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DISTRIBUTION OF CYTOCHROME P4501A1–INDUCING CHEMICALS IN SEDIMENTS OF THE DELAWARE RIVER–BAY SYSTEM, USA

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Abstract—The Delaware River–Bay system, USA, was the subject of a study by the National Oceanic and Atmospheric Administration that involved chemical and biological analyses, including the use of the biomarker P450 human reporter gene system (HRGS) to document the occurrence and distribution of cytochrome P450 (CYP) 1A1–inducing compounds. Sediment extracts from 81 locations along the Delaware River, Delaware Bay and immediate coastline were tested by utilizing HRGS as an inexpensive screening test, and were also analyzed for polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls, with selected stations analyzed for dioxins and furans. Benthic community degradation has been observed when benzo[*a*]pyrene equivalents (B*a*PEq) exceeded 60 μg/g. The average levels of B*a*PEq for the largely industrialized upper, middle, and lower regions of the Delaware River were 107, 62, and 5 μg/g, respectively, excluding outliers. Tributaries leading into river averaged 21 μg/g B*a*PEq, whereas the central Bay and open coast had relatively low values (2.0 and 0.5 μg/g B*a*PEq, respectively). The HRGS values were highly correlated with total PAHs measured in the same sediment samples ($r^2 = 0.81$). Overall, contamination levels consistently decreased from the upper and middle river sites as collection locations progressed down through the lower river and bay to the coast. Thus, despite the relatively high contaminant load in the river system, Delaware Bay and the immediate coastline seem to have relatively low levels of contaminants, and, therefore, impacts on the benthic organisms in the bay and coast would not be expected from these findings.

Keywords—Delaware River Polycyclic aromatic hydrocarbons Dioxins Human reporter gene system

INTRODUCTION

Delaware Bay is one of the largest estuaries on the East Coast of the United States, with a vast drainage area that is located in the states of Delaware, New Jersey, New York, and Pennsylvania. Although impairment of coastal environmental quality and degradation of coastal ecosystems due to population increase and industrialization in the region were recognized in the 1930s, concerns about the widespread occurrence of toxicants in the bay are relatively more recent [1]. Several sediment toxicity studies have been carried out in the bay during the past 10 years, focusing on specific sources of contaminants or areas of concern [2–4].

As part of its environmental stewardship mission, the National Oceanic and Atmospheric Administration (NOAA) conducts studies to determine the extent and severity of environmental contamination and its associated biological effects in coastal waters and estuaries of the United States. These studies primarily focus on sediment contamination and are based on the sediment quality triad approach [5,6]. The approach consists of measurements of sediment contaminant levels, sediment toxicity, and parameters of benthic community structure. In 1997, NOAA initiated a geographically comprehensive study to examine the spatial extent, patterns, and severity of sediment toxicity in the Delaware River and Bay. Typically, the amphipod mortality test, the sea urchin fertilization impairment test, and the Microtox® test (AZUR Environmental, Carlsbad, CA, USA) have been used [7,8]. However, in recent years, a human reporter gene system (HRGS) has been added to determine the presence of contaminants that induce the cytochrome P450 (CYP) 1A1 gene. In an investigation of San Diego Bay, California, USA, Fairey et al. [9] found that sediments exhibiting HRGS responses of 60 μg/g benzo[*a*]pyrene (B*a*P) equivalents (B*a*PEq) and greater contained infaunal communities that were classified as degraded, accounting for 50% of the stations that were sampled for both parameters.

The induction of CYP 1A1 in the CYP gene family has been widely used as a biomarker for exposure to toxic or carcinogenic organic compounds in the aquatic environment. Organic compounds such as dioxins, furans, coplanar polychlorinated biphenyls (PCBs), and the higher molecular weight polycyclic aromatic hydrocarbons (PAHs) are known to bind to intracellular cytosolic protein referred to as the aryl hydrocarbon receptor. Upon binding, this complex is translocated to the nucleus of the cell, where it interacts with xenobiotic response elements in the promoter region of the CYP 1A1 gene and causes transcription of the P450 enzyme system.

The induction of the CYP gene family, specifically CYP 1A1, in response to PAHs has been extensively studied in fish [10–12]. These studies have demonstrated that high levels of P450 in fish are correlated with increased sediment contamination, especially by PAHs, and histological and reproductive effects on the fish. In recent NOAA studies, HRGS responses have correlated well ($r^2 \sim 0.7$–0.8) with the concentrations of total PAHs in sediments, and even better ($r^2 \sim 0.85$) with the concentrations of PAHs containing between four and six benzene rings (molecular weights ranging from 228 to 302) [13]. The HRGS assay has also been used to detect PAH contam-

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ination in sediments from the coastal waters of Korea [14,15]. In addition, HRGS B*a*PEq obtained in analysis of extracts of mussel tissue were highly correlated ($r^2 = 0.9$) with the presence of four- to six-ring PAHs reported from chemical analytical data [16]. Strong correlations have been demonstrated between HRGS responses and subsequent high-resolution gas chromatography–mass spectrometry analyses of chlorinated compounds (dioxins and furans) in contaminated soil samples [17,18].

The P450 HRGS utilizes a human liver cancer cell line, which has been stably transfected with a plasmid containing CYP 1A1 promoter regions fused to the firefly luciferase gene. When the cells are exposed to compounds that induce CYP 1A1, luciferase is produced and can be detected by a simple assay that measures relative light units with a luminometer. The potencies of several PAHs and coplanar PCBs for this test system have been described [19]. The HRGS (also known as U.S. Environmental Protection Agency method 4425 [20]) has gained acceptance as a rapid and inexpensive in vitro approach to screening solvent extracts of environmental samples of soil, sediment, tissue, and water. As an alternative to chemical analysis of all samples in an environmental assessment, the HRGS detects the total amount of compounds that act via the aryl hydrocarbon receptor to induce CYP 1A1. As such, the HRGS provides a measure of the inducing chemicals in a sample with the potential to cause harmful health effects. When only dioxins, furans, and coplanar PCBs are the concern, extracts are first cleaned of PAHs with silica gel and carbon columns (extraction method for U.S. Environmental Protection Agency method 8290 [www.epa.gov/epaoswer/hazwaste/test/8290.pdf]).

The objectives of this paper are to document the occurrence and distribution of CYP 1A1–inducing compounds in surficial sediment of the Delaware River–Bay system; relate the HRGS responses to the levels of PAHs, coplanar PCBs, and dioxins and furans in the sediment; and infer likely adverse biological effects associated with these chemicals.

MATERIALS AND METHODS

Sampling

The study area extended from Trenton, New Jersey, USA, to the coastal area outside the mouth of the bay, encompassing approximately 2,350 km^2. Sampling sites were selected on a random basis within well-defined sampling strata, whose boundaries were established in consideration of regional bathymetry, hydrography, and surficial sediment distribution, and to include areas of concern as expressed by state and local resource managers (Fig. 1). The total number of sampling sites was 81.

Surface sediment samples were collected by NOAA in September 1997, by using a Kynar-coated modified van Veen grab. Each sample was visually inspected to determine if the surface of the sample was undisturbed and contained fine-grained material. If acceptable, 2 to 3 cm of the surficial sediment was removed with a sterile high-impact styrene scoop and placed into a polyethylene bucket. When enough material had been collected, the sample was homogenized with a polyethylene paddle and distributed among the various sample containers. Samples to be tested by HRGS P450 were sent to the Columbia Analytical Services laboratory in Jacksonville (FL, USA) for extraction.

The extraction procedure was done according to the U.S. Environmental Protection Agency method 3550 (www.epa.gov/epaoswer/hazwaste/test/3550b.pdf) and the dichloromethane

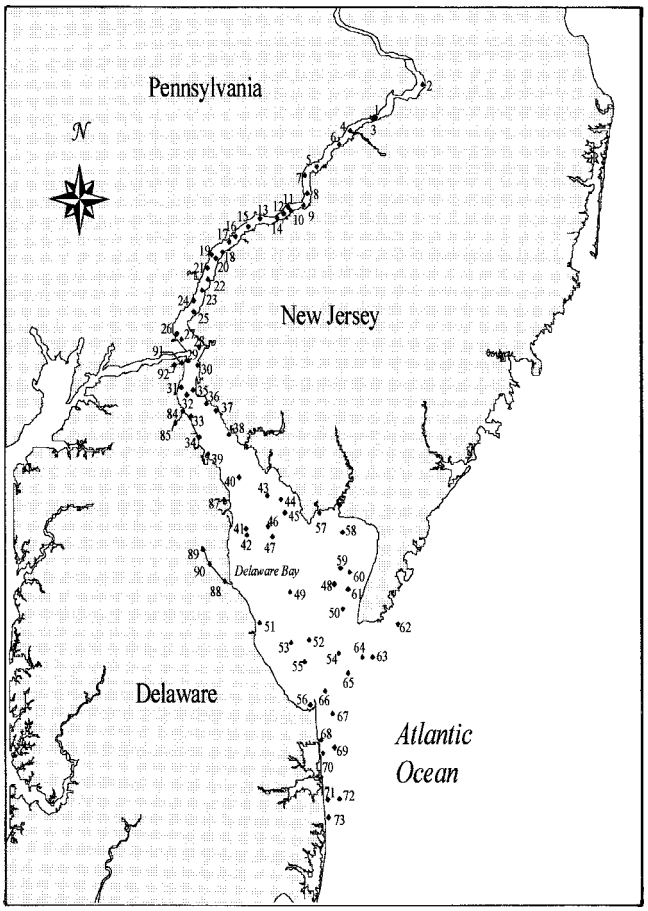

Fig. 1. Map of Delaware River, Delaware Bay, USA, and immediate coastline with labeled station numbers.

was evaporated to 2 ml. The dichloromethane extracts were exchanged into dimethylsulfoxide to better facilitate the HRGS P450 assay. Two 1-ml vials were prepared from each extract for the purpose of testing one vial by HRGS and the second vial by Microtox [21]. Additional sediment from each sample location also was prepared and used to test sea urchin gamete survival at three pore-water concentrations according to methods described by Carr [22]. A small subsample of each of the 81 sediments was used to measure percent solids. For purposes of data interpretation and description, samples were placed into six different categories based on their location within the river, bay, or ocean (Table 1). The location of each sample can be found on the map in Figure 1. Because stations 54 and 66 are located in the middle of the mouth of the central bay and are subject to a tidal regime characteristic of stations along the coastline, they were considered part of the open coast region (Table 1).

Test methods

The HRGS cell culturing methodology used in this study has been described previously [23–24]. Human liver cancer cells (101L cells) were grown as monolayers in an atmosphere of 5% CO_2 and 100% humidity at 37°C in Eagle's minimum essential media (Mediatech, Herndon, VA, USA), supplemented with 10% fetal bovine serum, 2% L-glutamine, 1% sodium pyruvate, and 0.4 mg/ml of genticin (all from Sigma, St. Louis, MO, USA). Cells were split upon reaching a maximum of 90% confluence, and discarded after 25 passages.

Table 1. Regional divisions and locations of sampling sites in the United States

Region	Stations	Location	Size (km^2)	Salinity (ppt)	Sediment type	Characteristics
Upper river	1–7	Trenton, NJ–Camden, NJ/Philadelphia, PA	30	0.1–1	Silt–clay with sand	Severely impacted, tidal fresh
Middle river	8–25	Camden, NJ/Philadelphia, PA–Wilmington, DE	99	1–3	Silt–clay with sand	Tidal fresh and mixing zone—high turbidity, low biological productivity; upstream portion is severely impacted
Lower river	26–39	Wilmington, DE–due W of Millville, NJ	250	3–15	Silt–clay with sand	Mixing zone—high turbidity, low biological productivity
Tributaries	84, 85, 87–92	Middle and lower river regions	NA[a]	5–20	Silt–clay	Mixing zone—high turbidity
Central bay	40–61 (excluding 54)	Due W of Millville, NJ–mouth of Atlantic Ocean	1,535	15–30	Silt–clay, some gravel	Mixing zone—high biological productivity
Open coast	54, 62–73	Coastline immediately N and S, and across mouth of the bay	433	>30	Sand with gravel	Seawater zone

[a] NA = not available.

In preparation for testing, cells were subcultured into six-well plates at a density of 2.5×10^5 cells/well and allowed to grow for 72 h in the environment described above to reach a density of approximately 1×10^6 cells/well. Triplicate test wells were inoculated with 10 μl of a given sample extract before incubating the plates at 37°C for 16 h. Standards, consisting of a 1 ng/ml solution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in dimethylsulfoxide, a 300 ng/ml B*a*P spike in dimethylsulfoxide, and a dimethylsulfoxide solvent blank, were also applied to wells in triplicate. At the end of the exposure, the cells were rinsed with Hank's balanced salt solution, and lysed with 200 μl of luciferase 3× cell lysis buffer (Promega, Madison, WI, USA). Plates were scraped and the cell lysates were centrifuged at 6,000 rpm for 10 s. Fifty-microliter aliquots of the supernatant were then applied to a 96-well plate, followed by 100 μl of substrate A (buffer and cofactors). Reactions were initiated by timed injection from the luminometer of 100 μl of substrate B (luciferin). Luminescence was measured in relative light units with a ML2250 Luminometer (Dynatech Laboratories, Chantilly, VA, USA). Luciferase assay buffers were purchased from Pharmingen (San Diego, CA, USA). When extracts were tested at two time periods, two replicates of each extract were applied to duplicate plates at the same time, so that one plate was incubated for 6 h, and the other for 16 h [23].

Means of the dimethylsulfoxide solvent blanks were used as a fold induction baseline and set equal to 1, whereas the fold induction of each sample extract and reference inducer (2,3,7,8-tetrachlorodibenzo-*p*-dioxin and B*a*P) was normalized by dividing the mean relative light units produced by the sample by the mean relative light units produced by the solvent blank. As a quality assurance–quality control measure, a long-term control chart for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been logged with each test run since the development of the test. For the Delaware study, 13 test runs with 1.0 ng/mL of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin produced a mean of 117.2-fold induction, a standard deviation of 15.0, and a coefficient of variation of 12.8%. These numbers illustrate the low variability of cellular response to the reference inducer and solvent blank. The test results for a sample were acceptable if both the coefficient of variation was less than 20% and the fold induction was less than 100. Any samples not meeting these criteria were retested; if the fold induction exceeded 100, the samples were first diluted and then retested. Fold induction from the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard over 100 is acceptable, because the concentration response curve for dioxin does not flatten out above 100, as does the curve for PAHs, which possibly is caused by toxicity. Only acceptable tests and final dilutions were used to determine fold induction and B*a*PEq.

Analysis

The HRGS fold induction response at 16 h of exposure was used to produce a quantitative estimate of the levels of CYP 1A1–inducing compounds in all sample extracts. All standard curves and analyses of environmental samples have been produced from the standard 16-h time interval. During this study a standard spike of 300 ng B*a*P/ml was used with the tests, and these produced an average 14.4-fold induction with a standard deviation of 2.93. The HRGS determination of B*a*PEq in all previous studies of coastal sediments for NOAA has utilized a conversion factor of 60-fold equal to 1 part per

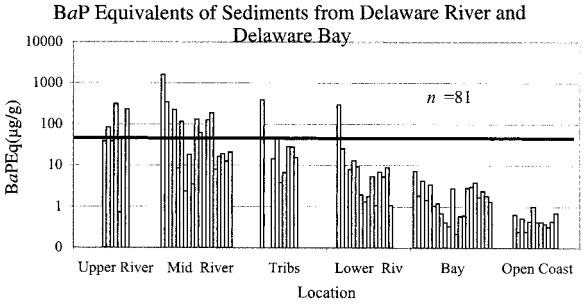

Fig. 2. Distribution of benzo[*a*]pyrene equivalents (B*a*PEq) taken from 81 sediment samples along the Delaware River, Delaware Bay, and Delaware, USA, coast. Categories were based on hydrogeography, salinity, sediment type, and location. Samples exceeding 60 μg B*a*PEq/g represented by the line would be expected to exhibit degraded benthic communities [9].

million of B*a*P, based on previous concentration–response curves [13]. The HRGS B*a*PEq are calculated as follows:

$$\text{HRGS B}a\text{PEq} = (\text{fold induction}/60)\cdot[(V_e/V_a)/W_d]$$

where V_e is the total extract volume, V_a is the volume of extract applied to cells, and W_d is the dry weight of sample. The above equation yields HRGS B*a*PEq in μg/g dry weight, and assumes that all CYP 1A1–inducing compounds in the samples are PAHs. Although the data were expressed as B*a*PEq, the measured responses at 16 h of exposure may have included induction from coplanar PCBs, dioxins, or furans. However, expressing the HRGS results as B*a*PEq is appropriate because chemical findings have shown that PAHs generally are the dominant class of organic contaminants within estuarine and marine sediments.

To determine if samples with the highest levels of induction were due to chlorinated hydrocarbons, two time interval tests were performed on selected samples. Laboratory analysis and testing have found that HRGS responses over time can predict if induction is mainly a result of PAHs or chlorinated hydrocarbons [25]. This is done by means of two-time-interval testing at 6 h and 16 h. If 16-h responses drop by approximately 60 to 80% in relation to 6-h responses (a 6-h:16-h response ratio of 5 or greater), the induction can be assumed to be a result of PAHs (cells metabolize PAHs). Conversely, if the fold induction increases or stays the same from 6 h to 16 h (response ratio of 1 or less), a major portion of the response can be attributed to chlorinated compounds such as dioxins, furans, and coplanar PCBs. Response ratios between one and five indicate approximately equal contribution from PAHs and chlorinated organic compounds.

Chemical methods

Chemical analysis methods for quantifying PAHs and their alkylated homologues by gas chromatography–mass spectrometry in this study were performed by NOAA contractors according to the appropriate methods under the National Status and Trends Program [26]. Also following procedures described for this program, PCB analyses were quantitatively determined by NOAA contractors through capillary gas chromatography with an electron-capture detector and dioxins and furans were measured by high-resolution gas chromatography and mass spectrometry. Toxic equivalency quotients (TEQs) were determined from the analyses of dioxins and furans, by using the World Health Organization (WHO) [27] TEQs. The TEQ values for coplanar PCBs were not calculated, because only 5 (77, 126, 169, 105, and 118) of the 12 (nonortho- and monoortho-) congeners evaluated by the WHO [27] were included in the analyses of 24 samples and only 2 (105 and 118) were measured in all samples. Of the five samples tested for dioxins and furans, only one was analyzed for the content of three (of the four) coplanar PCBs.

RESULTS

Distribution of P450 HRGS responses

The HRGS B*a*PEq for each portion of the Delaware River–Bay system, as defined in Table 1, are plotted on a log scale in Figure 2. Values ranged widely among the 81 samples, varying from 1,584 μg B*a*PEq/g in sample 11 to 0.22 μg B*a*PEq/g in sample 51. The mean for all samples was 57.4 μg B*a*PEq/g, with an upper 99% confidence interval of 112 μg B*a*PEq/g and a standard deviation of 190.8 μg B*a*PEq/g. The highest sediment value observed in this investigation was located on the eastern bank of the Delaware River, across from the Philadelphia (PA, USA) airport in close proximity to several other high samples. The lowest value was found in the central bay region. The vast majority of samples with high B*a*PEq values were found along the upper and middle portions of the river. These sections of the river contained 10 of the 12 sites having higher than 60 μg B*a*PEq/g. The mean for the upper river section, which contained no outliers was 106.7 μg B*a*PEq/g, whereas the middle river average changed from 162.6 to 62.3 μg B*a*PEq/g when outliers were excluded (Table 2). The lower portion of the river, comprised of 14 sampling locations, had a mean of 27.7 μg B*a*PEq/g (5.3 μg B*a*PEq/g excluding outliers) and contained only one site with a value above 60 μg B*a*PEq/g. The central bay and open coastal areas around the mouth of the bay showed primarily background levels, ranging from 0.2 to 7.2 μg B*a*PEq/g (Fig. 2).

Table 2 lists the number of samples, the means, and standard

Table 2. Summary of human reporter gene system responses to regional samples[a]

Region	Stations	*n*	Mean B*a*PEq (μg/g)	SD (μg/g)	CV (%)
Upper river	1–7	7	106.6	116.4	109.2
Middle river	8–25	18	162.6	367.1	225.8
Lower river	26–39	14	27.7	78.2	282.3
Tributaries	84, 85, 87–92	8	67.7	133.8	197.6
Central bay	40–61 (excluding 54)	21	2	1.7	85.0
Open coast	54, 62–73	13	0.7	0.7	100.0

[a] B*a*PEq = benzo[*a*]pyrene equivalents; SD = standard deviation; CV = coefficient of variation (in percent).

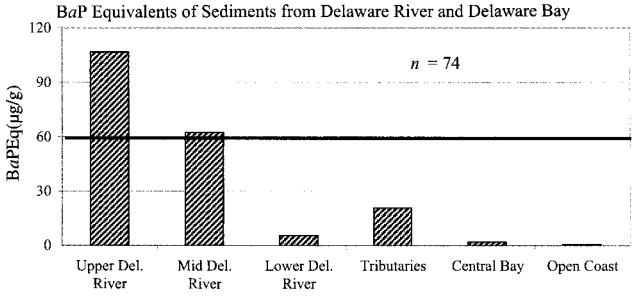

Fig. 3. Average benzo[*a*]pyrene equivalents (B*a*PEq) values among six regions of Delaware River basin (USA). Seven values determined to be outliers within their locations were excluded from this figure.

deviations of the 6 selected sections of the Delaware River–Bay system. The means for the different sections of the watershed have been plotted in Figure 3 to illustrate the relationships between regions of the system and the dramatic decrease in contamination from the upper and middle river toward the open coast. To reduce the effect of extremely high or low values skewing the regional averages in Figure 3, outliers were removed within each set of data. Outlier values were determined by multiplying the interquartile range by 1.5 and then subtracting or adding the value of the first or third quartile, respectively, within each region. By this method, samples 11, 13, 89, 29, 26, 40, and 66 were found to be outliers and were excluded from Figure 3.

6- and 16-h testing

To determine if the 11 samples producing the highest HRGS responses contained significant levels of chlorinated hydrocarbons, two-time-period tests were performed (Fig. 4). These samples required high dilution to bring their fold induction values within the test range. At 16 h, fold induction should be less than 100, and at 6 h it should be below about 50. Samples 4 and 11 initially required dilutions of 1:300. Fold induction values decreased by 75 to 85% in every sample from initial 6-h values to final 16-h values with the exception of sample 11. Sample 11 decreased by just 39% and yielded a response ratio of 1.65, which could indicate the presence of chlorinated hydrocarbons. However, because the 6-h response exceeded 50-fold, a spillover of PAHs or oversaturation effect could have occurred. In this case, the response ratio is skewed toward a low value because of insufficient time for the cells to metabolize the high amount of PAHs present. For this reason, samples 11, 13, and 29 (the samples with the highest 16-h values) were diluted once more and retested (Fig. 4). In each case, the 6-h responses decreased somewhat, whereas the 16-h responses all decreased markedly as a result of the additional dilution. These findings indicated that both PAHs and chlorinated hydrocarbons (dioxins, furans, and coplanar PCBs) were present in the samples.

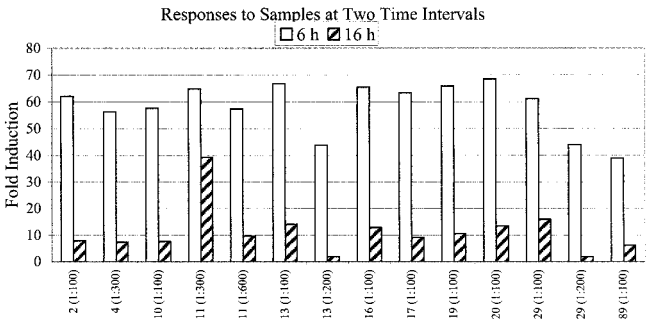

Fig. 4. Fold induction responses of the 11 highest samples at 6-h and 16-h test periods, with dilution factors in parentheses.

Chemical analyses

Jones and Anderson [19] previously established HRGS toxic equivalency factors (TEFs) for eight of the PAHs analyzed. By using the concentrations of each of these PAHs and their respective HRGS TEFs, the chemical B*a*PEq (expected HRGS response) values were calculated for each sample. Table 3 summarizes information on the total concentrations of all measured PAHs, the P450 HRGS responses to extracts of these sediment samples (in μg B*a*PEq/g), and calculations of predicted HRGS responses from chemical analyses. The HRGS B*a*PEq are in general about an order of magnitude higher than the chemical B*a*PEq, which is likely due to induction from compounds (alkylated PAHs) other than the eight inducing PAHs previously studied [19].

Table 4 illustrates results of the PCB congener analyses performed by the NOAA contractor on all samples. Although the analysis of noncoplanar PCB congeners was performed on all 81 samples, coplanar PCB congeners were analyzed in only 24 randomly chosen samples. Coplanar PCB analyses included PCB congeners 77, 126, and 169, but not 81, which has been shown to induce HRGS at a lowest concentration of any PCB [19]. Samples 1, 3, 4, 7, 13, and 91 were the only samples analyzed in which the levels of coplanar PCBs (at about 0.3 ng/g and greater) would be expected to induce the P450 HRGS.

Dioxin and furan chemical analyses were performed on five samples that were suspected of containing significant amounts of chlorinated hydrocarbons, based on two-time-period HRGS testing. The chemical TEQs (in parts per trillion; pg/g) for samples 10, 11, 20, 29, and 89 are based on the potency of the 17 dioxin and furan compounds that induce the CYP 1A1 promoter gene (Table 5). The TEQ values were derived from the concentration of each chemical and the WHO [27] TEFs. The HRGS TEFs are not necessarily equivalent to the WHO TEFs, because the latter were derived from data on many species and a wide variety of biological responses. However, two years of daily testing with HRGS (D. McCoy, unpublished data) have shown that fold induction is equivalent to the WHO TEQ (in pg/ml) of the test solution. Among the five samples, sample 29 had the highest TEQ value (48.3 pg/g), followed by samples 20 and 89 (26.9 and 20.5 pg/g TEQ, respectively). Samples 10 and 11 contained only small quantities of dioxins and furans (0.3 and 3.8 pg/g TEQ, respectively), which would likely be responsible for only a small percentage of the observed induction.

Correlations with analytical chemistry data

The correlation of P450 HRGS data with the subsequent chemical analyses (Table 3) of the total measured PAHs in the sediment samples is shown in Figure 5. A strong correlation value ($r^2 = 0.81$) suggests that total measured PAHs are the primary contributors to CYP 1A1 induction, despite the fact that other compounds such as coplanar PCBs, dioxins, and furans also were present (Tables 4 and 5) and may have contributed to the overall HRGS response. In Figure 6, the HRGS responses (except samples 11 and 17) are plotted against the predicted responses (chemical B*a*PEq), based on the conversion of data on the specific PAHs known to induce the CYP 1A1 gene in this assay, and the magnitude of the expected

Table 3. Measured and calculated human reporter gene system (HRGS) responses and total polycyclic aromatic hydrocarbons (TPAHs)

Sample	HRGS B*a*PEq[a] (μg/g)	Chemical B*a*PEq (μg/g)	TPAH (μg/g)
1	38.4	9.83	8.5
2	83.3	3.80	6.1
3	37.9	11.62	9.1
4	313.0	20.30	17.2
5	0.7	0.36	0.4
6	47.6	4.12	4.5
7	225.5	9.40	14.0
8	226.6	9.03	12.9
9	8.4	2.80	3.5
10	115.1	4.70	6.5
11	1,584.0	5.48	10.0
12	2.4	0.74	0.9
13	344.7	8.61	18.1
14	18.0	7.88	5.4
15	3.4	0.33	0.4
16	130.6	4.91	11.1
17	62.3	1.7[b]	1.6[b]
18	47.4	2.77	2.8
19	124.2	5.24	6.1
20	183.1	6.31	14.6
21	7.9	3.93	4.0
22	16.3	1.01	1.3
23	18.8	2.61	3.7
24	12.5	2.38	2.8
25	20.4	1.70	2.4
26	25.2	1.08	2.2
27	7.9	1.06	2.0
28	13.0	0.83	1.2
29	298.4	4.98	12.7
30	9.3	1.32	2.2
31	1.9	0.03	0.2
32	1.3	0.01	0.4
33	1.7	0.01	0.4
34	5.3	0.65	1.0
35	1.1	0.27	0.5
36	6.9	0.64	1.1
37	5.2	0.57	1.0
38	8.9	0.27	0.5
39	1.1	0.07	0.1
40	7.2	0.57	0.6
41	1.8	0.10	0.2
42	4.2	0.27	0.3
43	1.5	0.49	0.5
44	3.4	0.22	0.2
45	1.0	0.03	0.0
46	1.2	0.05	0.1
47	0.7	0.02	0.0
48	0.4	0.05	0.1
49	0.3	0.04	0.0
50	2.7	0.11	0.2
51	0.2	0.00	0.0
52	0.6	0.02	0.0
53	0.6	0.01	0.0
54	0.6	0.07	0.1
55	2.8	0.04	0.1
56	2.9	0.30	0.4
57	3.7	0.52	0.7
58	1.7	0.12	0.1
59	2.3	0.05	0.1
60	1.8	0.59	0.6
61	1.3	0.01	0.0
62	0.2	0.01	0.0
63	0.5	0.01	0.0
64	0.2	0.00	0.0
65	0.4	0.00	0.0
66	3.0	0.05	0.1
67	1.0	0.02	0.1
68	0.4	0.01	0.0
69	0.4	0.01	0.0
70	0.4	0.00	0.0
71	0.3	0.02	0.0
72	0.4	0.00	0.0
73	0.7	0.01	0.0
84	14.3	0.82	1.3
85	48.1	0.92	1.5
87	3.8	0.06	0.3
88	6.7	0.96	1.2
89	396.9	12.33	10.9
90	28.3	1.74	1.7
91	27.8	1.40	2.1
92	15.5	0.98	2.0

[a] B*a*PEq = benzo[*a*]pyrene equivalents.
[b] Chemistry provided was a factor of 10 higher than listed and was suspect.

response (HRGS TEFs). The correlation coefficient in Figure 6 ($r^2 = 0.63$) is not as high as that of Figure 5, because a few high HRGS values are not explained by the concentrations of inducing PAHs. Samples with significant amounts of dioxins, furans, or coplanar PCBs would be expected to fall above the trend line in Figure 6. Of the three samples located substantially above the trend line (samples 13, 29, and 89), only sample 13 was analyzed for coplanar PCBs and was found to contain more than four times the amount of any other analyzed sample (2 ng/g coplanar congeners + 660 ng/g other congeners; Table 4). As noted above (Table 5), samples 29 and 89 contained significant amounts of dioxins and furans (TEQs of 48 and 20 pg/g, respectively), which may explain the high HRGS response. Sample 17, with suspect analytical chemical data, was not included in Figure 6. The highest HRGS value (sample 11 at 1,584 μg B*a*PEq/g) was not plotted in Figure 6 because it would have expanded the scale significantly. This sample was found to contain about 4 pg TEQ/g of dioxins or furans and 10 μg/g of PAHs, but was not analyzed for coplanar PCBs, which may have also contributed to the high induction observed. Some samples were well below the trend lines of Figures 5 and 6, and the best explanation is likely differences in both the known and unknown inducing PAH compounds in these extracts. Some suppression or toxicity is possible, considering the interactions between chemicals in these complex mixtures.

Correlations with biological analyses

A recent report from NOAA [28] describes the full amount of biological and chemical data gathered on samples taken from the Delaware River–Estuary system. In its analysis, the report compares P450 HRGS results with both chemical data and the findings of other bioassays and benthic community descriptions. In the estuarine strata, the HRGS responses were negatively correlated ($p < 0.01$) with the total number of taxa as well as the diversity index of benthic organisms, whereas in the freshwater strata this high correlation was only associated with the number of taxa. The B*a*PEq also were negatively correlated with sea urchin fertilization success (Spearman rank correlation of −0.663 to sea urchin sperm exposed to 50% pore water) and to the 50% effects concentration of Microtox [28]. Not surprisingly, the results of 10-d amphipod mortality tests did not correlate with B*a*PEq, because HRGS

Table 4. Coplanar and total polychlorinated biphenyl (PCB) congeners. Coplanar PCBs only were measured in those samples listed

Sample	Coplanar PCBs (ng/g)	Noncoplanar PCBs (ng/g)	Total PCBs (ng/g)
1	0.320	142.99	143.31
2		20.35	20.35
3	0.446	237.18	237.63
4	0.401	188.51	188.91
5		11.63	11.63
6		52.37	52.37
7	0.589	229.52	230.11
8		252.49	252.49
9		53.65	53.65
10	0.005	92.20	92.21
11		112.57	112.57
12	0.013	13.32	13.33
13	2.064	660.61	662.67
14	0.371	30.24	30.61
15	0.021	17.29	17.31
16		466.23	466.23
17		112.58	112.58
18	0.214	46.68	46.89
19		138.78	138.78
20		319.42	319.42
21	0.348	100.80	101.15
22	0.111	40.20	40.31
23		110.77	110.77
24		15.49	15.49
25		70.50	70.50
26		54.77	54.77
27		3.73	3.73
28		34.38	34.38
29		32.41	32.41
30		60.50	60.50
31		2.94	2.94
32	0.004	17.28	17.28
33		2.24	2.24
34		26.26	26.26
35		5.28	5.28
36		33.13	33.13
37	0.182	28.16	28.34
38		17.46	17.46
39	0.018	3.68	3.70
40		9.61	9.61
41		3.64	3.64
42		4.56	4.56
43	0.017	3.54	3.56
44		4.54	4.54
45		1.50	1.50
46		1.74	1.74
47		2.10	2.10
48		3.72	3.72
49		1.46	1.46
50		2.76	2.76
51		0.27	0.27
52		0.91	0.91
53		0.88	0.88
54	0.018	1.90	1.92
55		1.88	1.88
56		6.61	6.61
57		21.93	21.93
58		3.43	3.43
59		1.12	1.12
60	0.053	14.50	14.55
61		1.11	1.11
62		0.34	0.34
63		0.33	0.33
64	0.002	0.18	0.18
65	0.003	0.43	0.43
66		2.28	2.28
67		2.95	2.95
68		0.54	0.54
69		0.43	0.43
70	0.003	0.19	0.19
71	0.003	0.28	0.28
72		0.25	0.25
73		0.37	0.37
84		38.05	38.05
85		37.67	37.67
87		12.04	12.04
88		30.66	30.66
89		279.07	279.07
90		71.29	71.29
91	0.487	59.95	60.44
92	0.254	28.51	28.76

measures the levels of hydrophobic toxic organic compounds, which, in situ, may be released slowly over time.

As already described above, a strong correlation was found between the HRGS and PAHs, but because metals co-occurred with PAHs, this correlation also was high. Because the HRGS assay is conducted on organic solvent extracts of sediments, only the organometals such as tributyl tin would be expected to be present in the extract applied to the cells.

DISCUSSION

Samples that generated an HRGS response equal to or above 60 μg B*a*PEq/g are an indication of areas in which environmental degradation is expected [9]. This is based on the levels of CYP 1A1–inducing compounds observed in a San Diego Bay (CA, USA) study that were highly correlated with degradation of the benthic community [9,13]. This degradation was based on low species diversity as well as the presence of significant numbers of opportunistic species in tandem with the absence of nonopportunistic species. Both the upper and middle Delaware River regions had a significant number of sites that approached or exceeded the 60 μg B*a*PEq/g level. Of the total 81 samples tested in this project, 13 (16%) were above 60 μg B*a*PEq/g. Also, of the 47 samples taken from the Delaware River and its tributaries, 28% were above 60 μg B*a*PEq/g, including 42% of the samples from the upper and middle river areas. These regions are located in close proximity to major metropolitan and urban areas, with a large number of refineries along the river's banks. Therefore, not surprisingly, nearly all of the samples in the Delaware River watershed with high HRGS values were located in the middle and upper reaches of the river, adjacent to the urban areas of Philadelphia (PA, USA), Wilmington (DE, USA), and Trenton (NJ, USA).

Based on the analyses of previous HRGS data from several coastal areas [13], detrimental biological effects may occur among benthic, epibenthic, or bottom-feeding fish communities when B*a*PEq levels are above 37 μg B*a*PEq/g. This estimate is based on the mean upper 99% confidence interval derived from more than 912 samples from 12 different study locales (Table 6). Five of the samples from this investigation (6%) were greater than 37 but less than 60 μg B*a*PEq/g.

Samples taken from the marshes and inner portions of small tributaries along the middle portion of the river also had elevated contaminant levels, including sample 89, which had a B*a*PEq level of 396.8 μg B*a*PEq/g. Lower flow rates, decreased water volume, industrial discharge, and runoff from both Dover Air Force Base, and the city of Dover (DE, USA) might be contributing to pockets of high contamination in these areas. These data would be consistent with those of other

Table 5. Chemical toxic equivalency quotients (TEQs) of dioxins and furans contained in Delaware Bay, USA, samples (pg/g; parts per trillion)[a]

Analyte	WHO TEFs	Samples									
		10	TEQ	11	TEQ	20	TEQ	29	TEQ	89	TEQ
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1.0	ND	0.00	ND	0.00	ND	0.00	ND	0.00	ND	0.00
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	1.0	ND	0.00	ND	0.00	4.12	4.12	ND	0.00	ND	0.00
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	0.1	ND	0.00	ND	0.00	7.36	0.74	ND	0.00	ND	0.00
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	0.1	0.31	0.03	3.56	0.36	12.4	1.24	5.53	0.55	26.90	2.69
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	0.1	ND	0.00	3.09	0.31	8.67	0.87	8.72	0.87	23.93	2.39
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	0.01	14.09	0.14	62.13	0.62	222.04	2.22	137.98	1.38	599.94	6.00
Octachlorodibenzo-*p*-dioxin	0.0001	118	0.01	1,103.98	0.11	4,902.63	0.49	4,530.75	0.45	13,272.54	1.33
2,3,7,8-Tetrachlorodibenzofuran	0.1	0.46	0.05	ND	0.00	ND	0.00	9.86	0.99	15.88	1.59
1,2,3,7,8-Pentachlorodibenzofuran	0.05	0.50	0.03	1.23	0.06	8.09	0.40	25.27	1.26	ND	0.00
2,3,4,7,8-Pentachlorodibenzofuran	0.5	ND	0.00	2.7	1.35	12.83	6.42	24.07	12.04	8.14	4.07
1,2,3,4,7,8-Hexachlorodibenzofuran	0.1	ND	0.00	3.27	0.33	29.97	3.00	124.67	12.47	7.98	0.80
1,2,3,6,7,8-Hexachlorodibenzofuran	0.1	ND	0.00	2.55	0.26	17.63	1.76	58.33	5.83	9.06	0.91
2,3,4,6,7,8-Hexachlorodibenzofuran	0.1	ND	0.00	1.93	0.19	14.39	1.44	30.18	3.02	ND	0.00
1,2,3,7,8,9-Hexachlorodibenzofuran	0.1	ND	0.00	ND	0.00	ND	0.00	ND	0.00	ND	0.00
1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.01	1.95	0.02	25.95	0.26	407.29	4.07	787.78	7.88	72.27	0.72
1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.01	ND	0.00	ND	0.00	14.66	0.15	114.76	1.15	ND	0.00
Octachlorodibenzofuran	0.0001	ND	0.00	39.44	0.00	512.65	0.05	4,488.92	0.45	128.73	0.01
Total chemical TEQ (pg/g)			0.27		3.85		26.96		48.33		20.51

[a] WHO = World Health Organization; TEF = toxic equivalency factor; ND = not detected at 0.5 pg/g.

NOAA studies done in south and central Puget Sound (WA, USA) and Florida, USA, in which areas of high contamination were frequently found in creeks, canals, inlets, and back bays that were often subjected to the aforementioned phenomena [13].

The lower river region also contained elevated contaminant levels (mean = 27.8 μg B*a*PEq/g), although not to the extent of the upper or middle river or tributary regions. The main reason for this is likely that it is situated further away from the urban sprawl and refineries upstream. Only one sample (sample 29) exhibited greater than 60 μg B*a*PEq/g and if this value is considered an outlier, the overall mean for the lower river drops to 6.8 μg B*a*PEq/g.

In contrast to the upper and middle river and tributary regions, and to a lesser extent the lower river, the central bay and open coast sites contained concentrations of chemicals producing low induction levels. Of the 34 samples taken from the central bay or open coast regions, none were above 10 μg B*a*PEq/g, and 50% were below 1 μg B*a*PEq/g. Analyses of previous data from many regions [13] indicate that biological effects would not be expected at B*a*PEq levels less than 11 μg B*a*PEq/g. At these low levels, relatively little contamination with respect to dioxins, furans, coplanar PCBs, and PAHs would be present.

In comparison to other recent studies that utilized the HRGS method, the Delaware River and Bay study produced a mean similar to that of southern Puget Sound, and higher than several other regions (Table 6) [13]. The Delaware system had a greater percentage of samples above 60 μg B*a*PEq/g than most regions previously studied. Aside from samples 20, 29, and 89, which had elevated amounts of dioxins and furans, and sample 13 with high PCBs, nearly all of the HRGS induction appears to be the result of PAH contamination. However, because only 24 of the 81 samples were analyzed for PCBs, and only 5 samples were analyzed for dioxins and furans, the possibility exists that contributions from chlorinated hydrocarbons may have been responsible for other high induction values not explained by PAHs.

Overall, contamination levels consistently decreased from the upper and middle river sites on down through the lower river and central bay to the open coast. Of the 2,347 km^2 covered in this investigation, examination of the HRGS data indicates that 63% of the system (1,825 km^2) did not contain levels of sediment chemicals found to be linked to moderate

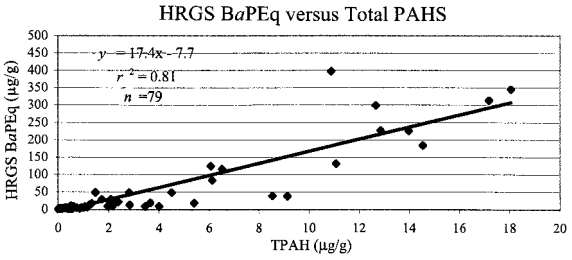

Fig. 5. Correlation between human reporter gene system (HRGS) benzo[*a*]pyrene equivalents (B*a*PEq) and total polycyclic aromatic hydrocarbons (PAHs). Sample 11 was excluded because of the very high value (1,584) and sample 17 was excluded because of suspect chemical data.

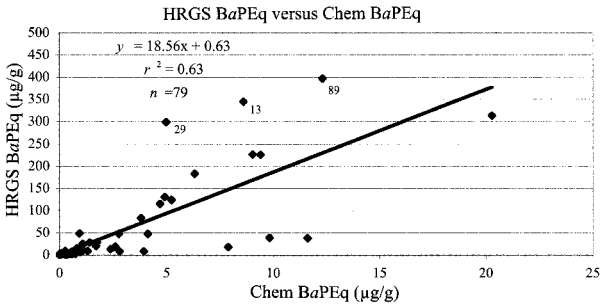

Fig. 6. Correlation between observed human reporter gene system (HRGS) benzo[*a*]pyrene equivalents (B*a*PEq) and the expected B*a*PEq, based on the presence of specific polycyclic aromatic hydrocarbons (PAHs) and their potency in the HRGS assay [19]. Samples 11 and 17 were excluded. Samples above the trendline would be expected to contain compounds other than PAHs. Labeled stations were found to contain significant concentrations of polychlorinated biphenyls and dioxins and furans. Stations below the trend-line may contain fewer unidentified compounds, which induce cytochrome P450 1A1.

Table 6. Distribution of data from P450 human reporter gene system (HRGS) analyses of sediment samples from regional surveys. Values listed represent HRGS responses (μg benzo[*a*]pyrene equivalents/g) and percentages of samples exceeding estimated thresholds[a]

Locations in United States	No. stations	Mean	SD	99% CI	Lower CI	Upper CI	No. stations >60	% Stations >60	Total area (km^2)
Southern California bays	29	5.2	6.2	2.5	2.7	7.7	0	0.0	5.0
Galveston Bay, TX	75	5.2	6.0	1.8	3.4	7.0	0	0.0	1,351.1
Biscayne Bay, FL (1996)	121	8.2	8.5	2.0	6.2	10.2	0	0.0	271.4
Northern Puget Sound	100	11.1	27.3	7.0	4.0	18.1	4	4.0	806.2
Central Puget Sound	100	37.5	48.3	12.5	25.1	50.0	19	19.0	737.5
Southern Puget Sound	100	52.8	207.7	53.5	0.0	106.3	14	14.0	857.7
Sabine Lake, TX	65	14.5	21.1	6.7	7.8	21.2	5	7.7	245.9
Winyah Bay, SC	9	16.3	14.3	14.0	2.3	30.2	0	0.0	7.3
Delaware River and Bay	81	57.0	190.8	23.8	58.7	112.0	13	16.0	2,346.8
Chesapeake Bay (1998)	63	25.9	31.0	10.1	15.8	36.0	6	9.5	2,266.0
Chesapeake Bay (1999)	69	8.3	11.9	37	4.6	12.0	1	1.4	NA
San Francisco Bay (2000)	100	15.7	14.9	3.8	11	18.7	2	2.0	1,200.0
Mean values	Total = 912	21.5	49.0	11.8	11.8	35.8	5.3	6.1	917.7
Charleston Harbor[b]	20	30.1	26.8	17.3	12.7	47.4	3	15.0	6.6
San Diego Bay[b]	30	31.4	35.5	16.2	15.2	47.6	15	50.0	5.5
Columbia River[c]	23	0.9	1.9	1.02	0	1.9	0	0.0	NA
Willamette River[c]	52	63.5	171.3	61.2	2.3	124.6	11	21.2	NA
Southern California Bight (1998)[c]	268	18.5	58.8	8.8	9.7	27.3	15	5.6	3,808.0
Total samples	1,305								

[a] SD = standard deviation; CI = confidence interval; NA = estimate of area not currently available.
[b] Samples from the NOAA Projects on Charleston Harbor and San Diego Bay, USA, were selected from a larger group of samples, and therefore are not representative of the random sampling approach used in other NOAA surveys.
[c] Projects funded by sources other than NOAA.

or strong biological effects. Much of the low contaminant levels in the central bay likely can be attributed to a high rate of tidal flushing in concert with an up-estuary movement of sediment transport within areas of strong tidal flow. The deep channels in the bay run northwest from the mouth of the bay. At the mouth of the bay, Cape May shoals restrict the entrance to the bay on the north side and tidal flow velocities are strongest in the channels. As a consequence, net sediment flux is actually in the upstream direction in the main bay area because ebb tidal velocities are relatively weaker due to the shoreline configuration and Coriolis effects [29]. Further upstream, the estuary narrows significantly and the influence of lateral water movement on depositional patterns is reduced. Sediment-bound contaminants associated with fine-grained, high organic content materials possibly are prevented from migrating to the lower estuary by the circulation system. Thus, despite the relatively high contaminant loads in the river system leading into it, the Delaware Bay and immediate coastline seem to have relatively low levels of contaminants and likely exhibit healthy populations of marine organisms.

It is important to keep in mind that because the solvent extracts used in HRGS testing remove all hydrophobic contaminants from the samples and does not take bioavailability into consideration, the values derived by HRGS may represent a worst-case scenario. The HRGS values may not always correlate well with acute toxicity studies, but other studies have shown that impacts on epibenthic and infaunal species exposed to sediment-bound compounds can be significant when considered over the course of months or years [30]. We believe the best use of the HRGS assay is an initial screening of all stations in a sediment survey and a subsequent comparison to measured PAHs. Those stations with HRGS responses well above the HRGS–total measured PAHs correlation curve should then be investigated for contamination from chlorinated CYP 1A inducers (dioxins, furans, and coplanar PCBs), thus concentrating the expensive analytical costs on the key stations.

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