Biomonitoring of POPs and POPs-like Chemicals in East Asia -Phase1&2-

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Introduction

The Stockholm Convention entered into force in May 2004 which is a global treaty to protect human health and the environment from Persistent Organic Pollutants (POPs). The purpose of the treaty is to globally regulate the use, release and emission of POPs and signed countries need to take POPs measures including seven East Asian countries; Cambodia, Indonesia, Laos, Malaysia, Philippine, Thailand and Vietnam. The World Bank has planned Capacity Building Program on Risk Management, which plans variety of project to strengthen the capacity of the seven countries. Hiyoshi has done Pilot project using biomonitoring tool, CALUX[1] (Chemically Activated LUciferase eXpression) to demonstrate use of CALUX since it is difficult for developing countries to use expensive instruments such as HRGC/MS for environmental monitoring of POPs. Among the seven countries, Laos was selected as not many researches were done in the past. Samples were collected by local experts and in Phase 1, 39 soil and 6 fish samples were collected. In Phase 2, 41 soil and 2 fish samples were collected from potential hotspot and control sites and also 36 breast milk samples were collected from sites around potential hotspots and in each phase, collected samples were analyzed and evaluated at a local laboratory.

Materials and methods

Sampling

Phase1 ; In this research, soil samples are collected from total of 39 points as shown in Table1. 1) Sites potentially contaminated with dioxins (effects of agent orange); 19 samples from 5 southern cities; 2) Site potentially contaminated with PCBs (effects of transformer); 10 samples from Vientiane capital city and 4 other cities; 3) to 6) Around Vientiane; 5 samples from Landfill area, 1 sample from iron recycling plant, 2 samples from paper manufacturing plant, 2 samples from Army area. Also, 6 seafood samples (fish and clam) were collected from Vientiane capital city and 3 other cities, which also are shown in Table1.

Phase1/Validation: Furthermore 5 soil samples were sent to Japan to confirm CALUX data with HRGC/HRMS.

Phase2: In this research among all Laos areas we have selected potential contaminated sites and called them as "hotspot" and sites which is a few ten meters from the site as "control site" .Soil samples were collected and the spread of contamination was studied. We have selected several "hotspot" from 5 cities; 1) Vientiane Capital, 2)Savannaket, 3)Saravane, 4)Sekong, 5)Attapeu and also collected samples from "control sites" as background. Total of 41 samples were analyzed and the results are shown in Table 1. After obtaining informed consent, breast milk samples, were collected from mothers who live around the "hotspots" to confirm the pollution level. We have selected several "hotspot" areas from five cities in Laos and collected 36 samples from the "hotspots". The cities are; 1) Vientiane Capital, 2) Savannaket, 3) Saravane, 4) Sekong, 5) Attape shown in Table1. Two fish samples were collected from Vientiane capital city as shown in Table 2.

Analysis

CALUX Assay ; Through pretreatment process, each soil and seafood and human milk samples were separated into two, PCDD/Fs and dioxin-like PCBs (DL-PCBs) fragment. After substituting into DMSO solution, cell culture media were added and applied to CALUX assay[2]. Since there is no facility for cell cultivation or subculture in Laos, it will be difficult to do such process. Therefore, plates were prepared, sealed, and put in a box (1400 CASE PERICAN® with purge valve) in Japan, and shipped to Laos and measured dioxins using portable plate system. By using a water bath, this box could also be used as a simple CO_2 incubator. CO_2 gas was blown for ten seconds and the plate was reactivated for 1 to 2 hours. Then the cell was dosed and cultivated in the simple incubator and the media was removed. Finally, the luciferase activity (RLU) induced by luciferase

Table1. List of sampling points (Above: Phase1, Below: Phase2)

	Polluted Sample						
	Soil(Bs)						Fish(Bf)
	1(Dioxin)	2(PCB)	3(Land fill)	4(Steel)	5(Paper)	6 (Nongchem)	
Sekong	1,2,3	-	-	-	-	-	3,4
Attapeu	4,5,6	-	-	-	-	-	1
Saravane	7,8,910,11,12,13	-	-	-	-	-	2
Savannaket	14,15,16	1	-	-	-	-	-
Khammoune	17,18,19	2	-	-	-	-	-
Champassak	-	3	-	-	-	-	-
Vientiane pro.	-	4,5	-	-	-	-	-
Vientiane cap.	-	6,7,8,9,10	1,2,3,4,5	1	1,2	1,2	5,6

	Polluted Sample				Clean Site (Reference)	
	Site(Village name)	Soil(Bs)	Fish(Bf)	Milk(Bm)	Site(Village name)	Soil(Bs)
Vientiane cap.	Phontong	1,2,3	-	-	-	-
	Nongchem	4,5	1,2	1	Near by contraminated site	6
	Sokphalouang	41	-	-	-	-
Savannaket	Dong Gnai	35	-	2,3,4	Near by contraminated site	36
	Chakiphin	37	-	5,6,7	Near by contraminated site	38
	Saleo	39	-	8	Near by contraminated site	40
Saravane	Pacheucheun	7	-	33,35	Near by contraminated site	14
	Pasia	8	-	9	Near by contraminated site	15
	Poren	9	-	10,11	Near by contraminated site	16
	Doub	10	-	12,13	Near by contraminated site	17
	Kape	11	-	14,15,16	Near by contraminated site	18
	Cho tai	12	-	18,19	Near by contraminated site	19
	Phobeui	13	-	34,36	Near by contraminated site	20
Sekong	Dak Seng	21	-	20,21	Near by contraminated site	25
	Dak Lann	22	-	17	Near by contraminated site	26
	Dak Pork	23	-	22,23	Near by contraminated site	27
	Dak Choung	24	-	24,25	Near by contraminated site	28
Attapeu	Phoo home	33	-	26,27,28	Near by contraminated site	34
	Palai	31	-	29,30	Near by contraminated site	32
	Phoo home III	29	-	31,32	Near by contraminated site	30

Figure1. Sampling point

assay system was measured using a luminometer.

HRGC/HRMS ; The extraction and cleanup of the soil samples for HRGC/HRMS followed previously published protocols (Japan guideline method for soil measurements relating to DXNs by Japan Ministry of Environment)on. Briefly, 10 grams of ground composite spiked with 13C-labelled surrogate dioxin and DL-PCB standards. Samples were extracted with an organic solvent. Extracts were subjected to a sequential cleanup using several column chromatography steps. The analysis of the 17 active PCDDs/DFs and 12 DL-PCBs (4 non-ortho and 8 mono-ortho PCBs) by HRGC/HRMS was carried out using SIM with a Micromass Autospec Ultima HRMS equipped with a Hewlett-Packard 6890 GC. The TEQs for PCDDs/DFs and DL-PCBs were calculated using WHO-TEF values (1998).

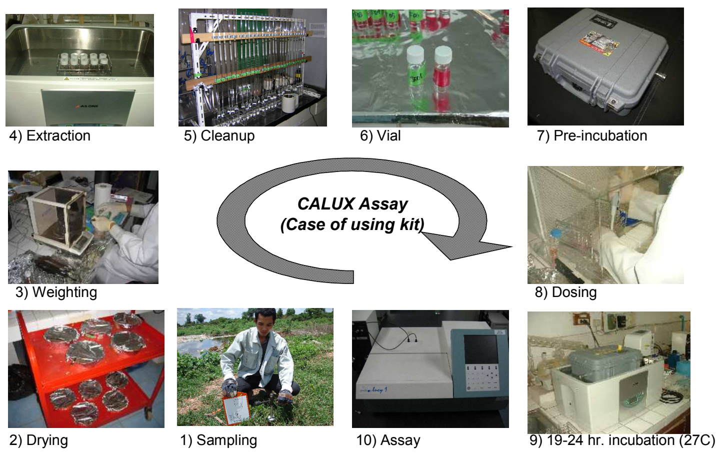

Figure2. CALUX flow (kit style: soil)

Results and Discussion

Phase1 ; Sites potentially contaminated with dioxins: Agent Orange effect were not confirmed but PCDDs/Fs effect were found in all the areas. Sekong area (Bs1-1 and Bs1-2) had a high level (110pg/g and 88pg/g (unit:pg/gs shows actual concentration using CALUX.)) as compared to other site. Sites potentially contaminated with PCBs: Since PCDDs/Fs exist in PCB transformers, PCDDs/Fs are also found in the samples. Significantly high DL-PCBs contaminations were found from samples collected from Vientiane capital city (Bs2-7 and Bs2-8) and further research and measurement will be necessary for the sites. Around Vientiane: Some point from Landfill showed high level of dioxins. Not significant contaminations were found from Metal recycling plant and paper manufacturing plant. Samples collected from Nongsuim area (Bs6-1 and Bs6-2) were significantly high, which exceeds Japanese soil standard level. For these points, further research on surrounding area to specify contaminated area and further analysis by HRGC/MS to specify source of contaminant will be necessary. For seafood samples: PCDD/Fs level were very low and DL-PCBs level were below detection level. No DL-PCBs were detected from collected samples therefore contaminations from leach of effluent from landfill were not found from area around landfill (Bf-5 and Bf-6). Since landfill is increasing in area around Vientiane, further research is necessary for each site and for seafood. Due to weather condition we could not collect samples from southern inland area, which are said to be the dioxin hotspot, and we will need additional work for the places.

Phase1/Validation: CALUX results tend to show higher toxicity because of the effects of brominated dioxins and other chemical substances. As CALUX uses original biological method to evaluate toxicity, CALUX TEQ differs from HRGC/HRMS TEQ. Five soil samples from Laos was analyzed using both CALUX assay and HRGC/HRMS .The HRGC/HRMS results were compared by CALUX actual value and a converted CALUX value which is very similar to HRGC/HRMS value (TEQ concentration).This converted CALUX value which is very similar to HRGC/HRMS value (TEQ concentration) is obtained by multiplying the conversion factor with the CALUX actual value. Through the study we found that CALUX gives a relative value and so can be used for monitoring. In addition by using conversion factor, CALUX can be converted into HRGC/HRMS result hence it can be used as semi-quantification and used as a method to determine the level. Furthermore it is necessary to collect more data.

Phase2: As an additional study for Phase1, samples were collected from a total of 34 places, potential Agent Orange contaminated areas (four cities: Sekong, Attapeu, Savannaket) and control site. CALUX cannot be used to determine Agent Orange but as a screening test we found PCDD/Fs effect at all places. The result of Sekong hotspot Bs24 and control area Bs27 were 210 pg/g and 110pg/g respectively which is higher as compared to other areas. There were no effect of PCB found as all the results were below or near detection limit (ND=0.89pg/g) except for Sekong hotspot, Bs24 (2.7pg/g). We have selected 4 sites around Vientiane (Phontong and Sokphalouang) as these areas are being concerned for PCB pollution to human and environment by storage and disposal of PCB. Since dioxins are contained in PCB as impurity, effects of PCDDs/Fs were also found. Especially level of Sokphalouang, Bs4 (PCDDs/Fs: 760pg/g, DL-PCBs: 1000pg/g) and we suspect serious pollution in the area. In the Phase 1, we have selected dioxin sampling area and high levels of dioxin were found from two places. In this Phase 2 we have selected total 3 places, 2 hotspots and 1 control site, around the contaminated place found in Phase 1. Very high levels of dioxin were found from the two hotspots which exceeds Japanese soil environmental standard: Bs4(PCDD/Fs: 2800000pg/g, DL-PCBs: 1100000pg/g) and Bs5(PCDDs/Fs: 5000pg/g, DL-PCBs: 33pg/g). In addition the level of dioxin found from control site Bs6: PCDD/Fs: 40pg/g、DL-PCBs: 2.9pg/g) were also relatively high compared to other spot which reveal possibility of effect from the hotspot. Further analysis by HRGC/HRMS is necessary to determine area of pollution and to identify source of pollution. Two fish samples were collected from Vientiane city (Nongchem and Nongsa). Detection limit is 0.31pg-CALUX TEQ/g wet. Only PCDDs/Fs were found from both the samples but the levels were very low at around detection limit and both DL-PCBs were below detection limit. We suspect that since the area is closed ocean no DL-PCBs were detected. No pollution was found from Nongchem and Nongsa but since it is located close to Nongchem high level of soil contamination was found. Periodic monitoring to avoid spread of contamination will be necessary. We have collected total 36 breast milk samples from people living in the area of hotspot and surrounding area, Vientiane, Saravane, Sekong, Attapeu, and Savannaket. Detection limit was 0.16pg/g wet using 20g sample amount. All PCDDs/Fs results showed above detection limit but most of the results for DL-PCBs were below detection limit. In the urban samples it was less and the contamination was likely to be more from food and environment than PCB pollution. As compared to the situation in a soil hotspot (Nongchem and Sekong) it is thought that the source of pollution is not Agent Orange or any other specific pollution source.

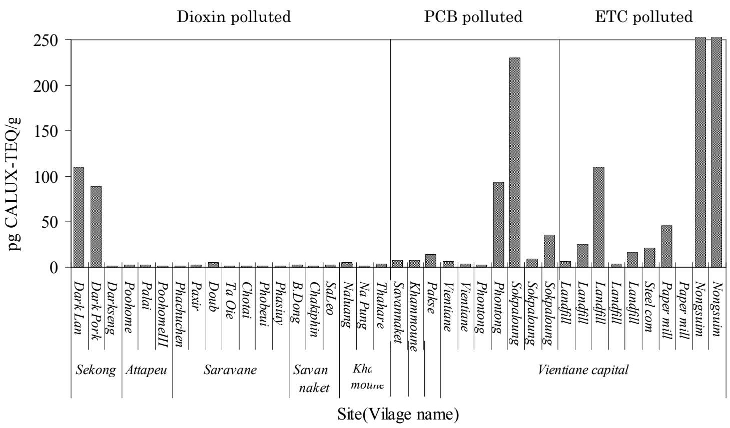

Figure3. Result of Soil sample(Phase1)

Table3. Result of Soil sample (CALUX vs. HRGCMS)

-		CALUX Actual Value			CALUX Toxicity Equivalence Quantity			HRGC/HRMS			
		PCDD/Fs	DL-PCBs pg /g	DXNs	PCDD/Fs	DL-PCBs pg-TEQ/g	DXNs	PCDD/Fs	DL-PCBs pg-TEQ/g	DXNs	Ratio
No.1	Bs1-2	88	0	88	30	0	30	23	0.0016	23	1.3
No.2	Bs1-19	1.8	1.5	3.3	0.62	4.4	5.0	0.21	0.0079	0.22	23
No.3	Bs2-2	3.8	3.8	7.6	1.3	11	12	0.13	0.21	0.34	37
No.4	Bs2-8	130	100	230	45	290	340	40	170	210	1.6
No.5	Bs3-3	110	2.9	110	38	8.5	50	90	9.9	100	0.50
No.6	Bs6-1	11000	110	11000	3800	320	4120	3400	190	3600	1.1

Toxicity Equivalence Quantity (pg-TEQ/g)
=Actual Value (pg/g) x correction factor

PCDD/Fs；correction factor＝0.346×actual value
DL-PCBs；correction factor＝2.939×actual value
DXNscorrection factor＝PCDD/Fs TEQ＋DL-PCBsTEQ

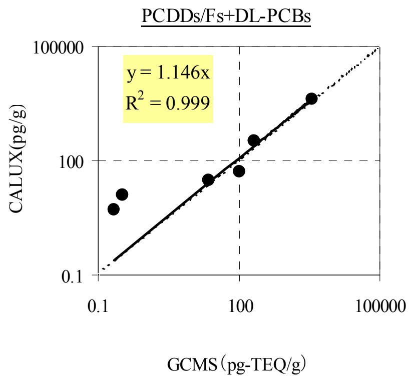

Table4. Result of human milk (Phase2)

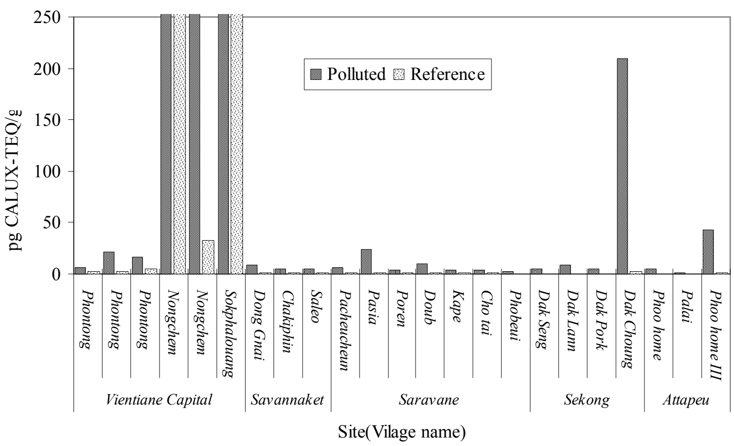

Figure5. Result of Soil sample(Phase2)

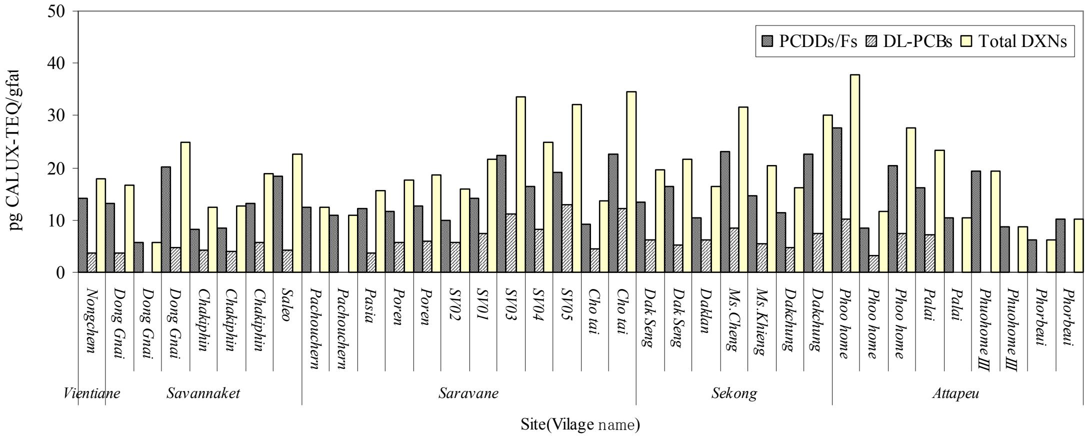

Figure3. Result of Human milk sample (Phase2)

Although we have limited target matrix to soil and seafood and human milk this time, it was meaningful that we could do consistent analysis in the laboratory in a developing country. We had demonstrated that we could perform 81 soil and 8 seafood and 36-human milk analysis in two weeks. The important points in doing this research in developing countries were: 1) Understanding and cooperation of local researcher: We needed cooperation in sample collecting and providing laboratory local researcher and request from the World Bank or international organization and local staff's understanding were fundamental. 2) Secured appropriate laboratory: Frequent technical trade and support made smooth cooperation, contract, or proceeding documentation for shipping. 3) Secured technique: It was fundamental to establish CALUX cell, portable plate, simple incubator that can be used in developing countries. Even if we could get understanding and cooperation, if the method of analysis is not simple and quick, the technique cannot be established in developing countries. In the future, if we could give training to local staffs, invest on equipment and secure route for procurement, CALUX can be used as POPs monitoring tool and sustainable application could be expanded. We hope that it will be a POPs measure in the future and the local staffs would play an important role in the monitoring of POPs.

Based on the results obtained, further study has been done in Phase 3 in East Asian countries: Laos, Thailand, Vietnam, and Cambodia. In Phase 3, screening was done by CALUX and HRGC/HRMS analyses were done for some of the remarkable samples to obtain more detail information and the results will be announced as poster presentation at the Dioxin conference 2009.

Acknowledgement

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References

1. M. Denison, A. Brouwer, G. Clark, U.S patent # 5, 854, 010, 1998.

2. Nakamura T, Nakamura M, Suzuki S, Takahashi M, Fujino J, Yabushita H, Yamamoto T, Brown DJ, Nakai K, Satoh H (2002) Organohalogen Compounds58,381-384