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DEVELOPMENT OF A QUICK & EFFICIENT ANALYSIS METHOD OF DIOXINS USING ELISA

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Introduction

High-resolution gas chromatography / high-resolution mass spectrometry (HRGC/HRMS) is commonly used for identifying dioxin congeners. It has advantages in separating a number of congeners and quantifying each one with high sensitivity and precision. HRGC/HRMS, however, has disadvantages as well. It takes a long time for measurement, is expensive and needs highly advanced skills to analyze complicated specimens. Due to such disadvantages, a quick, simple and inexpensive method of dioxin analysis is in demand.

Recently enzyme-linked immuno-sorbent assay (ELISA) methods[1,2] and Ah-receptor dependent assay methods [3-5] have emerged as quick and simple analytical tools. Traditionally, ELISA methods were meant to detect 2,3,7,8-TeCDD, which is the most toxic but only one of many dioxin congeners. However, 2,3,7,8-TeCDD shows a poor correlation with toxicity equivalency (TEQ). We have discovered that other congeners of dioxins such as PentaCDF and HexaCDF display good correlations with TEQ.[6] We have successfully developed a unique and stable anti-dioxin monoclonal antibody that widely reacts with dioxin congeners, including PentaCDF and HexaCDF. Using this antibody, we have developed a quick, simple and highly sensitive ELISA method designed for dioxins having good correlations with TEQ.

Materials and Methods

Sampling

For the test, we used samples of exhaust gas, fly ash and soil from municipal-waste incineration plants. Dioxins in the exhaust gas were captured by a silica-fiber and XAD-2 resin with a vacuum pump and a gas meter located downstream, by means of isokinetic sampling (gas flow of approximately $3m^3$/4hr). Soil samples were collected from six different locations surrounding the incineration plants. Fly ash samples came from the electrostatic precipitator (EP) ashes and the bag filter ashes collected at eight such plants.

Clean-up Procedure for ELISA and HRGC/HRGCM

Solid samples (silica filter, XAD-2, fly ash and soil) were Soxhlet-extracted with toluene. The extract was washed with H_2SO_4 until the sulfuric acid layer became colorless. It was loaded to multi-layered silica gel chromatography column (Na_2SO_4 / 10%$AgNO_3$-silica / silica gel) to remove various types of polycyclic aromatic hydrocarbons (PAHs). After removing PAHs from the samples, halves of each extract were completely dried and reconstituted with 2ml Dimethyl

sulfoxide (DMSO), and subjected to ELISA analysis. The other halves were cleaned up by an activated carbon chromatography column. Each final extract was dried and reconstituted with decane, and analyzed by HRGC/HRMS.

Antigen coated micro plate and Standard substance

2,4,5-Trichlorophenol linked Bovine Serum Albumin (TCP-BSA) was diluted with phosphate-buffer-with- saline (PBS) based coating buffer and dispensed on 96 wells of polystyrene micro plate and incubated in a cold room over night. After coating, the buffer was discarded, and each well was washed with PBS/Tween20. Afterwards, all wells were blocked with blocking solution containing skim milk constituents. As a standard substance for calibration, we adopted 2,4,5-trichlorophenol-glycylglycine (TCP-gly-gly) that was synthesized using activated TCP hapten and glycylglycine.

Estimation of samples

Each 100µl of the sample that was cleaned up and reconstituted with DMSO was thoroughly mixed with an equal volume of PBS. Then, 200µl solution of the anti-dioxin monoclonal antibody was added. 100µl of this mixture was dispensed in the wells of micro plate coated with TCP-BSA. After each mixture is placed in the wells, the whole micro plate was incubated in a cold room (2-8 °C) for an hour, and all wells were washed 3 times in a plate washer. 100µl of a solution of goat antibody labeled with horseradish peroxidase (HRP) was dispensed into each washed well. The plate was incubated for an hour at room temperature (around 25 °C), and all wells were washed in the washer. Next, we added 100µl of Tetramethylbenzidine (TMB) solution to each well and developed a color at 25 °C for 30 minutes. Finally 100µl of 0.5N H_2SO_4 solution was added into each well to stop the developing reaction, and light absorption of each well was measured at the wavelength of 450nm. Experiments were performed in triplicate (n=3) for every sample. The calibration curve was obtained using TCP-gly-gly as the standard substance, although other ELISA systems generally use highly toxic 2,3,7,8-TeCDD.

Results and Discussion

Cross-reactivity

Table.1 show the cross-reactivity of typical chemical compounds to the anti-dioxin monoclonal antibody that is used for the ELISA. The antibody widely reacts with PentaCDF and HexaCDF, but hardly does so with many other compounds that exist in the samples as impurities. The correlation between each 2,3,7,8-substituted PCDDs/PCDFs concentration and TEQ by HRGC/HRMS is also shown in the Table 1. These data had been recovered from the archives of test results of exhaust gas at Takuma's Research Center accumulated over a long period. With HRGC/HRMS, each congener concentration was measured independently, and the sum of each value multiplied by the toxicity equivalency factor (TEF) was considered the total TEQ. This result suggests that the sum of concentrations of PentaCDF and HexaCDF can be substituted for the TEQ and it is not necessary to measure all congeners that have TEF. Studies of fly ash samples and soil samples revealed nearly the same results as those of exhaust gas samples. The antibody that enables this type of measurement is very suitable for determination of TEQ. Moreover, it has such high sensitivity as to detect 1,2,3,7,8-PeCDF down to the level of 0.15 ng/ml. This is good enough to measure dioxins in the ash, soil, and exhaust gas to the limits mandated by the government.

Table 1 Cross-reactivity of typical chemical compounds by ELISA

PCDDs/Fs	WHO-TEF	Cross-Reactivity (% j	Coefficient of correlation with TEQ
2,3,7,8-TeCDD	1	1.1	0.20
1,2,3,7,8-PeCDD	1	29.9	0.32
1,2,3,4,7,8-HxCDD	0.1	24.8	0.43
1,2,3,6,7,8-HxCDD	0.1	18.0	0.46
1,2,3,7,8,9-HxCDD	0.1	**39.8**	0.45
1,2,3,4,6,7,8-HpCDD	0.01	17.0	0.51
1,2,3,4,6,7,8,9-OCDD	0.0001	<0.1	0.48
2,3,7,8-TeCDF	0.1	1.5	0.40
1,2,3,7,8-PeCDF	0.05	**100.0**	**0.98**
2,3,4,7,8-PeCDF	0.5	16.1	**0.99**
1,2,3,4,7,8-HxCDF	0.1	**45.9**	**0.95**
1,2,3,6,7,8-HxCDF	0.1	**36.6**	**0.97**
1,2,3,7,8,9-HxCDF	0.1	**42.7**	0.69
2,3,4,6,7,8-HxCDF	0.1	**43.8**	**0.80**
1,2,3,4,6,7,8-HpCDF	0.01	17.9	**0.82**
1,2,3,4,7,8,9-HpCDF	0.01	28.7	0.65
1,2,3,4,6,7,8,9-OCDF	0.0001	8.0	0.62

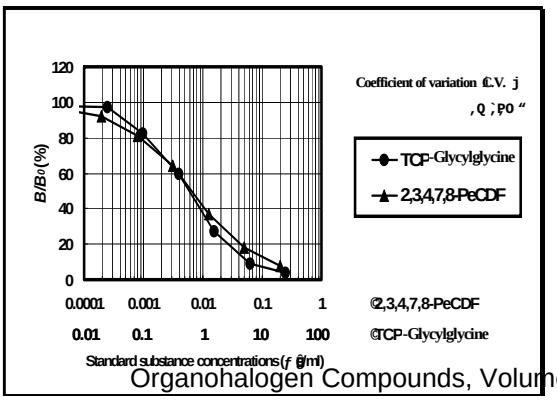
Other compounds	Cross-Reactivity (% j
PAHs	
Anthracene	< 0.1
Naphthalene	< 0.1
Fluoranthene	< 0.1
Pyrene	< 0.1
Fluorene	< 0.1
Benz[a]anthracene	< 0.1
Benzo[b]fluoranthene	< 0.1
Benzo[k]fluoranthene	< 0.1
Benzo[ghi]perylene	< 0.1
Chrysene	< 0.1
Indeno[1,2,3-cd]pyrene	< 0.1
Phenanthrene	< 0.1
Coplanar-PCBs	
3,3',4,4'-TeCB	< 0.1
3,3',4,4',5-PeCB	< 0.1
3,3',4,4',5,5'-HxCB	< 0.1
Chlorobenzene & Chlorophenol	
m-Dichlorobenzene	< 0.1
1,2,3-Trichlorobenzene	< 0.1
2,4-Dichlorophenol	< 0.1
3,4-Dichlorophenol	< 0.1
2,4,5-Trichlorophenol	< 0.1
Chlorobiphenyl	
p-chlorobiphenyl	< 0.1

Calibration curve

The calibration curve of TCP-gly-gly is shown in Figure 1. Also shown is another curve by 2,3,4,7,8-PeCDF as a standard substance for TEQ. They indicate that TCP-gly-gly can be used as a standard substance for calibration curve, although its reactivity is almost one-one hundredth of 2,3,4,7,8-PeCDF.

Correlation

Using this ELISA method, we measured TEQ of samples of exhaust gas (n=19), fly ash (n=8), and soil (n=6). As a result, fairly good correlations were observed between each ELISA value and corresponding TEQ measured by HRGC/HRMS. Coefficients of correlation were R^2=0.98 (exhaust gas), R^2=0.99 (fly ash), and R^2=0.97 (soil). As the summation of the measured results, Figure 2 shows the correlation between ELISA values and TEQ values of exhaust gas samples by HRDC/HRMS method. The test has proven that our ELISA method is effective for the measurement of dioxins in these three kinds of samples.



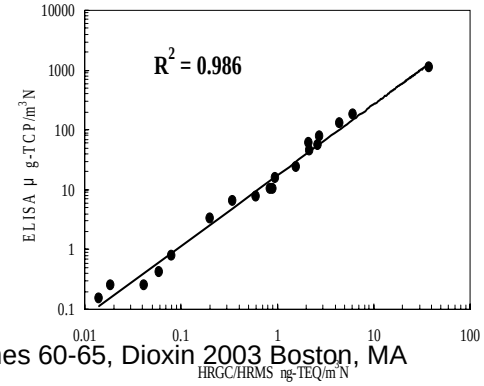


Figure 1 The comparison between two standard curves PentaCDF and TCP-Glycylglycine as a standard

Figure 2 Correlation between ELISA values and HRGC/HRMS values of exhaust gas samples

Conclusion

There is a strong correlation between the ELISA results and HRGC/HRMS-measured TEQ of environmental samples such as exhaust gas, fly ash, and soils. The cost per sample of ELISA analysis is low, and it is capable of multiple analyses of many samples. Therefore, the ELISA method is suitable where frequent measuring is required for daily combustion control, and for screening polluted soils. Furthermore, the ELISA method, by using TCP-gly-gly, not only improves safety of all experimental processes, but also attains excellent stability by enabling storage of every reagent in an ordinary refrigerator at 2-8 °C. It also enables transportation of all reagents without special handling.

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