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Simultaneous detection of multiple hydroxylated polychlorinated biphenyls from a complex tissue matrix using gas chromatography/isotope dilution mass spectrometry

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ABSTRACT

In this study, we developed a comprehensive, highly sensitive, and robust method for determining 53 congeners of three to eight chlorinated OH-PCBs in liver and brain samples by using isotope dilution gas chromatography (GC) coupled with electron capture negative ionization mass spectrometry (ECNI-MS). These results were compared with those from GC coupled with electron ionization high-resolution mass spectrometry (EI-HRMS). Clean-up procedures for analysis of OH-PCBs homologs in liver and brain samples involve a pretreatment step consisting of acetonitrile partition and 5% hydrated silica-gel chromatography before derivatization. Recovery rates of tri- and tetra-chlorinated OH-PCBs in the acetonitrile partition method followed by the 5% hydrated silica-gel column (82% and 91%) were higher than conventional sulfuric acid treatment (2.0% and 3.5%). The method detection limits of OH-PCBs for each matrix obtained by GC/ECNI-MS and GC/EI-HRMS were 0.58–2.6 pg g^{-1} and 0.36–1.6 pg g^{-1} wet wt, respectively. Recovery rates of OH-PCB congeners in spike tests using sample matrices (10 and 50 pg) were 64.7–117% (CV: 4.7–14%) and 70.4–120% (CV: 2.3–12%), respectively. This analytical method may enable the simultaneous detection of various OH-PCBs from complex tissue matrices. Furthermore, this method allows more comprehensive assessment of the biological effects of OH-PCB exposure on critical organs.

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of chlorinated hydrocarbons. They have a diverse range of applications in various materials, such as electrical equipment, paint, and carbon paper. The production and use of PCBs was restricted or banned globally due to their persistent, bioaccumulative, and toxic properties [1].

PCBs are formed by oxidative metabolism by the cytochrome P450 (CYP450) mono-oxygenases enzyme system in the liver [2]. The metabolism of PCBs results in the formation of a relatively large number of hydroxylated PCB congeners (OH-PCBs), which might be associated with disrupted thyroid homeostasis and neurodevelopmental deficits [3], [4–6]. Theoretically, there are 837 mono-hydroxylated PCB congeners with a substitution of 1–9 chlorine atoms, and each congener could have a specific toxicological effect [7], [8]. It has been proposed that the mechanism involved in the disruption of thyroid hormone (TH) homeostasis is the competitive binding of OH-PCBs with transthyretin (TTR), the TH transport protein, in blood [3,9]. It has been demonstrated that the binding affinity of OH-PCBs to TTR is much stronger than that of the parent PCBs [10]. TH plays critical roles in the development of the central nervous system and brain functions [11]. A recent study using reporter gene assays demonstrated that extremely low doses of OH-PCBs (i.e., 10^{-10} Mol) suppress the 3,5,5′-triiodothyronine (T3)-induced transcriptional activation of the TH receptor [12]. These studies indicate that the brain, which is commonly the target tissue for OH-PCBs, and the liver which plays a major role in the metabolism of PCBs, are suitable for understanding the toxicity and kinetics of OH-PCBs.

OH-PCB concentrations and distributions in human serum and wildlife blood have previously been investigated [13–20]. Although OH-PCBs have been detected in the blood of several wildlife species such as marine mammals and birds [15–18,21], detailed information regarding OH-PCBs levels in the animal brain and liver are still scarce. In addition, these studies on wildlife investigated only a small number of OH-PCB congeners (mainly penta- to octa-chlorinated OH-PCB congeners) or were limited to global data as opposed to

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individual congener information. Recently, species-specific accumulation data, such as data of tri- to penta-chlorinated OH-PCBs in the blood of dolphins [18], octa-chlorinated OH-PCBs in polar bear (*Ursus maritimus*) plasma [22], and hepta- to octa-chlorinated OH-PCBs in terrestrial mammal blood [20] have been reported. These studies suggested that the levels and profiles of OH-PCBs in animal blood vary by species and that several animals may be at a risk from these metabolites including congeners which are not found in human are present. However, because of difficulties in detecting low-chlorinated (e.g., 3–4 chlorine atoms) OH-PCBs in biological tissues, comprehensive investigations on the levels of various OH-PCB homologs in the brain and liver are still limited [23,24], In fact, because tri- and tetra-chlorinated OH-PCBs are unstable and characterized by low recovery rates, conventional pretreatment procedures (e.g., sulfuric acid treatment or multilayer silica-gel column) [15,23,24] cannot be used for their analysis. Thus, to analyze lower-chlorinated OH-PCBs, mild pretreatment methods before derivatization are required. In this study, we tested an acetonitrile partition method coupled to a deactivated silica-gel column as pretreatment before derivatization and compared this new method with conventional sulfuric acid treatment.

To perform this analysis, a high degree of separation of OH-PCBs from the complex environmental matrix is required and the chosen method must have high selectivity, sensitivity, and precision. To meet these requirements, many previous environmental OH-PCBs studies utilized a gas chromatography (GC)-electron capture detector (ECD) [25]. Other studies used GC/electron ionization high-resolution mass spectrometry (GC/EI-HRMS) [18,26] GC/electron capture negative ionization mass spectrometry (GC/ECNI-MS) [5,15,27] or liquid chromatography/mass spectrometry (LC/MS) [26,28]. Although LC/MS is characterized by high sensitivity and selectivity, its resolution might not be adequate to separate many OH-PCB congeners. Although GC-ECD is a sensitive technique and is easy to handle, a complete purification of the samples prior to the chromatographic separation of individual substances is needed because the internal standards cannot be spiked. Although GC/HRMS can provide highly sensitive and selectively, the instrumentation is too expensive for conducting routine or high-throughput analyses and its operation requires specialized technical skills. In contrast, GC/ECNI-MS is a sensitive and selective instrument particularly suitable for the analysis of halogenated compounds. The ECNI mode allows the use of $^{13}C_{12}$-labeled internal standards for a more precise determination of the target compounds. So far, it has been used for the analysis of numerous contaminants, including OH-PCBs, in various environmental matrices [5,15,27]. Nevertheless, the effectiveness of GC/ECNI-MS for the analysis of tri- to tetra-chlorinated OH-PCBs has not been clarified yet and analytical methods using GC/ECNI-MS and the GC/EI-HRMS were not compared for complex biological samples.

In this study, we developed a comprehensive, highly sensitive, and robust method for determining OH-PCBs, including various homologs (3–8 chlorine atoms), in liver and brain samples by using isotope dilution GC/ECNI-MS and/or GC/EI-HRMS. The current method incorporates several modifications and improvements for a more sensitive and selective analysis of a wide range of OH-PCB homologs in complex biological tissues.

2. Materials and methods

2.1. Chemicals and reagents

Fifty-three OH-PCBs (tri- to octa-, methoxylated derivatives: MeO-PCBs) isomers were used for identification and quantification. Thirteen compounds were synthesized by thermal diazo-coupling between a chlorophenol and a chloroaniline diazonium salt [29,30], 8 compounds were obtained from AccuStandard, Inc. (New Haven, CT), and 31 from Wellington Laboratories Inc. (Guelph, ON, Canada) (Table S1).

Dichloromethane (DCM), *n*-hexane, methanol, ethanol, methyl *tertiary*-butyl ether (MTBE), decane, and silica gel (Wako-gel S1) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Trimethylsilyldiazomethane (TMSDM) for derivatization was supplied by Tokyo Chemical Industry (Tokyo, Japan). Anhydrous sodium sulfate (purity > 99%) and acetone were obtained from Nacalai Tesque (Kyoto, Japan). Silica gel was baked at 130 °C for 3 h prior to use. Five percent hydrated silica gel (5% H_2O deactivated) was prepared by slowly adding an appropriate amount of Milli-Q water (Millipore Corp., Bedford, MA) to activated silica at room temperature.

2.2. Sample collection

Brain samples of finless porpoise (*Neophocaena phocaenoides*) carcasses stranded or caught during 2005–2010 along the Japanese coast were transported and stored at −25 °C at local universities and aquariums until biometric measurements and dissections were conducted [18]. Liver samples of Baikal seals (*Phoca sibirica*) were collected from Lake Baikal in 2005 [31]. Permission was obtained from the Lake Baikal Basin Committee for Protection, Reproduction of Fish Resources and Fishing Control (known by its Russian acronym BAIKALRYBVOD) under the annual seal culling quota. The animals were shot and immediately dissected. The brain and liver samples were stored frozen in the environmental specimen bank (*es*-BANK) of Ehime University, Matsuyama, Japan, at −25 °C [32].

2.3. Sample preparation

The OH-PCB extraction procedure used in this study is similar to that described in a previous report [18]. $^{13}C_{12}$-labeled OH-PCBs (1 ng of each 4′OH-CB29, 4′OH-CB61, 4′OH-CB79, 4OH-CB107, 4′OH-CB120, 4′OH-CB159, 4′OH-CB172, and 4OH-CB187) were spiked as surrogate internal standards. The liver and brain samples (2.5 g) were denatured with 3 mL of 6 M hydrochloric acid. After adding 2-propanol (9 mL), the target compounds were extracted thrice with 50% methyl *t*-butyl ether (MTBE)/hexane by a homogenizer (11,000 rpm, 10 min) (POLYTRON PT 2100: Kinematica, Luzernerstrasse, Switzerland). After centrifugation, the organic phases were combined and washed with 5% NaCl prepared in hexane-washed water. The resulting organic phase was evaporated by a rotary evaporator and re-dissolved in hexane. Potassium hydroxide (KOH; 1 M) in 50% ethanol/water (20 mL) was added and the solution was shaken to ensure mixing. The partition step of the neutral and phenolic fractions (alkaline phases) was repeated and the alkaline phases were combined.

In the next step, the KOH solution phase containing OH-PCBs was acidified (pH 2) with sulfuric acid. Then, OH-PCBs were extracted twice with 50% MTBE/hexane (60 mL). The phases were separated, and the organic phase was combined and evaporated by a rotary evaporator. A mixture of acetonitrile:hexane (1:1 v/v; 10 mL) was later added and shaken. Again, the partition step of the neutral and phenolic fractions was repeated, and the alkaline phases were combined. To the acetonitrile solution phase containing OH-PCBs was added 100 mL of hexane-washed water, and the solution was acidified to pH 2 with sulfuric acid. Then, OH-PCBs were again extracted twice with 50% MTBE/hexane (60 mL). Subsequently, the phases were separated, and the organic phase was combined and evaporated by a rotary evaporator.

The solvent-evaporated residue was dissolved in hexane and passed through a glass column packed with 3 g hydrated silica gel (Wako-gel S-1, 5% H_2O deactivated). The OH-PCBs fraction was eluted with 50% DCM/hexane (100 mL), concentrated, and dissolved in hexane (1 mL). Each treated analyte in hexane was

derivatized (methylation; overnight at 20 °C) using methanol and trimethylsilyldiazomethane (Tokyo Chemical Industry, Tokyo, Japan). The derivatized solution was treated by gel permeation chromatography (GPC) using a column packed with 50 g of Bio-Beads S-X3 (Bio-Rad Laboratories, Richmond, CA). Fifty percent DCM/hexane was used as the mobile phase at a flow rate of 5 mL min^{-1}. The derivatized OH-PCB solution was passed through 3 g of activated silica gel packed in a glass column. The methoxylated PCBs (MeO-PCBs) fraction was eluted with 140 mL of 10% DCM/hexane and concentrated to near dryness. Then, $^{13}C_{12}$-labeled CB77 and CB157 (1 ng g^{-1}) dissolved in up to 50 μL of decane were injected as surrogates for the GC/MS analysis.

2.4. Comparison of the recovery rates of OH-PCBs from sulfuric acid treatment and deactivated silica-gel column

After the extraction and partition procedures, we compared the recovery rates of $^{13}C_{12}$-labeled OH-PCB congeners obtained using a 5% hydrated silica-gel column ($n=5$) and sulfuric acid treatment ($n=5$) before the derivatization procedure.

$^{13}C_{12}$-labeled OH-PCBs (1 ng of each 4′OH-CB29, 4′OH-CB61, 4′OH-CB79, 4OH-CB107, 4′OH-CB120, 4′OH-CB159, 4′OH-CB172, and 4OH-CB187) were spiked as surrogate internal standards to 1 ml of hexane. The solvent passed through a glass column packed with 3 g hydrated silica gel (Wako-gel S-1, 5% H_2O deactivated). The analytes were eluted with a 50% DCM/hexane mixture (100 mL), concentrated, and dissolved in hexane (1 mL).

Part of the solvent used to test the clean-up efficiency of the sulfuric acid treatment method. Sulfuric acid (98%, 3 mL) was added to the evaporated residue after the KOH partition procedure. After shaking, the supernatant was washed with hexane-washed water and dissolved in hexane (1 mL).

After each treated analyte in hexane was derivatized, silica-gel clean-up was performed and the samples were analyzed by GC/EI-HRMS.

2.5. GC column

In this study, the separation of OH-PCB congeners was performed by GC/MS using two capillary columns (i.e., DB5-MSUI (40 m × 0.18 mm ID × 0.18 μm film) for GC-ECNI/MS and DB-5 MS (60 m × 0.25 mm ID × 0.25 μm film) for GC-HRMS) from Agilent (Agilent Technologies Inc., CA, USA).

The column oven temperature program for the analysis of MeO-PCBs congeners with DB5-MSUI (40 m) and DB-5 MS (60 m) were shown in Table 1.

2.6. GC/ECNI-MS conditions

The parameters used for the GC/ECNI-MS are shown in Table 1. MeO-PCBs analysis was run on a Shimadzu QP2010 ultra quadrupole mass spectrometer equipped with a Shimadzu GC2010 plus gas chromatograph and an AOC-20i autosampler (Shimadzu Inc., Kyoto, Japan). GC separation was achieved using a DB5-MSUI fused silica capillary column (40 m × 0.18 mm ID × 0.18 μm film) (Agilent Technologies Inc, Tokyo, Japan). The identification and quantification of the 53 native MeO-PCB congeners were achieved by monitoring the four most intense ions [selected ion monitoring (SIM) analysis] of the molecular ion cluster ($[M]^-$, $[M+2]^-$, $[Cl]^-$ and $[M\text{-}CH_3]^-$) with the ECNI-MS detector (Table S1).

2.7. GC/EI-HRMS conditions

The parameters used in this study for GC/EI-HRMS are similar to those previously described (Table 1) [17,18]. To compare the sensitivity of different analysis methods, MeO-PCBs were also analyzed using an MS-800D high-resolution mass spectrometer (JEOL, Tokyo, Japan) coupled with an Agilent 6890 gas chromatograph and a 7683B autosampler (Agilent Technologies Inc, Tokyo, Japan.). GC separation was achieved using a DB-5MS fused silica capillary column (60 m × 0.25 mm ID × 0.25 μm film) (Agilent Technologies Inc, Tokyo, Japan). The identification and quantification of the 53 native MeO-PCB isomers was achieved by SIM analysis of the molecular ion cluster ($[M]^+$, $[M+2]^+$, and $[M+2\text{-}COCH_3]^+$) with the EI-HRMS detector (Table S1).

2.8. Method validation

Multilevel calibration curves (1–100 μg L^{-1}) in the linear response interval of the detector were created for the quantification of OH-PCBs, and a good correlation ($R^2 > 0.95$–0.99) was achieved with both GC/ECNI-MS and GC/EI-HRMS. OH-PCBs were quantified using the isotope dilution method with the corresponding $^{13}C_{12}$-internal standards in all protocols. The recovery rates of $^{13}C_{12}$–OH-PCBs in the liver sample were as follows: 4′OH-CB29 (64–83%), 4′OH-CB61 (92–105%), 4′OH-CB79 (72–92%), 4′OH-CB107 (72–94%), 4′OH-CB120 (85–108%), 4′OH-CB159 (78–91%), 4′OH-CB172 (89–116%), and 4OH-CB187 (77–92%). The recovery rates of $^{13}C_{12}$-OH-PCBs in the brain sample were as follows: 4′OH-CB29 (71–86%), 4′OH-CB61 (83–99%), 4′OH-CB79 (69–81%), 4OH-CB107 (73–97%), 4′OH-CB120 (77–94%), 4′OH-CB159 (78–89%), 4′OH-CB172 (89–111%), and 4OH-CB187 (82–95%). The method repeatability was in the range 0.6–9.8% coefficient of variation (CV) for $^{13}C_{12}$-labeled OH-PCBs. The identification of the target analytes was based on the comparison of the relative retention times to the internal standards used for quantification, ion chromatograms, and intensity ratios of the monitored ions. The peaks with heights within 15% of the theoretical ratio of two reference ions and with a signal-to-noise (S/N) ratio greater than 10 were quantified as MeO-PCB isomers. Owing to the lack of an octa-chlorinated $^{13}C_{12}$–OH-PCB internal standard, octa-chlorinated OH-PCB isomers were quantified using $^{13}C_{12}$-labeled 4′OH-CB172. Procedural blanks were analyzed simultaneously with every batch of four samples to check for interferences or contamination from solvent and glassware. No peaks were detected in the chromatograms of the blank samples. The limit of quantification was defined as the amount of the target compound that resulted in an S/N of 10:1. When OH-PCBs (MeO-PCBs after methylation) were analyzed using the GC/MS scan mode, OH-PCB congeners were characterized by the distinctive fragment daughter ions $[M3COCH_3]^+$ and $[M3CH_3Cl]^+$.

The instrument detection limits (IDLs) were defined as three times the standard deviation (SD) of five replicate injections of a low concentration standard solution of OH-PCBs (5 pg). The method detection limits (MDLs) were calculated from S/N=3 in low-concentration samples.

3. Results and discussions

3.1. GC column selection

For the detection and separation of a wide range of OH-PCBs homologs, two types of GC capillary columns were tested, DB-5MSUI (40 m × 0.18 mm ID × 0.18 μm film) and DB-5MS (60 m × 0.25 mm ID × 0.25 μm film). The retention times of the first and the last OH-PCB congeners eluted in the 40 m and 60 m columns were 16.5 and 36.3 min and 8.84 and 22.5 min, respectively (Figure S1). Interestingly, the experimental time was reduced by nearly 15 min when the DB-5MSUI column was used.

Next, we established the relative retention times (RRTs) of the 53 OH-PCBs in the two capillary columns. RRTs were calculated

Table 1
Optimized GC/MS parameters.

Optimization parameters	GC/ECNI-MS	GC/EI-HRMS
Gas chromatography	Shimadzu GC-2010 plus	Agilent 6890N
Injection port temperature (°C)	300	300
Mass spectrometry	Shimadzu QP2010 Ultra	JMS-800D
Ion source temperature (°C)	200	250
Interface temperature (°C)	300	300
Ionization mode	ECNI	EI
Measurement mode	SIM	SIM
Filament emission (eV)	70	37
Column	DB5-MSUI (40 m × 0.18 mm ID × 0.18 μm film)	DB-5 MS (60 m × 0.25 mm ID × 0.25 μm film)
Temperature program	130 °C (1 min) −20 °C min^{-1} −180 °C − 2 °C min^{-1} −260 °C − 5 °C min^{-1} −300 °C (4 min)	130 °C (1 min) −20 °C min^{-1} −210 °C (1 min) − 2 °C min^{-1} −260 °C (1 min) − 5 °C min^{-1} −300 °C (10 min)
Resolution	Unit mass	10,000
Reagent gas	Methane	–

from the retention time of $^{13}C_{12}$-labeled 4OH-CB187 internal standard. The calculated RRTs of the OH-PCBs congeners in the tested columns are summarized in Table 2. The elution order of the OH-PCBs and the separation ability of the two columns were similar. For instance, the peaks of 4OH-CB146 and 3OH-CB153 were resolved, whereas 4OH-CB107/4′OH-CB108 and 4′OH-CB101/4′OH-CB120 were co-eluted in each column (Table 2). These results suggested that the experimental time could be reduced by using the shorter narrow bore column. However, not all the congeners were separated in these columns. A better separation of OH-PCBs is needed in the future for the identification and clarification of residue profiles of OH-PCBs.

3.2. Optimization of GC/ECNI-MS conditions

The optimized experimental parameters of GC/ECNI-MS are shown in Table 1. The injection port temperature of GC was increased from 180 to 300 °C. The highest peak abundance of the OH-PCB congeners was observed at an injection port temperature of 300 °C (Figure S2A). The peak abundance at lower temperatures (i.e., 240 °C) was about 10% less than that at higher temperature. The CV of the peak area for seven replicate injections improved from 10% at 240 °C to 5% at 300 °C. The ion source temperature was increased from 180 to 230 °C (Figure S2B). The reproducibility at 200 °C was good, as demonstrated by the CV values of 3.7 and 10% of the relative peak areas and sensitivity of the investigated OH-PCBs respectively. LODs for all OH-PCBs in GC/ECNI-MS and GC/EI-HRMS were 0.060–4.8 pg (tri-: 0.65–4.8, tetra-: 0.94–2.0, penta-: 0.10–0.80, hexa-: 0.060–0.63, hepta-: 0.080–0.33, and octa-chlorinated OH-PCBs; 0.088–0.16 pg) and 0.048–0.25 pg (tri-: 0.11–0.25, tetra-: 0.058–0.14, penta-: 0.070–0.17, hexa-: 0.048–0.12, hepta-: 0.065–0.11 and octa-chlorinated OH-PCBs: 0.11–0.15 pg), respectively (Table 2). The sensitivities of the GC/ECNI-MS and GC/EI-HRMS methods relative to penta- to octa-chlorinated OH-PCBs were comparable. However, concerning the tri- to tetra-chlorinated OH-PCBs, the performance of GC/ECNI-MS was lower than that of GC/EI-HRMS. Owing to the low intensity of the molecular ion of some tri- and tetra-chlorinated OH-PCBs, the baseline noise was relatively higher in GC/ECNI-MS than in GC/EI-HRMS. However, IDLs of lower chlorinated OH-PCBs were higher or comparable to those obtained with liquid chromatography/time-of-flight mass spectrometry (LC/ToF-MS) and a GC-ECD [25,26].

3.3. Recovery test and detection limit

The extraction procedure used in this study is similar to a method previously described for which good recovery rates were achieved [18]. The recovery data of the target analytes using the 5% hydrated silica-gel column and the sulfuric acid treatments before the derivatization procedure are summarized in Table 3. After the sulfuric acid treatment, the recovery rates of tri- and tetra-chlorinated OH-PCBs were insufficient (2.0–6.3%), indicating that these OH-PCBs were degraded by sulfuric acid. In contrast, using the acetonitrile partition method followed by the 5% hydrated silica-gel column, 82–102% of $^{13}C_{12}$-labeled OH-PCBs were recovered with CV values between 2.2 and 5.6%. These results suggest that acetonitrile partition and the 5% hydrated silica-gel column are effective in the purification and enrichment of OH-PCBs from brain and liver extracts before the derivatization procedure. It has been formerly reported that this chromatography step was also effective in blood analysis [18]. Based on these findings, the resulting protocol comprised acidification and supersonic wave extraction followed by KOH partition, acetonitrile partition, 5% hydrated silica-gel purification, TMSDM derivatization, GPC and silica-gel clean-up.

The method for analyzing OH-PCBs from the brain and liver was optimized by spiking $^{13}C_{12}$-OH-PCBs internal standards into the samples (2.5 g). Recoveries for the $^{13}C_{12}$-labeled internal standard in the brain samples were as follows: 4′OH-CB29 (71–86%), 4′OH-CB61 (83–99%), 4′OH-CB79 (69–81%), 4OH-CB107 (73–97%), 4′OH-CB120 (77–94%), 4′OH-CB159 (78–89%), 4′OH-CB172 (89–111%), and 4OH-CB187 (82–95%). The CV of the analyses executed in five replicates was between 4.2 and 8.2% for all the $^{13}C_{12}$-labeled OH-PCBs. In addition, recoveries for the $^{13}C_{12}$-labeled internal standard in the liver samples were as follows: 4′OH-CB29 (64–83%), 4′OH-CB61 (92–105%), 4′OH-CB79 (72–92%), 4OH-CB107 (72–94%), 4′OH-CB120 (85–108%), 4′OH-CB159 (78–91%), 4′OH-CB172 (89–116%), and 4OH-CB187 (77–92%). The CV of the analyses executed in five replicates was between 5.4 and 9.6% for all the $^{13}C_{12}$-labeled OH-PCBs. These results suggest that the optimized method provides excellent accuracy for the measurement of OH-PCBs in brain and liver tissues.

To determine the recovery rates before the samples were homogenized, two different concentrations (i.e., 10 pg, low dose; 50 pg, high dose) of 27 OH-PCBs congeners were spiked into 2.5 g of liver samples. The results are summarized in Table 4. The recovery rates of OH-PCB congeners in 10 and 50 pg spikes were 64.7–117% (CV: 4.7–14%) and 70.4–120% (CV: 2.3–12%), respectively. These results indicate that better extraction and clean-up efficiency was proved and isotope dilution methods could correct values.

MDLs of individual OH-PCBs in GC/ECNI-MS and GC/EI-HRMS were 0.58–2.6 pg g^{-1} and 0.36–1.6 pg g^{-1} wet wt for each matrix, respectively (Table 2). Owing to their high IDL values, MDLs of tri- to tetra-chlorinated OH-PCBs were relatively higher than those of penta- to octa-chlorinated OH-PCBs in GC/ECNI-MS. These results are in agreement with those reported in the

Table 2
OH-PCB congeners analyzed, relative retention times (RRTs), relative response factors (RRF), instrument detection limits (IDL, pg) and method detection limits (MDL, pg g^{-1}).

BZ no.	IUPAC	RRT[a] (60 m)	RRT (40 m)	IDL[b] (HRMS, pg)	IDL (ECNI-MS, pg)	MDL[c] (HRMS, pg g^{-1})	MDL (ECNI-MS, pg g^{-1})
4′OH-CB18	4OH-2,2′,5′-trichlorobipheyl	0.513	0.471	0.11	1.1	1.0	1.1
3′OH-CB31	3OH-2′,4,5′-trichlorobiphenyl	0.523	0.477	0.12	4.0	0.70	1.4
3′OH-CB28	3OH-2′,4,4′-trichlorobiphenyl	0.537	0.553	0.16	1.4	1.1	1.3
3OH-CB25	3OH-2,3′,4-trichlorobiphenyl	0.566	0.565	0.24	2.2	0.49	1.3
4OH-CB26	4OH-2,3′,5-trichlorobiphenyl	0.580	0.598	0.23	2.4	0.94	1.5
4′OH-CB26	4OH-2′,3,5′-trichlorobiphenyl	0.593	0.620	0.22	4.3	0.49	2.0
4OH-CB31	4OH-2,4′,5-trichlorobiphenyl	0.594	0.621	0.23	4.8	0.77	2.1
4′OH-CB25	4OH-2′,3,4′-trichlorobiphenyl	0.595	0.623	0.15	0.65	1.4	1.2
3′OH-CB74	3OH-2′,4,4′,5′-tetrachlorobiphenyl	0.597	0.625	0.063	0.94	0.82	0.96
3′OH-CB53	3OH-2,2′,5′,6-tetrachlorobiphenyl	0.605	0.633	0.058	2.0	0.68	1.4
4′OH-CB20	4OH-2′,3,3′-trichlorobiphenyl	0.624	0.651	0.25	3.8	0.50	1.9
4′OH-CB72	4OH-2′,3,5,5′-tetrachlorobiphenyl	0.645	0.670	0.065	1.0	1.6	1.8
4′OH-CB65	4OH-2′,3′,5′,6′-tetrachlorobiphenyl	0.671	0.695	0.070	1.1	1.2	1.7
4′OH-CB63	4OH-2′,3′,5′,6′-tetrachlorobiphenyl	0.683	0.703	0.060	1.2	0.98	1.7
4′OH-CB121	4OH-2′,3,4′,5,6′-pentachlorobiphenyl	0.695	0.718	0.088	0.53	1.5	1.8
3OH-CB66	3OH-2,3′,4,4′-tetrachlorobiphenyl	0.700	0.723	0.093	1.0	1.4	1.7
4′OH-CB35	4OH-3,3′,4′-trichlorobiphenyl	0.711	0.734	0.25	4.8	1.1	2.3
4OH-CB70	4OH-2,3′,4′,5-tetrachlorobiphenyl	0.768	0.786	0.058	1.1	0.18	1.1
4′OH-CB61	4OH-2′,3′,4′,5′-tetrachlorobiphenyl	0.770	0.792	0.078	1.3	0.47	0.93
4OH-CB79	4OH-3,3′,4′,5-tetrachlorobiphenyl	0.778	0.796	0.14	1.2	0.36	1.0
4′OH-CB101	4OH-2,2′,4′,5,5′-pentachlorobiphenyl	0.811	0.826	0.12	0.11	0.98	1.8
4′OH-CB120	4′OH-2,3′,4,5,5′-pentachlorobiphenyl	0.812	0.826	0.10	0.13	0.95	1.2
2′OH-CB114	2′OH-2,3,4′,5,6-pentachlorobiphenyl	0.824	0.838	0.14	0.15	NA[d]	NA
3OH-CB118	3OH-2,3′,4,4′,5-pentachlorobiphenyl	0.866	0.878	0.090	0.10	NA	NA
4OH-CB107	4OH-2,3,3′,4′,5-pentachlorobiphenyl	0.871	0.881	0.085	0.16	0.88	1.6
4′OH-CB108	4′OH-2,3,3′,4,5′-pentachlorobiphenyl	0.871	0.881	0.11	0.30	0.80	1.5
4′OH-CB97	4′OH-2,2′,3,4′,5′-pentachlorobiphenyl	0.876	0.891	0.070	0.80	NA	NA
4OH-CB134	4OH-2,2′,3,3′,5,6-hexachlorobiphenyl	0.879	0.891	0.12	0.11	NA	NA
3′OH-CB184	3′OH-2,2′,3,4,4′,6,6′-heptachlorobiphenyl	0.886	0.898	0.093	0.31	NA	NA
4′OH-CB165	4OH-2′,3,3′,5,5′,6′-hexachlorobiphenyl	0.892	0.901	0.056	0.06	NA	NA
3OH-CB153	3OH-2,2′,4,4′,5,5′-hexachlorobiphenyl	0.897	0.905	0.063	0.63	0.78	1.5
4OH-CB146	4OH-2,2′,3,4′,5,5′-hexachlorobiphenyl	0.901	0.910	0.060	0.40	0.70	1.1
4′OH-CB127	4′OH-3,3′,4,5,5′-pentachlorobiphenyl	0.950	0.953	0.17	0.58	NA	NA
3′OH-CB138	3OH-2,2′,3′,4,4′,5-hexachlorobiphenyl	0.957	0.962	0.048	0.24	NA	NA
4′OH-CB130	4OH-2,2′,3,3′,4′,5-hexachlorobiphenyl	0.963	0.967	0.068	0.41	NA	NA
4′OH-CB106	4OH-2′,3,3′,4′,5′-pentachlorobiphenyl	0.969	0.972	0.093	0.24	NA	NA
4OH-CB163	4OH-2,3,3′,4′,5,6-hexachlorobiphenyl	0.976	0.978	0.058	0.12	NA	NA
4OH-CB178	4OH-2,2′,3,3′,5,5′,6,6′-heptachlorobiphenyl	0.982	0.982	0.075	0.21	NA	NA
3′OH-CB182	3′OH-2,2′,3,4,4′,5,6′-heptachlorobiphenyl	0.990	0.990	0.078	0.080	NA	NA
3′OH-CB183	3OH-2,2′,3′,4,4′,5,6′-heptachlorobiphenyl	0.990	0.990	0.083	0.19	NA	NA
4OH-CB187	4OH-2,2′,3,4′,5,5′,6-heptachlorobiphenyl	1.000	1.000	0.080	0.14	0.70	1.5
4′OH-CB159	4OH-2′,3,3′,4′,5,5′-hexachlorobiphenyl	1.018	1.022	0.058	0.23	0.81	0.58
4OH-CB162	4OH-2,3,3′,4′,5,5′-hexachlorobiphenyl	1.027	1.034	0.065	0.20	NA	NA
4OH-CB202	4OH-2,2′,3,3′,5,5′,6,6′-octachlorobiphenyl	1.041	1.056	0.11	0.14	NA	NA
4OH-CB177	4OH-2,2′,3,3′,4′,5,6-heptachlorobiphenyl	1.043	1.062	0.065	0.18	NA	NA
4′OH-CB201	4′OH-2,2′,3,3′,4,5′,6,6′-octachlorobiphenyl	1.051	1.074	0.13	0.16	NA	NA
3′OH-CB180	3′OH-2,2′,3,4,4′,5,5′-heptachlorobiphenyl	1.063	1.094	0.098	0.12	NA	NA
4′OH-CB172	4OH-2,2′,3,3′,4′,5,5′-heptachlorobiphenyl	1.066	1.100	0.10	0.20	1.3	0.96
4OH-CB193	4OH-2,3,3′,4′,5,5′,6-heptachlorobiphenyl	1.078	1.118	0.11	0.33	NA	NA
3′OH-CB203	3′OH-2,2′,3,4,4′,5,5′,6-octachlorobiphenyl	1.110	1.171	0.13	0.088	NA	NA
4′OH-CB198	4′OH-2,2′,3,3′,4,5,5′,6-octachlorobiphenyl	1.111	1.174	0.15	0.11	NA	NA
4′OH-CB199	4OH-2,2′,3,3′,4′,5,5′,6-octachlorobiphenyl	1.116	1.182	0.12	0.13	NA	NA
4′OH-CB200	4′OH-2,2′,3,3′,4,5,6,6′-octachlorobiphenyl	1.127	1.199	0.12	0.13	NA	NA

[a] RRTs were calculated relative to the retention time of 4OH-BDE187.
[b] IDLs were defined as three times the standard deviation (SD) of five replicate injections of a low concentration OH-PCBs standard solution (5 pg).
[c] MDLs were calculated from S/N=3 in low concentration samples.
[d] NA=not analyzed.

Table 3
Comparison of the recovery rates (mean and coefficient of variation, CV) of $^{13}C_{12}$–OH-PCBs congeners obtained with different clean-up procedures (analysis was repeated five times).

	Acetonitrile partition with 5% H_2O deactivated silica-gel		Sulfuric acid treatment	
	Mean	CV	Mean	CV
$^{13}C_{12}$–4′OH-CB29	82	5.6	2.0	0.37
$^{13}C_{12}$–4′OH-CB61	91	5.0	3.5	2.6
$^{13}C_{12}$–4′OH-CB120	88	2.2	104	3.4
$^{13}C_{12}$–4′OH-CB159	85	3.2	102	3.0
$^{13}C_{12}$–4′OH-CB172	102	3.8	98	1.4
$^{13}C_{12}$–4OH-CB187	90	3.9	95	1.5

Table 4
Recovery concentrations (mean and coefficient of variation, CV) of the OH-PCBs congeners spiked (low and high concentration) into liver samples obtained by performing the entire analytical method (analysis was repeated five times).

BZ no.	IUPAC	10 pg add	CV	50 pg add	CV
4′OH-CB18	4OH-2,2′,5′-trichlorobipheyl	9.16	14	44.8	8.3
4′OH-CB20	4OH-2′,3,3′-trichlorobiphenyl	8.03	12	42.9	2.3
3OH-CB25	3OH-2,3′,4-trichlorobiphenyl	7.73	11	39.6	12
4′OH-CB25	4OH-2′,3,4′-trichlorobiphenyl	6.47	11	40.2	1.6
4OH-CB26	4OH-2,3′,5-trichlorobiphenyl	7.78	12	38.3	11
4′OH-CB26	4OH-2′,3,5′-trichlorobiphenyl	8.71	12	44.4	7.6
3′OH-CB28	3OH-2′,4,4′-trichlorobiphenyl	7.81	6.2	41.5	6.2
3′OH-CB31	3OH-2′,4,5′-trichlorobiphenyl	6.72	10	35.2	10
4OH-CB31	4OH-2,4′,5-trichlorobiphenyl	8.31	8.6	42.9	6.7
4′OH-CB35	4OH-3,3′,4′-trichlorobiphenyl	8.47	12	47.5	10
3′OH-CB53	3OH-2,2′,5′,6-tetrachlorobiphenyl	9.96	7.9	54.7	8.6
4′OH-CB61	4OH-2′,3′,4′,5′-tetrachlorobiphenyl	9.41	11	49.2	7.6
4′OH-CB63	4OH-2′,3′,5′,6′-tetrachlorobiphenyl	8.69	9.1	53.8	7.5
4′OH-CB65	4OH-2′,3′,5′,6′-tetrachlorobiphenyl	8.48	11	49.5	10
3OH-CB66	3OH-2,3′,4,4′-tetrachlorobiphenyl	9.81	13	58.5	8.2
4OH-CB70	4OH-2,3′,4′,5-tetrachlorobiphenyl	10.5	12	49.1	8.9
4′OH-CB72	4OH-2′,3,5,5′-tetrachlorobiphenyl	11.1	10	53.8	7.2
3′OH-CB74	3OH-2′,4,4′,5′-tetrachlorobiphenyl	8.69	9.8	50.1	11
4OH-CB79	4OH-3,3′,4′,5-tetrachlorobiphenyl	9.81	8.8	58.4	5.4
4OH-CB107	4OH-2,3,3′,4′,5-pentachlorobiphenyl	11.1	8.3	43.6	3.1
4′OH-CB120	4′OH-2,3′,4,5,5′-pentachlorobiphenyl	10.7	7.6	48.1	9.5
4′OH-CB121	4OH-2′,3,4′,5,6′-pentachlorobiphenyl	9.36	7.1	42.6	7.5
4OH-CB146	4OH-2,2′,3,4′,5,5′-hexachlorobiphenyl	11.4	11	57.3	4.3
3OH-CB153	3OH-2,2′,4,4′,5,5′-hexachlorobiphenyl	10.2	10	52.1	7.1
4′OH-CB159	4OH-2′,3,3′,4′,5,5′-hexachlorobiphenyl	11.4	5.3	52.5	5.9
4′OH-CB172	4OH-2,2′,3,3′,4′,5,5′-heptachlorobiphenyl	9.24	4.7	60.2	6.5
4OH-CB187	4OH-2,2′,3,4′,5,5′,6-heptachlorobiphenyl	11.7	8.2	55.5	6.5

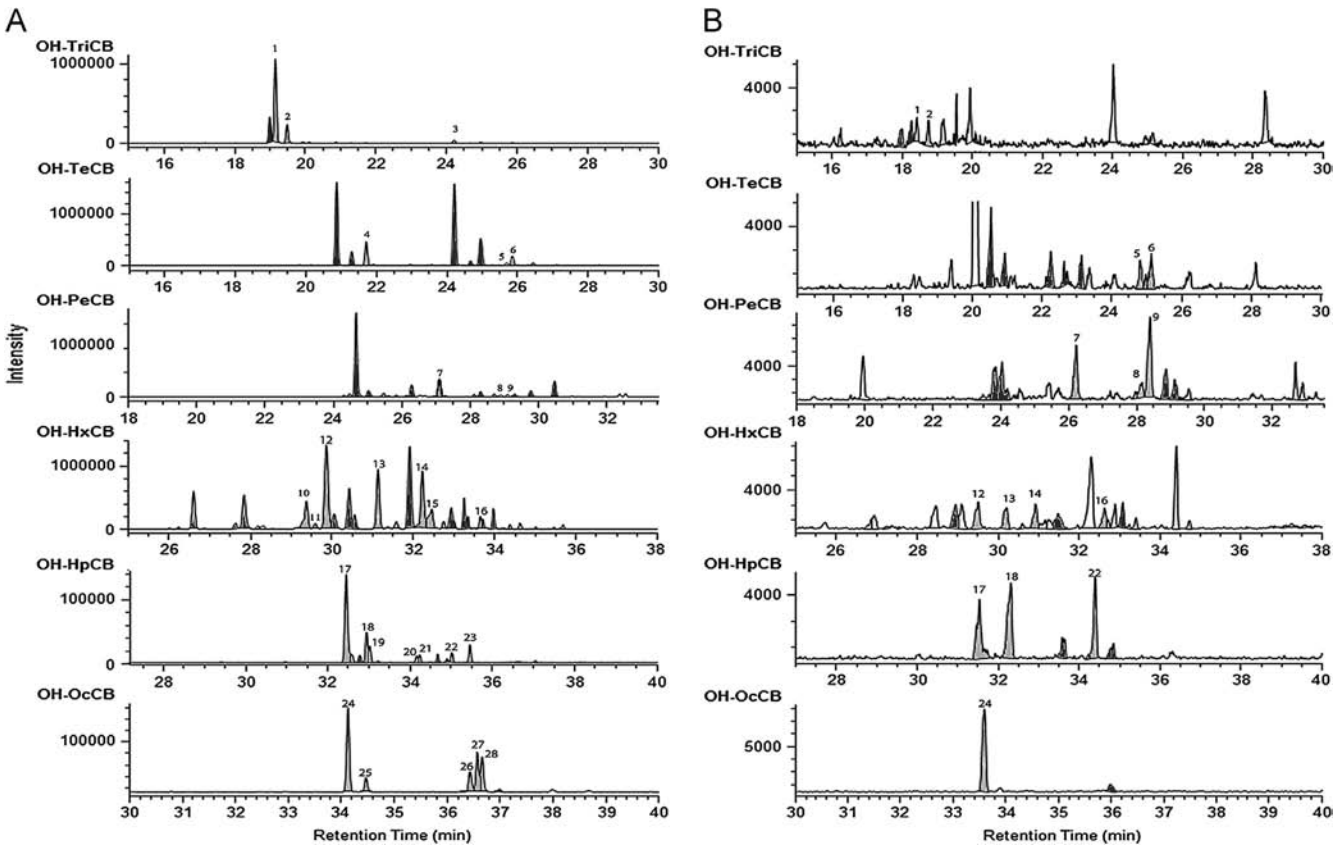

Fig. 1. SIM chromatograms of OH-PCBs (MeO-PCBs after methylation) in the liver of Baikal seals (A) and brain of finless porpoises (B). Identified peaks are shown in gray: (1) 4OH-CB26, (2) 4′OH-CB25/4′OH-CB26/4OH-CB31, (3) 4'OH-CB35, (4) 4'OH-CB63, (5) 4OH-CB61, (6) 4′OH-CB79, (7) 4′OH-CB101/4′OH-CB120, (8) 3OH-CB118, (9) 4OH-CB107/4′OH-CB108, (10) 4′OH-CB97, (11) 4′OH-CB165, (12) 3OH-CB153, (13) 4OH-CB146, (14) 3′OH-CB138, (15) 4′OH- CB159, (16) 4OH-CB162, (17) 4OH-CB178, (18) 3′OH-CB182/183, (19) 4OH-CB187, (20) 3′OH-CB180, (21) 4′OH-CB172, (22) 4OH-CB193, (23) 4OH-CB202, (24) 4′OH-CB201, (25) 4′OH-CB198/3′OH-CB203, (26) 4′OH-CB199, and (27) 4′OH-CB200. Unknown peaks are shown inblack, IF=interference (peaks with more than 50% of the theoretical ratio of two reference ions).

Table 5
Comparison of concentrations (pg g^{-1}) of OH-PCBs in liver of Baikal seals and brain of finless porpoises between GC/HRMS and GC/ECNI-MS

	Liver from Baikal seal (n=3, GC/HRMS)				Liver from Baikal seal (n=3, GC/ECNI-MS)				Brain from Finless porpoise (n=3, GC/HRMS)				Brain from Finless porpoise (n=3, GC/ECNI-MS)			
	05BS-30	05BS-41	05BS-44	Mean	05BS-30	05BS-41	05BS-44	Mean	FP1	FP2	FP3	Mean	FP1	FP2	FP3	Mean
4OH-CB26	100	< MDL	120	77	91	< MDL	110	67	0.3	1.8	0.67	0.93	< MDL	2.5	< MDL	0.83
4′OH-CB35	< MDL*	< MDL	15	5.0	< MDL	< MDL	15	5.0	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4′OH-CB25/4′OH-CB26/4OH-CB31	59	2.5	5.2	22	59	< MDL	5.2	21	0.48	2.8	1.1	1.5	< MDL	3.4	1.4	2.4
Total OH-TriCBs	160	2.5	140	101	150	< MDL	130	93	0.79	4.6	1.8	2.4	< MDL	5.9	1.4	2.4
4OH-CB61	88	29	160	92	93	35	140	89	0.94	1.2	1.1	1.1	1.0	1.2	1.2	1.2
4′OH-CB79	16	6.2	14	12	20	7.6	10	13	2.1	2.1	1.4	1.9	2.1	2.2	1.6	2.0
3′OH-CB53	2.3	1.2	< MDL	1.2	2.3	1.6	< MDL	1.3	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4′OH-CB63	34	9.2	60	35	30	8.2	58	32	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Total OH-tetraCBs	140	46	240	140	150	50	210	140	3.1	3.3	2.8	3.2	3.1	3.5	2.8	3.1
4OH-CB97	160	97	< MDL	86	160	110	< MDL	90	17	58	22	32	14	66	22	34
3OH-CB118	62	27	57	49	67	31	59	52	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4OH-CB120/4OH-CB101	690	460	870	670	700	440	890	680	10	16	6.9	11	9.6	22	6.3	13
4OH-CB108/4OH-CB107	26	23	13	20	33	21	16	23	2.9	8.1	3.5	4.8	3.5	9.5	3.7	5.6
Total OH-PentaCBs	940	610	940	830	940	600	970	840	30	82	32	48	33	76	29	46
4OH-CB146	66	91	150	100	74	85	130	96	4.2	3.4	3.7	3.8	4.5	4.1	4.2	4.3
4OH-CB162	19	20	50	29	21	25	50	32	2.7	4.2	2.2	3.1	2.3	4.4	2.6	3.1
4OH-CB134	100	< MDL	210	100	110	< MDL	170	93	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
3OH-CB138	230	230	590	350	260	230	600	360	3.3	< MDL	5.9	3.1	3.2	< MDL	5.9	3.0
4OH-CB165	68	55	120	81	78	56	110	81	1.5	0.41	0.67	0.9	2.1	< MDL	< MDL	0.7
3OH-CB153	98	68	< MDL	55	110	68	< MDL	59	1.4	0.34	0.72	0.8	2.2	< MDL	< MDL	0.7
Total OH-HexaCBs	580	460	1100	710	650	460	1100	740	13	8.4	13	13	14	8.5	12	16
4OH-CB187	27	43	77	49	29	49	81	53	8.0	2.4	2.5	4.3	8.3	2.7	2.5	4.5
3OH-CB180	6.8	8.3	21	12	5.6	10	18	11	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4OH-CB172	28	17	52	32	36	21	48	35	5.3	3.3	3.7	4.1	6.1	3.5	4.1	4.6
4OH-CB193	47	17	80	48	55	11	80	49	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4OH-CB178	13	12	40	22	13	16	51	27	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4OH-CB177	10	< MDL	32	14	11	< MDL	22	11	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
3OH-CB182/3OH-CB183	12	13	38	21	16	17	44	26	3.3	< MDL	0.73	1.3	3.0	< MDL	< MDL	1.0
Total OH-HeptaCBs	140	110	340	200	170	120	340	210	17	5.7	6.9	10.4	17	6.2	6.6	10
4OH-CB202	15	22	51	29	21	22	46	30	12	8.9	4.6	8.5	15	9.5	4.2	9.6
4OH-CB199	4.2	10	22	12	5.1	9.6	21	12	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4OH-CB201	2.6	2.6	7.5	4.2	3.5	3.2	8.1	4.9	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
4OH-CB200	1.1	< MDL	1.9	1.0	< MDL	< MDL	2.3	0.77	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
3OH-CB203/4OH-CB198	3.4	6.4	14	7.9	4.2	6.2	14	8.1	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL	< MDL
Total OH-OctaCBs	26	40	97	55	34	41	92	55	12	8.9	4.6	8.5	15	9.5	4.2	9.6
Total OH-PCBs	2000	1300	2900	2000	2100	1300	2800	2100	76	110	62	86	80	120	59	91

* < MDL=below method detection limit (See Table 2).

literature for biota samples using GC/EI-HRMS (MDLs: 1.0 pg g^{-1} wet wt) [26], GC/ECNI-MS (MDLs: 10 pg g^{-1} wet wt) [15,26], GC/ECD (MDQs: 50 pg g^{-1} wet wt) [25,33], and LC/QToF-MS and LC-MS/MS (MDLs: 2.0–5.0 pg g^{-1} wet wt) [26,28]. Thus, GC/ECNI-MS and GC/EI-HRMS systems were found to be equally well suited for determination of pg-levels of penta- to octa-OH-PCBs in biological samples.

3.4. Application to liver and brain samples

The method developed in this study was applied to analyze the OH-PCB content in unspiked livers of Baikal seals and brains of finless porpoises. The total ion chromatograms (Fig. 1) and concentrations of the target compounds in the samples analyzed by GC/EI-HRMS and GC/ECNI-MS are shown in Table 5.

The amounts of almost all the OH-PCB congeners in tissue samples were comparable irrespective of analysis technique used. Therefore, these results indicate that both GC/ECNI-MS and GC/EI-HRMS are suitable for the simultaneous analysis of tri- to octa-chlorinated OH-PCBs in liver and brain samples.

In this study, 29 OH-PCBs congeners were identified in liver samples of Baikal seals (Fig. 1A) and their concentrations varied between 1.0 and 670 pg g^{-1} wet wt (Table 5). The dominant species in the liver tissues were OH-penta-PCBs followed by OH-hexa-PCBs, OH-hepta-PCBs, OH-tetra-PCBs, OH-tri-PCBs, and OH-octa-PCBs. This profile is quite similar to the profile of PCBs in blubber samples of Baikal seals [34]. The predominant congeners were 4′OH-CB101/4′OH-CB120 followed by 3′OH-CB138, 4OH-CB134, 4OH-CB146 and 4′OH-CB61. In a former report, the levels of 4OH-CB107, 4OH-CB146, and 4OH-CB187 in the liver samples of harbor seals [23] and in the blood samples of gray seals [13] were higher than what we found. The potential parent PCBs of 4′OH-PCB107 and 4OH-CB101/4OH-CB120 are CB105, CB118, and CB101, respectively [14,15,18,35,36]. In the blubbers of Baikal seals, the concentration of CB101 was three orders of magnitude higher than those of CB105 and CB118, indicating that the concentration profiles of the metabolites reflected those of the corresponding parent PCBs. Among the tri- to hepta-chlorinated OH-PCB congeners, some unknown species were detected in the liver of Baikal seals. It has been already reported that some unknown OH-PCB congeners were discovered in wildlife blood, liver, and brain [15,17,24]. Since these unknown species are likely metabolic products of PCBs and seem to help in the elucidation of the transport kinetics of OH-PCBs, their identification is of primary importance.

Fifteen OH-PCB congeners were identified in the brain samples of finless porpoises (Fig. 1B) at concentrations of 0.30–58 pg g^{-1} wet wt (Table 5). The dominant homologs of the brain samples in the order of the concentration level were OH-penta-PCBs followed by OH-hexa-PCBs, OH-hepta-PCBs, OH-octa-PCBs, OH-tetra-PCBs and OH-tri-PCBs with the predominant species being 4OH-CB97 followed by 4′OH-CB101/4′OH-CB120, 4OH-CB146, 4′OH-CB172, and 4OH-CB162. Specifically, OH-PCB congeners with the OH-group substituted on the *para*-position were the predominant congeners in the brain samples of wildlife. In this study, a larger number of OH-PCB congeners, in particular tri- and tetra-chlorinated OH-PCBs, were detected compared with those in recent studies [24,37]. To the best of our knowledge, there are no reports describing the identification of these lower-chlorinated congeners in the brain of marine mammals. Owing to their structural similarity to TH, these congeners are characterized by a strong binding affinity to TTR in blood [10,38]. For these reasons, the disrupting effects of these molecules on the TH in the central nervous system are a critical concern. In addition to the tri- and tetra-chlorinated OH-PCBs, some unknown congeners were also discovered in the brain of finless porpoises. Since they might have a high transfer potential into the brain, the identification of these predominant unknown OH-PCBs is needed in the future. The concentrations determined using GC/ECNI-MS and GC/EI-HRMS were in agreement (Table 5) with differences of approximately 20%, except for low concentration congeners.

The use of this analytical method would provide the simultaneous detection of a wide range of OH-PCB homologs within liver and brain. Moreover, this method enables a more comprehensive assessment of the biological effects of OH-PCBs exposure in critical organs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.10.031.

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