COMPARISON OF DIFFERENT CLEAN-UP, EXTRACTION AND DETECTION TECHNIQUES FOR THE DETERMINATION OF DL-PCBs AND PCDD/Fs IN SAMPLES FROM HIYOSHI CORPORATION IN JAPAN AND SOIL AROUND A WASTE LANDFILL IN CHINA

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Introduction

The Stockholm Convention came to be legally effective in China since Nov. 2004, therefore related emission control and governance standards of persistent organic pollutants (POPs) should be legislated to implement the convention plan in China[1]. However, general invest+igation on environmental background contamination database is lack of, especially the survey on POPs contamination of soil is very limited in China. Sanitary landfill is one of the main methods of waste disposal, but less attention has been paid to soil contamination in landfill, compared with incinerator plants [2-4]. Nine soil samples were collected surrounding the landfill, including sites lying in the lower terrain of the landfill, soil covered on the waste and sites on the natural mountains around the landfill. CALUX (Chemically Activated LUciferase gene eXpression) bioassay is considered to be a fast, sensitive and inexpensive tool for the analysis of a high number of samples [5], therefore the use of this technology in routine analysis of soil samples may be a valuable alternative for HRGC/MS. Two kind of clean-up process were operated in CALUX assay, i.e. manual and semi-auto, two dioxin-extraction methods were compared in CALUX assay, i.e. soxhlet and ultrasonic; as well as two dioxin analysis methods were compared in this work, i.e. CALUX assay and HRGC/MS analysis. Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) were analyzed in this work.

Materials and methods

The samples in this work were divided into two parts. The first six samples, including four soil and two fly ash samples, which are of high concentration, were obtained from Hiyoshi Corporation in Japan. The left nine samples, which are of respectively low concentration, were collected from a municipal solid waste landfill in east China. The soil samples were collected by mixing five different aliquots (each in four main directions of 5m to the center) within a 25m^2 area. The sampling was carried out by inserting a cylindrical steel corer down to a 20cm depth. Approximately 2 kg of soil was taken and mixed at each site. The soil samples were subsequently dried in an electric thermostatic blast box under 100℃ within 3h. Then they were ground and passed through a 2-mm sieve. About 500 g soil of each sample was homogenized through a 60-mesh sieve, and was stored in brown vials away from light. Sources and numbers of nine soil samples are described in Table 1.

For Hiyoshi samples, two kind of clean-up process, i.e. conventional manual and semi-auto, were operated after extraction for CALUX assay. Semi-auto process was operated in a new developed device which was under trial. New-produced acid-silica gel and XCARB columns were used for clean-up instead of manual filled columns. For the nine soil samples collected from the landfill in China, two dioxin-extraction methods, i.e. soxhlet and ultrasonic, were applied in CALUX assay. Both CALUX assay and HRGC/MS were compared in analyzing Hiyoshi samples and Chinese samples.

For Soxhlet Extraction (SxE), overall 7g (dry matter) of soil samples were taken out, and the SxE solution was divided into two parts: the first half was prepared for CALUX assay; the other half 3.5g of SxE samples was refrigerated before HRGC/MS analysis. For ultrasonic extraction (UsE), 3.5g of soil samples were dissolved in 10ml 20%methanol/toluene, and then the solution was shaken and ultrasonic extracted for 5min. The supernatant after UsE was passed through a celite column, the above operations were conducted two times, and the mixed solution was prepared for CALUX assay. The extractions both from SxE and UsE were further loaded onto an acid-silica gel and XCARB (XDS Inc., USA) bigeminal columns for clean-up. The DL-PCBs and PCDD/Fs fractions were then eluted with different solution only onto XCARB column respectively. The CALUX assay was carried out using a recombinant H1L6.1 cell line. The cells were seeded on 96-well microplates, and after

24h of incubation, cells were exposed in duplicate to the purified sample extracts and 2,3,7,8-TCDD standard solution for 20-24h of incubation. Then luciferase activity was measured using an Ensipre luminometer (Perkin Elmer). Duplicate ultrasonic sampling for CALUX assay has been done.

Four of the nine refrigerated 3.5g of SxE samples were taken out for HRGC/MS analysis. Clean-up and analytical determinations were described as the previous research [6]. Each sample was spiked with a mixture of ^{13}C-labelled PCDD/Fs compound stock solution (10μl, 100ng/ml) and clean-up standard (25μl, 8ng/ml) before pickling. The extracts from SxE were subsequently passed through multilayer silica gel column and alkaline alumina column following USEPA Method 1613. The extracts were blow down to 40μl by nitrogen (N_2), and 5μl internal standard solution (200ng/ml) were added before sample were subjected to PCDD/Fs analysis by using HRGC/HRMS (JEOL JMS-800D) with a DB-5MS column (60m×0.25mm×0.25μm).

Results and discussion

The TEQ of DL-PCBs and PCDD/Fs processed by manual and semi-auto clean-up pretreatment are written as “CALUX-M” and “CALUX-A”, respectively. The results show that CALUX-M and CALUX-A of the six samples from Hiyoshi Corporation are well correlated; the ratio between CALUX-M and CALUX-A is in the range of 0.8 to 1.2 (table not show in this paper). Taking the average of CALUX-M and CALUX-A as the CALUX-TEQ of the six samples, the TEQ of DL-PCBs and PCDD/Fs determined by HRGC/MS is written as “WHO-TEQ”. The ratio between CALUX-TEQ and WHO-TEQ were 1.8~2.9 for soil samples and 3.3~3.6 for fly ash (Fig. 1), so generally, CALUX-TEQ showed higher values than WHO-TEQ[7].

TEQ values of the nine samples collected from a municipal solid waste landfill in China are listed in Table 2. The TEQ of total DL-PCBs and PCDD/Fs determined by CALUX assay using ultrasonic and soxhlet extraction are named as “CALUX-UsE” and “CALUX-SxE”, respectively. The TEQ of total DL-PCBs and PCDD/Fs determined by HRGC/MS with WHO-TEF (2006) is named as “HRGC/MS”. Soil samples collected from low-lying sites (No. 1~2) and fresh soil covered on solid waste (No. 3~5) showed lower values than soil located on the mountains surrounding the landfill (No. 6~9). Average CALUX-UsE and CALUX-SxE values of samples No. 1~5 are 1.2 pg-TEQ/g and 1.6 pg-TEQ/g, respectively; while for samples No. 6~9 are 21 pg-TEQ/g and 19 pg-TEQ/g respectively. The TEQ concentrations in all soil samples were at background level[3], providing data reference for the establishment of POPs control directory in China.

In comparison of soxhlet and ultrasonic extraction by CALUX assay, the ratio of UsE to SxE is centralized in the range of 0.6~1.2, the correlation coefficient is 0.956 (Fig. 2). These results show that both soxhlet and ultrasonic extraction meet the standard measurement in the pretreatment of soil in CALUX assay. The coefficient of variation (C.V.) values of the repeated UsE samples are below the required standard value of 30% except the first four samples with lower concentration below 1.8 pg-TEQ/g. Stability of the pretreatment of lower concentration samples warrants further repeat.

CALUX-SxE and HRGC/MS of the four soil samples from landfill were 12~24 pg-TEQ/g and 9～17 pg-TEQ/g respectively, these results show that CALUX and HRGC/MS are highly correlated in soil samples within low concentration. The ratio between CALUX-TEQ and WHO-TEQ was 1.3 ± 0.5, which is close to the result of 1.4 ± 0.7 in the previous research[8].

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Table 1 Sources and numbers of the nine soil samples collected from a landfill in China

Sources of nine soil samples in China	Soil collected from low-lying sites of the landfill	Fresh soil covered on waste	Soil located on the mountains surrounding the landfill
No. of soil samples	No.1, No.2	No.3, No.4, No.5	No.6, No.7, No.8, No.9

Table 2 TEQ Summary of the nine soil samples using UsE and SxE methods by CALUX assay and HRGC/MS

Sample No.	Ultrasonic Extraction (UsE)					Soxhlet Extraction (SxE)		Ratio	
	CALUX 1^{st}	CALUX 2^{nd}	Ave.	S.D.	C.V.	CALUX SxE	HRGC/MS	CALUX-UsE /SxE	CALUX-SxE/ HRGC/MS
1	0.55	1.5	1.0	0.70	67	1.5		0.7	
2	0.27	3.4	1.8	2.2	120	3.0		0.6	
3	1.5	0.31	0.93	0.87	94	1.3		0.7	
4	0.08	1.4	0.75	0.94	126	1.1		0.7	
5	1.0	1.6	1.3	0.36	28	1.2		1.1	
6	26	21	24	3.3	14	20	11	1.2	1.8
7	6.6	9.7	8.2	2.2	27	12	17	0.7	0.7
8	20	22	21	1.6	7.4	21	16	1.0	1.3
9	28	31	29	2.4	8.1	24	19	1.2	1.3

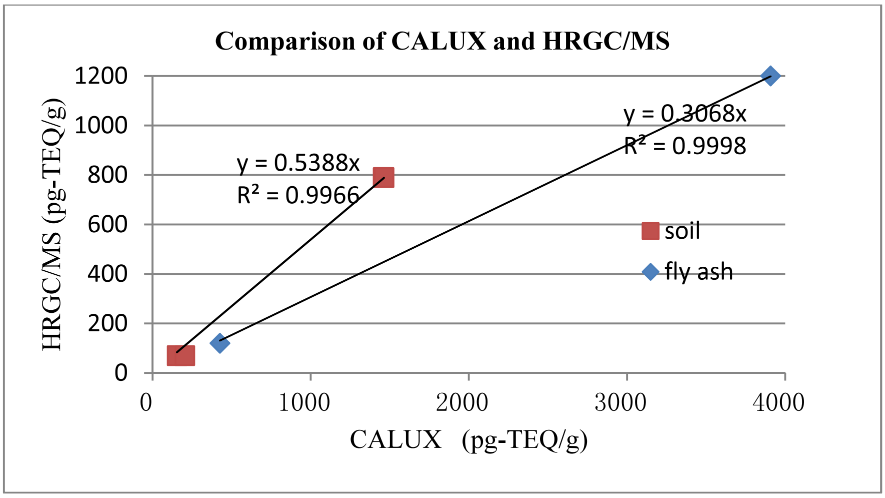

Fig. 1 Comparison of CALUX-TEQ and WHO-TEQ for four soil samples and two fly ash samples obtained from Hiyoshi Corporation in Japan.

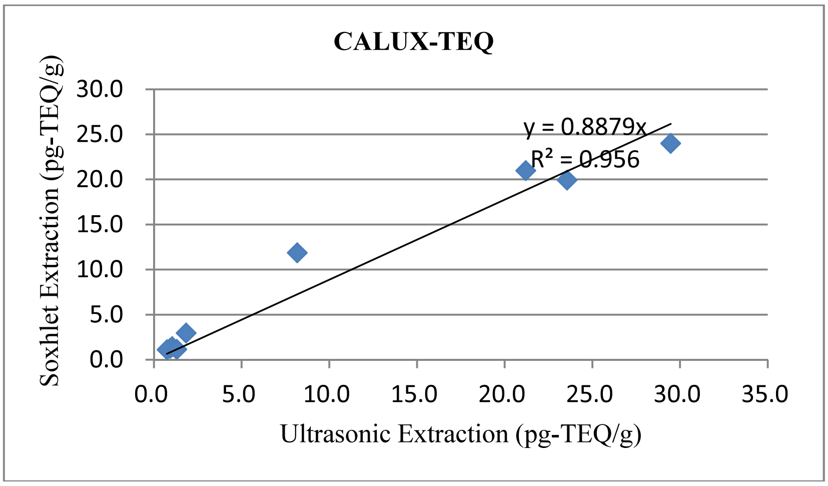

Fig. 2 Comparison of ultrasonic and soxhlet extraction by CALUX assay for the nine samples collected from a municipal waste landfill in China