ANALYTICAL METHOD OF PBDES AND PCBS FROM SMALL VOLUME HUMAN BLOOD

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Introduction

Polychlorinated biphenyls (PCBs) were widely used as the insulating material and the heat transfermedium, while polybrominated diphenyl ethers (PBDEs) are used as the flame retardants. These chemicals are known to persist in the environment and are called as the persistent organic pollutants (POPs). The contamination of POPs has been spreading in worldwide environment, leading to the accumulation of these chemicals in food chain. As almost all peoples have contaminated with these chemicals, babies also will have a risk to be exposed to these chemicals via maternal placenta. We have studied some methodologies to evaluate the human exposure to POPs using HRGC/HRMS as the instrumental analysis[1,2)]. However these methods need to use large volume of sample, i.e. 30 mL to 100 mL of whole blood, limiting the application of these methods to only adult and/or healthy subjects.

In order to conduct the epidemiological survey for children and old peoples, it is necessary to develop/improve the analytical method with which to conduct the analysis in smaller volume of blood samples because the blood volume from these subjects are normally allowed to take less than 5mL. For these purposes, we developed the Small Volume method (SV-method) using HRGC/HRMS for the PCBs and PBDEs analysis. The SV method for PCB can start the analysis only with 1.0 mL of whole blood and that for PBDE with 1.5 mL of whole blood, respectively; these methods showed the ultra high sensitivity. However the SV-method can not determine all isomer of PCBs and PBDEs but only predominant isomers of those chemicals. In the Healthy volunteers examination, the SV-PCB determined 13 isomers and the SV-PBDE method determined 4 isomers, respectively. By the way, SV-PCB and SV-PBDE method determined almost all levels in the sample; SV-PCB is 75 % as compared with sum of all PCB isomers level, SV-PBDE is 82 % as compared with sum of all PBDE isomers level.

In summary, SV-method using HRGC/HRMS can determine approximate and predominant isomers levels of PCBs and PBDEs, making it possible to evaluate the human exposure to these chemicals. In this paper, the method of SV-PCB and SV-PBDE were described with precision date.

Materials and Methods

Chemicals

Authentic PCBs, PBDEs, $^{13}C_{12}$ –PCBs and $^{13}C_{12}$ -PBDEs were purchased from Cambridge Isotope Laboratories, Inc. (MA, U.S.A.). Solvents (n-hexane, methylene chloride and ethanol) and reagents (various silica gels and KOH) purchased from Wako Chemicals (Osaka, Japan). Anhydrous Na_2SO_4 purchased from Kanto Chemical Co. (Tokyo, Japan). All solvents and reagents used were of dioxin-analysis grade.

Samples

The human whole blood samples corrected from healthy volunteer, it and banked whole blood samples were stored at –20 until analysis.

Preparation of samples

1. SV-PCB

About 1.0mL of whole blood sample with 100 pg $^{13}C_{12}$-PCBs were degraded with 1mL of 2M-KOH/EtOH containing 10 % H_2O for 2 hr with shaking at room temperature. After degradation, the solution containing PCBs was partitioned and extracted with 1.0 ml of n-hexane (3 times). The extract was washed by high purity water at 2 times and dried up with sodium sulfate. Dried solution was cleaned up on a multi-layer column containing Na_2SO_4 (0.9 g), silica (0.5 g), $AgNO_3$-silica (1.8 g), silica (0.5 g), 44 %(W/W) H_2SO_4-silica (2.7 g), silica (0.5 g) and Na_2SO_4 (0.9 g). Before loading of the sample, the column was washed with 60 ml of n-hexane. The sample was loaded on the column and was eluted with 100 mL of 10 % methylene chloride / n-hexane at a flow-rate of 2.5 mL/min. About 10 µl of n-decane was added to the first fraction containing the PCBs, and evaporated at 40 on a rotary evaporator to about 0.5 mL.

PCBs fraction was evaporated on a rotary evaporator to *ca.* 0.5 mL and transferred to a GC autosampler vial tube. The remaining solvent was evaporated under the stream of nitrogen. The walls of the flask were flushed out with small volumes of methylene chloride, typically decreasing from 20 to 40 µl. This solution was added to 50 pg of the each syringe spike ($^{13}C_{12}$-PCBs). 1 µL of the sample was injected to analyze for PCBs.

2. SV-PBDE

About 1.5mL of whole blood sample with 40pg $^{13}C_{12}$-PBDEs were degraded with 1mL of 2M-KOH/EtOH containing 10 % H_2O for 2 hr with shaking at room temperature. After degradation, the solution containing PCBs was partitioned and extracted with 1.0 ml of n-hexane (3 times). The extract was washed by high purity water at 2 times and dried up with sodium sulfate. Dried solution was cleaned up on a multi-layer column containing Na_2SO_4 (0.9 g), silica (0.5 g), $AgNO_3$-silica (1.8 g), silica (0.5 g), 44 %(W/W) H_2SO_4-silica (2.7 g), silica (0.5 g) and Na_2SO_4 (0.9 g). Before loading of the sample, the column was washed with 100 ml of n-hexane. The sample was loaded on the column. First effluent was eluted with 50 mL of n-hexane, second effluent was eluted with 150 mL of 10 % methylene chloride / n-hexane. The second was collected to analysis, about 5 µl of n-decane was added, and evaporated at 40 on a rotary evaporator to about 0.5 mL.

PBDEs fraction was evaporated on a rotary evaporator to *ca.* 0.5 mL and transferred to a GC autosampler vial tube. The remaining solvent was evaporated under the stream of nitrogen. The walls of the flask were flushed out with small volumes of methylene chloride, typically decreasing from 20 to 40 µl. This solution was added to 50 pg of the each syringe spike ($^{13}C_{12}$-PBDEs). 1 µL of the sample was injected to analyze for PBDEs.

Analysis of PCBs and PBDEs

HRGC/HRMS which consisted of an AutoSpec-Ultima (Micromass, U.K.) and a HP-6890 Series gas chromatograph (Agilent Technologies, Inc., U.S.A.) was used for analysis. The used column for SV-PCB analysis was HT8-PCB capillary column, 0.25 mm I.D. x 60 m (Kanto Chemical Co., INC., Japan). On SV-PBDE analysis, DB-17HT capillary column, 0.25 mm I.D. x 30 m (J&W Scientific, USA) was used. The column temperature program for SV-PCB was maintained at 120 , heated to 180 at a rate of 20 /min., heated to 270 at a rate of 2 /min., heated to 310 at a rate of 50 /min., and maintained at 310 for 6 min. and the column temperature program for SV-PBDE was maintained at 180 (2 min.), heated to 240 at a rate of 3 /min., heated to 320 at a rate of 20 /min and maintained at 320 for 10 min.. The injection temperature was 290 , ion source temperature was maintained at 290 , and the carrier gas (helium) rate was 1.0 ml/min.. The ionizing energy and accelerating voltage were 40 eV and 8 kV, respectively. The resolution was about 11,000 throughout the work, and carried out according to an SIM.

Results and Discussion

The data from the SV-method were quantitatively compared with that from the conventional method (Table 1). These data were collected from whole blood samples from a few healthy subjects. As for the PBDEs, the SV-method showed almost the same quantitative result in spite of differing 10 times of sample volume. The difference was observed in high level isomers, which was about 10% of increase in PCB209 level. However the difference of the level observed in PCB will be acceptable as a screening method.

On the comparison of the total level between the SV-method and the conventional method, SV-methods were 78% or more of their conventional method. The SV-method obtained less about 78% of the total amount, which may lead a problem. However we considered that the SV-method is useful because the SV-method requires the smaller sample volume such as 1.0 to 1.5ml.

The precision of the SV-methods has been examined and the comparison showed that it has practically enough precision to meet the demand for the evaluation of human exposure. In summary, SV-PBDE and SV-PCB methods reported in this study will provide the practical tools for the evaluation of human exposure to PBDEs and PCBs.

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References

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Table 1. Comparision of the quantitative levels between SV-method and conventional method.

Congeners		Conventional method PBDE	SV-PBDE
	IUPAC No.	(pg/ml)	(pg/ml)
2,2',4,4'-TetraBDE	#47	2.5	2.5
2,2',4,4',6-PentaBDE	#100	1.1	1.3
2,2',4,4',5-PentaBDE	#99	1.1	1.2
2,2',4,4',5,5'-HexaBDE	#153	3.5	3.2
ΣPBDEs		**10.5**	**8.2**
Using whole blood volume (ml)		30.0	1.5

Congeners			Conventional method PCB	SV-PCB
	(BZ#)	TEF	(pg/ml)	(pg/ml)
2,4,4',5-TetraCB	#74	***	25.5	22.9
2,3',4,4',5-PentaCB	#118	0.0001	81.0	97.9
2,2',4,4',5-PentaCB	#99	***	29.3	28.5
2,2',3,4,4',5'-HexaCB	#138	***	125.0	139.0
2,2',3,4',5,5'-HexaCB	#146	***	36.7	39.6
2,2',4,4',5,5'-HexaCB	#153	***	301.0	323.8
2,3,3',4',5,6-, 2,3,3',4',5',6-HexaCB	#163,164	***	52.0	60.7
2,3,3',4,4',5-HexaCB	#156	0.0005	25.1	28.1
2,2',3,3',4,4',5-HeptaCB	#170	0.0001	49.6	52.2
2,2',3,4,4',5,5'-HeptaCB	#180	0.00001	212.0	234.8
2,2',3,4,4',5,6'- , 2,2',3,4',5,5',6-HeptaCB	#182,187	***	97.0	99.3
ΣPCBs			**1381.0**	**1126.6**
Using whole blood volume (ml)			15.0	1.0