DETERMINATION OF POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN DRIED BLOOD SPOTS AND VERY LOW VOLUME BLOOD SAMPLES

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Introduction

Persistent organic pollutants such as PCBs and PBDEs exist in the environment for many years after disposition and bioaccumulate in biota including humans[1-2]. Human biomonitoring methods typically use 1 ml of blood for exposure evaluation to persistent organic pollutants. In recent years, the dry blood spot (DBS) sampling technique has been extensively used not only in clinical and disease studies but also in human biomonitoring for environmental chemicals. DBS requires only a few drops of blood the collection, shipping and storage of samples is easy. Published studies demonstrated the feasibility of DBS techniques for assessing human prenatal exposure to perchloride[3], polyfluoroalkyl chemicals[4, 5], and *p,p'*-DDE[6].

In this study, we developed a procedure to measure low levels of PCBs and PBDEs in DBS. The objectives included evaluation of filter paper background interferences, and comparing the DBS technique with a method using very low volume samples, one drop of blood processed through routine liquid-liquid extraction (LLE). We concluded that DBS or very low volume samples could be used to measure PCBs and PBDEs in biomonitoring program.

Materials and methods

The target analytes (100 µg/ml in isooctane) include five dioxin-like PCBs (dl-PCBs, 77, 105, 118, 126, 169), six marker PCBs (28, 52, 101, 153, 138, 180), three other PCBs (194, 206 209), and five PBDEs (50 µg/ml in nonane, PBDE 47, 99, 100, 153, 183). All 19 native compounds and the corresponding 19 $^{13}C_{12}$ labeled congeners were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA). A set of calibration standard solutions within the concentration range of 0.05-10 ng/ml were prepared in nonane by combining native, ^{13}C-labeled internal standards (5 ng/ml each), and an injection internal standard $^{13}C_{12}$-PCB 128 (5 ng/ml). Hexane and acetone (pesticide grade) were from Fluka (Steinheim, Germany), and formic acid was from EMD (Darmstaldt, Germany). NIST human serum standard reference material (NIST SRM 1957) was used for method proficiency testing.

Three QC materials were prepared in Defib Sheep Blood (Hemostat Laboratories, CA, USA). Pool I was sheep blood blank sample, Pool II was spiked with PCBs and PBDEs at levels of 0.05 ng/ml, and Pool III was spiked at levels of 0.16 ng/ml. From each pool, an aliquot of 50 µl was spotted five times on a piece of filter paper. These blood spot samples were air dried and stored at -20°C in zip-lock plastic bags.

Each DBS (about 50 µl blood) was cut into small pieces, added to 500 µl of a mixture of formic acid and

acetone (2/3, v/v), spiked with 20 µl $^{13}C_{12}$ labeled IS solution, and sonicated. Another 1.0 ml of a mixture of hexane and dichloromathane (8/2, v/v) was added. The extracts were reduced to near dryness and 10 µl of internal standard solution $^{13}C_{12}$-PCB 128 (5 ng/ml in nonane) was added and the mixtures were vortexed.
One DBS volume (50 µl) of blood samples were processed through routine liquid-liquid extraction[7]. The stability of the target analytes in DBS was studied by measuring PCBs and PBDEs in spiked DBS at levels of 0.1–0.2 ng/ml over 30 days. The absorption of the target analytes into the filter paper during the transportation and storage was examined by measuring PCBs and PBDEs in one piece of blank filter paper, which was precleaned with a mixture of hexane and dichloromethane and then wrapped in aluminum foil at room temperature for 30 days. All samples were analyzed in a Trace GC Ultra (Thermo Scientific, Santa Clara, CA, USA) coupled to an DFS high resolution mass spectrometer (Thermo Scientific, Santa Clara, CA, USA)

Results and discussion
The result of examining background contamination was that filter paper itself, which was used to prepare the DBS, contained certain levels of PCBs and PBDEs. The levels of PCBs and PBDEs seem to decrease over time. The levels of PCBs and PBDEs in the 1987 filter paper was over ten times higher than those in the other filter paper sources, which were marked for use in 1996, 2003, 2005, 2007, and 2009. However, there was no obvious difference for PCBs and PBDEs levels in the filter papers from 1996 to 2009. The background PCBs and PBDEs levels were possibly from the paper manufacture processes or subsequent handling. If such contaminations are avoided, DBS method would be good sampling techniques for the determination of PCBs and PBDEs. In order to check whether PCBs and PBDEs levels are different at different sites on the filter paper, we randomly punched five small pieces in five different sites on one filter paper (15×21 cm). One-way ANOVA (SPSS 17.0) analysis showed that there was no obvious background difference between the different sites on the filter paper. Relative deviation (%) of the background PCBs and PBDEs among the five sampling points was within ±22%.
During blood spotting on the filter paper, the filter paper might function as paper chromatography so that PCBs and PBDEs could disperse around a central DBS. To examine such paper chromatography effect, a piece of blank filter paper was punched at a distance of 0, 1, 2, 3, and 4 mm from the immediate margin of a DBS. The results were that there was no obvious difference for PCBs and PBDEs levels between these sampling points. However, at the zero distance site, PCB 126 and 169 and BDE 153 were detected where they were normally not detected in filter paper suggesting dispersion of PCBs and PBDES in the filter paper happened within 1mm from the peripherial of the DBS. Therefore, the levels of PCBs and PBDEs in DBS must be corrected from the blank filter paper, measured at least 1 mm from the DBS.
Stability test demostrated that the PCBs and PBDEs in DBS were stable for at least 30 days at the room temperature. Furthermore, no obvious absorption of PCBs and PBDEs from the enviroment was noticed during the same time period. The results from background contamination, stability and absorption experiments further suggested that the contamination of PCBs and PBDEs in the filter paper possibly happened during the manufacture process.
In order to examine the method performance, we compared the results in spiked DBS with the very low volume samples using one drop of blood in liquid form. Typically, 1.0 ml of blood sample was used for the analysis of

halogenated organic pollutants by using liquid-liquid extraction[8]. However, extensive sample preparation is required to remove matrix interferences. In this study, we used one drop of blood (50 µl) taken through LLE without additional clean-up. The method was applied to sheep blood samples spiked at 0.05 ng/ml and 0.16 ng/ml. The results from the DBS method and very low volume method are comparable (Table 1). The recoveries of only a few congeners are exceptional. For example, at the spiked level of 0.05 ng/ml, the recoveries of PCBs 28 and 101 were 132% and 158%, respectively; and the RSD of PCB 52 and 101 were 47% and 30%, respectively. The higher spiked level of 0.16 ng/ml overrides the effect from the background and background fluctuation of filter paper, and the recoveries and RSDs of all congeners were within the ranges of 81-140% and 6-26%. Moreover, the measured PCBs and PBDEs levels from DBS and one drop of blood (50 µl) were in the range of three times the uncertainty certified in proficiency test (PT) sample (NIST SRM 1957) (Table 1). This shows that both the DBS method and the very low sample volume method provided acceptable accuracy for the analytes tested, except that DBE-183 was difficult to quantify due to interfering peaks that co-eluted.

Table 1. Percentage recoveries (Rec), relative standard deviations (RSD), and proficiency test by using spiked DBS and DBS volume (DBSV) of blood (50 µl)

Congeners	Low spiked level (ng/ml) (0.05) (n=8)				High spiked level (ng/ml) (0.16) (n=8)				PT (ng/kg) (n=2)		
	DBSV		DBS		DBSV		DBS		DBS	DBSV	Certified value
	Rec	RSD	Rec	RSD	Rec	RSD	Rec	RSD			
CB-28	71	12	132	13	106	5	105	10	11.5	9	9.3±1.2
CB-52	111	20	103	47	127	8	81	11	0	0	/
CB-101	83	20	158	30	126	10	98	15	0	0	/
CB-153	74	12	84	11	119	8	114	10	59	64	57±3.3
CB-138	99	11	74	7	125	7	117	8	38.5	44	37±5.4
CB-180	86	9	68	7	115	8	119	17	57	54	54±1.3
CB-77	80	9	68	5	107	6	118	6	0	0	/
CB-105	83	16	86	14	123	5	118	10	8.5	5	4.1±3.1
CB-118	84	9	77	8	119	5	124	6	14	13	19±2.7
CB-126	76	11	67	4	108	4	120	10	0	0	/
CB-169	78	10	70	10	117	5	131	9	0	0	/
CB-194	81	9	76	7	111	7	115	22	13	12	12±0.5
CB-206	88	16	81	7	105	9	117	25	8	9	7.51±0.4
CB-209	75	9	68	5	93	14	106	26	3	4	3.6±0.63
BDE-47	74	19	107	16	121	13	93	8	269	286	272±14
BDE-100	71	18	75	18	111	5	115	10	55	52	51±2.5
BDE-99	99	20	108	18	104	5	106	12	76.5	71	77.8±1.7
BDE-153	94	12	109	11	110	8	140	14	65	59	62.1±3
BDE-183	88	13	120	9	109	7	115	14	0	6	3.4±2.3

In conclusion, the DBS method generated good method accuracy and precision in the measurement of PCBs and PBDEs except for a few congeners. To make the method useful for all congeners, further studies are required for confirmation of the contamination sources in filter papers and seeking a method to eliminate or mitigate the contamination . The method makes it feasible for us to examine the large number of archived DBS for biomonitoring of PCBs and PBDEs. In addition, the very low sample volume method using one drop of blood also could be used for PCBs and PBDEs analysis. Therefore, the collection of one drop of blood may serve as an alternative low-cost sampling technique and hence a high through-put analytical technique compared to the traditional larger volume blood sample in biomonitoring programs.

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