POPS ANALYSIS CAPACITY DEVELOPMENT AND MONITORING IN 10 ASIAN COUNTRIES

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Abstract
The United Nation University (UNU) has been implementing a capacity development project on chemical analysis and monitoring of environmental pollutants since 1996. The project has undertaken monitoring of various organic pollutants in the environment in ten participating countries in Asia using gas chromatography/mass spectrometry. Shimadzu Corporation prepared the analytical procedures and quality control protocols that suit the capacities and resources of the institutes participating in the monitoring project. The procedures, quality control protocols and data gathered from fish samples in this UNU project are presented here.

Introduction
UNU's capacity development project on environmental pollutant analysis using quadrupole gas chromatography coupled to a quadrupole mass spectrometry (GC/MS) by project participating countries has been implemented since 1996 with support from Shimadzu Corporation[1]. In total, more than 66 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, rice, fish, and air). In the previous project phases,

various target environmental pollutant chemicals have been analyzed ranging from Volatile Organic Compounds (VOCs) to Persistent Organic Compounds (POPs) as reported elsewhere[2].
Since the Stockholm Convention entered into force in 2004, existing regional networks engaged in POPs monitoring like this UNU project have been recognized as important data sources on the global POPs levels. In this paper, the project's monitoring results, in a selection of countries, as well as the quality assurance and quality control aspects of the project activities, are reported and discussed.

Materials and Methods
In the current 4th phase (2005-2008), biological samples have been analyzed. POPs in shrimps (2006) and in fish samples (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 fish analytical procedure is shown in Fig. 1. The chemical analysis was carried out using Shimadzu GC/MS QP5050A and GC/MS QP2010 systems.

Quality assurance and quality control
The project conducted an inter-laboratory study in 2002. The result was reported previously[2]. A closer look at the inter-laboratory data revealed 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-$^{13}C_{12}$ cleanup spike recovery ratio.
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-$^{13}C_{12}$ cleanup spike recovery ratios have been checked with 70-130% as its acceptable range. One of the two internal standards, phenanthrene-d_{10} and chrysene-d_{12}, were chosen for the quantification of

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 % Acetone/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-d10 and Chrysene-d12 (10 ppm) into the Centrifuge Tube-3
	Measure to 1 ml by n-Hexane
Analysis by GCMS	Inject 2 µl to GCMS

Fig. 1 Project analytical procedure for fish samples

each POPs depending on its GC elution time. To determine instrument detection limit (IDL), five 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[3].

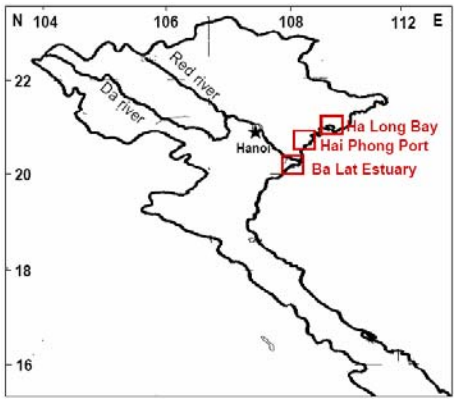

Fig. 2 Three sampling locations in Vietnam

Results and Discussion

Fish sampling data in 2007

Due to the wide-ranging capabilities of the laboratory facilities at the project participating institutes, international data comparison must be performed with caution. Each country reported about 15 organochlorine pesticide POPs in fish above their corresponding method detection levels determined by project participants.

In the Philippines, nine fish individuals (*Lates calcarifer*) of various sizes were purchased from fishermen with the assistance of the Fisheries Aquatic Resources Management Council (FARMC) officer in Tagkawayan, Quezon in July, 2007. The fish samples were classified according to their size and weight as small, medium and large which corresponded to three composite samples for analysis. Water samples were collected about 1.5 kilometers from the shoreline. One fish sample was collected in Roxas City and 7 fishes were collected in Estancia, Ilo-ilo in September. The fishes from Estancia were classified into 2 groups by length and size. Water samples were collected about 2 kilometers from the shoreline. No POPs were detected from the water and fish samples over quantitative limits (MDL).

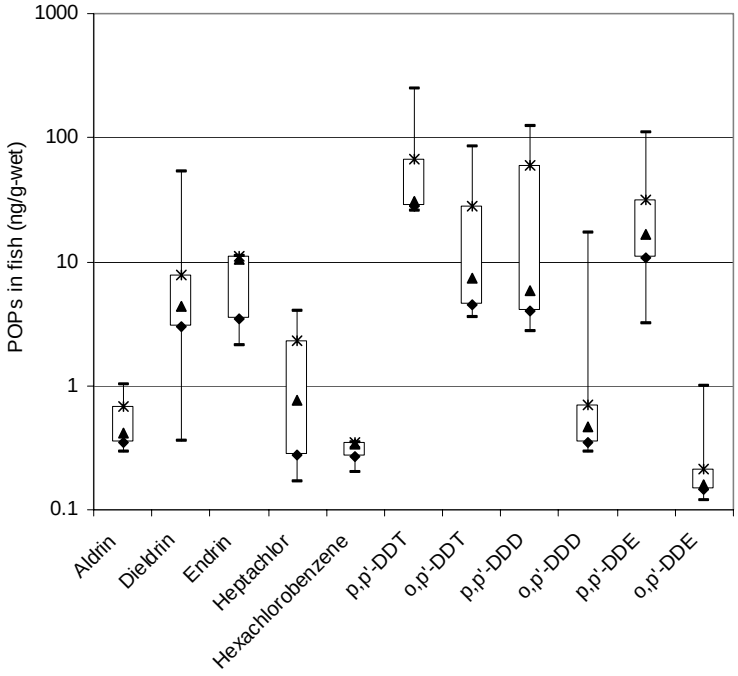

Fig. 3 POPs detected in fish sampled in Vietnam.
Method detection limit (MDL, ng/g-wet weight) for the compounds were: Aldrin 0.14; Dieldril 0.28; Endrin 2.04; Heptachlor 0.11; Hexachlorobenzene 0.12; p,p´-DDT 0.18; o,p´-DDT 0.18; p,p´-DDD 0.73; o,p´-DDD 0.19; p,p´-DDE 0.11; o,p´-DDE 0.11.

In Vietnam, Ha Long Bay, Hai Phong Port, and Ba Lat Estuary were selected as sampling locations, as indicated in Fig. 2. Ha-Long Bay has very diverse ecosystems and is recognized as a UNESCO World Heritage site in Vietnam. It has very important resources for tourism as well as aquaculture and commercial activities. Next to Ha Long Bay is Hai Phong Port, which has facilitated industrial activities for more than one hundred years. These anthropogenic activities may cause pollution of the local aquatic environment and of Ha Long Bay. Discharges from the port site may bring considerable load of

contaminants such as POPs to the coastal waters. Known as one of the main regions in producing rice for the domestic consumption and export, Thai Binh province has annually utilized a large quantity of pesticides and herbicides to protect crops from pest and weeds. Ba Lat Estuary is the final repository where receiving water from channels and streams that contain industrial and agricultural wastes from manufacturing and agricultural activities of Hanoi city and Thai Binh province end up.

Table 1. DDT and its metabolites detected in fish samples from three sampling locations in Vietnam (ng/g-wet)

Fish samples	p,p-DDT	o,p-DDT	p,p'-DDD	o,p-DDD	p,p'-DDE	o,p'-DDE	DDT/DDE
BL-F11	2.6E+01	4.1E+00	6.9E+00	4.8E-01	1.8E+01	1.3E-01	1.6
BL-F12	2.6E+01	3.6E+00	5.9E+00	2.9E-01	1.7E+01	ND	1.8
BL-F15	4.6E+01	1.2E+01	2.7E+00	5.0E-01	3.2E+00	1.6E-01	17
BL-F16	2.8E+01	4.6E+00	3.2E+00	3.2E-01	7.3E+00	ND	4.5
BL-F17	2.9E+01	7.3E+00	4.1E+00	4.1E-01	1.1E+01	ND	3.5
BL-F18	2.8E+01	4.6E+00	3.9E+00	3.2E-01	1.1E+01	1.2E-01	2.8
BL-F19	3.0E+01	5.9E+00	4.2E+00	3.7E-01	1.2E+01	ND	3.0
HL-F11	2.5E+02	6.4E+01	1.2E+02	1.7E+01	3.6E+01	1.6E-01	8.5
HP-F13	8.7E+01	8.4E+01	5.8E+01	4.7E-01	2.7E+01	1.8E-01	6.2
HL-1-TR	6.3E+01	2.4E+01	1.2E+02	1.4E+00	1.1E+02	1.0E+00	0.8
HL-1-TH	7.0E+01	3.2E+01	6.1E+01	8.9E-01	3.5E+01	2.5E-01	2.9

Fish species sampled in Vietnam were Black porgy (*Sparus macrocephalus*), Yellowfin seabream (*Sparus latus*), and Waeigieu seaperch (*Psammoperca*). Among 11 fish samples analyzed, Mirex and cis-/trans-Chlordane were not detected at all. Other compounds detected are shown in Fig. 3. Recent inputs of p,p'-DDT to the water bodies may be indicated by higher ratios of DDT/DDE than 0.5 in Table 1.

Different DDT/DDE profiles in fish tissues were found in data obtained in the Republic of Korea. While in both sampling locations of Tongyeong and Gadukdo that are about 60km apart (Fig. 4) DDT and its metabolites were the dominant compounds among the detected POPs, p,p'-DDT was detected only in Gadukdo, which implied recent inputs of p,p'-DDT to the environment.

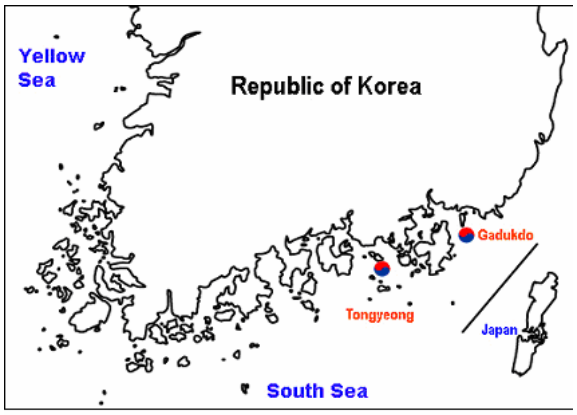

Fig. 4 Two sampling locations in the Republic of Korea

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3. UNU Quality Assurance Document For "Environmental Monitoring and Governance in the Asian Coastal Hydrosphere", Draft Version, April 2007.