INTERIM REPORT I

ON

RESPONSE OF THE P450 HUMAN REPORTER GENE SYSTEM (HRGS) ASSAY TO EXTRACTS OF SEDIMENTS COLLECTED FROM SAINT LUCIE BAY, FLORIDA IN 2001

TO

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INTRODUCTION

Background and Rationale

Both NOAA and the state of Florida resource agencies were concerned with the possible contamination of sediments in Saint Lucie Bay, Florida. Sediments were collected from 20 stations in May of 2001 and sent to various laboratories for both biological and chemical analyses.

Preparation of Sample Extracts

Sediment samples (21) were extracted using EPA Method 3540 (soxhlet). Briefly, approximately 20 g of sediment from each station were extracted to yield 1 mL dichloromethane (DCM) containing the organic compounds, and a sub-sample of sediment was used to determine percent solids. Extracts were split into a small (1g wet; 5%) portion for MicroTox, and the larger (19g wet 95%) portion for P450 Human Reporter Gene System (HRGS) analysis (EPA Method 4425). The Mirotox fraction was evaporated to near dryness and exchanged into 1 mL of DMSO. The 4425 fraction was then exchanged into a DMSO mixture (2:1:1) with toluene and isopropyl alcohol, for a final volume of 1 mL. Two 1 mL vials were prepared from each sample and one was shipped to the Columbia Analytical Services laboratory in Vista, CA for analysis by Method 4425 and the other to the NOAA laboratory in Charleston, SC for MicroTox testing.

METHODS

4425 Analysis

The basic methodology of the P450 Reporter Gene System (HRGS) has been described elsewhere (APHA 1998, ASTM, 1999, EPA 1999). Ten μL of sample extracts were applied to two replicate exposure wells and incubated for 16 hours. Cells were then washed, lysed, and the solution centrifuged. Fifty μL of the supernatant was then applied to a 96-well plate, followed by 100 μL of a cofactor solution, and then 100 μL of the enzyme substrate luciferin. Luminescence was then measured as relative light units (RLU) using a ML 2250 Luminometer. A solvent blank and reference inducers (2,3,7,8-TCDD and B[a]P) were used with each sample test run.

Equivalency Calculations

Benzo[a]pyrene Equivalents (B[a]PEq) were calculated for all sample extracts. The B[a]PEq is a measure of the CYP1A1-inducing PAHs, plus any coplanar PCBs, dioxins or furans that may be present in the sample and are calculated as follows:

$$B[a]PEq\ (\mu g/g) = ((\text{fold induction} / 60) * (\text{volume factor}/ \text{dry weight}))* d.f.$$

Fold induction is calculated as mean relative light units (RLU) produced by the sample divided by mean RLU produced by the solvent blank. The factor of 60 represents the approximate fold induction produced by 1.0 μg of B[a]P/mL. The volume factor (100) represents the total extract volume (1 mL) divided by the volume of extract applied to the cells (10 μL). Dividing by the dry weight of each sample, calculated using percent solids of the 19 g samples, yields B[a]PEq in μg/g dry weight. If a dilution is used, the B[a]PEq value is multiplied by the dilution factor.

A standard curve for a dioxin/furan mixture has demonstrated that fold induction per mL is equal to the dioxin Toxic Equivalents (TEQ_{HRGS}) in pg/g dry weight. Therefore, the equation to express the data as only chlorinated inducers (in ng/g) is as follows:

$$TEQ_{HRGS}\ (ng/g) = ((\text{fold induction }) * (\text{volume factor}/ 1000 * \text{dry weight}))* d.f.$$

RESULTS

The first observation regarding the extracts received in Vista from the Columbia Analytical Services (CAS) laboratory in Jacksonville, FL was that the extracts from the Saint Lucie samples were lighter in color than most sediment extracts tested thus far. Since the same wet weight of 20 g was used as previously, the lighter color could be the results of larger particle size, lower organic matter, or lower concentrations of PAHs. Based on experience, it was determined that 10 μL, instead of the 2 μL used previously, would be applied from each extract to the test system.

The information sheet lists the NOAA and CAS sample numbers for the 21 samples, as well as the method blanks, laboratory control samples, and the standard reference material (SRM). The data sheet shows the raw data from testing of the samples on June 12 and 15, 2001. In the second test samples that produced a coefficient of variation greater than 20% were re-tested, along with SRM samples that required dilution.

An overview of the distribution of HRGS responses, on a basis of μg/g of B[a]P equivalents is shown in the enclosed graph. None of the samples produced responses of 60 μg/g B[a]PEq or greater, which has been associated with degraded benthic communities. In addition, no samples were at the level of response (about 32 μg/g of B[a]PEq) where biological effects might be expected to occur (Anderson et al. 1999a). All but 3 samples were below 11 μg/g of B[a]PEq, where impacts on the benthos would not be expected.

Quality Control and Quality Assurance

Also shown on the data sheet are the responses to the standard inducing chemicals (dioxin; TCDD and B[a]P). Fifty parts per trillion (ppt) of TCDD produced fold induction values of 60.3 and 67.9 on the two test periods. Three-hundred ppb of B[a]P produced fold induction values of 10.5 and 12.2 on the two test periods. The solvent blanks produced B[a]P equivalent values of 2.0 and 0.5 μg/g B[a]PEq, approximately the level of the lowest sediment samples. The laboratory control samples (20g) were spiked with 2.5 ng TEQ of a dioxin/furan mixture, which should produce a calculated TEQ of 0.125 ng/g (ppb). The data sheet shows that the calculations produced values of 0.092 and 0.124 ng/g TEQ for these two LCS samples (73 & 99 %).

CONCLUSIONS

Since this report provides data on only the 4425 testing of the sediments, it is too early to reach any conclusions regarding the significance of the findings or possible explanations for the results. In comparison to several previous studies, the samples from Saint Lucie Bay, Florida contain much lower amounts of the types of chemicals that induce the CYP1A1 gene (PAHs, coplanar PCBs, dioxins, furans). The 7.0 μg B[a]PEq/g mean and 10.4 upper 99 % CI observed in this study are nearly as low as the South Florida Study conducted in 1999.

It is certainly possible that chemicals other than those that attached to the Ah receptor are present in these samples, and are producing toxic effects on the biota. We will be interested to learn what the many other tests conducted have found. Since we do not know which inducers have produced the responses measured above the upper 99 % CI (3 samples), we recommend testing these samples for levels of inducing compounds. These findings will determine if the observed responses are from PAHs or chlorinated organics in the sediments.

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APPENDIX