POPS ANALYSIS AND MONITORING IN THE ASIAN COASTAL HYDROSPHERE

Iino F[1], Sebesvari Z[2], Renaud F[2], Kitsuwa T[3], Morita M[4], Shibata Y[4], Huang Y[5], Rajendran B R[6], Syafrul H[7], Shim W J[8], Mustafa Ali Mohd[9], Muhammad Aslam Tahir[10], Santiago E[11], Suthamanuswong B[12], Lee H K[13], Viet P H[14], Boonyatumanond R[12]

[1] United Nations Univeristy, Environmental and Sustainable Development Programme (UNU-ESD), 5-53-70 Jingumae, Shibuya, Tokyo 150-8925, Japan
[2] United Nations University, Institute for Environment and Human Security (UNU-EHS), UN Campus, Hermann-Ehlers-Str. 10, D-53113 Bonn, Germany
[3] Shimadzu Corporation, 1-3 Kandanishiki-cho, Chiyoda, Tokyo 101-8448, Japan
[4] National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki, 305-8506, Japan
[5] Shino-Japan Friendship Center for Environmental Protection, No. 1 Yuhui Nanlu, Chaoyang District, Beijing 100029, P. R. China
[6] School of Environmental Sciences, Bharathidasan University, Tiruchirappalli-620024 Tamil Nadu, India
[7] Environmental Impact Control Facility (SARPEDAL), Kompleks PUSPIPTEK Jl. Raya Puspiptek, Serpong, TANGERANG 15314, Indonesia
[8] South Sea Institute, KORDI, 391, Jangmok-myon, Jangmok-ri, Geoje, Gyungnam 656-830, Korea
[9] Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[10] Pakistan Council of Research in Water Resources, Ministry of Science & Technology, Kyaban-e-Johar Sector H-8/1, Islamabad, Pakistan
[11] Research and Analytical Services Laboratory, Natural Sciences Research Institute, University of the Philippines, Diliman Quezon City, 1101, Philippines
[12] Environmental Research & Training Center, Technopolis, Tambon Klong 5, Klong Luang, Pathumthani 12120, Thailand
[13] Department of Chemistry, National University of Singapore, 3 Science Drive 3, 117543, Singapore
[14] Research Centre for Environmental Technology and Sustainable Development (CETASD), Hanoi University of Science, Vietnam National University, T3 Building, 334 Nguyen Trai Street, Thanh Xuan District, Hanoi, Viet Nam

Abstract
The United Nation University's (UNU) has been implementing a capacity development project on chemical analysis of environmental pollutants since 1996. The project has undertaken monitoring of various organic pollutants in the environment in ten participating countries in Asia using a quadruple type gas chromatograph with mass spectrometer. Shimadzu Corporation prepared the analytical procedures and quality control protocols that suit the capacities and resources of the institutes participating in the monitoring projects. An inter-laboratory calibration study was conducted to check the project data variability. The procedures, quality control protocols and data gathered from water, sediment, soil, and biological samples under this UNU project are presented.

Introduction
The UNU's capacity development project on environmental pollutant analysis using a quadruple type gas-chromatograph mass spectrometer (GC/MS) by project participating countries has been implemented since 1996 with support from Shimadzu Corporation. In total, more than 56 research staff from participating governmental institutions and universities in ten countries (China, India, Indonesia, Korea, Malaysia, Pakistan, Philippines, Singapore, Thailand, and Viet Nam) have been trained on sample pretreatment and data analysis using GC/MS for a wide variety of samples (water, biota, sediment, and food, fish scale and air). Various target environmental pollutant chemicals have been analyzed ranging from Volatile Organic Compounds (VOCs) to Persistent Organic Compounds (POPs) as shown in Table 1.

Since the Stockholm Convention entered into force in 2004, the expectations from this capacity development monitoring project have been increasing. Existing regional networks engaged in POPs monitoring like this UNU project could be important data sources on the global POPs levels. In this paper, the project's monitoring results as well as the quality assurance and quality control aspects of the project activities are discussed.

Materials and Methods

Table 1 summarizes sample species and target chemicals as well as surrogates and internal standards used for each year's analysis during the previous three phases. Every year different pollutants / environmental media have been chosen for monitoring. In the current 4th phase, biological samples have been analyzed. POPs in shrimps (2006) and in sea bass (2007) were monitored. Shimadzu Corporation has verified and provided the sample pretreatment and analytical procedures that have been customized to meet capacities and resources available at the participating institutes. The sea bass analytical procedure is shown in Fig. 1. A project quality assurance and quality control document has been prepared to ensure the project data quality. In this analytical procedure, phenanthrene-d_{10} and chrysene-d_{12} were used as internal standards (syringe spikes), and DDT-$^{13}C_{12}$ was used as a surrogate (a clean-up spike). The chemical analysis was carried out using Shimadzu Corporation Shimadzu-GCMS QP5050A and Shimadzu-GCMS QP2010.

Sea bass	Remove the head, bones and internal organs of 100 g Sea bass
↓	
Grind	Mince the Sea bass by Food – processor
↓	
Extraction	Put 5 g of the minced Sea bass into the Cup of Homogenizer
↓	Put 100 µl of 0.2 % BHT, 50 ml of Acetonitrile and 10 µl of DDT-^{13}C (2 ppm) into the Cup of Homogenizer
	Homogenize for 5 min
Filtering	Filter the homogenized sample into 100 ml Flask-1
↓	Rinse the Cup by 20 ml of Acetonitrile Filter the rinsed solvent into 100ml Flask-1
Extraction-1	Put the filtered solvent into 500ml Funnel-1
↓	Put 350 ml of water, 17 g of NaCl and 40 ml of n-Hexane: Ethyl acetate (3:2) into 500 ml Funnel-1
	Shake Funnel-1 for 10min and stay
Extraction-2	Put the lower layer (water solution) into 500 ml Funnel-2
	Put the upper layer (organic solution) into 100 ml Flask-2
	Put 40 ml of n-Hexane: Ethyl acetate (3:2) into 500 ml Funnel-2
↓	Shake Funnel-2 for 10 min and stay
	Dump the lower layer (water solution) in Funnel-2
	Add the upper layer (organic solution) into 100ml Flask-2
Hydration	Put 3 g of Na_2SO_4 (anhydrous.) into 100 ml Flask-2
↓	Set 15 min
	Put the hydrated solution in Flask-2 into 200 ml Round-bottomed Flask-1
Concentration-1	Concentrate the solution in 200ml Round-bottomed Flask-1 to a few ml by Rotary Evaporator at 35 ℃
↓	Put 20 ml of n-Hexane into 200 ml Round-bottomed Flask-1
	Shake 200 ml Round-bottomed Flask-1
Concentration-2 (Change to n-Hexane)	Concentrate the solution in 200 ml Round-bottomed Flask-1 to less 2 ml by Rotary Evaporator at 35 ℃ Put the solution in 200 ml Round-bottomed Flask-1 into 10 ml Centrifuge Tube-1
↓	Concentrate the solution in 10 ml Centrifuge Tube-1 to less 2 ml by N2 Gas at 40 ℃ (Do not dry up)
	Measure to 2 ml by n-Hexane
2 g NH2 Column	Elute 25 ml of n-Hexane into 2 g NH2 Column Cartridge for conditioning *1
↓	Elute the sample solution in the centrifuge Tube-1 to 2 g NH2 Column Cartridge
Elution	Rinse 10ml Centrifuge Tube by 1 ml of n-Hexane twice
↓	Put 2 ml of the rinsed solution and 50 ml of n-Hexane into 200 ml Round-Bottomed Flask-2
Concentration-3	Concentrate the solution in 200 ml Round-bottomed Flask-2 to less 1 ml by Rotary Evaporator at 35 ℃
	Put the solution in 200 ml Round-bottomed Flask-2 into 10 ml Centrifuge Tube-2
↓	Concentrate the solution in 10 ml Centrifuge Tube-2 to less 1 ml by N2 Gas at 40 ℃ (Do not dry up)
	Measure to 1 ml by n-Hexane
1 g Silica Column	Elute 10 ml of 2 % Acetine/n-Hexane in 1 g Silica Column Cartridge for conditioning
	Elute the sample solution in the centrifuge Tube-2 to 1 g Silica Column Cartridge
↓	Rinse 10 ml Centrifuge Tube-2 by 0.5 ml 2 % Acetone/n-Hexane twice
	Elute 1 ml of the rinsed solution and 8 ml of 2 % Acetone/n-Hexane into 10 ml Centrifuge Tube-3
Concentration-4	Concentrate the solution in 10 ml Centrifuge Tube-3 to less 1 ml by N2 Gas at 40 ℃ (Do not dry up)
↓	Put 5 µl of Phenanthren-d_{10} and Chrysene-d_{12} (10 ppm) into the Centrifuge Tube-3
	Measure to 1 ml by n-Hexane
Analysis by GCMS	Inject 2 µl to GCMS

Fig. 1 Example of analytical procedures for fish-species customized to meet resources available at the project participating institutes

Results and Discussion

Inter-laboratory calibration study in 2002

Eight project member institutes participated in the calibration study using two reference water samples with different POPs compositions that were prepared by Shimadzu Corporation. Table 2 shows the statistical analysis of the reported results. Unfortunately, the original data collected in determining the reference material concentrations are missing. Therefore, following the ISO Guide 43 to analyze inter-lab data with unknown value samples, z-scores were calculated as follows.

z-score = (each lab's average value – Median)/NIQR where NIQR stands for Normalized Interquartile Range that was calculated as IQR x 0.7413. IQR is a difference between Upper quartile and Lower quartile. The number of 0.7413 is an inverse number of the normal distribution's IQR. All z-scores were lower than 2 indicating all data were within the acceptable range of inter-

laboratory variability. However, a closer look at the inter-lab data and NIQR values reveals that some countries faced difficulties getting accurate concentrations of Aldrin, Endrin, and *p,p'*-DDT. In addition, some countries did not meet the acceptable range of DDT-$^{13}C_{12}$ recovery data.

Quality assurance and quality control

To ensure the quality of the analytical activities, quality control indicators such as blank tests, injection repeatability tests and standard addition recovery tests were conducted by all project members as necessary, and DDT-$^{13}C_{12}$ recovery data have been collected for all samples with 70-130% as an acceptable range. One of the two internal standards, phenanthrene-d_{10} and chrysene-d_{12}, were chosen in quantifying each POPs chemical depending on its capillary column elution time. To determine instrument detection limit, five times to eight times injections for the injection repeatability test were recommended. The number of repetition determines the coefficient to use in calculating detection limits, as can be seen below. IDL = t (n-1, 0.01) x σ, where t (n-1, 0.01) is a value of *t*-distribution at α = 0.01 for one tail. More details are described in the UNU Project Quality Assurance Document.

POPs levels in water and sediment

Due to the wide-ranging capabilities of the laboratory facilities at the project participating institutes, international data comparison must be performed with caution. The project, however, has provided domestic data on POPs in water,

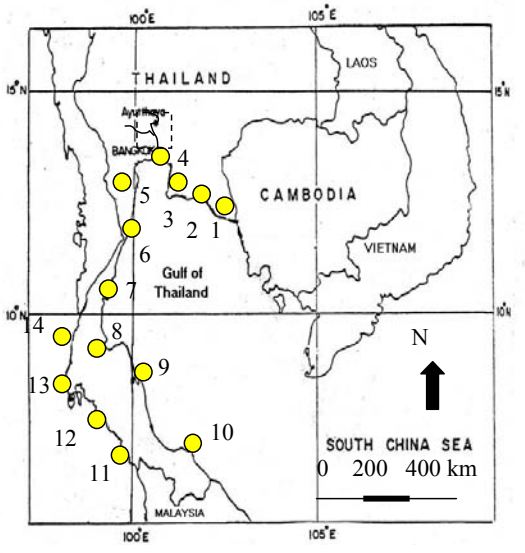

Fig. 2. Sampling locations for water and sediment samples in Thailand in 2005

Table 1. Target chemicals and media examined in this project during the last three phases (1996 - 2004)

		First Phase (1996 - 1998)						Second Phase (1999 - 2001)				Third Phase (2002 - 2004)			
		1996	1997		1998			1999	2000	2001		2002	2003	2004	
		Pesticides	VOCs	TBTs	VOCs	Aldehydes		EDC-like Pesticides	EDC-like Phenols	EDC-like Phthalates		Pesticides POPs	Pesticides POPs	Pesticides POPs	
Target Media		Rice	Tap/River Water	Fish Scales	Indoor/Ambient Air	Indoor/Ambient Air		River Water	River Water	River Water		River Water	River Water/Sediment	River Water/Sediment	
Target Chemicals		Fenitrothion(MEP)	1,1-Dichloroethylene	Tri Buthyl Tin	Trichloromethane	Formaldehyde		α-BHC	Bisphenol-A	Di methyl phthalate		Hexachlorobenzene	Hexachlorobenzene	Hexachlorobenzene	
		Malathion	Dichloroethane	Tri Phenyl Tin	1,1,1-Trichloroethane	Acetaldehyde		β-BHC	4-t-Butylphenol	Di ethyl phthalate		Heptachor	Heptachor	Heptachor	
		Chlorpyrifos	*c*-1,2-Dichloroethene		Tetrachloromethane			γ-BHC	4-n-Butylphenol	Di i-propyl phthalate		Aldrin	Aldrin	Aldrin	
		p,p'-DDT	*t*-1,2-Dichloroethene		Benzene			δ-BHC	4-n-Pentylphenol	Di allyl phthalate		*r*-Chlordane	*r*-Chlordane	*r*-Chlordane	
			Trichloromethane		1,2-Dichloroethane			Aldrin	4-n-Hexylphenol	Di n-propyl phthalate		*c*-Chlordane	*c*-Chlordane	*c*-Chlordane	
			1,1,1-Trichloroethane		Trichloroethene			Dieldrin	4-n-Heptylphenol	Di i-butyl phthalate		Dieldrin	Dieldrin	Dieldrin	
			Tetrachloromethane		1,2-Dichloropropane			*p,p'*-DDE	4-t-Octylphenol	Di n-butyl phthalate		Endrin	Endrin	Endrin	
			Benzene		Bromodichloromethane			Endrin	4-n-Octylphenol	Di n-pentyl phthalate		*p,p'*-DDT	*p,p'*-DDT	*p,p'*-DDT	
			1,2-Dichloroethane		*c*-1,3-Dichloropropene			*p,p'*-DDD	4-Nonylphenol	Butyl benzyl phthalate					
			Trichloroethene		Toluene			*p,p'*-DDT	2,4-Dichlorophenol	Di n-hexyl phthalate					
			1,2-Dichloropropane		*t*-1,3-Dichloropropene				Pentachlorophenol	Di butoxy ethyl phthalate					
			Bromodichloromethane		1,1,2-Trichloroethane					Di cyclohexyl phthalate					
			c-1,3-Dichloropropene		Tetrachloroethene					Di phenyl phthalate					
			Toluene		Dibromochloromethane					Di n-heptyl phthalate					
			t-1,3-Dichloropropene		*m,p*-Xylene					Di 2-ethyl hexyl phthalate					
			1,1,2-Trichloroethane		*o*-Xylene					Di n-octyl phthalate					
			Tetrachloroethene		Tribromomethane					Di 2-ethyl hexyl adipate					
			Dibromochloromethane		*p*-Dichlorobenzene										
			m,p-Xylene												
			o-Xylene												
			Tribromomethane												
			p-Dichlorobenzene												
Surrogate				Tri Pentyl Tin					Bisphenol-A d_{16}	Di n-pentyl phthalate-d_4		*p,p'*-DDT-$^{13}C_{12}$	*p,p'*-DDT-$^{13}C_{12}$	*p,p'*-DDT-$^{13}C_{12}$	
Internal Standards			*p*-Bromofluorobenzene	Tetra Butyl Tin		Diphenylamine		Phenanthrene-d_{10}	Naphthalene-d_8	Di n-butyl phthalate-d_4		Pyrene-d_{10}	Pyrene-d_{10}	Pyrene-d_{10}	
								Pyrene-d_{10}	Phenanthrene-d_{10}	Di 2-ethyl hexyl phthalate-d_4					
Instruments		GCMS-QP5050A							Pyrene-d_{10}	GCMS-QP5050A		GCMS-QP2010			

sediment, soil, and biological samples at different locations. In Thailand between 2004-2005, for example, *p,p'*-DDT was found up to 8.5 ng/L in water and 40 ng/g in sediment, while *trans*-Chlordane was found up to 8.9 ng/L in water and 0.96 ng/g in sediment collected from coastal areas and river basins (Figure 2).

Table 2. Data from inter-laboratory calibration exercise conducted in 2002

Countries (A-H)	A	B	C	D	E	F	G	H				
					Z-scores				Average	Median	SD	NIQR
Hexachlorobenzene	1.77	1.01	0.29	1.15	0.52	0.32	0.19	0.14	8.0	7.6	2.1	2.3
Heptachlor	0.10	0.51	0.86	0.27	1.10	1.04	0.05	0.46	18.2	18.3	3.1	3.4
cis -Chlordane	0.67	0.27	1.46	0.03	0.16	0.30	0.48	0.78	19.5	19.2	2.0	1.8
Dieldrin	0.49	1.50	0.60	0.01	0.87	0.57	0.58	0.17	29.1	30.2	3.7	4.0
Aldrin	0.83	0.35	0.71	0.66	0.43	0.36	0.95	0.03	53.1	52.1	15.8	23.9
Dieldrin	0.20	1.15	0.70	0.10	0.56	0.26	1.09	0.25	54.8	55.5	6.9	7.9
Endrin	1.05	0.19	1.06	0.19	0.87	1.61	0.03	0.08	82.6	79.9	45.4	50.6
p,p'-DDT	0.42	0.04	0.66	0.77	0.68	1.24	0.03	0.84	54.1	51.4	13.7	18.2

|z|=<2: Satisfiable, 2<|z|<3: Doubtful, |z| >=3: Unsatisfiable, SD: Standard Deviations, NIQR: Normalized InterQuartile Range

Shrimp sampling data in 2006

Some organochlorine pesticide POPs in wild shrimps were reported above their corresponding method detection levels determined by some project participants. In the Philippines, *trans*-/*cis*-Chlordanes and *p,p'*-DDT were detected from a substantial number of the samples originating from Bay Laguna (Table 3). In China, Hexachlorobenzene and *p,p'*-DDE were detected from all samples taken from Donting and Tai Lakes. Some countries have experienced unsatisfactory ranges of DDT-$^{13}C_{12}$ recovery data.

Table 3 Concentration of organochlorine pesticides(in ng/g wet weight) in shrimps from Bay, Laguna.

Bay, Laguna, 1st sampling	BLS1	BLS2	BLS3	BLS4	BL S5	BL S6	EMDL
	Body &head *M,rosenbergii daquete*	Body & Head, *M idella*	Body, Head and scale, Small but mature	Body *M idella*	Body & Head Big *Caridina sp*	Body & Head Light colored	
trans-Cchlordane	0.64	**1.43**	**1.06**	**1.26**	0.72	**1.22**	1
cis-Chlordane	**1.19**	**1.68**	**1.36**	**0.92**	**0.92**	**0.97**	0.8
p,p'-DDE				**2.23**			0.2
p,p'-DDT	**4.21**	**7.7**	**1.31**	**5.40**	**3.77**	**1.99**	1
α HCH	**0.70**						0.2
Endosulfan1	**1.92**	**3.78**				0.70	1
Methoxychlor	1.22		0.78		6.67		1
Trans Nonachlor	**1.02**	**1.44**	**1.26**	0.90	0.83	0.80	1
%Rec,*p,p'*-DDT$^{13}C_{12}$	82	103	84	108	116	113	

Contributions to the Stockholm Convention's effectiveness evaluation

UNEP's Guidance for a Global Monitoring Programme for POPs (1st edition, 2004) recommended air and human breast milk samples as priority targets to detect a long term trend of the POPs global level. These samples have not been analyzed in this project. However, biological samples as well as water monitoring data could serve as supporting information on the effectiveness of the Stockholm Convention that will take place in 2008.

References

1. UNU Quality Assurance Document For "Environmental Monitoring and Governance in the Asian Coastal Hydrosphere", Draft Version, April 2007.
2. UNEP's Guidance for a Global Monitoring Programme for POPs (1st edition, 2004), http://www.chem.unep.ch/gmn/default.htm, accessed on 19 April, 2007.