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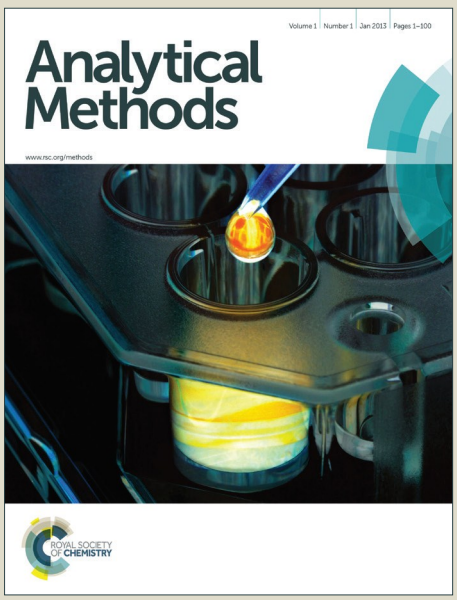

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Development of polychlorinated biphenyls screening method with 3 µL of blood

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Kazutoshi Nose[a], Hisatoshi Yabushita[a], Tetsuya Hirai[a] and Tomiko Tachikawa[a]

In the present study, we developed a method of screening for polychlorinated biphenyls (PCBs) by using 3 µL whole-blood samples accurately metered with a capillary tube on filter paper. Elution from the sample and miniaturization of the clean-up were optimized. The analysis of the target PCB congeners in the samples were demonstrated successfully by high-resolution gas chromatography – high-resolution mass spectrometry. This method will be useful for increasing the number of samples tested and performing high-throughput analyses requiring minimal sample volume and analysis time. For three predominant PCB peaks (IUPAC #153, #138/158/163/164, and #180/193), the results between our screening method and conventional method were compared by Deming regression analysis. The results yielded slopes of 0.95 (95% confidence interval ; CI, 0.758 to 1.14), 1.15 (0.875 to 1.43) and 1.01 (0.847 to 1.16) were respectively observed. Then the y-intercepts of -6.93 (-23.18 to 9.33), -15.89 (-35.04 to 3.26) and -8.06 (-18.40 to 2.28) were respectively observed. A correlation coefficient of 0.9625 was observed between the results for the sum of these three peaks obtained with the screening method and the total tri- to deca-CB concentration results obtained with the conventional method. Other analytical aspects, such as limits of detection, contamination of the blanks used in the screening method (including information on the presence of some PCB congeners), and comparison of the concentrations with standard reference materials, are also reported. A larger sample size is required to statistically prove the validity of the new screening method; however, the method appears promising for screening for human PCB exposure by using as dried blood spot (DBS) samples.

Introduction

Polychlorinated biphenyls (PCBs) are so widespread in nature and in foods (including breast milk) that virtually every child is at risk of exposure to measurable levels[1–3]. Because epidemiologic studies have reported that PCBs influence brain development[4], evaluation of human exposure to them is important. Generally, such evaluations are performed by analyzing blood levels. Quantification of PCBs in blood usually requires laborious sample preparation and large sample volumes. The recent movement to encourage children's environmental health studies is pushing the world's researchers to utilize chemical analysis of biological samples (such as blood and urine) to investigate the effects of chemical exposure on children's health and development[5-8]. In Japan, a birth cohort study involving 100,000 parent–child pairs was launched in 2011[9]. In such studies, we often face difficulties collecting from children samples that are large enough for multiple chemical analyses. Epidemiological studies also require the analysis of large sets of samples for a number of analytes. Such demands have challenged analytical chemistry to develop inexpensive, high-throughput, and low-volume analytical procedures. We therefore need to develop both analytical methods and monitoring techniques. Recently, the use of dozens of microliters of blood samples as dried blood spots (DBSs) to analyze hazardous chemicals has been reported[10-15]. DBS analysis using 20 to 50 µL of serum or blood has already been performed for PCBs. Although the number of target congeners is limited, one of the advantages of DBS analysis is that it needs a minimal sample volume and thus facilitates population screening of blood samples. However, when blood samples are collected by using skin puncture, it is hard to obtain dozens of microliters. There are 209 PCB congeners, and only specific ones are usually detectable in blood samples[16-19]. We therefore developed a method designed to quantify potential PCB congeners in only 3 µL of whole blood on a filter paper. Theoretically, a blood volume of the order of 3 µL is absorbed by a punched-out piece of paper 1/8-inch in diameter[20,21]. A DBS of this volume could be subjected to multiple uses. For example, surplus samples remaining after hazardous chemical analysis could be valuable for use in neonatal screening for congenital diseases[22]. The use of 3 µL of whole blood on a DBS could be a solution to the above-mentioned challenges. However, it is difficult to determine the exact volume of blood in a piece of punched-out filter paper. The blank background of the filter paper must also be taken into account. Here, we applied 3 µL whole blood samples accurately metered with a capillary tube to filter paper. We then investigated the possibility of screening for PCBs by using these small blood samples.

[a] Diagnostic Division, Otsuka Pharmaceutical Co., Ltd., 224-18 Ebisuno Hiraishi Kawauchi-cho Tokushima, 771-0195, Japan. E-mail: nosek@otsuka.jp; Fax: +81-88-665-3613; Tel: +81-88-665-1721

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Experimental

Chemicals and reagents

An isotope-labeled standard solution of PCBs, as well as EC-5366 calibration standard, EC-5367 internal standard, and ED-910 injection internal standard, was obtained from Cambridge Isotope Laboratories, Inc. (CIL; Andover, MA, USA). EC-5433 native standard mixture was also supplied to evaluate the method detection limit (MDL) and method quantification limit (MQL). Formic acid of RoHS (Restriction of Hazardous Substances) analysis grade was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). A 22% sulfuric acid–impregnated silica gel was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Dichloromethane (DCM) and *n*-hexane solvents of dioxin analytical grade were purchased from Kanto Chemical Co. and Wako Pure Chemical Industries.

Sample and sample preparation

A total of 10 whole blood samples collected from healthy donors aged 19 to 58 years into ethylenediaminetetraacetic acid dipotassium salt dihydrate tubes were purchased from Tennessee Blood Service (Memphis, TN, USA). No other information associated with the blood (such as race, area of residence, or gender) was available. The samples were processed and tested in accordance with the appropriate FDA regulation and guidelines at an FDA facility. They were then stored at −20 °C until use in the analysis. A standard reference material (SRM 1957, Organic Contaminants in Non-fortified Human Serum, NIST, Gaithersburg, MD, USA), which was reconstituted by adding 10.7 mL of *n*-hexane-washed distilled water, was also analyzed. The materials were then analyzed by using two different methods, namely our newly developed screening method and a conventional method.

For sample preparation for the newly developed screening method, 3 µL of blood was placed on a 1/8-inch circular filter paper (Whatman-903, GE Healthcare Life Sciences, Little Chalfont, UK) by using a Microcaps (Drummond Scientific Co., Broomall, PA, USA) glass capillary tube. The paper was then dried overnight in air[12,15]. Before the sample was applied to the filter paper, the paper was pretreated with DCM to remove any contaminating PCBs. After being dried in air, the sample was placed in a Spitz-type glass tube. It was then spiked with isotope-labeled homologs as internal standards and treated with 10 µL of formic acid[23,24] for 10 min. The sample was then extracted with 450 µL of 50% DCM-*n*-hexane (v/v) under sonication for 30 min. The extract was loaded onto a simplified glass Pasteur pipette column containing 50 mg of 22% sulfuric acid–impregnated silica gel. The column was washed with 50% DCM-*n*-hexane (v/v) before use. It was then eluted with 500 µL of 5% DCM-*n*-hexane (v/v). The eluate was concentrated to 20 µL under a gentle nitrogen flow. Each analysis was run in triplicate.

Instrumental analysis of PCBs in filter paper samples

A total of 1.5 µL of each final solution was injected into the column by using an Agilent 7683 autosampler (Agilent, Santa Clara, CA, USA) with the splitless mode. In the case of the calibration standards, 0.5 µL of solution was injected to avoid saturation. Analysis was performed by high-resolution gas chromatography – high-resolution mass spectrometry (HRGC-HRMS: Agilent 6890 series GC, Agilent, USA – Autospec Ultima, Waters, Milford, MA, USA) with selected ion monitoring mode. HRMS was performed in electron impact ionization mode at a resolution R of >10,000 (10% valley definition). The column used was a DB-5MS UI fused silica capillary column, 20 m, 0.18-mm i.d., 0.18-µm film thickness (J&W, Agilent, Santa Clara, CA, USA). Column temperature was maintained at 120 °C for a 1.5-min hold; increased to 200 °C at a rate of 15 °C/min, to 224 °C at a rate of 4 °C/min, and to 300 °C at a rate of 20 min °C/min; and finally maintained at 300 °C for 1 min (total operation time less than 17 min/run). The interface and ion-source temperature was 290 °C, and the carrier gas (helium) flow rate was 1 mL/min. The ionizing current was 800 µA, the ionizing energy was 38 eV, and the accelerating voltage was 8 kV. IUPAC numbers are used to represent PCB congeners throughout this manuscript. The tri- to deca- CB congeners #18, #28/31, #43/52/73, #74, #66/80, #90/101, #99, #105, #106/118, #110, #114, #123, #128, #138/158/163/164, #146, #153, #156, #157, #167, #170/190, #172/192, #177, #178, #180/193, #182/187, #183, #189, #194, #195, #196/203, #201, #206, and #209 were monitored. Congeners that were coeluted in family groups under GC conditions are shown separated by a slash (/) in the article. Fig. 1 shows chromatograms of the EC-5366 calibration standard. Some tetra- to hexa- CB congeners that were not candidates for quantification are included in the chromatograms.

A concentration conversion factor of 1.055 was applied to convert volume base to gravimetric base. The conventional analytical method was applied to 2-mL blood samples[18] and the results from the two techniques were compared.

Calibration

Calibration standards (EC-5366 CDC Calibration Solution; CIL) CS1 to CS4 were used at concentrations of 0.2, 0.5, 1, and 2.5 ng/mL for calibration. Several congeners, namely #138 and #158 and #196 and #203, which coelute under GC conditions, were prepared at half concentrations.

Limit of detection and quantification

Instrumental detection limit (IDL) for the screening method was evaluated by using EC-5366 CDC PCB calibration solution CS1 (0.2 ng/mL; CIL). The IDL for each individual PCB congener was defined as three times the standard deviation (SD) of five injections on signal to noise (S/N) ratio. The instrumental quantification limit (IQL) was calculated at 10 times the SD.

MDL and MQL were also evaluated. EC-5433 comprehensive native PCB mixture (CIL) was used for testing. Each congener was added at an absolute amount of 0.1 pg to blank paper

Table 1 IDL, IQL, MDL, and MQL values and detection levels (median and maximum) in 10 blood

Homologue	Congener	IDL (pg/g-blood)	IQL (pg/g-blood)	MDL (pg/g-blood)	MQL (pg/g-blood)	Median* (pg/g-blood)	Max* (pg/g-blood)
Tri-CB	#18	7.8	26	10	33	<MDL	<MDL
	#28/31	5.9	20	8.4	28	(8.9)	(25)
Tetra-CB	#43/52/73	3.4	11	8.1	27	(9.8)	(24)
	#74	6.2	21	8.7	29	(10)	(24)
	#66/80	5.6	19	8.8	29	<MDL	(18)
Penta-CB	#90/101	6.2	21	7.4	25	<MDL	(19)
	#99	7.1	24	18	59	<MDL	(28)
	#110	7.5	25	17	57	<MDL	<MDL
	#123	7.9	26	15	49	<MDL	(32)
	#108/118	4.5	15	11	35	(17)	38
	#114	6.4	21	14	48	<MDL	<MDL
	#105	3.9	13	14	47	<MDL	<MDL
Hexa-CB	#146	6.7	22	-	-	(10)	(30)
	#153	5.6	19	10	34	71	230
	#138/158/163/164	6.0	20	9.3	31	49	140
	#128	6.1	20	8.6	29	<MDL	(9.6)
	#167	6.9	23	8.9	30	<MDL	<MDL
	#156	7.7	26	15	49	<MDL	(21)
	#157	8.2	27	8.9	30	<MDL	<MDL
Hepta-CB	#178	4.4	15	11	37	<MDL	(13)
	#182/187	10	35	13	44	(25)	63
	#183	7.5	25	-	-	<IDL	(23)
	#177	7.5	25	-	-	<IDL	(12)
	#172/192	7.7	26	-	-	<IDL	(54)
	#180/193	8.0	27	8.1	27	51	140
	#170/190	5.7	19	10	34	(17)	55
	#189	8.1	27	10	35	<MDL	<MDL
Octa-CB	#201	4.4	15	-	-	(15)	(49)
	#196/203	4.4	15	14	46	<MDL	(39)
	#195	6.5	22	12	41	<MDL	45
	#194	8.5	28	14	46	<MDL	(41)
Nona-CB	#206	4.3	14	7.6	25	<MDL	(22)
Deca-CB	#209	3.3	11	8.5	28	<MDL	90

* Values in parentheses are provided for reference use only

filter. After preparation, samples were analyzed in septuplicate. The MDL and MQL were calculated in the same way as the IDL and IQL, respectively.

Statistical analysis

Comparison between the conventional and screening method was calculated with Deming regression for two independent methods. We calculated about detectable three peaks (#153, #138/158/163/164, and #180/193), individually. Agreement was considered to be good if the 95% confidence interval (CI) of the intercept and slope from the Deming regression included 0 and 1, respectively. The analyses were performed with the software package SAS R9.3 (SAS Institute Japan Ltd.).

The correlation of total tri- to deca-CB concentration values obtained by the conventional method relative to sum of the detectable three peaks values obtained by the screening method was compared using Pearson's correlation analysis. The test was performed with SPSS 11.0J for Windows (SPSS Japan Inc.).

Results and discussion

Calibration and linearity

All compounds showed linearity in the range between 0.2 and 2.5 ng/mL, with regression coefficients ranging between 0.9952 and 1.0000.

Limit of detection and quantification and sample current values

Each limit value was calculated by using definitions based on the SD of the S/N ratio. Table 1 shows the IDL, IQL, MDL, and MQL values and the levels detected (median and maximum) in 10 blood samples. The rates of recovery of isotope-labeled compounds ranged from 89% to 125%. Detection values in parentheses in the table are between the MDL and the MQL and are provided for reference only. Units were converted to pg/g-blood.

Table 2 Congener levels in untreated Whatman-903 filter paper blanks (10 sheets each out of three lots)

Homologue	Congener	Filter Paper Lot W111			Filter Paper Lot W113			Filter Paper Lot W121		
		AV* (pg/g-blood)	SD (pg/g-blood)	%RSD	AV* (pg/g-blood)	SD (pg/g-blood)	%RSD	AV* (pg/g-blood)	SD (pg/g-blood)	%RSD
Tri-CB	#28/31	40	25	64	56	26	47	49	13	27
Tetra-CB	#43/52/73	(25)	16	64	36	15	42	(15)	2.2	15
	#66/80	(9.8)	2.6	26	(9.9)	3.0	31	(13)	2.8	22
Penta-CB	#90/101	(8.6)	6.4	75	(11)	8.1	73	<MDL	-	-

* Values in parentheses are provided for reference use only

The estimated IDL ranged from 3.3 to 10 pg/g-blood and the IQL ranged from 11 to 35 pg/g-blood. The estimated MDL ranged from 7.4 to 18 pg/g-blood, and the MQL ranged from 25 to 59 pg/g-blood. As the results of screening method, the sensitivities were comparatively about 5 to 12 times lower than the conventional method. In our analysis of a total of 10 blood samples and one SRM, the main detectable congeners that exceeded the MQL were #153 (eight out of 10 samples and the SRM), #138/158/163/164 (seven samples and the SRM), and #180/193 (eight samples and the SRM). Moreover, #170/190 (three samples) and #182/187 and #209 (one sample each) were detected. The detection levels of the other congeners were generally below the MQL.

Filter paper background

DBS analysis requires specific care to control the blank in sample preparation[15]. Blanks, as well as contamination levels on the treated Whatman-903 filter paper, were evaluated by using 10 sheets for each out of three lots. Detectable levels of some tri- to penta-CB congeners were found individually in the absence of any treatment (Table 2). The estimated levels detected ranged widely and depended to a large extent on the production lot. To test further for PCB background levels, we washed the punched-out filter paper and extracted the solvent by liquid-liquid extraction for 10 min before sample preparation. After washing-out of the solvent, most of the PCB congeners fell to negligible levels, the exception being #28/31. The levels of any persistent congeners generally ranged between the MDL and the MQL. Complete elimination of congeners from the blanks is difficult; filter paper washing enabled only a substantial decrease in background levels.

Table 3 Comparison of the values of three peaks, as measured by using the two methods, with certified NIST SRM values

Congener	Conventional method AV ± SD (pg/g-serum)	Screening method AV ± SD (pg/g-serum)	NIST certified values[a] (pg/g-serum)
#153	55±4.5	69±8.4	58.2±0.9
#138/158/163/164	41±4.7	46±7.5	(36.9±9.0)[b]
#180/193	43±3.2	49±10	54.5±0.5

[a] Weighted mean of mass fractions ± expanded uncertainty about the mean, with coverage factor, k = 2

[b] Value is shown for #138 only

Comparison between the screening and conventional methods

Total tri- to deca- CBs levels ranged from 34 to 890 pg/g-blood (median 410), as analyzed by using the conventional method. PCBs #153, #138/158/163/164, and #180/193, which are generally the most abundant congeners in human tissues, accounted for 29% to 49% of the total. Detection levels were relatively low and demonstrated a tendency to be related to the pattern of composition[2,3,5,12,15,18]. Judging from these results and the quantification limits, this new screening method is applicable to PCB screening in human blood.

Fig. 2 shows typical chromatograms of tri- to deca-CBs in 3 µL of blood, as determined by using the new screening method. Fig. 3 shows the comparison between the conventional and screening method of three peaks. Congener concentrations below the MQL were not included in the regression analysis. In eight out of 10 samples the concentration of #153, comparison of the two measurements yielded the following Deming regression equation: y = -6.93+0.95x (95% CI -23.1 to 9.33). Seven out of 10 samples in the case of #138/158/163/164: y = -15.8+1.15x (95% CI -35.0 to 3.26). Eight out of 10 samples in the case of #180/193: y = -8.06+1.01x (95% CI -18.4 to 2.28). While there is an agreement between two methods, the results estimated by using the new method tended to be higher than those with the conventional method. The main cause of this trend may have been related to the chromatographic separation or the presence of background levels of congeners.

Fig. 4 shows the relationship between the sum of concentrations of the three peaks (as determined by using the new screening method) and the total tri- to deca-CBs concentration (as determined by using the conventional method) in human blood. The sum determined by using the new method ranged from 42% to 72% of the total detected by using the conventional method. This range may have occurred because of a difference in limit values between the two methods. With the conventional method, congeners undetectable by the screening method contribute to the total concentrations. This trend is marked in the case of low concentrations. While a correlation coefficient (r = 0.9625, P < 0.001) was observed between the sum of concentrations of

the three peaks and the total PCB concentrations, we need to examine on additional equivalence study.

Comparison of SRM values between the two methods

Table 3 compares SRM values, as analyzed by using the two different methods, and the NIST certified values. Analysis of #153 by the screening method gave higher concentrations than the NIST certified value or the value determined by the conventional method. This trend may have been due to factors such as the coelution of other PCB fragments. #138 was coeluted with #158, #163, and #164. In the analysis using the conventional method, #138 accounted for 75% of the total level of the family of four mixed congeners. The difference between this result and the NIST certified value was not significant. This result also corresponded to the value obtained by using the screening method. In the case of #180/193, both results were lower than the NIST certified value, but the difference between the screening method values and the NIST values was within 10%. The results for these congener peaks indicated that the screening method values were reasonable, within a limit of accuracy of about 20%.

Conclusions

We validated a method that we developed for PCB screening using 3 µL of blood by HRGC-HRMS. The values determined by using this method can be applied for screening by analyzing three predominant PCB peaks, namely #153, #138/158/163/164, and #180/193. Additionally, specific PCB exposure patterns may be identifiable in the monitoring of tri- to deca-CBs[25–28]. In this case, we detected only a few peaks with levels exceeding the MQL, namely #118, #182/187, #195, and #209. Occasionally, as has been reported in one study of schools[29], it is necessary to monitor PCB congeners such as 28/31, #52, and #101 to catch specific exposures. While we need to examine on additional study, the blood volume will be sufficiently pick out the common thread in such specific exposures without false negative. This method will be useful for increasing the number of samples tested and will enable high-throughput screening with minimal sample volumes and analysis times. Furthermore, our results suggest that this screening method will be applicable to real DBS samples or other persistent organic pollutants (POPs) candidates. However, because of the high detection limits and the difficulty in determining the exact volumes of blood in DBSs, further research will be required in future to fine-tune the method for successful use in bio-monitoring studies.

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Notes and references

1 Schecter A, Wallace D, Pavuk M, Piskac A, Päpke O, *J Toxicol Environ Health A*, 2002, **65**, 1937-1943.
2 Mori C, Kakuta K, Matsuno Y, Todaka E, Watanabe M, Hanazato M, Kawashiro Y, Fukata H, *Environ Sci Pollut Res Int.*, 2014, **21**, 6434-6469.
3 Tatsuta N, Nakai K, Murata K, Suzuki K, Iwai-Shimada M, Kurokawa N, Hosokawa T, Satoh H, *Environ Res.*, 2014, **133**, 321-326.
4 Schantz S. L, Widholm J. J, Rice D.C, *Environ Health Perspect.*, 2003, **111**, 357-576.
5 Jusko T. A, Sisto R, Iosif A. M, Moleti A, Wimmerová S, Lancz K, Tihányi J, Sovčiková E, Drobná B, Palkovičová L, Jurečková D, Thevenet-Morrison K, Verner M. A, Sonneborn D, Hertz-Picciotto I, Trnovec T, *Environ Health Perspect.*, 2014, **122**, 1246-1252.
6 Agay-Shay K, Martinez D, Valvi D, Garcia-Esteban R, Basagaña X, Robinson O, Casas M, Sunyer J, Vrijheid M, *Environ Health Perspect.*, 2015, **123**, 1030-1037.
7 Ulutaş O. K, Çok İ, Darendeliler F, Aydin B, Çoban A, Henkelmann B, Schramm K. W, *Environ Sci Pollut Res Int.*, 2015, **22**, 19896-19904.
8 Caspersen I. H, Kvalem H. E, Haugen M, Brantsæter A. L, Meltzer H M, Alexander J, Thomsen C, Frøshaug M, Bremnes N. M, Broadwell S. L, Granum B, Kogevinas M, Knutsen H. K, *Environ Res.*, 2016, **146**, 136-144.
9 Kawamoto T, Nitta H, Murata K, Toda E, Tsukamoto N, Hasegawa M, Yamagata Z, Kayama F, Kishi R, Ohya Y, Saito H, Sago H, Okuyama M, Ogata T, Yokoya S, Koresawa Y, Shibata Y, Nakayama S, Michikawa T, Takeuchi A, Satoh H, Working Group of the Epidemiological Research for Children's Environmental Health, *BMC Public Health.*, 2014, **14**, 25.
10 Kato K, Wanigatunga A. A, Needham L. L, Calafat A. M, *Anal Chim Acta.*, 2009, **656**, 51-55.
11 Patterson D. G. Jr, Welch S. M, Turner W. E, Sjödin A, Focant J. F, *J Chromatogr A*, 2011, **1218**, 3274-3281.
12 Lu D, Wang D, Ip HS, Barley F, Ramage R, She J, *J Chromatogr B Analyt Technol Biomed Life Sci.*, 2012, **891-892**, 36-43.
13 Ma W, Kannan K, Wu Q, Bell E. M, Druschel C. M, Caggana M, Aldous K. M, *Anal Bioanal Chem.*, 2013, **405**, 4127-4138.
14 Ma W, Gao C, Bell E. M, Druschel C. M, Caggana M, Aldous K. M, Louis G. M, Kannan K, *Environ Res.*, 2014, **133**, 204-210.
15 Batterman S, Chernyak S, *Sci Total Environ.*, 2014, **494-495**, 252-260.
16 Mimura, K., Tamura, M., Haraguchi, K., Masuda, Y., *Fukuoka Igaku Zasshi*, 1999, **90**, 202-209.
17 Masuzaki Y, Matsumura T, Morita M, Ito H, *Journal of Environmental Chemistry 12th Symposium on Environmental Chemistry Programs and Abstracts*, 2003, 42-43. (in Japanese).
18 Hirai T, Fujimine Y, Watanabe S, Nakano T, *Environ Geochem Health*, 2005, **27**, 65-73.
19 Satoh H, Nakai K, Matsumura T, Sasai S, Nakamura Y, Inaba Y, *30th international Symposium on Halogenated Persistent Organic Pollutants (DIOXIN2010)*, 2010, No.1622.
20 Adam B. W, Alexander J. R, Smith S. J, Chace D. H, Loeber J. G, Elvers L. H, Hannon W. H, *Clin Chem.*, 2000, **46**, 126-128.
21 Spliethoff H. M, Tao L, Shaver S. M, Aldous K. M, Pass K. A, Kannan K, Eadon G. A, *Environ Sci Technol.*, 2008. **42**, 5361-5367.
22 Mei J. V, Alexander J. R, Adam B. W, Hannon W. H, *J Nutr.*, 2001, **131**, 1631S-1636S.
23 Ramos J. J, Gómara B, Fernández M. A, González M. J, *J Chromatogr A.*, 2007, **1152**, 124-129.
24 Dasheng Lu, Dongli Wang, Ho Sai Simon Ip, Frank Barley, Robert Ramage, Jianwen She, *J. of Chromatography B.*, 2012, **891-892**, 36-43.

25 DeCaprio A. P, Johnson G. W, Tarbell A. M, Carpenter D. O, Chiarenzelli J. R, Morse G. S, Santiago-Rivera A. L, Schymura M. J, Akwesasne Task Force on the Environment, *Environ Res*., 2005, **98**, 284-302.
26 Wingfors H, Seldén A. I, Nilsson C, Haglund P, *Ann Occup Hyg.*, 2006, **50**, 65-73.
27 Fitzgerald E. F, Hwang S. A, Gomez M, Bush B, Yang B. Z, Tarbell A, *J Expo Sci Environ Epidemiol*., 2006, **17**, 269-78.
28 Hwang S. A, Yang B. Z, Fitzgerald E. F, Bush B, Cook K, *J Expo Anal Environ Epidemiol*, 2001, **11**, 184-92.
29 Gabrio T, Piechotowski I, Wallenhorst T, Klett M, Cott L, Friebel P, Link B, Schwenk M, *Chemosphere*, 2000, **40**, 1055-1062.

Figure Captions:

Fig. 1 Chromatograms of the EC-5366 calibration standard

Fig. 2 Typical chromatograms of tri- to deca-CBs in 3 µL of blood, as determined by using the new screening method

Fig. 3 Comparison between the conventional and screening method of three peaks

Fig. 4 Relationship between the sum of the levels of three peaks in human blood (see Fig. 3), as obtained by using the new screening method, and the total tri- to deca-CBs concentrations obtained by using the conventional method

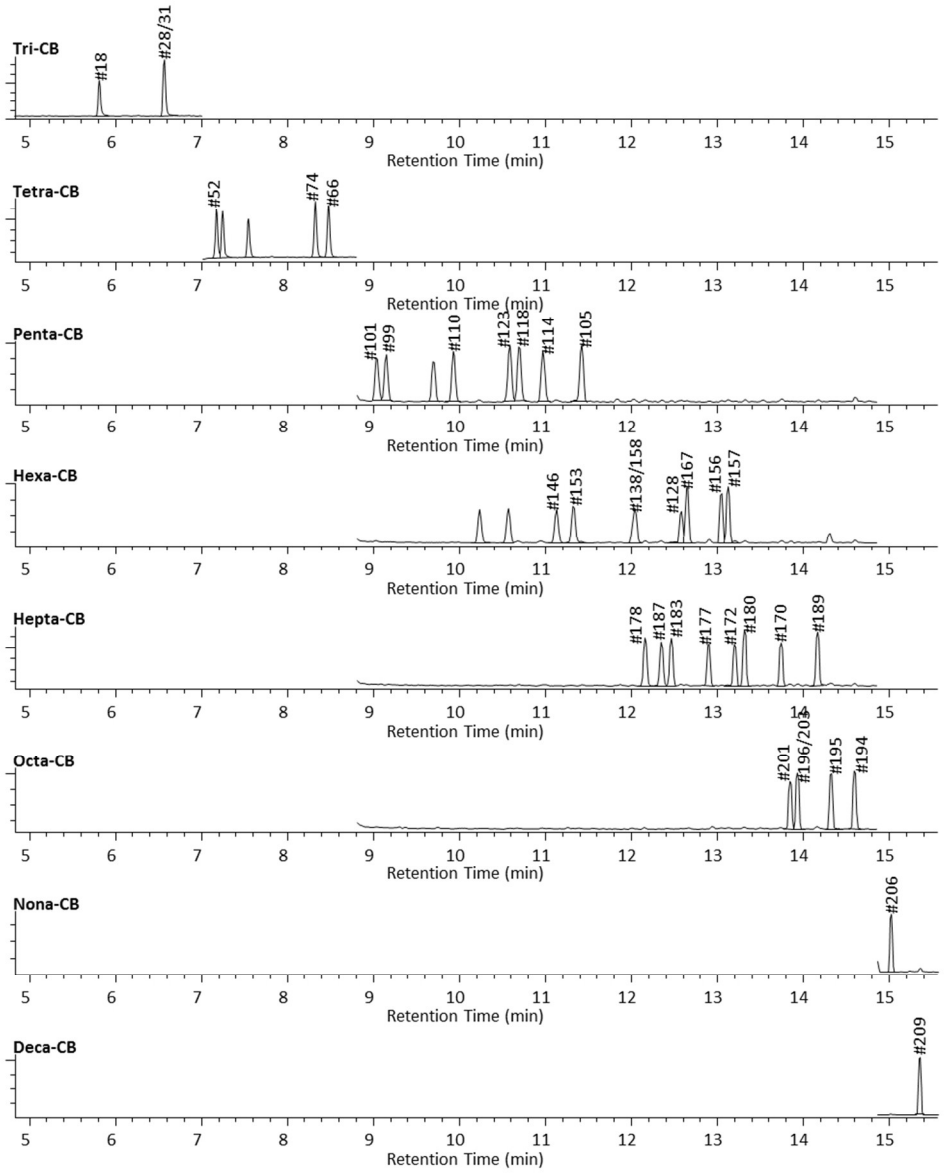

Fig. 1 Chromatograms of the EC-5366 calibration standard

170x210mm (150 x 150 DPI)

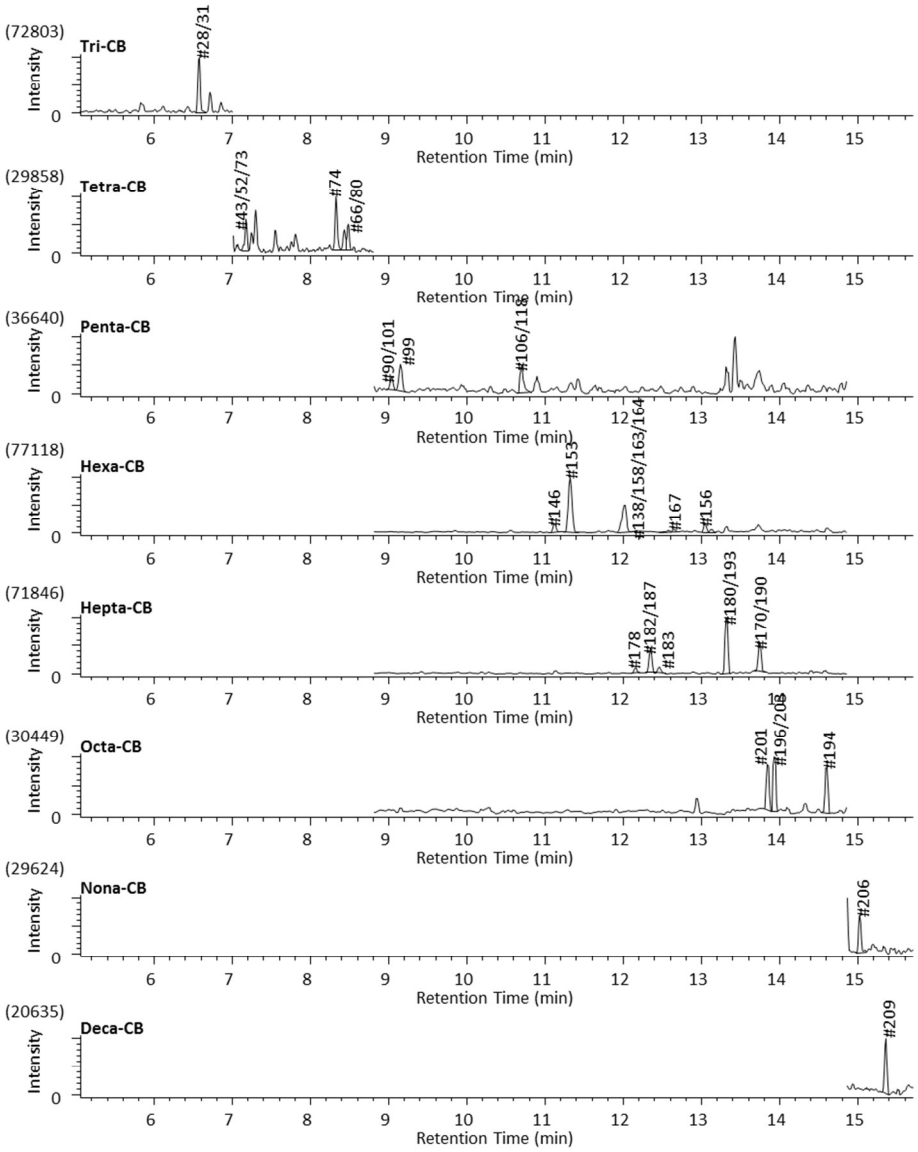

Fig. 2 Typical chromatograms of tri- to deca-CBs in 3 μL of blood, as determined by using the new screening method

170x210mm (150 x 150 DPI)

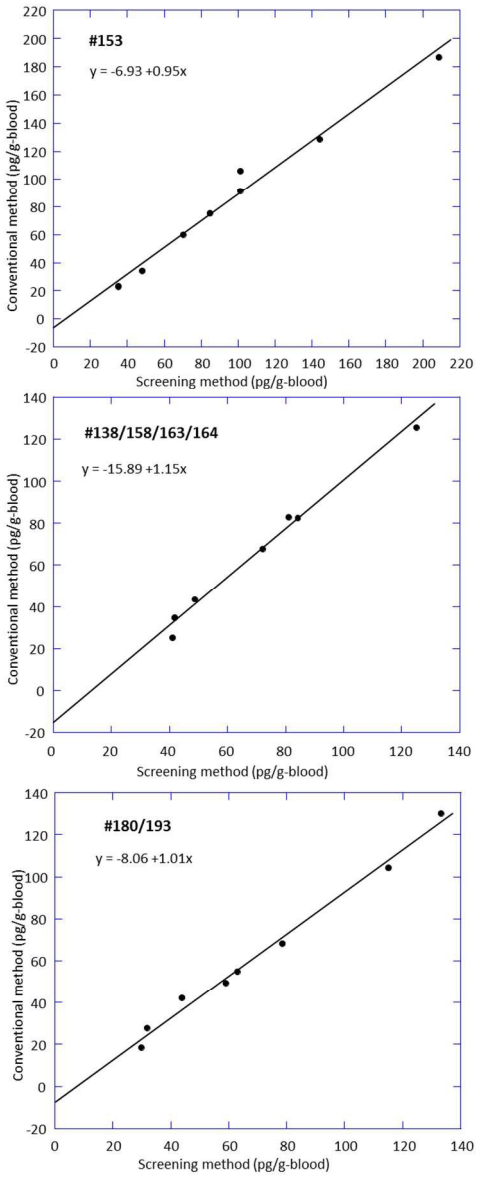

Fig. 3 Comparison between the conventional and screening method of three peaks

119x281mm (150 x 150 DPI)

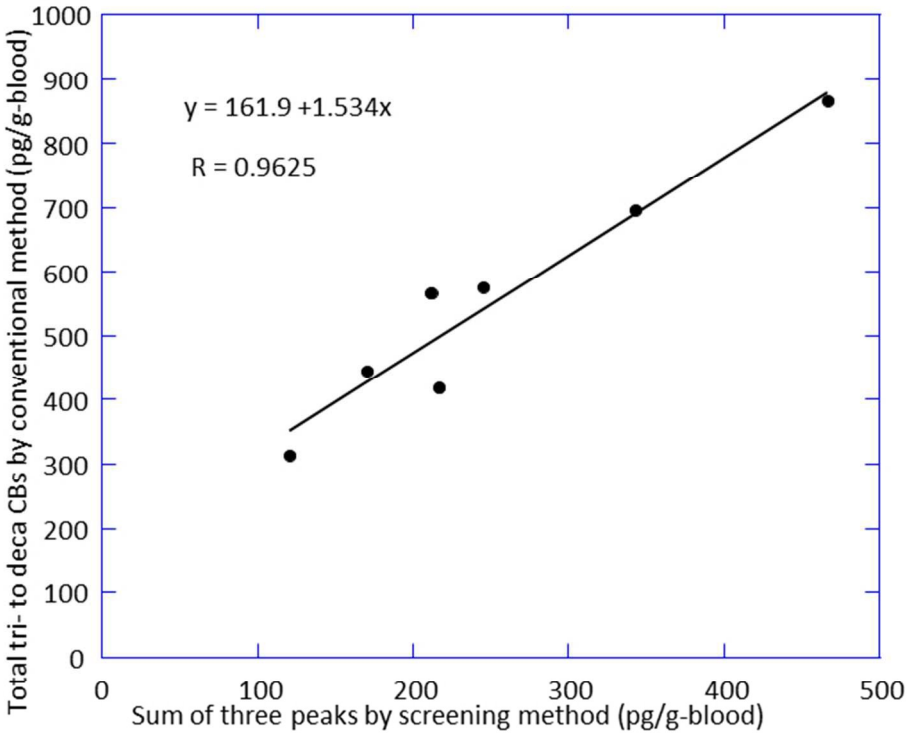

Fig. 4 Relationship between the sum of the levels of three peaks in human blood (see Fig. 3), as obtained by using the new screening method, and the total tri- to deca-CBs concentrations obtained by using the conventional method

122x98mm (150 x 150 DPI)