BIOASSAY DIRECTED INSTRUMENTAL ANALYSES FOR CHARACTERIZATION OF DIOXIN-LIKE AND ESTROGENIC COMPOUNDS IN KOREAN SEDIMENTS

Chul-Hwan Koh[1], Jong Seong Khim[1,2], Kyu-Tae Lee[1], Daniel L.Villeneuve[2], Kurunthachalam Kannan[2], and John P. Giesy[2]

[1]School of Earth and Environmental Sciences (*Oceanography Program*), College of Natural Sciences, Seoul National University, Seoul 151-742, Korea
[2]National Food Safety and Toxicology Center, Department of Zoology and Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824, USA

Introduction

Instrumental analysis is useful for identifying compounds and quantifying the concentrations of environmental contaminants. However, instrumental analysis alone provides little information regarding the integrated biological relevance of a complex mixture of compounds associated with environmental samples such as sediment. Used appropriately, bioassay directed fractionation and mass balance analyses are powerful tools to characterize the causative agents responsible for bioassay responses observed. Recent studies have indicated that sediment organic extracts from Korean coasts, elicit both dioxin-like and estrogenic responses significantly *in vitro,* although the chemical concentrations often did not explain the bioassay activities observed[1-5]. Thus, a combination use of bioassay and instrumental analysis is an important approach to assess sediment contamination since the sediment extracts may contain a myriad of potentially bio-active/toxic compounds, which were not analyzed by instrumental methods. Risk assessment based solely on the instrumental results may underestimate the potential hazard of sediment contamination. The objective of this presentation is to overview the bioassay directed instrumental analyses and mass balance approaches. Additionally, the current status of organic contamination in Korean sediment was summarized.

Methods

Instrumental Analysis: Sediment samples were collected from more than 100 locations on Korean coastal areas (Lake Shihwa, Masan, Ulsan, and Onsan Bay) during 1998-1999 (Figure 1). Sediment samples were Soxhlet extracted using 400 mL dichloromethane and extracts were passed through 10 g of activated Florisil packed in a glass column (10 mm i.d.) for fractionation. Three fractions (F1, F2, and F3) were collected by polarity using solvents appropriate for both instrumental analyses and bioassay. Reverse phase high performance liquid chromatography (HPLC) with fluorescence detection was used to quantify nonylphenol (NP), octylphenol (OP) and bisphenol A (BPA). Polycyclic aromatic hydrocarbons (PAHs) were quantified using a Hewlett Packard 5890 series II gas chromatograph equipped with a 5972 series mass spectrometer detector. Polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides were quantified using a gas chromatograph (Perkin Elmer series 600) equipped with ^{63}Ni electron capture detector (GC-ECD). Further details of the instrumental analysis are presented elsewhere[1,2,4].

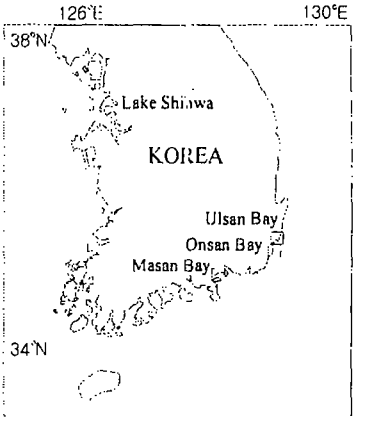

Figure 1. Map of the study areas in Korea

***In Vitro* Bioassay and Mass Balance Analysis**: Each sample was tested as raw extract (RE) and three fractionated extract (FE) using *in vitro* bioassays (H4IIE-luc recombinant cells for dioxin like activity and MVLN recombinant cells for estrogenic activity). Luciferase and protein assays were conducted after 72 h of exposure. Sample responses, expressed as mean RLU over three replicate wells, 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.) or 17-β-estradiol (E2, %-E2-max.) standard curves generated on the same day. Where appropriate, sample potency relative to the TCDD or E2 standard, was estimated. Mass balance analysis (or potency balance analysis) was used to examine whether or not the known composition of a sample (identified by instrumental analysis) could account for the magnitude or potency of biological response observed. Two types of mass balance analysis (potency-based and magnitude based) were applied to aid in discussion and hypothesis generation regarding the probable causes of dioxin-like and estrogenic activity associated with samples. Further details of the bioassay procedures and mass balance analysis have been presented elsewhere[1,3,5].

Results and Discussion

Instrumental Analysis: Sediment samples were analyzed for PCBs, OC pesticides, PAHs, NP, OP, BPA. Generally, the relative abundance of organic contaminants in Korean sediment was in the order, NP > PAHs > PCBs ≈ OP > BPA > OC pesticides (Table 1). Spatial distributions of these contaminants in each area suggested that their sources were independent of each other. However, there were some localized zones (estuary, harbor, and ship building area) of relatively greater concentrations

of PAHs and APs. Although the mean concentrations of PCBs and PAHs in Korean sediments were less than the suggested sediment quality guidelines (SQG), concentrations in some locations were close to or above the SQG for toxic effects in benthic organisms[6,7].

Table 1. Concentrations (ng/g dry wt) of target organic contaminants in sediment from Lake Shihwa, Masan, Ulsan, and Onsan Bay, Korea

Locations	PCBs	DDTs	PAHs	NP	OP	BPA
Lake Shihwa	<1.00-12.3	0.45-1.94	<10.0-30.8	20.2-1820	4.69-50.5	-
(n=11, Feb, 1998)[a]	(7.18)[b]	(1.29)	(23.3)	(616)	(17.9)	(-)
Masan Bay	10.3-127	0.40-12.6	41.5-1100	113-3890	3.97-179	2.70-50.3
(n=28, May, 1998)	(38.4)	(3.45)	(353)	(510)	(18.1)	(11.5)
Ulsan Bay	<1.00-76.7	<0.01-41.9	<10.0-3100	<1.00-1040	<1.00-120	<1.00-53.5
(n=32, May, 1999)	(17.6)	(3.35)	(408)	(97.9)	(17.8)	(10.9)
Onsan Bay	<1.00-56.2	0.01-7.58	<10.0-573	<1.0-860	<1.0-11.0	<1.00-204
(n=23, May, 1999)	(15.6)	(1.29)	(89.0)	(86.0)	(5.62)	(27.1)

[a] Values in parenthesis indicate the total number of sediment samples and the date of collection.
[b] Values in parenthesis indicate the mean concentration of compounds.

Table 2. Potency based (TCDD-EQ) and magnitude based (%-TCDD-max.) mass balance analysis for PAH compounds associated with sediment samples from Ulsan Bay, Korea

Sampling Location	TEQ_{PAH}[a]	$TCDD\text{-}EQ_{50}$[b]	%-TCDD-Max	
	pg TEQ_{PAH}/g	Pg TCDD-EQ/g	Calculated[c]	Observed[d]
U1	1.24	52.2	5.50	72.6
U2	0.20	NA[e]	<0.00	NA
U3	0.27	NAC[f]	<0.00	30.8
U4	0.91	NAC	4.23	43.1
U5	0.75	NA	3.16	NA
U6	7.80	93.2	16.6	74.2
U7	11.6	129	19.8	83.4
U8	0.76	NA	2.57	NA
U9	3.84	43.8	11.6	67.0
U10	0.30	NA	<0.00	NA
U11	0.52	NA	0.66	NA
U12	0.37	NA	<0.00	NA
U13	0.94	NAC	4.29	43.0
U14	0.17	NAC	<0.00	24.3
U15	0.52	NAC	0.99	44.1
U16	14.5	74.1	22.5	71.9

[a] Instrumentally-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (TEQs) of PAHs associated with sediment samples. Refer to the TEQ_{PAH} generated from assay specific REP-50 value and selected PAH concentrations (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, and dibenz(a,h)anthracene)[8].
[b] Bioassay-derived TCDD equivalents (TCDD-EQs) of PAHs sediment fraction (pg TCDD/g dry wt). Refer to the dioxin equivalents generated from one-point estimates made for response of 50%-TCDD-max.
[c] Regression of TEQ_{PAH} against the TCDD standard curve was used to predict the magnitude of bioassay response.
[d] Observed bioassay response (%-TCDD-max.) of sediment fraction F2 samples contained PAHs.
[e] NA: not analyzed.
[f] NAC: not available data for the calculation of TCDD-EQs, i.e., dose response curve could not obtained in the full dose response bioassay.

***In Vitro* Bioassay and Mass Balance Analysis**: In general, sediment organic extracts elicited significant bioactivity in both dioxin-like and estrogenic responses *in vitro*. Dioxin-like activity has consistently been associated primarily with compounds present in F2 and F3 samples. For example, magnitudes of response observed for sediment F2 samples (Ulsan Bay) were much greater than magnitudes predicted based on the maximal potential TEQ_{PAH} in the samples (Table 2). Empirical bioassay results and mass balance analyses suggested that the target compounds quantitated by instrumental analysis accounted for only a portion of the mechanism specific biological activity of Masan and Ulsan Bay sediment extracts. The F2 responses may be explained by the PCDDs, PCDFs etc. and/or derivatives of PAHs in F2. Future studies should employ high-resolution gas chromatography equipped with mass spectrometry for identification and/or quantification of these compounds to address the potential contribution of these known aryl hydrocarbon receptor (AhR) agonists. F3 responses were more perplexing. Prototypical dioxin-like compounds would not be expected to partition to F3. Current evidence suggests that the dioxin-like compounds present in F3 are relatively polar and acid labile[3]. Little other information is known so far. Most of estrogenic activities associated with F3 samples could not be explained by the concentrations of known estrogen receptor (ER) agonists such as alkylphenols and BPA. Based on qualitative and quantitative mass balance analysis, known concentrations of prototypical xenoestrogens can account for only a portion of estrogenic activities observed in F3 samples. These results suggest that F3 samples contained unidentified or non-detectable bioactive compounds that contributed to the MVLN responses. Overall, additional bioassay directed fractionation and instrumental quantification will be required to identify the active agents, both dioxin-like and estrogenic compound present in F2 and F3 samples.

Acknowledgements
This work was supported from National Institute of Environmental Research (NIER), Ministry of Environment, Korea (Sediment Organic Compounds Bioassay Study; SORGBIOS 98-2000). The chemical and bioassay analyses were performed at MSU. We thank colleagues from the Benthos Lab at Seoul National University, Korea and the Aquatic Toxicology Lab at Michigan State

University, USA.

References

1. Khim J.S., Villeneuve D.L., Kannan K., Lee K.T., Snyder S.A., Koh C.H., and Giesy J.P. R.J. (1999) *Environ Toxicol Chem* 18:2424-2432.
2. Khim J.S., Villeneuve D.L., Kannan K., Koh C.H., and Giesy J.P. (1999) *Environ Sci Technol* 33:4199-4205.
3. Khim J.S., Kannan K., Villeneuve D.L., Koh C.H., and Giesy J.P. (1999) *Environ Sci Technol* 33:4206-4211.
4. Khim J.S., Lee K.T., Villeneuve D.L., Kannan K, Giesy J.P.,and Koh C.H. (2000) *Arch Environ Contam Toxicol* 40:141-150.
5. Khim J.S., Lee K.T., Villeneuve D.L., Kannan K, Giesy J.P., and Koh C.H. (2000) *Arch Environ Contam Toxicol* 40:151-160.
6. Swartz R. (1999) *Environ Toxicol Chem* 18:780-787.
7. MacDonald D.D., Dipinto L.M., Christopher J.F., Ingersoll C.G., Long E.R., and Swartz R. (2000) *Environ Toxicol Chem* 19:1403-1413.
8. Khim J.S., Kannan K., Villeneuve D.L., Kang J., Koh C.H., and Giesy J.P. (2000) *Organohalogen Compounds* 46:455-458.