PARTITIONING, TOXICITY AND MUTAGENICITY OF IN-PLACE CONTAMINANTS OF SEDIMENTS FROM THE GRAND CALUMET RIVER AND INDIANA HARBOR, INDIANA

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ABSTRACT

PARTITIONING, TOXICITY AND MUTAGENICITY OF IN-PLACE CONTAMINANTS OF SEDIMENTS FROM THE GRAND CALUMET RIVER, INDIANA

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The presence of a wide variety of in-place chemical contaminants in sediments from the Grand Calumet River-Indiana Harbor Canal, Indiana Area of Concern, their chemical partitioning between environmental compartments, and their potential toxicological effects were the primary focus of this study. The partitioning behavior of non-polar organic chemicals (NPOCs) present in both sediment and sediment pore waters was investigated to determine the importance of NPOC binding to dissolved organic carbon (DOC) in sediment pore water. Better concordance was observed between estimated and actual field partition coefficients with a three-phase partitioning model. These results highlight the potential importance of the DOC-binding phenomenon in determinations of the bioavailability and effects of NPOCs present in sediment pore waters.

The Microtox® assay, 48 h Daphnia magna and Ceriodaphnia dubia tests and a 10-d Chironomus tentans test were used in a toxic units approach to assess the toxicity of sediments and sediment pore waters and to conduct a preliminary identification of the potential toxicants. Based on the results of these analyses, ammonia, polycyclic aromatic hydrocarbons, metals, petroleum hydrocarbons and bicarbonate ion were the major contaminants of concern to benthic invertebrates within the study

area. Separate experiments to determine the mechanism of bicarbonate ion toxicity to D. magna suggested that toxicity was due to the inhibition of the active uptake of Cl^- from water. Therefore, pore water alkalinity should be considered when interpreting the results of aqueous phase toxicity tests with cladocerans and, perhaps, other species of invertebrates and fish.

Evaluation of the comparative mutagenicity of solvent extracts of sediments from the study area was conducted with the Ames and Mutatox® assays. Extracts were mutagenic with metabolic activation in both assays, however, few samples contained direct acting mutagens. The lack of mutagenicity in Mutatox® assays of pore waters indicates that short-term human exposure to mutagens in pore waters and sediments is likely to be non-problematic. Greater concern is required for the potential ecological and human health effects due to food chain transfer of mutagenic compounds in sediments from the study area.

This dissertation is dedicated to the memory of my father and to my son, Ian, whose insatiable curiosity about the world around him is a constant source of wonder.

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TABLE OF CONTENTS

TABLE OF CONTENTS - continued

TABLE OF CONTENTS - continued

LIST OF TABLES

LIST OF TABLES - continued

LIST OF FIGURES

LIST OF FIGURES - continued

GENERAL INTRODUCTION

Many rivers, harbors and connecting channels of the Great Lakes have sediments which contain a wide array of chemical compounds, including organic xenobiotics (Pranckevicius 1986, Fallon and Horvath 1985) and metals (Pranckevicius 1986, Fallon and Horvath 1985, Hamdy and Post 1985). Due to municipal, industrial and non-point source waste discharges and the tendency of many chemicals to become associated with sediments (Knight 1984, Cairns et al. 1984), contaminated sediments are particularly problematic near densely populated, industrialized urban areas, such as the area surrounding the Grand Calumet River-Indiana Harbor (GCR-IH) International Joint Commission (IJC) Area of Concern (AOC) in northwest Indiana. Rodgers et al. (1985) have summarized the recent environmental status, relative to anthropogenically-introduced chemical contamination, in these area of the Great Lakes.

The drainage basin of the Grand Calumet River comprises 43,000 acres within the Calumet Lake plain in northwest Indiana which have been greatly affected by development activities. The Calumet Lake plain occupies an area of low relief which historically was formed by the bottom of Lake Chicago and three subsequent lakes prior to the formation of Lake Michigan (IDEM 1988). High linear coastal sand dunes and many low beach ridges characterized the Grand Calumet River basin prior to the beginning of industrial development in the late 1800's. The wetland areas between the beach ridges has subsequently been filled with sand from the ridges and

dunes and slag from the area's many steel mills (Reshkin et al. 1975, Hartke et al. 1975).

The hydrology of the Grand Calumet River basin is complex as a result of modifications which were initially begun by Indians living in the area (i.e. a channel to connect the Grand Calumet River with the river Draining Lake Calumet). Additional alterations in the hydrology of the basin were made by various local and federal agencies to facilitate industrial development and protect the water intake of Chicago (and southern Lake Michigan) from contaminants in the many municipal and industrial effluent discharges to the river. Surface water in the system may flow east toward the Marquette Park lagoons, west to the Mississippi River or north to Lake Michigan depending on the local weather conditions and municipal/industrial effluent discharge rates and volumes. Flow in the east branch of the Grand Calumet River may be comprised of up to 93 % municipal and industrial effluent while municipal waste treatment effluent may account for 100 % of the flow in the west branch (U.S. EPA 1985, Crawford and Wangsness 1987). The direction of flow in the west branch also has been observed to change from 34 cfs to the east to 42 cfs to the west over a 24 hour period (IDEM 1988).

The severely contaminated condition of the GCR-IH AOC is based on water quality problems with conventional pollutants, metals, and organic chemicals, contaminated sediments, impacted aesthetics and biota, and fish consumption advisories (IDEM 1988). The AOC covers the entire length of the east branch and the first two miles of the west branch of the Grand Calumet River, the Indiana Harbor Canal, Indiana Harbor and the Lake Michigan nearshore zone of the harbor.

The presence of a wide variety of in-place chemical contaminants in

sediments from the AOC and their potential effects on benthic macroinvertebrate populations were the primary focus of this study. A wasteload allocation study of the Grand Calumet River conducted by HydroQual, Inc (1984) identified the presence of numerous priority pollutants in sediments from 10 sampling locations within the GCR-IH system. Concentrations of 13 metals (As, Cd, Cr, Cu, Pb, Hg, Ni, Ag, Se, Zn, Sb, Be, Tl) and cyanide were analyzed with increased concentrations of at least one metal (generally more than one) or cyanide observed in each of the 10 samples. Concentrations of 21 pesticides were generally below 0.05 μg/g and were always below 5.0 μg/g. Various PCB Aroclors (1016, 1221, 1232, 1242, 1260) also were present at concentrations below 1.0 μg/g although Aroclors 1248 and 1254 were present at concentrations as high as 17.0 and 6.9 μg/g, respectively. Forty-seven base neutral priority pollutants also were analyzed and concentrations of 24 of these generally observed to be below 0.025 μg/g. Nine of the remaining base neutral compounds exhibited concentrations > 100 μg/g in sediment from at least one location. These nine compounds were polynuclear aromatic hydrocarbons (PAHs) with maximum observed concentrations in sediments collected from locations adjacent to steel mill effluent discharges. High concentrations of other base neutral compounds also were observed near a municipal wastewater treatment plant effluent discharge. Comparison of the results of the sediment chemical analyses conducted by HydroQual, Inc (1984) with results from earlier studies demonstrated little change in concentrations of chemicals in sediments from the Grand Calumet River (HydroQual, Inc. 1984).

Previous assessments of the effects of chemicals in the water column and sediments of the GCR-IH AOC on indigenous fauna have relied heavily on

surveys of benthic macroinvertebrate and fish community structure (U.S. EPA 1985, Polls and Dennison 1984, U.S. ACOE 1985, 1986). Effects on plankton (Cook 1966, Gannon and Beeton 1969) and other wildlife, including birds and mammals (U.S. ACOE 1985, 1986), also have been conducted on a limited basis. Elevated contaminant levels in sediment and water have been implicated in the lack of aquatic and terrestrial organisms in the GCR-IH system (IDEM 1988). Sediments from Indiana Harbor and Canal have been demonstrated to be toxic to benthic macroinvertebrates during laboratory sediment toxicity tests (Gannon and Beeton 1969, U.S. ACOE 1987). Concern also has been raised over the potential for bioaccumulation of metals and organic chemicals by terrestrial species if harbor sediments were dredged and moved to an upland disposal site (U.S. ACOE 1987). To date, however, no such comprehensive sediment toxicity evaluations have been conducted on sediments collected from the east and west branches of the Grand Calumet River.

Any meaningful assessment of sediment contamination and toxicity in the GCR-IH system must include an evaluation of the contaminant-associated toxicity of sediments to aquatic life including benthic macroinvertebrates. The data needed (and which is currently lacking) for such an assessment includes synoptic measures of chemical concentrations in sediments and interstitial (pore) waters, physical characteristics of the sediments (particle size, total organic carbon, etc) and measures of sediment and interstitial water toxicity to relevant benthic test species (U.S. EPA 1985).

Currently, great interest also exists among regulators, legislators and the scientific community in the development of sediment quality criteria (Shea 1989). Numerous approaches have been proposed for the

development of criteria (Anonymous 1985), including the use of the equilibrium partitioning theory (Pavlou 1987). Empirical observations from various studies (Adams et al. 1985, Ziegenfuss et al. 1986, Adams 1987, Connell et al. 1988, Lake et al. 1990 Swartz et al. 1990) support the hypothesis that neutral organic chemical bioavailability, toxicity and bioaccumulation are more closely related to pore water chemical concentrations of contaminants than to total (bulk) sediment concentrations. These observations engendered and continue to fuel the interest in the equilibrium partitioning theory as a mechanism for predicting potential exposure to, or bioaccumulation of, contaminants by benthic biota.

The information presented above led to the selection of the GCR-IH AOC as a study area for the evaluation of the usefulness of several alternative invertebrate and microbial assays for the comparative assessment of sediment and/or sediment pore water toxicity and mutagenicity. The specific objective of chapter one was to conduct a field evaluation of the equilibrium partitioning theory and the ramifications of non-polar organic compound binding to dissolved organic carbon relative to the development of sediment quality criteria using the equilibrium partitioning theory. Chapter two presents comparative toxicity data from four toxicity tests commonly used to assess sediments or sediment pore waters. It also tests the hypothesis that the toxicity of sediment pore waters can be predicted based on toxic units calculated from laboratory-derived, chemical-specific dose response relationships. Chapter three examines the toxicity of bicarbonate ion to the cladocerans Daphnia magna and Ceriodaphnia dubia, evaluates the potential for bicarbonate toxicity in sediment pore waters and presents a potential

mechanism for the observed effects produced by the bicarbonate ion. The final chapter presents a comparative evaluation of several different assays for determining effects, other than acute toxicity, which may be linked to exposure to contaminated sediments.

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CHAPTER 1

A Field Evaluation of Equilibrium Partitioning in Sediments from the Grand Calumet River and Indiana Harbor, Indiana

Introduction

Currently, great interest exists among regulators, legislators and the scientific community in the development of sediment quality criteria (1). Numerous approaches have been proposed for the development of these criteria (2), including the use of the equilibrium partitioning theory (EqP) (3,4). Empirical observations from various studies (4-10) support the hypothesis that non-polar organic chemical (NPOC) bioavailability, toxicity and bioaccumulation are more closely related to pore water (interstitial water) or organic carbon-normalized bulk sediment chemical concentrations of contaminants than to total (bulk) sediment concentrations. These observations engendered and continue to fuel the interest in the EqP as a mechanism for predicting potential exposure to, or bioaccumulation of, NPOCs by benthic biota.

The equilibrium partitioning theory predicts contaminant bioavailability in pore water by assuming that a thermodynamic equilibrium is established between the solid phase sediment and pore water concentrations of NPOCs (4). According to the theory, pore water concentrations and tissue residues of these compounds should be predictable based on the bulk sediment concentration of the compound and the physical and chemical properties of the sediment and compound of interest (3,4,11).

For NPOCs, the equilibrium concentration in pore water is a result of partitioning between the solid and dissolved phases. This partitioning

process is primarily controlled by the concentration of the NPOC of interest in the bulk sediment, the fractional organic carbon content of the sediment and the proportionality constant for distribution of the NPOC between water and organic matrices. This constant is approximated by the octanol-water partition coefficient (K_{ow}) for the compound of interest (4,11). It should theoretically be possible to estimate the pore water concentration of a neutral organic chemical based on the following relationship between the solid (bulk) phase and pore water concentrations, C_s and C_p, respectively (Equation 1),

$$C_s \approx f_{oc} K_{ow} C_p \quad (1)$$

where f_{oc} = fractional organic carbon content of the sediment and K_{ow} = the octanol-water partition coefficient for the chemical of concern.
If C_{soc} is defined as the solid phase concentration of the NPOC of interest normalized to the fractional organic carbon content of the sediment (Equation 2),

$$C_{soc} \approx \frac{C_s}{f_{oc}} \quad (2)$$

then the concentration of NPOC in the pore water can be estimated from the concentration in the bulk sediment normalized to the fractional organic carbon content of the sediment and the K_{ow} for the NPOC (Equation 3).

$$C_p \approx \frac{C_{soc}}{K_{ow}} \quad (3)$$

However, determination of chemical concentrations in pore water, and thus quantification of potential chemical exposure via one of the major exposure routes for benthic organisms in contaminated sediments (5,12,13), may not be this simple. Numerous authors have proposed that a third phase, dissolved organic carbon (DOC), plays an important role in the partitioning behavior of NPOCs with a $\log_{10} K_{ow} > 3.0$ (14-20). Because DOC complexation is related to NPOC hydrophobicity, complexation will increase with an increase in the K_{ow}, and thus K_{oc} and K_{DOC}, of the NPOC. This phenomenon will result in a greater total concentration of NPOC in the pore water than would be estimated from equation 3.

An in-depth discussion of the ramifications of DOC-binding of NPOCs in sediment pore water is presented by DiToro et al. (4). The essence of the problem, however, involves the distribution of measured NPOC concentrations in pore water, i.e., what proportion of the total NPOC concentration is "dissolved" or "free" and what proportion is bound to DOC. This distinction is important because NPOC complexed to DOC may be partially or totally unavailable to cause effects on biota (21-22). Thus "dissolved" NPOC concentrations in pore water may most accurately reflect the true chemical exposure for benthic biota.

The relative concentrations of free and bound, soluble NPOC in pore water should be related to the organic carbon-normalized sediment concentration (C_{soc}) (Equations 4-6):

$$C_{soc} = K_{ow} C_p \quad (4)$$

where,

$$C_p = C_d + C_{DOC} \quad (5)$$

and,

$$C_d = \frac{C_p}{1 + m_{DOC} K_{DOC}} \quad (6)$$

if,

C_d = "dissolved" NPOC concentration in pore water

C_{DOC} = DOC-bound NPOC concentration in pore water

m_{DOC} = DOC concentration in pore water

K_{DOC} = DOC partition coefficient for NPOC

Knowledge of these values would permit an initial examination of the potential importance of DOC-binding of NPOCs in the field and provide a better understanding of the potential problems in the application of EqP for the development of sediment quality criteria.

If EqP can be verified, sediment quality criteria could be developed by applying water quality criteria to chemical concentrations in pore water and back-calculating permissible sediment concentrations based on sediment organic carbon content. This method of developing sediment quality criteria is frequently referred to as the equilibrium partitioning theory/water quality criteria (EqP/WQC) approach. To date, attempts to verify EqP have been restricted to laboratory evaluations of the behavior of a few NPOCs in sediments containing a limited range of organic carbon concentrations (8,10) or field evaluations with an equally restrictive number of compounds and sampling locations (9,11,23).

The objective of this study was to measure bulk sediment and pore water concentrations of a suite of NPOCs in samples collected from 10

locations on the Grand Calumet River near Gary, IN and to evaluate the potential ramifications of DOC-binding of NPOCs on the EqP by examining two general types of field data: 1) apparent versus estimated theoretical partition coefficients for NPOCs as a direct test of the $C_{soc} = K_{ow} C_p$ relationship, independent of DOC and 2) actual versus estimated theoretical partition coefficients for NPOCs when DOC-binding is accounted for by using the relationship $C_{soc} = K_{ow} (C_d + C_{DOC})$.

Materials and Methods

Chemical Analysis

Sediment samples were collected with a Ponar grab sampler from 10 locations along the Grand Calumet River in the northwestern corner of Indiana (Figure 1) between 1 September 1988 and 1989. The sample from each location was a composite of approximately 80-100 L of wet sediment from multiple Ponar grabs. Each sample was placed in coolers or plastic buckets lined with food-grade plastic bags, immediately transported to the laboratory and placed in 4°C storage.

Sediment samples were dried and percent moisture determined for each sample. Ten grams of dried sample were mixed with 10 g of anhydrous sodium sulphate and Soxhlet extracted for 24 h with pesticide-grade acetone/hexane (1:1, v/v). The extract was passed over a drying column containing anhydrous sodium sulphate and the column rinsed with approximately 100 ml of the acetone/hexane mixture to complete the transfer. The extract was transferred to a Kuderna-Danish concentrator and extract volume reduced to 1 ml. The final extract volume was adjusted to 10 ml with the acetone/hexane mixture. These procedures and all other

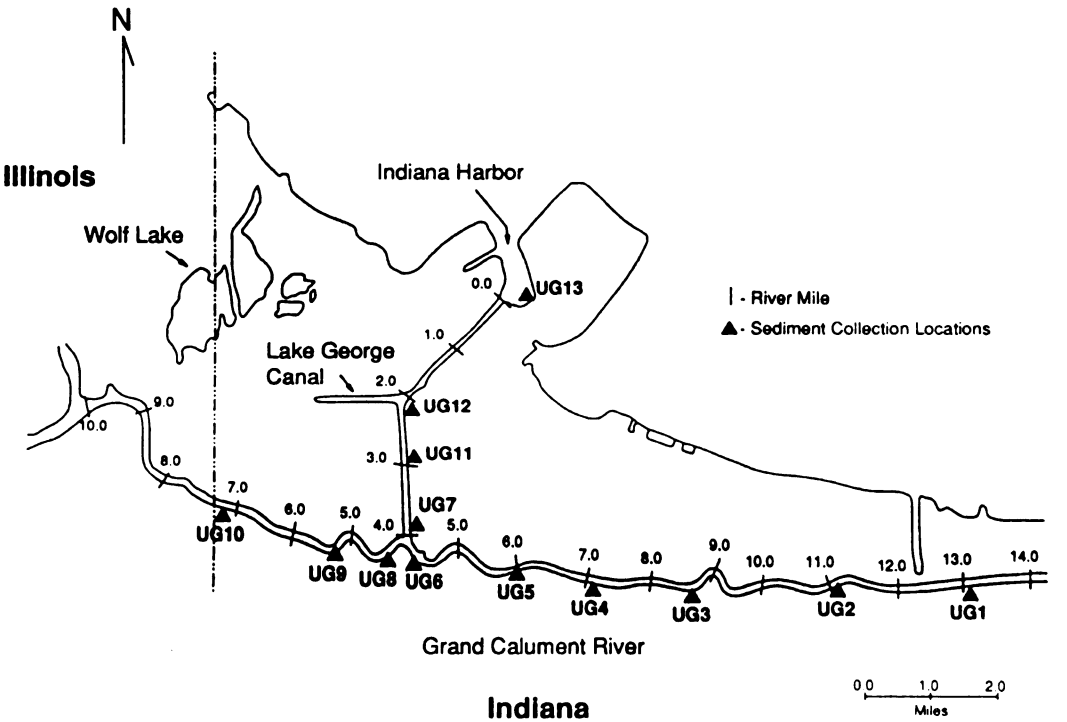

Figure 1. Sampling locations in the Grand Calumet River and Indiana Harbor, Indiana

aspects of sediment sample preparation followed U.S. EPA Method 3540 (24).

Pore waters were prepared by centrifuging 275-325 g of wet sediment in acid-rinsed 250 ml polycarbonate centrifuge tubes for 45 min at 6800 xg. Supernatants were filtered though a Whatman glass fiber filter (GF-F, 0.7 μ nominal pore size) in a Millipore stainless steel filtering funnel. Multiple centrifuge tubes were necessary to provide sufficient sample volume for all analyses. One liter of filtered pore water was collected, preserved with 5 ml/L of a 1 g/L solution of $HgCl_2$ to prevent microbial degradation, and stored in the dark at 4°C until required for analysis.

One liter of pore water was adjusted to a Ph of >11 with concentrated NaOH, extracted with three successive 60 ml portions of pesticide-grade methylene chloride in a separatory funnel and the extracts combined for analysis of NPOCs. If a large emulsion was observed, continuous liquid-liquid extraction was used to complete the sample extraction. Extracts were passed over an anhydrous sodium sulphate drying column and reduced to 1 ml in a Kuderna-Danish concentrator. Final extract volumes were adjusted to 10 ml with methylene chloride. Pore water extraction followed the protocols outlined in U.S. EPA Method 3510 or, for emulsions, U.S. EPA Method 3520 (24). Cleanup procedures for both sediment and pore water extracts followed the protocols outlined in U.S. EPA Methods 3620, 3630 or 3660 (24) and were dictated by the class of compounds quantified in subsequent analyses.

Identification and quantitation of chemical analytes were performed with gas chromatography/mass spectroscopy (GC/MS) techniques. U.S. EPA 600 Series methods (25) were used for GC analyses while compound confirmation was conducted with U.S. EPA GC/MS Methods 8240 and 8250 (24). GC/MS operating conditions were as follows for all analyses: electron

energy 70 eV, mass range 35-550 amu, scan time 1 sec/scan, transfer line temperature 250°C, source temperature 200-250°C, injector temperature 250-350°C, injector on column or Grob splitless, sample volume 1 μl and carrier gas He at 15 psi. Analytical detection limits for all analysis have been presented elsewhere (26). Bulk sediment and pore water chemical concentrations are reported as mg/kg and μg/L, respectively.

Bulk sediment total organic carbon (TOC) was measured with a LECO carbon analyzer dry combustion technique and the results reported as % TOC on a dry wt. sediment basis. Dissolved organic carbon (DOC) in the pore water was operationally defined as the material passing a 0.7 μm filter and was measured with an IO Corporation, Inc. Model 700 TOC analyzer after sample acidification. Pore water DOC is reported on a mg/L basis.

Equilibrium Partitioning Calculations

Apparent partition coefficients, K_p', for NPOCs were calculated from measured chemical concentrations (Equation 7):

$$K_p' = \frac{C_{soc}}{C_p} \qquad (7)$$

where:

$$C_{soc} = \frac{C_s}{f_{oc}}$$

while actual partition coefficients K_p, were calculated from Equation 8,

$$K_p = \frac{C_{soc}}{C_d} \quad (8)$$

where:

$$C_d = \frac{C_p}{1 + m_{DOC} K_{DOC}}$$

and; C_p = measured total NPOC concentration in pore water

C_s = measured NPOC concentration in bulk sediment

C_{soc} = organic carbon-normalized NPOC concentration in bulk sediment

f_{oc} = decimal fraction organic carbon in bulk sediment

C_d = calculated dissolved NPOC concentration in pore water

m_{DOC} = measured DOC concentrations in pore water

K_{DOC} = DOC partition coefficient for NPOC

Theoretical partition coefficients, K_t were calculated with the fractional organic carbon content of the sediment and the K_{ow} for the NPOC of interest (Equation 9).

$$K_t = f_{oc} K_{ow} \quad (9)$$

For all NPOCs, the $\log_{10} K_{oc}$ (i.e., pK_{oc}) was calculated (Equation 10) [27].

$$\log_{10} K_{oc} = 0.00028 + 0.983 \log_{10} K_{ow} \quad (10)$$

Measured pK_{ow} values, if available, were used in all calculations of pK_{oc}. Estimated pK_{ow} values were used if necessary, however, all values, either measured or estimated (Table 1), were obtained from the AQUIRE database (28). In calculations of potential DOC-binding by NPOCs and dissolved NPOC concentrations in pore water, K_{DOC} was assumed to be equal to K_{oc}.

Results

A total of 29 NPOCs analyzed for were present in both Grand Calumet River bulk sediments and pore waters (Table 1). Measured or estimated pK_{ow} values from AQUIRE and calculated pK_{oc} values for each chemical also are reported in Table 1. As a point of interest, the original organic chemistry analytical suite for both bulk sediments and pore waters was composed of 106 discrete compounds (26). The compounds present in sediments and pore waters from the study area included phenolic compounds, industrial solvents and degreasers, pesticides and polycyclic aromatic hydrocarbons (PAHs).

The concentrations of 29 NPOCs which were present in both bulk sediment and pore water samples from the study area are presented in Table 2. TOC and DOC concentrations in bulk sediment and pore water, respectively, are presented in Table 2, as well as calculated "dissolved" concentrations of the 29 NPOCs in pore water. Measured concentrations of the NPOCs in bulk sediment ranged from 10 μg/kg for tetrachloroethylene to over 100 mg/kg for benzo(a)pyrene. Measured total concentrations of the NPOCs in pore water ranged from 0.1 μg/L for some of the chlorinated pesticides (p,p′-DDT, dieldrin, lindane, chlordane) and industrial chemicals (e.g., hexachlorobenzene) to greater than 450 μg/L for the PAH,

naphthalene.

The pK_t, which represents the $\log_{10}$ ($f_{oc} \times K_{ow}$) was calculated for each NPOC and compared with the pK_p',which represents the apparent partition coefficient $\log_{10}$ (C_{soc}/C_p) between the organic carbon-normalized sediment and total pore water chemical concentrations and the pK_p, which represents the actual partition coefficient $\log_{10}$ (C_{soc}/C_d) based on the calculated "dissolved" chemical concentration in the pore water (Table 3). These results indicate that under the operationally defined conditions of $K_{DOC} = K_{oc}$ and DOC = <0.7 μm, the binding of NPOCs to DOC was an important factor determining the distribution of NPOCs in pore waters from the Grand Calumet River. As the pK_{ow} of a compound increased, so did the importance of DOC-binding in determining the pore water distribution of a given NPOC. Di-octyl phthalate was the NPOC with the greatest pK_{ow} in the data set. Calculations based on measured total pore water DOC and di-octyl phthalate concentrations indicated that less than .01% of the measured total concentration of di-octyl phthalate in pore water (1.7-27.7 μg/L) was actually present as "dissolved", un-bound chemical.

To assess the influence of DOC in the pore water on concentrations of all 29 NPOCs in pore water, pK_p' was plotted as a function of pK_t (Figure 2). A similar relationship was developed for only those NPOCs with a measured or estimated pK_{ow} value <3.0 (Figure 3) or >3.0 (Figure 4). Each point with confidence limits represents the mean value ± one standard deviation from 5-10 samples (see Table 3) for an individual chemical.

Little relationship was observed between pK_p' and pK_t since pK_t accounts for only approximately 9% (r=0.29, R=0.09) of the variance in

Table 1. Non-polar organic compounds analyzed for and present in Grand Calumet River sediments and pore waters. Measured (M) or estimated (E) octanol-water partition coefficients (K_{ow}) from the AQUIRE database were used to calculate estimated organic carbon partition coefficients (K_{oc}) with the following equation: $\log_{10} K_{oc} = 0.00028 + 0.983 \log_{10} K_{ow}$ (27).

Compound	CAS No.	$\log_{10} K_{ow}$ (M/E)	$\log_{10} K_{oc}$
Phenol	108-95-2	1.46(M)	1.44
2,4-Dinitrotoluene	121-14-2	1.98(M)	1.95
1-Chloro-2-nitrobenzene	88-73-3	2.24(M)	2.20
1,2,3-Trichloropropene	96-19-5	2.36(E)	2.32
1,1,1-Trichlorethane	71-55-6	2.49(M)	2.45
Chlorobenzene	108-90-7	2.84(M)	2.79
Styrene	100-42-5	2.95(M)	2.90
Ethylbenzene	100-41-4	3.15(M)	3.09
Naphthalene	91-20-3	3.30(M)	3.25
p-Chlorotoluene	106-43-4	3.33(M)	3.28
o-Dichlorobenzene	95-50-1	3.38(M)	3.33
Tetrachloroethylene	127-18-4	3.40(M)	3.35
Lindane	58-89-9	3.61(M)	3.55
Biphenyl	92-52-4	4.09(M)	4.02
Dieldrin	60-57-1	4.32(M)	4.25
Phenanthrene	85-01-8	4.46(M)	4.39
Heptachlor	76-44-8	4.61(E)	4.53
Pentachloronitrobenzene	82-68-8	4.64(M)	4.56

Table 1. (cont.)

Compound	CAS No.	$\log_{10} K_{ow}$ (M/E)	$\log_{10} K_{oc}$
Pyrene	129-00-0	4.88(M)	4.80
Fluoranthene	206-44-0	4.95(E)	4.87
Pentachlorophenol	87-86-5	5.24(M)	5.15
Hexachlorobenzene	118-74-1	5.31(M)	5.22
Chlordane	57-74-9	5.54(E)	5.45
Chrysene	218-01-9	5.66(E)	5.57
Benzo(a)anthracene	56-55-3	5.66(E)	5.57
Benzo(a)pyrene	50-32-8	5.97(M)	5.87
p,p'-DDT	50-29-3	6.36(M)	6.25
p,p'-DDE	72-55-9	6.51(M)	6.40
Di-octyl phthalate	117-81-7	8.71(M)	8.56

Table 2. Measured non-polar organic chemical (NPOC) concentrations in Grand Calumet River bulk sediment (BS), mg/kg, and pore waters (PW, Cp), μg/L. Calculated "dissolved" pore water NPOC concentrations (PW, Cd) are reported as μg/L and sediment TOC and pore water DOC are reported as % dry wt. and mg/L, respectively.

		Grand Calumet River Location Number									
Parameter		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
TOC (% dry wt)	BS	28.1	4.4	7.2	12.5	14.3	15.9	14.7	22.3	18.8	13.4
DOC (mg/L)	Cp	28.6	25.9	30.2	24.2	53.0	46.1	28.8	31.1	28.0	18.7
Pentachlorophenol	BS	2.16	4.01	2.03	0.97	4.36	2.00	2.69	2.64	2.09	4.52
PW	Cp	12.8	6.4	10.0	3.5	13.9	11.3	12.5	14.8	18.8	24.5
PW	Cd	2.5	1.4	1.9	0.8	1.6	1.5	2.5	2.7	3.8	6.7
Phenol	BS	0.86	0.89	0.48	0.25	4.03	0.84	1.55	0.91	0.07	5.10
PW	Cp	226.1	50.3	189.9	27.8	256.6	207.1	107.6	225.3	326.2	255.5
PW	Cd	226.1	50.3	189.9	27.8	256.6	207.1	107.6	225.3	326.2	255.5
Biphenyl	BS	0.56	1.27	1.49	0.18	3.61	0.71	2.04	1.22	6.45	0.78
PW	Cp	14.5	4.2	13.2	6.9	33.2	12.5	23.2	32.5	25.6	30.1
PW	Cd	11.2	3.3	10.0	5.5	21.3	8.4	17.8	24.4	19.8	25.0

Table 2. (cont.)

Parameter		Grand Calumet River Location Number									
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Hexachlorobenzene	BS	0.92	1.38	1.00	0.04	1.38	0.86	0.98	0.54	0.19	0.47
PW	Cp	0.9	0.2	0.8	0.1	4.1	--	1.1	3.0	2.1	2.0
PW	Cd	0.16	0.04	0.13	0.02	0.42	--	0.19	0.49	0.37	0.49
Naphthalene	BS	1.02	1.75	3.78	2.11	4.92	1.89	1.38	1.63	3.58	8.24
PW	Cp	452.4	40.8	321.4	134.7	76.2	120.0	413.6	306.6	375.2	357.7
PW	Cd	430.86	38.86	306.10	129.52	69.91	111.11	393.90	292.0	357.33	347.28
p,p′-DDT	BS	0.23	0.67	1.44	0.25	0.75	1.01	0.83	0.55	1.75	0.09
PW	Cp	0.2	0.1	--	0.1	0.9	--	--	0.8	2.4	--
PW	Cd	0.0038	0.0021	--	0.0023	0.0094	--	--	0.0141	0.0470	--
p,p′-DDE	BS	2.46	2.46	2.49	0.71	1.55	4.23	2.90	1.78	4.65	2.71
PW	Cp	1.6	2.5	2.6	5.2	2.6	2.7	4.8	4.3	6.9	5.8
PW	Cd	0.02	0.04	0.04	0.08	0.02	0.02	0.07	0.06	0.10	0.12
Dieldrin	BS	0.21	0.76	0.72	0.04	0.08	0.93	0.62	0.04	3.21	1.14
PW	Cp	0.6	0.1	1.4	0.7	1.2	0.1	0.1	5.1	1.0	0.8

Table 2. (cont.)

Parameter		Grand Calumet River Location Number UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
PW	Cd	0.40	0.07	0.92	0.49	0.62	0.06	0.07	3.29	0.67	0.60
Lindane	BS	0.34	0.21	1.83	1.43	0.29	0.38	0.64	0.79	3.16	0.26
PW	Cp	0.1	--	--	--	0.4	--	0.3	0.7	--	0.2
PW	Cd	0.09	--	--	--	0.34	--	0.27	0.63	--	0.19
Chlordane	BS	1.04	0.44	1.23	0.05	0.09	1.64	1.89	2.41	2.18	2.14
PW	Cp	0.1	0.1	0.2	0.6	--	--	--	3.3	0.6	0.6
PW	Cd	0.01	0.01	0.02	0.08	--	--	--	0.34	0.07	0.10
Heptachlor	BS	0.98	0.42	2.66	0.36	1.24	0.52	1.37	0.44	1.72	0.19
PW	Cp	4.6	1.7	0.5	4.4	1.5	4.6	4.1	4.5	0.5	0.8
PW	Cd	2.34	0.90	0.25	2.42	0.54	1.79	2.07	2.18	0.26	0.49
Chlorobenzene	BS	1.47	2.47	2.09	1.04	0.93	1.18	3.56	1.83	6.82	1.05
PW	Cp	0.8	0.1	0.6	0.9	1.1	0.6	2.5	0.2	2.9	0.9
PW	Cd	0.78	0.10	0.59	0.89	1.07	0.58	2.45	0.20	2.84	0.89

Table 2. (cont.)

Parameter		Grand Calumet River Location Number UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
p-Chlorotoluene	BS	21.43	5.28	6.41	5.70	8.22	5.35	10.24	7.32	1.74	5.27
PW	Cp	23.2	10.0	11.1	5.0	16.4	5.4	12.4	54.6	14.6	20.0
PW	Cd	22.10	9.52	10.47	4.76	14.91	4.95	11.81	51.51	13.90	19.23
o-Dichlorobenzene	BS	2.86	4.20	2.98	1.66	2.91	2.10	2.99	1.49	--	2.21
PW	Cp	0.2	0.9	0.4	0.8	0.6	--	--	0.6	0.5	--
PW	Cd	0.19	0.86	0.38	0.76	0.54	--	--	0.56	--	--
Ethylbenzene	BS	4.19	2.17	4.72	2.34	3.61	4.48	4.00	1.84	0.05	10.28
PW	Cp	3.5	2.8	2.9	0.4	12.5	0.5	10.5	9.5	15.8	12.2
PW	Cd	3.37	2.72	2.79	0.39	11.68	0.47	10.10	9.13	15.19	11.96
Styrene	BS	4.17	1.66	2.77	0.49	0.65	0.68	1.94	1.85	6.49	6.85
PW	Cp	5.2	5.1	5.9	1.9	13.8	2.8	1.6	5.9	15.6	17.5
PW	Cd	5.10	5.00	5.78	1.86	13.27	2.69	1.57	5.78	15.29	17.33
Tetrachloroethylene	BS	0.02	--	--	0.01	--	0.01	0.02	--	0.02	0.01
PW	Cp	0.7	0.2	1.2	0.4	0.9	0.4	0.2	4.6	2.8	2.4

Table 2. (cont.)

Parameter		Grand Calumet River Location Number									
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
PW	Cd	0.66	--	--	0.38	--	0.36	0.19	--	2.64	2.31
Di-octyl phthalate	BS	0.23	0.78	1.20	--	1.04	1.21	0.15	2.61	0.41	--
PW	Cp	5.8	4.8	2.3	2.2	14.6	3.6	1.7	27.7	8.5	8.6
PW	Cd	0.0006	0.0005	0.0002	--	0.0008	0.0002	0.0001	0.002	0.0008	--
Pentachloro-nitrobenzene	BS	0.15	2.04	0.33	0.17	0.03	0.22	0.16	2.65	0.38	0.07
PW	Cp	0.6	0.2	0.4	0.3	2.8	0.2	0.3	1.5	0.7	1.1
PW	Cd	0.29	0.10	0.19	0.16	0.96	0.07	0.15	0.70	0.35	0.65
Pyrene	BS	3.68	5.11	4.81	1.59	7.26	3.05	4.05	1.72	4.57	4.20
PW	Cp	10.1	4.6	9.9	6.8	28.7	3.3	6.4	18.6	19.8	19.0
PW	Cd	3.62	1.76	3.43	2.70	6.64	0.85	2.28	6.31	7.17	8.76
Fluoranthene	BS	0.34	0.66	1.22	0.42	0.99	0.06	0.18	0.48	0.31	6.83
PW	Cp	30.5	25.9	27.4	10.3	35.9	9.8	8.8	66.6	32.1	17.4
PW	Cd	9.84	8.93	8.51	3.71	7.34	2.23	2.82	20.24	10.49	7.34

Table 2. (cont.)

		Grand Calumet River Location Number									
Parameter		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Benzo(a)pyrene	BS	27.21	20.26	16.88	8.44	45.66	8.94	1.85	83.62	100.21	32.51
PW	Cp	1.4	0.6	0.2	0.2	5.6	2.4	0.2	5.8	3.3	2.8
PW	Cd	0.06	0.03	0.01	0.01	0.14	0.07	0.01	0.24	0.15	0.19
Phenanthrene	BS	2.04	4.32	5.06	0.66	5.28	1.28	3.61	2.15	4.04	2.55
PW	Cp	230.6	99.2	176.5	55.8	145.4	66.4	27.4	188.2	245.4	186.8
PW	Cd	136.44	60.86	102.02	35.09	63.77	31.32	16.12	107.54	146.07	128.83
Chrysene	BS	2.09	3.56	3.46	0.83	4.21	1.15	2.96	2.64	5.21	2.57
PW	Cp	8.4	3.6	2.8	2.9	23.3	1.7	6.0	31.9	9.0	9.4
PW	Cd	0.73	0.34	0.23	0.29	1.14	0.09	0.52	2.57	0.80	1.20
Benzo(a)anthracene	BS	2.63	1.68	2.14	0.58	1.43	1.66	0.53	2.66	1.65	0.17
PW	Cp	9.5	4.6	7.1	2.4	18.9	10.4	0.6	26.4	16.2	22.5
PW	Cd	0.83	0.44	0.59	0.24	0.93	0.58	0.05	2.13	1.44	2.87

Table 3. Log_{10} apparent field partition coefficients, K_p, (C_{soc}/C_p); actual field partition coefficients Kp, (C_{soc}/C_d) and theoretical partition coefficients K_t (f_{oc} x K_{ow}) for non-polar organic chemicals (NPOCs) measured in Grand Calumet River sediments and pore waters.

		Grand Calumet River Location Number											
Parameter	Coefficient	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
Pentachlorophenol	Kc	4.69	3.88	4.10	4.34	4.40	4.44	4.41	4.59	4.51	4.37	4.37	0.23
	Kp′	2.78	4.15	3.45	3.35	3.34	3.05	3.17	2.90	2.77	3.19	3.22	0.41
	Kp	3.49	4.81	4.17	3.99	4.28	3.92	3.86	3.64	3.47	3.70	3.93	0.41
Phenol	Kc	0.91	0.10	0.32	0.56	0.62	0.66	0.63	0.81	0.73	0.59	0.59	0.23
	Kp′	1.13	2.60	1.55	1.86	2.04	1.41	1.99	1.26	0.06	2.17	1.61	0.71
	Kp	1.13	2.60	1.55	1.86	2.04	1.41	1.99	1.26	0.06	2.17	1.61	0.71
Hexachlorobenzene	Kc	4.76	3.95	4.17	4.41	4.47	--	4.48	4.66	4.58	4.44	4.44	0.25
	Kp′	3.56	5.20	4.24	3.51	3.37	--	3.78	2.91	2.68	3.24	3.61	0.75
	Kp	4.31	5.89	5.03	4.20	4.36	--	4.55	3.69	3.44	3.85	4.37	0.74
Naphthalene	Kc	2.75	1.94	2.16	2.40	2.45	2.50	2.47	2.65	2.57	2.43	2.43	0.23
	Kp′	0.90	2.99	2.21	2.10	2.65	2.00	1.36	1.38	1.71	2.24	1.95	0.63
	Kp	0.93	3.01	2.23	2.12	2.69	2.03	1.38	1.40	1.73	2.25	1.98	0.63

Table 3. (cont.)

Parameter	Coefficient	Grand Calumet River Location Number											
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
p,p´-DDT	Kc	5.81	5.00	--	5.46	5.52	--	--	5.71	5.63	--	5.52	0.29
	Kp´	3.61	5.18	--	4.30	3.77	--	--	3.49	3.59	--	3.99	0.65
	Kp	5.33	6.86	--	5.94	5.75	--	--	5.24	5.30	--	5.74	0.62
p,p´-DDE	Kc	5.96	5.15	5.37	5.61	5.67	5.71	5.68	5.86	5.78	5.64	5.64	0.23
	Kp´	3.74	4.35	4.12	3.03	3.62	3.99	3.61	3.27	3.55	3.54	3.68	0.39
	Kp	5.64	6.15	5.94	4.85	5.73	6.12	5.45	5.12	5.39	5.23	5.56	0.43
Dieldrin	Kc	3.77	2.96	3.18	3.42	3.48	3.52	3.49	3.67	3.59	3.45	3.45	0.23
	Kp´	3.10	5.24	3.85	2.66	2.67	4.77	4.63	1.55	4.23	4.03	3.67	1.15
	Kp	3.27	5.39	4.04	2.81	2.96	4.99	4.78	1.74	4.41	4.15	3.85	1.14
Lindane	Kc	3.06	--	--	--	2.77	--	2.78	2.96	--	2.74	2.86	0.14
	Kp´	4.08	--	--	--	3.71	--	4.16	3.70	--	3.97	3.92	0.21
	Kp	4.12	--	--	--	3.78	--	4.21	3.75	--	4.01	3.97	0.20
Chlordane	Kc	4.99	4.19	4.40	4.64	--	--	--	4.89	4.82	4.67	4.66	0.28
	Kp´	4.57	5.00	4.93	2.82	--	--	--	3.52	4.29	4.43	4.22	0.79

Table 3. (cont.)

Parameter	Coefficient	Grand Calumet River Location Number											
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
	Kp	5.57	6.00	5.93	3.70	--	--	--	4.50	5.22	5.20	5.16	0.82
Heptachlor	Kc	4.05	3.25	3.46	3.70	3.76	3.81	3.77	3.95	3.88	3.73	3.74	0.23
	Kp'	2.88	3.75	4.87	2.82	3.76	2.85	3.36	2.64	4.26	3.25	3.44	0.72
	Kp	3.17	4.03	5.17	3.08	4.21	3.26	3.65	2.96	4.55	3.46	3.75	0.72
Chlorobenzene	Kc	2.29	1.48	1.70	1.94	2.00	2.04	2.01	2.19	2.11	1.97	1.97	0.23
	Kp'	3.82	5.75	4.68	3.97	3.77	4.09	3.97	4.61	4.10	3.94	4.27	0.60
	Kp	3.83	5.75	4.69	3.97	3.78	4.11	3.99	4.61	4.11	3.94	4.28	0.60
p-Chlorotoluene	Kc	2.78	1.97	2.19	2.43	2.49	2.53	2.50	2.68	2.60	2.46	2.46	0.23
	Kp'	3.52	4.08	3.90	3.96	3.54	3.79	3.75	2.78	2.80	3.29	3.54	0.46
	Kp	3.54	4.10	3.93	3.98	3.59	3.83	3.77	2.80	2.82	3.31	3.57	0.46
o-Dichlorobenzene	Kc	2.83	2.02	2.24	2.48	2.54	--	--	2.73	--	--	2.47	0.30
	Kp'	4.71	5.03	5.01	4.22	4.53	--	--	4.05	--	--	4.59	0.40
	Kp	4.73	5.05	5.04	4.24	4.58	--	--	4.08	--	--	4.62	0.40
Ethylbenzene	Kc	2.60	1.79	2.01	2.25	2.31	2.35	2.32	2.50	2.42	2.28	2.28	0.23

Table 3. (cont.)

Parameter	Coefficient	Grand Calumet River Location Number											
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
	Kp'	3.63	4.25	4.35	4.67	3.31	4.75	3.41	2.94	1.23	3.80	3.63	1.04
	Kp	3.65	4.26	4.37	4.68	3.33	4.78	3.43	2.96	1.24	3.81	3.65	1.04
Styrene	Kc	2.40	1.59	1.81	2.05	2.11	2.15	2.12	2.30	2.22	2.08	2.08	0.23
	Kp'	3.46	3.87	3.81	3.31	2.52	3.18	3.92	3.15	3.34	3.47	3.40	0.42
	Kp	3.46	3.88	3.82	3.32	2.53	3.20	3.92	3.16	3.35	3.47	3.41	0.41
Tetrachloroethylene	Kc	2.85	--	--	2.50	--	2.60	2.57	--	2.67	2.53	2.62	0.13
	Kp'	2.01	--	--	2.30	--	2.20	2.83	--	1.58	1.49	2.07	0.50
	Kp	2.03	--	--	2.32	--	2.24	2.85	--	1.61	1.51	2.09	0.49
1,2,3-Trichloro-propene	Kc	1.80	1.00	1.22	1.46	1.52	1.56	1.53	1.71	--	--	1.48	0.26
	Kp'	3.77	4.85	4.30	4.04	3.75	3.15	4.56	--	--	--	4.06	0.57
	Kp	3.77	4.85	4.30	4.04	3.75	3.15	4.56	--	--	--	4.06	0.57
1,1,1-Trichloroethane	Kc	1.94	1.13	1.35	--	--	1.69	--	1.84	--	--	1.59	0.34
	Kp'	1.16	2.96	1.59	--	--	2.82	--	2.31	--	--	2.17	0.78
	Kp	1.16	2.96	1.59	--	--	2.82	--	2.31	--	--	2.17	0.78

Table 3. (cont.)

Parameter	Coefficient	Grand Calumet River Location Number											
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
Di-octyl phthalate	Kc	8.16	7.35	7.56	--	7.86	7.91	7.87	8.06	7.98	--	7.84	0.27
	Kp′	2.15	3.57	3.86	--	2.70	3.33	2.78	2.63	2.41	--	2.93	0.60
	Kp	6.17	7.55	7.92	--	6.96	7.58	7.01	6.77	6.44	--	7.05	0.60
1-Chloro-2-nitrobenzene	Kc	1.69	--	1.10	1.34	1.40	1.44	1.41	1.59	1.51	--	1.44	0.18
	Kp′	1.95	--	3.99	2.48	1.82	2.53	2.12	2.81	1.50	--	2.40	0.77
	Kp	1.95	--	3.99	2.48	1.82	2.53	2.12	2.81	1.50	--	2.40	0.77
2,4-Dinitrotoluene	Kc	1.43	0.62	0.84	--	1.14	1.18	1.15	--	1.25	1.11	1.09	0.25
	Kp′	3.25	3.36	3.39	--	2.39	3.28	2.53	--	2.73	2.00	2.87	0.53
	Kp	3.25	3.36	3.39	--	2.39	3.28	2.53	--	2.73	2.00	2.87	0.53
Pentachloro-nitrobenzene	Kc	4.09	3.28	3.50	3.74	3.80	3.84	3.81	3.99	3.91	3.77	3.77	0.23
	Kp′	2.95	5.37	4.06	3.66	1.87	3.84	3.56	3.90	3.46	2.68	3.54	0.93
	Kp	3.26	5.67	4.38	3.93	2.34	4.30	3.86	4.23	3.76	2.91	3.86	0.91
Pyrene	Kc	4.33	3.52	3.74	3.98	4.04	4.08	4.05	4.23	4.15	4.01	4.01	0.23

Table 3. (cont.)

Parameter	Coefficient	Grand Calumet River Location Number											
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
	Kp'	3.11	4.40	3.83	3.27	3.25	3.76	3.63	2.62	3.09	3.22	3.42	0.50
	Kp	3.56	4.82	4.29	3.67	3.88	4.35	4.08	3.09	3.53	3.55	3.88	0.51
Fluoranthene	Kc	4.40	3.59	3.81	4.05	4.11	4.15	4.12	4.30	4.22	4.08	4.08	0.23
	Kp'	1.60	2.76	2.79	2.51	2.29	1.59	2.14	1.51	1.71	3.47	2.24	0.65
	Kp	2.09	3.23	3.30	2.96	2.97	2.25	2.64	2.03	2.20	3.84	2.75	0.61
Benzo(a)pyrene	Kc	5.42	4.61	4.83	5.07	5.13	5.17	5.14	5.32	5.24	5.10	5.10	0.23
	Kp'	4.84	5.89	6.07	5.53	4.76	4.37	4.80	4.81	5.21	4.94	5.12	0.55
	Kp	6.21	7.19	4.37	6.83	6.36	5.90	6.10	6.19	6.55	6.11	6.18	0.74
Phenanthrene	Kc	3.91	3.10	3.32	3.56	3.62	3.66	3.63	3.81	3.73	3.59	3.59	0.23
	Kp'	1.50	3.00	2.60	1.98	2.40	2.08	2.95	1.71	1.94	2.01	2.22	0.51
	Kp	1.73	3.21	2.84	2.18	2.76	2.41	3.18	1.95	2.17	2.17	2.46	0.51
Chrysene	Kc	5.11	4.31	4.52	4.76	4.82	4.87	4.83	5.01	4.94	4.79	4.80	0.23
	Kp'	2.95	4.35	4.23	3.36	3.10	3.63	3.53	2.56	3.49	3.31	3.45	0.54
	Kp	4.01	5.38	5.32	4.36	4.41	4.91	4.59	3.66	4.54	4.20	4.54	0.55

Table 3. (cont.)

Parameter	Coefficient	Grand Calumet River Location Number											
		UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10	$\bar{X}$	± SD
Benzo(a)anthracene	Kc	5.11	4.31	4.52	4.76	4.82	4.87	4.83	5.01	4.94	4.79	4.80	0.23
	Kp′	2.99	3.92	3.62	3.29	2.72	3.00	4.78	2.65	2.73	1.75	3.15	0.82
	Kp	4.05	4.94	4.83	4.29	4.03	4.26	4.86	3.75	3.78	2.65	4.14	0.68
Biphenyl	Kc	3.53	2.73	2.95	3.19	3.25	3.29	3.26	3.44	3.36	3.22	3.22	0.23
	Kp′	2.14	3.84	3.20	2.32	2.88	2.55	2.78	2.23	3.13	2.29	2.74	0.54
	Kp	2.25	3.94	3.32	2.42	3.07	2.73	2.89	2.35	3.24	2.36	2.86	0.54

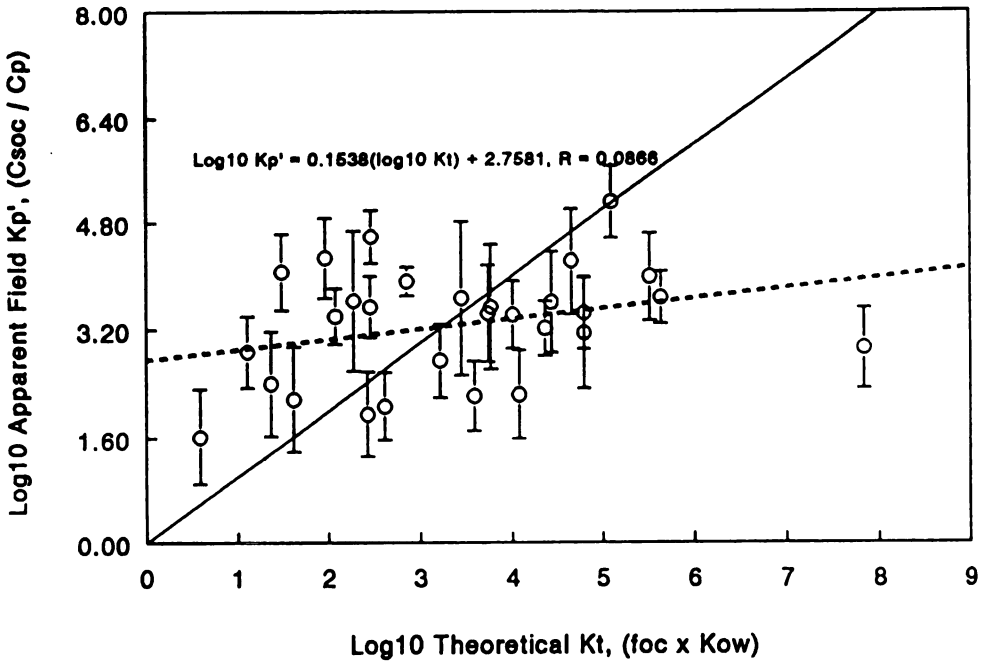

Figure 2. Log_{10} K_t, (f_{oc} x K_{ow}), versus log_{10} apparent field K_p, (C_{soc}/C_p), for all non-polar organic compounds analyzed for and present in Grand Calumet River sediments and pore waters. The relationship between K_t and K_p, data is plotted (dashed line) and shown in the equation while the solid line represents a theoretical relationship with r = 1.0.

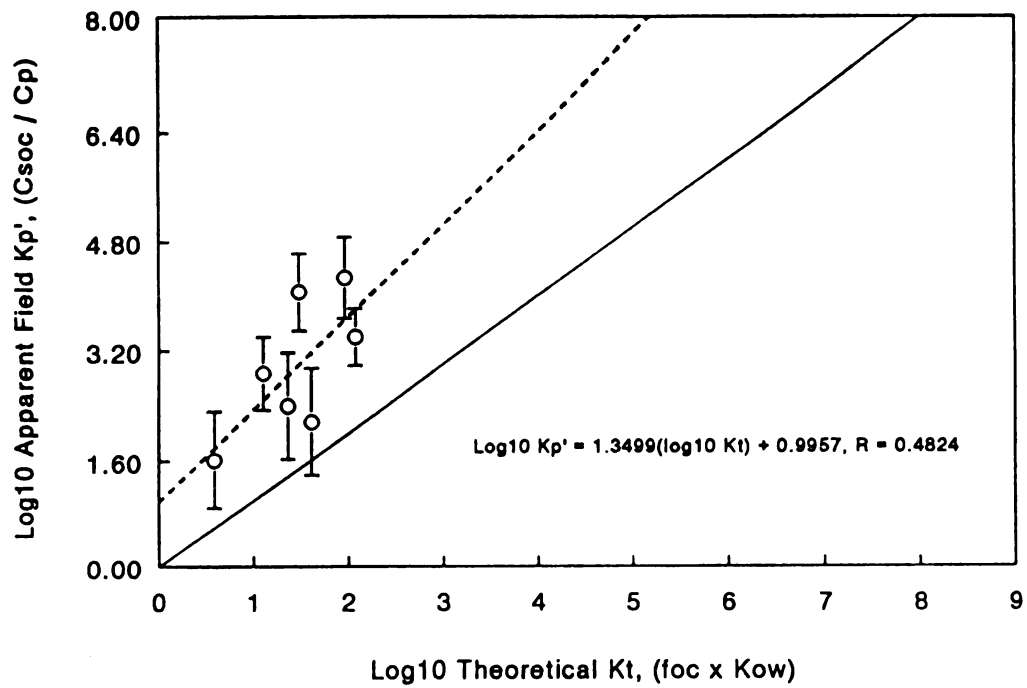

Figure 3. Log_{10} K_t (f_{oc} x K_{ow}), versus log_{10} apparent field K_p, (C_{soc}/C_p), for all non-polar organic compounds with log_{10} K_{ow} values <3.0 analyzed for and present in Grand Calumet River sediments and pore waters. The relationship between K_t and K_p, data is plotted (dashed line) and shown in the equation while the solid line represents a theoretical relationship with r = 1.0.

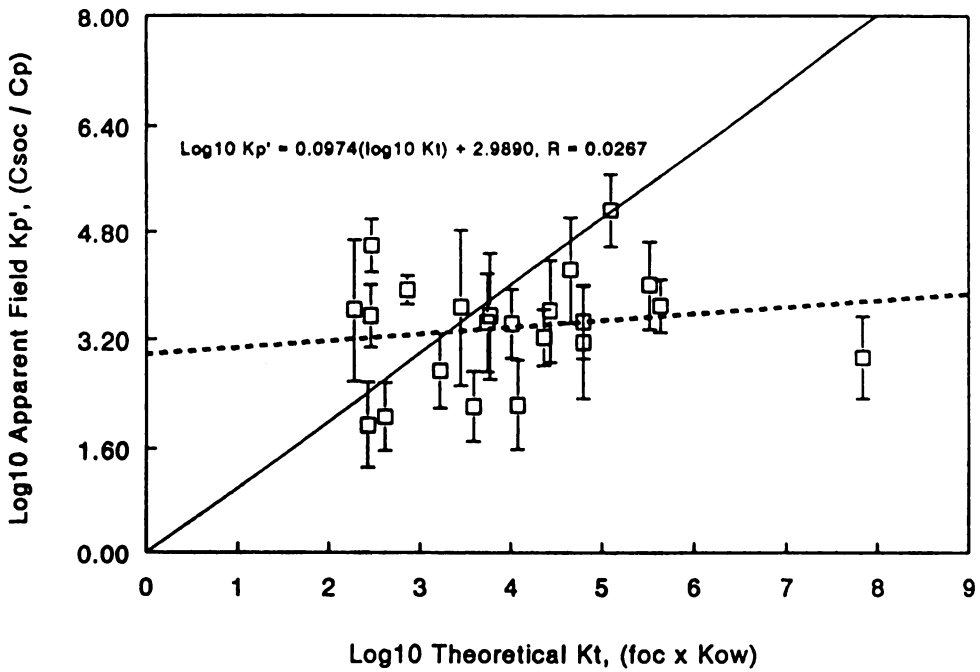

Figure 4. Log_{10} K_t (f_{oc} x K_{ow}), versus log_{10} apparent field K_p, (C_{soc}/C_p), for all non-polar organic compounds with log_{10} K_{ow} values >3.0 analyzed for and present in Grand Calumet River sediments and pore waters. The relationship between K_t and K_p, data is plotted (dashed line) and shown in the equation while the solid line represents a theoretical relationship with r = 1.0.

pK_p'. This is also indicated by the divergence of the dashed line which best fits the data from the solid line representing unity or perfect correspondence between pK_p' and pK_t. When only the data for NPOCs with pK_{ow} values <3.0 were plotted (Figure 3), better agreement was observed between theoretical predictions and observed results. The line which best fits the data has an intercept of 0.99 and a slope of 1.35. The relationship pK_t accounts for 48% of the variance in pK_p, (r=0.69, R=0.48). The NPOCs with pK_{ow} values >3.0 exhibited little relationship between pK_t and pK_p' since pK_t explained <3% of the variance in pK_p, (r=0.16, R=0.03).

If the data for NPOCs with pK_{ow} values >3.0 are corrected for binding to DOC and plotted, there is much better concordance between pK_t and the actual partition coefficient, pK_p (Figure 5). After correction, pK_t accounted for 58% of the variance in pK_p (r=0.76, R=0.58) as opposed to accounting for only <3.0% of the variance in pK_p' before correction for binding to DOC. If the original data for NPOCs with pK_{ow} values <3.0 are added back into the data set, the relationship between pK_t and pK_p further improves and approximately 62% of the variance in pK_p can be accounted for by pK_t (Figure 6).

The original data on pK_p' versus pK_t from this study as well as similar data from other field studies on PAHs (18, 23) and laboratory sediment-spiking studies with industrial chemicals and pesticides (29) are compared in Figure 7. These data fit the general pattern observed for the Grand Calumet River data presented here, however, it appears that binding to DOC was potentially more important in determining NPOC distribution in pore waters from the studies by Socha and Carpenter (18) and Oliver (29) than in the study by Kadeg and Pavlou (23). This should be the case since

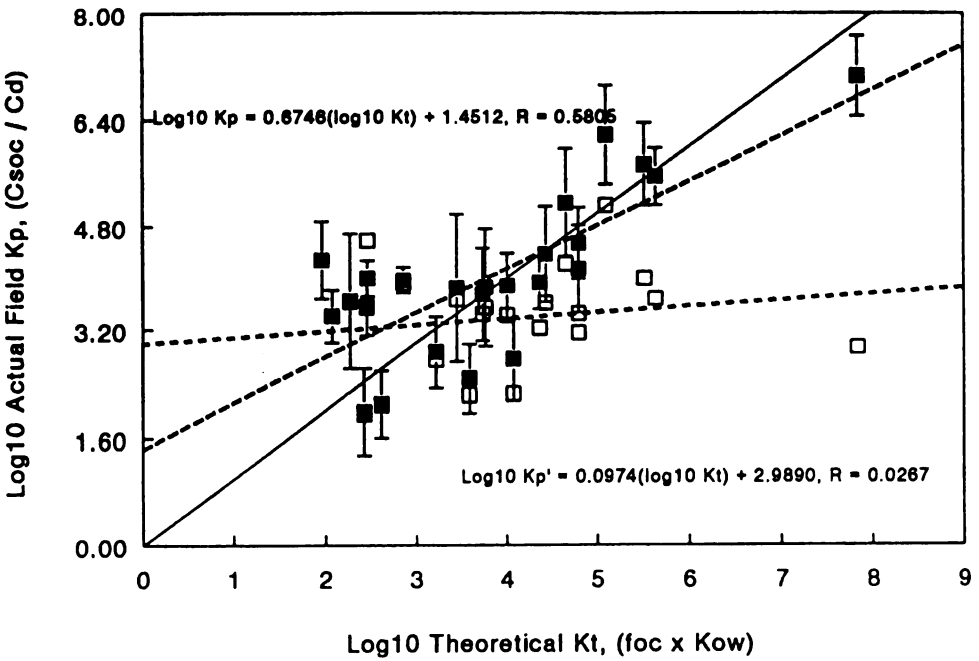

Figure 5. Log_{10} K_t, (f_{oc} x K_{ow}), versus log_{10} apparent field K_p, (C_{soc}/C_p), open squares) and actual K_p, (C_{soc}/C_d), closed squares) for all non-polar organic compounds with log_{10} K_{ow} values >3.0 analyzed for and present in Grand Calumet River sediments and pore waters. The equation in the upper left corner presents the relationship between K_t and K_p for these compounds while the equation in the lower right corner presents the relationship between K_t and K_p'.

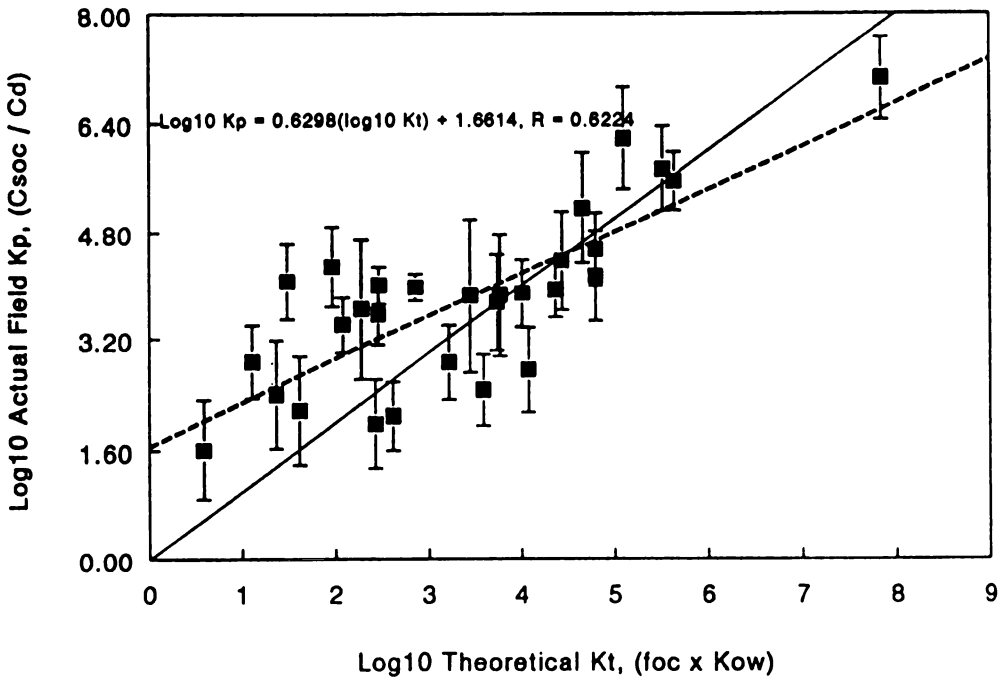

Figure 6. Log_{10} K_t, (f_{oc} x K_{ow}), versus log_{10} K_p, (C_{soc}/C_d), for all non-polar organic compounds analyzed for and present in Grand Calumet River sediments and pore waters.

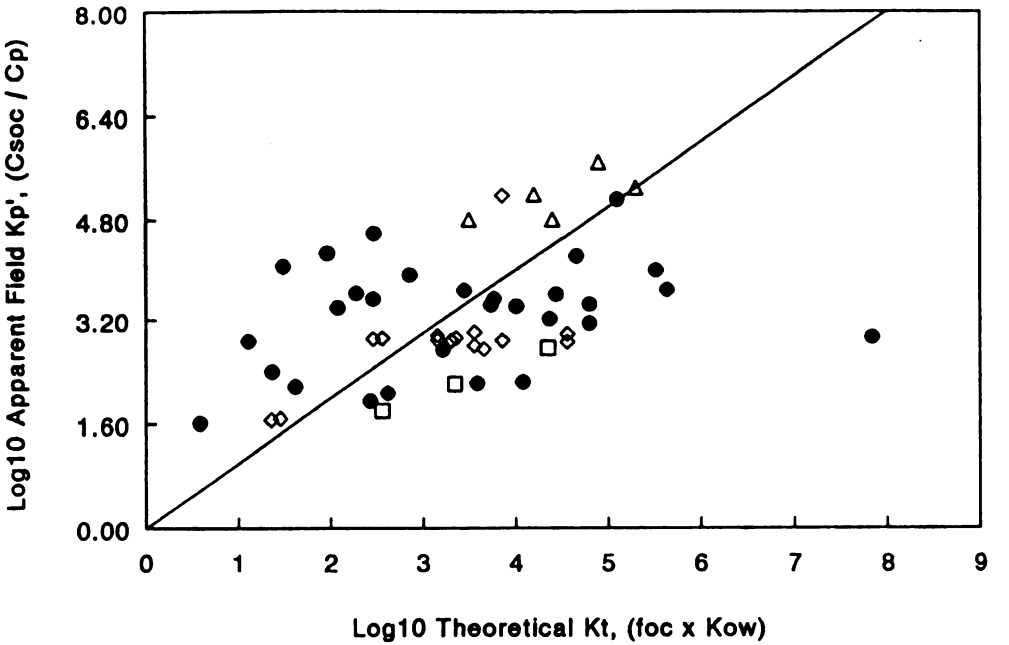

Figure 7. Comparative $\log_{10} K_t$, (f_{oc} x K_{ow}), versus apparent field K_p, (C_{soc}/C_p) data from this study (closed circles), Kadeg and Pavlou (23, open triangles), Socha and Carpenter (18, open squares) and Oliver (29, open diamonds).

Kadeg and Pavlou (23) determined "dissolved" concentrations of PAHs in pore water.

Discussion

The potential for binding of NPOCs to DOC in pore water is an issue of importance in the development of exposure models for the effects of NPOCs on aquatic organisms and, in particular, benthic macroinvertebrates. If DOC-bound NPOCs are unavailable to cause effects on biota as proposed by McCarthy and Jimenez (21) and Landrum et al (22), then these exposure models must account for DOC-binding of NPOCs in pore water to accurately predict exposure concentrations for NPOCs. Currently, equilibrium partitioning theory (EqP) is being proposed as the theoretical basis for the development of sediment quality criteria (SQC). Draft SQC criteria documents have been prepared by the U.S. Environmental Protection Agency (EPA) for five NPOCs, endrin, dieldrin, phenanthrene, fluoranthene and acenaphthene. These criteria development efforts have made no effort to account for DOC-binding of NPOCs in pore water because, to date, there has been little empirical field evidence conclusively demonstrating the importance of DOC-binding in determining the distribution, and thus bioavailability, of NPOCs in pore waters from contaminated sediments. NPOC binding to DOC may be of the greatest importance in attempts to extrapolate SQC which are reported on an organic carbon-normalized bulk sediment concentration basis (μg NPOC/gm organic carbon) to permissible concentrations of NPOCs in pore water. Binding to DOC could increase NPOC concentrations in pore water to values greater than permissible based on extrapolation from SQC while the "dissolved" (i.e, bioavailable)

concentration of the NPOC in pore water could actually be acceptable if binding to DOC was accounted for in the calculation of acceptable NPOC concentrations in pore water. A major strength of EqP as an exposure model and potential basis for the development of SQC is that including a correction for DOC-binding of NPOC requires only a simple extension of the partitioning theory to DOC and is consistent with the thermodynamic principals underlying EqP (4).

Current EqP models are best described as two phase because they account for NPOC partitioning between two sorbent phases, sediment organic carbon and pore water. The existence of a third sorbent phase, DOC, also would explain the particle concentration effect which has been reported by some researchers (26, 30). Experimental limitations make it difficult to measure empirically the "dissolved" and DOC-bound concentrations of NPOCs in pore water, however, Voice et al (14) and Gschwend and Wu (15) have previously proposed that dissolved ligands and colloids, respectively, were the agents causing the observed particle concentration effects.

The results of this investigation of the DOC-binding of NPOCs in pore waters from the Grand Calumet River has demonstrated the potential importance of this phenomenon in determinations of the bioavailable fraction of NPOCs in pore water. Frequently, critics of the EqP method of predicting exposure concentrations have decried the lack of relationship between predicted and measured pore water concentrations of NPOCs. This conclusion would follow after a cursory examination of the data presented in Figure 2. This observation is made even more apparent for NPOCs with $\log_{10} K_{ow}$ >3.0 if the data are divided into NPOCs with $\log_{10} K_{ow}$ values <3.0 (Figure 3) and >3.0 (Figure 4).

The importance of NPOC binding to DOC in pore water has previously

been reported for two limited groups of NPOCs, PCB congeners (16) and PAHs (18), respectively. Both studies reported that NPOC concentrations in pore waters from field collected sediments were greater than predicted due to partitioning to DOC. Correction for DOC-binding resulted in "dissolved" NPOC concentrations in pore waters which were in better agreement with predicted values. Chiou et al. (17) also have reported that the apparent water solubilities of organic chemicals, including pesticides, increased linearly with an increase in DOC (DOM) concentration while Brusseau (31) reported the enhanced sorption of three non-ionic compounds to two aquifer materials of low organic carbon content. The mechanism for this effect appeared to be increased DOC concentration as a result of experimental additions of tetrachloroethane.

This study demonstrates the applicability of the EqP predictions to a much greater range of compounds, if DOC-binding is taken into account. However, one of the criticisms of our approach to determining the "dissolved" concentrations of NPOCs in Grand Calumet River pore waters may be the assumption that $K_{DOC} = K_{OC}$. Most studies have reported K_{OC} values < K_{OW} values but few studies have evaluated K_{DOC} values in relationship to K_{OC}. Although several studies have reported K_{DOC} values $<K_{OC}$, several also have reported that NPOCs bind to organic sorbents in particulate and dissolved form to a similar extent (32, 34). An important factor in these evaluations may be determining that the sediment organic matter and the pore water dissolved organic matter are of the same origin (i.e. terrestrial, aquatic plant, etc.) and physical/chemical characteristics (i.e. hydrophobicity, CHNO composition, etc). Numerous examples exist of altered partitioning of NPOCs to different types of organic matter which was due to differences in the chemical composition of the organic matter

(32-35).

Although NPOCs with pK_{ow} >3.0 are generally of the greatest interest because of their propensity to resist environmental degradation and to be bioconcentrated by biota, the behavior of the NPOCs with pK_{ow} <3.0 also is of interest in the Grand Calumet River data set. Based on the data presented in Table 2 and Figure 3, it appears that these NPOCs either occurred at greater than expected concentrations in sediments from the study sites or at concentrations less than expected in the study site pore waters. The former possibility was reported by Boyd and Sun (36) for soils and was hypothesized to be due to residual petroleum hydrocarbons and polychlorobiphenyl oils acting as a sorptive phase with approximately 10 times the partitioning strength of native soil organic matter. Sediments from the Grand Calumet River do contain significant amounts of petroleum hydrocarbons (26). However, based on our unpublished comparisons of measured versus predicted pore water concentrations of the NPOCs in pore water, it appears that a much more plausible explanation is that these compounds may be volatilized from the pore water preferentially by the vacuum filtration step during pore water preparation.

Equilibrium partitioning theory appears to be a viable basis for predicting the partitioning behavior of NPOCs under field conditions if consideration is given to the potential for DOC-binding of NPOC. Based on comparison of actual partition coefficients, pK_p, versus estimated partition coefficients, pK_t, for the NPOCs measured in Grand Calumet River sediments and pore waters, binding to DOC in pore water is not important for NPOCs with pK_{ow} values <3.0. NPOCs with pK_{ow} values >4.0 can be expected to demonstrate significant potential for binding to DOC in pore water. If SQC are to be based on water quality criteria extrapolated to

"dissolved" pore water chemical concentrations, binding to DOC in pore water must be accounted for in determining "dissolved" pore waters concentrations of the chemicals of interest, otherwise the application of SQC to determine permissible concentrations of NPOCs in pore water will overestimate the potential for adverse effects.

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CHAPTER 2

Toxicity of Sediments and Sediment Pore Waters from the Grand Calumet River-Indiana Harbor, IN Area of Concern.

Introduction

The sediments of many rivers, harbors and connecting channels of the Great Lakes contain a wide array of chemical compounds, including organic xenobiotics (Pranckevicius 1986, Fallon and Horvath 1985) and metals (Pranckevicius 1986, Fallon and Horvath 1985, Hamdy and Post 1985). Due to municipal, industrial and non-point source waste discharges and the tendency of many chemicals to become associated with sediments, contaminated sediments are particularly problematic near densely populated, industrialized urban areas, such as the area surrounding the Grand Calumet River-Indiana Harbor (GCR-IH) in northwest Indiana. Due to contaminants and nutrients in the water and sediments from this area, it has been designated an Area of Concern (AOC) by the International Joint Commission (IJC 1985). The environmental status, relative to anthropogenically-introduced chemical contamination, in these areas of the Great Lakes has been summarized elsewhere (Rodgers et al. 1985). The severely contaminated condition of the GCR-IH AOC is based on water quality problems with conventional pollutants, metals, and organic chemicals; contaminated sediments, impacted aesthetics and biota, and fish consumption advisories (IDEM 1988). The presence of a wide variety of in-place chemical contaminants in sediments from the AOC and their potential effects on biota were the primary focus of this study.

Assessing the degree of contamination of sediments consists of basically two aspects: 1) determination of which contaminants are present

and 2) evaluation of the potential effects of these contaminants on biota (Bishop 1987). The potential toxicity of sediments to benthic organisms can be determined by surveying the number and types of indigenous organisms present in a sediment (Chapman 1986). However, the absence of a particular macroinvertebrate species in a sediment does not necessarily indicate that the sediment is toxic. Toxicity of sediment to benthic invertebrates also can be estimated by quantifying all of the toxic compounds and elements associated with the sediment by analyzing: 1) bulk sediment, 2) sediment pore water (Jenne et al. 1980, Batley and Giles 1980), 3) an elutriate of the sediment (Brannon et al. 1980, Laskowski-Hoke and Prater 1980), or 4) various organic or acid extracts designed to selectively remove particular classes of toxic substances (Samoiloff et al. 1983). The potential toxicity of a sediment to benthic organisms then can be predicted by comparing the observed concentrations of chemicals in 1, 2, 3, or 4 above to dose-response relationships determined under laboratory conditions for each individual toxicant. However, a number of parameters, such as organic carbon content and particle size distribution, can affect the availability of both metals and organic chemicals in sediments to benthic organisms (Babich and Stotzky 1977, Laxen 1985, Oliver 1985). In addition, potential interactions due to the presence of a complex chemical mixture in a sediment are not known. Toxicity tests, however, can be used to provide a direct assessment and to integrate the effects of the biologically active fraction of all of the toxic chemicals present in a sediment, pore water, elutriate or organic solvent extract. If laboratory toxicity data are available for specific chemicals present in the samples, the results of toxicity tests can be compared with measured chemical concentrations in a "toxic units" (TU) approach (Sprague

and Ramsay 1965) to help determine potential causes of the observed toxicity.

This investigation tested the relative sensitivities of several simple toxicity tests with bacteria and invertebrates in order to provide a framework for conducting rapid, comprehensive surveys of potentially toxic sediments and to conduct an assessment of sediment toxicity in the Grand Calumet River, IN. The tests chosen for use in the toxicity assessment of sediment pore waters were the Microtox® test and 48 h acute tests with Daphnia magna and Ceriodaphnia dubia while a 10-d test with Chironomus tentans was chosen to assess the toxicity of solid phase sediments.

The Microtox® test is a bacterial luminescence toxicity test developed by Beckman, Inc. in 1977 (Bulich 1984) as a rapid screening alternative to standard acute toxicity testing with fish or invertebrates. This test is based on the reduction in bioluminescence of the marine bacterium (Photobacterium phosphoreum) (NRRL B-11177) by toxic chemicals. Comparisons of the Microtox® test and acute toxicity tests with both fish and invertebrates for a large number of pure compounds and complex mixtures has demonstrated good general agreement between the fathead minnow, Pimephales promelas, and Daphnia magna acute tests and the Microtox® test, both within- and among-laboratories (Green et al. 1985). The use of both sucrose and NaCl osmotic adjustment techniques in the Microtox® test also has been demonstrated to be of use in determining the nature of the compound causing the toxicity when testing complex mixtures (Hinwood and McCormick 1987, Ankley et al. 1990a, Hoke et al. 1992a).

Cladoceran species have numerous advantages for aquatic

toxicity testing, including their sensitivity to a wide variety of environmental contaminants (Maki 1979, LeBlanc 1980). Daphnia magna and D. pulex have been most frequently used in aquatic toxicity tests and a large data base exists for the effects of pure compounds on D. magna (Hunter et al 1990). In addition, the response of D. magna has been correlated with the responses of other species to toxicants (Nebeker et al. 1983, Nebeker et al. 1986). Acute lethality tests with D. magna also seem appropriate for use in sediment pore water toxicity tests because it is one of the species used to establish surface water quality criteria (U.S. EPA 1980a).

Ceriodaphnia dubia occurs in natural zooplankton assemblages of the Great Lakes and has recently gained wide-spread acceptance as a test species for acute and chronic toxicity testing of effluents and various other types of aqueous extracts (Mount and Norberg 1984, DeGraeve and Cooney 1987, Mount and Anderson-Carnahan 1988a, b; Mount 1989, Ankley et al. 1990b, Oris et al. 1991, Kszos and Stewart 1991). Several investigations have evaluated of the comparative sensitivity of C. dubia and other daphnid species to water quality variables and pure chemicals (Cowgill et al. 1985, Takahashi et al. 1987, Winner 1988, 1989; Mokry and Hoaglund 1990, Cowgill and Milazzo 1990, 1991a, b). The sensitivity of C. dubia also has been compared with the response of the Microtox® test to different wastewater fractions (Mazidji et al. 1990), with the responses of natural zooplankton and benthos communities to chronic copper stress (Moore and Winner 1989), with the response of fathead minnow tests for evaluating in-stream toxicity dynamics (Stewart et al. 1990) and with the responses of the Microtox® and fathead minnow tests to sediment elutriates (Hoke et al. 1990). Because the comparison of relative species

sensitivities to pore water from contaminated sediments was of interest, acute toxicity tests of sediment pore waters were conducted with both D. magna and C. dubia.

Chironomus tentans (Diptera:Chironomidae) is a representative of a group of insects known as the midges, which are widely distributed in freshwater sediments during their larval stage of development. This species spends almost all of its life cycle in a tunnel in the upper few centimeters of sediments (Sadler 1935). Chironomids often comprise a significant proportion of the benthic biomass and are important in the cycling of oxygen, nutrients and contaminant residues into and from the sediments due to bioturbation (Graneli 1979, Hargrave 1975, Matisoff et al. 1985). Chironomus tentans can be satisfactorily reared in the laboratory and has previously been used as a sediment toxicity test organism (Wentzel et al. 1977, 1978, Batac-Catalan and White 1982, Mosher and Adams 1982, Mosher et al. 1982, Giesy et al. 1988, 1990). In previous studies of Great Lakes sediments (Giesy et al. 1988), a reduction in C. tentans dry weight, relative to control, of approximately 30-40% in the whole sediment toxicity tests was observed for sediments which did not support viable communities of benthic invertebrates in the field.

The objectives of this study were as follows:

1) To evaluate the toxicity of sediments and sediment pore waters from 13 locations in the Grand Calumet River, IN (Figure 8) with the following toxicity tests and endpoints,

   i) Photobacterium phosphoreum, Microtox®, inhibition of bioluminescence in 5, 15, and 30 minute incubations with sediment pore water in assays using two forms of osmotic protection (NaCl, sucrose) for the bacteria.

   ii) Daphnia magna and Ceriodaphnia dubia, survival of 24 h old neonates during 48 h exposures to sediment pore water.

iii) Chironomus tentans, survival and inhibition of dry wt. gain, relative to control, following 10-d exposures to whole sediments.

2) To test the hypothesis that the toxicity of whole sediments or pore waters as determined by laboratory toxicity tests can be predicted from toxic units calculated based on the concentrations of individual or classes of compounds and known dose-response relationships.

3) To determine whether sediment toxicity is better predicted from concentrations of chemical residues in pore water than residues in bulk sediments.

Materials and Methods

Sample Collection

Sediment samples were collected from the Grand Calumet River, IN on 11 October and 22 November 1988; 10 March, 24 May, 30 October and 13 November 1989; and 12 May 1990. A Ponar grab sampler was used to collect sediment samples from 10 locations along the Grand Calumet River and three locations in the Indiana Harbor ship canal (Figure 8). At the time of sample collection, study sites were located by triangulation of local landmarks. The sample from each location was a composite of approximately 80-100 L of wet sediment from multiple Ponar grabs. Multiple grab samples were collected and composited to ensure sufficient sample volume for all necessary sub-sample collection. Compositing and homogenization of composite samples were done in a large stainless steel pan with stainless steel tools. Large debris was removed from the composite samples and two, 1-L aliquants (sub-samples) for quantification of metals and organic compounds in bulk sediments were placed in 1-L glass jars capped with washed aluminum foil under the lid. Samples for toxicity testing or pore water extraction were placed in coolers or plastic buckets lined with

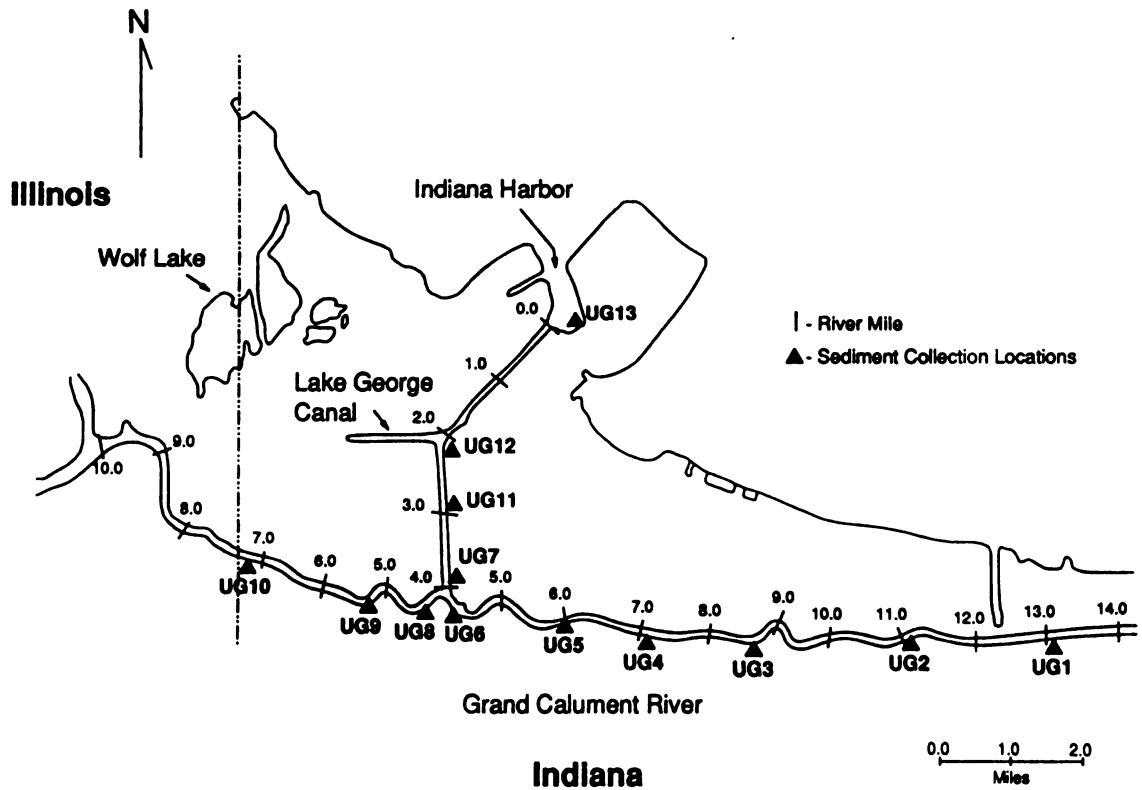

Figure 8. Sampling locations in the Grand Calumet River and Indiana Harbor, Indiana

food-grade plastic bags. Prior to sample collection, sample bottles were washed and solvent rinsed with hexane. After collection, sediment samples were placed on ice in coolers and transported to the laboratory, where they were maintained in a walk-in cooler at 4° C until processing and analysis.

Pore Water Extraction

Pore water was extracted from sediments by a combination of centrifugation and filtration as described by Hoke et al (1992a). The pore water extracts were placed in glass bottles, the bottles capped with aluminum foil-lined lids, and the pore water maintained in the dark at 4° C until used for assays (< seven days) or until extracted for subsequent chemical analysis. Pore water samples for metals analysis were preserved with a sufficient amount of concentrated HNO_3 to lower the sample pH to ≤ 2.0. Samples for quantification of organic compounds were preserved with 5 ml/L of a 1 g/L solution of $HgCl_2$ to prevent microbial degradation. The cleaning procedure for pore water sample bottles was identical to the procedure used for sediment sample bottles. Subsamples of sediments and pore water extracts were archived at 4° C.

Chemical Analysis

Organics

Organic chemical analyses were only conducted on samples collected from the 10 locations on the Grand Calumet River (Figure 8). In general, the analytical methods for the quantification of non-polar organic compounds in sediment and pore water followed the scheme presented below. Subsamples of sediment were dried at 105°C for 24 h and percent moisture determined for each sample. Ten grams of dried sample were mixed with 10

g of anhydrous sodium sulphate and Soxhlet extracted for 24 h with pesticide-grade acetone/hexane (1:1, v/v). The extract was passed over a drying column containing anhydrous sodium sulphate and the column rinsed with approximately 100 ml of the acetone/hexane mixture to complete the transfer. The extract was transferred to a Kuderna-Danish concentrator and extract volume reduced to 1 ml. The final extract volume was adjusted to 10 ml with the acetone/hexane mixture. These procedures and all other aspects of sediment sample preparation followed U.S. EPA Method 3540 (U.S. EPA 1986).

One liter of pore water was adjusted to a pH of >11, extracted with three successive 60 ml portions of pesticide-grade methylene chloride in a separatory funnel and the extracts combined for analysis of neutral organic compounds. If a large emulsion was observed, continuous liquid-liquid extraction was used to complete the sample extraction. Extracts were passed over an anhydrous sodium sulphate drying column and reduced to 1 ml in a Kuderna-Danish concentrator. Final extract volumes were adjusted to 10 ml with methylene chloride. Pore water extraction followed the protocols outlined in U.S. EPA Method 3510 or, for emulsions, U.S. EPA Method 3520 (U.S. EPA 1986). Cleanup procedures for both sediment and pore water extracts followed the protocols outlined in U.S. EPA Methods 3620, 3630 or 3660 (U.S. EPA 1986) and were dictated by the class of compounds quantitated in subsequent analyses.

Identification and quantitation of chemical analytes were performed with gas chromatography/mass spectroscopy (GC/MS) techniques. U.S. EPA 600 Series methods (U.S. EPA 1984) were used for GC analyses while compound confirmation was conducted with U.S. EPA GC/MS Methods 8240 and 8250 (U.S. EPA 1986). GC/MS operating conditions were as follows for

all analyses: electron energy 70 eV, mass range 35-550 amu, scan time 1 sec/scan, transfer line temperature 250 °C, source temperature 200-250 °C, injector temperature 250-350 °C, injector on column or Grob splitless, sample volume 1 μl and carrier gas He at 15 psi. Compounds were identified by comparison to library spectra and confirmed by comparison to authentic standards.

Metals

Concentrations of metals in whole sediments and pore waters were determined by a combination of inductively-coupled argon plasma (ICP) and atomic absorption (AA) spectroscopy. Whole sediments were digested in concentrated HNO_3 (Plumb 1981, APHA 1985). Standard additions were used to avoid matrix effects.

Miscellaneous parameters

A number of parameters were determined by standard techniques. Acid volatile sulphides (AVS) were measured in bulk sediments with a gravimetric technique (Di Toro et al. 1990, 1991). Total oil and grease was measured by the freon extraction method followed by gravimetric quantitation (Plumb 1981). Sediment TOC was measured with a LECO carbon analyzer dry combustion technique.

Selected contaminants (CN, H_2S, NH_3), total organic (TOC) and total inorganic carbon (TIC) in pore water were measured when samples were prepared for toxicity tests. Concentrations of cyanide and hydrogen sulfide were determined by standard methods (APHA 1985). Total ammonia was measured with an Orion® Model 701A ionanalyzer and an Orion® Model 95-12 ammonia electrode. TOC and TIC were measured with an IO Corporation, Inc. Model 700 TOC analyzer. Hardness, alkalinity, conductivity and Ph were measured in the 100% pore water concentration

during cladoceran toxicity tests. Reported values are the maximum values observed during the course of the 48 h toxicity tests.

Toxicity Tests

Photobacterium phosphoreum

The Microtox' bacterial luminescence assay was performed on sediment pore waters with the standard procedure (Bulich et al. 1981) and the alternate osmotic adjustment procedure developed by Hinwood and McCormick (1987). Reduction in bioluminescence of the bacterium P. phosphoreum was used as a measure of the toxicity of the sediment pore waters. A Microtox® Model 2055 Toxicity Analyzer (Microbics Co., Carlsbad, CA.) was used for all measurements. The calculated ratio of corrected light emitted to emitted light remaining after 5, 15 or 30 minutes was determined for each sample dilution and all results reported as the percent pore water causing a 50% inhibition of bioluminescence (EC_{50}). EC_{50} values were calculated with the linearized gamma distribution.

Daphnia magna and Ceriodaphnia dubia

Initial C. dubia cultures were obtained from the Eli-Lilly Co. (Greenfield, IN) while D. magna originated from laboratory cultures maintained by the Michigan Department of Natural Resources (East Lansing, MI). In the laboratory, C. dubia were either maintained in mass cultures in 1000 ml beakers containing 900 ml of culture water or in a brood board as previously described by Hoke et al (1990). Daphnia magna were maintained in mass cultures contained in 1000 ml beakers. Mass cultures of both species were initiated with one gravid female and maintained in a Scientific Products incubator at 25° C with a 16L:8D photoperiod (light

levels - 200 $\mu E/m^2$ sec). Brood boards were initiated with 60 gravid females and were maintained in the same incubator as the mass cultures. Feeding consisted of daily additions of Selenastrum capricornutum (200,000 cells/ml culture water) and Yeast-Cerophyl-Trout chow (YCT, 3 ml/1000 ml culture water). Thinning of the mass cultures was performed weekly by removing approximately 90% of the existing organisms and water with replacement of an equal volume of fresh culture water. Cultures were maintained for 14-d (brood boards) and 21-d (mass cultures) prior to initiation of new cultures for C. dubia and D. magna, respectively. Culture, control and diluent waters for all assays was 10% Perrier mineral water prepared as described by Hoke et al (1990). Two $\mu g/L$ of cyanocobalamin (vitamin B_{12}) and selenium (as sodium selenate) were added to the 10 % Perrier water mixture. The aerated 10% Perrier water mixture had the following chemical characteristics over the entire study period (mean $\pm$ SD): DO-7.9 $\pm$ 0.4 mg/l, Ph-8.1 $\pm$ 0.4, specific conductance-105 $\pm$ 26 $\mu mhos/cm^2$, hardness-60.3 $\pm$ 9.6 mg/L as $CaCO_3$ and alkalinity-59.3 $\pm$ 11.7 mg/L as $CaCO_3$.

The sediment pore water was prepared 24-48 h prior to assays and allowed to equilibrate to 25° C immediately before starting a test. Twenty mL of each test solution were dispensed into 10 replicates for each pore water dilution concentration or control using a device developed by Mount and Norberg (1984) for this purpose. Chemical characteristics of the initial pore water dilutions were measured at test initiation. All assays were conducted in a Scientific Products incubator at the same temperature, photoperiod and light level regimes used for culturing the test organisms.

Ceriodaphnia dubia neonates, < 12 hr old and not more than 8 h different in age, from the third brood of the originally isolated neonates were used to begin all assays. D. magna neonates <12 h old were collected by screening all neonates from the mass cultures and then again screening all neonates from the mass cultures within 12 h. Observations of mortality (lack of movement or respiration) were made at the termination of the 48 h tests with both cladoceran species. Acute 48 h LC_{50} values were calculated with the TOXCALC program. This program originated at U.S. EPA and was modified for use in the laboratory at Michigan State University. Unless noted above, all other culture and assay methods followed those presented by Mount and Norberg (1984) and U.S. EPA (1985a).

Chironomus tentans

Chironomus tentans were originally obtained from Dr. David White of the University of Michigan and have been continuously cultured in the laboratory at MSU for over five years. Cultures were maintained using the methods of Giesy et al (1988). Second instar individuals (12 d post-hatching) were used for all tests. Culture records indicate second instar C. tentans had a mean dry wt. of 0.503 mg (n=7, SD=0.136 mg); while recent historical test data from the laboratory indicate that individual control organisms had a mean dry wt. of 6.98 mg (n=20, SD=0.94 mg) at test termination (22 d post-hatching).

Tests were conducted with individual second instar C. tentans using the methods of Giesy et al. (1988) except that a 9:1 mixture of HPLC-grade deionized water and Perrier mineral water was used in each test instead of distilled water. Tests were conducted for 10 d at 22 ± 1° C with a 16L:8D photoperiod. At test termination, surviving larvae were recovered, rinsed with deionized laboratory water and placed in aluminum

weigh boats. Larvae were dried at 80° C for 24 h and each larvae weighed on a Mettler Model H54AR analytical balance. Results of C. tentans tests are reported as mean percent inhibition in dry wt. gain at each station relative to a control sediment.

Statistical Analysis

LC_{50} values from the toxicity tests were used for correlation analyses with the chemical data. Statistical Analysis System (SAS 1988) software was used to perform univariate Pearson product moment correlations among the results of toxicity tests and chemical analyses of sediments and pore waters. Correlation analysis results are reported only if they were significant at the $P \leq 0.05$ level.

Results

Chemical Analysis

Organics

Analyses were conducted to determine solid phase sediment concentrations for a total of 104 organic chemicals. Forty-one of these 104 compounds were not present in GCR sediments at concentrations above the limit of detection (LOD). Analyses were subsequently conducted to determine pore water concentrations of the 63 compounds observed at concentrations greater than the LOD in the sediments. Of these 63 compounds, only 44 were observed in pore water samples at concentrations greater than the LOD (Table 4). Concentrations of organic chemical analytes detected in either whole sediment or pore water are listed in Tables 5 and 6, respectively.

Table 4. Organic compounds analyzed for but not detected in sediments or sediment pore waters from the Grand Calumet River, IN. The limit of detection (LOD) is given for the matrix in which the compound was not detected.

Parameter	Sediment LOD mg/kg	Pore water LOD ug/L
m-Chlorophenol		0.1
m-Cresol	0.01	
p-Cresol	0.01	
2,3-Dichlorophenol	0.01	
2,5-Dichlorophenol	0.01	
2,6-Dichlorophenol		0.1
3,4-Dichlorophenol	0.01	
3,5-Dichlorophenol	0.01	
2,3-Dibromophenol	0.01	
2,4-Dibromophenol	0.01	
2,5-Dibromophenol	0.01	
2,6-Dibromophenol	0.01	
3,4-Dibromophenol	0.01	
3,5-Dibromophenol	0.01	
2,3,4,5-Tetrachlorophenol	0.01	
2,3,4,6-Tetrachlorophenol	0.01	
2,3,5,6-Tetrachlorophenol		0.1
2,3,4-Trichlorophenol	0.01	
2,3,5-Trichlorophenol	0.01	

Table 4. (continued).

Parameter	Sediment LOD mg/kg	Pore water LOD ug/L
2,3,6-Trichlorophenol	0.01	
2,4,5-Trichlorophenol	0.01	
2,4,6-Trichlorophenol		0.1
3,4,5-Trichlorophenol	0.01	
Mercaptobenzothiazole		0.1
1,2,3,4-Tetrachlorobenzene	0.01	
1,2,3,5-Tetrachlorobenzene	0.01	
1,2,4,5-Tetrachlorobenzene	0.01	
Benzidine	0.01	
3,4-Dichloroaniline		0.1
3,3-Dichlorobenzidine		0.1
p,p'-DDD		0.1
Aldrin	0.01	
Methoxychlor	0.01	
m-Dichlorobenzene		0.1
p-Dichlorobenzene		0.1
1,2-Dichloropropane		0.1
1,3-Dichloropropane	0.01	
1,3-Dichloropropene		0.1
Hexachloro-1,3-butadiene		
Hexachloroethane	0.01	
Pentachloroethane		0.1
1,2,3-Trichlorobenzene		0.1

Table 4. (continued).

Parameter	Sediment LOD mg/kg	Pore water LOD ug/L
1,2,4-Trichlorobenzene		0.1
1,3,5-Trichlorobenzene	0.01	
1,2,3-Trichloropropane		0.1
Trichloroethene	0.01	
Butylbenzylphthalate	0.02	
Diethylphthalate	0.02	
Dimethylphthalate	0.02	
Di-n-butylphthalate		0.5
Di-n-octylphthalate	0.02	
1-Chloro-2,4-dinitrobenzene	0.01	
1-Chloro-2,6-dinitrobenzene	0.01	
1-Chloro-3,4-dinitrobenzene	0.01	
1-Chloro-4-nitrobenzene	0.01	
2,6-Dinitrotoluene	0.01	
Nitrobenzene	0.01	
Cresyldiphenyl phosphate	0.01	
Trixylene phosphate	0.01	
2,3,7,8-Dibenzo-p-dioxin [1]		2.0
Dimethyl nitrosamine		0.3

1 - units for TCDD LOD = pg/L

Table 5. Concentrations of organic chemicals in bulk or whole sediments from the Grand Calumet River, IN. Results are reported as mg/kg dry wt., except for organic carbon, oil and grease (% dry wt.) and 2,3,7,8-dibenzo-p-dioxin (pg/kg).

	Grand Calumet River Site Number									
Parameter	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Organic Carbon (% dry wt)	28.1	4.4	7.2	12.5	14.3	15.9	14.7	22.3	18.8	13.4
Oil & Grease (% dry wt)	6.2	2.5	4.1	1.6	5.3	4.3	2.5	13.5	6.1	2.2
o-Chlorophenol	0.21	0.47	0.45	0.06	0.87	0.34	0.57	0.27	0.68	1.26
m-Chlorophenol	0.03	0.23	<0.01	<0.01	0.19	0.01	0.14	0.05	<0.01	<0.01
p-Chlorophenol	0.45	0.68	0.95	0.21	2.13	0.42	0.95	0.55	0.14	0.68
o-Cresol	0.36	0.32	0.48	0.05	0.65	0.62	0.51	0.12	0.10	0.01
2,4-Dichlorophenol	1.64	1.99	3.91	1.07	1.05	2.84	2.81	1.07	1.32	6.35
2,6-Dichlorophenol	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.85
4,6-Dinitro-o-cresol	0.67	0.85	1.77	0.38	0.38	0.60	0.69	0.34	2.08	1.92
2,4-Dinitrophenol	2.13	3.68	3.62	2.00	2.10	2.55	1.54	2.65	1.09	3.77
Hydroquinone	0.44	0.27	1.50	0.12	0.56	0.28	0.78	0.41	1.54	0.07

Table 5. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Pentachlorophenol	2.16	4.01	2.03	0.97	4.36	2.00	2.69	2.64	2.09	4.52
Phenol	0.86	0.89	0.48	0.25	4.03	0.84	1.55	0.91	0.07	5.10
2,3,5,6-Tetrachlorophenol	0.02	0.04	<0.01	0.08	0.07	0.02	0.01	0.01	<0.01	<0.01
2,4,6-Trichlorophenol	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.09
Biphenyl	0.56	1.27	1.49	0.18	3.61	0.71	2.04	1.22	6.45	0.78
Hexachlorobenzene	0.92	1.38	1.00	0.04	1.38	0.86	0.98	0.54	0.19	0.47
2-Mercaptobenzothiazole	1.27	1.34	3.41	1.62	1.04	1.20	1.06	1.51	1.20	0.16
Naphthalene	1.02	1.75	3.78	2.11	4.92	1.89	1.38	1.63	3.58	8.24
Polychlorobiphenyl (1248)	2.17	1.49	6.89	0.94	18.33	1.66	4.26	2.80	4.61	7.93
3,4-Dichloroaniline	0.03	0.09	0.68	0.05	0.09	0.01	0.15	0.04	<0.01	0.01
3,3-Dichlorobenzidine	<0.01	<0.01	<0.01	<0.01	0.21	<0.01	<0.01	<0.01	<0.01	<0.01
p-Nitroaniline	0.56	0.55	0.32	0.20	0.37	0.24	0.99	0.02	0.08	0.44

Table 5. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
p,p'-DDT	0.23	0.67	1.44	0.25	0.75	1.01	0.83	0.55	1.75	0.09
p,p'-DDE	2.46	2.46	2.49	0.71	1.55	4.23	2.90	1.78	4.65	2.71
p,p'-DDD	0.04	0.08	<0.01	<0.01	0.62	0.17	0.01	0.23	0.01	<0.01
Dieldrin	0.21	0.76	0.72	0.04	0.08	0.93	0.62	0.04	3.21	1.14
Lindane	0.34	0.21	1.83	1.43	0.29	0.38	0.64	0.79	3.16	0.26
Chlordane	1.04	0.44	1.23	0.05	0.09	1.64	1.89	2.41	2.18	2.14
Toxaphene	2.45	2.09	0.95	1.88	2.65	3.10	3.67	7.38	3.32	2.05
Heptachlor	0.98	0.42	2.66	0.36	1.24	0.52	1.37	0.44	1.72	0.19
Acrylonitrile	3.21	3.74	3.10	1.39	2.50	0.26	1.88	0.94	0.09	4.25
Chlorobenzene	1.47	2.47	2.09	1.04	0.93	1.18	3.56	1.83	6.82	1.05
p-Chlorotoluene	21.43	5.28	6.41	5.70	8.22	5.35	10.24	7.32	1.74	5.27
o-Dichlorobenzene	2.86	4.20	2.98	1.66	2.91	2.10	2.99	1.49	<0.01	2.21
m-Dichlorobenzene	0.46	0.72	0.41	0.42	0.38	0.25	1.28	0.04	<0.01	0.42

Table 5. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
p-Dichlorobenzene	3.71	3.06	5.88	0.87	4.50	1.28	1.43	1.96	4.62	6.44
1,2-Dichloropropane	0.24	0.15	0.43	0.26	0.09	0.15	0.06	0.02	0.21	0.08
1,3-Dichloropropene	0.37	0.49	0.55	0.11	0.38	0.16	0.32	0.33	0.15	1.14
Ethylbenzene	4.19	2.17	4.72	2.34	3.61	4.48	4.00	1.84	0.05	10.28
Hexachloro-1,3-butadiene	0.22	0.43	0.28	0.29	0.02	0.20	0.33	0.46	0.20	0.05
Pentachloroethane	0.28	0.39	0.23	0.25	0.31	0.24	0.20	0.02	0.22	<0.01
Styrene	4.17	1.66	2.77	0.49	0.65	0.68	1.94	1.85	6.49	6.85
Tetrachloroethylene	0.02	<0.01	<0.01	0.01	<0.01	0.01	0.02	<0.01	0.02	0.01
1,2,3-Trichlorobenzene	0.03	0.08	0.08	0.01	<0.01	0.01	0.05	0.14	0.01	0.01
1,2,4-Trichlorobenzene	3.64	3.91	1.27	1.77	3.41	1.59	0.36	2.54	3.89	1.05
1,2,3-Trichloropropane	3.26	0.58	3.63	1.22	1.25	3.02	3.05	0.42	3.64	0.66
1,2,3-Trichloropropene	1.84	1.89	1.87	1.38	1.05	0.36	2.14	1.68	1.42	<0.01
1,1,1-Trichloroethane	0.02	0.06	0.03	<0.01	<0.01	0.51	<0.01	0.11	0.03	<0.01

Table 5. *(continued)*.

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Di-n-butyl phthalate	0.04	0.15	0.45	<0.02	<0.02	0.66	<0.02	0.17	<0.02	0.82
Di-n-octyl phthalate	0.23	0.78	1.20	<0.01	1.04	1.21	0.15	2.61	0.41	<0.01
1-Chloro-2-nitrobenzene	0.04	<0.01	0.07	0.06	0.02	0.07	0.08	0.13	0.01	<0.01
2,4-Dinitrotoluene	0.05	0.03	0.07	<0.01	0.06	0.03	0.01	<0.01	0.02	0.02
Pentachloronitrobenzene	0.15	2.04	0.33	0.17	0.03	0.22	0.16	2.65	0.38	0.07
Tricresyl phosphate	1.95	1.13	0.57	0.09	1.00	1.14	1.34	0.22	3.40	0.05
Pyrene	3.68	5.11	4.81	1.59	7.26	3.05	4.05	1.72	4.57	4.20
Fluoranthene	0.34	0.66	1.22	0.42	0.99	0.06	0.18	0.48	0.31	6.83
Benzo-a-pyrene	27.21	20.26	16.88	8.44	45.66	8.94	1.85	83.62	100.21	32.51
Phenanthrene	2.04	4.32	5.06	0.66	5.28	1.28	3.61	2.15	4.04	2.55
Chrysene	2.09	3.56	3.46	0.83	4.21	1.15	2.96	2.64	5.21	2.57
Benzo-k-fluoranthene	3.16	4.18	6.10	1.64	4.03	3.69	3.27	5.83	2.18	3.10
Benzo-b-fluoranthene	4.56	5.63	7.25	2.72	5.26	0.75	8.22	4.79	7.82	2.99

Table 5. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Benzo-a-anthracene	2.63	1.68	2.14	0.58	1.43	1.66	0.53	2.66	1.65	0.17
2,3,7,8-Dibenzo-p-dioxin	6.20	<2.00	<3.00	2.00	12.40	3.50	<1.00	3.50	7.30	7.30
Dimethyl nitrosamine	0.21	0.16	<0.01	<0.01	1.69	<0.01	0.27	0.82	<0.01	0.20

Table 6. Concentrations of organic chemicals in pore waters of sediments from the Grand Calumet River, IN. Results are reported as μg/L, except for total inorganic and organic carbon, which are reported as mg/L.

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Total Inorganic Carbon	73.3	34.4	26.1	94.9	71.4	123.9	27.2	154.4	39.7	74.9
Total Organic Carbon	28.6	25.9	30.2	24.2	52.8	46.1	28.8	31.3	28.0	18.7
o-Chlorophenol	23.5	13.5	21.6	5.2	63.2	5.8	19.2	40.5	100.4	88.6
p-Chlorophenol	1.4	0.7	0.9	0.2	5.9	1.3	2.0	3.7	15.2	17.5
o-Cresol	4.7	4.0	4.8	3.7	12.6	0.8	4.8	10.2	23.6	20.9
p-Cresol	8.3	0.3	6.3	6.2	16.2	2.5	2.6	9.1	10.3	24.2
2,4-Dichlorophenol	16.5	5.7	14.6	15.5	7.4	14.3	17.1	23.7	23.5	24.0
4,6-Dinitro-o-cresol	4.4	1.1	2.7	0.9	10.3	1.6	4.7	<0.1	1.6	1.5
2,4-Dinitrophenol	23.1	15.4	26.0	7.3	34.4	15.8	14.5	54.1	34.5	30.6
Hydroquinone	5.6	3.7	4.2	1.1	12.5	4.9	6.1	13.6	12.7	8.3
Pentachlorophenol	12.8	6.4	10.0	3.5	13.9	11.3	12.5	14.8	18.8	24.5

Table 6. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Phenol	226.1	50.3	189.9	27.8	256.6	207.1	107.6	225.3	326.2	255.5
Biphenyl	14.5	4.2	13.2	6.9	33.2	12.5	23.2	32.5	25.6	30.1
Hexachlorobenzene	0.9	0.2	0.8	0.1	4.1	<0.1	1.1	3.0	2.1	2.0
Naphthalene	452.4	40.8	321.4	134.7	76.2	120.0	413.6	306.6	375.2	357.7
Polychlorinated biphenyls (as Aroclor 1248)	0.5	1.2	0.7	0.7	0.9	0.2	0.4	2.1	10.7	8.9
p-Nitroaniline	4.7	1.3	4.2	5.1	8.3	3.5	4.4	9.6	8.1	7.6
p,p'-DDT	0.2	0.1	<0.1	0.1	0.9	<0.1	<0.1	0.8	2.4	<0.1
p,p'-DDE	1.6	2.5	2.6	5.2	2.6	2.7	4.8	4.3	6.9	5.8
Dieldrin	0.6	0.1	1.4	0.7	1.2	0.1	0.1	5.1	1.0	0.8
Lindane	0.1	<0.1	<0.1	0.1	0.4	<0.1	0.3	0.7	<0.1	0.2
Chlordane	0.1	0.1	0.2	0.6	<0.1	<0.1	<0.1	3.3	0.6	0.6
Toxaphene	3.2	7.2	2.6	3.1	6.7	0.3	1.2	7.5	5.6	6.4

Table 6. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Heptachlor	4.6	1.7	0.5	4.4	1.5	4.6	4.1	4.5	0.5	0.8
Acrylonitrile	0.1	0.4	<0.1	1.7	0.7	<0.1	0.2	0.2	1.8	1.4
Chlorobenzene	0.8	0.1	0.6	0.9	1.1	0.6	2.5	0.2	2.9	0.9
p-Chlorotoluene	23.2	10.0	11.1	5.0	16.4	5.4	12.4	54.6	14.6	20.0
o-Dichlorobenzene	0.2	0.9	0.4	0.8	0.6	<0.1	<0.1	0.6	0.5	<0.1
Ethylbenzene	3.5	2.8	2.9	0.4	12.5	0.5	10.5	9.5	15.8	12.2
Styrene	5.2	5.1	5.9	1.9	13.8	2.8	1.6	5.9	15.6	17.5
Tetrachloroethylene	0.7	0.2	1.2	0.4	0.9	0.4	0.2	4.6	2.8	2.4
1,2,3-Trichloropropene	1.1	0.6	1.3	1.0	1.3	1.6	0.4	<0.1	<0.1	<0.1
1,1,1-Trichloroethane	4.9	1.5	10.7	1.5	19.6	4.8	0.5	2.4	<0.1	0.8
Di-octyl phthalate	5.8	4.8	2.3	2.2	14.6	3.6	1.7	27.7	8.5	8.6
1-Chloro-2-nitrobenzene	1.6	0.5	0.1	1.6	2.1	1.3	4.1	0.9	1.7	4.3
2,4-Dinitrotoluene	0.1	0.3	0.4	0.2	1.7	0.1	0.2	1.1	0.2	1.5

Table 6. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Pentachloronitrobenzene	0.6	0.2	0.4	0.3	2.8	0.2	0.3	1.5	0.7	1.1
Tricresyl phosphate	12.9	11.5	22.6	0.3	2.5	4.6	5.6	0.5	6.8	9.7
Pyrene	10.1	4.6	9.9	6.8	28.7	3.3	6.4	18.6	19.8	19.0
Fluoranthene	30.5	25.9	27.4	10.3	35.9	9.8	8.8	66.6	32.1	17.4
Benzo-a-pyrene	1.4	0.6	0.2	0.2	5.6	2.4	0.2	5.8	3.3	2.8
Phenanthrene	230.6	99.2	176.5	55.8	145.4	66.4	27.4	188.2	245.4	186.8
Chrysene	8.4	3.6	2.8	2.9	23.3	1.7	6.0	31.9	9.0	9.4
Benzo-k-fluoranthene	2.3	0.1	1.8	0.6	1.8	0.4	1.8	1.8	5.7	4.5
Benzo-b-fluoranthene	5.9	2.8	6.6	4.1	10.3	4.9	0.6	9.7	12.2	11.4

Concentrations of the various compounds present in sediments varied greatly. Chemicals such as m-chlorophenol; 2,6-dichlorophenol; 2,4,6-trichlorophenol; 2,3,5,6-tetrachlorophenol; 3,4-dichloroaniline; 3,3-dichlorobenzidine; p,p'-DDD; tetrachloroethylene; 1,2,3-trichlorobenzene; 1,1,1-trichloroethane; di-n-butyl phthalate; 1-chloro-2-nitrobenzene and 2,4-dinitrotoluene were generally present in the low ug/kg (ppb) range (Table 5). Compounds exhibiting the greatest sediment concentrations were the various polycyclic aromatic hydrocarbons (PAHs), total polychlorinated biphenyls (PCBs, as Aroclor 1248), p,p'-DDE, toxaphene, p-chlorotoluene, ethylbenzene and p-dichlorobenzene. These compounds were generally present in the 2-20 mg/kg range although several of the PAHs were present at concentrations as great as 100 mg/kg (Table 5). Percent TOC ranged from 4.4 to 28.1% in the sediments while percent oil and grease ranged from 1.6 to 13.5% (Table 5).

Most compounds observed in sediment pore waters were present in the low ug/L range (i.e., 0-10 ug/L, Table 6). Notable exceptions included phenol and the PAHs, phenanthrene and naphthalene. These compounds were present at concentrations in pore water as great as 326, 245 and 452 ug/L, respectively (Table 6). Several other compounds such as p-chlorotoluene, fluoranthene, pyrene, 2,4-dichlorophenol, 2,4-dinitrophenol, pentachlorophenol and biphenyl were observed in pore water at concentrations in the 10-100 ug/L range. Pore water TOC concentrations ranged from 18.7 to 52.8 mg/L (Table 6).

Metals

Detectable concentrations of most metals analyzed for were present in all study site sediments (Table 7). Iron, magnesium and manganese were

Table 7. Concentrations of metals and acid volatile sulphide (AVS) in sediments from the Grand Calumet River, IN. Results are reported as gm/kg dry wt., except AVS, which is reported as μM S/gm dry wt.

	Grand Calumet River Site Number									
Parameter	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Benzo-a-anthracene	9.5	4.6	7.1	2.4	18.9	10.4	0.6	26.4	16.2	22.5
Cadmium	0.020	0.079	0.032	0.042	0.012	0.069	0.049	0.089	0.052	0.018
Chromium	0.009	0.327	0.393	0.344	0.438	0.923	0.814	1.225	0.904	0.097
Copper	0.007	0.226	0.153	0.099	0.242	0.241	0.271	0.387	0.544	0.257
Lead	0.017	0.912	0.403	0.538	0.803	1.526	1.815	3.940	1.916	1.306
Nickel	0.030	0.047	0.288	0.391	0.166	0.033	0.160	0.337	0.361	0.055
Zinc	0.108	2.834	0.069	1.542	0.218	0.486	5.230	0.793	0.450	0.125
Manganese	0.051	3.896	0.982	1.492	2.234	3.261	2.585	2.419	1.679	0.614
Magnesium	2.506	3.616	1.648	3.242	6.532	3.831	2.695	5.268	4.930	7.239
Iron	1.71	19.19	17.15	21.65	9.52	17.68	27.10	6.33	6.94	2.31
AVS [1]	126.9	0.41	11.97	6.57	0.34	19.55	87.52	81.90	549.5	158.6
Metal/AVS [2]	<0.1	144.2	1.5	6.3	65.8	1.9	1.3	0.8	0.1	0.1

Table 7. (continued).

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[1] Acid volatile sulphides

[2] (μM Cd + Cu + Pb + Ni + Zn/gm dry wt) / (μM S/gm dry wt.)

generally present in high mg/kg to low gm/kg concentrations in solid phase sediments (Table 7). Of the metals of toxicological concern in aquatic systems, zinc, lead and chromium were present at concentrations as great as 5.23, 3.94 and 1.22 gm/kg, respectively. Copper, nickel and cadmium concentrations were generally below 500 mg/kg.

Sediment AVS concentrations also are presented in Table 7 along with the molar metals (Cd + Cu + Pb + Ni + Zn)/AVS ratios for each sediment. The concentrations of metals in sediments used in the calculation of the molar metals/AVS ratios were not determined by sequential extraction during the AVS analysis as recommended by Di Toro et al. (1991). The molar metal/AVS ratios for the sediments ranged from <0.1 (i.e. no free metal) at site UG-1 to 144.2 (potentially a large amount of free metal) at site UG-2. Only three sites, UG-2,4 and 5 had molar metal/AVS ratios >2.0. Although the metals concentrations reported here were not determined by the sequential extraction (cold, weak acid; Di Toro et al. 1992), the harsher extraction technique used in the analyses reported here would result in conservative, worst case estimates of metal bioavailability.

Copper and zinc were the only metals of toxicological concern typically present in pore waters at concentrations greater than the ICP LOD for these metals (0.005 mg/L) (Table 8). Aluminum and arsenic were the only other two metals or metalloids of concern observed in any pore water sample at concentrations greater than their respective LODs of 0.100 and 0.050 mg/L (Table 8).

Table 8. Total concentrations of metals (mg/L) in pore waters of sediments from the Grand Calumet River, IN.

	Grand Calumet River Site Number									
Parameter	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Boron	0.407	0.148	0.815	0.316	0.152	0.156	0.243	0.929	0.227	0.313
Barium	0.376	0.091	0.202	1.000	0.074	0.242	0.316	0.854	0.249	0.243
Calcium	143.0	21.40	111.0	68.60	7.63	143.0	91.20	265.0	368.0	30.30
Copper	<0.005	0.060	0.180	0.061	0.017	0.007	0.073	0.008	0.025	<0.005
Cobalt	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Iron	0.436	0.620	0.375	0.387	0.162	1.060	0.588	1.78	50.10	0.050
Magnesium	43.20	4.27	27.70	23.50	29.40	87.30	14.10	88.80	69.30	30.30
Manganese	0.155	<0.010	0.251	0.096	<0.010	0.450	0.197	0.205	3.62	0.094
Molybdenum	0.119	0.114	<0.020	0.035	<0.020	<0.020	0.034	<0.020	<0.020	<0.020
Phosphorus	0.237	0.111	0.170	<0.100	<0.100	0.270	0.135	0.299	0.854	1.20
Zinc	0.019	0.106	0.028	0.134	0.007	0.490	0.081	0.074	0.114	0.028
Aluminum	0.120	<0.100	<0.100	<0.100	<0.030	0.623	<0.100	<0.100	15.00	<0.030
Tin	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050

Table 8. (continued).

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Thallium	<0.500	<0.500	<0.050	<0.500	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Chromium	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Cadmium	<0.005	<0.005	<0.010	<0.005	<0.010	<0.010	<0.010	<0.016	<0.010	<0.010
Mercury	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Lead	<0.020	0.054	0.023	<0.020	<0.020	<0.020	0.029	0.037	<0.020	<0.020
Selenium	<0.100	<0.100	<0.050	<0.100	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Arsenic	0.321	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Sodium	110.0	15.40	236.0	39.30	31.70	50.80	64.90	163.0	265.0	211.0
Potassium	61.00	10.70	94.80	13.40	NA [1]	6.48	9.06	38.80	32.50	NA
Nickel	<0.100	<0.100	<0.100	<0.100	<0.100	<0.100	<0.100	<0.100	<0.100	<0.100

[1] NA = not analyzed

Table 8. (continued).

Parameter	Grand Calumet River Site Number UG-11	UG-12	UG-13
Boron	0.133	0.226	0.194
Barium	0.373	0.182	0.935
Calcium	101.0	139.0	48.60
Copper	<0.005	<0.005	<0.005
Cobalt	<0.010	<0.010	<0.010
Iron	0.841	3.23	0.416
Magnesium	18.80	44.20	23.20
Manganese	0.449	0.598	0.114
Molybdenum	0.021	<0.020	0.105
Phosphorus	<0.100	<0.100	0.193
Zinc	0.035	0.036	0.132
Aluminum	<0.100	<0.100	<0.100
Tin	<0.050	<0.050	<0.050

Table 8. (continued).

Parameter	Grand Calumet Site Number UG-11	UG-12	UG-13
Thallium	<0.500	<0.500	<0.500
Chromium	<0.010	<0.010	<0.010
Cadmium	<0.005	<0.005	<0.005
Mercury	<0.050	<0.050	<0.050
Lead	<0.020	<0.020	<0.020
Selenium	<0.100	<0.100	<0.100
Arsenic	<0.050	<0.050	<0.050
Sodium	289.0	46.80	26.30
Potassium	8.30	19.10	13.80
Nickel	<0.100	<0.100	<0.100

[1] NA = not analyzed

Table 9. Concentrations of miscellaneous chemical compounds in pore waters of sediments from the Grand Calumet River, IN.

Parameter	Grand Calumet River Site Number									
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8	UG-9	UG-10
Hardness, mg/L $CaCO_3$	640	160	370	370	NA[1]	1200	460	1300	1040	2500
Alkalinity, mg/L $CaCO_3$	660	70	460	410	NA	290	490	1500	1040	180
Conductivity, μmhos/cm^2	2570	423	3420	830	NA	2420	1010	3465	3650	4680
Ph	8.2	8.9	8.4	8.7	NA	8.0	8.9	8.9	8.4	7.7
NH_3, un-ionized, mg/L	8.1	0.2	1.4	5.3	0.5	0.8	1.6	6.4	3.4	3.3
Cyanide, μg/L	216	194	174	177	99	177	177	187	179	189
Hydrogen sulfide, μg/L	0.2	0.9	0.9	0.4	1.2	1.1	0.4	1.4	1.5	1.2

[1] NA = not analyzed

Table 9. (continued).

Parameter	Grand Calumet River Site Number UG-11	UG-12	UG-13
Hardness, mg/L $CaCO_3$	640	700	360
Alkalinity, mg/L $CaCO_3$	620	1480	500
Conductivity, μmhos/cm^2	2230	2100	729
Ph	7.7	7.8	8.8
NH_3, un-ionized, mg/L	1.0	6.2	0.3
Cyanide, μg/L	20	20	20
Hydrogen sulfide, μg/L	NA	NA	NA

[1] NA = not analyzed

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Miscellaneous parameters

Alkalinity and hardness in several of the samples were elevated (1040-1500 mg/L as $CaCO_3$, Table 9) above the values commonly reported for surface waters while conductivity and Ph were within the ranges reported for surface waters (Cole 1988). Cyanide and H_2S were present in detectable, although relatively low, concentrations but unionized NH_3 concentrations ranged from 0.2-8.1 mg/L (Table 9).

Toxicity Tests

Photobacterium phosphoreum

Five, 15 and 30 minute EC_{50} values were calculated for sediment pore waters osmotically adjusted with both NaCl and sucrose (Table 10). Fifteen minute EC_{50} values for NaCl- and sucrose adjusted pore waters ranged from 0.3-93.8 % and 0.2-39.5 %, respectively. Pore waters osmotically adjusted with sucrose were generally equitoxic or less toxic than NaCl adjusted pore waters with the exception of pore waters from sediments collected at sites UG-7, 8, 9, and 10. Pore waters at these sites were from 1.3-2.4 times more toxic when osmotically adjusted with sucrose (Fig 9). Both sucrose and NaCl-adjusted Microtox® tests generally exhibited similar EC_{50} values at 5, 15 and 30 minutes. However, pore water from sites UG-2, 4 and 6 became more toxic with time in both sucrose- and NaCl-adjusted tests while pore water from sites UG-9 and 11 became more toxic with time only in the sucrose-adjusted tests.

Several significant correlations were observed between the results of the Microtox® pore water tests osmotically adjusted with NaCl and the results of the pore water organic chemical analyses. Correlation coefficients (r) between the Microtox® tests and o-, and p-chlorophenol,

Table 10. Results of toxicity tests conducted with bulk sediments or pore waters of sediments from the Grand Calumet River, IN. All data are expressed as either % pore water, or % response (e.g. C. tentans inhibition of dry wt gain relative to control).

Assay	Grand Calumet River Site Number							
	UG-1	UG-2	UG-3	UG-4	UG-5	UG-6	UG-7	UG-8
Microtox® 5 min EC_{50}, N[1]	3.3	10.8	14.2	7.7	0.6	24.6	44.5	25.2
Microtox® 15 min EC_{50}, N[1]	3.6	5.8	15.1	4.0	0.3	16.7	48.6	26.9
Microtox® 30 min EC_{50}, N[1]	3.9	5.3	17.7	3.2	0.3	16.1	66.7	33.9
Microtox® 5 min EC_{50}, S[1]	7.3	11.4	19.8	9.1	0.4	26.2	25.3	28.7
Microtox® 15 min EC_{50}, S[1]	8.6	6.6	22.3	5.3	0.2	15.1	28.6	20.8
Microtox® 30 min EC_{50}, S[1]	8.6	5.7	23.8	5.6	0.2	14.3	21.3	17.6
C. dubia, 48 h EC_{50}	24.6	>100	16.4	60.2	34.5	67.0	>100	3.2
D. magna, 48 h EC_{50}	34.4	>100	>100	16.2	67.6	>100	>100	5.5
C. tentans % reduction dry wt	100	89.2	100	100	95.0	99.0	37.0	98.0

Table 10. (continued).

Assay	Grand Calumet River Site Number				
	UG-9	UG-10	UG-11	UG-12	UG-13
Microtox® 5 min EC_{50}, N[1]	93.0	43.2	61.8	2.4	59.8
Microtox® 15 min EC_{50}, N[1]	93.8	47.7	48.1	1.9	53.4
Microtox® 30 min EC_{50}, N[1]	>100	58.3	44.5	1.7	54.1
Microtox® 5 min EC_{50}, S[1]	>100	25.8	>100	3.5	66.3
Microtox® 15 min EC_{50}, S[1]	39.5	27.2	>100	2.6	74.2
Microtox® 30 min EC_{50}, S[1]	30.9	29.3	54.5	2.9	70.4
C. dubia, 48 h EC_{50}	20.3	71.5	>100	14.7	41.7
D. magna, 48 h EC_{50}	67.6	70.6	>100	17.0	38.0
C. tentans % reduction dry wt	90.0	89.0	97.6	100	100

[1] N = NaCl, S = Sucrose

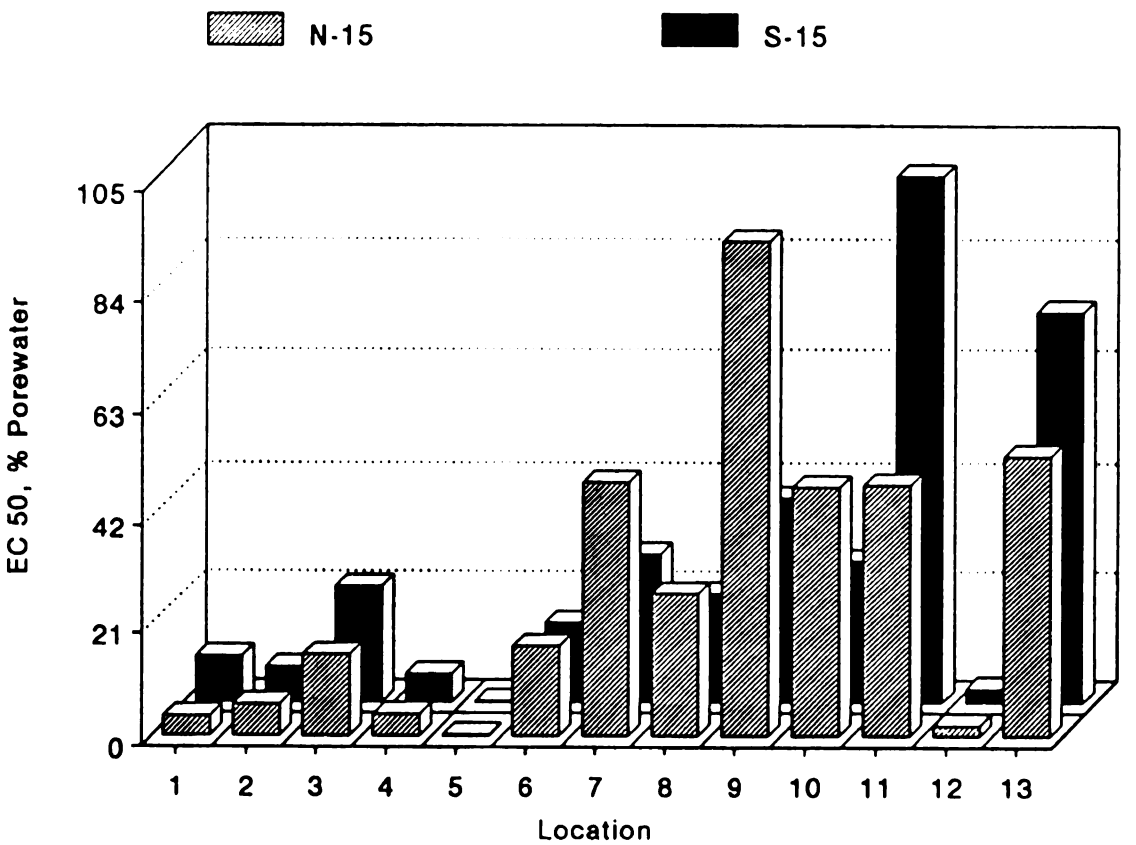

Figure 9. Comparative effects of NaCl versus sucrose osmotic adjustment on 15-min EC_{50} values for Microtox® assays of study site pore waters.

o-cresol, PCBs (as Aroclor 1248), p,p'-DDE, chloro- and ethylbenzene and benzo(k)fluoranthene ranged from 0.64-0.81. The results of sucrose-adjusted tests were also correlated with these same compounds in pore water. However, the total number of significant correlations was less for the sucrose-adjusted tests and the observed correlations were generally with EC_{50} values calculated after 15 and 30 minute exposures. The significance of these correlations was questionable due to the fact that if increasing chemical concentrations were related to greater toxicity (i.e., smaller EC_{50} value) the correlations should have been negative rather than positive. No biologically or statistically significant correlations were observed between the results of Microtox® test results and the results of the ICP metals or the miscellaneous chemical analyses of the pore waters.

Toxic units (Sprague and Ramsey 1965) were calculated for pore water concentrations of unionized ammonia, o- and p-chlorophenol, o-cresol, ethylbenzene, chlorobenzene, 2,4-dinitrophenol, 1,1,1-trichloroethylene, pentachlorophenol, phenol and naphthalene by dividing the pore water concentration by the 15-min EC_{50} value from NaCl-adjusted Microtox® tests of the pure chemical (Kaiser and Ribo, 1988). Ammonia, naphthalene and phenanthrene were the only compounds present at >0.1 TU in the pore water samples. The calculated TU units for these three compounds were then summed and compared to the measured TU units for each sample (Table 11). Measured TU were calculated by dividing 100% by the 15-min EC_{50} from NaCl-adjusted pore water Microtox® tests. The total calculated TU for the pore water samples ranged from 0.2 - 4.6 while the measured TU ranged from 1.1 - 333.3. Comparison of calculated versus measured TU for sucrose-adjusted

Table 11. Calculated and measured pore water toxic units (TU) for selected parameters based on pore water chemical concentrations and 15-min EC_{50} values from Microtox® tests of pore waters and pure chemicals. Microtox® tests were osmotically adjusted with NaCl.

Parameter	Pure Chemical 15 min EC_{50} (ug/L)	Grand Calumet River UG-Site Number												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Unionized Ammonia	2000.0[1]	4.1	0.1	0.7	2.7	0.3	0.4	0.8	3.2	1.7	1.7	0.5	3.1	0.2
Naphthalene	900[1]	0.5	0.1	0.4	0.2	0.1	0.1	0.5	0.3	0.4	0.4	NA[3]	NA	NA
Phenanthrene	49[2]	4.7	2.0	3.6	1.1	3.0	1.4	0.6	3.8	5.0	3.8	NA	NA	NA
Total Calculated TU[4]		9.3	2.2	4.7	4.0	3.4	1.9	1.9	7.3	7.1	5.9	0.5	3.1	0.2
Total Measured TU[5]		27.8	17.2	6.6	25.0	333.3	6.0	2.1	3.7	1.1	2.1	2.1	52.6	1.9

1 Kaiser and Ribo 1988

2 Kaiser and Palaerica 1991

3 NA = not available because pore water chemical concentrations were not measured.

4 Pore water chemical conc. + 15-min EC_{50} from Microtox® NaCl-adjusted test of pure chemical.

5 100% pore water + 15-min EC_{50} as % pore water from Microtox® NaCl-adjusted test of sample pore water.

tests was not conducted due to the paucity of data for sucrose-adjusted tests of pure chemicals.

D. magna and C. dubia

Pore water EC_{50} values for D. magna ranged from 5.5% pore water for site UG-8 to >100% pore water at sites UG-2, 6 and 7 (Table 10). Ceriodaphnia dubia 48 h EC_{50} values ranged from 3.2% pore water at site UG-8 to >100% at sites UG-2 and 7 (Table 10). With the exception of sites UG-3 and 4, where pore water was approximately 3-4 times more toxic to D. magna than to C. dubia, EC_{50} values indicated C. dubia were always as sensitive or more sensitive than D. magna to the toxic effects of the sediment pore waters.

Daphnia magna 48 h EC_{50} values were negatively correlated ($r = -0.65$) with concentrations of dieldrin in pore water while C. dubia 48 h EC_{50} values were negatively correlated with pore water concentrations of 2, 4-dinitrophenol, dieldrin, fluoranthene, phenanthrene and benzo(k)fluoranthene ($r = -0.70, -0.67, -0.72, -0.74$ and -0.65, respectively). No significant correlations were observed between concentrations of metals measured by ICP in pore water and the results of the acute daphnid tests. However, significant correlations were observed between pore water concentrations of unionized ammonia and alkalinity and the 48 h EC_{50} values for both D. magna ($r = -0.69, -0.61$) and C. dubia ($r = -0.60, -0.74$).

In a manner analogous to that used for the Microtox® tests, TU were calculated for pore water concentrations of unionized ammonia, alkalinity (bicarbonate ion), copper, zinc, naphthalene and phenanthrene based on 48 h LC_{50} values for tests with the pure compounds (Table 12). Forty-eight

Table 12. Calculated and measured pore water toxic units (TU) for selected parameters based on pore water chemical concentrations and 48 h LC_{50} values from D. magna and C. dubia acute toxicity tests of pore waters and pure chemicals.

D. magna Parameter	Pure Chemical 48 h LC_{50} (ug/L)	Grand Calumet River UG-Site Number												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Unionized Ammonia	2600[1]	3.1	0.1	0.5	2.0	0.2	0.3	0.6	2.5	1.3	1.3	0.4	2.4	0.1
HCO_3^-	921000[2]	0.7	0.1	0.5	0.4	NA[3]	0.3	0.5	1.6	1.1	0.2	0.7	1.6	0.5
Cu^{++}	69[4]	0.1	0.9	2.6	0.9	0.2	0.1	1.1	0.1	0.4	0.1	0.1	0.1	0.1
Zn^{++}	655[5]	0.0	0.2	0.0	0.2	0.0	0.7	0.1	0.1	0.2	0.0	0.1	0.1	0.2
Naphthalene	2160[6]	0.2	0.0	0.1	0.1	0.0	0.1	0.2	0.1	0.2	0.2	NA	NA	NA
Phenanthrene	700[6]	0.3	0.1	0.3	0.1	0.2	0.1	0.0	0.3	0.4	0.3	NA	NA	NA
Total Calc. TU[7]		4.4	1.4	4.0	3.7	0.6	1.6	2.5	4.7	3.6	2.1	1.3	4.2	0.9
Total Measured TU[8]		2.9	<1.0	<1.0	6.2	1.5	<1.0	<1.0	18.2	1.5	1.4	<1.0	5.9	2.6

Table 12. (continued).

C. dubia Parameter	Pure Chemical 48 h LC_{50} (ug/L)	Grand Calumet River UG-Site Number 1	2	3	4	5	6	7	8	9	10	11	12	13
Unionized Ammonia	Var.[9]	5.0	0.1	0.8	3.0	NA	0.5	0.9	3.5	2.0	2.7	0.8	4.7	0.2
HCO_3^-	780000[2]	0.8	0.1	0.6	0.5	NA	0.4	0.6	1.9	1.3	0.2	0.8	1.9	0.6
Cu^{++}	Var.[9]	0.0	0.3	0.9	0.3	NA	0.0	0.4	0.0	0.1	0.2	0.2	0.2	0.0
Zn^{++}	Var.[9]	0.2	1.1	0.3	1.4	NA	5.2	0.9	0.8	1.2	0.1	0.1	0.1	1.4
Total Calc. TU[7]		6.0	0.6	2.6	5.2	NA	6.1	2.8	6.2	4.6	3.2	1.9	6.9	2.2
Total Measured TU[8]		4.1	<1.0	6.1	1.7	NA	1.5	<1.0	31.3	4.9	1.4	<1.0	6.8	2.4

1 U.S. EPA 1985b

2 Hoke et al. 1992b

3 NA = not available because pore water chemical concentrations was not measured or no LC_{50} was determined.

4 U.S. EPA 1980b, 48 h LC_{50} at approximate water hardness of 200 mg/L as $CaCo_3$.

5 U.S. EPA 1980c, 48 h LC_{50} of approximate water hardness of 200 mg/L as $CaCO_3$.

6 Millemann et al. 1984.

Table 12. (continued).

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[7] Pore water chemical conc. ÷ 48 h LC_{50} from D. magna or C. dubia acute toxicity test of pure chemical.

[8] 100% pore water ÷ 48 h LC_{50} as % pore water from D. magna or C. dubia acute toxicity test of sample pore water.

[9] Var. = variable values which are pH dependent (Ankley et al. 1991).

hour C. dubia and D. magna LC_{50} values for unionized ammonia were obtained from U.S. EPA (1991) and U.S. EPA (1985b), respectively. Ceriodaphnia dubia LC_{50} values for unionized ammonia used for TU calculations were pH-specific based on the greatest pH value measured during the test of each pore water sample (U.S. EPA 1991) while the D. magna LC_{50} value was the mean of five separate LC_{50} values from tests conducted at an approximate pH of 8.0 (U.S. EPA 1985b). Forty-eight hour LC_{50} values for alkalinity (bicarbonate ion), copper and zinc, and the PAHs used in TU calculations were obtained from Hoke et al. (1992b), U.S. EPA (1980b,c); and Millemann et al. (1984), respectively. LC_{50} values for the metals were at an approximate water hardness of 200 mg/L as $CaCo_3$. Based on the pure chemical data for the two cladoceran species, the total calculated TU for the pore water samples ranged from 0.4 - 6.9, while the measured TU ranged from 1.0 - 18.2 (Table 12). Unionized ammonia and bicarbonate ion accounted for the greatest proportion of the total TU calculated for both D. magna and C. dubia (Table 12).

Chironomus tentans

Percent inhibition in dry weight gain in C. tentans exposed to sediments from the Grand Calumet River ranged from 37% at UG-7 to 100% at sites UG-1, 3 and 4. Only one site, UG-7, exhibited a % inhibition of dry weight gain of less than 90%. Because the range of response for C. tentans was very small if the data from site UG-7 were excluded from the analysis, no further attempts were made to correlate results with the results of chemical analyses or to calculate TU based on observed chemical concentrations.

Discussion

The sediments and sediment pore waters from a number of sites in the Grand Calumet River system of northwest Indiana contained detectable concentrations of a wide variety of organic chemicals and metals. Simple visual inspection of sediments from the system, however, led to the conclusion that one of the primary contaminants in the system was oil and grease. The concentrations of this broad category of petroleum hydrocarbons ranged from 1.6-13.5% on a sediment dry weight basis and undoubtedly, even in the absence of other contaminants, would exert a strong influence on the presence and/or distribution of benthic macroinvertebrates in the system. Previous studies have reported on the depauperate nature of the benthic macroinvertebrate community in the Grand Calumet River (IDEM 1988). The oil and grease content of the sediment also was likely at least partially responsible for the high mortality and inhibition of dry weight gain observed in the C. tentans test.

Petroleum hydrocarbons are a major contaminant of aquatic systems with as much as 6 million metric tons of these products introduced into aquatic ecosystems worldwide on a yearly basis (NAS 1975). Petroleum hydrocarbons are a mixture of hydrocarbons and trace elements which can effect almost all aquatic macroinvertebrates by direct exposure (Petrakis and Weiss 1980, Hoehn et al. 1974). Petroleum hydrocarbons may affect macroinvertebrates by coating gills or other body surfaces responsible for cutaneous respiration thus limiting oxygen exchange, by direct toxic action, by bioaccumulation of hydrocarbons or by blanketing the substrate and preventing colonization. The impact of a massive crude oil spill on

macroinvertebrate fauna in a stream system was described by Crunkilton and Duchrow (1990). A three orders of magnitude decrease in expected numbers of organisms was observed for a month after the spill. Species diversity and the number of sensitive species (mayflies, stoneflies) were decreased for almost a year after the spill. The authors observed that the visible presence of petroleum hydrocarbons in the stream substratum was an effective predictor of effects on the benthic macroinvertebrate community and that total flow volume and occurrence of scouring due to floods were the major factors controlling recovery. Schloesser et al. (1991) also observed that the visible presence of petroleum hydrocarbons in sediments was a good indicator of effects on Hexagenia limbata abundance in the upper Great Lakes connecting channels. In a study of the effects of oil/gas field produced water on the benthic macroinvertebrate community in a small gradient estuary, Nance (1991) reported that sediment oil content was a more important determinant of population abundance than salinity. A sediment hydrocarbon concentration of 2.5 mg/gm dry weight sediment (0.25%) was observed to cause a minor depression of macroinvertebrate abundance while 5.0 mg/gm (0.5%) was observed to cause major effects on abundance. Assuming the presence of no other contaminants, the observed oil and grease content of sediments in the Grand Calumet River (1.6-13.5%) alone appears to have been sufficient to prohibit the development of a viable benthic macroinvertebrate community in the sediments. Based on personal observations made by the authors during field sampling on the Grand Calumet River, it also appeared that petroleum hydrocarbon inputs to the system were a continuing, rather than simply a historical, problem.

Although petroleum hydrocarbons in sediments from the study area appeared to affect the results of the C. tentans bulk sediment tests, the

physical effects of this suite of compounds should have been less important in determining the results of the pore water tests with P. phosphoreum, D. magna and C. dubia. The limited water solubility of most petroleum hydrocarbons and the filtration step (Whatman GF-F, 0.7 μm nominal pore size) used in the preparation of sediment pore waters could have removed all but the most water soluble petroleum hydrocarbons (i.e. naphthalene, phenanthrene) from the pore waters. Therefore, the results of the pore water tests provided information on the toxicity of water soluble compounds which likely was not provided by the C. tentans test results due to the confounding presence of large amounts of insoluble petroleum hydrocarbons in the sediments.

The results of separate sucrose and NaCl osmotically-adjusted Microtox® tests on a sample may be helpful in determining the causes of observed sample toxicity (Hinwood and McCormick 1987, Ankley et al. 1990a, Hoke et al. 1992a). Changes in sample toxicity over the 30-min course of the full Microtox® test also may be helpful in identifying potential toxicants. Several ionic chemicals (certain metals, ammonia) have been observed to be more toxic in Microtox® tests osmotically adjusted with sucrose (Hinwood and McCormick 1987, Ankley et al. 1990a) while chlorine was more toxic in NaCl-adjusted tests (Ankley et al. 1990a). Some metals also exhibit a pattern of increasing toxicity with time while the toxicity of ammonia and many organic chemicals is constant over time. In general, little difference was observed in the toxicity of GCR pore waters in Microtox® tests osmotically-adjusted with sucrose or NaCl. This may indicate that ionic toxicants in the pore water were not responsible for the majority of the observed toxicity. Pore waters from UG-7, 8, 9 and 10 were more toxic in tests osmotically-adjusted with sucrose while pore

waters from UG-11 and UG-13 were more toxic in tests osmotically adjusted with NaCl. Measurable concentrations of Cu and Zn were present and pH and unionized ammonia also were greater in pore waters from site UG-7 through 10 (Tables 8, 9). Zn is known to be more toxic at higher pH (Ankley et al. 1991) and higher pH also shifts the ammonia equilibrium towards greater proportions of unionized ammonia which is the more toxic form of ammonia to most aquatic species (U.S. EPA 1985b). No increase in toxicity with time was observed in any Microtox® test conducted on these pore waters. These observations coupled with the known presence of municipal wastewater treatment plant discharges on this section of the GCR (IDEM 1988) strongly implicate unionized ammonia as one of the principal contaminants causing the effects observed in Microtox® tests of pore water from sites UG-7 through 10.

Greater concentrations of metals in pore water (i.e., UG-2,3,4) or greater concentrations of ammonia (i.e., UG-1,4) were observed but pore water from these sites also generally had lesser pH values. The increase in toxicity with time observed over the course of both NaCl-and sucrose-adjusted Microtox® tests of pore waters from sites UG-2,4 and 6 suggests, however, that metals were responsible for at least part of the observed toxicity. No explanation is evident for the greater toxicity in NaCl-adjusted tests of pore waters from sites UG-11 and 13 because complete chemical analyses were not conducted on sediments or pore waters from these sites.

Based on the calculated versus measured TU from Microtox® 15-min EC_{50} values for the study site pore waters, a large proportion of the observed toxicity was unaccounted for at most sites, except sites UG-7 through UG-10. The contribution of the PAH, phenanthrene, to the total

calculated TU for a given site was generally equal to or greater than the contribution of ammonia. Naphthalene TU were generally equal to or less than the unionized ammonia TU at each site. Copper and zinc TU from NaCl-adjusted Microtox® tests were generally ≤ 0.1 TU for each site (data not shown) and it was not possible to calculate fluoranthene TU because no Microtox® toxicity data exist for this compound. Calculated TU exceeded measured TU at sites UG-8,9 and 10, however, when the contribution of the PAHs was dropped from the calculated TU there was much better agreement between total calculated and measured TU. An additional factor (i.e., pore water bicarbonate ion concentration) discussed below appeared to cause toxicity in the cladoceran pore water assays. However, no effect on the Microtox® test was observed in a series of experiments designed to evaluate the effects of water hardness and alkalinity at concentrations up to 3000 mg/L as $CaCO_3$, respectively (data not shown). At sites UG-1 through UG-6 and UG-11 through UG-13, it appeared that unidentified compounds caused a large portion of the observed effects in the Microtox® tests.

In 48 h pore water tests, C. dubia was, with the exception of site UG-4, always equally or more sensitive to the effects of pore waters than was D. magna. The difference in response between the two species was generally small, perhaps indicating that the cladocerans were responding to the same contaminants with C. dubia being slightly more sensitive. At sites UG-3,5,6 and 9, however, C. dubia was approximately 1.3-6 times more sensitive to the effects of the pore waters than was D. magna. This variability also could have been the result of the effect of pH on the relative species sensitivity to chemicals. C. dubia sensitivity to unionized ammonia exhibits a greater pH dependence than does that of D.

magna (Ankley et al. 1991, U.S. EPA 1985b). Lack of sensitivity to pH effects on ammonia toxicity also has been observed for Hyalella azteca (Ankley et al. 1991). Sensitivity to some metals may exhibit a similar pattern since the zinc 48 h LC_{50} for C. dubia tested in very hard reconstituted water at a pH of 8.0-8.5 was 95 μg/L (Ankley et al. 1991) while the 48 h LC_{50} for D. magna tested under similar conditions was 655 μg/L (U.S. EPA 1980c). This factor alone could explain a large portion of the variation in the responses of the cladoceran tests, especially in light of the observations that the pore water pH values at sites UG-3,6 and 9 were 8.4, 8.0 and 8.4, respectively (pH was not measured on site UG-5 pore water) while pore water zinc concentrations were 28, 490 and 114 μg/L, respectively.

Total calculated TU for D. magna and C. dubia exceeded the measured TU at 8 and 10 of the 13 sites, respectively. However, total calculated TU for both species assumed that measured pore water concentrations of copper and zinc were present as the bioavailable free ion. This is unlikely due to the pH dependent nature of chemical speciation and the probable presence of many inorganic and organic ligands which could bind free metal ions in pore waters. A decrease in TU attributable to copper and zinc would result in a decrease in the total calculated TU for each pore water and bring total calculated and measured TU into closer agreement.

Toxic units attributable to phenanthrene and naphthalene in D. magna pore water tests were less than TU attributable to unionized ammonia and bicarbonate ion. The TU attributable to unionized ammonia and bicarbonate ion also accounted for the greatest proportion of the total calculated TU units in the C. dubia tests. Pore water concentrations of unionized

ammonia and alkalinity also were negatively correlated with the 48 h EC_{50} values from the cladoceran tests, which indicates that greater concentrations of these chemicals caused greater toxicity (lesser LC_{50} value) of the pore waters to the cladocerans.

Ammonia is known to be toxic to cladocerans (U.S. EPA 1985b) and several authors have recently reported on the effects of carbonate alkalinity on cladocerans (Cowgill and Milazzo 1991, Hoke et al. 1992b). In an investigation of the toxicity and potential mode of action of the bicarbonate ion, Hoke et al. (1992b) demonstrated that bicarbonate ion was toxic to both D. magna and C. dubia and that sufficient bicarbonate ion was present in several GCR-IHC pore waters to cause a large portion of the observed toxicity of the pore waters to these species. These observations reinforce the importance of unionized ammonia and alkalinity in the TU calculations for the cladocerans.

It was not possible to calculate the potential contribution of naphthalene and phenanthrene to the total measured TU for C. dubia tests because no 48 h reference toxicant LC_{50} data for these chemicals were available for C. dubia. The total calculated and measured TU for both cladoceran species at each site were generally observed to be in good agreement with the exception of site UG-8 where the total calculated TU for both species were much less than the total measured TU. One or more chemicals not measured during this study must have been present in the pore water from this site at concentrations sufficient to affect both cladoceran species.

The relative sensitivities of the tests used during this study were very different. The C. tentans test appeared to be affected primarily by the physical presence of petroleum hydrocarbons in the sediments, which

also obscured any potential impacts on this species of contaminants in the sediment pore waters. The cladoceran tests appeared to be affected primarily by concentrations of unionized ammonia and bicarbonate ion in the pore water. Unionized ammonia and the PAHs naphthalene and phenanthrene also were possibly important determinants of the toxicity of sediment pore waters in the Microtox® test. However, at several sites, observed toxicity in both the Microtox® and cladoceran tests could not be accounted for solely on the basis of the concentrations of the chemicals used in the TU calculations presented here.

Reference toxicant data necessary for calculation of TU for a wide variety of chemicals are not available for either the Microtox® or the *C. dubia* tests. The chemical analysis of study site sediments and pore waters included only 104 analytes. Although a large number of compounds were analyzed for in the samples, the potential for additional compounds to be present in the samples is great. Both of these factors limit the application of the TU approach, without a concurrent toxicity identification evaluation (TIE) effort Ankley et al. 1991), for determining the compounds causing the observed toxicity in tests of whole sediments and sediment pore waters.

In addition, the TU approach assumes additivity of chemical effects and complete bioavailability of all measured analytes, which may or may not be a valid assumptions in complex environmental matrices, depending on the chemicals under consideration. Antagonism and synergism are equally plausible consequences for the expression of the toxicity of chemical mixtures. If chemicals exhibit their effects via different modes of action, the assumption of additivity of effects will not be met and calculated TU could be over-estimates of chemical effects. This

phenomenon, in addition to the various factors affecting chemical bioavailability, the lack of reference chemical toxicity data and the incomplete nature of the chemical analyses previously discussed, contribute to the observed discrepancy between calculated and measured TU.

The results of the study presented here highlight the necessity and importance of the battery of tests approach for toxicity assessments which has been previously advocated by other investigators (Slooff et al. 1983, Millemann et al. 1984, Williams et al. 1986, Dutka and Kwan, 1988, Giesy and Hoke 1989). Each test responded in a unique way which emphasized the intrinsic sensitivity of the test, the differences in exposure routes among tests and the fate of individual chemicals or classes of chemicals in the samples. The study also demonstrated the advantages and limitations of a toxic units approach by itself for evaluating the potential causes of the observed toxicity in the various assays. A more utilitarian approach to assessments of contaminated sediments could incorporate the battery of assays approach and sediment TIE, including the evaluation of calculated versus measured TU, to identify and confirm the causes of observed sediment and pore water toxicity and, ultimately, guide site remediation efforts (Ankley et al. 1991).

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CHAPTER 3

Bicarbonate as a Potential Confounding Factor in Cladoceran Toxicity Assessments of Pore Water from Contaminated Sediments

Introduction

Toxicity assessment of contaminated sediments has become de rigueur in planning for remedial actions at International Joint Commission (IJC) Great Lakes "Areas of Concern" (AOC) (Hileman 1988) and at many U.S. EPA Superfund sites (Warren-Hicks et al. 1989). Sediments contaminated by a wide array of metals and organic chemicals are an acknowledged legacy of the explosive post-World War II growth in the chemical industry and the continued reliance on fossil fuels for energy production. Approaches traditionally used to evaluate contaminated sediments include: 1) chemical analyses for total sediment concentrations of compounds or elements of concern, 2) toxicity tests of bulk sediments or aqueous extracts of sediments using benthic and upper water column test species, respectively, and 3) field surveys of in-situ effects such as changes in benthic macroinvertebrate community structure or the occurrence of histopathological lesions in benthic fish species (for reviews see Giesy and Hoke 1989, Chapman et al. 1987). In the present study, we focus on the potential implications of, and one of the problems involved in, the use of pore water (interstitial water) toxicity tests with cladocerans as surrogates for bulk sediment tests with benthic invertebrates.

Pore water from contaminated sediment is used as an exposure medium for toxicity tests (Giesy and Hoke 1989). Comparisons have been made of the results of tests which evaluated the toxicity to Hyalella azteca and

Lumbriculus variegatus of bulk sediment, pore water and elutriate from the same samples (Ankley et al. 1991). These comparisons and the results of other studies (Nebeker et al. 1984, van de Guchte and Maas-Diepeveen 1988, Knezovich and Harrison 1988) suggest that pore water exposures with benthic species are good surrogates for bulk sediment exposures.

The underlying assumption in the use of pore water exposures as a surrogate for bulk sediment exposures is that organisms receive most of their exposure to toxic substances in sediments through contact with the pore water (Nebeker et al. 1984, Ankley et al. 1991). The relationship between pore water and bulk sediment exposures has not been clearly established (Cairns et al. 1984, Nebeker et al. 1984) and many physico-chemical processes can be expected to affect the partitioning of metals and xenobiotics from the solid phase into the pore water (Sinex et al. 1980, Knezovich et al. 1987, U.S. EPA 1989, Di Toro et al. 1990). Additional assumptions inherent in the use of pore water as a test medium are that basic chemical properties of pore water, such as major ion concentrations and/or ratios, are within the physiologically tolerated ranges of the test species and that the pore water extraction technique does not cause artifacts in the pore water chemistry. Recent reviews of the advantages and disadvantages associated with various methods of pore water preparation can be found in Adams (1991) and Schults et al. (1992).

During an assessment of the toxicity of sediments at the Grand Calumet River-Indiana Harbor Canal (GCR-IHC) IJC AOC, several observations caused concern over the effects of chemical properties of the pore waters on the results of toxicity tests with cladocerans. Elevated pore water alkalinity values (Table 13), gas bubble formation, white crystalline residue on the inside of test chambers and increases in pore water pH in

Table 13. Results of routine chemical analyses of sediment pore waters from the Grand Calumet River-Indiana Harbor Canal IJC AOC tested in 48-h acute assays with D. magna and C. dubia. Maximum measured pH, conductivity, hardness and alkalinity are reported from the 100% pore water exposure treatment for each location, as well as calculated free CO_2 and HCO_3^- concentrations in pore water based on measured pH, alkalinity, and temperature (25°C) from the cladoceran assays.

	Measured Values				Calculated Values	
Location	pH	Conductivity (μmhos/cm^2)	Hardness (mg/L, $CaCO_3$)	Alkalinity (meq/L)	CO_2 (free) (mmoles/L)	HCO_3^- (mmoles/L)
UG-1	8.00	2570	640	13.2	0.3	13.1
UG-2	8.30	423	160	1.2	0.0	1.2
UG-3	7.70	3420	460	5.2	0.2	5.2
UG-4	8.50	830	370	7.4	0.1	7.2
UG-6	8.00	2920	2340	5.8	0.1	5.8

Table 13. (continued).

Location	pH	Measured Values: Conductivity (μmhos/cm^2)	Measured Values: Hardness (mg/L, $CaCO_3$)	Measured Values: Alkalinity (meq/L)	Calculated Values: CO_2 (free) (mmoles/L)	Calculated Values: HCO_3^- (mmoles/L)
UG-7	7.60	1420	1040	10.0	0.6	10.0
UG-8	7.65	4300	2160	30.0	1.5	29.9
UG-9	7.86	3650	1040	20.8	0.7	20.8
UG-10	7.06	4680	2500	3.6	0.7	3.6
UG-11	7.74	2230	640	12.4	0.5	12.3
UG-12	7.60	2100	700	22.4	1.3	21.9

test vessels during cladoceran toxicity tests led to the hypothesis that the test results might be affected by the altered character of the carbonate-bicarbonate buffer system in the pore waters. At the time the investigation was begun, no information existed in the literature concerning the toxicity of bicarbonate ion to cladocerans although some information was available on the toxicity of CO_2 (Mount and Anderson-Carnahan 1988). It also was known that CO_2 could be used to anesthetize zooplankton prior to preservation (Gannon and Gannon 1975). Limited information also was available on the toxicity of bicarbonate ion to fish (Beatty 1959, Mitchum 1960).

This investigation was conducted to examine the toxicity of the bicarbonate ion to D. magna and C. dubia. The specific objectives of the study were: 1) to determine the effects of bicarbonate ion concentrations on D. magna and C. dubia using $NaHCO_3$ and NaCl as reference toxicants, 2) to compare calculated concentrations of HCO_3^- in sediment pore waters from the AOC study area to concentrations shown to cause adverse effects on D. magna and C. dubia in laboratory exposures, and, if HCO_3^--induced toxicity was observed, 3) to propose potential mechanisms for the observed HCO_3^--induced effects.

X-ray dispersive microanalysis previously has been used to study the role of various invertebrate tissues in metal storage and the effects of different environmental levels of metals on metal localization in invertebrates (Krantzberg and Stokes 1990, Ballan-Dufrancais et al. 1985). Investigations also have been conducted on diffusible ions in invertebrate osmoregulatory systems (Marshall and Wright 1973, Roomans 1988a), and in bulk specimens of skeletal muscle (Zierold and Schafer 1978). Because of its previous use in the examination of physiological levels of diffusible

ions, X-ray dispersive microanalysis was chosen as the simplest and most direct method for monitoring the potential effects of bicarbonate ion on the relative concentrations of several physiologically important elements in D. magna.

Materials and Methods

Toxicity of Na^+ and HCO_3^-

Forty-eight hour, acute tests (U.S. EPA 1985) were conducted with Baker reagent-grade $NaHCO_3$ and NaCl to establish the toxicity of HCO_3^- and Na^+ to D. magna and C. dubia. These exposures were initiated with <24 h old neonates from laboratory cultures of each species and also with six or seven-day old, sub-adult D. magna. Six or seven-day old D. magna were initially necessary to provide specimens of sufficient size for manipulation in the preparation procedures for the X-ray microanalysis and were specifically selected for further X-ray microanalysis because of their larger size. Test organisms were not fed and the temperature and photoperiod were maintained at 25°C and 16L:8D, respectively, during all tests. New $NaHCO_3$ and NaCl stock solutions were prepared for each test (3000 mg/L and 8000 mg/L nominal concentrations, respectively). A 0.56X dilution series was used to prepare dilutions of the stock solutions for testing. A minimum of five toxicant concentrations and a control were prepared for each test. Five replicates, each containing five organisms, were tested for each toxicant concentration or control. The laboratory culture/diluent water was prepared by mixing bottled Perrier® mineral water and HPLC-grade laboratory water (1:9 v/v) followed by vigorous aeration for 24 h to ensure equilibration of gases prior to culture or

test use (U.S. EPA 1989). Two μg/L each of cyanocobalamin (Vitamin B_{12}) and selenium, as sodium selenate, also were added to the culture/diluent water (Lanno 1989). Control survival in toxicity tests was always greater than 90%. Forty-eight hour LC50 values were calculated using the binomial test and are reported as nominal HCO_3^- or Na^+ concentrations in mmoles/L (Table 14).

Calculation of Free CO_2 and HCO_3^-

A series of twenty-six, 48-h acute pore water tests were performed with D. magna and C. dubia as part of the original AOC sediment toxicity assessment (Hoke et. al 1992). Data from these exposures subsequently were used to calculate theoretical equilibrium concentrations of free CO_2 and HCO_3^- in the pore waters for comparison with effects concentrations from the literature or the reference toxicant tests described above. Free CO_2 and HCO_3^- concentrations at equilibrium were calculated using temperature (25°C), pH and alkalinity values measured in the 100% pore water exposure treatment from the cladoceran tests (Table 13). Alkalinity was measured using a Hach kit (accuracy = ± 0.4 meq/L) and pH was measured using an Orion Model 701A ionanalyzer equipped with a glass Ross combination pH probe. The following equations (Harvey 1957) were used to calculate free CO_2 and HCO_3^- concentrations, respectively.

$$x = \alpha\beta \text{; where } \beta = \frac{H^2}{K_1(H + 2K_2)} \quad (1)$$

and

$$y = \frac{2H}{H+2K_2} \quad (2)$$

where:

$$H = [H^+]$$

$$\alpha = [HCO_3^-] + 2[CO_3^-] = \textit{Measured alkalinity}$$

$$x = [H_2CO_3], \textit{ including free } CO_2$$

$$y = [HCO_3^-]$$

$$K_1 = \frac{[HCO_3^-][H^+]}{[H_2CO_3]}$$

$$K_2 = \frac{[CO_3^{-2}][H^+]}{[HCO_3^-]}$$

X-ray Dispersive Microanalysis

To investigate the effects of HCO_3^- on the internal ion balance (Na, Si, Cl, Ca) of D. magna, several 48-h tests in which $NaHCO_3$ was used as a toxicant were initiated with 6 or 7-d old, sub-adult D. magna. Three experiments were conducted with $NaHCO_3$. Additional experiments were conducted with pore water collected from one of the original AOC sediment samples (Location UG-9) due to its high alkalinity and calculated HCO_3^- concentration, and with NaSCN, a metabolic inhibitor of Cl^- uptake (Epstein et al. 1973). Live D. magna were removed from the tests for subsequent X-ray analysis after 16-20 h exposure to $NaHCO_3$ or Location UG-9 pore water. Organisms were removed for analysis after 2 h of exposure to NaSCN. Forty-eight hour exposures to the highest concentrations of either $NaHCO_3$ or pore water were lethal to D. magna. Organisms to be analyzed by X-ray microanalysis were exposed for a shorter period of time because the variable of interest was change in elemental peak to background (P/B) ratios in living organisms as a result of exposure to $NaHCO_3$, pore water or NaSCN.

Table 14. Toxicity to D. magna and C. dubia of Na^+ as NaCl or $NaHCO_3$ and of HCO_3^- as $NaHCO_3$.

Species	Age	Compound	48 h LC50 (mmoles/L)	Mean ± SD LC50 (mmoles/L)
D. magna	<24 h	NaCl	85.7	81.3 ± 6.2
D. magna[1]	<24 h	NaCl	76.9	
D. magna[2]	4th instar/ adult	NaCl	57.9	--
C. dubia	<24 h	NaCl	14.3	13.5 ± 1.2
C. dubia	<24 h	NaCl	12.6	
D. magna	<24 h	$NaHCO_3$	16.6	15.1 ± 2.2
D. magna	<24 h	$NaHCO_3$	13.5	
D. magna	6 d	$NaHCO_3$	21.2	--
D. magna	7 d	$NaHCO_3$	26.3	20.6 ± 8.1
D. magna	7 d	$NaHCO_3$	14.9	
C. dubia	<24 h	$NaHCO_3$	13.8	12.8 ± 1.5
C. dubia	<24 h	$NaHCO_3$	11.7	

1 Mount and Anderson-Carnahan 1988.

2 Dowden 1961.

Whole D. magna (generally 5 per treatment) were removed from the tests and mounted on carbon planchets. A 1:1 (v/v) mixture of Tissue Tek and graphite was used to mount the daphnids on the carbon planchets and to attach the carbon planchets to electron microscopy stubs. The Tissue Tek-graphite mixture did not interfere with subsequent X-ray analysis of D. magna (R. Hoke, unpublished data). For preliminary experiments with post-$NaHCO_3$ exposure organisms, traditional cryogenic preparation and fracturing techniques (EM Scope 2000) were used with etching at -20°C for 15 min if ice crystals were present in the sample (Boekestein et al. 1980, Marshall 1988). Because these fracturing techniques were cumbersome and frequently displaced organisms from the planchets, in subsequent experiments organisms were mounted on planchets and then plunged into liquid nitrogen for 15 sec. The carapace of each D. magna was then gently sliced away with a razor blade to reveal the internal body structure and facilitate penetration of the X-ray beam.

X-ray microanalyses were performed with a JEOL JSM-35C scanning electron microscope equipped with a cryostage and a Tracor Northern Series II X-ray microanalysis system with software. Each measurement was acquired at an accelerating voltage of 20.0 KeV for a preset scanning time of 60 s. Results are reported as P/B ratios (Boekestein et al. 1980, Marshall 1988, Roomans 1988b). Elemental measurements were conducted at five randomly chosen locations within the head and upper body area for each of five D. magna from control and either, low and high, or low, medium and high experimental treatments. Absolute peak height or emission intensity data were not used due to the lack of analytical standards in an appropriate matrix. The same analytical constraints were operative among

all measurements conducted as part of each experiment; therefore, P/B ratios and changes in these ratios as a result of experimental treatments, relative to controls, were the data of interest in all experiments.

Data Analysis

X-ray dispersive microanalysis results were analyzed with a nested, fixed effects ANOVA design (Petersen 1985) using the Statistical Analysis System (SAS 1985) software. For the ANOVA, the X-ray microanalysis measurements were nested within experimental units (i.e. individual Daphnia magna). In the variance component analysis, three separate aspects of experimental variance were examined: 1) treatment variance, 2) organism (among daphnid within treatment) variance and measurement (among measurement within daphnid) variance.

Results

Toxicity of Na^+ and HCO_3^-

Forty-eight hour LC50 values for D. magna and C. dubia tests with $NaHCO_3$ and NaCl are presented in Table 14. Based on mean LC50 values, Ceriodaphnia dubia neonates (< 24-h old) were approximately six times more sensitive to Na^+ as NaCl than D. magna neonates of similar age. However, 24-h old C. dubia neonates were only slightly more sensitive (~ 1.2 X) than D. magna neonates to Na^+ as $NaHCO_3$. Little difference was observed in the toxicity of the two compounds to <24-h old C. dubia neonates. Daphnia magna neonates were approximately 5.4 x more sensitive to Na^+ as $NaHCO_3$ than as NaCl. Based on literature values for adults (Dowden 1961),

D. magna neonates were approximately 1.4 X less sensitive than adults to NaCl while adults were approximately 1.4 X less sensitive to $NaHCO_3$ (Table 14).

Calculation of Free CO_2 and HCO_3^-

The results of theoretical equilibrium concentration calculations for CO_2 and HCO_3^- in the AOC sediment pore water samples are presented in Table 13. Calculated concentrations of CO_2 ranged from 0.0-1.5 mmoles/L while calculated HCO_3^- concentrations ranged from 1.2-29.9 mmoles/L.

X-ray Dispersive Microanalysis

Mean P/B ratios (± SD) for Na^+, Si^{+4}, Cl^-, and Ca^{+2} in daphnids analyzed from each experiment are presented in Table 15. Within an experiment and within the individual treatments comprising an experiment, relative levels of Ca^{+2} were the most variable parameter measured based on standard deviation of the means while Si^{+4} was the least variable. The variability of relative levels of Na^+ and Cl^- among experiments was similar but Cl^- levels were generally more variable within an experiment. The results of variance component analyses from the ANOVA's of the X-ray microanalysis data are presented in Table 16. The proportion of the total variance accounted for by among treatment effects is presented as well as the variance accounted for by among organism (among daphnid) effects within an experimental treatment and the among measurement (measurements within an individual daphnid) variance. Variation in the Cl^- P/B ratio among treatments was statistically significant in each experiment. P/B

Table 15. Results of X-ray microanalysis experiments with D. magna. Values reported are mean (SD) P/B ratios for each experimental treatment. Significant treatment effects (indicated with asterisk) for elemental P/B ratios were based on a significant ANOVA for the individual element ($\alpha = 0.05$) followed by Bonferroni t-test which indicated a significant difference from either the control or lowest experimental treatment.

Experiment No.-Toxicant; No. Measurements per Daphnid, No.		Mean P/B Ratios			
Daphnids per Treatment	Treatment	Na^{+}	Si^{+4}	Cl^{-}	Ca^{+2}
No. 1 - $NaHCO_3$; 5,1	Control	0.30 (0.09)	0.23 (0.04)	0.67 (0.22)	0.82 (0.49)
	11.5 mM/L HCO_3^-	0.58* (0.15)	0.24 (0.09)	0.25* (0.11)	0.60 (0.48)
	35.9 mM/L HCO_3^-	0.41 (0.15)	0.25 (0.19)	0.27* (0.06)	0.68 (0.78)
No. 2 - $NaHCO_3$; 5,2	0.4 mM/L HCO_3^-	0.32 (0.19)	0.25 (0.12)	0.61 (0.13)	2.58 (1.25)
	11.5 mM/L HCO_3^-	0.47 (0.19)	0.21 (0.09)	0.72 (0.20)	2.49 (1.17)
	35.9 mM/L HCO_3^-	0.47 (0.29)	0.20 (0.15)	0.00* (0.07)	1.59 (0.98)

Table 15. (continued).

Experiment No.-Toxicant; No. Measurements per Daphnid, No. Daphnids per Treatment	Treatment	Mean P/B Ratios Na^{+}	Si^{+4}	Cl^{-}	Ca^{+2}
No. 3 - $NaHCO_3$; 5,5	Control	0.28 (0.17)	0.13 (0.11)	0.33 (0.18)	0.96 (0.89)
	6.4 mM/L HCO_3^{-}	0.18 (0.11)	0.12 (0.06)	0.27 (0.14)	0.96 (0.86)
	11.5 mM/L HCO_3^{-}	0.36 (0.19)	0.18 (0.20)	0.76* (0.45)	2.03* (1.32)
No. 4 - UG-9	Control	0.29 (0.20)	0.16 (0.10)	0.62 (0.32)	2.92 (1.88)
Pore Water; 5,5	32% Pore Water	0.26 (0.19)	0.07* (0.06)	0.38* (0.25)	2.60 (2.15)
	100% Pore Water	0.20 (0.15)	0.17 (0.14)	0.32* (0.11)	2.40 (1.26)
No. 5 - NaSCN; 5,5	Control	0.29 (0.12)	0.11 (0.10)	0.76 (0.22)	0.58 (0.44)
	500 μm/L NaSCN	0.16 (0.13)	0.14 (0.15)	0.32* (0.13)	0.34 (0.33)
	1500 μm/L NaSCN	0.23 (0.11)	0.16 (0.07)	0.40* (0.17)	0.59 (0.52)
	5000 μm/L NaSCN	0.29 (0.11)	0.18 (0.11)	0.44* (0.17)	0.63 (0.44)

Table 16. Variance component analyses of results from analysis of variance of X-ray microanalysis data.

Experiment No.[1], Toxicant	Source	df	% Total Variance[2] Na^{+}	Si^{+4}	Cl^{-}	Ca^{+2}
No. 2,	Total	29	100.0	100.0	100.0	100.0
$NaHCO_3$	Treatment	2	9.8	0.1	86.9*	7.3
	Organism	3	0.0	0.0	0.6	10.1
	Measurement	24	90.2	99.9	12.5	82.6
No. 3	Total	74	100.0	100.0	100.0	100.0
$NaHCO_3$	Treatment	2	18.7*	0.0	40.5*	21.5*
	Organism	12	19.3*	19.5*	24.6*	11.6
	Measurement	60	62.0	80.5	34.9	66.9
No. 4	Total	74	100.0	100.0	100.0	100.0
UG-9 Pore	Treatment	2	1.1	19.3*	24.8*	0.0
Water	Organism	12	5.4	0.0	18.3*	40.2*
	Measurement	60	93.5	80.7	56.9	59.8
No. 5	Total	99	100.0	100.0	100.0	100.0
NaSCN	Treatment	3	13.8	0.0	53.2*	5.2
	Organism	16	17.5*	44.9*	8.3*	0.0
	Measurement	80	68.7	55.1	38.5	94.8

1 Variance component analysis was not possible on the results of experiment 1 due to inadequate replication.

2 * denotes a significant ANOVA F value at $\alpha = 0.05$.

ratios for Na^{+} and Ca^{++} also were significantly different among treatments in the No. 3- $NaHCO_3$ experiment, as was the Si^{+4} P/B ratio in the Location UG-9 pore water experiment. Variation among organisms treated alike also was significantly different for Na^{+}, Si^{+4} and Cl^{-} in the No.3- $NaHCO_3$ and NaSCN experiments and for Cl^{-} and Ca^{++} in the Location UG-9 pore water experiment. Treatment effects accounted for 1.1-18.7, 0.0-19.3, 24.8-86.9 and 0.0-21.5 % of the total experimental variation in P/B ratios for Na^{+}, Si^{+4}, Cl^{-} and Ca^{++}, respectively. With the exception of experiment No. 4 with the UG-9 pore water, treatments effects accounted for the majority of the observed variation in each experiment. Among daphnid variation in P/B ratios within a given experimental treatment always was the smallest component of the observed experimental variation. Variance due to differences in measured P/B ratios within an individual daphnid generally accounted for 12-57% of the total observed experimental variation in P/B ratios.

Discussion

Sediment pore water has been hypothesized to be the primary route of exposure to sediment contaminants for benthic macroinvertebrates (Nebeker et al. 1984, Knezovich and Harrison 1988). Pore water also has been demonstrated to be a reasonable surrogate test fraction for the assessment of bulk sediment toxicity (Ankley et al. 1991). Little research has been conducted, however, on the physical or chemical properties of pore water, other than contaminants, which potentially could affect the results of pore water toxicity tests. Numerous characteristics of pore water (major

ion content, ratios, etc.) potentially can confound the interpretation of results from pore water toxicity assays.

From these results, it appears that HCO_3^- (or bicarbonate alkalinity) is one factor which can affect toxicity test results with some sediment pore waters, irrespective of the effects of other substances present in the sample. Several indirect lines of evidence lead to this conclusion. The first is that HCO_3^- itself may be partially responsible for the toxicity observed in $NaHCO_3$ reference toxicant assays, as evidenced by the observation that Na^+, as $NaHCO_3$, is approximately six times more toxic to <24-h old D. magna than is Na^+ as NaCl. This observation assumes that Cl^- does not protect against Na^+ toxicity. This assumption is supported by recent research which indicates that the toxicity of sodium compounds is due to the anion and not Na^+. Therefore, not only is Cl^- not protective, but it appears that it is responsible for the toxicity of NaCl (D. Mount, ENSR, Inc.,Ft. Collins, CO, pers. comm., Gulley et al. 1991). Recent research by Cowgill and Milazzo (1991) also has reported on alkalinity toxicity to cladocerans. These authors conducted a series of experiments with $NaHCO_3$ as a reference toxicant to determine the chronic toxicity of alkalinity, as mg/L $CaCO_3$, to D. magna and C. dubia. They also reported alkalinity 48 h LC50 values for D. magna and C. dubia of approximately 800 and 500 mg/L as $CaCO_3$, respectively. These values compare well with the mean 48-h D. magna and C. dubia LC50 values of 921 mg/L (15.1 mmoles/L) and 781 mg/L (12.8 mmoles/L) as $CaCO_3$, respectively (Table 2) reported in this study.

A second line of evidence is based on theoretical equilibrium calculations of HCO_3^- concentrations in the AOC pore waters. Based on these calculations and the cladoceran LC50 values for HCO_3^- determined in

this study, concentrations of HCO_3^- sufficient to produce observable toxicity were present in several of the AOC sediment pore water samples (Locations UG-8,9,11,12). Based on the lack of effects on C. dubia at CO_2 concentrations of 0.014 M/L (600 mg/L) in acute tests (Mount and Anderson-Carnahan 1988), the calculated CO_2 concentrations in the AOC sediment pore water samples were not great enough to cause observable effects. The final evidence for the toxic effects of HCO_3^-, and its proposed mode of action, comes from the results of the X-ray microanalysis.

The choice of X-ray dispersive microanalysis of elemental concentrations in D. magna tissues was based on the hypothesized mode of action for HCO_3^-. It is well known that freshwater fish actively regulate internal concentrations of several ions by exchanging them for other ions contained in the external medium (Maetz and Garcia-Romeu 1964, Garcia-Romeu and Maetz 1964, Garcia-Romeu et al. 1969, de Renzis and Maetz 1973, de Renzis and Bornancin 1977, Payan 1978). The existence of a similar active regulatory mechanism in D. magna for metabolically-produced H^+ and external Na^+ was proposed by Potts and Fryer (1979). They hypothesized that freshwater crustaceans obtain a large portion of their Na^+ by active uptake from water. Higher rates of Na^+ turnover (0.163 h^{-1}) were found for D. magna by Potts and Fryer (1979) compared to rates for freshwater fish or crayfish weighing 10-100 g (0.001-0.005 h^{-1}) but rates were believed to be consistent with the greater cladoceran surface/volume ratios. Similar Na^+ turnover rates for D. magna were observed by Stobbart et al. (1977) in experiments in which they demonstrated that 1) Na^+ influx in D. magna followed a Michaelis-Menten relationship, 2) the Na^+ transport mechanism can be saturated at Na^+ concentrations $\geq$0.5 mM/L, and 3) the metabolic inhibitors KCN and dinitrophenol (DNP) inhibited Na^+ uptake at

less than acutely lethal concentrations. They also suggested that the presence of a Michaelis-Menten type uptake relationship for Na^+ and the effects of KCN and DNP on Na^+ influx indicated that Na^+ uptake is actively regulated in D. magna. Although the presence of actively-regulated HCO_3^--Cl^- exchange similar to that found in freshwater fish has never been demonstrated in D. magna, it has been suggested that the NH_4^+-Na^+ and HCO_3^--Cl^- exchange processes may occur frequently in freshwater animals (Maetz and Garcia-Romeu 1964).

The results of the experiments with $NaHCO_3$, AOC Location UG-9 pore water and the metabolic inhibitor NaSCN can be interpreted as supportive of the hypothesis that the exchange process for HCO_3^--Cl^- is actively regulated in D. magna and that the mode of action for HCO_3^- toxicity is the inhibition of this exchange process which results in decreased internal concentrations of Cl^-. This study and others (Gulley et al. 1991, Cowgill and Milazzo 1991) have demonstrated that HCO_3^- can be toxic to D. magna and C. dubia. This study also has demonstrated that D. magna from HCO_3^- treatments which caused acute toxicity exhibited decreased internal Cl^- concentrations, relative to controls, based on mean P/B ratios from X-ray microanalysis. Calculations of the theoretical equilibrium concentration of HCO_3^- in AOC Location UG-9 pore water demonstrated that the sample contained sufficient HCO_3^- to cause acute toxicity based on the 48-h LC50 values determined in the $NaHCO_3$ reference toxicant assays. X-ray microanalysis demonstrated that organisms from the AOC Location UG-9 pore water toxicity test exhibited decreased internal Cl^- concentrations, relative to control, in the 32% and 100% pore water exposures. The metabolic inhibitor NaSCN also caused statistically significant ($\alpha = 0.05$) reductions in internal concentrations of Cl^- across

all NaSCN treatments; indicating that the uptake of Cl^- from the external environment is actively regulated in D. magna.

The nested ANOVA experimental design facilitated the evaluation of the variance in measured elemental P/B ratios due to 1) experimental treatment, 2) among daphnid differences within a treatment, and 3) among measurement differences within an individual daphnid (Table 4). With the exception of experiment No. 4 with AOC Location UG-9 pore water, treatment effects accounted for the majority of the variation in measured P/B ratios for Cl^- in D. magna. In experiment No 4. with the Location UG-9 pore water, treatment effects accounted for 25% of the observed variation in Cl^- P/B rations. Among measurement differences within the same daphnid accounted for the next greatest proportion of the experimental variance in P/B ratios for Cl^- while among daphnid differences always accounted for the smallest proportion of the experimental variance in P/B ratios for Cl^-. This analysis highlights the importance of the experimental treatments on P/B ratios for Cl^- in D. magna. It further suggests that in future investigations of this type, greater experimental precision could be achieved by decreasing the number of daphnids analyzed per experimental treatment but increasing the number of X-ray microanalysis measurements conducted on an individual daphnid.

Additional circumstantial support for the hypothesis that HCO_3^--Cl^- exchange is actively regulated in other aquatic organisms may be found in the field studies of Montezuma Well, Arizona conducted by Blinn and Sanderson (1989) and Dehdashti and Blinn (1991). In this ecosystem, high concentrations of CO_2 (>550 mg/L) and alkalinity (>600 mg/L as $CaCO_3$) were hypothesized to be responsible for the lack of fish and benthic macroinvertebrates of the orders Trichoptera, Lepidoptera, Megaloptera and

Anisoptera. Limited collections of benthic macroinvertebrates in the orders Chironomidae and Ephemeroptera were attributed to the same phenomenon (Blinn and Sanderson 1989). Planktonic rotifers and cladocerans also were absent from this ecosystem (Dehdashti and Blinn 1991). Additional field studies in the Intermountain Region of the U.S. Forest Service concluded that the number of macroinvertebrate taxa in 164 samples from 20 different streams was inversely correlated ($r = -0.67$, $p = 0.001$) with ambient stream alkalinity as $CaCO_3$ (Winget and Mangum 1979). Similarly, McCarraher and Thomas (1968) observed that in alkaline lakes of Nebraska, fathead minnows (Pimephales promelas) flourished when carbonate alkalinity was below 800 ppm and total alkalinity was below 1,800 ppm. However, carbonate or total alkalinity in excess of these values was reported to greatly impair reproduction and abundance of this species.

The proposed existence in D. magna of an actively-regulated exchange process for HCO_3^- and Cl^- and the hypothesis that disruption of this process is the mode of action for bicarbonate toxicity have several practical implications. Bicarbonate concentrations should be routinely measured during toxicity tests of aqueous samples, such as pore waters or effluents. If bicarbonate concentrations are sufficient to produce toxicity, this observation should be considered when interpreting the test results. The importance of bicarbonate toxicity also needs to be addressed in toxicity identification evaluation (TIE) procedures (Mount and Anderson-Carnahan 1988, 1989, Mount 1989), both for effluents and sediment pore waters, because current TIE methodology would not identify high bicarbonate concentrations as the specific cause of observed toxicity. Instead, the observed toxicity could be wrongly ascribed to the effects of total dissolved solids (TDS). This would implicate cations or

cation ratios in the sample as the cause of observed effects when, in fact, the effects were due to the anion HCO_3^-. Finally, since active regulatory processes for HCO_3^--Cl^- also exist in freshwater fish and amphibians, and may exist for other aquatic invertebrates, TIE procedures performed with these species also may be affected by high bicarbonate concentrations in the test solution.

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CHAPTER 4

Mutagenicity and 2,3,7,8-Tetrachlorodibenzo-p-dioxin Equivalents in Organic Solvent Extracts of Sediments from the Grand Calumet River, Indiana

Introduction

The importance of sediments as both a sink and source for chemicals of environmental concern has become obvious during the preceding 20 years. As a result, an increasing amount of research effort has been devoted to investigations of the fate and effects of chemicals in sediments. Most research on the effects of chemicals in sediments has focused on the potential acute toxicity of in-place pollutants to benthic species. A lesser amount of research effort has been devoted to the potential for chronic toxicity or bioaccumulation of sediment-associated chemicals and the potential mutagenicity of bulk sediments or various extracts of sediments. The driving force behind research on the mutagenic effects of pure chemicals or complex environmental mixtures in the past has been the potential for human health effects. Although humans have little direct exposure to sediments or extracts of sediments, we are potentially exposed to these complex mixtures indirectly via aquatic food chains (i.e. ingestion of aquatic species exposed to contaminated sediments). Therefore, any chemical in sediments which is mutagenic and can be bioaccumulated could potentially cause both ecological and human health effects. Research also has been completed or is on-going which suggests that, for certain chemicals, concentrations of mutagenic chemicals in the environment which may be protective of human health may produce mutagenic effects in aquatic biota and organisms which consume aquatic biota.

Chemical analysis can only provide a limited idea of the potential effects (i.e. toxicity, mutagenicity, etc.) of exposure to a complex mixture such as a bulk sediment or extract of a sediment, due to the potential for interactive effects (antagonism, synergism); the potential for effects at concentrations of chemicals which are below the detection limits for the analytical method; and the incompleteness of the chemical analysis (i.e. it is not possible to analyze for all chemical compounds potentially present in a sample). Therefore, the best measure of potential biological effects is a direct measure of some form of biological activity after exposure to the complex mixture (Durant et al. 1992, Tillett et al. 1991).

Evaluations of the mutagenicity of extracts of soils or bulk sediments have been conducted with the Ames assay (Donnelly et al. 1991, Maccubbin et al. 1991, Ersing 1987, Maccubbin 1986, West et al. 1986 a,b, 1988, Durant et al. 1992, Fabacher et al. 1988, Sato et al. 1983 and Maccubbin and Ersing 1991), a human lymphoblast assay (Durant et al. 1992) and the Mutatox® assay (Kwan et al. 1990, Dutka et al. 1991). The study by Durant et al. (1992) compared mutagenicity of organic extracts of sediments in both the Ames and human lymphoblast assays. To date, no comparison of mutagenicity in complex environmental samples such as organic extracts of sediments has been made between the Ames and Mutatox® assays although Johnson (1992) investigated the relative mutagenicity of pure chemicals in the two assays.

The Salmonella typhimurium mutagenicity assay, more commonly known as the Ames assay (Ames et al. 1975, Maron and Ames 1983) is a rapid method of screening for potential mutagenicity, and thus carcinogenicity, of chemicals and complex mixtures. The Ames assay is based upon the use

of selected mutant strains of the bacterium S. typhimurium and the particular vulnerability of these strains to mutations at the histidine locus. These bacteria carry a mutation for histidine dependency and are reverted back to histidine prototropy by the mutagenic chemical. The inclusion of liver microsomes containing biochemically induced enzymes is required for activation of certain chemicals to their reactive forms. Bacterial strains TA-98 (sensitive to frame-shift mutations) and TA-100 (sensitive to base-pair substitution) have separate mutations which increase the assay sensitivity to a greater range of chemical agents when a sample is tested with both bacterial strains (Maron and Ames 1983).

The Mutatox® assay employs a dark mutant of the luminescent marine bacterium, Photobacterium phosphoreum which undergoes a forward mutation after exposure to a variety of mutagenic compounds. The Mutatox® assay is based on a similar assay developed by Ulitzur (1986) and is capable of detecting base substitutions (point mutations), base additions or deletions (frameshift mutations), intercalations of DNA, or the inhibition of DNA synthesis. Over 100 compounds representing a dozen chemical classes, including volatile chemicals and complex mixtures, have been tested using the dark mutant strain of P. phosphoreum (Ulitzur 1986). A number of these compounds also have been tested and categorized by the National Toxicology Program (Tennant et al. 1987). For these chemicals, correlative data from long-term animal studies and four short-term assays (Ames assay, Chinese hamster ovary chromosome aberrations and sister chromatid exchange, mouse lymphocyte assay) exist for comparative purposes. As in the Ames assay, it also is possible to conduct the Mutatox® procedure with and without metabolic activation to compare mutagenic activity of parent compounds and potential metabolic products.

Although analytical techniques exist for the detection of minute quantities of polychlorinated hydrocarbons (PCH), these procedures can be extremely costly and time-consuming, particularly when samples may theoretically contain up to 209 different polychlorinated biphenyl (PCB) congeners and 75 different polychlorinated dibenzodioxin (PCDD) or dibenzofuran (PCDF) isomers and congeners (Safe 1987). The most toxic PCB and PCDD congeners are those which are planar, or nearly planar (Greenlee and Neal 1985). The toxic properties of different planar PCH compounds appear to be expressed via a common mode of action, and therefore, it is possible to calculate the biological potencies of complex mixtures of PCHs by expressing their toxicity relative to the most toxic PCH known, 2,3,7,8-TCDD (Bradlaw and Casterline 1979, Eadon et al. 1986, Safe 1987). TCDD-equivalents can be assigned to complex mixtures of PCHs by measuring the ability of the PCH mixture to induce cytochrome p-450-dependent ethoxyresorufin-o-deethylase (EROD) activity in H4IIE rat hepatoma cell cultures and expressing the magnitude of the response relative to induction observed with TCDD (Bradlaw and Casterline 1979, Casterline et al. 1983, Safe 1987).

The H4IIE rat hepatoma cell assay is also useful because it serves as both a qualitative check and a supplement to the results of the Ames assay. Several investigators have reported a suppression of mutagenic potential in the Ames assay between PAHs and other components of crude oil (Hermann et al. 1981, Hermann 1980, Petrilli et al. 1981, Carver et al. 1985, Haugen and Peak 1983). The cause of the suppression of mutagenic potential was demonstrated to be inhibition of the hepatic microsomal monooxygenase (MO) system (Carver et al. 1985). Therefore induction of

EROD activity would preclude suppression of mutagenic potential due to MO inhibition in samples also tested in the Ames assay.

The objectives of this study were: 1) to compare the mutagenicity of organic solvent extracts of bulk sediments with the Ames and Mutatox® assays, 2) to evaluate the mutagenicity of aqueous extracts of sediments in the Mutatox® assay, 3) to evaluate EROD induction in the H4IIE assay after exposure to organic solvent extracts of bulk sediments and 4) to compare assay results to a limited set of organic chemical analyses of bulk sediment.

Materials and Methods

Sample Collection

Sediment samples were collected from the Grand Calumet River, IN on 11 October and 22 November 1988; 10 March, 24 May, 30 October and 13 November 1989; and 12 May 1990. A Ponar grab sampler was used to collect sediment samples from 10 locations along the Grand Calumet River and three locations in the Indiana Harbor ship canal (Figure 10). At the time of sample collection, study sites were located by triangulation of local landmarks. The sample from each location was a composite of approximately 80-100 L of wet sediment from multiple Ponar grabs. Multiple grab samples were collected and composited to ensure sufficient sample volume for all necessary sub-sample collection. Compositing and homogenization of composite samples were done in a large stainless steel pan with stainless

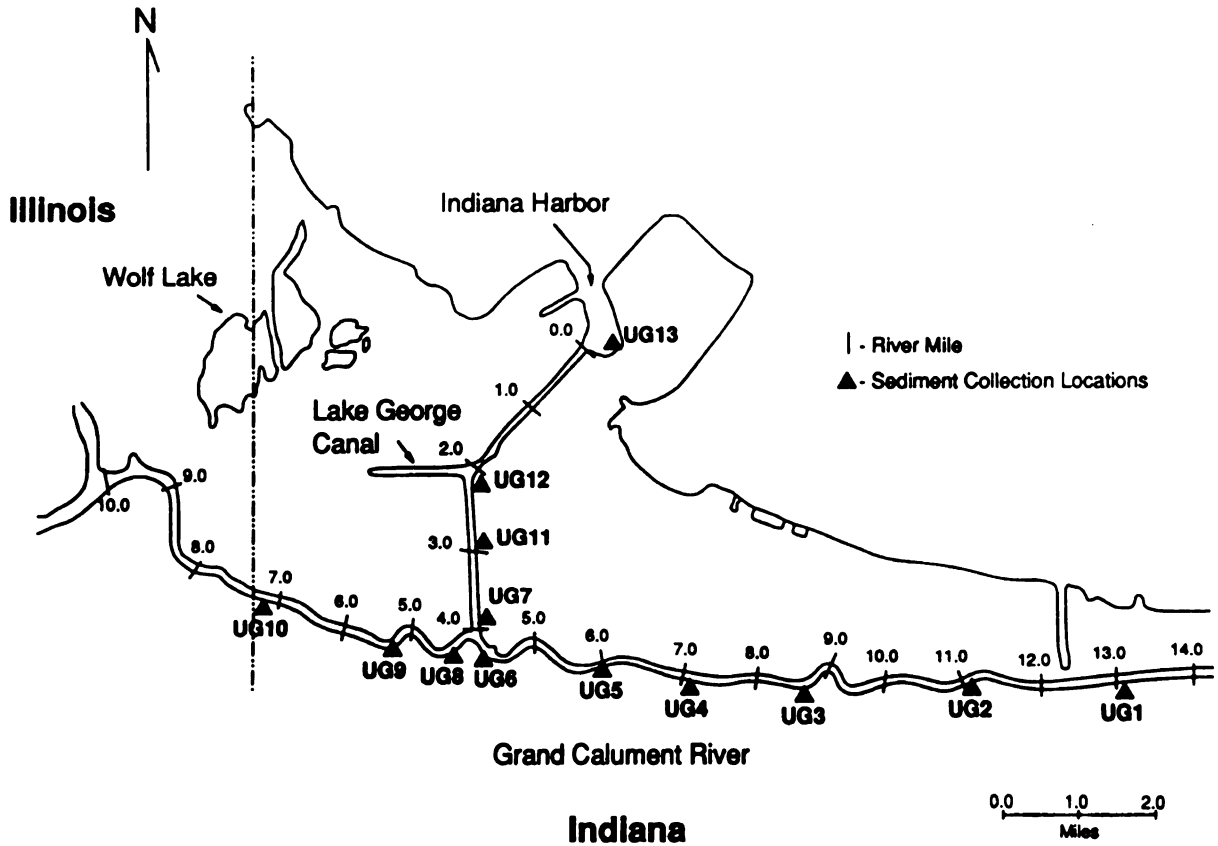

Figure 10. Sampling locations in the Grand Calumet River and Indiana Harbor, Indiana

steel tools. Large debris was removed from the composite samples and two, 1-L aliquants (sub-samples) for quantification of metals and organic compounds in bulk sediments were placed in clean 1-L glass jars capped with solvent rinsed aluminum foil under the lid. Samples for toxicity testing or pore water extraction were placed in coolers or plastic buckets lined with food-grade plastic bags. After collection, sediment samples were placed on ice in coolers and transported to the laboratory, where they were maintained in a walk-in cooler at 4° C until processing and analysis.

Pore Water Extraction

Pore water was extracted from sediments by a combination of centrifugation and filtration as described by Hoke et al (1992a). The pore water extracts were placed in clean, solvent-rinsed glass bottles, the bottles capped with aluminum foil-lined lids, and the pore water maintained in the dark at 4° C until used for assays (< seven days).

Ames Assay

The Ames test was conducted according to the methods and procedures described by Maron and Ames (1983). The complete sample extraction and Ames test methods used in this study have previously been described by Maccubbin and Ersing (1991), however, a brief summary is presented below. A subsample of each sediment sample was air-dried at 80° C to determine moisture content. A wet sediment sub-sample was homogenized and a 30-50 g sample mixed with anhydrous sodium sulphate to absorb the water from the sample. The sample was then placed in a cellulose extraction thimble and extracted for two successive 24 h periods with 300 mL isopropyl alcohol

and dichloromethane, respectively. The extracts were combined, the volume reduced to 50 mL and residue content determined by drying three, one mL aliquants of the combined extracts in tared aluminum weigh boats. An appropriate amount of extractant was then solvent-exchanged into dimethyl sulfoxide (DMSO) to give a final organic residue content of 10 mg/ml (Maccubbin and Ersing 1991).

Sediment extracts were diluted to provide a dose series of 1000 μg, 600 μg, 200 μg, 100 μg and 60 μg residue per plate for testing. One hundred μl of organic extract from each sediment were mixed with 100 μl of an overnight culture of bacteria (tester strain TA98 or TA100) and 2 ml of melted agar containing 5 mM histidine and biotin (Ames et al. 1975, Maron and Ames 1983). Molten top agar was then poured onto a minimal glucose agar base plate and the plates incubated at 37° C for 2 days. The existence of compounds requiring metabolic activation was evaluated by adding 0.5 ml of buffer solution containing rat liver homogenate (S9 from Aroclor treated rats, Litton Bionetics, Charleston, SC.) and co-factors to the top agar prior to plating. Spontaneous mutation rates, solvent,and S9 effects were evaluated with plates containing bacteria only, DMSO only, and DMSO + S9 only as negative controls. Tester strain sensitivity was monitored with benzo(a)pyrene, sodium azide and daunomycin as positive controls. Each extract and control treatment was tested in triplicate. The number of His + revertant colonies/μg residue were determined after incubation and converted to His+ revertants/mg dry weight sediment based on the residue content of each sediment. Toxic effects were evaluated by decreases in mutagenicity with increases in dosage and loss of the background "lawn" of cell growth that occurred due to the small amount of histidine present in the culture media. Dose response data were evaluated

over the linear portion of the dose response curve according to the two-fold rule (Chu et al. 1987). Sediment was classified for mutagenic potential based on dose dependent increases in the reversion rate.

Mutatox® Assay

The Mutatox® assay was conducted on aliquots of the organic sediment extracts tested in the Ames and H4IIE tests. The dry Mutatox® medium was reconstituted with distilled water and the pH adjusted to 6.8 with 5 N KOH. Direct test medium was prepared by adding one mL of the reconstituted dark mutant P. phosphoreum to 100 mL of reconstituted medium. Twenty-six cuvettes (10 sample plus 3 control × 2 replicates each) were set-up, labelled from 1-13 (A and B) and one mL of test medium added to each number one tube. One-half (0.5) mL of test medium was added to each tube numbered 2-13. A 0.2 mL aliquot of the organic sediment extract was added to the number 1 tubes, the solution mixed and serially diluted by transferring 0.5 mL of mixed solution from cuvette 1 through cuvette 10 for each set of replicates. The samples were then placed on a rotary shaker set at 70 ± 10 rpm and incubated at 23 ± 2° C for 16 h. Light output of the dark mutant (i.e. mutagenesis) was measured at 16, 18 and 20 h for both the direct and S9-activated mutagenesis tests. The measurements of light emission used the same methods as those described for the Microtox® test (Bulich 1981). A positive mutagenic response was classified as a light peak of 100 or more at three times the reagent control light output. A non-reactive chemical also was expected to exhibit a negative response in both direct and S9-activated tests.

H4IIE Assay

The H4IIE rat hepatoma cells were obtained from the American Type Culture Collection (ATCC No. CRL 1548). The cell culture medium used was a supplemented Dulbecco's Modified Eagle's Medium (D-MEM) (Tillett et al. 1991). Phenol red was not used in the medium because it may adversely affect liver cell cultures and may potentiate induction in the H4IIE cells. H4IIE cells were kept in continuous culture in the laboratory and the cultures regularly restarted from frozen cells to insure the cultures were not altered. Standard sterile tissue culture techniques were used to maintain the integrity of the cell line and prevent contamination of the cells.

Cell culturing and harvesting were performed according to the procedures described in Tillitt et al. (1991). The cells were dosed with aliquots of the same organic extracts of the sediment samples used in the Ames and Mutatox® assays. DMSO was used as the carrier solvent with the same quantity (mass) of extract delivered to separate plates in different volumes (<100 μl) of DMSO. There was no effect on the basal EROD activity of the H4IIE cells of DMSO volumes between 10 and 100 μL/plate.

Triplicate plates were tested at each of 4-5 doses of the sediment extracts along with a concurrent TCDD standard curve (4 doses in triplicate). Bioassay to bioassay variations in experimental technique were taken into account with a standard TCDD curve analyzed with the sediment extracts. The calculation of TCDD-equivalents does not reflect these among-experiment variations (Tillitt et al. 1991). Plates were incubated for 72 hours and the cells rinsed with PBS and scraped from the plates while in a Tris-sucrose (0.05 - 0.2 M) buffer. The collected cells were centrifuged for 10 minutes, resuspended in the Tris-sucrose buffer,

and duplicate analyses performed of protein content (Lowry et al. 1951) and enzyme induction. The indirect EROD assay was chosen to monitor induction of cytochrome P-4501A1 monooxygenase activity (Pohl and Fouts 1980). The EROD assay has good sensitivity, is specific for the induction of P-4501A, and correlates well to aryl hydrocarbon hydroxylase (AHH) activity in this cell line (Bandiera et al. 1984). EROD assays were conducted in polycarbonate tubes in a shaking water bath incubator maintained at 37° C. The assay protocol followed that described by Tillitt et al. (1991).

Fluorescence of samples was determined on a SLM 4800 spectrofluorometer at emission and excitation wavelengths of 585 and 550 nm, respectively. The machine was calibrated with a standard rhodamine B solution and fluorescence in samples read relative to this internal standard. Each reading was an average of 20 automatic scans. A resorufin standard was also run on each experiment for calibration to a resorufin standard curve and calculation of specific enzyme activities. EROD activity was calculated and reported as picomoles resorufin/mg protein/minute for each treatment combination.

Chemical Analysis

A suite of 106 organic chemical compounds and metals were analyzed for in sediments from the Grand Calumet River and Indiana Harbor, IN. The complete analytical methods and results for all analytes have previously been reported by Hoke et al. (1992).

Statistical Analysis

All data analyses were performed with SAS statistical software (SAS 1985). Relative potencies of the samples were estimated with the slope-ratio assay (Finney 1978). The relative potency was derived from the slope on the linear portion of the dose-response curve for the sample in comparison to the slope of the TCDD standard curve. Sample potency was calculated as:

$$\text{Sample Slope/TCDD Slope} \qquad (1)$$

with final units of pg TCDD/uL of extract. TCDD-equivalents (EQ) on a pg/gm dry wt sediment basis were estimated based on the known mass of residue extract on a dry wt sediment basis. TCDD-EQ attributable to measured total PCBs (as Aroclor 1248) and TCDD in each sediment sample were calculated and compared to EROD TCDD-EQ. For calculation purposes, one μg of total PCBs (as Aroclor 1248) was assumed to be equivalent to 10 pg TCDD-EQ (Tillitt et al. 1991). Pearson product moment correlation analysis (SAS 1985) was used to compare the results of both the Ames and Mutatox® mutagenicity assays with the results of chemical analyses of bulk sediments.

Results

Mutatox® Assay

Mutagenicity in the Mutatox test without S9 activation only occurred in the organic extract of sediment from site UG-1 and was equivocal in the extract from site UG-3 (Table 17). Organic extracts of sediments from all

sites were mutagenic when tested with S9 activation (Table 17). The extracts from sites UG-3 and UG-7 were the most and least mutagenic with S9 activation, respectively. In general, organic extracts of sediments from the western branch of the Grand Calumet River were less mutagenic when tested with S9 activation than were extracts from the eastern branch. No mutagenicity was observed in any sample when aqueous extracts (pore waters) from study site sediments were tested in the Mutatox® assay with and without S9 activation. The relationship of the results of the Mutatox® assays of organic solvent extracts of sediments also was evaluated in the relationship to the results of the organic chemistry of the sediments which have previously been presented elsewhere (Hoke et al. 1992). No significant correlations were observed between the results of the Mutatox® assay and the organic chemical analyses of the sediments.

Ames Assay

Organic extracts of sediments from sites UG-3 and 8 were mutagenic when analyzed without S9 activation using tester strain TA98 while extracts from sites UG-3 and 5 were mutagenic without S9 activation when analyzed with tester strain TA100 (Table 17). Organic extracts of sediments from all sites were mutagenic when analyzed with S9 activation using both tester strains TA98 and TA100 (Table 17). With metabolic activation, the number of strain TA98 revertants per mg dry wt sediment ranged from 1 at site UG-4 to 102 at site UG-3 while the number of TA100 revertants ranged from 10 per mg dry wt sediment at site UG-4 to 1710 at site UG-3 (Table 17).

A number of statistically significant correlations ($p \leq 0.05$) were observed between the Ames assay results and the organic chemical analyses

Table 17. Results of Ames and Mutatox® assays of organic solvent extracts of sediments from the Grand Calumet River and Indiana Harbor, IN. Extracts were tested with and without S9 metabolic activation in both assays.

Location No.	% Moisture[1]	%Extractable[1] (dry wt)	Ames - No. Revertants[2] TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	Mutatox® -S9	Mutatox® LDC(µl)[5] +S9
UG-1	50.6	7.4	NM[3]	19	NM	429	0.2	0.10
UG-2	38.2	3.1	NM	4	NM	45	NM	0.30
UG-3	31.0	7.5	5	102	38	1710	E[5]	0.04
UG-4	32.7	0.8	NM	1	NM	10	NM	0.30
UG-5	59.1	11.2	NM	20	45	179	NM	0.60
UG-6	50.8	6.3	NM	10	NM	17	NM	0.60
UG-7	53.5	7.5	NM	7	NM	45	NM	2.50
UG-8	65.4	19.7	2	20	NM	238	NM	0.60
UG-9	82.3	11.0	NM	8	NM	ND[4]	NM	1.25
UG-10	51.2	6.7	NM	44	NM	322	NM	0.6

Table 17. (continued).

Location No.	% Moisture[1]	%Extractable[1] (dry wt)	Ames - No. Revertants[2] TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9	Mutatox® LDC(µl)[5] -S9	Mutatox® LDC(µl)[5] +S9
UG-11	ND	ND	NM	NM	3	ND	ND	
UG-12	ND	ND	NM	8	64	ND	ND	
UG-13	ND	ND	ND	ND	3	ND	ND	

1 Mean of triplicate determinations.

2 No. of revertants/mg dry wt sediment based on No. revertants/µg extractable residue corrected for spontaneous revertants.

3 Not mutagenic at doses tested.

4 Not done.

5 LDC = lowest detectable concentration.

of the sediment (Table 18). No significant correlations were observed between the results of the Ames assays and concentrations of metals in bulk sediment (data not shown).

H4IIE Assay

Based on EROD induction, the greatest number of TCDD-EQ (pg/gm dry wt sediment) were observed for extracts of sediments from sites UG-1, 3 and 8 (Table 19). TCDD-EQ for other sites were generally an order of magnitude less. Concentrations of TCDD-EQ in bulk sediments from the study sites ranged from approximately 4,700 to greater than 500,000 pg/gm dry wt sediment.

The proportion of the total TCDD-EQ from the H4IIE assay attributable to measured concentrations of total PCBs (as Aroclor 1248) and TCDD in bulk sediments from the study sites was small (i.e. <0.5%). Pearson product moment correlation analysis of TCDD-EQ and results of chemical analyses of the bulk sediments indicated significant correlations existed between TCDD-EQ and bulk sediment concentrations of 2,4-and 2,6-dichlorophenols ($r = 0.70, 0.66$); 2,4,6-trichlorophenol ($r = 0.66$); acrylonitrile ($r = 0.66$); p-dichlorobenzene ($r = 0.66$); 1,3-dichloropropene; ethylbenzene ($r = 0.76$) and fluoranthene ($r = 0.69$).

Discussion

Although investigations of the mutagenicity of sediments from the Great Lakes have been limited, several authors have reported that organic solvent extracts of sediments from various locations were mutagenic when tested in the Ames assay (Maccubbin 1986, Maccubbin and Ersing 1991,

Table 18. Pearson product moment correlation coefficients from analyses of Ames assays with S9 activation and organic chemical analyses of sediment from the Grand Calumet River, IN. Correlations reported were statistically significant at $p \leq 0.05$.

Parameter	Tester Strain	
	TA98 +	TA100+
Hydroquinone	--	0.83
Mercaptobenzothiazole	0.64	0.79
3,4-Dichloroaniline	0.86	0.91
Heptachlor	0.62	0.82
p-Dichlorobenzene	0.68	--
1,2-Dichloropropane	--	0.74
2,4-Dinitrotoluene	0.63	0.68

Table 19. TCDD-EQ from the H4IIE assay in comparison to TCDD-EQ based on measured concentrations of total PCBs (as Aroclor 1248) and TCDD in bulk sediments from the Grand Calumet River and Indiana Harbor, IN.

Location No.	EROD TCDD-EQ (pg/gm dw)	Total PCBs as Aroclor 1248 (μg/gm)	TCDD-EQ as Total PCBs[1] (pg/gm dw)	TCDD (pg/gm dw)	Unaccounted for TCDD-EQ (pg/gm dw)
UG-1	565,828.3	2.17	21.7	6.4	565,800.2
UG-2	10,636.5	1.49	14.9	0.0	10,621.6
UG-3	331,798.3	6.89	68.9	0.0	331,729.4
UG-4	4,729.9	0.94	9.4	2.0	4,718.5
UG-5	45,140.0	18.33	183.3	12.4	44,944.3
UG-6	25,869.5	1.66	16.6	3.5	25,849.4
UG-7	89,476.1	4.26	42.6	0.0	89,433.5
UG-8	213,620.2	2.80	28.0	3.5	213,588.7
UG-9	21,021.6	4.61	46.1	7.3	20,968.2
UG-10	71,305.5	7.93	79.3	7.3	71,218.9

[1] Assuming 10 pg TCDD-EQ/μg dry wt total PCBs

Maccubbin et al. 1991, West et al. 1986a,b, 1988, Fabacher et al. 1988). Several authors also have reported that solvent extracts of sediments were mutagenic in the Mutatox® assay (Kwan et al. 1990, Dutka et al. 1991) but, to date, little or no comparative data exist for results of Ames and Mutatox® assays performed on solvent extracts of the same sediments.

The data from this study indicate that both the Ames and Mutatox® assays yield similar results when organic solvent extracts of sediments are tested with S9 microsomal metabolic activation. Correlation of the number of TA98 or TA100 revertants with the Mutatox® results in samples tested with S9 activation was weak ($r = -0.35$ to -0.40). Little concordance was observed in the determination of mutagenicity for solvent extracts of sediments tested without S9 activation in both assays although the extract from site UG-3 was mutagenic in all direct assays. Ames assays using tester strains TA98 and TA100 without activation indicated extracts from sites UG-3 and 8, and UG-3 and 5 were mutagenic, respectively. Mutatox® assays without activation indicated the extract from site UG-1 was mutagenic while the results for the extract from site UG-3 was equivocal.

Differences in observed results among assays and tester strains during assays without metabolic activation are most likely due to the individual strain or assay sensitivity to different chemicals and the type of genetic mutation caused by the chemical. Ames assay tester strain TA98 detects mutagens which cause frameshift mutations while tester strain TA100 detects those causing point mutations (Maron and Ames 1983). The Mutatox® assay detects compounds which cause frameshift or point mutations as well as compounds which inhibit DNA synthesis and DNA-intercalating agents (Johnson 1992).

Previous investigations have reported the detection of direct acting mutagens in complex mixtures by the Mutatox® assay (Kwan et al. 1990). In the only direct comparison of the Ames and Mutatox® assays conducted to date, Johnson (1992) tested the sensitivity of the two assays with metabolic activation to eight progenotoxins. Both assays exhibited similar sensitivities to the eight compounds with lowest detected concentrations in the low microgram range for both assays. However, pyrene was not detected by the Ames assay but caused a strong positive response in the Mutatox® assay (Johnson 1992).

Interestingly, no statistical correlations were observed between the results of the Mutatox® assay with activation and organic chemical analyses of sediments from the study sites. However, a number of statistically significant correlations were observed between the organic chemical analyses and the results of Ames assays with tester strains TA98 and/or TA100. Chemicals for which concentrations in bulk sediments were significantly correlated with Ames assay results with tester strain TA98 included mercaptobenzothiazole; 3,4-dichloroaniline, heptachlor, p-dichlorobenzene and 2,4-dinitrotoluene. Ames assay results with tester strain TA100 were significantly correlated to chemical concentrations in bulk sediments of hydroquinone; mercaptobenzothiazole; 3,4-dichloroaniline; heptachlor; 1,2-dichloropropane and 2,4-dinitrotoluene. In pure compound assays, mercaptobenzothiazole, hydroquinone; 2,4-dichlorophenol and heptachlor have been reported as non-mutagenic in Ames assay while 1,2-dichloropropane and 2,4-dinitrotoluene have been reported to be mutagenic in the assay (Ashby and Tennant 1991, Tennant and Ashby 1991).

Extracts of sediments from the Detroit River (Maccubbin et al. 1991), the Buffalo River (Ersing 1987) and the Black River (Fabacher et al. 1988, West et al. 1986a,b, 1988) within the Great Lakes basin have previously been reported to elicit mutagenicity in the Ames assay. PAHs have been the most frequently implicated compounds in the observed mutagenicity. West et al. (1986a,b) identified polycyclic aromatic hydrocarbons (PAH), nitrogen heterocycles of PAHs (PANH) and alkylated forms of both PAH and PANHs as the primary causes of the observed mutagenicity. Maccubbin et al. (1991) also indirectly implicated PAHs as the potential cause of mutagenicity observed in organic solvent extracts of sediment from the Detroit River tested with the Ames assay. When these investigators fractionated the solvent extracts of the samples and then tested the individual fractions of the original extract, they observed a higher total number of revertants for the fractions than for the original extract. This phenomenon suggests that mutagenicity was inhibited or suppressed, possibly due to the presence of other PAHs (Hermann et al. 1981, Hermann 1980, Haugen and Peak 1983, Petrilli et al. 1981, Carver et al. 1985). The mechanism for this suppression or inhibition was demonstrated to be related to inhibition of the hepatic microsomal MO system in the S9 (Carver et al. 1985). Additional S9 was recommended for Ames assays of complex mixtures containing petroleum hydrocarbons to ensure adequate metabolic activation of PAHs (Carver et al. 1985).

The Ames assay protocol used to test solvent extracts of sediments from the Grand Calumet River and Indiana Harbor, IN included the addition of a large volume of S9 mixture to each extract (Maron and Ames 1983). Therefore, suppression of mutagenicity due to PAHs should have been minimal. Hoke et al. (1992) reported that individual PAH concentrations

in the bulk sediments were as great as 100 mg/kg. This may account for the large number of revertants observed for the samples.

Solvent extracts of other sediments from the Great Lakes have been reported to cause 80-12,000 revertants/gm dry wt of sediment (Ersing 1987, Maccubbin et al 1991) while extracts of Grand Calumet River sediments tested with metabolic activation caused from 1000-1,710,000 revertants/gm dry wt sediment. Direct acting mutagens in Grand Calumet River sediments caused 2000-45,000 revertants/gm dry wt sediments.

The importance of PAHs in the Grand Calumet River and Indiana Harbor, IN. can also be observed in the results of the H4IIE assays. Significant amounts of TCDD-EQ were detected in solvent extracts of sediments from the study area. Measured concentrations of other compounds which can cause EROD induction in environmental samples (e.g., PCDDs, PCDFs, PCBs) did not account for the observed TCDD-EQ from the H4IIE assay (i.e. <0.5%). It must be noted, however, that total PCBs as Aroclor 1248 and TCDD were the only representatives of the PCDDs, PCDFs and PCBs measured in bulk sediments from the Grand Calumet River. A stronger, albeit circumstantial, case can be made that PAHs in bulk sediments from the study area were responsible for the observed TCDD-EQ. PAHs are known to cause EROD induction in the H4IIE assay (Whitlock et al. 1974, Xu and Bresnick 1990, Corcos and Weiss 1988) and large concentrations of several PAHs have been measured in sediments from the study area (Hoke et al. 1992). It is also likely that large concentrations of both unmeasured parent PAHs and their degradation products exist in sediments from the study area. PAHs also are likely responsible for a portion of the mutagenicity observed in the Ames and Mutatox® assay even though no

statistically significant correlations were observed between assay results and PAH concentrations in bulk sediments.

As evidenced in the results of the Ames and Mutatox® assays, numerous mutagenic compounds exist in sediments from the Grand Calumet River and Indiana Harbor, IN. Black et al (1985) demonstrated that neoplasia in fish could be induced by repeated applications of solvent extracts of sediments from the Buffalo River, NY which contained numerous mutagens including PAHs. However a long latency period was necessary to demonstrate evidence of carcinoma. Thus a need arose for short-term tests of genotoxicity such as the Ames and Mutatox® tests. Although these assays can provide an indication of the potential mutagenicity of complex environmental mixtures, more research needs to be conducted on the representativeness of the exposure route used in these assays (solvent extracts). The absence of mutagenicity in Mutatox® assays of pore waters from the study site sediments indicates that short-term direct human exposure to mutagens in pore waters and sediments are likely to be non-problematic. Greater concern is required for the potential for both ecological and human health effects due to bioaccumulation (bioconcentration and biomagnification) of mutagenic compounds present in sediments from the Grand Calumet River and Indiana Harbor, IN.

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SUMMARY

At the present time, the GCR-IHC system has severely degraded sediments which contain a multitude of chemicals. The most important acute toxicity problems for benthic macroinvertebrates within the system appear to be petroleum hydrocarbons, metals, ammonia and PAHs. Remedial action plans for the area which propose to foster the development of healthy instream communities of invertebrates and fish must of necessity provide a strategy for dealing with both source control and existing concentrations of these contaminants in sediments.