RAPID DETERMINATION OF DIOXINS IN MUNICIPAL WASTE INCINERATION ASH AND CONTAMINATED SOIL USING TIME-RESOLVED FLUOROIMMUNOASSAY

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Introduction

The most important requirements regarding dioxins in Japan are to decrease dioxins generated at waste incinerators and to remedy dioxin-contaminated soil around some incineration facilities. A rapid and easy method for analyzing dioxins in fly ash, bottom ash, exhaust gas and contaminant soil is needed to control the dioxins effectively. Quantitative determination of dioxins concentration by high resolution gas chromatography/ high resolution mass spectrometry (HRGC/HRMS) is highly sensitive and accurate for each dioxin isomer, but is expensive and time consuming. With this background, immunoassay as a simple, rapid method of measuring dioxins has been applied to quantitatively determine dioxins in various contaminated substances.

It was described previously[1] that the values measured by immunoassay were higher than those by HRGC/HRMS in the analysis of fly ash taken from municipal solid waste incinerators (MSWIs). This finding suggests that coexisting substances in samples could interfere with the immuno-reaction. However, there have been few attempts to solve this problem and to apply immunoassay to other materials such as contaminated bottom ash and soil that might contain more interfering substances than the fly ash. In this study, the viability of a newly improved immunoassay system for the analysis of dioxins in fly ash, bottom ash and contaminated soil was examined. The system consists of an efficient and rapid extraction using an accelerated solvent extractor (ASE), an improved cleanup, and time-resolved fluoroimmnoassay (TRFIA) analysis.

Methods and Materials

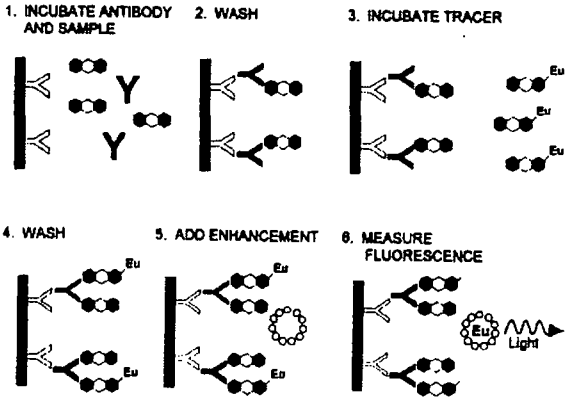
Samples: 21 fly ash samples, 4 bottom ash samples and 6 soil samples from or around MSWIs were analyzed.

Extraction: Dioxins in samples were extracted using Accelerated Solvent Extractor (ASE-200, DIONEX)[2]. 1-4 g of fly ash samples, 20-40 g of bottom ash samples and 40-100 g of soil samples were prepared and the extraction was performed at 150 degree Celsius at 2000 psi for 30 minutes in toluene containing 5%(v/v) glacial acetic acid.

Cleanup: Samples of concentrated crude extracts were applied respectively to a multi-layer silica gel column which was filled from bottom to top with 0.18 g of silica gel, 0.6 g of 2% potassium hydroxide-impregnated silica gel, 0.18 g of silica gel, 0.9 g of 44% sulfuric acid-impregnated silica gel, 1.2 g of 22% sulfuric acid-impregnated silica gel, 0.18 g of silica gel, 0.6 g of 10% silver nitrate-impregnated silica gel and 1.2 g of

sodium sulfate. Samples were eluted with 50 ml of n-hexane, respectively. The elutes were applied respectively to an alumina column which was filled from bottom to top with 2.5 g of alumina (Activated, Basic, Activity 1) and 1.0 g of sodium sulfate. Samples were eluted with 15 ml of n-hexane containing 50% dichloromethane after washing with 10 ml of n-hexane and 10 ml of n-hexane containing 2% dichloromethane. After the elutes were evaporated to dry up, samples were dissolved in 0.1 ml of methanol.

TRFIA analysis: The immunoassay for dioxin was performed using Hybrizyme DELFIA™ TCDD Test kit based on competitive TRFIA by HYBRIZYME Corporation. TRFIA is a solid phase fluoroimmnoassay. During incubation with a sample and TeCDD antibody, any TeCDD that is present binds to the antibody. A second antibody, which binds with the TeCDD antibody, is attached to the microtiter plate wells, and traps the Ab-TeCDD complex. A wash step removes any interfering substances that may be present in the sample. A europium-labeled dioxin compound (TeCDD tracer) is then allowed to bind to any TeCDD antibody binding sites that are empty. A second wash separates antibody–bound and free tracers. Following the second wash step, the addition of enhancement solution forms highly fluorescent chelates with the bound europium ions. The amount of fluorescence measured is inversely proportional to the concentration of TeCDD in the cleanup sample. 2,3,7,8-TeCDD (Cambridge Isotope Laboratories, Inc.) in methanol was used as a calibration standard for assay.



HRGS/HRMS analysis: Extraction, cleanup, HRGS/HRMS analysis and calculation of the toxic equivalent quantity (TEQ) were carried out according to JIS[3]. TEQ was calculated based on WHO-TEF (1998).

Results and Discussion

In the preliminary experiment, it was confirmed that the efficiency of dioxin extraction using ASE is equivalent to that of Soxhlet extraction based on the JIS method[3] that took 16 hours. Cleanup using the multi-layer silica gel column and the alumina column was able to provide a cleaned-up sample suitable for TRFIA. This newly improved cleanup method is a simplified one based on the JIS method[3]. 10% silver nitrate-impregnated silica gel was effective to remove sulfuric compounds and also alumina was effective to remove nonpolar compounds in samples.

Table 1 shows cross-reactivity in TRFIA for each isomer among PCDDs and PCDFs compared with 2,3,7,8-TeCDD. The antibody used in TRFIA has strong cross-reactivity for 2,3,7,8-TeCDD, but rarely reacts with dioxin isomers which have over 5 chlorine residues. Nevertheless, TRFIA has unique specificity for dioxins; a fairly good correlation between TEQs estimated by HRGS/HRMS analysis and values obtained by TRFIA (Fig. 1) was obtained as well as correlations between 2,3,7,8-TeCDD concentrations determined by HRGS/HRMS and the values done by TRFIA (Fig. 2) and between 2,3,7,8-TeCDF by HRGS/HRMS and TRFIA (Fig. 3). The slope of the linear regression equation between TEQs by HRGS/HRMS and TRFIA ($y=0.10x+0.81$) is 0.10. This means TRFIA covers the reaction to several isomers of dioxins that have TEF. The ratios of TRFIA values to TEQ values in the soil and bottom ash samples in the range of low concentration were slightly higher than those for fly ash samples. However, it is concluded that TEQ values can be predicted from TRFIA values by the linear regression equation having a strong correlation between TEQ and TRFIA values for semi-quantitative screening and monitoring. In total, the analysis took 250 minutes from the extraction to the assay. These results suggest that this TRFIA is effective for screening and monitoring dioxins in fly ash, bottom ash and contaminated soil.

Acknowledgments

We would like to thank HYBRIZYME Corporation for their kind advice and providing TRFIA kits.

References

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2. Richter B.E., Ezzell J.L., Knowles D.E. and Hoefler F. (1997) Chemosphere 34, 975
3. JIS Handbook 2000, 10, 2080 ISBN4-542-12987-X

Table 1. Cross-reactivity of TRFIA for PCDDs and PCDFs (2,3,7,8-TeCDD was used as a calibration standard)

PCDDs	Cross-reactivity %	PCDFs	Cross-reactivity %
2-CDD	0.9	2,8-DCDF	0
2,3-DCDD	7.3	2,3,7,8-TeCDF	33
2,7-DCDD	14	1,2,7,8-TeCDF	0.1
2,3,7-TriCDD	28	1,2,3,7,8-PeCDF	0
2,3,7,8-TeCDD	100	2,3,4,7,8-PeCDF	0.2
1,2,7,8-TeCDD	0.1	1,2,3,7,8,9-HxCDF	0
1,2,8,9-TeCDD	0	1,2,3,6,7,8-HxCDF	0
1,2,3,7,8-PeCDD	0	2,3,4,6,7,8-HxCDF	0
1,2,3,7,8,9-HxCDD	0	1,2,3,4,7,8-HxCDF	0
1,2,3,6,7,8-HxCDD	0	1,2,3,4,6,7,8-HpCDF	0
1,2,3,4,7,8-HxCDD	0	1,2,3,4,6,7,8,9-OCDF	0

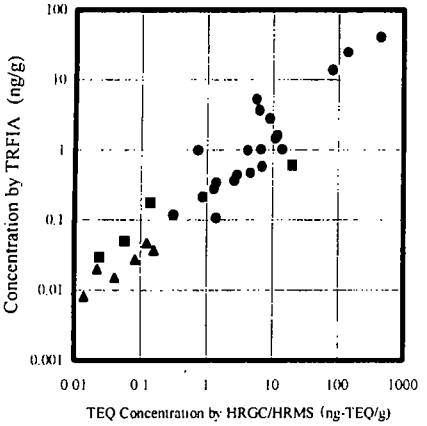

Figure 1. Relationship between dioxins measured by HRGC/HRMS(TEQ) and TRFIA.
(●) represents fly ash sample extracts, (■) represents bottom ash sample extracts and (▲) represents soil sample extracts.
y=0.10x + 0.81, R=0.96, n=31.

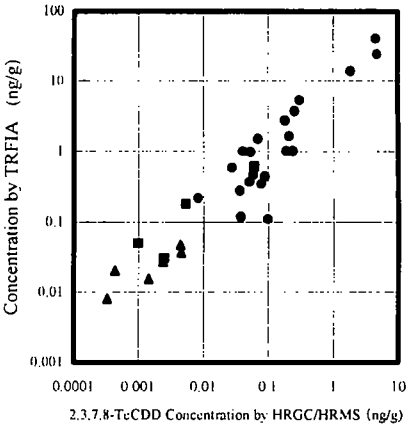

Figure 2. Relationship between dioxins measured by HRGC/HRMS(2,3,7,8-TeCDD) and TRFIA.
(●) represents fly ash sample extracts, (■) represents bottom ash sample extracts and (▲) represents soil sample extracts.
y=6.8x + 0.33, R=0.96, n=31.

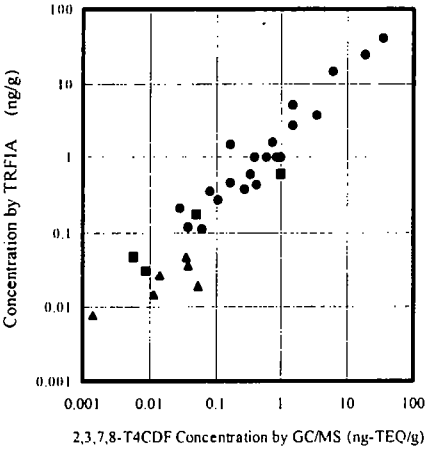

Figure 3. Relationship between dioxins measured by GC/MS(2,3,7,8-T4CDF) and TRFIA.
(●) represents fly ash sample extracts, (■) represents burnt residue sample extracts and (▲) represents soil sample extracts.
y=1.2x + 0.48, R=0.99, n=31.