Technical Report D-95-1
April 1995

US Army Corps of Engineers
Waterways Experiment Station

Long-Term Effects of Dredging Operations Program

A Comparison of the Ames Assay and Mutatox in Assessing the Mutagenic Potential of Contaminated Dredged Material

by A. Susan Jarvis

Prepared for Headquarters, U.S. Army Corps of Engineers

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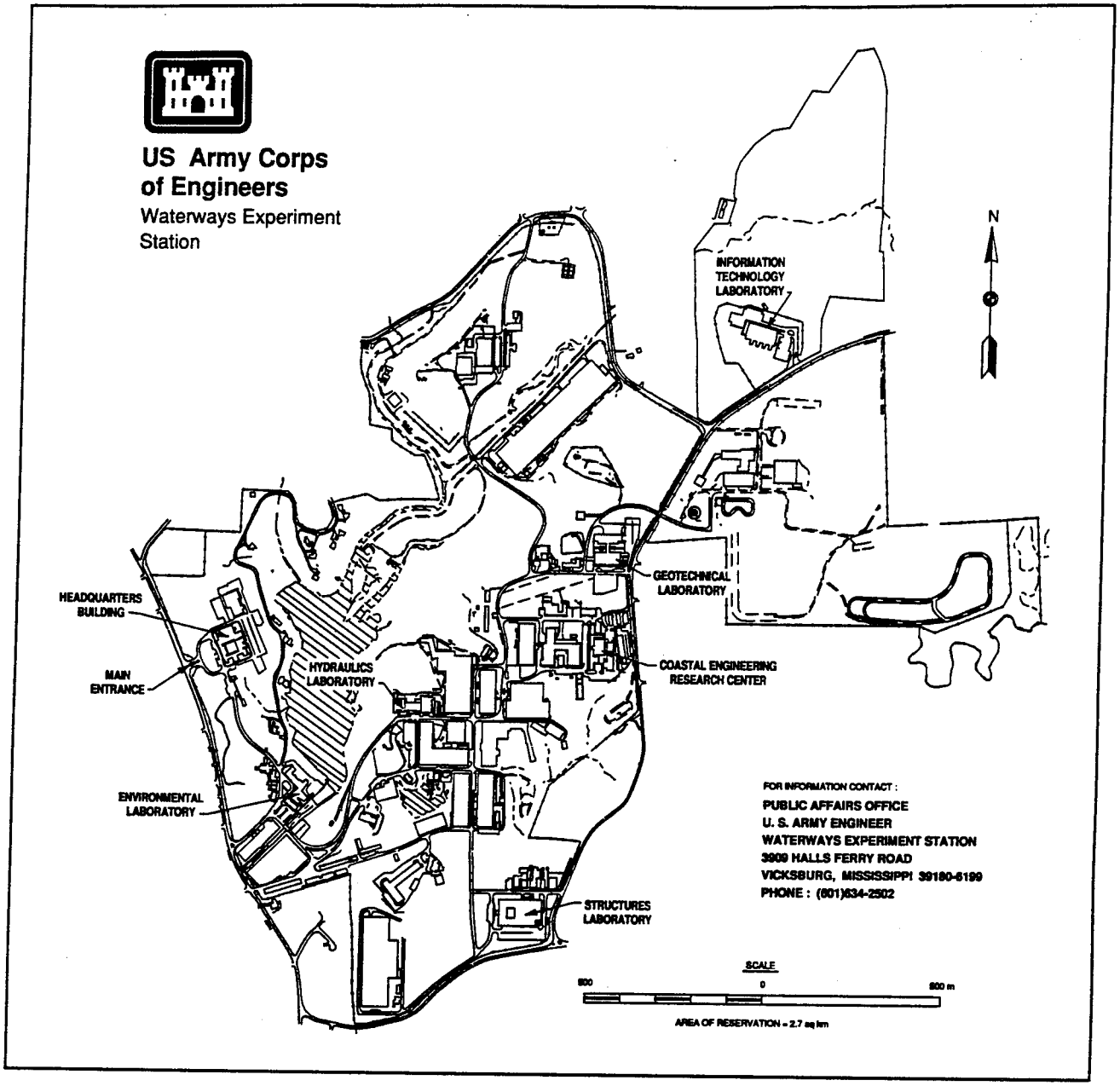
Final report

Approved for public release; distribution is unlimited

Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000



Waterways Experiment Station Cataloging-in-Publication Data

Jarvis, A. Susan.

A comparison of the Ames Assay and Mutatox in assessing the mutagenic potential of contaminated dredged material / by A. Susan Jarvis ; prepared for U.S. Army Corps of Engineers.

89 p. : ill. ; 28 cm. — (Technical report ; D-95-1)

Includes bibliographic references.

1. Marine sediments — Toxicology — Testing. 2. Dredging spoil — Environmental aspects. 3. Mutagenicity testing. I. United States. Army. Corps of Engineers. II. U.S. Army Engineer Waterways Experiment Station. III. Long-Term Effects of Dredging Operations Program (U.S.) IV. Title. V. Series: Technical report (U.S. Army Engineer Waterways Experiment Station) ; D-95-1.

TA7 W34 no.D-95-1

US Army Corps of Engineers
Waterways Experiment Station

Environmental Effects of Dredging Programs

Long-Term Effects of Dredging Operations

Report Summary

A Comparison of the Ames Assay and Mutatox in Assessing the Mutagenic Potential of Contaminated Dredged Material (TR D-95-1)

ISSUE: Aquatic sediments are known to act as repositories for contaminants. Sediments often are sources of contamination when disturbed by events such as storms, floods, construction, site remediation or dredging, and disposal activites. Some of the contaminants found in sediments may have genotoxic potentially inducing effects on individual genes of organisms, which may lead to cancer, birth defects, or death. Although many tests are available to determine genotoxicity of single chemicals to certain animals and humans, little applied research has been conducted concerning complex mixtures such as occur in sediments.

RESEARCH: This research was undertaken to test the hypothesis that the Ames assay, a well-validated procedure, and the Mutatox genotoxicity assay, a relatively new test, will give similar results in assessing the mutagenic potential of contaminated dredged material.

SUMMARY: In a side-by-side comparison of the Ames assay (TA98+S9) and Mutatox, 80 percent of the sediment extracts had similar responses, both positive and negative. Overall, Mutatox compared favorably with the Ames assay and shows promise as a screening tool to assess sediment genotoxicity when used with Ames assay as a confirmation.

AVAILABILITY OF REPORT: The report is available on Interlibrary Loan Service from the U.S. Army Engineer Waterways Experiment Station (WES) Library, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199; telephone (601) 634-2355.

To purchase a copy, call the National Technical Information Service (NTIS) at (703) 487-4780. For help in identifying a title for sale, call (703) 487-4780. NTIS report numbers may also be requested from the WES librarians.

About the Authors: Ms. Susan A. Jarvis is a research biologist in the WES Environmental Laboratory. For further information about the Long-Term Effects of Dredging Operations Program, contact Mr. Thomas R. Patin, Program Manager, at (601) 634-3444.

April 1995

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

The work reported herein was conducted by the U.S. Army Engineer Waterways Experiment Station (WES) for the Headquarters, U.S. Army Corps of Engineers (HQUSACE), and Northeast Louisiana University, Monroe, LA, as partial fulfillment of requirements for a Master of Biology degree. Financial support was provided by HQUSACE through the Long-term Effects of Dredging Operations (LEDO) Program, Work Unit 32726, "Genotoxicity of Contaminated Dredged Material." The LEDO Program is managed through the Environmental Effects of Dredging Programs, Dr. R. M. Engler, Manager.

This report in thesis form was prepared by Ms. A. Susan Jarvis of the Fate and Effects Branch (FEB), Environmental Processes and Effects Division (EPED), Environmental Laboratory (EL), WES. The author gratefully acknowledges the support provided by numerous individuals at Northeast Louisiana University. Especially helpful were Drs. Harold C. Bounds, Benny Blaylock, Kim M. Tolson, and Paul Ferguson.

The work was performed under the general supervision of Dr. Bobby L. Folsom, Jr., Chief, FEB, EPED. The Chief of EPED was Mr. Donald L. Robey, and Director of EL was Dr. John W. Keeley.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

This report should be cited as follows:

> Jarvis, A. S. (1995). "A comparison of the Ames assay and Mutatox in assessing the mutagenic potential of contaminated dredged material," Technical Report D-95-1, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

INTRODUCTION

Aquatic sediments are known to act as repositories for contaminants. Sediments often are sources of contamination when disturbed by such things as storms, floods, construction, site remediation or dredging, and disposal activities (Reilly et al. 1990). Some of the contaminants found in sediments may have genotoxic potentially inducing effects on individual genes of organisms which may lead to cancer, birth defects, or death (Jarvis et al. 1993). Although there are many tests that will determine genotoxicity of single chemicals to certain animals and humans (Huggett et al. 1992), there has been little applied research conducted concerning complex mixtures such as occur in sediments. Areas of sediment genotoxicity considered important are mutagenicity, carcinogenicity, and teratogenicity.

This thesis will consider the area of mutagenicity. Mutagenicity occurs when the DNA of an organism is damaged causing an error in the genetic code that may be transmitted to the next generation. Mutations may be caused by chemical, physical, or biological means. Mutations can occur in any cell and can result in either minor phenotypic changes or in major genotypic changes to essential processes that may cause death to the cell or the organism (Hartl 1991). *In vitro* and *in vivo* tests have been developed that detect chemically-caused mutations. The *in vitro* methods include the Ames assay, Chinese Hamster Ovary test, Syrian Hamster Embryo test, and a newly introduced test, Mutatox™. These tests generally indicate changes in an organism's genetic integrity due to exposure to genotoxic chemicals.

The Ames assay, a well validated test, has been used for approximately 20 years and has been applied and modified in testing many environmental mixtures. The Mutatox™ genotoxicity assay is relatively new and is still being validated. Recently, Mutatox™ has been used in studies with complex environmental mixtures, especially sediments.

This thesis research was undertaken to test the hypothesis that the Ames assay and the Mutatox™ genotoxicity assay will give similar results in assessing the mutagenic potential of contaminated dredged material.

LITERATURE REVIEW

Ames Assay

Background and Theory

The *Salmonella*/mammalian-microsome mutagenicity assay, also commonly known as the Ames test or Ames assay, is the most widely validated and accepted *in vitro* bacterial assay used to screen chemicals and environmental samples for mutagenic agents (Williams 1985). Many years of research by Dr. Bruce Ames and colleagues went into the development of the Ames assay. In the early 1950's, Ames conducted graduate research in biochemical genetics, i.e., the study of gene products, enzymes, and proteins. At that time, little was known about how genes functioned. He experimented with the bread mold, *Neurospora crassa*, to determine the process of how a cell makes histidine. Ames discovered that a series of chemical reactions carried out by the cell to make histidine were controlled by the mold's genes and that there was an ordered activity of those genes (Day 1987).

He continued to work with bread mold but later changed to the bacterium, *Salmonella typhimurium*, because of its simplicity and faster growth rate. After approximately 15 years, Ames discovered the chemical events that activate or inactivate genes involved in the synthesis of histidine. In 1964, Ames began to develop a simple test using the *Salmonella* bacterial strains he had developed to screen new synthetic chemicals for their ability to cause mutations in genes (Day 1987).

Ames' original *Salmonella typhimurium* tester strain was designated LT2. The *Salmonella* tester strains listed in Table 1 have been derived from the original culture. These *Salmonella* strains have been the

primary strains developed to screen for general mutagenicity. Also in Table 1, differences in important characteristics for each bacterial strain are listed.

This series of *Salmonella* strains was known as histidine auxotrophs because they have a mutation that will not allow them to produce the amino acid histidine which is required for survival (Zeiger 1985). On a histidine-free agar, only cells that can revert spontaneously to "wild-type" bacteria will form colonies. Each *Salmonella* strain has a range of spontaneous reverse mutation values which is relatively constant. When these selected strains of *Salmonella* were exposed to mutagenic compounds, their reversion rate was significantly increased (Williams 1985). When this occurred, the bacteria regained the ability to synthesize histidine and were known as histidine prototrophs (Zeiger 1985).

The tester strains were able to detect base-pair substitution (BPS) and/or frameshift (FS) mutagens. A base-pair substitution mutation is the simplest form of mutation where one nucleotide in DNA is replaced with a different nucleotide and is classified as either a transition or transversion. Transitions are those base substitutions that change the pyrimidine base for another pyrimidine base or that change a purine base for another purine base while maintaining their purine:pyrimidine alignment in the two strands. Transversions are the remaining substitutions in which the placement of the purine and pyrimidine bases are reversed in the two strands. At any given DNA site, there are three possible substitutions which contain one transition and the others are transversions (Ripley 1991).

Table 1. Primary *Salmonella* Testing Strains for General Mutagenicity

Strains	His Mutation[2]	DNA Target[2]	Other Mutations			Mutation Detected[2]
			LPS[1]	Excision Repair[1,2]	R Factor[1,2]	
TA 1535	G46	-CCC- -GGG-	*rfa*	*uvr*B	none	BPS
TA100	G46	-CCC- -GGG-	*rfa*	*uvr*B	pKM101	BPS+FS
TA1537	C3076	-CCCC- -GGGG-	*rfa*	*uvr*B	none	FS
TA97	D6610	-CCCCCC- -GGGGGG-	*rfa*	*uvr*B	pKM101	FS
TA1538	D3052	-CGCGCGCG- -GCGCGCGC-	*rfa*	*uvr*B	none	FS
TA98	D3052	-CGCGCGCG- -GCGCGCGC-	*rfa*	*uvr*B	pKM101	FS
TA102	G428	-TAA- -ATT-	*rfa*	+	pKM101 + pAQ1	BPS+FS
TA104	G428	-TAA- -ATT-	*rfa*	*uvr*B	pKM101 + pAQ1	BPS+FS

Source: [1] Maron and Ames (1983); [2] Zeiger (1985).

Silent substitutions are mutations that change the nucleotide sequence without changing the amino acid and are not detected by changes in phenotype (Hartl 1991). A missense mutation is a type of base substitution changing a codon into one that codes for a different amino acid. A frameshift mutation may consist of an addition or deletion of one or more bases, altering the expression of that particular DNA segment distal to the mutation. Because the bases are read in a series of three, an addition or subtraction of a base can shift the reading frame resulting in a random sequence of codons. The protein distal to the mutation will bear no resemblance to the native protein (Jarvis et al. 1993). The biological properties of the protein may be changed when the sequence of amino acids in a protein is changed (Hartl 1991).

Various histidine mutations are contained in the bacterial strains chosen for the tester series, and these mutations revert to the wild-type using different chemical and molecular mechanisms. For instance, TA1535 and TA100 contain the missense mutation (G46) and chemicals that induce base-pair substitution induce reversion. In addition, TA100 contains the plasmid pKM101 which carries SOS repair genes. The SOS repair response is a complex set of processes which occurs in bacteria. An important feature of the SOS repair system is lack of activity until induced by DNA damage. The SOS repair system has appeared to be a radical repair system that is designed to save the cell when there is persistent DNA damage. The system is induced only after a delay in which incomplete replication of DNA has occurred. This system includes a bypass system that allows DNA replication to occur across pyrimidine dimers or other DNA distortions which cause problems in replication.

The DNA strands formed by SOS repair are often defective. Once activated, the SOS system has filled in spaces in the DNA without copying the template so that errors are not declared (Atlas 1984). The proofreading system of DNA polymerase is relaxed to permit polymerization to continue across the damage, even though the helix is deformed (Hartl 1991). Frameshift mutations are also detected by TA100 because of this added effect of the pKM101 plasmid, a defective bacterial virus (Zeiger 1985). Chemicals that cause base-pair substitutions also induce the reversion of the ochre mutation (G248). This mutation is contained in TA102 and TA104 in addition to the plasmid pKM101 which increases these bacterial strains' sensitivity to frameshift mutagens. A second plasmid, pAQ1, is located on the *his* G248 mutation which is a multicopy plasmid. This plasmid allows multiple mutation copies increasing target sites for the mutagen and increasing the sensitivity of the bacterial strain. The strain TA102 detects mutagens such as formaldehyde, hydroperoxides, X-rays, ultraviolet (UV) light, and cross-linking agents such as mitomycin C. These mutagens are not identified by the standard strains TA100, TA98, and TA97 (Maron and Ames 1983). The DNA target as noted in Table 1 is different for each of the two above mentioned histidine mutations and would be expected to be mutated by different classes of chemicals (Zeiger 1985).

Reversions of the *his* mutations C3076, D3057, and D6610 are induced by chemicals that are frameshift mutagens. The bacterial strain TA1537 contains C3076 but has been replaced in general usage by the strain TA97 which contains D6610. The strain TA1537 proved to be less sensitive even though both strains have a similar mutagenic specificity (Maron and

Ames 1983). The strains TA1538 and TA98 contain D3052 and detect frameshift mutagens. The bacterial strain TA1538 has detected specific aromatic frameshift mutagens such as 4-nitro-o-phenylenediamine and has been dropped as a tester strain because of overlap with TA98 (Maron and Ames 1983). There are some adjunct strains TA94, TA2637, and TA1978 developed by Ames to identify certain mutagens that are not detectable by the more common tester strains and are discussed in more detail by Maron and Ames (1983).

Two other mutations were added to increase sensitivity of the *Salmonella* bacterial strains to mutagenic compounds. One of the mutations, designated as *uvrB* mutation, caused loss of the accurate DNA repair system known as the excision repair system. The excision repair system is a widespread multi-step enzymatic process by which a portion of a damaged DNA strand is removed from a duplex molecule and replaced by resynthesis, using the undamaged strand as a template (Hartl 1991). This deletion also includes the biotin gene, so it is necessary to add biotin for the cells to grow. The *uvrB* mutation is confirmed by exhibiting UV sensitivity in bacterial strains containing the mutation. The *uvrB* mutation is quite stable and not easily lost from the bacterial strains (Ames et al. 1975; Maron and Ames 1983; Williams 1985).

The deep rough (*rfa*) character, another mutation, caused a partial loss of the lipopolysaccharide (LPS) barrier that covers the bacteria's surface. The cells became more permeable to large molecules that normally would not enter because of the removal of most of the cell wall (Maron and Ames 1983). The *rfa* mutation in a strain was confirmed by exhibiting its sensitivity to crystal violet.

As Ames further developed the Ames test, he found that known carcinogens such as benzo[*a*]pyrene (B[*a*]P) and 2-acetylaminofluorene, also important known mutagens, were not detected in the testing. Relying on information that the liver breaks down toxic substances, he added rat liver homogenate to test plates to imitate how the liver metabolizes chemicals *in vivo* (Day 1987). Results of this study indicated that 18 of 20 different aromatic-type carcinogens were activated to frameshift mutagens when the rat liver homogenate was used (Ames et al. 1973). None of these compounds had caused detectable frameshift mutations without the rat liver homogenate, thus the activation would have been caused by the liver enzymes. An important feature of mammalian metabolism was incorporated into the *in vitro* assay (Ames et al. 1975).

Many chemical compounds are not directly mutagenic but can be converted into mutagens when metabolized by higher animals such as fish or man (Jarvis and Reilly 1992). One of the enzyme systems in the liver, Cytochrome P450 system, is a group of oxidative enzymes which are involved in the detoxification process of harmful compounds produced by metabolism. Cytochrome P450 uses high energy electrons that are transferred from NADPH by a reductive enzyme in the endoplasmic reticulum (ER) membrane to add hydroxyl groups to potentially harmful water-insoluble hydrocarbons dissolved in the bilayer. Other enzymes in the ER membrane then add negatively charged water-soluble molecules, e.g., sulfates, to these hydroxyl groups. After a series of these types of reactions, a water-insoluble metabolite that would ordinarily accumulate in the cell membranes is made to be sufficiently water-

soluble to leave the cell and be excreted by the organism (Alberts et al. 1989). The microsomal enzymes that are used *in vitro* are taken from the livers of rats induced with a commercial mixture of polychlorinated biphenyl Aroclor 1254. The liver homogenate, which is usually a 9000 g supernatant fraction commonly called S9, provides the P450-mixed function oxidase metabolic pathways that are present in an intact liver (Zeiger 1985). The S9 can activate a chemical, thus making it a mutagen. The S9 is necessary since microorganisms do not have the mixed function oxidation capabilities found in mammals (Jarvis and Reilly 1992).

Validation and Utility

Ames and his colleagues first validated the Ames test in determining the mutagenicity of 300 chemicals of known carcinogenicity and noncarcinogenicity (McCann and Ames 1976; McCann et al. 1975). The groups of chemicals included aromatic amines, alkyl halides, polycyclic aromatics, esters, epoxides, carbamates, nitro aromatics, miscellaneous aliphatics and aromatics, nitrosamines, fungal toxins and antibiotics, azo dyes and compounds, miscellaneous heterocycles and nitrogen compounds, and compound mixtures such as cigarette smoke condensate. Since this initial validation, over 5000 chemicals have been tested using the Ames assay, and the data has been reported in the Environmental Mutagen Information Center Index (Maron and Ames 1983).

Approximately 80 to 90% of all compounds that test positive by the Ames assay subsequently are shown to be carcinogens that affect higher animals such as fish or man (Maron and Ames 1983). Some researchers had problems with the high percentage of correlation between mutagenicity

and carcinogenicity reported by McCann et al. (1975). Zeiger (1985) believed that the studies were based on biased data because the carcinogens and noncarcinogens were already known. Reported correlations were influenced by the inadequate identification of non-carcinogens and testing of a limited class of chemicals that were known carcinogens or expected to be positive (Zeiger 1985). In recent years, the percentage of correlation between mutagenicity and carcinogenicity has diminished to approximately 60%. Tennant et al. (1987) found in the database used for this assessment that the number of compounds suspected of being nongenotoxic carcinogens and promoting agents were particularly significant. Ramel (1988) stated that, when the fraction of nongenotoxic chemicals rises in a database, the correlation between carcinogenicity and short-term tests goes down.

The Ames assay has been extensively used as a rapid screening procedure for the determination of mutagenic and potential carcinogenic hazards of pure chemicals, commercial products, and complex environmental mixtures (Williams 1985). The complex environmental mixtures have included air (Butler 1985; Houk et al. 1992), water (Doerger et al. 1992; Houk 1992), soil (Maggard et al. 1987) and sediments (West et al. 1986, 1988; Grifoll et al. 1990; Lan et al. 1991), and hazardous wastes generated from industries (Bessi et al. 1992; Houk 1992).

Sediment

Many researchers have used the Ames assay to identify potential mutagens contained in complex environmental mixtures such as sediment or soil since the initial validation study of McCann et al. (1975). Kinae

et al. (1981) utilized the Ames test to identify mutagenic substances from three different sediment samples taken at pulp and paper mill effluent sites. The sediment extracts were tested using three strains of *Salmonella typhimurium* with and without S9 metabolic activation. Results indicated only one of the three sediment extracts was mutagenic. Twenty-eight organic compounds were identified in this sediment extract by using gas chromatography-mass spectrometry (GC-MS) analysis.

Allen et al. (1983) used the Ames assay to assess dredged material in the Lake Michigan area to determine if this data could provide information on the environmental impact of dredged material. At that time, no bioassay for mutagenic potential had been used in assessing dredged material. Results of this study indicated there were sediments in several areas on the Southwestern shore of Lake Michigan that contained mutagenic compounds.

The Ames assay was used with minor modifications in the amount of agar to test river sediments from a highly industrialized area for mutagenicity (West et al. 1986, 1988). Catfish from these waters had been observed to have a high incidence of liver tumors. The Ames assay indicated mutagenic activity in the fractions of sediment extract that contained 4 to 6-ringed polyaromatic hydrocarbons (PAHs), especially fractions that contained 5-ringed PAHs such as B[*a*]P and dibenz[*a*, *h*]anthracene (West et al. 1986).

The Ames test was utilized by Maggard et al. (1987) to measure the mutagenic potential of spiked soil samples in evaluating the efficiency of the blender extraction method. Also, the blender extraction and soxhlet extraction methods were compared using waste-amended soil.

Results of the Ames test indicated that the blender extraction method was efficient in testing an indirect-acting, nonpolar mutagen and a polar, direct-acting mutagen. In comparing the efficiency of the blender method and the soxhlet extraction method, there were significantly greater levels of mutagenic activity detected by the blender method in fractions of the waste-amended sediment than the Soxhlet method.

Fabacher et al. (1988) used the Ames assay along with two other assays and a polycyclic aromatic compound characterization in assessing five sediments from the Great Lakes region where fish had tumors. A conclusion from this study suggested that, due to variable results in the Ames test, the more complex a mixture becomes, the utility of Ames decreases.

The Ames test was applied in screening Welsh soils of varying contamination to assess mutagenic activity. Jones and Peace (1989) studied the mutagenic potential of soils of varying degrees of contamination for selected land use. Results from this study indicated a general trend towards increased mutagenic activity in soils with elevated PAH levels.

The Ames assay has been combined with mass spectrometric techniques to assess river and marine sediments in a study to correlate chemical composition and mutagenicity of the sediment (Grifoll et al. 1990). The mass spectrometric methods used in this study were electron impact and positive and negative ion chemical ionization coupled to gas chromatography. In assessing the chemical fractions of the sediments, negative ion chemical ionization provided the sensitivity for screening

known mutagenic compounds in aquatic sediments. There were problems with a lack of correlation between chemical composition and mutagenic activity in fractions where mutagenic PAHs were identified and this was attributed to antagonistic interactions with other identified compounds. A multiple-step fractionation which included lipidic separation from organic extracts was recommended to possibly enhance the accuracy of the mutagenic assessment of aquatic sediments (Grifoll et al. 1990).

Metcalfe et al. (1990) utilized the Ames assay and two other carcinogenicity assays, the DNA adduct assay and the sac fry microinjection assay, to assess genotoxicity in three sediment extracts from sites where fish tumors were widespread. Results indicated mutagenicity in one of the sediment extracts as well as induction of malignant hepatic tumors in rainbow trout and induction of DNA adducts in mammalian cells in an *in vitro* assay. The chemical analysis detected high concentrations of PAH compounds in this sediment extract. The researchers reported that other aromatic compounds present could have possibly caused the mutagenicity and carcinogenicity.

Lan et al. (1991) used the Ames test to detect mutagenicity of river sediment samples that gave a positive response in the SOS chromotest. The SOS chromotest used *Escherichia coli* and its SOS repair response to DNA damaging agents. The SOS chromotest with metabolic activation indicated a higher positive genotoxic response in the industrial-influenced sites over the agriculturally influenced sites. Without metabolic activation, the SOS chromotest indicated a negative genotoxic response. When the three positive response samples from the SOS chromotest were tested using TA98 and TA100 with S9 metabolic

activation, the results indicated a negative mutagenic response. The results suggested that the SOS chromotest was more sensitive than the Ames test in identifying progenotoxic agents at certain concentrations. Some of the sediment extracts tested using the Ames test appeared to be also toxic.

Durant et al. (1992) conducted Ames testing and human lymphoblast mutation assay studies on sediment samples collected in a watershed that had been continually contaminated with organic and inorganic wastes from industries since before the mid-nineteenth century. The researchers found that there was a lack of correlation between the two species' responses to possible mutagens. The Ames test indicated that 20 out of 32 sediment samples gave a positive mutagenic response, while the human cells only indicated two out of 32 gave a positive mutagenic response. This response was consistent with wide differences in sensitivities that had been observed in chemical mutagens. The researchers found that the sediment mutagenicity was not significantly correlated with proximity to known hazardous waste sites, present or former industry, or with toxic metals concentration. The conclusions of this study were that mutagens were wide spread throughout the watershed or reflected an origin that was not associated with identified industrial releases.

The Ames assay was used by Fernandez et al. (1992) in directing the chemical characterization in a three-level bioassay-directed chemical fractionation of coastal sediments. The preparation of organic extract fractions of coastal sediments included gel permeation chromatography (GPC), normal phase (NP-LC), and reversed phase liquid chromatography (RP-LC). The Ames test was conducted on the resulting fractions using

TA98 with S9 metabolic activation. As GPC was applied to the sediment extracts, most of the genotoxic compounds were recovered in a single fraction. This fraction was further separated by NP-LC into 35 fractions. The intermediate fractions recovered were separated again by RP-LC into 10 subfractions. Mutagenicity was indicated in the 10 subfractions and identified as nitrated arenes which contain four to six aromatic rings. Four to six-ringed PAHs were identified in the low-polarity NP-LC fractions. Polar-substituted nitroarenes and azaarenes were identified in the polar fractions as mutagenic. The distribution of the identified mutagenic compounds in the coastal sediments elucidated the perplexity of directly assessing coastal environments.

Mutatox™

Background and Theory

The development of a sensitive, simple bioluminescence test (BLT) for detection of mutagenic compounds was reported in 1980 by Ulitzur et al. Mutagenicity was determined by the ability of test compounds to cause an increase in the reversion rate of a dark mutant strain of a luminous bacteria to the luminescent state. A spontaneous, dark variant strain (8SD18) of a luminous bacteria, *Photobacterium leiognathi* (BE8), was selected because of characterization of very low levels of *in vivo* luminescence and cellular luciferase content (Nealson and Hastings 1979) and exhibited a low frequency of spontaneous reversion (10^{-6}) to the luminescent state. Luciferase is an enzyme that catalyzes the oxidation of luciferin, a species-specific pigment in luminescent organisms that produces almost heatless light when undergoing oxidation. Studies (Ulitzur et al. 1980; Ulitzur and Weiser 1981; Weiser et al. 1981)

measuring luminescence with a scintillation counter were conducted using three groups of genotoxic agents to check the response of the test system.

In one study, known base-pair substitution and frameshift mutagens were tested using minute amounts both with and without metabolic activation (S9). The presence of both base-pair substitution and frameshift mutagens were detected using the bioluminescence test, and the reversion frequency to luminescence was increased so that it was possible to detect some mutagens at 100 times lower than the Ames assay (Ulitzur et al. 1980).

In a second study, chemicals that were considered DNA intercalating agents were tested (Ulitzur and Weiser 1981). Intercalation occurs when a molecule other than the nucleotide is included in the DNA structure, causing the wrong structure to be copied. This group of chemicals caused nearly complete restoration of the *in vivo* luminescence of the dark-variant strain but did not increase the reversion rate at the genetic level. This group of chemicals was also able to induce luminescence in wild-type cells in the absence of an added inducer (Ulitzur and Weiser 1981).

In a third study conducted by Weiser et al. (1981), DNA damaging agents and DNA-synthesis inhibitors were tested using the method described by Ulitzur et al. (1980). Although there was a high level of *in vivo* luminescence of the treated dark-mutation cell, the DNA damaging agents and DNA-synthesis inhibitors did not result in genetically luminous revertants. These genotoxic agents were shown to be capable of inducing only phenotypic reversion of the dark-variant cells and that

possibly they could cause inactivation of the repressor of the luciferase operon through SOS repair system.

In these initial studies, the genetic system of the luminous bacteria was not known. Ulitzur et al. (1980) assumed from the high level of reversion that the mutational target involved a forward mutation rather than a reversion system. This assumption was supported by the observation that both base-pair substitution and frameshift mutagens act as effective mutagens. A working model of the effect of chemicals on the bacteria was hypothesized by Ulitzur et al. (1980).

Ulitzur (1986) reported that increasing evidence suggested the transduction of the luminescence operon was under continuous repression probably by an intercistronic repressor. He theorized that restoring the luminescence of the repressed dark mutant was accomplished by three independent events: (1) blocking the formation of the repressor; changing its, or the operator site's structure, (2) inactivating the repressor of the luminescence system, and (3) changing the configuration of the DNA, allowing unrepressed transcription of the luciferase operon. Different genotoxic agents caused the above-mentioned events. Direct mutagens were expected to cause the first event, while DNA-damaging agents such as UV irradiation and DNA synthesis inhibitors were found to be associated with the second event. In restoring the luminescence of the dark mutant, the strongest and fastest way was through DNA intercalating agents such as caffeine interacting with DNA bases to cause configurational changes. There was a difference in the timing of the beginning of the induced luminescence in the dark mutant which was exhibited in the different end points and mechanisms of action. Another

difference with these groups of genotoxic agents was that the direct mutagens initiate the appearance of genetically stable revertants while the SOS inducing agents and the DNA intercalating agents resulted in only phenotypic reversion of the luminescence.

In 1988, the Microbics Corporation continued the development of the bioluminescence test and trade-marked it as the Mutatox™ genotoxicity test system. At this time, Microbics developed an engineered strain of *Photobacterium phosphoreum* with selective capability of screening genotoxic chemicals or complex mixtures (Bulich, Pers. comm.).

Validation

Elmore and Fitzgerald (1990) reviewed data from a coded validation study of 66 National Toxicology Program chemicals with known carcinogenic activity using Mutatox™ and compared these data to those data obtained from other genotoxic assay studies. These researchers found that Mutatox™ did not appear as predicative of carcinogenicity as the Ames test because of sensitivity and specificity responses to 52 chemicals. When comparing data from assays such as the sister chromatid exchange, chromosomal aberrations, and mouse lymphoma assay to Mutatox™ or the Ames test, the researchers found that the Ames test produced approximately the same sensitivity but was more specific than Mutatox™. Teratogenic data from 28 of the 66 chemicals was evaluated and compared to the Mutatox™ and Ames test data for these chemicals. Mutatox™ appeared to be twice as sensitive as the Ames in indicating teratogens. This indication may be attributed to the fact that Mutatox™ can detect chemicals that alter gene expression.

Sediment Studies

Several researchers (Kwan and Dutka 1990; Kwan et al. 1990; Dutka et al. 1991) have used the Mutatox™ test in assessing complex environmental mixtures. Kwan and Dutka (1990) utilized Mutatox™ and two other tests to determine toxicity and genotoxicity in organic and aqueous extracts from river sediments. Mutatox™ indicated the presence of genotoxic chemicals in both water-extracted and solvent-extracted (DMSO, methanol) sediments. Mutatox™ was utilized in its first field application in a series of tests to assess contaminated river water and sediment samples (Kwan et al. 1990; Dukta et al. 1991). Results of this study indicated that Mutatox™ was extremely responsive in identifying genotoxic chemicals in the water and sediment extract samples. Mutatox™ also indicated that the genotoxic chemicals were distributed throughout the studied watershed (Dutka et al. 1991).

Johnson further evaluated the Mutatox™ assay in two studies (1992a, 1992b). The first study (1992a) evaluated Mutatox™ with S9 activation in testing a series of known progenotoxic and nongenotoxic chemicals. All eight progenotoxic chemicals indicated genotoxic activity, with seven out of the eight demonstrating a dose response. With more than three positive responses in each dilution series of the Mutatox™ testing, the seven progenotoxic chemicals were classified as genotoxic. The six nongenotoxic chemicals indicated no genotoxic response. Optimum testing conditions for conducting Mutatox™ were determined using two progenotoxic chemicals, 2-aminoanthracene and B[*a*]P. The test conditions considered were S9 concentrations, preincubation temperatures, and incubation periods. The results of this validation

testing indicated optimal conditions were 0.4 mg/ml rat S9 concentration with preincubation at 37° C for 30 minutes which would be followed by an 18 (16-24) hour incubation period at 23° C. Also in this study, the Ames test and Mutatox™ were compared in testing the above-mentioned groups of chemicals. Sensitivity, specificity, and predicative value of each test were compared and Mutatox™ demonstrated that it could be a valuable screening tool in detecting genotoxic compounds in different sources.

Johnson (1992b) conducted a second study using Mutatox™ with S9 activation (S9) to determine genotoxic potential in Great Lakes sediment samples. Results indicated that 23 out of 28 study sites were genotoxic, four sites were considered suspect and one site was a negative response. Again, the Ames test and Mutatox™ were compared in studying the sediment samples. Results for both tests compared in indicating genotoxic agents in 27 of the 28 study sites.

Ho and Quinn (1993) used Mutatox™ in assessing the bioassay-directed fraction of organic contaminants from a contaminated estuarine sediment. Chemical fractionation by polarity of the sediment extracts was accomplished by silica gel chromatography followed by C_{18} reverse phase high performance liquid chromatography (HPLC). Each of the fractions were analyzed to determine the presence or absence of mutagenic compounds. Mutatox™ was conducted with S9 activation and exhibited a positive mutagenic response in four of the eight HPLC fractions. The GC-MS analysis of the four mutagenic fractions detected known mutagenic PAH such as benzo[*a*]pyrene in two of the four fractions.

The GC-MS analysis of the other two fractions indicated no known mutagenic compounds.

Continuing Research

The Microbics Corporation is continuing research and further development of Mutatox™. The bacterial test strain currently used in the Mutatox™ test system is a dark mutant (M169) of *Vibrio fisheri*, a marine bioluminescent bacterium (Bulich 1992). Bulich (1992) of Microbics Corporation has conducted research focusing on the analysis of the steps which lead to the reappearance of luminescence in this dark strain. The main lesion responsible for the low light of this strain has not been clarified at this time. After the dark variant of *V. fisheri* goes through usual induction of the *lux* gene system, the generated luminescence is approximately 10^6 times lower than the wild-type strain. The main lesion of the dark variant is thought to come from a lesion in the regulatory system, rather than a mutation of one of the *lux* structural genes. This assumption was reinforced when the entire *lux* gene system of *V. fisheri* was introduced into M169 cells with the assistance of a RP4-*lux* plasmid, and the M169 cells did not luminesce. When the RP4-*lux* plasmid was re-transferred into a wild-type strain of *E. coli*, the bacteria yielded extremely luminescent cells. Observations were made that when M169 cells are deprived of nitrogen, they go through complete induction of the *lux* gene system. A similar observation has been made in *E. coli* strains that carry the entire *lux* gene system of *V. fisheri*. The complete induction of the *lux* system was credited to the effect of starvation on the formation of certain proteins called groESL and sigma-32 (HtpR). Based upon this

information, theories of how different genotoxic agents could restore *lux* gene expression in the M169 strain of *V. fisheri* were advanced. These theories proposed by Microbics Corporation in a model include: "activation of the SOS repair system by DNA damaging agents or DNA synthesis inhibitors; formation of auxotrophic mutants for amino acids which can lead to cell starvation and thus activation of HtpR and GroESL proteins; production of *lexA* mutants indicating lower affinity to the *lux*-DNA binding site; formation of *lux* mutants with an altered *LexA* DNA binding site; mutational events that would increase the cellular pool of HtpR and GroESL proteins; and by-pass the *lux* regulatory control system by DNA intercalating agents" (Bulich 1992).

MATERIALS AND METHODS

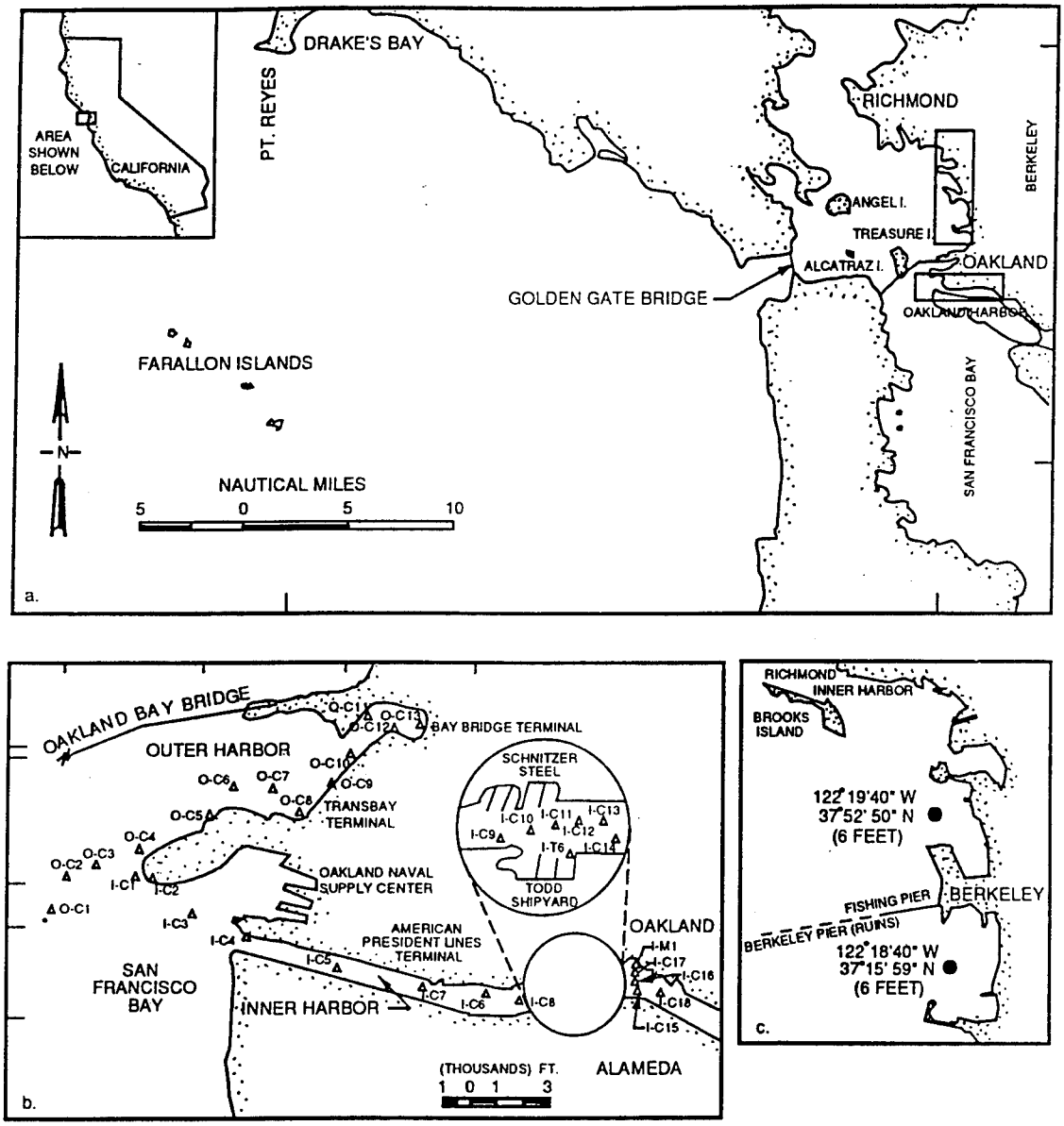
Sediment Collection and Handling

Oakland Harbor Sediments

Four sediments from the Oakland Harbor, San Francisco, CA, were collected by Battelle/Marine Sciences Laboratory of Sequim, Washington, using a 30.5 cm Vibratory Hammer Corer. A single core section at each station for the sediments was used to prepare a composite sample of each sediment. Locations of the sediment collection sites in Central San Francisco Bay are shown in Figure 1. Figure 1a depicts the entire areas that were sampled for the four composited sediments. In Figure 1b, Oakland Inner (IC) consisted of composited cores from 18 stations, the Oakland Outer (OC) consisted of composited cores from 13 stations, and the Oakland Hot consisted of composited cores from two stations (IT-6 and IM-1). The total volume of each composited sediment was 208 L. The cores were mixed in an epoxy-lined drum aboard the boat and stored at 4° C.

The Oakland Reference sediment was collected in a shoal area of the East Bay at a depth of 2-3 m as shown in Figure 1c. A benthic sled device was used because it could be adjusted to skim the top few centimeters of sediment when towed behind a small boat. Sediments were collected at two sites and composited in an epoxy-lined 208 L drum.

Composites collected from the Oakland Inner, Outer, and Hot locations were shipped by refrigerated truck to Waterways Experiment Station (WES), Vicksburg, MS. The Oakland Reference sediment composite was shipped to WES in insulated coolers by overnight air freight. All the Oakland sediments received at WES were stored at 4° C until use.



Source: McFarland et al. (In Press)
Figure 1. Location of the Sediment Collection Sites in Central San Francisco Bay. (a) Overview (b) Oakland Inner, Outer, and Hot Sediment Collection Sites (c) Oakland Reference Sediment Collection Sites.

New York Sediments

Four sediments were collected in the New York/New Jersey waterways using a clamshell grab aboard a boat. Maps were unavailable that would describe the sediment collection sites. The first sediment was collected at Gowanus Creek off the 23rd Street Pier (New York City) at a depth of 8.23 m. Sediment from the Red Hook Channel of the Upper New York Bay, south of the Statue of Liberty, was collected from a depth of 14.33 m. Sediment from Trembley Point of Arthur Kill was then sampled from a depth of 5.79 m. The reference sediment was collected from Sandy Hook, NJ, from a depth of 25.30 m. All sediment samples were placed in new 18.92 L polyethylene buckets, placed in ice chests and shipped to WES by overnight delivery. Upon arrival at WES, each of the four sediments was sieved to remove debris, composited, and stored at 4° C until use.

Hamlet City Lake Sediment

Hamlet City Lake sediment from Hamlet City, NC, was collected using a crane and drag-line bucket system. A map was unavailable to describe the collection sites. Fifteen 208 L barrels of Hamlet City Lake sediment were collected from the north side of the lake. This area includes a section of the lake near a railroad and former railroad work yard and is east of a sewage treatment plant. During the sediment collection, the crane moved along the shoreline approximately 68.62 m. The drag-line removed surface sediment to an approximate depth of 0.76 m. The barrels were transported in a refrigerated truck to WES, composited, and stored at 4° C until use in laboratory tests.

Chicago Confined Disposal Facility (CDF)

Sediment samples were taken from Station 4 of the Chicago CDF, a freshwater area at the mouth of the Calumet River, Chicago, IL. A map was unavailable to describe the sediment collection sites. Two geographic locations at Station 4 were sampled to obtain hand cores. Station 4A was approximately 5 m inshore under water, and Station 4B was approximately 1.5 m inland of Station 4A. A 5 cm diameter aluminum core tube was pushed into the sediment at each location. The top 10 cm of the core were placed into glass jars, stored on ice, and shipped by overnight express to WES. The sediment samples were stored at 4° C until use.

Reagents

All solvents used were gas chromatography grade or pesticide grade and obtained from Baxter Scientific, McGaw Park, IL; Fisher Scientific, Pittsburgh, PA; or Sigma Chemical Company, St. Louis, MO. All reagents used in buffers were American Chemical Society grade or better and obtained from Sigma Chemical Company or Aldrich Chemical Company, Milwaukee, WI, unless otherwise indicated.

Sediment Extraction

Six of the 10 sediments including the four New York sediments (Gowanus Creek, Arthur Kill, Red Hook, and Sandy Hook), Hamlet City Lake sediment, and the Oakland Outer sediment were extracted and analyzed by the Analytical Laboratory Group (ALG) of the Environmental Laboratory (EL), WES. The four Oakland Harbor sediments (Oakland Inner, Outer, Reference and Hot) were extracted and analyzed by Battelle Pacific Northwest Laboratories (BPNL) of Richland, WA. The Chicago CDF sediment

was extracted by the Aquatic Contaminants Team (ACT), WES and analyzed by the ALG, WES.

Soxhlet Extraction

Wet sediment was extracted according to EPA Method 3540 (USEPA 1979) using 1:1 acetone:hexane (ALG) or dichloromethane (DCM) (BPNL). A 30 g aliquot of each sediment sample was weighed into cellulose thimbles (Thomas Scientific, Swedesboro, NJ) and combined with 30 g precleaned dried sodium sulfate (Thomas Scientific). The soxhlets were cycled at four cycles/hr for approximately 20 hrs. After cooling, the extraction solvent remaining in each apparatus was transferred into its boiling flask.

Drying of Extracts

The extracts were then passed through sodium sulfate drying columns to remove any remaining water (EPA Method 3540) and were collected in Zymark concentration tubes (Zymark Corporation). The columns were rinsed with 100 ml of the extraction solvent to complete the volumetric transfer.

Concentration

The extracts were concentrated on a Zymark TurboVap automatic evaporator (Zymark Corporation, Hipkinton, MA). The TurboVap was set at 4 psi using ultrapure (grade 5) nitrogen (Nordan Smith Welding Supplies, Vicksburg, MS), and the water bath was set at 35° C. The extracts were concentrated to a final volume of approximately 3 ml.

Silica Gel Clean-up

The concentrated extracts were cleaned for PAH analysis using silica gel columns (Warner 1976). The BPNL used alumina and copper for

the clean-up step. One 25 cm chromatography column was assembled for each sediment extract. A slurry of 15 g silica gel (Supelco, Inc., Bellefonte, PA), and 30 ml hexane was rinsed into each column and allowed to settle. A 40 ml portion of DCM followed by 40 ml hexane was then eluted through the column at a rate of approximately 1 drop/sec. A 4 cm layer of prepared sodium sulfate was placed on the column to remove any excess water from the extract, and hexane was eluted to 0.5 cm above the sodium sulfate level.

The concentrated extract was quantitatively pipetted onto the prepared columns. After penetration of the columns by the extract, 40 ml hexane was added to each and was eluted into a waste beaker to remove saturated hydrocarbons. Eighty-six milliliters of 1:4 DCM:hexane were eluted into a clean Zymark tube in preparation for evaporation. The cleaned extracts were concentrated to a final volume of less than 1.0 ml for use in chemical analysis.

Sediment Chemical Analysis

The 10 sediments were analyzed for the 16 PAHs shown in Figure 2. The ALG, WES analyzed the Oakland Outer, Hamlet City Lake, Gowanus Creek, Red Hook, Arthur Kill, and Sandy Hook sediments as well as the Chicago CDF sediment extract. PAHs were analyzed by GS-MS according to EPA Method 8270 (USEPA 1986) using a Hewlett Packard HP 5880 GC equipped with an HP 5970 MS detector. Oven temperature was programmed to increase at a rate of 6° C/min from 35° C to the final temperature of 325° C. The carrier gas was Helium at approximately 25 cm/sec flow-rate. The column used was a Hewlett Packard Ultra 2 column 25 m,

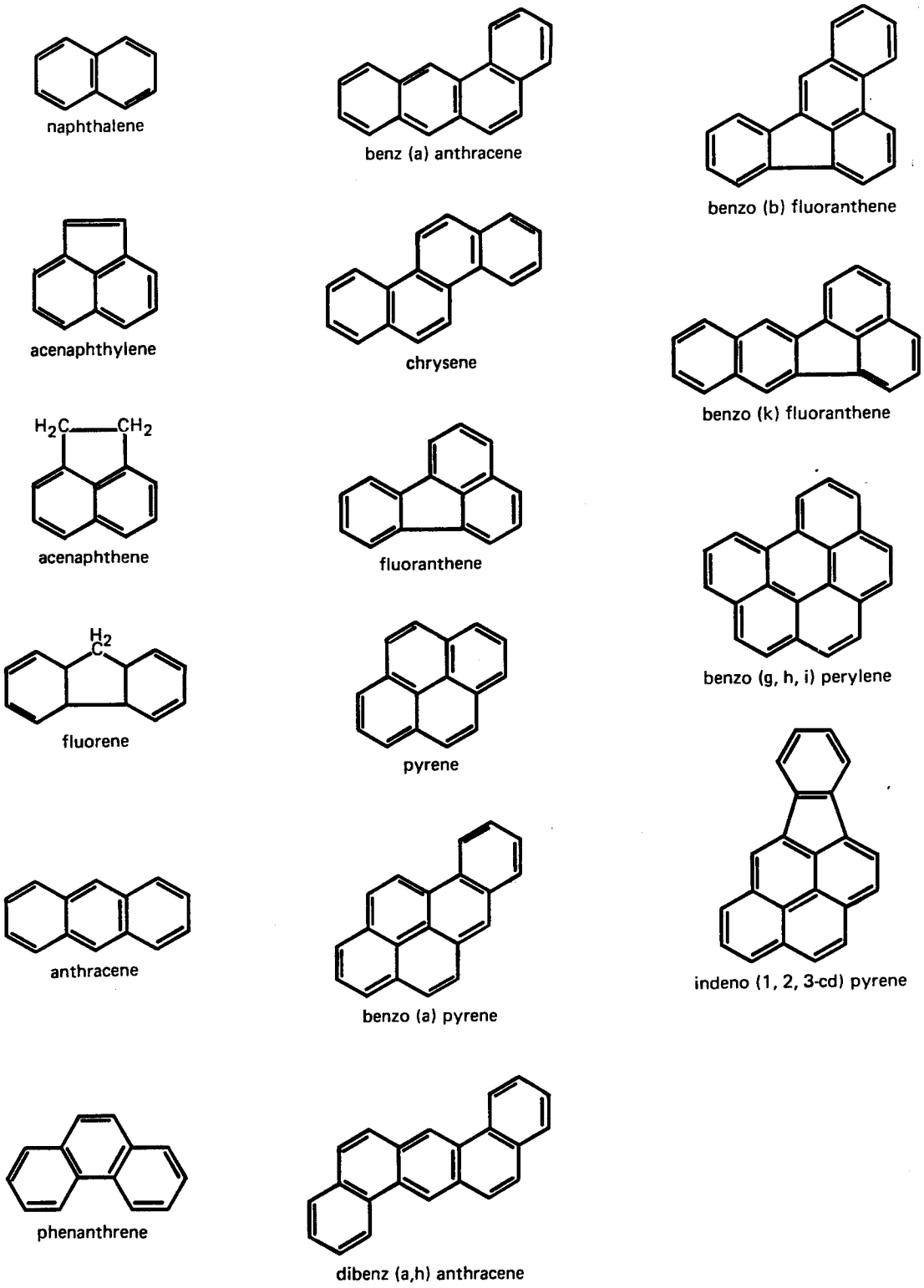


Figure 2. Names and Structures of the 16 PAHs analyzed

0.32mm i.d., and 0.52 μm film thickness. The EPA method 8000 was used as quality assurance/quality control (QA/QC) guidance for PAH analysis for all sediments except Chicago CDF (USEPA 1986).

The BPNL analyzed the four Oakland Harbor sediments, Reference, Inner, Outer, and Hot. The methods used were similar to ALG, WES with the following exceptions: a) the extracts were analyzed for PAH compounds following EPA Method 8270 (USEPA 1986) using GC-MS on a Hewlett Packard HP 5890 GC and an HP 5970 MS detector; b) the column used was a J&W DB-5 30 m x 0.25 mm i.d. x 0.25 μm film thickness.

Sediment Extraction for the Ames Assay

The 10 sediments were extracted using a modification of EPA Method 3540 (USEPA 1986). Six 30 g aliquots of each sediment sample were weighed into cellulose thimbles, and each was combined with 30 g precleaned dried sodium sulfate. The six soxhlets were cycled at four cycles/hr for approximately 20 hrs. The extracts were then passed through sodium sulfate drying columns to remove any remaining water and were collected in Zymark concentration tubes. The extracts were concentrated on a Zymark TurboVap automatic evaporator, combined and concentrated to 60 ml final volume. A 30 ml aliquot was removed, split into three 10 ml portions, and stored in teflon-capped amber glass vials for Ames mutagenicity testing as crude samples.

The remaining 30 ml of extract was concentrated to approximately 12 ml on the TurboVap and cleaned using silica gel as described previously. The cleaned extracts were then concentrated again and combined to a final volume of 30 ml and divided into three 10 ml samples.

The two sets of sediment extracts (cleaned and crude) were solvent exchanged into filter-sterilized dimethylsulfoxide (DMSO) for use in the Ames plate incorporation test. To prepare the 1:1 transfer, a 1 ml portion of extract was transferred by volumetric pipet to a 5 ml microvial and concentrated to near-dryness under ultra-pure nitrogen. A 1 ml aliquot of DMSO was added volumetrically, and the sample was reduced to a final volume of 1.0 ml under nitrogen. The 4:1 transfer was prepared similarly using a starting volume of 4 ml extract and reducing to a final volume of 1.0 ml in DMSO. The completed transfers were stored in a microvial at -9° C in a freezer until use.

Weights of the sediment extract were determined by allowing triplicate 100 μl aliquots to evaporate from tared aluminum weighing pans and weighing the residue on a Cahn C-31 microbalance (Cahn Instruments, Cerritos, CA).

Ames Assay

Bacterial Cultures

Salmonella typhimurium strains TA100 and TA98 were obtained from Dr. Bruce Ames, University of California, Berkeley. Upon receipt, the bacterial tester strains were confirmed for their genetic mutations of histidine requirement, *rfa* character (crystal violet sensitivity), *uvrB* deletion (ultraviolet light sensitivity), and R-factor sensitivity (ampicillin response). Frozen permanent cultures (1×10^{-9} concentration) of each bacterial strain were prepared (Maron and Ames, 1983).

Plate Incorporation Test

The Ames plate incorporation test was performed as described in Maron and Ames (1983). Cultures of bacteria were prepared by adding 100 µl of bacteria taken from the thawed frozen permanent culture to 25 ml sterile oxoid broth (0.625 g Oxoid #2 medium [Unipath, Odgenburg, NY] in 25 ml distilled water) in a sterile 125 ml culture flask. The prepared flasks were incubated at 37° C in a shaking water bath for 12-16 hr. Culture tubes containing 2.0 ml of top agar (0.5 mM histidine/biotin, 0.6% agar, 0.5% NaCl in distilled water) were placed in a water bath at 45° C prior to and during testing. A 5% mixture of Arochlor 1254-induced rat liver S9 (Molecular Toxicology, Annapolis, MD) was prepared in 0.2 M phosphate buffer, pH 7.4, with 0.1 M nicotinamide adenine dinucleotide phosphate (NADP), 1 M glucose-6-phosphate (G-6-P), 0.4 M $MgCl_2$ and 1.65 M KCl and made up to volume with sterile water. The S9 preparation was filter-sterilized (x2) using sterile 0.45 µm and 0.22 µm Millipore Millex filter units (Millipore Corporation, Bedford, MA) and held on ice prior to and during testing.

The positive control for testing bacterial strain TA100 was sodium azide (2 mg/plate) and for TA98 was 2,4,7-trinitro-9-fluorenone (2 µg/plate). Benzo[*a*]pyrene (0.2 µg/plate) was used as a control to check the activity of the S9 in each series of tests with each bacterial strain.

Reagents were added to sterile culture tubes in the order (1) DMSO, (2) sediment extract or positive control, (3) 100 µl aliquot of bacteria, (4) 500 µl aliquot of S9 or phosphate buffer. The DMSO volumes were varied with volume of sediment extract to give a constant

addition of 50 μl. Five dose levels of the sediment extract were prepared. Dose levels 1-3 were composed of 2, 10 or 25 μl of the 1:1 solvent-exchanged extract and an extra addition of DMSO to give a total volume of 50 μl. The fourth dose level consisted of 50 μl of the 1:1 solvent-exchanged extract alone. The fifth dose consisted of 50 μl of the 4:1 solvent-exchanged extract, equivalent to 200 μl of the 1:1 extract. The culture tubes were vortexed after each addition, and the mixture combined with prepared top agar, vortexed, and poured on a minimal glucose agar (MGA) (1.5% agar, Vogel-Bonner medium E [Vogel and Bonner, 1956], 40% glucose in distilled H_2O) plate (1 tube/MGA plate). The MGA plates were prepared in triplicate at each of the five dose levels. After allowing one hour for solidification of the top agar layer, the plates were inverted and incubated for 48 h at 37° C in a Scientific Products Model I-83 incubator (Baxter Scientific, McGaw, IL).

Plates were examined for the presence of background lawn using a Cambridge Instruments Photozoom inverted microscope (Buffalo, NY) at 10X magnification. Revertant colonies on each plate were counted using a Dynatech Autocount colony counter (Dynatech Laboratories, Inc., Chantilly, VA). A mutagenic response was considered positive when two consecutive doses had average number of revertants twice the solvent control for that experiment or in the last non-toxic dose and demonstrated a linear dose response (Modified two-fold rule) (Chu et al. 1981).

Least-squares analysis was used to calculate the slope of the linear portion of the dose response curves, including the zero dose.

INSTAT (GraphPad Software, San Diego, CA), a statistics software package, was used to determine the least-squares analysis.

Mutatox™ Methods

Dr. Anthony Bulich of Microbics Corporation, Carlsbad, CA, conducted the standard protocol for Mutatox™ genotoxicity assay using 20 sediment extract samples sent from WES (Microbics Corporation 1993). The sediment extract samples (crude and clean) were a 1:1 sediment extract:DMSO solvent exchange prepared as above for the Ames assay. The Mutatox™ test was conducted with and without metabolic activation. A 0.5% S9 medium was used for testing with metabolic activation. The positive controls used in the Mutatox™ testing were B[*a*]P, aflatoxin, and 2-aminoanthracene for S9 metabolic activation samples. Phenol and nitrosoguanidine were used as positive controls for the direct-acting samples. A solvent control (DMSO) was also run with the sediment extract samples during testing.

Vials of Mutatox Medium and Mutatox S9 Medium were each prepared by adding 15 ml of cold (5° C) Mutatox Reconstitution Solution to each vial. Cuvette supports holding up to 50 cuvettes were set up to accommodate the extract samples. Each sediment extract sample had 10 cuvettes and was set up in a serial dilution. A 250 μl portion of either Mutatox Medium or Mutatox S9 Medium was pipetted into the first nine cuvettes for each sediment extract. A 500 μl aliquot of either Mutatox Medium or Mutatox S9 Medium was added to the 10th cuvette of each sediment sample. The sediment extract was added in 10 μl aliquots to 500 μl of test medium in the 10th cuvette. The samples were set up in a series of 10 in a 1:1 dilution.

A 1.1 ml portion of Mutatox Reconstitution Solution was added to two cuvettes to each receive a bacterial pellet. An aliquot of 10 μl of bacterial reagent was pipetted into each prepared cuvette having Mutatox Medium and Mutatox S9 Medium. When the series of samples were prepared in the Mutatox S9 Medium, these samples were mixed by shaking the prepared cuvette support block, covered with parafilm® and aluminum foil, and incubated for 45 minutes at 35° C. Both the Mutatox S9 Medium samples and the Mutatox Medium samples were incubated at 27° C for a total of 24 hours. The cuvettes in each medium were read at 16, 20, and 24 hours using the Microbics M500 Toxicity Analyzer. A sediment extract sample was considered genotoxic when light levels produced were two times or greater than the control. The data was interpreted using the modified two-fold rule (Chu et al. 1981).

RESULTS

Sediment Analysis

The concentrations of 16 PAHs measured in the four Oakland Harbor sediment are listed in Table 2. The concentrations of the PAHs for each sediment are reported in ng/g dry weight with the standard deviation. All four Oakland sediments contained each of the 16 PAHs analyzed. Mean concentrations of the 16 PAHs were higher in the Hot (apparently by one to three orders of magnitude) than in the other three sediments. The Oakland Hot sediment contained high concentrations of the 4 to 6-ringed PAHs. The concentrations of the 16 PAHs in the Oakland Reference and Outer sediments were generally comparable. Again, the 4 to 6-ringed PAHs had the highest concentrations in these two sediments. The Oakland Inner sediment generally had lower concentrations of the 16 PAHs than the other three sediments, but it still showed 4 to 6-ringed PAHs with higher concentrations than the 3-ringed compounds.

The concentrations of the 16 PAHs analyzed in the four New York Sediments are listed in Table 3. The concentrations of the PAHs for each sediment were measured in μg/g dry weight with the standard deviation (SD). Of the four New York sediments, Sandy Hook was the only sediment in which the concentration values of all 16 PAHs were below the instrument detection limit values. The PAH, dibenz[*a*,*h*]anthracene, had a concentration level that was below the instrument detection limit in the other three sediments. In the Red Hook sediment, naphthalene, acenaphthylene, acenaphthene, and fluorene had concentrations below the instrument detection limit. In the Arthur Kill sediment, the concentrations of naphthalene, acenaphthylene, acenaphthene, anthracene,

Table 2. PAH Chemical Analysis of Oakland Harbor Sediments

Concentration in ng/g dry wt (N = 5) ± Standard Deviation				
PAH	Oakland Reference	Oakland Inner	Oakland Outer	Oakland Hot
Naphthalene	19.21 ± 10.99	3.64 ± 0.45	19.86 ± 1.14	550.08 ± 85.16
Acenaphthylene	5.29 ± 8.24	1.34 ± 0.36	6.68 ± 1.43	69.31 ± 24.12
Acenaphthene	1.62 ± 0.04	1.80 ± 0.49	8.58 ± 0.94	1238.76 ± 248.37
Fluorene	1.62 ± 0.07	1.66 ± 0.40	9.71 ± 1.55	533.77 ± 178.89
Anthracene	27.33 ± 9.00	3.68 ± 1.16	33.29 ± 20.43	1766.12 ± 856.76
Phenanthrene	111.45 ± 36.74	11.34 ± 2.67	68.33 ± 12.23	5053.03 ± 1008.251
Benz[*a*]anthracene	115.77 ± 20.94	19.66 ± 3.94	58.77 ± 14.52	2408.59 ± 579.44
Chrysene	105.58 ± 20.05	21.76 ± 6.05	71.29 ± 19.38	3203.56 ± 733.22
Fluoranthene	242.46 ± 40.07	28.46 ± 6.38	133.34 ± 19.11	7122.13 ± 1479.86
Pyrene	251.72 ± 41.40	45.78 ± 10.60	210.92 ± 25.80	7329.70 ± 1458.67
Benzo[*a*]pyrene	193.32 ± 37.36	46.80 ± 16.84	122.60 ± 5.76	4306.25 ± 757.03
Dibenz[*a,h*]anthracene	5.09 ± 7.80	6.66 ± 1.50	13.23 ± 1.25	432.05 ± 89.94
Benzo[*b*]fluoranthene	223.11 ± 32.48	79.80 ± 17.45	193.13 ± 10.53	7368.13 ± 1347.63
Benzo[*k*]fluoranthene	223.11 ± 32.48	79.80 ± 17.45	193.13 ± 10.53	7368.13 ± 1347.63
Benzo[*g,h,i*]perylene	128.08 ± 33.67	51.28 ± 14.38	136.87 ± 24.77	3260.84 ± 685.01
Ideno[*1,2,3-c,d*]pyrene	127.16 ± 26.14	38.86 ± 13.35	125.87 ± 4.82	3600.52 ± 671.33

Table 3. PAH Chemical Analysis of New York Sediments

Concentration in μg/g dry wt (N = 3 or 4) ± Standard Deviation				
PAH	Sandy Hook (3)	Red Hook (3)	Arthur Kill (4)	Gowanus Creek (3)
Naphthalene	0.40[1] ± 0.01	0.32[1] ± 0.10	0.42[1] ± 0.30	0.80 ± 0.70
Acenaphthylene	0.40[1] ± 0.01	0.17[1] ± 0.22	0.67[1] ± 0.01	0.95[1] ± 0.04
Acenaphthene	0.40[1] ± 0.01	0.30[1] ± 0.08	0.53[1] ± 0.28	0.59[1] ± 0.25
Fluorene	0.40[1] ± 0.01	0.28[1] ± 0.04	0.54[1] ± 0.27	0.19[1] ± 0.12
Anthracene	0.40[1] ± 0.01	0.94 ± 0.07	0.44[1] ± 0.26	1.21 ± 0.51
Phenanthrene	0.40[1] ± 0.01	2.07 ± 0.46	0.65 ± 0.47	1.22 ± 0.44
Benz[*a*]anthracene	0.40[1] ± 0.01	1.57 ± 0.32	0.81 ± 0.26	4.77 ± 1.50
Chrysene	0.40[1] ± 0.01	2.13 ± 0.11	1.25 ± 0.38	5.87 ± 1.70
Fluoranthene	0.40[1] ± 0.01	2.47 ± 0.57	1.56 ± 1.14	5.77 ± 2.53
Pyrene	0.40[1] ± 0.01	3.83 ± 1.15	1.50 ± 0.79	7.97 ± 3.54
Benzo[*a*]pyrene	0.40[1] ± 0.01	0.99 ± 0.12	0.57[1] ± 0.14	2.06 ± 0.97
Dibenz[*a,h*]anthracene	0.40[1] ± 0.01	0.43[1] ± 0.01	0.67[1] ± 0.01	0.94[1] ± 0.04
Benzo[*b*]fluoranthene	0.40[1] ± 0.01	1.17 ± 0.31	0.74 ± 0.08	2.30 ± 0.82
Benzo[*k*]fluoranthene	0.40[1] ± 0.01	1.17 ± 0.31	0.74 ± 0.08	2.30 ± 0.82
Benzo[*g,h,i*]perylene	0.40[1] ± 0.01	0.35[1] ± 0.27	0.51[1] ± 0.24	0.84 ± 0.92
Ideno[*1,2,3-c,d*]pyrene	0.40[1] ± 0.01	0.28[1] ± 0.22	0.48[1] ± 0.25	0.81 ± 0.86

[1] All concentrations observed were below the detection limits and values identified are the instrument detection limits.

benzo[a]pyrene, benzo[*g,h,i*]perylene, fluorene, and ideno[*1,2,3,-c,d*]pyrene were below the instrument detection levels. The concentrations of acenaphthylene, acenaphthene, and fluorene were below the instrument detection limits in Gowanus Creek sediment. In Red Hook, Arthur Kill, and Gowanus Creek sediments, the 4 to 6-ringed PAHs generally had the highest concentrations of the 16 PAHs.

The PAH chemical analysis of the freshwater sediments, Chicago CDF and Hamlet City Lake, are listed in Table 4. The concentrations of the PAHs in the single replicate of the Chicago CDF sediment that was analyzed are reported in μg/g wet weight. Hamlet City Lake sediment was measured in μg/g dry weight with the standard deviation. In the Chicago CDF sediment, acenaphthylene and dibenz[*a,h*]anthracene had concentrations that were measured below instrument detection limits. In the Hamlet City Lake sediment, acenaphthylene, acenaphthene, and dibenz[*a,h*]anthracene had concentrations measured below the instrument detection limits. In both the Chicago CDF and Hamlet City Lake sediment, the PAHs, fluoranthene and pyrene, had the highest concentrations.

Ames Assay Results

Each of the 20 sediment extracts was tested using the Ames plate incorporation test with and without metabolic activation (S9) as previously described. The *Salmonella typhimurium* strains TA98 and TA100 were used in evaluating the sediment extracts for mutagenic potential.

Table 4. PAH Chemical Analysis of the Freshwater Sediments

PAH	Chicago CDF* (N = 1)	Hamlet City** (N = 4)
Naphthalene	0.21	0.19 ± 0.13
Acenaphthylene	<0.11	<1.8
Acenaphthene	0.08	<1.8
Fluorene	0.11	0.09 ± 0.10
Anthracene	0.15	0.07 ± 0.07
Phenanthrene	0.74	0.89 ± 1.07
Benz[*a*]anthracene	0.30	0.58 ± 0.88
Chrysene	0.32	0.85 ± 1.17
Fluoranthene	0.84	1.25 ± 1.77
Pyrene	0.94	1.33 ± 1.78
Benzo[*a*]pyrene	0.20	0.46 ± 0.76
Dibenz[*a,h*]anthracene	<0.11	<1.8
Benzo[*b*]fluoranthene	0.24	0.86 ± 1.36
Benzo[*k*]fluoranthene	0.19	0.46 ± 0.76
Benzo[*g,h,i*]perylene	0.09	0.62 ± 0.92
Ideno[*1,2,3-c,d*]pyrene	0.10	0.63 ± 1.05

* Conc in μg/g wet wt
** Conc in μg/g dry wt ± Standard Deviation

The slopes of the sediment extracts which indicated positive mutagenic responses for each bacterial strain with and without S9 metabolic activation are shown in Tables 5 through 7. Mean slope values and SD in revertants/μg of extract are shown in the first column of the tables. Slope values were not determined for sediment extracts that indicated a negative mutagenic response. For example, only 16 of the 20 extracts tested with TA98+S9 demonstrated a positive mutagenic response (Table 5). The number of data points used to determine the slope were listed in the second column of the table and toxicity (T) in doses was designated. The correlation coefficient (r) as well as the goodness of fit value (r^2) were given to assist in showing the linearity of the dose response. Generally, as the r values indicate in Tables 5 through 7, most of the sediment extracts had dose-response curves that were highly linear.

The Chicago clean sediment extract using TA100 without S9 activation showed a positive mutagenic response. The slope was calculated at 4 (0.54) revertants/μg extract using six data points. The r value was 0.96 with an r^2 value of 0.92. However, the other 19 sediment extracts indicated a negative mutagenic response when tested with TA100 without metabolic activation.

TA98+S9

A positive mutagenic response was demonstrated with TA98 with S9 activation in 16 of the 20 sediment extracts. The 16 extracts include: Gowanus Creek clean and crude (Figure 3a & b), Arthur Kill clean and crude (Figure 4a & b), Red Hook clean and crude (Figure 5a & b), Chicago

Table 5. Least Squares Linear Regression - TA98+S9

Sediment Extract	Revert/μg Extract	# of Data Points	Corr (r)	Goodness of Fit (r^2)
Oakland Inner-clean	12(0.8)	6	0.99	0.98
Arthur Kill-crude	7(1.9)	6	0.87	0.76
Oakland Outer-clean	5(0.2)	6	0.99	0.99
Oakland Hot-crude	5(3)	6	0.60	0.36
Oakland Reference-clean	4(0.5)	6	0.97	0.94
Red Hook-clean	4(0.4)	6	0.97	0.94
Oakland Hot-clean	3(1.9)	6	0.66	0.43
Red Hook-crude	3(0.3)	4(T)	0.98	0.98
Oakland Inner-crude	1(9.6)	5(T)	0.99	0.99
Arthur Kill-clean	1(0.1)	6	0.97	0.95
Gowanus Creek-crude	0.96(4)	6	0.99	0.99
Hamlet City-crude	0.89(7)	6	0.99	0.97
Chicago CDF-clean	0.79(0.2)	6	0.91	0.83
Oakland Outer-crude	0.23(8)	6	0.81	0.66
Gowanus Creek-clean	0.16(5)	6	0.84	0.70
Chicago CDF-crude	0.14(0.1)	6	0.89	0.78

T = Toxicity exhibited in doses.

Table 6. Least Squares Linear Regression - TA98-S9

Sediment Extract	Revert/μg Extract	# of Data Points	Corr (r)	Goodness of Fit (r^2)
Red Hook-clean	4(0.4)	4(T)	0.99	0.98
Chicago CDF-clean	4(0.2)	6	0.99	0.99
Oakland Reference-clean	3(0.4)	6	0.98	0.95
Oakland Hot-clean	2(0.4)	6	0.94	0.89
Oakland Inner-crude	1(4)	5(T)	0.99	0.99
Gowanus Creek-crude	0.93(3)	6	0.99	0.99
Arthur Kill-clean	0.9(0.1)	6	0.97	0.94
Hamlet City-crude	0.56(9)	6	0.95	0.90
Oakland Hot-crude	0.27(6)	4(T)	0.95	0.90
Gowanus Creek-clean	0.15(1.9)	6	0.96	0.94
Oakland Reference-crude	0.14(2)	6	0.93	0.88

T = Toxicity exhibited in doses.

Table 7. Least Squares Linear Regression - TA100+S9

Sediment Extract	Revert/μg Extract	# of Data Points	Corr(r)	Goodness of Fit(r^2)
Oakland Outer-clean	49(6.9)	6	0.91	0.83
Oakland Reference-clean	18(3)	6	0.96	0.92
Oakland Inner-clean	17(0.9)	6	0.99	0.99
Red Hook-clean	14(4)	4(T)	0.94	0.87
Chicago CDF-clean	11(1)	5(T)	0.99	0.98
Chicago CDF-crude	2(0.2)	5(T)	0.99	0.98
Arthur Kill-clean	2(0.5)	6	0.90	0.81
Oakland Inner-crude	1(0.3)	6	0.89	0.78
Oakland Reference-crude	1(0.2)	6	0.96	0.91
Oakland Outer-crude	1(0.7)	6	0.65	0.43
Gowanus Creek-clean	0.62(0.3)	6	0.76	0.58

T = Toxicity exhibited in doses.

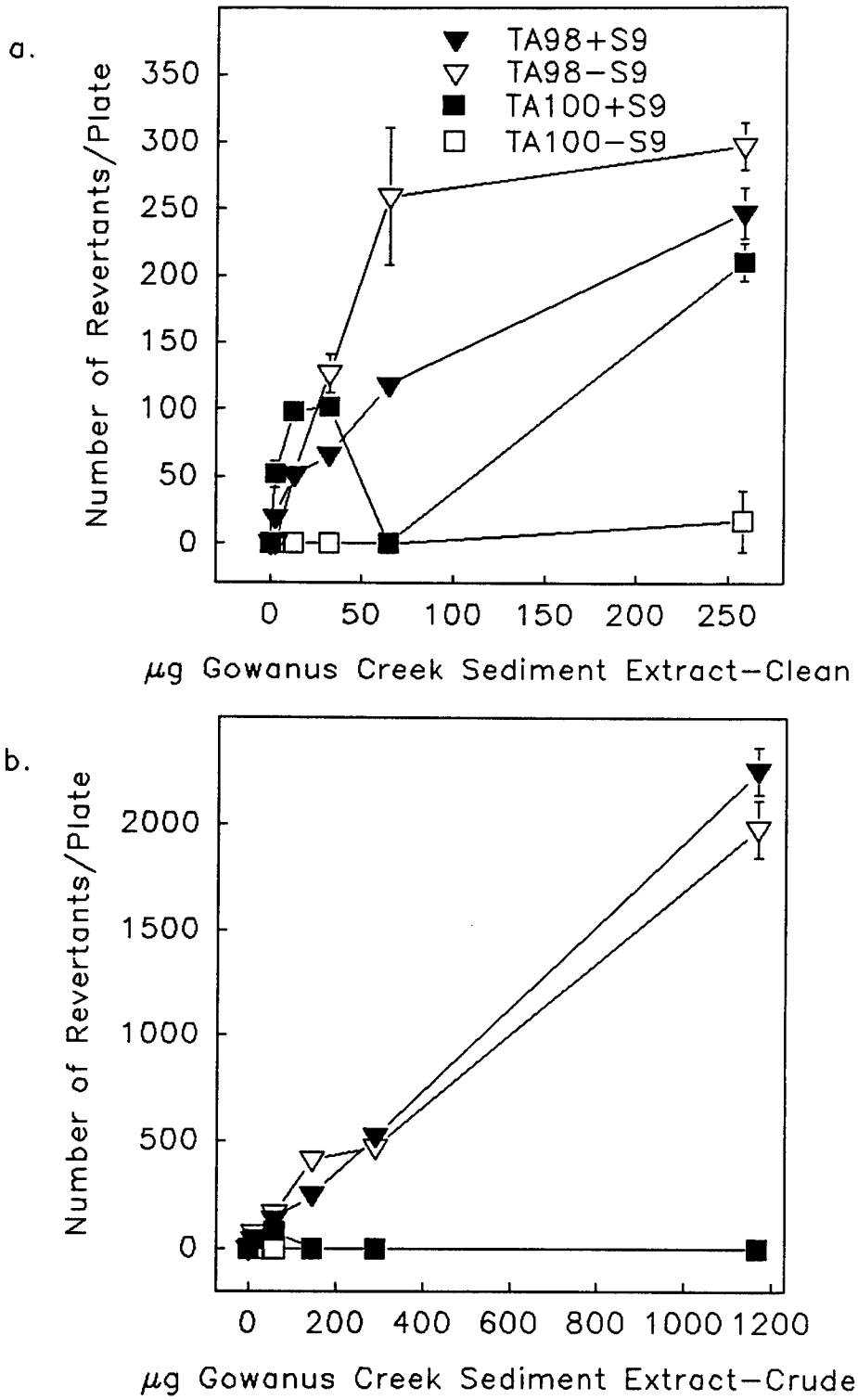
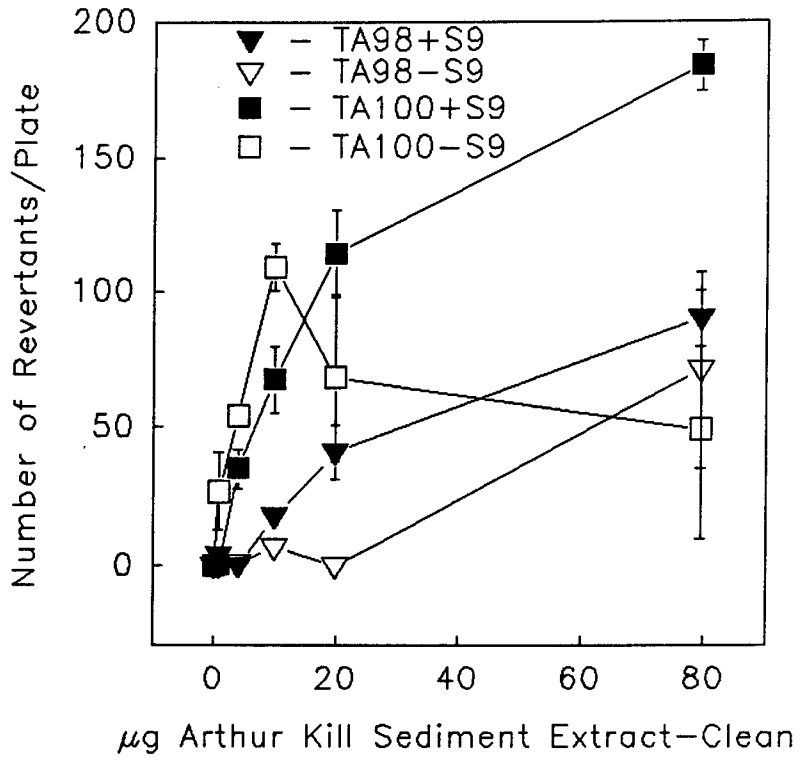


Figure 3a & b. Mutagenic evaluation of Gowanus Creek sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

a.



b.

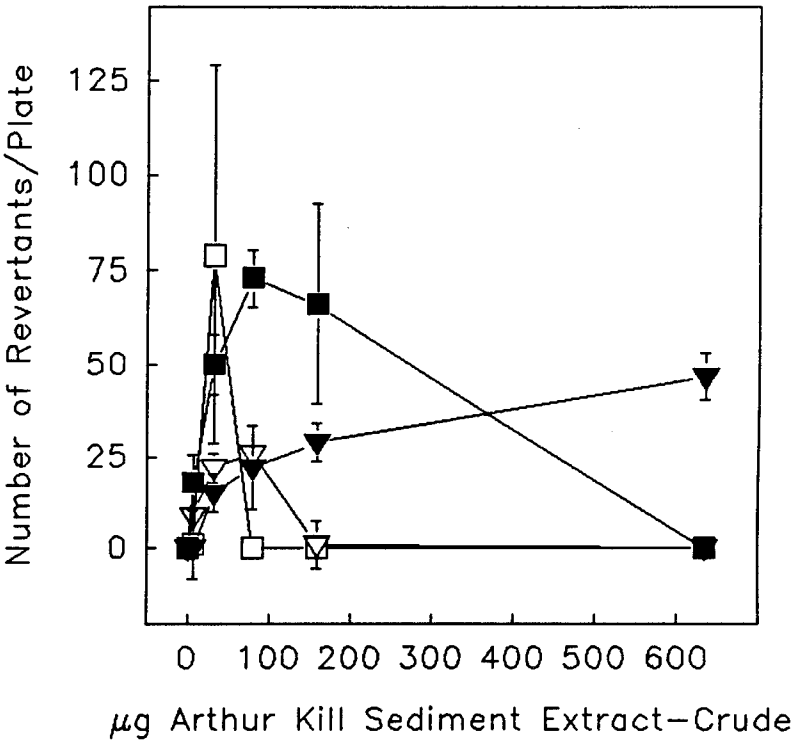


Figure 4a & b. Mutagenic evaluation of Arthur Kill sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

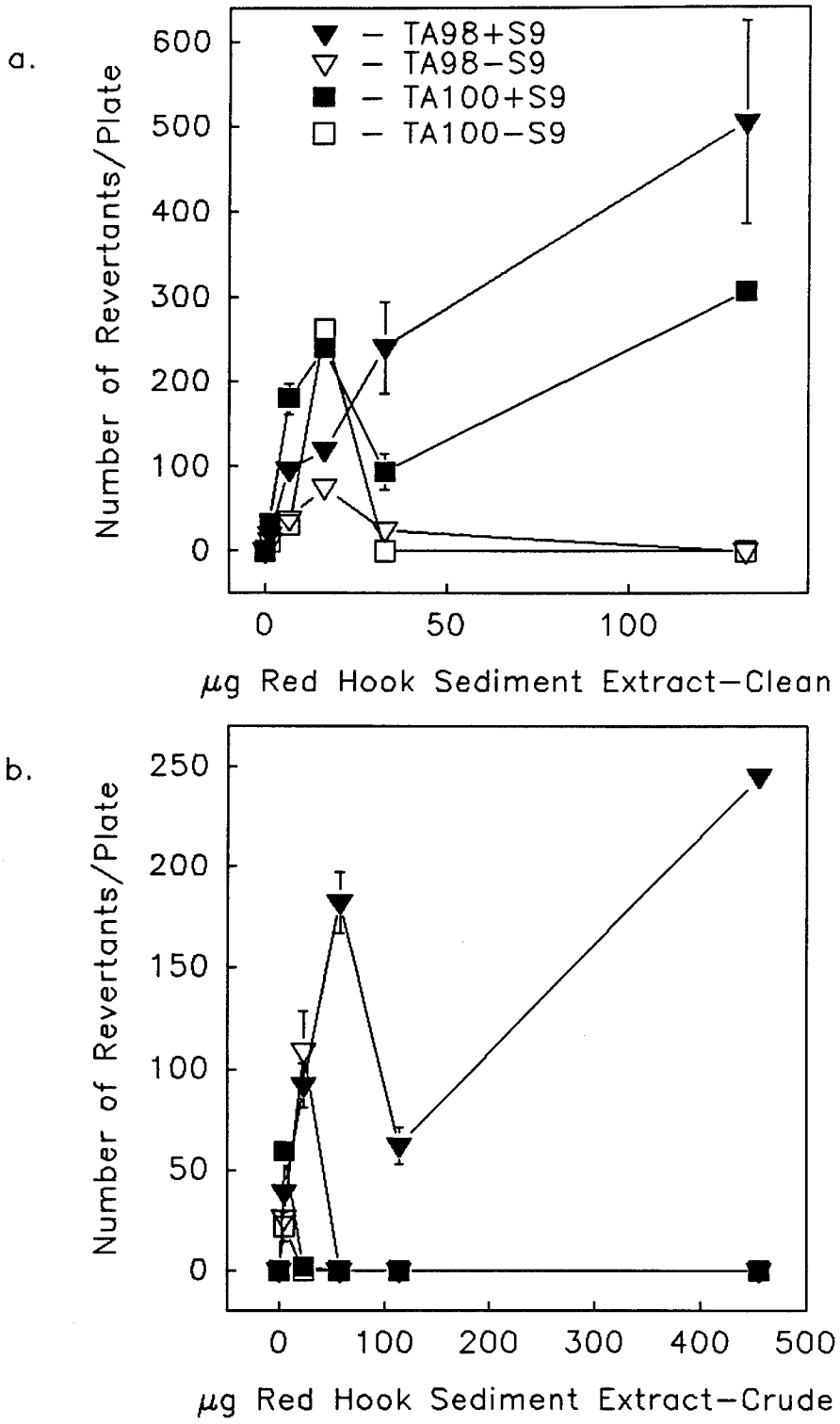


Figure 5a & b. Mutagenic evaluation of Red Hook sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

CDF clean and crude (Figure 6a & b), Hamlet City crude (Figure 7), Oakland Reference clean (Figure 8a), Oakland Inner clean and crude (Figure 9a & b), Oakland Outer clean and crude (Figure 10a & b), and Oakland Hot clean and crude (Figure 11a & b). The Gowanus Creek crude sediment extract exhibited a 108-fold increase in the highest dose of the net number of revertants over the control group, as shown in Figure 3b. Toxicity was shown in the highest dose of Arthur Kill crude sediment extract (Figure 4b). A negative mutagenic response was indicated with TA98+S9 in Sandy Hook clean and crude, Hamlet City clean, and Oakland Reference crude (Figure 8b) sediment extracts.

TA98-S9

Positive mutagenic activity was shown in 50% of the sediment extracts when tested with TA98 without S9 activation. The extracts exhibiting a positive response were: Gowanus Creek clean and crude (Figure 3a & b), Arthur Kill clean (Figure 4a), Chicago CDF clean and crude (Figure 6a & b), Hamlet City crude (Figure 7), Oakland Reference clean and crude (Figure 8a & b), Oakland Outer (Figure 10a & b), and Oakland Hot clean (Figure 11a). In the highest dose of the Gowanus Creek crude sediment extract, there was an approximately 76-fold increase in the net number of revertants over the control group (or zero dose) (Figure 3b). In the two highest doses of Red Hook clean and crude sediment extracts, toxicity was indicated (Figure 4a & b).

A negative mutagenic response was indicated with TA98-S9 in the following sediment extracts: Sandy Hook clean and crude, Arthur Kill crude (Figure 4b), Red Hook (Figure 5a & b), Hamlet City clean, Oakland

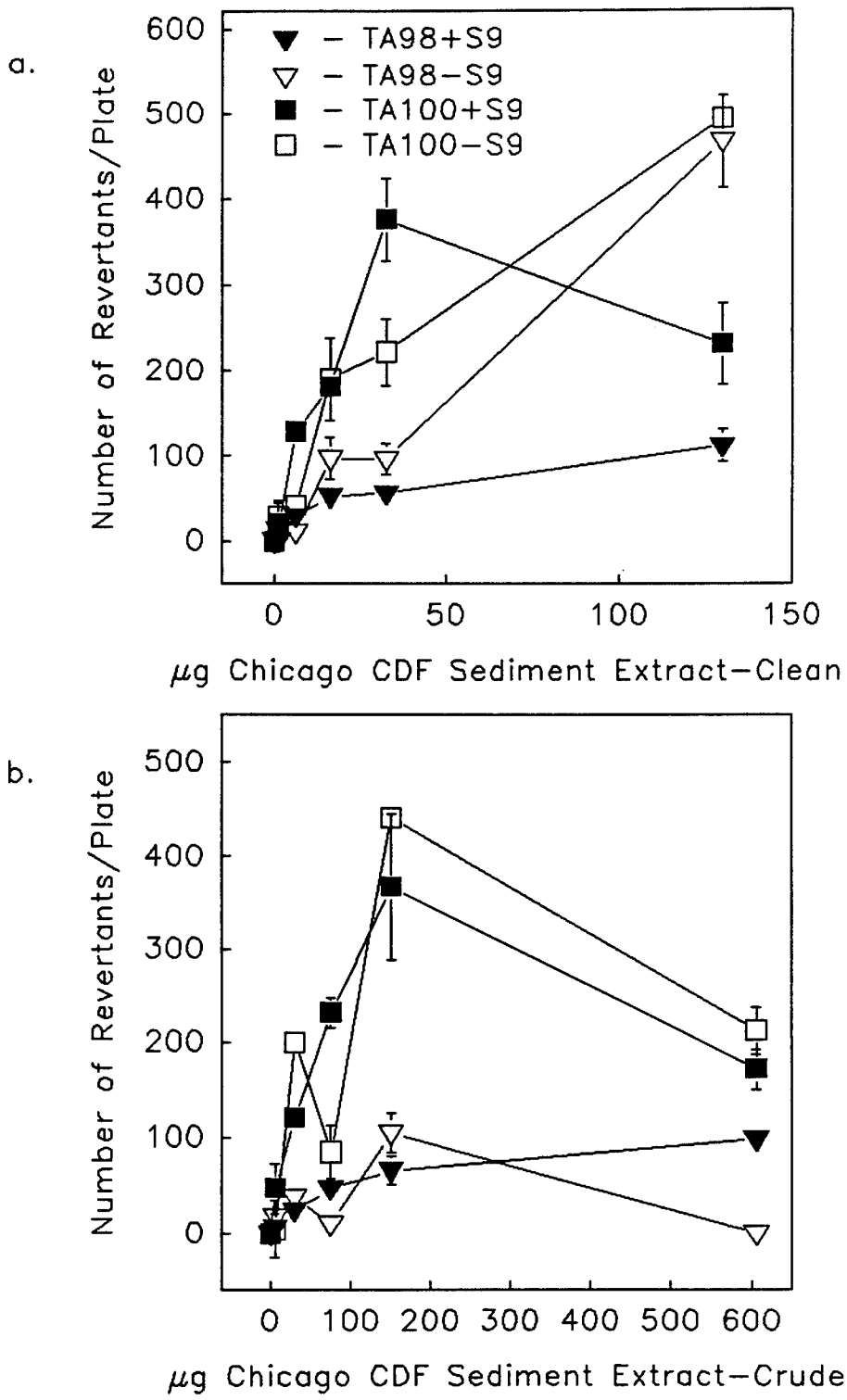


Figure 6a & b. Mutagenic evaluation of Chicago CDF sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

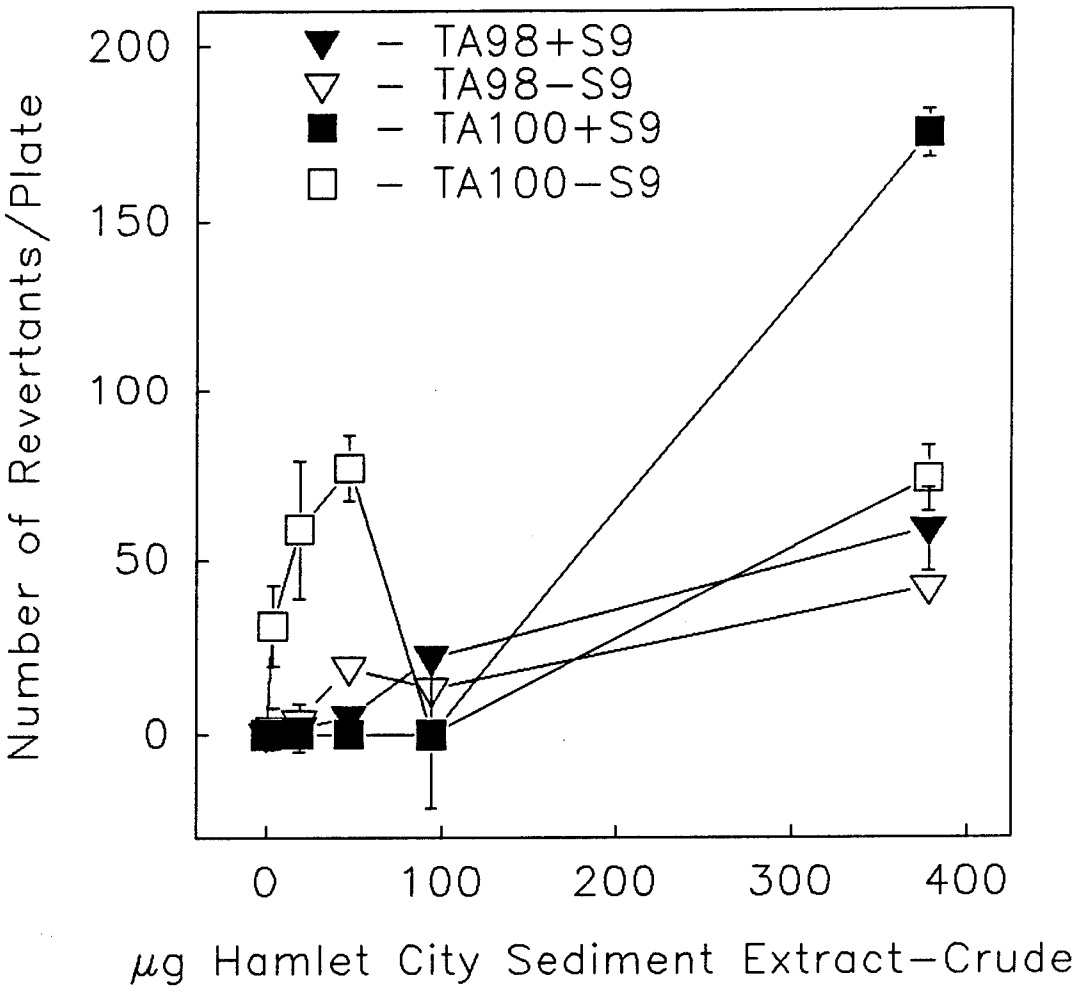


Figure 7. Mutagenic evaluation of Hamlet City sediment extract-crude using the Ames plate incorporation test. Data points represent mean and standard error.

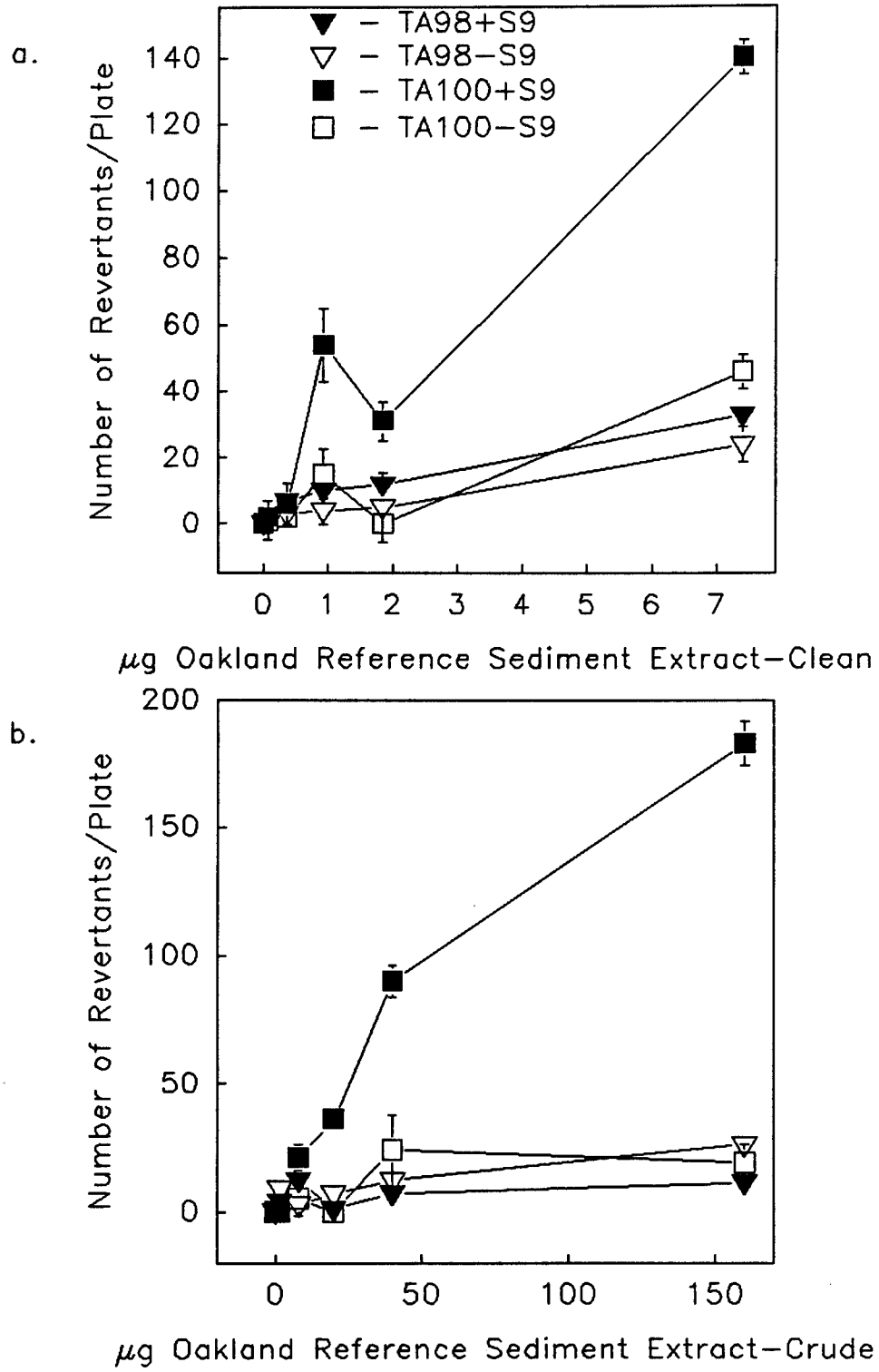


Figure 8a & b. Mutagenic evaluation of Oakland Reference sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

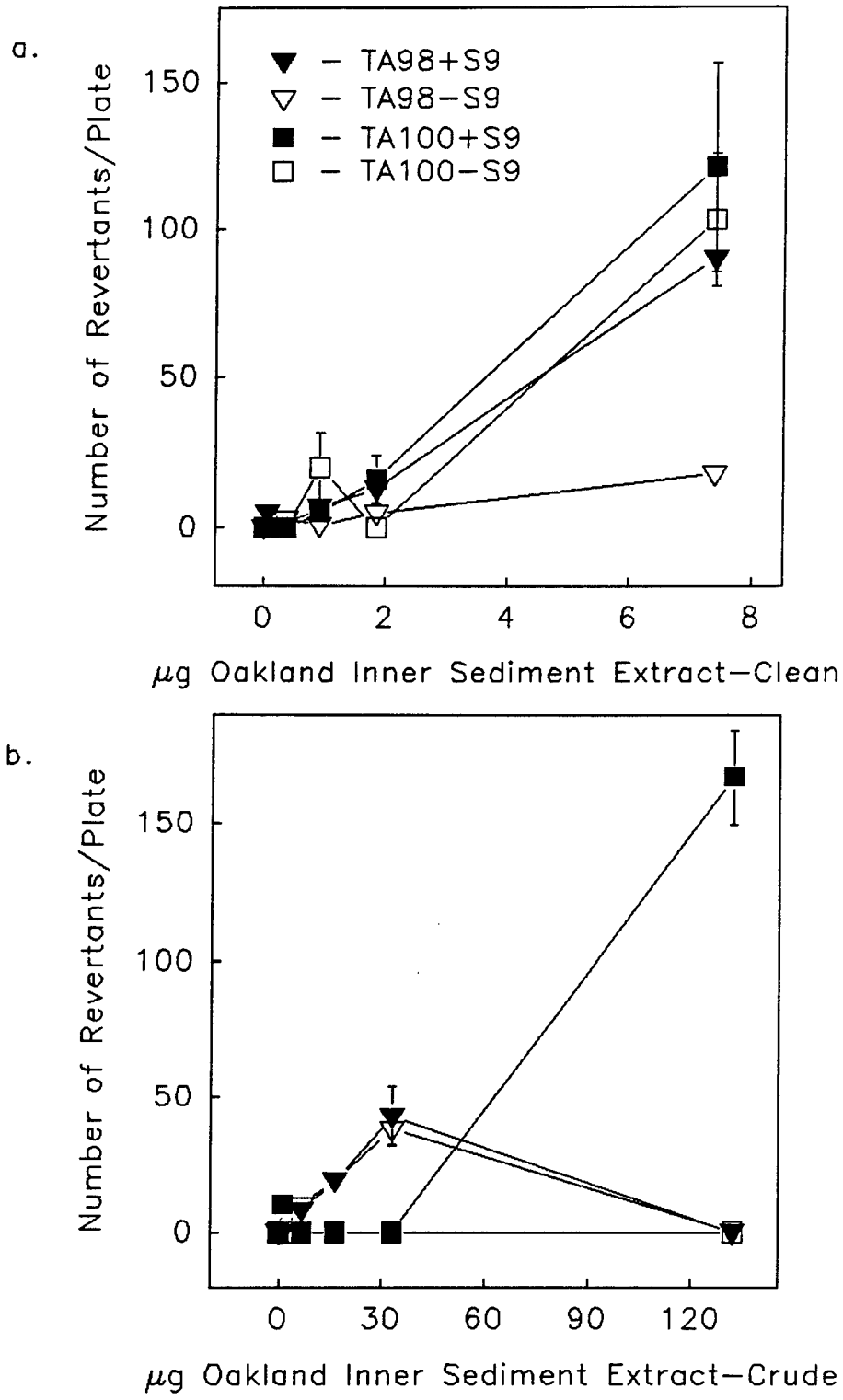


Figure 9a & b. Mutagenic evaluation of Oakland Inner sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

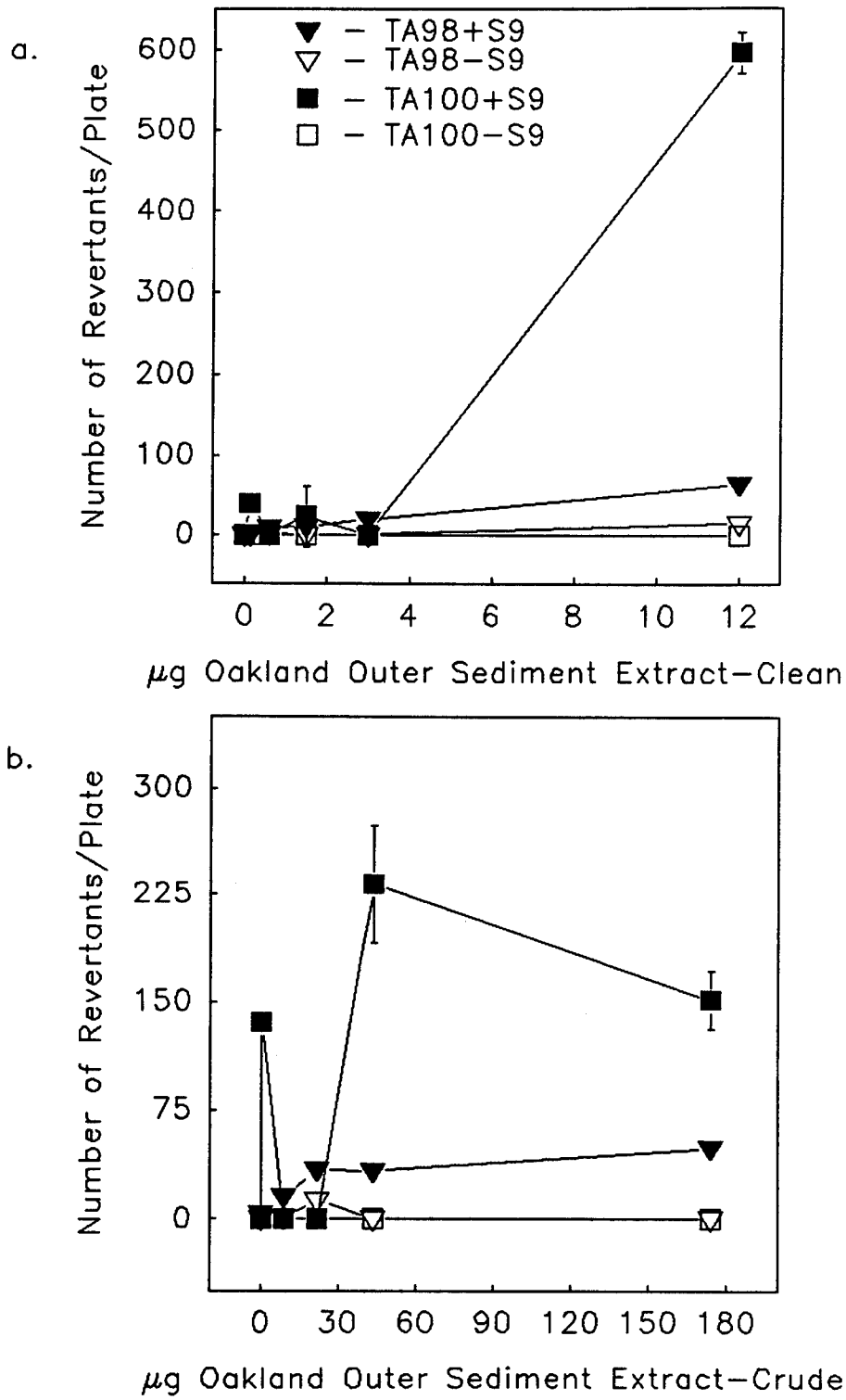


Figure 10a & b. Mutagenic evaluation of Oakland Outer sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

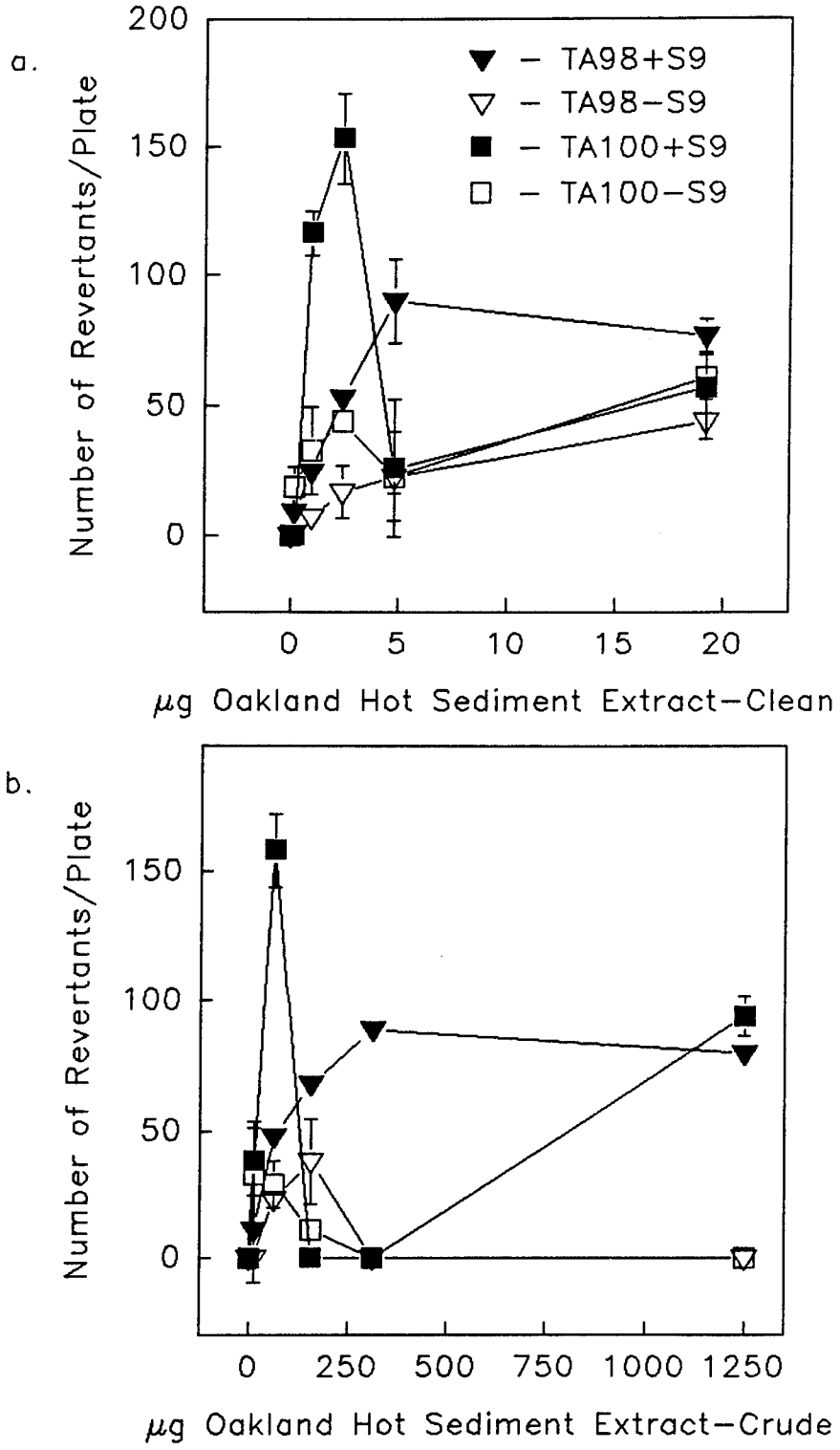


Figure 11a & b. Mutagenic evaluation of Oakland Hot sediment extract a) clean b) crude using the Ames plate incorporation test. Data points represent mean and standard error from triplicate plates.

Inner clean and crude (Figure 9a & b), Oakland Outer crude (Figure 10b) and Oakland Hot crude (Figure 11b).

TA100+S9

A positive mutagenic response was demonstrated in half of the sediment extracts when tested with TA100 with S9 activation. These extracts include: Gowanus Creek clean (Figure 3a), Arthur Kill clean (Figure 4a), Red Hook clean (Figure 5a), Chicago CDF clean and crude (Figure 6a & b), Oakland Reference clean and crude (Figure 8a & b), Oakland Inner clean and crude (Figure 9a & b), and Oakland Outer clean (Figure 10a).

A negative mutagenic response was exhibited in 10 of the 20 sediment extracts. This group includes: Gowanus Creek crude (Figure 3b), Sandy Hook clean and crude, Arthur Kill crude (Figure 4b), Red Hook crude (Figure 5b), Hamlet City clean and crude (Figure 7), Oakland Outer crude (Figure 10b), and Oakland Hot clean and crude (Figure 11a & b). Toxicity was exhibited in the two highest doses of Arthur Kill crude (Figure 4b), Red Hook crude (Figure 5b), Oakland Inner crude (Figure 9b), and Oakland Hot clean and crude (Figure 11a & b) sediment extracts.

TA100-S9

Only 10% of the sediment extracts, Chicago CDF clean and crude, indicated a positive mutagenic response when tested with TA100 without S9 activation.

Eighteen of 20 extracts demonstrating a negative mutagenic response when tested with TA100-S9 were: Gowanus Creek clean and crude (Figure 3a & b), Sandy Hook clean and crude, Arthur Kill clean and crude (Figure 4a & b), Red Hook clean and crude (Figure 5a & b), Hamlet City clean and

crude (Figure 7), Oakland Reference clean and crude (Figure 8a & b), Oakland Inner clean and crude (Figure 9a & b), Oakland Outer clean and crude (Figure 10a & b), and Oakland Hot clean and crude (Figure 11a & b). Toxicity was shown in the two highest doses of Arthur Kill crude (Figure 4b), Red Hook clean and crude (Figure 5a & b), Oakland Inner crude (Figure 9b), and Oakland Hot crude (Figure 11b).

Mutatox™ Results

The twenty sediment extract samples were tested with and without S9 metabolic activation. None of the twenty sediment extracts showed a positive mutagenic response when tested without metabolic activation. Eleven of the 20 sediment extracts indicated a positive mutagenic response, which included: Gowanus Creek clean and crude, Arthur Kill clean and crude, Red Hook clean, Chicago CDF clean and crude, Hamlet City clean, Oakland Reference clean and Oakland Hot clean and crude (Figures 12a & b through 18a & b). Negative mutagenic responses were indicated in the following group of sediment extracts: Sandy Hook clean and crude, Red Hook crude, Hamlet City crude, Oakland Reference crude, Oakland Inner clean and crude, and Oakland Outer clean and crude.

Comparison of Ames Assay and Mutatox™ Results

Table 8 presents a side by side comparison of the Ames assay results and the Mutatox™ genotoxicity test results. Sixteen sediment extracts out of 20 compared favorably in similar mutagenic responses (negative and positive) with Mutatox™ and TA98 results when tested with S9 activation. Half of the sediment extracts compared favorably when tested using Mutatox™ and TA98 without S9 activation. Fifty percent of the sediment extracts compared with similar results with Mutatox™ and

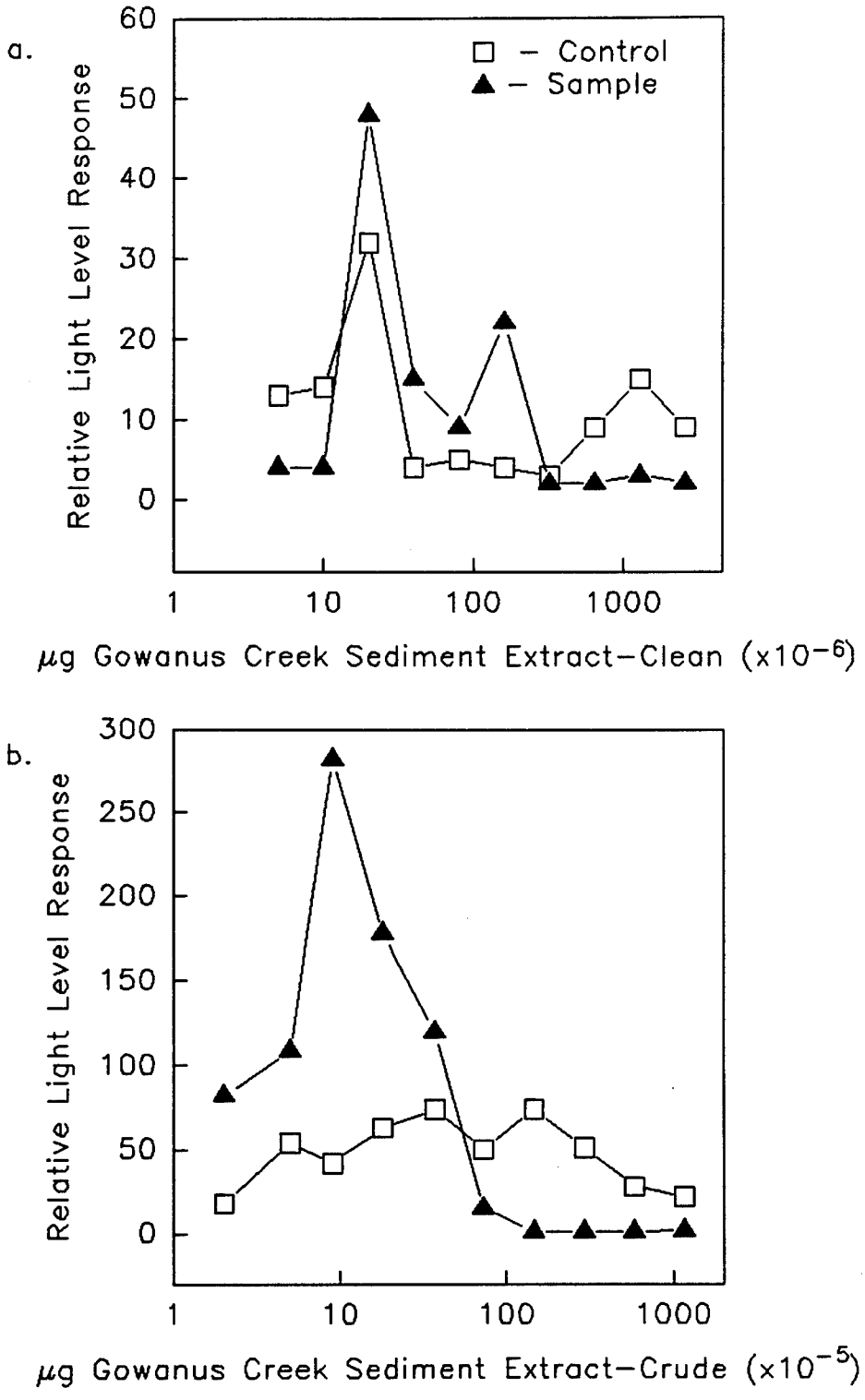


Figure 12a & b. Mutagenic evaluation of Gowanus Creek sediment extract a) clean b) crude using Mutatox™. Data points represent relative light level response taken at the most genotoxic time period.

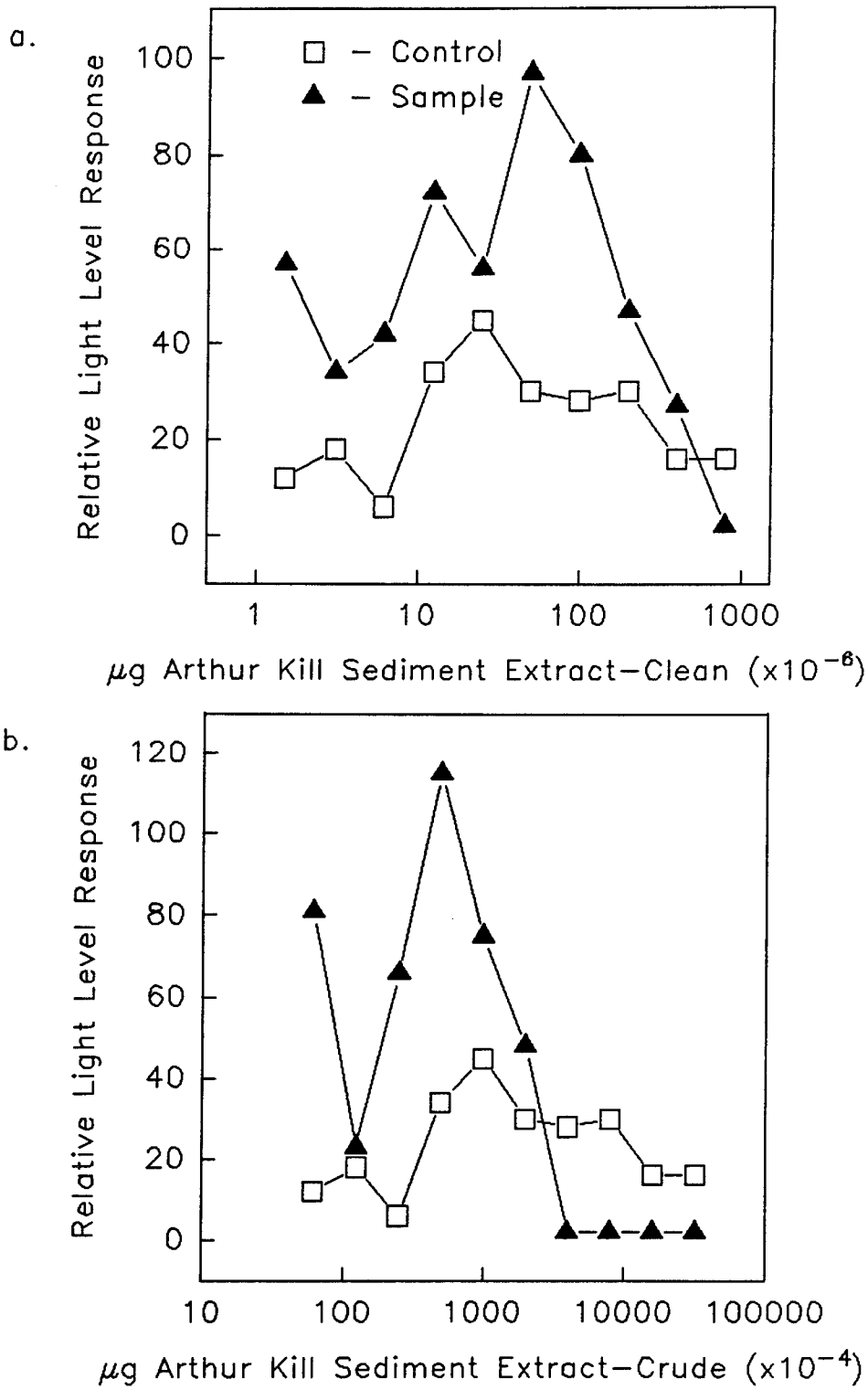


Figure 13a & b. Mutagenic evaluation of Arthur Kill sediment extract a) clean b) crude using Mutatox™. Data points represent relative light level response taken at the most genotoxic time period.

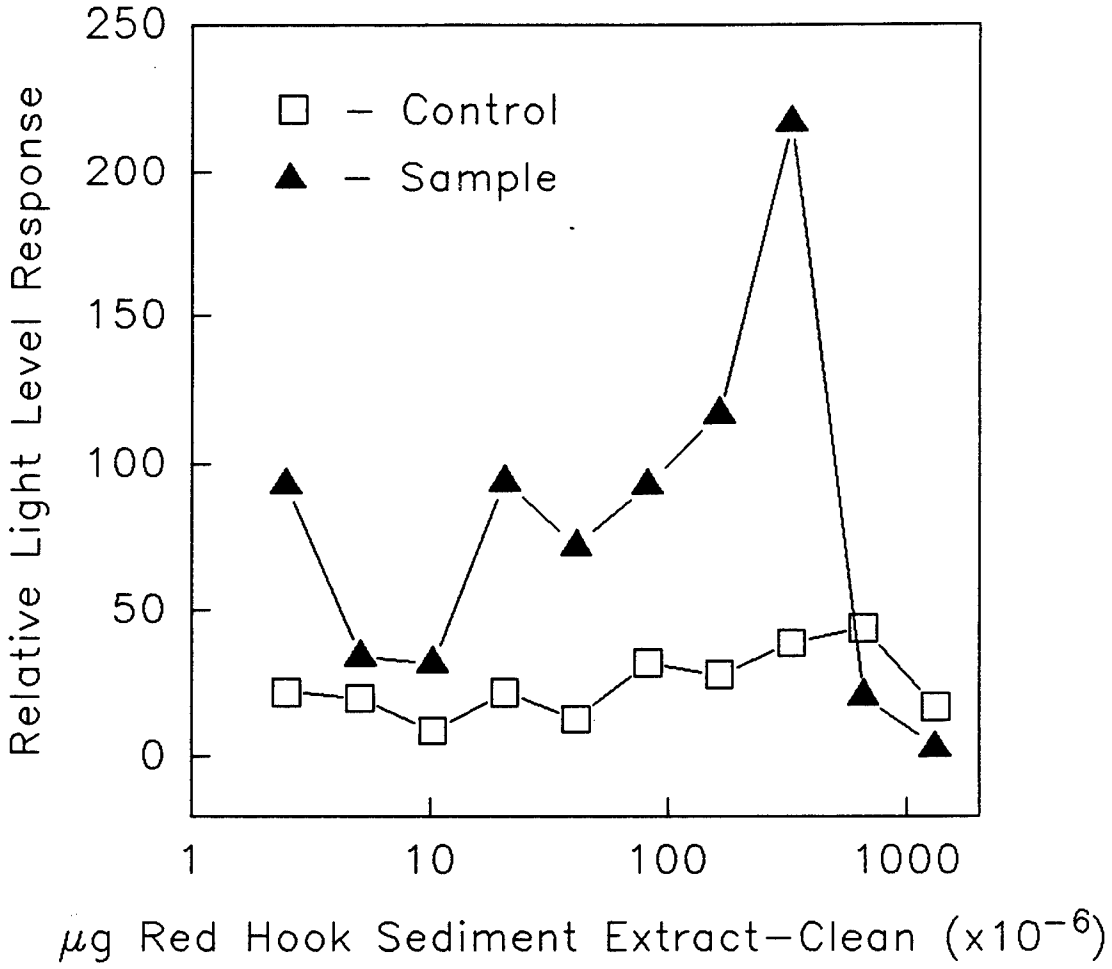


Figure 14. Mutagenic evaluation of Red Hook sediment extract-clean using Mutatox™. Data points represent relative light level response taken at the most genotoxic time period.

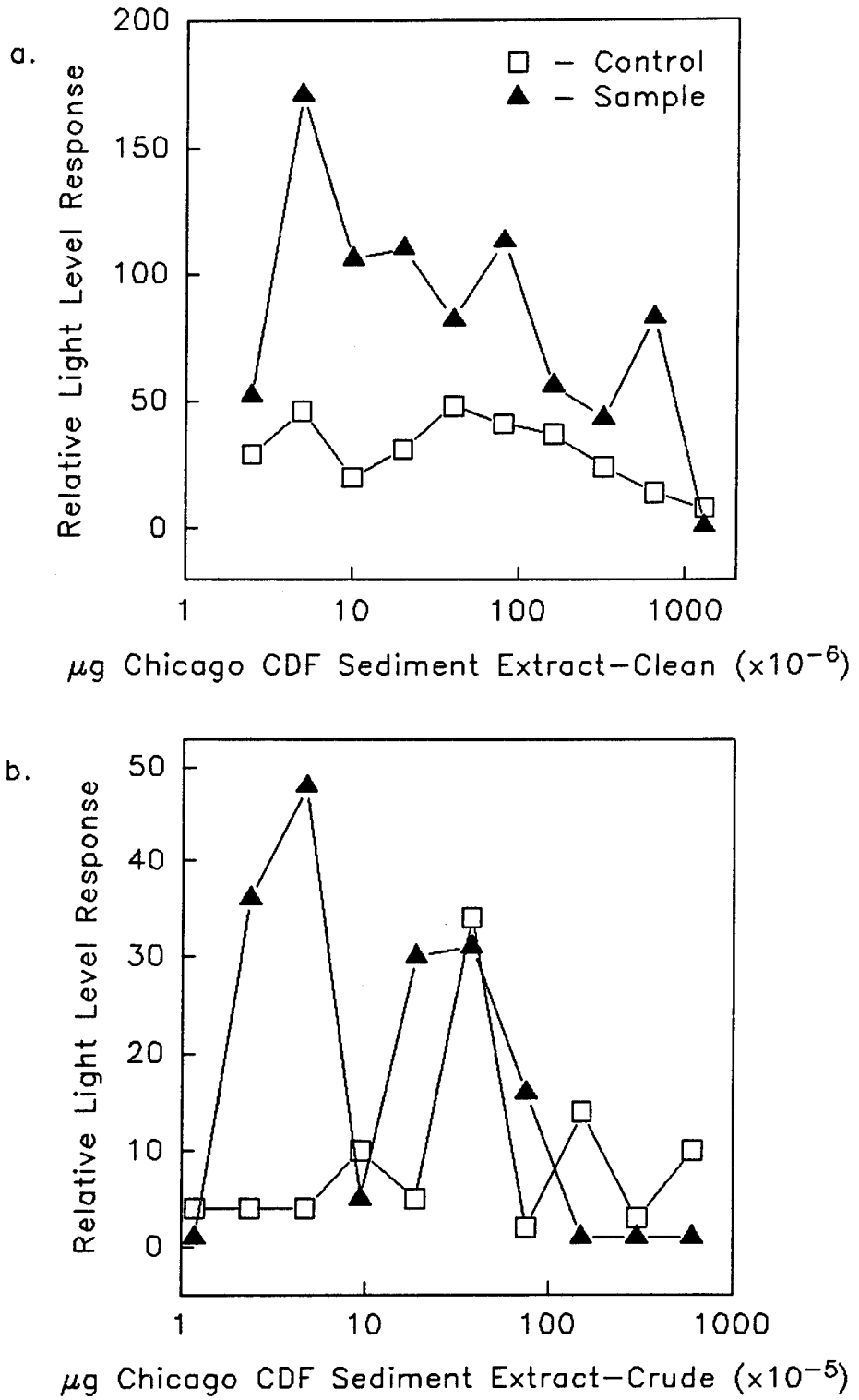


Figure 15a & b. Mutagenic evaluation of Chicago CDF sediment extract a) clean b) crude using Mutatox™. Data points represent relative light level response taken at the most genotoxic time period.

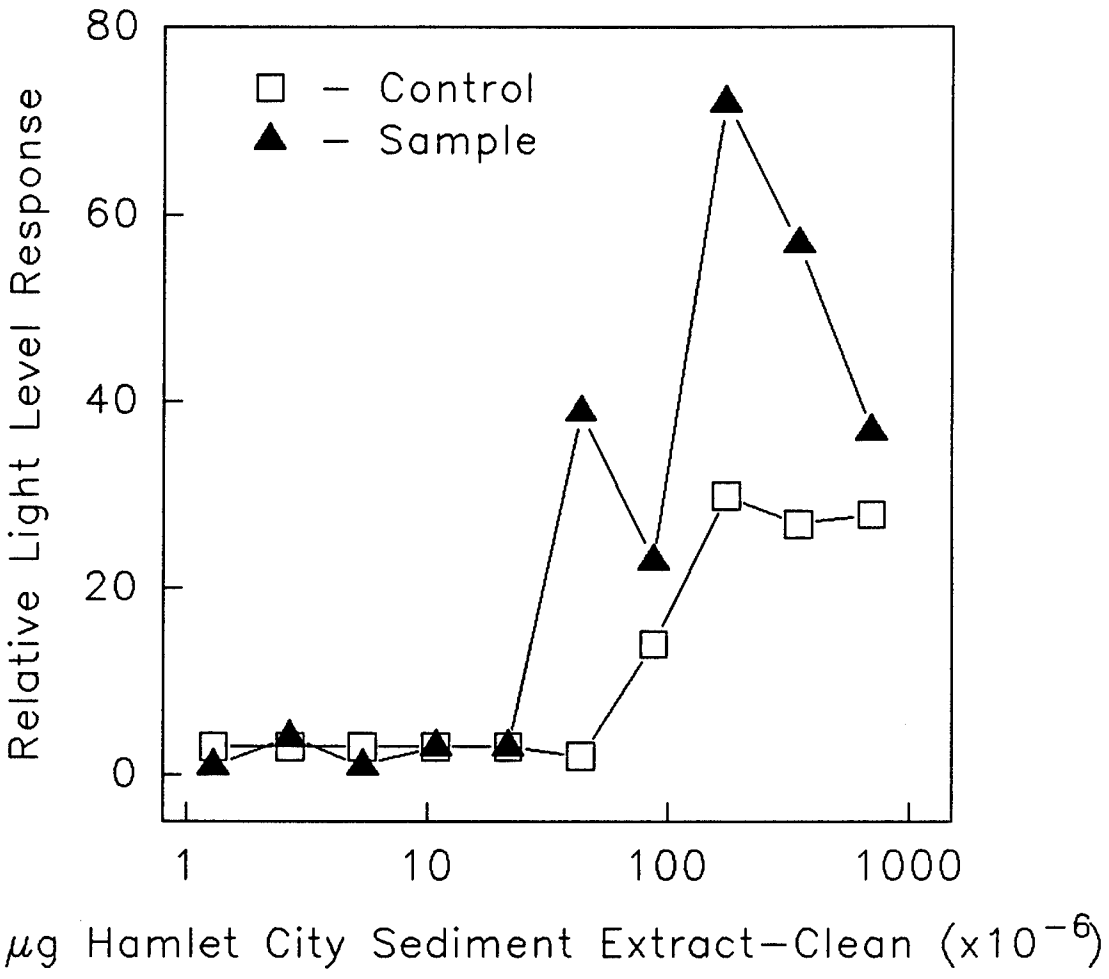


Figure 16. Mutagenic evaluation of Hamlet City sediment extract-clean using Mutatox™. Data points represent relative light level response taken at most genotoxic time period.

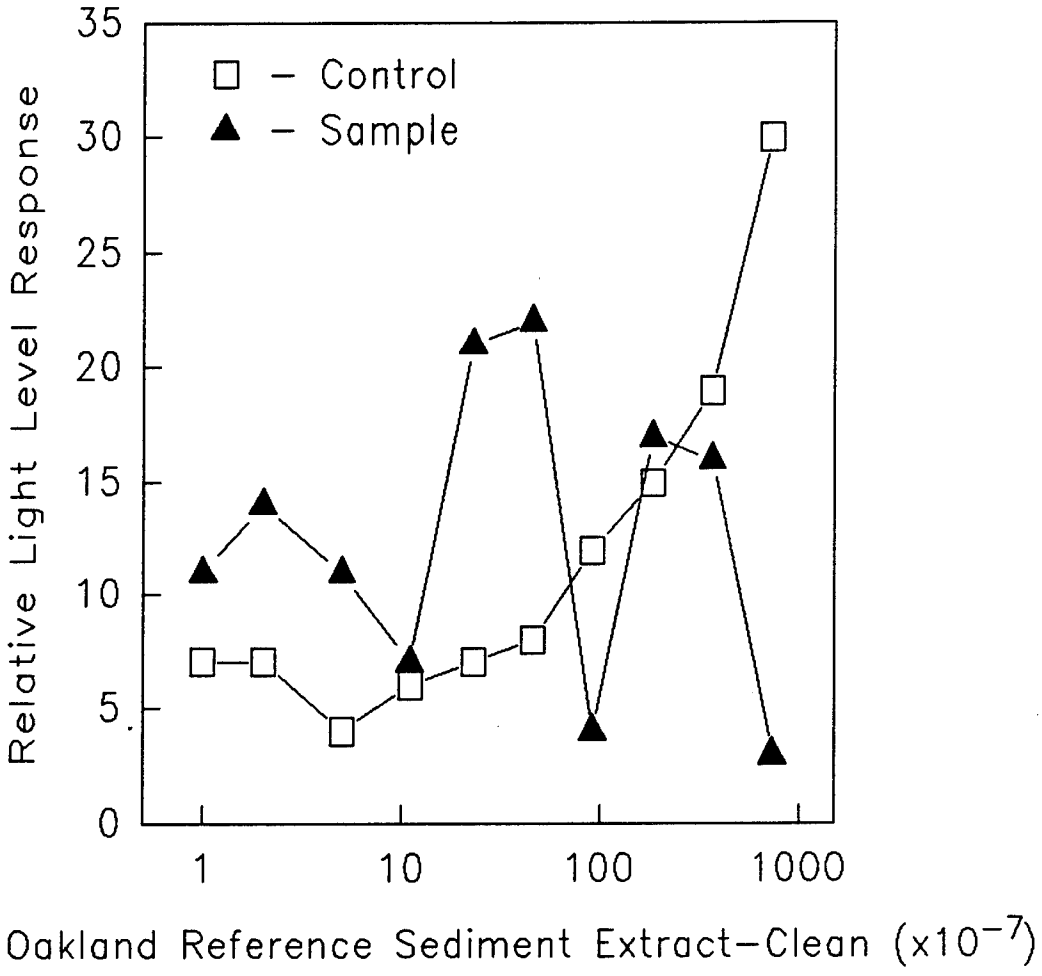


Figure 17. Mutagenic evaluation of Oakland Reference sediment extract-clean using Mutatox™. Data points represent relative light response taken at the most genotoxic time period.

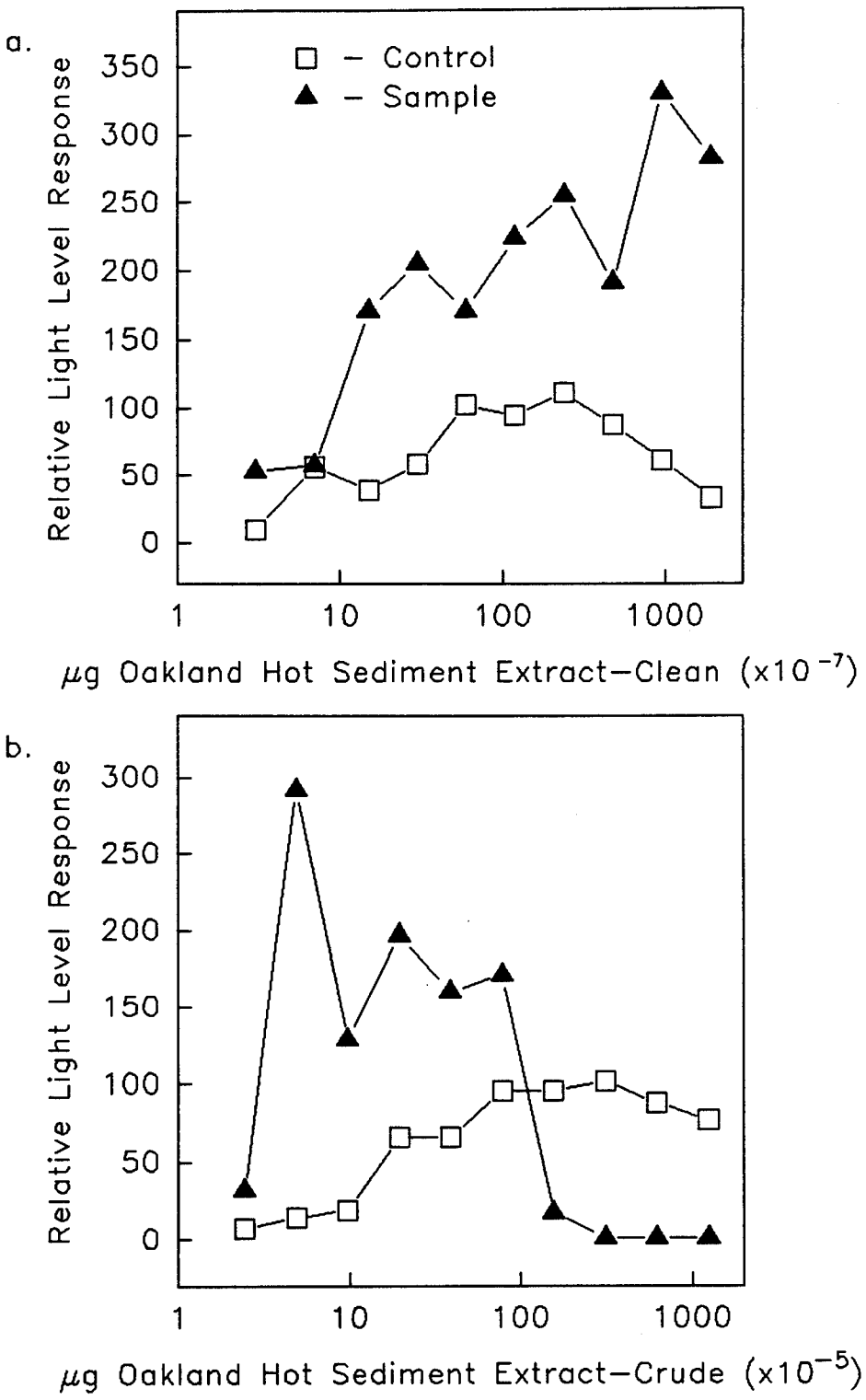


Figure 18a & b. Mutagenic evaluation of Oakland Hot sediment extract a) clean b) crude using Mutatox™. Data points represent relative light level response taken at the most genotoxic time period.

Table 8. Comparison of Ames Assay and Mutatox™ Results

	S9 Metabolic Activation			No Metabolic Activation		
Sediment Extract	TA98	Mutatox	TA100	TA98	Mutatox	TA100
Gowanus Creek-clean	+	+	+	+	-	-
Gowanus Creek-crude	+	+	-	+	-	-
Sandy Hook-clean	-	-	-	-	-	-
Sandy Hook-crude	-	-	-	-	-	-
Arthur Kill-clean	+	+	+	+	-	-
Arthur Kill-crude	+	+	-	-	-	-
Red Hook-clean	+	+	+	-	-	-
Red Hook-crude	+	-(+)	-	-	-	-
Chicago CDF-clean	+	+	+	+	-	+
Chicago CDF-crude	+	+	+	+	-	+
Hamlet City-clean	-	+	-	-	-	-
Hamlet City-crude	+	-	-	+	-	-
Oakland Reference-clean	+	+	+	+	-	-
Oakland Reference-crude	-	-(+)	+	+	-	-
Oakland Inner-clean	+	-	+	-	-	-
Oakland Inner-crude	+	-	+	-	-	-
Oakland Outer-clean	+	-(+20μl)	+	+	-	-
Oakland Outer-crude	+	-(+)	-	-	-	-
Oakland Hot-clean	+	+	-	+	-	-
Oakland Hot-crude	+	+	-	-	-	-

+ = positive mutagenic response; - = negative mutagenic response.
( ) = second set of test results; 20 μl initial dose was used and obtained positive results.

TA100 with S9 metabolic activation. Eighteen out of 20 sediment extract results compared favorably when Mutatox™ and TA100 without metabolic activation were tested. The doses of sediment extracts for Mutatox™ were very small in comparison to the Ames assay doses.

DISCUSSION

A positive mutagenic response was detected in nine out of the 10 sediments studied using both tester strains, TA98 and TA100, in the Ames assay while Mutatox™ detected a positive mutagenic response in eight sediments. The nine sediments included Gowanus Creek, Arthur Kill, Red Hook, Chicago CDF, Hamlet City, Oakland Reference, Inner, Outer, and Hot and were from highly industrialized harbor areas with varying degrees of PAH contamination. In the chemical analysis of these nine sediments, the 4 to 6-ringed PAHs of the 16 PAHs analyzed generally had the highest concentrations. Mutatox™ did not indicate a positive mutagenic response in Oakland Inner sediment. Sandy Hook sediment demonstrated no mutagenic response in either bacterial tester strain of the Ames assay or in Mutatox™ and had no detectable quantities of PAHs. When McCann et al. (1975) conducted Ames testing of 300 chemicals, these 4 to 6-ringed PAHs, benzo[*a*]pyrene, chrysene, benz[*a*]anthracene, and dibenz[*a,h*]anthracene, were identified as mutagenic with S9 metabolic activation. Cerniglia and Heitkamp (1989) reported other 4 to 6-ringed PAHs, fluoranthene and indeno[*1,2,3-c,d*]pyrene, as mutagenic. Most PAHs are not mutagenic by themselves and require metabolic activation to form one or more active metabolites.

Generally, when testing with TA98+S9, both the clean and crude sediment extracts that demonstrated a positive mutagenic response had similar responses. Although TA98+S9 demonstrated a positive mutagenic response in Gowanus Creek sediment extract, there was an order of magnitude difference in the number of revertants between the clean and crude extracts (268 to 2275 revertants, respectively) at the highest

dose. The effects of silica gel clean-up are shown in this difference, and contaminants were possibly being removed in the sample clean-up procedure. The other nine sediments, when divided into the two extracts-clean and crude, did not demonstrate the magnitude of order difference similar to Gowanus Creek. When Gowanus Creek sediment extracts were tested using TA98-S9, there was also an order of magnitude difference in the positive mutagenic response (318 to 2009 revertants, respectively) at the highest dose. Again, the sample clean-up procedure probably removed mutagenic contaminants. When tested with TA98+S9, Gowanus Creek crude demonstrated a 108-fold increase (21 to 2275 revertants) in the highest dose (1168 μg) of net number of revertants over the control group. When tested with TA98-S9, the highest dose demonstrated an approximately 76-fold increase (27 to 2010 revertants) of net number of revertants over the control group.

Other researchers (West et al. 1986; Grifoll et al. 1988, 1990; Fernandez et al. 1992) found when testing sediment extracts that were fractionated, the 4 to 6-ringed PAH were present when a positive mutagenic response resulted and TA98+S9 proved to be most sensitive in detecting mutagenic activity in PAH-contaminated sediments. Grifoll et al. (1990) had tested various *Salmonella* tester strains and found that TA98+S9 had the best sensitivity in detecting mutagens in polar fractions.

In some instances, TA98 (50%) and TA100 (10%) indicated direct-acting mutagens in the 20 sediment extracts (clean and crude). The TA98 detected direct-acting mutagens in Gowanus Creek clean and crude, Arthur Kill clean, Chicago CDF clean and crude, Hamlet City crude, Oakland

Reference clean and crude, Oakland Outer clean, and Oakland Hot crude sediment extracts. The TA100 detected direct-acting mutagens in only Chicago CDF clean and crude sediment extracts.

Mutatox™ did not detect any direct-acting mutagens in the 20 sediment extracts. Johnson (1992b) and Ho and Quinn (1993) found a similar result when using Mutatox™ in testing PAH-contaminated sediments. Johnson (1992b) demonstrated 96% of the sediments tested showed positive mutagenic responses with S9 activation. Ho and Quinn (1993) had results that indicated 50% of the sediment fractions were mutagenic with S9 activation. The results of this thesis study indicated 75% of the sediment extracts were mutagenic when tested using Mutatox™ with S9 activation.

Sediments are complex mixtures and, because of the complexity, simple determination of mutagenicity may not be possible. Toxicity factors and suppression of mutagenic responses may occur through interactions between contaminants, thus possibly masking mutagenicity.

Toxicity was observed in the highest doses of Arthur Kill-crude sediment extract when tested with TA98 and TA100 with and without metabolic activation. Toxicity was also observed in Red Hook clean and crude sediment extracts when tested with TA98-S9 and Red Hook-crude with TA100+S9. The background lawn of each test plate was examined for evidence of toxicity, and minimal growth was observed in the highest dose of these test plates as compared to the control plate. Maron and Ames (1983) suggested that if a massive cell death had occurred, there would be a sparse background lawn on the test plates as compared to the control plates which is what happened in these treatments. In that

case, more histidine is available to the surviving bacteria, and the cells will undergo more cell divisions and appear as small colonies. These colonies could be mistaken for revertants if the researcher has not observed the absence of the background lawn. Another indicator of toxicity is when there is a decrease in the number of revertants with increasing dose (Zeiger and Pagano 1984). This could be a possible explanation in Chicago CDF-crude when tested with TA100±S9 and Oakland Inner-crude when tested with TA98±S9. Visible effects of toxicity were not observed in the Mutatox™ testing.

Grifoll et al. (1988) discovered when testing chemical fractions of coastal sediments, that the whole organic extract was sometimes inactive or toxic at certain doses when fractions of the sediment extract would indicate mutagenicity.

When apparent toxicity such as thinning of the background lawn has not been observed, but there were less revertants than in the last dose, some contaminants are possibly causing a nonlethal toxic effect (Zeiger and Pagano 1984). When tested with TA98+S9, Oakland Reference-clean demonstrated a positive mutagenic response, while Oakland Reference-crude did not. There were chemicals possibly inhibiting the number of *his*$^+$ revertant colonies of *Salmonella* on the test plate in the Oakland Reference-crude sediment extract that were removed in the silica gel chromatography clean-up. In complex mixtures, there may be inhibitors that can cause a reduction in revertants of a test mixture so that the mixture appears nonmutagenic when in fact it is mutagenic. This type of results leads to false negatives in the Ames assay. Thus, Mutatox™ may be better at reducing false negatives than Ames. There was no toxicity

indicated in any of the Mutatox™ results. Mutatox™ did not detect mutagenicity in the crude extracts of Red Hook, Hamlet City, Oakland Reference, Oakland Inner, and Oakland Outer while TA98 did detect mutagenicity in these extracts. There was suppression possibly occurring in the Mutatox™ testing in these treatments. Red Hook, Oakland Reference, and Oakland Outer crude sediment extracts were retested because of unclear results and had a positive response in the second test.

The Ames assay and Mutatox™ compared reasonably well in testing the PAH-contaminated sediments. Eighty percent of the sediment extracts had similar mutagenic responses (negative and positive) when tested with Mutatox™ and TA98 with S9 activation, while 50% of the sediment extracts had similar mutagenic responses when tested with Mutatox™ and TA100 with S9 activation. Without S9 activation, 50% of the sediment extracts had similar mutagenic responses when tested with Mutatox and TA98 while 80% of the sediment extracts had similar mutagenic responses when tested with Mutatox™ and TA100. In a study conducted on 28 Great Lake sediments, Johnson (1992b) compared Ames assay and Mutatox™ results and found they were in 96% site agreement.

The Ames assay is considered a reverse-mutation assay because the bacterial strains are mutated in one of the genes of the histidine biosynthetic pathway and, as a result, cannot manufacture histidine. An additional mutation is needed to revert the cells to histidine independence (Houk 1992). This second mutation takes place when the *Salmonella* strains are exposed to certain chemical classes in testing. Mutatox™ is a bioluminescence test where certain test compounds cause a

forward mutation in a dark mutant strain (M169) of *Vibrio fisheri*, and it becomes luminescent again (Ulitzur 1986; Bulich 1992). The mode of action involved in Mutatox™ is still not completely understood. Although the Ames assay and Mutatox™ have different mechanisms of action, both tests detect some of the same classes of mutagens, which include base-pair substitution and frameshift mutagens. In addition, Mutatox™ detects intercalating agents, DNA-damaging agents, and DNA-synthesis inhibitors.

The endpoints measured in each assay were different. Light levels were quantitated using a luminometer in Mutatox™ while revertant colonies on agar plates are counted either manually or with an automated counter in the Ames assay. If the luminometer is calibrated properly, the light readings will provide less human error in determination of the reading. Light levels are less difficult to read and not as tedious to determine and may be more sensitive than counting plates. When counting colonies manually or with an automated counter, there may be a large degree of human error. Counting colonies manually is a tedious and time consuming effort, and every colony cannot possibly be seen. When using an automated counter, there is a degree of compensation given in the total count because of the sensitivity and size setting of the instrument cannot account for every revertant colony on the plate. In the Ames assay, the background lawn must also be checked for toxicity problems which adds an additional step in determining the results of testing.

Before testing begins, confirmation of the Ames *Salmonella* tester strains is necessary to determine if the bacteria still possess their

genetically-engineered characteristics. There are many tester strains in the Ames assay which can be used in testing for mutagenicity. Mutatox™ has only one tester strain, *Vibrio fisheri*, and confirmation is not required each time it is used. The Ames assay is labor intensive and requires technical expertise such as microbiological training and aseptic techniques to obtain dependable results while the Mutatox™ is considered technician-friendly. In the Ames assay, bacterial cultures must be incubated overnight before the test can be initiated, and the next morning, test solutions are prepared before beginning the test. In Mutatox, freeze-dried aliquots of the bacterial culture can be prepared, and testing can begin the same day. When using the Ames test, there were cytotoxicity problems encountered in testing the sediment extracts. Mutatox™ did not demonstrate the same kind of toxicity problems in this thesis research.

The Ames assay has been validated and used for over 20 years and has a large data base of information on many chemicals and environmental mixtures. There have been many modifications of the Ames assay developed that have been applied to complex mixtures and other chemical classes. Mutatox™ is a relatively new assay and is still undergoing research. Mutatox™ is in the process of being validated and has a small database of information (10 years of use) on single chemicals and complex mixtures. In the last few years, Mutatox™ has been used for the testing of environmental samples with favorable results.

Both the Ames test and Mutatox™ detected mutagenic activity in most of the same sediment extracts but with different levels of sample concentration. Mutatox™ was able to detect mutagenicity in sediment

extract concentrations six to seven orders of magnitude lower than the Ames test.

Generally in bacterial assays, problems can arise when using S9 as an exogenous metabolizing system. The contaminant may bind to the S9 protein and not be available in the test (Gatehouse 1987). The concentration of the S9 can also cause potential problems for the demonstration of mutagenic potential of contaminants. If too high a concentration of S9 is used, it will mask the mutagenicity, while with too small a concentration of S9, a mutagenic response will not be demonstrated.

The cost of Ames assay materials can be relatively inexpensive to start-up (approximately $3000) depending on existing lab facilities. The initial start-up of Mutatox™ is quite expensive ($23,000) because of the special equipment needed which includes a luminometer. When testing on a routine basis, Mutatox™ becomes quite affordable when testing a number of samples (approximately $200.00 when testing 50 samples at one time). In running an Ames test, with four samples and two bacterial strains, the average cost would be approximately $1500.00. The Mutatox™ uses a very small concentration of the test material in a series of ten dilutions as compared to the Ames assay, utilizing a three order of magnitude dilution series. Mutatox™ results are obtained in a total of 24 hours, whereas Ames assay results are obtained in 48-72 hours depending on possible problems with reversion rates and the particular chemical or mixture being tested.

Generally, in the testing of sediments, both the Ames assay and Mutatox™ apparently require S9 metabolic activation to demonstrate a

positive mutagenic response, especially when testing sediments contaminated with PAHs.

Other researchers (Durant et al. 1992; Fabacher et al. 1988; Fernandez et al. 1992; Grifoll et al. 1988, 1990; Johnson 1992b; Lan et al. 1991; Metcalfe et al. 1990; West et al. 1986) found a suite of short-term mutagenicity tests were more successful in detecting mutagenic potential of contaminated sediments than using the Ames assay alone. Mutatox™ shows promise of being an ideal screening tool in assessing potential mutagenicity in sediments when using Ames as a final confirmation. Although technically more difficult to use, the Ames assay is more established and has a large database of mutagenic information. The advantages of Mutatox™ include speed, economy of testing after the initial startup, ease of testing (no technical expertise necessary-user friendly), and increased sensitivity with Mutatox™.

CONCLUSIONS

- When preparing sediment extracts for bulk-sediment analysis and genotoxicological testing, consideration of the sediment clean-up method is advised. Some traditional methods such as silica gel chromatography may remove mutagenic contaminants. Alternative clean-up methods should be considered according to the possible contaminants in the sediment when preparing sediments for chemical analysis and biological testing.

- The Ames assay and Mutatox™ results compared similarly when testing PAH-contaminated sediments (80% or 16/20 sediment extracts).

- Sediments are complex environmental mixtures and have problems of possible toxicity and suppression associated with the contaminants when conducting genotoxicological testing such as the Ames assay.

- The Ames assay is a more established mutagenicity test than Mutatox™, but is also more technically difficult to perform than Mutatox™.

- Mutatox™ has demonstrated that it is a useful screening tool in assessing mutagenic potential of contaminated sediments. It is a fast, economical, and technically simple test to use. Since Mutatox™ is relatively new, a suggested method of sediment assessment involves using Mutatox™ as a primary screening test with the Ames test being used as a confirmation test in a testing series of sediments.

LITERATURE CITED

Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. Molecular biology of the cell. 2nd ed. New York: Garland Publishing, Inc.; 1989.

Allen, H. E.; Noll, K. E.; Nelson, R. E. Methodology for assessment of potential mutagenicity of dredged materials. Environ. Technol. Letters 4:101-106; 1983.

Ames, B. N.; Durston, W. E.; Yamasaki, E.; Lee, F. D. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. USA. 70(8):2281-2285; 1973.

Ames, B. N.; McCann, J.; Yamasaki, E. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. 31:347-364; 1975.

Atlas, R. M. Microbiology Fundamentals and Applications. New York: Macmillan Publishing Company; 1984.

Bessi, H.; Ferard, J. F.; Vasseur, P.; Colin, F.; Belkhadir, E. Genotoxicity of hazardous leacheates from solid wastes evaluated for environmental impact with the Ames test. Environmental. Toxicol. Water Qual. 7:71-86; 1992.

Bulich, A. A. The mode of action of genotoxic agents in the restoration of light in the Mutatox™ system. Paper presented at SETAC 13th Annual Meeting. 1992. Cincinnati, OH. Available from: Microbics Corporation, Carlsbad, CA.

Butler, J. P. Separation and characterization of biologically significant chemical classes in airborne particulate organic matter. New York: Institute of Environmental Medicine, New York University Medical Center; 1985. Dissertation.

Cerniglia, C. E.; Heitkamp, M. A. Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. In: Varanasi, U., editor. Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. Boca Raton, FL: CRC Press, Inc.; 1989:41-68 (Table 1).

Chu, K. C.; Patel, K. M.; Lin, A. H.; Tarone, R. E.; Linhart, M. S.; Dunkel, V. C. Evaluating statistical analyses and reproducibility of microbial mutagenicity assays. Mutat. Res. 85:119-132; 1981.

Day, L. Bruce N. Ames. In: World Book, INC. 1988 Science Year, The World Book Annual Science Supplement. Chicago. 1987.

Doerger, J. U.; Meier, J. R.; Dobbs, R. A.; Johnson, R. D.; Ankley, G. T. Toxicity reduction evaluation at a municipal wastewater treatment plant using mutagenicity as an endpoint. Arch. Environ. Contam. Toxicol. 22:384-388; 1992.

Durant, J. L.; Hemond, H. F.; Thilly, W. G. Determination of mutagenicity in sediments of the Aberjona watershed using human lymphoblast and *Salmonella typhimurium* mutation assays. Environ. Sci. Technol. 26:599-608; 1992.

Dutka, B. J.; Kwan, K. K.; Rao, S. S.; Jurkovic, A.; Liu, D. River evaluation using ecotoxicological and microbiological procedures. Environ. Monitor. Assess. 16:287-313; 1991.

Elmore, E.; Fitzgerald, M. P. Evaluation of the bioluminescence assays as screens for genotoxic chemicals. Mutation and the Environ. 340:379-387; 1990.

Fabacher, D. L.; Schmitt, C. J.; Besser, J. M.; Mac, M. J. Chemical characterization and mutagenic properties of polycyclic aromatic compounds in sediment from tributaries of the Great Lakes. Environ. Toxicol. Chem. 7:529-543; 1988.

Fernandez, P.; Grifoll, M.; Solanas, A. M.; Bayona, J. M.; Albaiges, J. Bioassay-directed chemical analysis of genotoxic components in coastal sediments. Envir. Sci. Technol. 26:817-829; 1992.

Gatehouse, D. Guidelines for testing of environmental agents-Critical features of bacterial mutation assays. Mutagenesis. 2(5):397-409; 1987.

Grifoll, M.; Solanas, A. M.; Bayona, J. M. Characterization of genotoxic components in sediments by mass spectrometric techniques combined with *Salmonella*/microsome test. Arch. Environ. Contam. Toxicol. 19:175-184; 1990.

Grifoll, M.; Solanas. A. M.; Pares, R. Assessment of mutagenic activity of coastal sediments off Barcelona. Toxicity Assessment. 3:315-329; 1988.

Hartl, D. L. Basic Genetics. 2nd ed. Boston: Jones and Bartlett Publishers; 1991.

Ho, K. T. Y.; Quinn, J. G. Bioassay-directed fractionation of organic contaminants in an estuarine sediment using the new mutagenic bioassay, Mutatox™. Environ. Toxicol. Chem. 12:823-830; 1993.

Houk, V. S. The genotoxicity of industrial wastes and effluents. Mutat. Res. 277:91-138; 1992.

Houk, V. S.; Goto, S.; Endo, O.; Claxton, L. D.; Lewtas, J.; Matsushita, H. Detection of direct-acting mutagens in ambient air: A comparison of two highly sensitive mutagenicity assays. Environ. Mol. Mutagen. 20(1):19-28; 1992.

Huggett, R. J.; Kimerle, R. A.; Mehrle, P. M., Jr.; Bergman, H. L., editors. Biomarkers: biochemical, physiological, and histological markers of anthropogenic stress. Boca Raton: Lewis Publishers; 1992.

Jarvis, A. S.; Reilly, F. J., Jr. Initial development of methods in determining sediment genotoxicity. Environmental effects of dredging. Vol D-92-2. July 1992. Vicksburg, MS: U.S. Army Corps of Engineers Waterways Experiment Station. 1992. p. 1-6. Available from: Department of the Army, Waterways Experiment Station, Corps of Engineers, Vicksburg, MS.

Jarvis, A. S.; Reilly, F. J., Jr.; Lutz, C. H. A primer in genotoxicity. Environmental effects of dredging. Vol D-93-3. August 1993. Vicksburg, MS: U.S. Army Corps of Engineers Waterways Experiment Station. 1993. p. 1-6. Available from: Department of the Army, Waterways Experiment Station, Corps of Engineers, Vicksburg, MS.

Johnson, B. T. An evaluation of a genotoxicity assay with liver S9 for activation and luminescent bacteria for detection. Environ. Toxicol. Chem. 11:473-480; 1992a.

Johnson, B. T. Potential genotoxicity of sediments from the Great Lakes. Environ. Toxicol. Water Qual. 7:373-390; 1992b.

Jones, K. C.; Peace, E. A. The Ames mutagenicity assay applied to a range of soils. Chemosphere 18(7-8):1657-1664; 1989.

Kinae, N.; Hashizume, T.; Makita, T.; Tomita, I.; Kimura, I.; Kanamori, H. Studies on the toxicity of pulp and paper mill effluents I. Mutagenicity of the sediment samples derived from kraft paper mills. Water Res. 15:17-24; 1981.

Kwan, K. K.; Dutka, B. J. Simple two-step sediment extraction procedure for use in genotoxicity and toxicity bioassays. Toxic. Assess. 5:395-404; 1990.

Kwan, K. K.; Dutka, B. J.; Rao, S. S.; Liu, D. Mutatox test: A new test for monitoring environmental genotoxic agents. Environ. Pollut. 65:323-332; 1990.

Lan, Q.; Dickman, M.; Alvarez, M. Evidence of genotoxic substances in the Niagara River watershed. Environ. Toxicol. Water Qual. 6:1-15; 1991.

Maggard, L. A.; Brown, K. W.; Donnelly, K. C. The efficiency of two standardized procedures for extracting mutagenic chemicals from soils. Chemosphere 16(6):1243-1255; 1987.

Maron, D. M.; Ames, B. N. Revised methods for the *Salmonella* mutagenicity test. Mutat. Res. 113:173-215; 1983.

McCann, J.; Ames, B. N. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals: Discussion. Proc. Natl. Acad. Sci. USA. 73(3):950-954; 1976.

McCann, J.; Choi, E.; Yamasaki, E.; Ames, B. N. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. USA. 72(12):5135-5139; 1975.

McFarland, V. A.; Clarke, J. U.; Lutz, C. H.; Jarvis, A. S.; Mulhearn, B.; Reilly, F. J., Jr. Bioaccumulation potential of contaminants from bedded and suspended Oakland Harbor deepening project sediments to San Francisco Bay flatfish and bivalve mollusks. Miscellaneous Paper D-94-XX. In Press. Vicksburg, MS: U.S. Army Corps of Engineers Waterways Experiment Station. In Press. p. 1-98. Available from: Department of Army, Waterways Experiment Station, Corps of Engineers, Vicksburg, MS.

Metcalfe, C. D.; Balch, G. C.; Cairns, V. W.; Fitzsimons, J. D.; Dunn, B. P. Carcinogenic and genotoxic activity of extracts from contaminated sediments in western Lake Ontario. Sci. Total Envir. 94:125-141; 1990.

Microbics Corporation. Mutatox™ Manual. Carlsbad, CA. 1993. 18p.

Nealson, K. M.; Hastings, J. W. Bacterial bioluminescence: Its control and ecological significance. Microbiol. Rev. 43:496-518; 1979.

Ramel, C. Short-term testing-are we looking at the wrong endpoints? Mutat. Res. 205:13-24; 1988.

Reilly, F. J., Jr.; McFarland, V. A.; Jarvis, A. S. A low-cost combined chemical/biological procedure for the analysis of contaminated sediments. U.S. Army Corps of Engineers; Committee on Water Quality. Coastal & Inland Water Quality Seminar Proceedings; 1990 February 6-7; Las Vegas, NV. 287-299.

Ripley, L. S. Mechanisms of Gene Mutations. In: Li, A. P.; Heflich, R. H., eds. Genetic toxicology. Boca Raton, FL: CRC Press, Inc.; 1991:p. 13-40.

Tennant, R. W.; Margolin, B. H.; Shelby, M. D.; Zeiger, E.; Haseman, J. K.; Spalding, J.; Caspary, W.; Resnik, M.; Stasiewicz, S.; Anderson, B.; Minor, R. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science 236:933-941; 1987.

Ulitzur, S. Bioluminescence test for genotoxic agents. Methods Enzymol. 133:264-274; 1986.

Ulitzur, S.; Weiser, I. Acridine dyes and other DNA-intercalating agents induce the luminescence system of luminous bacteria and their dark variants. Proc. Natl. Acad. Sci. USA. 78:3338-3342; 1981.

Ulitzur, S.; Weiser, I.; Yannai, S. A new, sensitive and simple bioluminescence test for mutagenic compounds. Mutat. Res. 74:113-124; 1980.

USEPA. Test methods for evaluating solid waste: Physical/chemical methods. EPA-600/4-79-020 Methods 413.2, 418.1. Environmental Monitoring and Support Laboratory, Cincinnati, OH. 1979.

USEPA. Test methods for evaluating solid waste: Physical/chemical methods SW-846. U.S. Document No. 955-001-00000. U.S. Environmental Protection Agency, Washington, DC. 1986.

Vogel, H. J., Bonner, D. M. Acetylornithinase of *Escherichia coli*: Partial purification and some properties. J. Biolo. Chem. 218:97-106; 1956.

Warner, J. S. Determination of aliphatic and aromatic hydrocarbons in marine organisms. Anal. Chem. 48(3):578-583; 1976.

Weiser, I.; Ulitzur, S.; Yannai, S. DNA-damaging agents and DNA-synthesis inhibitors induce luminescence in dark variants of luminous bacteria. Mutat. Res. 91:443-450; 1981.

West, W. R.; Smith, P. A.; Booth, G. M.; Lee, M. L. Isolation and detection of genotoxic components in a Black River sediment. Environ. Sci. Technol. 22:224-228; 1988.

West, W. R.; Smith, P. A.; Booth, G. M.; Wise, S. A.; Lee, M. L. Determination of genotoxic polycyclic aromatic hydrocarbons in a sediment from the Black River (Ohio). Arch. Environ. Contam. Toxicol. 15:241-249; 1986.

Williams, L. R. Quality assurance considerations in conducting the Ames test. In: Taylor, J. K.; Stanley, T. W., eds. Quality assurance for environmental measurements. ASTM STP 867. Philadelphia: American Society for Testing and Materials.; 1985:p. 260-271.

Zeiger, E. The *Salmonella* mutagenicity assay for identification of presumptive carcinogens. In: Milman, H. A.; Weisburger, E. K., eds. Handbook of carcinogen testing. Park Ridge, NJ.; 1985:p. 83-99.

Zeiger, E.; Pagano, D. A. Suppressive effects of chemicals in mixture on the *Salmonella* plate test response in the absence of apparent toxicity. Environ. Mutagenesis 6:683-694; 1984.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1995	Final report

4. TITLE AND SUBTITLE

A Comparison of the Ames Assay and Mutatox in Assessing the Mutagenic Potential of Contaminated Dredged Material

5. FUNDING NUMBERS

6. AUTHOR(S)

A. Susan Jarvis

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Engineer Waterways Experiment Station
3909 Halls Ferry Road, Vicksburg, MS 39180-6199

8. PERFORMING ORGANIZATION REPORT NUMBER

Technical Report D-95-1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Corps of Engineers, Washington, DC 20314-1000

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

Available from National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The Ames assay and Mutatox were evaluated to compare their ability to identify the genotoxic potential of dredged sediments. The Ames assay has been used extensively in the testing of environmental contaminants. Mutatox, a bacterial bioluminescence test, was developed as a genotoxicity bioassay. Ten sediments with varying degrees of contamination were soxhlet extracted. These extracts were divided into crude and clean samples. Cleaned samples were prepared using silica-gel chromatography resulting in 20 extract samples. Both the Ames test (TA98 and TA100) and Mutatox were conducted with and without S9 metabolic activation. TA98+S9 and TA100+S9 indicated a positive mutagenic response in 80 and 50 percent, respectively, of the sediment extracts. Half of the extracts indicated a positive mutagenic response with TA98-S9, while only 10 percent did so with TA100-S9. Mutatox indicated a positive mutagenic response with S9 activation in 75 percent of the extracts and no mutagenic response in any of the sediment extracts without metabolic activation. In a side-by-side comparison of the Ames assay (TA98+S9) and Mutatox, 80 percent of the sediment extracts had similar responses, both positive and negative. Fifty percent of the sediment extracts had similar responses when tested with TA100+S9 and Mutatox. Mutatox compared favorably with the Ames assay and shows promise as a screening tool to assess sediment genotoxicity when used with Ames assay as a confirmation.

14. SUBJECT TERMS

Ames assay
Mutagenic potential
Mutatox
Sediment

15. NUMBER OF PAGES
89

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		