

ANALYSIS ON PCDD/Fs IN SOIL AROUND WASTE INCINERATION PLANTS IN CHINA USING CALUX ASSAY WITH HRGC/HRMS

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) are a group of toxic related compounds, known as dioxins¹. Dioxins are not produced intentionally, but they are by-products generated from processes when heat is applied to substances containing carbon, oxygen, hydrogen and chlorine². The major and important source of these compounds at present is waste incineration, with most being formed in combustion processes or cooling stage of flue gas¹⁻⁴. Dioxins is then emitted to the ambient air without being fully captured by flue gas purification equipment, and may accumulate in the soil in surrounding environment. Recently, a great number of waste incineration plants have been constructed in China, including municipal solid waste and hazardous waste incinerators. Moreover, the toxicity and pollution condition of dioxins is arousing more and more attention from the common people. The monitoring of dioxins in soil around waste incinerators must to be conducted at present. In order to understand the dioxins levels in the surrounding soil of some typical waste incineration plants, one of analysis methods, that is CALUX assay, was carried out to analyze the concentrations of PCDD/Fs in this study. Additionally, TEQs of PCDD/Fs obtained by the CALUX assay were compared with WHO-TEQ obtained by traditional chemical analysis--HRGC/HRMS.

Materials and methods

The soil samples in this work were divided into two part. The first five soil samples, which are of high concentration, were obtained from Hiyoshi Corporation in Japan. The left twenty-four sample, which are of respectively low concentration, were collected from three different kinds of municipal solid or hazardous waste incineration plants in Zhejiang Province, China. The second part of soil samples were collected by inserting a cylindrical steel corer down to a 10cm depth. Approximately 1.5 kg of soil was taken at each site. The soils were subsequently dried in a ventilated room. 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 refrigerated until analysis.

For HRGC/HRMS analysis, about 10g (dry matter) of soil samples were taken out for extraction, clean-up procedure and analytical determination, described as the previous research⁵. A fully automated ASE 300 system (Dionex, Sunnyvale, CA, USA) was used for sample extraction. Each sample was spiked with a mixture of ¹³C-labelled PCDD/Fs compound stock solution (5μl) and clean-up standard (5μl) before extraction. The extracts from ASE were subsequently passed through multilayer silica gel column following USEPA Method 1613. The extracts were blow down to 20μl by nitrogen (N₂), and 5μl internal standard solution 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).

Soil samples (3.50-3.54g) dissolved in 10ml 20%methanol/toluene, and then the solution was shaken

and ultrasonic extracted for 5min, which were prepared for CALUX assay. The extraction was passed through a celite column, and then the above operations were conducted two times. The mixed solution was further loaded onto an acid-silica gel and XCARB (XDS Inc., USA) bigeminal columns. The DL-PCBs and PCDD/Fs fractions were then eluted with different solution only onto XCARB column respectively, and the PCDD/Fs fraction was selected for CALUX assay in this study. 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. The luciferase activity was measured using a Lucy I luminometer (Antos, Salzburg, Austria).

Results and discussion

The TEQ of PCDD/Fs determined by CALUX assay and HRGC/HRMS with WHO-TEF(2005) are written as "CALUX-TEQ" and "WHO-TEQ", respectively. CALUX-TEQ and WHO-TEQ of five soil samples from Hiyoshi Corporation were 20.8 ~ 7795.2 pg-TEQ/g and 9.7 ~ 6760.0 pg-TEQ/g respectively (not shown in this paper), as shown in Fig.1. These results show that CALUX-TEQ and WHO-TEQ are highly correlated in soil samples. The ratio between CALUX-TEQ and WHO-TEQ were 1.4 ± 0.7 , so generally, CALUX-TEQ showed higher values than WHO-TEQ⁶.

WHO-TEQ of the twenty-four soil samples collected from three waste incineration plants in China were in the range of 1.72 to 31.44 pg-TEQ/g (not shown in this paper), with a mean value of 6.09 pg-TEQ/g. Schuhmacher et al.⁷ investigated the baseline level of PCDD/Fs in soil in Catalonia (Spain), and PCDD/Fs levels ranged from 0.13 to 24.2 pg I-TEQ/g, with a mean value of 1.68 pg I-TEQ/g. In addition, the WHO-TEQ values observed in the present study are basically consistent with those found in Barcelona (Spain; 6.78 ~ 16.62 pg I-TEQ/g)⁸ and Buchon (Korea; 1.25 ~ 74.98 pg I-TEQ/g)⁹. Furthermore, CALUX-TEQ of the twenty-four soil samples were in the range of 1.71 to 44.23 pg-TEQ/g, and CALUX-TEQ and WHO-TEQ values are compared graphically in Fig.2. It is obvious that CALUX-TEQ is significantly correlated with WHO-TEQ of the twenty-four samples from China, and the correlation coefficient is 0.7639, which is lower than that of the five samples from Hiyoshi Company. Notably, maybe PCDD/Fs concentration in the samples from Hiyoshi Company is extremely higher than that from China, and the concentration distributes between 9.7 to 6760.0 pg-TEQ/g, which is more dispersive, while the distribution from China is centralized in the range of 1.72 ~ 31.44 pg-TEQ/g. Therefore, the correlation of the second part samples (China) is not very satisfactory, but the CALUX assay for PCDD/Fs determination has been increasingly recognized as a useful tool. Moreover, the development of a correlation database between WHO-TEQ and CALUX-TEQ for soil samples is required in the future.

The CALUX assay is conducted by the reaction of specific cell line with PCDD/Fs congeners, so CALUX-TEQ values depend on the biological responsiveness to specific PCDD/Fs isomers. To examine the factors affecting the discrepancy found by TEQ determined by bioassay and chemical analysis, relative potency (REP) values for individual PCDD/Fs isomers were applied (the data were shown in another paper)¹⁰. REP-TEQ was calculated by multiplying the concentration of each isomer measured by HRGC/HRMS with its CALUX-REP. Interestingly, CALUX-REP was

found to be close to WHO-TEQ, except that the REP values of 1,2,3,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8,9-HpCDF and OCDF were much higher than WHO-TEF values. It is indicated that biological responsiveness of CALUX assay is much more active to PCDFs, which is a possible factor of differences between CALUX-TEQ and WHO-TEQ. Furthermore, it is speculated that the presence of other AhR agonists is another possible factor of discrepancy. Polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) and a few polychlorinated naphthalene (PCNs) congeners also have dioxin-like activities¹¹.

PCDD/Fs homologues were shown in Table 1. OCDD was the predominant homologue, and the other major homologues are HpCDD, HpCDF and OCDF. Due to the soil samples from different kinds of waste incineration plants, the ratios of PCDFs to PCDDs in soil samples vary greatly, which are the important indicator to ascertain the possible reaction routes for PCDD/Fs formation. For example, [PCDF]/[PCDD] ratios of S20 ~ S24 from the same plant are above one, which indicates that the main route for PCDD/Fs formation is de novo synthesis, and when [PCDF]/[PCDD] ratios of S11 ~ S19 from the same plant are below one, the main route is precursor condensation.

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Reference

1. B.R. Stanmore, (2004), *Combust. Flame*, 136, 398–427.
2. Government of Japan, Dioxins, 2009.
3. H. Fiedler, (2007), *Chemosphere*, 67, S96.
4. E. Finocchio, G. Busca, M. Notaro, (2006), *Appl. Catal.*, B62, 12–20.
5. Li XD, Yan M, Chen T, Lu SY, Yan JH, Cen KF, (2010), *Journal of Hazardous Materials*, 179, 783-789.
6. US Environmental Protection Agency, Test methods for evaluating solid waste, physical/chemical methods. USEPA, 2007.
7. M. Schuhmacher, M.C. Rodriguez-Larena, M.C. Agramunt, J. Diaz-Ferrero, J.L. Domingo, (2002), *Chemosphere*, 48, 187-193.
8. M. Schuhmacher, S. Granero, J. Rivera, L. Muller, J.M. Llobet, J.L. Domingo, (2000), *Chemosphere*, 40, 593-600.
9. J.E. Oh, S.D. Choi, S.J. Lee, Y.S. Chang, (2006), *Chemosphere*, 64, 579-587.
10. Brown D.J., Chu M. van, Overmerir L., Chu A., Clark G.C., (2001), *Organohalogen Compd.*, 53, 211-214.
11. Jun Hasegawa, Keerthi S. Guruge, Nobuyasu Seike, Yuji Shirai, Toshiaki Yamata, Masafumi Nakamura, Hiroshi Handa, Noriko Yamanaka, Shigeru Miyazaki, (2007), *Chemosphere*, 69, 1188-1194.

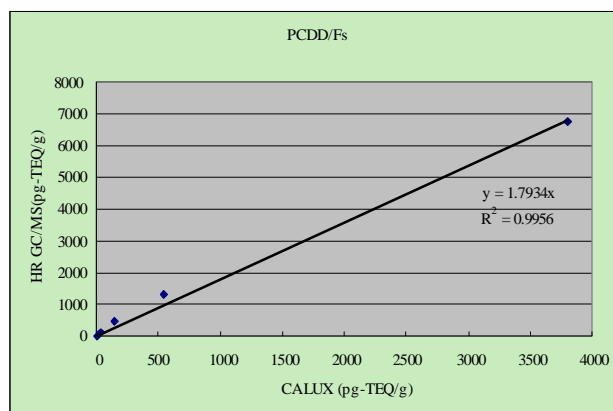


Fig. 1 Comparison of CALUX-TEQ and WHO-TEQ for five soil samples from Hiyoshi Corporation in Japan.

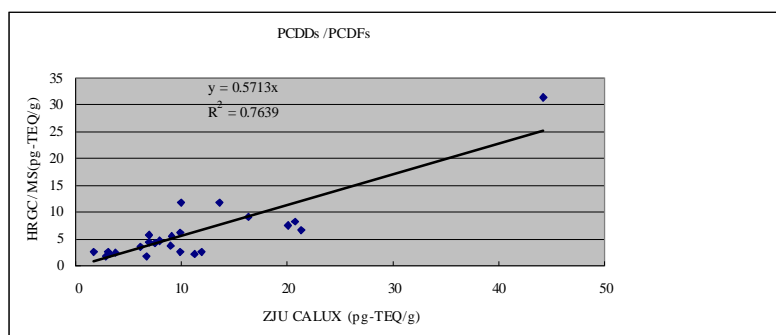


Fig. 2 Comparison of CALUX-TEQ and WHO-TEQ for twenty-four soil samples collected from three waste incineration plants in China.

Table 1 PCDD/Fs homologues in soil samples collected from China

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
TeCDD	0.15	0.25	0.13	0.25	0.00	0.17	0.15	0.15	0.09	0.06	1.76	0.34
PeCDD	0.77	0.95	0.51	0.69	0.00	1.11	1.01	1.18	1.33	0.97	9.15	2.76
HxCDD	2.58	2.23	4.32	2.99	3.24	5.77	5.28	6.99	8.13	3.16	44.41	20.15
HpCDD	4.10	5.03	37.62	18.82	26.90	19.40	14.20	24.60	35.60	8.54	684.54	355.25
OCDD	61.68	78.11	1392.68	776.79	816.79	61.20	65.80	62.20	71.10	39.50	27182.50	9840.81
TeCDF	0.97	1.46	1.15	1.56	1.23	1.09	1.09	0.88	1.07	0.78	1.17	0.15
PeCDF	2.43	2.49	1.73	3.00	1.62	3.72	3.98	3.43	5.06	1.87	2.83	0.42
HxCDF	4.35	4.52	4.16	5.28	4.07	12.41	11.74	15.21	21.21	5.37	4.19	1.13
HpCDF	4.23	4.35	4.41	5.81	4.29	21.75	20.12	27.10	32.54	9.57	7.95	1.21
OCDF	5.06	8.23	7.50	3.67	0.00	10.70	11.20	14.50	22.30	6.99	16.96	6.45
	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
TeCDD	0.31	0.65	0.11	0.41	0.39	0.22	0.69	0.09	0.27	0.00	0.21	0.03
PeCDD	0.49	3.84	0.45	2.45	1.83	1.43	3.71	0.00	1.82	0.53	0.18	0.50
HxCDD	3.99	15.61	2.89	9.60	9.49	8.53	16.74	0.81	1.02	0.96	1.86	1.29
HpCDD	108.06	130.08	73.75	99.46	127.45	168.90	104.20	6.95	4.26	10.68	4.22	9.00
OCDD	11317.50	4956.19	8391.78	4868.26	5424.01	5965.92	1066.52	771.71	226.05	1463.27	44.04	1019.55
TeCDF	2.01	2.37	1.41	2.04	0.70	0.18	1.65	4.51	1.01	5.95	1.10	3.59
PeCDF	3.55	6.63	3.03	4.48	1.59	0.57	5.31	2.38	6.54	6.40	3.46	3.53
HxCDF	8.96	11.85	7.97	8.45	2.63	1.22	8.85	5.97	7.89	13.91	6.97	7.58
HpCDF	15.11	14.81	14.21	14.07	5.23	1.66	12.81	7.28	25.29	19.09	10.96	10.64
OCDF	8.10	2.53	4.43	3.13	3.80	3.56	0.99	9.55	221.54	350.67	7.71	14.19