SENSITIVE DETERMINATION METHOD OF DIOXINS AND RELATED COMPOUNDS IN HUMAN BLOOD

Yuko Masuzaki*, Tohru Matsumura*, Tatsuya Hattori*, Shingo Kimura†, Hiroshi Noda†, Shunji Hashimoto§ and Masatoshi Morita§

* Shin-Nippon Meteorological and Oceanographical Consultant Co.Ltd.
1334-5 Riemon, Ohigawa, Shida, Shizuoka 421-0212, JAPAN
†Environment Agency of Japan
1-2-2 Kasumigaseki, Chiyoda-ku, Tokyo 100-8975, JAPAN
§National Institute for Environmental Studies
16-2 Onogawa , Tsukuba, Ibaraki 305-0053, JAPAN

Introduction

The effect of dioxins and related compounds to human health is still one of the greatest concern. It is serious problem in Japan because of high density distribution municipal and industrial wastes incinerators, which may be the current principal source of those compounds.

The sample such as blood, milk and adipose fat tissue has been used for analysis of those compounds in human (1-7). Among these, human blood has advantage over the other because sampling is relatively easier than the other samples. However, the concentration of those compounds in blood is quite low that measurement is not easy. The measurements require accuracy and sensitivity, and it is not rare that the data reported vary widely. The source of error can be in insufficient sensitivity of method, poor purification and recovery and contamination.

Present here is an improved analytical method, which can be applied to relatively small volume samples of blood.

Materials and Methods

Sample: Whole blood and serum pool samples stored at –30 ºC in National Institute for Environmental Studies (NIES, Japan) were used for the examination. Serum sample was divided into two portions and one of them was fortified with selected native PCDD and PCDF isomers listed in table 2. Analysis of PCDDs and PCDFs was made by Shin-Nippon Meteorological and Oceanographical Consultant Co.Ltd (SNMOC) and NIES.

Preparation for analysis: All glassware was soaked in 2 M-KOHaq/ethanol overnight and washed. All organic solvent was distilled by sub-boiling system. KOH solution and sulfuric acid were washed with distilled hexane. Silica gel was washed with methanol using an ultra sonic washer, dried and deactivated. Active carbon was washed with distilled acetone and toluene using a Soxhlet-extractor for a week and dried. All process was handled in a clean room or in a clean bench.

Extraction and purification for sample: Sample, typically 50 g, was weighed accurately and spiked with ^{13}C labeled standard mixture (5 pg of tetra, penta, hexa, and hepta chlorinated isomer, 10pg of octachlorinated isomer). They were mixed with 50ml of 2 M-KOHaq/methanol (3:2 v/v) and left at room temperature for one night. The solution was extracted 3 times with hexane using 20 ml at

each time. The combined hexane extracts were rinsed with 50 ml of water twice and washed with concentrated sulfuric acid for 3 to 5 times. The hexane layer was rinsed with water 3 times and then dried by passing through anhydrous sodium sulfate in a glass funnel. The solution was concentrated and subjected to clean up process using a coupled column of silica gel (1.5g of Silicagel 60, Merck) or multi-layer silica gel (22%-H_2SO_4/silica gel, 44%-H_2SO_4/silica gel, $AgNO_3$ /silica gel from top to bottom) and of active carbon (0.5 g of active carbon impregnated-silica gel, Wako Pure Chemical, Japan).
After cleaning-up, the sample was spiked another ^{13}C labeled compounds (syringe spike) and made up to a small volume (10 µl for splitless injection or 100 µl for OPTIC2)

Measurement: The GC/MS analysis was performed on a JMS-700 (JEOL, Japan) or Ultima (Micromass, UK) coupled to a HP 6890 gas chromatograph (Hewlett Packard, US). The sample solution was introduced into the HP 6890/JMS-700 by a FOCUS and an OPTIC2 large volume sample injecting system (Atas-USA, US) equipped with an At-Column (GL Science, Japan). A CP-SIL 8CB-MS (30m length, 0.25 mm ID, 0.25 µm film thickness, Chrompak, US) or a BPX-5 (30m length, 0.25 mm ID, 0.25 µm film thickness, SGE, Australia) low breading capillary column was used to reduce noise on chromatograms.
The mass profiles of the selected ions were obtained during GC elution. Identification was based on examination of isotopic ratio of M^+ and $(M+2)^+$ or $(M+2)^+$ and $(M+4)^+$ and retention time of the GC separation. Only one channel was used for monitoring of ^{13}C labeled isomers to minimize cycling time. The area of the mass profile peaks of the quantification ions was used for the quantitative analysis. Quantified values were calculated by the internal standard methods for PCDDs and PCDFs.

Results and Discussion
Sensitivity is the most important factor to detect low level dioxins in a limited volume of blood samples. 5 fg of 2,3,7,8-TCDD was detected over the detection limit (S/N=3) and linear calibration curve was obtained between 5 fg and 1 pg. In real samples, however, 10 fg was a reasonable detection limit because of increased noise level in the chromatogram.
SIM chromatograms of typical sample is shown in figure 1. PCDD, PCDF and PCB isomer was identified as a significant peak. Among PCDDs and PCDFs, only 2,3,7,8- isomers were detected. Those isomer compositions is similar to other reports (8-10). Background levels on this method is shown in table 1.
Variation of measured concentrations of PCDDs and PCDFs on 17 isomers in 4 whole blood samples by the method was average of 14 %. Variation of planar PCBs was average of 8.6 %.
The results of serum samples from two laboratories (NIES and SNMOC) by the same method were shown in table 2. The difference of results is small and the average of recovery of spiked native PCDDs and PCDFs was 86.1 %. In addition, the reproducibility of measurement on total of 10 samples was also fairly good.
By using the method, It was possible to reduce the sample volume. Using 10 ml of blood sample, 2,3,7,8-TCDD was detected at the level of 1 pg/g lipid.

References
1. Ono, M., Wakimoto, T. and Tatsukawa R.; *Chemosphere*, **1986**, 15, 1629
2. Patterson, Jr., D.G., Hampton, L., Lapeza, Jr.,C.R., Belser, W.T., Green, V., Alexander, L. and Needham, L.L.; *Anal. Chem*., **1987**, 59, 2000

Table 1 Concentrations (pg/g)* of PCDDs, PCDFs and planar PCBs in blank sample.

compound		compound (IUPAC No.)	
2,3,7,8-TCDD	<0.00012	3,3',4,4'-TeCB (#77)	0.0012
1,2,3,7,8-PeCDD	<0.00022	3,4,4',5-TeCB (#81)	0.0049
1,2,3,4,7,8-HxCDD	<0.000076	3,3',4,4',5-PeCB (#126)	0.0037
1,2,3,6,7,8-HxCDD	<0.000076	3,3',4,4',5,5'-HxCB (#169)	0.028
1,2,3,7,8,9-HxCDD	<0.000076	2,3,3',4,4'-PeCB (#105)	0.0021
1,2,3,4,6,7,8,-HpCDD	0.0015	2,3,4,4',5-PeCB (#114)	0.0076
OCDD	0.0090	2,3',4,4',5-PeCB (#118)	0.0016
2,3,7,8-TCDF	<0.000076	2',3,4,4',5-PeCB (#123)	0.0021
1,2,3,7,8-PeCDF	0.00061	2,3,3',4,4',5-HxCB (#156)	0.0036
2,3,4,7,8-PeCDF	0.00055	2,3,3',4,4',5'-HxCB (#157)	0.00072
1,2,3,4,7,8-HxCDF	<0.00013	2,3',4,4',5,5'-HxCB (#167)	0.0042
1,2,3,6,7,8-HxCDF	<0.00013	2,3,3',4,4',5,5'-HpCB (#189)	<0.00018
1,2,3,7,8,9-HxCDF	<0.00013		
2,3,4,6,7,8-HxCDF	<0.00013		
1,2,3,4,6,7,8-HpCDF	0.0015		
1,2,3,4,7,8,9-HpCDF	<0.00013		
OCDF	<0.00048		

* calculated of 50 g sample

Table 2 Concentrations (pg/g whole) of PCDDs and PCDFs in serum reference samples.

compound	fortified amount (pg/g)	serum (fortified)		serum (not fortified)		recovery of standard (%)
		mean (n=10)	*RSD (%)*	*mean (n=7)*	*RSD (%)*	
2,3,7,8-TCDD	0.015	*0.024*	*23.3*	*0.0088*	*18.9*	99.0
1,2,3,7,8-PeCDD	0.074	*0.083*	*20.3*	*0.025*	*29.9*	77.3
1,2,3,4,7,8-HxCDD	0.074	*0.083*	*30.9*	*0.029*	*20.7*	73.4
1,2,3,6,7,8-HxCDD	0.074	*0.38*	*19.5*	*0.27*	*31.4*	144
1,2,3,7,8,9-HxCDD	0.074	*0.089*	*14.8*	*0.031*	*33.0*	77.8
1,2,3,4,6,7,8,-HpCDD	0.074	*0.32*	*10.3*	*0.26*	*25.5*	73.2
OCDD	0.147	*2.5*	*10.2*	*2.4*	*26.7*	105
2,3,7,8-TCDF	0.015	*0.014*	*17.7*	*<0.003*		96.0
1,2,3,7,8-PeCDF	0.074	*0.059*	*14.2*	*<0.003*		80.0
2,3,4,7,8-PeCDF	0.074	*0.091*	*12.3*	*0.027*	*5.9*	85.7
1,2,3,4,7,8-HxCDF	0.074	*0.096*	*17.0*	*0.040*	*34.6*	75.7
1,2,3,6,7,8-HxCDF	0.074	*0.084*	*13.1*	*0.024*	*24.4*	81.0
1,2,3,7,8,9-HxCDF	0.074	*0.062*	*21.9*	*<0.005*		83.6
2,3,4,6,7,8-HxCDF	0.074	*0.058*	*12.8*	*0.0080*	*17.7*	68.0
1,2,3,4,6,7,8-HpCDF	0.074	*0.17*	*10.3*	*0.088*	*14.8*	106
1,2,3,4,7,8,9-HpCDF	0.074	*0.057*	*15.2*	*<0.005*		76.6
OCDF	0.147	*0.12*	*13.9*	*<0.01*		80.7

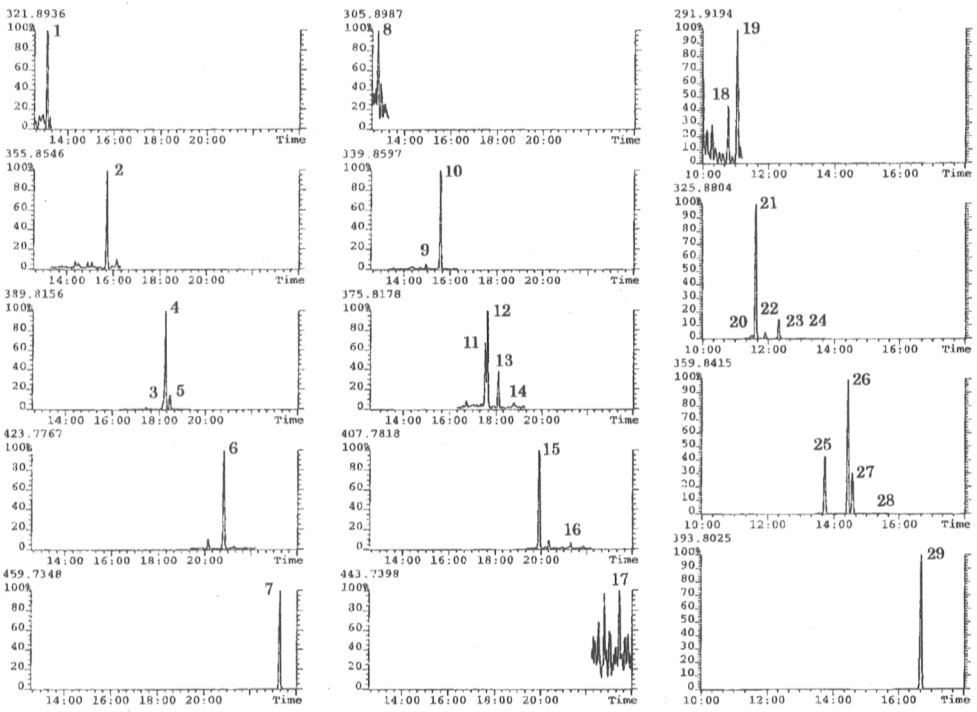

Figure 1 SIM chromatograms of PCDDs, PCDFs and planar PCBs in human blood.

3. Ryan, J.J. Levesque, D., Panopio, L.G., Sun, W.F., Masuda, Y. and Kuroi, H.;, *Environ. Contam.*
Toxicol., **1993**, 24, 504
4. Fürst, P., Meemken, Chr., Krüger, H.-A. and Groebel, W.; *Chemosphere*, **1987**, 16, 1983
5. Hayward, D.G., Charles, J.M., de Bettancourt, C.V., Stephens, S.E. and Stephens, R.D.; *Chemosphere*, **1989**, 18, 455
6. Pruim, H.J., Slot, P.C., Olie, K,. van der Slikke, J.W. and Koppe, J.G.; *Chemosphere*, **1992**, 25, 307
7. Hashimoto, S., Yamamoto, T., Yasuhara, A. and Morita, M.; *Chemosphere*, **1995**, 31, 4067
8. Papke, O., Ball, M. and Lis, A.; *Chemosphere*, **1994**, 29, 2355
9. Deml, E., Mangelsdorf, I. and Greim, H.; *Chemosphere*, ,**1996**, 33, 1941
10. Wittsiepe, J., Schrey, P., Ewers, U., Wilhelm, M. and Selenka, F.; *Dioxin'98*, *18th Symposium*

on Halogenated Environmental Organic Pollutants, Stockholm, Sweden, **1998**, 38, 211