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Notes

Measurement of Benzophenones in Human Urine Samples by Stir Bar Sorptive Extraction and Thermal Desorption-Gas Chromatography–Mass Spectrometry

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Determination of benzophenones (BPs) in human urine samples by stir bar sorptive extraction (SBSE) and thermal desorption (TD)-gas chromatography-mass spectrometry (GC-MS) is described. As analytes, BP, its metabolites benzhydrol (BP-OH) and 2-hydroxybenzophenone (2OH-BP), and its derivatives 2-hydroxy-4-methoxybenzophenone (BP-3) and 2-hydroxy-4-methoxy-4'-methylbenzophenone (BP-10) were selected. After enzymatic hydrolysis, a polydimethylsiloxane (PDMS) stir bar was placed in a urine sample diluted 1:1 with water and stirred for 60 min at room temperature. The limit of quantification (LOQ) of BPs is 0.2 – 0.5 ng ml^{-1} (ppb). The method showed linearity over the calibration range (0.2 – 10 or 0.5 – 10 ng ml^{-1}), and the correlation coefficients were equal to or higher than 0.993 for all of the analytes. The average recoveries of BPs were equal to or higher than 98.7% (RSD: 1.5 – 4.8%, $n = 6$).

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Introduction

Benzophenone (BP) and its derivatives are the most commonly used sunscreen agents in cosmetics. However, various studies have revealed the estrogenic activity of BPs.[1,2] The migration of BPs from multilayer plastic-paper material intended for food packaging and the contamination of food samples with BPs have recently been reported.[3,4] The fact that healthy humans may be exposed to BPs *via* a variety of daily activities indicates that the assessment of human exposure to BPs is an important issue.

It has been reported that when a human ingests BP, it is excreted in urine as a metabolite, such as benzhydrol (BP-OH) glucuronide.[5] Thus, it is thought that human exposure can be presumed by measuring these compounds in human urine samples. However, because the concentration of BPs in human urine is at the sub ng ml^{-1} level, a method with both high sensitivity and high accuracy is required.

Several analytical methods for the determination of BPs in human urine samples have been reported, including liquid chromatography (LC) with diode array detection (DAD),[3] and tandem mass spectrometry (MS-MS).[5,6] On the other hand, gas chromatography-mass spectrometry (GC-MS) is useful for the determination of BPs in human urine samples.[3]

The sample preparation method known as solid-phase micro extraction (SPME) has been achieved the determination of BPs in human urine samples.[7,8] However, because the limit of detection (LOD) of 2-hydroxy-4-methoxybenzophenone (BP-3) was 5 ng ml^{-1}, the sensitivity of the above SPME method remains low. In 1999, a sorptive extraction technique that uses a stir bar coated with polydimethylsiloxane (PDMS) was developed,[11] and is known as stir bar sorptive extraction (SBSE). Its main advantages compared to a general sample preparation technique are high sensitivity and a wide application range that includes volatile aromatics, halogenated solvents, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, preservatives, odor compounds and organotin compounds.[12] Rodil and Moeder have reported that the determination of UV filters contains BP-3 in a water sample by SBSE method.[13] In addition, we have reported that the trace analysis of BPs in river water samples by the SBSE method,[9,10] and liquid-phase microextraction (LPME) method.[14]

The aim of this study was to determine trace amounts of BPs in human urine samples by the SBSE and thermal desorption (TD)-GC-MS methods. Emphasis was placed on the determination of BP, its metabolites BP-OH and 2-hydroxybenzophenone (2OH-BP), and its derivatives BP-3 and 2-hydroxy-4-methoxy-4'-methylbenzophenone (BP-10). The developed method was applied to human urine samples.

Experimental

Materials and reagents

Benzophenone (BP) and benzophenone-d_{10} (BP-d_{10}) as internal standard were purchased from Kanto Chemical Inc. (Tokyo, Japan). Benzhydrol (BP-OH) and 2-hydroxybenzophenone (2OH-BP) were purchased from Wako Pure Chemical, Inc. (Osaka, Japan). 2-Hydroxy-4-methoxybenzophenone (oxybenzone, BP-3) was purchased from Sigma-Aldrich Co. (St. Louis, MO). 2-Hydroxy-4-methoxy-4'-methylbenzophenone (BP-10) was purchased from Lancaster Synthesis (Morecambe, England). The chemical structures are shown in Fig. 1. *E. coli*

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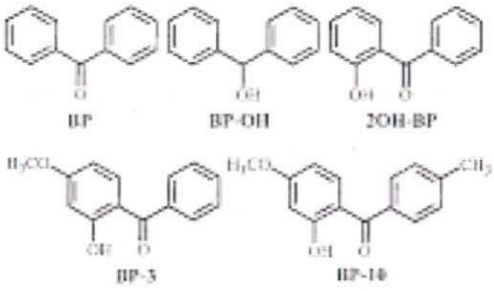

Fig. 1 Chemical structures of BPs.

β-glucuronidase (25000 units/0.4 ml, 62500 units ml^{-1}) and *H. pomatia sulfatase* (3540 units ml^{-1}) were purchased from Sigma-Aldrich Co. Prior to use, the β-glucuronidase was added to 0.1 M ammonium acetate to make a total concentration of 10000 units ml^{-1}. Other reagents were purchased from Wako Pure Chemical, Inc. The water-purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA).

Standard solutions

Stock solutions (1.0 mg ml^{-1}) of BP, BP-OH, 2OH-BP, BP-3 and BP-10 standards were dissolved in methanol. Standard solutions used for calibration were prepared by the addition of purified water. The calibration curve was performed daily for all samples containing an internal standard (5 ng ml^{-1}) using the SBSE and TD-GC-MS methods. For quantification, the internal standard method was used.

SBSE tools

Stir bars coated with a 0.5-mm-thick PDMS layer (24 µl; Twister™) were obtained from Gerstel (Mülheim an der Ruhr, Germany). The stir bars were conditioned for 1 h at 300°C in a flow of helium. Then, the stir bars were stored in new 2 ml vials until immediately prior to use. The stir bars could be used more than 50 times with appropriate reconditioning. For extraction, a 10 ml headspace vial from Agilent Technologies (Palo Alto, CA) was used.

Instrumentation

TD was performed with a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS A autosampler and a Gerstel Cooled Injection System (CIS) 4 programmable temperature vaporization (PTV) inlet. GC-MS was performed with an Agilent 6890N gas chromatograph equipped with a 5973N mass-selective detector with an ultra ion source (Agilent Technologies).

The TDS 2 temperature was programmed from 20°C (held for 1 min) to 250°C (held for 5 min) at 60°C min^{-1}. The desorbed compounds were cryofocused in CIS 4 at –150°C. Then, the CIS 4 temperature was programmed from –150 to 300°C (held for 10 min) at 12°C s^{-1} to inject trapped compounds into the analytical column. Injection was performed in the solvent vent mode. Separations were conducted on a DB-5ms fused silica column (30 m × 0.25 mm i.d., 0.25 µm film thickness, J & W Scientific, Agilent Technologies). The oven temperature was programmed from 40°C (held for 1 min) to 190°C at 5°C min^{-1} and from 190°C to 280°C (held for 3 min) at 15°C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.2 ml min^{-1}. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron ionization (EI) at 70 eV.

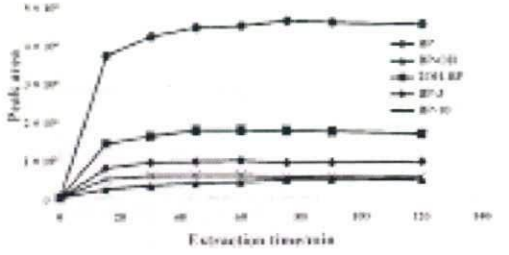

Fig. 2 Extraction time profiles of BPs in human urine sample subjected to the SBSE method.

Table 1 Figures of merit of SBSE and TD-GC-MS

Compound	SIM[a] (*m/z*)	LOD[b] ng ml^{-1}	LOQ[c] ng ml^{-1}	Range/ ng ml^{-1}	Correlation coefficient, r
BP	182, 105	0.1	0.5	0.5 - 10	0.999
BP-OH	184, 105	0.05	0.2	0.2 - 10	0.998
2OH-BP	197, 121	0.1	0.5	0.5 - 10	0.997
BP-3	227, 151	0.1	0.5	0.5 - 10	0.993
BP-10	241, 227	0.1	0.5	0.5 - 10	0.994

a. The underlined number is the *m/z* of the ion used for quantification.
b. LOD: limit of detection (*S/N* = 3).
c. LOQ: limit of quantification (*S/N* > 10).

The ions selected for ion monitoring are given in Table 1.

Urine samples

Urine was collected from six healthy volunteers (I, II, III, IV, V, and VI). All samples were stored at 4°C prior to use.

Sample preparation

One milliliter of a urine sample spiked with BP-d_{10} (5 ng ml^{-1}) was pipetted into a 10-ml vial. Then, 1.0 M ammonium acetate (100 µl, pH 6.8) was added. After adding β-glucuronidase (10 µl, 10000 units ml^{-1}) and sulfatase (10 µl, 3540 units ml^{-1}), the sample was gently mixed. A hydrolysis process to release free BPs was accomplished by incubating at 37°C for 3 h. One milliliter of water was added to the sample and SBSE was performed at room temperature for 60 min while stirring at 500 rpm. After extraction, the PDMS stir bar was thermally desorbed, and subjected to GC-MS thereafter.

Results and Discussion

Optimization of extraction time

One important parameter affecting SBSE was the extraction time. To optimize the extraction time, 1 ml of human urine sample (5 ng ml^{-1} BPs standard solutions) and 1 ml of purified water were mixed and used. The extraction time profiles (0 - 180 min) of BPs in the human urine sample that was subjected to SBSE were determined by TD-GC-MS, and are shown in Fig. 2. BPs reached equilibrium after approximately 60 min. Therefore, this condition was used for the determination of BPs in human urine samples.

Figures of merit of SBSE and TD-GC-MS for the determination of BPs

The calculated LODs of BPs were 0.05 to 0.1 ng ml^{-1} for

Table 2 Recoveries of BPs in human urine samples

Compound	Amount spiked			
	1 ng ml^{-1}		10 ng ml^{-1}	
	Recovery, %	RSD, %[a]	Recovery, %	RSD, %[a]
BP	101.5	2.4	98.7	1.5
BP-OH	99.1	4.3	99.4	4.8
2OH-BP	101.7	3.0	100.0	3.4
BP-3	99.7	3.2	100.7	2.2
BP-10	99.3	4.0	100.5	2.3

a. The recoveries and precision were also examined by replicate analysis ($n = 6$) of human urine samples.

Table 3 Concentrations of BPs in human urine samples

Compound	Human urine sample/ng ml^{-1}					
	I	II	III	IV	V	VI
BP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP-OH	3.7	0.5	0.4	<LOQ	0.4	<LOQ
2OH-BP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BP-3	1.2	0.5	<LOQ	<LOQ	<LOD	<LOD
BP-10	<LOD	<LOQ	<LOD	<LOD	1.5	<LOQ

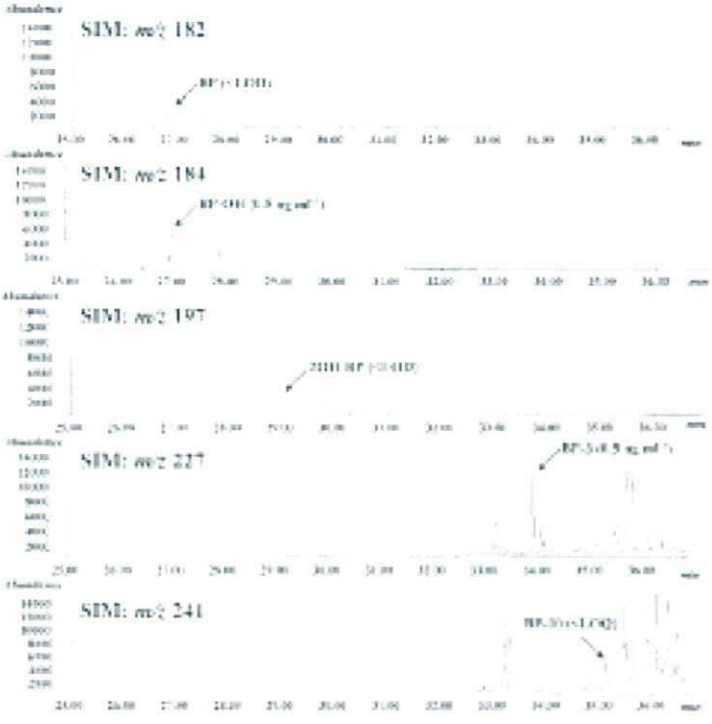

Fig. 3 SIM chromatograms of BPs in human urine sample (II).

SBSE and TD-GC-MS, with the signal to noise (S/N) ratio being 3. In addition, the limits of quantification (LOQs) of BPs when $S/N > 10$ were 0.2 to 0.5 ng ml^{-1}. The method showed good linearity and the correlation coefficients (r) were equal to or higher than 0.993 for all of the analytes. The figures of merit of the present method are summarized in Table 1. A comparison of the SBSE method with the SPME method,[10] used in previous studies revealed that the SBSE method was superior to the SPME method in terms of sensitivity.

The recovery and precision of the method were assessed by replicate analysis ($n = 6$) of human urine samples spiked at 1 and 10 ng ml^{-1} levels. Non-spiked and spiked samples were subjected to SBSE and TD-GC-MS. The recovery was calculated by subtracting the results for non-spiked samples from those for spiked samples. The results were obtained by using calibration curves acquired from standard solutions containing the internal standard. The recovery and the precision were 98.7 to 101.7% (RSD: 1.5 to 4.8%, $n = 6$) for human urine samples (Table 2). Therefore, the method enables the precise determination of standards, and may be applicable to the determination of trace amounts of BPs in human urine samples.

Determination of BPs in human urine samples

A total of six human urine samples were analyzed for BPs using the presented method, the results are given in Table 3. BP-OH (<LOQ – 3.7 ng ml^{-1}), BP-3 (<LOD – 1.2 ng ml^{-1}), and BP-10 (<LOD – 1.5 ng ml^{-1}) were detected in the samples with the present method. On the other hand, BP and 2OH-BP were not detected in the urine samples examined. Typical SIM chromatograms of the human urine sample (II) are shown in Fig. 3. SBSE and TD-GC-MS enabled the successful determination of trace amounts of BPs in the urine sample. The BP levels in the

urine samples were very low and could not be quantified by SPME-GC-MS.[11]

Conclusions

The determination of trace amounts of BPs in human urine samples using SBSE and TD-GC-MS was described. The proposed method has many practical advantages, including a small sample volume (1 ml) and simplicity of extraction; it is also solvent-free and has high sensitivity. The LODs of BPs were of sub ng ml^{-1} level. In addition, the present method showed good linearity and high correlation coefficients using the internal standard. The recovery was 98.7 to 101.7% and the precision was RSD: 1.5 to 4.8% ($n = 6$) for human urine samples spiked at 1 and 10 ng ml^{-1} levels.

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Short communication

Determination of urinary triclosan by stir bar sorptive extraction and thermal desorption–gas chromatography–mass spectrometry

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ABSTRACT

We have developed an analytical method for the determination of urinary 5-chloro-2-(2,4-dichlorophenoxy)phenol (triclosan), which utilizes stir bar sorptive extraction (SBSE) and thermal desorption (TD)–gas chromatography–mass spectrometry (GC–MS). Human urine sample is deconjugated by treatment with β-glucuronidase and sulfatase. A stir bar coated with polydimethylsiloxane (PDMS) is added to the urine sample in a vial and the sample is stirred for 60 min at room temperature (25 °C). Then, the PDMS stir bar is subjected to TD–GC–MS. The detection limit of triclosan is 0.05 ng mL^{-1}. The method shows linearity over the calibration range (0.1–10 ng mL^{-1}) and the correlation coefficient (r) is higher than 0.993 for triclosan standard solution. The average recoveries of triclosan in human urine sample are 102.8–113.1% (RSD: 2.4–6.2%). This simple, sensitive, and selective analytical method may be used in the determination of trace amounts of triclosan in human urine samples.

1. Introduction

Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol, is widely used as a broad-spectrum antimicrobial agent. As an additive in plastics, it prevents growth of microorganisms and suppresses the formation of stain and odor, thereby extending the polymer's shelf life [1]. For this reason, it has been considered that triclosan in polymeric materials come in contact with food [2,3]. As healthy humans may be exposed to triclosan via a variety of daily activities, exposure assessment of triclosan in human is an important issue.

The urinary excretion of orally ingested triclosan as free triclosan and glucuronide and sulfate conjugates has been reported [4]. Thus, it is thought that human exposure can be evaluated by measuring triclosan in urine sample. To this end, high-sensitivity and high-accuracy analytical methods are required.

Many analytical methods for the determination of triclosan in biological samples have been reported, including liquid chromatography (LC) with diode array detection (DAD) [5], electrochemical detection (ECD) [6] or tandem mass spectrometry (MS–MS) [7]. On the other hand, gas chromatography (GC) with MS or MS–MS was initially used for the determination of triclosan in environmental analysis [8–11].

Liquid–liquid extraction (LLE) [6] and online solid-phase extraction (SPE) [7] have been developed for the determination of triclosan in urine sample. Recently, a new sorptive extraction technique that uses a stir bar coated with polydimethylsiloxane (PDMS) was developed [12] and is known as stir bar sorptive extraction (SBSE). Its main advantage is its wide application range that includes volatile aromatics, halogenated solvents, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, endocrine disruptors, preservatives, odor compounds, and organotin compounds [13–15]. Silva and Nogueira determined triclosan in biological and environmental matrices with the SBSE-liquid desorption (LD)-LC-DAD method [5]. In LC analysis, LD was used to desorb the analyte from the PDMS stir bar. On the other hand, thermal desorption (TD) is often used to desorb the analyte from the PDMS stir bar. Because TD enables injection of the entire quantity of desorbed analyte into an analytical instrument such as GC–MS, high-sensitivity analysis is expected.

The aim of this study was to determine trace amounts of triclosan in human urine samples by SBSE and TD–GC–MS.

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2. Experimental

2.1. Materials and reagents

Triclosan was purchased from Wako Pure Chemical, Inc. (Osaka, Japan). Benzophenone-d_{10} (BP-d_{10}, used as internal standard) was purchased from Kanto Chemical Inc. (Tokyo, Japan). *E. coli* β-glucuronidase (25,000 units/0.4 mL, 62,500 units mL^{-1}) and *H. pomatia* sulfatase (3540 units mL^{-1}) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Prior to use, β-glucuronidase was added to 0.1 M ammonium acetate to make a total concentration of 10,000 units mL^{-1}. Other reagents were purchased from Wako Pure Chemical, Inc. The water purification system used was a Milli-Q gradient A 10 with an EDS polisher (Millipore, Bedford, MA, USA).

2.2. Standard solution

A concentrated solution (1.0 mg mL^{-1}) of triclosan was prepared by dissolving in methanol. Seven-point calibration (0.1, 0.2, 0.5, 1, 2, 5, and 10 ng mL^{-1}) was performed daily in duplicate analysis for all samples containing the internal standard using the SBSE method.

2.3. Instrumentation

TD was performed with a Gerstel TDS 2 thermodesorption system equipped with a Gerstel TDS A autosampler and a Gerstel Cooled Injection System (CIS) 4 programmable temperature vaporization (PTV) inlet. GC–MS was performed with an Agilent 6890N gas chromatograph equipped with a 5973N mass-selective detector (Agilent Technologies).

Stir bars coated with a 0.5-mm thick PDMS layer (24 μL: Twister™: a magnetic stirring rod is placed inside a glass jacket and coated with PDMS) were obtained from Gerstel (Mülheim an der Ruhr, Germany). The stir bars were conditioned for 1 h at 300 °C in a flow of helium. Then, the PDMS stir bars were kept in new 2 mL vials until immediately prior to use. The stir bars could be used more than 50 times with appropriate re-conditioning. For the extraction, a 10 mL headspace vial from Agilent Technologies (Palo Alto, CA, USA) was used.

2.4. TD–GC–MS conditions

TDS 2 temperature was programmed to increase from 20 °C (held for 1 min) to 240 °C (held for 5 min) at 60 °C min^{-1}. The desorbed compounds were cryofocused in CIS 4 at −150 °C. After the desorption, CIS 4 temperature was programmed to increase from −150 °C to 300 °C (held for 10 min) at 12 °C s^{-1} to inject the trapped compounds into the analytical column. CIS 4 is a type of PTV device. Once an analyte is trapped by means of temperature control, the entire quantity is subjected to GC–MS. Injection was performed in the solvent vent mode. Separations were conducted on a DB-5ms fused silica column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies). Oven temperature was programmed to increase from 40 °C (held for 1 min) to 190 °C at 5 °C min^{-1} and from 190 °C to 280 °C (held for 3 min) at 15 °C min^{-1}. Helium was used as the carrier gas at a flow rate of 1.2 mL min^{-1}. The mass spectrometer was operated in the selected ion-monitoring (SIM) mode with electron ionization (ionization voltage: 70 eV). Monitoring ions are shown in Table 1.

2.5. Human urine samples

Urine was collected from six healthy volunteers (I, II, III, IV, V, and VI). All samples were stored at 4 °C prior to use.

2.6. Sample preparation

One milliliter of urine sample spiked with BP-d_{10} was pipetted into a 10 mL vial. Then, 1.0 M ammonium acetate (100 μL, pH 6.8) was added. After β-glucuronidase (10 μL, 10,000 units mL^{-1}) and sulfatase (10 μL, 3540 units mL^{-1}) were added, the sample was gently mixed. Glucuronidase and sulfatase hydrolysis to release free triclosan was accomplished by incubating at 37 °C for 3 h. After enzymatic de-conjugation, 1 mL of purified water was added to the vial. A PDMS stir bar was added and the vial was crimped with a Teflon-coated silicone septum cap. SBSE was performed at room temperature for 60 min while stirring at 500 rpm. After the extraction, the stir bar was easily removed, rinsed with purified water, dried with lint-free tissue, and placed inside a glass TD tube. The TD tube was placed inside the TD system where the stir bar was thermally desorbed and the desorbed analyte was subjected to GC–MS thereafter.

3. Results and discussion

3.1. Optimization of GC–MS conditions

In the mass analysis of standard solutions using electron impact ionization (EI)-MS, *m/z* 288 was observed as the main peak of triclosan. The mass spectrometer was operated in the SIM mode. Three ions were monitored (*m/z* 288, 218 for triclosan; and *m/z* 192 for BP-d_{10}. The underlined and none underlined number are the *m/z* of the ion used for quantitation and qualitative analysis, respectively).

3.2. Optimization of SBSE conditions

One important parameter affecting SBSE was the extraction time. To determine the optimum extraction time, 1 mL of human urine sample (5 ng mL^{-1} triclosan standard solution) and 1 mL of purified water were mixed and used. The extraction time profile (0–180 min) of triclosan in the human urine sample that was subjected to SBSE and TD–GC–MS is shown in Fig. 1. Triclosan reached equilibrium after approximately 60 min. Therefore, this condition was used for the determination of triclosan in human urine samples.

Table 1
Figures of merit of SBSE and TD–GC–MS.

Compound	SIM[a] (*m/z*)	LOD[b] (ng mL^{-1})	LOQ[c] (ng mL^{-1})	Range (ng mL^{-1})	Correlation coefficient (*r*)	Amount spiked			
						0.5 ng mL^{-1}		5 ng mL^{-1}	
						Recovery (%)	RSD (%)[d]	Recovery (%)	RSD (%)[d]
Triclosan	288, 218	0.05	0.1	0.1–10	0.993	102.8	2.4	113.1	6.7

[a] The underlined number is the *m/z* of the ion used for quantification.
[b] LOD: limit of detection (S/N = 3).
[c] LOQ: limit of quantification (S/N > 10).
[d] Recoveries and precision were also examined by replicate analysis (*n* = 6) of human urine samples.

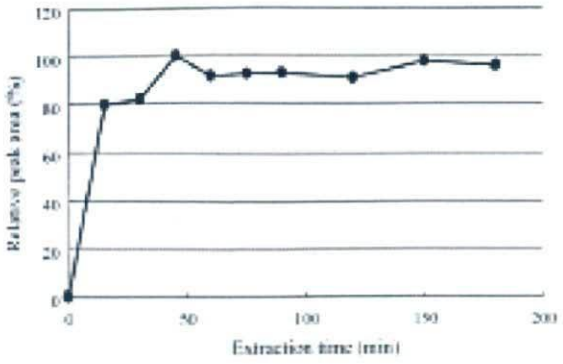

Fig. 1. Optimization of extraction time.

3.3. Figures of merit of SBSE and TD–GC–MS for determination of triclosan

The calculated detection limit (LOD) is 0.05 ng mL^{-1} for SBSE and TD–GC–MS, with the ratio of the compound's signal to the background signal (S/N) being 3. In addition, the limit of quantification (LOQ) when S/N > 10 is 0.1 ng mL^{-1} for triclosan. Some different lots of human urine were used for calculation of LOD and LOQ. The blank and internal standard only samples were checked. The interference was not observed. The method shows linearity over the calibration range (0.1–10 ng mL^{-1}) and the correlation coefficient (r) is higher than 0.993 for triclosan standard solution. The figures of merit of the present method are summarized in Table 1. The LOD of triclosan in urine sample previously determined by LLE-LC-ECD [6] and the online SPE–LC–MS–MS method [7] was 1.0 and 2 ng mL^{-1}, respectively. Compared to those studies, approximately 10–20 times higher sensitivity was achieved in this study.

The recovery and within-day precision of the method were assessed by replicate analysis (n = 6) of human urine samples fortified at 0.5 and 5 ng mL^{-1} levels. Non-spiked and spiked samples were subjected to SBSE and TD–GC–MS. Recovery was calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were obtained by using calibration curves obtained from standard solutions with internal standard. The recovery and precision were 102.8–113.1% (RSD: 2.4–6.7%) for human urine samples (Table 1). Therefore, the method enables the precise determination of standards and may be applicable to the

Table 2
Concentrations of triclosan in human urine samples.

Compound	De-conjugation	Human urine					
		I	II	III	IV	V	VI
Triclosan (ng mL^{-1})	Without de-conjugation	0.17	0.15	0.15	0.15	0.52	0.15
	De-conjugation	2.62	1.25	1.13	4.30	13.98	1.26
Ratio of conjugate (%)[a]		93.5	88.2	86.8	96.4	96.3	87.9

[a] Ratio of conjugate was calculated by (Total−Free)/Total*100.

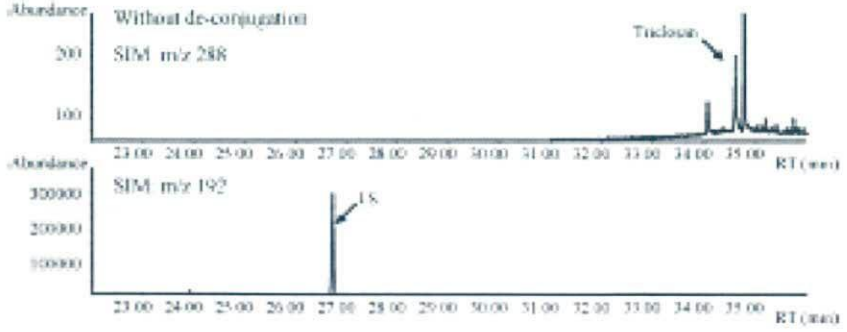

Fig. 2. Typical SIM chromatogram of triclosan in human urine sample (I) by SBSE–TD–GC–MS without de-conjugation. Triclosan: 0.17 ng mL^{-1}.

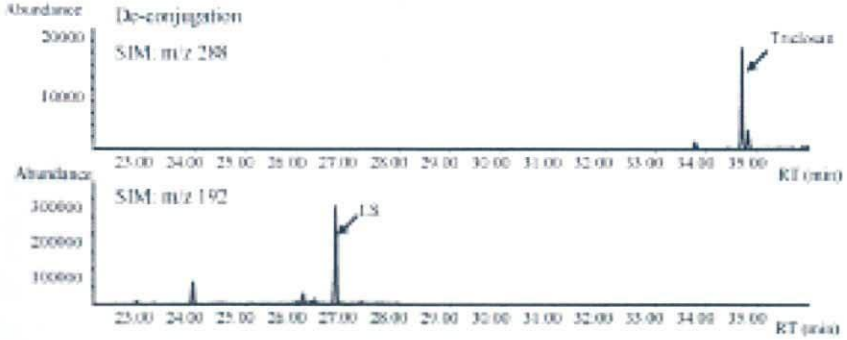

Fig. 3. Typical SIM chromatogram of triclosan in human urine sample (I) by SBSE–TD–GC–MS with de-conjugation. Triclosan: 2.62 ng mL^{-1}.

determination of trace amounts of triclosan in human urine samples.

3.4. Determination of triclosan in human urine samples

A total of six human urine samples were analyzed for triclosan using the present method and the results are shown in Table 2. For quality control, the urine samples spiked with standard were analyzed between the measurements of six human urine samples. In the human urine samples without de-conjugation, 0.15–0.52 ng mL^{-1} triclosan was detected. On the other hand, in the human urine sample treated with β-glucuronidase and sulfatase, 1.13–13.98 ng mL^{-1} triclosan was detected by the present method. Typical chromatograms of human urine sample (Volunteer 1) by SBSE–TD–GC–MS without de-conjugation and with de-conjugation are shown in Figs. 2 and 3, respectively. The ratio of conjugate in human urine sample was 86.8–96.4%. Most triclosan existed in human urine sample as conjugate. The comparatively high concentration of triclosan was determined in the sample. It was thought that the volunteers may be exposed to triclosan via a variety of daily activities.

SBSE and TD–GC–MS enabled the successful determination of trace amounts of triclosan in human urine sample. Because the previous methods [6,7] were low sensitivity, it was considered that triclosan in human urine sample might not be determined. The proposed method has many practical advantages, including small sample volume (1 mL) and simplicity of extraction; it is also solvent-free and has high sensitivity.

4. Conclusions

The determination of trace amounts of triclosan in human urine samples using SBSE and TD–GC–MS was performed. The detection limit for triclosan was 0.05 ng mL^{-1}. In addition, the present method showed good linearity and high correlation coefficient using the internal standard. The recovery was high (102.8–113.1%) and the precision was good (RSD: 2.4–6.7%) for human urine samples fortified at 0.5 and 5 ng mL^{-1} levels. This simple and highly sensitive method is expected to have potential applications in various aqueous samples.

Acknowledgements

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Polybrominated Diphenyl Ethers in Human Serum and Sperm Quality

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Abstract Polybrominated diphenyl ethers (PBDEs) are widely used flame retardants; currently, they are identified as ubiquitous environmental contaminants. Several studies indicate that PBDEs might affect male fertility. We present the results of a pilot study on the relationship between human serum PBDEs and sperm quality. The PBDE levels in Japan are comparable to those found in European countries. Strong inverse correlations were observed between the serum concentration of 2,2′,4,4′,5,5′-hexabromodiphenyl ether and sperm concentration ($r = -0.841$, $p = 0.002$) and testis size ($r = -0.764$, $p = 0.01$). Extensive studies on the relationship between PBDEs and sperm quality are required.

Keywords Polybrominated diphenyl ethers · Flame retardants · Human serum · Sperm

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Polybrominated diphenyl ethers (PBDEs) are used as flame retardants in the production of common consumer products such as electronics, furniture, and textiles. PBDEs are currently recognized as environmental pollutants of global concern because their levels in the environment and in humans have increased markedly over the past several decades (Meironyté et al. 1999; Ikonomou et al. 2002; Akutsu et al. 2003). Since PBDEs are somewhat structurally similar to thyroid hormones such as thyroxine (T4), it was speculated that PBDEs might mimic thyroid hormones and disrupt thyroid homeostasis. Several studies indicate that exposure to PBDEs can decrease the circulating levels of T4 in laboratory animals (Fowles et al. 1994; Zhou et al. 2002) and can cause permanent neurological effects similar to those associated with thyroid hormone deficiencies (Eriksson et al. 2001; Viberg et al. 2004). In addition, several PBDEs possess weak estrogenic/antiestrogenic activities (Meerts et al. 2001). The proliferation and differentiation of Sertoli cells and sperm production are regulated by thyroid and sex hormones. Thus, PBDEs might affect male reproductive health by interfering with the thyroid- and sex-hormone functions. Kuriyama et al. (2005) have reported that developmental exposure to a single low dose (60 μg/kg body weight) of 2,2′,4,4′,5-pentabromodiphenyl ether (PeBDE-99) decreased the

sperm count in male Wistar rats. However, no previous studies have examined the relationship between human PBDE levels and sperm quality.

We participated in an international project examining the sperm quality of fertile males and found that the sperm concentration of Japanese males was lower than that of European males (Iwamoto et al. 2006). The examination of sperm quality and an estimation of the concentration of chemicals in the serum would be required to reveal the correlation between chemical exposure and the sperm quality in Japanese males. The aim of this pilot study was to measure PBDEs in serum samples from young Japanese males and to examine the relationship between serum PBDE levels and sperm quality.

Materials and Methods

This study was performed in accordance with the protocols which were approved by the ethical committees of the St. Marianna University School of Medicine and Osaka Prefectural Institute of Public Health. Written informed consent was obtained from all study participants. Blood serum and sperm samples were collected on a monthly basis in the year 2003 from 45 young Japanese males at the Department of Urology, St. Marianna University School of Medicine. The participants were instructed to abstain from ejaculation for at least 48 h prior to sperm collection. The blood samples were collected in vacuum tubes, and the serum fractions were separated by centrifugation. The serum samples were stored at −80°C until analysis. Of the 45 sample sets, 10 were randomly selected for this study. For PBDE analysis, 10 pooled serum samples (0.5 g × 12 months; total, 6 g per person) were prepared, and each pool was regarded as a representative sample of each set. The mean ± standard deviation (SD) of the age of the 10 participants was 22 ± 1 years (range, 18–22 years). The mean ± SD abstinence period was 3.1 ± 0.4 days (range, 2.6–3.8 days). In addition, 2 brands of commercially pooled human serum ("L-Consera N" and "L-Seitrol I," Nissui Pharmaceutical, Tokyo, Japan) were used as in-house reference materials.

Standard mixture solutions of native PBDEs (BDE-AAP-A-15X) were purchased from AccuStandard (New Haven, CT, USA), and $^{13}C_{12}$-labeled PBDEs (MBDE-MXC) were purchased from Wellington Laboratories (Ontario, Canada). In this study, 29 PBDE congeners with 3–7 bromine atoms were monitored. The PBDE numbers are assigned according to the International Union of Pure and Applied Chemistry nomenclature for polychlorinated biphenyls. Acetone, acetonitrile, and *n*-hexane of pesticide analysis grade; ammonium sulfate of biochemistry grade; 44% sulfuric acid-impregnated silica gel; and *n*-nonane of dioxin analysis grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q cartridge system (Millipore, Bedford, MA, USA).

Sperm analyses were performed at the Department of Urology, St. Marianna University School of Medicine, according to World Health Organization criteria as described elsewhere (World Health Organization 1999; Iwamoto et al. 2006).

Serum samples were analyzed at Osaka Prefectural Institute of Public Health. The serum sample (6 g) was extracted using ethanol/*n*-hexane (1:3 v/v, 14 mL) in a 50 mL test tube after adding $^{13}C_{12}$-labeled surrogate standards ($^{13}C_{12}$-2,4,4′-tribromodiphenyl ether ($^{13}C_{12}$-TrBDE-28), $^{13}C_{12}$-2,2′,4,4′-tetrabromodiphenyl ether ($^{13}C_{12}$-TeBDE-47), $^{13}C_{12}$-2,2′,4,4′,5-pentabromodiphenyl ether ($^{13}C_{12}$-PeBDE-99), $^{13}C_{12}$-2,2′,4,4′,5,5′-hexabromodiphenyl ether ($^{13}C_{12}$-HxBDE-153), $^{13}C_{12}$-2,2′,4,4′,5,6′-HxBDE ($^{13}C_{12}$-HxBDE-154), and $^{13}C_{12}$-2,2′,3,4,4′,5′,6-heptabromodiphenyl ether ($^{13}C_{12}$-HpBDE-183); 10 pg for each congener) and 3.6 mL saturated ammonium sulfate solution. The test tube was shaken for 30 min and then centrifuged for 10 min at 3,000 rpm. The *n*-hexane phase was collected, and the aqueous phase was re-extracted twice with 12 mL *n*-hexane. The 3 *n*-hexane phases were combined and washed with 12 mL water. After evaporation of the solvent, the lipid content was determined gravimetrically using a semimicro balance (Sartorius RC210P, Goettingen, Germany). The lipid was dissolved in *n*-hexane and transferred to a column of 44% sulfuric acid-impregnated silica gel (3 g). The column was eluted with 30 mL *n*-hexane, and the eluate was evaporated to 2 mL. The *n*-hexane solution was transferred to a test tube and partitioned with *n*-hexane-saturated acetonitrile (4 mL) 3 times by shaking the test tube for 10 min and then centrifuging for 10 min at 3,000 rpm. The acetonitrile phase was combined and then evaporated to dryness. The residue was redissolved in *n*-hexane and transferred to a microconcentration tube. After addition of the injection standard ($^{13}C_{12}$-3,3′,4,4′,5-PeBDE ($^{13}C_{12}$-PeBDE-126)) and keeper solvent (10 µL *n*-nonane), the extract was finally evaporated to approximately 10 µL under a gentle stream of nitrogen. The serum extract was assayed by a gas chromatography/mass spectrometry (GC/MS) system (Agilent 6890A GC coupled with JEOL JMS-GC mateII, Tokyo, Japan) using a fused silica capillary column (Rtx-1MS, 15 m length, 0.25 mm i.d., 0.1 µm film thickness; Restek, Bellefonte, PA, USA). For each compound, 2 ions of the molecular ion or fragment ion cluster were monitored. Quantitation was based on the isotope dilution method using $^{13}C_{12}$-labeled internal standards. The PBDE concentrations were adjusted for total serum lipids and were expressed in units of nanogram per gram lipid weight (ng/g lw). TeBDE-47, PeBDE-99, PeBDE-100, and HxBDE-153 were of interest because they are dominant in human serum.

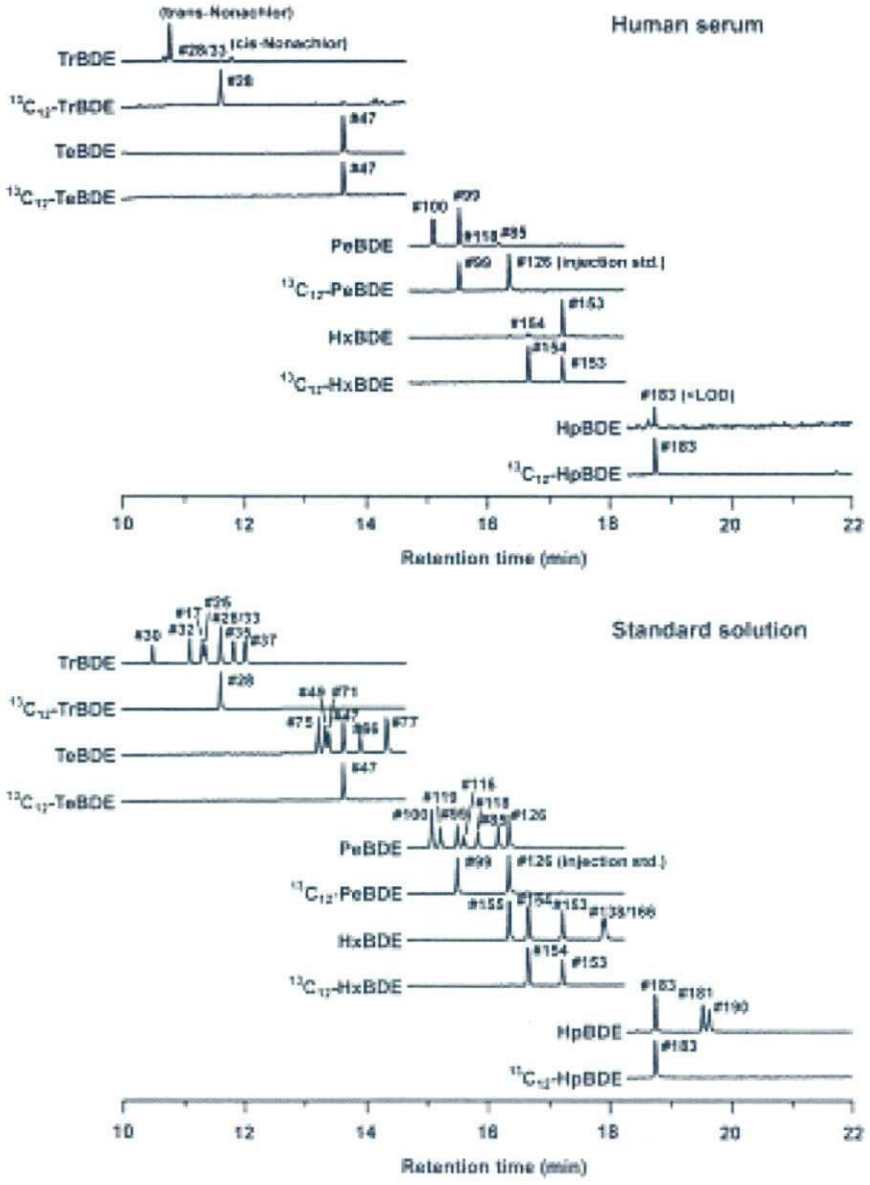

Fig. 1 Chromatograms of PBDEs in human serum (participant No.2) and standard solution (1–2.5 ng/mL each)

We validated the serum extraction procedure prior to beginning sample analysis by analyzing 4 replicate samples of pooled serum fortified with target analytes at 0.04–0.1 ng/g serum. The mean percent recovery of 7 representative PBDE congeners (TrBDE-28, TeBDE-47, PeBDE-99, PeBDE-100, HxBDE-153, HxBDE-154, and HpBDE-183) ranged from 91% to 107%, and the relative standard deviation (RSD) ranged from 2% to 10%. The limit of detection (LOD) and limit of quantification (LOQ) were defined as 3 times and 10 times the SD values obtained from the analysis of the 7 procedural blank samples (6 g of water), respectively. However, for congeners that could not be detected in the blanks, the values that were 3 times and 10 times the SD values obtained from the analysis of 5 replicates of the lowest calibration standard were used as LOD and LOQ. The LOD values for all the PBDE congeners were below 0.3 ng/g bw. In the analysis of 3 split unfortified serum samples, the RSD values for all the detected congeners were below 10%.

Results and Discussion

Of the 29 PBDE congeners monitored, 4 congeners (TeBDE-47, PeBDE-99, PeBDE-100, and HxBDE-153) were mainly detected in human serum samples (Fig. 1). The concentrations of the detected PBDE congeners in the serum samples (n = 10) are shown in Table 1. The median levels of the individual PBDE congeners were as follows: TeBDE-47, 1.4 ng/g lw; PeBDE-99, 0.21 ng/g lw; PeBDE-100, 0.24 ng/g lw; and HxBDE-153, 0.72 ng/g lw. The levels of total PBDEs in Japanese human serum samples were almost the same as those reported in European countries but were 1 order of magnitude lower than those reported in USA (Hites 2004). Significant positive correlations were observed between the concentrations of TeBDE-47 and PeBDE-99 ($r = 0.988$, $p < 0.001$), TeBDE-47 and PeBDE-100 ($r = 0.938$, $p < 0.001$), and PeBDE-99 and PeBDE-100 ($r = 0.915$, $p < 0.001$). In contrast, no significant correlations were observed between the concentration of HxBDE-153 and those of the other 3 congeners ($r = 0.306–0.390$, $p = 0.26–0.39$). The absence of a significant correlation between HxBDE-153 and the other 3 dominant congeners (TeBDE-47, PeBDE-99, and PeBDE-100) implies that the main sources and/or biological properties of HxBDE-153 were different from those of the other 3 congeners. It has been reported that the technical mixtures of pentaBDE (DE-71 and Bromkal 70-5DE) and octaBDE (DE-79 and Bromkal 79-8DE) both contained HxBDE-153 in the range 5.32%–5.44% w/w and 0.15%–8.66% w/w, respectively (La Guardia et al. 2006). The congeners TeBDE-47, PeBDE-99, and PeBDE-100 have been found in pentaBDE as the major components, but they have not been found in octaBDE (La Guardia et al. 2006). These 3 congeners and HxBDE-153 have never been found in a technical decaBDE mixture (Saytex 102E and Bromkal 82-0DE) (La Guardia et al. 2006). Therefore, TeBDE-47, PeBDE-99, and PeBDE-100 are mainly sourced from pentaBDE, although HxBDE-153 is sourced from both pentaBDE and octaBDE. In the early 1990s, Japanese manufacturers voluntarily stopped the production and use of pentaBDE because its potency to accumulate in the biota and produce toxic polybrominated dibenzofurans/dioxins under thermal stresses was a cause of concern. However, the production and use of octaBDE were continued in Japan until 2002 (Ministry of the Environment, Japan 2006). Therefore, many consumer products containing octaBDE in the Japanese indoor environment might continue to exist. Thus, with regard to octaBDE components such as HxBDE-153 and HpBDE-183, inhalation and dermal exposure might be important exposure routes for the Japanese people. Geyer et al. (2004) have predicted elimination half-lives of PBDEs in the human adipose tissue; the predicted half-lives of individual congeners in an adult male were as follows: TeBDE-47, 1.9 years; PeBDE-99, 3.5 years; PeBDE-100, 2.4 years; and HxBDE-153, 7.8 years. It is expected that the half-lives of Te-HxBDEs increase with the number of bromine atoms per molecule, and the half-life of HxBDE-153 is much longer than those of other dominant congeners detected in human serum. Further research is needed to examine the difference between the elimination half-lives and toxicity of individual PBDE congeners in animals and humans.

Table 1 Concentrations of PBDEs in serum samples from 10 Japanese males (ng/g lw)

Congener	Participant No									
	1	2	3	4	5	6	7	8	9	10
TrBDE-17	tr <0.04	tr <0.05	nd <0.01	nd <0.01	nd <0.02	nd <0.01	nd <0.02	nd <0.01	nd <0.02	nd <0.02
TrBDE-28/33	tr <0.2	0.37	0.16	tr <0.2	0.16	0.24	tr <0.2	0.17	tr <0.2	tr <0.2
TrBDE-37	tr <0.02	tr <0.09	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01	nd <0.01
TeBDE-49	nd <0.02	nd <0.03	0.09	tr <0.07	tr <0.08	0.07	nd <0.02	0.09	nd <0.03	tr <0.09
TeBDE-47	1.3	5.9	1.5	0.96	1.6	1.8	0.54	2.9	0.93	0.81
TeBDE-66	nd <0.04	nd <0.05	nd <0.04	nd <0.04	nd <0.04	nd <0.04	nd <0.04	tr <0.2	nd <0.05	nd <0.05
PeBDE-100	0.23	0.67	0.24	0.21	0.24	0.40	0.13	0.31	0.21	0.25
PeBDE-99	0.21	1.1	0.21	0.16	0.25	0.21	0.10	0.49	0.15	0.20
PeBDE-118	0.02	0.03	tr <0.02	tr <0.02	0.02	0.03	tr <0.02	tr <0.02	0.03	0.03
PeBDE-85	tr <0.07	tr <0.09	tr <0.07	nd <0.02	tr <0.08	nd <0.02	nd <0.02	tr <0.07	nd <0.03	nd <0.02
HxBDE-155	nd <0.02	tr <0.07	tr <0.05	tr <0.05	nd <0.02	tr <0.06	nd <0.02	nd <0.02	tr <0.07	nd <0.02
HxBDE-154	tr <0.06	0.08	0.05	0.05	tr <0.06	0.06	tr <0.06	tr <0.06	tr <0.07	tr <0.07
HxBDE-153	0.76	0.96	1.1	0.56	0.58	0.68	0.37	0.52	0.91	0.79
HpBDE-183	nd <0.1	nd <0.2	tr <0.4	tr <0.4	tr <0.4	tr <0.4	nd <0.1	nd <0.1	tr <0.5	nd <0.2
Sum of 4 PBDEs*	2.5	8.6	3.1	1.9	2.7	3.1	1.1	4.2	2.2	2.1

Abbreviations: tr, trace; nd, not detected. * Sum of TeBDE-47, PeBDE-100, PeBDE-99, and HxBDE-153

The sperm concentration and testis size of the 10 participants are shown in Table 2. The sperm concentration of these participants ranged from 25–115 million/mL. No participant had a sperm concentration below 20 million/mL, the minimum fertility standard established by the World Health Organization (World Health Organization 1999). Strong inverse correlations were observed between the serum HxBDE-153 concentration and sperm concentration ($r = -0.841$, $p = 0.002$; Fig. 2) and testis size ($r = -0.764$, $p = 0.01$). However, no significant relationships were observed between the serum concentrations of the other congeners and the sperm concentration (r ranged from −0.187 to −0.099, $p = 0.605$–0.786) or testis size (r ranged from −0.216 to −0.054, $p = 0.548$–0.883). Researchers have hypothesized that endocrine-disrupting chemicals with thyroid-hormonal or sex-hormonal activities might adversely affect male fertility. The thyroid-disrupting and estrogenic/antiestrogenic activities of PBDEs have been reported in several studies (Meerts et al. 2001; Zhou et al. 2002). In addition, considerable evidence regarding the reproductive effects of PBDEs is available from in vivo studies. Kuriyama et al. (2005) have reported that developmental exposure to a single low dose (60 μg/kg body weight) of PeBDE-99 decreased the sperm count in male Wistar rats. Although the levels of PBDEs found in our study are relatively low, we observed significant inverse associations between the serum concentration of HxBDE-153 and the sperm concentration and testis size; this suggests an association between the serum HxBDE-153 concentration and human sperm quality. The lack of a significant relationship among other individual PBDE congeners and sperm parameters might indicate a difference in bioactivity between the congeners. The relationship between PBDEs and sperm quality is a complicated problem and needs further study.

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Table 2 Sperm concentration and testis size of 10 Japanese males

	Participant No									
	1	2	3	4	5	6	7	8	9	10
Sperm concentration (million/mL)[a]	40	55	38	108	83	74	115	78	25	30
Testis size (mL)[b]	36	36	40	50	46	42	51	54	29	33

[a] Annual average of monthly data. [b] Total of right and left testes

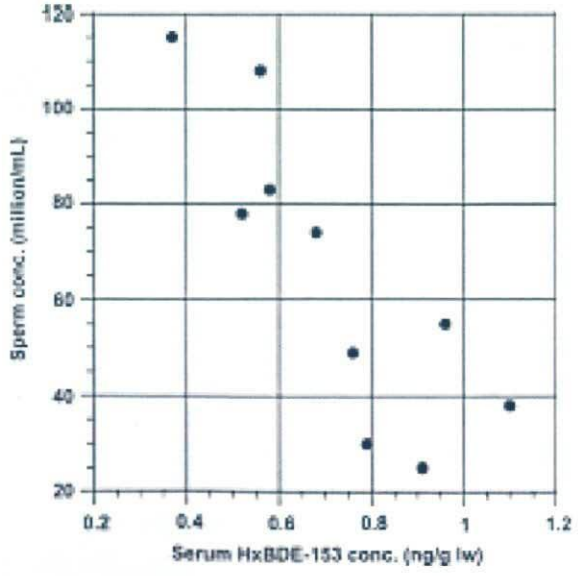

Fig. 2 Relationship between the serum HxBDE-153 concentration and sperm concentration

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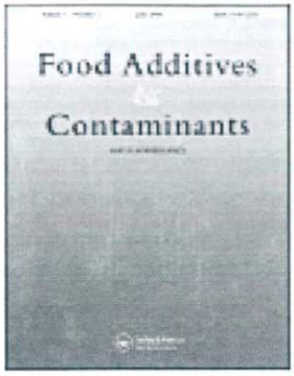

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Dietary intake estimations of polybrominated diphenyl ethers (PBDEs) based on a total diet study in Osaka, Japan

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This study presents the results of a total diet study performed for estimating the dietary intake of polybrominated diphenyl ethers (PBDEs) in Osaka, Japan. The concentrations of 36 PBDEs were measured in samples from 14 food groups (Groups I–XIV). PBDEs were detected only in Groups IV (oils and fats), V (legumes and their products), X (fish, shellfish, and their products), and XI (meat and eggs) at concentrations of 1.8, 0.03, 0.48, and 0.01 ng g^{-1}, respectively. For an average person, the lower bound dietary intakes of penta- and deca-formulations were estimated to be 46 and 21 ng day^{-1}, respectively. A high proportion of the decabrominated congener (DeBDE-209) was observed in Group IV. To confirm the presence of DeBDE-209 in vegetable oils, an additional analysis was performed using 18 vegetable oil samples. Of these, seven contained ng g^{-1} levels of DeBDE-209.

Keywords: polybrominated diphenyl ethers; total diet study; vegetable oil; decabromodiphenyl ether

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardants in a variety of consumer products such as electronics, furniture, and textiles. They are structurally similar to polychlorinated biphenyls (PCBs). There are theoretically 209 PBDE congeners from mono- to decabromo congeners. Technical mixtures of PBDEs are classified into three grades of penta-, octa- and decabromo diphenyl ethers (pentaBDE, octaBDE, and decaBDE, respectively) according to their average bromine content. The general compositions of these technical mixtures are as follows: pentaBDE, 0–1% tri-, 24–38% tetra-, 50–62% penta-, and 4–8% hexabromo congeners; octaBDE, 10–12% hexa-, 43–44% hepta-, 31–35% octa-, 9–11%, and nona-, 0–1% decabromo congeners; and decaBDE, 0.3–3% nona-, and 97–98% decabromo congeners (WHO/IPCS 1994).

Because PBDEs are not chemically bound to the materials, they can leak into the environment during the production, use, and disposal of the product. PBDEs are persistent and lipophilic in nature and, thus, they accumulate in the food chain (Darnerud et al. 2001; Burreau et al. 2006). PBDEs have been shown to alter thyroid homeostasis and hepatic enzyme activity in animal studies (Zhou et al. 2002). The reference doses of penta-, octa-, and decaBDEs for human oral chronic exposure are 2, 3, and 10 μg kg-body weight (b.w.)$^{-1}$ day^{-1}, respectively (US Environmental Protection Agency n.d. a–c). PBDEs have been recognized as environmental pollutants of global concern because their levels in the environment and humans have increased markedly over the past several decades (Meironyté et al. 1999, Ikonomou et al. 2002, Akutsu et al. 2003, Sjödin et al. 2004). Fish and other fatty foods have been recognized as sources of PBDE contamination in humans (Bocio et al. 2003, Joint FAO/WHO Expert Committee on Food Additives (JECFA) 2005).

The present authors have reported the PBDE levels detected in marine fish (Akutsu et al. 2001) and dietary supplements (Akutsu et al. 2006a, b) from samples collected in Japan. Other researchers have also reported varying PBDE levels in a variety of Japanese food items (Ohta et al. 2002, Ashizuka et al. 2004, Wada et al. 2005). However, human exposure to PBDEs has not been sufficiently documented through a total diet study (TDS). The aim of the study was to estimate the dietary intake of PBDEs by a TDS performed on a local scale in Osaka, Japan. Further, the results of an additional analysis of 18 vegetable oil samples are discussed.

Materials and methods

Sample collection

A total of 125 types of food samples were purchased from two supermarkets in Osaka, Japan, in 2006.

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Table 1. Information regarding the 14 food groups in the total diet study in Osaka, 2006.

Group no.	Food composition	Food variety (No. of food items)	Lipid content (%)	Daily intake per capita ($g d^{-1}$)
I	Rice and rice products	2	0.28	334
II	Grains, seeds, and tubers	15	2.9	179
III	Sugar and confectioneries	6	20	34.2
IV	Oils and fats	4	93	11.4
V	Legumes and their products	6	8.2	53.8
VI	Fruits	11	0.16	123
VII	Brightly coloured vegetables	13	0.29	100
VIII	Other vegetables, mushrooms, and seaweeds	13	0.28	184
IX	Beverages	7	0.01	577
X	Fish, shellfish, and their products	23	7.7	89.4
XI	Meat and eggs	8	18	124
XII	Milk and dairy products	5	6.7	157
XIII	Seasonings and other processed foods	11	8.8	91.3
XIV	Drinking water	1	0	2.50

Daily intake was estimated for an average consumer in Osaka based on the reports of the National Nutrition Survey, 2001–2003.

TDS samples were prepared based on the official food classification and consumption data obtained from the National Nutrition Survey, which was conducted by the Ministry of Health and Welfare of Japan from 2001 to 2003 (Ministry of Health, Labour and Welfare, Japan 2003, 2004, and 2006). These food samples were cooked or prepared for consumption in a typical manner and were then weighed according to relative consumption amounts. The weighed foods were mixed and homogenized to form 14 food-group composites. These food groups were designated as Groups I–XIV, as shown in Table 1. In addition, 18 bottled vegetable oil samples obtained from rapeseed, corn, safflower, sesame, olive, and soybean were purchased from two supermarkets in Osaka in 2006 (Table 2). In addition, one brand of bottled cod liver oil that was previously analysed for PBDEs was used as the in-house reference material (Akutsu et al. 2006b). Since the samples considered included only the leading brands available in the Japanese market, this was not a comprehensive survey of all the available brands.

Chemicals

Native and isotopically labelled standard solutions of PBDEs were purchased from AccuStandard (New Haven, CT, USA) and Wellington Laboratories (Ontario, Canada). In this study, 36 PBDE congeners having three to ten bromine atoms were monitored; tri-, tetra-, penta-, hexa-, hepta-, octa-, nona-, and decabromo congeners were abbreviated as TrBDE, TeBDE, PeBDE, HxBDE, HpBDE, OcBDE, NoBDE, and DeBDE, respectively. PBDE numbers were assigned according to the International Union of Pure and Applied Chemistry (IUPAC) PCB nomenclature (US Environmental Protection Agency n.d. d). The monitored congeners included eight TrBDEs (#17, #25, #28, #30, #32, #33, #35, and #37); six TeBDEs (#47, #49, #66, #71, #75, and #77); seven PeBDEs (#85, #99, #100, #116, #118, #119, and #126); five HxBDEs (#138, #153, #154, #155, and #166); three HpBDEs (#181, #183, and #190); three OcBDEs (#196, #197, and #203); three NoBDEs (#206, #207, and #208); and one DeBDE (#209). Most of these were representative congeners found in technical penta-, octa-, and decaBDE products (La Guardia et al. 2006). The nine isotopically labelled PBDEs ($^{13}C_{12}$-TrBDE-28, $^{13}C_{12}$-TeBDE-47, $^{13}C_{12}$-PeBDE-99, $^{13}C_{12}$-HxBDE-153, $^{13}C_{12}$-HxBDE-154, $^{13}C_{12}$-HpBDE-183, $^{13}C_{12}$-OcBDE-197, $^{13}C_{12}$-NoBDE-207, and $^{13}C_{12}$-DeBDE-209) were used as surrogate internal standards, and $^{13}C_{12}$-PeBDE-126 was used as an injection standard. Acetone, acetonitrile, and *n*-hexane of pesticide-analysis grade and 44% sulfuric acid-impregnated silica gel and *n*-nonane of dioxin-analysis grade were purchased from Wako Pure Chemicals (Osaka, Japan).

PBDE measurements

The TDS sample (5 g) was digested with 1 mol l^{-1} KOH aqueous-ethanolic solution (50 ml) for 2 h at room temperature after adding $^{13}C_{12}$-labelled surrogate internal standards. Subsequently, alkaline hydrolysate was extracted twice with 50 ml *n*-hexane. The extract was purified with sulfuric acid-impregnated silica gel (3 g) by using 30 ml *n*-hexane as an eluent. The eluate was concentrated, and then spiked with a $^{13}C_{12}$-labelled injection standard. The additional vegetable oil samples (0.5 g) were diluted with 10 ml *n*-hexane after adding $^{13}C_{12}$-labelled surrogate internal standards and partitioned three times with 20 ml *n*-hexane-saturated acetonitrile. The acetonitrile phase was combined and evaporated to dryness. The residue was treated with the sulfuric acid-impregnated silica

Table 2. Information regarding the vegetable oil samples.

No.	Oil composition	Country of origin	Bottle material
1	Rapeseed oil	NA	PET
2	Rapeseed oil	NA	PET
3	Rapeseed oil	NA	PE, EVOH
4	Rapeseed oil	Japan	PET
5	Rapeseed oil	Japan	Glass
6	Rapeseed oil	Japan	Glass
7	Blended oil (rapeseed, soybean)	NA	PE, EVOH
8	Blended oil (rapeseed, soybean)	NA	PE, EVOH
9	Corn oil	NA	PET
10	Corn oil	NA	PET
11	Corn oil	NA	PE, EVOH
12	Safflower oil	NA	PET
13	Safflower oil	NA	PET
14	Sesame oil	NA	Glass
15	Sesame oil	NA	Glass
16	Blended oil (perilla, sesame)	NA	Glass
17	Olive oil	Spain	Glass
18	Blended oil (unspecified)	NA	PET

Abbreviations: NA, not available; PET, polyethylene terephthalate; PE, polyethylene; EVOH, ethylene-vinyl alcohol copolymer.

gel (3 g) and then spiked with the $^{13}C_{12}$-labelled injection standard. The cleaned extract was assayed with a 6890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a JMS-GCmate II mass spectrometer (JEOL, Tokyo, Japan). Separation was carried out in a DB-1MS fused-silica capillary column (15 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies) using helium as a carrier gas (flow rate = 1 ml min^{-1}). The injector temperature was 250°C, and the injection volume was 1 μl (splitless). The column temperature was programmed from 100°C (2 min) to 310°C (3 min) at a rate of 10°C min^{-1}. The mass spectrometer was operated in an electron ionization mode with selected ion monitoring (35 eV; resolution = 1000). The ion source and interface temperatures were 280 and 310°C, respectively. Quantitation was based on an isotope dilution method by using $^{13}C_{12}$-labelled internal standards.

The identification of PBDE congeners was performed by comparing their relative retention times and isotope ratios with those of authentic calibration standards. A deviation of the ion intensity ratios within ±20% of the mean values of the calibration standards was considered acceptable. Procedural blank samples, matrix-spiking samples, and in-house reference samples were prepared and analysed in parallel with the study samples. Quality control standards were measured after every ten samples to check for instrumental drift. The limit of detection (LOD) for each congener was defined as three times the mean procedural blank value. The LODs of individual PBDE congeners varied from 0.01 to 0.1 ng g^{-1}. In the analysis of three split in-house reference samples, the coefficients of variation for all the ten detected PBDE congeners (#28/33, #47, #49, #66, #99, #100, #153, #154, and #155) were below 15%. The mean per cent recoveries of representative PBDE congeners ranged from 80% to 110% with relative standard deviations of less than 10% ($n = 3$; spiking level = 0.1–1 ng g^{-1}). The accuracy of the calibration standard mixtures and reliability of our performance for PBDE measurements, including DeBDE-209, were indicated by the satisfactory results in the intercalibration study on organobromine compounds (Takahashi et al. 2006).

Results and discussion

Example chromatograms of the standard solution and TDS samples are shown in Figure 1. Of the 36 monitored congeners, the levels of a total of 11 congeners from the TDS samples (#28/33, #47, #49, #66, #99, #100, #153, #154, #155, and #209) were detected above the LOD levels. The PBDE concentrations in the TDS samples are shown in Table 3. PBDEs were detected only in food groups IV (oils and fats), V (legumes and their products), X (fish, shellfish, and their products), and XI (meat and eggs) at concentrations of 1.8, 0.03, 0.48, and 0.08 ng g^{-1}, respectively (assuming not detected (ND) = 0). A high proportion of DeBDE-209 – a major constituent of decaBDE – was observed in the group IV food samples. The concentration and congener profile of PBDEs in food group X were similar to previous results observed in Japanese marine fish (Akutsu et al. 2001).

Figure 2 reveals the lower-bound intake of PBDEs (assuming ND = 0). For an average person (54 kg b.w.), we estimated the lower bound of the dietary intakes of the penta- and decaBDEs to be 46 and 21 ng day^{-1}, respectively. (PentaBDE was assumed to be the sum of 26 PBDEs: #17, #25, #28, #30, #32, #33, #35, #37, #47, #49, #66, #71, #75, #77, #85, #99, #100, #116, #118, #119, #126, #138, #153, #154, #155, and #166. decaBDE was assumed to be the sum of four PBDEs: #206, #207, #208, and #209.) Further, we estimated the middle- (ND = 0.5 LOD) and upper-bound intakes (ND = LOD) to be 330 and 610 ng day^{-1} for pentaBDE and 310 and 600 ng day^{-1} for decaBDE, respectively. The middle- and upper-bound values of penta- and decaBDEs may grossly overestimate the actual exposure levels because of the great proportion of undetected data in most food groups. The comparison data of the estimated dietary intake of PBDEs in different countries are shown in Table 4. The lower bound of the PBDE dietary intake value estimated in this study was almost comparable with that reported in the UK (91 ng day^{-1}) (Harrad et al. 2004), the USA

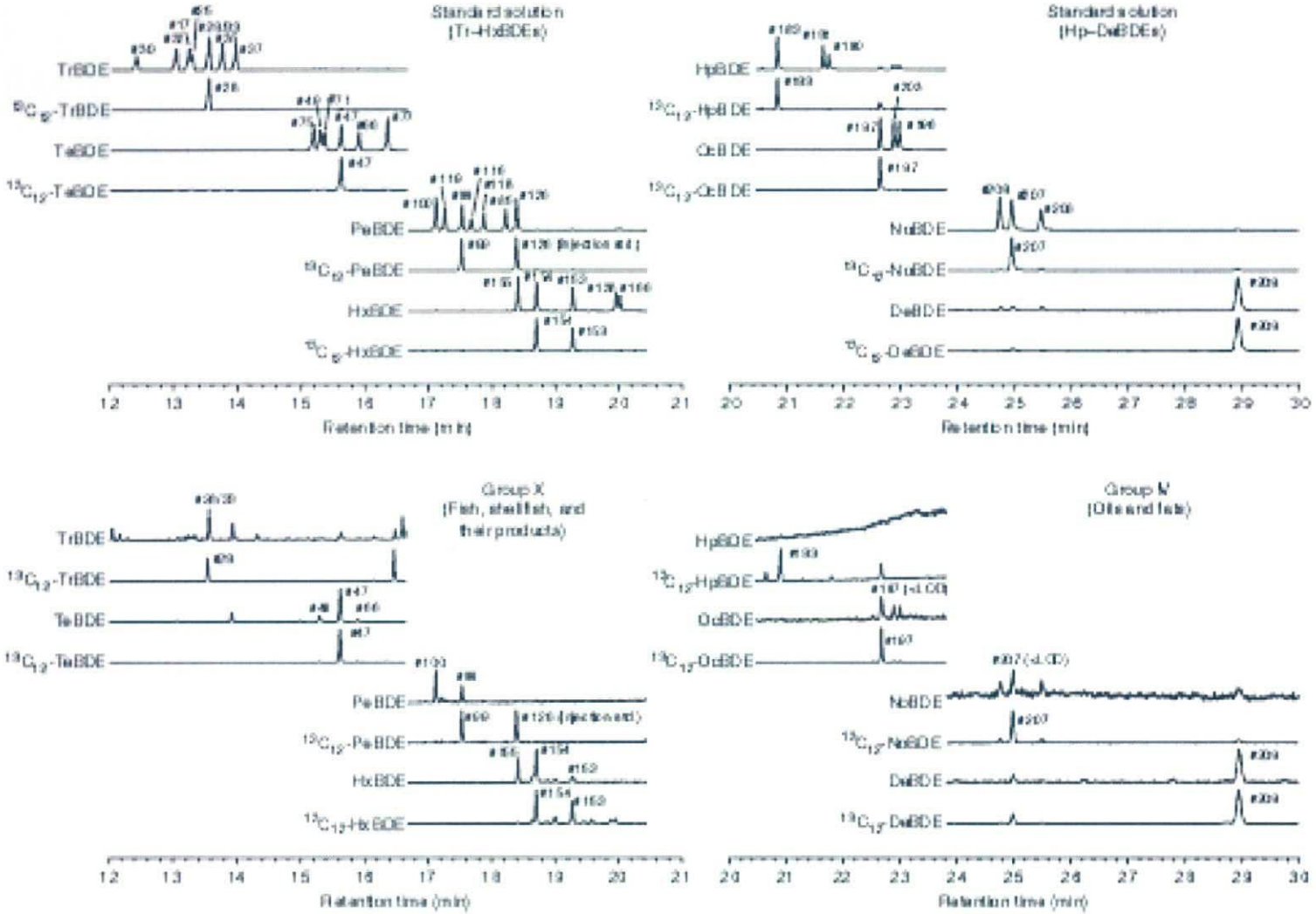

Figure 1. Example chromatograms of standard solution and food samples.

Table 3. Concentrations of PBDEs in foods (ng g^{-1}).

Group No.	TrBDE-28/33	TeBDE-49	TeBDE-47	TeBDE-66	PeBDE-100	PeBDE-99	HxBDE-155	HxBDE-154	HxBDE-153	DeBDE-209	Total PBDEs (sum of 36 congeners)
IV	0.01	ND (0.01)	0.01	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	1.8	1.8
V	ND (0.01)	ND (0.01)	0.01	ND (0.01)	ND (0.01)	0.02	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.1)	0.03
X	0.04	0.06	0.22	0.03	0.04	0.03	0.02	0.03	0.01	ND (0.1)	0.48
XI	ND (0.01)	ND (0.01)	0.01	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.01)	ND (0.1)	0.01

Note: Only congeners detected in at least one sample are shown in the table. PBDEs were not detected in food group nos. I, II, III, VI, VII, VIII, IX, XII, XIII, and XIV. The concentrations of total PBDEs were calculated by assuming the nondetected values as zero. Abbreviations: ND, not detected. Limits of detection are shown in parentheses.