Chemosphere 287 (2022) 132300

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Application of 96-well plate SPE method for analysis of persistent organic pollutants in low volume blood serum samples

Jiří Palát [a], Petr Kukučka [a,*], Garry P. Codling [a,b], Elliott J. Price [a], Petr Janků [c,d], Jana Klánová [a]

[a] RECETOX Centre, Masaryk University, Kamenice 753/5, 62500, Brno, Czech Republic
[b] Toxicology Centre, University of Saskatchewan, 44 Campus Dr, SK, S7N 5B3, Saskatoon, Canada
[c] Department of Gynecology and Obstetrics, University Hospital Brno and Faculty of Medicine, Masaryk University Brno, Czech Republic
[d] Department of Nursing and Midwifery, Faculty of Medicine, Masaryk University Brno, Czech Republic

HIGHLIGHTS

- Validated SPE method for blood serum analysis of selected POPs was developed.
- Method uses low volumes of blood serum (200–250 μL).
- Use of 96 well plate format allows future automation of sample preparation.
- Method is applicable to large scale human biomonitoring.

ARTICLE INFO

Handling Editor: Jerzy Falandysz

Keywords:
POPs
NFRs
Blood serum
Well-plate SPE
Chromatography-mass spectrometry

ABSTRACT

Though many persistent organic pollutants (POPs) are closely regulated the human population is still exposed to these ubiquitous chemicals from the environment and diet. Safe management and human biomonitoring of POPs is necessary to understand the risk of exposure. Within human biomonitoring the mass of sample is often limited, therefore robust methods using smaller sample amounts are necessary. This study developed a 96-well plate solid phase extraction (SPE) method for determination of selected POPs: polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and non-persistent novel flame retardants (NFRs) in low volume blood serum. Non-destructive clean-up coupling Oasis HLB extraction plate with Phree phospholipid removal plate was employed. Extraction efficiency was determined at low and high concentrations in certified reference materials NIST SRM 1957 and 1958, respectively. Target compounds deviated from certified values on average by 15% and 21% for SRM 1957 and SRM 1958, respectively. Observed limit of detections (LODs) ranged from 0.36 pg/mL (PCB 180) to 66.07 pg/mL (δ-HCH). The applicability for real samples is demonstrated on 48 samples from pregnant women enrolled in the pilot phase of the CELSPAC: TNG study. In total, 30 target compounds were detected in at least one sample. The method developed here provides a fast and reliable analysis of human blood serum with possibility to introduce automation for the sample preparation procedure.

1. Introduction

Persistent organic pollutants (POPs) comprise a wide range of compounds that are stable, bio-accumulative, prone to long-range transport and toxic for the living organisms, including humans (Lohmann et al., 2007). Although the manufacture and use of POPs was globally restricted in the first decade of this century via adoption of the Stockholm Convention (Buccini, 2003), their persistence means they remain ubiquitous in the environment and present a continual exposure risk.

Typical POPs include polychlorinated biphenyls (PCBs), organochlorinated pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs). PCBs were manufactured primarily for use as dielectric fluids in transformers, additives in pigments, and in building materials (Erickson and Kaley, 2011), OCPs as plant protection chemicals and insecticides (Blus, 1995) and PBDEs for use as flame retardants, particularly in consumer products (Alaee et al., 2003; Sjödin et al.,

* Corresponding author.
E-mail address: kukucka@recetox.muni.cz (P. Kukučka).

https://doi.org/10.1016/j.chemosphere.2021.132300
Received 28 June 2021; Received in revised form 6 September 2021; Accepted 17 September 2021
Available online 20 September 2021

2003), until their replacement with novel flame retardants (NFRs) (Covaci et al., 2011). Nowadays, diet represents the main source for human exposure to most POPs (Fernandes et al., 2019; Guo et al., 2019).

Monitoring the concentrations of POPs in humans is vital for exposure risk assessment and observing the change in POPs concentration over time required to assess the effectiveness of measures taken to reduce population exposure (Angerer et al., 2007; Magulova and Priceputu, 2016). Human biomonitoring of POPs is often conducted in blood, providing a snapshot of the contaminants within the entire circulatory system. Blood serum POPs concentration has been associated with a multitude of adverse health effects, including cardio-metabolic disorders (Gasull et al., 2018; Ha et al., 2007; Lee et al., 2007), stroke (Lim et al., 2018), obesity and diabetes (Airaksinen et al., 2011; Lee et al., 2006; Yang et al., 2017) and cancer (Lim et al., 2017; Park et al., 2020; Wielsøe et al., 2017).

Traditionally, the measurement of POPs in blood has been conducted via liquid-liquid (LLE) extraction and/or conventional solid phase extraction (SPE). These methods usually require large sample volumes (>1 mL) and time-consuming clean-up with high solvent consumption (Salihovic et al., 2013). Since population studies often comprise large numbers of samples but in limited volume, the development of rapid analytical methods to measure POPs from small volumes of blood is valuable.

Recently, SPE approaches using small sample volumes have been developed. For example, Koponen et al. analysed 13 POPs in 200 μL of serum/plasma however, the method used column-based SPE which is time-consuming with high solvent consumption (Koponen et al., 2013). Miniaturized SPE well plates can considerably improve sample throughput. A fully automated on-line SPE approach enabled the quantification of 24 POPs from 200 μL plasma/serum via gas chromatography-high resoution mass spectrometry (GC-HRMS) (Wittsiepe et al., 2014). However, the method employed on-line procedures and large volume injections which may not be accessible for all laboratories. Alternatively, Stubleski et al. developed an off-line 96-well plate SPE method for extraction of POPs from 150 μL plasma and serum samples (Stubleski et al., 2018). The developed method provided good results for a wide range of common environmental contaminants but included a sulphuric acid-impregnated silica gel clean-up, limiting the analysis to persistent compounds.

Herein, a well-plate SPE extraction with less destructive clean-up has been developed by coupling a Phree phospholipid removal plate with Oasis HLB plate to permit analysis of selected POPs (7 PCBs, 12 OCPs, 10 PBDEs, HBCD), plus 6 non-persistent NFRs in blood serum. Validation of the method using standard reference materials and application to 48 samples from pregnant women enrolled in the pilot phase of the Central European Longitudinal Studies of Parents and Children: The Next Generation (CELSPAC: TNG) study is presented. Detection was via gas chromatography coupled with atmospheric pressure chemical ionization tandem mass spectrometry (GC-APCI-MS/MS), gas chromatography coupled with electron ionization tandem mass spectrometry (GC-EI-MS/MS) and liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

2. Materials and methods

2.1. Sample information and storage

Study samples and data were obtained from the CELSPAC: TNG. CELSPAC: TNG is designed as a new prospective birth cohort which will follow up 10 000 children from their prenatal period to adolescence with the aim of assessing environmental factors potentially affecting children's health. CELSPAC: TNG study is based on previous efforts of Masaryk University, especially the Czech part of the WHO-initiated European Longitudinal Study on Pregnancy and Childhood (ELSPAC) (Piler et al., 2017).

In the current study, samples from 48 mothers enrolled in the pilot phase of CELSPAC: TNG were analysed. The pilot was initiated to evaluate feasibility of the protocol for collection, processing and storing of biological samples including venous blood. The Ethical committee of University Hospital Brno, Czech Republic approved this study (No. 20140409-01). All mothers gave their written informed consent.

Venous blood was collected to S-Monovette® serum gel collection tubes in the 38th week of pregnancy. Serum was prepared by centrifugation (2500 g for 10 min at 20 °C), aliquoted (250 μL) into polypropylene (PP) cryogenic tubes (2 mL; CryoGen 1D ClearLine, Biosigma, Italy) and stored at −80 °C.

Lipid content was measured as part of the biobanking procedures and herein, blood serum concentrations of POPs are adjusted to lipids using previously published method (Rylander et al., 2006). This method calculates lipid concentrations using total cholesterol and triglycerides serum concentrations. Total cholesterol and triglycerides were determined enzymatically in blood serum in an accredited laboratory for clinical biochemistry.

2.2. Chemicals and reference materials

Dichloromethane (DCM; pesticide residue analysis (PRA) grade; J.T. Baker, USA), *n*-hexane (Hex; PRA grade; J.T. Baker, USA), nonane (PRA grade; LGC Promochem GmbH, Germany), acetonitrile (MeCN; LC-MS grade; Biosolve B.V, Netherlands), methanol (MeOH; LC-MS grade; Biosolve B.V, Netherlands), isopropanol (IPA; LC-MS grade; Honeywell Riedel de Haën, Germany), acetone (Ace; PRA grade; J.T. Baker, USA)) and Type I water (ultrapure; >18 MΩ-cm; purified on-site) were used.

SPE comprises Oasis HLB 96-well plate (60 mg; Waters, USA) and Phree phospholipid removal 96-well plate (30 mg; Phenomenex, USA). Anhydrous sodium sulphate (Na_2SO_4; Merck, Germany) was used for removal of residual water. Ammonium acetate for mobile phase modifier was from Sigma Aldrich, USA.

Analytical standards of native PCBs and OCPs were obtained from LGC (UK), ^{13}C-labelled PCBs and OCPs were obtained from Cambridge Isotope Labs (USA), native and ^{13}C-labelled BFRs were obtained from Wellington Labs (Canada) and Cambridge Isotope Labs (USA).

Unless indicated otherwise, non-fortified human serum certified reference material NIST SRM 1957 (n = 10), fortified human serum certified reference material NIST SRM 1958 (n = 10), bovine serum (Sigma Aldrich, n = 10) and ultrapure water procedural blanks (n = 6) were used to calculate performance characteristics: limits of detection (LOD), trueness (tested by comparison of measured data with certified reference values), precision (expressed as standard deviation, SD and relative standard deviation, RSD of repeated analysis) and recoveries.

2.3. Sample extraction

Samples (200 μL) were allowed to stabilize at ambient temperature (~20 °C) for 60 min and transferred to 2 mL amber glass vials and fortified with ^{13}C internal standards (^{13}C-NFRs and ^{13}C-PBDEs: 400 pg, ^{13}C-PCBs and ^{13}C-OCPs: 800 pg, ^{13}C-BDBPE: 2000 pg, ^{13}C-BDE 209: 800 pg, ^{13}C-HBCD: 2000 pg) in 40 μL IPA and vortexed for 20 s. Protein precipitation was performed by addition of 600 μL MeCN. These samples were then extracted by 96-well plate solid phase extraction (SPE; 60 mg Oasis HLB) using DCM:Hex (1:19 (v/v)). Additionally, quality controls of six solvent blanks and ten replicates of SRM 1957 and SRM 1958 serum were extracted.

Prior to extraction, wells of the Oasis HLB plate were preconditioned with 1 mL DCM:Hex (1:19 (v/v)), followed by 1 mL MeOH and finally 1 mL ultrapure water. Serum samples were transferred to individual wells using disposable glass Pasteur pipettes. Three rinses of the pipette and amber vials with 200 μL 75% MeCN in water were performed and added to the respective well. After loading samples, vacuum was applied to pull the supernatant through (waste), at a speed of ~1 drop per second. To remove any remaining liquid, the plate was centrifuged for 15 min at 2400 g and further dried under a stream of nitrogen using a positive

pressure manifold for 30 min (~103,4 kPa). Prior to elution, the Phree plate was prepared by the addition of 0.1 g prebaked anhydrous Na_2SO_4 to each well and wells prewashed with 1 mL DCM:Hex (1:19 (v/v)).

The Oasis HLB plate was then stacked atop the Phree plate, and samples were eluted by addition of 1.2 mL DCM:Hex (1:19 (v/v)) and eluate collected in glass inserts. All SPE steps were performed with initial use of a vacuum pump to promote flow followed by elution under gravity at a rate of approximately one drop per second to maximise the contact time with the sorbent.

The eluates were evaporated under nitrogen to ~600 μL and transferred with three rinses of ~150 μL DCM into GC vials. After that 20 μL of nonane was added as keeper and extracts were evaporated under nitrogen to ~20 μL volume. Syringe standards (^{13}C-BDE 77 and ^{13}C-BDE 138: 1000 pg, ^{13}C-PCB 162: 500 pg in 20 μL nonane) were added and extracts concentrated under nitrogen to final volume of ~40 μL for analysis of POPs and NFRs. Following analysis of POPs and NFRs, the extracts were evaporated to dryness under N_2 and reconstituted in 50 μL of Ace:MeCN (1:19 (v/v)) for HBCD analysis.

2.4. Instrumentation

2.4.1. GC-APCI-MS/MS

Gas chromatography atmospheric pressure chemical ionization tandem mass spectrometry was undertaken on an Agilent 7890 GC (Agilent technologies, USA) coupled to a Waters Xevo TQ-S MS (Waters, UK). The MS was operated under dry source conditions (charge transfer) in multiple reaction monitoring (MRM) mode.

PCBs and OCPs were separated on a 30 m × 0.25 mm x 0.25 μm Rxi-5Sil MS column (Restek, USA). The injection was 1 μL pulsed splitless at 280 °C. Helium was used as carrier gas at constant flow 1.5 mL min^{-1}. The oven temperature programme was 80 °C (1 min hold), then 12 °C min^{-1} to 250 °C, followed by 5 °C min^{-1} to 280 °C and finally 45 °C min^{-1} to 320 °C (5 min hold). Further instrumental conditions are shown in Table S1, Table S2 and Table S3.

BFRs were separated on a 15 m × 0.25 mm x 0.1 μm Rtx-1614 column (Restek, USA). The injection was 2 μL splitless at 280 °C. Helium was used as carrier gas at constant flow 1.5 mL min^{-1}. The oven temperature programme was 80 °C (1 min hold), then 20 °C min^{-1} to 250 °C, followed by 1.5 °C min^{-1} to 260 °C (2 min hold) and finally 25 °C min^{-1} to 325 °C (5 min hold). Further instrumental conditions are shown in Table S1, Table S2 and Table S3.

Quantitative analysis was performed using Waters TargetLynx (ver. 4.1; Waters, UK), with automated LOD and LOQ calculation based on signal to noise (S/N) ratios, 3:1 for LOD and 9:1 for LOQ, noise measured as peak-to-peak. Results for BDE 66 and BDE 85 were expressed relative to ^{13}C-BDE 47 and ^{13}C-BDE 99, respectively.

2.4.2. GC-EI-MS/MS

Gas chromatography electron ionization tandem mass spectrometry was undertaken on an Agilent 8890 GC (Agilent technologies, USA) coupled to a 7000D Agilent TQ MS (Agilent technologies, USA). The MS was operated in multiple reaction monitoring (MRM) mode.

HCHs were separated on a 60 m × 0.25 mm x 0.25 μm Rxi-5Sil MS column (Restek, USA). The injection was 3 μL splitless at 280 °C. Helium was used as carrier gas at constant flow 1.5 mL min^{-1}. The oven temperature programme was 80 °C (1.5 min hold), followed by 40 °C min^{-1} to 200 °C (18 min hold) and finally 5 °C min^{-1} to 305 °C (2 min hold).

Quantitative analysis was performed using Agilent MassHunter (ver. 10.1; Agilent technologies, USA), with automated LOD and LOQ calculation based on signal to noise (S/N) ratios, 3:1 for LOD and 10:1 for LOQ, noise measured as peak-to-peak. Further instrumental conditions are shown in Table S2, Table S3 and Table S4. Results for each HCH isomer were expressed relative to ^{13}C-γ-HCH.

2.4.3. HPLC-MS/MS

Liquid chromatography tandem mass spectrometry was undertaken on an Agilent 1290 Infinity LC (Agilent Technologies, Germany) coupled to AB Sciex Qtrap 5500 (AB Sciex, Canada). HBCD isomers were separated on a Luna C18 column (3 μm; 100 × 2 mm; Phenomenex, USA) equipped with Phenomenex SecureGuard C18 guard column (4 × 2 mm; Phenomenex, USA) at 30 °C. The mobile phase consisted of (A) ultrapure water containing 1 mM ammonium acetate and (B) MeCN containing 1 mM ammonium acetate. Elution with a flow rate of 0.30 mL min^{-1} was carried out from 50% B (initial) to 90% B over 7 min, with a non-linear gradient. At 7.1 min, the column was returned to initial condition and equilibrated for 3 min prior to next injection. Injection volume was 5 μL and autosampler set at 10 °C. Negative electrospray ionization (ESI-) was used for detection with capillary voltage 4 kV; desolvation temperature 450 °C; curtain gas 35 psi, Gas 1 40 psi, Gas 2 40 psi.

Quantitative analysis was performed using Analyst (ver. 1.7.2; AB Sciex, Canada) with LOD calculated as 3 times standard deviation of blanks and LOQ calculated as 10 times standard deviation of blanks. Further instrumental conditions are shown in Table S2, Table S3 and Table S5. Results for β-HBCD were expressed relative to both ^{13}C-α-HBCD and ^{13}C-γ-HBCD.

3. Results and discussion

3.1. Method optimization and validation

The analysis of trace amounts of environmental pollutants in limited volume of human matrices, such as blood serum, requires effective and reliable sample preparation procedure. The aim of this study was based upon extending the offline Oasis HLB SPE well-plate extraction method of Stubleski et al. (2018), to a wider range of compounds amenable whilst maintaining applicability of the procedure for low sample volumes. In general, 96-well plate SPE has low solvent consumption and provides high throughput of the samples resulting in lower risk of contamination from blanks. Furthermore, the use of 96 well plate format of SPE has potential to be automated using pipetting robots to provide a faster sample preparation procedure in future.

During validation of the sample preparation procedure, different sample precipitation protocols using acetonitrile were tested: i) in glass vial precipitation, ii) precipitation directly in-well of SPE plate and iii) precipitation in PP cryogenic tubes were compared:

i) In vial precipitation is described in section 2.3 and reported herein; ii) for in-well precipitation, serum samples were quantitatively dispensed into preconditioned wells of the well plate using disposable Pasteur pipette, fortified with ^{13}C internal standards and protein precipitated directly in-well. After that, all samples were aspirated by automated multichannel pipette. The elution and clean-up step were performed in the same way as described in section 2.3. Recoveries obtained via in-well precipitation (Table S6) were up 50% lower for NIST SRM standards compared to the precipitation in vial, with the exception for ^{13}C-BDE 99, which were on average 8% greater for in-well precipitation (Table S7); iii) Precipitation in PP cryogenic tubes was also tested as these tubes are used for CELSPAC:TNG specimen banking. The majority of standards showed reduced recovery compared to glass vials (Table S8 and Table S9) yet retain adequate detectability to enable accurate quantification. As such, there is potential for direct protein precipitation of sera aliquots in these PP cryogenic tubes, which could reduce sample handling times in future.

Due to the lipid content in blood serum samples, an appropriate clean-up approach is needed. Notably, Stubleski et al. tested Oasis Prime HLB SPE plates on the premise that it's greater ability to remove phospholipids, proteins and salts may enable to avoid the sulphuric-acid based clean-up required with Oasis HLB (Stubleski et al., 2018). However, it was observed that more non-polar organic solvents (e.g. Hex: DCM) were not able to pass through.

Phree phospholipid removal plates are similarly advertised to provide fast and effective removal of phospholipids, proteins and salts. Phree cartridges have previously been used for clean-up of large volume

Ace:Hex (1:19 (v/v)) extracts of POPs (Pirard and Charlier, 2018). As such, coupling the Oasis HLB and Phree 96 well-plates was trialled. The well-plate format favourably enables a rapid single-step extraction and clean-up with low solvent consumption. However, automated positive pressure SPE manifolds are typically not capable to process stacked plates with ease.

3.2. Linearity

For respective POPs, linearity of the GC-MS/MS instrument was determined by analysing a 6-point calibration curve in the concentration range 0.1–100 ng/mL (for some compounds extended to 0.03–300, 1–500 and 1–1000 ng/mL) (Table 1). Linear regression was plotted as the ratio of the peak areas (analyte/respective mass labelled standard) versus the concentration of the analyte. The coefficient of determination (R^2) ranged from 0.95 to 1.00 for all analytes, except EHTBB (R^2 = 0.89). High response variations and poor linearity have previously been observed for EHTBB (Pirard and Charlier, 2018). Calibration curves were used to calculate relative response factors (RRFs) for each of the target compounds (Table 1). The mean value of RRF for each compound was used to quantify POPs concentration in SRMs and serum samples.

3.3. Limit of detection

In the case when the target compound was present in the blank samples (Table S10), the limit of detection (LOD) was calculated as three times the standard deviation of the concentration in ultrapure water procedural blanks. The lowest method LOD was observed for PCB 180 (0.36 pg/mL) and highest for δ-HCH (66.07 pg/mL) (Table 1). The mean value for compounds detected in procedural blanks was subtracted. All method LODs are summarized in Table 1. For other compounds, the detection limit was defined instrumentally (iLOD) as three times the signal to noise ratio in procedural blank. Potential effect of matrix residues on iLOD is given in Table S11. Low average iLOD values obtained from blood serum samples and SRM materials show high efficiency of clean-up procedure with minimal influence of matrix residues on results.

LODs from this method were compared with previously published methods. The LODs given in Table S11 are comparable or better than those obtained elsewhere (Koponen et al., 2013; Salihovic et al., 2012; Stubleski et al., 2018). Higher background of HCB concentration (S10) resulted in higher LOD value compared to those published by Koponen et al. (2013) but lower compared to those recently published by Stubleski et al. for 96-well plate format (Stubleski et al., 2018). However, the referenced studies use slightly different LOD calculation approaches. Therefore, values of average procedural blank concentration plus three times standard deviation and value of three standard deviation were included for transparent comparison (Table S12).

3.4. Recovery, reproducibility and repeatability

Recovery of the sample preparation method was assessed based upon measures of mass labelled standards introduced at the beginning of the sample preparation (Table 2). The corresponding recoveries in non-fortified NIST SRM 1957 ranged from 40% to 112% (RSD <9%) for PCBs; from 18% to 85% (RSD <16%) for OCPs and from 75% to 155% (RSD <13%) for PBDEs with exception of ^{13}C-BDE 209 (8%; RSD <21%) (Table 2). BDE-209 is a thermally labile compound, photosensitive and also sensitive to GC conditions, which could overall contribute to lower recovery (Covaci et al., 2003). In particular, poor response has been observed when using split/splitless injection (Björklund et al., 2004), as conducted here. Moreover, recovery was also tested on non-persistent NFRs, where the recoveries ranged from 14% to 73% (RSD <25%) in non-fortified NIST SRM 1957 and 27%–78% (RSD <40%) in fortified NIST SRM 1958.

The reproducibility of the 96-well plate method was tested by comparing measured and certified concentration values of POPs at low

Table 1
Relative response factors (RRF), coefficients of determination (R^2) and method LODs obtained using the 96-well plate method.

Analyte [a]	Range (ng/mL)	Calibration curve RRF	RRF RSD (%)	R^2 [b]	^{13}C-labelled standard	Method LOD (pg/mL) [c]
PCB 28	0.1–100	1.1	15.0	0.98	^{13}C-PCB 28	4
PCB 52	0.1–100	1.0	12.6	0.98	^{13}C-PCB 52	4
PCB 101	0.1–100	1.0	15.2	0.98	^{13}C-PCB 101	–
PCB 118	0.1–100	1.1	19.0	0.97	^{13}C-PCB 118	1
PCB 153	0.1–100	1.2	10.4	0.99	^{13}C-PCB 153	3
PCB 138	0.1–100	1.0	10.3	0.99	^{13}C-PCB 138	2
PCB 180	0.1–100	1.3	12.7	0.98	^{13}C-PCB 180	0
PeCB	0.1–100	0.9	7.4	0.99	^{13}C-PeCB	22
HCB	0.1–100	0.8	19.1	0.96	^{13}C-HCB	23
α-HCH	1–1000	1.9	4.9	1.00	^{13}C-γ-HCH	30
β-HCH	1–1000	1.3	6.0	1.00	^{13}C-γ-HCH	30
γ-HCH	1–1000	1.4	6.7	1.00	^{13}C-γ-HCH	36
δ-HCH	1–1000	1.1	15.9	1.00	^{13}C-γ-HCH	66
o,p'-DDE	0.1–100	0.8	12.3	0.98	^{13}C-*o,p'*-DDE	2
p,p'-DDE	0.1–100	0.7	15.3	0.97	^{13}C-*p,p'*-DDE	5
o,p'-DDD	0.1–100	1.0	23.9	0.95	^{13}C-*o,p'*-DDD	–
p,p'-DDD	0.1–100	1.2	20.4	0.96	^{13}C-*p,p'*-DDD	–
o,p'-DDT	0.1–100	1.2	16.4	0.98	^{13}C-*o,p'*-DDT	11
p,p'-DDT	0.1–100	1.2	20.6	0.96	^{13}C-*p,p'*-DDT	–
BDE 28	0.1–100	0.8	12.3	0.98	^{13}C-BDE 28	–
BDE 47	0.1–100	1.0	3.9	1.00	^{13}C-BDE 47	1
BDE 66	0.1–100	1.0	6.7	1.00	^{13}C-BDE 47	–
BDE 100	0.1–100	1.3	13.3	0.98	^{13}C-BDE 100	–
BDE 99	0.1–100	1.1	6.4	1.00	^{13}C-BDE 99	2
BDE 85	0.1–100	1.1	8.0	0.99	^{13}C-BDE 99	–
BDE 154	0.1–100	1.1	7.5	0.99	^{13}C-BDE 154	–
BDE 153	0.1–100	1.1	3.1	1.00	^{13}C-BDE 153	–
BDE 183	0.1–100	0.9	5.9	1.00	^{13}C-BDE 183	–
BDE 209	1–1000	1.5	13.2	0.98	^{13}C-BDE 209	–
α-HBCD	0.03–300	–	–	1.00	^{13}C-α-HBCD	30
β-HBCD	0.03–300	–	–	1.00	^{13}C-α-HBCD	10
γ-HBCD	0.03–300	–	–	1.00	^{13}C-γ-HBCD	30
PBBZ	1–500	0.7	9.3	0.99	^{13}C-PBBZ	2
HBB	1–500	0.7	8.3	0.99	^{13}C-HBB	7
EHTBB	1–500	0.6	33.3	0.89	^{13}C-EHTBB	2983
BTBPE	1–500	0.8	4.4	1.00	^{13}C-BTBPE	6
syn-DP	1–500	0.6	5.8	1.00	^{13}C-*syn*-DP	15
anti-DP	1–500	1.2	10.7	0.99	^{13}C-*anti*-DP	–

[a] Compounds measured via GC-APCI-MS/MS, except HCHs and HBCDs which were measured via GC-EI-MS/MS and LC-ESI-MS/MS, respectively.

[b] 6-point calibration curve with $1/x^2$ weighting except for HCHs and HBCDs where 8-point and 9-points calibration curves without weighting were used, respectively.

[c] The LOD calculated as the average plus 3 times the standard deviation of ultrapure water blank samples.

Table 2
The average percentage recovery (RSD%) of ^{13}C-labelled internal standards when spiked into various matrices with in glass vial precipitation.

Analyte	Percent Recovery			
	Ultrapure water (RSD%)	Bovine serum (RSD%)	NIST SRM 1957 (RSD%)	NIST SRM 1958 (RSD%)
^{13}C-PCB 28	51 (8)	40 (22)	51 (7)	53 (9)
^{13}C-PCB 52	54 (6)	48 (13)	47 (9)	52 (6)
^{13}C-PCB 101	45 (11)	46 (18)	40 (9)	49 (10)
^{13}C-PCB 118	92 (8)	90 (12)	112 (5)	106 (10)
^{13}C-PCB 153	71 (7)	62 (12)	78 (6)	77 (3)
^{13}C-PCB 138	72 (9)	66 (11)	77 (6)	77 (6)
^{13}C-PCB 180	76 (14)	76 (11)	80 (6)	78 (12)
^{13}C-PeCB	37 (10)	24 (12)	37 (5)	37 (8)
^{13}C-HCB	32 (15)	22 (13)	28 (5)	29 (7)
^{13}C-γ-HCH[a]	14 (19)	10 (28)	18 (16)	21 (15)
^{13}C-*o,p'*-DDE	45 (3)	43 (14)	48 (11)	50 (8)
^{13}C-*p,p'*-DDE	67 (14)	65 (13)	85 (7)	83 (14)
^{13}C-*o,p'*-DDD	44 (35)	42 (27)	61 (5)	48 (18)
^{13}C-*p,p'*-DDD	31 (15)	22 (23)	58 (9)	41 (20)
^{13}C-*o,p'*-DDT	45 (22)	47 (13)	60 (10)	48 (10)
^{13}C-*p,p'*-DDT	44 (12)	39 (15)	60 (11)	48 (15)
^{13}C-BDE 28	78 (32)	54 (13)	75 (8)	93 (15)
^{13}C-BDE 47	90 (17)	73 (13)	87 (8)	82 (3)
^{13}C-BDE 100	88 (14)	83 (11)	85 (13)	85 (8)
^{13}C-BDE 99	97 (20)	81 (12)	88 (13)	89 (8)
^{13}C-BDE 154	94 (18)	77 (12)	116 (11)	91 (9)
^{13}C-BDE 153	120 (15)	96 (13)	155 (11)	119 (10)
^{13}C-BDE 183	90 (20)	61 (17)	82 (7)	78 (8)
^{13}C-BDE 209	1 (99)	0.4 (64)	8 (21)	6 (16)
^{13}C-α-HBCD	74 (74)	14 (33)	93 (16)	108 (14)
^{13}C-γ-HBCD	21 (94)	11 (124)	44 (66)	39 (45)
^{13}C-PBBZ	49 (20)	42 (8)	46 (7)	50 (12)
^{13}C-HBB	55 (16)	46 (10)	54 (9)	59 (9)
^{13}C-EHTBB	33 (35)	23 (9)	23 (7)	32 (20)
^{13}C-BTBPE	95 (22)	77 (21)	73 (25)	78 (11)
^{13}C-*syn*-DP	36 (66)	17 (17)	17 (15)	27 (40)
^{13}C-*anti*-DP	34 (89)	11 (20)	14 (21)	33 (37)

and high concentration levels in NIST SRM 1957 (Table 3) and NIST SRM 1958 reference material, respectively (Table 4). Of the 15 POPs with certified or reference values in NIST SRM 1957, the majority deviated from their assigned values by less than 16% (average ~15%) (Table 3). Exceptions were the underestimation of PCB 118, BDE 85 and BDE 154 (deviation 27%, 31% and 25%, respectively) and overestimation of HCB (deviation 36%) (Table 3). Of the 27 POPs with certified or reference values in fortified NIST SRM 1958, the majority deviated by less than 30% (average ~21%) (Table 4). However, PCB 28 and HCB were overestimated (deviating 37% and 47%, respectively). It should be noted that PCB 28 and PCB 31 do not separate fully on the GC column used, so the increased deviation from certified value may be caused by contribution of co-eluting PCB 31. The underestimation of PCB 118, BDE 85 and BDE 154 may be caused by larger error at the lower concentrations, the concentrations of these PCB and BDE congeners in non-fortified blood serum are lower compared to other congeners. Alongside low recoveries of ^{13}C-BDE 209, the observed concentration of BDE-209 in fortified NIST 1958 reference material was underestimated by 30% (Table 4). Finally, p,p'-DDD and p,p'-DDT were largely underestimated, deviating 84% and 86% from certified values,

Table 3
Comparison of mean concentrations of target compounds quantified in non-fortified NIST SRM 1957 to reported concentrations.

Analyte	Certified concentration values in NIST SRM 1957 (pg/mL)		Measured concentration values in NIST SRM 1957 (pg/mL)		RSD (%)	Accuracy (%) [a]
	Mean	SD	Mean	SD		
PCB 28 [b]	8.6	1.1	9.3	1.7	18	108
PCB 52	not reported		5.7	4.2	73	–
PCB 101	not reported		3.4	1.1	31	–
PCB 118	18.9	1.2	13.8	1.7	12	73
PCB 153	58.2	0.9	51.8	5.2	10	89
PCB 138	36.9	9.0	37.8	3.3	9	103
PCB 180 [c]	46.0	0.5	45.7	2.5	5	99
PeCB	not reported		<LOD		–	–
HCB	29.7	3.5	40.4	7.2	18	136
α-HCH	not reported		<LOD		–	–
β-HCH	31.3	6.0	<LOD		–	–
γ-HCH	not reported		<LOD		–	–
δ-HCH	not reported		<LOD		–	–
o,p'-DDE	not reported		<LOD		–	–
p,p'-DDE	921.0	76.0	904.4	105.0	12	98
o,p'-DDD	not reported		<LOD		–	–
p,p'-DDD	not reported		<LOD		–	–
o,p'-DDT	not reported		<LOD		–	–
p,p'-DDT	not reported		2.4	0.6	25	–
BDE 28 [b]	20.0	2.4	17.1	0.9	5	85
BDE 47	268.0	14.0	231.4	7.0	3	86
BDE 66 [b]	6.7	0.1	<LOD		–	–
BDE 100	49.7	2.7	41.6	1.9	4	84
BDE 99	76.0	3.8	77.5	8.7	11	102
BDE 85 [b]	8.2	1.9	5.6	0.9	16	69
BDE 154 [b]	7.0	1.0	5.3	0.7	13	75
BDE 153	61.0	3.2	58.1	3.7	6	95
BDE 183	not reported		1.9	0.5	27	–
BDE 209	not reported		14.6	9.6	65	–
α-HBCD	not reported		<LOD		–	–
β-HBCD	not reported		<LOD		–	–
γ-HBCD	not reported		<LOD		–	–
PBBZ	not reported		<LOD		–	–
HBB	not reported		<LOD		–	–
EHTBB	not reported		<LOD		–	–
BTBPE	not reported		<LOD		–	–
syn-DP	not reported		<LOD		–	–
anti-DP	not reported		<LOD		–	–

[a] 100+(measured mean concentration - certified NIST SRM concentration)/(certified NIST SRM concentration)*100.

[b] Reference concentration value.

[c] Information concentration value.

Table 4
Comparison of mean concentrations of target compounds quantified in fortified NIST SRM 1958 with reported concentrations.

Analyte	Certified concentration values in NIST SRM 1958 (pg/mL)		Measured concentration values in NIST SRM 1958 (pg/mL)		RSD (%)	Accuracy (%) [a]
	Mean	SD	Mean	SD		
PCB 28	402	12	553	69	13	137
PCB 52	401	14	371	47	13	92
PCB 101	409	27	451	62	14	110
PCB 118	412	35	378	39	10	92
PCB 153	457	36	510	54	11	111
PCB 138	473	54	511	51	10	108
PCB 180	459	49	524	57	11	114
PeCB	not reported		420	53	13	–
HCB	442	46	649	64	10	147
α-HCH [b]	260	44	195	23	12	75
β-HCH [b]	278	47	324	38	12	117
γ-HCH [b]	315	43	282	40	14	89
δ-HCH	not reported		<LOD		–	–
o,p'-DDE	450	20	502	75	15	112
p,p'-DDE	1250	130	1285	239	19	103
o,p'-DDD	347	46	319	174	55	92
p,p'-DDD	416	17	65	12	19	16
o,p'-DDT	313	43	229	76	33	73
p,p'-DDT	293	12	40	7	17	14
BDE 28	462	19	529	68	13	115
BDE 47	651	29	744	121	16	114
BDE 66	440	41	536	84	16	122
BDE 100	475	27	447	41	9	94
BDE 99	492	15	608	65	11	124
BDE 85	475	27	555	44	8	117
BDE 154	441	39	465	68	15	106
BDE 153	455	54	522	65	13	115
BDE 183	453	42	508	66	13	112
BDE 209	417	5	290	61	21	70
α-HBCD	not reported		183	25	14	–
β-HBCD	not reported		<LOD		–	–
γ-HBCD	not reported		<LOD		–	–
PBBZ	not reported		11	2	15	–
HBB	not reported		652	88	14	–
EHTBB	not reported		<LOD		–	–
BTBPE	not reported		907	106	12	–
syn-DP	not reported		<LOD		-	–
anti-DP	not reported		<LOD		–	–

[a] 100+(measured mean concentration - certified NIST SRM concentration)/(certified NIST SRM concentration)*100.
[b] Reference concentration value.

respectively (Table 4). Minor underestimation of p,p'-DDT has been noted in other studies (Koponen et al., 2013), though reasons unclear. Moreover, the method performance was also tested by analysing non-persistent compounds in 10 replicates of fortified bovine serum control material, where larger inaccuracies for BDE 209 and *anti*-DP were observed (Table S9).

Repeatability of the 96-well plate method was determined by calculating relative standard deviation (RSD) of the certified compounds measured in NIST SRM 1957, NIST SRM 1958 and bovine serum. For the non-fortified NIST SRM 1957, the RSD was between 3% and 18% for all certified POPs (Table 3). For the fortified NIST SRM 1958, the relative standard deviation ranged from 8% to 21% for most of the certified POPs (Table 4). Two pesticides, o,p'-DDD and o,p'-DDT showed a higher RSD, 33% and 55%, respectively. In bovine serum, RSD ranged from 4% to 22% except BDE 209 (54%) (Table S13).

3.5. Application of the 96-well plate method

The 96-well plate method was applied to a subset of human serum samples (n = 48) from mothers in 38th week of pregnancy, collected for the pilot phase of CELSPAC: TNG (Table 5).

Of the targeted compounds, 75% were detected in at least one sample (7 PCBs, 7 OCPs, 10 PBDEs, 3 HBCDs and 3 NFRs) with PCB 138, PCB 153, PCB 180, p,p'-DDE, BDE-153 detected in all samples. PCB 28, HCB, p,p'-DDT and BDE 154 were detected in >50% samples whereas PeCB, HCHs and the majority of the NFRs were below LOD in all of the examined samples.

All seven PCBs were quantified in the samples, with greatest determined concentration of hexa and hepta-chlorinated PCB congeners (PCB 138, 9.2–150 ng/g l.w.; PCB 153, 20–277 ng/g l.w. and PCB 180 16–282 ng/g l.w.). Similar concentrations were reported in blood serum samples from recent Czech study (Svarcova et al., 2019). Concentrations determined for p,p'-DDE (27–632 ng/g l.w.) and other OCPs are similar to those observed in Spanish cohorts (Arrebola et al., 2013). Greater concentrations of p,p'-DDE, HCB and some higher chlorinated PCB congeners are in accordance with the greater persistence and stability of OCPs and PCBs in biological and environmental matrices (El-Shahawi et al., 2010).

Only three of ten PBDEs were found in >50% samples (BDE 100, <0.0062–1.5 ng/g l.w.; BDE 154, <0.0067–0.47 ng/g l.w. and BDE 153, <0.0089–2.1 ng/g l.w.) whilst other BFRs, such as HBCD isomers and novel flame retardants were below the LOD. Czech population has lower exposure to PBDEs as demonstrated in comparison of PBDEs contamination of indoor environment in Canada, Czech Republic and USA (Venier et al., 2016). In general, BFRs represent minor contaminants in human blood serum which results in lower detection frequencies in pilot and case studies (Fromme et al., 2016; Kalantzi et al., 2011; Sochorová et al., 2017), but are potentially detectable in large scale population studies in other countries (Dereumeaux et al., 2016; Sjödin et al., 2008; Zhao et al., 2021).

4. Conclusion

Through coupling of a 96-well phospholipid depletion plate with well plate SPE, a less destructive extraction and clean-up method which enables the analysis of selected POPs plus non-persistent NFRs in small volumes of blood serum has been developed. The accuracy of the method is satisfactory for the majority of the target compounds and the 96-well plate SPE format is sensitive enough to detect low concentrations of selected POPs and non-persistent NFRs. The applicability of the developed method was tested using blood serum samples from pregnant women enrolled in the pilot phase of the CELSPAC: TNG, with detection of 30 analytes (18 analytes above 20% detection frequency). When compared to other previously published methods, our method allows analysis of a wider range of compounds including those where non-destructive clean-up is favoured or needed (EHTBB, BTBPE). The method can be further extended by including a larger range of individual PCB congeners, pesticides and flame retardants. The method is simple, straightforward, has minimal solvent consumption and provides relatively high throughput suitable for human biomonitoring studies. For human biomonitoring, a large benefit of the developed 96-well plate method is the potential for automation by using pipetting robots which can increase sample throughput and minimise the impact of human factor errors.

CRediT author statement

Jiří Palát: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing – Original Draft; **Petr Kukučka:** Conceptualization, Methodology, Investigation, Writing-Review & Editing, Supervision; **Garry Codling:** Writing-Review & Editing; **Elliott James Price:** Writing-Review & Editing, Supervision; **Petr Janků:** Collection and provision of test samples; **Jana Klánová:** Writing-Review & Editing, Supervision, Funding acquisition.

Table 5
A summary of the minimum (Min), 25th percentile (25th), median, mean, 75th percentile (75th) and maximum (Max) concentration in the 48 human serum samples from the Pilot phase of CELSPAC: TNG study.

Analyte	Detection frequency (%) [a]	Min LOD (ng/g l. w.) [b]	Max LOD (ng/g l. w.) [b]	Min (ng/g l. w.)	25th (ng/g l. w.)	Median (ng/g l. w.)	Mean (ng/g l. w.)	75th (ng/g l. w.)	Max (ng/g l. w.)
PCB 28	73	0.45	0.90	0.51	0.70	0.93	1.10	1.30	2.90
PCB 52	17	0.55	1.10	0.70	0.78	0.84	1.10	1.50	1.80
PCB 101	48	0.04	0.54	0.12	0.30	0.37	0.39	0.48	0.72
PCB 118	100	0.05	0.60	0.46	1.70	2.50	2.70	3.20	7.80
PCB 153	100	0.08	0.15	20.00	35.00	53.00	62.00	65.00	280.00
PCB 138	100	0.06	0.12	9.20	21.00	29.00	37.00	38.00	150.00
PCB 180	100	0.29	0.58	16.00	32.00	42.00	51.00	53.00	280.00
PeCB	0	9.40	19.00	–	–	–	–	–	–
HCB	96	5.60	11.00	8.80	16.00	20.00	22.00	26.00	50.00
α-HCH	0	0.02	0.03	–	–	–	–	–	–
β-HCH	0	0.02	0.03	–	–	–	–	–	–
γ-HCH	0	0.02	0.04	–	–	–	–	–	–
δ-HCH	0	0.03	0.07	–	–	–	–	–	–
o,p'-DDE	27	0.03	0.07	0.04	0.08	0.10	0.22	0.35	0.89
p,p'-DDE	100	0.51	1.00	27.00	74.00	120.00	130.00	160.00	630.00
o,p'-DDD	6	0.03	0.36	0.05	–	–	–	–	0.36
p,p'-DDD	19	0.06	0.99	0.05	0.18	0.23	0.26	0.24	0.84
o,p'-DDT	19	0.03	0.30	0.03	0.14	0.18	0.21	0.21	0.56
p,p'-DDT	83	0.05	0.64	0.44	0.91	1.40	1.90	2.10	8.70
BDE 28	21	0.03	0.07	0.04	0.05	0.06	0.11	0.10	0.44
BDE 47	21	0.12	0.24	0.18	0.23	0.28	1.10	0.49	5.30
BDE 66	2	0.00	0.03	0.08	–	–	–	–	0.08
BDE 100	77	0.01	0.09	0.02	0.04	0.05	0.09	0.07	1.50
BDE 99	15	0.05	0.10	0.10	0.12	0.14	0.40	0.40	1.50
BDE 85	4	0.01	0.12	0.10	–	–	–	–	1.50
BDE 154	88	0.01	0.07	0.03	0.04	0.05	0.06	0.06	0.47
BDE 153	100	0.01	0.09	0.08	0.14	0.19	0.25	0.24	2.10
BDE 183	17	0.03	0.31	0.08	0.10	0.14	0.17	0.20	0.35
BDE 209	2	1.90	80.00	10.00	–	–	–	–	10.00
α-HBCD	19	0.59	1.20	11.00	22.00	26.00	26.00	32.00	49.00
β-HBCD	33	0.20	0.39	3.20	5.50	14.00	29.00	18.00	270.00
γ-HBCD	4	0.59	1.20	7.30	–	–	–	–	500.00
PBBZ	21	0.05	0.11	0.08	0.09	0.10	0.13	0.15	0.29
HBB	23	0.11	0.21	0.12	0.21	0.28	0.28	0.36	0.55
EHTBB	0	4.00	22.00	–	–	–	–	–	–
BTBPE	6	0.06	0.36	0.18	–	–	–	–	0.29
syn-DP	0	9.30	93.00	–	–	–	–	–	–
anti-DP	0	4.10	24.00	–	–	–	–	–	–

[a] Only minimum and maximum level were reported when detection frequency was <15% for the compound in samples.
[b] Reported LOD as a range (min, max) due to different lipid mass for each sample.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Authors thank to Research Infrastructure RECETOX RI (No. LM2018121) financed by the Ministry of Education, Youth and Sports, and the CETOCOEN Excellence project supported from the Teaming Action of the EU Horizon 2020 programme (857560) and Operational Programme Research, Development and Innovation (No. CZ.02.1.01/0.0/0.0/17_043/0009632). E.J.P. acknowledges support from the Czech Operational Programme Research, Development and Education – Project MSCAfellow4@MUNI (No. CZ.02.2.69/0.0/0.0/20_079/0017045). G.P.C. acknowledges support from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie (No. 839243).

The authors thank Lenka Andrýsková and Pavel Piler for sampling, provision and lipid content analysis of CELSPAC: TNG samples. Many thanks go to the participating families as well as the physicians and medical staff of The University Hospital Brno, and the entire study team. This study was funded by the Ministry of Health of the Czech Republic (FNBr, No. 65269705). Petr Šenk and Jakub Martiník are thanked for instrumental analysis of HBCDs and HCHs, respectively. The authors of this study (i.e. not the ELSPAC Scientific Council) are responsible for the content of this publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.132300.

References

Airaksinen, R., Rantakokko, P., Eriksson, J.G., Blomstedt, P., Kajantie, E., Kiviranta, H., 2011. Association between type 2 diabetes and exposure to persistent organic pollutants. Diabetes Care 34, 1972–1979. https://doi.org/10.2337/dc10-2303.

Alaee, M., Arias, P., Sjödin, A., Bergman, Å., 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. Environ. Int. 29, 683–689. https://doi.org/10.1016/S0160-4120(03)00121-1.

Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. Int. J. Hyg Environ. Health 210, 201–228. https://doi.org/10.1016/j.ijheh.2007.01.024.
Arrebola, J.P., Fernández, M.F., Olea, N., Ramos, R., Martin-Olmedo, P., 2013. Human exposure to p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) in urban and semi-rural areas in southeast Spain: a gender perspective. Sci. Total Environ. 458–460, 209–216. https://doi.org/10.1016/j.scitotenv.2013.04.001.
Björklund, J., Tollbäck, P., Hiärne, C., Dyremark, E., Östman, C., 2004. Influence of the injection technique and the column system on gas chromatographic determination of polybrominated diphenyl ethers. J. Chromatogr. A 1041, 201–210. https://doi.org/10.1016/j.chroma.2004.04.025.
Blus, L.J., 1995. Organochlorine pesticides. In: Hoffman, D.J., Rattner, B.A. (Eds.), Handbook of Ecotoxicology. Lewis Publishers, pp. 275–300. https://doi.org/10.1201/9781351069656-1.
Buccini, J., 2003. The development of a global treaty on persistent organic pollutants (POPs). In: Persistent Organic Pollutants. Springer-Verlag, Berlin/Heidelberg, pp. 13–30. https://doi.org/10.1007/10751132_2.
Covaci, A., Harrad, S., Abdallah, M.A.E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environ. Int. 37, 532–556. https://doi.org/10.1016/j.envint.2010.11.007.
Covaci, A., Voorspoels, S., de Boer, J., 2003. Determination of brominated flame retardants, with emphasis on polybrominated diphenyl ethers (PBDEs) in environmental and human samples - a review. Environ. Int. 29, 735–756. https://doi.org/10.1016/S0160-4120(03)00114-4.
Dereumeaux, C., Saoudi, A., Pecheux, M., Berat, B., de Crouy-Chanel, P., Zaros, C., Brunel, S., Delamaire, C., le Tertre, A., Lefranc, A., Vandentorren, S., Guldner, L., 2016. Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. Environ. Int. 97, 56–67. https://doi.org/10.1016/j.envint.2016.10.013.
El-Shahawi, M.S., Hamza, A., Bashammakh, A.S., Al-Saggaf, W.T., 2010. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. Talanta 80, 1587–1597. https://doi.org/10.1016/j.talanta.2009.09.055.
Erickson, M.D., Kaley, R.G., 2011. Applications of polychlorinated biphenyls. Environ. Sci. Pollut. Res. 18, 135–151. https://doi.org/10.1007/s11356-010-0392-1.
Fernandes, A.R., Mortimer, D., Rose, M., Smith, F., Steel, Z., Panton, S., 2019. Recently listed Stockholm convention POPs: analytical methodology, occurrence in food and dietary exposure. Sci. Total Environ. 678, 793–800. https://doi.org/10.1016/j.scitotenv.2019.04.433.
Fromme, H., Hilger, B., Albrecht, M., Gries, W., Leng, G., Völkel, W., 2016. Occurrence of chlorinated and brominated dioxins/furans, PCBs, and brominated flame retardants in blood of German adults. Int. J. Hyg Environ. Health 219, 380–388. https://doi.org/10.1016/j.ijheh.2016.03.003.
Gasull, M., Castell, C., Pallarès, N., Miret, C., Pumarega, J., Téllez-Plaza, M., López, T., Salas-Salvadó, J., Lee, D.H., Goday, A., Porta, M., 2018. Blood concentrations of persistent organic pollutants and unhealthy metabolic phenotypes in normal-weight, overweight, and obese individuals. Am. J. Epidemiol. 187, 494–506. https://doi.org/10.1093/aje/kwx267.
Guo, W., Pan, B., Sakkiah, S., Yavas, G., Ge, W., Zou, W., Tong, W., Hong, H., 2019. Persistent organic pollutants in food: contamination sources, health effects and detection methods. Int. J. Environ. Res. Publ. Health 16, 10–12. https://doi.org/10.3390/ijerph16224361.
Ha, M.H., Lee, D.H., Jacobs, D.R., 2007. Association between serum concentrations of persistent organic pollutants and self-reported cardiovascular disease prevalence: results from the National Health and Nutrition Examination Survey, 1999-2002. Environ. Health Perspect. 115, 1204–1209. https://doi.org/10.1289/ehp.10184.
Kalantzi, O.I., Geens, T., Covaci, A., Siskos, P.A., 2011. Distribution of polybrominated diphenyl ethers (PBDEs) and other persistent organic pollutants in human serum from Greece. Environ. Int. 37, 349–353. https://doi.org/10.1016/j.envint.2010.10.005.
Koponen, J., Rantakokko, P., Airaksinen, R., Kiviranta, H., 2013. Determination of selected perfluorinated alkyl acids and persistent organic pollutants from a small volume human serum sample relevant for epidemiological studies. J. Chromatogr. A 1309, 48–55. https://doi.org/10.1016/j.chroma.2013.07.064.
Lee, D.H., Lee, I.K., Porta, M., Steffes, M., Jacobs, D.R., 2007. Relationship between serum concentrations of persistent organic pollutants and the prevalence of metabolic syndrome among non-diabetic adults: results from the National Health and Nutrition Examination Survey 1999-2002. Diabetologia 50, 1841–1851. https://doi.org/10.1007/s00125-007-0755-4.
Lee, D.H., Lee, I.K., Song, K., Steffes, M., Toscano, W., Baker, B.A., Jacobs, D.R., 2006. A strong dose-response relation between serum concentrations of persistent organic pollutants and diabetes: results from the National Health and Examination Survey 1999-2002. Diabetes Care 29, 1638–1644. https://doi.org/10.2337/dc06-0543.
Lim, J. eun, Lee, Sunju, Lee, Sunmi, Jee, S.H., 2018. Serum persistent organic pollutants levels and stroke risk. Environ. Pollut. 233, 855–861. https://doi.org/10.1016/j.envpol.2017.12.031.
Lim, J. eun, Nam, C., Yang, J., Rha, K.H., Lim, K.M., Jee, S.H., 2017. Serum persistent organic pollutants (POPs) and prostate cancer risk: a case-cohort study. Int. J. Hyg Environ. Health 220, 849–856. https://doi.org/10.1016/j.ijheh.2017.03.014.
Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Global fate of POPs: current and future research directions. Environ. Pollut. 150, 150–165. https://doi.org/10.1016/j.envpol.2007.06.051.
Magulova, K., Priceputu, A., 2016. Global monitoring plan for persistent organic pollutants (POPs) under the Stockholm Convention: triggering, streamlining and catalyzing global POPs monitoring. Environ. Pollut. 217, 82–84. https://doi.org/10.1016/j.envpol.2016.01.022.
Park, E.Y., Park, E., Kim, J., Oh, J.K., Kim, B., Hong, Y.C., Lim, M.K., 2020. Impact of environmental exposure to persistent organic pollutants on lung cancer risk. Environ. Int. 143, 105925. https://doi.org/10.1016/j.envint.2020.105925.
Piler, P., Kandrnal, V., Kukla, L., Andrýsková, L., Švancara, J., Jarkovský, J., Dušek, L., Pikhart, H., Bobák, M., Klánová, J., 2017. Cohort profile: the European longitudinal study of pregnancy and childhood (ELSPAC) in the Czech Republic. Int. J. Epidemiol. 46 https://doi.org/10.1093/ije/dyw091.
Pirard, C., Charlier, C., 2018. Simple and fast method for the measurement of legacy and novel brominated flame retardants in human serum. Chemosphere 211, 918–925. https://doi.org/10.1016/j.chemosphere.2018.08.012.
Rylander, L., Nilsson-Ehle, P., Hagmar, L., 2006. A simplified precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids. Chemosphere 62, 333–336. https://doi.org/10.1016/j.chemosphere.2005.04.107.
Salihovic, S., Mattioli, L., Lindström, G., Lind, L., Monica Lind, P., Bavel, B. van, 2012. A rapid method for screening of the Stockholm Convention POPs in small amounts of human plasma using SPE and HRGC/HRMS. Chemosphere 86, 747–753. https://doi.org/10.1016/j.chemosphere.2011.11.006.
Salihovic, S., Nilsson, H., Hagberg, J., Lindström, G., 2013. Trends in the analysis of persistent organic pollutants (POPs) in human blood. TrAC Trends Anal. Chem. (Reference Ed.) 46, 129–138. https://doi.org/10.1016/j.trac.2012.06.009.
Sjödin, A., Patterson, D.G., Bergman Åke, Å., 2003. A review on human exposure to brominated flame retardants - particularly polybrominated diphenyl ethers. Environ. Int. 29, 829–839. https://doi.org/10.1016/S0160-4120(03)00108-9.
Sjödin, A., Wong, L.Y., Jones, R.S., Park, A., Zhang, Y., Hodge, C., Dipietro, E., Mcclure, C., Turner, W., Needham, L.L., Patterson, D.G., 2008. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. Environ. Sci. Technol. 42, 1377–1384. https://doi.org/10.1021/es702451p.
Sochorová, L., Hanzlíková, L., Černá, M., Drgáčová, A., Fialová, A., Švarcová, A., Gramblička, T., Pulkrabová, J., 2017. Perfluorinated alkylated substances and brominated flame retardants in serum of the Czech adult population. Int. J. Hyg Environ. Health 220, 235–243. https://doi.org/10.1016/j.ijheh.2016.09.003.
Stubleski, J., Kukucka, P., Salihovic, S., Lind, P.M., Lind, L., Kärrman, A., 2018. A method for analysis of marker persistent organic pollutants in low-volume plasma and serum samples using 96-well plate solid phase extraction. J. Chromatogr. A 1546, 18–27. https://doi.org/10.1016/j.chroma.2018.02.057.
Svarcova, A., Lankova, D., Gramblicka, T., Stupak, M., Hajslova, J., Pulkrabova, J., 2019. Integration of five groups of POPs into one multi-analyte method for human blood serum analysis: an innovative approach within biomonitoring studies. Sci. Total Environ. 667, 701–709. https://doi.org/10.1016/j.scitotenv.2019.02.336.
Venier, M., Audy, O., Vojta, Š., Bečanová, J., Romanak, K., Melymuk, L., Krátká, M., Kukučka, P., Okeme, J., Saini, A., Diamond, M.L., Klánová, J., 2016. Brominated flame retardants in the indoor environment - comparative study of indoor contamination from three countries. Environ. Int. 94, 150–160. https://doi.org/10.1016/j.envint.2016.04.029.
Wielsøe, M., Kern, P., Bonefeld-Jørgensen, E.C., 2017. Serum levels of environmental pollutants is a risk factor for breast cancer in Inuit: a case control study. Environ. Heal. A Glob. Access Sci. Source 16, 1–16. https://doi.org/10.1186/s12940-017-0269-6.
Wittsiepe, J., Nestola, M., Kohne, M., Zinn, P., Wilhelm, M., 2014. Determination of polychlorinated biphenyls and organochlorine pesticides in small volumes of human blood by high-throughput on-line SPE-LVI-GC-HRMS. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 945–946, 217–224. https://doi.org/10.1016/j.jchromb.2013.11.059.
Yang, C., Kong, A.P.S., Cai, Z., Chung, A.C.K., 2017. Persistent organic pollutants as risk factors for obesity and diabetes. Curr. Diabetes Rep. 17 https://doi.org/10.1007/s11892-017-0966-0.
Zhao, X., Chen, T., Yang, B., Wang, D., Sun, W., Wang, Y., Yang, X., Wen, S., Li, J., Shi, Z., 2021. Serum levels of novel brominated flame retardants (NBFRs) in residents of a major BFR-producing region: occurrence, impact factors and the relationship to thyroid and liver function. Ecotoxicol. Environ. Saf. 208, 111467. https://doi.org/10.1016/j.ecoenv.2020.111467.