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Mutagenic Potential of Sediments from the Grand Calumet River

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The Grand Calumet River/Indiana Harbor Canal is one of the International Joint Commission's Great Lakes Areas of Concern (AOC). Like many other AOCs, the Grand Calumet River is in a heavily industrialized area and has a history of chemical contamination (Colten 1985). Many of the chemicals found in the industrial and municipal wastes that enter the waterway end up in sediments where they are concentrated to high levels. A number of chemicals that have been identified in the contaminated sediments found in AOCs are mutagenic or carcinogenic. Although these chemicals have been shown to cause harmful biological effects as single compounds, their effects as part of a complex mixture are not well known. In several AOCs, organic extracts of sediments have been shown to be mutagenic (Black et al. 1980; West et al. 1986a,b; Maccubbin et al. 1991).

In order to assess the potential genotoxicity of sediments from the Grand Calumet River, we determined the mutagenic potential of organic extracts of sediments. The sediment extracts were assayed in the Salmonella/microsome mutagenicity test (the Ames test, Ames et al. 1975). In the Ames test, all ten sediment samples assayed were found to be mutagenic. In general, chemicals found in the sediments required metabolic activation before a positive mutagenic response was observed.

MATERIALS AND METHODS

Sediment samples were collected from ten sites along the east and west branches of the Grand Calumet River (Figure 1). Multiple grab samples of surficial sediment were collected using a Ponar grab sampler and were composited and homogenized. A subsample of each composite was placed in a 1-L glass bottle and stored at 4°C until processing and analysis.

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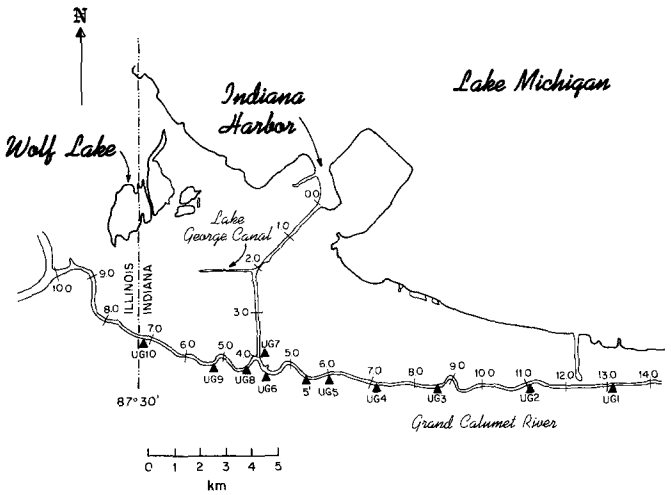

Figure 1. Sediment sampling sites in the Grand Calumet River. Sampling sites are designated by a number with the prefix UG. The other numbers along the river are miles from the mouth of the river at Indiana Harbor.

Wet sediment was extracted essentially as described by Furlong et al. (1988). After mixing the sample, 30-50 g of wet sediment was combined with anhydrous sodium sulfate to absorb the water resulting in a loose granular mixture. This mixture was placed in a cellulose extraction thimble that was then placed in a Soxhlet extraction unit. The sample was extracted for 24 hr with 300 mL isopropyl alcohol and then with dichloromethane for 24 hr. The extracted material from the two extraction steps was pooled and the volume was reduced to 50 mL. The residue content of the organic extract was determined by drying three 1-mL aliquots of extract in preweighed aluminum pans. The moisture content of the sediment was determined according to standard procedures for soil analysis (Black 1965). The percent extractable material on a dry weight basis was then calculated as follows:

$$100 \times \frac{\text{residue content of extract (mg/mL) X volume of extract (mL)}}{\text{dry weight of sediment extracted (mg)}}$$

Prior to assaying in the Ames test, a volume of extract was solvent exchanged into dimethylsulfoxide (DMSO) to give a stock solution of 10 mg residue/mL. Dilutions of the stock solution were made in DMSO to provide a range of residue concentrations to be tested in the Ames test. In general, the dose range was 60 - 1,000 µg residue/plate. In some instances, toxicity was

observed in this dose range and the samples were retested at lower residue concentrations.

The standard plate incorporation protocol was used for Ames tests (Maron and Ames 1983). Briefly, 100 µL of sediment extract was mixed with 100 µL of a 16 hr culture of bacterial tester (strain TA98 or TA100) and 2 mL of melted agar containing 5 mM histidine and biotin. The molten top agar was then poured onto a minimal glucose agar base plate and incubated at 37°C for 2d. To determine if the extract contained compounds requiring metabolic activation for mutagenicity, 0.5 mL of buffer solution containing rat liver homogenate and cofactors (S9) was added to the top agar prior to plating. Plates with bacteria only, DMSO only, or DMSO + S9 were included as negative controls to evaluate spontaneous mutation rates, mutation caused by the solvent carrier or by the S9 mixture. In addition, positive controls of benzo[a]pyrene (5 µg/plate) sodium azide (1 µg/plate) and daunomycin (6 µg/plate) were included to monitor the tester strain sensitivity (Maron and Ames 1983). Dilutions of extract and controls were assayed in triplicate. After incubation, the number of revertant colonies (His^+ revertants) was counted. For those samples in which a doubling of background was observed at one or more doses, His^+ revertants/µg residue were determined. His^+ revertants/µg were calculated from the linear portion of the dose response curve using linear regression analysis. Correlation coefficients were calculated to determine the degree of certainty of the linear regression. After calculating the His^+ revertants/µg, this value was converted to His^+ revertants/mg dry weight sediment based on the residue content of each sediment sample.

The tester strains used in individual assays were from a broth culture inoculated from a master plate. Whenever spontaneous reversion rates exceeded acceptable levels or whenever results from positive controls indicated loss of sensitivity, a new master plate was prepared from frozen stock cultures. Each new master plate was then tested for the correct phenotype as outlined by Maron and Ames (1983) before being used in assays.

RESULTS AND DISCUSSION

The moisture content and organic solvent-extractable material of sediments assayed in the Ames test are summarized in Table 1. In general, the sediments, prior to extraction, were comprised of small particles and were dark grey to black in color. Some sediments had separated into two phases during storage with an oily residue overlaying the sediment. The amount of organic solvent-extractable material in the sediments varied from 0.8 to 19.7% (Table 1). All organic extracts had the same general appearance ranging from light yellowish-brown to dark brown in

Table 1: Moisture Content and Organic Solvent-Extractable Material in Sediments from the Grand Calumet River.

Station	% Moisture[a]	% Extractable[b]
UG-1	50.6 ± 1.2	7.4 ± 0.10
UG-2	38.2 ± 0.4	3.1 ± 0.10
UG-3	31.0 ± 0.2	7.5 ± 0.10
UG-4	32.7 ± 0.2	0.8 ± 0.10
UG-5	59.1 ± 0.2	11.2 ± 0.03
UG-6	50.8 ± 0.8	6.3 ± 0.20
UG-7	53.5 ± 0.3	7.5 ± 0.10
UG-8	65.4 ± 0.4	19.7 ± 0.20
UG-9	82.3 ± 0.3	11.0 ± 0.40
UG-10	51.2 ± 0.6	6.7 ± 0.10

[a]Values are the mean ± S.D. of triplicate samples.
[b]Values are the mean ± S.D. of triplicate samples and are based on the dry weight of sediment.

color. A few extracts had a viscious, oily residue that separated from the bulk extract. However, when solvent exchanged into DMSO, the extracts were homogeneous with no undissolved material.

In general, mutagenic activity of sediment extracts was only observed with metabolic activation. Without metabolic activation, only three sediment samples caused a doubling of the background His^+ revertants and exhibited linear dose response curves (Table 2). Station UG-3 was mutagenic in both tester strains with levels of five His^+ revertants/mg and 38 His^+ revertants/mg for tester strains TA98 and TA100, respectively. Station UG-5 was mutagenic in strain TA100 (45 His^+ revertants/mg) and station UG-8 was mutagenic in strain TA98 (two His^+ revertants/mg).

In contrast to the results without metabolic activation, all of the sediments were mutagenic with metabolic activation (Table 3). Only station UG-9 was not mutagenic in both tester strains. For those stations that were mutagenic in both strains, more revertants/mg of sediment were observed with TA100. Station UG-3 was the most mutagenic in both strains with rates of 102 His^+ revertants/mg and 1710 His^+ revertants/mg for TA98 and TA100, respectively.

In this study we have demonstrated that the sediments from ten sites in the Grand Calumet River have chemicals that can be

Table 2: Mutagenicity Without Metabolic Activation of Organic Extracts of Sediments from the Grand Calumet River.

Station	Tester Strain[a]	His^+ Revertants/mg[b]	Correlation Coefficient	Significance[c]
UG-3	TA98	5	0.8515	>.99
	TA100	38	0.6867	>.99
UG-5	TA100	45	0.5274	>.95
UG-8	TA98	2	0.7618	>.99

[a]The background reversion rates for the solvent carrier control were 18.5 ± 5 revertants for TA98 and 107 ± 12 revertants for TA100. Positive control reversion rates were 2265 ± 370 revertants for TA98 (daunomycin, 6 μg/plate) and 984 ± 170 revertants for TA100 (sodium azide, 1 μg/plate).

[b]His^+ revertants/mg are on a dry weight of sediment basis and were determined from the His^+ revertants/μg residue. His^+ revertants/μg residue were calculated by linear regression analysis of dose response curves after correction for spontaneous revertants.

[c]Significance was determined from the correlation coefficient and the number of replicates included in the linear regression analysis and measures the degree of certainty in the linear correlation.

activated to mutagens. In addition, three sites also had direct acting mutagens that did not require metabolic activation. The observation of mutagenesis caused by extracts of sediments from contaminated waterways is not unique to the Grand Calumet River. Several studies of other AOCs that have chemically contaminated sediments have demonstrated that mutagenic compounds can be extracted from the sediments (Black et al. 1980; Maccubbin 1986; Ersing 1987; Maccubbin et al. 1991). Although comparisons to other AOCs is difficult because of qualitative and quantitative differences in the chemicals contaminating the sediments, sediments from the Grand Calumet River have a higher mutagenic potential than three other AOCs that we have studied. For example, more than 50% of the stations from the Grand Calumet River had His^+ revertant/mg values higher than the highest values (15.5 His^+ revertants/mg) observed in the Detroit River (Maccubbin et al. 1991). Moreover, no sediments from the Detroit River caused increases in His^+ revertants without metabolic activation. Similarly all but one station from the Grand Calumet River had higher His^+ revertants/mg values than those we have observed in the Buffalo River (1.2 - 3 His^+ revertants/mg, Maccubbin 1986; Ersing 1987) or the Black River (0.1 His^+ revertants/mg, Maccubbin 1986). However, in the case of the Buffalo and Black Rivers, other investigators using fractionation of sediment

Table 3: Mutagenicity with Metabolic Activation of Organic Extracts of Sediments from the Grand Calumet River.

Tester Strain[a]	Station	His+ Revertants/mg[b]	Correlation Coefficient	Significance[c]
TA98	UG-1	19	.7127	>.95
	UG-2	4	.9496	>.99
	UG-3	102	.8720	>.99
	UG-4	1	.8633	>.99
	UG-5	20	.7340	>.95
	UG-6	10	.8221	>.99
	UG-7	7	.8661	>.99
	UG-8	20	.8263	>.99
	UG-9	8	.9617	>.99
	UG-10	44	.8521	>.99
TA100	UG-1	429	.9132	>.99
	UG-2	45	.9593	>.99
	UG-3	1710	.9699	>.99
	UG-4	10	.9347	>.99
	UG-5	179	.9664	>.99
	UG-6	17	.7941	>.99
	UG-7	45	.8369	>.99
	UG-8	238	.6248	>.99
	UG-10	322	.9634	>.99

[a]Background reversion rates for solvent plus S9 control were 25 ± 6 His+ revertants for TA98 and 107 ± 17 His+ revertants for TA100. Positive control reversion rates were 288 ± 83 revertants for TA98 and 917 ± 220 revertants for TA100. Benzo[a]pyrene was used for the positive control with both tester strains.
[b]Same as "b" in Table 2.
[c]Same as "c" in Table 2.

extracts, have observed higher mutation rates (Black et al. 1980; West et al. 1986a, 1986b).

We have not fractionated the extracts from Grand Calumet River sediments and thus our determinations of mutagenicity may be underestimates of the true mutagenic potential. In addition, we have not characterized the sediments with respect to chemical composition and thus the cause of the mutagenic response is still unknown. However, previous analysis of sediments from the Grand Calumet River have documented high levels of polycyclic aromatic hydrocarbons, some of which are known mutagens in the Ames test. For example, the levels of benzo[a]pyrene as high as 200 µg/g have been measured in Grand Calumet River sediments (ISBH 1984). This compound alone, if assayed as a pure

compound, could account for much of the increase in His^+ revertants we observed. Interestingly, sediments from other AOCs that we have assayed for mutagenic potential had generally lower levels of polycyclic aromatic hydrocarbons and lower mutagenic potential. For example, the highest levels of total polycyclic aromatic hydrocarbons measured in Detroit River sediments was 130 μg/g with benzo[a]pyrene at 7.6 μg/g (Furlong et al. 1988). While it is possible that polycyclic aromatic hydrocarbons contribute to the mutagenic potential of the sediments, additional studies are needed to identify additional indirect and direct acting mutagens. These studies may identify and quantitate the mutagens contained in the sediments and possible adverse effects they may have on biota in the river and humans using the river.

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REFERENCES

Ames BN, McCann J, Yamasaki E (1975) Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res 31:347-364

Black CA (1965) Methods of soil analysis, vol 9 (Parts 1 and 2). American Society of Agronomy, Madison, Wisconsin

Black JJ, Holmes M, Dymerski PP, Zapisek WF (1980) Fish tumor pathology and aromatic hydrocarbon pollution in a Great Lakes estuary. In: Afgan BK, Mackay D (eds) Hydrocarbons and haologenated hydrocarbons in the aquatic environment. Environmental Science Research, vol 16, Plenum Press, New York, pp 559-565

Colten CE (1985) Industrial wastes in the Calumet area, 1869-1970. An historical geography. Illinois Department of Energy and Natural Resources, HWRIC RR001, Champaign, Illinois

Ersing N (1987) Mutagenic and carcinogenic potential of chemically contaminated sediments from the Buffalo River. MS Thesis, University of Buffalo, Buffalo, New York, 48 p

Furlong ET, Carter DS, Hites RA (1988) Organic chemical contaminants in sediments from the Trenton Channel of the Detroit River, Michigan. J Great Lakes Res 14:489-501

Indiana State Board of Health (1984) Grand Calumet River Waste Land Allocation Study. ISBH0010 prepared by HydroQual Inc. Mahway, New Jersey, 40 p

Maccubbin AE (1986) Mutagenicity of sediments from the Great Lakes ecosystem. In: Proceedings of the 29th conference of the International Association for Great Lakes Research, Scarbourgh, Ontario, Canada, p 58

Maccubbin AE, Ersing N, Frank ME (1991) Mutagenicity of sediments from the Detroit river. J Great Lakes Res, in press

Maron DM, Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutation Res 113:173-215

West WR, Smith PA, Booth GM, Wise SA, Lee ML (1986a) Determination of genotoxic polycylic aromatic hydrocarbons in a sediment from the Black River (Ohio). Arch Environ Contam Toxicol 15:241-249

West WR, Smith PA, Booth GM, Lee ML (1986b) Determination and genotoxicity of nitrogen heterocycles in a sediment from the Black River. Environ Toxicol Chem 5:511-519

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