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EPA Evaluation of Toxicity and Bioaccumulation of Contaminants in Sediments Samples from Waukegan Harbor, Illinois

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ABSTRACT

Waukegan Harbor in Illinois was designated as a Great Lakes Area of Concern due to high sediment concentrations of polychlorinated biphenyls (PCBs). The objective of this study was to evaluate sediment toxicity of 20 samples collected after remediation (primarily dredging) of Waukegan Harbor for PCBs. A 42-d whole-sediment toxicity test with the amphipod *Hyalella azteca* (28-d sediment exposure followed by a 14-d water-only exposure), a 28-d whole-sediment bioaccummulation test with the oligochaete *Lumbriculus variegatus*, and sediment-toxicity tests with Microtox® were conducted to evaluate sediments from Waukegan Harbor. Endpoints measured were survival, growth, and reproduction (amphipods), bioaccummulation (oligochaetes), and luminescent light emission (bacteria). Survival of amphipods was significantly reduced in 6 of the sediment samples relative to the control. Growth of amphipods (either length or weight) was significantly reduced relative to the control in all samples at Days 28 and 42. However, reproduction of amphipods identified only 2 samples as toxic relative to the control. Detection limits in the analysis of tissue samples from the bioaccumulation exposure of oligochaetes were too high to evaluate differences among sites. The Microtox® basic test identified the organic extracts of sediment from only one site as toxic, whereas, the Microtox® solid-phase test identified about 50% of the sites as toxic. A significant negative correlation was observed between reproduction of amphipods and the concentration of three PAHs normalized to total organic carbon. Sediment chemistry and toxicity data were evaluated using sediment quality guidelines (consensus-based Probable Effect Concentrations (PECs)). Results of these analyses indicate that sediment samples from Waukegan Harbor were toxic to *H. azteca* contaminated at similar contaminant concentrations as sediment samples that were toxic to *H. azteca* from other areas of the United States. The relationship between PECs and the observed toxicity was not as strong for the Microtox® test. The results of this study indicate that the first phase of sediment remediation in Waukegan Harbor successfully lowered concentrations of PCBs at the site. While the sediments were generally not lethal to amphipods, there are still sublethal effects of contaminants in the sediment at this site (associated with elevated concentrations of metals, PCBs and PAHs).

INTRODUCTION

Federal, state and provincial governments are required under The Great Lakes Water Quality Agreement to designate geographic Areas of Concern (AOCs) in the Great Lakes where conditions have caused or are likely to cause impairment of beneficial uses (ILEPA 1994). Due to high concentrations of polychlorinated biphenyls (PCBs) in and around the harbor, the U.S. Environmental Protection Agency (USEPA), the International Joint Commission (IJC) and Illinois Environmental Protection Agency (ILEPA) designated Waukegan Harbor, IL and 42 other sites in the Great Lake region as AOCs in 1981. Other contaminants of concern that were identified in Waukegan Harbor sediments included: (1) heavy metals, (2) total nitrogen, (3) volatile solids, (4) polynuclear aromatic hydrocarbons (PAHs) and (5) phenols (IJC 1988).

Land use in the Waukegan Harbor AOC is primarily industrial, but also includes several utilities. The primary sources of contaminants currently include discharges of industrial effluents, releases of municipal wastewater, and runoff from urban areas. There are no agricultural land uses in the watershed of the Waukegan Expanded Study Area (ILEPA 1994). Contaminant concentrations and toxicity of sediments from Waukegan Harbor has previously been monitored at various locations in the harbor (Ross et al. 1988; Burton et al. 1989; Ingersoll and Nelson 1990; Risatti et al. 1990; Lesnak 1997; ILEPA 1999). The results of the chemical analyses showed that Waukegan Harbor sediments were highly contaminated with PCBs, PAHs, heavy metals, and several other substances.

Burton et al.(1989), testing sediments from Waukegan Harbor that corresponded to sites in the current study, reported no significant toxicity to *H. azteca* in 48-hr whole-sediment exposures. However, Ingersoll and Nelson (1990) reported a significant reduction in survival and growth of *H. azteca* after 29-d of exposure to these sediments samples.

In response to concerns about sediment quality conditions, a Remedial Action Plan (RAP) was developed to address the issues related to the contaminants of concern in the harbor. Remediation actions in the harbor included: (1) removal of leaking underground storage tanks, (2) removal and securing of free tar at the Waukegan Tar Pit, (3) construction of Slip 4 in the northeast portion of the harbor to replace Slip 3 (Figure 1), (4) isolation of Slip 3 permanently from the harbor and its conversion into a containment cell, which was to be capped once sediment settling occurred, (5) dredging of contaminated sediments (about 5000 m^3 of PCB-contaminated sediment

was removed) from the harbor, and (6) treatment of sediments having PCB concentrations of above 500 µg/g using the Taciuk process, which removes over 97% of the PCBs from sediment by thermal treatment (USEPA 1993). Treated sediments were then placed in the containment cell (Slip 3).

Since the dredging process was completed in 1992, there has been no assessment of contamination or toxicity of sediments within the harbor. An assessment of current harbor sediments was designed to determined if remediation of the harbor was successful. Three approaches were used to assess the nature and extent of sediment contamination in Waukegan Harbor: (1) whole-sediment toxicity tests with the amphipod *Hyalella azteca* (USEPA 1999; ASTM 1998a), (2) whole-sediment bioaccummulation tests with the oligochaete *Lumbriculus variegatus* (USEPA 1999; ASTM 1998b), and (3) solid-phase sediment tests and basic toxicity tests with Microtox® (Johnson and Long 1998). In addition, the concentrations of chemicals of concern were measured in all of the sediment samples collected from the harbor.

METHODS AND MATERIALS

Description of Study Area

Waukegan Harbor is located on the western shore of Lake Michigan, about 60 km north of Chicago near the town of Waukegan, IL (Figure 1). The harbor is largely a manmade structure, which is about 15 ha in area with water depth ranging from 4.5 to 6.5 meters. The harbor bottom consists of three distinct layers of sediments: (1) a 1 to 3.2 meter layer of organic silt, (2) 2.7 meters of coarse sand, and (3) the natural clay harbor bottom (Mason and Hanger 1980).

Sample Collection, Handling, and Storage

Sediment samples were collected by personnel from the Illinois Environmental Protection Agency (ILEPA) from April 17 to 19, 1996 from 19 sites in Waukegan Harbor, IL (a second sample was collected from site WH-11 as a duplicate sample; (Figure 1). All sediment samples were collected using a petite ponar grab sampler (225 cm^2 area) from about the upper 6 cm of the sediment surface except for site WH-01. Site WH-01 was sampled to a depth of about 55 cm using a 58.4 cm vibrating core sampler. Samples were held in the dark on ice at 4^o C in high-density polyethylene containers before shipment to the Columbia Environmental Research Center (CERC) in Columbia, MO. The control sediment was a formulated sediment (80% sand and 1.5%

total organic carbon (TOC)) described in Kemble et al. (1999). All sediment toxicity and bioaccumulation tests were started within three months of sample collection from the field. Samples of sediment from multiple grabs were composited to obtain a minimum of 3 L of sediment/station (1 L for amphipod testing and Microtox® testing, 1 L for bioaccummulation testing, and 1 L for physical and chemical analyses of sediments). Sediments were not sieved to removed indigenous organisms; however, large indigenous organisms and large debris were physically removed (using forceps) during homogenization of samples in the laboratory.

Culturing of Test Organisms

Amphipods were mass cultured at 23° C with a luminance of about 800 lux using 80-L glass aquaria containing 50 L of CERC well water (hardness 283 mg/L as $CaCO_3$, alkalinity 255 mg/L as $CaCO_3$, pH 7.8; Tomasovic et al. 1995). Artificial substrates were placed in the amphipod culture aquaria (six 20 cm sections/aquarium of "coiled-web material"; 3M Corp., Saint Paul, MN). Known-age amphipods were obtained by isolating mixed aged adults in a 5-mm mesh sieve (#35 U.S. Standard size) inside a pan containing about 2 cm of well water. After 24 h, well water was sprinkled through the sieve, flushing <24-h old amphipods into the pan below. These <24-h old amphipods were then placed into 2-L beakers for 7 d before the start of the sediment exposure. Isolated amphipods were fed 10 ml of yeast-Cerophyl®-trout chow (YCT; USEPA 1999) and 10 ml of *Selenastrum capricornutum* (about 3 x 10^7 cells/ml) on the first day of isolation. Five ml of each food type was added to isolation cultures twice (about every other day) before the start of the sediment exposure (USEPA 1999). Oligochaetes were mass cultured in 80-L glass aquaria containing 50 L of well water using brown (unbleached) paper towels as substrate (USEPA 1999) and were removed directly from culture aquaria for testing (USEPA 1999; Brunson et al. 1998).

Sediment Exposures

- Sediment Preparation: Test sediments were homogenized in a stainless steel bowl using a plastic spoon and added to exposure beakers 1 d before test organisms were added (Day -1). Sediments from WH-04, WH-05, WH-09, WH-15, and WH-16 were not evaluated in the bioaccumulation exposure due to insufficient amounts of sediment. Formulated sediment was added to beakers on Day -1 and then hydrated with overlying

water (well water). Subsamples of sediment were then collected for pore-water isolation and physical and chemical characterizations. An oil sheen or petroleum odor was evident in all of the sediments except for samples from WH-05 and WH-09. Several of the sediment samples were observed to contain globs of oily material (WH-08, WH-11R, and WH-17).

- Amphipod Toxicity Exposures: Toxicity tests with *Hyalella azteca* were conducted for a total of 42 d (28 d of sediment exposure followed by 14 d of water only exposure; Ingersoll et al. 1998). Endpoints measured in the amphipod exposures included survival and growth (both length and weight) on Day 28, survival on Day 35, and survival and growth on Day 42, and reproduction (number of young/female produced from Day 28 to Day 42). The purpose for transferring surviving amphipods from sediment to water at Day 28 is to monitor reproduction. At about Day 28, amphipods used to start the exposures begin to go into amplexus followed by release of their first brood (Ingersoll et al. 1998).

  Amphipods were exposed to 100 ml of sediment with 175 ml of overlying water in 300-ml beakers (eight replicates/treatment; 4 replicates for Day 28 survival and growth and 4 replicates for Day 28 to 42 survival, growth and reproduction) at 23° C. The photoperiod was 16:8 h light:dark at an intensity of about 200 lux at the surface of the exposure beakers. Each beaker received 2 volume additions/d of overlying water starting on Day -1 (Zumwalt et al. 1994). One diluter cycle delivered 50 ml of water to each beaker (diluters cycled every 4 h ± 15 min). Tests were started on Day 0 by placing 10 amphipods (7-d old) into each beaker using an eyedropper. Amphipods in each beaker were fed 1.0 ml YCT (1.7 to 1.9 g/L) in a water suspension daily (USEPA 1999; ASTM 1998a). If excessive mold (>60% sediment surface) was observed on the sediment surface of any of the beakers in a treatment, feeding was withheld for that day in all of the beakers for that test treatment (feeding was withheld in the WH-12 treatment on Days 13 and 14; USEPA 1999; ASTM 1998a). Beakers were observed daily for the presence of animals, signs of animal activity (i.e., burrowing), and to monitor test conditions (mainly water clarity).

  On Day 28, amphipods were isolated from each beaker by pouring off most of the overlying water, gently swirling the remaining overlying water and upper layer of sediment and washing the sediment through a No. 50 (300-µm opening) US Standard stainless steel

sieve. The materials that were retained on the sieve were washed into a glass pan and the surviving amphipods were removed. Amphipods from 4 of the replicates were counted and preserved in 8% sugar formalin in a scintillation vial for subsequent length and weight measurements (Kemble et al. 1994; Ingersoll et al. 1998).

Amphipods from the remaining 4 replicates/treatment were placed in a 300-ml beaker containing 175 ml of overlying water and a 5 cm x 5 cm piece of Nitex® screen (Nylon (Nitex®) bolting cloth; 44% open area and 280-µm aperture; Wildlife Supply Company, Saginaw MI). In subsequent studies, Ingersoll et al. (1998) reported improved amphipods survival in water-only exposures when a nylon 3-M mesh substrate was substituted for the Nitex® screen. Each beaker received two volume additions of water and 1.0 ml of the YCT suspension daily. Reproduction of amphipods was then measured on Days 35 and 42 by counting the number of young in each of these water-only beakers. Production of young amphipods in these beakers was monitored by removing and counting the adults and young in each beaker. On Day 35, the adults were returned to the same water-only beakers. On Day 42 adult amphipods were preserved with sugar formalin for growth and sex determination (mature male amphipods were distinguished by the presence of an enlarged second gnathopod).

A Zeiss® Interactive Digital Analysis System in combination with a Zeiss SV8 stereomicroscope at a magnification of 25x was used to measure amphipods following methods described in Kemble et al. (1994). After measuring length, dry weight of test organisms was determined by combining all of the organism from each replicate in a pre-dried aluminum weigh pan and drying for 24 h at 60 to 90° C (Ingersoll et al. 1998).

- Oligochaete Bioaccumulation Exposures: Sediment preparation and test conditions for the oligochaete exposure were similar to those described for the amphipod exposure except for the following: (1) oligochaetes were exposed for 28 d in 4-L test containers containing 1 L of sediment and 3 L of overlying water (Brunson et al. 1998), (2) about 2.6 g of unblotted oligochaetes were transferred to each test beaker (this approach represents about 2 g of oligochaetes), (3) one replicate was tested for each sediment (samples from sites WH-04, WH-05, WH-09 WH-15, and WH-16 were not tested due to a insufficient volume of sediment), and (4) bioaccummulation was the endpoint evaluated. Three control

samples of oligochaetes (about 2.6 grams each) were collected at the start of the exposure. Control samples were blotted with a Kimwipe® paper tissue to remove excess water before weighing. Each control sample was then placed into a 125 ml glass jar and stored frozen until analyzed.

On Day 28 of the exposure, oligochaetes were isolated from each beaker by washing the sediment through a No. 18 (1.0-mm opening) followed by a No. 50 (300µm opening) US Standard stainless steel sieves Brunson et al. (1998). The material retained on each sieve was washed into several clear glass pans and all oligochaetes were removed from the debris using either an eyedropper or dental hook. *Lumbriculus variegatus* were separated from native oligochaetes based on behavior (native worms often form a tight, spring-like coil, whereas *L. variegatus* do not; USEPA 1999). Once isolated, *L. variegatus* from each beaker were cleaned of any detritus and held for a 24 h depuration period in 1-L water-only beakers to clear their gut contents (USEPA 1999; Note: Subsequent recommendations by USEPA (1999) recommend a shorter depuration period of 6 to 8 hours). After 24 h, surviving *L. variegatus* were isolated, cleaned of any remaining debris, and transferred to a tarred weigh boat. Samples were then blotted dry with a Kimwipe, weighed, placed in a 125 ml glass jar and frozen at -22° C until analysis by ILEPA. Due to the low number of native oligochaetes present in sediment samples collected from the field sites, tissue analysis was conducted on native oligochaete samples from only 4 sites (WH-02, WH-03, WH-12, and WH-14). Native oligochaete samples were processed similarly to the *L. variegatus* samples.

- Water Quality: About 170 ml of pore water was isolated from about 500 ml of sediment by centrifugation at 4° C for 15 min at 5200 rpm (7000 x G). Immediately after pore water was isolated, the following water quality parameters were measured: total sulfide, dissolved oxygen, pH, alkalinity, temperature, conductivity, total ammonia, and hardness (Kemble et al. 1993; 1997). Mean characteristics of porewater water quality (ranges in parentheses) are as follows: pH 7.43 (7.00 to 8.00); alkalinity 311 (210 to 466) mg/L; hardness 299 (236 to 380) mg/L; dissolved oxygen 4.1 (1.6 to 9.6) mg/L; conductivity 860 (599 to 3090) µs/cm @ 25° C; total ammonia 14.09 (0.39 to 63) mg/L; unionized ammonia 0.016 (0.002 to 0.065) mg/L; total sulfide 0.036 (<0.001 to 0.327)

mg/L; and hydrogen sulfide 0.010 (<0.001 to 0.097) mg/L (Appendix 1).

The following parameters were measured in overlying test water on Day -1 (the day before amphipods were placed into the beakers) and at the end of the toxicity test: dissolved oxygen, temperature, conductivity, pH, alkalinity, total hardness, and total ammonia. Methods used to characterize overlying water quality in the whole-sediment tests are described in Kemble et al. 1993; 1997. Dissolved oxygen, pH, and conductivity were also measured weekly in the overlying water. Temperature in the water baths holding the exposure beakers was measured daily. Overlying water pH, alkalinity, total hardness, conductivity and total ammonia measurements were similar among treatments, the control, and the in flowing test water. Dissolved oxygen measurements were at or above acceptable concentrations (2.5 mg/L; ASTM 1998a) in all treatments throughout the exposure (Appendix 2). An exception to this was dissolved oxygen concentrations were below 2.5 mg/L on Day 13 in the WH-12 sample (2.4 mg/L). However, dissolved oxygen concentrations were above 2.5 mg/L from Day 19 until end of the test. Means (ranges in parentheses) of overlying water quality for each parameter are as follows: pH 8.24 (8.02 to 8.40); alkalinity 264 (255 to 292) mg/L; hardness 298 (282 to 325) mg/L; dissolved oxygen 6.16 (4.19 to 6.72) mg/L; conductivity 641 (627 to 685) µs/cm @25° C; total ammonia 0.92 (0.25 to 2.81) mg/L; and unionized ammonia 0.010 (0.002 to 0.026) mg/L (Appendix 2).

- Microtox® Exposures: The analyses of whole-sediment and organic-sediment extracts were conducted according to the Microtox® basic and Microtox® solid-phase protocols and QA/QC performance standards (Microbics Corporation 1992). All essential test components, including analyzer, liquid reagents, and freeze-dried bacteria were obtained from AZUR Environmental. The Microtox® solid-phase toxicity test was performed on each whole-sediment samples and the Microtox® basic test was conducted on organic extracts of these samples following procedures used in testing Puget Sound sediments (Johnson 1999) and Pensacola Bay sediments (Johnson and Long 1998).

- Organic Extraction of Sediments: Organic extracts of sediment for the Microtox® basic test were prepared by Columbia Analytical Services, Inc., Kelso, WA using

procedures described in Johnson and Long (1998) and were then shipped to the CERC. The extractions and transfers were conducted under a laminar flow hood to limit exposure of the samples to light. All sediment samples and extracts were stored in the dark at 4EC. To prepare the organic extracts, excess water was decanted and shells, pebbles, wood and similar debris were discarded before the initial homogenization of the sediment samples. Each sediment sample was then centrifuged at 5EC for five minutes at 1000 x G. Water was removed by decanting with a Pasteur pipette. The moisture content of each sample was determined. Ten g of sediment were weighed, recorded, and placed into a 50 ml centrifuge tube and rinsed using dichloromethane (DCM). Sodium sulfate (15 g) was added to each centrifuge tube and mixed thoroughly. Spectral grade DCM (30 ml) was then added and mixed. The mixture was shaken for 10 seconds, vented, and tumbled overnight. Each sample was then centrifuged for 5 minutes at 1000 x G and the extract poured into a Kuderna-Danish flask. A Snyder column was attached to the flask, and the DCM extract was concentrated with steam to a final volume of < 2 ml. Acetone (5 ml) was added to the flask and the volume was concentrated to about 2 ml. This acetone procedure was then repeated. The extract was quantitatively transferred to a DCM-rinsed 10 ml volumetric flask using acetone to rinse the flask. The extract was evaporated and concentrated under a gentle flow of nitrogen gas and brought to a final volume of 1 ml by adding Dimethylsulfoxide (DMSO). Organic extracts were typically tested at concentrations from 1.5 to 50 mg equivalent wet weight of sediment/ml. A negative control (extraction blank) was prepared using DMSO, which was the carrier solvent used in the test.

- Microtox® Basic Test: A suspension of luminescent bacteria, *Vibrio fisheri*, formerly *Photobacterium phosphoreum*, (B-NRL 1117, Microbics Corp.) was thawed and hydrated. An aliquot of 10 µL of the bacterial suspension was transferred to a test vial containing the standard diluent (2% NaCl) and equilibrated to 15º C using a temperature-controlled photometer. The amount of light lost per sample was proportional to the toxicity of that test sample. Light loss was expressed as a gamma value and defined as the ratio of light lost to light remaining. The relative sensitivity of Microtox® has been reported by Kaiser and Palabrica (1991) and Johnson and Long (1998).

To determine sediment extract toxicity, each sample was diluted into four test concentrations. Because organic sediment extracts were obtained with DCM, a strong non-polar solvent, the final extract was evaporated and redissolved in DMSO to a final volume of 1 g wet weight/ml. Dimethylsulfoxide was compatible with the Microtox® system because of its low test toxicity and it's ability to solubilize a broad spectrum of non-polar organic compounds (Johnson and Long 1998). The log of gamma values from these four dilutions was plotted and compared with the log of the sample's concentrations. The concentration of the extract that inhibited luminescence by 50% after a 5 minute exposure period (EC_{50}) was determined and expressed as mg equivalent sediment wet weight. Data were reduced using the Microtox® Data Reduction software package (Microbics Corporation 1992). All EC_{50} values reported were 5-minutes readings with 95% confidence intervals. All tests were performed in triplicate.

- Microtox® Solid Phase: The solid-phase test (SPT), similar to the basic test in experimental design, exposes bioluminescent bacteria directly to sediment-bound contaminants in an aqueous suspension of the test sample. Sediment samples were first centrifuged at 5EC (1000 x G) to remove the excessive water and the remaining residual was then homogenized. A 300 mg aliquot of the sample was then placed with solid-phase NaCl diluent in a SPT tube, stirred with a vortex mixer, and used to prepare three controls and 12 tubes in a 1:2 dilution series. Glowing luminescent bacteria in stationary growth phase were then directly introduced into each SPT tube. This sample was blended with a vortex mixer for several seconds and incubated for 20 min at 15° C in a temperature-controlled water bath. (Note the 25 min total exposure period was only used for the SPT). After incubation a special filter column was inserted into the SPT tube to facilitate the separation of solid and liquid materials. The supernatant containing treated bioluminescent bacteria was transferred into standard cuvettes that were placed in a temperature-controlled luminometer for a 5 min stabilization period. The light emissions were then read with the luminometer. The standard dose-response curve method was used to determine a 50 percent loss of light in the test bacteria. The luminometer and supporting computer software with a standard log-linear model were used to calculate EC_{50} values. The

toxicological endpoint of the SPT was defined with an EC_{50} value expressed as sediment wet weight/ ml, µg/g, or as percent of sample/ml. All SPTs were performed in triplicate.

Physical Characterization of Sediment Samples

Physical characterization of sediments included: (1) percentage water (Kemble et al. 1993), (2) particle size analysis using a hydrometer (Foth et al. 1982; Gee and Bauder 1986; Kemble et al. 1993), and (3) total organic carbon using a coulometric titration method (Cahill et al. 1987; Kemble et al. 1993). All physical characterizations included analysis of duplicate samples. Differences in percentage water for duplicate samples ranged from 0% in sediment samples from WH-02 to 65% in sediment samples from WH-17. Duplicate samples of control sediment, sucrose standards and blanks were analyzed for sediment total organic carbon. Precision and accuracy of the coulometric technique used were tested against National Bureau of Standards and Standard Reference Materials (NBS-SRM) with an error of less than 0.03% of the excepted values (Cahill et al. 1987). Differences between duplicate TOC samples ranged from 9% in sediment samples from WH-18, to 43% in sediment samples from WH-11R.

Chemical Characterization of Sediment Samples

Chemical analyses of sediment samples included: (1) acid volatile sulfides (AVS) and simultaneously extractable metals, (2) total metals, and (3) Organochlorine Pesticides (OCs), Polychlorinated Biphenyls (PCBs), and Polycyclic Aromatic Hydrocarbons (PAHs).

- Acid-volatile Sulfides (AVS) and Simultaneously Extractable Metals (SEM): Sediments were subsampled for AVS and SEM at the start of the amphipod exposures. Concentrations of AVS in sediment samples were determined using a silver/sulfide electrode and concentrations of SEM were determined using atomic spectroscopy (Brumbaugh et al. 1994). Quality control for sediment samples analyzed for AVS and SEM determinations included a duplicate sample, procedural blanks, a reference sediment, and pre-extraction spikes. For each analyte analysis, spikes (post-extraction) and a calibration solution were also analyzed. Recoveries of pre-extraction blank spikes (method blanks) ranged from 79 to 105% for all SEM elements.

- Total Metals: Sediment samples were subsampled for total metals and shipped to ILEPA for analysis. The total metals analyses included: Ag, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Zn. Analysis of Waukegan Harbor sediment samples for total metals was conducted in accordance to ILEPA Quality Assurance requirements (ILEPA 1987).

- Organochlorine Pesticides (OCs), Polychlorinated Biphenyls (PCBs), and Polycyclic Aromatic Hydrocarbons (PAHs): A complete list of the PAHs and OCs analyzed for in the sediment samples are listed in Appendix 3. Sediment samples were subsampled in the field and shipped to ILEPA for organochlorine pesticide (OCs) and polychlorinated biphenyls (PCBs) analysis. Chemical analyses by ILEPA also included: organometals (butyltins and methyl mercury), and PAHs. Analysis of harbor sediment samples for OCs and PCBs were conducted in accordance to ILEPA Quality Assurance requirements (ILEPA 1987).

  Due to high detection limits for an initial analyses of PAHs in sediment samples, additional subsamples were analyzed for PAHs by Mississippi State University. Ten grams of sediment and five grams of Hydromatix were weighed and placed into a Pesticide Residue Quality (PRQ) beaker. Samples were stirred until the mixture became a flowable powder which left the sides of the beaker clean. The sample was then poured through a PRQ powder funnel into a PRQ Accelerated Solvent Extractor (ASE) 33-ml with a 2-cm glass fiber filter in the bottom cell cap. The ASE cell was tapped to settle the sample and more Hydromatrix was added to fill the cell. The funnel, spatula, and beaker were rinsed with no more than 6-ml total of petroleum (pet) ether and the rinses were added to the cell. The cells top cap was placed on the cell and hand tightened. Samples were extracted with the ASE according to EPA Method 3545 with the following extraction conditions: 5-min heating cycle, 2X2-min static cycles, 60% solvent flush, 60 sec purge cycle, 100^{o} C @ 1500 psi, 1:1 pet ether:acetone. A 500-ml separatory funnel was prepared with 200-ml PRQ water and 15-ml PRQ saturated sodium chloride. The sample extract was rinsed into the separatory funnel with 50 ml of 1:1 acetone:pet ether. The separatory funnel was shaken vigorously for one minute and the layers allowed to separate, the pet ether was removed, and the water fraction extracted again with 50-ml pet ether. The combined pet ether was washed twice with 50 ml of water and concentrated in a Kuderna-Danish flask

to the appropriate volume. The sample was dissolved in 4 ml of methylene chloride and 2 ml was injected into a Waters high pressure Gel Permeation Chromatography (GPC; EPA Method 3640A). The fraction was concentrated by Turbovap and then exchanged to hexane. The sample was transferred to a column containing 20 grams of 1% deactivated silica gel column (silica gel is added to the column in a pet ether slurry) topped with 5 grams neutral alumina. Aliphatic and polynuclear aromatic hydrocarbon residues were fractioned by eluting aliphatics from the column with 100 ml pet ether (Fraction I) followed by elution of aromatics using first, 100 ml 40% methylene chloride/60% pet ether, then 50 ml methylene chloride (Combined elutes, Fraction II). The silica gel fraction II containing aromatic hydrocarbons was concentrated, reconstituted in methylene chloride to a known volume, and quantified by gas chromatography and mass spectrometry (GCMS). Quality control for sediment samples analyzed for PAH determinations included a duplicate sample, procedural blanks, and matrix spikes. The average recovery of spikes was 85% and ranged from 19 to 228% for all PAHs.

Chemical Characterization of Tissues Samples

Chemical characterization of oligochaete tissues samples were conducted by ILEPA. Tissue analyses included: total lipids, total polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). About 1 g (wet wt) of oligochaete tissue was placed into a scintillation vial and Na_2SO_4 added to remove moisture. The following solvents were then added to the vial: (1) 10 ml of $MeCl_2$ for extraction, (2) 1 ml of the surrogate for PCB analysis, or (3) 1 ml of the surrogate for PAH analysis. A microtip sonication extraction was performed in the scintillation vial. A 1 ml sample of this extract was collected, and placed in a weigh boat. The 1 ml aliquot was dried and weighed, and was used to determine amount of lipid in the tissue sample. The remainder of the extract was filtered into a graduated test tube, measured and split in half. The portion of the extract for the PCB analysis was solvent exchanged to hexane, and the portion for the PAH analysis solvent exchanged to acetonitrile. Tissue samples were quantively analyzed using both gas chromatograph, mass spectrometry, and high pressure liquid chromatography.

Analysis of oligochaete tissue samples were conducted in accordance with ILEPA Quality Assurance requirements. Due to high detection limits for all analytes, differences among sites could not be evaluated. Results of these analytes are presented in Appendix 4 and not discussed further in this report.

Data Analysis and Statistics

- Amphipod Toxicity Exposures: Before statistical analyses were performed, data for percent survival were arcsin transformed. Dry weight data were log transformed before statistical analysis. Amphipod reproduction data (number of young/female) were square root transformed before statistical analysis. Data for 28-d amphipod length had a normal distribution and were not transformed before statistical analysis. Comparisons of mean survival, 28-d length mean body weight, and reproduction were made using a one-way analysis of variance (ANOVA) with mean separation by Fisher's protected least significant difference test at alpha = 0.05 (Snedecor and Cochran 1982). Variance among treatment means for Day 42 amphipod body length was heterogenous. Therefore, a rank analysis of variance was performed and mean differences determined using a T-test on ranked means (at " = 0.05). A sample was designated as toxic when mean survival, growth, or reproduction was significantly reduced in the site sediments relative to the control sediment. Spearman rank correlation procedures were also used to evaluate relationships between the responses of amphipods exposed to the field-collected sediments and the physical and chemical sediment characteristics, the water quality (pore water and overlying water) characteristics, or PAH and OCs data normalized to TOC. Statistical significance for the rank correlations was established at 0.0005 for all comparisons (except for TOC normalized samples which was 0.001) to minimize
  experiment-wise error (Bonferroni method; Snedecor and Cochran 1982). All statistical analyses were performed with Statistical Analysis System programs (SAS 1994).

- Sediment Quality Guideline Evaluations: Sediment chemistry and toxicity data were evaluated using consensus-based probable effect concentrations (PECs) reported in Ingersoll and MacDonald (1999) and MacDonald et al. (1999a). These consensus-based PECs were derived by compiling effects-based sediment quality guidelines (SQGs) that

define the concentration of contaminants above which adverse effects are likely to be observed in sediment-dwelling organism. The SQGs that were used to calculate the consensus-based PECs included: Effects range medians, (ERMs; Long and Morgan 1991), Toxic effect thresholds (TET; EC and MENVIQ 1992), Severe effect levels, SEL; Persaud et al. 1993), and Probable effect levels, (PEL; Ingersoll et al. 1996; Smith et al. 1996). Consensus-based PECs were calculated as the geometric mean of the existing SQGs with a similar narrative intent (Ingersoll and MacDonald 1999; MacDonald et al. 1999a). The consensus-based PECs were used in the present study because they provide a unifying synthesis of SQGs, reflect causative rather than correlative effects, and account for the effects of contaminant mixtures in sediment (MacDonald et al. 1999b; Swartz 1999). We chose to evaluate sediment toxicity relative to fourteen consensus-based PECs which correctly predicted >75% of the samples as toxic in Ingersoll and MacDonald (1999). These PECs (in ug/g dry weight of sediment) included: arsenic (33), cadmium (4.98), chromium (111), copper (149), lead (128), nickel (48.6), zinc (459), naphthalene (0.561), phenanthrene (1.17), benzo(a)pyrene (1.45), chrysene, (1.29) pyrene (1.52), sum DDE (0.0313), and total PCBs (0.676). Ingersoll and MacDonald (1999) also reported a PEC for benz(a)anthracene of 1.050 ug/g; however, this PAH was not analyzed for in the present study.

- Microtox® Exposures: Summary EC_{50} values are reported as the mean of three replicates, with variability expressed as coefficient of variations. A toxicity index was used to determine when a chemical contaminant is toxic in the Microtox® tests, that is when a potential pollutant is harmful to the bioluminescent bacteria. The organic extract of the control sediment, as well as a whole-sediment sample of this formulated control sediment (Kemble et al. 1999), were spiked with 10 µg/mg equivalent/ml pentachlorophenol (PCP) using procedures described in Johnson and Long (1998). Results of these spiking studies were used to develop a Toxicity Reference Index (TRI). A spiked sample with PCP had an EC_{50} value of 0.5 mg eq/ml for the basic test and 0.5% mg eq/ml for solid-phase Test and were each given the TRI number of 1.0. A sample with an EC_{50} value less than that of the spiked sample had a TRI number > 1.0 indicating the sample was more toxic than the model toxicant. Note, the lower the EC50 value the higher

the toxicity of the sample. For example, an organic extract with an EC_{50} value of 0.25 mg eq/ml would have a TRI number of 2 (spiked sample EC_{50} value/ test sample EC_{50} value = TRI number; 0.5/0.25 = 2.0) indicating that this sample was about two-fold more toxic than the PCP spiked sample. The TRI numbers generated for whole sediments were calculated similarly. A sediment sample was designated toxic using this single criteria of the TRI.

Pentachlorophenol was selected as a reference toxin because of its ubiquity, known toxicity, and high K_{ow} value. The EC50 value for each sample was compared with PCP number and placed in the TRI; samples with an index number > 1.0 was designated as toxic.

RESULTS AND DISCUSSION

Physical and Chemical Characteristics of Sediment Samples

Physical characteristics of the sediment samples are listed in Table 1. Water content ranged from 20% for sediment from WH-17 to 65% for sediment from WH-12. Sediment organic carbon content ranged from 1.6% in the control sediment to 7.8% in sediment from WH-08 (Table 1). Classification of the sediment samples for grain size varied from site to site (i.e., clay (WH-07, WH-12 and WH-17), loam (WH-10), silt loam (WH-19)) while the control sediment was a sandy loam (Table 1). Acid volatile sulfide concentrations ranged from 0.31 µmoles/g in the control sample to 39.40 µmoles/g in the WH-12 sample (Table 2).

Concentrations of simultaneously extracted metals in Waukegan Harbor sediment samples are listed Table 2. Sediment from sample WH-01 had the highest concentrations of extractable Cd, Ni, Pb, and Zn. Sample WH-07 had the highest concentration of SEM Cu (Table 2). However, the SEM-AVS molar concentration in the present study for all sediment samples was less than 0. This indicates the concentration of divalent metals listed in Table 2 may not have been high enough to cause the toxicity observed in the samples (Ankely et al. 1996).

Concentrations of total metals in Waukegan Harbor sediment samples are listed in Table 3. Sediment from site WH-01 had the highest concentrations of 7 of the 13 metals measured (Table 3). Sediment from site WH-12 had the highest concentrations of total As and total Cu. The highest concentration of Ni was measured in the WH-11 sediment sample.

Concentrations of organochlorine pesticides (OCs) in sediment samples are listed in Table 4. Before remediation, concentrations of PCBs in Waukegan harbor sediments ranged from 10 to 50 µg/g in the lower harbor to greater than 500 µg/g in Slip 3 of the upper harbor (Mason and Hanger 1980; Figure 2). Concentrations of total PCBs in sediment samples after remediation were all below 10 µg/g (Table 4; Figure 3). The highest concentration of total PCBs was 8.9 µg/g in the sediment sample from site WH-12 (Table 4). Slip 3 was not sampled in the present study. After the removal of about 5000 m^3 of PCB-contaminated sediments from Slip 3, the area was converted to a permanent containment cell to store treated sediments (USEPA 1993). Concentrations of other OCs analyzed for were below detection limits for all of the sediment samples with the following exceptions: (1) hexachlorobenzene concentrations from sites WH-10 (0.0012 µg/g) and WH-19 (0.0014 µg/g) and (2) the chlordane trans isomer concentration from the WH-18 sample (0.0017 µg/g).

Concentrations of polynuclear aromatic hydrocarbons (PAHs) in sediment samples are listed in Table 5. The highest concentrations of PAHs were observed in the WH-12 sediment sample and were generally lower in sediment samples from the southern portion of the harbor. Concentrations of PAHs in harbor sediments exceeded the Method Lower Limit of Quantitation (MLLQ; 0.03 µg/g) in every sediment sample for at least 20 of the 25 PAHs evaluated (concentrations of 19 of the 25 PAHs analyzed for exceeded the MLLQ in all 20 sediment samples; Table 5).

Sediment Exposures

- Amphipod Toxicity Exposures**:** Survival of amphipods after the 28-d exposure to sediment was significantly reduced compared to the control sediment in 6 of the 20 samples (Table 6). However, amphipod survival in 4 of these 6 samples was greater than the minimum control survival of 80% for test acceptability (USEPA 1999; ASTM 1998a; Table 6). Body length of amphipods at Day 28 was significantly reduced compared to the control in 19 of the 20 samples (Table 6; Appendix 5). Weight of amphipods at Day 28 was significantly reduced compared to the control in all of the sediment samples (Table 6; Appendix 6).

Survival of amphipods at Days 35 and 42 was significantly reduced compared to the control sediment in only one sample (WH-12; Table 6). Body length of amphipods at Day 42 was significantly reduced compared to the control in all of the samples (Table 6; Appendix 7). Weight of amphipods at Day 42 was significantly reduced compared to the control in 18 of the 20 samples (Table 6; Appendix 8). Only two sediment samples significantly reduced reproduction (number of young/female) compared to the control sediment (WH-02 and WH-10; Table 6; Appendices 9 and 10).

Indigenous organisms recovered at end of the 28-d sediment exposure included oligochaetes, clams, leeches, chironomids, ostracods, cyclops, and snails. Amphipods were observed in amplexus in all of the sediment treatments except for WH-08, WH-10, WH-11R, WH-14, and WH-19. Plant growth was observed in the WH-06, WH-07, WH-10, WH-11, WH-12 WH-13, WH-15, and WH-17 treatments from Day 4 to Day 28.

- Microtox® Exposures: Toxicological profiles of organic extracts for the Microtox® basic toxicity test of the 20 sediment samples from are listed in Table 7. Only one sample (WH-12) exceeded the TRI number of 1.0 and was designated as toxic with the Microtox® basic test. Toxicological profiles of 20 whole-sediment samples using the Microtox® solid-phase toxicity test are listed in Table 8. Over half of the samples were classified as toxic in the solid-phase test (TRI >1.0). Sediment toxicity ranged from 0.1 to 5.0 times greater than the PCP-spiked substrate. There was no correlation (Microsoft 1992) between whole-sediment clay content and toxicity (Figure 4); in contrast to the findings of Ringwood et al. (1997). For example, WH-16 had a TRI number of 5 with a 26.5% clay content as opposed to WH-06 with TRI number of 0.1 and a clay content of 36.3%; WH-16 had a lower clay content than WH-06, yet was 50 times more toxic. However, EC50 values were greatest when clay content was less than 40%. The results of the solid-phase test did not agree with results of the basic test toxicity assessment of organic extracts. These data indicate that there are differences in bioavailability of contaminants in the two types of samples. Alternatively, there maybe water soluble toxins in whole sediment that were recovered in the organic extraction of the sediment in the basic test. Similarly, neither

Microtox® exposure were consistent with the results of the amphipod tests. These data indicate that the amphipod exposures were more responsive than either of the Microtox® exposures.

Comparison of Sediment Characteristics to Toxicity Responses

Relationships of physical characteristics of sediments to toxicity were evaluated using Spearman Rank correlation. The results of this evaluation indicated that there were no significant correlations between survival, growth (length or weight) or reproduction (Table 6) and the measured physical characteristics of the sediment samples (Table 1). This finding is consistent with the results of earlier studies (USEPA 1999; ASTM 1998a) which showed that sediment particle size did not affect the response of *Hyalella azteca* in 28-d sediment exposures.

The relationship between chemical characteristics and biological responses was also evaluated using Spearman Rank correlation analysis. These analyses included the concentrations of contaminants on a dry-weight and a organic carbon-normalized basis. The results of these analysis showed that there was a significant correlation (negative) was observed between reproduction and the concentrations of three PAHs (dibenzothiophene, biphenyl, acenphthalene) normalized to total organic carbon concentrations. There were also a trend in correlations (all negative) with several other PAHs which had r values of 0.5 to 0.6. There were no significant correlations between sediment chemistry and survival or growth of amphipods. This lack of correlation may have resulted from the relatively narrow range in concentration of contaminants relative to the changes in survival, growth, or reproduction.

In addition to the correlation procedures described above, consensus-based probable effect concentrations (PECs) were used to evaluate relationships between sediment chemistry and toxicity. The number of PECs exceeded and mean PEC quotients were calculated for each sample evaluated in the present study (Table 9). The proportion of PECs exceeded was also calculated for each sediment sample from Waukegan Harbor and for sediment toxicity tests reported for *H. azteca* by Ingersoll et al. (1996, n = 62 samples), Kemble et al. (1998, n = 49 samples), and Ingersoll et al. (1998; n = 18 samples). A mean PEC quotient was calculated for each of these samples by first dividing the concentration of an individual chemical by its respective PEC, summing each of these individual values, and dividing the sum by the number of PECs for that sample (Canfield et al. 1996; Ingersoll et al. 1998; Long et al. 1998; Ingersoll and MacDonald

1999; MacDonald et al. 1999a). A total of 149 sediment samples were evaluated and 32% of these samples were designated as toxic. The mean quotients and proportion of PECs exceeded were then used to evaluate relationships between sediment chemistry and toxicity in the present study and in this historic database.

The frequency of toxicity to *H. azteca* increased in sediment samples with either an increase in the proportion of PECs exceeded or with an increase in the mean PEC quotient (Figure 5). For the entire database, only 6.8% of the samples were toxic to *H. azteca* below a mean PEC quotient of 0.1 (Table 10). Above a mean PEC quotient 0.6, 86% of the samples were toxic and between a quotient of 0.1 and 0.6, 22% of the samples were toxic. Similarly, only 10% of the samples were toxic when the proportion of the PECs exceeded was below 0.05. When the proportion of the PECs exceeded was above 0.2, 84% of the samples were toxic (Table 11). Between a proportion of 0.05 and 0.2 of the PECs exceeded, 40% of the samples were toxic to *H. azteca*. Consistent with these results, Long and MacDonald (1998) reported low probably of toxicity (12%) below a mean ERM quotient of 0.1 and a high probability of toxicity (74%) above a mean ERM quotient of 1.5 in sediment toxicity tests with marine amphipods. Similarly, McDonald et al. (1999a) report an incidence of toxicity of >85% at a mean PEC quotient above 0.5 for a variety of freshwater sediment tests.

For the Waukegan sediments, none of the samples had a mean PEC quotient below 0.1 or a proportion of PECs exceeded below 0.05. A total of 85% of the Waukegan samples that were toxic to *H. azteca* in the present study exceeded a mean PEC quotient of 0.6 or were above a proportion of 0.2 PECs exceeded. Therefore, the sediments from Waukegan Harbor that were toxic to *H. azteca* (toxicity identified based primarily on growth) were contaminated at similar concentrations to toxic sediments from other areas in the United States (Ingersoll et al. 1996; 1998; Kemble et al. 1998; Long and MacDonald 1998; MacDonald et al. 1999a).

Microtox® to Chemistry Comparisons

The relationship between PECs and the observed toxicity was not as clear for the Microtox® test as it was for the amphipod test (Tables 12 and 13). For example, above a mean PEC quotient 0.6, only 59% of the samples were identified as toxic in the solid-phase Microtox® test (Table 12). Similarly, when the proportion of the PECs exceeded was above 0.2, only 59% of the samples were toxic in the Microtox® test (Table 13). Between a proportion of 0.05 and 0.2 of

the PECs exceeded, 33% of the samples were toxic in the Microtox® test (Table 13). The PECs described in Ingersoll and MacDonald (1999) and MacDonald et al. (1999a) were derived using whole-sediment toxicity tests with benthic invertebrates. Therefore, it is not surprising to find lower correspondence between these PECs and the response of bacteria. Suspension of the sediment in the Microtox® solid-phase test may also influence the response of this test. Perhaps sediment quality guidelines developed specifically for the Microtox® test could be used to better evaluate relationships between sediment chemistry and toxicity data.

Sampling sites in the southern portion of the harbor generally had lower mean PEC quotients than sites in the northern part of the harbor (Figure 6). Mean PEC quotients ranged from 0.51 for the WH-02 sample to 2.40 for the WH-12 sample. Similarly, sites in the southern part of the harbor generally had fewer exceedances of the individual PECs (Table 9). However, at least one individual PEC reported in Ingersoll and MacDonald (1999) and MacDonald et al. (1999a) was exceeded in each sample. Exceedances ranged from 1 in the WH-02 sample to 8 in the WH-12 sample.

Despite the reduction in PCB levels throughout the harbor, total PCBs exceeded the PEC (0.68 µg/g) in all 19 sediment samples analyzed (WH-02 was not analyzed). Concentrations of naphthalene in 5 of 20 sediment samples exceeded the PEC. Concentrations of phenanthrene exceeded the PEC (1.17 µg/g) in 4 of the 20 sediment samples. Concentrations of chrysene and pyrene each exceeded their PEC in 2 of 20 of the sediment samples. Concentrations of BAP exceed the PEC in just 1 of the 20 samples. Concentrations of Sum DDE exceeded the PEC of 0.031 µg/g in 18 of 19 sediment samples. Similarly, concentrations of total Cd exceeded the PEC of 4.98 µg/g in 19 of the 20 sediment samples. Concentrations of total As exceeded the PEC of 33.0 µg/g in 5 of the 20 sediment samples. Concentrations of total Cu and Pb exceeded their PECs (149 and 128 µg/g respectively) in 2 of the 20 sediment samples. Concentrations of total Ni and Cr each exceeded their PEC in 1 of the 20 sediment samples. Zinc concentrations were below the PEC in all of the samples.

SUMMARY

Historical PCB concentrations in Waukegan Harbor sediments ranged from 10 µg/g to above 500 µg/g (Mason and Hanger 1980; Figure 2). Dredging of harbor sediments (about 5000 m^3 of PCB-contaminated sediment was removed) and other remedial activities within the harbor have reduced PCB levels in the harbor sediment to less than 10 µg/g. However, concentrations of PCBs, PAHs, and total metals in sediments remain elevated (above sediment quality guidelines).

Sediment samples from Waukegan Harbor were generally not lethal to amphipods. Amphipod survival identified only 6 of the 20 sediment samples as toxic (a significant reduction compared to the control sediment). However, amphipod growth was significantly reduced in all of the sediment samples compared to the control sediment on both Day 28 and Day 42. The Microtox® SPT identified 11 of the 20 sediment samples as toxic. With the exception of the WH-03 sample, the SPT identified all of the sites in which amphipod survival was significantly reduced compared to the control at Day 28. However, the Microtox® test did not identify samples that resulted in sublethal effects in the amphipod test.

Sediment chemistry and toxicity data were evaluated using consensus-based probable effect concentrations (PECs). Results of these analyses indicate sediments from Waukegan Harbor that were toxic to *H. azteca* were contaminated at similar concentrations as were sediments that were toxic to *H. azteca* from other areas in the United States. However, the relationship between PECs and the observed toxicity was not as strong for the Microtox® test.

Similarly, USEPA (1977) guidelines for the evaluation of Great Lake harbor sediment classifies sediment samples as moderately toxic if total PCB concentrations range from 1 to 10 µg/g. Based on these guidelines for total PCBs, 18 of the 19 sediment samples (WH-02 was not analyzed) from Waukegan Harbor would be classified as moderately toxic based on concentrations of total PCBs (Table 4) measured in harbor sediments. The results of this study indicate that the first phase of sediment remediation in Waukegan Harbor successfully lowered concentrations of PCBs at the site. Ingersoll and Nelson (1990) previously identified sediment samples from Waukegan Harbor as lethal to amphipods and midges. While the sediments were generally not lethal to amphipods in the present study, there are still sublethal effects of contaminants in the sediment at this site (associated with elevated concentrations of metals, PCBs and PAHs).

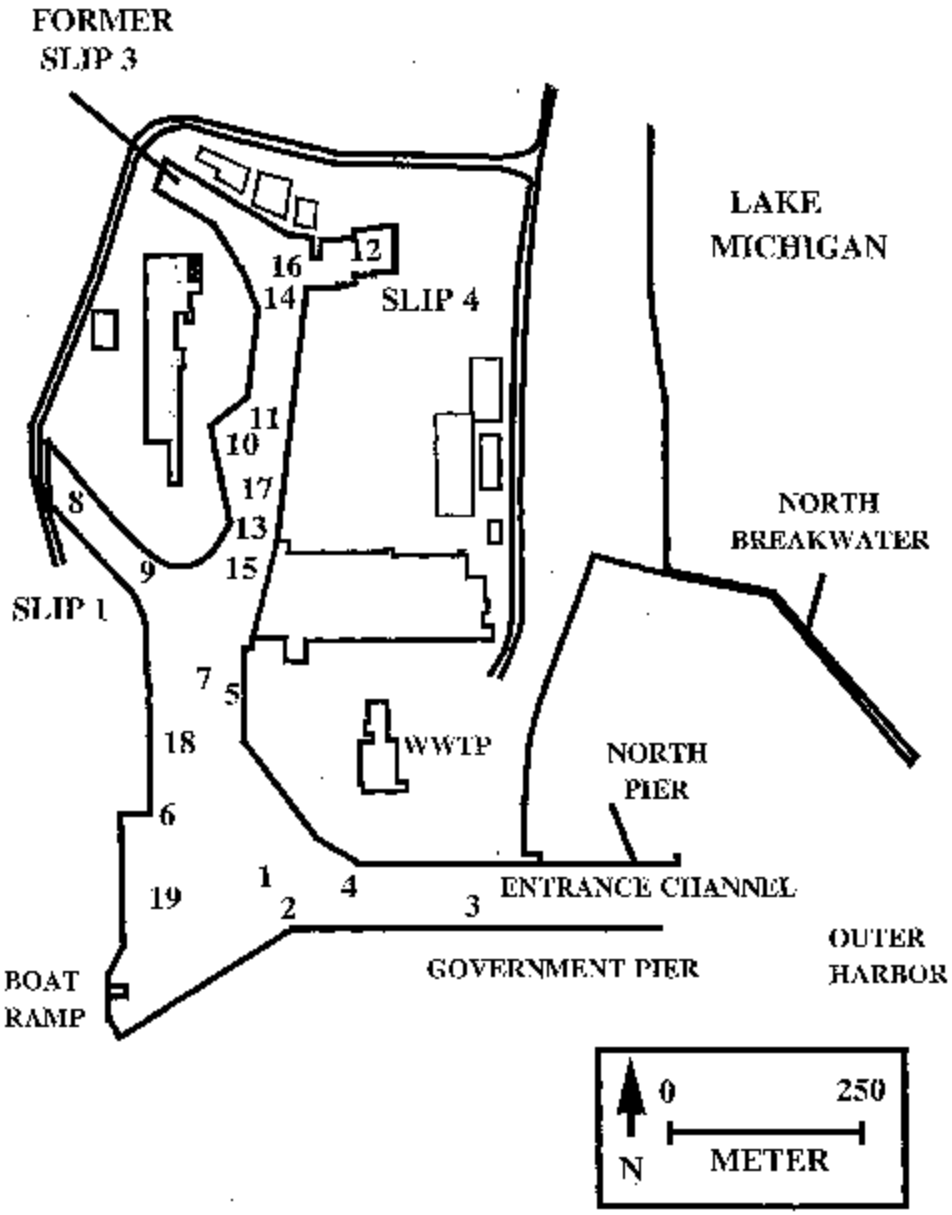

Figure 1 - Location of sampling stations in Waukegan Harbor, Illinois.
WWTP = Waukegan Water Treatment Plant

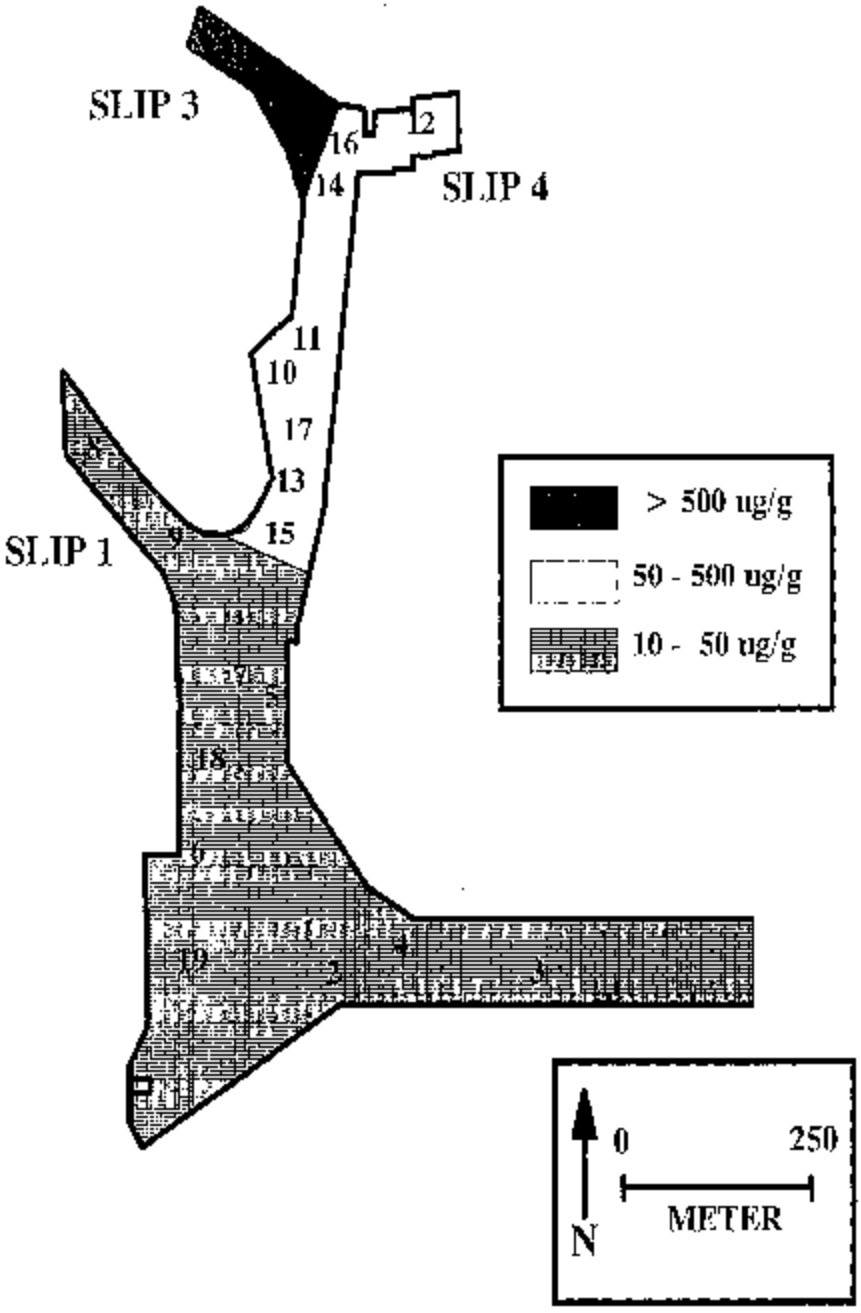

Figure 2 - Historical concentration of PCB contamination in Waukegan Harbor sediment samples (Mason and Hanger 1980).

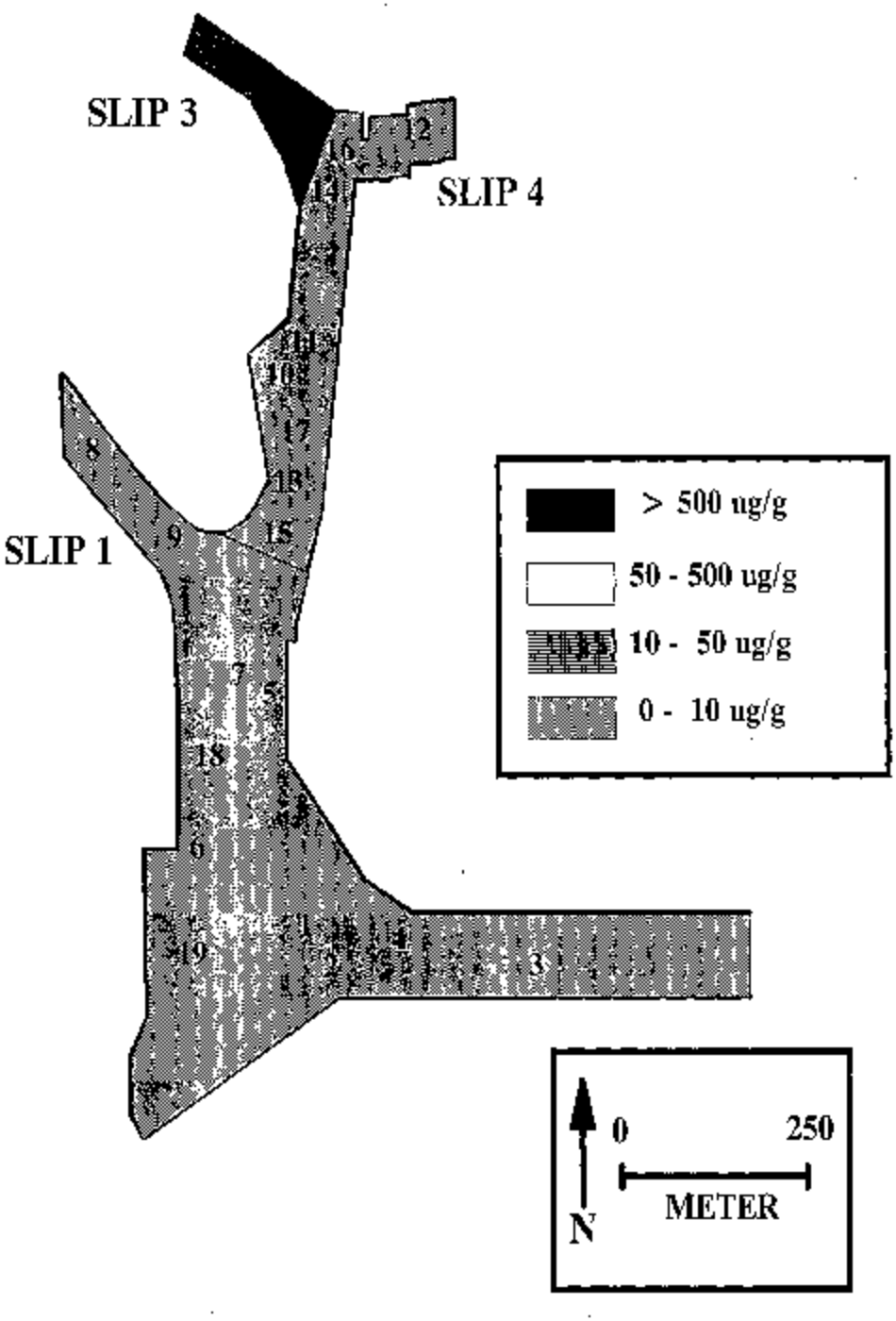

Figure 3 - Concentration PCB contamination in current sediment samples from Waukegan Harbor.

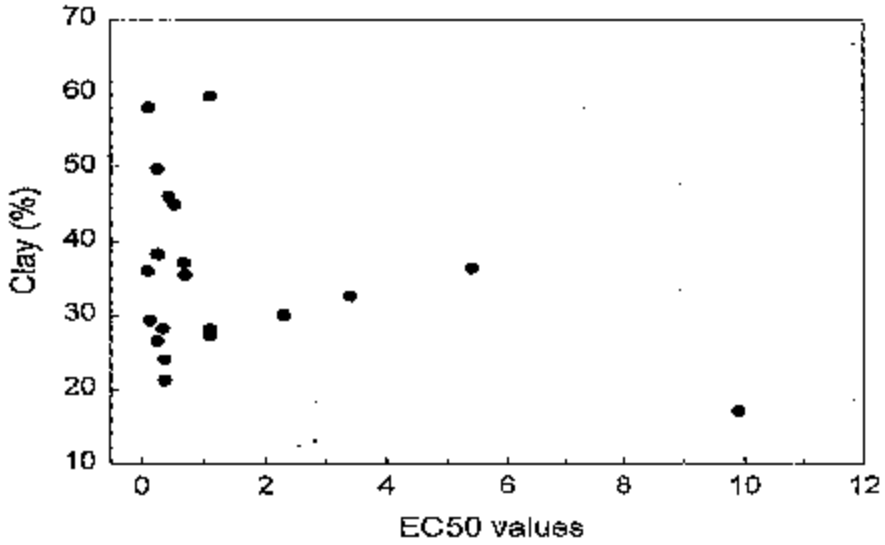

Figure 4 - Relationship between clay content and EC_{20} values in Microtox® solid-phase testing.

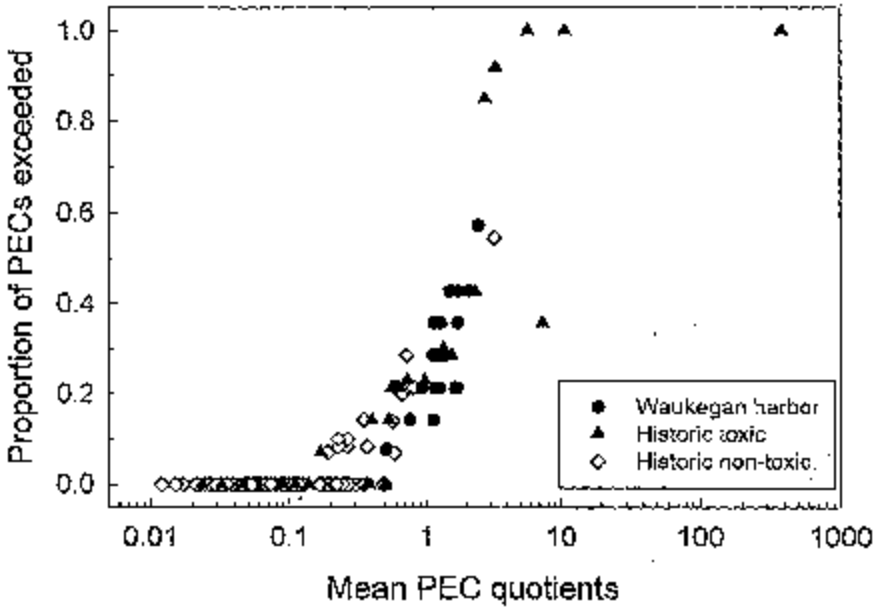

Figure 5 - Proportion of PEC exceeded compared to the mean PEC quotient for toxic or non-toxic samples from the present study and for a historical database with *H. aztecz* (Ingersoll et al. (1996, n = 62 samples), Kemble et al. (1998, n = 49 samples), and Ingersoll et al. (1998; n = 15 samples))

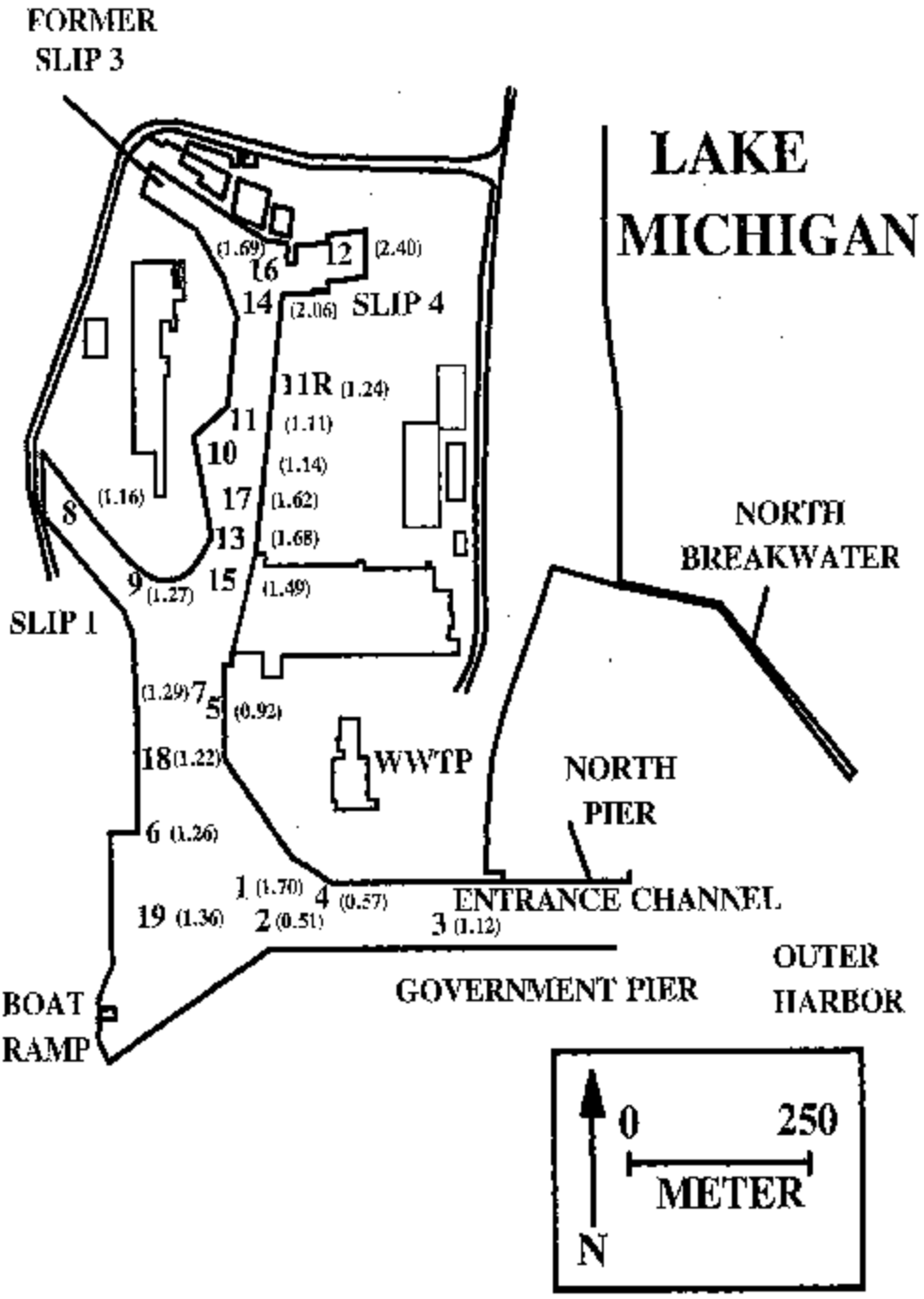

Figure 6 - Probable Effect Concentrations quotients by site for sediment samples from Waukegan Harbor.

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