Ah RECEPTOR REACTIVITY WITH PCDD/PCDF/CO-PCB USING Ah-IMMUNOASSAY™

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Introduction

It is known that in a living body, PCDD/PCDF/Co-PCB and dioxin-like compound combines with Ah (Aryl hydrocarbon) receptor to form a complex with ARNT (Ah Receptor Nuclear Translocator), which complex is carried to DNA and induces toxicity. Ah-Immunoassay™ is a technique to detect the reactivity of Ah receptor with PCDD/PCDF/Co-PCB and dioxin-like compound on an ELISA plate without using a living cell. It is useful for screening the biological toxicity of PCDDs, PCDFs, and Co-PCBs and dioxin-like compound.

In this paper, we evaluate the cross-reactivity of Ah Immunoassay and clarify the reactive characteristic of Ah Immunoassay with PCDDs, PCDFs, and Co-PCBs using actual samples.

Analytical Principle and Procedures of Ah Immunoassay

In Ah-Immunoassay (AhIA), Ah receptor, ARNT, and DNA pieces are used as the main reagents. When a sample solution containing PCDDs, PCDFs, or Co-PCBs are made to react with the main reagents, it combines with the Ah receptor and ARNT to form a complex. This complex combines with the DNA piece. Since the DNA piece also has a part which sticks to the binder on the well bottom wall of the ELISA plate, the PCDD, PCDF or Co-PCB is fixed by the complex and DNA piece. The amount of the complex thus fixed is measured by immunoassay that utilizes the antigen-antibody reaction. The Ah receptor used in the analysis was extracted from a liver cell of a mammal. The analysis takes approximately six hours in time. The sample to be analyzed is pretreated and dissolved in DMSO (dimethyl sulfoxide) solvent. The pretreatment consists of extraction of the principle by a Soxhlet extractor or high-speed solvent extractor and removal of the extremely reactive substances by sulfuric acid and a multilayer silica column.

Cross-Reactivity of AhIA

Cross-Reactivities of PCDDs/PCDFs and Co-PCBs to AhIA are shown in Table 1. Ah Immunoassay reacts with chemical compounds which are considered toxic. With PCDDs/PCDFs, its reactive characteristic is comparable to TEF.

Regularity of Reactivity and Assumed Reactivity of AhIA

At present, the PCDDs/PCDFs which have been specified as toxic compounds are only isomers having chlorine groups at positions 2, 3, 7, and 8. However, Ah receptor also reacts with certain isomers that have not been specified as toxic compounds. In this study, we measured reactivities of 18 types of PCDDs/PCDFs which have not been specified as toxic compounds using Ah Immunoassay. As a result, we found an interesting fact about the regularity of reactivity.

Namely, it is the fact that the compounds which have two or three chlorine groups on both ends

and a flat, straight axis, have the strongest reactivity with Ah Immunoassay . Concerning PCDDs, the isomer that has the strongest reactivity is 2,3,7,8-TCDD and 1,2,7,8-PeCDD as already known. With respect to PCDFs, since their oxygen groups are not symmetrical, the isomers that have the strongest reactivity are 2,3,4,7,8-PCDF and 2,3,4,6,7-PCDF which have chlorine groups at positions 3 and 7 which are close to the ends and at the neighboring positions 2 or 4 and 6 or 8. It was also found that as compared with the compound that has the strongest reactivity, the reactivity of isomers decreases as the number of chlorine groups increases.
The hypothetical regularity of reactivity obtained from the above findings is shown in Table 2 and Fig.1 & 2[1]. On the basis of this hypothetical regularity, we classified all the PCDDs/PCDFs that have four or more chlorine groups and assumed their reactivities.

Evaluation of Reactivity by AhIA Analysis
We applied an AhIA analysis to several samples of fly ash in which the concentrations of all PCDD/PCDF isomers are known. In addition, the concentration of each of the isomers was multiplied by the reactivity obtained by AhIA to calculate an AhIA value. The calculation results are shown in Table 3. From these results, it was found that the analyzed AhIA values were very close to the calculated AhIA values. This suggests that our assumption of AhIA reactivity is valid. It was also found that the correlation with TEQ was in the range 11 to 14 times for fly ash samples although the magnification varies according to the congeners distribution and isomers distribution.

Correlation of AhIA and TEQ Obtained with Various Samples
An AhIA analysis was applied to various samples, such as contaminated soil and exhaust gas, shown in Fig. 3 & 4. The correlation of AhIA and TEQ was about 18 times for the soil samples, 10 times for exhaust gas samples. The variation of magnification is considered due to the difference in composition, and the degree of cleanup procedures, i.e., removal of extremely reactive substances. On the whole, however, the magnifications were within the range 10 to 18, suggesting that AhIA activity and TEQ are correlated for real samples containing mixtures of dioxins and PCBs.

Acknowledgment
We appreciated Nisshin Environmental Planning Inc. for kindly giving us the soil samples and data.

Summary
1) Ah Immunoassay is a technique which can be used to detect the toxicity of endocrine disrupters containing PCDDs, PCDFs, and Co-PCBs and Dioxin-like compounds from the viewpoint of their reactivity with Ah receptor.
2) In addition to the isomers for which a TEF has been specified, there are isomers which show a toxic reaction in terms of reactivity with Ah receptor.
3) There is good correlation between AhIA and TEQ for various compounds, hence Ah Immunoassay can be used as a monitoring technique, such as for screening of biological toxicity.

References
1 Yasuo Kobayashi, Katsutoshi Ogiwara, Tshio Nakanishi, Evaluation of Reactivity of Dioxins(PCDDs/PCDFs) to Ah Receptor The 3rd Annual Meeting of Japan Society of Endocrine Disrupters Research

Table 1 Ah-Immunassay Cross-Reactivity

PCDDs			PCDFs			Co-Planar PCB		
Isomers	WHO-TEF	AhIA Cross-reactivity	Isomers	WHO-TEF	AhIA Cross-reactivity	Isomers	WHO-TEF	AhIA Cross-reactivity
2,3,7,8-TCDD	(1)	(1)	2,3,7,8-TCDF	0.1	0.3	3,4,4',5-TCB	0.0001	0.008
1,2,3,7,8-PeCDD	1	1	1,2,3,7,8-PeCDF	0.05	0.5	3,3',4,4'-TCB	0.0001	0.01
1,2,3,4,7,8-HxCDD	0.1	0.5	2,3,4,7,8-PeCDF	0.5	0.9	3,3',4,4',5-PeCB	0.1	0.3
1,2,3,6,7,8-HxCDD	0.1	0.5	1,2,3,4,7,8-HxCDF	0.1	0.5	3,3',4,4',5,5'-HxCB	0.01	0.06
1,2,3,7,8,9-HxCDD	0.1	0.4	1,2,3,6,7,8-HxCDF	0.1	0.6	2',3,4,4',5-PeCB	0.0001	0.002
1,2,3,4,6,7,8-HpCDD	0.01	0.2	1,2,3,7,8,9-HxCDF	0.1	0.6	2,3,4,4',5-PeCB	0.0001	0.0002
OCDD	0.0001	0.008	2,3,4,6,7,8-HxCDF	0.1	0.6	2,3,4,4',5-PeCB	0.0005	0.001
2,3,7-TriCDD		0.03	1,2,3,4,6,7,8-HpCDF	0.01	0.2	2,3,3',4,4'-PeCB	0.0001	0.0003
1,3,6,8-TCDD	-	ND	1,2,3,4,7,8,9-HpCDF	0.01	0.3	2,3,4,4',5,5'-HxCB	0.00001	0.0002
1,3,7,9-TCDD	-	ND	OCDF	0.0001	0.05	2,3,3',4,4',5-HxCB	0.0005	0.0004
1,2,3,7-TCDD	-	0.7	2,3,8-TriCDF	-	0.0005	2,3,3',4,4',5'-HxCB	0.0005	0.0005
1,2,3,8-TCDD	-	0.4	1,2,3,8-TCDF	-	0.002	2,3,3',4,4',5,5'-HpCB	0.0001	0.0002
1,2,3,4,7-PeCDD	-	0.2	2,3,4,7-TCDF	-	0.6			
1,2,4,7,8-PeCDD	-	0.5	2,3,4,8-TCDF	-	0.2			
1,2,3,4,6,7-HxCDD	-	0.4	1,2,3,4,8-PeCDF	-	0.1			
1,2,3,4,6,7,9-HpCDD	-	0.02	2,3,4,6,7-PeCDF	-	0.9			
			1,2,3,4,6,8-HxCDF	-	0.01			
			1,2,3,4,6,7-HxCDF	-	0.4			
			1,2,3,4,6,8,9-HpCDF	-	0.006			

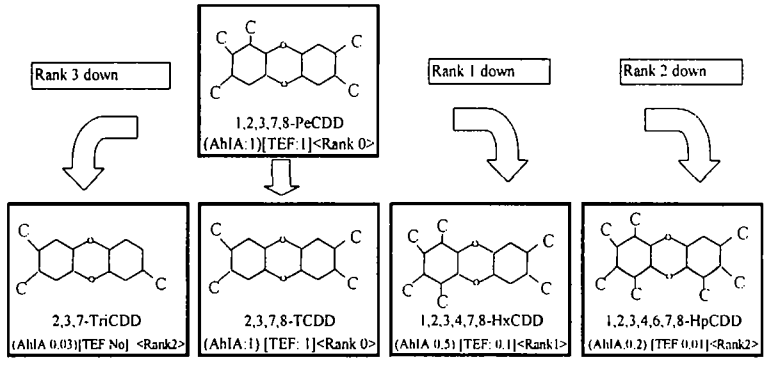

Fig. 1 Typical classified isomers of PCDDs

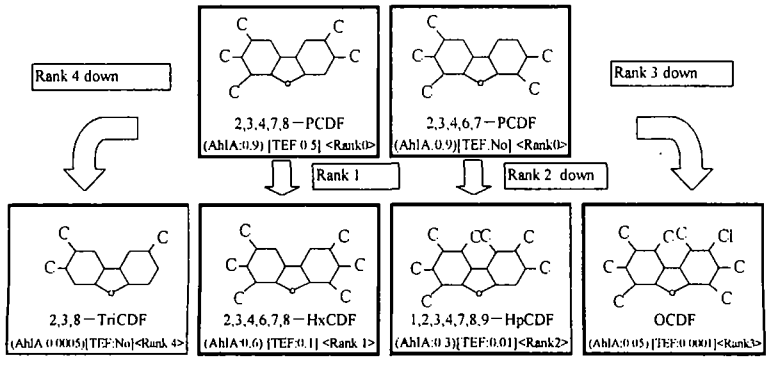

Fig. 2 Typical classified isomers of PCDFs

Table 2 Hypothetical Regularity of Ah-Immunoassay Reactiviy Ranks

No	Hypothetical Regularity
1	Basis Isomer with the most reactivity are ones with 2 and 3 chlorines at both end points. PCDDs→ 1,2,3,7,8-PeCDD PCDFs→2,3,4,7,8-PCDF and 2,3,4,6,7-PCDF
2	Reactivity is decreased as chlorines are added to or removed from Basic Isomer.
3	Reactivity depends on lateral position of chlorines.
4	Symmetric or non-symmetric configulation makes considerable influence on reactivity.

Table 3 valuation of AhIA Reactivity

Kind of Sample	Total of PCDDs/Fs & & Cod F pg/g	T Q (WHO) pgTEQ/g	Detected AhIA pgDEQ/g	Calculated AhIA pgDEQ/g	Ratio of AhIA /T Q -	Ratio of Calculated AhIA/ Detected A hIA -
Fly ash A	1,950,000	18,100	212,000	210,000	11.7	0.99
Fly ash B	153,000	2,240	30,900	26,400	13.8	0.86
Fly ash C	3,450,000	45,300	628,000	530,000	13.9	0.84

Samples of fly ash were obtained from different Garbage Incinerators.

Fig.3 Correlation of AhIA and TEQ in case of the Soil

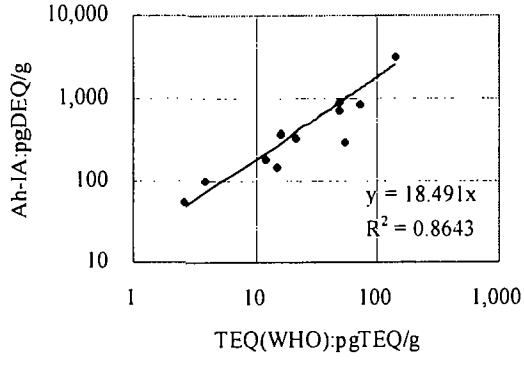

Fig.4 Correlation of AhIA ad TEQ in case of Exhaust Gas