Development of ELISA for Determination of Dioxins

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Introduction

Dioxins(Polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls(DL-PCB)) are one of the major concerns in environmental contaminants due to their teratogenic, reproductive, immunotoxic and carcinogenic effects.[1)] Currently, the relative toxicological/biological potency of a complex mixture of dioxin-like chemicals is assessed by the toxic equivalent factor (TEF) approach in which the concentration of the individual compounds present in the mixture are multiplied by their specific TEF and the sum of the values is expected as TCDD (2,3,7,8-tetrachlorodibenzo -*p*-dioxin) toxic equivalents (TEQs).[2)] The values of TEQs are usually determined by high resolution gas chromatography mass spectrometry (HR-GC-MS). This conventional analytical method is highly accurate and reliable. But it has drawbacks such that it requires expensive instrumentation and laborious operation including pretreatment and peak assignment process, resulting in a poor performance of measuring a number of samples. Therefore, simple, rapid and cost-effective analytical methods such as enzyme-linked immunosorbent assay (ELISA) are strongly needed for determination of dioxins. In this paper, we report a newly developed Dioxin ELISA, whose values were well approximate to those of HR-GC-MS in flue gas, fly and bottom ash samples based on TEQ values.

Materials and Methods

Reagents

Anti-dioxin monoclonal antibody (Dx02-B) and antigen-BSA (bovine serum albumin) conjugates (77-BSA) were obtained from Kyoto Electronics Manufacturing Co., Ltd. (Kyoto Japan). Dioxins, and other chemical reagents were purchased from Wako Pure Chemicals Industries (Osaka, Japan).

Pretreatment of samples

Twenty samples of flue gas and 10 samples each of bottom and fly ash were used in this experiment. The flue gas samples were collected with DioANA filter according to JIS K0311 (1999). The collected gas samples, and fly and bottom ash samples were treated with/without hydrochloric acid, and then extracted with accelerated solvent extraction method (ASE-200, Japan Dionex Co. Ltd., Osaka Japan) according to the instruction manual. The extracted samples were further purified with automated sample preparation system (SPD-600, Kyoto Electronics Manufacturing Co., Ltd., Kyoto Japan).

Immunoassay Procedure

Dioxins or pretreated dioxin samples were dissolved and diluted in 100% dimethylsulfoxide (DMSO), and added to dilution reagent to give a final concentration of 20 % DMSO solution prior to the ELISA assay. 25 uL of sample and 25 uL of diluted anti-dioxin monoclonal antibody were added for the assay to antigen-BSA coated microplate, and then incubated for 30 min at room temperature. After washing the plate, 50 uL of diluted anti-mouse IgG antibody-HRP conjugate were added, and then incubated for 60 min at room temperature. After washing the plate, 50 uL of coloring reagent was added and incubated for 30 min. The coloring reaction was stopped with 50 uL of stop reagent, and the absorbance was measured at 450nm.

B/B_0(%)=(absorbance at sample) / (absorbance at Dioxin=0) *100

Results and Discussion

Range of quantification

It was reported that 2,3,4,7,8-pentachloro dibenzofuran (2,3,4,7,8-PeCDF) was one of the main isomers, which highly contributed to TEQ values in environmental samples[3)]. Therefore, 2,3,4,7,8-PeCDF was used as standard. The typical standard curve was shown in Figure 1. The range of quantification (ROQ) was from 3.1 to 50 ng/mL in 100% DMSO. The ROQ was determined in five replicates, based on the definition of the concentration with less than 10 % RSD (relative standard deviation) according to Hayashi et al. [4)].

Cross reactivity against Dioxins

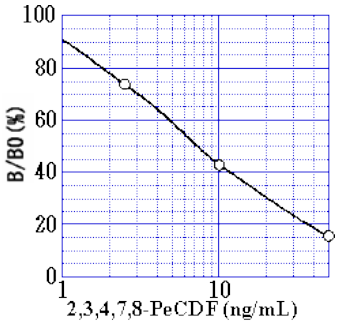

Figure 1 Standard curve of Dioxin ELISA

Cross reactivity pattern of Dioxin ELISA against isomer of dioxins, possessing TEF values, were shown in Table 1. This ELISA broadly reacted with 5 and 6 chlorinated dibenzo-*p*-dioxins and dibenzo-furans, which mainly contribute to the TEQ values obtained by HR-GC-MS in environmental samples.[3] These data indicates that the values obtained by Dioxin ELISA will well approximate to TEQ values obtained by HR-GC-MS with multiplying an appropriate coefficient in each sample matrices.

Table 1. Cross reactivity against dioxins

Isomer of dioxins	TEF	IC50 (ng/mL)	Cross reactivity (%)
2,3,7,8-TeCDD	1	280	3.93
1,2,3,7,8-PeCDD	1	42.0	26.2
1,2,3,4,7,8-HxCDD	0.1	>1000	<1.1
1,2,3,6,7,8-HxCDD	0.1	100	11.0
1,2,3,7,8,9-HxCDD	0.1	40.0	27.5
1,2,3,4,6,7,8-HpCDD	0.01	>1000	<1.1
OCDD	0.0001	>1000	<1.1
2,3,7,8-TeCDF	0.1	30.0	36.7
1,2,3,7,8-PeCDF	0.05	20.0	55.0
2,3,4,7,8-PeCDF	**0.5**	**11.0**	**100**
1,2,3,4,7,8-HxCDF	0.1	130	8.46
1,2,3,6,7,8-HxCDF	0.1	30.0	36.7
1,2,3,7,8,9-HxCDF	0.1	45.0	24.4
2,3,4,6,7,8-HxCDF	0.1	17.0	64.7
1,2,3,4,6,7,8-HpCDF	0.01	150	7.33
1,2,3,4,7,8,9-HpCDF	0.01	47.0	23.4
OCDF	0.0001	>1000	<1.1
3,3',4,4'-TeCB #77	0.0001	75.0	14.7
3,4,4',5-TeCB #81	0.0001	42.0	26.2
3,3',4,4',5-PeCB #126	0.1	28.0	39.3
3,3',4,4',5,5'-HxCB #169	0.01	56.0	19.6
2,3,3',4,4'-PeCB #105	0.0001	>1000	<1.1
2,3,4,4',5-PeCB #114	0.0005	>1000	<1.1
2,3',4,4',5-PeCB #118	0.0001	>1000	<1.1
2',3,4,4',5-PeCB #123	0.0001	>1000	<1.1
2,3,3',4,4',5-HxCB #156	0.0005	>1000	<1.1
2,3,3',4,4',5'-HxCB #157	0.0005	>1000	<1.1
2,3',4,4',5,5'-HxCB #167	0.00001	>1000	<1.1
2,3,3',4,4',5,5'-HpCB #189	0.0001	>1000	<1.1

Conversion coefficients to calculate from ELISA values to TEQ value

In order to obtain conversion coefficient from ELISA value (2,3,4,7,8-PeCDF equivalent) to TEQ value, 20 samples of flue gas and 10 samples each of fly and bottom ash samples, and a dioxin mixed sample were determined with HR-GC-MS and ELISA. The dioxin mixture was prepared according to the typical isomer composition rate in flue gas, which is reported by the Ministry of theEnvironment, Japan[5]. Table 2 shows the conversion coefficients in above-mentioned samples, which were determined from the slopes of comparison data between ELISA and HR-GC-MS (data not shown).

Table 2. Conversion coefficients

Sample matrices	Conversion coefficients
Pure dioxin mixture	0.222
Flue gas	0.0514
Fly ash	0.0554
Bottom ash	0.0481

Comparison between HR-GC-MS and ELISA based on TEQ values

Twenty of flue gas samples and 10 each of fly and bottom samples were determined with HR-GC-MS and ELISA. The 2,3,4,7,8-PeCFD equivalent values of ELISA were converted to TEQ values with multiplying conversion coefficients in each sample matrix (shown in Table 2), and then compared with TEQ values obtained with HR-GC-MS. As shown in Figure 3, good correlations were observed in all sample matrices with the slopes of 0.988 for Flue gas, 0.996 for fly ash and 0.997 for bottom ash, and correlation coefficients (R) of 0.976 for Flue gas, 0.985 for fly ash and 0.991 for bottom ash. These data suggest that the newly developed Dioxin ELISA gives accurate results as TEQ values by calculating with established conversion coefficients for each matrix. Using this ELISA, 40 quantitative results can be obtained in less than 3 hours after sample preparation. This system is ideally suited for monitoring of a number of dioxin-like chemicals in environment.

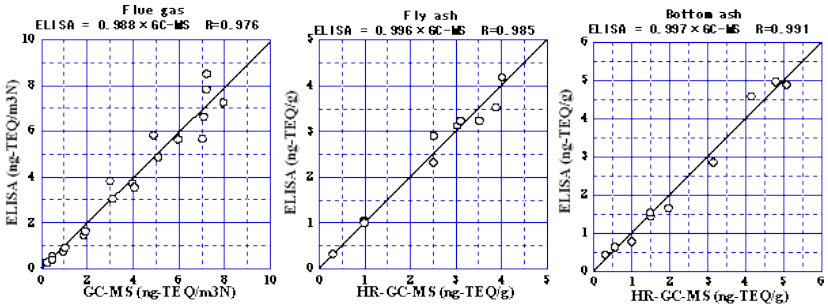

Figure 3 Comparison between HR-GC-MS and ELISA based on TEQ values

References

1) Okuyama M., Kobayashi N., Takeda W., Anjo T., Matsuki Y., Goto J., Kambegawa A. and Hori S. (2004) Anal. Chem., 76, 1948.

2) Garrison P. M., Tullis K., Aarts J., Brouwer A., Giesy J. P. and Denison M. S. (1996) Fundamental andAppl. Toxicol. 30, 194.

3) Shibayama M., Hinoue T. and Takasuga T. (2002) 13th annual meeting of Japan Society of Waste Management of Experts proceedings, 760.

4) Hayashi Y., Matsuda R. and Maitani T. (2004) Anal. Chem., 76, 1295.

5) Ministry of the Environment Japan homepage:

http://www.env.go.jp/chemi/dioxin/sokutei/sokutei_hou/

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