

CORRELATION BETWEEN RESPONSE OF HUMAN CELL LINE AND DISTRIBUTION OF SEDIMENT POLYCYCLIC AROMATIC HYDROCARBONS AND POLYCHLORINATED BIPHENYLS ON PALOS VERDES SHELF, CALIFORNIA, USA

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Abstract—Sections of a sediment core collected from Station 7C near the County Sanitation Districts of Los Angeles County, California, USA, outfall were analyzed for total organic carbon (TOC), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). In addition, the biological responses to extracts from the same samples were determined using the P450 reporter gene system (RGS) assay. The profiles of TOC, PAHs, and PCBs were consistent in illustrating the predischARGE baseline and the effects of improving wastewater treatment. Induction of the CYP1A1 gene using P450 RGS was correlated with total PAHs ($r^2 = 0.47$) and better correlated with the B[a]P toxic equivalency quotients ($r^2 = 0.63$) calculated using toxic equivalency factors (TEFs) derived from P450 RGS analyses of individual high molecular weight (4 to 6 rings) 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, etc.). Coplanar PCBs in the amounts of 0.9 to 3.1 $\mu\text{g/g}$ were found in three core sections analyzed; hence, the induction of RGS was likely produced by both 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 analyses.

Keywords—CYP1A1 P450 Polycyclic aromatic hydrocarbons Polychlorinated biphenyls Sediment

INTRODUCTION

The distribution of contaminants in the vicinity of four major municipal wastewater outfalls on the Southern California Bight (SCB) has been well documented [1,2]. Since about 1972, studies have shown that the sediments near the County Sanitation Districts of Los Angeles County (CSDLAC), California, USA, ocean outfall off Whites Point on the Palos Verdes Shelf (PVS) contain elevated concentrations of chlorinated pesticides (DDTs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs), as well as other pesticides and several trace metals [3–6]. In addition to the chemical analyses conducted as part of the annual monitoring program since 1972, several projects have evaluated the toxicity of sediments collected from this region [7]. These investigations and the on-going monitoring program of CSDLAC have described the distribution of infaunal species at various distances from the outfall. In recent years, the concentrations of contaminants in the surface sediments have decreased, while populations of benthic species have improved considerably [6].

The chemicals of concern in this paper are the specific high molecular weight PAHs and coplanar PCBs that are known to bind to the Ah receptor (AhR) and subsequently mediate induction of the CYP1A1 gene, resulting in the production of cytochrome P4501A1 [8]. This biochemical event is widely used as an indicator of exposure to potentially harmful contaminants. In vitro systems that utilize a reporter gene whose expression is induced by AhR ligands are increasingly used for their ease and sensitivity [9,10]. The P450 reporter gene

system (RGS) can detect the presence of CYP1A1-inducing compounds in solvent extracts of environmental samples, such as sediment, soil, and tissue [11]. The RGS cell line (101L) is stably transfected with a luciferase reporter gene under control of a human CYP1A1 promoter sequence.

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 [12,13]. Some of the compounds that induce CYP1A1 are toxic, carcinogenic, and have been shown to bioconcentrate and biomagnify. Exposure to these compounds has been shown to produce physiological, reproductive, and histopathological effects in birds, mammals, and fish species. Analytical chemical analyses of many environmental samples have been required due to concern over possible contamination of water, food, wildlife, soil, and aquatic sediment with such compounds. Screening tools such as P450 RGS can be used to help direct analytical efforts by identifying samples that contain CYP1A1-inducing compounds. The objective of this study was to combine the analytical chemistry expertise from the Southern California Coastal Water Research Project (SCCWRP) and the biomarker capability of Columbia Analytical Services (CAS, Kelso, WA, USA) to compare the assessment of historically contaminated sediments.

MATERIALS AND METHODS

Sample collection and chemical analyses

A sediment core (~80 cm) was collected by SCCWRP at station 7C (Fig. 1) on October 10, 1995, using a modified gravity corer [14] and was transported frozen to the SCCWRP laboratory. The core was later thawed and cut into 1-cm in-

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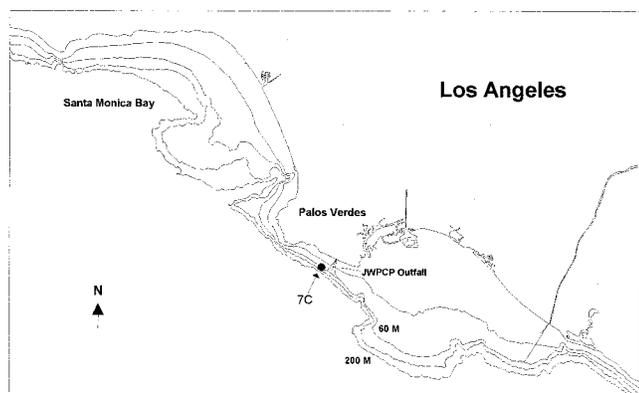


Fig. 1. Map of the Los Angeles, California, USA, area showing the Palos Verdes region and the location of sampling station 7C on the shelf near the County Sanitation Districts of Los Angeles County outfall.

tervals using a stainless steel hand saw. Sections were stored at -20°C prior to additional treatment. 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) 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, Hopkinton, MA, USA). Appropriate amounts of internal standards were added to the final extract before instrumental analysis.

Procedures for measuring PAHs and PCBs in SCCWRP core samples were described in previous publications [15,16]. 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 multicomponent peaks, which are found in a mixture of Aroclors 1242, 1248, 1254, and 1260 (1:1:1:1, weight). Concentrations of total organic carbon (TOC) were determined using an analytical procedure given previously [17].

Columbia Analytical Services conducted PCB congener analyses on three extracts (7–8, 18–19, and 29–30 cm) using U.S. Environmental Protection Agency (U.S. EPA) SW846 method 8082 [18]. Gas chromatographic analyses of these extracts used the two-column confirmation method, where retention times of 64 congeners were determined by the use of standard solutions for individual compounds. Fifteen of the 64 congeners were non-, mono- and di-*ortho* coplanar compounds. Only those congeners found in the extracts at detectable levels (20 ng/g) are discussed.

P450 RGS analyses

The specific method for P450 RGS testing was described in previous publications [11,19,20]. The test system is based

Table 1. Use of toxic equivalency factors (TEFs) for P450 reporter gene system (RGS) to calculate the chemical equivalents of benzo[*a*]pyrene for core sections

Chemical	TEF ^a	7C1-1		7C1-29	
		ng/g	Product	ng/g	Product
Benzo[<i>a</i>]anthracene	0.4	27	11	0	0
Chrysene	0.4	56	23	349	140
Benzo[<i>b</i>]fluoranthene	3	56	169	613	1,839
Benzo[<i>k</i>]fluoranthene	25	41	1,019	421	10,513
Benzo[<i>a</i>]pyrene	1	70	70	750	750
Indeno[1,2,3- <i>cd</i>]pyrene	3	0	0	0	0
Dibenzo[<i>a,h</i>]anthracene	4	0	0	0	0
Benzo[<i>g,h,i</i>]perylene	0.02	0	0	0	0
Total B[<i>a</i>]PEq ($\mu\text{g/g}$)			1.29		13.24

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

on a transgenic cell line developed and tested previously [21–24]. Solvent extracts of core sections prepared by SCCWRP for PCB analyses were added to individual wells (six-well plates) containing approximately one million cells and were exposed for the normal duration (16 h). Selected extracts were also analyzed using RGS 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 units, RLU) of the cell lysate from each of three replicate wells was determined for each sample, the solvent control, and reference toxicant (TCDD, 2,3,7,8-dioxin), using a ML2250 Luminometer (Dynatech Laboratories, Chantilly, VA). The mean RLUs of the control wells was set equal to unity, and 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 on the RGS concentration–response curve of B[*a*]P. Chemical B[*a*]PEq were calculated as the sum of the products of the analytical concentration of each RGS-inducing PAH and its RGS TEF (Table 1).

RESULTS

Sediment profiles of contaminants and biological responses

One of the basic measures for the amount of deposition from the wastewater discharge via the CSDLAC outfall is the level of TOC in the sediments at various depths. As shown in Figure 2, sediment TOC was quite constant at $\sim 1\%$ 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 above 60 cm and reached a high of $\sim 9\%$ at 27 cm, and then decreased to about 3.5% at the surface.

The depth profiles of total PAHs and PCBs showed similar patterns to that of TOC (Fig. 2). Baseline was again apparently reached at about 60 cm. Both classes of chemicals showed maximum peaks around 29 cm and then gradually decreased to the low values at 60 cm. The rates of decrease toward the surface were also similar, leveling off at about 18 to 20 cm and remaining at approximately the same concentration (1 $\mu\text{g/g}$) up to the surface. The maximum concentration of total PAHs was ~ 6.9 $\mu\text{g/g}$, while that of the sum of PCBs was ~ 23 $\mu\text{g/g}$. The decrease from 29 cm to the surface likely reflects

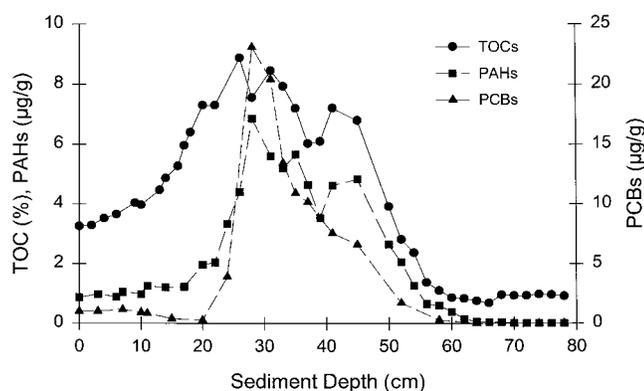


Fig. 2. Sediment profiles of TOC, PAHs, and PCBs at station 7C. Depth is in centimeters, PCBs and PAH are in micrograms per gram, and TOC is in percent.

the extensive waste treatment initially employed in 1973 through 1974.

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 toxic equivalency factors (TEFs) for the compounds that induce this system (Table 1). Similar to the sediment profiles of contaminants discussed above, the lowest RGS value (7 $\mu\text{g B[a]PEq/g}$) was also found at 62 cm. Moving up the core, the RGS responses increased sharply to nearly 140 $\mu\text{g B[a]PEq/g}$ at 47 cm, and then there was considerable variation in the responses up to 18 cm. Decreased levels of induction were observed in sections nearer the surface until the RGS response decreased back to about 40 $\mu\text{g B[a]PEq/g}$ in the top 4 cm. The value of 60 $\mu\text{g B[a]PEq/g}$ (horizontal line, Fig. 3) indicates a threshold above which degradation of the benthic community in sediment samples from San Diego Bay was observed [25]. Although the relatively high level of inducing compounds at depths of about 7 to 47 cm varied considerably, RGS responses remained above 60 $\mu\text{g B[a]PEq/g}$. We also conducted P450 RGS testing on a short core (~25 cm long) collected from the same station by CAS. Comparable sections of the long and short cores yielded similar results.

Recent studies by Jones and Anderson have described the relative potency of specific PAHs to induce the RGS assay [26]. Table 1 shows how the data from two core sections on

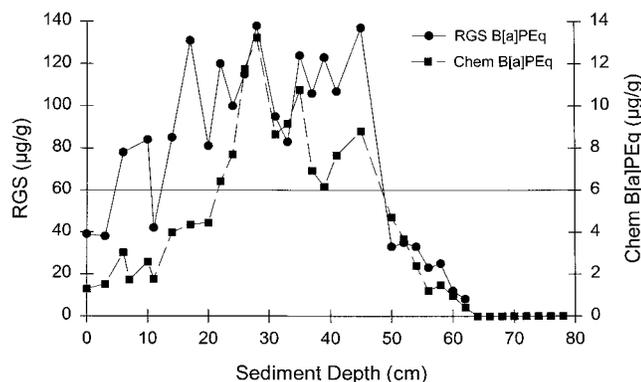


Fig. 3. P450 reporter gene system (RGS) responses and chemically derived B[a]P equivalents ($\mu\text{g/g}$) with depth in the 7C sediment core. Chemical B[a]P equivalents were derived from the concentrations of nine specific PAHs and the RGS toxic equivalency factors for each [26].

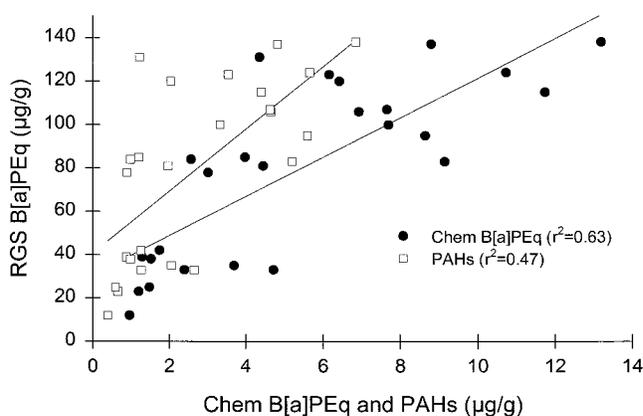


Fig. 4. Correlations between P450 reporter gene system responses in equivalents of benzo[a]pyrene (B[a]PEq) and both total PAHs and chem B[a]PEq values.

individual PAHs, which induce CYP1A1 as luciferase in the RGS assay, were converted to chemically derived B[a]P Equivalents. The 13.24- $\mu\text{g/g}$ value for the 29-cm section was the highest value among all the sections (Fig. 3). The chemically derived B[a]PEq values were compared to RGS derived B[a]PEq, measured in extracts of those sections 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 better than that between RGS and total concentrations of all measured PAHs (r^2 of 0.63 vs 0.47; Fig. 4). The correlation between RGS and total PAH concentration was improved slightly, as would be expected, when the lower molecular weight PAHs (two and three 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 chemical analyses of PAHs.

Time-dependent experiments

Recent research ([27] and unpublished data) has shown that the RGS response to PAHs reaches a peak at 6 h, at which time the response begins to decrease, as metabolic enzymes produced by the cells begin to degrade these compounds. Chlorinated hydrocarbons, not readily degraded, exhibit a continuously increasing induction of this system, reaching a maximal level at 16 h [27]. By conducting tests at both time intervals with selected samples, it was possible to assess the relative contribution of these two classes of hydrocarbons.

Each of the six samples tested at both time periods 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 dominant inducers, then the response at 6 h should have been stronger than that at 16 h [27]. One chemical group that could contribute to the RGS fold induction would be coplanar PCBs. Since the analytical methods used by SCCWRP did not separate co-occurring congeners, a few samples were sent to CAS for additional analyses to identify coplanar PCBs. Core sections from 7 and 18 cm contained about the same congeners and total concentration (0.9–1.4 $\mu\text{g/g}$), while the most contaminated portion of the core (29 cm) contained a larger number of coplanar PCBs, and the total concentration was 3.2 $\mu\text{g/g}$ (Table 2).

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

Congener	7 to 8 cm	18 to 19 cm	29 to 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

DISCUSSION AND CONCLUSION

This study, like several past programs, evaluated the historical record of solids deposition near the CSDLAC outfall by obtaining a sediment core and analyzing sections from the surface down to predischARGE depths. While data were available on the distribution of DDTs and several metals with depth [6,7,28], characterization of specific PAHs and PCBs at various depths had not been completely conducted. The RGS biomarker screening test was used here to test for potential AhR-mediated carcinogenicity and toxicity of PAHs, PCBs, and other CYP1A1 inducers in the same core section extracts chemically analyzed.

Distributions of TOC, PAHs, and PCBs in the 7C sediment core were highly correlated (Fig. 2). For instance, 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 predischARGE baseline at about 60 cm and the effects of initial extensive wastewater treatment at about 29 cm down the sediment core (Fig. 2). The RGS responses to the same extracts used for chemical analyses exhibited a pattern very similar to those of the chemical contaminants (Fig. 3). There was a weak correlation between the RGS results and total PAH measurements ($r^2 = 0.47$; Fig. 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 ($r^2 = 0.63$). A recent study [29] compared the RGS responses to extracts of 450 marine and estuarine sediment samples to total PAHs in the sediments and observed an overall correlation of 0.54 (r^2), but for seven of the nine regions investigated, the correlations were between 0.7 and 0.87 (r^2). It seems clear that the primary contaminants in many harbors, bays, and coastal systems are PAHs.

The biological significance of the RGS response was best demonstrated by the correlation 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 and greater [25]. Of the 17 stations evaluated by both biological methods, 11 of 11 above 60 μg B[a]PEq/g were degraded, while only one of the remaining six samples was judged to be degraded, and it was collected in a boat channel. As shown in Figure 2, sections of the core from a depth of about 7 cm to 45 cm produced an RGS response of 60 μg B[a]PEq/g and greater. Toxicity testing conducted by Swartz et al. [7] with sections of cores taken from nearby stations in 1985 showed significant amphipod toxicity from sections at approximately the same

depth (10–35 cm). More recent deposits (<7 cm) are well below this level of induction and marine monitoring data, as well as toxicity testing, have demonstrated that contaminants in the surface sediments are not producing effects on the biota.

The results of testing at two time periods suggest that chlorinated hydrocarbons contributed significantly to the observed RGS responses. Although DDTs are abundant in these sediments, previous studies have shown that there are no detectable effects from DDTs on the RGS responses [21]. Total PCBs measured by SCCWRP reached a peak of 24 $\mu\text{g}/\text{g}$ at 29 cm below the sediment surface and then decreased sharply to about 1 $\mu\text{g}/\text{g}$ from 20 cm to the surface. Analyses of only coplanar PCBs also indicated that concentrations from the surface down to about 20 cm were approximately 1 $\mu\text{g}/\text{g}$ and then increased to over 3 $\mu\text{g}/\text{g}$ at 29 cm (Table 2). It is likely that the coplanar PCBs identified were important contributors to the RGS responses, and this is one possible reason that the correlation with total PAHs was not stronger. However, we cannot be certain that other chlorinated hydrocarbons that induce the CYP1A1 gene (dioxins and furans) were not present, as these chemical analyses were not conducted. One advantage of using the RGS screen in the monitoring of marine sediments is that even low concentrations (10 ng/kg) of dioxins would elicit a response that can be used to direct chemical analysis efforts and potentially avoid paying the high price for chemical characterization.

The findings of this study demonstrate that the biomarker P450 RGS provides an assessment of the CYP1A1-inducing compounds in sediment samples, which correlates with the very detailed and time-consuming chemical analyses of samples for specific PAHs and PCB congeners. This assay does not identify contamination from pesticides, which can contribute to sediment toxicity. Whether evaluating organic contaminants in surface sediments from various locations or sections of cores, 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 purpose of most monitoring programs is to follow the levels of contaminants over time to determine if there is potential toxicity to the biota, it is appropriate to utilize a biological screening approach that only responds 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 and also compounds for which there is 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. By using the two-time-interval RGS test, it is also possible to make decisions regarding the need for either a PAH or a PCB analysis or both.

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