FINAL REPORT
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3/31/06

1. Project Title and EPA Grant Number:

New Approaches for Assessing Mutagenic Risk of Contaminants in the Long Island Sound Environment.
EPA Grant Number X-982582-00

2. Grantee Organization and Contact Name:

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3. Public Summary:

Urban sediments represent a reservoir of persistent contaminants that may pose a threat to both ecosystem and human health. To help evaluate these risks, testing approaches are needed that not only assess acute mortality but potential chronic effects that may reduce the fitness of affected populations. In the present study we utilized a relatively new technique, a transgenic fish mutation frequency assay primarily developed for biomedical research, to directly evaluate genotoxic potential of coastal sediments from New York City and around Long Island Sound (LIS) in the United States. Several national surveys characterizing chemical contamination in sediment and biota in U.S. estuarine waters have identified a number of sites in this area as being among the most contaminated in country (Long *et al*., 1993, Gronlund *et al.* 1991, Wolf *et al.,* 1994).

Twenty-five composit sediment samples were collected from sites in coastal embayments along both the Connecticut and New York shores of Long Island Sound, several sites in the middle of the Sound, several areas around New York Harbor and the lower Hudson River. Most of these sites were locations being sampled as part of EPA National Coastal Assessment. Sediments were first screened using the Microtox™ and Mutatox™ bacterial luminescent assays for overall toxicity and mutagenic potential of organic extracts of the sediment samples. Twelve of the sediments tested were significantly more toxic than a reference sediment in the central portion of eastern LIS, and 7 samples scored positive on the Mutatox™ test. Rank order of the toxicities indicated that the five most toxic sediment samples were from the Housatonic River near Sunnyside, Jamaica Bay, Raritan Bay, the Thames River north of Norwich, and Port Jefferson Harbor. The Mutatox™ assay is a more qualitative assay that indicates only whether a sample is genotoxic or not. Seven samples were classified as being mutatoxic. These included those from the Hudson River, Rikers Island,

Little Neck Bay, Port Jefferson Harbor, the Housatonic River near Shelton and near Sunnyside, and the Connecticut River in Hamburg Cove. Three of the seven of the samples scored as being mutatoxic were not toxic on the Microtox™ assay. Conversely eight samples that were significantly toxic in the Microtox™ assay were not mutagenic. These data indicate that not all chemicals contributing to cytotoxicity in these sediment extracts are mutagenic, and that some chemicals capable of causing mutations do not contribute to cytotoxicity. From a regional perspective these data also indicate that a relatively large number of these sediment should toxic potential but that sediment toxicity is very patchy.

The primary goal of this study was to test the utility of using embryos of a recently developed strain of the Japanese medaka (*Oryzias latipes*) carrying a lambda *cII* transgene to directly test the mutation potential of environmental samples. Because of budget constraints, not all the sites identified as either being toxic or mutatoxic in the bacterial screening tests could be evaluated using the medaka embryo assay. Sites to be evaluated with the embryos were picked to represent toxic sites from New York Harbor and the lower Hudson River, and sites along both the Connecticut and New York shores of Long Island Sound. Medaka embryos were incubated directly on sample sediments for 10 days and then hatched and reared in clean water for 60 days. Mutation frequency was assessed in the target transgene recovered from liver samples of these fish using selective plating techniques, and DNA from mutant plaques sequenced to determine mutation spectrum. Sediments from only one site near Rikers Island caused significant elevations in mutation frequency. The sediments at this site are remarkable for their extremely high levels of polycyclic aromatic hydrocarbons (PAHs). Indeed the spectrum or type of mutations observed in these fish were similar to that produced by exposure to PAHs in laboratory tests. Fractionation experiments designed to separately test the mutagenicity of different groups of contaminants found in the Rikers Island sediment confirmed that PAHs contributed significantly to the mutations observed. However, these studies also indicated that unidentified polar contaminants also contribute.

This project was the first to our knowledge to use transgenic fish embryos to directly evaluate the mutagenicity of mixtures of contaminants in sediment samples. The approach turned out to be extremely successful. Not only did the medaka embryos survive well on sediment, they provided conclusive and repeatable data quantifying both the magnitude and type of mutations caused by exposure to mutatoxic sediments. With modifications, this test could be used relatively efficiently to evaluate environmental samples. The results illustrate the utility of transgenic embryos to quantify and characterize mutations induced by exposure to environmental mutagens, providing much more valuable information that can be obtained by the bacterial screening tests. That only one of six toxic sites evaluated in LIS led to increased mutation frequency in the medaka embryo test indicates that the mutagenic risk of sediment contaminants to vertebrate

organisms is generally low in LIS, although the risk at more contaminated urban sites should be further evaluated.

4. Project Period:

9/1/02-8/31/05

5. Project Description:

The goals of this study were to assess the utility of a newly developed transgenic fish model, the Japanese medaka (*Oryzias latipes*), carrying a lambda *cII* transgene, to directly test the mutation potential of environmental samples. We first screened sediment collected from coastal embayments from around Long Island Sound and the New York/New Jersey Harbor Estuary for toxicity and mutagenicity using the luminescent bacterial tests Microtox™ and Mutatox™. Many of the sediments tested were collected as part of EPA's National Coastal Assessment. A subset of these sediments were then used in the medaka test where newly fertilized embryos were grown directly on sediment throughout most of their development, hatched in clean water and grown up to sufficient size to harvest liver tissue for DNA. Mutation frequency was assessed in the transgene mutation target and mutant plaques produced sequenced to determine mutation spectrum. Additional work was conducted on the site with the highest mutation frequency, notable for the extremely high levels of PAHs found there (Long *et al*. 1993). Repeat analysis of freshly collected sediment from this site confirmed its mutagenicity. A fractionation study indicated that PAHs in extracts of this sediment contributed significantly to mutagenicity, although more polar compounds also contributed. Sequence analysis of mutations observed confirmed this finding.

This study expanded data being collected in coastal embayments from around Long Island Sound by EPA's National Coastal Assessment by providing additional measures of cytotoxicity and mutagenicity assessed by the bacterial Microtox™ and Mutatox™ screening assay, giving managers a more complete picture of potential toxicity of sediments from this area than was available before. In addition, some sites were also assessed in the medaka embryo tests. The results of this study support the use of the transgenic medaka embryos as a valuable tool to assess sediment mutagenicity in that quantitative and repeatable results can be obtained relatively easily. This approach is easily amenable to assay directed fractionation studies aimed at identification of contaminants responsible for effects observed.

6. Activities & Accomplishments:

The project commenced once funding decisions were made in July of 2002 when a collaboration was set up with Lawrence Swanson of Stony Brook University and Paul Stacey of CT Department of Environmental Protection to obtain splits of sediment samples collected as part of the NY and CT portion of

EPA's National Coastal Assessment. These coastal samples were augmented with samples collected near the center of Long Island Sound to serve as reference samples. In addition some archived samples from NY City that had been tested in a previous study and found to be both toxic and mutagenic were included as positive controls.

A graduate student, Douglas Potts, was hired to complete Mutatox™ analysis of sediments collected during the Fall 2002 semester. The graduate student was assisted by a foreign exchange student, Sandra Leuke. Unfortunately reagents for Mutatox™ analysis were back ordered and did not arrive until the end of October.

Preliminary runs to optimize protocols were completed during November and December, with full replicated runs completed for both Mutatox™ and Microtox™ between January and March 2003. Results indicated that almost half (12 out of 25) of the sediments tested were significantly more toxic than the reference site in the Microtox™ test with seven sediments showing a positive response in the Mutatox™ test. There was not particularly good agreement between the two tests, indicating that these test are responding to different properties of the organic extractable material in the sediments. Because such a large number of sites tested positive in at least one of the bacterial tests, and few tested positive in both, these results were not terribly useful in selecting a limited number of sites for further study with the medaka embryo tests. Largely based on background information on levels of chemical contamination and location, we picked a representative suite of six potentially toxic sites and one reference site to examine with the medaka embryo test.

Seven sediments were sent to Brookhaven National Laboratory (BNL) to conduct the medaka embryo tests in March 2003. Due to problems associated with growing larvae in preliminary studies, after exposure, embryos were shipped to Richard Winn's laboratory at the University of Georgia for grow out and further analysis. There they were grown in clean water for 60 days and their livers harvested and DNA extracted. Determination of mutation frequency on samples collected began in July and was completed in September. Mutant plaques were cloned and sent back to BNL for sequencing.

Based on results obtained we decided to focus on Rikers Island sediment for the fractionation study in year two and fresh sediments were collected from the LIS reference site and Rikers Island in October 2003. A large sample of these sediments was extracted and split into compounds class specific fractions using column chromatography by graduate student Robin Barnes.

Co-PI Lynn Mendelman began working on sequencing the mutant plaques during the spring of 2004. During this time an honors college student, Alexander Bogler completed a related project determining uptake and metabolism of radioactive benzo[α]pyrene by medaka embryos. We also carried out a dosing

study where medaka embryos were exposed to reference sediment dosed with two concentrations of benzo[α]pyrene as well as to fresh Rikers Island and reference sediment. Winn's lab completed mutation frequency analysis of samples from these experiments in July 2004, and conducted the embryo exposures to sediments dosed with compound class specific fractions in October 2004. Mutation frequency analysis was completed in February 2005, and sequencing of mutant plaques completed in April.

Chemical analysis of reference, BaP dosed, and Rikers Island whole and fractionated sediments for PAHs were completed in Bruce Brownawell's laboratory during the spring of 2005.

Although some of the experiments and analyses conducted as part of this project were delayed due to back-ordered supplies and availability of support staff, all major objectives of the project were met. The results conclusively support the value of this approach in assessing mutagenicity of coastal sediments.

7. Modeling:

Modeling was not part of this project.

8. Summary of Findings:

The findings of this project are presented in the figures and tables located in the attached document *McElroy LIS Final Report Figures & Tables.*

Sediment Collection:

Sediment collection was accomplished primarily through contacts made with Lawrence Swanson at Stony Brook University and Paul Stacey at CT Department of Environmental Protection who are in charge of their state's portions of EPA's National Coastal Assessment (NAC). Their staff kindly preserved a portion of the sediments collected for NCA analysis in pre-cleaned glass jars provided by us. Sediments from 18 stations from LIS, Jamaica Bay, Raritan Bay and the lower Hudson River were obtained from the NCA. These samples were augmented with sediment collected from the central portion of LIS to serve as less impacted reference sites, and some sediments collected from two sites in NY City that had been studied in previous work as positive controls. Station descriptions and locations are given in Table 1, and their positions shown in Figure 1. All sediments were collected by Smith MacIntyre grabs, with only the top 5 cm of sediment being saved. Collections from each site consisted of a composite sample of at least three individual grabs. After collection all sediments were sieved to at least 1 mm to remove debris and macrofauna, and stored in the

dark at 4°C until tested. Sub-samples taken for sediment contaminants concentrations were frozen until analysis.

Microtox™ and Mutatox™ Screening Assays

To provide a more complete picture of sediment properties, we decided to run the Microtox™ bacterial luminescent assay of cellular toxicity on all samples. Compared to Mutatox™, this assay is relatively quick and easy to run and provide quantitative information on the relative toxicity of each sample in the set. We ran the assay following directions supplied by the manufacturer (Azur Environmental). We had some trouble conducting the Mutatox™ assay on these sediments. Necessary bacterial isolates were back-ordered for several months delaying the start of the project. Some batches of bacteria showed either high light output on solvent controls or low output on positive control and could not be used. In almost all the runs we observed unexplained light output at early time points requiring that made these data unusable. To overcome these difficulties, we adopted a stringent requirement for determination of a positive output in the test, accepting a test as positive only if light output of more than 2 times the solvent control and a light value of 50 or greater were obtained in two independent (using different vials of bacteria) runs of the test. If a sample scored positive in only one of two runs, a third run was conducted.

Results from Microtox™ and Mutatox™ analysis are shown in Table 2. Direct acute toxicity as measured by the Microtox™ test was observed in 12 out of 25 sediment extracts analyzed with mean EC50s being significantly below that of the Eastern Sound site A reference sample (Table 2). Mean EC50s of a few additional sediment extracts appeared to be toxic, but either because the computer program failed to calculate confidence intervals on both runs, or because the sample could only be analyzed once, these were not included in the group showing significant differences. Rank order of the EC50s indicated that the five most toxic sediment samples were from the Housatonic River near Sunnyside, Jamaica Bay, Raritan Bay, the Thames River north of Norwich, and Port Jefferson Harbor. This Housatonic River sample had an EC50 37 times lower than the reference site (0.034 as compared to 1.254 mg/ml for the 5 minute test), with EC50s of the five most toxic sites all being at least 15 times lower than that of the reference site. Although extracts of sediment collected from the Housatonic River near Sunnyside showed the lowest average LC50 in duplicate runs, variability in the dose response of the second run resulted in extremely large error estimates that resulted in this site not meeting the criteria for a significant difference from the reference site in both runs. Nine of the NCA sites were significantly toxic in the Microtox™ test, despite the fact that none of these sites were classified as being toxic in the *Ampelisca abdita* tests conducted on these same sediments as part of the NCA (pers com. P.Stacey & L. Swanson). These data suggest that either the Microtox™ test is more sensitive than the amphipod test, or the organic sediment extracts used in this study for the

Microtox™ test included compounds not bioavailable in the whole sediments evaluated in the amphipod test.

Toxicity observed appeared to be quite patchy as even among the deep water, supposedly reference, sites in the middle of LIS, EC50s ranged by a factor of 10. In the CT River where three samples sites were evaluated, toxicity also varied by a factor of 5 or more.

Data from the Mutatox™ assay are also summarized in Table 2. Because this assay does not provide a dose response curve, no EC50 could be calculated. Reversion to the light emitting phenotype observed at one concentration tested can be reversed due to cytotoxicity at a higher concentration. We also observed large batch-to-batch variation in spontaneous reversions in negative control samples, which is why we only scored an extract as being genotoxic if positive results were obtained in two separate tests. Seven samples were classified as being mutatoxic. These included those from Hudson River, Rikers Island, Little Neck Bay, Port Jefferson Harbor, the Housatonic River near Shelton and near Sunnyside, and the Connecticut River in Hamburg Cove. Three of the seven samples scored as being mutatoxic were not toxic on the Microtox™ assay. Conversely eight samples that were significantly toxic in the Microtox™ assay were not mutagenic. These data indicate that not all chemicals contributing to cytotoxicity in these sediment extracts are mutagenic, and that some chemicals capable of causing mutations do not contribute to cytotoxicity. These data also indicate a fairly widespread but patchy occurrence of toxic sediments throughout the area, at least as measured using these bacterial screening assays.

Medaka Embryo Assay

Due to resource constraints, not all sediments testing positive on the Mutatox™ assay could be tested using the medaka embryo assay. Samples were picked to provide a broad geographic coverage of the area, two from the Connecticut shore of LIS (the Housatonic near Shelton and the Connecticut River near Hamburg Cove), the New York shore of LIS (Oyster Bay and Little Neck Bay), two from the New York City area (Rikers Island, and Hudson River), and a reference area (Eastern LIS A). With the exception of those from the reference site, all sediments chosen for further analysis were either toxic or mutatoxic in the Microtox™ or Mutatox™ tests. A schematic of the conceptual framework for the medaka embryo assay is provided in Figure 2, and is described briefly in McElroy *et al.* (2006). Methods used for analysis of mutation frequency and spectrum are provided in Winn *et al.* (2000). Embryo survival on the test sediments from LIS was excellent (Table 3) indicating supporting the use of medaka embryo tests to evaluated sublethal effects of sediment contaminants.

Mutation frequency data from the *cII* transgene recovered from liver tissue samples of medaka grown up from sediment-exposed embryos are shown in

Figure 3 and Table 4. Analysis of variance showed significant differences between all sites tests ($p<0.001$), and the post hock Tukey-Kramer test showing that mutation frequency was significant higher in the fish exposed to Rikers Island sediment as compared to all treatment groups. Mean mutation frequency for the reference group was 3.85 $\pm$ 0.35 SEM x 10^{-5}, whereas that of the Rikers Island exposed fish was 10.3 $\pm$ 1.6 SEM x 10^{-5}. Mean values for all but one of the other treatments were higher than the reference group but were not statistically different from the reference group. on. As shown in the bar and whisker plot representation of the same data (Figure 4), in all groups except the reference group, some individuals had mutation frequencies higher than 95% of the distribution. This was particularly noticeable in the Rikers Island treatment where there were 3 out of 14 individuals with mutation frequencies in excess of 15 X 10 (Table 4). These data indicate that while the average mutation frequency was not significantly elevated, some individual fish from each group exposed to contaminated sediments showed highly elevated mutation frequencies. Evaluation of the types of mutations observed in these individuals might provide valuable information on the types of mutation stress encountered.

Additional information can be obtained by sequencing the recovered transgene to determine exactly what base pair substitution, additions or deletions have occurred in the *cII* mutation target. Since the entire sequence of the cII gene is known, any changes in the nucleotide (A=adenine, T= thymidine, C= cytosine, and G=guanine) sequence can be readily identified. A detailed description of this approach can be found in Winn et al. (2005). To obtain an initial picture of the types of mutations observed in response to exposure to sediments, mutant plaques from mutation assay were sequenced and compared between the reference site and the Rikers Island site. Data on the incidence of transitions, transversions and frame shift mutation are shown in Table 5. Mutant plaques from fish exposed to Rikers Island sediment showed what appears to be a higher proportion of G/C to T/A, G/C to C/G, and A/T to T/A transversions in the *cII* sequence than those from the LIS reference fish. These types of mutations are similar to those observed in mutation studies of both fish and other vertebrate models induced by the PAH benzo[α]pyrene (BaP) (Jian *et al*., 1991; Monroe et al, 1998; Winn & Norris, 2005).

The results of the first year activities on this project clearly demonstrated that the transgenic medaka embryo test could be successfully used to assess mutagenicity in whole sediment samples from the environment. Further, it demonstrated that most of the sediments from LIS and even NY City were not significantly mutatoxic in a vertebrate test. Other than Riker's Island, only the site the Housatonic River showed any signs of elevated mutation frequency, and these were not statistically significant. The Microtox™ and Mutatox™ screening tests conducted on organic extracts of the sediments indicated a much greater percentage of sediments evaluated were either cytotoxic, mutatoxic or both. Many sites were toxic in the Microtox™ test, less in the Mutatox™ test, and they were often not the same sites. Of the NCA sediments analyzed, data obtained

from NCA on levels of PAHs, PCBs, pesticides, and metals in these sediments indicated that none of them showed any significant levels of contaminants nor were identified as being toxic in the *Ampelisca abdita* amphipod test (pers Com. P Stacey, L. Swanson). These findings indicate that although relatively inexpensive, screening sediment toxicity with the Microtox™ or Mutatox™ bacterial assays may be overprotective with regard to either invertebrate or vertebrate responses.

Year two activities were focused on providing additional information to support use of the medaka test and to further characterize mutagenicity at the Rikers Island site. Fresh sediments were collected from the eastern central LIS reference site as well as from Rikers Island. It should be noted that the Rikers Island sediment used in year 1 was archived from a previous study and had been stored a 4 ^{o}C for five years when the embryo test was first conducted. The fresh sediments were tested again, along with sediment dosed with two concentrations of the model mutagen, BaP (20 and 54 ug/gdw as measured by gas chromatographic analysis),. In addition, using radiolabeled BaP, we conducted a small test to determine the extent to which medaka embryos absorb PAHs from sediments during a ten-day exposure, and to determine the extent to which embryos are capable of transforming accumulated BaP to more polar, and likely more mutagenic metabolites. Finally a large batch of sediment from the Rikers Island and reference sites were extracted with organic solvents, and the extract partitioned into compound class specific groups using column chromatography using methods developed by McElroy et al. (2000). The whole extract and the fractionated extracts were then amended onto reference sediment and these dosed sediments tested using the medaka embryo assay. As before, in addition to mutation frequency, mutant plaques were sequenced to provide data on the types of mutations formed.

Embryo survival was again excellent in this second sediment exposure experiments with 85 to 97 percent of the embryos surviving, and 6665 to 79% of the embryos hatching (Table 6). Hatching success appeared to be lowest among the embryos exposed to Rikers Island sediment, but these differences were not tested statistically. The *cII* mutant frequencies for the Reference Site, Rikers Island, BaP Low (20 ug/gdw), and BaP high (50 ug/gdw) treatments were 3.8 $\pm$ 0.3 X 10^{-5}, 6.4 $\pm$ 0.5 X 10^{-5}, 4.4 $\pm$ 0.4X 10^{-5}, and 6 $\pm$ 0.5 X 10^{-5}, respectively (Table 7, Figure 5). The mutant frequency in livers from the Rikers Island treatment was 1.7-fold greater than the Reference Site. The fish livers from the BaP High treatment exhibited a 1.6-fold induction of mutants compared to the Reference Site, while fish from the BaP Low treatment had a 1.2 fold induction in mutant frequency over the Reference Site. The frequencies from both Rikers Island and BaP High treatments were significantly different than those exposed to sediments from the Reference Site. There was no significant difference in the mutant frequency in fish exposed to the Reference Site and BaP Low (Figure 5).

Sequencing of *cII* mutant plaques recovered was again conducted to obtain information about the likely causes of mutations observed. These data are shown in Table 8 and Figure 6. A:T to C:G transversions comprised the predominant class of mutations (Mutation Frequency (Mf) = 0.8 X 10^{-5}), followed by G:C to A:T and A:T to G:C transitions (Mf = 0.7 X 10^{-5} for both) for the Reference Site. In contrast Rikers Island exhibited a high percentage of A:T to G:C (Mf = 1.4 X 10^{-5}) and G:C to A:T (Mf = 1.3 X 10^{-5}) transitions as well as G:C to T:A (Mf = 0.9 X 10^{-5}) and G:C to C:G (Mf = 0.7 X 10^{-5}) transversions . Mutations from the BaP High treatment were comprised predominately of G:C to A:T transitions (Mf = 2.0 X 10^{-5}) followed by insertions (Mf = 0.8 X 10^{-5}) and A:T to G:C transitions (Mf = 0.7 X 10^{-5}). Overall, G:C to A:T transitions, G:C to T:A, G:C to C:G, A:T to T:A transversions, and +1 frame shift mutations increased for both Rikers Island and B[a]P High over the Reference Site. Rikers Island also exhibited an increase in A:T to G:C transitions compared to both Reference Site and BaP High treatment. There was an overall increase in G:C mutation frequencies for both Rikers Island (Mf = 2.9 X 10^{-5}; 46%) and B[a]P High (Mf = 3.1 X 10^{-5}; 54%), approximately 2-fold, over the Reference Site (Mf = 1.4 X 10^{-5}; 36%). The mutation spectrum observed for the Rikers Island and BaP dosed sediments are consistent with those acquired previously by Winn's group in experiments where adult fish were dosed with BaP (Winn et al. 2005), and are consistent with results obtained in year one with different batches of both reference and Rikers Island sediments. These data illustrate how mutation spectrum data can be used to help identify the types of contaminants responsible for mutations observed.

Fractionation Study with Medaka Embryos

In an attempt to further identify the types of compounds responsible for the elevated mutation frequency observed at the Rikers Island site, a fractionation study was conducted where silica/alumnia column chromatography was used to partition the organic extract of a large sample of sediment from Rikers Island into four fractions based on polarity and size using methods previously developed (McElroy et al. 2000). Using this scheme, the F1 fraction primarily contains saturated hydrocarbons and the unresolved mixture of petroleum hydrocarbons, the F2 fraction primarily PCBs and pesticides, the F3 fraction, the bulk of the higher molecular weight PAHs, and the F4 fraction more polar compounds such as nonylphenol, steroids and drugs. Once prepared, these extract fractions were dosed back onto reference sediment at concentrations roughly two times that originally observed, in order to compensate for loss during the extraction, separation, concentration, and sediment dosing procedures. Briefly, if an extract was created from 1 kg of sediment, it was dosed back onto 0.5 kg of reference sediment. Chemical analysis of PAH and nonylphenol concentrations in the sediments prepared confirmed that the fractionation and sediment dosing scheme worked as planned, and that PAH levels were extremely high in the Rikers Island sediment (Table 9). Twelve sediment treatments were evaluated in this phase of the study: whole Rikers Island or

Reference sediments as before, reference sediments dosed with extracts of the Rikers Island or reference sediment, and reference sediments doses with fractions 1-4 of Rikers Island or reference sediment. Unlike previous assays, where no significant embryo mortality was observed when comparing reference to test sediments, in this experiment medaka embryos exposed to reference sediment dosed with fraction 3 of the Rikers Island sediment did not hatch (Table 10). Due to the significant delay in hatch observed in this group, a subset of the embryos were frozen, to allow DNA analysis to be conducted in this group. It is interesting to note that several developmental abnormalities were observed in embryos from this group included tube heart and cyclopia (merged eyes) as illustrated in Figure 7. The remainder of the embryos were grown up in clean water as before, and mutation frequency and spectrum evaluated either in DNA extracted from the livers as fish, or in the case of the Rikers Fraction 3 exposure, DNA extracted from frozen embryos. Mutation frequencies for this experiment are shown in Table 11 and Figure 8.

The delayed hatch and embryo toxicity observed in the embryos exposed to the PAH fraction of the sediment extract provided an unexpected additional beneficial outcome for this study. The fact that we were able to quantify elevated mutation frequency in DNA extracted from embryos indicates that the mutations produced are off sufficient magnitude to be detectable by the end of embryonic development (ten days), and do not necessarily need to be amplified by tissue proliferation observed in juvenile and adult fish. This also suggests that embryos are much more sensitive than adult fish to environmental mutagens, further supporting their use as a testing model. These results indicate that in future work with this model, the lengthy fish grow out stage to produce liver tissue of sufficient size for dissection may be unnecessary. Personnel effort and aquarium space associated with the two month grow out period utilized in our experiments represented the most significant component of the budget associated with the mutation frequency and spectrum determination. If this phase is unnecessary, significant time and cost savings could be achieved, making this assay more amenable for large-scale environmental testing.

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As is apparent from Figure 8 and Table 11, mutation frequency observed in fish grown up from embryos exposed to reference sediments, or reference sediments amended with either whole extracts or fractionated extracts of reference sediment were similar to what had been observed in previous experiments, and were not different from each other indicating that the sediment extraction, fractionation, and sediment re-dosing procedure did not in itself cause mutations in medaka embryos. Looking at mutation frequency results from the Rikers Island sediments and fractions, it can also be seen that Whole Rikers Island sediment and the whole organic extract yielded values similar to each other and to previous tests with this sediment, indicating that organic chemicals were most likely responsible for the mutagenicity of the intact sediment. In contrast, exposure to reference sediments dosed with fractions 1 and 2 of the Rikers sediment extract yielded mutations frequencies that were significantly

lower than all other tests with the Rikers Island sediment indicating that material in these two fractions did not contribute significantly to the mutations observed.

These data provide direct evidence that polycyclic aromatic hydrocarbons are indeed contributing significantly to the increase in mutation frequency observed, however they also indicate that as yet unidentified material in the polar fraction are also contributing to the elevation in mutation frequency observed.

Conclusions:

The results of this study provided baseline information on cytotoxicity and mutagenicity of a relatively large number sediment samples collected around LIS. Bacterial screening tests conducted on organic extracts of sediments indicated that toxicity was patchy but fairly widespread. These results are in contrast to preliminary reports of whole sediment toxicity tests conducted as part of EPA's National Coastal Assessment where no toxicity was observed. These data indicate that toxicity tests conducted on sediment extracts may over-estimate actual toxicity produced by intact sediments. Application of the new transgenic medaka embryo test indicated increased mutagenic potential at only the most contaminated sites evaluated, Rikers Island, an urban site containing very high levels of PAHs. Data from mutation spectrum analysis and a fractionation study confirmed that PAHs in this sediment contributed significantly to mutations observed, although other polar contaminants were also playing a role. Further work should focus on other areas of the Harbor Estuary with known levels of PAH inputs, and in other areas receiving significant contaminant inputs where the embryos test could be used to assess the toxicity of a wide range of environmental contaminants.

The approach of using λ transgenic Medaka to determine the mutagenic potential of sediment by assessing its mutation frequency and mutation spectrum is a novel method. This technique allows whole sediments to be assessed directly without chemical modification. Through direct contact with the sediment, the embryo accumulates only the bioavailable fraction of contaminants associated with the sediments. As a metabolically competent organism, the embryo also has the ability to bioactivate and potentially detoxify contaminants. As such this method allows both environmentally and physiologically realistic exposure scenarios. Furthermore the assay provides direct evidence of mutagenicity in a vertebrate organism as well as the potential to identify potential classes of responsible contaminants through the spectrum of specific mutations produced.

Embryo tests have potential use in environmental assessment to evaluate a wide range of both lethal and sublethal endpoints including mutagenesis and other biochemical, development, and molecular responses resulting from

exposure to contaminated sediments. Coupled with fractionation studies, such as the one conducted here, embryo tests can also provide definitive data on the specific classes of contaminants responsible for effects observed.

Presentations/Publications/Outreach:

Platform talks on this work have been presented at three national or international meetings.

> *New Approach for Assessing Mutagenic Risk of Contaminated Sediments*. Presented at the Society of Toxicology and Chemistry annual meeting in Austin Texas in November, 2003
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> *Use of Transgenic Medaka to Evaluate Mutagens in Urban Sediments*. Presented at the 13th Pollutant Responses in Marine Organisms conference in Alessandria Italy in June, 2005.
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> *Use of Transgenic Medaka to Evaluate Environmental Mutagens*. Invited talk at a National Institutes of Environmental Health sponsored workshop on Aquatic Models of Human Disease at the University of Georgia in November, 2005.

In addition three manuscripts are being prepared describing the results of this study.

> McElroy , A.E., Bogler , A. D. Weisbaum, D., Norris, M., Mendelman, L.V., Setlow, R., & Winn, R. 2006. Uptake, metabolism, mutant frequencies and mutational spectra in λ transgenic medaka embryos exposed to benzo[α]pyrene dosed sediments. In Press. *Marine Environmental Research.*
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