A New Approach to Measurement of Toxicity of Dioxins in Human Blood and Maternal Breast Milk Using Ah-Immunoassay

Yasuteru Usuki[1], Yoshinori Fujimine[1], Tetsuya Hirai[1], Yasuo Kobayashi[2], Takao Yamada[2], Toshio Nakanishi[2], Toru Takeuchi[3], Osamu Tsutsumi[3], T.Colin Campbell[4] and G.D. Wheelock[4].

[1] Otsuka Assay Laboratories, Otsuka Pharmaceutical Co., Ltd, Tokushima, Japan
[2] Technology Development Laboratory, Kubota Corporation, Tokyo, Japan
[3] Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, Tokyo, Japan
[4] Paracelsian Inc., U.S.A

Introduction

It is important to measure the toxicity of Dioxins in human blood or maternal breast milk from the viewpoint of menace to human beings. Presently, the toxicity of dioxins are measured using high-resolution Gas Chromatograph/ high-resolution Mass Spectrometer (GC/MS) by detecting all isomers assigned with TEF and summing the toxic equivalents. GC/MS requires much cost and time, although it can detect them with high sensitivity. Consequently, measurement of toxicity of dioxins has been limited to a small number of cases. It is most reasonable to measure the toxicity, using bioassay method with low cost and short time.

Ah-Immunoassay™ is a bio-assay detection method which is based on the principle of toxicity appearance of dioxins by detecting reactivity of Ah receptor with dioxins on an ELISA plate without using a living cell. It is useful specially for screening the biological toxicity of dioxins. In this paper, we report the correlation between Ah-Immunoassay and GS/MS, measuring the toxicity of dioxins contained in lipid in human blood and maternal breast milk.

Samples

The samples used for the test were a total of six, 3 each of blood and maternal breast milk, shown in the table 1.

Table 1 Test Samples

No.	Kind	Volume(mL)
1	Blood	150
2	Blood	150
3	Blood	150
4	Milk	45
5	Milk	50
6	Milk	50

Method for extracting lipid in blood and maternal breast milk and

The lipid in blood was extracted in accordance with modified method by Patterson et al, shown in table 2 [1)]. The method is

Table.2 Test Results (*DEQ is equivalent to 2,3,7,8-TCDD for Ah-Immunoassay).

No.	Sample	Amount of lipid extracted (mg)	GC/MS (pg-TEQ/g-lipid)	Ah-Immunoassay (pg-*DEQ/g-lipid)	Raio Of DEQ/TEQ (-)
1	Blood	207.2	35.3	352.3	9.9
2	Blood	171.2	32.8	280.4	8.5
3	Blood	130.1	25.4	215.2	8.4
4	Milk	305.4	18.9	85.1	4.5
5	Milk	449.3	26.8	151.3	5.6
6	Milk	171.7	10.7	285.4	26.6

shown in the left side of fig.1. The lipid in maternal breast milk was extracted in accordance with the Tentative Manual for Measuring Dioxins in Maternal Breast Milk of the Ministry of Labor and Welfare Japan shown in the right side of fig.1.

Clean-up Operation

Clean-up Operation was done in accordance with the procedures shown in Fig. 2.

Clean-uped samples in hexane were divided into three portions for Ah-Immunoassay and GC/MS. For Ah-Immunoassay test, the hexane in sample was evaporated to dryness by nitrogen spray, then dissolved with DMSO

Ah-Immunoassay Procedures

Procedures of Ah-Immunoassay method are shown in Fig.3. Each of portioned clean-uped samples 1 and 2 were prepared for Ah-Immunoassay.

Results and Discussion

The results by GC/MS and Ah-Immunoassay are shown in Table 2 and Fig.4.

Results of 6 cases were investigated, although number of data is small. It was found that as to No. 6 sample of milk, the lipid extraction amount was 171.7 mg, indicating an extremely low concentration as compared to other milk samples. In addition, the TEQ of GC/MS was as low as 10.7pg-TEQ/g-lipid. This case might be irregular, though the further investigation is necessary. Each correlation was obtained, excluding No.6 sample.

The correlation between the toxic equivalents (TEQ) of GC/MS and Ah-Immunoassay data (DEQ) was obtained and summarized in fig.3 or 4. As a result, the good correlation was found, expressed by gradient of 9.1 with correlation coefficient of 0.91for blood samples and by gradient of 5.3 with correlation coefficient of 0.93 for milk samples, respectively. The ratios of blood samples and milk samples are 8.4 to 9.9 and 4.5 to 5.6, respectively.

Tested samples in this report were as small as 6 cases, and it is not adequate to examine the correlation precisely. In the future, the number of cases must be increased for further investigation.

References

1. Patterson, D.G., Furst, P., Henderson, L.O., Isaacs, S.G., Alexander, L.R., Turner, W.E., Needman, L.L., Hannon, H., : Chemosphere, 1989, 19, 135-142

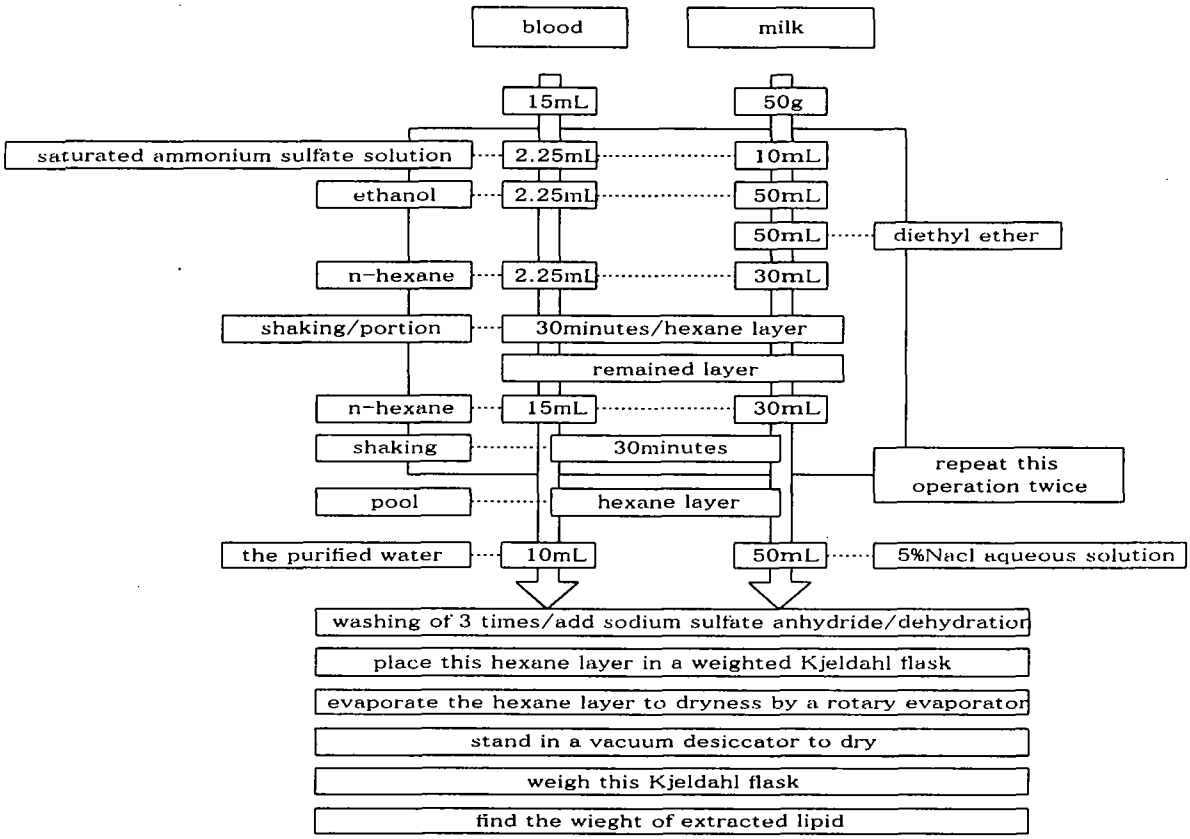

Fig.1 Method for extractingfat in blood and in maternal breast milk

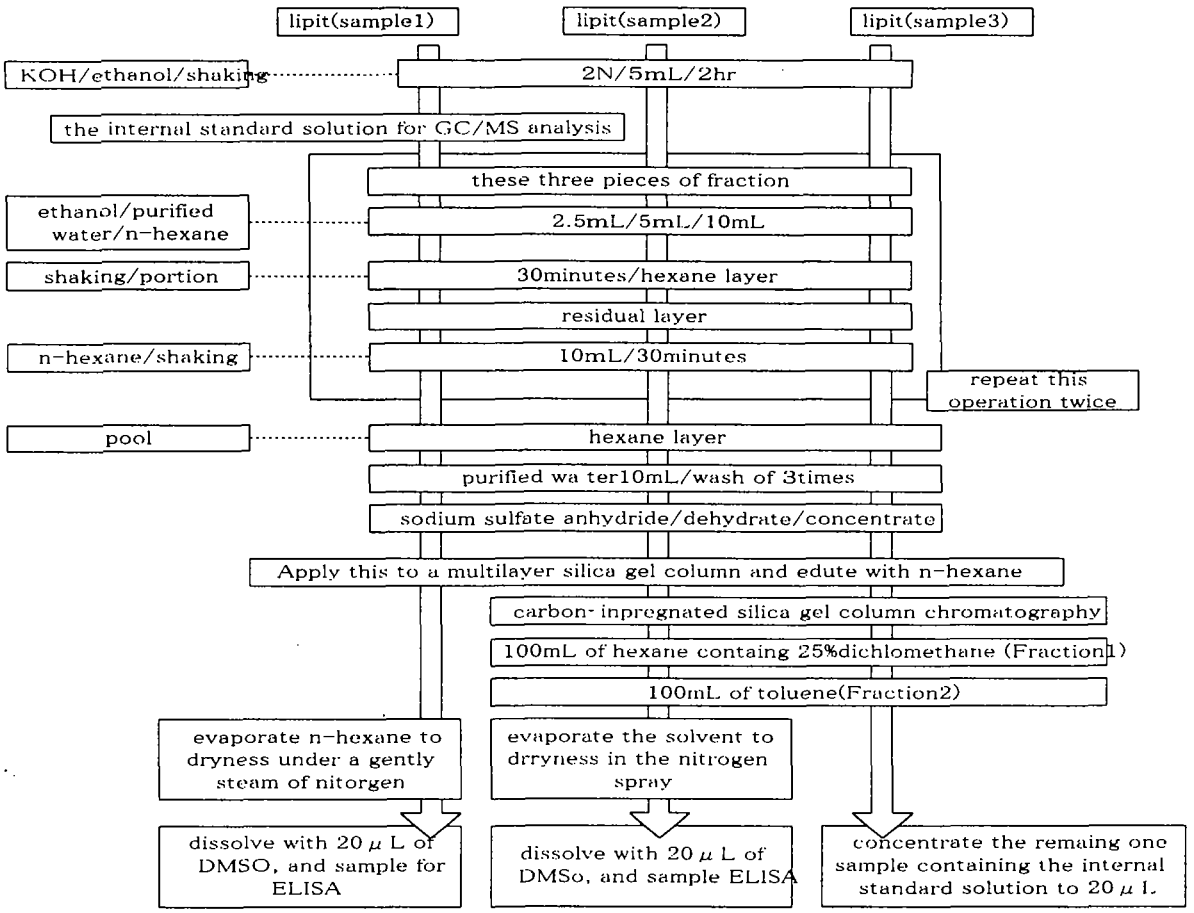

Fig.2 Procedures of clean-up operation

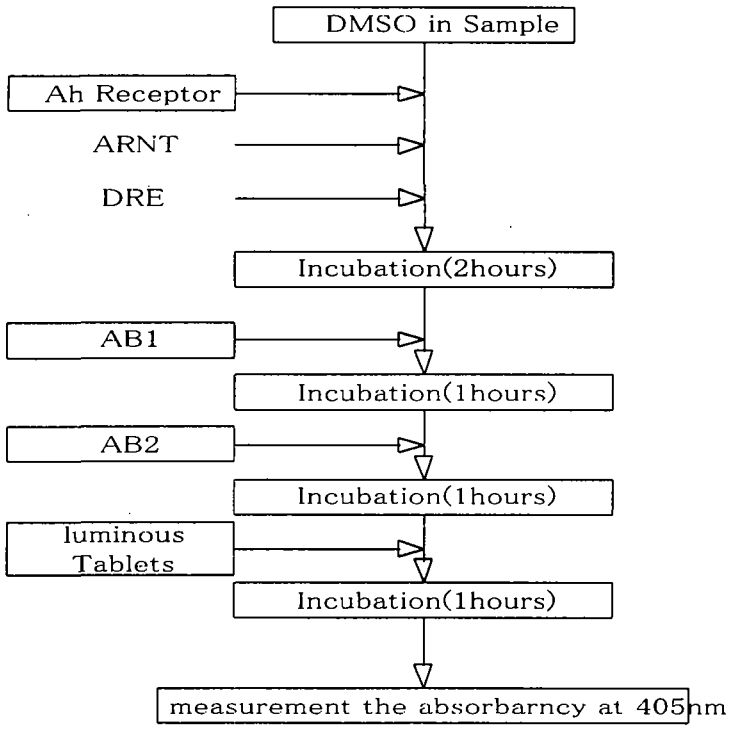

Fig.3 Procedures of Ah-Immnoassy methoc

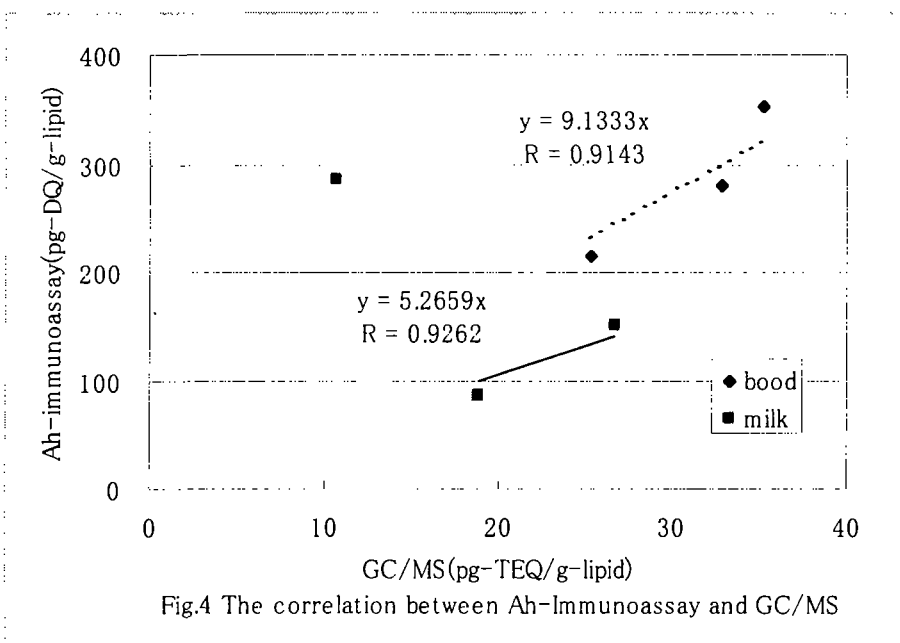

Fig.4 The correlation between Ah-Immunoassay and GC/MS