COMBINATORIAL BIO/CHEMICAL ANALYSIS OF PCDD/Fs IN AGRICULTURAL SOILS FROM CHONGMING ISLAND IN SHANGHAI, CHINA

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Abstract
In this study, we have compared PCDD/Fs levels (expressed as toxic equivalent quantities (TEQs)) in agricultural soil samples in Chongming Island by two analytical approaches: an enzyme-linked immunoassay (EIA) analysis and high resolution GC/MS (HRGC/HRMS) analysis. The PCDD/F concentrations in all 31 soil samples were at background level (7.30-16.7pg EIA-TEQ/g from EIA analysis, and 0.526–1.99 pg WHO-TEQ/g from HRGC/HRMS analysis). Although all the samples were overestimated by the EIA analysis compared to HRGC/HRMS analysis. The absence of false-negatives showed by the EIA analysis suggested the usefulness of this method for preliminary sample screening (prior to HRGC/HRMS analysis) and preliminary characterization of potentially contaminated sites.
Keywords: PCDD/Fs; EIA; HRGC/HRMS; Screening

1. Introduction

Governmental agencies in many countries have adopted HRGC/HRMS as the standard method for dioxin and furan analysis in environmental matrices for its reproducibility and low susceptibility to matrix interferences. For example, the EPA in the United States has method 8290 describing procedures for the analysis of PCDD and PCDFs by HRGC/HRMS (Roy et al et al., 2002). However, HRGC/HRMS analysis is time consuming and expensive. As a consequence, routine monitoring is prohibitively expensive and is infrequently performed.

As an alternative, these bio-analytical detection methods such as CALUX (chemically activated luciferase gene expression), aryl hydrocarbon (Ah) immunoassays, EROD (7-ethoxyresorufin- O-deethylase) and enzyme immunoassays (EIA) have been used for rapid screening of various matrices such as food, sediments, soil and fly ash. Gene assay [Method 4425] and an immunoassay [Method 4025] have been approved by the United States Environmental Protection Agency for screening extracts of environmental samples for planar organic compounds and dioxins respectively(JECFA, 2002). EIA analysis has also been proved useful for environmental monitoring studies (Van Emon and Lopez-Avila, 1992; Van Emon, 2001; Roda et al., 2006; Trang et al., 2007).

In our previous study, we have developed a combined strategy of screening with the EIA analysis and the HRGC/HRMS confirmatory method to investigate PCDD/Fs of soil in Shanghai (Li et al., 2009). And a regional screening value 19.0 pg EIA-TEQ/g was used as the screening bound, corresponding to the value of 10.0 pg TEQ/g, which is the maximum value of the background upper value coming from available literature.

In this work, we selected Chongming Island as an example area to verify our combined strategy of screening with the EIA analysis and the HRGC/HRMS confirmatory method. We have compared PCDD/Fs levels in all the 31 soil samples, detected by two analytical approaches: HRGC/HRMS analysis and EIA analysis.

2. Materials and methods

2.1 Sampling sites

Shanghai has the third largest island of China, Chongming Island, which covers an area of 1000km^2. Chongming Island is the greatest agricultural area in Shanghai which covers about 30% cultivated land. Although Chongming Island is acclaimed as "'last virgin territory" in Shanghai, Chongming Island is also surrounded by numbers of rapid development industrial areas, such as Pudong new area, Baoshan District in Shanghai and Taicang City in Jiangsu Province. And the mainland's commercial capital has drawn up a blueprint to develop Chongming, covering more than 1,400 square kilometers. The environmental issues in Chongming Island still need to be concerned. Fig 1 showed the sampling sites map.

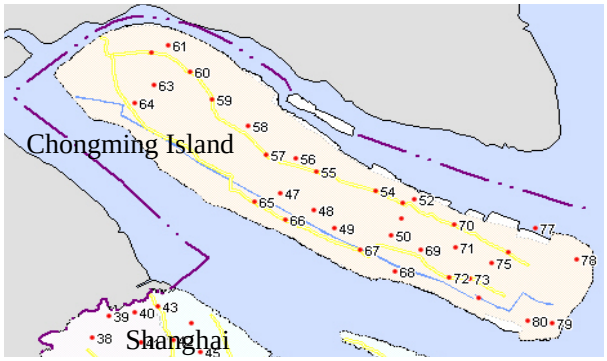

Fig.1 Map of sampling site

2.2 Sample collection and pre-treatment

The soil samples were collected during the spring (from April to May) in 2007. The sampling location is open and not excessively covered by the crop. Three top soil samples were taken within a 5-10 meters radius at the depth of 0-20 cm at each site using a pre-cleaned steel spoon. These three samples were then mixed together in a glass bottle being transported to the laboratory.

The samples were dried at room temperature for about 10-14 days. Dried soil were crushed by ceramic cut mill and then filtered through the stainless steel sieve with an aperture of 1*1 mm. The moisture of these homogenous soil samples were less than 3%. Finally the samples were labeled and stored in glass flasks at room temperature of 20 ℃ till analysis.

2.3 EIA analysis

The analytical procedure is based on the modified 4025 approved by USEPA using an SP3 sample preparation kit and a DF1 immunoassay kit (Cape-technologies, ME, USA). 5g Soil samples was mixed with 10g sodium sulfate and was then extracted using the solvent of 1:1 (v/v) hexane: acetone by shaking 3 hours. The supernatant of the extraction was then evaporated via nitro blow with tetradecane as the keeper. The residue was re-dissolved in hexane and loaded onto a coupled acid-silica: activated carbon mini-column. The samples and the standard were added to the antibody coated tube in which the sample diluents had been added. Then the contents in the tube were removed and the tubes were washed with Triton solvent. Subsequently the competitor-HRP was added allowing its binding to the free anti-PCDD/Fs site on immobilized antibodies for 15 minutes. After removed the contents in the tube and washed the tube completely, the HRP substrate was added to the tube. Colorless substrate was converted to blue color in proportion to the amount of bound enzyme after 30 minutes reaction time. The optical density value was read immediately after 0.5ml stop solution had been added to each tube.

2.4 HRGC/HRMS analysis

About 10 g (dry matter) of soil sample were used for PCDD/F analysis. Each sample was spiked with a mixture of $^{13}C_{12}$- labelled PCDD/F compound stock solution (10 µl) before extraction. The extracts from the ASE were subsequently followed by N2 concentration, acid silica bed, multilayer silica gel column and florisil column clean-up procedure following the Method of USEPA 1613. 10 µl of $^{13}C_{12}$- labelled PCDD/Fs internal standard solution were added before sample were subjected to analysis by high-resolution gas chromatography coupled with a high-resolution mass spectrometry (HRGC/HRMS) (Autospec Premier, Waters)) with a DB-5MS column (60m×0.25mm×0.25µm). And the Recoveries of internal standards, as determined against external standard, generally varied between 47.2 and 78.5%, and were all satisfied with the Method of USEPA 1613.

3. Results and discussion

3.1 The results from EIA analysis and HRGC/HRMS analysis

The concentrations of 17 2,3,7,8-PCDD/Fs congeners were determined in 31 soil samples collected from Chongming island by HRGC/HRMS and EIA analysis respectively. Table 1 summarizes the WHO-TEQ and EIA-TEQ concentrations of PCDD/Fs in these 31 samples. The total 2,3,7,8-PCDD/Fs concentrations ranged from 0.53~1.99 pg WHO-TEQ/g, with an average value of 1.07 pg WHO-TEQ/g from HRGC/HRMS analysis. While the total concentrations ranged from 7.30-16.7 pg EIA-TEQ/g, with an average value of 11.5pg EIA-TEQ/g from EIA analysis. The low SD values were observed from both analytical methods, indicating that

no oblivious pollution sources in Chongming Island. The EIA result indicated that all the soil samples were below the regional screening value 19.0g EIA-TEQ/g, obtained by previous study (Li et al., 2009). And the HRGC/HRMS analysis results validated that all the soil samples were actually below the maximum value of the background upper value coming from available literature (10.0 pg TEQ/g). Although all the samples were overestimated by the EIA analysis compared to HRGC/HRMS analysis. The absence of false-negatives showed by the EIA analysis suggested the usefulness of this method for preliminary characterization of potentially contaminated sites.

Table 1 The results from EIA analysis and HRGC/HRMS analysis

Sample	Sample TEQ obtained by (pg/g)		Ratio of EIA/	Sample	Sample TEQ obtained by (pg/g)		Ratio of EIA/
	HRGC/MS	EIA	HRGC/HRMS		HRGC/MS	EIA	HRGC/HRMS
47	0.78	9.6	12.26	65	1.52	16.7	11.01
48	0.78	12.1	15.43	66	1.99	9.9	4.98
50	0.63	9.5	15.14	67	1.56	14.7	9.45
51	0.84	12.3	14.61	68	1.07	15.1	14.11
52	1.32	9.9	7.48	69	0.91	13	14.34
54	0.60	9.4	15.78	70	0.79	12.2	15.39
55	1.44	13.3	9.22	71	0.94	7.3	7.74
56	1.29	13.1	10.14	72	1.60	11.1	6.93
57	0.78	11.5	14.68	73	1.40	14.7	10.50
58	1.30	13.6	10.46	74	0.98	11.4	11.64
59	0.73	9.5	13.09	75	1.87	11.1	5.93
60	0.53	10.8	20.54	76	0.76	8.2	10.79
61	0.74	9.7	13.05	77	0.69	11.4	16.53
62	0.78	9.1	11.60	79	1.10	7.4	6.70
63	0.95	14.4	15.22	80	1.14	8.3	7.27
64	1.47	10.7	7.29				

3.2 The comparison of TEQ levels derived from EIA and HRGC/HRMS analysis

Table 1 showed results obtained using different analytical methods on a total of 31 agricultural soil samples. The mean TEQs determined by EIA analysis was approximately ten times higher than WHO-TEQ determined by HRGC/HRMS analysis. Such a tendency has been observed in other investigations that have analyzed environmental samples by both Biotechnology and chemical method (US Environmental Protection Agency, 2007; Tomoaki T et al., 2008; Croes K et al., 2013).

Fig 2 compares the TEQs obtained using the EIA analysis and the HRGC/HRMS analysis. The correlation coefficients between the EIA-TEQ and WHO_{2005}-TEQ were 0.10 for all the 31 soil samples. Fig 3 showed after exclusion of 8 outliers, the correlation coefficients between the EIA-TEQ and WHO_{2005}-TEQ were much higher (0.63). Most of reports comparison between the EIA analysis and the HRGC/HRMS analysis results assessed the relationship between the two measurements by calculating the correlation coefficients. However high correlation does not necessarily mean the two measures are interchangeable and comparable. Moreover, the typical hypothesis test against a correlation coefficient of 0 is not as useful when the two measures are expected to be correlated (Bland and Altman, 1986).

No correlation was found between the abundance of any of the 17 congeners included in TEQ calculations (following HRGC/HRMS analysis) and the EIA analysis. This has led us to conclude that the differences between the TEQs obtained by the two methods are likely due to the presence, in the analyzed soil extracts, of interfering compounds that are not included in the calculations of TEQs (Roy et al et al., 2002). We therefore suggest that competitive binding of interfering compounds to the antibody may have been a confounding factor in our study.

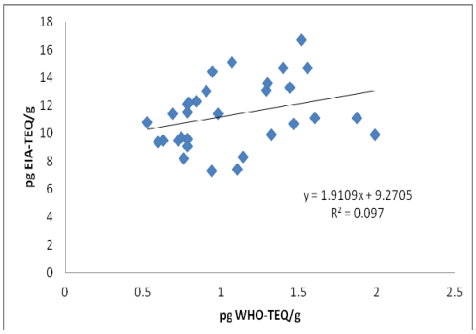

Fig 1 Correlation study between EIA and HRGC/HRMS analysis

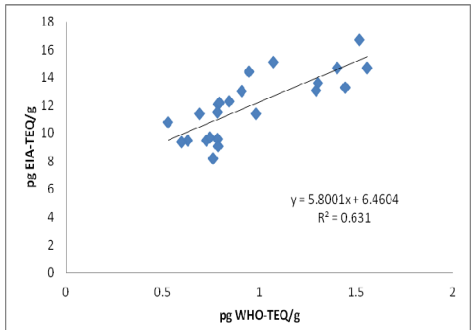

Fig 2 Correlation study between EIA and HRGC/HRMS analysis after exclusion of outliers

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