# Application of P450RGS (reporter gene system) as a bioindicator of sediment PAH contamination in the vicinity of Incheon Harbor, Korea

Gi Beum Kim, Jack W. Anderson, Kristen Bothner, Jong-Hyeon Lee, Chul-Hwan Koh and Shinsuke Tanabe

**Sixty-seven sediment samples were collected from Kyeonggi Bay, Korea, including the mouth of Han River, Incheon Harbor, the Namdong industrial complex, and the open sea. Collections were conducted in December, 1995 and samples were maintained frozen (–20 °C) until analysis. Dichloromethane extracts were analysed for their content of CYP1A1-inducing compounds with a P450RGS (reporter gene system) assay, and for polycyclic aromatic hydrocarbons (PAHs). Sediment samples were also analysed for organic carbon (OC) content and grain size, to aid in evaluating the relationship between contamination and physical nature of the sediments. The responses of the P450RGS assay to the sediment extracts were expressed as μg of benzo[*a*]pyrene toxic equivalents per g dry weight (μg $g^{-1}$ BaPTEQ), and these values correlated well ($r^2$=0.624) with total PAHs. BaPTEQ values were also highly correlated with the OC content of the sediments. The determination of P450RGS BaPTEQ is a useful tool, because it is both a rapid and inexpensive means of assessing the potential toxicity of organic compounds in environmental sediment samples. These values represent an estimate of the levels of compounds in the sediment that are potentially available to organisms through chronic exposure to pore water or ingestion of benthic species. We believe BaPTEQ values are more useful than tables of specific PAH concentrations, if the purpose of the investigation is to either obtain a rapid screening of an area or to develop some form of ecological or human health risk assessment.**

Keywords: *CYP1A1, P450RGS BaPTEQ, PAH, bioindicator, screening method.*



## Introduction

The majority of polycyclic aromatic hydrocarbons (PAHs) in the environment are derived from anthropogenic sources such as pyrolysis or organic material (Suess 1976), coal gasification (Hayatsu *et al.* 1975), oil refinery operations, incineration of industrial and domestic wastes (Andelman and Snodgrass 1972), and natural processes such as diagenesis of sediment organic matter to form fossil fuels (LaFlamme and Hites 1979). Land-derived PAHs are transported to the sea by two major routes: aeolian transport of fossil fuel and wood combustion products and riverine transport of combined PAH sources (e.g. stormwater runoff, municipal sewage effluents, and industrial input) (Boehm and Farrington 1984). Ports and harbours have often become contaminated with PAHs through stormwater runoff, many small spills of oil cargos or fuels, and bilge pumping or ship servicing operations. Once PAHs have been introduced to a harbour or coastal zone, they accumulate in sediments because of their hydrophobicity and partitioning to organic carbon-coated particles (Means *et al.* 1980). The concentrations of PAHs in sediment have been known to range from a few ng $g^{-1}$ to many μg $g^{-1}$ (Meador *et al.* 1995). Some of the PAH compounds measured in environmental programmes are mutagenic and carcinogenic (Varanasi *et al.* 1987, Meyers *et al.* 1991, Balch *et al.* 1995) and some have been reported to cause reproductive toxicity (Hose *et al.* 1982). There have been some recent sediment PAH studies (Huntley *et al.* 1995, Pereira *et al.* 1996, Yunker *et al.* 1996). However, only a small portion of all the two- to six-ring aromatic hydrocarbons found in petroleum, and likely present in contaminated sediments, are quantified in environmental programmes. Many of the unidentified and untested aromatic hydrocarbon compounds may also possess the ability to induce the CYP1A1 gene.

PAHs have been known to induce EROD (ethoxyresorufin *O*-deethylase), B[*a*]PH (benzo[*a*]pyrene hydroxylase), EH (epoxide hydrolase) in various animals (Suteau *et al.* 1988, Goksøyr *et al.* 1994, Roos *et al.* 1996). These and the other previous investigations (Stegeman and Lech 1991, Collier *et al.* 1995) have suggested the determination of xenobiotic biotransformation enzymes as useful tools in identifying contaminated compartments of the environment. Flatfish and bivalves have been frequently used as sentinel organisms to monitor the levels of PAHs in a region or portion of a harbour or coastline (Mix and Schaffer 1983, Cocchieri *et al.* 1990, Porte and Albaigés, 1993).

There are some recent publications on the application of bioindicators to the assessment of sediments (Aarts *et al.* 1995, Garrison *et al.* 1996, Murk *et al.* 1996). To our knowledge, however, the only studies showing that a biological test is useful in screening marine sediment samples for PAHs are those by Anderson and colleagues (Anderson 1995a, b, Anderson *et al.* 1995, 1996a). These studies used the P450 Reporter Gene System (RGS) assay, which is based on the response of a human cell line (101L) with the luciferase plasmids attached at the CYP1A1 site on the chromosome (Postlind *et al.* 1993). The P450RGS assay was used to rank the levels of inducing compounds in marine sediments, ranging from clean coastal areas to contaminated harbour sites.

The objectives of this study are to determine if sediment samples collected from various sites on the west coast of South Korea could be ranked from high to low contamination by use of the RGS assay, and to determine the correlation with the subsequent chemical characterization of split samples.

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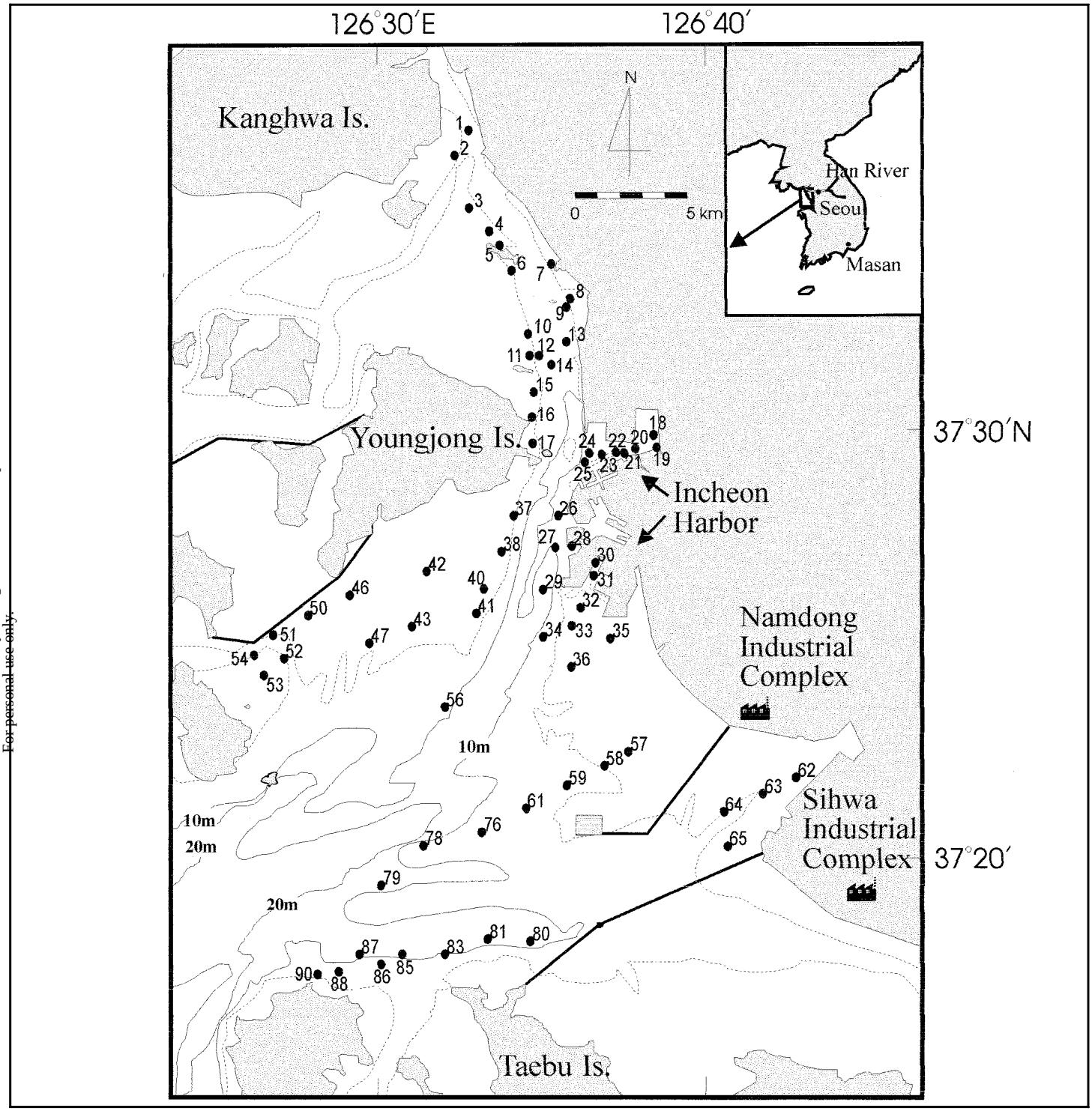


**Figure 1.** Map showing sampling sites around Kyeonggi Bay, Korea.

## MATERIALS AND METHODS

### *Sample sampling and characterization*

#### Sample collection and storage

In December, 1995, 67 sediment samples were collected from the mouth of Han River in the North, southward past Incheon Harbor and Namdong industrial complex, to the open sea (Figure 1). The positioning of the vessel at each sampling station was obtained from a global positioning system. All samples were collected by Van Veen Grab. After collection, the samples were kept in a freezer at –20 °C or lower, until analysis.

#### Extraction of PAHs from sediments

After air-drying, samples were ground by mortar and pestle and sieved using a 0.5 mm sieve. The extraction of PAHs from about 10 g of dry sediment was carried out by using a Microwave (MES 1000, CEM Corporation, Matthews, NC, USA) extraction system at the Skidaway Institute of Oceanography (Savannah, GA, USA). The volume of dichloromethane (DCM) extracts was reduced to 2 ml under the flow of nitrogen gas. The 2 ml sample was split, so that 1 ml was used in P450RGS assay and the other was later used fo

| Ring number | Compounds |
|---|---|
| 2-ring | Naphthalene (128), 1- and 2-methylnaphthalene (141+142), biphenyl (154), 2,6-dimethylnaphthalene (141+156), 2,3,5,-trimethylnaphthalene (155+170) |
| 3-ring | Acenaphthylene (152), acenaphthene (152+153+154), fluorene (165+166), phenanthrene and anthracene (178), 1-methylphenanthrene (191+192) |
| 4-ring | Fluoranthene and pyrene (202), benzo[*a*]anthracene and chrysene (228) |
| 5-ring | Benzo[*b*]- and benzo[*k*]fluoranthene (252), benzo[*e*]- and benzo[*a*]pyrene (252), perylene (252) |
| 6-ring | Indeno[1,2,3-*cd*]pyrene and benzo[*g*,*h*,*i*]perylene (276), dibenz[*a*,*h*] anthracene (278) |

**Table 1.** PAHs identified in this investigation.

Figures in parentheses after compound's name show *m/z*, i.e. GC/ITMS quantitation ion(s).

| Station | Organic C (%) | Clay (%) | PAHs (ng $g^{-1}$) | BaPTEQ ($\mu g\ g^{-1}$) | Station | Organic C (%) | Clay (%) | PAHs (ng $g^{-1}$) | BaPTEQ ($\mu g\ g^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.23 | 9 | 143 | 4.0 | 35 | 0.14 | 5 | 42 | 0.4 |
| 2 | 0.25 | NA | 40 | 1.0 | 36 | 0.07 | NA | 13 | 0.6 |
| 3 | 0.37 | 4 | 81 | 3.7 | 37 | 0.25 | 18 | 77 | 0.7 |
| 4 | 0.22 | 5 | 47 | 1.9 | 38 | 0.08 | 10 | 33 | 1.2 |
| 5 | 0.30 | 14 | 67 | 2.2 | 40 | 0.17 | 5 | 39 | 1.3 |
| 6 | 0.20 | 10 | 56 | 2.2 | 41 | 0.18 | 2 | 15 | 0.7 |
| 7 | 0.75 | 35 | 143 | 4.0 | 42 | 0.20 | 3 | 63 | 0.8 |
| 8 | 0.31 | NA | NA | 3.1 | 43 | 0.42 | 30 | 67 | 1.8 |
| 9 | 0.13 | 6 | 59 | 1.6 | 46 | 0.05 | 4 | 56 | 1.0 |
| 10 | 0.33 | NA | 38 | 1.1 | 47 | 0.17 | 8 | 41 | 1.0 |
| 11 | 0.09 | 11 | 228 | 1.3 | 50 | 0.17 | 4 | 34 | 0.9 |
| 12 | 0.27 | 4 | 74 | 3.1 | 51 | 0.29 | 18 | 85 | 1.3 |
| 13 | 0.05 | 4 | 46 | 0.7 | 52 | 0.31 | 6 | 61 | 1.8 |
| 14 | 0.15 | NA | 29 | 0.4 | 53 | 0.22 | 5 | 75 | 1.3 |
| 15 | 0.21 | 23 | 104 | 2.0 | 54 | NA | 4 | 167 | 3.6 |
| 16 | 0.37 | 6 | 99 | 2.7 | 56 | 0.24 | 27 | 59 | 1.5 |
| 17 | 0.30 | 11 | 129 | 1.3 | 57 | 0.06 | 2 | 26 | 0.5 |
| 18 | 1.20 | 30 | 1430 | 16 | 58 | 0.10 | 3 | 34 | 1.7 |
| 19 | 0.86 | 23 | 1128 | 21 | 59 | 0.05 | 1 | 10 | 0.4 |
| 20 | 0.72 | 35 | 286 | 7.7 | 61 | 0.06 | 1 | 10 | 0.6 |
| 21 | 0.68 | 46 | 237 | 11 | 63 | 0.30 | 21 | 57 | 1.2 |
| 22 | 0.45 | 30 | 174 | 4.9 | 64 | 0.17 | 16 | 9 | 0.7 |
| 23 | 0.32 | 22 | 170 | 5.1 | 65 | 0.09 | 16 | 24 | 0.5 |
| 24 | 0.33 | 18 | 107 | 2.1 | 76 | 0.35 | 8 | 72 | 1.4 |
| 25 | 0.31 | 6 | 167 | 5.0 | 79 | 0.14 | 11 | 41 | 0.5 |
| 26 | 0.17 | NA | 371 | 3.5 | 80 | 0.44 | 25 | 107 | 2.0 |
| 27 | 0.35 | 13 | 108 | 7.5 | 81 | 0.25 | NA | 53 | 1.0 |
| 28 | 0.52 | 32 | 154 | 14 | 83 | 0.26 | 13 | 30 | 0.5 |
| 29 | 0.28 | 18 | 39 | 1.7 | 85 | 0.29 | 6 | 83 | 0.8 |
| 30 | 0.54 | 67 | 236 | 13 | 86 | 0.29 | 18 | 33 | 0.4 |
| 31 | 0.48 | 8 | 215 | 3.6 | 87 | 0.29 | NA | 57 | 0.8 |
| 32 | 0.14 | 7 | 45 | 2.0 | 88 | 0.25 | NA | 47 | 0.7 |
| 33 | 0.07 | 0 | 85 | 6.9 | 90 | 0.38 | 15 | 77 | 1.9 |
| 34 | 0.12 | 17 | 163 | 3.7 | | | | | |

**Table 2.** BaPTEQ values, total PAH concentration, and content of organic carbon and clay in sediment samples used in this study.

Key: NA, not analysed.



### Analysis of PAHs

Sulphur was removed from the remaining 1 ml solution by adding an *activated copper*. This solution was then passed through a 12 g activated silica gel-packed wet column for clean-up. The column was eluted with 250 ml of 50% hexane/dichloromethane. This eluate was concentrated to 1 ml and subjected to GC (Varian STAR 3400 CX) equipped with MS (Varian SATURN 3). The mean recovery of certified PAHs in a standard marine sediment (SRM1941a, NIST) extracted with the Incheon Harbor sediment was 86%. The precise description of PAH analysis and the recovery data for specific compounds is published separately (Kim *et al.* 1996a). The list of PAH compounds analysed in this study is shown in Table 1, and the total PAH values are listed in Table 2.

For sediment grain size analysis, organic materials were removed from bulk sediment by treatment with hydrogen peroxide. By using a 62 μm sieve, sediment samples were divided into coarse and fine size fractions. The fraction larger than 62 μm was analysed at an interval of 1 ϕ by sieving and the fine fraction was analysed by the pipetting method. The mean size was calculated by the method of Folk and Ward (1957). Organic carbon (OC) content was determined by a back-titration method (Walkley and Black 1934).

### *P450RGS assay*

The protocol used in this study has been described in detail elsewhere (Anderson *et al.* 1995, 1996a). In essence, 10 μl of solvent extracts were added to two replicate wells, each with approximately one-million 101L cells in 2 ml of medium. These cells have a stably integrated plasmid that contains the human CYP1A1 promoter and 5′-flanking sequences fused to the firefly luciferase gene (Postlind *et al.* 1993). After 16 h incubation, the medium was aspirated and cells were rinsed with saline, and then spiked with 100 μl of lysic buffer. After allowing 15 min in a refrigerator (6 °C) for lysing, the contents of each well were scraped and transferred to a micro-centrifuge tube (2500 rpm, 1 min). Ten μl of supernatant was transferred into the wells of a 96-well plate. After addition of substrate A (reaction enhancer) and substrate B (luciferin) (both from Analytical Luminescence Lab. Ann Arbor, MI 48108, USA), light output was measured for 10 s at 25 °C with a luminometer (Dynatech, ML 2250, Chantilly, VA, USA). Relative light units (RLU) were converted to P450RGS fold induction by dividing the mean RLUs for each sample by that of the solvent (DCM). The coefficients of variation (% CV) were generally less than 20% of the mean, but samples with very low levels of inducing compounds produced values up to 80%.

### *Calculation of benzo[a]pyrene toxic equivalents from P450RGS (P450RGS BaPTEQ)*

The fold induction per gram in each sample was calculated by using the final extraction volume and the volume applied to the cells. Figure 2(a) shows the dose–response curve of benzo[*a*]pyrene, in which fold induction of about 30 is produced by a concentration of 500 ng $ml^{-1}$ benzo[*a*]pyrene. The conversion of data from fold induction to BaPTEQ could have been done by dividing by 30 and then multiplying by 0.5, which is of course the same as only dividing by 60 (Anderson *et al.* 1996a). In addition, Figure 2(b) revealed a linear dose–response relationship using a PAH mixture. The maximum amount (140 ng $ml^{-1}$) applied to the 101L cells of our study is well below the maximum concentration used in testing a PAH mixture. These data demonstrate that the approach used in converting the responses to BaPTEQ is valid.

### *Calculation of benzo[a]pyrene toxic equivalents from chemical analyses of PAHs (chemical BaPTEQ)*

At present only seven specific PAHs have been examined for P450RGS responses at multiple concentrations, which is necessary to estimate a toxic equivalent factor (TEF). These seven compounds are typically considered to be carcinogens

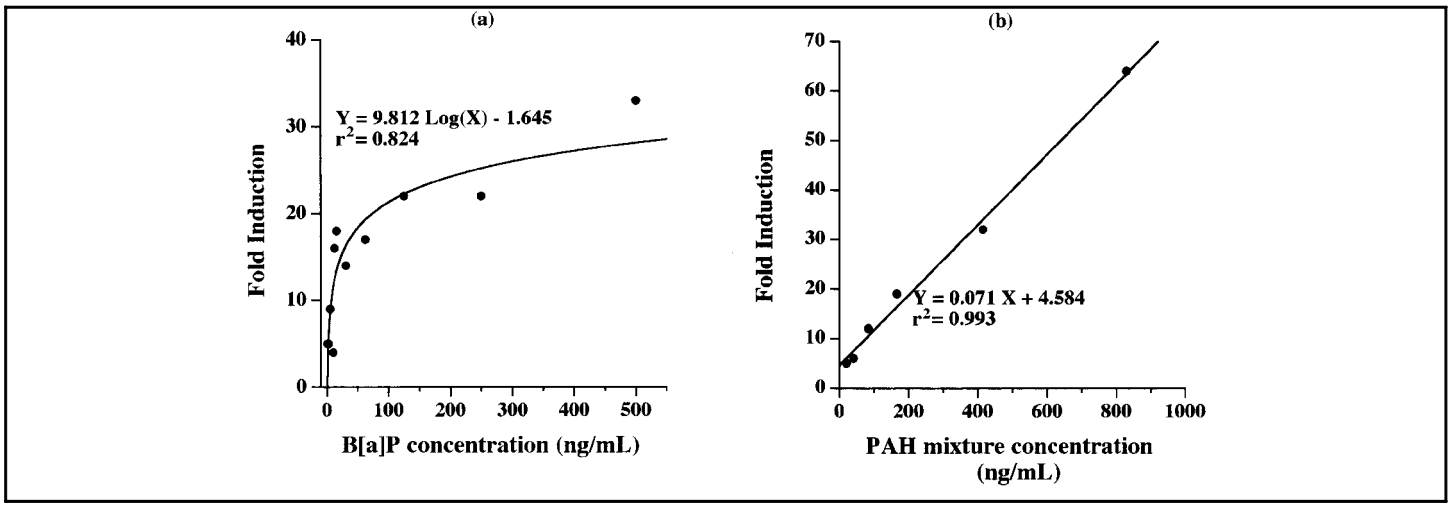


**Figure 2.** (a) Dose–response curve for concentrations of benzo[*a*]pyrene applied to the 101L cells and the induction of P450RGS. (b) Linearity in the P450RGS induction from application of increasing concentrations of a PAH mixture. The percent composition of individual compound is as follows: naphthalene (4.5), 2-methylnaphthalene (10.1), 1-methylnaphthalene (13.8), biphenyl (4.4), acenaphthylene (1.3), acenaphthene (0.5), fluorene (1.1), phenanthrene (4.1), anthracene (1.6), fluoranthene (4.8), pyrene (8.9), benz[*a*]anthracene (4.5), chrysene (4.8), benzo[*b*]fluoranthene (4.1), benzo[*k*]fluoranthene (4.1), benzo[*e*]pyrene (4.9), benzo[*a*]pyrene (6.4), perylene (6.7), indeno[1,2,3-*cd*]pyrene (3.2), dibenz[*a,h*] anthracene (1.4), benzo[*g,h,i*]perylene (4.1), dibenzothiophene (0.7).

| Chemical | USEPA TEF[a] | California EPATEF[b] | Proposed TEF[c] | P450RGS TEF[d] |
|---|---|---|---|---|
| Dibenz[*a,h*]anthracene | 1 | 0.4 | 5 | 20 |
| Benzo[*b*]fluoranthene | 0.1 | 0.1 | 0.1 | 10 |
| Benzo[*k*]fluoranthene | 0.01 | 0.1 | 0.1 | 5 |
| Indeno[1,2,3-*cd*]pyrene | 0.1 | 0.1 | 0.1 | 4 |
| Benz[*a*]anthracene | 0.1 | 0.1 | 0.1 | 1 |
| Benzo[*a*]pyrene | 1 | 1 | 1 | 1 |
| Chrysene | 0.01 | 0.01 | 0.01 | 0.02 |

**Table 3.** Toxic equivalent factor (TEF) for seven compounds on a basis of benzo[a]pyrene.

[a]USEPA (1993); [b]CalEPA (1994); [c]Nisbet and LaGoy (1992); [d]Anderson et al. (1996a).

(Menzie *et al.* 1992). Table 3 lists the TEF values for each compound described in an earlier publication (Anderson *et al.* 1996a). The concentration of a substance which produces a 10-fold induction is compared with that of benzo[*a*]pyrene to produce the BaPTEQ values. The chemical BaPTEQ was the sum of the products of individual compound concentrations and the TEF for those compounds. All the statistical analyses were conducted using Pearson's sample correlation included in SPSS software for Macintosh computers.

# Results

## *Distribution of PAHs based on P450RGS BaPTEQ*

The mean value of P450RGS BaPTEQ for 67 samples was 3 μg $g^{-1}$ and the range was from 0.4 to 21 μg $g^{-1}$ (Table 2). The values higher than 5 μg $g^{-1}$ were all found in the Incheon Harbor area (stations 18–36). Intermediate values, ranging from 2 to 5 μg $g^{-1}$, were observed near the mouth of Han River and areas surrounding Incheon Harbor. Most sediment samples in the open sea area exhibited BaPTEQ values less than 2 μg $g^{-1}$ (Figure 3). The distribution of P450RGS BaPTEQ values suggests that source of PAHs which induced CYP1A1 in the P450RGS assay was primarily Incheon Harbor, and possibly some contaminants may have come down the Han River. Sediments from the innermost areas of the harbour (stations 18 and 19) exhibited the highest levels (16 and 21 μg $g^{-1}$ BaPTEQ) of inducing compounds. In the northern portion of Incheon Harbor, there was a gradient of decreasing BaPTEQ values from the highest innermost stations (18 and 19), through a series of stations (20–25, 37) to the open sea.

## *Factors affecting P450RGS BaPTEQ*

PAHs often correlate with organic carbon (OC) content (Johnson *et al.* 1985, Larsen *et al.* 1986, Jones *et al.* 1989). P450RGS BaPTEQs in this study also exhibited a high correlation with OC content (Table 4, Figure 4(a)). Even after

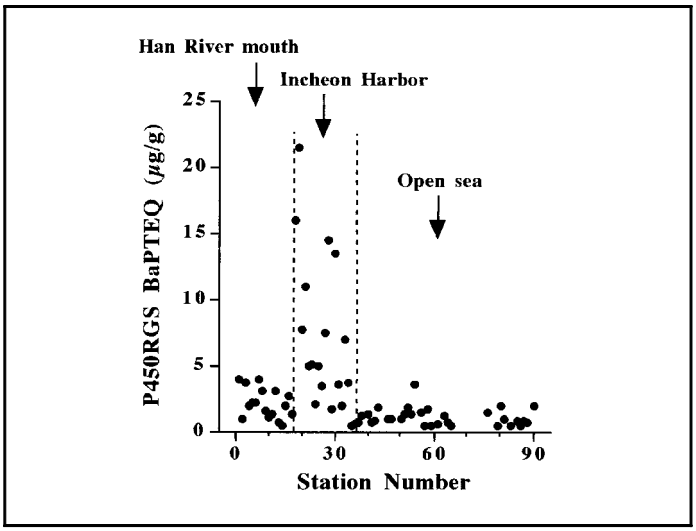


**Figure 3.** Station distribution of the P450RGS responses

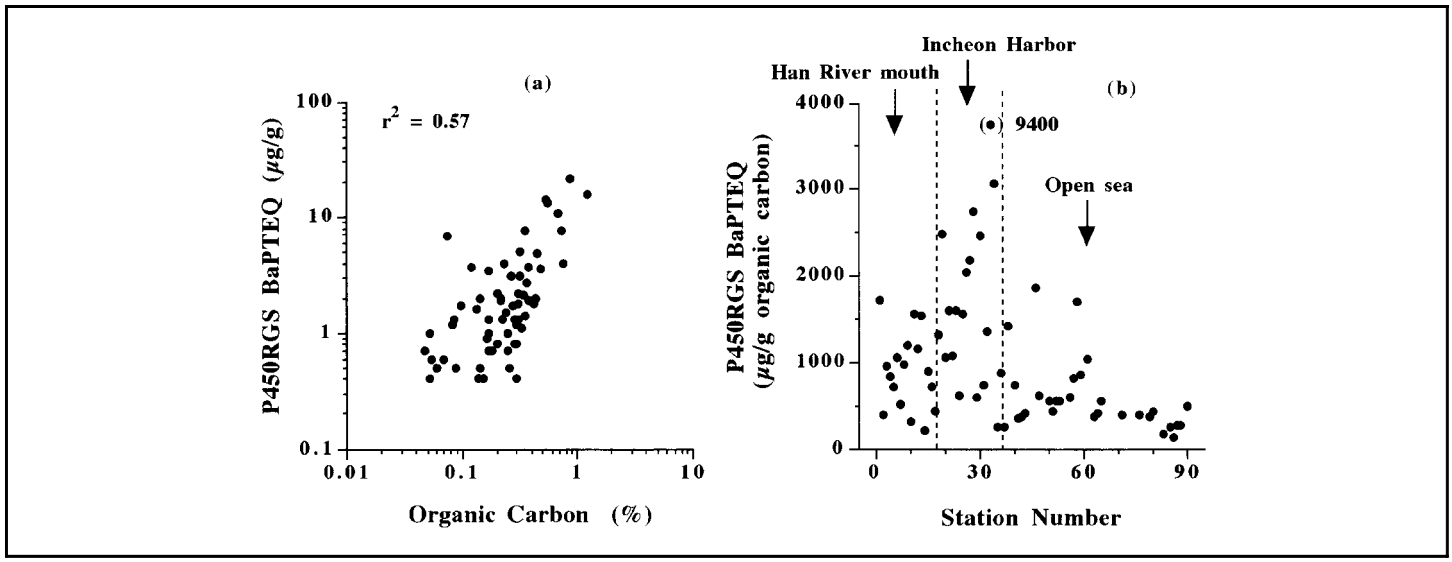


**Figure 4.** (a) Correlation between organic carbon content and P450RGS BaPTEQ (Pearson's sample correlation). (b) Station distribution of the P450RGS BaPTEQ normalized to sediment organic carbon content.



OC normalization of P450RGS BaPTEQ, Incheon Harbor area (stations 18–36) still exhibited higher values than the area of Han River mouth (stations 1–17) and the open sea (stations 37–90) (Figure 4(b)). Station 33 exhibited the highest P450RGS BaPTEQ value, which may be due to a very low OC content (0.07%) and clay content (0%) in addition to high BaPTEQ based on dry weight (Table 2). Incheon Harbor appears to be one of the main deposition areas for PAHs, which induce the CYP1A1 gene in the P450RGS assay. Sand content was negatively correlated with P450RGS BaPTEQs, while clay content exhibited a high positive correlation. As might be expected, the sediments with high clay content also contained high OC, providing a greater surface area for the absorption of PAHs that induced CYP1A1.

# Discussion

### Comparison of P450RGS BaPTEQ in various sites

It is difficult to directly compare the total PAH findings of this investigation with many other studies, because the lists of specific PAHs investigated by researchers often vary. Normally only 10–20 specific PAHs, among over several hundred PAH compounds are identified and quantified. In this investigation, 24 specific PAHs were measured (Table 1). Figure 5 shows a comparison of only the P450RGS BaPTEQs obtained in this study with those values reported at the other sites (Anderson 1995a, b, Anderson *et al.* 1996b). The mean value of P450RGS BaPTEQ in this study area was 3–20 times lower than those reported in the investigation of some regions in the USA, indicating that the effect of PAHs on benthic marine organisms in the present study area would be less than that of sediments in the other areas.

| | | Sand | Silt | Clay | Mean grain size | Organic carbon |
|---|---|---|---|---|---|---|
| P450RGS BaPTEQ | [a]n | 58 | 58 | 57 | 58 | 60 |
| | [b]$r^2$ | 0.20 | 0.067 | 0.35 | 0.22 | 0.57 |
| | [c]p | <0.005 | >0.05 | <0.001 | <0.001 | <0.001 |

**Table 4.** Correlation coefficients and statistical significance of P450RGS BaPTEQ with other factors in sediment.

[a]Number of sample: [b]Correlation coefficient (Pearson's sample correlation); [c]Statistical significance.

### Rapid decrease of PAHs with increasing distance from Incheon Harbor

In general, there was a decrease of P450RGS BaPTEQ with increasing distance from Incheon Harbor to the sea. This pattern is quite similar to previous results (Marcomini *et al.* 1986, Lipiatou and Saliot 1991), but the rate of decrease in values was more rapid than that observed at other sites. This steep increase of P450RGS BaPTEQ values may be due to the rapid dilution of contaminated particulate by high velocity currents, a high spring tide range (798 cm) at Incheon Harbor (Yi 1972), and rapid incorporation of PAHs on the suspended particles followed by sedimentation (Bjørseth *et al.* 1979, Wijayaratne and Means 1984).

### Interference of P450RGS from other contaminants

There are many compounds and metal elements which can be extracted from sediment by dichloromethane along with PAHs. They may consist of TCDD (tetrachlorinated dibenzo-*p*-dioxin), coplanar PCBs (polychlorinated biphenyls), TBT (tributyltin), other organo-metals, and pesticides.

Kyeonggi Bay, known to be a contaminated area in Korea, exhibited mean levels for copper and lead of 32 $\mu g\ g^{-1}$ and 31 $\mu g\ g^{-1}$ respectively (Lee *et al.* 1985). These levels are apparently low compared with the values for copper and lead of 210 $\mu g\ g^{-1}$ and 190 $\mu g\ g^{-1}$ respectively, in sediment tested previously, which did not interfere with the analysis of P450RGS (Anderson *et al.* 1995). Only two papers have reported TCDD and PCBs levels in K[illegible] sediments (Im *et al.*

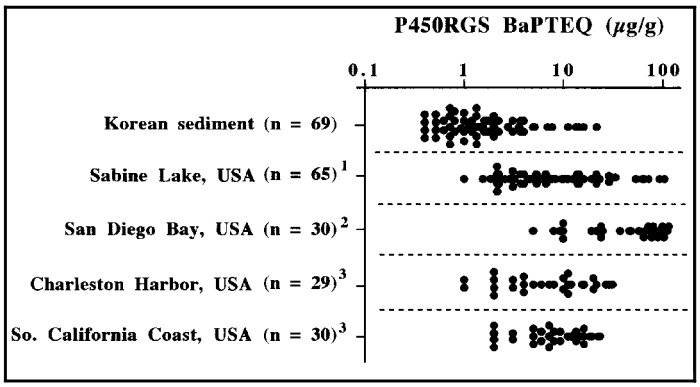


**Figure 5.** Comparison of Korean P450RGS BaPTEQ to those from other regions. [1]Anderson (1995a)*. [2] Anderson *et al.* (1996b). [3] Anderson (1995b). *See Acknowledgements.

1994, 1995). These studies reported PCB levels of 8–210 ng $g^{-1}$ and TCCD levels of 0.016–0.21 ng $g^{-1}$ in Masan Bay, a semi-enclosed bay consisting of harbour and industrial area. Lee *et al.* (unpublished data) detected a maximum PCBs level of 580 ng $g^{-1}$ in sediment samples taken in this study. Only coplanar PCBs produced induction of P450RGS (Anderson *et al.* 1995) and we have not attempted to identify these specific congeners. Anderson *et al.* (1995) found no evidence of induction from a pesticide mixture at concentrations as high as 4 μg $ml^{-1}$. Lee *et al.* (unpublished data) found relatively low levels of pesticides in the sediments analysed in this investigation (∑DDT; up to 34 ng $g^{-1}$, ∑HCH; 1.5 ng $g^{-1}$, ∑CHLs; 130 ng $g^{-1}$). TBT has a strong inhibitory effect *in vitro* and *in vivo* on microsomal cytochrome P450 in marine organisms and rat (Rosenberg *et al.* 1980, Rosenberg and Drummond 1983, Fent and Stegeman 1991, 1993, Kim *et al.* 1996b). Kim *et al.* (unpublished data) detected a maximum of TBT level of 84 ng $g^{-1}$ in Incheon Harbor sediments. In recent studies P450RGS induction was found to be inhibited only by sediment TBT concentrations of greater than 750 ng $g^{-1}$ (Anderson *et al.* unpublished data). Considering the levels of these contaminants found in these sediments, it is probable that the P450RGS induction observed was primarily due to PAHs.

### Possible availability of P450RGS as bioindicator of PAHs

In this study, we analysed PAHs and P450RGS in split samples. Figure 6(a) shows the relationship of P450RGS BaPTEQ and total PAH concentrations. A statistically significant correlation ($p<0.001$, $r^2=0.624$) was found between the two variables, indicating P450RGS is useful for screening levels of PAHs in sediments. The same pattern was also found when P450RGS BaPTEQ was plotted against chemical BaPTEQ, based on the chemical analyses of PAHs (Figure 6(b)). The chemical BaPTEQ could explain about 1–10% of P450RGS BaPTEQ. This is not surprising, since this chemical value was calculated using just seven compounds for which P450RGS BaPTEF values were known (Table 3). Some of these PAHs exhibited concentrations less than the mean detection level and thus were not included in chemical BaPTEQ calculation. This result is similar to the previous investigation, where the sum of the individual PAH monitored by GC–MS could explain less than 1% of the total PAH concentration produced by two spectroscopic (UV/F) techniques (Hellou *et al.* 1991).

### Sensitive and usefulness of P450RGS BaPTEQ

Table 3 lists the most recent P450RGS TEF values for seven PAH compounds. Four compounds produce significant CYP1A1 induction at concentrations lower than benzo[*a*]pyrene. Delistraty (1996) reviewed over 20 papers that proposed new TEF values for each of the PAH compounds listed in Table 3. Most TEF values for PAHs were less than that of benzo[*a*]pyrene based on various types of studies on mammals. Regardless of reasons for differences in the observed potency of various PAHs, the 101L cell line and the reporter gene system (P450RGS) is very sensitive to a number of PAHs frequently found in environmental samples. This fact makes

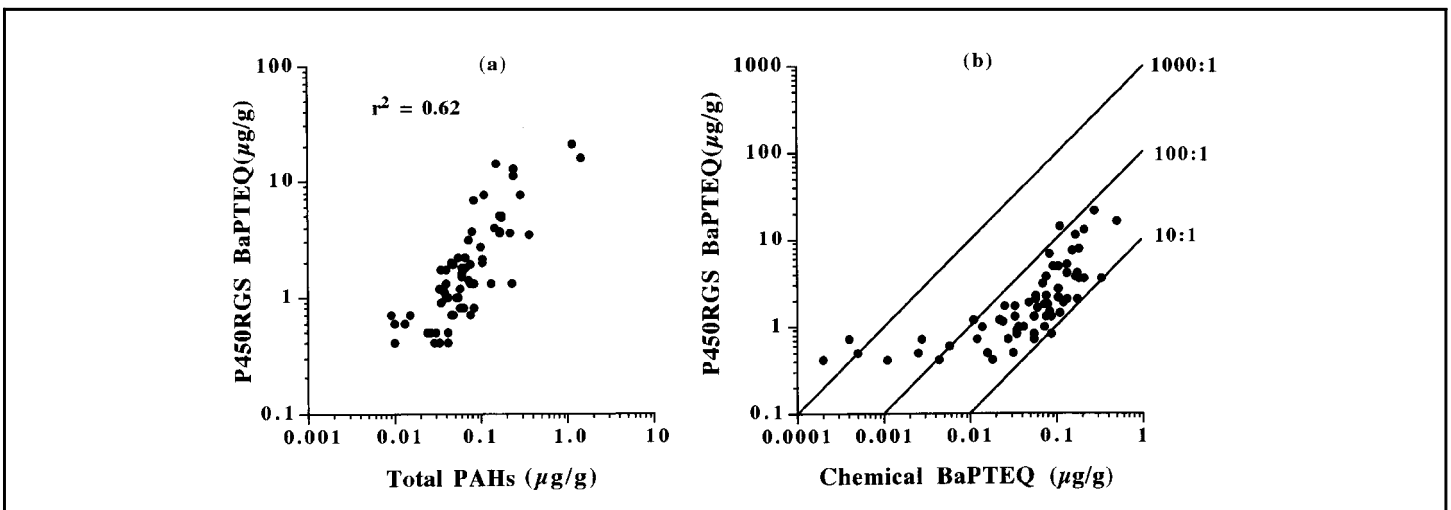


**Figure 6.** (a) Correlation between total PAHs and P450RGS BaPTEQ (Pearson's sample correlation). (b) Comparison of chemically-derived BaPTEQ estimates to P450RGS BaPTEQ values. Procedures for producing the chemical and P450RGS BaPTEQ values are described in Materials and Methods.

the use of the assay in screening samples for PAHs even more appropriate.

In addition to sensitivity, this method can reduce the total cost of monitoring programmes and environmental assessment studies and shorten analysis time compared with previous methods, such as HPLC (Marcomini *et al.* 1986, Witt 1995), GC/FID (Maliszewska-Kordybach 1993), GC/MS (Johnson *et al.* 1985, Evans *et al.* 1990). There is no need to remove interference materials from the extraction solution. After extraction, over 100 samples can be analysed for P450RGS in a little as 1 week. We believe it is desirable to first utilize this type of screening procedure, in order to prioritize samples for subsequent detailed chemical analyses.

## Conclusion

The P450RGS BaPTEQs responses were dependent mainly on PAH concentration, the distance from Incheon Harbor, and OC content. There was a steep decrease of P450RGS BaPTEQ values with increased distance from shore. This may be due to the rapid transport of particles containing PAHs by high velocity currents, large differences between spring and neap tide in the vicinity of Incheon Harbor, and rapid incorporation of PAHs on the suspended particles followed by sedimentation. The P450RGS method used in this investigation was found to be very simple and effective for screening of PAHs in sediments. The highest levels of response were from samples taken in the inner portions of an inlet and a harbour, where deposition of contaminants would be expected. The P450RGS data, obtained in a short time, correlated well with the subsequent chemical characterization of PAHs in the samples ($p<0.001$, $r^2=0.624$). Assessment of the potential ecological impacts of sediments contaminated with PAHs is enhanced by obtaining data on P450RGS responses in the form of BaPTEQs. While attempts have been made to develop sediment quality criteria for specific PAHs, these are based on responses of organisms to sediments containing different mixtures of PAHs, chlorinated hydrocarbons, and heavy metals. Comparison of the levels of P450RGS BaPTEQ at various sites seem to be more meaningful from a toxicological and biochemical standpoint than those of the total PAH concentrations.



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