STUDY OF THE INFLUENCE OF CO-PCBS IN DIOXIN SCREENING BY AH-IMMUNOASSAY®

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Introduction

There are immunoassay method that uses antibody to dioxin and the bioassay method that utilizes Ah receptor as biological methods for dioxin analysis. Ah-Immunoassay® (Ah-I) is a variation of the latter method utilizing Ah receptor extracted from a liver cell of a guinea pig[1]. The sample preparation method for Ah-I to analyze dioxins inexpensively in environmental samples has been developed actively, and it was reported that with a suitable sample clean-up procedure, a strong correlation between Ah-I values and TEQ values determined by GC/MS was obtained[2]. On the other hand, it has been reported that when the concentrations of Co-PCBs in samples are considerably high, there is a tendency that the ratio of Ah-I values to the TEQ values becomes small[3].

With the aim of clarifying the reason for that, we studied the fundamental influence of the Co-PCB isomers on the Ah-I response and its influence in environmental samples. This paper describes the results of our study.

Materials and Methods

Preparing mixed standards in DMSO

We requested Cambridge Isotope Laboratories (CIL) to prepare a 32 pg/ul 2,3,7,8-TCDD standard in DMSO. Concerning the PCB standard in DMSO, a required amount of the PCB standard purchased in the form of nonan or toluene was evaporated naturally to dryness in a draft chamber. And then redissolved into 1-5ml of DMSO under 10-min sonication in 40 C water bath, in which the concentrations are all resulted in 10000 pg/ul. 5ul of the 2,3,7,8-TCDD standard was mixed with 15ul of each Co-PCB standard, and the concentrations of TCDD and Co-PCB are 8 pg/ul and 7500 pg /ul, respectively.

Preparing environmental samples with and without mono-ortho PCBs

150ul of each solution of mono-ortho PCB #123, #118, #114, #105, #156, #157, and #189 were mixed. On the another hand, as Ah-Immunoassay samples, fly ash, soil, and flue gas were extracted, cleaned up and then redissolved in 40ul of DMSO as shown in Table1. Then 6ul of each environmental sample except fly ash2 was diluted 10-fold with 54ul of the mixed standards of the mono-ortho PCBs described above and that of fly ash2 was diluted 100-fold with 594ul. Also, for comparison, each of the environmental samples was diluted with DMSO as same as diluted with the mixed standards.

Ah-I conditions

We followed the instructions given in the operation manual issued by Kubota Co. and Paracelsian Inc.

Table1. Information of environmental samples

Sample	Extraction method	Clean-up method	Sample amount for clean-up procedure (g or Nm3)
Fly ash 1	After HCl treatment,	Sulfuric acid treatment and four-layered	0.10
Fly ash 2	ASE by toluene	silica gel cartridge column (SUPELCO,	0.10
Soil 1	ASE by toluene	Cat.No.118J029)	2.0
Soil 2			2.0
Flue gas 1	Soxhlet	multilayer silica column for JIS method	0.025
Flue gas 2			0.031

Table2. Concentrations of environmental samples added mono-ortho PCBs mixture

Sample	PCDD/DFs + non-orthoPCB (pg/ul)	Mono-orhto PCB (pg/ul)	PCDD/DFs + non-orthoPCB (pg-TEQ/ul)	Mono-orhto PCB (pg-TEQ/ul)	Added mono-ortho PCB (pg/ul)	Added mono-ortho PCB (pg-TEQ/ul)
Fly ash 1	191	1.9	1.8	0.00046	9000	2.4
Fly ash 2	(0.29)*	0.30	0.19	0.000073	9900	2.7
Soil 1	56	0.75	1.0	0.000034	9000	2.4
Soil 2	112	1.4	1.3	0.00038	9000	2.4
Flue gas 1	106	0.80	0.86	0.00019	9000	2.4
Flue gas 2	103	0.99	1.6	0.00022	9000	2.4

* Sum of the concentrations of only 17 PCDD/DFs and 4 non-ortho PCB isomers with WHO-TEF

Non-ortho PCB:#81,#77,#126 and #169, mono-ortho PCB: #118,#114,#105,#167,#156,#157, #189, added mono-ortho PCB: #118,#114,#105,#156,#157,#189

Results

The results of Ah-I of each of the mixed standards are shown in Table3, in which the Ah-I values are calculated by the following formula:

(Absorbance for the mixed standard - absorbance for DMSO)/(Absorbance for 2,3,7,8-TCDD, 16 pg/well - absorbance for DMSO) x 100 (%)

The results of Ah-I of each environmental sample diluted with and without mono-ortho PCBs are shown in Table4, in which the Ah-I values are calculated by the following formula:

(Absorbance for sample - absorbance for DMSO)/(Absorbance for 2,3,7,8-TCDD, 64 pg/well - absorbance of DMSO) x 100 (%)

Table3. Ah-I results of mixed standards

Conc. of PCB	Each PCB + 2,3,7,8-TCDD									
(pg/well)	#81	#77	#169	#123	#118	#114	#105	#167	#156	#157	#189

15000	88 %	69 %	160 %	125 %	52 %	67 %	68 %	233 %	131 %	43 %	66 %
1500	145 %	112 %	143 %	141 %	60 %	89 %	60 %	233 %	104 %	76 %	74 %
150	82 %	115 %	134 %	130 %	85 %	95 %	98 %	167 %	142 %	113 %	78 %
15	87 %	96 %	121 %	131 %	100 %	87 %	93 %	158 %	122 %	101 %	76 %
0	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %	100 %

* Concentration of 2,3,7,8-TCDD was constant with 16pg/well.

Response value of only 2,3,7,8-TCDD congener was set arbitrarily to 100 %.

Table 4. Ah-I results of environmental samples with and without mono-ortho PCBs

Dilution	Fly ash 1(%)			Fly ash 2(%)			Soil 1(%)			Soil 2(%)			Flue gas 1(%)			Flue gas 2(%)		
	A	B	B/A	A	B	B/A	A	B	B/A	A	B	B/A	A	B	B/A	A	B	B/A
1	39	11	27	13	5	40	31	10	31	43	5	11	83	45	55	53	22	42
2	18	6	34	5	3	67	11	6	50	16	2	12	57	34	60	26	15	58
4	8	4	43	2	2	119	5	3	71	8	1	18	33	25	77	12	9	69
8	4	2	51	0	2	427	1	4	289	4	1	24	17	15	90	7	6	86

A: original, B: added mono-ortho PCBs mixture into A

Response value of 2,3,7,8-TCDD standard with 64pg/well for quantification was set arbitrarily to 100 % on A and B.

Discussion

As shown in Table 3, there are the inhibitory effect of Ah-I with the addition of PCB #118, #114, #105, #157, and #189. But the effect is apparent in high ratio of PCB/TCDD amount; for instance, fewer than 70 % value of the Ah-I response to only TCDD is obtained in condition under the addition of over 1500pg/well of PCB, in which the ratio of PCB/TCDD is about over 100-fold.

Also, as shown in Table 4, in environmental samples we could confirm the inhibition. To compare the Ah-I response on top dose without addition of PCBs, the addition of 18000 or 19800pg/well PCBs caused the 11-55 % response to only TCDD, in which the ratio of added PCBs amount to original PCDD/DFs and non-ortho PCBs is about 50-160-fold. But Since the cross reactivities of some PCDD/DFs in these samples are N.D.. So, if the 2,3,7,8-TCDD equivalency quantity of samples without additional PCBs by Ah-I means the total amount of dioxins binding Ah receptors, the ratio of added PCBs amount to the total dioxins is about 350-1800-fold which corresponds with the result of the mixed standards.

Since the original mono-ortho PCBs amount on the fly ashes and the flue gases is about 1/10000 of the amount added in this experiment, there should be little inhibitory effect on Ah-I on those original samples. In Figure 1 and 2, analytical examples of total 117 soils on our previous study are shown, which collected from one landfill and prepared by the same method in Table 1. As shown Figure 1, max value of ratio of mono-ortho PCBs/total Dioxins is 0.95-fold and as shown Figure 2, max value of ratio of mono-ortho PCBs/2,3,7,8-TCDD equivalency quantity by Ah-I is 107-fold. Both values are less than those of the condition of the experiment described above, and on the range shown in Figure 1 and 2 there is no tendency that ratio of Ah-I values/the TEQ values becomes small when the ratio of mono-ortho PCBs increase.

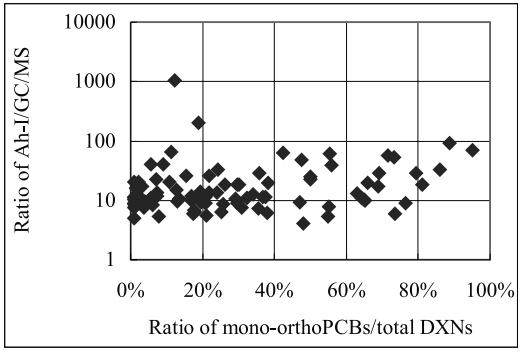

Figure 1. Comparison of Ah-I/TEQ value and ratio of mono-ortho PCB/total DXNs on soil samples

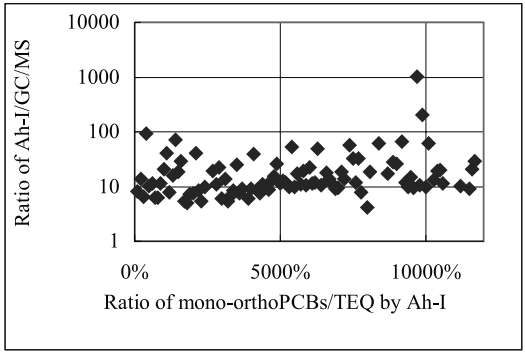

Figure 2. Comparison of Ah-I/TEQ value and ratio of mono-ortho PCB/Ah-I on soil samples

Conclusion

It was found that some mono-ortho PCBs had remarkable inhibitory effect on Ah receptor activation by TCDD, or cleaned-up extraction form environmental samples when there is much higher concentration of the PCBs than PCDD/DFs and non-orhto PCBs. This fact reveals that separation of mono-ortho PCB is necessary for the Ah-I analysis of PCB polluted sample and biological samples in which Co-PCB is highly concentrated, to obtain a strong correlation between the Ah-I results and the TEQ values determined by GC/MS. It seems that the effect of mono-orhto PCB appears under the condition of over 100-fold of the ratio of mono-ortho PCBs/total dioxins. So, it was found that on fly ash, flue gas and soil samples, the separation does not need for those low concentrations of mono-ortho PCBs.

References

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