S. IAN HARTWELL

Columbia Analytical Services inc.

January 12, 1998

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NOAA\ORCA
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7600 Sand Point
Seattle, WA 98115

Jawed Hameedi
DOC/NOAA/NOS/ORCA/CMBAD
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SSUM-4, N/ORCA21
Silver Spring, MD 20910

Dear Ed and Jawed:

The enclosed revised report includes the most recent testing by P450 RGS of the most highly contaminated samples using our two-time-interval assay. We have shown that samples containing only PAHs will always decrease in induction between 6 and 16 hours, as the P450 produced is degrading the compounds over time. Since the chlorinated chemicals which induce this system (coplanar PCBs, dioxins, furans) are not readily degraded, the response to these compounds is always greater at 16 hours than at 6 hours. Mixtures of the two types of inducing compounds, as explained in the report, will give intermediate responses. Figure 4 shows that the RGS response to NOAA # 11 is predominately from chlorinated hydrocarbons, but the other 12 samples tested at two time periods contain lower levels of chlorinated hydrocarbons. Significant amounts of both classes of inducing compounds are present in each of the 13 samples.

Thank you for providing the additional funds to screen these samples at both time periods, and I will be pleased to answer any questions you may have regarding the latest results. We look forward to working with you both on another NOAA project in the future.

Sincerely,

Jack W. Anderson
Senior Aquatic Toxicologist

RESPONSE OF THE P450 RGS ASSAY TO EXTRACTS OF SEDIMENTS COLLECTED FROM DELAWARE BAY

TO

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FROM

JACK ANDERSON and JENNIFER JONES
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Revised January 12, 1998

RESPONSE OF THE P450 RGS ASSAY TO EXTRACTS OF SEDIMENTS COLLECTED FROM DELAWARE BAY

ABSTRACT

The induction of the cytochrome P450 gene family, specifically CYP1A1, in response to toxic and/or carcinogenic organic compounds is widely used as a biomarker for exposure to contaminants in the aquatic environment. Organic compounds, including dioxins, furans, coplanar PCBs, and high molecular weight PAHs, are known to bind to an intracellular cytosolic protein referred to as the aryl hydrocarbon (Ah) receptor. This complex is translocated to the nucleus of the cell, where it interacts with xenobiotic response elements in the promoter of the CYP1A1 gene and causes transcription of the P450 enzyme system. CYP1A1 induction has been used in the development of toxic equivalency factors (TEFs) for polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and PCB congeners as a measure of their potency relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. TEFs have been developed by EPA for PAHs as a measure of carcinogenic potential compared to benzo[*a*]pyrene.

The P450 Reporter Gene System (RGS) measures induction of the CYP1A1 protein via the reporter gene, firefly luciferase, to determine the presence of inducing compounds in environmental samples. As such, RGS gives an actual assessment of the potential of contaminants on sediments at a specific site to produce chronic and/or carcinogenic effects on flatfish if they were to occupy the site. It also gives an indication of the chronic effects on benthic organisms at the site, as an RGS response above 60 μg of benzo[*a*]pyrene equivalents per g was highly correlated with degradation of infaunal communities in San Diego Bay, CA.

This testing program was designed to determine the potential toxicity and carcinogenicity of sediments collected from Delaware Bay. The results of this project illustrate the ability of P450 RGS to identify, rapidly and inexpensively, the most toxic and hazardous samples within a harbor, bay or coastal section. Using the entire data set (n = 81), 13 sediment samples were identified as containing concentrations greater than 60 μg B[*a*]PEq/g. The mean for all samples was 57 μg B[*a*]PEq/g, and there was a great deal of variation with the standard deviation of 191 μg B[*a*]PEq/g. Using the 95% confidence value of 41.5, 11 samples were found to be significantly higher in potential toxicity, as they were above the upper 95% confidence interval (99 μg B[*a*]PEq/g). The sample that was by far the highest in CYP1A1 inducing compounds (1,584 μg B[*a*]PEq/g) was tested at both 6 and 16 hours of exposure, and the observed responses indicated the presence of chlorinated organics (coplanar PCBs, dioxins, furans). The 13 samples producing the highest RGS responses at 16 hours were diluted and tested at both 6 and 16 hours to provide an estimate of the relative contributions from PAHs and chlorinated organics. Even the 1:300 dilution of sample #11 showed a prominent contribution from the slower acting chlorinated hydrocarbons. The other 12 samples exhibited responses which indicated the primary inducing compounds were PAHs, but chlorinated organics were also contributing to the total CYP1A1 induction measured.

INTRODUCTION

The induction of the cytochrome P450 gene family, specifically CYP1A1, in response to toxic and/or carcinogenic organic compounds is widely used as a biomarker for exposure to contaminants in the aquatic environment. Organic compounds, including dioxins, furans, coplanar PCBs, and high molecular weight PAHs, are known to bind to an intracellular cytosolic protein referred to as the aryl hydrocarbon (Ah) receptor. This complex is translocated to the nucleus of the cell, where it interacts with xenobiotic response elements in the promoter of the CYP1A1 gene and causes transcription of the P450 enzyme system. CYP1A1 induction has been used in the development of toxic equivalency factors (TEFs) for polychlorinated dibenzo-dioxins (PCDDs), polychlorinated dibenzo-furans (PCDFs), and PCB congeners as a measure of their potency relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Safe 1994). Similarly, TEFs have been developed for PAHs as a measure of carcinogenic potential compared to B[*a*]P (U.S. EPA 1993).

The induction of the cytochrome P450 gene family, specifically CYP1A1, in response to PAHs has been extensively studied in fish (Stegeman and Hahn 1994, Collier *et al*, 1995, 1996). These studies have demonstrated that higher levels of P450 in fish are correlated with increased sediment contamination, especially by PAHs, and histological and reproductive effects on the fish.

The P450 Reporter Gene System (RGS) measures induction of the CYP1A1 protein via the reporter gene, firefly luciferase, to determine the presence of inducing compounds in environmental samples (Anderson *et al* 1995, 1996, 1997). As such, RGS gives an actual assessment of the potential of contaminants on sediments at a specific site to produce chronic and/or carcinogenic effects on flatfish if they were to occupy the site. It also gives an indication of the chronic effects on benthic organisms at the site, as an RGS response above 60 µg of benzo[*a*]pyrene equivalents per g was highly correlated with the degradation of infaunal communities in San Diego Bay (Fairey *et al* 1996).

In recent NOAA studies, RGS responses have correlated very well (r^2 about = 0.7 - 0.8) with the concentrations of total PAHs in sediments, and even better (r^2 about = 0.85) with the concentrations of high molecular weight PAHs (Anderson and Jones 1997). Strong correlations have also been demonstrated with subsequent analyses of chlorinated compounds (dioxins/furans) in soil samples. In addition, RGS benzo[*a*]pyrene equivalents obtained in analysis of extracts of mussel tissue were highly correlated (r^2 = 0.9) with the presence of 4- to 6 -ring PAHs reported from chemical analytical data.

This testing program was designed to determine the potential toxicity and carcinogenicity of sediments collected from Delaware Bay. P450 RGS analysis by Columbia Analytical Services (CAS) was used to determine the induction of CYP1A1 in a human liver cancer cell line (101L).

METHODS

The basic methodology of the P450 Reporter Gene System (RGS) has been described elsewhere (Anderson *et al* 1995, 1996). RGS has been approved as Standard Method 8070 (APHA 1996) and an ASTM Standard Guide E1853-96 (ASTM 1997) is available. Included in the Appendix are the raw data from the RGS assays, the Quality Assurance data on sample spiking, etc., information on sample weights, and chain-of-custody documents. A disk with the Excel file for this project, in PC and Mac format is enclosed with this report.

Approximately 20 grams of sediment from each station were extracted by EPA method 3550 to produce 1 mL of dichloromethane (DCM) containing the extractable organic compounds. These extracts were then exchanged into 2 mL of DMSO. One mL of this DMSO was shipped to Tom Johnson of USGS for Microtox testing and the other was used in RGS testing. In the Carlsbad CAS laboratory we applied 10 µL of the DMSO to the cells in three replicate wells containing 2 mL of culture medium. After 16 hours of incubation, the cells were washed, then lysed, the solution centrifuged, and 50 µL of the supernatent was measured for luminescence. Solvent blanks and two reference toxicants (2,3,7,8-dioxin and B[*a*]P) were used with each sample test run.

Equivalency Calculations

This report assigns Benzo[*a*]pyrene Equivalents (B[*a*]PEq) and Toxic Equivalency Quotients (TEQ) for each of the 81 sediment extracts. The B[*a*]PEq values are a measure of potential inducing PAHs in the sample and are calculated as follows:

B[a]PEq (µg/g) = (fold induction / 60) * (volume factor / dry weight)

Fold induction is calculated as mean relative light units (RLUs) produced by the sample divided by mean RLUs produced by the solvent blank. Sixty is the fold induction produced by 1.0 µg of B[a]P/mL. The volume factor brings the amount of sample applied up to the total extract volume. Because 10 µL of the 2mL extracts were used in these tests, the volume factor used here was 200. Final division by the dry weight, which was calculated using per cent solids of the 20 gram samples, yields B[*a*]PEq's in µg/g.

The TEQs are a measure of dioxin equivalents and are calculated using:

TEQ (ng/g) = (fold induction * volume factor) / (1000 * dry weight)

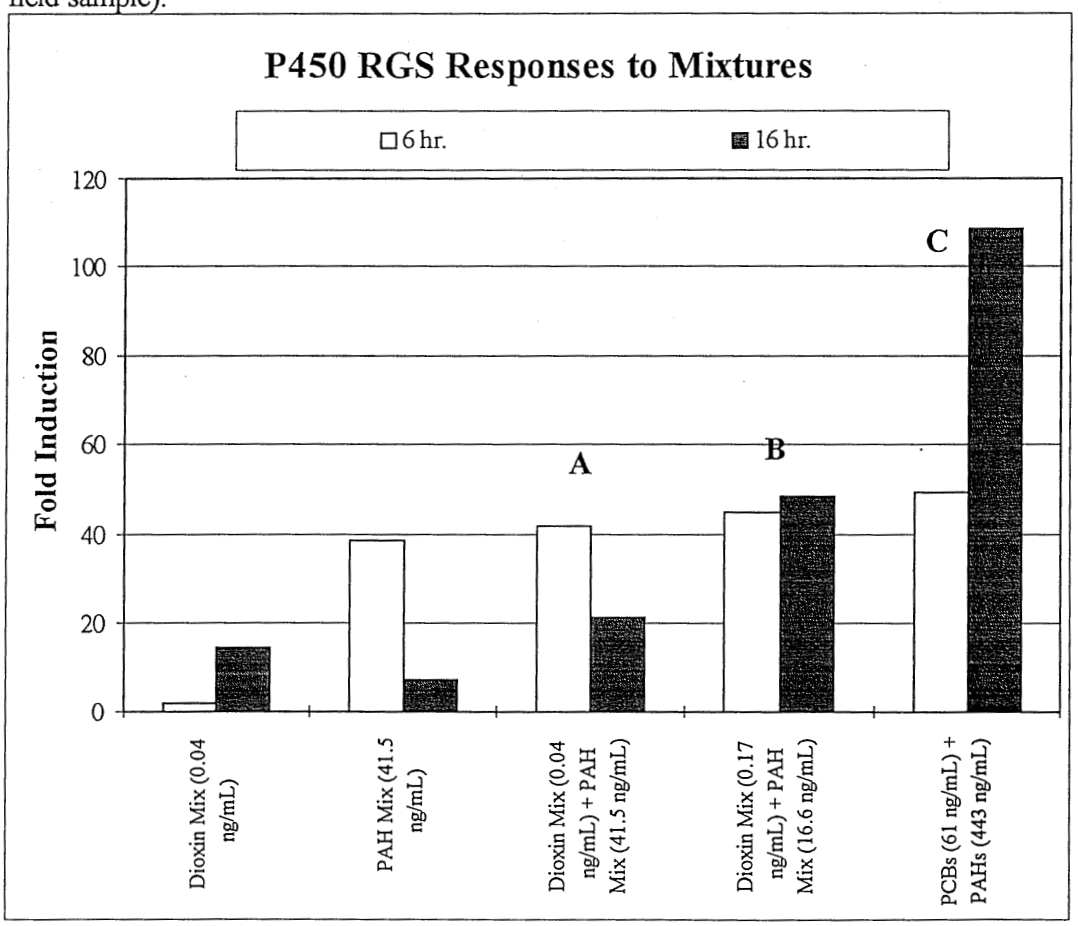
Using a concentration-response curve developed for a dioxin/furan mixture, the RGS fold induction is linear and equal to the concentration of the mixture in pg/mL. By multiplying the fold induction produced by the sample by the volume factor (200), then dividing by 1000 (to convert pg to ng) and the dry weight of the sample, we arrive at a TEQ in ng/g.

Statistical Analysis

Microsoft Excel 5.0 was used to determine the mean RGS response, the standard deviation and the 95% confidence interval of the B[*a*]PEq values of all 81 samples. This interval was then used to determine which samples were significantly greater than the mean.

P450 RGS Time Course Experiments

Because PAHs are metabolized more readily by the 101L cells than are chlorinated compounds, the RGS response to PAHs reaches a maximum at 6 hours, whereas the response to chlorinated compounds peaks at 16 hours. Comparison of the RGS responses at the two time periods enables the identification of the types of contaminants and their relative contributions in an environmental sample. Studies indicate that when only PAHs are present, the 6h response is always at least 5 times greater than the 16h response, whereas when only chlorinated compounds are present, the 16h response is always greater than the 6h response. When both PAHs and chlorinated compounds are present, the responses vary according to the relative proportion of the chlorinated compounds. In the figure below, (A) shows that the response will decrease less than 5 times with low concentrations, stay the same with moderate concentrations (B), or increase with high concentrations of chlorinated compounds (C, actual field sample).



Samples judged to contain levels of inducing compounds found in previous studies to be associated with degraded benthic communities (60 μ B[a]PEq/g), were selected for RGS analyses at two time intervals.

Quality Control and Quality Assurance

QA/QC procedures used during the course of RGS analysis included the spiking of samples with B[*a*]P, and monthly luciferase standard curves. To insure that each sample result was within the linear range of RGS response, the sample was diluted and retested if the fold induction was greater than 100. In addition, when the coefficient of variation (as per cent of the mean) of three replicates was greater than 20%, the sample was retested.

RESULTS

The results of this project are summarized in the following tables and figures. A disk is enclosed with Excel PC and Mac files that can be used to conduct any comparisons NOAA would like to perform. The text of this report will primarily provide an overview of the findings. The sample information, raw data from the tests, and the quality assurance data may be found in the Appendix. The first table in the Appendix lists the percent solids and the subsequent dry weights of each of the 81 samples sent to the Columbia Analytical Services laboratory in Kelso, Washington, for extraction and solids measurements.

The second table in the Appendix shows the results of 9 different runs of the RGS assay, where the luminometer relative light unit (RLU) readings for each replicate of the extracts, as well as dichloromethane (DCM), dimethyl sulfoxide (DMSO), 2,3,7,8-tetrachloro-dibenzodioxin (TCDD), and benzo[*a*]pyrene (B[*a*]P) are listed. The % CV, as well as the variability of the TCDD/solvent ratios will be discussed below under QA/QC data. As in all other testing, three replicate wells were used to produce the RGS response for each sediment extract. The mean of these three replicates was used to produce fold induction values, which were then converted to B[*a*]PEQs and TEQs. Testing at two time intervals utilizes two replicates at each time period.

When the coefficient of variation of the mean (% CV) was greater than 20%, the sample was retested, and the average of the test B[*a*]PEQs was then used as the final B[*a*]PEq value. In addition, when the fold induction was greater than 100, the sample was diluted 1:10 or greater in DMSO and retested. In 13 cases a 1:10 dilution was adequate to reduce the fold induction to less than 100. NOAA sample # 29 required a 1:50 dilution and a 1:100 dilution was necessary to reduce the induction of NOAA # 11 to the appropriate range. Only the results from the final dilution assays were used to produce the equivalency values, shown in the summary table and figures in the following section of the report.

Table 1 summarizes the findings of the P450 RGS Analysis of the 81 samples from Delaware Bay. Samples are listed by both the CAS number and the Client (NOAA) number. Table 1 includes both the μg of B[*a*]PEq /g and the Toxic Equivalency (TEQ in ng/g) for each sample, as the former is appropriate for PAH contamination and the TEQ is the usual manner of expressing the potential toxicity of coplanar PCBs or dioxin contamination. Figure 1 shows the distribution of the 81 samples, demonstrating that about 25 % were less than 1.0 μg B[*a*]PEq/g. Of the remaining 75 %, about one-half were between 1 and 10 μg B[*a*]PEq/g, and the other half were between 10 and 1000 μg B[*a*]PEq/g. Figure 2 shows that there were 10 samples of greater than 100 μg B[*a*]PEq/g, with the most contaminated sample (NOAA #11) reaching 1,584 μg B[*a*]PEq/g. Statistical analyses of the data produced a mean of 57.4 μg B[*a*]PEq/g, with a standard deviation of 190.8 μg B[*a*]PEq/g. The 95 % confidence interval was 41.5, so the samples above 99 μg B[*a*]PEq/g were significantly higher than the mean. As noted above there were 10 samples that fell into this category. Another way of examining the data would be to evaluate those samples that were greater than 60 μg B[*a*]PEq/g, which has in the past been observed to be the level at which effects on the benthos were observed. Figure 3 shows the 13 samples (in a log plot) which fall into this category.

To evaluate whether the very high induction observed in NOAA sample #11 was the result of PAH or chlorinated hydrocarbon contamination, a two-time-interval assay was conducted. The response at 16 hours was 2.5 times the response at 6 hours of exposure. These findings clearly indicate that chlorinated hydrocarbons (coplanar PCBs, dioxins, furans) are making a major contribution to the induction measured for this sample.

After approval by the NOAA office, additional two-time-interval testing was conducted. Figures 4A and 4B show the results of these tests on 1:10 and 1:100 dilutions of the 13 samples producing the highest CYP1A1 induction at 16 hours. Since 1:10 dilutions produced relatively high induction, it was determined that all samples should be diluted to 1:100. While 1:10 dilutions of samples 16 and 20 produced higher RGS responses at 16 than 6 hours, further dilution decreased the relative amount of induction at 16 hours for all samples except #11. This sample was diluted 1:300 and again tested at two time intervals (Figure 4C). The significance of these findings will be considered in the Discussion section.

Spiking Studies

In the Appendix are the results of the spiking of samples with 300 ng/mL of B[*a*]P. The last column gives the % additivity, calculated as the observed fold induction of the spiked sample divided by the fold induction expected by adding the induction from the unspiked sample to that of the toxicant. The variability in % additivity appears to be related to the variability in RGS response to specific mixtures of toxicants. Percent recovery was greater than 100% for samples 10 and 37. The response of sample 10 was possibly high because of some interaction between the B[a]P spike and chlorinated hydrocarbons in the extract. Sample 37 was low in B[a]P equivalents (5.2 μg/g), so was not tested at two time intervals but the presence of chlorinated compounds could have contributed to the high recovery (145%) observed.

Table 1. Summary of P450 RGS Equivalency Values of Delaware Bay Sediment Extracts

Client I.D.	B[a]P Eq. (µg/g dry)	TEQ (ng/g dry)
11	1583.99	95.04
89	396.85	23.81
13	344.65	20.68
4	313.01	18.78
29	298.41	17.90
8	226.59	13.60
7	225.52	13.53
20	183.08	10.98
16	130.58	7.83
19	124.19	7.45
10	115.12	6.91
2	83.28	5.00
17	62.29	3.74
85	48.14	2.89
6	47.59	2.86
18	47.36	2.84
1	38.41	2.30
3	37.86	2.27
90	28.32	1.70
91	27.80	1.67
26	25.18	1.51
25	20.41	1.22
23	18.84	1.13
14	17.95	1.08
22	16.32	0.98
92	15.49	0.93
84	14.33	0.86
28	12.97	0.78
24	12.54	0.75
30	9.25	0.56
38	8.89	0.53
9	8.40	0.50
21	7.93	0.48
27	7.87	0.47
40	7.20	0.43
36	6.86	0.41
88	6.65	0.40
34	5.34	0.32
37	5.20	0.31
42	4.17	0.25
87	3.78	0.23

Client I.D.	B[a]P Eq. (µg/g dry)	TEQ (ng/g dry)
57	3.74	0.22
15	3.42	0.21
44	3.38	0.20
66	3.02	0.18
56	2.95	0.18
55	2.78	0.17
50	2.73	0.16
12	2.36	0.14
59	2.34	0.14
31	1.93	0.12
41	1.81	0.11
60	1.79	0.11
33	1.73	0.10
58	1.66	0.10
43	1.46	0.09
61	1.32	0.08
32	1.30	0.08
46	1.19	0.07
39	1.08	0.06
35	1.05	0.06
45	1.04	0.06
67	0.99	0.06
5	0.72	0.04
73	0.70	0.04
47	0.68	0.04
54	0.62	0.04
53	0.60	0.04
52	0.58	0.03
63	0.52	0.03
65	0.44	0.03
72	0.43	0.03
68	0.42	0.03
69	0.42	0.03
48	0.41	0.02
70	0.37	0.02
49	0.33	0.02
71	0.32	0.02
64	0.24	0.01
62	0.24	0.01
51	0.22	0.01

Figure 1. Distribution of 81 Samples from Delaware Bay

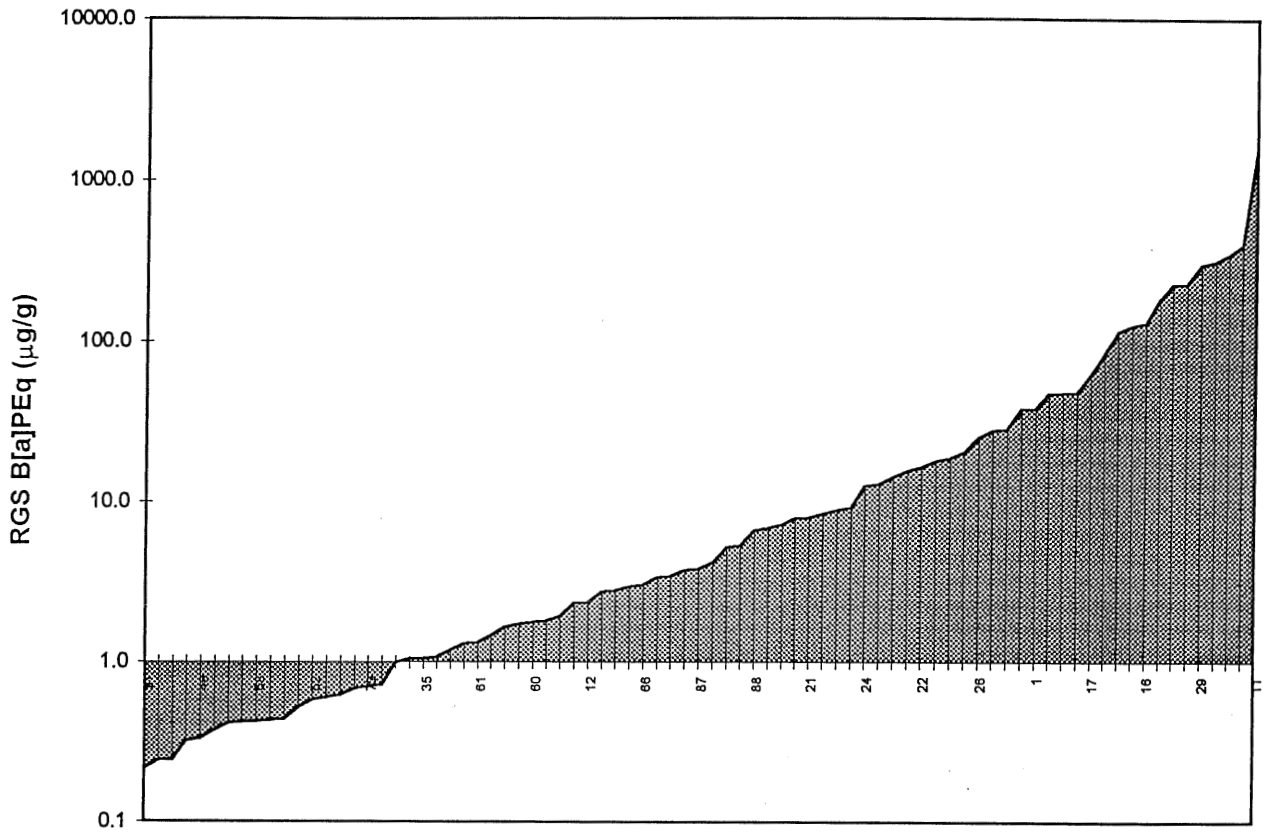


Figure 2. P450 RGS B[a]P Equivalents in Delaware Bay Extracts

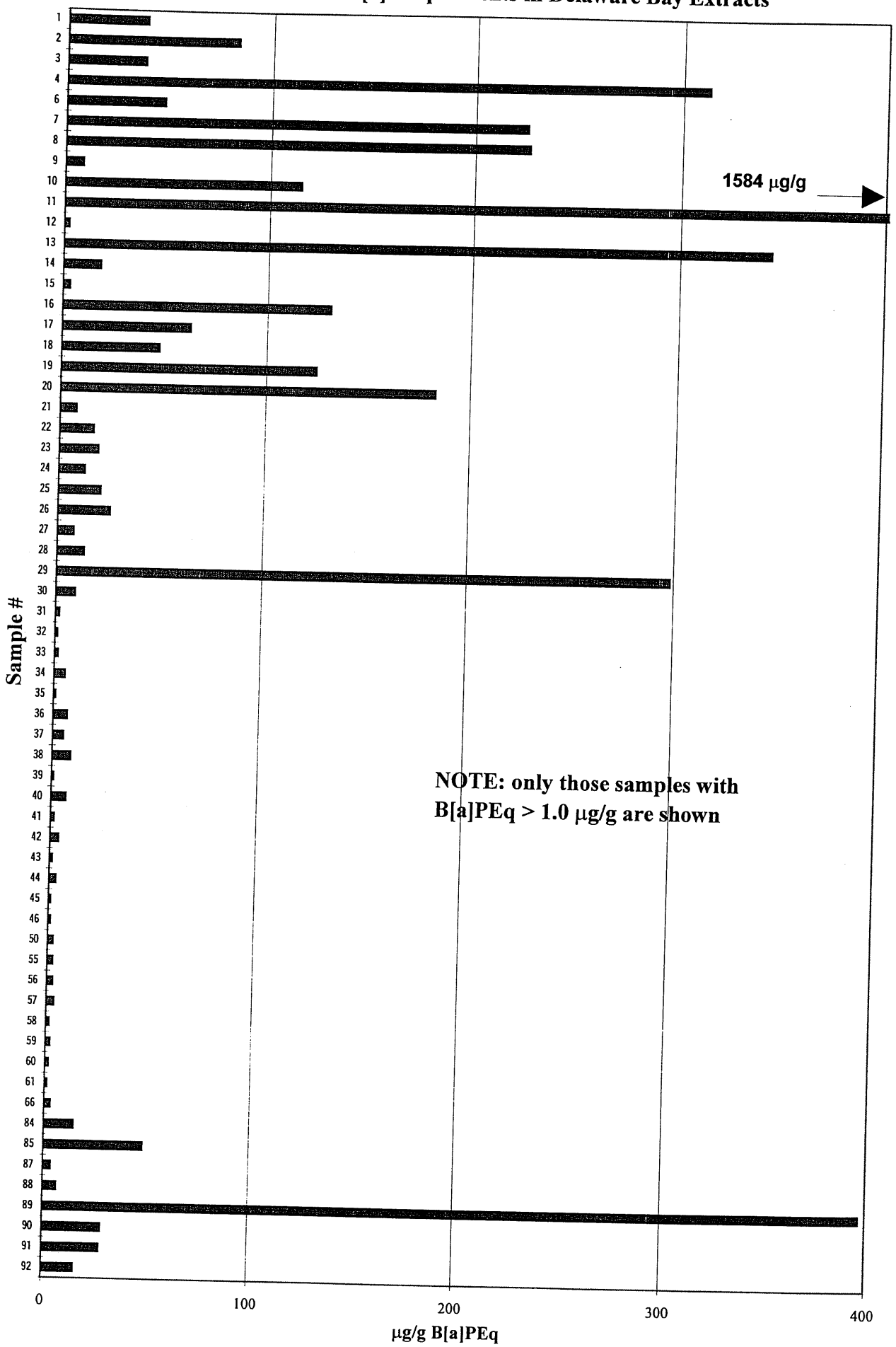


Figure 3. Log Plot of Samples of 60 ug/g B[a]PEq and Greater

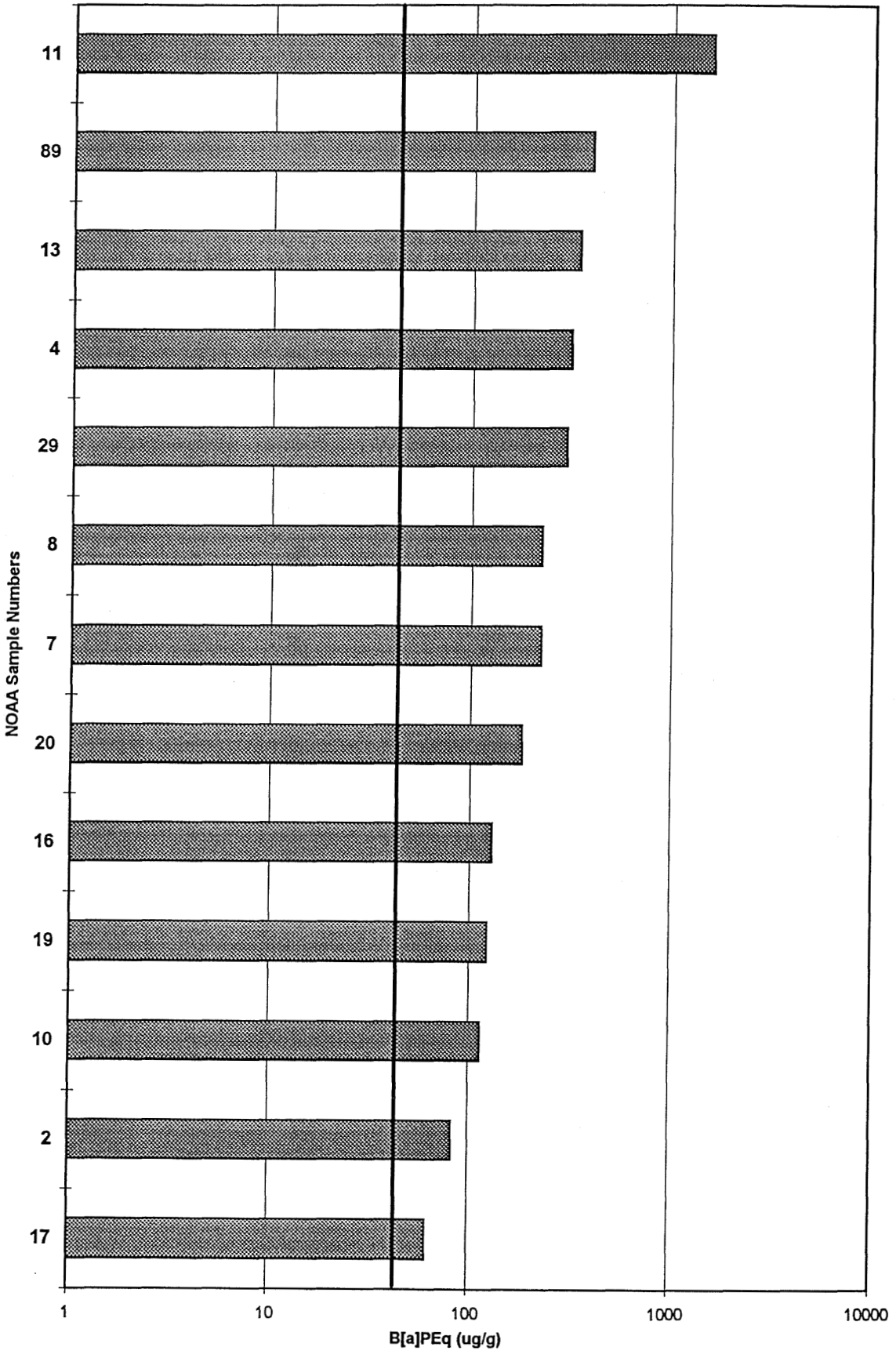


Fig 4A. P450 RGS Responses to Delaware Bay Sediment Extracts - 1:10 Dilutions

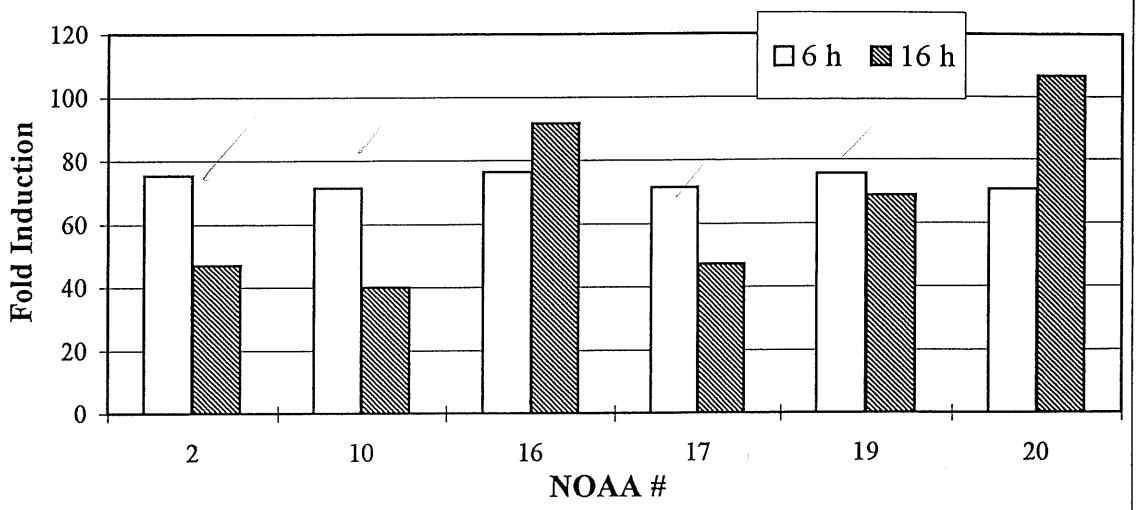


Fig 4B. P450 RGS Responses to Delaware Bay Sediment Extracts - 1:100 Dilutions

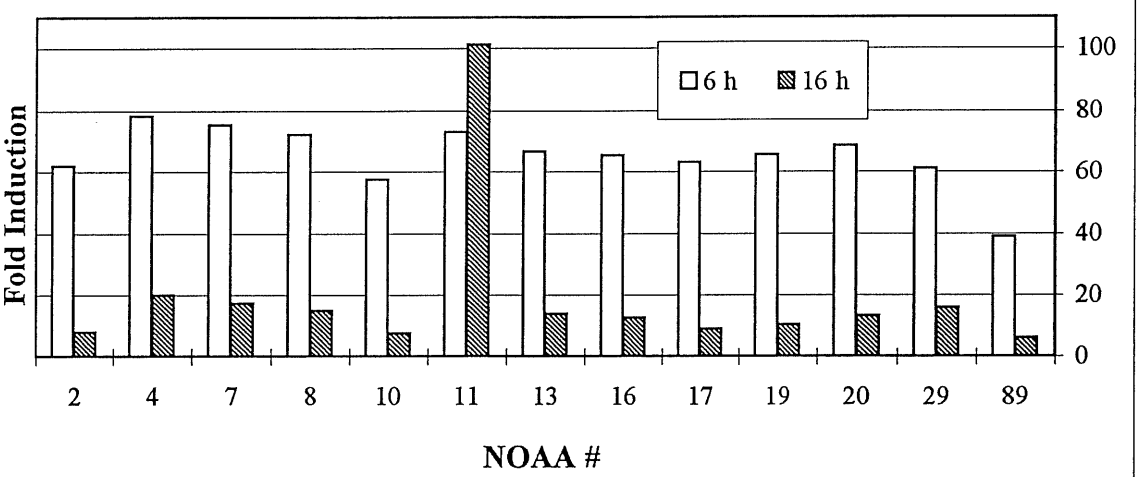
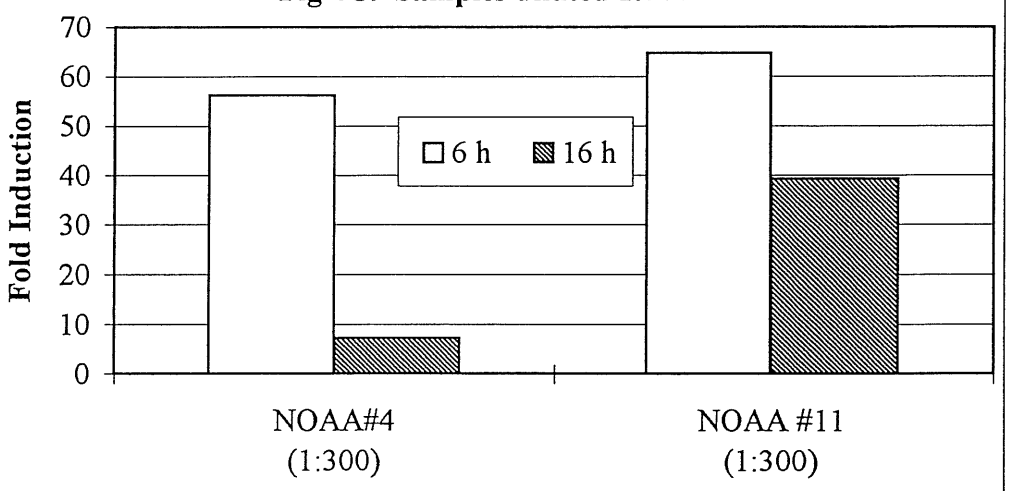


Fig 4C. Samples diluted 1:300



Quality Control and Quality Assurance

Also in the Appendix is a table summarizing the coefficients of variation (% CV) of the TCDD, DCM, DMSO, B[*a*]P, and the mean % CV of the samples tested in each of the test runs. Shown are the means, standard deviations and coefficients of variation for TCDD/DCM and TCDD/DMSO ratios and the B[*a*]P folds. The mean fold induction for 300 ng/mL B[a]P was 14.3, which is very near the average of 13.5 ± 4.4 found in our past concentration-response studies using B[*a*]P. Ratios of TCDD to both solvents were also very near the running average for these responses. Two figures demonstrate the monthly assessment of the ability of the luminometer to produce a linear concentration-response curve and to detect levels of a standard luciferase down to 2 picograms.

DISCUSSION

Unlike the recently completed Northern Puget Sound Study, the samples collected in this project have produced a very wide range of P450 RGS responses (0.2 to 1,584 µg B[*a*]PEq/g). Without knowing anything about the collection sites, it is difficult to discuss relationships between various sets of samples. It is interesting and perhaps coincidental, that the first 37 samples extracted by our CAS Kelso laboratory were, in general, at the low end (0.2-7.2 µg B[*a*]PEq/g) of the data set. We will be most interested in learning about the collection sites and helping to determine any patterns in contamination that may exist. Since the responses of the most contaminated sample (NOAA # 11) tested at two time intervals indicated significant amounts of PCBs or other chlorinated hydrocarbons were present, we requested and obtained approval to conduct this type of testing on the other 12 samples which produced a response above 60 µg B[*a*]PEq/g.

The findings of this latter phase of the program requires some explanation. It is clear that regardless of the dilution (up to 1:300), sample #11 gives a strong signal of contamination from chlorinated hydrocarbons. At the 1:100 dilution, it is more difficult to assess the other 12 samples. Six of these tested at 1:10 exhibited responses indicating the presence of both PAHs and chlorinated hydrocarbons. With greater dilution, it appears that these chlorinated compounds have been reduced to such a low level that they are no longer being represented in the 16 hour response. The PAHs are clearly more concentrated than the chlorinated hydrocarbons in these 12 samples. However, the data from 1:10 dilutions indicated that there were significant amount of chlorinated hydrocarbons contributing to the overall induction.

We would like to use our results in aiding to interpret the distribution of the two classes of contaminants in the samples collected from Delaware Bay. Knowledge of the location of the samples in this estuary may help us to interpret our findings. Based on the findings presented in Figure 4, sample 11 was clearly shown to contain substantial amounts of chlorinated compounds. We would expect to see a contribution of these compounds to samples taken in the vicinity of sample 11. It is possible that the other 12 samples shown in Figure 4 were collected in that area, but at this time we have no knowledge of the sampling plan.

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APPENDIX

Delaware Bay Sediment Extracts						
Extracts received 10/7/97						
Client I.D.	CAS #	Sample Wt (g)	% Solids	Dry Wt (g)	Final Extract Vol (mL)	
62	K6874-1	20.67	81.0	16.7	2	
63	2	20.23	80.2	16.2	2	
68	3	20.24	78.6	15.9	2	
64	4	20.22	82.2	16.6	2	
65	5	20.54	79.7	16.4	2	
67	6	20.10	71.0	14.3	2	
69	7	20.47	81.3	16.6	2	
70	8	20.18	81.7	16.5	2	
72	9	20.45	82.3	16.8	2	
73	10	20.43	79.5	16.2	2	
71	11	20.62	83.8	17.3	2	
66	12	20.63	68.6	14.2	2	
55	13	20.20	74.6	15.1	2	
50	14	20.26	71.3	14.4	2	
54	15	20.52	74.0	15.2	2	
56	16	20.11	68.9	13.9	2	
53	17	20.15	76.9	15.5	2	
51	18	20.69	85.5	17.7	2	
59	19	20.41	74.1	15.1	2	
48	20	20.48	79.5	16.3	2	
	1002 MB	20.31				
Extracts received 10/15/97						
Client I.D.	CAS #	Sample Wt (g)	% Solids	Dry Wt (g)	Final Extract Vol (mL)	
61	6874-21	20.56	78.0	16.0	2	
60	22	20.83	70.4	14.7	2	
58	23	20.21	73.4	14.8	2	
52	24	20.16	82.9	16.7	2	
40	25	20.56	68.8	14.1	2	
46	26	20.63	82.0	16.9	2	
57	27	20.58	32.4	6.7	2	
49	28	20.36	77.5	15.8	2	
47	29	20.74	75.8	15.7	2	
45	30	20.14	76.8	15.5	2	
44	31	20.17	74.2	15.0	2	
43	32	20.50	78.3	16.1	2	
42	33	20.49	73.3	15.0	2	
41	34	20.86	74.0	15.4	2	
39	35	20.61	79.9	16.5	2	
33	36	20.40	51.1	10.4	2	
32	37	20.24	47.4	9.6	2	
25	38	20.82	42.8	8.9	2	
22	39	20.31	69.8	14.2	2	
23	40	20.38	41.1	8.4	2	
	1013 MB	20.36				

Extracts received 10/23/97						
Client I.D.	CAS #	Sample Wt (g)	% Solids	Dry Wt (g)	Final Extract Vol (mL)	
24	6874-41	20.31	52.9	10.7	2	
21	42	20.56	41.1	8.5	2	
17	43	20.70	82.2	17.0	2	
16	44	20.77	71.1	14.8	2	
12	45	20.37	69.8	14.2	2	
19	46	20.37	59.4	12.1	2	
20	47	20.88	54.9	11.5	2	
18	48	20.69	74.8	15.5	2	
5	49	20.20	78.7	15.9	2	
4	50	20.40	52.3	10.7	2	
7	51	20.56	58.3	12.0	2	
8	52	20.57	55.7	11.5	2	
6	53	20.58	74.0	15.2	2	
3	54	20.73	32.7	6.8	2	
2	55	20.86	68.9	14.4	2	
1	56	20.82	38.1	7.9	2	
14	57	20.80	80.6	16.8	2	
15	58	20.32	76.9	15.6	2	
9	59	20.15	81.3	16.4	2	
10	60	20.21	54.3	11.0	2	
11	61	20.24	75.8	15.3	2	
13	62	20.73	46.6	9.7	2	
	1018MB	20.13				
	1018MB	20.83				
Extracts received 11/1/97						
Client I.D.	CAS #	Sample Wt (g)	% Solids	Dry Wt (g)	Final Extract Vol (mL)	
26	K7531-1	20.3	41.9	8.5	2	
27	2	20.6	57.6	11.9	2	
28	3	20.9	64.1	13.4	2	
30	4	20.2	43.2	8.7	2	
29	5	20.9	51.8	10.8	2	
91	6	20.7	35.4	7.3	2	
92	7	20.1	24.7	5.0	2	
34	8	20.7	56.1	11.6	2	
38	9	20.2	62.8	12.7	2	
37	10	20.6	54.2	11.2	2	
35	11	20.2	78.1	15.8	2	
31	12	20.8	60.2	12.5	2	
88	13	20.6	39.2	8.1	2	
89	14	20.9	14.3	3.0	2	
90	15	20.2	30.8	6.2	2	
36	16	20.8	47.9	10.0	2	
84	17	20.1	39.3	7.9	2	
85	18	20.4	29.4	6.0	2	
87	19	20.8	45.3	9.4	2	
	1029MB	20.1				

P450 RGS Data for Delaware Bay Study

P450 RGS Analysis of NOAA/Delaware Bay Extracts										
October 19-20, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.090	0.097	0.079	0.089	0.009	10.2	1		
						TCDD/DMSO		**138.2**		
DCM	2 uL	0.124	0.129		0.127	0.004	2.8	1		
TCDD	1 ng/mL	12.173	12.005	12.573	12.250	0.292	2.4	96.5		
Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
K6874-1	0.099	0.114	0.111	0.108	0.008	7.3	1.2	16.7	0.24	0.01
2	0.216	0.239	0.217	0.224	0.013	5.8	2.5	16.2	0.52	0.03
3	0.170	0.184	0.186	0.180	0.009	4.8	2.0	15.9	0.42	0.03
4	0.108	0.105	0.111	0.108	0.003	2.8	1.2	16.6	0.24	0.01
					ave	5.2				
October 20-21, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.066	0.074	0.070	0.070	0.004	5.7	1		
						TCDD/DMSO		**113.8**		
DCM	5 uL	0.096	0.101	0.088	0.095	0.004	3.7	1		
TCDD	1 ng/mL	8.047	8.197	7.655	7.966	0.280	3.5	83.9		
Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
K6874-5	0.142	0.160	0.151	0.151	0.009	6.0	2.2	16.4	0.44	0.03
6	0.270	0.301	0.319	0.297	0.025	8.4	4.2	14.3	0.99	0.06
7	0.140	0.150	0.152	0.147	0.006	4.4	2.1	16.6	0.42	0.03
8	0.122	0.138	0.127	0.129	0.008	6.3	1.8	16.5	0.37	0.02
9	0.149	0.152	0.155	0.152	0.003	2.0	2.2	16.8	0.43	0.03
10	0.228	0.241	0.246	0.238	0.009	3.9	3.4	16.2	0.70	0.04
11	0.108	0.114	0.127	0.116	0.010	8.3	1.7	17.3	0.32	0.02
						5.6				
October 23-24, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.057	0.061	0.064	0.061	0.004	5.8	1		
						TCDD/DMSO		**127.5**		
DCM	5 uL	0.095	0.092	0.079	0.089	0.002	2.4	1		
TCDD	1 ng/mL	7.032	7.611	8.558	7.734	0.770	10.0	86.9		
B[a]P	300 ng/mL	0.945	1.082	1.026	1.018	0.069	6.8	11.4		
Applied 10 uL of samples										
samples #15 and #25 spiked with 300 ng/mL B[a]P								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
6874-12	0.907	0.713	0.730	0.783	0.107	13.7	12.8	14.2	3.02	0.18
13	0.776	0.792	0.729	0.766	0.033	4.3	12.6	15.1	2.78	0.17
14	0.779	0.720	0.668	0.722	0.056	7.7	11.8	14.4	2.73	0.16
15	0.154	0.201	0.165	0.173	0.025	14.2	2.8	15.2	0.62	0.04
16	0.719	0.793	0.731	0.748	0.040	5.3	12.3	13.9	2.95	0.18
17	0.170	0.180	0.160	0.170	0.010	5.9	2.8	15.5	0.60	0.04

Recorded By Jennifer Jones (JJ)
Date 1/9/98

Reviewed By WJ
Date 1/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
18	0.068	0.073	0.069	0.070	0.003	3.8	1.1	17.7	0.22	0.01
19	0.669	0.661	0.615	0.648	0.029	4.5	10.6	15.1	2.34	0.14
20	0.104	0.131	0.133	0.123	0.016	13.2	2.0	16.3	0.41	0.02
21	0.393	0.395	0.377	0.388	0.010	2.5	6.4	16.0	1.32	0.08
22	0.496	0.471	0.471	0.479	0.014	3.0	7.9	14.7	1.79	0.11
23	0.493	0.461	0.395	0.450	0.050	11.1	7.4	14.8	1.66	0.10
24	0.158	0.197	0.173	0.176	0.020	11.2	2.9	16.7	0.58	0.03
25	1.961	1.896	1.733	1.863	0.117	6.3	30.5	14.1	7.20	0.43
spike 15	0.776	0.810	0.901	0.829	0.065	7.8	13.6			
spike 25	2.539	2.493	2.357	2.463	0.095	3.8	40.4			
						7.4				
October 27-28, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.059	0.063	0.054	0.059	0.005	7.7	1		
						TCDD/DMSO		**125.3**		
DCM	2 uL	0.078	0.078	0.074	0.077	0.002	3.0	1		
TCDD	1 ng/mL	7.304	7.006	7.751	7.354	0.375	5.1	95.5		
Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
6874-26	0.356	0.361	0.351	0.356	0.005	1.4	6.0	16.9	1.19	0.07
27	0.417	0.417	0.491	0.442	0.043	9.7	7.5	6.7	3.74	0.22
28	0.090	0.096	0.091	0.092	0.003	3.5	1.6	15.8	0.33	0.02
29	0.187	0.205	0.176	0.189	0.015	7.7	3.2	15.7	0.68	0.04
30	0.274	0.286	0.296	0.285	0.011	3.9	4.8	15.5	1.04	0.06
31	0.856	0.925	0.902	0.894	0.035	3.9	15.2	15.0	3.38	0.20
						5.0				
October 30-31, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.084	0.093	0.088	0.088	0.005	5.1	1		
						TCDD/DMSO		**117.4**		
DCM	5 uL	0.117	0.131	0.102	0.117	0.010	8.5	1		
TCDD	1 ng/mL	9.952	10.391	10.769	10.371	0.409	3.9	88.6		
B[a]P	300 ng/mL	1.661	1.792	1.695	1.716	0.068	4.0	14.7		
Applied 10 uL of samples										
sample #35 spiked with 300 ng/mL B[a]P								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
6874-32	0.603	0.663	0.588	0.618	0.040	6.4	7.0	16.1	1.46	0.09
33	1.592	1.789	1.579	1.653	0.118	7.1	18.8	15.0	4.17	0.25
34	0.693	0.789	0.726	0.736	0.049	6.6	8.4	15.4	1.81	0.11
35	0.411	0.561	0.437	0.470	0.080	17.1	5.3	16.5	1.08	0.06
36	0.454	0.507	0.469	0.477	0.027	5.7	5.4	10.4	1.73	0.10
37	0.296	0.338	0.356	0.330	0.031	9.3	3.8	9.6	1.30	0.08
38	4.447	5.078	4.876	4.800	0.322	6.7	54.5	8.9	20.41	1.22
39	6.017	6.699	5.603	6.106	0.553	9.1	69.4	14.2	16.32	0.98
40	3.809	4.601	4.090	4.167	0.402	9.6	47.3	8.4	18.84	1.13
41	2.829	3.757	4.082	3.556	0.650	18.3	40.4	10.7	12.54	0.75

Recorded By JJ
Date 1/9/98

Reviewed By JM
Date 1/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
42	1.614	2.026	1.666	1.769	0.224	12.7	20.1	8.5	7.93	0.48
spike #35	1.424	1.357	1.112	1.298	0.164	12.7	14.7			
						10.1				
November 3-4, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.060	0.057	0.054	0.057	0.003	5.3	1		
						TCDD/DMSO		**131.3**		
DCM	5 uL	0.086	0.091	0.071	0.083	0.004	4.3	1		
TCDD	1 ng/mL	7.290	7.463	7.705	7.486	0.208	2.8	90.2		
B[a]P	300 ng/mL	1.053	1.058	1.014	1.042	0.024	2.3	12.6		
Applied 10 uL of samples										
sample #45 spiked with 300 ng/mL B[a]P								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
6874-43	6.981	8.511	7.310	7.601	0.805	10.6	**133.3**	17.0	26.12	1.57
44	8.963	10.678	7.457	9.033	1.612	17.8	**158.5**	14.8	35.77	2.15
45	0.495	0.587	0.637	0.573	0.072	12.6	10.1	14.2	2.36	0.14
46	6.685	8.214	7.907	7.602	0.809	10.6	**133.4**	12.1	36.74	2.20
47	9.398	10.214	9.634	9.749	0.420	4.3	**171.0**	11.5	49.73	2.98
48	6.393	6.524	5.180	6.032	0.741	12.3	**105.8**	15.5	22.79	1.37
49	0.163	0.195	0.228	0.195	0.033	16.6	3.4	15.9	0.72	0.04
50	10.219	11.139	7.993	9.784	1.618	16.5	**171.6**	10.7	53.63	3.22
51	9.481	9.999	10.019	9.833	0.305	3.1	**172.5**	12.0	47.97	2.88
52	7.786	8.904	9.359	8.683	0.809	9.3	**152.3**	11.5	44.32	2.66
53	6.217	6.339	6.952	6.503	0.394	6.1	**114.1**	15.2	24.97	1.50
54	4.082	4.715	4.367	4.388	0.317	7.2	77.0	6.8	37.86	2.27
55	8.216	8.844	8.685	8.582	0.327	3.8	**150.6**	14.4	34.92	2.10
56	5.034	5.551	5.045	5.210	0.295	5.7	91.4	7.9	38.41	2.30
57	5.583	4.734	5.121	5.146	0.425	8.3	90.3	16.8	17.95	1.08
Spike #45	0.856	0.910	1.207	0.991	0.189	19.1	17.4			
						10.2				
REDO all samples except #45, 49, 54, 56, 57; Folds >100 (redo 10 samples-dilute 1:10)										
November 6-7, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.099	0.102	0.096	0.099	0.003	3.0	1		
						TCDD/DMSO		**112.7**		
DCM	5 uL	0.117			0.117			1		
TCDD	1 ng/mL	11.483	11.395	10.598	11.159	0.488	4.4	95.4		
Applied 10 uL of samples diluted 1:10										
								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
6874-43 (1:10)	3.060	3.236		3.148	0.124	4.0	31.8	17.0	62.3	3.7
44 (1:10)	5.134	6.320		5.727	0.839	14.6	57.8	14.8	130.6	7.8

Recorded By _____
Date 1/9/98

Reviewed By _____
Date 1/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
46 (1:10)	4.516	4.410		4.463	0.075	1.7	45.1	12.1	124.2	7.5
47 (1:10)	6.031	6.435		6.233	0.286	4.6	63.0	11.5	183.1	11.0
48 (1:10)	1.947	2.407		2.177	0.325	14.9	22.0	15.5	47.4	2.8
50 (1:10)	10.096	9.741		9.9185	0.251	2.5	100.2	10.7	313.0	18.8
51 (1:10)	7.782	8.275		8.0285	0.349	4.3	81.1	12.0	225.5	13.5
52 (1:10)	7.409	8.012		7.7105	0.426	5.5	77.9	11.5	226.6	13.6
53 (1:10)	2.199	2.106		2.1525	0.066	3.1	21.7	15.2	47.6	2.9
55 (1:10)	3.456	3.654		3.555	0.14	3.9	35.9	14.4	83.3	5.0
58	1.626	1.448	1.693	1.589	0.127	8.0	16.1	15.6	3.4	0.2
59	1.359	3.172	1.720	2.084	0.960	**46.1**	21.0	16.4	4.3	0.3
60	6.580	9.119	12.040	9.246	2.732	**29.5**	93.4	11.0	28.4	1.7
need to redo # 59 and 60 ; CV > 20%										
November 10-11, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.056	0.060	0.056	0.057	0.002	4.0	1		
						TCDD/DMSO		**126.1**		
DCM	5 uL	0.082	0.077	0.067	0.075	0.004	4.7	1		
TCDD	1 ng/mL	7.206	7.388	7.098	7.231	0.147	2.0	96.4		
B[a]P	300 ng/mL	0.833	0.991	1.137	0.987	0.152	15.4	13.2		
Applied 10 uL of samples										
samples #6874-60 and #7531-10 spiked with 300 ng/mL B[a]P								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
redo59	2.280	2.307	2.476	2.354	0.106	4.5	41.3	16.4	8.4	0.5
redo60	6.201	7.634	8.637	7.491	1.224	16.3	**131.4**	11.0	39.9	2.4
61	7.794	7.670	7.220	7.561	0.302	4.0	**132.7**	15.3	28.8	1.7
62	9.851	9.404	10.030	9.762	0.322	3.3	**171.3**	9.7	59.1	3.5
K7531-1	3.434	3.904	3.647	3.662	0.235	6.4	64.2	8.5	25.2	1.5
2	1.453	1.509	1.829	1.597	0.203	12.7	28.0	11.9	7.9	0.5
3	2.957	2.907	3.047	2.970	0.071	2.4	52.1	13.4	13.0	0.8
4	1.099	1.420	1.622	1.380	0.264	19.1	24.2	8.7	9.3	0.6
5	16.693	17.830	17.067	17.197	0.579	3.4	**301.7**	10.8	92.9	5.6
6	3.177	3.663	3.612	3.484	0.267	7.7	61.1	7.3	27.8	1.7
7	1.125	1.395	1.426	1.315	0.166	12.6	23.1	5.0	15.5	0.9
8	1.056	1.059	1.066	1.060	0.005	0.5	18.6	11.6	5.3	0.3
9	1.887	1.976	1.925	1.929	0.045	2.3	33.8	12.7	8.9	0.5
10	0.768	1.119	1.089	0.992	0.195	19.6	17.4	11.2	5.2	0.3
11	0.242	0.312	0.296	0.283	0.037	12.9	5.0	15.8	1.1	0.1
12	0.359	0.436	0.444	0.413	0.047	11.4	7.2	12.5	1.9	0.1
13	0.80	0.962	0.994	0.919	0.104	11.3	16.1	8.1	6.7	0.4
14	5.302	5.530	6.580	5.804	0.682	11.7	**101.8**	3.0	113.6	6.8
15	2.667	2.967	3.406	3.013	0.372	12.3	52.9	6.2	28.3	1.7
16	0.975	1.168	1.361	1.168	0.193	16.5	20.5	10.0	6.9	0.4
17	1.826	1.966	2.017	1.936	0.099	5.1	34.0	7.9	14.3	0.9
18	4.441	5.118	5.252	4.937	0.435	8.8	86.6	6.0	48.1	2.9
spike 6874-60	9.133	8.928	10.338	9.466	0.762	8.0	166.1			

Recorded By JP
Date 1/9/98

Reviewed By JM
Date 1/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
spike7531-10	2.687	2.791	2.094	2.524	0.376	14.9	44.3			
						9.5				
Redo 60, 61, 62, 5, 14 because fold > 100										
November 13-14, 1997		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.078	0.079	0.080	0.079	0.001	1.3	1		
						TCDD/DMSO		**107.5**		
DCM	5 uL	0.096	0.101	0.099	0.099	0.004	3.6	1		
TCDD	1 ng/mL	8.425	8.533	8.523	8.494	0.060	0.7	85.8		
B[a]P	300 ng/mL	1.823	2.216	1.831	1.957	0.225	11.5	19.8		
Applied 10 uL of samples										
samples #6874-60-62 and #7531-5 and 14 diluted 1:10 with DMSO: sample #7531-5 also diluted 1:50										
sample 7531-19 spiked w/ 300 ng/mL B[a]P								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
874-60 (dil 1:10)	3.080	2.951	2.951	2.994	0.074	2.5	37.9	11.0	115.1	6.9
874-61 (dil 1:10)	21.736	24.674	20.462	22.291	2.160	9.7	**282.2**	15.3	613.0	36.8
874-62 (dil 1:10)	8.116	8.053	7.503	7.891	0.337	4.3	99.9	9.7	344.7	20.7
7531-5 (dil 1:10)	10.060	10.052	9.549	9.887	0.293	3.0	125.2	10.8	385.3	23.1
7531-5 (dil 1:50)	1.441	1.672	1.481	1.531	0.123	8.1	**19.4**	10.8	298.4	17.9
531-14 (dil 1:10)	2.860	2.798	2.775	2.811	0.044	1.6	35.6	3.0	396.9	23.8
7531-19	0.765	0.855	0.912	0.844	0.074	8.8	10.7	9.4	3.8	0.2
7531-19 (1:10)	0.194	0.209	0.217	0.207	0.012	5.6	2.6	9.4	9.3	0.6
spike 7531-19	1.370	2.140	2.205	1.905	0.464	24.4	24.1			
need to redo #61 at 1:100						7.5				
P450 RGS Analysis of NOAA/Delaware Bay Extracts: time course exp.										
November 26-27, 1997										
16 hours		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.089	0.096	0.097	0.094	0.004	4.6	1		
						TCDD/DMSO		**97.5**		
DCM	2 uL	0.122	0.120	0.118	0.120	0.001	1.2	1		
TCDD	1 ng/mL	9.247	9.256	8.995	9.166	0.148	1.6	76.4		
Applied 10 uL of sample 6874-61 diluted 1:100								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
687461 (dil 1:10	6.563	7.229	6.767	6.853	0.341	5.0	72.9	15.3	1584.0	95.0
6 hours		RLU1	RLU2	RLU3	Mean	St.Dev	CV%	FOLD		
DMSO	10 uL	0.098	0.108	0.107	0.104	0.006	5.3	1		
						TCDD/DMSO		**30.2**		
DCM	2 uL	0.123	0.099	0.098	0.107	0.017	15.9	1		

Recorded By JD
Date 1/9/98

Reviewed By JM
Date 1/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
TCDD	1 ng/mL	3.195	3.152	3.118	3.155	0.039	1.2	29.5		
Applied 10 uL of sample 6874-61 diluted 1:100								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
6874-61 (dil 1:10	3.102	3.094	2.993	3.063	0.061	2.0	29.5	15.3	639.9	38.4
January 1-2, 1998										
16 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.056	0.054	0.055	0.001	2.6	1			
					TCDD/DMSO		**170.8**			
DCM	2 uL	0.069	0.068	0.069	0.001	1.0	1			
TCDD	1 ng/mL	9.486	9.304	9.395	0.129	1.4	136.2			
Applied 10 uL of samples (all diluted 1:10)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-43 (1:10)	2.496	2.697	2.597	0.142	5.5	47.2	17.0	92.5	5.5	
44 (1:10)	4.675	5.416	5.046	0.524	10.4	91.7	14.8	207.1	12.4	
46 (1:10)	3.881	3.675	3.778	0.146	3.9	68.7	12.1	189.2	11.4	
47 (1:10)	5.823	5.893	5.858	0.049	0.8	106.5	11.5	309.7	18.6	
55 (1:10)	2.471	2.710	2.591	0.169	6.5	47.1	14.4	109.2	6.6	
60 (dil 1:10)	2.108	2.290	2.199	0.129	5.9	40.0	11.0	121.4	7.3	
6 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.085	0.087	0.086	0.001	1.6	1			
					TCDD/DMSO		**63.6**			
DCM	2 uL	0.085	0.092	0.089	0.005	5.6	1			
TCDD	1 ng/mL	4.727	6.210	5.469	1.049	19.2	61.4			
Applied 10 uL of sample 6874-61 diluted 1:100							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-43 (1:10)	5.820	6.459	6.140	0.452	7.4	71.4	17.0	139.9	8.4	
44 (1:10)	6.147	7.005	6.576	0.607	9.2	76.5	14.8	172.6	10.4	
46 (1:10)	6.828	6.183	6.506	0.456	7.0	75.6	12.1	208.4	12.5	
47 (1:10)	5.837	6.308	6.073	0.333	5.5	70.6	11.5	205.3	12.3	
55 (1:10)	6.260	6.691	6.476	0.305	4.7	75.3	14.4	174.6	10.5	
60 (dil 1:10)	6.165	6.093	6.129	0.051	0.8	71.3	11.0	216.5	13.0	
January 4-5, 1998										
16 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.046	0.048	0.047	0.001	3.0	1			
					TCDD/DMSO		**219.3**			
DCM	2 uL	0.067	0.076	0.072	0.006	8.9	1			
TCDD	1 ng/mL	9.955	10.663	10.309	0.501	4.9	143.2			

Recorded By JJ
Date 1/9/98

Reviewed By JMG
Date 1/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
Applied 10 uL of samples (all diluted 1:100)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-43 (1:100)	0.419	0.426	0.423	0.005	1.2	9.0	17.0	176.1	10.6	
44 (1:100)	0.545	0.652	0.599	0.076	12.6	12.7	14.8	287.4	17.2	
46 (1:100)	0.528	0.455	0.492	0.052	10.5	10.5	12.1	288.1	17.3	
47 (1:100)	0.616	0.632	0.624	0.011	1.8	13.3	11.5	386.1	23.2	
50 (1:100)	0.892	1.003	0.948	0.078	8.3	20.2	10.7	629.8	37.8	
51 (1:100)	0.820	0.824	0.822	0.003	0.3	17.5	12.0	486.4	29.2	
52 (1:100)	0.710	0.696	0.703	0.010	1.4	15.0	11.5	435.2	26.1	
55 (1:100)	0.337	0.400	0.369	0.045	12.1	7.8	14.4	181.8	10.9	
60 (1:100)	0.386	0.318	0.352	0.048	13.7	7.5	11.0	227.5	13.6	
6 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.073	0.066	0.070	0.005	7.1	1			
					TCDD/DMSO		**79.9**			
DCM	2 uL	0.085	0.108	0.097	0.016	16.9	1			
TCDD	1 ng/mL	5.602	5.500	5.551	0.072	1.3	57.2			
Applied 10 uL of samples (all diluted 1:100)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-43 (1:100)	4.329	4.515	4.422	0.132	3.0	63.2	17.0	1237.5	74.3	
44 (1:100)	4.243	4.911	4.577	0.472	10.3	65.4	14.8	1475.9	88.6	
46 (1:100)	4.969	4.231	4.600	0.522	11.3	65.7	12.1	1810.3	108.6	
47 (1:100)	4.664	4.923	4.794	0.183	3.8	68.5	11.5	1991.3	119.5	
50 (1:100)	5.065	5.900	5.483	0.590	10.8	78.3	10.7	2447.0	146.8	
51 (1:100)	5.539	5.011	5.275	0.373	7.1	75.4	12.0	2095.6	125.7	
52 (1:100)	4.999	5.137	5.068	0.098	1.9	72.4	11.5	2106.3	126.4	
55 (1:100)	4.240	4.431	4.336	0.135	3.1	61.9	14.4	1436.4	86.2	
60 (1:100)	4.341	3.710	4.026	0.446	11.1	57.5	11.0	1746.8	104.8	
January 5-6, 1998										
16 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.054	0.044	0.049	0.007	14.4	1			
					TCDD/DMSO		**195.3**			
DCM	2 uL	0.058	0.074	0.066	0.011	17.1	1			
TCDD	1 ng/mL	9.448	9.688	9.568	0.170	1.8	145.0			
Applied 10 uL of samples (all diluted 1:100)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-61 (1:100)	4.94	4.996	4.968	0.040	0.8	101.4	15.3	2202.8	132.2	
62 (1:100)	0.615	0.754	0.685	0.098	14.4	14.0	9.7	482.0	28.9	
7531-5 (1:100)	0.795	0.759	0.777	0.025	3.3	15.9	10.8	488.2	29.3	

Recorded By _____
Date 1/9/98

Reviewed By _____
Date 4/12/98

P450 RGS Data for Delaware Bay Study

Applied 10 uL of samples								Dry Wt.	[a]P Eq	TEQ
Sample I.D.	RLU1	RLU2	RLU3	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry
14 (1:100)	0.277	0.314	0.296	0.026	8.9	6.0	3.0	672.6	40.4	
6 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.073	0.069	0.071	0.003	4.0	1			
					TCDD/DMSO		**69.5**			
DCM	2 uL	0.093	0.108	0.101	0.011	10.6	1			
TCDD	1 ng/mL	4.659	5.205	4.932	0.386	7.8	48.8			
Applied 10 uL of samples (all diluted 1:100)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-61 (1:100)	5.051	5.319	5.185	0.190	3.7	73.0	15.3	1586.7	95.2	
62 (1:100)	4.504	4.973	4.739	0.332	7.0	66.7	9.7	2302.9	138.2	
7531-5 (1:100)	4.471	4.205	4.338	0.188	4.3	61.1	10.8	1881.2	112.9	
14 (1:100)	2.715	2.809	2.762	0.066	2.4	38.9	3.0	4338.7	260.3	
January 8-9, 1998										
16 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.061	0.059	0.060	0.001	2.4	1			
					TCDD/DMSO		**173.9**			
DCM	2 uL	0.070	0.084	0.077	0.010	12.9	1			
TCDD	1 ng/mL	10.384	10.487	10.436	0.073	0.7	135.5			
Applied 10 uL of samples (all diluted 1:300)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-50 (1:300)	0.404	0.470	0.437	0.047	10.7	7.3	10.7	682.7	41.0	
6874-61 (1:300)	2.267	2.443	2.355	0.124	5.3	39.3	15.3	2558.3	153.5	
6 hours		RLU1	RLU2	Mean	St.Dev.	CV%	FOLD			
DMSO	10 uL	0.085	0.078	0.082	0.005	6.1	1			
					TCDD/DMSO		**68.7**			
DCM	2 uL	0.117	0.119	0.118	0.001	1.2	1			
TCDD	1 ng/mL	5.687	5.507	5.597	0.127	2.3	47.4			
Applied 10 uL of samples (all diluted 1:300)							Dry Wt	B[a]P Eq	TEQ	
Sample I.D.	RLU1	RLU2	Mean	St.Dev.	CV%	FOLD	(g)	ug/g dry	ng/g dry	
6874-50 (1:300)	4.480	4.732	4.606	0.178	3.9	56.2	10.7	5264.8	315.9	
6874-61 (1:300)	5.517	5.100	5.309	0.295	5.6	64.7	15.3	4219.7	253.2	

Recorded By _____
Date 1/9/98

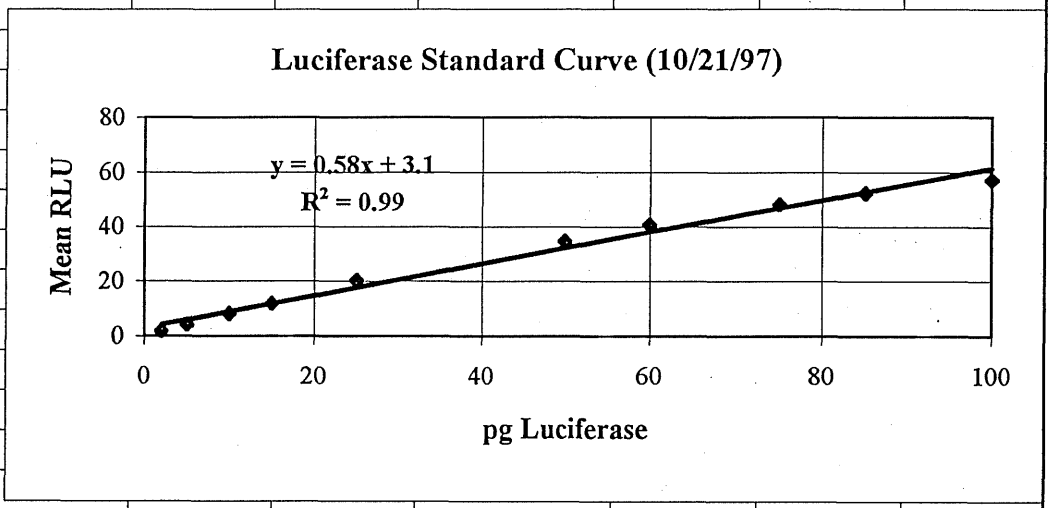
Reviewed By _____
Date 1/12/98

QA /QC Data

Spiking of NOAA/ Delaware Bay Samples								
								Recovery
Date	CAS ID #	NOAA#	Toxicant	Spike conc	Sample Fold	Tox.Fold	Spike Fold	(% additive)
10/24/97	6874-15	54	B[a]P	300 ng/mL	2.8	11.4	13.6	95
10/24/97	6874-25	40	B[a]P	300 ng/mL	30.5	11.4	40.4	96
10/31/97	6874-35	39	B[a]P	300 ng/mL	5.3	14.7	14.7	74
11/4/97	6874-45	39	B[a]P	300 ng/mL	10.1	12.6	17.4	77
11/11/97	6874-60	10*	B[a]P	300 ng/mL	131.4	13.2	166.1	115
11/11/97	7531-10	37	B[a]P	300 ng/mL	17.4	13.2	44.3	145
11/14/97	7531-19	87	B[a]P	300 ng/mL	10.7	19.8	24.1	79
	* Also tested for 2-time periods							

Quality Control of NOAA: Bioeffects RGS Testing-Delaware Bay								
	CV%	CV%	CV%	CV%	mean CV%			
Date	TCDD	DCM	DMSO	B[a]P	of Samples	TCDD/DCM	TCDD/DMSO	B[a]P Fold
10/20/97	2	3	10	NT	5	96.5	138.2	NT
10/21/97	4	4	6	NT	6	83.9	113.8	NT
10/24/97	10	2	6	7	7	86.9	127.5	11.4
10/28/97	5	3	8	NT	5	125.3	95.5	NT
10/31/97	4	8	5	4	10	88.6	117.4	14.7
11/4/97	3	4	5	2	10	90.2	131.3	12.6
11/11/97	2	5	4	15	9	96.4	126.1	13.2
11/14/97	1	4	1	11	8	85.8	107.5	19.8
11/27/97	2	1	5	NT	5	76.4	97.5	NT
					Mean	92.2	117.2	14.3
					Std.Dev.	13.9	15.0	3.3
					CV (%)	15.0	12.8	22.8

10/21/97						
lot #70416	Exp:10/97	Specific Activity: 20,511 RLU/pg				
pg Luciferase	RLU1	RLU2	pg Luciferase	Mean		
2	1.862	1.868	2	1.865		
5	4.392	4.142	5	4.267		
10	8.314	8.05	10	8.182		
15	12.148	11.699	15	11.924		
25	19.982	20.356	25	20.169		
50	33.415	36.075	50	34.745		
60	40.292	41.255	60	40.774		
75	47.799	48.577	75	48.188		
85	52.072	52.711	85	52.392		
100	57.348	56.983	100	57.166		



11/14/97						
lot #70922	Exp:1/98	Specific Activity: 27,848 RLU/pg				
pg Luciferase	RLU1	RLU2	pg Luciferase	Mean		
2	3.162	3.879	2	3.521		
5	7.274	7.388	5	7.331		
10	15.237	15.157	10	15.197		
15	21.291	21.357	15	21.324		
25	35.453	35.409	25	35.431		
50	58.572	61.53	50	60.051		
60	69.08	70.72	60	69.900		
75	82.4	86.46	75	84.430		
85	93.03	92.14	85	92.585		
100	100.57	103.39	100	101.980