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EVALUATION OF AH-IMMUNOASSAY® AS A SCREENING METHOD FOR DIOXINS AND CO-PCBS IN ENVIRONMENTAL SAMPLES

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Introduction

We have been focusing on development of clean-up procedures to practically apply Ah-Immunoassay ® (Ah-I) to various kinds of environmental samples in coporation with national institutes and private laboratories since Kubota introduced Ah-I technology on January 2000. This paper describes evaluation of Ah-I, using accumulated data and knowledge through several activities for validation. Japan has severer regulations and controls on exhaust and presence of PCDDs/DFs and dioxin-like co-PCBs into and in environments. Maximum levels have been already set in force for atmosphere and public water, exhausted flue gas, incineration fly ashes, wastewater, soil and sediment in river and bays.

Ah-Immunoassay method

The Ah-I is a cell-free system that comprehensively analyzes total toxicity potential contributed by dioxin and dioxin-like compounds. Analysis of toxicity is functionally performed by measuring their collective ability to bind to a cytosolic aryl-hydrocarbon receptor protein (AhR). This activated AhR protein then binds an exogenous Arnt protein to form an activated protein complex, which is able to bind an ELISA plate-bound oligonucleotide "dioxin responsive element(DRE)" and is then detected by an immunoassay-based color reaction. The Ah-I measures toxic dioxins and dioxin-like compounds, based on toxicity appearance mechanism, in an inexpensive and simple-to-use format without the need for live cell culture or radioactivity. A lower detection limit of 1.0 pg DEQ and high sensitivity makes this technology for screening large sample bases and quantitative analysis. Cross-reactivity of Ah-I most similar to TEFs indicates theoretical basis for good relationship with WHO-TEQ[1)]

Clean-up procedures for Ah-I

Clean-up procedures for environmental samples are schematically shown Fig.1. It is essential to extract dioxins and dioxin-like compounds from samples at high efficiency and high recovery and remove other toxic compounds such as PAHS for screening method of DXNs. ASE(Dionex) was used for extraction of samples in a solid state. Subsequently, 98% H2SO4 treatment was applied to the samples containing relatively much organic compounds such as soil or sediment. Multi-layered column mainly composed of Na2SO4 10%AgNo3, and 44%H2SO4 embedded silica agents was used for all samples after extraction to remove various matrix. In case of low level contaminated samples such as wastewater and sediment, fraction operation using alumina column was added at final stage to obtain effective results along with 44% H2SO4 column. Sample quantity was determined in consideration of detection levels indicated in table 1.

Sample sources are as follows.

1) Samples of soil are from different 2 final disposal sites, 2 old factory sites and unknown sites probably contaminated with dioxins.
2) Sources of ash samples are fly ashes from different municipal garbage incinerators.
3) Flue gas samples were gained from combustion test of various simulated RDFs using a laboratory-scale fluidized-bed incinerator in Ritumeikan University and unknown incinerators.
4) Sources of wastewater samples are 5 different sites.
5) Sources of sediment samples are 2 different sites identified (No.1,2) and all different sites(No.3).

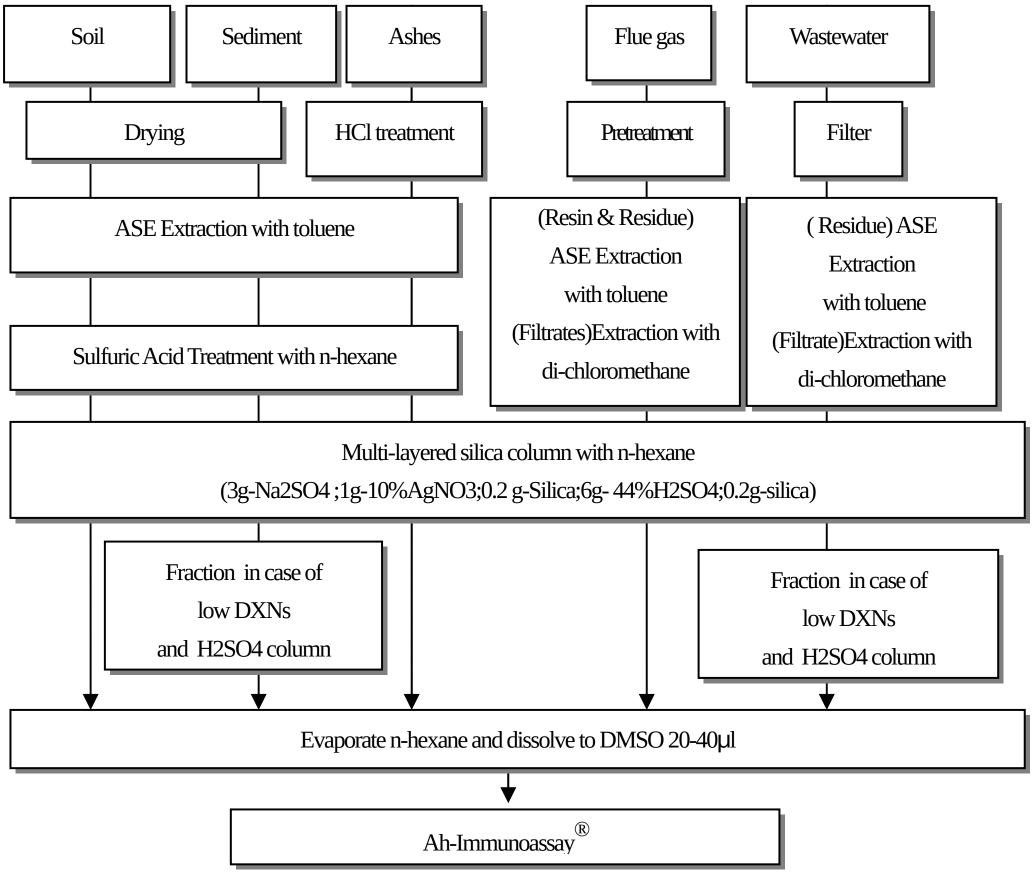


Fig.1 Schematic diagram of clean-up procedures

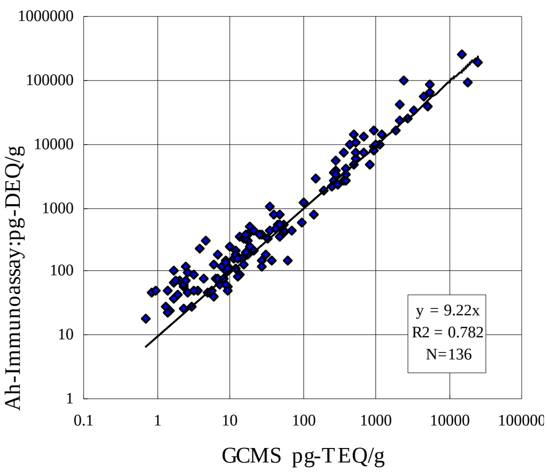
Results and evaluation

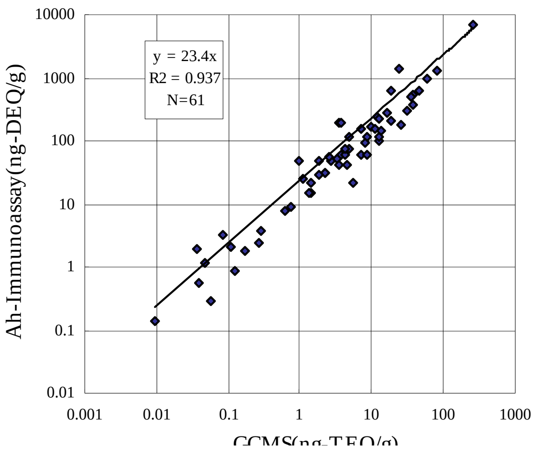
Many environmental samples were assayed by Ah-I according to the above clean-up procedures and operation manual prepared by Paracelsian Inc. Results are shown in Fig.2 for soil samples, Fig.3 for ash samples, Fig.4 for flue gas samples, Fig.5 for wastewater and Fig.6 for sediment samples. The correlation data obtained from mathematical analysis are shown in Table 2.

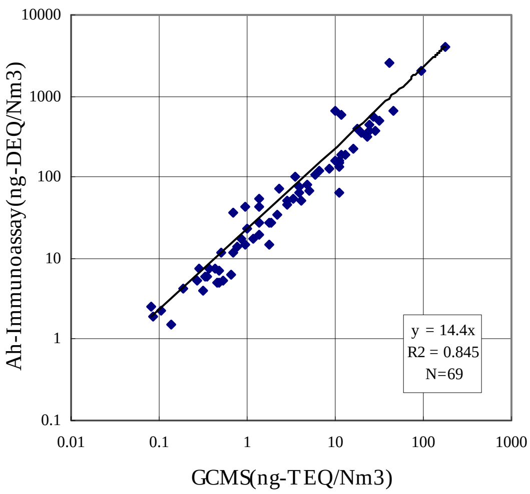
1) In general, remarkably good correlations with TEQs were observed. The averages of DEQs/TEQs are

16.2, 20.9 and 20.2 with high coefficient factors or small CV of the averages for soil, ash and flue gas samples respectively.

2) Soil samples show very good correlation with TEQs except low levels below 10 pg-TEQ/g.
3) Referring to sediment samples, No.3 sediment shows CV of 86% because the sources of No.3 samples are different from each other. Considering good co-relationship of No.1&2, it is understandable that DXNs isomer patterns vary largely, depending on sites.
4) In case of wastewater, relatively good co-relation with TEQs is obtained, though the level of dioxins is so low and difficult to be detected even by GCMS. The average of DEQs /TEQs is 25.8. It is relatively







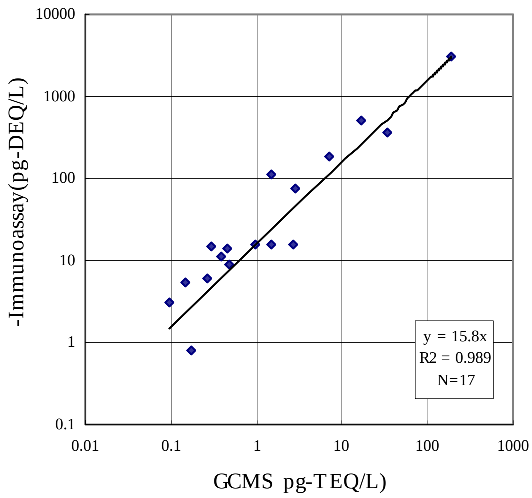


Fig.4 Results of Flue gas samples by Ah-Immunoassay Fig.5 Results of wastewater samples by Ah-Immunoassay

higher than the average of the other samples. It is noted that the average of DEQs/TEQs shows 16–21 times. This bias is firstly explained from difference of cross-reactivity of Ah-I and the WHO-TEF, since Ah-I utilizes Ah receptor of guinea pig most sensitive to dioxin-like compounds. Secondly, the isomers similar to 2,3,7,8-substituted isomers present in large amounts in environmental samples increase Ah-I reaction. Thirdly, there may exist unidentified toxic compounds such as bromated dioxins and chlorinated PAHs. Moreover, higher recovery by ASE and determination method of Ah-I results by using linear evaluation will effect the bias upwards. The further discussion and investigation on this matter will need.

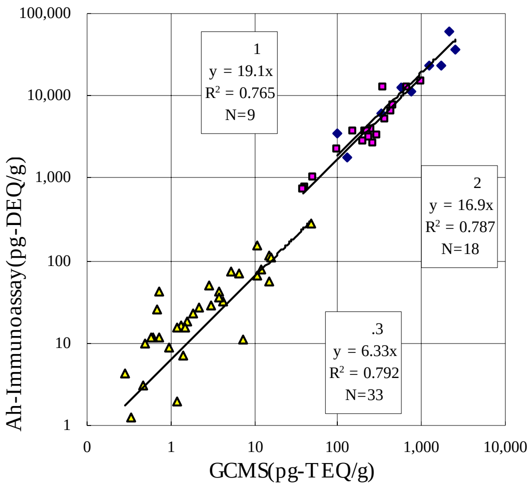


Fig.6 Results of sediment samples by Ah-Immunoassay

Table1 Sample quantity

Kind	Density to be measured	Sample quantity	
		Minimum	Practical
Soil	1000 pgTEQ/g	0.06 g	2 g
Sediment	150 pgTEQ/g	0.4 g	2 g
Ahs	3000 pgTEQ/g	0.02 g	1 g
Wastewater	10 pgTEQ/L	4 L	
Water	1 pgTEQ/L	20 L	
Flue gas	100 pgTEQ/nM3	0.8 Nm3	

Table 2 Summary of correlation between Ah-I and TEQ

Sample kind	Soil	Ash	Flue gas	Waste water	Sediment		
					No.1	No.2	No.3
correlation magnification	9.22	23.4	14.4	15.8	19.1	16.9	6.33
coefficient factor	0.782	0.937	0.845	0.989	0.765	0.787	0.792
Average of DEQs/TEQs	16.2	20.9	20.2	25.8	20.0	17.9	13
CV of Average of DEQs/TEQs	72%	71%	55%	67%	36%	34%	86%
Number of data	136	61	69	17	9	18	33

Conclusion

Strong correlation between DEQs by Ah-I and TEQs was obtained for various kinds of environmental samples. It proves that the Ah-I is useful as a screening method for dioxin and dioxin-like compounds in environmental samples. Additional development will be continuously conducted to improve clean-up procedures to be applied to very low level samples.

References

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