RF-064 **Study on contamination by candidates of persistent organic pollutants in the Asia-Pacific region and development of new monitoring methods for these compounds (Abstract of the Final Report)**

Contact person Shin Takahashi
Associate Professor, Division of Environmental Chemistry
Center for Marine Environmental Studies
Ehime University
Bunkyo-cho 2-5, Matsuyama 790-8577, Japan
TEL&FAX: +81-89-927-8196
e-mail: shint@agr.ehime-u.ac.jp

Total Budget for FY2006-FY2007 19,304,000Yen (**FY2007**; 9,307,000Yen)

Key Words Brominated Flame Retardants, Polybrominated Diphenyl Ethers, Hexabromocyclododecanes, Bioassay, Dioxin-like Compounds

1. Introduction

The Stockholm Convention on Persistent Organic Pollutants (POPs) has entered into force since May, 2004. The convention prohibits the production and use of certain POPs such as PCBs and aims to eliminate dioxins. Recently, environmental issues relating to brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) have become a matter of great concern due to their persistent and bioaccumulative properties and potential toxic risk to humans. These compounds can be considered as 'candidate POPs' because their physico-chemical properties and environmental behavior seem to be similar to those of legacy POPs such as PCBs. The growth of interest in PBDEs has been exponential as their apparent increase in the environment over the last few decades in Europe and North America. However, there is still little information on contamination by BFRs in the Asia-Pacific region. Significant contamination by BFRs may arise from rapid industrialization in some Asian countries. Besides, advantages of bioassays, i.e. the extremely high sensitivity, rapid and easy clean-up/work-up, small sample size and reduced cost compared to instrumental methods, are expected to facilitate environmental monitoring and risk assessment of POP-related compounds in Asian developing countries.

2. Research Objectives

Based on such a background, we have started monitoring studies on the contamination status of candidate POPs such as PBDEs and HBCDs in the Asia-Pacific region and development of new monitoring methods for POP-related compounds. In the sub-theme one, we aimed to develop a new analytical method for BFRs and to elucidate contamination status of BFRs as well as other POPs such as PCBs by using 'bioindicators' such as mussels, human breast milk and higher

trophic animals from various Asian countries and North Pacific. Temporal trends of contamination by BFRs in some Asian regions were also investigated by using archived marine mammal and sediment core samples. In the sub-theme two, a bioassay technology, Dioxin Responsive-Chemical Activated LUciferase gene eXpression (DR-CALUX), was developed for the determination of candidate POPs and dioxin-related compounds and applied for monitoring sediment and soil samples from Japan and Asian developing countries. Toxicity Identification and Evaluation (TIE) approach was also examined to identify and prioritize causative compounds in combination with chemical analysis by HRGC/HRMS. Furthermore, we examined 1) photolytic debromination of BFRs such as technical DecaBDE (including decabrominated diphenyl ether as a major compound) and decabromodiphenyl ethane (DeBDethane) and subsequent formation of brominated dioxins/furans (PBDD/DFs) in plastics and 2) thermal degradation/formation of BFRs and dioxin related compounds during incomplete combustion of waste printed circuit boards (P-CBs) to assess the potential sources and emission of BFRs and dioxin related compounds in uncontrolled waste treatment processes in developing countries.

3. Research Methods

Mussel and human breast milk samples archived in our specimen bank (*es*-BANK) were used as bioindicators to elucidate the pollution status and distribution of BFRs and other POPs in the Asia-Pacific region. The muscle, liver or fat samples of higher trophic animals such as raccoon dog, common cormorant, jungle crow and various raptors from Japanese inland and coastal regions and offshore species such as striped dolphin, melon headed whale, albatross and northern fulmar from North Pacific were analyzed to understand bioaccumulation features of BFRs and their distribution in ecosystems. To clarify the temporal trends, the archived fat tissue samples of northern fur seals from the Pacific coast of northern Japan and cetaceans stranded along the coasts of Japan and South China as well as sediment core samples from Tokyo Bay were employed for the chemical analysis. Surface sediment and sediment core samples from Osaka Bay and soil samples from waste dumping sites in Asian developing countries such as India, Cambodia and Vietnam were employed for DR-CALUX and HRGC/HRMS.

PBDEs and HBCDs were determined by GC/MS (LRMS&DFMS) and LC-MS/MS, respectively, with carbon isotope labeled surrogates (i.e. internal standard methods). Dioxin like POPs such as PCDD/Fs, PCB, PBDD/Fs, PXDD/Fs and PBDEs in sediments and soils were analyzed by HRGC/HRMS. A cell-based bioassay: DR-CALUX was applied to the sediment samples for quantitative screening of dioxin-like POPs including the above substances. For the CALUX assay, the target compounds are ligands which bind to AhR in recombinant H4IIE-*luc* cells and the endpoint is based on AhR-mediated firefly (*Photinus pyralis*) luciferase gene expression. Using the data from the chemical analysis, Chemical-TEQ values were calculated and compared to CALUX-derived TEQ values (CALUX-TEQs) to account for contribution of POPs and its candidates to overall CALUX-TEQs.

For the sunlight irradiation experiment, three different plastic powder samples including

high impact polystyrene (HIPS) with technical DecaBDE, HIPS with technical DeBDethane, and used TV casing were transferred to quartz tubes and placed in a temperature-controlled glass room from September 2006 to January 2007. PBDEs, DeBDethane, and PBDD/Fs in the samples collected at 0, 7, 14, 28, 56, 112, and 224 days after exposure were quantified by a HRGC/HRMS.

Combustion experiment of waste P-CBs was performed using the thermal treatment plant equipment at National Institute for Environmental Studies, Japan. PBDEs, DeBDethane, tetrabromobisphenol A (TBBPA), HBCDs, polybromobenzenes (PBBzs), polybromophenols (PBPhs), PBDD/Fs, PXDD/Fs and PCDD/Fs were analyzed in the samples of waste P-CBs, flue gas and ash. In addition to these compounds, tri-alkyl/aryl phosphates (PFRs) were examined in waste P-CBs. Quantification was performed by LC/MS/MS for TBBPA and HBCDs, GC/LRMS for PFRs, and HRGC/HRMS for other compounds.

4. Results and Discussion

Contamination Status and Temporal Trend in the Asia-Pacific Region

New analytical method for the determination of HBCDs by LC-MS/MS was combined with our previous method for PBDEs. QA/QC for the new procedure of BFR analysis was confirmed by analyzing a reference sample (animal fat) and comparing with reported values for HBCDs and PBDEs in an interlaboratory study[1)]. Our data obtained by the new method were within the range of reported values from other laboratories.

Our results of Asia-Pacific Mussel Watch Project showed a widespread contamination by PBDEs in the coastal waters of Asia with higher concentrations in mussels from Hong Kong and Korea (Fig. 1). Our another study focused on cetaceans also showed the highest PBDEs concentrations in samples from Hong Kong [2)]. These results suggest the existence of significant pollution sources of PBDEs even in some Asian developing nations. The highest concentrations of PBDEs found in mussels and cetaceans from coastal waters in Hong Kong and Korea seem to be comparable to those in European countries. HBCDs were also detected in all the mussels from Korea. To our knowledge, this is the first report on the detection of HBCDs in Asian countries except for Japan. No significant relationship was observed between the concentrations of PBDEs and HBCDs in mussel samples, indicating different pollution source for these compounds.

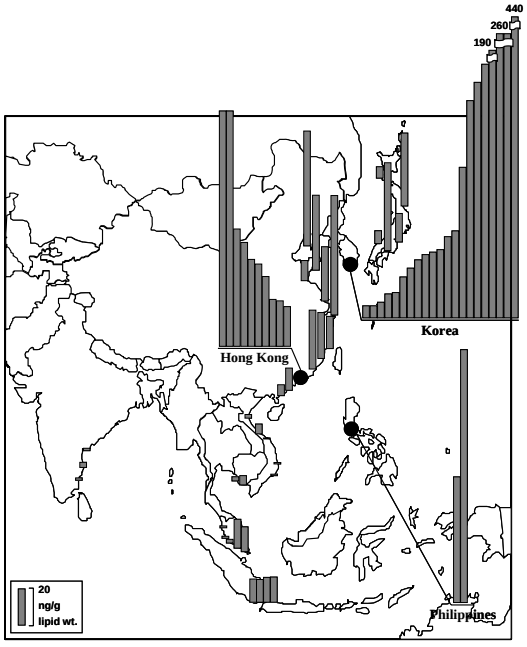

Fig. 1. PBDEs concentrations in mussels from Asia-Pacific region

PBDEs were detected in all the milk samples from Japan, China, the Philippines, Malaysia, Korea, Indonesia, Cambodia, Vietnam and India. The results indicate that, similar to that in developed nations, human exposure to PBDEs is also occurring in Asian developing countries. Nevertheless, the concentrations of PBDEs in human milk were much lower than those of PCBs and DDTs. The extent of contamination by PBDEs in human milk from Asian countries seems to be comparable to those reported from Europe, but one or two orders of magnitude lower than levels found in North America. Although relatively high concentrations of PBDEs were found in milk samples from China and the Philippines, spatial difference in PBDE levels between the countries was relatively small compared with those found in marine organisms. In addition, composition profiles of PBDEs in the human milk were rather different among countries and those from marine organisms, suggesting various sources and routes of human exposure to PBDEs.

PBDEs and PCBs were detected in all the samples of higher trophic animals from Japanese inland and coastal regions and offshore waters of North Pacific. Among these animals, raptors such as Steller's sea lion and goshawk showed the highest concentration of PBDEs (Fig. 2). In addition, relatively high concentrations of PBDEs were found in jungle crow with higher proportions of higher brominated isomers such as BDE 209. These results indicate the biomagnification of PBDEs through the foodweb and significant exposure to technical DecaBDE in the terrestrial/coastal ecosystem. In comparison with PCBs, higher PBDE/PCB concentration ratios were observed in Japanese coastal and inland animals than in offshore species, suggesting differences in transport behavior and/or biotransformation capacity between PBDEs and PCBs as well as the input of PBDEs into the terrestrial environment. Besides, HBCDs were detected in almost all the animal samples analyzed in this study, indicating widespread contamination of this BFR. Higher concentrations were found in offshore species of cetaceans and fish eating bird, common cormorant, than in a terrestrial animal, raccoon dog, indicated significant biomagnification of HBCDs in the aquatic foodweb and its long range transport to offshore waters.

Analytical results of the archived samples of northern fur seals from the Pacific coast of northern Japan indicated peak concentration of PBDEs

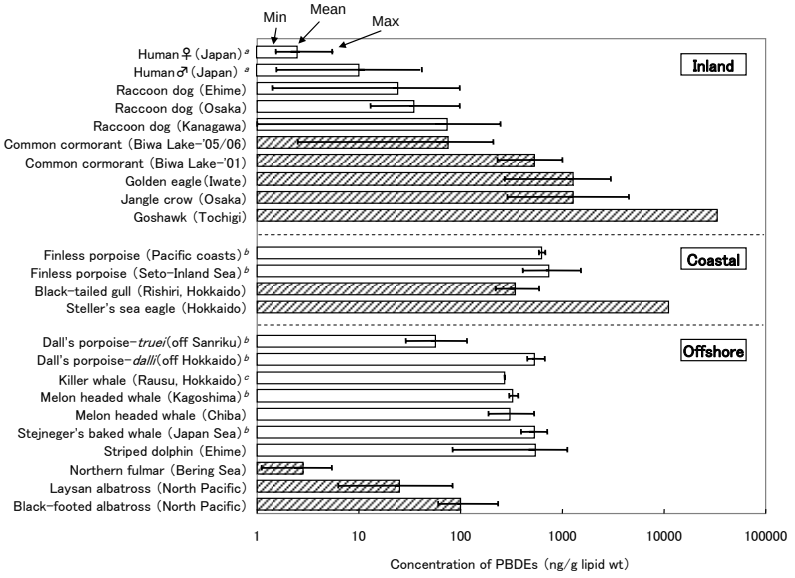

Fig. 2. Comparison of PBDE concentrations among higher trophic animals from Japanese inland and coastal regions and offshore waters of North Pacific (white and slanting bars indicate the data on mammals and avian species, respectively).

around 1991-1994, and slight decrease in the late 1990s[3)], while, noteworthily, a significant increasing trend in the concentrations of HBCDs was found in fur seals collected in 1990s. The concentrations of HBCDs were almost close to those of PBDEs in fur seals collected during the late 1990s. Increasing trends of BFRs, particularly HBCDs, during the last few decades were also found in cetaceans stranded along the coasts of Japan and in sediment core samples from Tokyo Bay (Fig. 3). On the other hand, finless porpoises from South China showed no apparent trend for HBCDs but a significant increase in PBDE levels during the last decade was observed. Considering the highest levels of PBDEs in finless porpoises from South China among cetacean species from other Asian waters, contamination by BFRs in this region would become more serious in future.

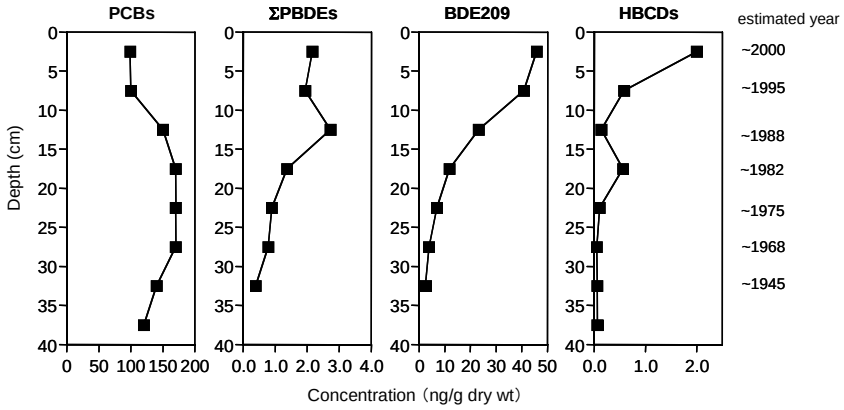

Fig. 3. Temporal trends in the concentrations of PCBs, ΣPBDEs (sum of mono to nona-BDEs), BDE 209 (deca-BDE) and HBCD in a sediment core sample from Tokyo Bay.

Monitoring and Toxic Identification Evaluation by Using Bioassay and Behavior in Photolytic and Combustion Processes

The combinatorial bio/chemical investigation of sediments from Osaka Bay, Japan was conducted to clarify the horizontal and vertical distribution profiles of POPs related compounds in the sediments (Fig. 4). For surface sediments, a significant correlation (r^2 = 0.96) was observed between World Health Organization-toxicity equivalent (WHO-TEQ) and the bioassay-TEQ (CALUX-TEQ) values. On the other hand, CALUX-TEQ values were 1-5-fold more than WHO-TEQ values in all the surface and core samples. CALUX-TEQ values were calculated for PBDE and PBDD/F concentrations, employing their CALUX toxicity equivalent factors (CALUX-TEFs). The estimated CALUX-TEQ values obtained for the brominated compounds accounted for 11% on average (range 4.7 – 31%) of the experimentally obtained CALUX-TEQ values in the investigated surface sediments. The same integrated approach was conducted for the evaluation of dioxin-like compounds in soil samples (at dumping sites and reference locations) from Cambodia, Vietnam and India. Chemical analysis indicated that PCDD/Fs dominated the chemical TEQ profiles in the investigated soil, which were in the range of 0.14－1,700 pg/g dry weight as WHO-TEQ. For these samples, CALUX-TEQ was between <1.4－2,300 pg/g dry wt and the ratio/chemical-TEQ ratio was in the range of 0.5 to 2, which showed good performance of DR-CALUX for WHO-TEQ prediction.

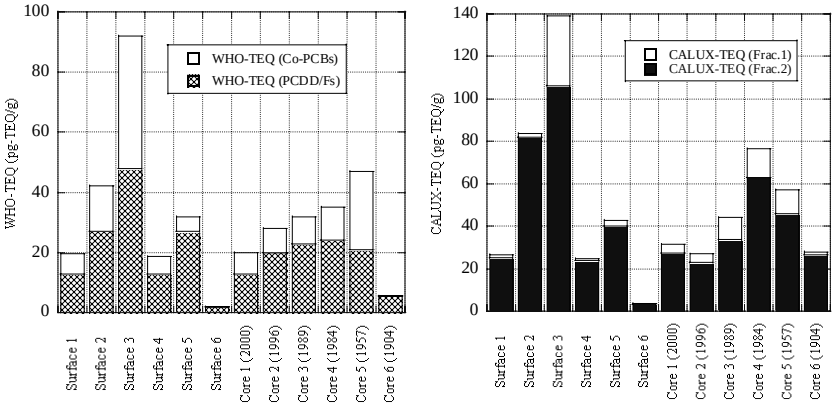

Fig. 4. WHO-TEQ (left) and CALUX-TEQ (right) values in surface and core sediments from Osaka Bay.

During the photolytic study, BDE 209 in pulverized HIPS+DecaBDE samples degraded with a half-life of 51 days. In contrast, no marked loss of DeBDethane occurred throughout the experimental period of 224 days. During BDE 209 photolysis in HIPS+DecaBDE samples, partial debromination to nona- and octa-BDEs was observed, however, environmentally relevant PBDE congeners such as BDE 47, 99, and 100 were not formed. Formation of PBDFs was clearly apparent in the flame-retarded plastics that we investigated. In the HIPS+DecaBDE samples, the PBDF concentration increased by about 40 times after 1 week of exposure, with a concomitant decrease in BDE 209. In the TV casing, tetra- to octa-BDF congener concentrations increased continuously during the experiment (Fig. 5). Although the concentrations of PBDFs found in the plastic matrices tested were one to four orders of magnitude lower than those of PBDEs, more attention should be paid to the fact that PBDFs are formed by sunlight exposure during normal use as well as disposal/recycling processes of flame-retarded consumer products.

PBDEs were found at the highest concentrations in waste P-CBs, followed by DeBDethane > PBPhs > PFRs > TBBPA > PBBzs > PBDD/Fs > PCDD/Fs. HBCDs and PXDD/Fs were not detected. Bromine content in these compounds was only 2.6% of the total bromine content in waste P-CBs. PBPhs in recent waste P-CBs (used in this study) were at higher levels than those in old ones, while levels of PBDEs, PBBzs, TBBPA and PBDD/Fs were extremely lower. Major isomers of PBPhs have been used as a raw material and an

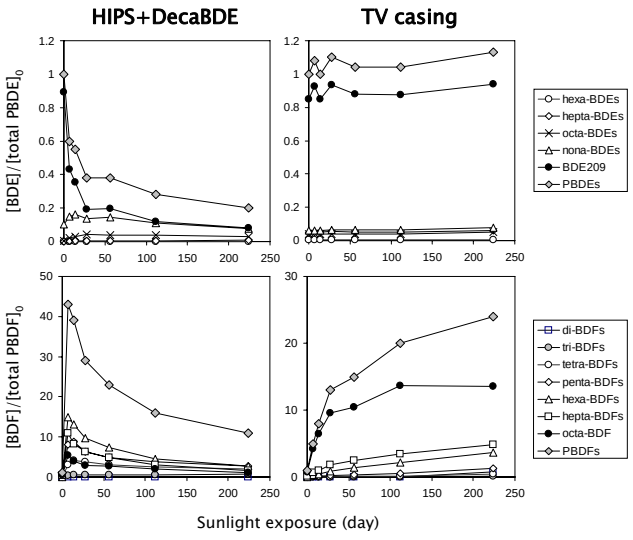

Fig. 5. Photolytic decomposition/formation behavior of PBDEs and PBDFs in HIPS+DecaBDE and TV casing. [A]: molar concentrations of compound A

end-cap for some BFRs, such as brominated epoxy resins and bis(tribromophenoxy)ethane. These results imply that BFRs with high molecular weight and reactive types might have been applied in recent P-CBs and their mount devices. Compounds, which were used as BFRs, were decomposed during incomplete combustion (primary combustion). In contrast, thermal formation was observed for other brominated organic and dioxin-related compounds (Fig. 6). After incomplete combustion, the much higher amounts of most compounds were found in flue gas than in ash. Most amounts of brominated organic compounds and PCDD/Fs, which were remained in flue gas after incomplete combustion, were decomposed and/or removed during flue gas treatment (Fig. 6). These results indicate that emission of brominated organic compounds and PCDD/Fs to the environment can be decreased by appropriate management of flue gas treatment. Amounts of some compounds, such as PBDD/Fs, PXDD/Fs and PCDD/Fs, in ashes were comparable or higher than inputs, implying that it was important to understand the behavior of these compounds during waste treatment and recycling of ashes.

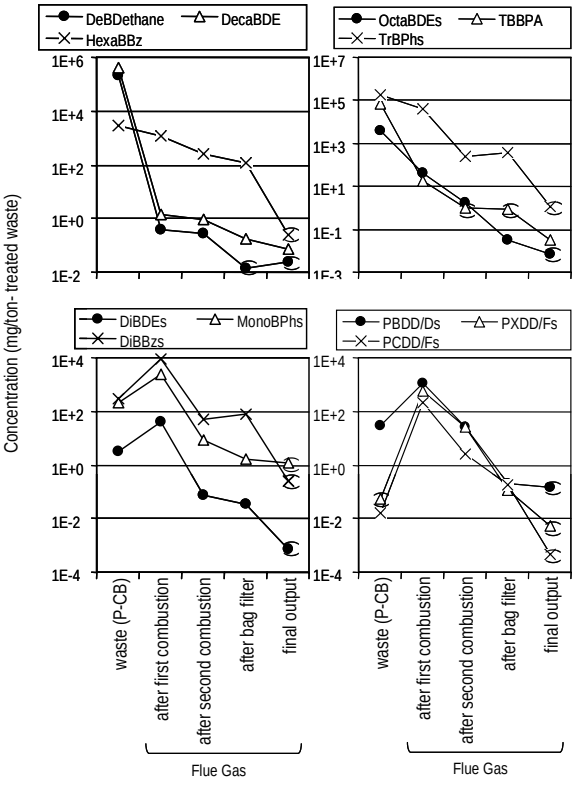

Fig. 6. Behavior of BFRs and dioxin related compounds during waste combustion and flue gas treatment processes (the symbols in parenthesis are below the detection limits).

References

1) Takahashi, S., Sakai, S., Watanabe, I. (2005) An intercalibration study on organobromine compounds: brominated flame retardants and related dioxin-like compounds in waste TV cabinet and animal fat. *Organohalogen Compd*., 67: 494-497.

2) Kajiwara, N., Kamikawa, S., Ramu, K., Ueno, D., Yamada, T.-K., Subramanian, A., Lam, P. K. S., Jefferson, T. A., Prudente, M., Chung, K.-H. and Tanabe, S. (2006) Geographical distribution and temporal trends of polybrominated diphenyl ethers (PBDEs) in cetaceans from Asian waters. *Chemosphere*, 64: 287-295.

3) Kajiwara, N., Ueno, D., Takahashi, A., Baba, N., Tanabe, S. (2004) Polybrominated diphenyl ethers and organochlorines in archived northern fur seal samples from the Pacific coast of Japan, 1972-1998. *Environ. Sci. Technol.*, 38: 3804-3809.

Major Publications

1) Takigami, H., Behnisch, PA., Shiozaki, K., Ohno, M., Sakai, S.I. (2006) Bioassay

monitoring of waste PCB samples during chemical destruction treatments. *Water Sci Technol.*, 53: 43-50.

2) Hirobe, M., Goda, Y., Okayasu, Y., Tomita, J., Takigami, H., Ike, M., Tanaka, H. (2006) The use of enzyme-linked immunosorbent assays (ELISA) for the determination of pollutants in environmental and industrial wastes. *Water Sci Technol*., 54: 1-9.
3) Ramu, K., Kajiwara, N., Isobe, T., Takahashi, S., Kim, E.Y., Min, B.Y., We, S.U., Tanabe, S. (2007) Spatial distribution and accumulation of brominated flame retardants, polychlorinated biphenyls and organochlorine pesticides in blue mussels (*Mytilus edulis*) from coastal waters of Korea. *Environ. Pollut.* 148: 562-569.
4) Minh, N. H., Isobe, T., Ueno, D., Matsumoto, K., Mine, M., Kajiwara, N., Takahashi, S., Tanabe, S. (2007) Spatial distribution and vertical profile of polybrominated diphenyl ethers and hexabromocyclododecanes in sediment core from Tokyo Bay, Japan. *Environmental Pollution,* 148: 409-417.
5) Ramu, K., Kajiwara, N., Sudaryanto, A., Isobe, T., Takahashi, S., Subramanian, An., Ueno, D., Zheng, G. J., Lam, P. K. S., Takada, H., Zakaria, M. P., Viet, P. H., Prudente, M., Tana, T. S., Tanabe, S. (2007) Asian mussel watch program: contamination status of polybrominated diphenyl ethers and organochlorines in coastal waters of Asian countries. *Environ. Sci Technol.,* 41: 4580-4586.
6) Isobe, T., Ramu, K., Kajiwara, N., Takahashi, S., Lam, P. K. S., Jefferson, T. A., Zhou, K., Tanabe, S. (2007) Isomer specific determination of hexabromocyclododecanes (HBCDs) in small cetaceans from the South China Sea – Levels and temporal variation. *Mar. Pollut. Bull*., 54: 1139-1145.
7) Tanabe, S., Kunisue, T. (2007) Persistent organic pollutants in human breast milk from Asian countries. *Environ. Pollut.,* 146: 400-413.
8) Go Suzuki, Hidetaka Takigami, Kazutoshi Nose, Shin Takahashi, Misuzu Asari, and Shin-ichi Sakai. (2007) Dioxin-Like and Transthyretin-Binding Compounds in Indoor Dusts Collected from Japan: Average Daily Dose and Possible Implications for Children. *Environ. Sci. Technol.*, 41: 1487 -1493.
9) Takigami, H., Etoh, T., Nishio, T., Sakai, S. (2008) Chemical and bioassay monitoring of PCB-contaminated soil remediation using solvent extraction technology. *J. Environ. Monitor.*, 10: 198-205.
10) Tanabe, S., Ramu, K., Isobe, T., Takahashi, S. (2008) Brominated flame retardants in the environment of Asia-Pacific: an overview of spatial and temporal trends. *J. Environ. Monitor.*, 10: 188-197.
11) Sudaryanto, A., Kajiwara, N., Takahashi, S., Muawanah, Tanabe, S. (2008) Geographical distribution and accumulation features of PBDEs in human breast milk from Indonesia. *Environ. Pollut.*, 151: 130-138.
12) Kunisue, T., Takayanagi, N., Isobe, T., Takahashi, S., Nakatsu, S., Tsubota, T., Okumoto, K., Bushisue, S., Shindo, K., Tanabe, S. (2008) Regional trend and tissue distribution of brominated flame retardants and persistent organochlorines in raccoon dogs (*Nyctereutes*

procyonoides) from Japan. *Environ. Sci. Technol.*, 42: 685-691.

13) Kunisue, T., Higaki, Y., Isobe, T., Takahashi,S., Subramanian, A., Tanabe, S. (2008) Spatial trends of polybrominated diphenyl ethers in avian species: Utilization of stored samples in the Environmental Specimen Bank of Ehime University (*es*-Bank). *Environ. Pollut.* (published online: 26 November 2007, doi:10.1016/j.envpol.2007.10.013)