DIOXIN SCREENING IN ENVIRONMENTAL SAMPLES USING THE AH-IMMUNOASSAY®

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Introduction

The Ah-Immunoassayâ (Ah-I) is a cell-free system that comprehensively analyzes total toxicity potential contributed by dioxin and dioxin-like compounds (1). It does so by measuring their collective ability to bind to a cytosolic aryl hydrocarbon receptor protein (AhR) isolated from tissue cells. This bound or 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 response element." This complex is then detected by an immunoassay-based color reaction. The unique aspect of the Ah-I technology is the ability to determine the potential biological response to the dioxin-like compounds present in a sample. The Ah-I is able to measure toxic dioxin-like compounds such as 2,3,7,8-substituted isomers 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 reproducibility make this technology ideal for screening large sample bases and quantitative analysis. The Kubota Corporation has been developing actual application technologies of the Ah-I for environmental samples(2). This paper describes the application to environmental samples and correlation of Ah-I DEQ results and CG/MS TEQ.

Materials and Methods

When screening dioxins, the clean-up procedures for the Ah-I are important. Specifically poly-cyclic aromatic hydrocarbons (PAHs) compounds are the main substances to be removed. They are present in large amounts in environmental samples. It was determined that many types of PAHs were eliminated by 95% or more using a multi-layered sulfated silica column. The elimination of PAHs in the sample following clean-up procedures led to the present method for extraction of dioxin and dioxin-like compounds from soil. Dried soil (1-2 grams) was extracted with toluene using automated solvent extraction (ASE), Dionex. Following a solvent exchange with 40 ml hexane, the sample was reacted with 10 ml 98 % sulfuric acid until completely oxidized. The sample was loaded onto a four-layered silica column. The collected fraction was subjected to a second identical chromatography step. The column was mainly 44% H_2SO_4 and 6g silica. The resulting fraction in hexane was evaporated to dryness and reconstituted in 20-40 ul DMSO. Analysis using the Ah-I per the directions by Paracelsian, Inc., was performed using 2 ul of sample per ELISA well .

Results

The cross-reactivity of the Ah-I to 2,3,7,8-substituted PCDDs/PCDFs, co-PCBs, other PCDDs/

PCDFs, bromide dioxins (PBDDs/PBDFs) and PAHs is shown in Table 1. The cross-reactivity agrees well with WHO-TEF values for 2,3,7,8-substituted isomers and co-PCBs. A strong cross-reactivity to PAHs was observed using the Ah-IÒ technology. These compounds are suspected carcinogens, such as benzo (b) fluoranthene and benzo (k) fluoranthene. show toxicity similar to chlorinated PCDDs/ PCDFs.

Soil samples were collected from three different locations and the results are shown in Figure 1. The samples were extracted by accelerated solvent extraction method (ASE) and cleaned up as described above. The samples show a remarkably good correlation with the GC/MS determined TEQ values. The correlation magnification and coefficient factor (r^2) are 9.8 and 0.75, respectively, through the wide range from 1 pg TEQ/g to more than 20,000 pg TEQ/g. The results of incinerated ashes are shown in Figure 2. The correlation magnification and coefficient factor (r^2) are 13 and 0.68, respectively. These results reveal the good correlation between Ah- I DEQ values and TEQ values for environmental samples.

Table 1 Cross-Reactivity of Typical Chemical Compounds by Ah-Immunoassay

PCDDs/Fs	WHO-TEF	Cross-Reactivity
2,3,7,8-TCDD	(1)	(1)
1,2,3,7,8-PeCDD	1	0.8
1,2,3,4,7,8-HxCDD	0.1	0.5
1,2,3,6,7,8-HxCDD	0.1	0.5
1,2,3,7,8,9-HxCDD	0.1	0.5
1,2,3,4,6,7,8-HpCDD	0.01	0.2
OCDD	0.0001	0.003
2,3,7,8-TCDF	0.1	0.06
1,2,3,7,8-PeCDF	0.05	0.07
2,3,4,7,8-PeCDF	0.5	0.9
1,2,3,4,7,8-HxCDF	0.1	0.08
1,2,3,6,7,8-HxCDF	0.1	0.6
1,2,3,7,8,9-HxCDF	0.1	0.6
2,3,4,6,7,8-HxCDF	0.1	0.5
1,2,3,4,6,7,8-HpCDF	0.01	0.06
1,2,3,4,7,8,9-HpCDF	0.01	0.07
OCDF	0.0001	0.008
3,4,4',5-TetraCB((#81)	0.0001	0.03
3,3',4,4'-TetraCB(#77)	0.0001	0.01
3,3',4,4',5-PentaCB(#126)	0.1	0.3
3,3',4,4',5,5'-HexaCB(#169)	0.01	0.06
2',3,4,4',5-PentaCB(#123)	0.0001	0.002
2,3',4,4',5-PentaCB(#118)	0.0001	0.0002
2,3,4,4',5-PentaCB(#114)	0.0005	0.001
2,3,3',4,4'-PentaCB(#105)	0.0001	0.0003
2,3',4,4',5,5'-HexaCB(#167)	0.00001	0.0001
2,3,3',4,4',5-HexaCB(#156)	0.0005	0.001
2,3,3',4,4',5'-HexaCB(#157)	0.0005	0.002
2,3,3',4,4',5,5'-HeptaCB(#189)	0.0001	0.0002

PCDDs/Fs with no TEF	Cross-Reactivity
(PCDDs)	
2,3,7-TriCDD	0.03
1,2,3,4-TCDD	0.005
1,2,7,8- TCDD	0.9
1,2,8,9-TCDD	0.3
1,3,6,8-TCDD	ND
1,3,7.9-TCDD	ND
1,2,3,7-TCDD	0.7
1,2,3,8-TCDD	0.4
1,2,3,4,7-PeCDD	0.2
1,2,3,8,9-PeCDD	0.7
1,2,4,7,8-PeCDD	0.5
1,2,3,4,6,7-HxCDD	0.4
1,2,3,4,6,9-HxCDD	0.001
1,2,3,4,6,7,9-HpCDD	0.02
(PCDFs)	
2,3,8-TriCDF	0.0005
1,2,3,4-TCDF	0.006
1,2,3,8-TCDF	0.002
1,2,3,9-TCDF	0.006
1,2,6,9-TCDF	0.01
1,2,7,8-TCDF	0.06
1,2,8,9-TCDF	0.007
1,3,4,6,8-PCDF	0.01
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
1,2,3,4,8,9-HxCDF	0.2
1,2,3,8,9-PCDF	0.07

PBDDs/Fs & PAHs	Cross-Reactivity
(PBDDs/Fs)	
2,3,7,8-TeBDD	0.5
1,2,3,7,8-PeBDD	0.3
OBDD	0.004
2,3,7,8-TeBDF	0.5
1,2,3,7,8-PeBDF	0.2
2,3,4,7,8-PeBDF	0.3
1,2,3,4,7,8,-HxBDF	0.03
(PAHs)	
Benzo(b)fluoranthene	1
Benzo(j)fluoranthene	0.3
Benzo(k)fluoranthene	1
Benzo(a)fluorene	0.001
Benzo(b)fluorene	0.001
Benzo(a)pyrene	0.2
Benzo(e)pyrene	ND
Indeno(1,2,3-cd)pyrene	0.8
Dibenzo(a,c)anthracene	0.3
Dibenzo(a,h)anthracene	1
Dibenzo(a,h)pyrene	0.1
Dibenzo(a,i)pyrene	0.6
Picene	0.9
3-Methyl-cholanthrene	0.8
Benzo(a)anthracene	0.001
Naphthalene	ND
Phenanthrene	ND
Anthracene	ND
Fluoranthene	ND
Pyrene	ND

Discussion

It is noted that there is a 10–13 discrepancy between the determined Ah-I and CG/MS values. There are several avenues by which this discrepancy could occur. The first comes from the difference of cross-reactivity and the WHO-TEF. The Ah receptor used in the Ah-I kit is extracted from the guinea

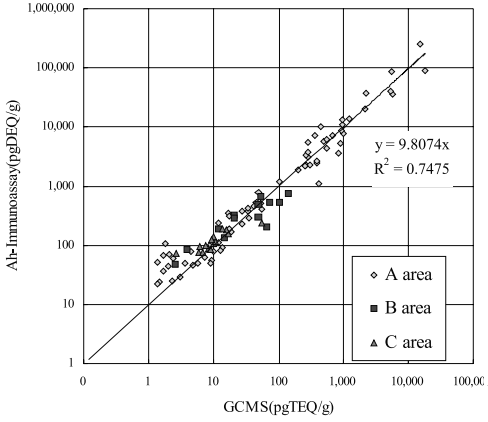

Figure 1. Comparison of Ah-1® and GC/MS values from soil samples

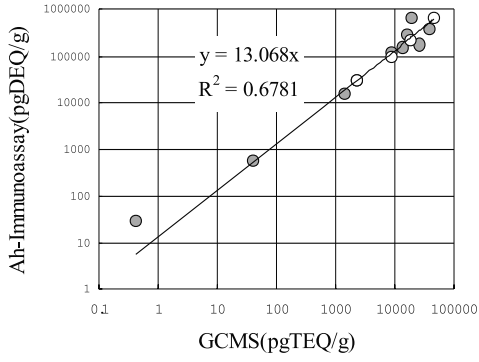

Figure 2. Comparison of Ah-1® and GC/MS values from ash samples

Table 2 Actually assayed and Theoretical Ah-I values.

Sample Kind		GCMS	Actual AhIA	Theoretical AhIA	Actual/ Theoretical	Theoretical /GCMS
		pgTEQ/g	pgDEQ/g	pgCEQ/g	-	-
Ash	1	18,095	212,000	209,866	11.7	11.6
	2	2,236	30,863	26,391	13.8	11.8
	3	45,331	628,220	530,255	13.9	11.7
	Average				13.1	11.7
	CV(%)				7.6%	0.7%
Soil	1	5,461	85,839	43,228	15.7	7.9
	2	491	5,684	3,474	11.6	7.1
	3	952	11,069	6,203	11.6	6.5
	4	919	8,784	6,384	9.6	6.9
	5	15,190	248,436	130,301	16.4	8.6
	6	5,559	36,045	28,219	6.5	5.1
	7	681	7,068	4,868	10.4	7.2
	8	444	9,963	4,139	22.5	9.3
	9	960	11,305	7,282	11.8	7.6
	Average				12.9	7.4
	CV(%)				36.3%	16.6%

pig, which is most sensitive to dioxin-like compounds. Moreover, cross-reactivity of 6,7 chlorinated PCDDs/PCDFs, which share the quantitatively main portions among TEQ is also 5-6 times that of the WHO-TEF values. The same holds true for co-PCB compounds. Secondly, the existence of isomers which are similar to 2,3,7,8-substrated isomers but not assigned TEF values could result in an increased DEQ analysis. It is known that the Ah receptor reacts with isomers having at least 3 positions among 2,3,7,8 such as 1,2,3,7 TCDD. Several isomers are widely contained in the environmental samples. These isomers are neither decomposed nor removed in the extraction and clean-up processes and are recovered together with 2,3,7,8-PCDD/PCDF isomers in the solvent. As shown in Table 1, we have assayed many pure compounds. Using these cross-reactivity and data and assumed cross-reactivity based on structural rule of toxicity appearance. It is possible to estimate all theoretical reactivity by the Ah-I. After selecting several samples, all of which isomers are known in quantity by GC/MS, we calculated theoretical Ah-I values and compared them with the measured Ah-I values. The results are shown in Table 2. It was found that the measured Ah-I values reflect the calculated Ah-I values very

accurately, although there are a few discrepancies between them. The last reason of the discrepancies above-mentioned may come from reaction by unidentified compounds. For example bromide dioxins have a strong toxicity similar to that of chlorinated dioxins. In recent research in Japan about 10 % of bromide dioxins were contained in ash and exhaust gas from municipal incinerators. Taken together, the previous three hypotheses can reasonably explain the 10-13 magnification of Ah-I DEQ results in comparison to TEQ values.

Conclusion

There is a strong correlation between the Ah-I DEQ results and TEQ values determined by GC/MS. It was found that the correlation magnification for environmental samples is in the range of 10 to 13 on average. These magnifications are reasonably explainable based on the unique biological nature of the Ah-I technology, such as a wide compound range of detection and the ability to detect yet unknown and potentially toxic substances. Successful validation of the Ah-I technology with GC/MS proves that the Ah-I is useful as a screening method for dioxin and dioxin-like compounds in environmental samples. Moreover, the Ah-I technology is powerful in its sensibility of detection to a large range of compounds such as bromide dioxin with strong toxicity like PCDDs/Fs without the use of live cells or radioactivity. The Ah-I can be used as 1) a rapid and in-expensive screening method for TEQ values, 2) a binding index of the Ah receptor, and 3) means of measuring new total toxicity.

References

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