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Correlation Between the Response of a Human Cell Line (P450 RGS) and the Distribution of Sediment PAHs and PCBs on the Palos Verdes Shelf, California

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ABSTRACT

The applicability of the P450 Reporter Gene System (RGS) assay in the assessment of sediment contamination was examined by correlating biological responses with detailed chemical compositions obtained from the same samples. Sections of a sediment core collected from the Palos Verdes Shelf (PVS) were analyzed for total organic carbon (TOC), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). The biological responses to extracts from the same samples were determined using the P450 RGS assay. The profiles of TOC, PAHs, and PCBs were consistent in illustrating the pre-discharge baseline and the effects of more efficient wastewater treatment and source control. Induction of CYP1A1 using the P450 RGS assay was correlated with total PAHs (r^2=0.47) and more closely correlated with the B[a]P Toxic Equivalency Quotients (r^2=0.63). These quotients were calculated using toxic equivalency factors derived from P450 RGS analysis of individual high molecular weight (4-6-ring) PAHs. Results from short (6 h) and long (16 h) exposures using the RGS assay suggested that a portion of the induction was from the slower acting chlorinated hydrocarbons (coplanar PCBs, dioxins, furans, and so forth). Coplanar PCBs in the amounts of 0.9 to 3.1 µg/g were found in three core sections analyzed; hence, the induction of RGS was likely produced by PAHs and coplanar PCBs. Since dioxins and furans were not analyzed, their contributions to the RGS responses are unknown. The RGS assay may be used to screen samples for potential toxicological importance before conducting costly chemical analysis.

INTRODUCTION

The PVS is one of the most extensively studied coastal areas in the U.S., largely in response to concerns about its sediment contamination with chlorinated pesticides (DDTs), PCBs, PAHs, and trace metals (e.g., McDermott *et al.* 1974, Young *et al.* 1976, Young and Heesen 1978, Stull *et al.* 1986, Eganhouse and Gossett 1991, Lee 1994, Stull *et al.* 1996). These investigations focused primarily on direct measurements of chemical contaminants, a process that is time-consuming and costly. In addition, these investigations did not link chemical analyses directly to biological responses.

A more efficient and cost-effective approach to the assessment of contaminated sediments is the use of the P450 RGS assay. In this assay, the luminescent reporter gene produces the luminescent enzyme, luciferase, as a function of the concentrations and potencies of chemicals in the sample. Certain specific high molecular weight PAHs and coplanar PCBs are known to bind to the Ah receptor (AhR) and subsequently mediate induction of

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the CYP1A1 gene, resulting in the production of cytochrome P4501A1 (Whitlock 1990). This biochemical event has been used as an indicator of exposure to potentially harmful chemicals. In-vitro systems that utilize a reporter gene whose expression is induced by AhR ligands are increasingly used for their ease and sensitivity (El-Fouly *et al.* 1994, Hahn *et al.* 1996). The P450 RGS assay can detect the presence of CYP1A1-inducing compounds in solvent extracts of environmental samples such as sediment, soil, and tissue (ASTM 1997). Previous studies have shown that fish collected from sites contaminated with PAHs or PCBs exhibit high levels of CYP1A1 and associated activity in their livers (Gooch *et al.* 1989, Stein *et al.* 1992). The P450 RGS assay provides a viable, low-cost alternative for assessing sediment contamination, as well as an opportunity for demonstrating a correlation between chemical contamination and biological effects.

The P450 RGS assay has been successfully used to identify "hot-spot" areas of contamination when applied to over 450 surface sediment samples collected by the National Oceanic and Atmospheric Administration (NOAA) along the Atlantic, Gulf, and Pacific coasts of the U.S. (Anderson *et al.* 1999). This assay has not been used previously to evaluate historical levels of inducing chemicals by testing sections of a core sample. The goal of this study was to test the applicability of the P450 RGS method for the assessment of historically contaminated sediments. Specifically, sections of a sediment core collected from the PVS were analyzed for TOC, PAHs, and PCBs; and the biological responses to extracts from these samples were determined using the P450 RGS assay.

METHODS

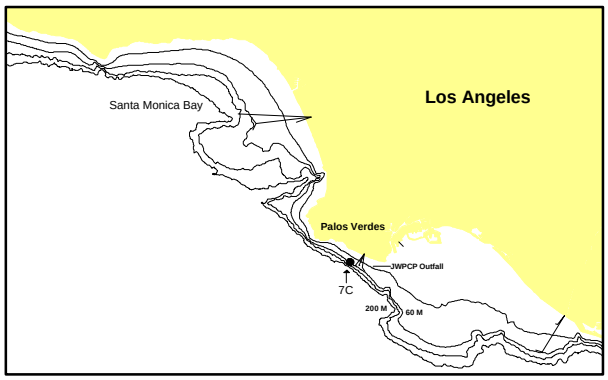
Sample Collection and Chemical Analysis

A sediment core (~80 cm) was collected from Station 7C near the outfall of the Joint Water Pollution Control Plant (JWPCP) off Palos Verdes (Figure 1) on October 10, 1995, using a modified gravity corer (SCCWRP 1982). This core was frozen and transported to the laboratory, then later thawed and sectioned every centimeter using a stainless steel hand saw. Sectioned sediments were stored in glass bottles at -20°C prior to extraction. Selected sections down to 80 cm in depth were analyzed. An aliquot of each sample (~40 g) was centrifuged, mixed with anhydrous sodium sulfate, spiked with surrogate standards, and extracted successively three times (16, 6, and 16 h) with methylene chloride (100 mL each extraction) using a roller table. The combined extract was solvent-exchanged to hexane and subjected to sulfur removal (using activated copper granules) and chromatographic column clean-up/fractionation (1:2 alumina:silica gel glass column). The fraction containing PCBs and PAHs was concentrated to 1 mL using a Zymark 500 TurboVap concentrator (Zymark Corporation, Hopkinton, MA). Appropriate amounts of internal standards were added to the final extract before instrumental analysis.

Quantitation of PAHs and PCBs has been described in previous publications (Zeng and Vista 1997, Zeng *et al.* 1997). Total PAHs include naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, 2,6-dimethylnaphthalene, acenaphthylene, acenaphthene, 2,3,6-trimethylnaphthalene, fluorene, phenanthrene, anthracene, 2-methylphenanthrene, 1-methylphenanthrene, 3,6-dimethylphenanthrene, fluoranthene, pyrene, 2,3-benzofluorene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, 9,10-diphenylanthracene, indeno[1,2,3-cd]pyrene, dibenzo[a,h]anthracene, and benzo[g,h,i]perylene. Total PCBs include approximately 120 PCB congeners with 64 singly eluting congeners and 28 multi-component domains found in a mixture of Aroclors 1242, 1248, 1254, and 1260 (1:1:1:1, weight). Concentrations of TOC were determined using an analytical procedure described previously (SCCWRP 1994).

Additional measurements of a selected number of potentially toxic PCB congeners (coplanar PCBs) were conducted by Columbia Analytical Services (Kelso, WA) on three extracts (7-8 cm, 18-19 cm, and 29-30 cm) using the U.S. Environmental Protection Agency SW846 Method 8082 (U.S. EPA 1990). Gas chromatographic

FIGURE 1. Map of the Palos Verdes Shelf showing the sampling station, 7C, near the Joint Water Pollution Control Plant outfall.



analyses of these extracts used the two-column confirmation method, where retention times of 64 congeners were determined using standard solutions for individual compounds. Fifteen of the 64 congeners were non-, mono-, and di-ortho coplanar. Only those congeners found in the extracts at detectable levels (20 ng/g) were addressed in this study.

P450 RGS Analysis

The methodology used for P450 RGS testing has been described in previous publications (Anderson *et al.* 1996a, APHA 1996, ASTM 1997). The test system is based upon a transgenic cell line developed and tested previously (Anderson *et al.* 1995, Anderson *et al.* 1996b, Anderson *et al.* 1996c, Kim *et al.* 1997). Solvent extracts of sediment samples prepared for chemical analysis were added to individual wells (6-well plates), containing approximately one million cells, and exposed for the normal duration (16 h). Selected extracts were also analyzed using the RGS assay at a shorter exposure time (6 h). Volumes of solvent successfully tested were 2 to 10 µL, which produce a low background (solvent blank) induction when applied to the 2 mL of culture medium. In each assay, the luminescence (in relative light unit (RLU)) of the cell lysate from each of three replicate wells was determined for each sample, the solvent control, and the reference toxicant (TCDD, 2,3,7,8-dioxin), using a ML2250 Luminometer (Dynatech Laboratories, Chantilly, VA). The mean RLUs of the control wells were set to unity. The mean RLUs of samples and standards were converted to fold induction by dividing by the mean RLUs of the solvent (control). This biochemical response detects the presence of CYP1A1-inducing compounds in the extract. Final results were expressed as equivalents of benzo[a]pyrene (B[a]PEq) per dry gram of sample, based upon the RGS concentration-response curve of B[a]P. Chemical B[a]PEq was calculated as the sum of the products of the analytical concentration of each RGS-inducing PAH and its RGS toxic equivalency factor (TEF).

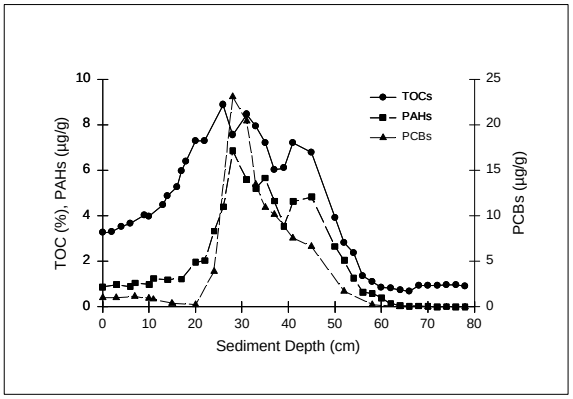
RESULTS

Sediment Profiles of Contaminants and Biological Responses

Concentrations of TOC in sediments generally represent the amount of bulk organic materials and therefore can be used to identify the input history related to a specific source. As shown in Figure 2, sediment TOC concentration was constant at ~1% in sections from 60 to 80 cm, apparently representing a pre-discharge baseline level in the coastal marine environment. The TOC concentration increased steadily from 60 cm upward, reaching a high of ~9% at 27 cm and then decreasing to about 3.5% at the surface.

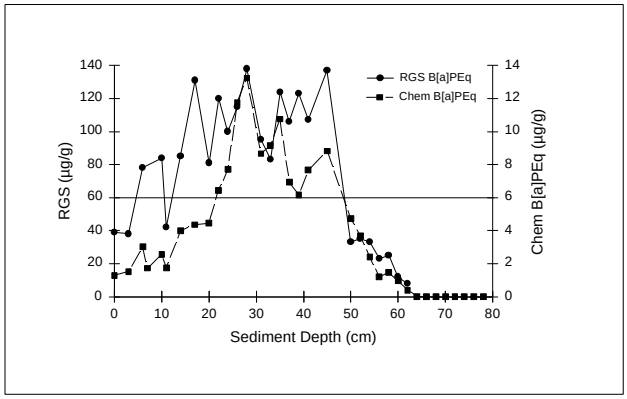
The depth profiles of total PAHs and PCBs showed patterns similar to TOC (Figure 2). Baseline was reached at approximately 60 cm. Both classes of chemicals showed maximum peaks at approximately 29 cm, and then gradually decreased to the low values at 60 cm. The rates of decrease toward the surface for PAHs and PCBs were also similar to TOC, leveling off at 18 to 20 cm and remaining at approximately the same concentration (1 µg/g) up to the surface. The maximum concentration of total PAHs was ~6.9 µg/g, while that of total PCBs was ~23 µg/g. The decreases in PAHs and PCBs from 29 cm to the surface can be attributed to the extensive waste treatment methods initially employed in 1973-74 and continuing to the present, as well as better source control in general. Changes in the mass emissions of PAHs and PCBs have been described previously (Schafer 1989).

FIGURE 2. Sediment profiles of TOC, PAHs, and PCBs at Station 7C.



The RGS responses to various sediment sections are depicted in Figure 3, along with the expected responses from conversion of chemical data to B[a]P equivalents, using RGS TEFs for the compounds that induce this system (Table 1). Similar to the sediment profiles of contaminants discussed above, the lowest RGS value (7 µg B[a]PEq/g) was also found at 62 cm. The RGS responses increased sharply at shallower depths, to nearly 140 µg B[a]PEq/g at 47 cm. Considerable variation in the responses was evident from 47 cm to 18 cm. Decreased levels of induction were observed in sections nearer the surface, with the RGS response decreasing to approxi-

FIGURE 3. P450 RGS responses and chemically derived equivalents in Station 7C sediments.



mately 40 µg B[a]PEq/g in the top 4 cm. The value of 60 µg B[a]PEq/g (horizontal line, Figure 3) is a threshold above which degradation of the benthic community in sediment samples from San Diego Bay has been observed (Fairey *et al.* 1996). Although the relatively high levels of inducing compounds at depths of 7 to 47 cm varied considerably, RGS responses remained above 60 µg B[a]PEq/g.

Recent studies (Jones and Anderson 1998a) have described the relative potency of specific PAHs to induce the RGS assay. Table 1 shows how the data on individual PAHs (sampled from two core sections), which induce CYP1A1 and produce luciferase in the RGS assay, were converted to chemically derived B[a]P equivalents. The 13.24 µg /g value for the 29-cm section was the highest value among all of the sections (Figure 3). The chemically derived B[a]PEq values were compared to RGS derived B[a]PEq, measured in extracts of those sections addressed in Figure 3. While both the RGS and chemically derived values showed peaks at 27 cm, the RGS assay produced multiple peaks, remaining high over a longer portion of the core, and showed considerable variability.

The correlation between RGS results and the chemically derived B[a]P equivalents was slightly closer than that between RGS and total concentrations of all measured PAHs (correlation coefficients, r^2=0.63 vs. r^2=0.47; Figure 4). The correlation between RGS and total PAH concentration was improved slightly, as would be expected, when the lower molecular weight PAHs (2 and 3 aromatic rings) were not included in the regression (r^2=0.50 vs. r^2=0.47). The biological response was about nine times greater than that calculated from the chemical analysis of PAHs.

Time-Dependent Experiments

Recent research (Jones and Anderson 1998b) has shown that the RGS response to PAHs reaches a peak at 6 h. At that time, the response begins to decrease as metabolic enzymes produced by the cells begin to degrade these compounds. Chlorinated hydrocarbons (e.g., coplanar PCBs, dioxins, furans, and so forth), which are not readily degraded, exhibit a continuously increasing induction of this system and reach a maximum level at 16 h. By conducting tests at 6 h and 16 h with selected samples, the relative contributions of these two classes of hydrocarbons could be assessed. Each of the six samples tested at both time intervals produced approximately 2.5 times higher RGS response at 16 h than at 6 h, suggesting a significant contribution from chlorinated hydrocarbons. If PAHs had been the domi-

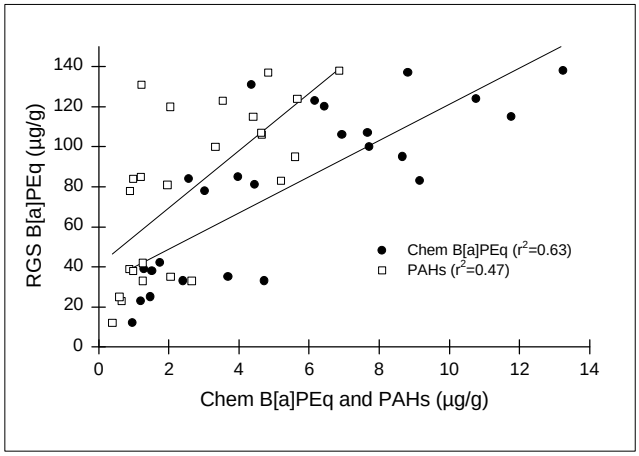
TABLE 1. Use of toxic equivalency factors (TEFs) for RGS to calculate the chemical B[a]PEq values for core sections.

		0-1 cm		29-30 cm	
Chemical	TEF[a]	(ng/g)	Product	(ng/g)	Product
Benzo[a]anthracene	0.4	27	11	0	0
Chrysene	0.4	56	23	349	140
Benzo[b]fluoranthene	3	56	169	613	1,839
Benzo[k]fluoranthene	25	41	1,019	421	10,513
Benzo[a]pyrene	1	70	70	750	750
Indeno[1,2,3-cd]pyrene	3	0	0	0	0
Dibenzo[a,h]anthracene	4	0	0	0	0
Benzo[g,h,i]perylene	0.02	0	0	0	0
Total B[a]PEq (µg/g)			1.29		13.24

[a]TEF values based on relative potency in the RGS assay [26].

nant inducers, then the response would have been stronger at 6 h than at 16 h (Jones and Anderson 1998b). One chemical group that has the potential to contribute to the RGS fold induction is the coplanar PCBs. Since the analytical methods used in the present study did not separate co-eluting congeners, some of the sample extracts were sent to Columbia Analytical Services for additional analysis to identify coplanar PCBs. Core sections from 7-8 and 18-19 cm contained approximately the same congeners and total concentrations (0.9 to 1.4 µg/g). The most contaminated portion of the core (29-30 cm), which contained a larger number of coplanar PCBs, had a total concentration of 3.2 µg/g (Table 2).

FIGURE 4. P450 RGS responses as B[a]PEq vs. Chem B[a]PEq and total PAHs.



DISCUSSION

This study, like several past programs, evaluated the historical record of solids deposition on the PVS near the JWPCP outfall by analyzing sections of a sediment core taken from the surface down to pre-discharge depths. While data were available on the distribution of DDTs and several metals with depth (Stull *et al.* 1986, Stull *et al*. 1996, Swartz *et al.* 1991), a similar characterization of specific PAHs and PCBs had not been conducted. The RGS biomarker screening test was used to test for the potential AhR-mediated carcinogenicity and toxicity of PAHs, PCBs, and other CYP1A1 inducers in the same core section extracts that were chemically analyzed.

Distributions of TOC, PAHs, and PCBs in the Station 7C sediment core were highly correlated (Figure 2). For example, the coefficient of correlation (r^2) between PAH and PCB concentrations estimated from Figure 2 was 0.82 with a 99% confidence. Measurements of these three chemical classes were consistent in predicting the pre-discharge baseline at approximately 60 cm and the effects of initial extensive wastewater treatment at approximately 29 cm down the sediment core (Figure 2). The RGS responses to the same extracts used for chemical analysis exhibited a very similar pattern (Figure 3). A weak correlation (r^2=0.47) was observed between the RGS results and total PAH measurements (Figure 4). When the concentrations of individual CYP1A1-inducing PAHs were used to calculate chemically derived B[a]P equivalents (Table 1), the correlation was improved notably (r^2=0.63). The RGS responses to extracts of 450 marine and estuarine sediment samples collected in several NOAA projects were recently compared (Anderson *et al.* 1999) to the concentrations of total PAHs in these samples. An overall correlation coefficient of r^2=0.54 was obtained. For seven of the nine regions investigated, the correlations were between 0.70 and 0.87 (r^2). This information would indicate that the primary chemical contaminants present in the sediments of many harbors, bays, and coastal systems are PAHs.

The biological significance of the RGS response was best demonstrated by the correlation observed by Fairey *et al.* (1996) between the degradation of the benthic community in sediment samples from San Diego Bay and RGS assay values of 60 µg B[a]PEq/g or higher. Of the 17 stations evaluated in the previous study using both biological methods, 11 of 11 sediment samples with RGS assay values above 60 µg B[a]PEq/g were shown to have degraded benthic communities, whereas only 1 of the remaining 6 samples below 60 µg B[a]PEq/g was judged to be a degraded site (and this sample was collected in a boat channel). In the present study, core sections from depths of approximately 7 to 45 cm produced an RGS response of 60 µg B[a]PEq/g or higher (Figure 3). Toxicity tests conducted by Swartz *et al*. (1991) on core sediments taken from nearby stations in 1985 showed significant amphipod toxicity at approximately the same depths (10 to 35 cm). More recent deposits (< 7 cm) are well below this level of induction. While RGS responses are clearly correlated with the concentrations of inducing chemicals in the samples, additional investigation is required into the relationship between high CYP1A1 induction and biological effects.

The results of testing at two time intervals (6 h and 16 h) suggest that chlorinated hydrocarbons contributed significantly to the observed RGS responses. Although DDTs are abundant in these sediments, a previous study (Anderson *et al.* 1995) found no detectable RGS induction from DDTs. Total PCBs reached a peak of 24 µg/g at 29 cm below the sediment surface, and then decreased sharply to about 1 µg/g from 20 cm to the surface (Figure 2). The analysis of three samples for only coplanar PCBs

also indicated that concentrations reached a peak of over 3 µg/g at 29 cm below the sediment surface, and then decreased sharply to approximately 1 µg/g from 20 cm to the surface (Table 2). It is likely that the coplanar PCBs identified were important contributors to the RGS responses, which may be one of the reasons why the correlation of RGS responses with concentrations of total PAHs was not stronger. However, other chlorinated hydrocarbons that induce the CYP1A1 gene (such as dioxins and furans) were not measured and may also have contributed to the RGS responses. One advantage of using RGS screening in monitoring marine sediments is that low concentrations (10 ng/kg) of dioxins elicit a response that is sufficient to direct chemical analysis efforts, thus avoiding the high costs of chemical characterization.

TABLE 2. Concentrations (ng/g) of coplanar PCB congeners in three sediment sections.

Congener	7-8 cm	18-19 cm	29-30 cm
77			156
123			158
118	279	140	1,087
114			139
105	149	71	612
167			52
156	36		116
169	257	235	352
180	116	70	358
189	559	351	161
Total	1,396	866	3,192

The findings of this study demonstrate that the biomarker P450 RGS provides a viable assessment of the CYP1A1-inducing compounds in sediment samples that correlates with the time-consuming and costly chemical analysis of samples for specific PAHs and PCB congeners. This assay does not identify contamination from chlorinated pesticides, which can contribute to sediment toxicity. Nevertheless, this screening approach allows investigators to concentrate their chemical analytical efforts on the most significant samples, particularly when the same extract is also analyzed for pesticides.

Since the objective of most monitoring programs is to determine the temporal trend of contamination levels and potential toxicity to biota, it is appropriate to utilize a biological screening approach that responds only to the chemicals of known biological significance. Within the very long lists of PAH and PCB analytes are compounds seldom, if ever, found in marine sediments, as well as compounds of no toxicological significance. It seems logical and cost-effective to first screen a sample for potential toxicological importance before spending the time and money to produce a complete chemical characterization. Chemical analyses of specific PAHs and PCB congeners are approximately $300 each and dioxin/furan analyses are approximately $1,000. At a cost of $150, the two-time-interval RGS test is is a cost-effective tool for making decisions regarding the need for either a PAH or a PCB analysis, or both.

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