J. Great Lakes Res. 22(3):591–601
Internat. Assoc. Great Lakes Res., 1996

Mutagenicity of Great Lakes Sediments

Diana M. Papoulias and Denny R. Buckler

National Biological Service
Midwest Science Center
4200 New Haven Road
Columbia, Missouri 65201-9634

ABSTRACT. *The Ames/salmonella assay optimized for use with sediment extracts was used to assess the mutagenic potential of contaminated sediments collected from the Saginaw River, the Buffalo River, and Indiana Harbor as part of the Assessment and Remediation of Contaminated Sediments Program undertaken by the Great Lakes Program Office of the U.S. Environmental Protection Agency. Ames assays were conducted with 35 organic chemical extracts prepared from sediment samples collected from a total of 29 stations in the three areas of concern. Prior to the assay extracts required pre-treatment by gel permeation chromatography, but no further fractionation was performed. Mutagenicity was detected in both grab and core sediment samples from every station. Of the four bacterial strains tested, only TA98 consistently identified mutagenicity.*

INDEX WORDS: *Ames assay, mutagenicity, Great Lakes, sediment.*

INTRODUCTION

The presence of chemical contaminants in the Great Lakes is well-documented. In 1980 the International Joint Commission (IJC) estimated that about 2,500 chemicals were in common use in the Great Lakes Basin ecosystem (International Joint Commission 1980). A comprehensive review of fish-tissue residues prepared by the IJC's Great Lakes International Surveillance Plan (Hesselberg and Seelye 1982) tentatively identified 476 organic compounds. Most of these organic compounds are also present in Great Lakes sediments, and extracts of these sediments have tested positive in mutagenicity assays (Allen *et al.* 1983, Metcalfe *et al.* 1990, Maccubbin and Ersing 1991). Baumann (1984) and Baumann and Whittle (1988) suggested a link between high levels of industrial contaminants and frequency of tumors in fish populations. Recent study of contaminated sediments from tributaries of the Great Lakes using Medaka (*Oryzias latipes*) supports the hypothesis that chemical contaminants in lake sediment are associated with neoplasms in fishes near contaminated sites (Fabacher *et al.* 1991).

Short-term bioassays are a fundamental component of tier testing to assess the genotoxicity (DNA-damaging properties) of chemical contaminants (Barfknecht and Naismith 1984, International Joint Commission 1980, Lovell 1989). The bioassays are typically performed *in vitro* over a period of hours to weeks and encompass a wide range of genetic endpoints. In general, short-term assays identify specific genotoxic contaminants or those in complex mixtures of contaminants, provide baseline data for monitoring changes in environmental conditions, and predict potential long range genotoxic health effects (Epler 1980). Genotoxicity assays encompass a broad range of tests that detect damage at the genetic level, while the mutagenicity endpoint specifically identifies those changes to the DNA that are heritable. Mutagenicity assays are relatively rapid and inexpensive and are especially useful for establishing priorities for more definitive chemical analysis and validation testing with whole animals (Ashby 1988, Ashby and Tennant 1988, also see Benigni 1990).

In this investigation, part of a series of extensive sediment studies, the Ames/*Salmonella* Test (Maron and Ames 1983) was used to screen organic chemical extracts of contaminated sediments for mutagens from three areas of concern (AOC): Indiana Harbor, the Buffalo River, and the Saginaw River. These are three of the 42 Areas of Concern (AOC) in the Great Lakes basin as designated by the International Joint Commission.

MATERIALS AND METHODS

Summary of Samples Tested

Sediment samples were collected at 29 stations from 1988 to 1990 from the three ARCS sites (Ingersoll *et al.* 1993). Sediment grab samples were assayed from seven stations (3,4,5,6,7,8,10) in Indiana Harbor and 10 stations (1,2,3,4,5,6,7,8,9,10) in the Buffalo River. Sediment grab samples were assayed from a total of 12 stations (1,2,3,4,5,6,7,8,9,10,16,24) collected during two surveys of the Saginaw River. In addition, various depths of core samples at Saginaw Stations 2, 5, and 6 were assayed. Maps and more detailed descriptions of sampling locations are provided in Ingersoll *et al.* (1993).

Assay Procedures

Methylene chloride extracts of test sediments and a reference sediment were prepared by Battelle Northwest as follows: sub-samples of about 100 to 200 g wet sediment were dried with sodium sulfate, triple-extracted with methylene chloride, and concentrated by roto-evaporation to 11 mL (Eric Crecelius, Battelle, Sequim, WA, personal communication; USEPA 1986, method SW-46). Upon receipt at MSC, extracts were stored in amber glass vials with Teflon-lined caps at 4°C until processed.

Processing of methylene chloride extracts included gel permeation chromatography (GPC) followed by transfer into dimethyl sulfoxide (DMSO) (Papoulias *et al.* 1996). Samples collected from GPC were concentrated by roto-evaporation to about 5 mL. Five mL of DMSO were then added and the remaining methylene chloride:cyclopentane was evaporated under a gentle stream of nitrogen. Procedures were conducted under yellow lights to reduce possible photo-degradation. Resulting extracts, representing 5 or 10 g-equivalents dry sediment/mL extract, were kept in the dark at room temperature in 8-mL amber glass vials with Teflon® cap liners.

The Ames assay procedure generally followed Maron and Ames (1983) for the plate incorporation test with preincubation. Briefly, 100 μL of cultured test strain was combined with 500 μL of either phosphate buffer or activating S9 enzyme mixture. To this, 100 μL of extract or DMSO solvent was added and the entire mixture was incubated at 37°C for 20 to 30 min in a dry block heater. After incubation, 2 mL of top agar containing trace histidine and biotin was added, and the mixture was poured onto agar plates. Plates were incubated in the dark at 37°C and the resulting colonies were counted at 72 h. Plates with fewer than 100 colonies were counted manually; all other plates were mechanically enumerated with an electric counter as described by Johnson (1990).

The experimental design was developed in preliminary studies so as to optimize dose and S9 levels thereby reducing interference due to cytotoxicity (Papoulias *et al.* 1996). Three replicates of three nominal concentrations of sediment extract: 5 or 10, 50 or 100 and 500 or 1,000 mg-equivalents dry sediment per plate, and three concentrations of S9: 0, 1, 3 mg-equivalents protein per plate were tested with four strains of *Salmonella typhimurium*, TA97a, TA98, TA100 and TA102 (Table 1). Preparation of reagents and solutions used in the Ames assay was as described by Maron and Ames (1983) except that the 30% S9 mixture (3 mg/plate) contained twice the normal amount of NADP co-factor with a corresponding decrease in water (Gatehouse 1987). A positive mutagenic response was indicated when the number of revertants on test plates was greater than or equal to two times the number of colonies on the DMSO solvent control plate.

In order to discriminate between prototroph (revertant) and auxotroph (non-revertant) colonies, an additional step was included to confirm histidine independence. Plates indicating potential mutagenicity (e.g., ≥ two times their respective controls) were evaluated by lightly stamping the colonies with a velvet-covered disk and then transferring the colonies to new minimal glucose plates containing biotin but no histidine. If the pattern and number of transferred colonies resembled that on the original plate, the response was scored as mutagenic. When relatively few colonies transferred, the original plates contained primarily auxotrophs resulting from cytotoxicity.

Quality Control and Quality Assurance

The integrity and sensitivity of the test organisms used in the Ames test was assessed by confirmation of genotypes, measurement of spontaneous reversion frequency, and confirmation of reversion properties of the tester strains using the known mutagens 2,4,7-trinitro-9-fluorenone; 2-aminofluorene; methyl methanesulfonate; and benzo[a]pyrene (Maron and Ames 1983). In each test series, the results of the diagnostic tests confirmed strain genotype and sensitivity (Table 2). Bacterial culture

TABLE 1. Ames assay test strain characteristics (Maron and Ames 1983).

Strain	Mutation rfa[1]	Mutation uvrB[2]	Mutation HIS[3]	Specificity	Spontaneous Revertants
TA97a	+	–	hisD6610	frameshift	90–180
TA98	+	–	hisD3052	frameshift	30– 50
TA100	+	–	hisG46	base-pair	120–200
TA102	+	+	hisG428	excision repair	240–320

[1]causes partial loss of the bacteria's lipopolysaccharide barrier
[2]causes deletion of a gene coding for the DNA excision repair system
[3]mutation at the histidine operon

TABLE 2. Summary of results of Quality Assurance/Quality Control tests for Ames assay. Solvent and Positive Control numbers are expressed as the mean number of revertant colonies per petri plate.

Strain	Solvent Control[1]	Positive Control[1,2]	Positive Control[1,3]	Density Estimate[1]	Crystal Violet	Ampicillin	Tetracycline
97a	173 (80–325)	521 (216–996)	926 (545–1,496)	6.3×10^9 (0.1–16)	NG[4]	G[5]	NG
98	32 (9–65)	1,828 (323–2,421)	1,641 (955–2,353)	5.2×10^9 (0.3–9.2)	NG	G	NG
100	170 (84–283)	846 (436–1,394)	551 (335–990)	3.0×10^9 (0.3–5.4)	NG	G	NG
102	357 (166–531)	1,669 (661–2,559)	465 (295–662)	8.8×10^9 (0.3–16)	NG	G	G

[1]Mean for all AOCs and range of means for individual AOCs (in parentheses). Test acceptable at ± 2 S.D.
[2]Positive control is direct–acting.
[3]Positive control requires activation.
[4]NG = no growth
[5]G = growth

densities and spontaneous reversion frequencies were within acceptable limits and acceptable reversion frequencies were obtained with each combination of strain and positive mutagen (Maron and Ames 1983).

Additional quality control included determination of S9 protein content, (18.7 ± 2.5 mg/mL; Bradford 1976) and ethoxyresorufin-o-deethylase (EROD) activity (7.05 ± 0.91 nmol/mg/min; Pohl and Fouts 1980). A most-probable-number test was used to estimate population densities of *Salmonella* cell cultures. Procedural blanks from the extraction process and reference samples (extracts from a fine silt- and clay-particle size sediment obtained from local undisturbed agricultural soil (Ingersoll and Nelson 1990)) were prepared and assayed along with AOC sediments. Procedural blanks and reference samples were assayed using the same experimental matrix using three doses, three S9 concentrations, and four bacterial strains. For the reference sediments, cytotoxicity was observed in 22 (92%), 4 (17%), and 2 (8%) of the 24 tests conducted at each of the high, medium, and low doses, respectively. Of the six toxic responses at the lower doses, only one was observed when S9 was present. Mutagenicity was detected in only 3 of the 72 total test combinations of dose, S9, and bacterial strain—and only by TA98 with S9. For the procedural blanks, only 7 of 72 tests (10%) showed cytotoxicity; in all cases these were at the high dose and cytotoxicity was eliminated with the addition of some concentration of S9. Mutagenicity was not observed with the procedural blanks.

Data Interpretation and Presentation

Means and standard deviations of the number of colonies per plate were calculated from the three

replicate plates. Test results were categorized as no-effect, toxic, and mutagenic. Plates were scored as "Mutagenic" if mean revertants on extract exposed plates were greater or equal to two times the mean number of spontaneous revertants on solvent control plates. Plates were scored as "Toxic" if mean revertant values on extract exposed plates were less than half the mean spontaneous revertants on solvent control plates, or if cytotoxicity was detected through the confirmation step. Those plates that were neither identified as cytotoxic nor mutagenic were scored as "No-Effect."

Using this approach, we obtained a total of 1332 scores for the samples assayed in this project, excluding reference samples, procedural blanks, and positive and negative controls (for detailed results, see Papoulias and Buckler 1993). In an effort to reduce the volume of tabular data presented in this manuscript to a manageable amount, we have chosen to consolidate information as follows. First, toxicity results of sample extracts are only reported for the high dose without S9. Secondly, mutagenicity results are simply reported as with or without S9 (metabolic activation) rather than reporting results at specific dilutions and S9 concentrations. Finally, positive mutagenic responses with S9 are further categorized according to whether the response indicated the presence of promutagens (i.e., occurred at non-toxic dilutions or after addition of excess S9). Thus, we report the total number of original sediment samples (identified by station number) that were scored as toxic or mutagenic for a given bacterial strain.

RESULTS

Mutagenicity was detected in both grab and core samples from every station tested. Of the four strains, only TA98 consistently identified mutagenicity. In general, addition of S9 reduced toxicity and improved the test's ability to detect mutagenicity.

Indiana Harbor

Without S9, sediment samples from all stations were cytotoxic to all strains of bacteria, except Station 10 with strain TA102, while mutagenicity was only detected at Station 7 and with only strain TA98 (Table 3). With S9, strains TA98 and TA100 detected mutagenicity in samples from all seven stations. Strains TA97a and TA102 were effective in identifying mutagenicity at six stations. An observation of mutagenicity with metabolic activation was made for each station (bacterial strains combined) at a non-toxic dilution or at a toxic dilution for which toxicity was removed with S9. Overall, samples from all stations were toxic and mutagenic with one or more bacterial strains.

Buffalo River

Without S9, sediment samples from all stations were cytotoxic to all strains of bacteria (Table 4).

TABLE 3. Number of sediment samples showing toxic (T) and mutagenic (M) responses for 7 Indiana Harbor stations. Station numbers [3,4,5,6,7,8,10] are identified in brackets.

	Strain			
Response	TA97a	TA98	TA100	TA102
Toxic at High Dose w/out S9	7 [all]	7 [all]	7 [all]	6 [3,4,5,6,7,8]
Mutagenic at Any Dose w/out S9	0 [none]	1 [7]	0 [none]	0 [none]
Mutagenic at Any Dose w/ S9	6 [3,4,6,7,8,10]	7 [all]	7 [all]	6 [3,4,5,6,7,8]
• Mutagenic at Non-Toxic Dilutions	2 [3,7]	3 [3,6,7]	2 [3,5]	5 [3,5,6,7,8]
• Mutagenic w/ Excess S9[1]	1 [4]	5 [3,4,5,6,8]	4 [3,4,7,10]	2 [4,7]

[1]Mutagenicity observed after addition of S9 in excess of the amount necessary to remove toxicity.

TABLE 4. Number of sediment samples showing toxic (T) and mutagenic (M) responses for 10 Buffalo River stations. Station numbers [1,2,3,4,5,6,7,8,9,10] are identified in brackets.

Response	Strain			
	TA97a	TA98	TA100	TA102
Toxic at High Dose w/out S9	10 [all]	10 [all]	10 [all]	10 [all]
Mutagenic at Any Dose w/out S9	0 [none]	1 [6]	1 [3]	0 [none]
Mutagenic at Any Dose w/ S9	7 [1,2,4,6,7,8,9]	10 [all]	6 [1,2,4,5,6,8]	0 [none]
• Mutagenic at Non-Toxic Dilutions	2 [8,9]	5 [4,5,6,7,8]	1 [2]	0 [none]
• Mutagenic w/ Excess S9[1]	1 [2]	0 [none]	4 [4,5,6,8]	0 [none]

[1]Mutagenicity observed after addition of S9 in excess of the amount necessary to remove toxicity.

Without S9, mutagenicity was only detected at Stations 3 and 6, with strains TA100 and TA98, respectively. With S9, strain TA98 detected mutagenicity in samples from all ten stations. Strains TA97a and TA100 identified mutagenicity at seven and six stations, respectively. Strain TA102 did not detect mutagenicity at any station. An observation of mutagenicity with metabolic activation was made for seven of 10 stations (bacterial strains combined) at a non-toxic dilution or at a toxic dilution for which toxicity was removed with S9. Overall, samples from all stations were toxic and mutagenic with one or more bacterial strains.

Saginaw River

Grab samples from all stations, except Station 24 with strain TA100, were cytotoxic without S9 (Table 5). Without S9, mutagenicity was only detected at Stations 2 and 24 with strains TA98 and TA100, respectively. With S9, strain TA98 detected mutagenicity at all stations. Strains TA97a, TA100, and TA102 detected mutagenicity at two (2 and 6), three (2, 6, and 24), and one (24) stations, respectively. An observation of mutagenicity with metabolic activation was made for 10 of 12 stations (bacterial strains combined) at a non-toxic dilution or at a toxic dilution for which toxicity was removed with S9. Overall, the grab samples taken from all stations were toxic and mutagenic to one or more bacterial strains.

Core samples that were taken at various depths from three of the Saginaw River stations were toxic to all strains, except Sample 2d (taken at 51-89 cm deep at Station 2) which was not toxic to strain TA100 (Table 6). Sample 2d was observed to be mutagenic with strain TA100. Mutagenicity was not detected for any other station without S9. With S9, strain TA98 detected mutagenicity in all samples; TA97a, TA100, and TA102 identified Sample 2d as mutagenic; and TA97a and TA100 detected mutagenicity with Sample 5a. An observation of mutagenicity with metabolic activation was made for four of six samples (bacterial strains combined) at a non-toxic dilution or at a toxic dilution for which toxicity was removed with S9. Overall, all core samples were shown to be both toxic and mutagenic with one or more bacterial strain.

DISCUSSION

Extracts of sediment samples from every station for the three ARCS sites were both cytotoxic and mutagenic for at least one combination of dilution, S9, and bacterial strain.

Use of S9 with single compounds allows distinction of classes of mutagens that are direct acting from compounds requiring metabolic activation (promutagens). Compounds that are mutagenic are also cytotoxic at high doses. With single compounds, the sample must be sufficiently diluted to remove toxicity before mutagenicity can be ob-

TABLE 5. Number of sediment samples showing toxic (T) and mutagenic (M) responses for 12 Saginaw River stations. Station numbers [1,2,3,4,5,6,7,8,9,10,16,24] are identified in brackets.

Response	Strain			
	TA97a	TA98	TA100	TA102
Toxic at High Dose w/out S9	12 [all]	12 [all]	11 [1,2,3,4,5,6,7,8,9,10,16]	12 [all]
Mutagenic at Any Dose w/out S9	0 [none]	1 [2]	1 [24]	0 [none]
Mutagenic at Any Dose w/ S9	2 [2,6]	12 [all]	3 [2,6,24]	1 [24]
• Mutagenic at Non-Toxic Dilutions	0 [none]	7 [1,2,3,5,8,9,16]	2 [6,24]	0 [none]
• Mutagenic w/ Excess S9[1]	0 [none]	2 [4,8]	1 [2]	1 [24]

[1]Mutagenicity observed after addition of S9 in excess of the amount necessary to remove toxicity.

TABLE 6. Number of sediment samples showing toxic (T) and mutagenic (M) responses for 3 Saginaw River stations (core survey). Station numbers [2,5,6] are identified in brackets. Letters following station numbers identify core depth.

Response	Strain			
	TA97a	TA98	TA100	TA102
Toxic at High Dose w/out S9	6 [all]	6 [all]	5 [2c;5a,b;6a,b]	6 [all]
Mutagenic at Any Dose w/out S9	0 [none]	0 [none]	1 [2d]	0 [none]
Mutagenic at Any Dose w/ S9	2 [2d,5a]	6 [all]	2 [2d,5a]	1 [2d]
• Mutagenic at Non-Toxic Dilutions	1 [5a]	2 [5a,6b]	2 [2d,5a]	0 [none]
• Mutagenic w/ Excess S9[1]	0 [none]	1 [2c]	1 [5a]	1 [2d]

[1]Mutagenicity observed after addition of S9 in excess of the amount necessary to remove toxicity.
a = 0–61 cm deep; b = 61–122 cm deep; c = 20–51 cm deep; d = 51–89 cm deep

served. Subsequently, addition of S9 can be used to discriminate between direct-acting mutagens and those requiring metabolic activation. It is more difficult to distinguish between these categories of compounds with complex mixtures (Ball *et al.* 1990, Pederson and Siak 1981). Complex mixtures may contain both mutagenic and non-mutagenic compounds at cytotoxic concentrations. Sample dilution to remove toxicity may conceal the presence of mutagens due to their co-dilution. For the present study with Great Lakes sediment extracts, addition of S9 was necessary to both reduce sample toxicity and activate promutagens (see Papoulias *et al.* 1996), resulting in a loss of discrimination between direct-acting and promutagenic compounds in many cases.

Mutagenicity attributable to direct-acting mutagens (dilution without S9) could be detected in only six of the samples (Indiana Harbor, Station 7; Buf-

falo River, Stations 3 and 6; Saginaw River, Stations 2 and 24; Saginaw River, Core Sample 2d). Expression of a mutagenic response after addition of S9 to non-cytotoxic dilutions, or addition of S9 beyond that necessary for elimination of cytotoxicity indicated the presence of promutagens. Using this criterion, all of the samples containing direct-acting mutagens (except Buffalo River Station 3) were also shown to contain promutagens. In addition, all samples from Indiana Harbor and most of the samples from the other areas of concern were shown to contain promutagens. Undetected direct-acting mutagens may have also been present in these samples. If present, their effects were likely masked by co-dilution or S9 metabolism. For the few remaining samples (Buffalo River Stations 1, 3, and 10; Saginaw River Station 7, and 10 grab samples; and Saginaw River Station 5b and 6a core samples), cytotoxicity was not sufficiently reduced through dilution or addition of S9 to characterize the classes of mutagens present.

The bacterial strains varied in their ability to detect mutagenicity with environmental samples obtained from the ARCS sites. Strain TA98 detected mutagenicity most frequently. With S9, strain TA98 detected mutagenicity in 100% of the samples, while strains TA100, TA97a, and TA102 detected mutagenicity in 62%, 59%, and 28% of the samples, respectively. Only strains TA98 and TA100 detected mutagenicity without S9. In a comparison by Zeiger *et al.* (1985), strains TA98 and TA100 positively identified 89% of the mutagenic chemicals in the NTP (National Toxicity Program) database.

Mutagenicity detected in samples from the ARCS sites could be primarily attributable to polyaromatic hydrocarbons (PAHs). Chemical analysis of the sediment samples indicates that as a class, PAHs comprised the greatest percent by weight of the total identified organics (Table 7; methods reviewed in Nelson *et al.* 1993). High molecular weight PAHs were more abundant than low molecular weight PAHs. Of the classes of contaminants present in our samples, the high molecular weight PAHs contained the greatest number of individual compounds known to be mutagenic. It should be noted, however, that many of the other sediment contaminants identified are reported to cause DNA damage in other organisms (Table 7).

Other investigations have identified PAHs as the most likely contaminants causing positive Ames results in sediment samples from other sites (Maccubbin and Ersing 1991, Abe *et al.* 1989, Grifoll *et al.* 1988, Odense *et al.* 1988, Oishi and Takahashi 1987, Sato *et al.* 1983, Waldron and White 1989, Marvin *et al.* 1993). Crude extracts of freshwater sediments from Hamilton Harbor, Lake Ontario, Canada and marine sediments from Long Island Sound, USA contained concentrations of high molecular weight PAHs similar to those in the present study and were also mutagenic, while control samples were not (Metcalfe *et al.* 1990, Gardner *et al.* 1987). Furthermore, PAH-contaminated sediment reportedly was associated with induction of neoplasia in oyster and flounder in Black Rock Harbor, Connecticut (Gardner *et al.* 1991), and in walleye and brown bullheads in tributaries of the Great Lakes (Baumann 1991).

One goal of mutagenicity testing is to predict the carcinogenic and intergenerational effects a compound may have on organisms of concern. Mutagenicity is only one of many genotoxicity end-points that can contribute information towards this goal. Furthermore, detection of a mutation in the *Salmonella* organisms in the Ames assay is dependent upon optimization of the test parameters. For these reasons, it is generally accepted that a tiered approach using a variety of tests and end-points is the most effective for detecting potential gene and chromosome damage (Heinze and Poulsen 1983).

The present studies demonstrate the utility of the Ames assay for screening crude extracts of complex mixtures. By varying S9 concentration and extract dilution, cytotoxicity was controlled so that mutations could be observed. However, use of the assay with these samples did not always allow discrimination between promutagenic and direct-acting responses because of the dual role of S9 (metabolic activation and toxicity reduction). Nor was the assay particularly useful for ranking the mutagenic potential of sediments from the stations tested in this study. This may be due to the sensitivity of the test, or the relatively high degree of contamination found in all samples. Fractionation of samples before testing may allow a greater degree of discrimination and aid in identifying the compounds which are causing the observed mutagenicity. Increasing the number of dilutions would provide additional information on the mutagenic potency of sediments at these stations. Additionally, the rigorous chemical extraction methods applied in this study preclude an evaluation of the bioavailability of mutagenic compounds in these sediments. The use of passive membrane sampling techniques (Huckins *et al.* 1990), and development of methods for testing elutriates and pore waters, may be useful for this purpose.

TABLE 7. Summary of chemistry data for grab samples from the AOCs and the reference sediment with literature reported results of Ames assay with individual chemicals.

	Buffalo River (n = 10) range	Indiana Harbor (n = 7) range	Saginaw River (n = 12) range	Reference Sediment (n = 2) range	Reported Ames Assay Mutagenicity	Notes	Citation
Pesticides (μg/kg)							
Aldrin	N.D.	N.D.–330	N.D.–360	N.D.	—	1	NIOSH (1992)
(Alpha) Hexachlorocyclohexane	N.D.	N.D.	N.D.–390	N.D.		1	USDHHS (1987)
(Beta) Hexachlorocyclohexane	N.D.–150	N.D.–290	N.D.	N.D.		1	USDHHS (1987)
(c) Hexachlorocyclohexane	N.D.	N.D.	N.D.	N.D.		1	USDHHS (1987)
(Gamma) Chlordane	N.D.	N.D.–170	N.D.–140	N.D.	—	1	NIOSH (1992)
(Alpha) Chlordane	N.D.	N.D.	N.D.	N.D.	—	1	NIOSH (1992)
4,4 DDD	N.D.–75	N.D.–70	N.D.–13	N.D.		1, 3	NIOSH (1992)
4,4 DDE	N.D.	N.D.–210	N.D.–140	N.D.	—	1	NIOSH (1992)
4,4 DDT	N.D.–100	N.D.–100	N.D.–180	N.D.	—	1	NIOSH (1992)
Dieldrin	N.D.–120	N.D.–343	N.D.–96	N.D.	—	1	NIOSH (1992)
Endo Sulfan I	N.D.	N.D.–41	N.D.–12	N.D.		3	
Endo Sulfan II	N.D.–80	N.D.–160	N.D.–130	N.D.		3	
Endo Sulfan sulfate	N.D.	N.D.	N.D.	N.D.		3	
Endrin	N.D.	N.D.–44	N.D.–45	N.D.	—	1	NIOSH (1992)
Endrin aldehyde	N.D.–60	N.D.– 65	N.D.–6	N.D.		3	
Heptachlor	N.D.	N.D.–320	N.D.–140	N.D.		3	NIOSH (1992)
Heptachlor epoxide	N.D.–30	N.D.–320	N.D.–190	N.D.		2	NIOSH (1992)
Lindane	N.D.	N.D.	N.D.–17			3	
Toxaphene	N.D.	N.D.	N.D.	N.D.	+	1, (a)	NIOSH (1992)
Methoxychlor	N.D.	N.D.	N.D.	N.D.	—	1, (a)	NIOSH (1992)
Endrin ketone	N.D.	N.D.–51	N.D.–16			3	
PAHs Low molecular weight PAHs (μg/kg)							
1,4 Dichlorobenzene	< 36–810	31–930	< 4–130		—	1	NIOSH (1992)
Napthalene	< 57–20,000	3,600–24,000	< 6–55	13.5	—	(a)	NIOSH (1992)
2–Methylnapthalene	< 67–2,400	930–21,000	< 6–63	N.D.–3	+	1	NIOSH (1992)
Dimethyl pthalate	< 86	< 110	N.D.–110		+	1	NIOSH (1992)
Dibenzofuran	< 71–1,600	930–26,500	< 7–38			1, 3	NIOSH (1992)
Fluorene	< 71–3,400	790–30,500	< 7–69	N.D.	—	1	NIOSH (1992)
Phenanthrene	< 36–10,000	3,400–151,500	N.D.–1,000	< 10	—	1	NIOSH (1992)
Anthracene	< 34–4,300	1,400–215,000	< 8–70	N.D.	—	1, (a)	NIOSH (1992)
High molecular weight PAHs (μg/kg)							
Fluoranthene	< 55–7,500	4,800–80,000	N.D.–1,200	7–10	+	1	NIOSH (1992)
Pyrene	< 68–6,700	5,500–46,500	N.D.–1,800	29	—	1, (a)	NIOSH (1992)
Butyl Benzl Phthalate	< 180–15,000	< 240–16,000	< 18–3,000		—		NIOSH (1992)
Benzo (a) anthracene	< 21–3,500	4,200–32,000	N.D.–690	N.D.	+	1	NIOSH (1992)
Bis (2–Ethylhexyl) phthalate	< 880–59,000	3,800–290,000	170–13,000		+	1	NIOSH (1992)
Chrysene	< 27–4,000	5,200–33,000	N.D.–600	4–5	+	1	NIOSH (1992)
Di-n-octypthalate	< 84–38,000	< 240–37,000	< 19–2,200		+		NIOSH (1992)
Benzo-b-fluoranthene	< 30–7,000	5,600–26,000	< 7–600	8–9	+	1	NIOSH (1992)
Benzo-k-fluoranthene	< 41–9,500	4,200–23,000	< 9–400	N.D.	+		NIOSH (1992)
Benzo(a)pyrene	< 27–5,800	5,700–31,000	< 6–440	N.D.–2	+	1	NIOSH (1992)
Indeno 123 CD pyrene	< 100–3,800	5,300–22,000	< 10–220	2	+	1	NIOSH (1992)
Benzo (ghi) perylene	< 130–3,800	6,300–31,000	< 13–310	2		2	NIOSH (1992)

Continued

TABLE 7. Concluded.

	Buffalo River (n = 10) range	Indiana Harbor (n = 7) range	Saginaw River (n = 12) range	Reference Sediment (n = 2) range	Reported Ames Assay Mutagenicity	Notes	Citation
Dioxins (ng/kg)					—		Schoeny (1982)
TCDD	N.D.–12	32–230	6–230				
PeCDD	N.D.–74	N.D.–950	N.D.–150				
HxCDD	N.D.–190	350–8,700	33–460				
HpCDD	12–2,000	980–22,000	74–2,100				
OCDD	53–12,000	2,300–46,000	340–26,800				
Furans (ng/kg)					—		Schoeny (1982)
TCDF	N.D.–1.5	170–3,700	230–22,000				
PeCDF	N.D.–83	76–1,350	120–8,700				
HxCDF	N.D.–110	220–3,600	200–2,100				
HpCDF	3–640	380–8,200	120–4,000				
OCDF	< 3.9–780	250–17,300	89–2,800				
PCB (µg/kg)					—		Schoeny (1982)
PCB 1016	N.D.	N.D.	N.D.				
PCB 1221	N.D.	N.D.	N.D.				
PCB 1232	N.D.	N.D.	N.D.				
PCB 1242	N.D.	3,000–43,000	N.D.–60,000	N.D.			
PCB 1248	N.D.	N.D.	N.D.	N.D.			
PCB 1254	N.D.–1,000	N.D.–4,450	N.D.–7,900	N.D.			
PCB 1269	N.D.	N.D.	N.D.–300				

Notes: (a) = Reference sediment data from MSC historical records not Battelle.
[1]Demonstrated mutagenicity in tests other than Ames assay.
[2]Data inconclusive.
[3]Information not available.

ACKNOWLEDGMENTS

We thank B. Steadman for assistance in the design of our experimental approach, Dr. B.T. Johnson and L. Arroyo for technical assistance in the conduct of the studies and E. Head for editorial assistance. This project was funded in part by the United States Environmental Protection Agency under Interagency Agreement No. DW 14933874. The contents of this document do not necessarily reflect the view and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Submitted: 15 April 1996
Accepted: 9 July 1996