Temporal trend and age-dependent serum concentration of phenolic organohalogen contaminants in Japanese men during 1989-2010

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Abstract

The temporal trend in serum concentrations of phenolic organohalogen contaminants (POCs) were investigated in two age groups of men from Kyoto, Japan, from 1989 to 2010. These concentrations and trends were compared with neutral contaminants including polychlorinated biphenyls (PCBs) and pesticides. Serum concentrations of pentachlorophenol (PenCP) and 4-hydroxy-PCB187 were age-dependent and decreased to approximately one-half during the two decades, whereas no contamination trends were observed for 2,4,6-tribromophenol (TriBP), tetrabromobisphenol A (TBBPA) and 6-hydroxy-2,2',4,4'-tetrabromodiphenyl ether (6-OH-BDE47). 6-OH-BDE47 was found in all samples (up to 3000 pg/g wet weight), whereas TBBPA was detected in 17 of 60 serum samples (up to 950 pg/g wet weight). The concentrations of TriBP, TBBPA and 6-OH-BDE47 were not correlated to those of PenCP or 4-OH-PCB187 in either age group, suggesting the different kinetics on exposure routes and fate between these brominated and chlorinated POCs.

Capsule abstract

>Pentachlorophenol and 6-hydroxy-tetrabromodiphenyl ether were predominant in serum.
>Age-dependent decrease of serum neutral organohalogen concentrations during the two decades.

1. Introduction

Despite the use of persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), having either been phased-out or restricted, they are still found in the environment, in both neutral and phenolic forms. Phenolic organohalogen contaminants (POCs) of concern may include pentachlorophenol (PenCP), hydroxylated PCBs (OH-PCBs) and hydroxylated PBDEs (OH-PBDEs) and brominated flame retardants (BFRs), such as 2,4,6-tribromophenol (TriBP) and tetrabromobisphenol A (TBBPA). These POCs have endocrine-disrupting effects and may influence neurological, reproductive and sexual development (Lans et al., 1993; Meerts et al., 2000; Montañó et al., 2013; Suzuki et al., 2008). However, limited information is available on human exposure to these POCs in contrast to traditional neutral organohalogens.

PenCP, which is the predominant POC in human serum, has similar concentrations to persistent pesticide i.e., 4,4'-DDE (Meijer et al., 2008; Rylander et al., 2012). PenCP has been used as an herbicide in agricultural chemicals and preservatives in Japan until 1990 (Suzuki et al., 2008). The usage of PenCP in the past might have led to its ubiquitous presence because of its persistence and, consequently, to contamination of the indoor environments (Ge et al., 2007; Inoue et al., 2006; Suzuki et al., 2008). OH-PCBs are another group of POCs that have been commonly detected in human tissues. PenCP and certain OH-PCBs have shown the capability of interacting in human blood with thyroid hormones (Meerts et al., 2002; Otake et al., 2007), which play an important role in fetal growth and development (Zheng et al., 2012). Previous studies have suggested that PenCP and OH-PCBs are responsible for adverse effects on thyroid or sex hormone homeostasis (Meerts et al., 2002) and are transferred from mother to fetus via the placenta during pregnancy (Meijer et al., 2008).

Of brominated POCs, TriBP and TBBPA are now used as flame retardants in many industrial and computer products. In Asian countries, the total production of both BFRs accounted for 85,900 tons

in 1999 of which 36% are produced in Japan (Watanabe and Sakai, 2003). TriBP is not used directly as a BFR but as an intermediate for brominated epoxy resins made from TBBPA (9500 tons/year in 2001) (Suzuki et al., 2008; Watanabe and Sakai, 2003). TriBP is also **naturally** produced by marine bacteria or plants and **has been detected** in fish (Haraguchi et al., 2010; Whitfield et al., 1999). Household materials containing TriBP may exist as indoor sources, whereas seafood may be a dietary source of TriBP. TBBPA, in contrast, is a reactive BFR that is released into the environment via many household electrical products and their disposal. Because of the short half-life of these phenolic BFRs in air, water, sediment and in humans (Covaci et al., 2009), their environmental distribution has been limited. However, phenolic BFRs have been reported to be thyroid hormone agonists and **estrogenic in vitro** (Kitamura et al., 2002; Meerts et al., 2001), and thus have potential for posing a threat to human health even at a low-dose exposure.

Exposure to hydroxylated PBDEs (OH-PBDEs) may be a more recent concern. Two congeners, e.g., 2'-OH-BDE68 and 6-OH-BDE47, have been frequently detected in blood of fish (Marsh et al., 2004), marine mammals (Marsh et al., 2005) and humans (Eguchi et al., 2012; Fujii et al., 2012). The ortho-substituted OH-PBDEs are proposed to be of natural origin, whereas meta- or para-substituted OH-PBDE congeners detected in other studies may be possible metabolites of PBDEs (Athanasiadou et al., 2008). OH-PBDEs have been shown to have a higher affinity for human transthyretin (TTR) than PBDEs in liver (Meerts et al., 2000). However, the formation process and exposure routes of OH-PBDEs remain unclear.

Previous studies have suggested that the body burden of legacy neutral contaminants, such as PCBs or chlorinated pesticides, has decreased from 1973 to 1996 (Masauda et al., 2005), but there is much less information available on the temporal trends of POCs in human tissues. Therefore, retrospective monitoring of POCs is required to judge the development of pollution and to assess the fate of contaminants in humans.

The aim of the present study was to investigate the temporal trends of serum POC concentration in Japanese populations from 1989 to 2010. Target chemicals consisted of representative congeners, **1** PenCP and 4-OH-PCB187; 2) TriBP and TBBPA; and 3) 6-OH-BDE47. To avoid possible

variation of body burdens by age and gender, this study was restricted to male subjects in two groups, aged in their 20s (21–29 years) and >50 years of age, at different sampling periods. Furthermore, the temporal trends in serum POC concentrations were compared with those of legacy neutral contaminants including α -endosulfan.

2. Materials and methods

2.1. Sample collection

Human serum samples were obtained from the Kyoto University Human Specimen Bank using a standardized protocol (Koizumi et al., 2005). Individual serum samples (1–2 mL each, n=60) were collected from healthy volunteers in the Kyoto area that consisted of two groups of males, aged in their 20s and >50 years of age, in 1989, 1999 and 2010 (Table 1). The Ethics Committee of Kyoto University approved the protocol of the present study (E25), and appropriate written informed consent was obtained from all of the participants. Samples were stored in clean screw-capped plastic containers at -20°C until the time of analysis.

2.2. Chemicals

Three internal standards, $^{13}\text{C}_9$ -labeled α -endosulfan, $^{13}\text{C}_6$ -labeled PenCP, and $^{13}\text{C}_{12}$ -labeled 4-hydroxy-2,2',4,4',5,5'-hexachlorobiphenyl (4-OH-[$^{13}\text{C}_{12}$]PCB187), were used for the determination of target POCs. Pesticide standard solution (unlabeled pesticide mix #1037; 2 $\mu\text{g}/\text{mL}$) was purchased from Kanto Chemical Co., Ltd., (Tokyo, Japan). The standards of two PCB congeners (PCB-153 and PCB-187) and α -endosulfan were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). PenCP, TriBP, 2,2-bis(3,5-dibromo-4-hydroxyphenyl)propane (TBBPA), 4-hydroxy-PCB187, 6-hydroxy-BDE47 and their O-methylated analogs were purchased from Wellington Laboratories Japan Inc. (Tokyo, Japan). These standards were used for the calibration, recovery, and quantification of the target compounds. Silica-gel (Wako gel S-1) used for purification was obtained from Wako Pure Industries Ltd. (Osaka, Japan) and was heated at 130°C for 3 hours prior to use. All solvents used (i.e., ethanol, diethylether, *n*-hexane and dichloromethane) were of

pesticide-grade quality, and purchased from Kanto Chemical Co. Inc. Japan. Diazomethane was prepared by 1-methyl-3-nitro-1-nitrosoguanidine (Kanto Chemical Co., Inc. Japan).

2.3. *Extraction, clean-up and fractionation procedures*

The methodology used to analyze neutral and phenolic contaminants in serum samples was based on lipid extraction, gel permeation chromatography (GPC) and silica-gel column cleanup, and gas chromatography-mass spectrometry with electron capture negative ionization (GC/MS/ECNI) analysis as previously described (Fujii et al., 2012a). Briefly, each 1-mL serum sample was spiked with three internal standards, namely [$^{13}\text{C}_6$]PenCP and 4-OH- $^{13}\text{C}_{12}$]PCB187 for phenolic analytes (0.2 ng of each) and α - $^{13}\text{C}_9$]endosulfan for neutral OCs (2 ng). The sample was extracted with *n*-hexane, after adding formic acid (0.1% v/v), ethanol, and diethyl ether. A combined extract was dissolved in dichloromethane (DCM):*n*-hexane (1:1), and then subjected to GPC with a Bio-Beads S-X3 column (Bio-Rad Laboratories, Hercules, CA, USA). The gel material (35 g) was packed in a glass column (55 cm $\times$ 27 m i.d.) with DCM:*n*-hexane (1:1) as the eluting solvent at a flow rate of 4 mL/min. The first 96-mL fraction of the eluate contained lipids and was discarded. Subsequently, the next 68 mL fraction was collected. The eluate was concentrated and partitioned between 1 M KOH:ethanol (7:3) and *n*-hexane. The phenolic contaminants in the KOH solution were back-extracted twice with 20% diethylether in *n*-hexane. The phenolic fraction was derivatized to O-methylated analogs by diazomethane in diethyl ether. The neutral and methylated phenolic fractions were purified with a silica-gel column (0.2 g, Wako gel S-1) by an elution of 15 mL DCM:*n*-hexane (12:88, v/v). Each fraction was concentrated to 200 μL and spiked with ^{13}C -labeled PCB-153 to determine the recoveries of internal standards and to correct the volume of samples prior to GC/MS analysis.

2.4. *Instruments and quantification*

Fifteen analytes were measured by GC/MS/ECNI using an Agilent GC/MSD 5973i (Agilent Technologies, Santa Clara, CA, USA) coupled with a 6890N gas chromatograph. The GC/MS

conditions and target ions for the determination of analytes are summarized in Supplementary Table S1. Quantification of the compounds was based on signals in the mass chromatograms and on comparison with ^{13}C -PCB153 that was used as a syringe **standard**. The concentrations of all analytes were reported as picogram per gram **wet weight (pg/g ww)**.

2.5. Quality control and quality assurance

The extraction, cleanup, and fractionation steps were evaluated by measuring the absolute recoveries of the compounds ($^{13}\text{C}_{12}$ -labeled internal and native surrogate standards). **For recovery tests, two concentrations (2.0 and 10.0 ng/mL) of 11 analytes were spiked into cow milk, passed through the entire analytical procedure and determined based on GC/MS-selected ion monitoring.** Procedural blanks were analyzed simultaneously with every batch of 10 samples to check for interference or contamination from solvents and glassware. The recoveries were between 81% and 94% with relative standard deviations of <10% ($n = 5$). The limits of quantification (LOQ), defined as 10-fold that of the noise level, ranged from 1 to 50 pg/g **ww** (Supplementary Table S1). When the **concentrations** of the target chemicals were less than their LOQs, we allocated half of the LOQ as the value for analysis. The calibration (2 to 100 ng/mL of each analyte) was linear and characterized by good correlation coefficients (>0.99). The quality of the method under validation was verified by Standard Reference Materials (non-fortified human serum, SRM1974, NIST) for PCBs and selected pesticides. Data from the current study were within 15% of the certified values of SRM1974.

2.6. Statistical analysis

The data were analyzed using SPSS version 16.0 for Windows 2007 (SPSS Inc., Chicago, IL, USA). Steel-Dwass test and Mann-Whitney U-test were used to examine differences in the target chemical concentrations in three sampling years and between two age groups. Spearman's rank correlation coefficients were used to evaluate the relationship between neutral and phenolic OCs. Probability values of less than 0.05 were considered to indicate statistical significance.

3. Results and discussion

3.1 Legacy persistent organohalogen pollutants (POPs)

Within the current study group of legacy organohalogens, serum concentrations of the representative congeners, 4,4'-DDE, PCB-153, *trans*-nonachlor, hexachlorobenzene (HCB) and α -endosulfan are summarized in Table 2. During the two decades from 1989 to 2010, the mean serum concentrations were significantly decreased from 5620 to 1620 pg/g ww for 4,4'-DDE, from 1070 to 680 pg/g ww for PCB-153, and from 180 to 86 pg/g ww for *trans*-nonachlor. These contaminants were significantly higher in the >50 years of age group than in the 20 years of age group ($p<0.01$) in each sampling year. For legacy POPs, older people in 2010 has been exposed to these contaminants for more decades, and when the contamination was decreasing, age-difference may influence the trend. Such age-dependency in the concentrations of legacy POPs has also been observed in blood samples in 1999 ($n=151$) where the concentrations of pesticides were positively correlated with age (20s to 50s) (Masuda et al., 2005).

α -Endosulfan was detected in all serum samples (mean, 160 pg/g ww in 1989) at comparable concentrations to *trans*-nonachlor, and then were gradually reduced to 85 pg/g ww in 2011. The concentrations of α -endosulfan were positively correlated to those of PCBs, *trans*-nonachlor and HCB ($p<0.01$) but not to those of 4,4'-DDE (Table 3). Unlike 4,4'-DDE, no decreasing trend was observed for α -endosulfan between 1999 and 2010. This trend is most likely due to continuous local usage of endosulfans until its agricultural registration had expired in 2010. A recent dietary survey has reported an exponential increase in α - and β -endosulfans in diets from China and Korea (Desalegn et al., 2011). However, no such increasing trend appeared in foods from Japan in that study. The present concentrations of endosulfans and residue profiles of pesticides in serum of Japanese men may be correlated to those in Japanese breast milk which has been contaminated with endosulfans at similar concentrations to other pesticides (Fujii et al., 2012b).

3.2. PenCP and OH-PCBs

Serum PenCP concentrations ranged from 89 to 1670 pg/g ww with a mean of 650 pg/g ww in

1989 and then decreased to 140 pg/g ww in 2010 (Table 2). No age-dependency in these concentrations was observed. The mean serum PenCP concentrations were at comparable concentrations to *trans*-nonachlor but not significantly correlated to each other (Table 3). Based on previous studies, the current concentrations are lower than those of European women (Glynn et al., 2011; Meijer et al., 2008; Rylander et al., 2012).

Unlike other pesticides, such as 4,4'-DDE and *trans*-nonachlor, PenCP concentrations in the 20 years of age group in 1989 ranged from 37 to 1670 pg/g ww, which were an order of magnitude greater than those of the >50 years of age group (Fig. 1). Such large variation of PenCP in young ages has also been observed in Norwegian children (Thomsen et al., 2002). This may be attributed to the combined exposure routes, dietary ingestion of contaminated seafood (Ge et al 2007; Guvenius et al., 2003; Sjödin et al., 2001), and dust ingestion via inhalation (Inoue et al., 2006; Suzuki et al., 2008). It should not be excluded that PenCP may exist as a de-methylated metabolite of pentachloroanisole (Ikeda and Sapienza, 1995) or as a hydroxylated metabolite of hexachlorobenzene in serum (To-Figueras et al., 1997).

For the monitoring of hydroxylated PCBs, we measured 4-OH-PCB187 that has been detected as one of major components in human blood (Rylander et al., 2012; Sandau et al., 2002). Serum concentrations of 4-OH-PCB187 in both age groups ranged from <LOQ to 800 pg/g ww (mean 120 pg/g ww), which are comparable to previous results from Japanese women (15–43 pg/g ww, Kawashiro et al., 2008; 12–370 pg/g ww, Nomiya et al., 2010), and those from eastern Slovakia (273 pg/g ww, Park et al., 2009), Latvia (50 pg/g ww), and Sweden (120 pg/g ww, Sjödin et al., 2001).

The concentration of 4-OH-PCB187 was positively correlated to that of the parent compound PCB-187 ($r=0.747$, $p<0.01$, Fig. 2) but not significantly to that of PenCP ($r=0.178$, $p>0.05$, Table 3). This finding was also observed in other studies (Rylander et al., 2012). No significant differences in the ratio were observed between the 20 and >50 years of age groups. The mean concentration ratio of OH-PCB187 to PCB-187 was 1.4 in 1989, but it declined to 0.6 in 1999 and 2010. These findings suggest that OH-PCBs may be eliminated faster than the parent PCBs in individuals with lower

concentrations of PCBs. This association indicates that the variation of 4-OH-PCB187 would depend on the extent of dietary exposure to the parent PCBs. This same phenomenon has been observed by Dirtu et al. (2009).

3.3. TriBP and TBBPA

Serum TriBP concentrations ranged from 46 to 960 pg/g ww (mean 248 pg/g ww) and showed slightly increasing trends in both age groups during the two decades (Fig. 1). Unlike the chlorinated pesticide, no age-dependency was observed for TriBP. Compared with other studies, the current TriBP concentrations were one order of magnitude greater than those of pregnant women (mean 22 pg/g ww; Kawashiro et al., 2008) or those of Norwegians during 1977–1999 (0.077–26 pg/g ww; Thomsen et al., 2002), whereas concentrations were comparable to those of Indians (72–1200 pg/g ww, mean 360 pg/g ww, Eguchi et al., 2012).

Serum TriBP concentrations were not correlated to those of the other POCs (Table 3). This may be explained by the combined exposure to TriBP from different sources. One possible exposure source would be via dietary seafood because TriBP is a naturally produced flavor in marine algae and fish (Haraguchi et al., 2010; Whitfield et al., 1999), which can be bioconcentrated in higher trophic organisms (Whitfield et al., 1999). Another possible source may be house dust contaminated with TriBP, which was used as a BFR intermediate and released into the air by leakage (Watanabe and Sakai, 2003) and indoor dust (Suzuki et al., 2008). Because of the lack of data on other TriBP exposure routes, we have no conclusion regarding the relative importance of diet or house dust.

TBBPA was detected in 28% of the studied samples (17 out of 60) with concentrations ranging from <LOQ (50 pg/g ww) to 950 pg/g ww (Table 2). The highest concentration was observed in the 20 years of age group in 1999. None of volunteers were occupationally exposed to TBBPA. The mean concentration of TBBPA in the detected samples (n=17) was 98 pg/g ww, which is higher than that of the Fukuoka residents investigated in 2000 (7 pg/g ww, Nagayama et al., 2000) and that of pregnant women from Japan (26 pg/g ww, Kawashiro et al., 2008). As the present mean values of TBBPA were based on only 17 samples (28% detection frequency), the present TBBPA

concentrations may be overestimated. However, our results in 1999 and 2010 are comparable to the serum concentrations from Belgium (80 pg/g ww; Dirtu et al., 2008) or France (154 pg/g ww; Cariou et al., 2008). This is the first study to report the temporal trend of TBBPA using human blood samples in Japan. This study could not clarify the relationship between concentrations of TriBP and TBBPA because of the small sample size utilized in this study (Table 3).

The exposure routes of TBBPA are possibly via diet and house dust, which is similar to that of TriBP. The Norwegian population was exposed to TBBPA via diet as a major source (Thomsen et al., 2001). However, plasma concentrations of TBBPA were higher in the workers at the dismantling plant (Thomsen et al., 2002) than that of the non-occupationally-exposed workers. Consistent with its phenolic structure, TBBPA can be rapidly conjugated in human liver and subsequently excreted in bile (Schauer et al., 2006). No age-dependency in serum concentrations of TBBPA may be because of its short half-life (two days) in human plasma (Hagmar et al., 2000). Therefore, the occurrence of TBBPA in human serum is likely to reflect recent exposure rather than past exposure (Covaci et al., 2009).

3.4. OH-PBDEs

Of phenolic PBDE congeners, 6-OH-BDE47 was predominantly detected in all serum samples investigated. The concentrations ranged from 25 to 3075 pg/g ww (mean 451 pg/g ww), which was 20-fold greater than those of BDE-47 (Table 2). The 6-OH-BDE47 concentrations were comparable to those of PenCP or 4-OH-PCB187. It was not significantly correlated to those of BDE-47 or to the other chlorinated OCs (data not shown). No age- or time-related differences were observed from 1988 to 2010 (Fig. 1). Other isomeric hydroxy- and methoxy-PBDEs have been found in human blood from India and Nicaragua (Athanasiadou et al., 2008; Eguchi et al., 2012), but we could not detect any metabolites in our samples. The *ortho*-substituted 6-OH-BDE47 detected in this study is considered to be of natural origin, although it can be also formed by human P460 *in vitro* (Erratico et al., 2013). The *meta*- and *para*-substituted OH-tetraBDEs detected in the other studies are most likely metabolites of parent PBDEs (Athanasiadou et al., 2008). The occurrence of 6-OH-BDE47 in

serum in this study may be a result of dietary exposure to 6-OH-BDE47 itself via seafood (Marsh et al., 2004; Wang et al., 2012) or demethylation of 6-MeO-BDE47 by microsomes of mammals (Wan et al., 2009). The toxicological implication of human exposure to OH-PBDEs remains unknown, but some studies have shown that 6-OH-BDE47 exhibits thyroid-disrupting effects and a higher affinity to human TTR than BDE-47 *in vitro* (Liu et al., 2011; Meerts et al., 2001).

4. Conclusions

Serum concentrations of legacy neutral contaminants in Japanese men showed a decreasing trend in two age groups from 1989 to 2010. Although the concentrations of PenCP and 4-OH-PCB187 showed a decreasing trend, TriBP and 6-OH-BDE47 appeared to be slightly increasing. TBBPA was detected in 28% of serum samples, whereas 6-OH-BDE47 was present at the highest concentrations of POCs investigated in all samples. No age-dependency was observed for all POCs tested. The concentrations of TriBP and 6-OH-BDE47 were not correlated with those of PenCP, OH-PCBs and chlorinated pesticides. These findings suggest that the kinetics on exposure routes and fate of the studied brominated POCs are different from chlorinated POCs and legacy POPs.

Acknowledgments

This study was supported mainly by Special Coordination Funds for Promoting Science and Technology (No. 1300001) sponsored by the Japan Science and Technology Agency and a Grant-in-Aid for Health Sciences Research from the Ministry of Health, Labour and Welfare of Japan (H21-Food-003 and H24-Food-002). The funding bodies had no role in the study design, data collection, or analysis, nor in decisions concerning the preparation and publication of the manuscript. The authors have no financial conflicts of interest.

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Fig. 1. Temporal trend in serum concentration (pg/g **wet weight**) of organohalogens in two age groups during 1989–2010 from Kyoto. Box plot: box, 25th–75th percentiles; center line, median value; whiskers, min-max values; dots, outliers; circle, extremes.

Fig. 2. Correlation between PCB-187 and 4-OH-PCB187 in serum concentration (pg/g wet weight) from Kyoto. Spearman's rank correlation coefficient, $r=0.747$, $p<0.01$.

Table 1 Information of serum samples collected from volunteers in Kyoto during 1989–2010.

Sample group	Region	M/F	n	Year of sampling	Mean age (range)
Serum89A	Kyoto	M	10	1989	20.6 (20-22)
Serum89B	Kyoto	M	10	1989	51.0 (50-52)
Serum99A	Kyoto	M	10	1999	24.1 (21-28)
Serum99B	Kyoto	M	10	1999	54.2 (50-59)
Serum10A	Kyoto	M	10	2010	21.5 (21-23)
Serum10B	Kyoto	M	10	2010	68.0 (65-69)

Table 2 Serum concentrations in Japanese men (20 and >50 age groups) from Kyoto during 1989–2010.

Contaminants	LOQ (pg/g ww)	Freq (%)	AM concentration (pg/g wet weight)						p value
			Sampling year			Age		p value	
			1989 (n=20)	1999 (n=20)	2010 (n=20)	20s (n=30)	>50s (n=30)		
Neutral OCs									
4,4'-DDE	50	100	5620* (330-12000)	3260 (390-8890)	1620 (470-6000)	<0.001	1970 (330-10900)	5030* (620-13000)	<0.001
HCB	2.0	100	490* (130-1230)	230 (120-450)	260 (43-660)	<0.001	250 (43-710)	400* (130-1230)	0.009
PCB-153	10	100	1070 (100-2830)	640 (200-1390)	680 (100-2140)	0.051	390 (100-1050)	1200* (330-2830)	<0.001
PCB-187	10	100	150 (13-560)	104 (21-240)	135 (20-480)	0.383	60 (13-200)	200* (49-560)	<0.001
trans-nonachlor	10	100	180 (10-714)	90 (12-190)	86 (4.1-530)	0.051	41 (4.1-190)	200* (42-710)	<0.001
α-endosulfan	10	100	160 (28-600)	59 (14-110)	85 (39-150)	0.004	61 (42-710)	140* (14-600)	0.002
Phenolic OCs									
PenCP	1.0	100	650* (89-1670)	300 (150-1060)	140 (37-850)	<0.001	430 (37-1670)	290 (50-1060))	0.917
4-OH-PCB187	20	92	220 (23-800)	67 (<LOQ -300)	76 (<LOQ -340)	0.792	42 (<LOQ -140)	200 (6.6-800)	0.241
TriBP	2.0	100	150 (46-370)	240 (110-380)	350* (62-960)	<0.001	250 (46-960)	250 (67-500)	0.149
TBBPA	50	28	NR (5%) (<LOQ -940)	NR (45%) (<LOQ -950)	NR (35%) (<LOQ -420)	NR	NR (33%) (<LOQ -950)	NR (23%) (<LOQ -420)	NR
6-OH-BDE47	20	100	200 (52-680)	550 (25-1590)	610 (34-3075)	0.032	360 (25-1580)	550 (52-3080)	0.182
Ratio									
4-OH-CB187/PCB-			1.4	0.6	0.6		0.7	1.0	

AM, arithmetic mean; LOQ, limit of quantification; n, number of samples; Freq%, % proportion of numbers that were >LOQ.

*Significantly higher than the other group ($p < 0.05$, Steel-Dwass test and Mann-Whitney U-test). p values, Spearman's rank correlation.

Concentrations below LOQ were treated as 1/2 LOQ for arithmetic mean. NR: not reported due to low detection frequency (<50%).

Table 3 Spearman's rank correlation coefficients between concentrations of analytes in human serum (n=60).

	HCB	PCB-153	PCB-187	<i>trans</i> -nonachlor	α -endosulfan	TriBP	PenCP	4-OH-PCB187	TBBPA	6-OH-BDE47
4,4'-DDE	0.519*	0.778**	0.651**	0.683**	0.329	-0.241	0.388	0.646*	-0.260	-0.204
HCB		0.588*	0.516*	0.585*	0.367	-0.089	0.226	0.518*	0.069	-0.186
PCB-153			0.951**	0.770**	0.585*	0.053	0.091	0.793**	-0.115	-0.069
PCB-187				0.736**	0.538*	0.191	-0.067	0.747**	-0.020	-0.026
<i>trans</i> -nonachlor					0.455*	0.132	0.160	0.629**	0.011	0.014
α -endosulfan						0.162	-0.007	0.474*	-0.140	-0.144
TriBP							-0.411	-0.105	0.412	0.340
PenCP								0.178	-0.159	-0.245
4-OH-PCB187									-0.258	-0.082
TBBPA										0.131

*P<0.05, **P<0.01