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THE MEASUREMENT OF TOXICITY OF DIOXINS IN HUMAN BLOOD USING THE AH- IMMUNOASSAY

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Introduction

It is important to measure the toxicity of dioxins in human blood to help eliminate the negative effect on human health . Presently, the toxicity of dioxins is measured using high- resolution Gas Chromatography/high- resolution Mass Spectrometery (GC/MS) by detecting all isomers assigned with a TEF value (2,3,7,8- TeCDD Toxicity Equivalency Factor) and summing the toxic equivalents. GC/MS requires high cost and a long turnaround time, although it can detect dioxin isomers with high sensitivity. Consequently, measurement of the toxicity of dioxins has been limited to a very small number of cases. It is more reasonable to measure the toxicity, using a bioassay or immunoassay method with a low cost and short turnaround time. The Ah- Immunoassay® is a bioassay detection method based upon the toxic potential of dioxins by detecting the reactivity of the Ah- receptor with dioxins in an ELISA plate format without using living cells. It is especially useful for screening the biological toxicity of dioxins. In this paper, we will report the correlation between the Ah- Immunoassay and GS/MS, measuring the toxicity of dioxins contained in the lipid fraction of human blood.

Methods and Materials

Sample: The number of samples used for this report was 22 (13 whole blood and 9 serum).
Method for extracting lipid in blood and clean- up operation: According to the Tentative Manual for Measuring Dioxins in Blood by the Ministry of Labor and Welfare Japan (Figure 1 and Figure 2), we first extracted the lipid and then cleaned- up the samples.
Sample preparation for the Ah- immunoassay: For the Ah- Immunoassay test, the hexane layer was evaporated to dryness by nitrogen spray, then dissolved with 20µL DMSO.
Ah- Immunoassay Procedure: Procedure of the Ah- Immunoassay method is shown in Figure 3.

Results and Discussion

The level of dioxins in human blood that were measured by GC/MS and the Ah- Immunoassay are shown in Table 1. The correlation between the toxic equivalents (TEQ) of GC/MS and Ah-

Immunoassay data (DEQ) are summarized in Figure 4. This figure details the significant correlation expressed by gradient of 0.812 with a correlation coefficient of 0.851. One justification of the correlation test revealing meaningful results, is that the principle of Ah- immunoassay is based upon the toxicity mechanism of dioxins in human cells. That is the toxicity mechanism of dioxins in human cells is controlled by the Ah- receptor and ARNT (Ah- receptor Nuclear Translocator). In living cells, dioxins which pass the cell membrane bind to the Ah- receptor, which subsequently binds ARNT forming a stable complex. The complex will bind to a specific DNA sequence termed DRE (Dioxin Response Element) inducing several enzymes and bring about the toxicity. The principle of the Ah- immunoassay is based upon the toxicity mechanism, which can detect the total toxicity of Ah receptor binding compounds, such as dioxins, comprehensively [1)]. Furthermore the crossreactivity values of the Ah- immunoassay to dioxins are very similar to the WHO TEF values of dioxins [2)]. These observations support the results of the correlation test.

Finally, we have tested the validity of the Ah- immunoassay for screening purposes using a contingency table. When the cut off value of dioxins in human blood was set at 50pg- TEQ/g- lipid, we defined the Ah- immunoassay cut off value at 30pg- DEQ/g- lipid, based upon the results of the correlation test. According to this result, we derived the contingency table shown in Table 2. Among the 22 samples, we observed the correct hitting ratio at 15/22 (68.1%), the false positive ratio at 4/22 (18.2%) and the false negative ratio at 3/22 (13.7%), respectively. A possible reason for the high false negative ratio is that the blank sample for these 3 samples also revealed a high value. Therefore, when using the Ah- immunoassay for a screening tool, one must note this high ratio of false negatives. In addition, there is a need to develop the new extraction and clean- up method which confers a low blank value.

Conclusion

These results clearly show the validity for the measurement of dioxins using the Ah- immunoassay. In addition, this presents a new method for measuring dioxins in human blood.

blood 30mL or serum 15mL saturated ammonium sulfate solution 12mL (in the case of serum 6mL) 25% ethanol/hexane 30mL (in the case of serum 15mL)
↓ 30min sonication, 30min shaking
water layer
↓ hexane 30mL, 30min shaking, 2 times
pool hexane layer
hexane washed water 20mL, 3 times
sodium sulfate anhydride
place hexane layer in a weighted kjeldahl flask
evaporate hexane layer by rotary evaporator stand in a vacuumed dissector
weigh the kjeldahl flask and find the weight of extracted lipid

Figure 1. The method of lipid extraction from human blood.

extracted lipid, 2N KOH/ethanol 10mL
↓ 2hr shaking
ethanol 5mL, hexane washed water 10mL, hexane 20mL
↓ 30min shaking
water layer
↓ add hexane 20mL, 30min shaking, 2 times
pool hexane layer
hexane washed water 20mL, 3 times
H_2SO_4 10mL
hexane washed water 20mL, 3 times
sodium sulfate anhydride
concentration
multilayer silica gel chromatography
Ah- immunoassay Sample
↓
carbon- dispersed silica gel chromatography
GC/MS Sample

Figure 2. The Clean- up procedure for dioxins from extracted lipid.

Sample in DMSO
Ah-receptor, ARNT, DRE ↓
Incubation (2 hours)
AB1 ↓
Incubation (1 hour)
AB2 ↓
Incubation (1 hour)
Substrate ↓
after 1 hr incubation, absorbance reading at 405nm

Figure 3. Procedure of the Ah- immunoassay.

Table 1. The level of dioxins in human blood

Sample No.	GC/MS (pg-TEQ/g-lipid)	Ah- immunoassay (pg-DEQ/g-lipid)	Sample No.	GC/MS (pg-TEQ/g-lipid)	Ah- immunoassay (pg-DEQ/g-lipid)
1	57	10.4	12	56	77.0
2	91	48.5	13	46	49.5
3	84	38.7	14	28	17.6
4	173	85.9	15	38	0.0
5	176	280.1	16	45	44.9
6	397	304.3	17	120	55.5
7	71	0.0	18	122	0.0
8	120	69.4	19	301	204.6
9	84	90.9	20	48	0.0
10	150	145.9	21	28	33.6
11	187	176.9	22	34	30.4

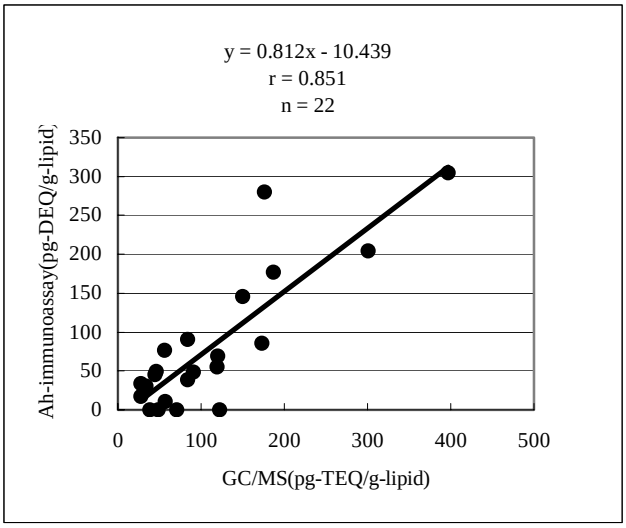

Figure 4. The correlation between GC/MS and the Ah- immunoassay.

		Ah-immunoassay	
		30pg-DEQ/g-lipid<	30pg-DEQ/g-lipid>
GC/MS	50pg-TEQ/g-lipid<	3	4
	50pg-TEQ/g-lipid>	3	12

Table 2. Contingency table for the Ah- immunoassy and GC/MS.

References

1. Yasuo Kobayashi and Toshio Nakanishi, (2002) Journal of Water Re-use Technology, 27, 3
2. Geoffrey D. Wheelock, Kelly R. Hurst and John G. Babish, (1996) Toxicology Methods 6, 41