

# Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-TCDD equivalents (TEQs) in sediment from the Hyeongsan River, Korea

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**“Capsule”:** *A combination of in vitro bioassay and chemical analysis identified that PCDDs/Fs are responsible for the dioxin-like activity present in the extracts of Hyeongsan River sediment.*

## Abstract

Sediment, pore water and water samples from the Hyeongsan River, Korea were analyzed for several classes of halogenated aromatic hydrocarbons (HAHs) and their dioxin-like activities were evaluated using the in vitro H4IIE-luc bioassay. Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs) were detected in sediments from all six sampling locations with mean concentrations of  $2.8 \times 10^2$  pg/g, 190 pg/g, and 61.4 ng/g, dw, respectively. Polycyclic aromatic hydrocarbons (PAHs) were predominated by 4–6 ring compounds with concentrations in the range of 5.30–7680 ng/g, dw. Chemical profiles of target analytes in sediment and water samples revealed that there was a gradient of concentrations along the river from upstream to downstream, which suggested that the primary source was a wastewater reservoir adjacent to a sewage treatment plant (STP). TEQs derived by summing the product of concentrations of individual congeners by their respective relative potencies (REPs or TEFs) ranged from  $4.3 \times 10^{-1}$  to  $1.1 \times 10^3$  pg/g, dw. Raw Soxhlet extracts from all six sampling locations induced significant dioxin-like responses in the H4IIE-luc bioassay. TCDD-EQs derived from H4IIE bioassay ranged from  $7 \times 10^{-3}$  to  $1.5 \times 10^3$  pg/g, dw, which were significantly correlated with TEQs ( $r^2 = 0.994$ ,  $p < 0.05$ ). Among the three Florisil fractions tested, PCDD/Fs in fraction (F2) induced the greatest magnitude of response (range: 24–83%-TCDD-max.) in the H4IIE-luc assay. Comparison of the TEQ and TCDD-EQ suggested little non-additive interaction between fractions and AhR-active and inactive compounds. Concentrations of individual congeners as well as TEQs and TCDD-EQs suggest inputs from the industrial center waste stream in the Hyeongsan River.

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## 1. Introduction

The combination of instrumental and bioassay analyses has been useful in the assessment of halogenated aromatic hydrocarbons (HAHs) such as PCDDs, PCDFs, and planar PCBs in environmental samples (Tillitt et al., 1991; Gardinali and Wade, 1998; Khim et al., 1999a; Hilscherova et al., 2000). These compounds can cause reproductive and developmental toxicity, immunotoxicity, hepatotoxicity, and cancer in humans and wildlife (Safe, 1990; Giesy and Snyder, 1997). The toxic responses of HAHs are based on their ability to interact with and activate the arylhydrocarbon receptor (AhR). Compounds other than HAHs such as polycyclic aromatic hydrocarbons (PAHs) found in environmental samples may also contribute to toxic effects via AhR-mediated pathway (Poland and Knutson, 1982; Willett et al., 1997). The effects of complex mixtures of HAHs and PAHs can be evaluated by determining total dioxin-like activity in samples. This can be done in two ways. First, the total dioxin-like potency of a sample can be calculated as the sum of the product of the concentration of each known AhR-active congener multiplied by its relative potency (REP) or toxic equivalency factor (TEF). Alternatively, bioassays can be used to determine the dioxin-like potency of complex mixtures, which may contain unidentified compounds or compounds for which no TEFs are available.

A variety of *in vitro* bioassay systems have been developed. Some, like the H4IIE-luc assay applied in this study, are based on the AhR-dependent mechanism for the detection of dioxin-like HAHs. H4IIE-luc cells are rat hepatoma cells that were stably transfected with a luciferase reporter gene under control of dioxin responsive elements (Sanderson et al., 1996). H4IIE-luc cell bioassay has been successfully applied to evaluate both individual HAHs or PAHs and mixtures of compounds that act through AhR pathway (Khim et al., 1999a,b; Kannan et al., 2000; Hilscherova et al., 2001). Used appropriately, bioassay analysis of sample extracts provides a powerful tool to characterize causative agents in complex mixtures of contaminants.

This study employed a combination of instrumental analyses and an *in vitro* bioassay to evaluate dioxin-like (AhR-mediated) potency of complex mixtures of contaminants present in sediment from an industrialized area. The purpose of this study was to measure concentrations of dioxin equivalents (TCDD-EQs) by use of the H4IIE-luc cell bioassay and compare them to instrumentally derived dioxin equivalents (TEQs) contributed by PCDD/Fs, coplanar PCBs, and certain AhR-active PAHs in sediment and water samples. This type of analysis has been applied previously to identify and characterize dioxin-like compounds in sediment samples (Khim et al., 1999b, 2001a; Koh et al., 2002). Concentrations of PCDDs, PCDFs, PCBs and PAHs

were determined by instrumental analyses and total TCDD-EQs were determined by use of bioanalytical (Bioassay) techniques, based on a genetically altered cell system. Responses of the bioassay to raw extract (RE) and purified fractions from Florisil and carbon columns were used to further characterize dioxin-like activity associated with sediments. Toxicity identification evaluation (TIE) was applied to develop hypotheses regarding which specific compounds or classes of compounds were responsible for AhR-mediated activity observed in the sediments.

## 2. Materials and methods

### 2.1. Sampling

Sediment and water samples were collected from six locations (Y1–Y6) in the Hyeongsan River and Yeongil Bay, Korea in February 2001 (Fig. 1). Sampling was designed to determine potential sources of contaminants from inland regions (Y1–Y5) to coastal areas (Y6). Sampling location Y2 was a wastewater reservoir adjacent to a sewage treatment plant (STP). Wastewater from the Posco industrial complex enters the reservoir and is temporarily stored there before treatment. Dissolved water (DF: dissolved fraction), pore water (PW), particulate matter (PM), and sediment samples were collected from both locations Y2 and Y3, directly downstream of where the treated effluent enters the Hyeongsan River. This was done to investigate the chemical composition and biological effects of sewage samples before and after sewage treatment. Surface sediment (0–5 cm) samples were collected using a grab sampler and pebbles and twigs were removed. Samples were freeze-dried and ground with a mortar and pestle and stored in pre-cleaned high-density polyethylene bottles at  $-20^{\circ}\text{C}$  until extraction. Water samples (about 16 L) were collected from locations Y2 and Y3 in clean 4 L glass bottles and pore water samples (about 1 L) were obtained from corresponding sediment samples using a pressurized squeezing method. Water samples were further separated into PM and DF using glass fiber filters (GF/F) and membrane filters (final pore size:  $0.45\ \mu\text{m}$ ). Particulate matter samples were freeze-dried and stored in a pre-cleaned aluminum foil at  $-20^{\circ}\text{C}$  until extraction.

### 2.2. Sample preparation

Detailed descriptions of the sample extraction and fractionation procedures have been reported earlier (Khim et al., 1999c; Kannan et al., 2001; Koh et al., 2002). Briefly, sediment and PM samples were Soxhlet extracted for 20 h using dichloromethane concentrated using a rotary evaporator and treated with activated

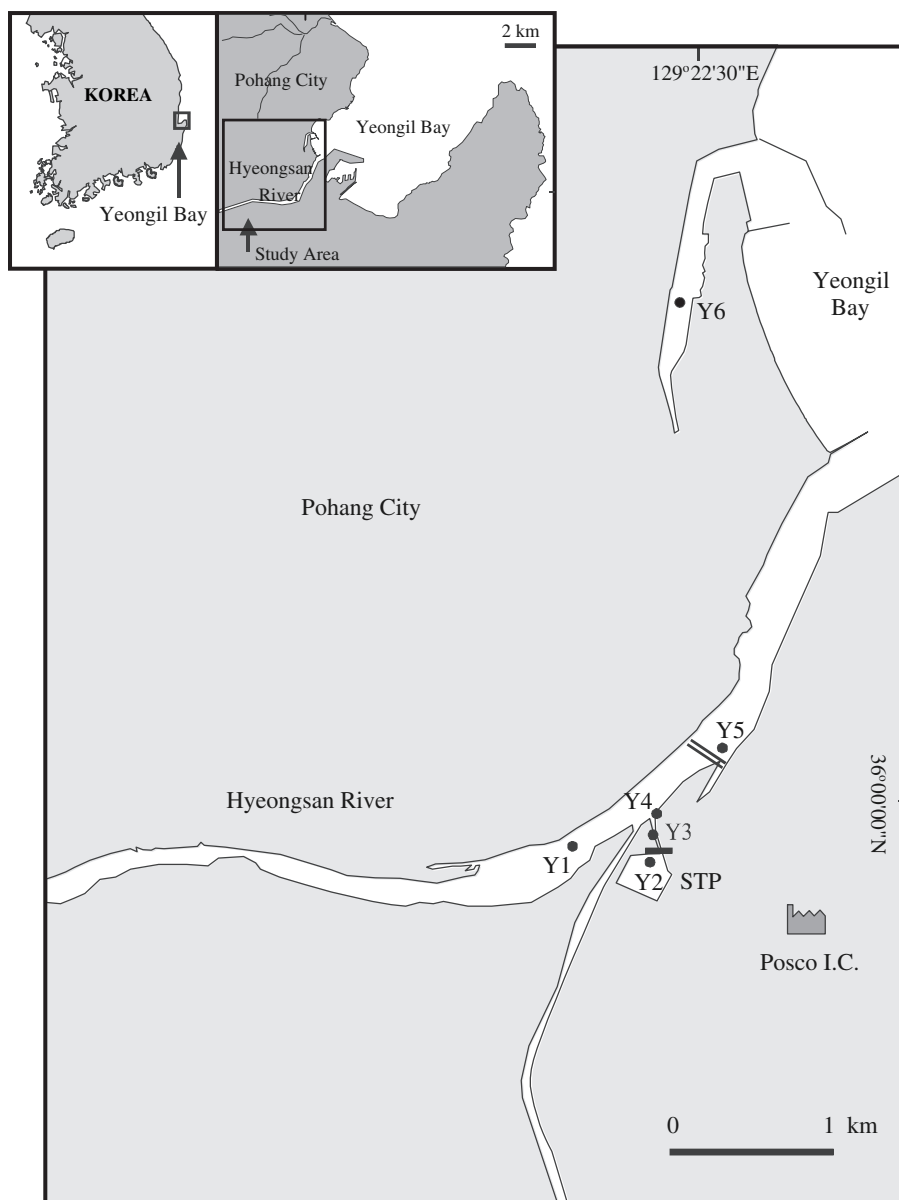


Fig. 1. Map of the Hyeongsan Bay study area in Korea. Sediment samples were collected at locations Y1–Y6. Pore water and water samples were also collected from the locations near sewage treatment plant (Y2 and Y3).

copper granules to remove sulfur from the extracts. Aqueous phase samples such as water and pore water were passed through Empore™ (3M Corporation, St. Paul, MN, USA) solid phase disk (C18 (octadecyl): non-polar, SDB-XC (styrenedivinylbenzene): polar) and then extracted with hexane and DCM appropriately (15). Raw Soxhlet extracts were concentrated to 2 ml. RE (1 ml) was used for Florisil column fractionation to separate PCBs, PAHs, alkylphenols (APs) and 0.8 ml of RE was used for fractionation of PCDD/Fs from PCBs. Three Florisil fractions were collected according to polarity and used for both instrumental analyses and bioassay. The first fraction (F1) eluted with 100 ml of hexane contained PCBs, HCB and *p,p'*-DDE. Remaining OC pesticides and PAHs were eluted in the second

fraction (F2) using 100 ml 20% DCM in hexane. NP, OP and BPA were eluted in the third fraction (F3) with 100 ml 50% DCM in high purity methanol. Spike-recovery tests with PCDD/Fs suggested that they eluted in both F2 and F3, despite their relative non-polarity (Khim et al., 1999c). Thus, PCDD/Fs fractions were obtained after a series of silica, alumina, and silica impregnated activated carbon clean-up. Although non-target compounds (unidentified components of the sediment extract) were expected to partition to the three Florisil fractions, such separation could not be confirmed for non-target compounds. Procedural blanks were analyzed with every set of six samples to check for interference or contamination arising from solvents and/or glassware.

### 2.3. Chemical analysis

PCBs and OC pesticides were quantified using a gas chromatograph (Perkin Elmer series 600) equipped with  $^{63}\text{Ni}$  electron capture detector (GC-ECD). An equivalent mixture of 98 individual PCB congeners (AccuStandard) with known composition and content and OC pesticides mixture (CLP-023R, CLP-024R, AccuStandard) were used as a standard. Sixteen priority PAHs were quantified using a Hewlett Packard 5890 series II gas chromatograph equipped with a 5972 series mass spectrometer detector (GC-MSD). Reverse phase high performance liquid chromatography (HPLC) with fluorescence detection was used to quantify NP, OP, and BPA. Seven 2,3,7,8-substituted PCDDs and ten PCDFs were analyzed by a high resolution gas chromatography interfaced with a high resolution mass spectrometer (HRGC). A Hewlett Packard 5890 series II HRGC connected to a JEOL JMS SX 102A HRMS was used for the identification and quantifications of individual congeners. Concentrations of individually resolved peaks of 98 PCBs, 16 PAHs, and 17 PCDD/Fs were summed to obtain total PCBs, PAHs, and PCDD/Fs concentrations, respectively. The detection limits for PCBs, PAHs, and APs were 1.0, 10, 1.0 ng/g, dw, respectively. Detection limits for seventeen 2,3,7,8-substituted PCDD/Fs were 0.01 pg/g, dw.

### 2.4. Cell culture and in vitro bioassay

Each sample was tested as both RE and Florisil fractionated extract (F1–F3) in H4IIE-luc in vitro bioassays. Cells were seeded into 96-well plates and exposed to 2.5  $\mu\text{l}$  of extract for 72 h. Combination doses were delivered as separate 2.5  $\mu\text{l}$  doses of each fraction in the combination (total volume delivered ranging from 5.0 to 7.5  $\mu\text{l}$ ). A minimum of three solvent control and three blank wells were analyzed with each 96-well plate, and all samples were tested in triplicate. Sample dose–response curves consisted of six concentrations prepared by serial dilution of the RE. Luciferase and protein assays were conducted after 72 h of exposure using methods described previously (Khim et al., 1999a,b, 2001a; Koh et al., 2002). Sample responses, expressed as relative luminescence units (RLU), were converted to relative response units, expressed as a percentage of the maximum response observed for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; %-TCDD-max.) standard curves generated on the same day. Where appropriate, sample potency relative to the TCDD standards, was estimated. Further details of cell culture condition and in vitro bioassay have been described extensively in earlier publications (Khim et al., 1999a,b, 2001a; Koh et al., 2002). Cell viability was determined based on a live–dead ratio (Hilscherova et al., 2001).

### 2.5. Mass balance analysis

This study used mass balance analysis to determine probable causes of dioxin-like activity associated with samples from the Hyeongsan River. Instrumentally derived dioxin equivalents (TEQs) were calculated by multiplying the concentrations of known AhR agonists (i.e., PCDD/Fs, coplanar PCBs, and certain PAHs) by published assay specific relative potencies and summing the products for each agonist present in the sample (not done on a per fraction basis) of interest. Where it was possible to obtain sample dose–response relationships by testing samples at multiple dilutions, bioassay-derived dioxin equivalents (TCDD-EQs) were derived. These were estimated directly from sample dose–response curves using methods described elsewhere (Khim et al., 1999b; Villeneuve et al., 2000). Instrumentally derived values (TEQs) were then compared to the bioassay-derived relative potency estimates (TCDD-EQs) (Villeneuve et al., 2000; Sanderson and Giesy, 1998).

## 3. Results and discussion

### 3.1. Sediment characteristics

Total organic carbon (TOC) content and sediment grain size (expressed as Mz) were determined (Table 1). Sediment from upstream and downstream of the Hyeongsan River (Y1, Y4, Y5) consisted predominantly of gravel and sand (mean: 97%), whereas the locations Y2, Y3, and Y6 composed primarily of silt and clay (mean: 75%). TOC concentrations varied from 0.27 to 5.8% with a significant correlation to sediment grain size ( $r^2 = 0.99$ ,  $p < 0.05$ ). TOC concentrations as great as 5.8% were measured in sediments from the wastewater reservoir, immediately downstream of the STP (Y2 and Y3). A significant correlation between TOC content and concentrations of PCDD/Fs ( $r^2 = 0.90$ ,  $p = 0.004$ ) was observed; whereas, concentrations of PCBs ( $r^2 = 0.50$ ,  $p = 0.117$ ) and PAHs ( $r^2 = 0.58$ ,  $p = 0.079$ ) were not correlated with sediment TOC.

Table 1  
Total organic carbon (TOC) contents, grain size composition, and mean grain size (Mz) of sediment samples from Hyeongsan River, Korea

Sampling location	TOC <sup>a</sup> (%)	Grain size composition (%)				Mz <sup>a</sup> ( $\phi$ )
		Gravel	Sand	Silt	Clay	
Y1	0.27	11	89	<0.01	<0.01	0.90
Y2	5.8	<0.01	7.0	54	39	7.1
Y3	3.3	<0.01	64	19	17	4.3
Y4	0.45	5.9	94	<0.01	<0.01	1.3
Y5	0.56	24	68	3.9	3.4	0.99
Y6	3.1	<0.01	4.5	37	58	8.6

<sup>a</sup> TOC and Mz of Hyeongsan Bay sediment were significantly correlated with  $r^2$  of 0.99 ( $p < 0.01$ ).

## 3.2. PCDD/Fs, PCBs, and PAHs

Nearly all tetra- through octa-chlorinated PCDD/Fs were found in Hyeongsan River sediments and their concentrations ranged from 2.6 to  $1.2 \times 10^3$  pg/g, and from 2.2 to  $6.4 \times 10^2$  pg/g, dw, respectively (Table 2). Non-, mono-, di-*ortho* PCBs were also detected in some locations ranging from  $<1.0 \times 10^{-2}$  to  $1.0 \times 10^1$  ng/g, dw (Table

2). Maximum concentrations of total PCDD/Fs and PCBs were detected at locations Y2 and Y3, respectively, which are near the STP (Table 2, Fig. 1). Concentrations of total PCDDs, PCDFs and PCBs in sediments were similar to those previously reported sediment PCDD/Fs ( $1.8 \times 10^1$ – $6.5 \times 10^4$  pg/g, dw,  $n = 30$ ) and PCBs ( $<1.00$ – $5.8 \times 10^2$  ng/g, dw,  $n = 164$ ) from Korean coasts (Moon et al., 2001; Im et al., 2002; Koh et al., 2002).

Table 2  
Concentrations of PCDD/DFs<sup>a</sup> (pg/g, dw) and PCBs<sup>b</sup> (ng/g, dw) in sediment from Hyeongsan River, Korea

Compounds	Y1	Y2	Y3	Y4	Y5	Y6	Mean
<i>PCDDs</i>							
2,3,7,8-D	<0.01	6.7	2.5	0.15	0.20	<0.2	2.4
1,2,3,7,8-D	0.14	930	70	2.7	2.5	1.5	170
1,2,3,4,7,8-D	0.05	2.6	4.5	0.23	0.12	1.1	1.4
1,2,3,6,7,8-D	0.07	40	12	0.56	0.28	2.4	9.1
1,2,3,7,8,9-D	0.01	1.7	1.3	0.09	<0.01	0.46	0.71
1,2,3,4,6,7,8-D	0.30	32	50	2.8	1.3	21	18
OCDD	2.0	140	90	11	6.8	230	80
<i>PCDFs</i>							
2,3,7,8-F	0.33	70	70	3.3	2.7	5.9	26
1,2,3,7,8-F	<0.01	<0.01	<0.01	<0.01	<0.01	3.5	3.5
2,3,4,7,8-F	0.28	180	360	9.8	13	1.9	94
1,2,3,4,7,8-F	0.14	15	29	1.2	0.70	8.6	9.1
1,2,3,6,7,8-F	0.10	14	19	1.0	0.35	3.6	6.4
2,3,4,6,7,8-F	0.13	12	27	1.4	0.46	2.6	7.2
1,2,3,7,8,9-F	0.02	<0.01	0.68	0.10	<0.01	<0.20	0.27
1,2,3,4,6,7,8-F	0.50	58	78	4.8	1.4	18	27
1,2,3,4,7,8,9-F	0.10	8.2	9.5	1.9	<0.01	0.61	4.1
OCDF	0.55	46	40	3.9	1.0	6.6	16
Non- <i>ortho</i> PCBs (IUPAC <sup>c</sup> no.)							
81	<0.001	0.010	0.013	<0.001	<0.001	0.001	0.008
77	0.002	1.66	2.32	0.029	0.023	0.143	0.697
126	<0.001	0.021	0.028	0.002	0.001	0.007	0.012
169	<0.001	0.001	0.005	<0.001	<0.001	0.001	0.002
Mono- <i>ortho</i> PCBs							
105	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
114	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
118	<0.01	1.1	1.7	<0.01	0.12	0.58	0.87
123	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
156	<0.01	0.14	0.08	<0.01	<0.01	0.08	0.10
157	<0.01	1.5	0.04	<0.01	<0.01	0.04	0.51
167	<0.01	0.09	0.08	<0.01	<0.01	0.06	0.08
189	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Di- <i>ortho</i> PCBs							
170	<0.01	4.7	0.52	0.01	0.03	0.45	1.1
180	<0.01	1.0	0.60	0.06	0.10	0.95	0.54
PCDDs	2.6	1200	230	18	11	260	290
PCDFs	2.2	400	630	27	20	52	190
Non- <i>ortho</i> PCBs	0.002	1.70	2.37	0.031	0.025	0.153	0.712
Mono- <i>ortho</i> PCBs	<0.01	2.8	1.9	<0.01	0.12	0.76	1.4
Di- <i>ortho</i> PCBs	<0.01	5.7	1.1	0.08	0.13	1.4	1.7
Total PCBs <sup>d</sup>	<1.0	100	170	8.8	3.1	26	62

<sup>a</sup> Detection limit for PCDDs and PCDFs was 0.01 for Y1–5 and 0.2 for Y6 samples.

<sup>b</sup> Detection limit for non-*ortho* PCBs was 0.001 and for mono-*ortho* and di-*ortho* PCBs were 0.01 for all samples.

<sup>c</sup> IUPAC is International Union of Pure and Applied Chemists.

<sup>d</sup> Total PCBs obtained from the sum of 98 individual PCB congeners. Detection limit for total PCBs was 1.0.



Although concentrations of PCDD/Fs and PCBs were greatest in sediment from locations Y2 and Y3, the patterns of relative concentrations were different (Fig. 2a,b). The total concentration of PCDDs was threefold greater than that of PCDFs at location Y2 (before treatment), while concentrations of PCDDs were threefold less than that of PCDFs at location Y3 (after treatment) (Table 2). Tetrachloro dibenzo-*p*-dioxin (TeCDD) and pentachloro dibenzo-*p*-dioxin (PeCDD) homologues decreased after treatment of the sewage. Relative concentrations of octachloro dibenzo-*p*-dioxin (OCDD) observed at some locations, such as Y1 and Y4–Y6 indicated that the likely sources of PCDD in the Hyeongsan River were primarily due to atmospheric input from industrial complexes or combustion related sources in this area. The homologue composition of PCDFs was similar between locations Y2 and Y3 (Table 2). Pentachloro dibenzofurans (PeCDF) contributed the greatest proportions, accounting for 44 and 57% of the total PCDF concentrations at locations Y2 and Y3, respectively. Among PCB congeners, lesser chlorinated congeners such as tri- and tetra-CBs were the most prevalent homologues accounting for approximately 50% of the total PCB concentrations in Hyeongsan River sediments (Fig. 2b). Previous studies have also reported the presence of lesser chlorinated PCB congeners in sediments collected from Ulsan and Onsan

Bays near Pohang City (Khim et al., 2001b; Koh et al., 2002).

The greatest concentration of total PAHs of  $7.7 \times 10^3$ , 680 ng/g, dw, was found at Y2 while the next greatest concentration of  $2.4 \times 10^3$  ng/g, dw, was found at Y3 (Table 3). Industrial and STP waste from the Posco Industrial Complex and Pohang City are considered to be potential sources of PAHs (Fig. 1). Several studies have reported that concentrations of total PAHs in marine and riverine sediment in Korea range from  $<10$  to  $3.0 \times 10^3$  (mean:  $2.2 \times 10^2$ ,  $n = 164$ ) ng/g, dw (Khim et al., 1999a,c, 2001b; Koh et al., 2002). Total PAH concentrations were threefold less after the treatment of sewage. Four-ring aromatic hydrocarbons, such as fluoranthene and pyrene, were the predominant PAHs in Hyeongsan River sediment (Figs. 2c and 3). Ratios of specific PAHs, such as fluoranthene to pyrene (Fluo/Py) and indeno[1,2,3-*cd*]pyrene to benzo[*g,h,i*]perylene (IP/BP), were calculated to evaluate potential sources of PAHs (Baumard et al., 1998). The ratio of Fluo/Py at locations Y2, Y3, Y4, and Y6 (range: 1.0–1.2) was greater than 1.0, while at other locations (range:  $7.9\text{--}8.2 \times 10^{-1}$ ), the ratios were less than 1.0. The ratios of IP/BP were greater than 1.0 at locations Y2, Y3, Y4, and Y6 (range: 1.0–1.5). These results suggested that the sources of PAHs to the Hyeongsan River were both petrogenic and pyrolytic.

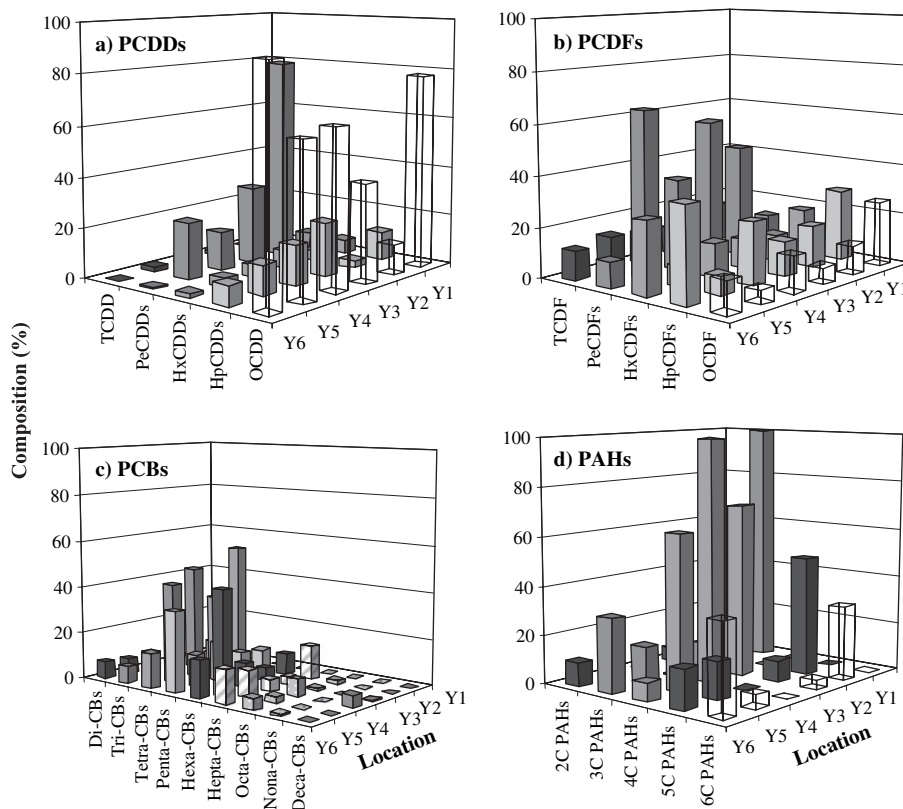


Fig. 2. Homologue composition (%) of polychlorinated dibenzo-*p*-dioxins (PCDDs; (a)), furans (PCDFs; (b)), polychlorinated biphenyls (PCBs; (c)), and polycyclic aromatic hydrocarbons (PAHs; (d)) in sediment from Hyeongsan River, Korea.

Table 3  
Concentrations (ng/g, dw) of 16 priority polycyclic aromatic hydrocarbons (PAHs) in sediment from Hyeongsan River, Korea

Compound	Y1	Y2	Y3	Y4	Y5	Y6	Mean
Naphthalene	<0.50	140	260	<0.50	<0.50	22	140
Acenaphthalene	<0.50	3.7	13	0.64	<0.50	<0.50	5.9
Acenaphthene	<0.50	88	130	<0.50	0.71	5.6	56
Fluorene	<0.50	100	75	0.65	<0.50	8.0	46
Phenanthrene	1.5	480	230	4.9	3.5	56	130
Anthracene	<0.50	93	66	0.87	0.55	1.1	32
Fluoranthene	1.7	1200	400	8.2	6.7	88	280
Pyrene	2.1	980	330	7.5	8.2	86	240
Benzo[ <i>a</i> ]anthracene	<0.50	500	140	1.2	0.98	13	130
Chrysene	<0.50	700	170	2.5	1.9	48	190
Benzo[ <i>b</i> ]fluoranthene	<0.50	1000	180	1.3	1.5	38	240
Benzo[ <i>k</i> ]fluoranthene	<0.50	460	130	2.1	1.2	<0.50	150
Benzo[ <i>a</i> ]pyrene	<0.50	630	120	<0.50	2.0	<0.50	250
Indeno[1,2,3- <i>cd</i> ]pyrene	<0.50	670	100	<0.50	<0.50	51	270
Dibenzo[ <i>a,h</i> ]anthracene	<0.50	10	1.7	<0.50	<0.50	0.50	4.2
Benzo[ <i>g,h,i</i> ]perylene	<0.50	650	88	<0.50	1.7	34	190
Total PAHs <sup>a</sup>	5.3	7700	2400	30	29	450	2200

<sup>a</sup> Total PAHs is sum of 16 priority components.

### 3.3. Sources of organic contaminants

To evaluate chemical composition and fate of trace organic contaminants during the sewage treatment process, several classes of organic compounds such as PCBs, PAHs, OC pesticides, NP, OP, BPA were analyzed in sediment, pore water, and water samples collected at locations Y2 and Y3. Almost all target analytes were detected in sediment and dissolved water from the

Hyeongsan River (Table 4). PAHs and NP were the predominant compounds in sewage samples followed by BPA, OP, PCBs, and OC pesticides. The Hyeongsan River receives  $3.0 \times 10^5$  t of wastewater daily from several cities and industrial complexes and about 78% of that wastewater is from municipal sewage discharge. The daily sewage treatment capacity of Pohang City is less than  $9.0 \times 10^4$  t. Thus, more than 60% of the sewage is discharged directly into the Hyeongsan River. The lack

Table 4  
Concentrations of PCBs, OC pesticides, PAHs, NP, OP, and BPA in sediment, pore water and water (particulate matter and dissolved water) from locations near sewage treatment plant in Hyeongsan River, Korea

Compound	Y2 (before sewage treatment)				Y3 (after sewage treatment)			
	Solid phase		Aqueous phase		Solid phase		Aqueous phase	
	Sediment (ng/g)	PM <sup>a</sup> (ng/g)	PW <sup>b</sup> (ng/L)	DF <sup>c</sup> (ng/L)	Sediment (ng/g)	PM (ng/g)	PW (ng/L)	DF (ng/L)
PCBs <sup>d</sup>	100	<1.00	<1.00	9.5	170	<1.00	39	8.6
HCB <sup>e</sup>	0.95	2.1	<0.01	0.07	2.0	<0.01	7.9	0.16
HCHs <sup>f</sup>	<0.01	<0.01	79	73	1.3	90	1000	34
CHLs <sup>g</sup>	0.08	<0.01	<0.01	0.16	0.09	<0.01	<0.01	<0.01
DDTs <sup>h</sup>	1.1	<0.01	29	9.2	1.3	<0.01	35	10
PAHs <sup>i</sup>	7700	120	2600	2300	2400	190	1300	2100
NP <sup>j</sup>	6800	21400	440	73	4400	6200	630	30
OP <sup>k</sup>	99	<1.00	<10.0	<10.0	64	<1.00	<10.0	<10.0
BPA <sup>l</sup>	15	<1.00	400	890	<1.00	<1.00	<10.0	300

<sup>a</sup> PM: particulate matter of water.

<sup>b</sup> PW: pore water samples were obtained from corresponding sediment samples using pressure method.

<sup>c</sup> DF: dissolved fraction of water.

<sup>d</sup> PCBs: Polychlorinated biphenyls, sum of 98 individual congeners.

<sup>e</sup> HCB: Hexachlorobenzene.

<sup>f</sup> HCHs: sum of  $\alpha$ -,  $\beta$ -,  $\gamma$ -hexachlorocyclohexanes.

<sup>g</sup> CHLs: sum of  $\alpha$ -,  $\gamma$ -chlordanes.

<sup>h</sup> DDTs: sum of *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE.

<sup>i</sup> PAHs: Polycyclic aromatic hydrocarbons, sum of 16 priority components.

<sup>j</sup> NP: Nonylphenol.

<sup>k</sup> OP: Octylphenol.

<sup>l</sup> BPA: Bisphenol A.

of sewage treatment facilities and un-permitted discharges could be responsible for the relatively great concentrations of PAHs and NP in the riverine sediments. The likely sources of organochlorine pesticides were from agricultural use and livestock complexes located upstream of the Hyeongsan River.

PAHs in sediments collected at locations Y2 (wastewater reservoir) and Y3 (downstream of STP) were predominated by 4 and 5 ring aromatic hydrocarbons such as fluoranthene, pyrene, chrysene, and benzo[*b*]fluoranthene. Concentrations of these PAHs downstream of the STP were three- to sixfold less than those detected in the wastewater reservoir. PAH concentrations in the dissolved fraction (water samples) did not decrease markedly after sewage treatment (Figs. 1 and 3). This indicates that physical treatment by settling the particles was likely the major mechanism of PAH reduction in sewage treatment plant. PAH concentrations in pore water and water samples were generally  $1 \times 10^2$  to  $1 \times 10^3$ -fold less than those of corresponding sediment samples. Naphthalene was the predominant PAH accounting for 80% of total concentration of PAHs in surface and pore water (Table 4, Fig. 3). This is consistent with the lesser capacity of aqueous phases to absorb hydrophobic and greater-molecular weight PAH compounds. In general, concentrations of PCBs and OC pesticides in aqueous samples were less than 100 ng/L for both pore water and dissolved water samples. Among a number of OC pesticides that were screened, concentrations of HCHs ( $3.4 \times 10^1$ – $1.0 \times 10^3$  ng/L) in aqueous samples were the greatest (Table 4).

### 3.4. Potential for biological and ecological effects

Based on concentrations of instrumentally determined dioxin-like compounds, 2,3,7,8-TCDD equivalents (TEQs) were estimated using human/mammalian toxic equivalency factor as suggested by the World Health Organization (WHO) for PCDD/Fs, non- and mono-*ortho*-PCB congeners (Van den Berg et al., 1998) and relative potencies specific to H4IIE-luc assay for selected PAHs (Villeneuve et al., 2002). Concentrations of total TEQs ranged from  $4.3 \times 10^{-1}$  to  $1.1 \times 10^3$  pg/g, dw (Table 5). Greater than 70% of the total TEQ concentrations in most sediment samples were contributed by PCDD/Fs. The contributions to TEQs decreased in the order of PCDFs > PCDDs > dioxin-like PAHs > non-*ortho* PCBs > mono-*ortho* PCBs. This suggests that PCDD/Fs were responsible for the H4IIE-luc responses observed in sediment RE samples. Total concentrations of TEQ in sediment from locations Y2 and Y3 exceeded the sediment quality guideline (SQG) of  $1.4 \times 10^{-2}$  to  $2.1 \times 10^2$  pg/g, dw, reported for dioxin equivalents (Iannuzzi et al., 1995). Consensus SQGs for organic compounds including 13 individual PAHs, total PAHs, *p,p'*-DDE, total DDTs, and total PCBs were suggested to provide a unifying synthesis of existing SQGs (Swartz, 1999; MacDonald et al., 2000). SQGs such as effect range low (ERL) and threshold effects concentration (TEC) for PAHs and PCBs were applied to evaluate the quality of sediment in the Hyeongsan River (Long et al., 1995). Only one location (Y2) exceeded the ERL guideline of  $4.0 \times 10^3$  ng/g, dw, for total PAHs. Most of the individual PAHs at Y2

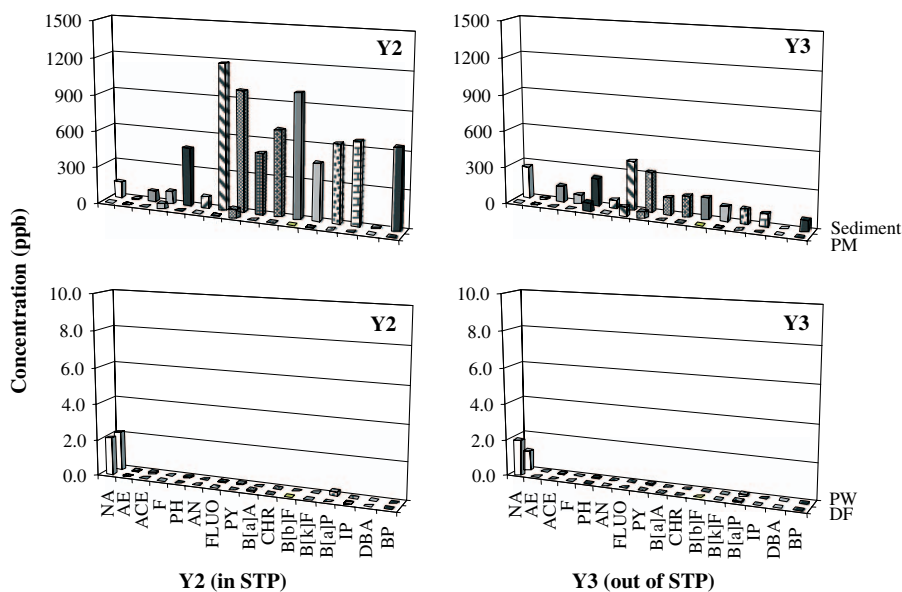


Fig. 3. Concentrations (ppb) of 16 individual polycyclic aromatic hydrocarbons (PAHs) in solid phase (sediment, particulate matter (PM); ng/g, dw) and aqueous phase (pore water (PW), dissolved water (DF); µg/L) samples from Hyeongsan River, Korea. NA, naphthalene; AC, acenaphthene; ACE, acenaphthylene; F, fluorene; PH, phenanthrene; AN, anthracene; FLUO, fluoranthene; PY, pyrene; B[a]A, benz[a]anthracene; CHR, chrysene; B[b]F, benzo[b]fluoranthene; B[k]F, benzo[k]fluoranthene; IP, indeno[1,2,3-*cd*]pyrene; DBA, dibenz[a,h]anthracene; BP, benzo[g,h,i]perylene.



Table 5

Instrumentally derived dioxin equivalents (TEQs) of PCDDs, PCDFs, PCBs, PAHs and bioassay derived dioxin equivalents (TCDD-EQs) for dioxin-like compounds associated with sediment samples from Hyeongsan River, Korea

TEQs conc.	Y1	Y2	Y3	Y4	Y5	Y6
TEQ <sub>PCDD</sub> <sup>a</sup>	0.16	937	74.9	3.0	2.8	2.1
TEQ <sub>PCDF</sub> <sup>a</sup>	0.22	100	197	5.7	6.9	3.4
TEQ <sub>PCB</sub> <sup>a</sup>	0.05	3.7	3.37	0.17	0.16	0.95
TEQ <sub>PAH</sub> <sup>a</sup>	<0.01	88	21.9	0.31	0.20	1.1
Total TEQs <sup>b,d</sup>	0.43	1130	298	9.1	10	7.5
TCDD-EQs <sup>c,d</sup>	0.01	1520	303	14	27	45
TCDD-EQs <sup>c</sup>	<0.01–0.41	1230–1880	265–360	11.7–15.8	26.5–26.8	14.7–135

<sup>a</sup> Instrumentally-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) of PCDD, PCDF, PCB, and dioxin-like PAH associated with sediment samples (pg TCDD/g, dw). Toxic equivalent factors or H4IIE-luc specific relative potency values for PCDDs/DFs, PCBs and AhR active PAHs (Van den Berg et al., 1998; Villeneuve et al., 2002).

<sup>b</sup> Total TEQs are sum of TEQ<sub>PCDD</sub>, TEQ<sub>PCDF</sub>, TEQ<sub>PCB</sub>, and TEQ<sub>PAH</sub>.

<sup>c</sup> Bioassay-derived dioxin equivalents (TCDD-EQs) of sediment raw extract (pg TCDD-EQ/g, dw).

<sup>d</sup> Total TEQs and TCDD-EQs were significantly correlated with  $r^2$  of 0.99 ( $p < 0.01$ ).

<sup>e</sup> TCDD-EQ uncertainty range (TCDD-Eqs). Uncertainty due to deviations from parallelism to the TCDD standard curve (Villeneuve et al., 2000).

location exceeded the ERLs whereas low molecular weight PAHs such as acenaphthene and fluorene were 5.5- and 5.3-fold greater than the ERL values, respectively. The consensus TEC value of  $2.9 \times 10^2$   $\mu\text{g/g}$ , OC (organic carbon) for total PAHs was not exceeded at any of the locations. Only one location (Y6) exceeded the ERLs for organochlorine pesticides whereas three locations exceeded the limit of  $2.3 \times 10^1$  ng/g, dw, for total PCBs. Concentrations of total PAHs and PCBs were generally less than the suggested SQGs, although their concentrations in some locations (Y2, Y3) were close to or slightly greater than the SQGs for toxic effects in benthic organisms.

### 3.5. Potency estimates of dioxin-like activity

Extracts (RE) from all six sampling locations induced significant dioxin-like responses in the H4IIE-luc bio-

assay (Fig. 4). Bioassay-derived dioxin equivalents (TCDD-EQ) were estimated directly from H4IIE-luc bioassay dose–response curves (not shown) generated by analyzing six dilutions of sediment RE. Estimates of concentrations of TCDD-EQs were derived using methods detailed elsewhere (Sanderson and Giesy, 1998; Villeneuve et al., 2000). Sample efficacy was generally less than TCDD and some deviation from parallelism to the TCDD standard curve was observed (Putzrath, 1997; Villeneuve et al., 2000). Nonetheless, except in the case of Y1, the dose–response curves were fairly complete and the uncertainty in the TCDD-EQs caused by deviations from parallelism to the TCDD standard curve is shown (Table 5). Thus, the TCDD-EQs derived should provide a reasonable approximation of the relative potency of the extracts.

Bioassay-derived TCDD-EQs were compared to instrumentally derived TEQs in a mass (potency) balance analysis (Sanderson and Giesy, 1998) in order to help assess whether the known composition of the samples could account for the potency of the responses observed in the H4IIE-luc bioassay (Table 5). Response magnitudes of sediment RE samples were less than the maximum response produced by a 1500 pM TCDD standard and ranged from 13 to 88%-TCDD-max. (Fig. 4). TCDD-EQ estimates for the Hyeongsan River sediment samples ranged from  $7.0 \times 10^{-3}$  to  $1.5 \times 10^3$  pg TCDD-EQ/g, dw (Table 5). This was similar to the range of TEQ concentrations ( $4.3 \times 10^{-1}$  to  $1.1 \times 10^3$  pg/g, dw) calculated by multiplying congener specific concentrations of AhR-active PCDD/Fs, PCBs, and PAHs by their WHO TEFs or H4IIE-luc-derived REPs (Table 5). The rank order of dioxin-like potency, based on TCDD-EQs estimates, was  $Y2 > Y3 > Y6 > Y5 > Y4 > Y1$ . The same rank order was observed for total TEQs in sediment samples except for Y6 ( $Y2 > Y3 > Y5 > Y4 > Y6 > Y1$ ). The lesser rank order of total TEQs in sediment from Y6 could be due to the lesser

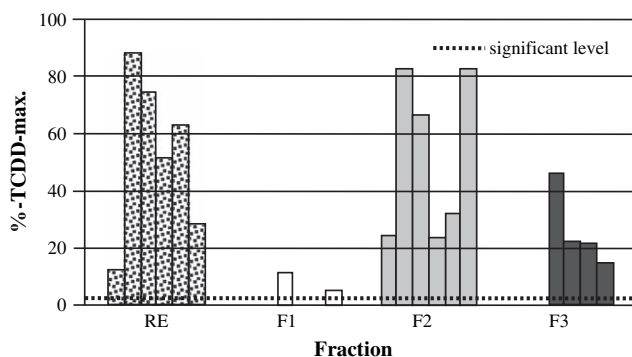


Fig. 4. Luciferase induction in the H4IIE-luc cell bioassay elicited by Hyeongsan River samples (single dose of sediment raw extract (RE) and fraction (F1, F2, F3) samples). Response magnitude presented as percentage of the maximum response observed for a 1500 pM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard (%-TCDD-max.). Horizontal line equals 3 standard deviations (expressed in %-TCDD-max.) above the mean solvent control response (set to 0%-TCDD-max.).

concentrations of more potent AhR agonists such as 2,3,7,8-TCDD and 1,2,3,7,8-TCDD relative to those in sediments from Y2 and Y3. RE TCDD-EQs were significantly correlated with total TEQs ( $r^2 = 0.99$ ,  $p < 0.01$ ). Based on potency estimates of RE samples, the H4IIE-luc results for the raw extracts appear consistent with the analytical characterization of the Hyeongsan River samples.

Given the uncertainty in the TCDD-EQ estimates (Table 5), and the potential variability/uncertainty in the TEQ estimates, there were no significant differences between the bioassay-derived TCDD-EQs and instrumentally derived TEQs. This supports the hypothesis that concentrations of known AhR agonists quantified in the Hyeongsan River sediment extracts can account for the potency observed in the H4IIE-luc bioassay. The hypothesis is further supported by the combination dose experiments which generally conformed to an additive model, with the exception of non-specific toxic effects on the cells. The results of this study suggest that all the AhR-active compounds contributing to the TEQ in sediments from the Hyeongsan River region were accounted for by PCDD/F, PCBs and PAHs.

Previous bioanalytical studies of Korean sediment extracts generally did not report mass (potency) balance (Khim et al., 1999a, 2001a). In previous studies, however, analytical characterization was limited primarily to PCBs and PAHs. Therefore, PCDD/Fs were in the category of “unidentified/unquantified AhR agonists” for the purpose of those studies. In the Hyeongsan River, PCDD/Fs were the primary source of total TEQs in the sediment extracts (Fig. 5). PAHs contributed less than 20% and in most cases less than 10% of the total TEQs (Fig. 5). PCBs contributed less than 15% of the total TEQs (Fig. 5).

### 3.6. Bioassay-directed fractionation

Three Florisil fractions of each extract were analyzed in order to examine the bioassay responses to specific classes of compounds separated based on polarity. F1 samples contained nearly all the PCB-derived TEQs ( $TEQ_{PCB}$ ), but very few PAH ( $TEQ_{PAH}$ ) or PCDD/F-derived TEQs ( $TEQ_{PCDD/F}$ ). As has been observed in previous studies of dioxin-like activity in Korean sediments, F1 samples induced little or no H4IIE-luc response (Khim et al., 1999a,b, 2001a; Koh et al., 2002). Thus, potency estimates of F1 samples were not reported. The efficacy of a single dose of F1 provided the only suitable indication of potential biological activity. Samples from locations Y3 and Y6 induced significant responses, but both were less than 15%-TCDD-max. Sediments from Y3 and Y6 had the second and third greatest concentrations of  $TEQ_{PCB}$ , respectively. Sediments from Y4, Y5, and Y1 had  $TEQ_{PCB}$  concentrations that were at least fivefold less. However, sample Y2 had

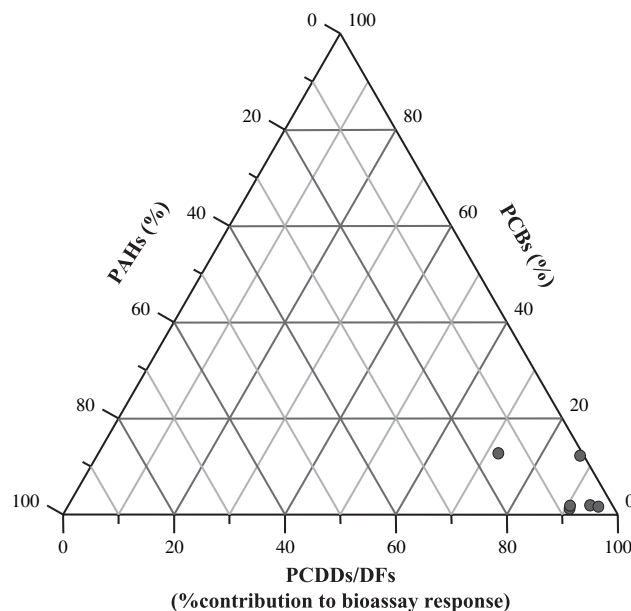


Fig. 5. Contribution (%) of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs; *X* axis), polychlorinated biphenyls (PCBs; *Y* axis), and polycyclic aromatic hydrocarbons (PAHs; *Z* axis) to total 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (TEQs) in raw sediment extracts from Hyeongsan River, Korea.

greater  $TEQ_{PCB}$  concentrations than did Y3 and Y6, but did not elicit a significant response in the bioassay. It is not clear why the Y2 F1 sample did not elicit a response. In general, however, the low response of F1 reflected the fact that  $TEQ_{PCB}$  contributed less than 15% of the total TEQs in each sample, and this was consistent with previous studies of Korean sediments (Khim et al., 1999a,b, 2001a; Koh et al., 2002).

Among the three fractions tested, the Florisil fraction 2 (F2) induced the greatest magnitude of response in the H4IIE-luc assay. This was consistent with previous studies of dioxin-like activity caused by Korean sediment extracts (Khim et al., 1999a,b, 2001a; Koh et al., 2002). Laboratory spike-recovery tests have shown that PAHs partition into F2 (Khim et al., 1999c). PCDD/Fs partition primarily to F2, but some may carry over into F3, as well (Khim et al., 1999c). Magnitudes of induction, elicited by F2 samples, ranged from 24 to 83%-TCDD-max. Without additional fractionation or chemical treatment to separate the effects of PAHs and PCDD/Fs, it was not possible to determine the proportion of response contributed by PAHs versus PCDD/Fs. No cytotoxic effects were associated with F2 samples.

Based on a single dose of F3 samples tested, four of the six F3 samples induced moderate responses (15–46%-TCDD-max.) in the H4IIE-luc bioassay (Fig. 4). Cytotoxicity and/or stressed cell morphology was observed for two of the six F3 samples (Y2 and Y6; Fig. 4); F3 of Y1 did not induce a significant response.

Overall, bioassay results for F3 samples were consistent with previous studies of Korean sediments that found both dioxin-like activity and cytotoxicity associated with Florisil fraction 3 (Khim et al., 1999b, 2001a). It is not clear, from the results of this study, whether the activity was due to PCDD/Fs that carried over into F3, or whether the activity was caused by unidentified, relatively polar, compounds present in Hyeongsan River sediment. Concentrations of  $TEQ_{PCDD/F}$  were certainly great enough to induce some H4IIE-luc response, even if only a fraction of the total carried over into F3 (Table 5). However, fraction-specific concentrations of  $TEQ_{PCDD/F}$  were not determined. Therefore, the identity of the AhR agonists present in F3 samples remains unclear. PCDD/Fs do not appear responsible for the cytotoxic effects of the F3 extracts. Although PCDD/Fs may have occurred in F3, even the concentrations present in the raw extract would not be expected to cause cytotoxicity.

### 3.7. Fate and distribution

Sampling location Y2 was a wastewater reservoir adjacent to a STP. Dissolved phase, pore water, particulate matter, and sediment samples were collected from both Y2 and Y3, which were directly downstream

of where the treated STP enters the Hyeongsan River. Thus, comparison of Y2 and Y3 samples can provide some insight regarding the effects of the sewage treatment process on the efficacy and fate of dioxin-like activity. Prior to sewage treatment (Y2), the greatest dioxin-like activity (52%-TCDD-max.) was observed in the non-polar fractions of sediment extracts (Fig. 6). The two particulate matter fractions induced the least response (<0 and 8%-TCDD-max.), while the dissolved and pore water fraction induced responses ranging from about 14 to 39%-TCDD-max. Following sewage treatment, the response induced by the sediment non-polar fraction was similar (69%-TCDD-max.), while the activity induced by the sediment polar fraction was greater (Fig. 6). It should be noted that the response of the in-plant polar fraction may have been masked by cytotoxic effects. The responses induced by the particulate, pore water, and dissolved fractions were relatively unchanged after sewage treatment relative to before. Overall, the results suggest that sewage treatment has relatively little effect on the dioxin-like activity of the wastewater being treated.

The profile of sediment responses suggests the treated effluent coming from the Posco industrial center as a likely source of dioxin-like contaminants in the Hyeongsan River. The raw extract of a sediment sample

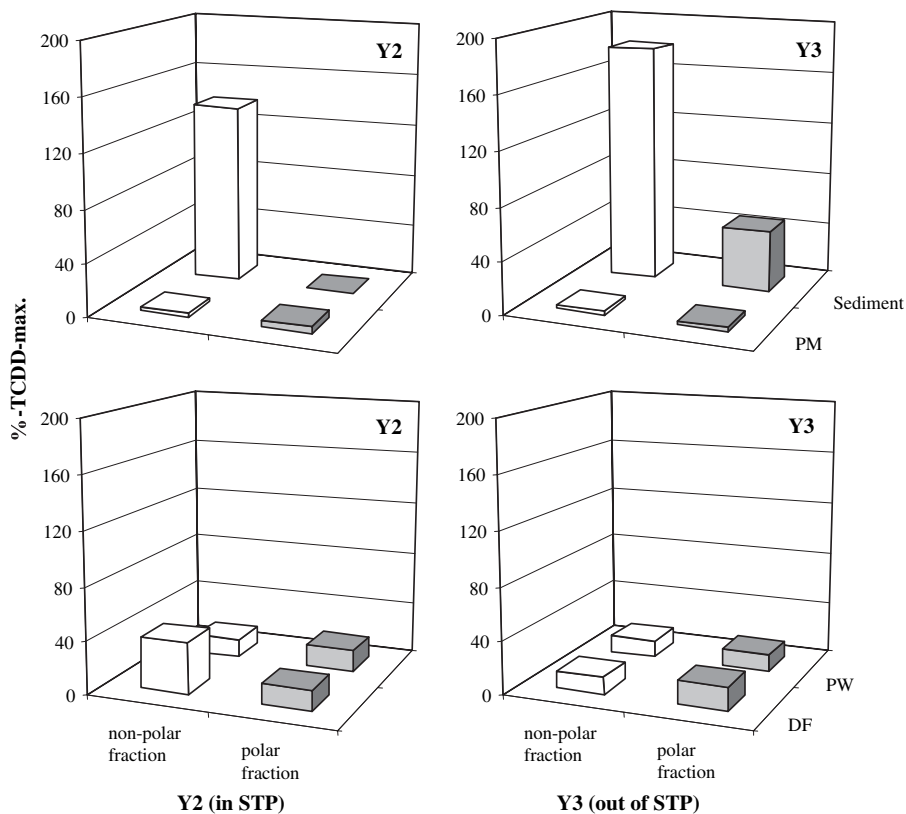


Fig. 6. Luciferase induction in the H4IIE-luc cell bioassay elicited by sewage samples (sediment, particulate matter (PM), pore water (PW), dissolved water (DF) from locations Y2 and Y3). Response magnitude presented as percentage of the maximum response observed for a 1500 pM 2,3,7,8-tetrachlorodibenzo-*p*-dioxin standard (%-TCDD-max.).

collected upstream of the Hyeongsan River induced relatively little activity (Fig. 4). A raw extract of sediment from the wastewater reservoir caused the greatest bioassay response, while raw extracts from downstream sites induced responses that were greater than the response produced by extracts of sediment from Y1, but less than that caused by Y2 (Fig. 4). The profile of sediment F3 responses also suggests an increase in polar AhR-active compounds downstream of the STP effluent (Fig. 4). Overall, the bioassay response profile is consistent with the analytical profile suggesting inputs from the Posco industrial center waste stream as likely sources of AhR agonists and dioxin-like contamination in the Hyeongsan River.

#### 4. Conclusion

Varying concentrations of PCDDs/DFs, PCBs, and PAHs were detected in sediment and water samples from Hyeongsan River. Mass balance analysis comparing instrumentally derived TEQs to bioassay-derived TCDD-EQs suggested that the known contaminant composition associated with the Hyeongsan river sediment samples could account for the dioxin-like potency observed in the H4IIE-luc bioassay. The evidence suggests that PCDD/Fs were responsible for dioxin-like potency observed, while PCBs and PAHs contributed relatively small fractions to the overall activity. These results suggest that future studies should focus efforts on characterizing the occurrence and distribution of PCDD/Fs in sediment, before initiating extensive bioassay-directed fractionation in search of other, relatively polar, AhR agonists.

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