus multisetosus "m"	0	0	28	34	0	0	0	0	20	0
Tubifex tubifex	0	0	0	0	0	0	0	65	0	0
UIW/OCC[2]	2249	6766	4377	5212	6172	16590	11698	14322	1706	2829
UW/CC[3]	73	81	56	67	70	0	0	130	46	99

[1]unidentifiable naidid
[2]unidentifiable, without capilliform chaetee (mostly Tubificidae)
[3]unidentifiable, with capilliform chaetee (mostly Tubificidae)

Table 5.8 Mean abundance values (number/m^2, n=5/station) for Chironomidae genera collected from Buffalo River, NY, October 1989.

	STATION									
Taxa	01	02	03	04	05	06	07	08	09	10
Tanypodinae										
Coelotanypus sp.	-	-	28	26	-	-	-	4	-	-
Procladius sp.	45	8	71	325	11	110	26	174	57	261
Tanypus sp.	-	-	-	-	-	-	-	-	-	11
Tanytarsini										
Tanytarsus	-	-	-	-	-	-	-	-	-	404
Chironomini										
Chironomus sp.	76	53	-	-	-	-	-	-	64	355
Cladopelma sp.	4	34	-	-	-	-	-	-	15	4
Cryptochironomus sp.	30	15	52	34	-	4	-	4	140	284
Dicrotendipes sp.	4	-	-	-	-	-	-	-	-	155
Glyptotendipes sp.	34	-	-	-	-	-	-	-	-	-
Microchironomus sp.	-	-	-	-	-	-	-	-	4	-
Polypedilum sp.	4	-	-	-	-	-	-	-	8	1297
Orthocladinae										
Cricotopus sp.	-	-	-	-	-	-	-	-	-	-
Total	197	116	151	386	11	114	26	181	287	2771
Taxa Richness	7	4	3	3	1	2	1	3	6	8

Table 5.9 Mean abundance values (numbers/m^2, n=5/station) for Mollusca genera and species collected from Buffalo River, NY, October 1989.

Taxa	01	02	03	04	05	06	07	08	09	10
	STATION									
Gastropoda										
Valvatidae										
Valvata lewisi	23	-	5	163	-	57	23	-	-	-
Valvata tricarinata	-	-	5	-	-	-	-	-	-	-
Bithyniidae										
Bithynia tentaculata	4	-	-	23	-	-	-	-	-	-
Ancylidae										
Laevapex fucus	-	-	5	4	-	-	-	-	-	-
Hydrobiidae										
Cincinnatia cincinnatiensis	-	-	57	185	4	-	-	-	-	-
Total	27	0	72	375	4	57	23	0	0	0
Taxa Richness	2	0	4	4	1	1	1	0	0	0
Bivalvia										
Sphaeriidae										
Musculium sp.	11	-	70	11	8	15	4	8	-	-
Pisidium sp.	30	-	109	45	-	53	57	30	8	-
Sphaerium sp.	-	-	33	15	4	19	19	30	-	-
Sp. unidentified	-	-	-	-	-	-	4	-	-	-
Unionidae										
Anodonta imbecillis	4	-	-	4	-	-	-	-	4	-
Anodonta grandis	4	-	5	-	-	-	-	-	-	-
Eliptio complanata	-	-	-	4	-	-	-	-	-	-
Total	49	0	217	79	12	87	84	68	12	0
Taxa Richness	4	0	4	5	2	3	4	3	2	0

Table 5.10 Percentage contribution of each taxa to the total number of taxa collected from the first survey of Saginaw River, MI, December 1989.

	STATION						
Taxa	02	03	04	06	07	09	10
Oligochaeta	96	98	98	98	98	80	92
Chironomidae	4	2	1	1	1	19	7
Bivalvia	0	<1	<1	1	0	0	0
Gastropoda	0	0	0	0	0	0	0
Ephemeroptera	0	0	0	0	0	0	0
Odonata	0	0	0	0	0	0	0
Pelcoptera	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	0
Trichoptera	0	0	0	0	0	0	<1
Coleoptera	0	0	0	0	0	0	<1
Diptera	<1	<1	<1	0	<1	<1	<1
Hirudinea	0	0	0	0	0	0	0
Amphipoda	0	0	0	0	<1	<1	0

Table 5.11 Mean abundance values (number/m^2, n=5/station) for aquatic Oligochaeta species collected from the first survey of Saginaw River, MI, December 1989.

	STATION						
Taxa	02	03	04	06	07	09	10
Naididae							
Dero digitata	0	0	0	0	0	15	0
UN[1]	0	0	0	0	0	0	0
Tubificidae							
Aulodrilus pigueti	0	0	0	0	0	0	0
Aulodrilus limnobius	0	0	0	0	0	0	0
Aulodrilus pluriseta	0	0	0	0	0	0	0
Ilyodrilus templetoni	32	98	0	34	30	15	0
Limnodrilus cervix	32	265	174	59	101	62	109
Limnodrilus cervix variant	90	70	109	34	30	21	0
Limnodrilus claparedianus	0	0	0	19	0	0	0
Limnodrilus clap.-cerv. complex	0	0	0	0	0	0	0
Limnodrilus hoffmeisteri	809	1318	391	232	536	268	35
Limnodrilus maumeensis	0	0	22	0	0	7	0
Limnodrilus udekemianus	0	0	0	0	0	0	0
Limnodrilus sp.	122	133	221	86	286	109	71
Quistadrilus multisetosus "l"	0	0	22	8	0	0	0
Quistadrilus multisetosus "m"	0	0	0	0	0	0	0
Tubifex tubifex	0	0	0	8	0	0	0
UIW/OCC[2]	5295	5056	2680	1189	4972	998	585
UW/CC[3]	32	35	0	8	0	0	13

[1]unidentifiable naidid
[2]unidentifiable, without capilliform chaetee (mostly Tubificidae)
[3]unidentifiable, with capilliform chaetee (mostly Tubificidae)

Table 5.12 Mean abundance values (number/m^2, n=5/station) for Chironomidae genera collected from the first survey of Saginaw River, MI, December 1989.

	STATION						
Taxa	02	03	04	06	07	09	10
Tanypodinae							
Coelotanypus sp.	-	-	-	-	-	-	-
Procladius sp.	227	121	45	-	83	280	38
Tanypus sp.	-	-	-	-	-	-	-
Tanytarsini							
Tanytarsus	-	-	-	-	-	-	-
Chironomini							
Chironomus sp.	4	-	-	-	-	79	-
Cladopelma sp.	-	-	-	-	-	-	-
Cryptochironomus sp.	-	-	-	11	-	4	24
Dicrotendipes sp.	4	-	-	-	-	-	-
Glyptotendipes sp.	-	-	-	-	4	-	-
Microchironomus sp.	-	-	-	-	-	-	-
Polypedilum sp.	-	-	-	-	-	-	-
Orthocladinae							
Cricotopus sp.	4	-	-	-	-	-	-
Total	238	121	45	11	87	363	61
Taxa Richness	4	1	1	1	2	3	2

Table 5.13 Mean abundance values (numbers/m^2, n=5/station) for Mollusca genera and species collected from the first survey of Saginaw River, MI, December 1989.

	STATION						
Taxa	02	03	04	06	07	09	10
Gastropoda							
Valvatidae							
Valvata lewisi	-	-	-	-	-	-	-
Valvata tricarinata	-	-	-	-	-	-	-
Bithyniidae							
Bithynia tentaculata	-	-	-	-	-	-	-
Ancylidae							
Laevapex fucus	-	-	-	-	-	-	-
Hydrobiidae							
Cincinnatia cincinnatiensis	-	-	-	-	-	-	-
Total	0	0	0	0	0	0	0
Taxa Richness	0	0	0	0	0	0	0
Bivalvia							
Sphaeriidae							
Musculium sp.	-	-	-	4	-	-	-
Pisidium sp.	-	-	-	12	-	-	-
Sphaerium sp.	-	4	8	-	-	-	-
Sp. unidentified	-	-	-	-	-	-	-
Unionidae							
Anodonta imbecillis	-	-	-	-	-	-	-
Anodonta grandis	-	-	-	-	-	-	-
Eliptio complanata	-	-	-	-	-	-	-
Total	0	4	8	16	0	0	0
Taxa Richness	0	1	1	2	0	0	0

Table 5.14 Percentage contribution of each taxa to the total number of taxa collected from the third survey of Saginaw River, MI, June 1990.

	STATION						
Taxa	01	02	05	06	08	16	24
Oligochaeta	69	99	94	95	69	90	86
Chironomidae	31	<1	5	5	30	9	13
Bivalvia	0	<1	<1	0	0	<1	0
Gastropoda	0	0	0	0	0	0	0
Ephemeroptera	0	0	0	0	0	0	0
Odonata	0	0	0	0	0	0	0
Pelcoptera	0	0	0	0	0	0	0
Hemiptera	0	0	0	0	0	0	0
Megaloptera	0	0	0	0	0	0	0
Trichoptera	0	0	0	0	0	0	0
Coleoptera	0	<1	0	0	0	0	<1
Diptera	0	<1	<1	0	<1	<1	0
Hirudinea	0	0	0	0	0	0	0
Amphipoda	0	0	0	0	0	0	0

Table 5.15 Mean abundance values (number/m^2, n=5/station) for aquatic Oligochaeta species collected from the third survey of Saginaw River, MI, June 1990.

	STATION						
Taxa	01	02	05	06	08	16	24
Naididae							
Dero digitata	0	0	14	0	0	0	0
UN[1]	0	0	0	0	0	14	0
Tubificidae							
Aulodrilus pigueti	0	0	0	0	0	0	0
Aulodrilus limnobius	0	0	0	0	0	0	0
Aulodrilus pluriseta	0	19	14	0	0	0	0
Ilyodrilus templetoni	20	82	160	0	0	60	0
Limnodrilus cervix	138	190	69	18	21	198	30
Limnodrilus cervix variant	282	291	446	68	92	308	181
Limnodrilus claparedianus	0	0	0	0	0	0	0
Limnodrilus clap.-cerv. complex	0	0	0	0	0	0	0
Limnodrilus hoffmeisteri	158	243	257	68	98	240	188
Limnodrilus maumeensis	124	34	106	23	71	120	42
Limnodrilus udekemianus	0	0	0	0	0	0	0
Limnodrilus sp.	460	209	92	5	11	120	12
Quistadrilus multisetosus "l"	20	0	0	0	0	0	0
Quistadrilus multisetosus "m"	59	0	0	0	0	0	0
Tubifex tubifex	0	0	0	0	0	0	0
UIW/OCC[2]	3505	2633	1634	121	527	2487	352
UW/CC[3]	180	34	69	0	0	0	0

[1]unidentifiable naidid
[2]unidentifiable, without capilliform chaetee (mostly Tubificidae)
[3]unidentifiable, with capilliform chaetee (mostly Tubificidae)

Table 5.16 Mean abundance values (number/m^2, n=5/station) for Chironomidae genera collected from the third survey of Saginaw River, MI, June 1990.

	STATION						
Taxa	01	02	05	06	08	16	24
Tanypodinae							
Coelotanypus sp.	-	-	-	-	8	-	-
Procladius sp.	144	15	125	4	147	234	87
Tanypus sp.	-	-	-	-	4	-	8
Tanytarsini							
Tanytarsus	-	-	-	-	8	-	-
Chironomini							
Chironomus sp.	2045	4	19	4	178	34	26
Cladopelma sp.	-	-	-	-	-	-	-
Cryptochironomus sp.	-	4	-	8	4	113	4
Dicrotendipes sp.	-	-	-	-	-	-	-
Glyptotendipes sp.	-	-	-	-	-	-	-
Microchironomus sp.	-	-	-	-	11	8	-
Polypedilum sp.	-	-	-	-	-	-	-
Orthocladinae							
Cricotopus sp.	-	-	-	-	-	-	-
Total	2204	23	144	15	360	389	125
Taxa Richness	2	3	2	3	7	4	4

Table 5.17 Mean abundance values (numbers/m^2, n=5/station) for Mollusca genera and species collected from the third survey of Saginaw River, MI, June 1990.

	STATION						
Taxa	01	02	05	06	08	16	24
Gastropoda							
Valvatidae							
Valvata lewisi	-	-	-	-	-	-	-
Valvata tricarinata	-	-	-	-	-	-	-
Bithyniidae							
Bithynia tentaculata	-	-	-	-	-	-	-
Ancylidae							
Laevapex fucus	-	-	-	-	-	-	-
Hydrobiidae							
Cincinnatia cincinnatiensis	-	-	-	-	-	-	-
Total	0	0	0	0	0	0	0
Taxa Richness	0	0	0	0	0	0	0
Bivalvia							
Sphaeriidae							
Musculium sp.	-	8	11	-	-	4	-
Pisidium sp.	-	-	-	-	-	-	-
Sphaerium sp.	-	-	-	-	-	-	-
Sp. unidentified	-	-	-	-	-	-	-
Unionidae							
Anodonta imbecillis	-	-	-	-	-	-	-
Anodonta grandis	-	-	-	-	-	-	-
Eliptio complanata	-	-	-	-	-	-	-
Total	0	8	11	0	0	4	0
Taxa Richness	0	1	1	0	0	1	0

Table 5.18 Comparison of total invertebrate abundance (number/m^2) values for each area of concern (AOC).

Taxa	Study				
	BR-01 (n=49)	IH-01 (n=35)	IH-01[1] (n=30)	SR-01 (n=34)	SR-03 (n=32)
Abundance (number)					
Oligochaeta					
Mean	8726	73391	3309	3938	2429
Median	7333	3364	1994	3572	2325
Range	170-28047	132-970704	132-14062	0-8505	0-7163
Std. Dev.	6669	222599	3563	2596	1909
Chironomidae					
Mean	426	1	0	135	465
Median	132	0	0	95	170
Range	0-9148	0-38	0	0-548	0-3553
Std. Dev.	1325	6.4	0	152	793
Total					
Mean	9323	73437	3357	4094	2783
Median	8108	3515	2003	3695	2476
Range	189-28558	283-970704	284-14099	0-9072	0-10716
Std. Dev.	6680	222592	3591	2623	2519
Abundance (percentage)					
Oligochaeta					
Mean	91	97	96	95	86
Median	95	99	99	98	90
Range	39-100	37-100	37-100	69-100	47-100
Std Dev.	13	11	12	8	13
Chironomidae					
Mean	7	.	.	5	14
Median	2	.	.	2	10
Range	0-60	.	.	0-30	0-53
Std. Dev.	12	.	.	7	13

[1] Station 10 not included in analysis.

Table 5.19 Comparison of mean abundance (number/m^2) values among stations within an AOC using Duncans Multiple Range test. Values with the same letter within ans AOC are not significantly different ($P \leq 0.05$).

AOC	Station	Oligochaeta	Station	Chironomidae	Station	Total
BR-01	06	19157 A	10	2771 A	06	19418 A
BR-01	08	16220 A	04	306 B	08	16447 A
BR-01	07	14496 A	09	287 B	07	14700 AB
BR-01	04	8407 B	01	197 B	04	9461 BC
BR-01	02	7394 BC	08	181 B	03	7536 BC
BR-01	03	7059 BCD	03	151 B	02	7530 CD
BR-01	05	6403 BCD	06	114 B	05	6445 CD
BR-01	10	3092 CD	02	110 B	10	6067 CD
BR-01	01	2714 CD	07	26 B	01	3016 D
BR-01	09	1984 D	05	11 B	09	2294 D
IH-01	10	493887 A	10	4 A	10	493917 A
IH-01	04	5935 B	04	0 A	04	6025 B
IH-01	05	5198 BC	03	0 A	05	5307 BC
IH-01	03	3765 BC	06	0 A	03	3791 BC
IH-01	08	2903 BC	07	0 A	08	2907 BC
IH-01	06	1501 BC	08	0 A	06	1501 BC
IH-01	07	552 C	05	0 A	07	609 C

Table 5.19 Continued.

AOC	Station	Oligochaeta	Station	Chironomidae	Station	Total
SR-01	03	6974 A	09	363 A	03	7129 A
SR-01	02	6418 A	02	238 AB	02	6664 A
SR-01	07	5954 A	03	121 BC	07	6056 A
SR-01	04	3621 B	07	87 C	04	3686 B
SR-01	06	1682 C	10	61 C	09	1890 C
SR-01	09	1478 C	04	45 C	06	1705 C
SR-01	10	813 C	06	11 C	10	888 C
SR-03	01	4944 A	01	2204 A	01	7152 A
SR-03	02	3735 AB	16	389 B	16	3977 B
SR-03	16	3538 B	08	360 B	05	3047 B
SR-03	05	2862 B	05	144 B	02	2888 BC
SR-03	08	820 C	24	125 B	08	1157 CD
SR-03	24	805 C	02	23 B	24	941 D
SR-03	06	302 C	06	15 B	06	321 D

Table 5.20 Comparison of Chironomidae deformities (%) for each area of concern (AOC).

AOC/STATION	n	OCCURRENCE OF DEFORMITIES (%)
BR-01-01	52	8
BR-01-02	29	7
BR-01-03	32	6
BR-01-04	102	5
BR-01-05	3	0
BR-01-06	30	7
BR-01-07	7	14
BR-01-08	48	4
BR-01-09	76	17
BR-01-10	210	7
MEAN	10	7
IH-01-10	2	100
SR-01-02	63	14
SR-01-03	32	15
SR-01-04	12	25
SR-01-06	3	0
SR-01-07	23	13
SR-01-09	96	16
SR-01-10	6	6
MEAN	7	13
SR-03-01	334	2
SR-03-02	6	14
SR-03-05	38	23
SR-03-06	4	25
SR-03-08	94	16
SR-03-16	103	21
SR-03-24	33	16
MEAN	7	17

Overall occurance among individual replicate grabs

Buffalo River (19/42)	45
Indiana Harbor (1/1)	100
Saginaw River 1 (17/29)	59
Saginaw River 3 (23/30)	77

CHAPTER 6: AMES MUTAGENICITY ASSAY

Papoulias, D. and D.R. Buckler

INTRODUCTION

The presence of chemical contaminants in the Great Lakes is well-documented. In 1980 the International Joint Commission (IJC) estimated that about 2,500 chemicals were in common use in the Great Lakes Basin ecosystem (International Joint Commission 1980). A comprehensive review of fish-tissue residues prepared by the IJC's Great Lakes International Surveillance Plan (Hesselberg and Seelye 1982) tentatively identified 476 organic compounds. Most of these organic compounds are also present in Great Lakes sediments, and extracts of these sediments have tested positive in mutagenicity assays (Allen et al. 1983, Metcalfe et al. 1990, Maccubbin and Ersing 1991). Baumann (1984) and Baumann and Whittle (1988) suggested a link between high levels of industrial contaminants and frequency of tumors in fish populations. Recent study of contaminated sediments from tributaries of the Great Lakes using Medaka (*Oryzias latipes*) supports the hypothesis that chemical contaminants in lake sediment are associated with neoplasms in fishes near contaminated sites (Fabacher et al. 1991).

Short-term bioassays are a fundamental component of tier testing to assess the genotoxicity (DNA-damaging properties) of chemical contaminants (Barfknecht and Naismith 1984, Lovell 1989, International Joint Commission 1988). The bioassays are typically performed *in vitro* over a period of hours to weeks and encompass a wide range of genetic endpoints. In general, short-term assays identify specific genotoxic contaminants or those in complex mixtures of contaminants, provide baseline data for monitoring changes in environmental conditions, and predict potential long range genotoxic health effects (Epler 1980). Genotoxicity assays encompass a broad range of tests that detect damage at the genetic level, while the mutagenicity end-point specifically identifies those changes to the DNA that are heritable. Mutagenicity assays are relatively rapid and inexpensive and are especially useful for establishing priorities for more definitive chemical analysis and validation testing with whole animals (Ashby 1988, Ashby and Tennant 1988; also see Benigni 1990).

In this investigation, part of a series of extensive sediment studies, the Ames/*Salmonella* Test (Maron and Ames 1983) was used to determine the mutagenicity of organic chemical extracts of contaminated sediments from three priority areas: Saginaw River, Indiana Harbor, and Buffalo River.

MATERIALS AND METHODS

SUMMARY OF SAMPLES TESTED

Sediment samples were collected at 29 stations from 1988 to 1990 from the three AOCs. At each AOC, splits of the collected sediment were used to prepare blind replicate samples. For additional details on stations and sample collection, see the section in Chapter 1 dealing with Sediment Collection, Handling, and Storage and the LLRS 1991 Final Report.

Indiana Harbor

Grab samples from each of 7 stations from the first survey of Indiana Harbor were assayed. The stations included: 3, 4, 5, 6, 7, 8, and 10. A replicate sample was prepared from Station 7. See Table 2.1 for complete station identification numbers.

Buffalo River

Grab samples from each of 10 stations from the first survey of the Buffalo River were assayed. The stations included: 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. A replicate sample was prepared from Station 6. See Table 2.1 for complete station identification numbers.

Saginaw River

Saginaw River was sampled twice. Grab samples from each of 7 stations from the first survey of Saginaw River were assayed. The stations included: 2, 3, 4, 6, 7, 9, and 10. A replicate sample was prepared from Station 2. Grab samples from each of 7 stations from the third survey of Saginaw River were assayed. Sampling of two of the initial stations was repeated. The stations included: 1, 2, 5, 6, 8, 16, and 24. Two replicate samples of Station 1 were included. In addition, various depths of core samples from Stations 2, 5, and 6 collected during the third survey were assayed. See Table 2.1 for complete station identification numbers.

SEDIMENT PHYSICAL AND CHEMICAL CHARACTERIZATIONS

Sediment physical characterization included (1) organic carbon, (2) inorganic carbon, (3) percentage water, and (4) particle size. Sediment chemical characterization included (1) metals (Ag, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Se, Zn), (2) organometals (butyltins and methyl mercury), (3) acid volatile sulfide (AVS) and simultaneously extracted metals (SEM), (4) chlorinated pesticides, (5) PCB congeners, (6) polychlorinated dioxins and furans, and (7) select PAHs. For additional details on physical and chemical characterizations, see Chapters 2 and 3.

EXPOSURE PROCEDURES

Disposable gloves, tyvek suits, sleeve protectors, and safety glasses were used for handling potentially genotoxic substances. All toxicological

transfers were conducted in a laminar flow hood under yellow light. Toxicant disposal was pursuant to National Fisheries Contaminant Research Center (NFCRC) Safety Plan.

Methylene chloride extracts of test sediments and a control sediment were prepared by Battelle Northwest as follows: sub-samples of about 100 to 200 g wet sediment were dried with sodium sulfate, triple-extracted with methylene chloride, and concentrated by roto-evaporation to 11 mL (Eric Crecilius, Battelle, Sequim, WA, personal communication). Upon receipt at NFCRC, extracts were stored in amber glass vials with wax septa caps at 4°C until processed.

Processing of methylene chloride extracts included gel permeation chromatography (GPC) followed by transfer into dimethyl sulfoxide (DMSO). This chromatographic system involves a mixture of size exclusion and adsorption to remove interfering materials from the sediment extracts. A chromatographic column was packed with 60 g of Bio-beads[R] SX-3 (200 to 400 mesh) and was wetted with 100 mL of a 50:50 (v:v) mixture of methylene chloride and cyclopentane. Before application of the methylene chloride sediment extracts, the GPC was calibrated with a standard solution containing di(2-ethylhexyl) phthalate (DEHP), Dacthal, and pyrene. The potential interferents (alkanes, phthalate esters, fatty acids and their esters, biogenic pigments, and tannic acids) elute earlier than do the standards. Analytes that are traditionally targeted for mutation assays, such as PCBs, most organochlorine pesticides, phenols, chlorinated dioxins and furans, and neutral and nitrogen-containing PAHs, elute from the gel later than the standards.

Samples collected from GPC were concentrated by roto-evaporation to about 5 mL. Five mL of DMSO was then added and the remaining methylene chloride:cyclopentane was evaporated under a gentle stream of nitrogen. Procedures were conducted under yellow lights to reduce possible photo-degradation. Resulting extracts, representing 5 or 10 g-equivalents dry sediment/mL extract, were kept in the dark at room temperature in 8-mL amber glass vials with teflon cap liners.

The Ames assay procedure generally followed Maron and Ames (1983) for the plate incorporation test with preincubation. Briefly, 100 μL of cultured test strain was combined with 500 μL of either phosphate buffer or activating S9 enzyme mixture. To this, 100 μL of extract or DMSO solvent was added and the entire mixture was incubated at 37°C for 20 to 30 min in a dry block heater. After incubation, 2 mL of top agar containing trace histidine and biotin was added, and the mixture was poured onto agar plates. Plates were incubated in the dark at 37°C and the resulting colonies were counted at 72 h. Plates with fewer than 100 colonies were counted manually; all other plates were mechanically enumerated with an electric counter as described by Johnson (1990).

The experimental design consisted of 3 replicates of 3 nominal concentrations of sediment extract: 5 or 10, 50 or 100 and 500 or 1000 mg-equivalents dry sediment/plate; and 3 concentrations of S9: 0, 1, 3 mg-equivalents protein/plate; tested with 4 strains of Salmonella typhimurium, TA97a, TA98, TA100 and TA102 (Table 6.1). Preparation of reagents and solutions used in the Ames assay was as described by Maron and Ames (1983) except that the 30% S9 mixture (3 mg/plate) contained twice the normal amount of NADP co-factor with a corresponding decrease in water (Gatehouse 1987). A positive mutagenic response was indicated when the number of revertants on

test plates was greater than or equal to 2 times the number of colonies on the DMSO solvent control plate.

In order to discriminate between prototroph (revertant) and auxotroph (non-revertant) colonies, an additional step was included to confirm histidine independence. Plates indicating potential mutagenicity (e.g., $\geq$ 2 times their respective controls) were evaluated by lightly stamping the colonies with a velvet-covered disk and then transferring the colonies to new minimal glucose plates containing biotin but no histidine. If the pattern and number of transferred colonies resembled that on the original plate, the response was scored as mutagenic. When relatively few colonies transferred, the original plates contained primarily auxotrophs resulting from cytotoxicity.

STATISTICAL ANALYSES

Means and standard deviations of the number of colonies/plate were calculated from the three replicate plates. Test results were summarized based on three categories: no effect, cytotoxic, and mutagenic. Plates were scored as "Mutagenic" if mean revertants on extract exposed plates were greater or equal to two times the mean number of spontaneous revertants on solvent control plates. Plates were scored as "Cytotoxic" if mean revertant values on extract exposed plates were less than half the mean spontaneous revertants on solvent control plates, or if cytotoxicity was detected through the confirmation step. "No-effect" describes those plates that were neither identified as cytotoxic nor mutagenic. Means and standard deviations are provided in Appendices 6.1 to 6.13.

QUALITY CONTROL AND QUALITY ASSURANCE

Precision in the Ames Salmonella microsome test was assessed through replication (triplicate minimum), inclusion of splits of sediment samples which were run blind, and by making three counts per plate with an electronic colony counter.

The integrity and sensitivity of the test organisms used in the Ames test was assessed by confirmation of genotypes, measurement of spontaneous reversion frequency, and confirmation of reversion properties of the tester strains using the known mutagens 2,4,7-trinitro-9-fluorenone; 2-aminofluorene; methyl methanesulfonate; and benzo[a]pyrene (Maron and Ames 1983).

Additional quality control included determination of S9 protein content, (~20 mg/ml; Bradford 1976) and EROD activity (~7.05 nmol/mg/min; Pohl and Fouts 1980). A most-probable-number test was used to estimate population densities of Salmonella cell cultures. Procedural blanks (no sediment) from the extraction process were prepared and control samples (extracts from a fine silt- and clay-particle size sediment obtained from local undisturbed agricultural soil (Ingersoll and Nelson 1990) were prepared and assayed along with AOC sediments. See also the Quality Assurance Project Plan for "Assessment and Remediation of Contaminated Sediments (ARCS) Assistance", National Fisheries Contaminant Research Center.

RESULTS

Mutagenicity was detected in both grab and core samples from every station tested. Of the four strains, only TA98 identified 100% of the stations as mutagenic.

INDIANA HARBOR

Without Metabolic Activation

Mutagenicity was detected at Station 7 with strain TA98 (Table 6.2, Appendix 6.1). No effect of the extracts was observed at the lowest dose (5 mg-equiv sediment) at all stations except Stations 8 and 10 with strains TA98 and TA100. Cytotoxicity tended to increase with increasing dose of sediment extract and all but Station 10 samples were cytotoxic at the highest dose.

With Metabolic Activation

Mutagenicity was detected at each Indiana Harbor station with strains TA98 and TA100 at one or more concentrations of sediment extract or S9 (Table 6.3, Appendix 6.2). Strain TA97a detected mutagenicity at every station except 5 and 7, and strain TA102 detected mutagenicity at every station except Station 10.

BUFFALO RIVER

Without Metabolic Activation

A no-effect response was obtained for all Buffalo River stations (except Stations 4 and 6) at the lowest concentration of sediment extract, but all sample extracts were cytotoxic at the highest concentration (Table 6.4, Appendix 6.3). Only Station 3, with strain TA100 at 100 mg-equiv sediment indicated mutagenicity.

With Metabolic Activation

All stations tested with strains TA97a, TA100 and TA102 responded with no effect at the lowest concentration of sediment extract at both concentrations of S9 tested (Table 6.5, Appendix 6.4). No mutagenicity was detected with strain TA102, while either strain TA97a or TA100 indicated mutagenicity at every station except Station 3. All stations were mutagenic with strain TA98 generally at more than one concentration of sediment extract and S9.

SAGINAW RIVER

Without Metabolic Activation

In the first survey samples from Saginaw River, Station 2 was mutagenic with strain TA98 (Table 6.6, Appendix 6.5). Except for Station 2, samples from all stations indicated a no-effect response for all strains at the lowest

extract concentration and a cytotoxic response at the highest concentration. Overall, results from the third survey of Saginaw River were similar (Table 6.7, Appendix 6.6). Station 2, although mutagenic in the first set of samples was not mutagenic in the set of samples from the third survey. A positive mutagenic response was observed for Station 24 with strain TA100.

No-effect and cytotoxicity patterns were generally the same for the core samples as for surficial grab samples from the third survey collection (Table 6.8, Appendix 6.7). Mutagenicity was detected only in one section of core from Station 2 with strain TA100.

With Metabolic Activation

Assay results for all stations from the first survey samples were similar with no mutagenicity evident for strains TA97a or TA102 (Table 6.9, Appendix 6.8). With strain TA100 this pattern varied only at Station 6 which was mutagenic. In contrast, with strain TA98 mutagenicity was detected at each station. Station 6 with strain TA100 was again mutagenic in grab samples from the third survey. Mutagenicity was detected by TA97a at Station 8 in this survey, but not in the first (Table 6.10, Appendix 6.9). Similar to the first survey results, no stations from the third survey were mutagenic with strain TA102. Mutagenicity was not observed at Station 1 with TA98, however as in the first survey results, mutagenicity was detected at all other stations with this strain.

Core samples tested with S9 were mutagenic for all strains but TA102 for at least one section of the core (Table 6.11, Appendix 6.10).

QUALITY CONTROL AND QUALITY ASSURANCE

At the high dose, the control sediment was cytotoxic to most strains with and without metabolic activation (Tables 6.12 and 6.13, Appendices 6.11 and 6.12). Additionally, there were indications of cytotoxicity and mutagenicity at lower doses. Only strain TA98 detected the mutagenicity. The procedural blank (no sediment) was cytotoxic at the highest dose (100 μL of undiluted sample per plate) with no S9. Additions of S9 eliminated the cytotoxic response; mutagenicity was not observed. Results for blind replicate split samples were similar to their complimentary original samples except for the second samples from Saginaw River which were more cytotoxic than the original samples (Tables 6.14 and 6.15, Appendices 6.11 and 6.12).

In each test series, the results of the diagnostic tests confirmed strain genotype and sensitivity (Table 6.16, Appendix 6.13; Maron and Ames 1983). Bacterial culture densities and spontaneous reversion frequencies were within acceptable limits (Maron and Ames 1983). Acceptable reversion frequencies were obtained with each combination of strain and positive mutagen (Maron and Ames 1983). See also the Data Verification Report for Assessment and Remediation of Contaminated Sediment Program, Report Number 4, Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S.E.P.A.

DISCUSSION

Sediments are sinks for many anthropogenic contaminants. As such, organic extracts of contaminated sediments often contain a wide variety of chemicals which makes mutagenicity testing of sediments complex. The individual components of chemical extracts of environmental samples can be both cytotoxic and mutagenic. A predominance of cytotoxic compounds and synergistic interactions among individual compounds may mask the expression of a mutagenic response (Epler 1980, Carver et al. 1985). Consequently, a test may not detect mutagenicity. Class fractionation of samples before testing can reduce problems of cytotoxicity and aid in the identification of mutagenic components (Alfheim et al. 1984). However, individual components of the sample may be modified or lost and synergistic effects may be altered (Epler 1980). Fractionation may also significantly increase the number of samples to be tested.

Due to the cytotoxicity of methylene chloride to the bacterial test strains, the crude sediment extracts could be used only after transfer to an Ames compatible solvent. Initially, a direct transfer to DMSO and other Ames solvents, resulted in separation of samples into two distinct phases- a clear or slightly amber-colored solution with a thicker dark-colored solution on the surface. Since these samples were not homogenous and components could be trapped in the upper layer, the crude methylene chloride extracts were pretreated with gel permeation chromatography (GPC). Gel permeation chromatography is a size exclusion process that removes large biogenic molecules (e.g., lipids, tannins) which can interfere with the complete transfer of material from crude extracts to DMSO.

Optimizing the transfer of complex mixtures containing mutagenic components into DMSO does not ensure a positive response with the Ames assay. Extracts of sediments collected from polluted areas are often quite cytotoxic (Metcalf 1990, Fabacher et al. 1988) and excessive cytotoxicity can prevent the expression of mutagenicity (Epler 1980). Dilution can remove cytotoxicity, but with a concomitant reduction of potentially mutagenic compounds. Dilution is particularly ineffective when the sample is highly cytotoxic and only slightly mutagenic (Maron and Ames 1983).

Cellular microsomal and cytosolic enzymes catalyze the biotransformation of a variety of chemicals. Biotransformation may result in polar metabolites that are biologically inactive and readily excretable, or may produce metabolites that are reactive intermediates that can damage DNA. Exogenous S9 enzyme preparations are routinely used in the Ames assay to more closely approximate the chemical metabolizing abilities of eucaryotes. Maron and Ames (1983) suggest rat liver as the source of S9, but other species (Mueller et al. 1980, Johnson 1990, Blevins 1991) may provide better results depending on the compounds to be tested. The concentration of S9 must also be considered. Again, Maron and Ames (1983) recommends a 4 and 10% S9 mixture with the caveat that it may be necessary to define the optimal concentration for a particular sample.

Coupling extract dilution with S9 and optimizing the S9 concentration, allowed detection of mutagenicity at more stations than would have been possible had the samples only been diluted to non-cytotoxic concentrations then assayed with the standard concentrations of S9. Extracts of sediment samples from every station for the three AOCs were both cytotoxic and

mutagenic to the bacteria. At 7 of 29 stations mutagenicity could not be identified at dilutions sufficient to eliminate cytotoxicity. Diluting to non-cytotoxic concentrations likely co-diluted mutagenic components. At these stations, cytotoxicity reduction by use of S9 enzymes was required to observe mutagenicity. Moreover, for 3 of the 29 stations, an increase in S9 concentration from 1 to 3 mg-equiv. protein per plate was necessary for detection of mutagenicity. This procedure of combining extract dilution with additions of increasing concentrations of S9, identified mutagenicity in the complex contaminant mixtures from each station in the AOCs.

Use of S9 with single compounds allows distinction of classes of mutagens that are direct acting from compounds requiring metabolic activation. It is more difficult to identify these classes of mutagens with complex mixtures (Ball et al. 1990, Pederson and Siak 1981). For the purpose of this study, mutagens were identified as direct-acting when a positive response was observed in the absence of S9. Promutagens were identified when addition of S9 to non-cytotoxic dilutions, or addition of S9 beyond that necessary for elimination of cytotoxicity, resulted in a positive response. Mutagenicity in 2 of the samples could be attributed to promutagens alone. Two other samples contained both promutagens and direct acting mutagens. At the remaining 25 stations, cytotoxicity was not sufficiently reduced through dilution or addition of S9 to completely characterize the classes of mutagens in the sample. For example, the positive response from Indiana Harbor Station 6 with strain TA98 at 5 mg-equiv. sediment, can be attributed to promutagens since there was no observed effect except with metabolic activation (Tables 6.2 and 6.3). If the sample contains direct-acting mutagens, they have been diluted out at this concentration. At 50 mg-equiv. sediment the sample is cytotoxic and upon addition of S9 we observe a mutagenic response. This positive response may be due to reduction of cytotoxicity alone (allowing the expression of direct-acting mutagens), reduction of cytotoxicity coupled with activation of promutagens, or a combination of both.

Two strains detected mutagenicity more frequently. Strain TA98 with S9 detected mutagenicity at every station. Strain TA98 without S9 detected mutagenicity at 3 stations. Of the other 3 strains, positive responses were most commonly observed with TA100. In a comparison by Zeiger et al. (1985), strains TA98 and TA100 positively identified 89% of the mutagenic chemicals in the NTP (National Toxicity Program) database. While strain TA98 detected mutagenicity at no and low concentrations of S9, the other strains generally required high concentrations of S9 for a mutagenic response.

Chemical analysis (Table 6.17; also see Chapter 4) of whole sediment from the three AOCs and the control sediment indicates that as a class, PAHs comprise the greatest percent by weight of the total identified organics. High molecular weight PAHs were more abundant than low molecular weight PAHs. Of the classes of compounds tested, PAHs contain the greatest number of compounds known to be mutagenic in the Ames assay. Within this class, the high molecular weight PAHs are more likely to cause mutation in the Ames assay. It should be noted, however, that many of the other sediment contaminants identified are reported to cause DNA damage in other organisms.

Mutagenicity detected in samples from the AOCs could be primarily attributable to the PAH compounds. Other investigations have identified sediment PAHs as the most likely contaminant causing a positive Ames result (Maccubbin and Ersing 1991, Abe et al. 1989, Grifoll et al. 1988, Odense et al. 1988, Oishi and Takahashi 1987, Sato et al. 1983, Waldron and White 1989).

Crude extracts of freshwater sediments from Hamilton Harbor, Lake Ontario, Canada and marine sediments from Long Island Sound, USA. contained concentrations of high molecular weight PAHs similar to those in the present study and were also mutagenic while control samples were not (Metcalfe et al. 1990, Gardner et al. 1987). Furthermore, PAH contaminated sediment reportedly caused induction of neoplasia in oyster and flounder (Gardner et al. 1991).

A ranking was develped to describe the relative cytotoxicity and mutagenicity among stations and AOCs. A no-effect response at all three doses was given a value of 1; no-effect for the two lowest doses with mutagenicity or cytotoxicity only at the highest dose was given a value of 2; no-effect at only the lowest of the three doses received a value of 3; and mutagenicity or cytotoxicity at all three doses received a value of 4. The values from each of the four strains tested for each sample were summed to obtain a score from 4 (low) to 16 (high) for each station. Figures 6.1 and 6.2 compare the cytotoxicity and mutagenicity of the stations. For each AOC, the procedural blank (no sediment) had a lower score than any of the stations. For Indiana Harbor Station 6 had the highest mutagenicity score and Stations 5, 8, and 10 had the lowest mutagenicity scores. Stations 4 and 6 had the highest scores and Station 3 the lowest for Buffalo River. Saginaw River Stations 1 and 4 received the high and low scores, respectively. No distinct relationship between cytotoxicity and mutagenicity scores is apparent, although the Saginaw station with the highest mutagenicity score (Station 1) also has the highest cytotoxicity score and Station 4 scored lowest for both cytotoxicity and mutagenicity. The range of mutagenicity and cytotoxicity scores and the modes for all stations for each AOC are depicted in Figures 6.3 and 6.4. Mutagenicity scores for Indiana Harbor were distributed over a narrower range than those for Buffalo and Saginaw Rivers. In general, Indiana Harbor stations scored at the upper end of the range of all stations combined while Buffalo and Saginaw River stations scored in the middle to lower end of the range.

The information provided through the use of these station ranks needs to be evaluated with caution. The control sediment is considerably lower in potential types and quantities of mutagenic compounds than the lowest ranking station from the AOCs (Table 6.17). Yet the control sediment out-ranks 7 of the 29 stations for mutagenicity. A test with such a high degree of sensitivity is probably most useful for detecting presence or absence of mutagens and its utility for more detailed discrimination is questionable. The ultimate goal of mutagenicity testing is to identify the carcinogenic and intergenerational effects a compound may have on organisms of concern. Mutagenicity is only one of many genotoxicity end-points that can contribute information towards this goal. Furthermore, detection of a mutation in the Salmonella organisms in the Ames assay is dependent upon optimization of the test parameters. For these reasons, it is generally accepted that a tiered approach using a variety of tests and end-points is most valuable for detecting potential gene and chromosome damage (Heinze and Poulsen 1983).

These studies demonstrate the utility of the Ames assay for screening crude extracts of complex mixtures. By varying S9 concentration and extract dilution, cytotoxicity was controlled so that mutations could be observed. However, due to the assay's sensitivity, it is not able to discriminate among discrete mutagenic responses. Fractionation of samples before testing may allow a greater degree of discrimination and aid in identifying those compounds which are causing the observed mutagenicity. Additionally, the Ames

test as applied in this study does not address the issue of bioavailability of mutagenic compounds in sediments. Development of methods to test elutriates and pore waters and studies utilizing passive membrane bag samplers may be useful for this purpose.

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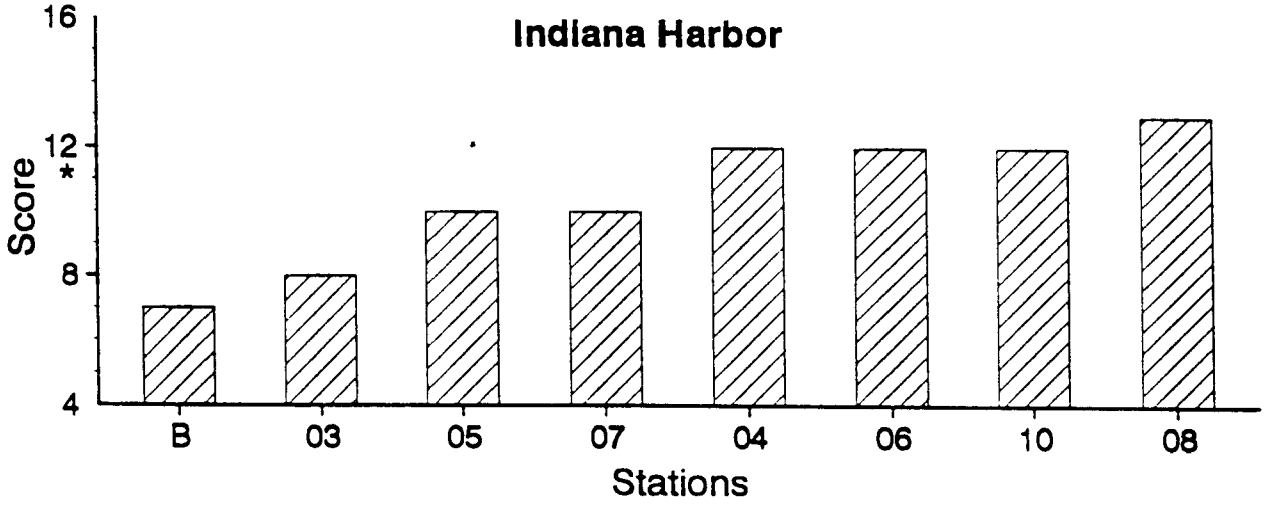
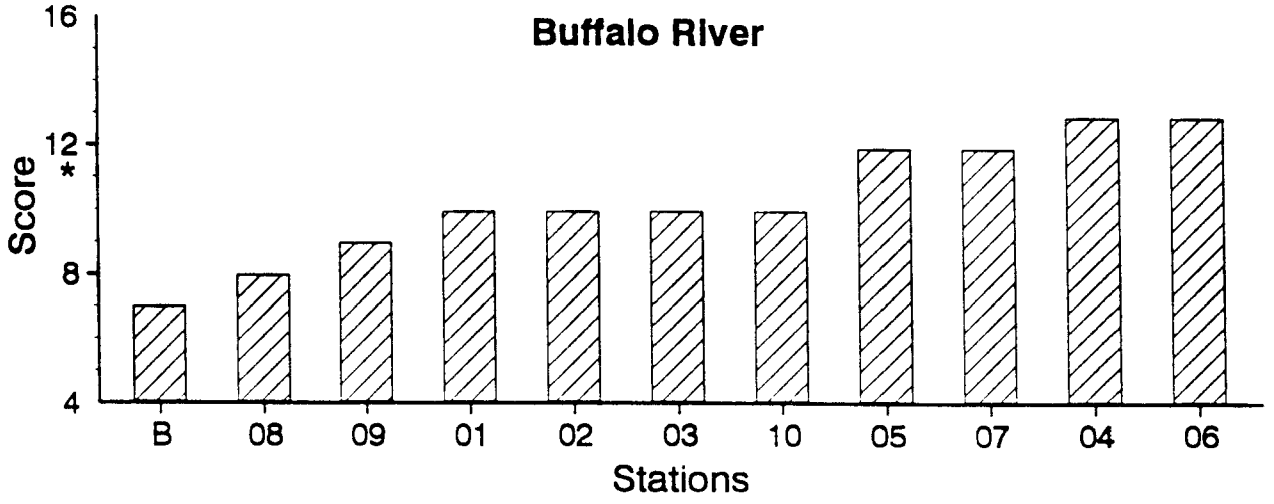
6.1 Toxicity Scores for AOC Stations.
6.2 Mutagenicity Scores for AOC Stations.
6.3 Summary of Toxicity Score Results for AOCs.
6.4 Summary of Mutagenicity Score Results for AOCs.

Figure 6.1

Toxicity





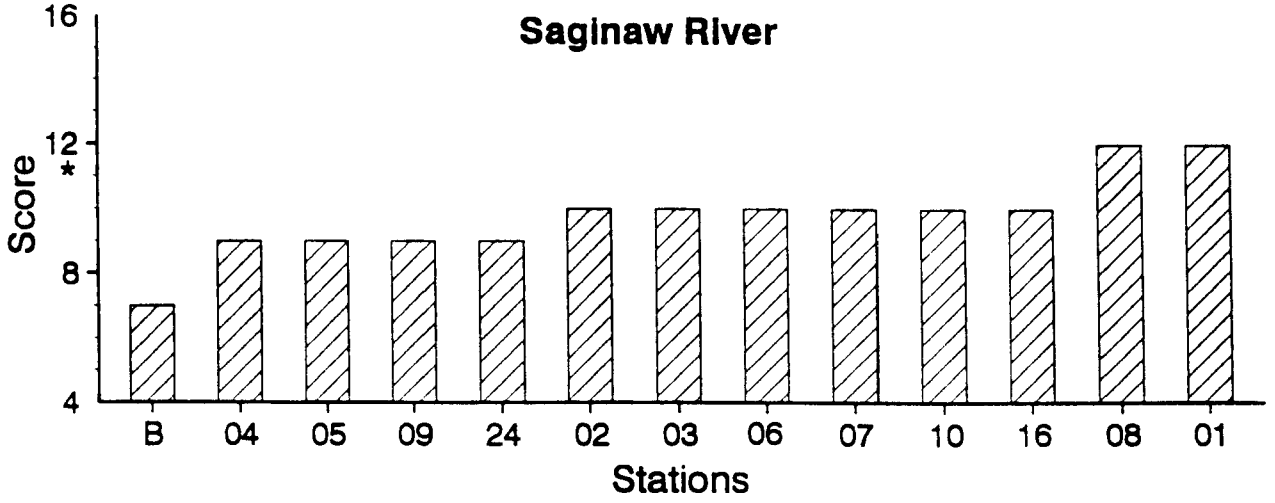
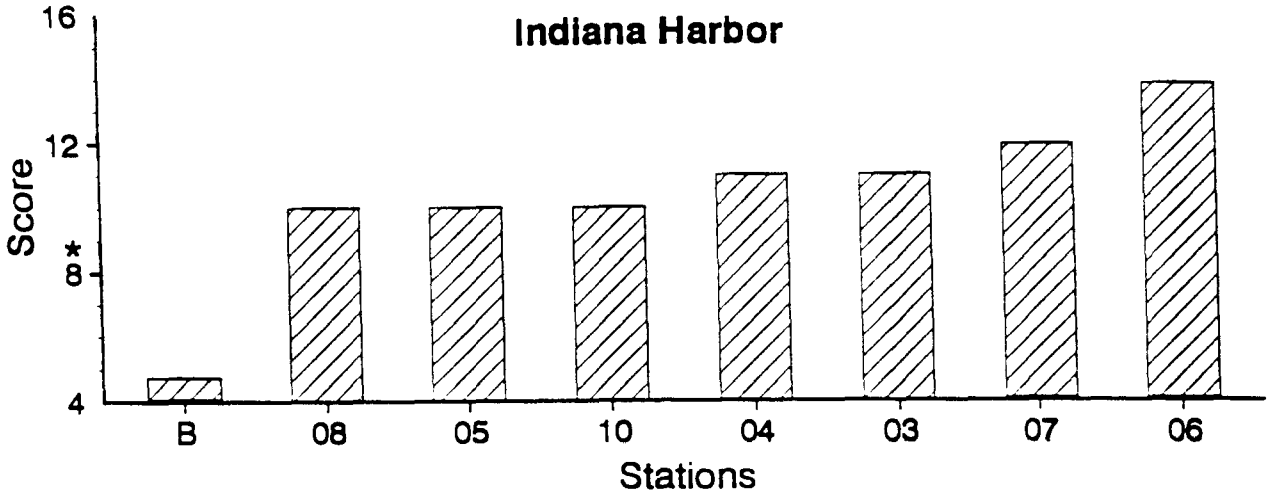
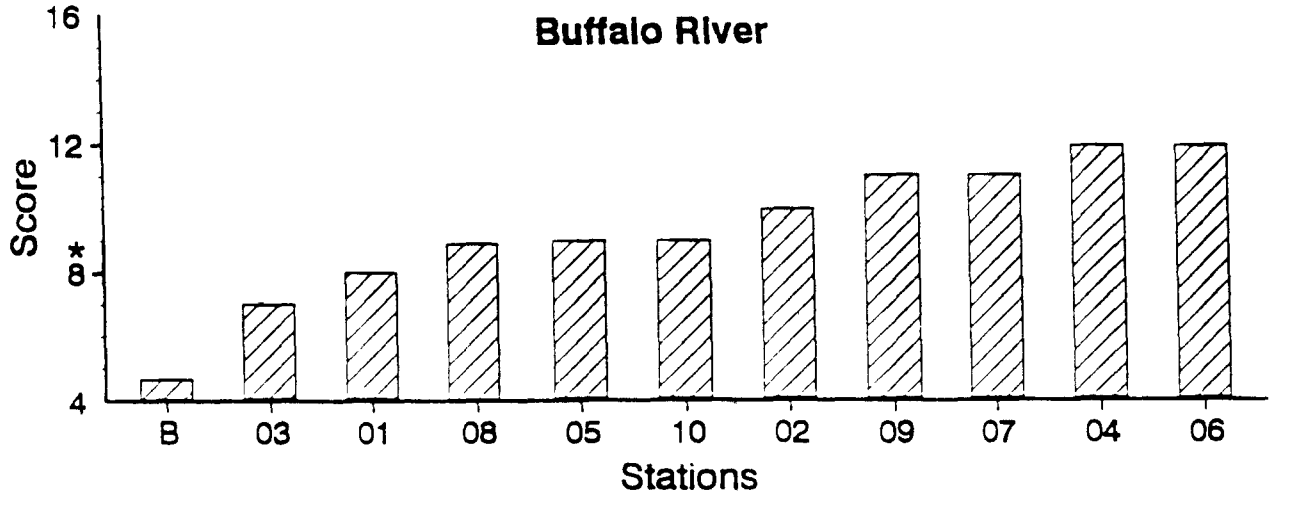


Figure 6.2

Mutagenicity





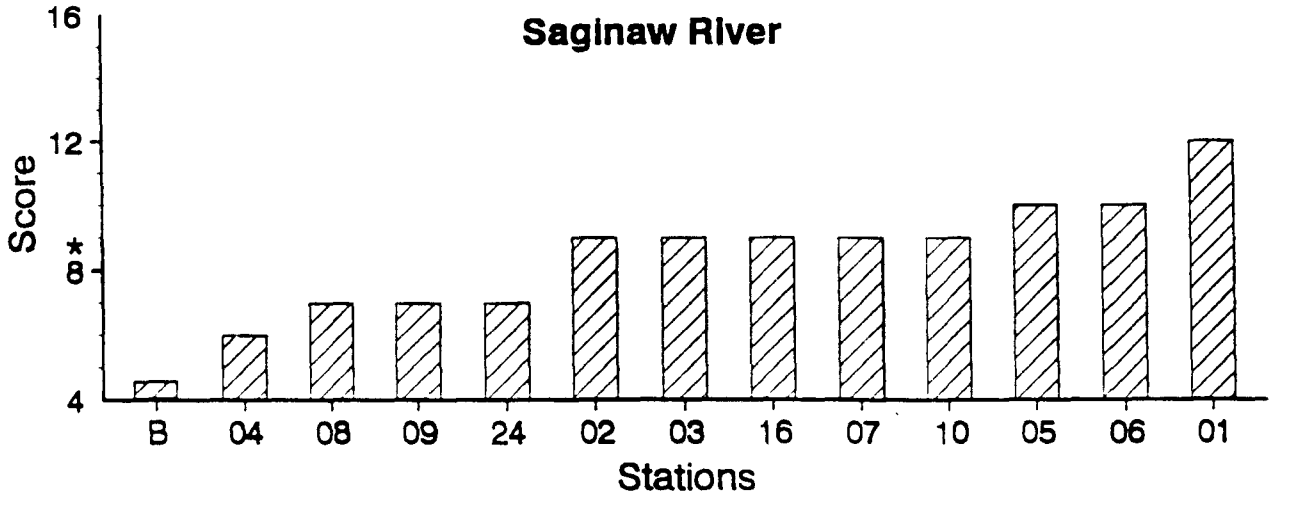


Figure 6.3

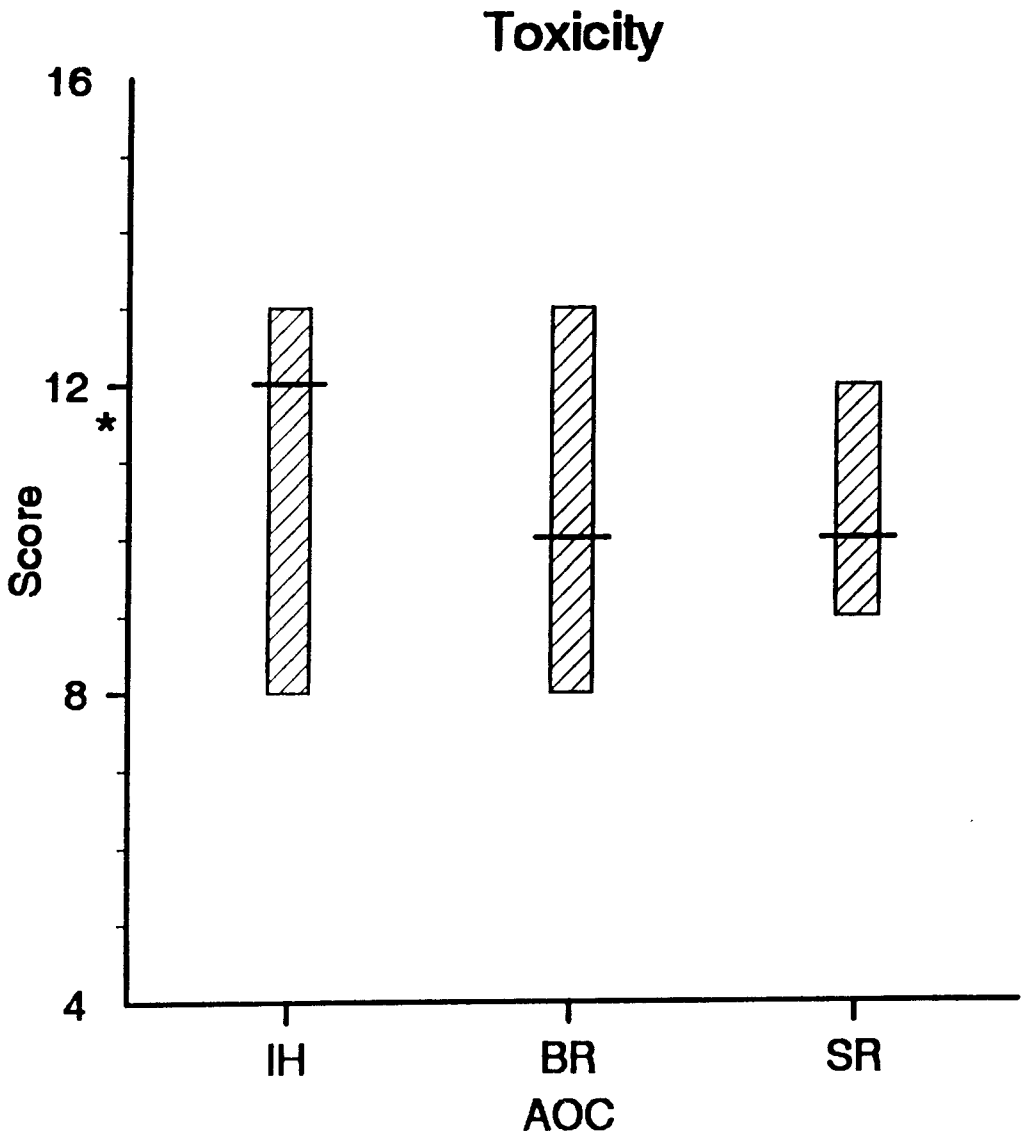


Figure 6.4

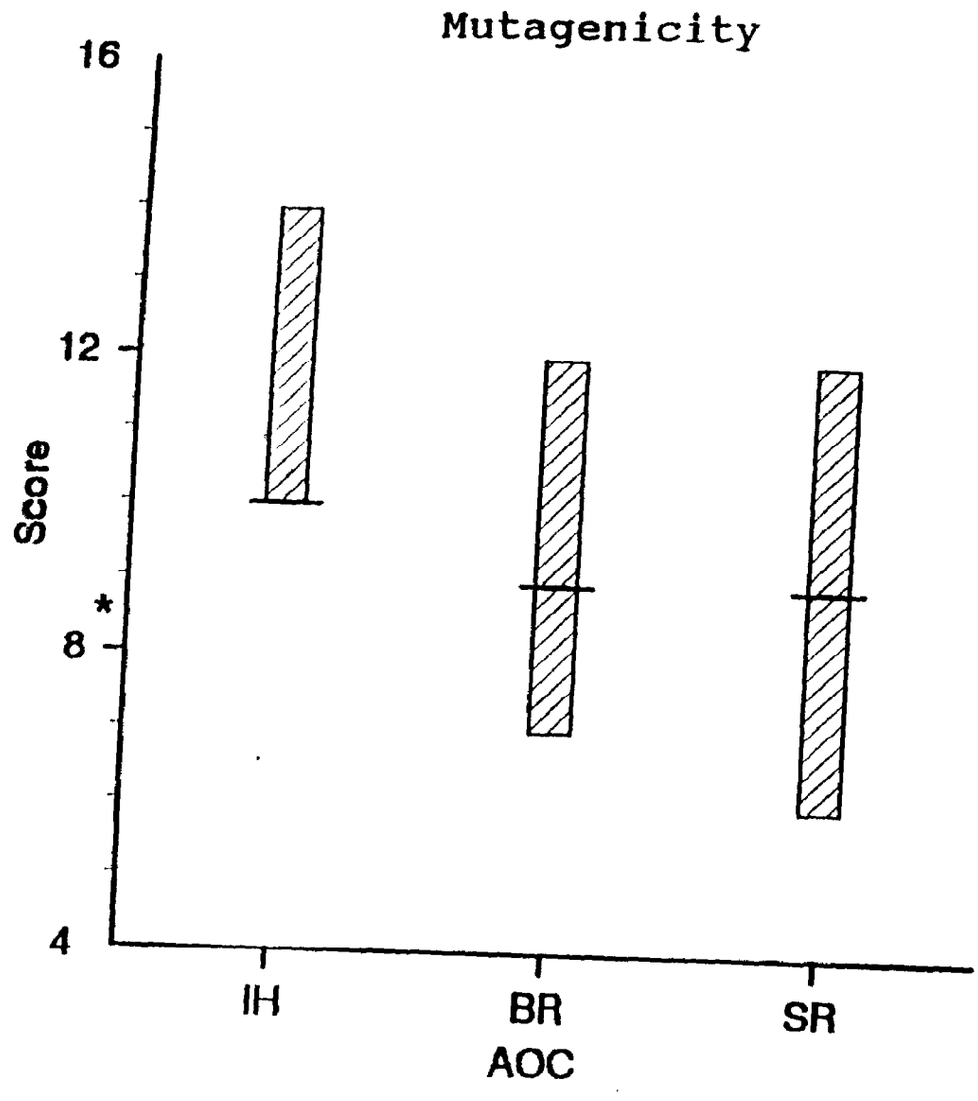


Table 6.1 Ames Assay test strain characteristics (Maron and Ames 1983).

Strain	Mutation			Specificity	Spontaneous Revertants
	rfa[1]	uvrB[2]	HIS[3]		
TA97a	+	-	hisD6610	frameshift	90-180
TA98	+	-	hisD3052	frameshift	30-50
TA100	+	-	hisG46	base-pair	120-200
TA102	+	+	hisG428	excision repair	240-320

[1] causes partial loss of the bacteria's lipopolysaccharide barrier
[2] causes deletion of a gene coding for the DNA excision repair system
[3] mutation at the histidine operon

Table 6.2 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and no S9 for 7 sites in Indiana Harbor. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	Station 3 $\bar{X}$	4 $\bar{X}$	5 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	8 $\bar{X}$	10 $\bar{X}$
TA97a	5	NE	NE	NE	NE	NE	NE	NE
TA97a	50	NE	T	T	T	NE	T	T
TA97a	500	T	T	T	T	T	T	T
TA98	5	NE	NE	NE	NE	NE	T*	T
TA98	50	NE	T	T	T	M	T*	T
TA98	500	T	T	T	T	T	T*	T
TA100	5	NE	NE	NE	NE	NE	NE	T
TA100	50	NE	T	NE	T	T	T	T
TA100	500	T	T	T	T	T	T	T
TA102	5	NE	NE	NE	NE	NE	NE	NE
TA102	50	NE	T	NE	T	T	T	NE
TA102	500	T	T	T	T	T	T	NE

* only 2 replicates

Table 6.3 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 stations in Indiana Harbor. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 3 $\bar{X}$	4 $\bar{X}$	5 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	8 $\bar{X}$	10 $\bar{X}$
TA97a	5	1	NE	NE	NE	NE	NE	NE	NE
TA97a	5	3	NE	NE	NE	NE	NE	NE	NE
TA97a	50	1	M	NE	NE	M	NE	NE	M
TA97a	50	3	NE	NE	NE	NE	NE	NE	NE
TA97a	500	1	M	NE	NE	M	T	M	M
TA97a	500	3	NE	M	NE	NE	T	NE	NE
TA98	5	1	NE	NE	NE	M	NE	M*	NE
TA98	5	3	NE	NE	NE	NE	NE	M*	NE
TA98	50	1	M	NE	M	M	NE	NE*	M
TA98	50	3	M	M	M	M	M	M*	M
TA98	500	1	NE	T	NE	NE	T	NE*	T
TA98	500	3	M	T	M	M	T	NE*	T
TA100	5	1	NE	NE	NE	NE	NE	NE	NE
TA100	5	3	NE	NE	NE	NE	NE	NE	NE
TA100	50	1	M	NE	M	M	NE	NE	NE
TA100	50	3	M	M	M	M	M	NE	M
TA100	500	1	NE	T	NE	M	T	M	NE
TA100	500	3	M	T	NE	M	T	M	NE
TA102	5	1	NE	NE	NE	M	NE	NE	NE
TA102	5	3	M	NE	NE	M	M	M	NE
TA102	50	1	NE	NE	M	M	NE	NE	T
TA102	50	3	M	M	M	M	M	NE	T
TA102	500	1	NE	T	M	T	T	NE	T
TA102	500	3	NE	M	M	M	T	NE	T

* only 2 replicates

Table 6.4 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and no S9 for 10 stations in Buffalo River. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	Station 1 $\bar{X}$	2 $\bar{X}$	3 $\bar{X}$	4 $\bar{X}$	5 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	8 $\bar{X}$	9 $\bar{X}$	10 $\bar{X}$
TA 97a	10	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA 97a	100	T	T	T	T*	T	T	T	NE	NE	T
TA 97a	1000	T	T	T	T	T	T	T	T	T	T
TA 98	10	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA 98	100	T	T	T	T	T	T	T	NE	T	T
TA 98	1000	T	T	T	T	T	T	T	T	T	T
TA 100	10	NE	NE	NE	T	NE	T	NE	NE	NE	NE
TA 100	100	NE	NE	M	T	T	T	T	NE	NE	T
TA 100	1000	T	T	T	T	T*	T	T	T	T	T
TA 102	10	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA 102	100	NE	NE	NE	T	T	T	T	NE	NE	NE
TA 102	1000	T	T	T	T	T	T	T	T	T	T

* only 2 replicates

Table 6.5 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 10 stations in Buffalo River. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Stations 1 $\bar{X}$	2 $\bar{X}$	3 $\bar{X}$	4 $\bar{X}$	5 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	8 $\bar{X}$	9 $\bar{X}$	10 $\bar{X}$
TA97a	10	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA97a	10	3	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA97a	100	1	NE	NE	NE	M	NE	M	M	M	M	NE
TA97a	100	3	NE	M	NE	M	NE	M	NE	NE	NE	NE
TA97a	1000	1	M	T	T	T	NE	T	T	T	T	T
TA97a	1000	3	M	NE	NE	T	NE	T	T	M	T	T
TA98	10	1	NE	NE	NE	M	M	M	M	NE	NE	NE
TA98	10	3	NE	NE	NE	NE	NE[1]	NE	M	NE	T	NE
TA98	100	1	M	M	M	M	M	M	M	M	M	M
TA98	100	3	M	M	M	M	M	M	M	M	M	M
TA98	1000	1	M	T	T	T	T	T	T	T	T	T
TA98	1000	3	M	T	M	T	T	T	T	M	T	T
TA100	10	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA100	10	3	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA100	100	1	NE	M	NE	NE	NE	NE	NE	NE	NE	NE
TA100	100	3	NE	M	NE	M	M	M	NE	NE	NE	NE
TA100	1000	1	T	T	T	T	T	T	T	NE	T	T
TA100	1000	3	M	T	T	T	NE	NE	T	M	T	T
TA102	10	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA102	10	3	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA102	100	1	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA102	100	3	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
TA102	1000	1	NE	NE	NE	T	NE	T	T	T	T	T
TA102	1000	3	NE	NE	NE	T	T	T	T	NE	T	T

[1] only 2 replicates

Table 6.6 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and no S9 for 7 stations from the first survey (grab) of Saginaw River. "T" = toxicity, "NE" = no-effect, "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	Station 2 $\bar{X}$	3 $\bar{X}$	4 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	9 $\bar{X}$	10 $\bar{X}$
TA97a	10	NE	NE	NE	NE	NE	NE	NE
TA97a	100	NE	T	NE	T	NE	NE	NE
TA97a	1000	T	T	T	T	T	T	T
TA98	10	M	NE	NE	NE	NE	NE	NE
TA98	100	T	NE	T	T	T	NE	T
TA98	1000	T	T	T	T	T	T	T
TA100	10	NE	NE	NE	NE	NE	NE	NE
TA100	100	T	T	NE	NE	T	T	T
TA100	1000	T	T	T	T	T	T	T
TA102	10	NE	NE	NE	NE	NE	NE	NE
TA102	100	NE	NE	NE	NE	NE	NE	NE
TA102	1000	T	T	T	T	T	T	T

Table 6.7 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and no S9 for 7 stations the third survey (grab) of Saginaw River. "T" = toxicity, "NE" = no-effect, "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	Station 1 $\bar{X}$	2 $\bar{X}$	5 $\bar{X}$	6 $\bar{X}$	8 $\bar{X}$	16 $\bar{X}$	24 $\bar{X}$
TA97a	10	NE	NE	NE	NE	T	NE	NE
TA97a	100	T	T	NE	NE	T	T	T
TA97a	1000	T	T	T	T	T	T	T
TA98	10	NE	NE	NE	NE	NE	NE	NE
TA98	100	T	T	T	NE	T	T	T
TA98	1000	T	NE	T	T	T	T	T
TA100	10	NE	NE	NE	NE	NE	NE	NE
TA100	100	T	NE	NE	NE	T	NE	NE
TA100	1000	T	T	T	T	T	T	M
TA102	10	NE	NE	NE	NE	NE	NE	NE
TA102	100	T	NE	NE	NE	NE	NE	NE
TA102	1000	T	T	T	NE	T	T	T

Table 6.8 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 8 core samples from the third survey of Saginaw River. Toxicity determined by visual inspection or replicate plating is denoted with a "T".

Strain	Dose (mg-equiv. sediment)	Station 5[1] $\bar{X}$	5[2] $\bar{X}$	6[1] $\bar{X}$	6[2] $\bar{X}$	2[3] $\bar{X}$	2[4] $\bar{X}$	6[5] $\bar{X}$	6[6] $\bar{X}$
TA97a	10	NE	NE	NE	NE	NE	NE	NE	NE
TA97a	100	NE	T	T	NE	T	NE	T	T
TA97a	1000	T	T	T	T	T	T	T	T
TA98	10	NE	NE	NE	NE	NE	NE	NE	NE
TA98	100	T	T	T	NE	T	NE	T	T
TA98	1000	T	T	T	T	T	T	T	T
TA100	10	NE	NE	NE	NE	NE	NE	NE	NE
TA100	100	NE	T	T	NE	T	NE	T	T
TA100	1000	T	T	T	T	T	M	T	T
TA102	10	NE	NE	NE	NE	NE	NE	NE	T
TA102	100	NE	T	T	NE	NE	NE	NE	T
TA102	1000	T	T	T	T	T	T	T	T

[1] core from 0 - 2 feet deep
[2] core from 2 - 4 feet deep
[3] core from 8 - 20 inches deep
[4] core from 20 - 35 inches deep
[5] core from 5 - 13 inches deep
[6] core from 13 - 32 inches deep

Table 6.9 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 stations from first survey (grab) of Saginaw River. "T" = toxicity, "NE" = no-effect, "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 2 $\bar{X}$	3 $\bar{X}$	4 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	9 $\bar{X}$	10 $\bar{X}$
TA97a	10	1	NE	NE	NE	NE	NE	NE	NE
TA97a	10	3	NE	NE	NE	NE	NE	NE	NE
TA97a	100	1	NE	NE	NE	NE	NE	NE	NE
TA97a	100	3	NE	NE	NE	NE	NE	NE	NE
TA97a	1000	1	T	T	T	T	T	T	T
TA97a	1000	3	T	T	NE	T	T	NE	T
TA98	10	1	NE	NE	NE	NE	NE	NE	NE
TA98	10	3	NE	NE	NE	NE	NE	NE	NE
TA98	100	1	M	M	NE	M	M	M	M
TA98	100	3	M	M	M	M	M	M	M
TA98	1000	1	T	T	NE	T	T	T	T
TA98	1000	3	T	T	M	T	T	M	T
TA100	10	1	NE	NE	NE	NE	NE	NE	NE
TA100	10	3	NE	NE	NE	NE	NE	NE	NE
TA100	100	1	NE	NE	NE	NE	NE	NE	NE
TA100	100	3	NE	NE	NE	M	NE	NE	NE
TA100	1000	1	T	T	NE	T	T	NE	T
TA100	1000	3	T	T	NE	T	T	NE	T

Table 6.9 Continued.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 2 $\bar{X}$	3 $\bar{X}$	4 $\bar{X}$	6 $\bar{X}$	7 $\bar{X}$	9 $\bar{X}$	10 $\bar{X}$
TA102	10	1	NE	NE	NE	NE	NE	NE	NE
TA102	10	3	NE	NE	NE	NE	NE	NE	NE
TA102	100	1	NE	NE	NE	NE	NE	NE	NE
TA102	100	3	NE	NE	NE	NE	NE	NE	NE
TA102	1000	1	T	T	T	T	T	T	T
TA102	1000	3	NE	T	NE	T	T	T	T

Table 6.10 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 stations from third survey (grab) Saginaw River. "T" = toxicity, "NE" = no-effect, "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 1 $\bar{X}$	2 $\bar{X}$	5 $\bar{X}$	6 $\bar{X}$	8 $\bar{X}$	16 $\bar{X}$	24 $\bar{X}$
TA97a	10	1	NE	T	NE	NE	NE	NE	NE
TA97a	10	3	NE	NE	NE	NE	NE	NE	NE
TA97a	100	1	T	NE	NE	NE	NE	NE	NE
TA97a	100	3	T	NE	NE	NE	NE	NE	NE
TA97a	1000	1	T	T	T	M	NE	T	T
TA97a	1000	3	T	T	T	NE	NE	NE	NE
TA98	10	1	NE	NE	NE	NE	NE	NE	NE
TA98	10	3	NE	NE	M	NE	M	M	NE
TA98	100	1	T	NE	M	NE	NE	M	M
TA98	100	3	T	NE	M	NE	M	M	NE
TA98	1000	1	T	M	T	M	T	T	T
TA98	1000	3	T	M	T	M	M	M	M
TA100	10	1	NE	NE	NE	NE	NE	NE	NE
TA100	10	3	NE	NE	NE	NE	NE	NE	NE
TA100	100	1	T	T	NE	NE	NE	NE	NE
TA100	100	3	T	T	NE	NE	NE	NE	NE
TA100	1000	1	T	T	T	NE	NE	T	NE
TA100	1000	3	T	T	T	NE	NE	T	M

Table 6.11 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 8 core samples from third survey of Saginaw River. Toxicity determined by visual inspection or replicated plating is denoted with a "T".

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 5[1] $\bar{X}$	5[2] $\bar{X}$	6[1] $\bar{X}$	6[2] $\bar{X}$	2[3] $\bar{X}$	2[4] $\bar{X}$	6[5] $\bar{X}$	6[6] $\bar{X}$
TA97a	10	1	NE	NE	NE	T	NE	NE	NE	NE
TA97a	10	3	NE	NE	NE	T	NE	NE	NE	NE
TA97a	100	1	NE	NE	NE	T	NE	NE	NE	NE
TA97a	100	3	M	NE	NE	T	NE	NE	NE	M
TA97a	1000	1	T	T	T	T	NE	M	T	T
TA97a	1000	3	NE	T	T	T	NE	M	T	T
TA98	10	1	M	NE	NE	NE	NE	NE	NE	NE
TA98	10	3	M	NE	NE	NE	NE	NE	NE	NE
TA98	100	1	M	M	M	M	M	NE	NE	M
TA98	100	3	M	M	M	M	M	NE	NE	M
TA98	1000	1	T	T	T	T	NE	M	T	T
TA98	1000	3	M	T	T	T	M	M	T	T

Table 6.11 Continued.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 5[1] $\bar{X}$	5[2] $\bar{X}$	6[1] $\bar{X}$	6[2] $\bar{X}$	2[3] $\bar{X}$	2[4] $\bar{X}$	6[5] $\bar{X}$	6[6] $\bar{X}$
TA100	10	1	NE	NE	NE	NE	NE	NE	NE	NE
TA100	10	3	NE	NE	NE	NE	NE	NE	NE	NE
TA100	100	1	NE	NE	NE	NE	NE	NE	NE	NE
TA100	100	3	M	NE	NE	NE	NE	NE	NE	M
TA100	1000	1	NE	T	T	T	NE	NE	T	T
TA100	1000	3	M	T	T	T	NE	M	T	T
TA102	10	1	NE	NE	NE	NE	NE	NE	NE	NE
TA102	10	3	NE	NE	NE	NE	NE	NE	NE	NE
TA102	100	1	NE	NE	NE	NE	NE	NE	NE	NE
TA102	100	3	NE	NE	NE	NE	NE	NE	NE	NE
TA102	1000	1	T	T	T	T	NE	NE	T	T
TA102	1000	3	NE	T	T	T	NE	M	T	T

[1] core from 0 - 2 feet deep
[2] core from 2 - 4 feet deep
[3] core from 8 - 20 inches deep
[4] core from 20 - 35 inches deep
[5] core from 5 - 13 inches deep
[6] core from 13 - 32 inches deep

Table 6.12 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and no S9 for 4 Quality Assurance and Quality Control samples. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	Sample			
		BR-CON	SR-CON	IH-BLANK[a]	SR-BLANK[a]
		$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$
TA97a	10	NE	T	NE	NE
TA97a	100	T	T	NE	NE
TA97a	1000	T	T	T	T
TA98	10	NE	T	NE	NE
TA98	100	T	NE	NE	NE
TA98	1000	T	T	T	T
TA100	10	NE	NE	NE	NE
TA100	100	T	NE	NE	NE
TA100	1000	T	T	T	NE
TA102	10	NE	NE	NE	NE
TA102	100	NE	NE	NE	NE
TA102	1000	T	T	T	NE

[a] Procedural blanks do not contain sediment.

Table 6.13 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 4 Quality Assurance Quality Control samples. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Sample			
			BR-CON	SR-CON	IH-BLANK[a]	SR-BLANK[a]
			$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$
TA97a	10	1	NE	NE	NE	NE
TA97a	10	3	NE	NE	NE	NE
TA97a	100	1	NE	NE	NE	NE
TA97a	100	3	NE	NE	NE	NE
TA97a	1000	1	T	NE	NE	NE
TA97a	1000	3	T	NE	NE	NE
TA98	10	1	NE	NE	NE	NE
TA98	10	3	NE	M	NE	NE
TA98	100	1	NE	M	NE	NE
TA98	100	3	NE	M	NE	NE
TA98	1000	1	T	T	NE	NE
TA98	1000	3	T	T	NE	NE
TA100	10	1	NE	NE	NE	NE
TA100	10	3	NE	NE	NE	NE
TA100	100	1	NE	NE	NE	NE
TA100	100	3	NE	NE	NE	NE
TA100	1000	1	T	T	T	NE
TA100	1000	3	T	T	NE	NE

Table 6.13 Continued

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Sample			
			BR-CON	SR-CON	IH-BLANK	SR-BLANK
			$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$
TA102	10	1	NE	NE	NE	NE
TA102	10	3	NE	NE	NE	NE
TA102	100	1	NE	NE	NE	NE
TA102	100	3	NE	NE	NE	NE
TA102	1000	1	T	T	NE	NE
TA102	1000	3	T	T	NE	NE

[a] Procedural blanks do not contain sediment.

Table 6.14 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and no S9 for replicate samples. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	Sample				
		IH-REP[d]	BR-REP[e]	SR1-REP[f]	SR2-REP1[g]	SR2-REP2[g]
		$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$	$\bar{X}$
TA97a	10[a]	NE	NE	NE	T	NE
TA97a	100[b]	NE	NE	T	T	NE
TA97a	1000[c]	T	T	T	T	T
TA98	10[a]	NE	NE	NE	NE	NE
TA98	100[b]	T	M	T	NE	NE
TA98	1000[c]	T	T	T	T	T
TA100	10[a]	T	NE	NE	NE	NE
TA100	100[b]	T	T	T	NE	NE
TA100	1000[c]	T	T	T	T	T
TA102	10[a]	NE	NE	NE	NE	NE
TA102	100[b]	T	NE	NE	NE	NE
TA102	1000[c]	T	T	T	T	T

[a] For sample IH-REP dose, is 5 mg-equiv. sediment
[b] For sample IH-REP dose, is 50 mg-equiv. sediment
[c] For sample IH-REP dose, is 500 mg-equiv. sediment
[d] Blind duplicate of IH-7
[e] Blind duplicate of BR-6
[f] Blind duplicate of SR-2 (first survey)
[g] Blind duplicate of SR-1 (third survey)

Table 6.15 Results of Ames assay (n=3) for 4 strains, 3 doses of extract, and 2 concentrations of S9 for replicate samples. "T" = toxicity, "NE" = no-effect, and "M" = mutagenicity.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Sample: IH-REP[d] $\bar{X}$	BR-REP[e] $\bar{X}$	SR1-REP[f] $\bar{X}$	SR2-REP1[g] $\bar{X}$	SR2-REP2[g] $\bar{X}$
TA97a	10[a]	1	M	NE	NE	NE	NE
TA97a	10[a]	3	NE	NE	NE	NE	NE
TA97a	100[b]	1	M	NE	M	NE	NE
TA97a	100[b]	3	M	NE	NE	NE	NE
TA97a	1000[c]	1	T	T	T	NE	NE
TA97a	1000[c]	3	T	T	T	NE	NE
TA98	10[a]	1	M	NE	NE	NE	NE
TA98	10[a]	3	M	NE	NE	NE	NE
TA98	100[b]	1	M	M	M	NE	NE
TA98	100[b]	3	M	M	M	NE	M
TA98	1000[c]	1	T	T	T	M	T
TA98	1000[c]	3	T	M	T	M	T
TA100	10[a]	1	NE	NE	NE	NE	NE
TA100	10[a]	3	NE	NE	NE	NE	NE
TA100	100[b]	1	M	NE	NE	NE	NE
TA100	100[b]	3	M	NE	M	NE	NE
TA100	1000[c]	1	T	NE	T	NE	NE
TA100	1000[c]	3	T	NE	T	NE	NE
TA102	10[a]	1	NE	NE	NE	NE	NE
TA102	10[a]	3	NE	NE	NE	NE	NE
TA102	100[b]	1	NE	NE	NE	NE	NE
TA102	100[b]	3	NE	NE	NE	T	NE
TA102	1000[c]	1	T	T	T	NE	NE
TA102	1000[c]	3	NE	T	T	T	NE

[a] For sample IH-REP, dose is 5 mg-equiv. sediment
[b] For sample H-REP, dose is 50 mg-equiv. sediment
[c] For sample IH-REP, dose is 500 mg-equiv. sediment
[d] Blind duplicate of IH-7
[e] Blind duplicate of BR-6
[f] Blind duplicate of SR-2 (first survey)
[g] Blind duplicate of SR-1 (third survey)

Table 6.16 Summary of results of Quality Assurance Quality Control tests for Ames Assay.

Strain	Solvent Control[1]	Positive Control[1,2]	Positive Control[1,3]	Density Estimate[1]	Crystal Violet	Ampicillin	Tetracycline	Completeness[6]
97a	173 (80-325)	521 (216-996)	926 (545-1496)	6.3×10^9 (0.1-16)	NG[4]	G[5]	NG	100%
98	32 (9-65)	1828 (323-2421)	1641 (955-2353)	5.2×10^9 (0.3-9.2)	NG	G	NG	100%
100	170 (84-283)	846 (436-1394)	551 (335-990)	3.0×10^9 (0.3-5.4)	NG	G	NG	100%
102	357 (166-531)	1669 (661-2559)	465 (295-662)	8.8×10^9 (0.3-16)	NG	G	G	100%

[1] Mean for all AOCs and range of means for individual AOCs (in parentheses). Test acceptable at ± 2 S.D.
[2] Positive control is direct-acting.
[3] Positive control requires activation.
[4] NG = no growth
[5] G = growth
[6] 7 samples from Indiana Harbor plus rep and blank samples; 10 samples from Buffalo River plus rep and control sediment samples; 13 grab samples and 7 core samples from Saginaw River plus rep, blank and control sediment samples.

Table 6.17 Summary of chemistry data for grab samples from the AOCs and the control sediment with literature reported results of Ames assay with individual chemicals.

	Buffalo River (n=10) range	Indiana Harbor (n=7) range	Saginaw River (n=12) range	Control Sediment (n=2) range	Reported Ames assay Mutagen-icity	Notes	Citation
Pesticides (ug/kg)							
Aldrin	N.D.	N.D. - 330	N.D. - 360	N.D.	-	1	NIOSH (1992)
(Alpha) Hexachlorocyclohexane	N.D.	N.D.	N.D. - 390	N.D.		1,2	USPHS (1989)
(Beta) Hexachlorocyclohexane	N.D. - 150	N.D. - 290	N.D.	N.D.		1,2	USPHS (1989)
(c) Hexachlorocyclohexane	N.D.	N.D.	N.D.	N.D.		1,2	USPHS (1989)
(Gamma) Chlordane	N.D.	N.D. - 170	N.D. - 140	N.D.	-	1	NIOSH (1992)
(Alpha) Chlordane	N.D.	N.D.	N.D.	N.D.	-	1	NIOSH (1992)
4,4 DDD	N.D. - 75	N.D. - 70	N.D. - 13	N.D.		1,3	NIOSH (1992)
4,4, DDE	N.D.	N.D. - 210	N.D. - 140	N.D.	-	1	NIOSH (1992)
4,4, DDT	N.D. - 100	N.D. - 100	N.D. - 180	N.D.	-	1	NIOSH (1992)
Dieldrin	N.D. - 120	N.D. - 343	N.D. - 96	N.D.	-	1	NIOSH (1992)
Endo Sulfan I	N.D.	N.D. - 41	N.D. - 12	N.D.		3	
Endo Sulfan II	N.D. - 80	N.D. - 160	N.D. - 130	N.D.		3	
Endo Sulfan sulfate	N.D.	N.D.	N.D.	N.D.		3	
Endrin	N.D.	N.D. - 44	N.D. - 45	N.D.	-	1	NIOSH (1992)
Endrin Aldehyde	N.D. - 60	N.D. - 65	N.D. - 6	N.D.		3	
Heptachlor	N.D.	N.D. - 320	N.D. - 140	N.D.		3	NIOSH (1992)
Heptachlor expoxide	N.D. - 30	N.D. - 320	N.D. - 190	N.D.		2	NIOSH (1992)
Lindane	N.D.	N.D.	N.D. - 17			3	
Toxaphene	N.D.	N.D.	N.D.	N.D.	+	1, (a)	NIOSH (1992)
Methoxychlor	N.D.	N.D.	N.D.	N.D.	-	1, (a)	NIOSH (1992)
Endrin ketone	N.D.	N.D. - 51	N.D. - 16			3	
PAHs							
Low Molecular Wt PAHs (ug/kg)							
1,4 Dichlorbenzene	<36 -810	31 - 930	<4 - 130		-	1	NIOSH (1992)
Napthalene	<57 - 20000	3600 - 24000	<6 - 55	13.5	-	(a)	NIOSH (1992)
2-Methylnaphthalene	<67 - 2400	930 - 21000	<6 - 63	N.D. - 3	+	1	NIOSH (1992)
Dimethyl pthalate	<86	<110	N.D. - 110		+	1	NIOSH (1992)
Dibenzofuran	<71 - 1600	930 - 26500	<7 - 38			1,3	NIOSH (1992)
Fluorene	<71 - 3400	790 - 30500	<7 -69	N.D.	-	1	NIOSH (1992)
Phenanthrene	<36 - 10000	3400 - 151500	N.D. - 1000	<10	-	1	NIOSH (1992)
Anthracene	<34 - 4300	1400 - 215000	<8 - 70	N.D.	-	1 (b)	NIOSH (1992)
High Molecular Wt PAHs (ug/kg)							
Fluoranthene	<55 - 7500	4800 - 80000	N.D. - 1200	7 - 10	+	1	NIOSH (1992)
Pyrene	<68 - 6700	5500 - 46500	N.D. - 1800	29	-	1 (a)	NIOSH (1992)
Butyl Benzie Phthalate	<180 - 15000	<240 - 16000	<18 - 3000		-		NIOSH (1992)
Benzo (a) anthracene	<21 - 3500	4200 - 32000	N.D. - 690	N.D.	+	1	NIOSH (1992)
Bis (2-Ethylhexyl) phthalate	880 - 59000	3800 - 290000	170 - 13000		+	1	NIOSH (1992)
Chrysene	<27 - 4000	5200 - 33000	N.D. - 600	4 - 5	+	1	NIOSH (1992)
Di-n-octypthalate	<84 - 38000	<240 - 37000	<19 - 2200		+		NIOSH (1992)
Benzo-b-fluoranthene	<30 - 7000	5600 - 26000	<7 -600	8 - 9	+	1	NIOSH (1992)
Benzo-k-fluoranthene	<41 - 9500	4200 - 23000	<9 - 400	N.D.	+		NIOSH (1992)
Benzo(a)pyrene	<27 - 5800	5700 - 31000	<6 -440	N.D.- 2	+	1	NIOSH (1992)
Indeno 123 CD Pyrene	<100 - 3800	5300 - 22000	<10 - 220	2	+	1	NIOSH (1992)
Benzo (ghi) perlyene	<130 - 3800	6300 - 31000	<13 - 310	2		2	NIOSH (1992)
Dioxins (ng/kg)					-		Schoeny (1982)
TCDD	N.D. - 12	32 - 230	6 - 230				
PECDD	N.D. - 7.4	N.D. - 950	N.D. - 150				
HXCDD	N.D. - 190	350 - 8700	33 - 460				
HPCDD	12 - 2000	980 - 22000	74 - 2100				
OCDD	53 - 12000	2300 - 46000	340 - 26800				
Furans (ng/kg)					-		Schoeny (1982)
TCDF	N.D - 1.5	170 - 3700	230 - 22000				
PECDF	N.D. - 83	76 - 1350	120 - 8700				
HXCDF	N.D. - 110	220 - 3600	200 - 2100				
HPCDF	3 - 640	380 - 8200	120 - 4000				
OCDF	<3.9 - 780	250 - 17300	89 - 2800				
PCB (ug/kg)					-		Schoeny (1982)
PCB 1016	N.D.	N.D.	N.D.				
PCB 1221	N.D.	N.D.	N.D.				
PCB 1232	N.D.	N.D.	N.D.				
PCB 1242	N.D.	3000 - 43000	N.D. - 60000	N.D.			
PCB 1248	N.D.	N.D.	N.D.	N.D.			
PCB 1254	N.D. - 1000	N.D. - 4450	N.D. - 7900	N.D.			
PCB 1269	N.D.	N.D.	N.D. - 300				

Notes: (a), Florissant data from NFCRC historical records not Battelle
1, demonstrated mutagenicity in tests other than Ames Assay.
2, data inconclusive
3, information not available

Appendix 6.1 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 7 stations in Indiana Harbor. Suspected toxicity is denoted by a "*", confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	Station 3		4		5		6		7		8		10	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**135**	**7**	**135**	**7**	**135**	**7**	**135**	**7**	**135**	**7**	**135**	**7**	**91**	**16**
TA97a	5	140	25	143	2	135	4	141	11	134	20	164	6	65	10
TA97a	50	135	3	4	5	171*	58	328*	117	126	25	430*	91	42*	4
TA97a	500	242*	132	0	-	0	-	0	-	0	-	463*	19	8	4
spont. revertants		**17**	**1**	**17**	**1**	**17**	**1**	**17**	**1**	**17**	**1**	**31**[1]	**-**	**32**	**11**
TA98	5	29	8	26	8	23	6	22	2	19	3	6[1]	-	11*	4
TA98	50	15	7	1	2	295*	59	458*	26	109	3	0	-	0	-
TA98	500	215	172	0	-	0	-	1	2	0	-	0	-	0	-
spont. revertants		**124**	**19**	**124**	**19**	**124**	**19**	**124**	**19**	**124**	**19**	**124**	**19**	**212**	**16**
TA100	5	130	12	133	26	137	17	98	8	127	9	104	15	153*	131
TA100	50	116	19	1	2	102	12	641*	218	496*	157	95*	9	72*	7
TA100	500	386*	263	0	-	45	38	58*	58	369*	49	685*	162	99*	36
spont. revertants		**228**	**29**	**228**	**29**	**228**	**29**	**228**	**29**	**228**	**29**	**228**	**29**	**394**	**18**
TA102	5	231	23	266	24	254	6	268	24	266	8	225	41	286	14
TA102	50	210	21	0	-	183	9	276*	52	312*	346	152*	35	226	22
TA102	500	249*	256	0	-	0	-	1	1	0	-	1129*	221	671	42

[1] only 2 replicates

Appendix 6.1 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 7 stations in Indiana Harbor. Suspected toxicity is denoted by a "*", confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	Station 3		4		5		6		7		8		10	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**135**	**7**	**135**	**7**	**135**	**7**	**135**	**7**	**135**	**7**	**135**	**7**	**91**	**16**
TA97a	5	140	25	143	2	135	4	141	11	134	20	164	6	65	10
TA97a	50	135	3	4	5	171*	58	328*	117	126	25	430*	91	42*	4
TA97a	500	242*	132	0	-	0	-	0	-	0	-	463*	19	8	4
spont. revertants		**17**	**1**	**17**	**1**	**17**	**1**	**17**	**1**	**17**	**1**	**31**[1]	**-**	**32**	**11**
TA98	5	29	8	26	8	23	6	22	2	19	3	6[1]	-	11*	4
TA98	50	15	7	1	2	295*	59	458*	26	109	3	0	-	0	-
TA98	500	215	172	0	-	0	-	1	2	0	-	0	-	0	-
spont. revertants		**124**	**19**	**124**	**19**	**124**	**19**	**124**	**19**	**124**	**19**	**124**	**19**	**212**	**16**
TA100	5	130	12	133	26	137	17	98	8	127	9	104	15	153*	131
TA100	50	116	19	1	2	102	12	641*	218	496*	157	95*	9	72*	7
TA100	500	386*	263	0	-	45	38	58*	58	369*	49	685*	162	99*	36
spont. revertants		**228**	**29**	**228**	**29**	**228**	**29**	**228**	**29**	**228**	**29**	**228**	**29**	**394**	**18**
TA102	5	231	23	266	24	254	6	268	24	266	8	225	41	286	14
TA102	50	210	21	0	-	183	9	276*	52	312*	346	152*	35	226	22
TA102	500	249*	256	0	-	0	-	1	1	0	-	1129*	221	671	42

[1] only 2 replicates

Appendix 6.2 Continued

Strain	Dose (g equiv. sediment)	S-9 (mg-equiv. protein)	Station 3 $\bar{X}$	3 SD	4 $\bar{X}$	4 SD	5 $\bar{X}$	5 SD	6 $\bar{X}$	6 SD	7 $\bar{X}$	7 SD	8 $\bar{X}$	8 SD	10 $\bar{X}$	10 SD
spont. revertants		**1**	**108**	**3**	**108**	**3**	**108**	**3**	**108**	**3**	**108**	**3**	**108**	**3**	**169**	**5**
spont. revertants		**3**	**108**	**36**	**108**	**36**	**108**	**36**	**108**	**36**	**108**	**36**	**108**	**36**	**168**	**9**
TA100	5	1	204	21	149	15	197	13	180	14	142	4	199	22	272	27
TA100	5	3	153	8	144	23	163	11	203	24	150	10	169	5	288	14
TA100	50	1	218	39	139	4	295	18	249	19	140	12	121	10	245	55
TA100	50	3	237	19	216	40	308	44	295	13	222	7	204	29	397	17
TA100	500	1	178	24	115	41	140	15	191	22	637*	252	235	42	191	39
TA100	500	3	303	39	265	346	188	17	256	18	981	170	255	76	188	15
spont. revertants		**1**	**239**	**12**	**239**	**12**	**239**	**12**	**239**	**12**	**239**	**12**	**239**	**12**	**400**	**50**
spont. revertants		**3**	**178**	**13**	**178**	**13**	**178**	**13**	**178**	**13**	**178**	**13**	**178**	**13**	**373**	**39**
TA102	5	1	292	11	346	11	336	60	505	68	406	14	356	13	446	13
TA102	5	3	366	26	305	15	286	8	639M	26	442	20	367	37	467	13
TA102	50	1	409	31	404	23	671M	40	625	85	282	16	349	48	509*	59
TA102	50	3	563	14	516M	29	794M	95	877	72	390M	25	313	14	635*	43
TA102	500	1	262	195	14	13	811	184	66	71	674*	317	249	32	376*	21
TA102	500	3	409	34	1565	106	426	99	411	41	407*	177	203	57	416*	153

[1] only 2 replicates

Appendix 6.2 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 stations in Indiana Harbor. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	S-9 (mg-equiv. protein)	Station 3		4		5		6		7		8		10	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**1**	**135**	**6**	**135**	**6**	**135**	**6**	**135**	**6**	**135**	**6**	**135**	**6**	**124**	**26**
spont. revertants		**3**	**203**	**9**	**203**	**9**	**203**	**9**	**203**	**9**	**203**	**9**	**203**	**9**	**212**	**41**
TA97a	5	1	194	28	185	22	196	17	238	39	181	12	262	38	232	21
TA97a	5	3	284	38	195	9	234	19	285	18	204	13	217	18	285	24
TA97a	50	1	298	15	218	6	252	35	354	7	221	18	206	14	270	12
TA97a	50	3	279	7	284	20	351	30	377	45	269	20	286	35	361	40
TA97a	500	1	298	24	76	22	127	23	317	9	803*	82	349	20	274	11
TA97a	500	3	290	10	472	171	336	13	393	64	557*	252	392	30	285	24
spont. revertants		**1**	**27**	**7**	**27**	**7**	**27**	**7**	**27**	**7**	**27**	**7**	**26**[1]		**39**	**6**
spont. revertants		**3**	**42**	**8**	**42**	**8**	**42**	**8**	**42**	**8**	**42**	**8**	**31**[1]		**44**	**6**
TA98	5	1	47	4	35	10	28	6	55M	3	36	5	70[1]		56	8
TA98	5	3	48	13	41M	4	29	5	71	21	35	2	96[1]		76	17
TA98	50	1	136	16	41	5	57	9	72	23	26	4	57[1]		104M	9
TA98	50	3	105M	8	119M	14	200M	127	145	23	111M	3	143[1]		181	8
TA98	500	1	37	6	11	11	21	2	46	2	703*	232	24[1]		110*	13
TA98	500	3	93M	20	331T	56	103M	2	178	79	453T	255	29[1]		117*	16

Appendix 6.3 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 10 stations in Buffalo River. Toxicity determined by visual inspection or replicated plating is denoted with a "T".

Dose (mg-equiv. sediment)	Station 1		2		3		4		5		6		7		8		9		10	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
STRAIN: TA97a																				
spont. reverts.:	**107**	**23**	**107**	**23**	**107**	**23**	**107**	**23**	**107**	**23**	**107**	**23**	**106**	**13**	**106**	**13**	**106**	**13**	**106**	**13**
10	128	16	134	5	133	7	89	12	98	23	133	13	210	25	182	3	152	5	139	2
1000	344*	95	238*	48	266*	158	17	18	10	9	174*	24	2	3	204	17	127	2	109*	46
1000	183*	48	0	-	2	4	0	-	11	19	0	-	292*	153	0	-	0	-	0	-
STRAIN: TA98																				
spont. reverts.:	**23**	**5**	**23**	**5**	**23**	**5**	**23**	**5**	**23**	**5**	**23**	**5**	**29**	**2**	**29**	**2**	**29**	**2**	**29**	**2**
10	20	4	29	8	30	1	20	3	18	5	16	3	29	3	24	2	27	8	23	4
100	108T	63	132*	111	169*	210	62T	87	0	-	101*	174	1	1	36	6	242T	172	207*	355
1000	75*	38	0	-	0	-	58*	100	115*	200	0	-	170*	127	0	-	0	-	95*	40
STRAIN: TA100																				
spont. reverts.:	**265**	**17**	**265**	**17**	**265**	**17**	**265**	**17**	**265**	**17**	**265**	**17**	**138**	**13**	**138**	**13**	**138**	**13**	**138**	**13**
10	158	9	240	11	253	13	90	4	168	25	95	7	179	27	138	10	130	6	142	8
100	503	165	219	92	658	71	131*	202	276*	264	259	152	25	30	85	19	158*	87	190*	277
1000	129	25	17	18	17	29	288*	90	26	1	0	-	62*	36	1	1	0	-	55*	96
STRAIN: TA102																				
spont. reverts.:	**340**	**18**	**340**	**18**	**340**	**18**	**340**	**18**	**340**	**18**	**340**	**18**	**361**	**47**	**361**	**47**	**361**	**47**	**361**	**47**
10	392	20	353	18	374	24	369	27	333	12	283	7	426	15	402	8	430	19	387	22
100	323	33	320	5	281	33	449*	103	150	34	153	24	294*	168	393	13	379	32	289	58
1000	141*	15	201*	258	10	10	88*	152	10	11	40*	35	1	1	295*	480	0	-	117*	107

Appendix 6.4 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 10 stations in Buffalo River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 1		2		3		4		5		6		7		8		9		10	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. reverts:		1	**164**	**18**	**164**	**18**	**164**	**18**	**164**	**18**	**164**	**18**	**164**	**18**	**143**	**6**	**143**	**6**	**143**	**6**	**143**	**6**
spont. reverts:		3	**182**	**10**	**182**	**10**	**182**	**10**	**182**	**10**	**182**	**10**	**182**	**10**	**180**	**10**	**180**	**10**	**180**	**10**	**180**	**10**
TA97a	10	1	182	5	184	8	174	13	212	6	210	10	213	16	229	33	206	14	190	10	148	13
TA97a	10	3	182	15	197	6	196	15	226	8	173	21	211	15	236	33	236	41	244	22	154	14
TA97a	100	1	297	33	312	10	259	5	328	12	277	11	330	12	336	43	336	31	365	5	238	82
TA97a	100	3	306	16	437	19	273	10	404	36	361	44	415	17	344	5	314	29	289	7	217	10
TA97a	1000	1	547	77	686*	118	720*	57	19*	27	132	9	337*	151	146*	15	201*	48	453*	281	434*	85
TA97a	1000	3	425	131	274	21	226	27	523*	452	138	10	129*	34	887*	183	799	22	201*	38	777*	570
spont. reverts:		1	**28**	**3**	**28**	**3**	**28**	**3**	**28**	**3**	**28**	**3**	**28**	**3**	**29**	**8**	**29**	**8**	**29**	**8**	**29**	**8**
spont. reverts:		3	**48**	**3**	**48**	**3**	**48**	**3**	**48**	**3**	**48**	**3**	**48**	**3**	**33**	**4**	**33**	**4**	**33**	**4**	**33**	**4**
TA98	10	1	43	8	48	11	39	3	53	6	70	9	56M	6	73	12	27	12	27	6	22	5
TA98	10	3	52	6	45	7	39	4	45	16	51[1]	-	40	8	67	6	60	34	230*	256	23	10
TA98	100	1	184	18	119M	6	148M	9	142M	17	89	3	215M	10	136	16	137	19	163	14	63	13
TA98	100	3	157M	19	167	14	141	7	216M	12	170M	5	226	31	130M	16	135M	15	138M	9	95M	36
TA98	1000	1	131M	9	847*	162	453*	313	599*	373	26	3	609*	194	42*	58	630*	410	710*	55	351	123
TA98	1000	3	194M	39	326*	22	243M	18	776*	420	77*	64	457T	90	600*	154	341M	31	507*	339	500*	688
spont. reverts:		1	**217**	**48**	**217**	**48**	**217**	**48**	**217**	**48**	**217**	**48**	**217**	**48**	**139**	**13**	**139**	**13**	**139**	**13**	**139**	**13**
spont. reverts:		3	**224**	**28**	**224**	**28**	**224**	**28**	**224**	**28**	**224**	**28**	**224**	**28**	**139**	**20**	**139**	**20**	**139**	**20**	**139**	**20**
TA100	10	1	282	18	277	20	248	7	263	6	276	19	270	18	182	9	155	7	148	4	202	26
TA100	10	3	262	29	257	21	260	29	237	7	251	6	266	22	170	12	150	11	167	13	152	15
TA100	100	1	407	14	451M	71	386	44	364	20	367	64	397	46	233	23	232	17	264	26	202	26
TA100	100	3	383	34	539M	16	390	26	449M	39	514M	13	460M	7	213	28	213	7	273M	19	188	9
TA100	1000	1	280T	6	490T	270	847T	128	260*	42	100	19	786*	21	575*	144	129	26	302*	417	480T	420
TA100	1000	3	460M	54	414*	296	224*	19	297	168	117	14	125	39	1067T	224	450M	82	828*	465	509*	205

Appendix 6.4 Continued

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 1 $\bar{X}$	1 SD	2 $\bar{X}$	2 SD	3 $\bar{X}$	3 SD	4 $\bar{X}$	4 SD	5 $\bar{X}$	5 SD	6 $\bar{X}$	6 SD	7 $\bar{X}$	7 SD	8 $\bar{X}$	8 SD	9 $\bar{X}$	9 SD	10 $\bar{X}$	10 SD
spont. reverts:		**1**	**394**	**23**	**394**	**23**	**394**	**23**	**394**	**23**	**394**	**23**	**394**	**23**	**426**	**21**	**426**	**21**	**426**	**21**	**426**	**21**
spont. reverts:		**3**	**299**	**35**	**299**	**35**	**299**	**35**	**299**	**35**	**299**	**35**	**299**	**35**	**468**	**14**	**468**	**14**	**468**	**14**	**468**	**14**
TA102	10	1	469	15	404	18	436	32	418	26	398	64	347	92	475	72	401	94	442	31	396	26
TA102	10	3	460	30	439	14	423	22	430	28	365	62	353	25	513	31	356	58	492	17	530	61
TA102	100	1	492	62	506	24	534	9	509	26	522	14	489	24	537	33	522	6	540	15	528	34
TA102	100	3	474	18	569	45	511M	7	591M	33	472	27	422M	38	609	51	471	63	550	38	536	44
TA102	1000	1	601	36	477	192	435	54	255*	39	241	45	891*	58	91	17	378*	57	842*	658	14	10
TA102	1000	3	539	72	337	31	364	46	124*	102	137	20	5	5	903*	597	642	59	584*	270	604*	390

[1] only 2 replicates

Appendix 6.5 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 7 stations from first survey of Saginaw River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity are denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	Station 2		3		4		6		7		9		10	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**136**	**15**	**136**	**15**	**136**	**15**	**136**	**15**	**136**	**15**	**216**	**48**	**136**	**15**
TA97a	10	120	21	154	7	135	12	141	32	132	3	203	9	114	10
TA97a	100	112	6	121*	97	140	4	4	7	101	9	152	6	101	8
TA97a	1000	0	-	9	10	0	-	0	-	0	-	0	-	0	-
spont. revertants		**19**	**3**	**19**	**3**	**19**	**3**	**19**	**3**	**19**	**3**	**34**	**1**	**19**	**3**
TA98	10	39	7	29	1	23	4	24	2	29	7	28	4	26	4
TA98	100	118*	29	29	34	57T	8	0	-	51*	7	20	6	364*	54
TA98	1000	0	-	96*	83	0	-	0	-	0	-	117*	113	0	-
spont. revertants		**139**	**12**	**139**	**12**	**139**	**12**	**139**	**12**	**139**	**12**	**165**	**41**	**139**	**12**
TA100	10	146	13	143	10	133	12	123	11	141	13	145	7	117	7
TA100	100	81*	5	382*	159	81	7	122	74	85*	4	198*	72	105*	40
TA100	1000	0	-	0	-	0	-	0	-	0	-	0	-	0	-
spont. revertants		**407**	**9**	**407**	**9**	**407**	**9**	**407**	**9**	**407**	**9**	**450**	**23**	**407**	**9**
TA102	10	456	10	403	8	396	9	443	29	460	20	375	15	370	10
TA102	100	379	6	256	4	417	16	226	7	365	26	401	48	358	17
TA102	1000	0	-	0	-	0	-	0	-	0	-	237*	217	15*	26

Appendix 6.6 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 7 stations from the third survey (grab) of Saginaw River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	Station 1		2		5		6		8		16		24	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**225**	**82**	**225**	**82**	**156**	**30**	**216**	**48**	**216**	**48**	**216**	**48**	**156**	**30**
TA97a	10	150	15	127	28	159	12	162	5	77	16	170	11	90	8
TA97a	100	29	30	69	11	130	13	222	15	85	2	107*	2	62	21
TA97a	1000	1	1	0	-	0	-	392*	226	3	5	27	31	0	-
spont. revertants		**60**	**7**	**60**	**7**	**22**	**6**	**34**	**1**	**34**	**1**	**34**	**1**	**22**	**6**
TA98	10	41	16	41	21	35	3	22	7	17	4	28	7	23	8
TA98	100	2	3	27	25	283*	344	28	2	15	3	205*	173	152*	49
TA98	1000	0	-	68	23	0	-	943T	60	322*	103	0	-	0	-
spont. revertants		**206**	**18**	**206**	**18**	**167**	**4**	**165**	**41**	**165**	**41**	**165**	**41**	**167**	**4**
TA100	10	229	13	239	18	171	11	124	11	116	16	157	16	185	33
TA100	100	1	1	209	29	115	7	167	18	78	5	307	38	154	24
TA100	1000	0	-	664	347	1	1	535*	5	166*	41	0	-	746	149
spont. revertants		**215**	**10**	**215**	**10**	**336**	**57**	**450**	**23**	**450**	**23**	**450**	**23**	**336**	**57**
TA102	10	241	70	176	6	418	22	433	19	388	34	416	20	178	11
TA102	100	0	-	182	79	371	36	390	21	352	13	446	59	175	34
TA102	1000	0	-	0	-	0	-	345	41	125*	23	0	-	342*	104

Appendix 6.7 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 8 core samples from the third survey of Saginaw River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	Station 5[1]		5[2]		6[1]		6[2]		2[3]		2[4]		6[5]		6[6]	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**156**	**30**	**156**	**30**	**225**	**82**	**156**	**30**	**216**	**48**	**156**	**30**	**225**	**82**	**156**	**30**
TA97a	10	221	21	106	18	168	18	94	17	146	44	181	10	167	85	108	32
TA97a	100	156	13	675*	267	3	5	89	14	79	13	195	21	3	1	494*	342
TA97a	1000	0	-	0	-	0	-	0	-	0	-	335*	353	0	-	0	-
spont. revertants		**22**	**6**	**22**	**6**	**60**	**7**	**22**	**6**	**34**	**1**	**22**	**6**	**60**	**7**	**22**	**6**
TA98	10	42	13	41	9	59	10	26	2	25	3	21	4	31	4	23	3
TA98	100	106*	14	95	66	6	4	31	14	187*	49	27	7	0	-	1043*	721
TA98	1000	1	2	0	-	0	-	149*	74	0	-	484*	443	0	-	0	-
spont. revertants		**167**	**4**	**167**	**4**	**206**	**18**	**167**	**4**	**165**	**41**	**167**	**4**	**206**	**12**	**167**	**4**
TA100	10	168	10	176	19	192	21	203	8	197	14	137	4	215	50	195	6
TA100	100	131	5	926*	335	52	79	161	16	282*	66	123	14	4	4	1115T	127
TA100	1000	224*	148	0	-	0	-	0	-	2	3	557M	53	0	-	360*	124
spont. revertants		**336**	**57**	**336**	**57**	**215**	**10**	**336**	**57**	**450**	**23**	**336**	**57**	**215**	**10**	**336**	**57**
TA102	10	384	35	194	51	271	12	198	19	285	52	346	74	242	33	164	9
TA102	100	395	44	130	21	172	158	174	25	408	18	435	14	141	19	80	11
TA102	1000	324	371	0	-	0	-	22	38	561*	78	1142*	7	0	-	0	-

[1] core from 0 - 2 feet deep
[2] core from 2 - 4 feet deep
[3] core from 8 - 20 inches deep
[4] core from 20 - 35 inches deep
[5] core from 5 - 13 inches deep
[6] core from 13 - 32 inches deep

Appendix 6.8 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 stations from the first survey of Saginaw River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity are denoted by a "T" and "M", respectively.

Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 2 $\bar{X}$	2 SD	3 $\bar{X}$	3 SD	4 $\bar{X}$	4 SD	6 $\bar{X}$	6 SD	7 $\bar{X}$	7 SD	9 $\bar{X}$	9 SD	10 $\bar{X}$	10 SD
						STRAIN TA97a									
spont. reverts.:	**1**	**161**	**12**	**161**	**12**	**161**	**12**	**161**	**12**	**161**	**12**	**183**	**8**	**161**	**12**
spont. reverts.:	**3**	**240**	**4**	**240**	**4**	**240**	**4**	**240**	**4**	**240**	**4**	**238**	**68**	**240**	**4**
10	1	203	6	194	5	197	5	184	8	230	8	210	37	147	7
10	3	248	12	239	9	255	10	213	18	261	12	181	45	208	12
100	1	293	10	256	11	263	5	294	12	268	20	293	7	167	16
100	3	278	14	259	26	259	18	347	21	272	8	284	22	175	30
1000	1	592*	192	79*	113	234*	95	332*	103	301*	81	198*	58	223*	180
1000	3	199*	19	482*	313	319	8	271*	432	471*	394	364	15	498*	320
						STRAIN TA98									
spont. reverts.:	**1**	**26**	**4**	**26**	**4**	**26**	**4**	**26**	**4**	**26**	**4**	**24**	**1**	**26**	**4**
spont. reverts.:	**3**	**28**	**4**	**28**	**4**	**28**	**4**	**28**	**4**	**28**	**4**	**17**	**8**	**28**	**4**
10	1	41	7	43	8	45	5	39	3	36	5	28	10	32	8
10	3	55	7	47	4	40	5	46	11	51	6	27	7	39	5
100	1	80	9	65M	7	50	3	84	13	71	7	54M	7	65M	6
100	3	86M	1	68	10	76M	2	123	10	76M	11	44	8	70	6
1000	1	24*	3	231T	231	23	6	300*	67	567T	99	34*	3	19*	8
1000	3	70*	6	73T	11	83M	17	614*	532	392T	466	73M	14	621T	502

Appendix 6.8 Continued

Dose (mg equiv. sediment)	S9 mg-equiv. protein)	Station 2 $\bar{X}$	2 SD	3 $\bar{X}$	3 SD	4 $\bar{X}$	4 SD	6 $\bar{X}$	6 SD	7 $\bar{X}$	7 SD	9 $\bar{X}$	9 SD	10 $\bar{X}$	10 SD
						STRAIN TA100									
spont. reverts.:	**1**	**152**	**16**	**152**	**16**	**152**	**16**	**152**	**16**	**152**	**16**	**140**	**8**	**152**	**16**
spont. reverts.:	**3**	**158**	**3**	**158**	**3**	**158**	**3**	**158**	**3**	**158**	**3**	**178**	**55**	**158**	**3**
10	1	188	14	179	19	182	10	186	7	178	25	149	15	140	5
10	3	191	14	183	10	177	4	173	15	164	14	152	13	149	7
100	1	252	21	242	15	273	15	278	12	214	18	194	8	221	4
100	3	234	17	273	20	243	14	367	16	193	16	173	20	220	15
1000	1	552T	442	208*	170	100	14	350*	39	412	102	129	34	436*	232
1000	3	130*	45	364*	373	253	13	665*	470	711T	126	233	41	725*	62
						STRAIN TA102									
spont. reverts.:	**1**	**469**	**42**	**469**	**42**	**469**	**42**	**469**	**42**	**469**	**42**	**388**	**16**	**469**	**42**
spont. reverts.:	**3**	**501**	**28**	**501**	**28**	**501**	**28**	**501**	**28**	**501**	**28**	**430**	**66**	**501**	**28**
10	1	438	36	480	25	507	23	502	17	482	9	405	27	404	35
10	3	485	57	481	18	484	8	522	26	546	15	466	50	420	59
100	1	563	16	448	92	525	15	561	32	542	19	439	11	457	17
100	3	533	29	467	35	498	75	611M	17	547	29	381	38	433	28
1000	1	166	93	6	6	1023T	91	378*	147	101	156	299	20	1186T	774
1000	3	467	62	555*	274	520	18	409*	124	588*	66	262T	402	1459*	982

Appendix 6.9 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 stations from third survey (grab) of Saginaw River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 1		2		5		6		8		16		24	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
						STRAIN TA97a									
spont. reverts.:	**1**	**238**	**61**	**238**	**61**	**165**	**27**	**183**	**8**	**183**	**8**	**183**	**8**	**165**	**27**
spont. reverts.:	**3**	**271**	**19**	**271**	**19**	**133**	**25**	**251**	**68**	**251**	**68**	**251**	**68**	**133**	**25**
10	1	222	39	102	13	198	13	155	34	132	18	212	19	148	3
10	3	192	19	155	11	224	28	189	71	169	10	219	29	144	24
100	1	204*	50	274	51	241	25	236	20	214	24	287	35	273	5
100	3	183	43	327	38	265	11	256	17	200	2	235	43	216	41
1000	1	0	-	6	2	18	11	482	10	116	8	777*	413	367*	217
1000	3	0	-	225	330	878*	321	361	8	326	23	289	19	228	77
						STRAIN TA98									
spont. reverts.:	**1**	**55**	**7**	**55**	**7**	**26**	**10**	**24**	**7**	**24**	**4**	**24**	**7**	**26**	**10**
spont. reverts.:	**3**	**54**	**5**	**54**	**5**	**22**	**8**	**17**	**8**	**17**	**8**	**17**	**5**	**22**	**4**
10	1	49	11	47	9	45	3	27	4	20	1	30	6	30	7
10	3	54	12	54	13	46M	8	19	2	28	8	46	17	11	8
100	1	266*	93	73	1	56	10	42M	11	47M	3	64M	12	86M	14
100	3	159	82	51	17	54	11	31	4	42	4	55	22	36	20
1000	1	0	-	116	24	328*	545	123M	21	53*	30	395*	354	460*	229
1000	3	0	-	207	23	865*	526	143M	23	119M	9	140M	37	139M	23

Appendix 6.9 Continued

Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 1 $\bar{X}$	1 SD	2 $\bar{X}$	2 SD	5 $\bar{X}$	5 SD	6 $\bar{X}$	6 SD	8 $\bar{X}$	8 SD	16 $\bar{X}$	16 SD	24 $\bar{X}$	24 SD
						STRAIN TA100									
spont. reverts.:	**1**	**207**	**12**	**207**	**12**	**163**	**32**	**140**	**8**	**140**	**8**	**140**	**8**	**163**	**32**
spont. reverts.:	**3**	**236**	**35**	**236**	**35**	**155**	**13**	**178**	**55**	**178**	**55**	**178**	**55**	**155**	**13**
10	1	253	27	226	63	162	6	160	1	138	12	162	9	178	21
10	3	214	15	244	11	175	6	147	13	172	23	151	13	167	26
100	1	331*	20	106	166	220	4	171	14	151	17	235	5	298	2
100	3	206*	32	108	186	213	9	156	3	149	7	245	6	267	15
1000	1	1	1	498	194	85	119	259	26	95	12	124*	34	174	2
1000	3	2	2	721	13	1010*	139	288	52	229	26	178*	60	349M	81
						STRAIN TA102									
spont. reverts.:	**1**	**334**	**28**	**334**	**28**	**287**	**26**	**388**	**16**	**388**	**16**	**388**	**16**	**287**	**26**
spont. reverts.:	**3**	**349**	**27**	**349**	**27**	**310**	**59**	**430**	**66**	**430**	**66**	**430**	**66**	**310**	**59**
10	1	327	12	271	36	508	47	412	33	412	10	420	8	222	41
10	3	288	12	262	48	561	52	322	23	499	28	552	57	257	33
100	1	767*	98	228M	33	495	40	328	89	427	11	467	17	307	38
100	3	536*	64	233	98	575	54	326	41	456	62	462	31	401	67
1000	1	503T	868	434	9	143	212	434	59	305	13	546*	109	258	36
1000	3	776	796	79	66	1167*	668	511	35	658M	11	378	87	621M	183

Appendix 6.10 Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 8 core samples from the third survey of Saginaw River. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 5[1] $\bar{X}$	SD	5[2] $\bar{X}$	SD	6[1] $\bar{X}$	SD	6[2] $\bar{X}$	SD	2[3] $\bar{X}$	SD	2[4] $\bar{X}$	SD	6[5] $\bar{X}$	SD	6[6] $\bar{X}$	SD
							STRAIN TA97a										
spont. reverts.:	**1**	**165**	**27**	**165**	**27**	**238**	**61**	**165**	**27**	**183**	**8**	**165**	**27**	**238**	**61**	**165**	**27**
spont. reverts.:	**3**	**133**	**25**	**133**	**25**	**271**	**19**	**133**	**25**	**238**	**68**	**133**	**25**	**271**	**19**	**133**	**25**
10	1	253	34	139	15	261	43	83	43	201	9	187	31	176	53	147	19
10	3	225	22	164	30	273	58	30	30	194	14	205	38	173	19	182	35
100	1	283	49	188	7	218	35	209	35	238	20	213	15	213	30	228	17
100	3	351	83	180	74	378	35	178	24	289	23	187	44	291	20	288	40
1000	1	481*	205	916*	498	149*	61	242	140	285	28	332	43	96*	4	1321*	269
1000	3	248	71	528	251	2		2293	872	344	58	362	39	1	1	2556*	320
							STRAIN TA98										
spont. reverts.:	**1**	**26**	**7**	**26**	**7**	**55**	**10**	**26**	**7**	**24**	**4**	**26**	**7**	**55**	**10**	**26**	**7**
spont. reverts.:	**3**	**22**	**5**	**22**	**5**	**54**	**8**	**22**	**8**	**17**	**8**	**22**	**5**	**54**	**4**	**22**	**5**
10	1	62	12	34	4	55	22	27	2	30	2	36	8	80	9	47	10
10	3	51M	7	33	15	73	6	32	11	25	2	37	2	53	8	39	2
100	1	76	17	109	11	132	12	74M	13	56M	8	40	3	104	9	132	37
100	3	106	47	44M	29	123	7	44M	16	59M	8	34	6	75	52	114M	26
1000	1	209*	88	1731*	425	0	-	813*	1360	34	8	52	13	1	1	1618*	924
1000	3	114M	5	1680*	1285	327T	180	1564*	1182	227M	21	114M	6	368*	294	2383*	241

Appendix 6.10 Continued

Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station 5[1] $\bar{X}$	SD	5[2] $\bar{X}$	SD	6[1] $\bar{X}$	SD	6[2] $\bar{X}$	SD	2[3] $\bar{X}$	SD	2[4] $\bar{X}$	SD	6[5] $\bar{X}$	SD	6[6] $\bar{X}$	SD
								STRAIN TA100									
spont. reverts.:	**1**	**163**	**32**	**163**	**32**	**207**	**12**	**163**	**32**	**140**	**8**	**163**	**32**	**207**	**12**	**163**	**32**
spont. reverts.:	**3**	**155**	**13**	**155**	**13**	**236**	**35**	**155**	**13**	**178**	**55**	**155**	**13**	**236**	**35**	**155**	**13**
10	1	196	5	245	60	246	38	213	32	194	38	142	11	227	36	223	33
10	3	186	7	231	44	250	10	204	57	126	8	150	21	213	4	167	12
100	1	302	20	312M	21	241	22	316	43	220	23	191	6	227	30	230	14
100	3	329M	49	248	48	338	23	275M	37	227	17	194	34	435	12	380M	59
1000	1	183	13	1432*	311	1	2	1129*	232	146	15	247	71	904*	124	604*	514
1000	3	318	35	1931*	405	0	1	2085T	158	314	60	341M	39	16	9	423*	65
								STRAIN TA102									
spont. reverts.:	**1**	**287**	**26**	**287**	**26**	**334**	**28**	**287**	**26**	**388**	**16**	**287**	**26**	**334**	**28**	**287**	**26**
spont. reverts.:	**3**	**310**	**59**	**310**	**59**	**349**	**27**	**310**	**59**	**430**	**66**	**310**	**59**	**349**	**27**	**310**	**59**
10	1	390	94	216	9	402	29	262	17	418	19	471	36	319	24	260	9
10	3	487	44	156	21	407	46	269	50	412	100	574	24	308	38	328	22
100	1	478	86	343	107	296	10	287	14	560	50	539	77	249	13	279	19
100	3	441	96	121	68	381	91	361	82	563	18	117	56	280	38	328	14
1000	1	670*	236	1248*	833	6	8	234	30	367	81	530	20	541*	584	1457*	185
1000	3	512	201	385*	44	232*	260	1895*	1003	676M	172	728M	31	562T	254	293	273

[1] core from 0 - 2 feet deep
[2] core from 2 - 4 feet deep
[3] core from 8 - 20 inches deep
[4] core from 20 - 35 inches deep
[5] core from 5 - 13 inches deep
[6] core from 13 - 32 inches deep

Appendix 6.11. Mean number of revertants (n=3) ± SD for 4 strains, 3 doses of extract, and no S9 for 7 Quality Assurance Quality Control samples. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	Station																	
		IH-REP[d]		BR-REP[e]		SR1-REP[f]		SR2-REP1[g]		SR2-REP2[g]		BR-CON		SR-CON		IH-BL[h]		SR-BL[h]	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. revertants		**91**	**16**	**106**	**13**	**136**	**15**	**225**	**82**	**216**	**48**	**106**	**13**	**216**	**48**	**91**	**16**	**225**	**82**
TA97a	10[a]	104	18	140	13	141	11	101	13	204	10	115	7	93	14	122	21	117	24
TA97a	100[b]	112	36	75	11	185*	147	60	7	191	16	89*	23	102	1	150	8	99	17
TA97a	1000[c]	0	-	0	-	157*	273	71	24	640*	130	0	-	14	1	546*	47	102	11
spont. revertants		**32**	**11**	**29**	**2**	**19**	**3**	**60**	**7**	**34**	**1**	**29**	**6**	**34**	**7**	**32**	**11**	**60**	**7**
TA98	10	19	6	24	2	17	4	57	9	30	7	26	6	14	5	27	8	40	15
TA98	100	0	-	82	17	580*	324	59	9	23	4	8	2	32	1	31	2	56	8
TA98	1000	0	-	0	-	0	-	175T	92	453*	354	0	-	155*	233	248*	250	236*	87
spont. revertants		**212**	**16**	**138**	**13**	**139**	**12**	**206**	**18**	**165**	**41**	**138**	**4**	**165**	**41**	**212**	**16**	**206**	**18**
TA100	10	84*	27	143	19	143	23	236	39	136	9	132	18	146	3	205	16	219	18
TA100	100	90*	21	360*	217	371*	262	164*	17	115	7	365*	498	161	10	186	25	204	14
TA100	1000	28*	22	0	-	0	-	21	18	313*	55	0	1	24	42	36	27	178	12
spont. revertants		**394**	**18**	**361**	**47**	**407**	**9**	**215**	**10**	**450**	**23**	**361**	**57**	**450**	**23**	**394**	**18**	**215**	**10**
TA102	10	296	11	360	10	403	21	147	24	333	27	379	15	367	50	288	14	219	20
TA102	100	172	22	408	3	218	16	148	52	375	13	180*	10	383	21	334	19	217	30
TA102	1000	0	-	0	-	0	-	326	300	487*	210	0	-	136*	162	625*	225	212	15

[a] For sample IH-REP, dose is 5 mg-equiv. sediment
[b] For sample IH-REP, dose is 50 mg-equiv. sediment
[c] For sample IH-REP, dose is 500 mg-equiv. sediment
[d] Blind duplicate of IH-7
[e] Blind duplicate of BR-6
[f] Blind duplicate of SR-2 (first survey)
[g] Blind duplicate of SR-1 (third survey)
[h] Procedural blanks do not contain sediment

Appendix 6.12 Mean number of revertants (n=3)± SD for 4 strains, 3 doses of extract, and 2 concentrations of S9 for 7 Quality Assurance Quality Control samples. Suspected toxicity is denoted by a "*". Confirmed toxicity and mutagenicity is denoted by a "T" and "M", respectively.

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	Station: IH-REP[d]		BR-REP[e]		SR1-REP[f]		SR2-REP1[g]		SR2-REP2[g]		BR-CON		SR-CON		IH-BL[h]		SR-BL[h]	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. reverts:		**1**	**124**	**26**	**143**	**18**	**161**	**12**	**238**	**61**	**183**	**8**	**143**	**18**	**183**	**8**	**124**	**26**	**238**	**61**
spont. reverts:		**3**	**212**	**41**	**180**	**10**	**240**	**4**	**271**	**19**	**251**	**68**	**180**	**10**	**251**	**68**	**212**	**41**	**271**	**19**
TA97a	10[a]	1	334	14	162	18	246	7	149	9	203	20	166	6	146	10	201	6	191	104
TA97a	10[a]	3	338	36	202	2	241	4	190	50	227	31	213	20	184	22	222	8	207	29
TA97a	100[b]	1	390	3	232	5	382	12	212	17	217	23	121	5	167	9	195	5	161	46
TA97a	100[b]	3	426	19	235	4	446	54	278	27	188	46	160	19	276	47	191	2	174	22
TA97a	1000[c]	1	169*	61	204*	54	63*	68	262	13	334	12	349*	253	144	30	124	8	255	7
TA97a	1000[c]	3	172*	24	293	20	3	6	352	39	415	64	721*	567	186	1	182	15	186	28
spont. reverts:		**1**	**39**	**6**	**29**	**3**	**26**	**4**	**55**	**10**	**24**	**4**	**29**	**3**	**24**	**4**	**39**	**6**	**55**	**10**
spont. reverts:		**3**	**44**	**6**	**33**	**3**	**28**	**4**	**54**	**4**	**17**	**8**	**33**	**3**	**17**	**8**	**44**	**6**	**54**	**4**
TA98	10	1	109	4	36	10	43	3	70	15	24	2	37	9	31	7	34	4	72	6
TA98	10	3	131	2	31	1	51	6	66	14	26	10	38	7	35	5	40	7	51	4
TA98	100	1	125	12	66	8	148	4	79	10	32	3	28	6	67M	7	40	1	66	13
TA98	100	3	245	24	75M	11	179	10	64	23	39	19	37	2	74	9	39	6	61	12
TA98	1000	1	111*	24	12*	3	39*	32	125	26	68	12	1039*	152	75*	34	24	9	68	23
TA98	1000	3	128*	30	130M	38	184*	304	114M	14	88T	41	105*	76	225*	473	64	5	67	9

Appendix 6.12 Continued

Strain	Dose (mg-equiv. sediment)	S9 (mg-equiv. protein)	IH-REP[d]		BR-REP[e]		SR1-REP[f]		SR2-REP1[g]		SR2-REP2[g]		BR-CON		SR-CON		IH-BL[h]		SR-BL[h]	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
spont. reverts:		**1**	**169**	**5**	**139**	**48**	**152**	**16**	**207**	**12**	**140**	**8**	**139**	**48**	**140**	**8**	**169**	**5**	**207**	**12**
spont. reverts:		**3**	**168**	**9**	**139**	**28**	**158**	**3**	**236**	**35**	**178**	**55**	**139**	**28**	**178**	**55**	**168**	**9**	**236**	**35**
TA100	10	1	333	22	144	28	165	6	217	29	200	41	152	0	163	10	167	30	237	13
TA100	10	3	305	47	145	15	188	8	202	45	168	19	149	13	153	14	159	8	201	21
TA100	100	1	356	18	217	23	251	28	242	37	163	6	86	17	262	18	194	21	259	27
TA100	100	3	519	60	181	16	344	49	217	55	153	11	101	23	229	25	161	5	263	28
TA100	1000	1	143	30	82*	3	214*	110	317	105	176	17	221	181	322*	191	55	14	321	50
TA100	1000	3	160*	14	202	31	72*	64	353	101	261	17	418T	372	168*	17	97	22	321	36
spont. reverts:		**1**	**400**	**50**	**426**	**23**	**469**	**42**	**334**	**28**	**388**	**16**	**426**	**23**	**388**	**16**	**400**	**50**	**334**	**28**
spont. reverts:		**3**	**373**	**39**	**468**	**35**	**501**	**28**	**349**	**27**	**430**	**66**	**468**	**35**	**430**	**66**	**373**	**39**	**349**	**27**
TA102	10	1	457	30	388	34	527	9	219	43	354	60	504	21	363	74	324	25	349	23
TA102	10	3	498	30	436	44	498	24	185	68	422	101	458	33	358*	85	236	40	312	20
TA102	100	1	434	24	454	49	557	40	246	21	368	23	255	8	391	52	355	18	361	27
TA102	100	3	671	71	481	69	606	23	146	71	419	102	262	32	394	69	222	123	264	30
TA102	1000	1	160	24	310*	69	146*	20	178	78	377	20	1214*	634	456*	44	220	16	363	40
TA102	1000	3	198	27	351*	65	7	7	227	197	592	164	1382T	991	879T	364	206	24	232	21

[a] For sample IH-REP, dose is 5 mg-equiv. sediment
[b] For sample IH-REP, dose is 50 mg-equiv. sediment
[c] For sample IH-REP, dose is 500 mg-equiv. sediment
[d] Blind duplicate of IH-7
[e] Blind duplicate of BR-5
[f] Blind duplicate of SR-2 (first survey)
[g] Blind duplicate of SR-1 (third survey)
[h] Procedural blanks do not contain sediment

Appendix 6.13 Mean number of revertants (n=3) ± SD for known direct-acting and promutagens for each test run.

Postive Mutagen	S9 (mg-equiv. protein)	Site IH-1 $\bar{X}$	IH-1 SD	IH-2 $\bar{X}$	IH-2 SD	BR-1 $\bar{X}$	BR-1 SD	BR-2 $\bar{X}$	BR-2 SD	SR1-1 $\bar{X}$	SR1-1 SD	SR2-1 $\bar{X}$	SR2-1 SD	SR2-2 $\bar{X}$	SR2-2 SD	SR2-3 $\bar{X}$	SR2-3 SD
							STRAIN TA97a										
spont. reverts.:	**0**	**135**	**7**	**91**	**16**	**107**	**23**	**106**	**13**	**136**	**15**	**216**	**48**	**156**	**30**	**225**	**82**
spont. reverts.:	**1**	**203**	**9**	**212**	**41**	**182**	**10**	**180**	**10**	**240**	**4**	**251**	**68**	**133**	**25**	**271**	**19**
TNF	0	535	91	664	162	462	32	345	5	577	72	971	36	247	34	426	169
2AF	1	833	36	649	147	717	30	857	17	918	18	1393	128	1020	80	1177	278
							STRAIN TA98										
spont. reverts.:	**0**	**17**	**1**	**32**	**11**	**23**	**5**	**29**	**8**	**19**	**3**	**34**	**1**	**22**	**6**	**60**	**7**
spont. reverts.:	**1**	**42**	**8**	**44**	**6**	**48**	**3**	**33**	**4**	**28**	**4**	**17**	**8**	**22**	**5**	**54**	**4**
TNF	0	1022	72	1092	64	1022	77	786	29	1008	60	2283	195	702	176	1333	283
2AF	1	1487	165	1647	31	1599	24	1714	123	1877	87	2211	201	1643	171	1745	188
							STRAIN TA100										
spont. reverts.:	**0**	**124**	**19**	**212**	**16**	**265**	**17**	**138**	**13**	**139**	**12**	**165**	**41**	**167**	**4**	**206**	**18**
spont. reverts.:	**1**	**108**	**36**	**168**	**9**	**224**	**28**	**139**	**20**	**158**	**3**	**178**	**55**	**155**	**13**	**236**	**35**
MMS	0	606	13	477	39	474	27	996	68	1050	61	747	25	1004	20	1379	13
BAP	1	177	5	538	14	477	11	370	32	401	27	629	62	558	43	914	73
							STRAIN TA102										
spont. reverts.:	**0**	**228**	**29**	**394**	**18**	**340**	**18**	**361**	**47**	**407**	**9**	**450**	**23**	**336**	**57**	**215**	**10**
spont. reverts.:	**1**	**178**	**13**	**373**	**39**	**299**	**35**	**468**	**14**	**501**	**28**	**430**	**66**	**310**	**59**	**349**	**27**
MMS	0	1007	73	1010	50	695	39	2197	87	2393	144	2104	59	1702	328	1755	280
2AF	1	302	12	516	18	473	34	430	35	650	12	527	83	460	100	381	56

CHAPTER 7: MUTATOX GENOTOXICITY ASSAY

Johnson, B.T.

INTRODUCTION

The Great Lakes of North America form the largest continuous mass of fresh water on earth. Lakes Michigan, Superior, Huron, Ontario, and Erie contain over 20 percent of the world's fresh water (Wetzel 1975). During the last two centuries, many cities with their supportive industries spread along their shorelines and tapped the natural wealth of the Great Lakes Basin (Wheeler and Kostbade 1990). The Lakes have become a conduit of world trade, a supporter of large fisheries, a dependable source of potable water, a playground for aquatic sports and recreational fisheries, and a repository for domestic and industrial anthropogenic wastes.

The presence of chemical contaminants in the Great Lakes is well-documented. A comprehensive review by Hesselberg and Seelye (1982) in conjunction with the International Joint Commission's (IJC) Great Lakes International Surveillance Plan tentatively identified 476 organic compounds in fish-tissue residues. Of these compounds, 53 were halogenated, 29 were isomers of PCBs, nine were chlorinated pesticides, five were polycyclic aromatic hydrocarbons, over 150 were oxygen-containing compounds such as phenols, esters, and carboxylic acids, and the remaining were aliphatic hydrocarbons. A study of sediment extracts with Medaka (Oryzias latipes) from tributaries of the Great Lakes (Fabacher et al. 1991) suggested that chemical contaminants can cause neoplasms in wild fishes. In 1980, the IJC estimated that about 2,500 chemicals were in common use in the Great Lakes Basin ecosystem (IJC 1980). Recently, a joint U.S.-Canadian committee investigating water quality concluded that humans inhabiting the Great Lakes Basin were exposed to and accumulated more toxic materials (including xenobiotics and inorganic pollutants) than anyone else on this continent (NRC 1985).

In 1987 the U.S. Congress under amendments to the Clean Water Act, in Section 118(c)(3), authorized the Great Lakes National Program Office (GLNPO) of the U.S. Environmental Protection Agency to implement a five-year study of chemical pollutants in Great Lake sediments. Data from this study will assist the Assessment and Remediation of Contaminated Sediment (ARCS) Project develop Remedial Action and Lake-wide Management Plans for removal of toxic materials from sediment. The present study, as part of this extensive sediment investigation, was designed to determine the potential genotoxicity (DNA-damage) of chemical contaminants in sediments from three Areas of Concern (AOC): Indiana Harbor in IN, Buffalo River in NY, and Saginaw River in MI with two procaryotic bioassays the Ames Salmonella Microsome Mutagenicity Test (Maron and Ames 1973) (Chapter 6) and the Mutatox Genotoxicity Assay (Johnson 1992). The Ames and the Mutatox tests both use the induced reversion of bacterial mutants to detect DNA-damaging substances. Unlike the Ames test, the transformation in the Mutatox assay may not always be mutagenic; therefore, the generic term genotoxicity is used to describe this assay.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

The objectives of this study were twofold: to validate the relative sensitivity and specificity of the activated Mutatox Genotoxicity Assay (Microbics Corp., Carlsbad, CA) using known progenotoxins and non-genotoxins in complex mixtures; and to use this assay to determine the potential genotoxicity of industrially contaminated sediments from selected Great Lakes Areas of Concern.

SAFETY PROCEDURES FOR GENOTOXICITY EXPERIMENTS

Disposable gloves, Tyvek^R suits, sleeve protectors, and safety glasses were used for handling potentially genotoxic substances. All toxicological transfers were conducted in a laminar flow hood under yellow light; treated samples were incubated in an enclosed vented hood. Toxicant disposal was pursuant to National Fisheries Contaminant Research Center (NFCRC) Safety Plan 1991.

CHEMICALS: PROGENOTOXINS, NON-GENOTOXINS, AND SOLVENTS

Progenotoxins 2-acetamidofluorene (2-AAF), 2-aminoanthracene (2-AA), and pyrene (PY) were purchased from Sigma Chemical Co. (St. Louis, MO), and 2-aminofluorene (2-AF), 2-aminonaphthalene (2-AN), benzo(α)pyrene (BαP), and 3-methylcholanthrene (3-MC) were obtained from Aldrich Chemical Co. (Milwaukee, WI). (A progenotoxin is defined as a chemical substance that must be metabolically activated to become a DNA-damaging agent.)

Non-genotoxins carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate), a carbamate insecticide; di-2-ethylhexyl phthalate, a plasticizer; malathion (O,O-dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate), an organophosphate insecticide; simazine (2-chloro-4,6-bis(ethylamino)-*s*-triazine), a triazine herbicide; and permethrin (3-(phenoxyphenyl)-methyl(I)-cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane carboxylate), a synthetic pyrethroid insecticide, were obtained from the chemical repository of NFCRC.

Solvents dimethyl sulfoxide (DMSO), spectrophotometric grade, and acetone were purchased from Burdwick Jackson Laboratory, Inc. (Muskegon, Michigan). All progenotoxins were dissolved in DMSO (unless indicated), stored frozen, and thawed at room temperature before use. Progenotoxin and non-genotoxin working solutions were prepared in acetone (acetone:DMSO, 10:1, V/V) and stored at 5°C in amber bottles with Teflon® cap liners.

SEDIMENT SAMPLES

Sediment samples were collected from 1988 to 1990 at twenty-eight stations in three industrial areas: Indiana Harbor, in IN, Buffalo River in NY, and Saginaw River in MI (Figures 1.1, 1.2, and 1.3). One-hundred g samples, either grab or core, were dried with anhydrous sodium sulfate, extracted with methylene chloride, subjected to gel-permeation chromatography cleanup, evaporated under nitrogen, and brought to volume in DMSO (Chapter 6). Polychlorinated biphenyls (PCBs), low and high molecular polyaromatic

hydrocarbons (PAHs), dioxins, and furans were present in sediment extracts from all priority stations (Chapter 4 for complete physical and chemical characterization of sediments). Working solutions of sediment extracts were prepared in acetone (acetone:DMSO, 10:1, V/V) and contained 1000 mg equivalent (eq) sediment dry weight/mL.

EXOGENOUS METABOLIC ACTIVATION

Metabolic activation of samples was accomplished with hepatic S9 from Aroclor-1254 induced rats (Microbiological Associates, Inc., Bethesda, MD). The S9 protein content was determined by the biuret method (Layne 1957); S9 preparations were stored at -80°C.

MUTATOX ASSAY

The Mutatox Assay uses rat liver S9 for exogenous metabolic activation of progenotoxins and a dark mutant strain of the luminescent bacterium Photobacterium phosphoreum for detection of genotoxins. DNA-damaging substances are detected by measuring the ability of a test extract or specific chemical to restore the luminescent state in the bacterial cells (Ulitzur et al. 1980). The degree of light increase indicates the relative genotoxicity of the sample.

Genotoxicity in single chemicals (Ulitzur et al. 1980; Weiser et al. 1981; Ulitzur and Weiser 1981; Ulitzur 1986; and Microbics Corp. unpublished data), as well as in complex mixtures was detected by Photobacterium without activation (Kwan et al. 1990) and with activation (Johnson 1992).

ACTIVATED MUTATOX ASSAY PROTOCOL

The Mutatox Assay System,[R] developed and packaged by Microbics Corp., Carlsbad, NM, consisted of reagents, freeze-dried bacteria, cuvettes, and an analyzer.

Preparation

Mutatox Assay Medium® (MAM), containing essential nutrients, growth factors, buffer, and cofactor (NADP-glucose-6-phosphate regenerating system; NADP 7.70 g/L and glucose-6-phosphate 3.40 g/L), was reconstituted with deionized water. A 100-mL aliquot of MAM was prepared in a 250-mL beaker using a magnetic stirrer and adjusted to 23 ±1°C. The rat hepatic S9 was thawed in a water bath at 23°C. An ampoule of freeze-dried luminescent bacteria (Photobacterium phosphoreum, Microbics Corp., lot #1089) was hydrated with 1.0 mL of MAM at 23°C.

MAM was immediately inoculated with the bacteria at a ratio of 100:1, stirred a few seconds, and incubated for 1 h at 23°C. Finally, rat hepatic S9 (40 mg protein/mL) was introduced into the MAM mixture at 0.4 mg/mL, stirred for 5 s and stirred again before dispensing it into sample cuvettes.

Test Procedure

Sediment extracts were tested at concentrations from 100 mg eq dry weight/mL to 0.19 mg eq dry weight/mL. Each test sample was serially double

diluted 9 times in the bacteria-S9-MAM mixture over a 100-fold dose range to elicit a normal dose response reaction. Ten cuvettes were placed in a row in a holder and numbered from 1 to 10; the process was repeated for each test sample. Using a 10-mL motorized electronic pipette (Rainin Instrument Co., Woburn, MA) that picks up and dispenses the liquid through a disposable polypropylene tip, 1.0 mL of the MAM mixture was dispensed into cuvette 1 and 0.5 mL into each cuvette 2 to 10. The test sample (0.1 mL) was added to cuvette 1 and the sample was briefly vortexed. With a disposable 1.0-mL pipette tip, 0.5 mL of the test sample was transferred from cuvette 1 to cuvette 2 (1:1 v/v) and vortexed a few seconds; the same procedure was repeated sequentially diluting the entire series. Finally, to maintain a similar surface-to-volume ratio in all cuvettes, 0.5 mL of the diluent from the last cuvette was discarded. Each test sample as well as control(s) was prepared in a standard dilution series as described. The treated cuvettes were covered with aluminum foil, preincubated in a water bath at 37°C for 30 min, and immediately placed in a dark incubator with vented hood at 23 ±1°C for 16 to 24 h. Aseptic techniques were not required because of the high salinity of test reagents and the relatively short incubation period of the test.

Test Endpoint

The response of the luminescent bacteria was determined by measuring the light intensity of each cuvette with a Model 500 Analyzer (Microbics Corp., Carlsbad, NM). A light value of 100 or more and at least three times the light intensity of the negative control was defined as a **GENOTOXIC RESPONSE**; sensitivity limits were the maximum peak concentration (MPC) and the lowest detected concentration (LDC) in each dilution series. The **DOSE-RESPONSE NUMBER** was defined as the number of genotoxic responses recorded at different concentrations per dilution series. A **DILUTION SERIES** with a dose-response number of three or more was designated genotoxic; with a dose-response number less than three, the series was designated suspect; and without a genotoxic response, the series was designated negative. The **TEST SAMPLE** was designated **GENOTOXIC** when at least three replicate series indicated a mean dose-response number of **three** or more, **SUSPECT** when at least three replicate series indicated a mean dose-response number less than three, or **NEGATIVE** when at least three replicate series contained no genotoxic response. Three replicate dilution series were conducted on different days before each test sample was finally identified. All final designations were made after the mean value of at least 30 data points (e.g., three replicate dilution series) over 100X concentration was determined.

ASSAY CONTROLS

Three controls -- positive, negative, and procedural -- were used in each assay. The positive controls consisted of four progenotoxins 2-AA, 2-AF, BαP, and PY (tested at 10-, 5-, 2.5-, 1.2-, and 0.6 μg/cuvette); these controls both determined the relative daily sensitivity of the assay and established an historical sensitivity baseline. An LDC of all positive controls (2-AA, 2-AF, BαP, and PY) ≤1.2μg/cuvette validated the sensitivity of the assay each day. When an LDC of any of the four positive controls was >1.2

μg/cuvette, the assay's sensitivity was considered suspect, the results were rejected, and this test-run was repeated. The negative control was the carrier solvent; this control identified the spontaneous light emission of the dark mutant strain. The procedural control was an organic extract from a fine silt and clay particle sediment obtained from local undisturbed agricultural soil (Ingersoll and Nelson 1990).

CYTOTOXICITY

The relative cellular toxicity of the test chemical (or complex organic extract) was determined by a turbidity test. Bacterial cytotoxicity of the sediment extract(s) and test chemical(s) was determined visually by comparing turbidity of bacterial growth in treatment and control tubes after 16 h. Phenol (1%) was used as a positive control.

No effort was made to determine the cytotoxicity of known genotoxins at concentrations >50 μg/test sample because the ecological relevancy of experimental high-dose effects is questionable. In addition, use of high concentrations of known genotoxins creates unnecessary handling hazards and disposal problems.

MUTATOX ASSAY WITH COMPLEX MIXTURES

The sensitivity and specificity of the activated Mutatox Assay with complex mixtures were determined by measuring genotoxic activity of known arylamine and PAH progenotoxins in sediment extracts (procedural control, see Assay Controls above) with and without exogenous metabolic activation, in non-genotoxic mixtures (model complex mixture), and in binary progenotoxic mixtures.

Progenotoxic Sensitivity

The relative sensitivity of the Mutatox Assay to detect model progenotoxins (2-AA, 2-AF, 2-AAF, 2-AN, BαP, 3-MC, and PY at concentrations ranging from 0.019 to 10 μg/cuvette) in environmental sediment extracts (methylene chloride) was determined by comparing procedural and negative controls (see Assay Controls above) and performing the assay with and without exogenous activation (rat S9). An additional procedural control with the model progenotoxins included heat inactivation of exogenous enzymes and omission of the regeneration system cofactor; rat S9 was heated 1 min in boiling water, cooled in crushed ice, brought to 23°C in a water bath, and then added to the bacteria-MAM mixture with or without NADP.

Non-genotoxic Specificity

A model complex mixture of potential non-genotoxic environmental pollutants (carbofuran, di-2-ethylhexyl phthalate, malathion, simazine, and permethrin) and model progenotoxins (2-AA or BαP) was tested to ascertain the specificity of the Mutatox Assay in detecting genotoxins when other chemical contaminants were also present in the test sample. Ten micrograms of each non-genotoxin were mixed in MAM reaction mixture with a magnetic stirrer for several minutes *before* preparing the dilution series; 10 μg of either 2-AA or

BαP were introduced and serially diluted as previously described (See Test Procedure).

Progenotoxin Interactions

Progenotoxins 2-AA, 2-AF, BαP, and PY were used to determine possible interfering interactions of progenotoxins and metabolites in Mutatox sensitivity. In paired permutations with each other, each progenotoxin was introduced at 10 μg (20μg total for the combination) into the standard dilution series as described above; the total acetone:DMSO carrier solvent did not exceed 100 μL/cuvette.

STATISTICAL ANALYSIS

Sample variability was measured by determining the standard deviation of the mean. Data were tested by analysis of variance with a Statistical Analysis System computer package (SAS Institute Inc. 1985). Mean differences were measured by one-way analysis of variance. If the overall F value was significant ($p \leq 0.05$), the mean differences were ascertained by using Fisher's least-significant-difference test.

RESULTS AND DISCUSSION

ASSAY VALIDATION: COMPLEX MIXTURES

Sensitivity

The activated Mutatox Assay detected all seven model progenotoxins in organic sediment extracts; each clearly demonstrated a dose-response with no observed cytotoxicity. Each progenotoxin was identified as a genotoxin when there were three or more responses in each dilution series (dose-response number ≥3). The Maximum Peak Concentration (MPC) and Lowest Dectected Concentration (LDC) were routinely found to be 5.0 μg/cuvette and <0.6 μg/cuvette for each progenotoxin (Table 7.1). In Figures 7.1 and 7.2, single data sets of 2-AA and BαP are shown in histograms; note the MPC, LDC, and dose-response number. The relative sensitivity of the activated Mutatox Assay to these arylamine and PAH progenotoxins (2-AA, 2-AF, 2-AAF, 2-AN, BαP, 3-MC, and PY) was established at ≤1 μg/cuvette.

Binary mixtures of four progenotoxins (2-AA + 2-AF, 2-AA + BαP, 2-AA + PY, 2-AF +BαP, 2-AF + PY, and BαP + PY) at concentrations of 10 to 0.6 μg/cuvette showed no evidence of inhibitory interactions (data not shown).

Exogenous Activation

Metabolic activation of complex mixtures was necessary for expression of bacterial genotoxicity; controls without S9 or with heat-killed S9 (60s in boiling water) showed no genotoxic activity in the assay (Table 7.2).

Specificity

The model complex mixture of carbofuran, di-2-ethylhexyl phthalate, malathion, simazine, and permethrin, representing five classes of potential aquatic contaminants, showed no genotoxic response or cytotoxicity at test doses of ≤10 μg/cuvette nor did the mixture interfere with the genotoxic expression of known progenotoxins (Table 7.2).

ENVIRONMENTAL COMPLEX MIXTURES: GREAT LAKES SEDIMENTS

Thirty-eight extracted samples collected from 28 stations in three Great Lakes AOCs were evaluated for genotoxicity with the activated Mutatox Assay.

Indiana Harbor

Grab samples from seven stations along the Indiana Harbor were collected in August 1989 (Table 2.1). All samples were genotoxic with an average 5.5 (0.8) dose-response number/station (Figure 7.3). The MPC detected ranged from 50 to 12 mg eq. sediment/mL and the LDC ranged from 0.7 to 0.09 mg eq. sediment/mL. In Figure 7.4, a data set of Station 6 is shown in histogram; note the MPC and LDC as well as dose-response number. Cytotoxicity was not observed in the turbidity tests; positive controls (2-AA, 2-AF, B(α)P and PY) were within acceptable sensitivity limits.

Buffalo River

Grab samples from ten stations along the Buffalo River were collected in October 1989 (Table 2.1). All samples were genotoxic with an average 5.0 (0.8) dose-response number/station (Figure 7.5). MPC detected ranged from 12 to 3 mg eq. sediment/mL and the LDC ranged from 0.75 to 0.37 mg eq. sediment/mL. No cytotoxicity was detected; positive controls were acceptable.

Saginaw River

Twenty-one grab and core samples from eleven stations along the Saginaw River were collected in December 1989 (first survey) and May 1990 (third survey). Ten of eleven staations showed evidence of genotoxic substances (Figure 7.6 to 7.8). Grab samples from Stations 2, 3, 5, 6, 16, and 24 were designated genotoxic and samples from Stations 4, 7, 8, and 10 were suspect (Figure 7.6 and 7.7). In the third survey of the Saginaw River, the grab sample from Station 1 failed to elicit a genotoxic response (Figure 7.7). The grab sample in this survey from Station 6 was negative (Figure 7.7), but the core sample was genotoxic (Figure 7.8). The average dose-response number/station for grab samples was 2.3 (1.5), about one-half the number for the Indiana Harbor and Buffalo River samples. The MPC detected ranged from 25 to 6 mg eq. sediment/mL and the LDC ranged from 0.75 to 0.18 mg eq. sediment/mL. Core samples collected from Stations 2, 5, and 6 during the third survey varied in depth from about 30 cm to 120 cm (Figure 7.8); all were genotoxic with an average dose-response number of 3.7 (Figure 7.8). This small core sampling suggested deep genotoxin penetration in the Saginaw sediment. No cytotoxicity was observed; positive controls were acceptable.

GENOTOXIN BIOAVAILABILITY

Most toxicological bioassays, procaryotic or eucaryotic, single cell or multicellular, lack an important ingredient: the element of in situ exposure to the real world. The best test can only simulate. The genotoxins recovered from Great Lakes sediments were mobilized with organic solvents, concentrated from large soil samples, and dissolved in compatible solvents. Therefore, the genotoxicity findings described in this manuscript must be prefaced with the word potential, e.g., existing in possibility, not in actuality. The bio-availability of genotoxins in freshwater sediments -- how they move in pore water, how they sorb onto sediment components, and how they move through the food-chain -- is poorly understood. This study, however, clearly showed that genotoxins were present in sediment as chemical contaminants and that they could possibly alter the expression of a cell's genome, an event that in most instances would be deleterious if not lethal to the organism.

MUTATOX AND AMES COMPARISON

The Mutatox Genotoxicity Assay compared favorably with the well-known and validated Salmonella Mutagenicity Test (Maron and Ames, 1983; Johnson 1992) (Table 7.3 and Chapter 6). The spectra of sensitivity with seven known or suspected arylamines and polycyclic aromatic hydrocarbon progenotoxins (2-AAF, 2-AA, 2-AF, 2-AN, B(α)P, 3-MC, and PY) indicated both qualitative and

quantitative similarities; the lowest detectable genotoxic concentrations were in the low microgram range (≤ 1) for both bacterial assays (Johnson 1992). The Mutatox Assay and the Ames Test both with exogenous metabolic activation (rat hepatic S9) detected genotoxins in complex mixtures extracted from the all sediments from Areas of Concern -- Indiana Harbor, Buffalo River, and Saginaw River. In parallel studies, Mutatox with 1% S9 and one tester strain and Ames with 10 to 30% S9 and four tester strains, the two genotoxic tests compared favorably detecting evidence of genotoxic substances in all AOCs with a 96% (27/28) station agreement (Chapter 6). Interestingly, the Great Lake sediment extracts were not cytotoxic to Photobacterium, but extremely toxic to Salmonella tester strains (Chapter 6). In the Ames test, however, confirmation tests were necessary to demonstrate reversion of the histidine-deficient tester strains from auxotrophic to prototrophic. When monitoring complex environmental mixtures, confirmation with the Ames Test can be tedious because sample extracts can cause cytotoxicity with the release of cellular nutrients.

SUMMARY

Genotoxins in complex organic mixtures were successfully detected by measuring the restored bioluminescence of a dark mutant strain of *Photobacterium*. Validation experiments showed that Mutatox was a sensitive, specific, and predictive genotoxicity test. In field studies, evidence of genotoxic substances was found in organic sediment extracts from all three AOCs: Indiana Harbor, Buffalo River, and Saginaw River. Within the three AOCs sampled, 27 stations showed evidence of genotoxins; 23 of 28 stations (82%) were designated genotoxic, four were suspect (14%), and one was negative (3%). Slightly over 80% (31/38) of all grab and core samples were genotoxic, about 13% (5/38) were suspect, and 5% (2/38) were negative. The Mutatox assay and Ames test compared favorably -- with 96% (27/28) station agreement -- in detecting evidence of genotoxic substances in all three AOCs. The activated Mutatox Assay seemed to be a good screening tool because of its simplicity, short-term duration, and sensitivity to detect genotoxins in complex environmental sediments from the Great Lakes.

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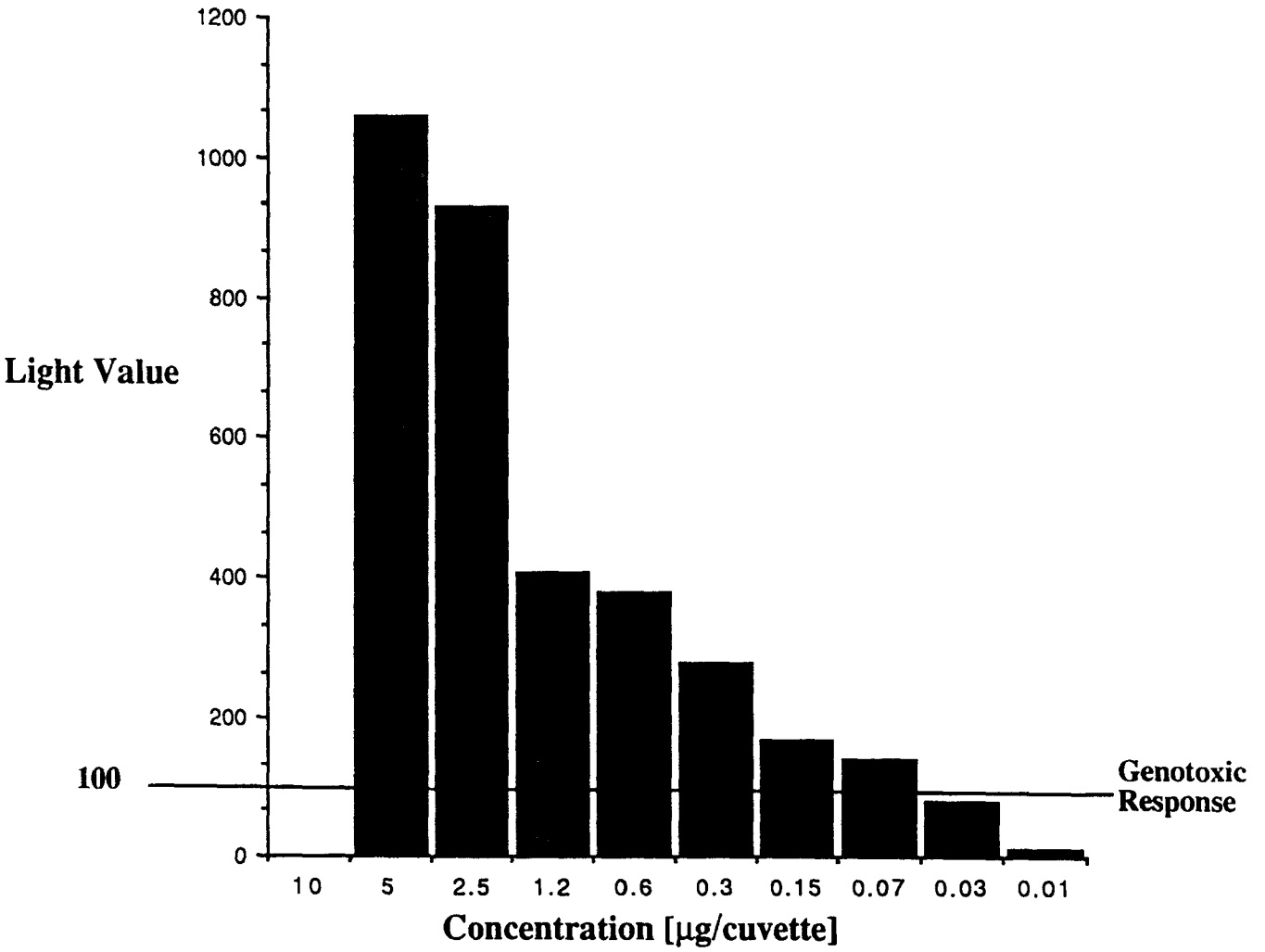

Figure 7.1 Genotoxicity of 2-aminoanthracene determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9).

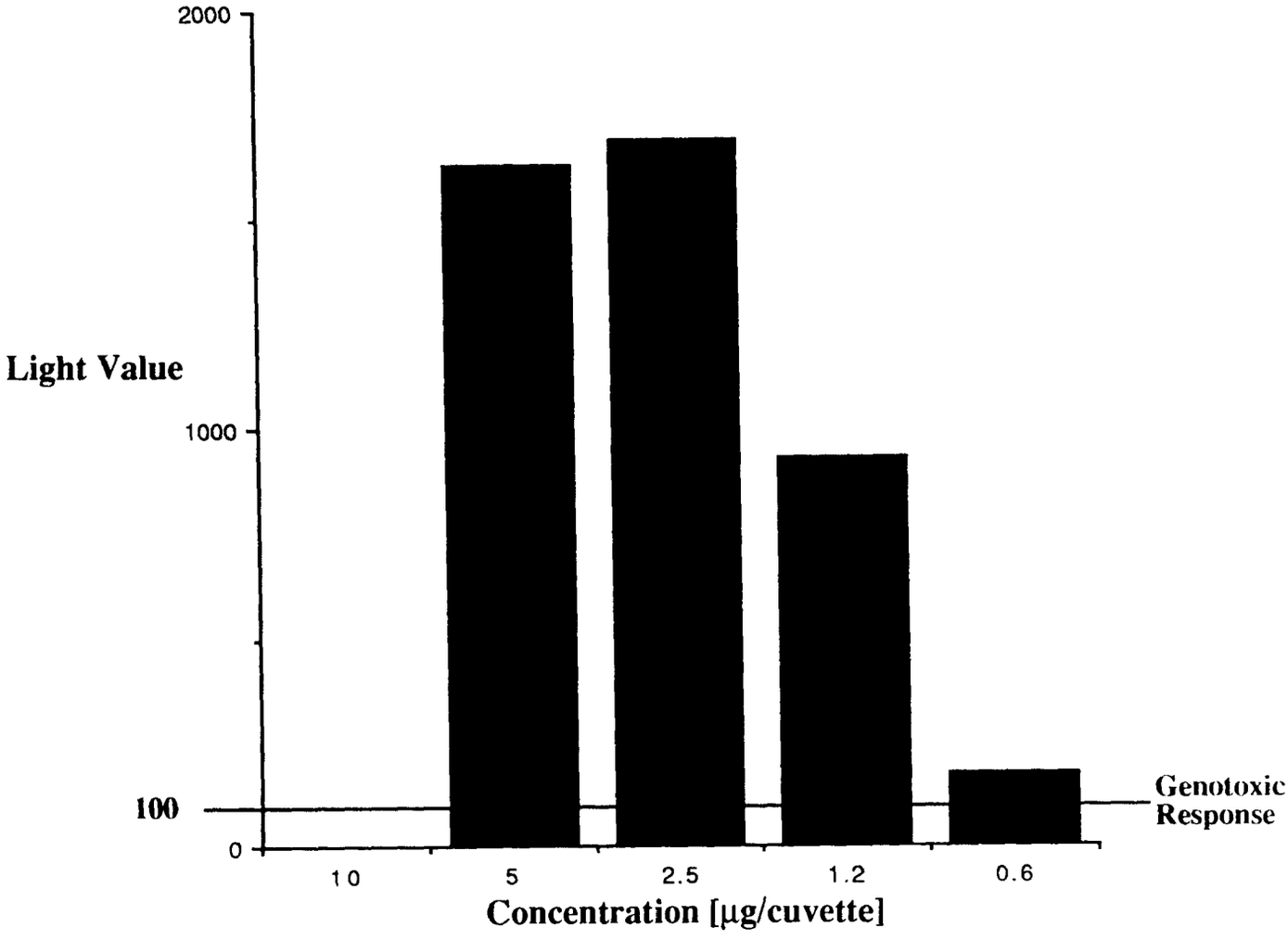

Figure 7.2 Genotoxicity of benzo(α)pyrene determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9).

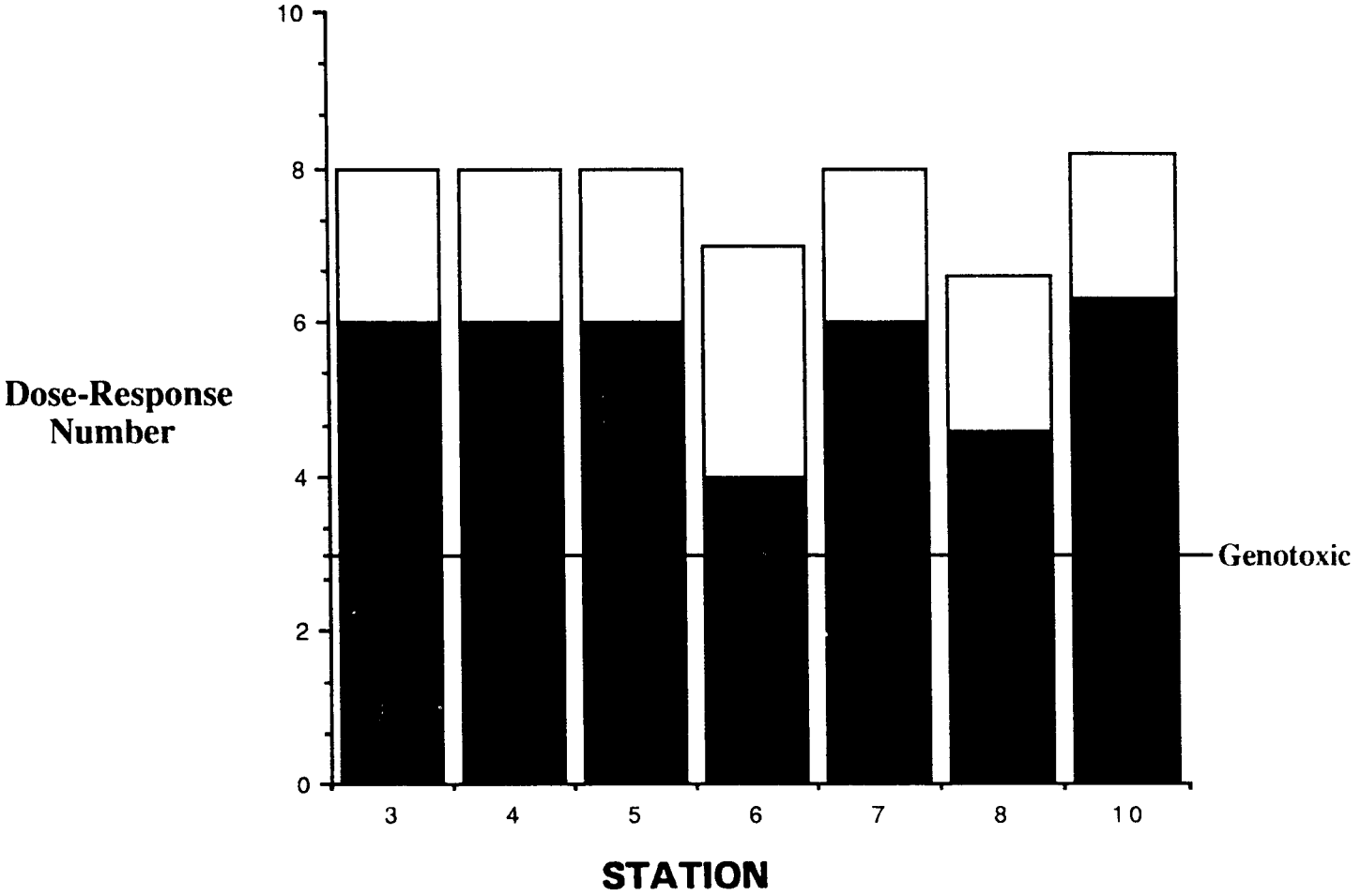

Figure 7.3 Genotoxicity of sediment extracts from Indiana Harbor determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9). Dose-response number = responses/dilution series; mean (dark bar) of three replicates with standard deviation (white bar).

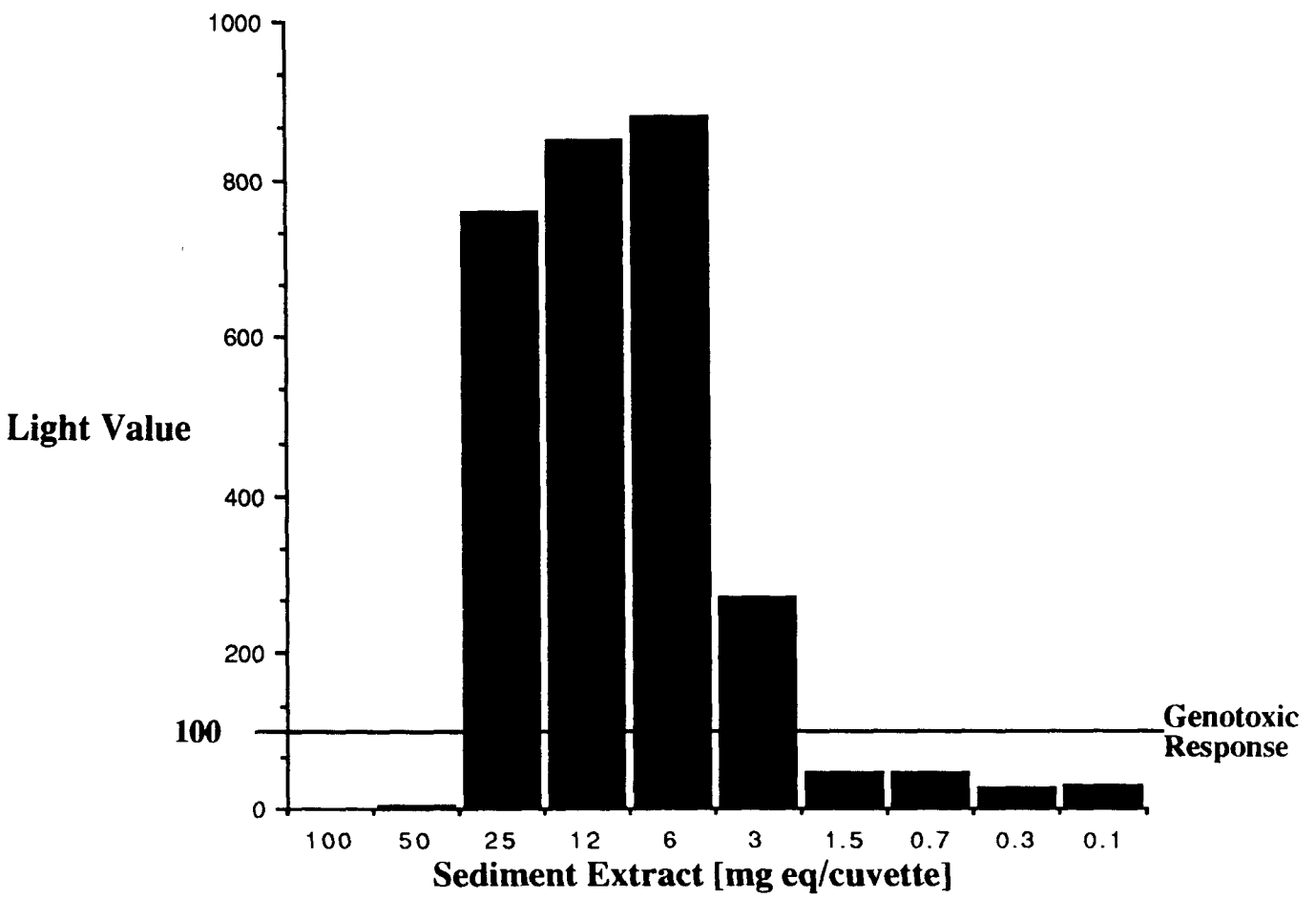

Figure 7.4 Genotoxicity of sediment extracts from Indiana Harbor determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9): single data set from Station 6.

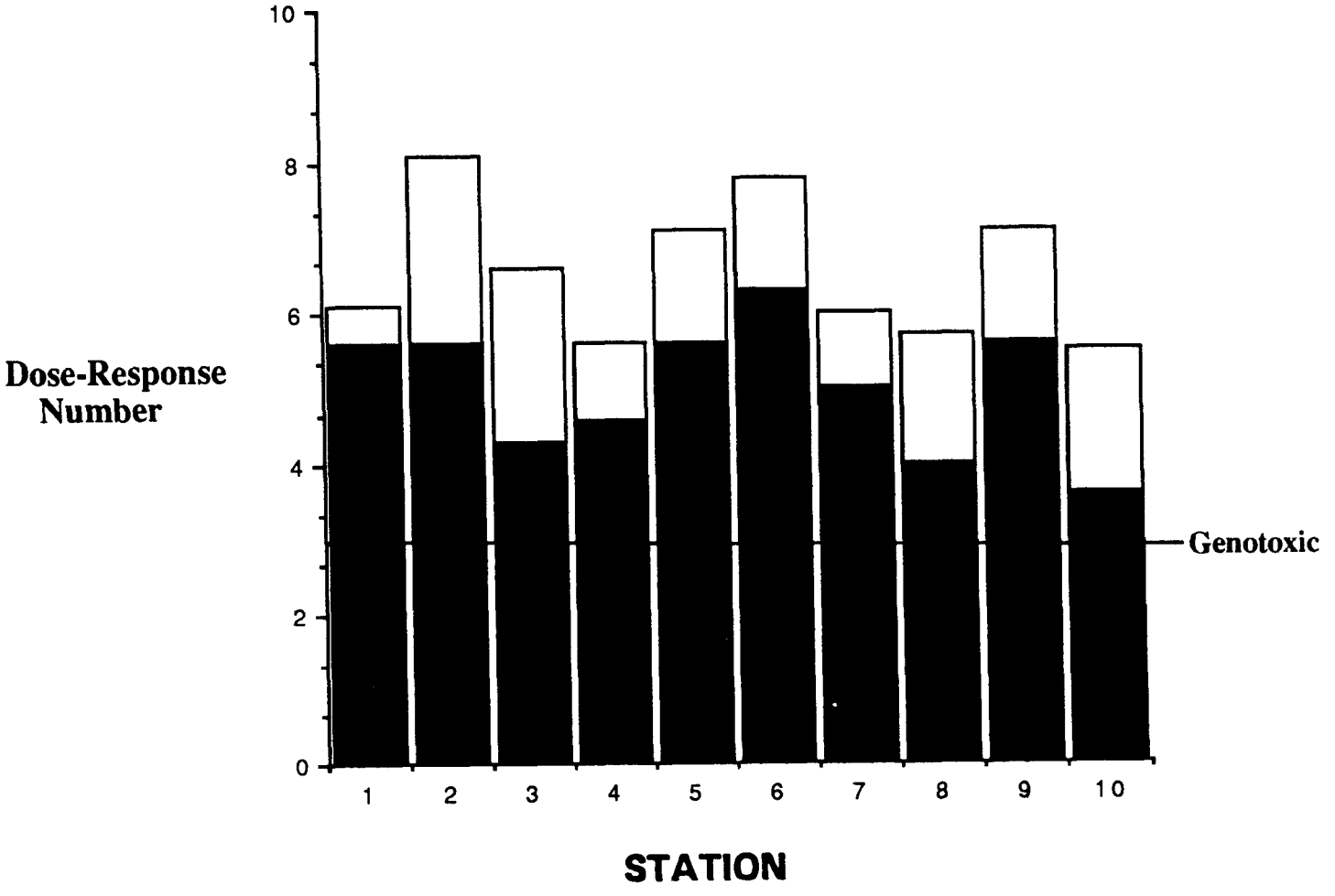

Figure 7.5 Genotoxicity of sediment extracts from Buffalo River determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9). Dose-response number = responses/dilution series; mean (dark bar) of three replicates with standard deviation (white bar).

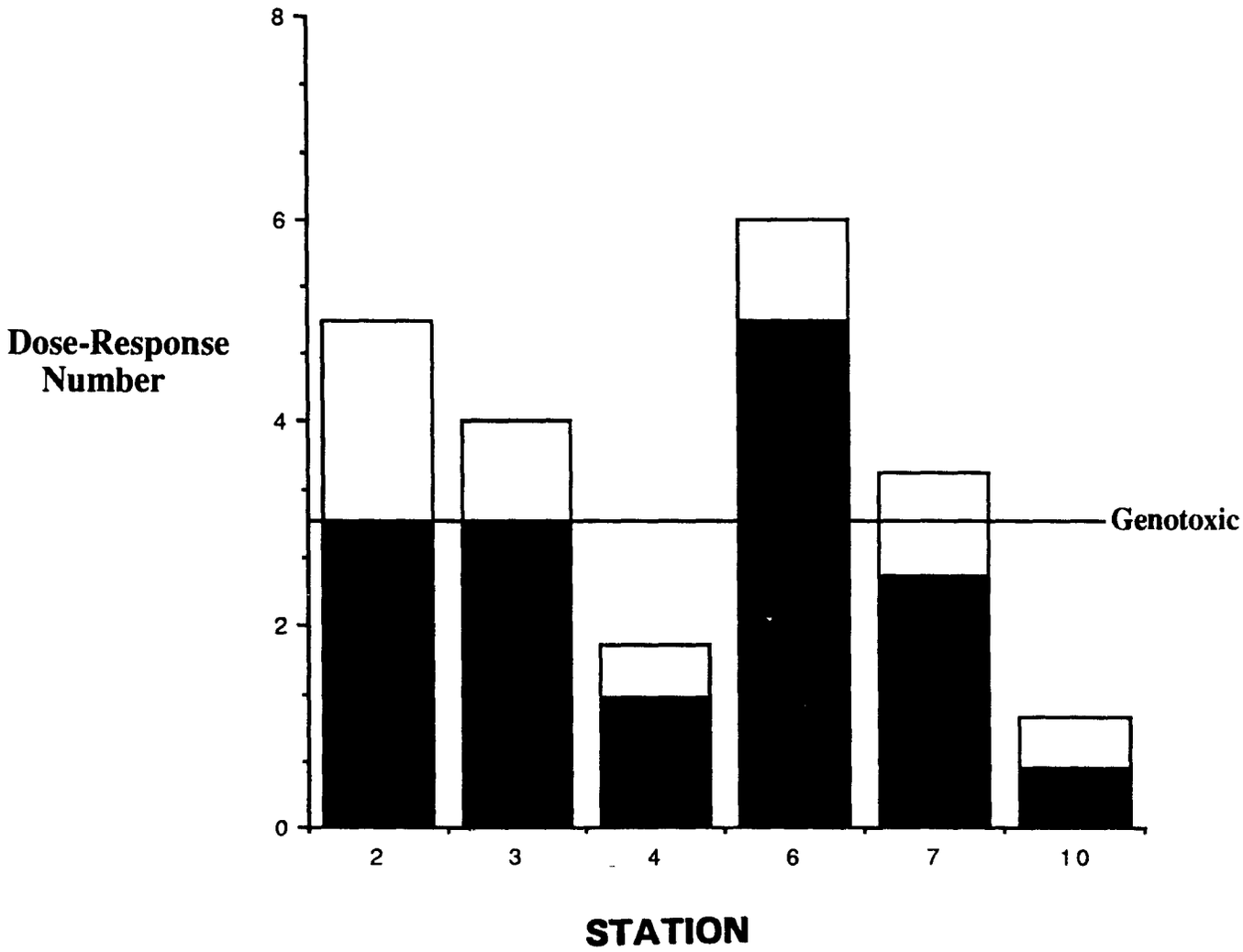

Figure 7.6 Genotoxicity of sediment extracts from the first survey of Saginaw River determined with the activated Mutatox Assay (Photobacterium-/rat hepatic S9). Dose-response number = responses/dilution series; mean (dark bar) of three replicates with standard deviation (white bar).

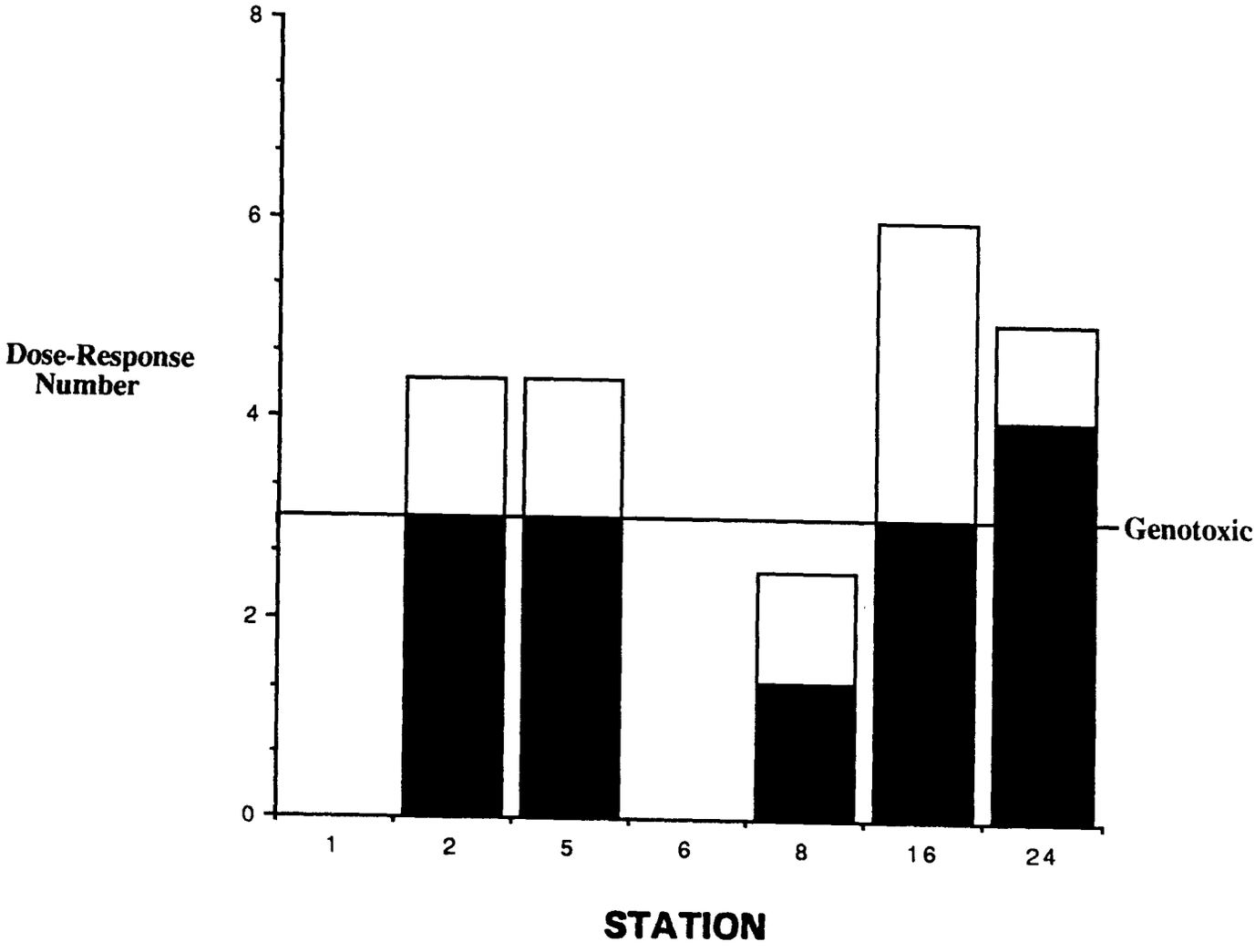

Figure 7.7 Genotoxicity of sediment extracts from the third survey of Saginaw River (core samples) determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9). Dose-response number = responses/dilution series; mean (dark bar) of three replicates with standard deviation (white bar).

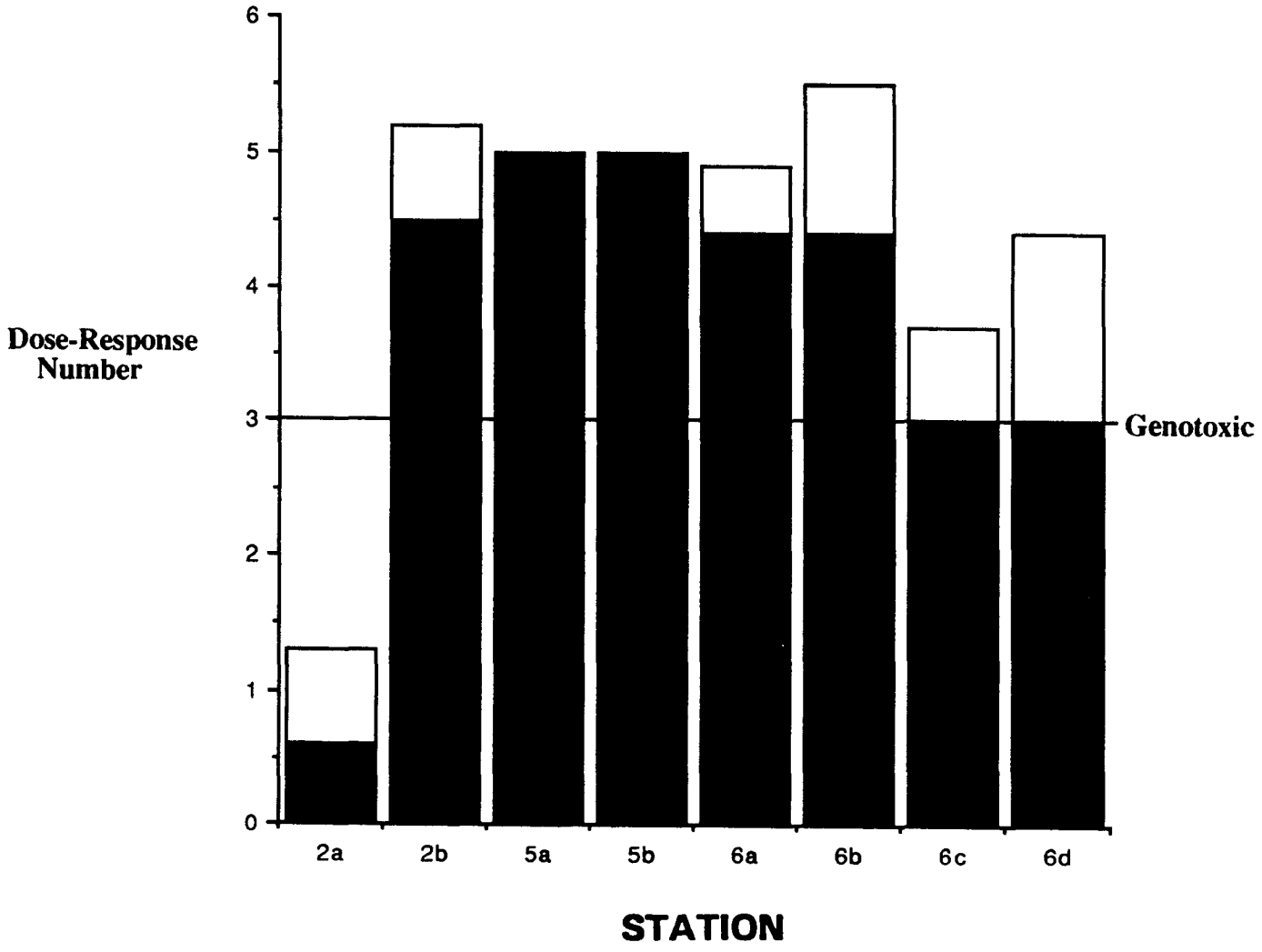

Figure 7.8 Genotoxicity of sediment extracts from the third survey of Saginaw River (grab sample) determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9). Station number and approximate core depth: 2a, 20 to 50 cm; 2b, 50 to 88 cm; 5a, 0 to 60 cm; 5b, 60 to 120 cm; 6a, 0 to 60 cm; 6b, 12 to 33 cm; 6c, 30 to 81 cm; 6d, 60 to 120 cm. For additional detail on core samples, see Table 2.1. Dose-response number = responses/dilution series; mean (dark bar) of three replicates with standard deviation (white bar).

Table 7.1 Genotoxicity[a] of arylamines and polycyclic aromatic hydrocarbons in sediment extracts determined with the activated Mutatox Assay (Photobacterium/rat hepatic S9).

PROGENOTOXIN	MPC (μg/cuvette)	LDC (μg/cuvette)	DOSE-RESPONSE NUMBER
2-ACETAMIDOFLUORENE	5.0	<0.6	5
2-AMINOANTHRACENE	5.0	<0.6	5
2-AMINOFLUORENE	5.0	<0.6	5
2-AMINONAPHTHALENE	5.0	<0.6	5
BENZO(α)PYRENE	5.0	<0.6	5
3-METHYLCHOLANTHRENE	5.0	<0.6	5
PYRENE	5.0	<0.6	5

[a]MPC = maximum peak concentration; LDC = lowest detected concentration; Dose-response number = number of genotoxic responses/dilution series; sediment extract = procedural control.

Table 7.2 Genotoxicity of progenotoxic chemicals[a] in complex mixtures determined with the activated Mutatox Assay (*Photobacterium*/rat hepatic S9).

CHEMICAL	TREATMENT		
	BOILING S9	WITHOUT S9	WITH S9
PROGENOTOXINS	ND[b]	ND	GENOTOXIC
NON-GENOTOXINS[c]	ND	ND	ND
PROGENOTOXINS + NON-GENOTOXINS	ND	ND	GENOTOXIC

[a]Progenotoxins: 2-acetamidofluorene, 2-aminoanthracene, 2-aminofluorene, 2-aminonaphthalene, benzo(α)pyrene, 3-methylcholanthrene, and pyrene.
[b]ND = not detected
[c]Non-genotoxins: complex mixture of carbofuran (carbamate insecticide), di-2-ethylphthalate (plasticizer), malathion (organophosphate insecticide), simazine (triazine herbicide), and permethrin (synthetic pyrethroid insecticide).

Table 7.3 Comparison of the Mutatox Assay and Ames Test[a].

	MUTATOX	AMES
TEST ORGANISM	Photobacterium	Salmonella
BACTERIAL REQUIREMENT	Single isolate	Usually one to four isolates
ENDPOINT	Light emission	Colony formation
EXOGENOUS ACTIVATION	Optional	Optional
TEST DURATION	16-24 h	48-72 h
TEST TEMPERATURE	23±1°C	37°C
RELATIVE SENSITIVITY[b]	≤1.0 μg/cuvette	≤1.0 μg/plate
STERILITY	Optional	Essential
PROCEDURE	Simple	Complex
INSTRUMENTATION	Luminometer or scintillation counter	Particle counter[c]
COST[d]	Low	High
SCIENTIFIC DEVELOPMENT	Validation phase	In common use[e]

[a]Photobacterium/activation and Salmonella/activation genotoxic assays.

[b]Rat S9 activation with 2-acetamidofluorene, aflatoxin B_1, 2-aminoanthracene, 2-aminofluorene, 2-aminonapthalene, benzo(α)pyrene, 3-methylcholanthrene, and pyrene.

[c]Particle counter is essential for enumeration of large samples.

[d]Labor and materials factor.

[e]Extensive literature and validation.

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