USE OF IN VITRO MICROBIAL ASSAYS OF SEDIMENT EXTRACTS TO DETECT AND QUANTIFY CONTAMINANTS WITH SIMILAR MODES OF ACTION

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ABSTRACT

Mutagenicity of organic solvent extracts of contaminated sediments from a Great Lakes Area of Concern (AOC) was evaluated with Ames and Mutatox® assays. Extract mutagenicity was evaluated with and without S9 metabolic activation in both assays. H4IE rat cell hepatoma assays also were conducted on the organic solvent extracts of the sediments. Little direct mutagenicity was observed in either the Ames or Mutatox® assays, however, a greater number of extracts were observed to be mutagenic after S9 metabolic activation. Although numerous potentially mutagenic compounds exist in sediments from this AOC, polycyclic aromatic hydrocarbons (PAHs) appear to be the likely cause of a major fraction of the observed mutagenicity in the Ames and Mutatox® assays. PAHs, as opposed to compounds with a mode of action similar to 2,3,7,8-TCDD, also appear responsible for the majority of the enzyme induction observed in the H4IE assay.

INTRODUCTION

The importance of sediments as both a sink and source for chemicals of environmental concern has become obvious during the last 20 years. As a result, the focus of research on contaminants has changed and 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. Less research has been devoted to the potential for chronic toxicity, or bioaccumulation of chemicals as a result of exposure to these complex mixtures or 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 typically have little direct exposure to sediments or extracts of sediments, potential exposure to complex mixtures may occur indirectly via aquatic food chains (i.e. ingestion of aquatic species

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exposed to contaminated sediments). Therefore, chemicals in sediments which are 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, in some cases, concentrations of mutagenic chemicals in the environment which may not affect human health may produce adverse effects on aquatic biota or wildlife.

Chemical analysis provides only 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 less than 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, a better 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; Tillitt 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; Erasing 1987; MacCubbin 1986; West et al. 1986 a,b, 1988; Durant et al. 1992; Fabacher et al. 1988; Sato et al. 1983; MacCubbin and Erasing 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 as determined by both the Ames and human lymphoblast assays. Little comparative information on mutagenicity of complex environmental samples such as organic extracts of sediments in the Ames and Mutatox® assays is available 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 mutagenicity of chemicals and complex mixtures. The Ames assay is based upon 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 prototrophy by the mutagenic chemical. The inclusion of liver microsomes (S9) often is used in the Ames assay to facilitate detection of chemicals which require metabolic activation to produce mutagenesis. Bacterial strains TA-98 and TA-100, which are sensitive to frame-shift and base-pair substitutions, respectively, can be tested in conjunction to increase the sensitivity of the assay to a greater range of chemicals (Maron and Ames 1983).

The Mutatox® assay employs a dark mutant of the luminescent marine bacteria 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 (frame-shift mutations) or the inhibition of DNA synthesis. Over 100 compounds representing a dozen chemical classes, including volatile chemicals and complex mixtures, have been tested for mutagenicity to 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 is possible to conduct the
Mutatox® procedure with and without metabolic activation by tissue fractions, such as mammalian S9, 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 costly and time-consuming, particularly when samples may theoretically contain up to 209 different polychlorinated biphenyl (PCB) congeners, 75 different polychlorinated dibenzoepinoxin (PCDD) or 135 dibenzofurans (PCDF) isomers and congeners (Safe 1987), as well as other compounds which may possess a similar mode of toxic action. 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 H4IE 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 H4IE 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. 1980; Hermann 1981; Petrilli et al. 1980; Carver et al. 1985; Hauges 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 assay 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 from the Grand Calumet River-Indiana Harbor, Indiana, USA.

MATERIALS AND METHODS

Sample Collection

Samples of sediment 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 Ponor 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 (Hoke et al. 1993). 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 Ponor 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 tongs. Large debris was removed
from the composite samples and two, 1-L aliquots (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 (Hoke et al. 1993). 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.

**Ames Assay**

The Ames assay was conducted according to the methods and procedures described by Maron and Ames (1983). The complete sample extraction and Ames assay methods used in this study have previously been described by Maccubbin and Ering (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 aliquots of the combined extracts in tared aluminum weigh boats. An appropriate amount of extract was then solvent-exchanged into dimethyl sulfoxide (DMSO) to give a final organic residue content of 10 mg/ml (Maccubbin and Ering 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 18 h. Light output of the dark mutant (i.e. mutagenesis) was measured at 16, 18 and 20 h for both the direct and 59-activated mutagenesis tests (T. Tung, Microbiics, Inc., pers. comm.). The measurements of light emission used the same methods as those described for the Microtox® test (Bulich et al. 1981). A positive mutagenic response was classified as a light output value of 100 or more which also was at least three times the reagent control light output. A non-reactive chemical (negative control) also was expected to exhibit a negative response in both direct and 59-activated tests.

**H4IIE Assay**

The H4IIE rat hepatoma cells were obtained from the American Type Culture Collection (ATCC No. CRL 1548). The culture medium used was a Dulbecco’s Modified Eagle’s Medium (D-MEM) base supplemented as described by Tillitt et al. (1991). H4IIE cells were kept in continuous culture and the cultures restarted regularly from frozen cells with standard sterile tissue culture techniques to maintain the integrity of the cell line and prevent contamination of the cells.

H4IIE cells were cultured in 75 sq. cm. tissue culture flasks in 25 ml of the modified D-MEM described above. Cells for assays were obtained using methods previously described by Tillitt et al. (1991). The cells were dosed with aliquots of the same organic extracts of the sediments used in the Ames 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 is no effect on the basal EROD activity of the H4IIE cells of DMSO volumes between 10 and 100 µl/plate (Tillitt et al. 1991).

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). Plates were incubated for 72 hr 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 min, 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-450-dependent monooxygenase activity (Pohl and Fouts 1980). The EROD assay has good sensitivity, is
specific for the induction of P-450IA and correlates well to aryl hydrocarbon hydroxylase (AHH) activity in this cell line (Bandiera et al. 1984). EROD assays were conducted as described in 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 pmol resorufin/mg protein/min.

Chemical Analysis

A suite of 106 organic chemical compounds and 22 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 been reported elsewhere (Hoke et al. 1993).

Statistical Analysis

All analyses of H4IIE assay results were performed with SAS statistical software (SAS 1985). Relative potencies of the samples were estimated with the slope-ratio method (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 (Equation 1) and expressed as pg TCDD/μl of extract.

\[
\text{Sample Slope/TCDD Slope} = \text{TCDD-pretreatment (EQ) on a pg/g 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 was used to compare the results of the Ames and Mutatox® assays with the results of chemical analyses of bulk sediments.}

RESULTS

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 1). Organic extracts of sediments from all sites were mutagenic when analyzed with S9 activation using both tester strains TA98 and TA100 (Table 1). With metabolic activation, the number of revertants per mg dry wt sediment for strain TA98 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 1). A number of statistically significant correlations (p ≤ 0.05) were observed between the Ames assay results and the organic chemical analyses of the sediments (Table 2). No significant
Table 1. 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.

<table>
<thead>
<tr>
<th>Location No.</th>
<th>% Moisture¹</th>
<th>% Extractable¹ (dry wt)</th>
<th>Ames - No. Revertants²</th>
<th>Mutatox³</th>
<th>LDC (µg)⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-99</td>
<td>+99</td>
<td>-99</td>
<td>+99</td>
</tr>
<tr>
<td>UG-1</td>
<td>50.6</td>
<td>7.4</td>
<td>NM</td>
<td>19</td>
<td>NM</td>
</tr>
<tr>
<td>UG-2</td>
<td>38.2</td>
<td>3.1</td>
<td>NM</td>
<td>4</td>
<td>NM</td>
</tr>
<tr>
<td>UG-3</td>
<td>31.0</td>
<td>7.5</td>
<td>5</td>
<td>102</td>
<td>38</td>
</tr>
<tr>
<td>UG-4</td>
<td>32.7</td>
<td>0.8</td>
<td>NM</td>
<td>1</td>
<td>NM</td>
</tr>
<tr>
<td>UG-5</td>
<td>59.1</td>
<td>11.2</td>
<td>NM</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>UG-6</td>
<td>50.8</td>
<td>6.3</td>
<td>NM</td>
<td>10</td>
<td>NM</td>
</tr>
<tr>
<td>UG-7</td>
<td>53.5</td>
<td>7.5</td>
<td>NM</td>
<td>7</td>
<td>NM</td>
</tr>
<tr>
<td>UG-8</td>
<td>65.4</td>
<td>19.7</td>
<td>2</td>
<td>20</td>
<td>NM</td>
</tr>
<tr>
<td>UG-9</td>
<td>82.3</td>
<td>11.0</td>
<td>NM</td>
<td>8</td>
<td>NM</td>
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<tr>
<td>UG-10</td>
<td>51.2</td>
<td>6.7</td>
<td>NM</td>
<td>44</td>
<td>NM</td>
</tr>
<tr>
<td>UG-11</td>
<td>ND</td>
<td>ND</td>
<td>N</td>
<td>M</td>
<td>NM</td>
</tr>
<tr>
<td>UG-12</td>
<td>ND</td>
<td>ND</td>
<td>NM</td>
<td>8</td>
<td>NM</td>
</tr>
<tr>
<td>UG-13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>NM</td>
</tr>
</tbody>
</table>

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 LDC = lowest detectable concentration.
⁴ Not mutagenic at doses tested.
⁵ Equivocal.
⁶ Not done.
Table 2. 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 \( p \leq 0.05 \).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TA98+</th>
<th>TA100+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroquinone</td>
<td>--</td>
<td>0.83</td>
</tr>
<tr>
<td>Mercaptobenzothiazole</td>
<td>0.64</td>
<td>0.79</td>
</tr>
<tr>
<td>3,4-Dichloroaniline</td>
<td>0.86</td>
<td>0.91</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>0.62</td>
<td>0.82</td>
</tr>
<tr>
<td>p-Dichlorobenzene</td>
<td>0.68</td>
<td>--</td>
</tr>
<tr>
<td>1,2-Dichloropropane</td>
<td>--</td>
<td>0.74</td>
</tr>
<tr>
<td>2,4-Dinitrotoluene</td>
<td>0.63</td>
<td>0.68</td>
</tr>
</tbody>
</table>

correlations were observed between the results of the Ames assays and concentrations of metals in bulk sediment (data not shown).

**Mutatox® Assay**

Mutagenicity in the Mutatox® assay 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 1). Organic extracts of sediments from all sites were mutagenic when tested with S9 activation (Table 1). 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. 1993). No significant correlations were observed between the results of the Mutatox® assay and the organic chemical analyses of the sediments.

**H4IE Assay**

Based on EROD induction, the greatest number of TCDD-EQ (pg/g dry wt sediment) were observed for extracts of sediments from sites UG-1, 3 and 8. 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/g dry wt sediment. The proportion of the total TCDD-EQ from the H4IE 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%, Table 3).
Table 3. TCDD-EQ from the H4IE 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.

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

1 Assuming 10 pg TCDD-EQ/µg dry wt total PCBs
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 Erasing 1991; 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, previously, little or no comparative data existed for results of Ames and Mutatox® assays performed on solvent extracts of the same sediments.

The correlation of the number of TA98 or TA100 revertants with the Mutatox® results in samples with S9 activation was weak (data not shown). 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 assay results with tester strains TA98 and TA100 without activation indicated extracts from sites UG-3 and 8, and UG-3 and 5 were mutagenic, respectively. Results of 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.

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 detectable 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).

In our study, 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 bulk sediment chemical concentrations 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 (Erasing 1987) and the Black River (Fabacher et al. 1988; West et al. 1986a,b, 1988) within the Great Lakes basin of the USA 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 greater total number of revertants for the fractions than for the original extract. This phenomenon suggests that mutagenicity was inhibited or suppressed in the whole extracts, possibly due to the presence of other PAHs (Herrmann et al. 1980; Herrmann 1981; Haugen and Peak 1983; Petrilli et al. 1980; 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). Thus, 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 used to test solvent extracts of sediments from the Grand Calumet River and Indiana Harbor, IN included the addition of sufficient S9 mixture to each extract to minimize suppression of mutagenicity due to PAHs (Maron and Ames 1983; Maccubbin and Erving 1991).

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

The prevalence of PAHs in the Grand Calumet River and Indiana Harbor, IN can also be observed in the results of the H4IEE 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) accounted for only a small proportion of the observed TCDD-EQ in the H4IEE 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 H4IEE assay (Whitlock et al. 1974; Xu and Brenneck 1990; Corcos and Weiss 1988) and great concentrations of several PAHs have been measured in sediments from the study area (Hoke et al. 1993). It is also likely that great concentrations of both unmeasured 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 Mutatex® assay even though no statistically significant correlations were observed between assay results and PAH concentrations in bulk sediments.

Numerous mutagenic compounds exist in sediments from the Grand Calumet River and Indiana Harbor, IN. Although the Ames and Mutatex® assays can provide an indication of the potential mutagenicity of complex environmental mixtures, more research needs to be conducted on the suitability of the exposure route (i.e., solvent extracts) used in these assays. Sediment assessment protocols could use the determination of the various effects caused by organic extracts of sediments as a "worst case" measure of organic contaminant bioavailability in contaminated sediments. The absence of mutagenicity in Mutatex® assays of pore waters from the study site sediments (data not shown) 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., USA.

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