Suspect-screening analysis of Environmental Chemicals s in paired Human Cerebrospinal Fluid and serum samples

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Chemicals and reagents

Reference standards of 28 target environmental chemicals (Table S1), including 13 per- and polyfluoroalkyl substances, three organophosphate esters, four personal care products, two photoinitiators, three bisphenols, two phthalate ester metabolites, and one antioxidant, were purchased from Wellington Laboratories (Guelph, Ontario, Canada), AccuStandard (New Haven, Connecticut, USA), or Toronto Research Chemicals (Toronto, Canada). Twenty-one isotopically labeled reference standards were used as internal standards and purchased from Wellington Laboratories (Guelph, Ontario, Canada), Dr. Ehrenstorfer, LGC Standards (Middlesex, UK), and Toronto Research Chemicals (Toronto, Canada) (Table S1). Methanol, ethyl acetate, hexane, and water were all high-performance liquid chromatography grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA). The sheep serum used for quality control was purchased from Future Biotechnology Company (Guangzhou, China).

Sample collection

Our study recruited 180 outpatients diagnosed with mental illness (n = 43), lumbar disc herniation (n = 48), spinal stenosis (n = 37), or viral meningitis (n = 52) from the Third Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) in 2022−2023. Basic demographic data of the participants are summarized in Table S2. The cerebrospinal fluid (CSF) samples were collected by a senior physician using a clinical lumbar puncture method. Paired blood was also collected from the participants by a specialized nurse in the same day as the CSF collection. Serum was collected after centrifugation of the blood samples. Field blanks were prepared by collecting deionized water with the same procedures. Collected samples were stored at −80°C for subsequent analysis. All participants completed an informed consent form. The study protocol was approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University.

Sample pretreatment

Pretreatment of serum and CSF samples followed the same method as described in Huang et al. (Huang et al, 2023) An aliquot of 0.2 mL of serum/CSF was spiked with 21 internal standards (5 ng) and extracted with 3 mL of a mixture of ethyl acetate and n-hexane (3:2, v/v) containing 0.6% formic acid. After the supernatant was collected, the extraction was repeated twice and the combined supernatant was combined, concentrated to near dryness, and reconstituted with 50 µL of methanol. The extract was frozen overnight at −80 °C, followed by centrifugation at 15000 rpm for 5 min. The supernatant was retained and reconstituted to 50 µL for instrumental analysis.

Compound identification with high-resolution mass spectrometer

A single pooled extract was prepared by combining 5 µL each of the 180 CSF or serum extracts. Compound identification in pooled extracts was conducted with a Vanquish ultra-high performance liquid chromatography (UHPLC) interfaced with a ThermoFisher Orbitrap 240 with a H-ESI ion source (Thermo Scientific, Waltham, MA, USA). The UHPLC was equipped with a Waters ACQUITY UPLC BEH shield RP 18 column (2.1 mm × 100 mm, 1.7 µm particle size; Waters, Milford, USA). The column oven temperature and flow rate were set at 40 °C and 0.4 mL/min, respectively. In positive mode，The mobile phase for positive mode analysis consisted of water containing 0.1% formic acid (A) and methanol (B). For negative mode, the mobile phases consisted of water containing 0.2 mM ammonium acetate (A) and methanol (B). Both modes shared the same gradient: 0–2 min 30% B; 2–7 min, 30% B ramped to 60% B; 7–23 min, 60% B ramped to 100% B; 23–27 min, 100% B; 27–27.1 min, 100% B decreased to 30% B; and 27.1–30 min, 30% B. Mass spectra analysis was conducted in data-dependent MS/MS spectra acquisition method. The ion source conditions were set as following: ion source type, H-ESI; spray voltage, 3500 v for positive mode, 2800 v for negative mode; sheath gas flow rate, 45 arbitrary units; aux gas flow rate, 10 arbitrary units; sweep gas flow rate, 1 arbitrary unit; ion transfer tube temp, 320°C; vaporizer temperature, 350°C. The following acquisition parameters were used for MS1 analysis: resolution, 120000; scan range 100–1700 m/z; RF lens (%), 70; AGC target, standard; maximum injection time mode, auto; spectrum data type, profile. Data-dependent MS/MS parameters included: collision energy type, normalized; collision energy mode, stepped; HCD collision energies (%), 20, 40, 100; resolution, 30000; maximum injection time mode, auto; AGC target, standard; number of dependents scans, 10; isolation window (*m/z*), 1.5; spectrum data type, profile; dynamic exclusion, custom.

Quantitative analysis of target compounds

Determination of 28 target compounds was conducted on a Shimadzu UHPLC coupled to an AB Sciex 7500 Q Trap triple quadrupole mass spectrometer (AB Sciex, Toronto, Canada). Chromatographic separation was achieved by using a Luna Omega 3 μm PS C18 (2) 100 Å column (100 mm × 3.0 mm; 3 μm particle size; Phenomenex, Torrance, CA). The mobile phase consisted of 0.2 mM ammonium acetate (A) and methanol (B). The flow rate was 400 μL/min, and the column temperature was kept at 40 °C. The following gradient was employed: 30% B (held for 2 min) and linearly ramped to 60% B in 5 min, followed by a linear increase to 100% B in 16 min and held for 4 min, and then changed to 30% B in 0.1 min and equilibrated for 2.9 min. The total run time was 30 min. The MS was equipped with a TurboIonSpray® electrospray ionization (ESI) probe and operated in multiple reaction monitoring (MRM) mode (positive and negative). The ESI source conditions were as follows: ion source Gas 1, 35; ion source Gas 2, 70; curtain gas, 40; collision gas, 9; source temperature, 320 °C; IonSpray voltage floating, 2000 V.

Quality assurance and control (QA/QC) procedures

Multiple procedures were performed to ensure data quality, including recovery test, matrix effect evaluation, monitoring of procedural blank contamination, and assessment of inter-batch variation. Considering the rarity of CSF samples, QA/QC tests were conducted only with sheep serum (Guangzhou Future Biotechnology Company, China) as the substitutive matrix for human serum and CSF.

Spiking tests were conducted to evaluate the recovery efficiency from sample treatments. An aliquot of 0.2 mL of sheep serum was spiked with 28 target analytes (1 ng) and internal standards (5 ng) and processed in five replicates with the aforementioned methodology, along with two matrix blanks (sheep serum spiked with internal standards only). The recovery efficiency (RE) was determined as following.

$$RE\ (\%) = 100 \times \frac{C_{matrix} - C_{mblk}}{C_{std}} \qquad \text{(Eq. 1)}$$

Where C_{matrix}, C_{mblk} and C_{std} represent the determined abundances of target chemicals in sheep plasma (spiked with target analytes), matrix blanks, and the solution of standard mixture, representatively. The recoveries of individual compounds were determined to range from 72 (± 15)% to 128 (± 5.6)%.

Matrix effects were evaluated for the target analytes in serum. In brief, sheep serum (with no standards spiked) was extracted with the aforementioned approach, and each extract was divided into two sub-samples with equal volume. Sub-sample A was spiked with 100 μL of a standard mixture of analytes and sub-sample B was spiked with 100 μL of methanol. An external standard solution (S) was prepared by mixing the 100 μL of analyte mixtures with 100 μL methanol. By comparing the response differences of the analytes in the sub-samples A and B to the responses of the analytes in the external standard, a matrix effect (ME) value was calculated as:

$$ME\ (\%) = 100 \times \frac{A_i - B_i}{S_i}$$ (Eq. 2)

Where A_i, B_i and S_i represent the chromatographic peak areas of the analyte (i) in sub-samples A and B and external standard solution (S), respectively. Matrix effect was determined to be 74 (± 5)% to 119 (± 7.9)% (Table S3).

A laboratory procedural blank was processed along with every 10 samples. A field blank was prepared for each batch of 50 samples by collecting high-performance liquid chromatography grade water with the similar procedures as that for serum/CSF collection. Only TBP, TPHP, TCIPP, MeP, M(i)BP, and BHT were detectable in the procedural or field blanks, with an average concentration ranging from 0.01 (± 0.009) to 0.13 (± 0.17) ng/mL. Concentrations of these compounds in serum/CSF samples were reported after subtracting the average contamination levels in the blanks. A QC sample (sheep serum spiked with known amounts of target compounds along with internal standards) was processed along with each batch of 50 samples. The inter-batch coefficients of variation in the recovery of target analytes ranged from 2.0% to 17.0%.

Table S1. Target chemical and corresponding internal standard, including its full name, abbreviation or commercial name, chemical formula, CAS number, and reference standard source.

Chemical	Full name	Chemical formula	CAS number	Supplier[a]
Per- and polyfluoroalkyl substances				
PFBA	perfluorobutanoic acid	$C_4HF_7O_2$	375-22-4	Wellington
PFHxA	perfluorohexanoic acid	$C_6HF_{11}O_2$	307-24-4	Wellington
PFHpA	perfluoroheptanoic acid	$C_7HF_{13}O_2$	375-85-9	Wellington
PFOA	perfluorooctanoic acid	$C_8HF_{15}O_2$	335-67-1	Wellington
PFNA	perfluorononanoic acid	$C_9HF_{17}O_2$	375-95-1	Wellington
PFDA	perfluorodecanoic acid	$C_{10}HF_{19}O_2$	335-76-2	Wellington
PFUdA	perfluoroundecanoic acid	$C_{11}HF_{21}O_2$	2058-94-8	Wellington
PFDoA	perfluorododecanoic acid	$C_{12}HF_{23}O_2$	307-55-1	Wellington
PFBS	perfluorobutanesulfonic acid	$C_4HF_9O_3S$	375-73-5	Wellington
PFHxS	perfluorohexanesulfonic acid	$C_6HF_{13}O_3S$	355-46-4	Wellington
PFHpS	perfluoroheptanesulfonic acid	$C_7HF_{15}O_3S$	375-92-8	Wellington
PFOS	perfluorooctanesulfonic acid	$C_8HF_{17}O_3S$	1763-23-1	Wellington
6:2 Cl-PFESA	6:2 chlorinated perfluoroalkyl ether sulfonic acid	$C_8F_{16}SO_4HCl$	756426-58-1	Wellington
Organophosphate esters				
TBP	tributyl phosphate	$C_{12}H_{27}O_4P$	126-73-8	AccuStandard
TPHP	triphenyl phosphate	$C_{18}H_{15}O_4P$	115-86-6	AccuStandard
TCIPP	tris(2-chloroisopropyl) phosphate	$C_9H_{18}Cl_3O_4P$	13674-84-5	AccuStandard
Personal care products				
MeP	methyl paraben	$C_8H_8O_3$	99-76-3	AccuStandard
EtP	ethyl paraben	$C_9H_{10}O_3$	120-47-8	AccuStandard
BuP	butyl paraben	$C_{11}H_{14}O_3$	94-26-8	AccuStandard
TCS	triclosan	$C_{12}H_7Cl_3O_2$	3380-34-5	AccuStandard
Photointiators				
MK	4,4 -bis(dimethylamino)benzophenone	$C_{17}H_{20}N_2O$	90-94-8	TRC
PI-651	2,2-dimethoxyphenyl acetophenone	$C_{16}H_{16}O_3$	24650-42-8	TRC
Bisphenols				
BPA	2,2-bis(4-hydroxyphenyl)propane	$C_{15}H_{16}O_2$	80-05-7	AccuStandard
BPF	4,4'-dihydroxydiphenylmethane	$C_{13}H_{12}O_2$	620-92-8	AccuStandard
BPS	4,4'-dihydroxydiphenylsulfone	$C_{12}H_{10}O_4S$	80-09-1	AccuStandard
Phthalate ester metabolites				

MMP	monomethyl phthalate	$C_9H_8O_4$	4376-18-5	AccuStandard
M(i)BP	mono-(iso)butyl phthalate	$C_{12}H_{14}O_4$	30833-53-5	AccuStandard
Antioxidants				
BHT	2,6-di-tert-butyl-4-hydroxytoluene	$C_{15}H_{24}O$	128-37-0	AccuStandard
Internal standards				
MPFBA	perfluoro[(13)C4]butanoic acid	$[^{13}C_4]HF_7O_2$	NA[b]	Wellington
MPFHxA	perfluoro[1,2-(13)C2]hexanoic acid	$[^{13}C_2]C_4HF_{11}O_2$	NA	Wellington
MPFHxS	perfluorohexane[(18)O2]sulfonic acid	$C_6HF_{13}[^{18}O_2]OS$	1585941-14-5	Wellington
MPFOA	perfluoro[1,2,3,4-(13)C4]octanoic acid	$[^{13}C_4]C_4HF_{15}O_2$	960315-48-4	Wellington
MPFNA	perfluoro[1,2,3,4,5-(13)C5]nonanoic acid	$[^{13}C_5]C_4HF_{17}O_2$	NA	Wellington
MPFDA	perfluoro[1,2-(13)C2]decanoic acid	$[^{13}C2]C_8HF_{19}O_2$	NA	Wellington
MPFUdA	perfluoro[1,2-(13)C2]undecanoic acid	$[^{13}C_2]C_9HF_{21}O_2$	960315-51-9	Wellington
MPFDoA	perfluoro[1,2-(13)C2]dodecanoic acid	$[^{13}C_2]C_{10}HF_{23}O_2$	NA	Wellington
MPFOS	perfluoro [1,2,3,4-(13)C4]octanesulfonic acid	$[^{13}C_4]C_4HF_{17}O_3S$	960315-53-1	Wellington
TBP-d_{27}	tri-n-butyl phosphate-d_{27}	$C_{12}[^2H_{27}]O_4P$	61196-26-7	Wellington
TPHP-d_{15}	triphenyl phosphate-d_{15}	$C_{18}[^2H_{15}]O_4P$	1173020-30-8	Wellington
TDCIPP-d_{15}	tris(1,3-dichloro-2-propyl) phosphate-d_{15}	$C_9[^2H_{15}]Cl_6O_4P$	1447569-77-8	Wellington
BHT-d_{21}	2,6-di(tert-butyl)-4-methyl-phenol-d_{21}	$C_{15}H_3[^2H_{21}]O$	64502-99-4	Dr. Ehrenstorfer
BPA-d_6	bisphenol A-d_6	$C_{15}H_{10}[^2H_6]O_2$	86588-58-1	TRC
BPF-d_{10}	bisphenol F-d_{10}	$C_{12}H_2[^2H_{10}]O_2$	1794786-93-8	TRC
BPS-13C_{12}	bisphenol S-$^{13}C_{12}$	$[^{13}C_{12}]H10O_4S$	1991267-29-8	TRC
MEPA-d_4	monoethyl phthalate-d_4	$C_{10}H_6[^2H_4]O_4$	1219806-03-7	TRC
MeP-d_4	methyl paraben-d_4	$C_8H4[^2H_4]O_3$	362049-51-2	TRC
EtP-d_4	ethyl paraben-d_4	$C_9H_6[^2H_4]O_3$	1219795-53-5	TRC
Bup-d_9	butyl-paraben-d_9	$C_{11}H_5[^2H_9]O_3$	1216904-65-2	TRC
TCS-d_3	triclosan-d_3	$C_{12}H_4[^2H_3]Cl_3O_2$	1020719-98-5	TRC
Internal standards				
M8PFOA	perfluoro[$^{13}C_8$]octanoic acid	$[^{13}C_8]HF_{15}O_2$	NA	Wellington
BPA-d_{16}	bisphenol A-d_{16}	$C_{15}[^2H_{16}]O_2$	96210-87-6	Sigma-Aldrich
CMP-d_{10}	coumaphos-d_{10}	$C_{14}H_6[^2H_{10}]ClO_5PS$	287397-86-8	TRC

[a]Suppliers: Wellington: Wellington Laboratories (Guelph, Ontario, Canada); AccuStandard: AccuStandard Inc. (New Haven, Connecticut, USA); TRC: Toronto Research Chemicals (Toronto, Canada); Dr. Ehrenstorfer: LGC Standards (Middlesex, UK); Sigma-Aldrich: Sigma-Aldrich, Inc. (St. Louis, Missouri, USA).
[b]NA: not available.

Table S2. Demographic characteristics of study participants.[a]

variables	n (%)	Mean ± SD	range
age (years)	180	39 ± 19	13-85
gender			
female	95 (52.8)		
male	85 (47.2)		
disease type			
mental illness	43 (23.9)		
lumbar disc herniation	48 (26.7)		
spinal stenosis	37 (20.6)		
or viral meningitis	52 (28.9)		

[a]Continuous variables are presented as mean ± SD. Category variables are presented as numbers (percentage)

Table S3. Quality assurance and control test results.

Chemical	Blank[a] (ng/mL, n = 18)	Recovery (%, n = 5)	Matrix effect (%, n=10)	Precision	
				Intra-batch (%, n=4)	Inter-batch (%, n=4)
Per- and polyfluoroalkyl substances					
PFBA		78.0 ± 5.4	100 ± 7.8	6.3	4.0
PFHxA		110 ± 4.4	86.1 ± 3.4	4.0	10.3
PFHpA		75.0 ± 29.1	92.3 ± 6.2	8.9	18.0
PFOA		103 ± 6.4	101 ± 10.3	6.2	11.0
PFNA		96.8 ± 5.3	97.7 ± 8.4	4.5	6.0
PFDA		99.8± 7.5	100 ± 3.9	12.1	2.0
PFUdA		108 ± 3.9	83.4 ± 3.8	6.0	2.0
PFDoA		109 ± 10.2	85.5 ± 7.7	5.0	5.2
PFBS		110 ± 10.1	103 ± 5.0	6.2	11.2
PFHxS		72.0 ± 15.0	74.0 ± 5.0	9.0	13.0
PFHpS		104 ± 8.3	101 ± 8.4	4.8	9.2
PFOS		90.5 ± 2.6	81.0 ± 6.2	3.0	7.0
6:2 Cl-PFESA		97.1 ± 8.8	79.5 ± 4.5	6.0	3.0
Organophosphate esters					
TBP	0.01 ± 0.009	128 ± 5.6	107 ± 5.2	5.2	2.0
TPHP	0.03 ± 0.05	80.1 ± 2.6	100 ± 6.7	7.0	2.0
TCIPP	0.04 ± 0.03	74.3 ± 4.0	111 ± 13.7	6.0	2.0
Personal care products					
MeP	0.06 ± 0.05	89.9 ± 12.0	119 ± 7.9	17.2	12.9
EtP		90.9 ± 6.0	108 ± 8.2	10.4	6.0
BuP		79.0 ± 5.9	111 ± 4.4	7.0	9.0
TCS		83.0 ± 9.7	79.4 ± 6.7	15.8	17.0
Photoiniators					
MK		111 ± 9.4	113 ± 6.8	14.0	12.0
PI-651		102 ± 6.7	91.0 ± 25.7	8.2	5.6
Bisphenols					
BPA		120 ± 16.1	103.3 ± 14.1	3.0	7.0
BPF		118 ± 14.0	109.6 ± 5.0	9.1	5.8

BPS		101 ± 3.6	115.5 ± 8.4	11.0	6.0
Phthalate ester metabolites					
MMP		93.0 ± 8.4	99.3 ± 8.6	6.0	7.0
M(i)BP	0.13 ± 0.17	108 ± 9.9	105 ± 13.0	12.1	10.3
Antioxidants					
BHT	0.11 ± 0.19	82.0 ± 5.8	108 ± 4.6	9.3	15.6

[a]No fill indicates no detectable blank contamination.

References

Huang W, Pan XF, Tang S, Sun F, Wu P, Yuan J, Sun W, Pan A, Chen D. Target exposome for characterizing early gestational exposure to contaminants of emerging concern and association with gestational diabetes mellitus. Environ Sci Technol. 2023 Sep 12;57(36):13408-13418, PMID: 37651547, https://doi: 10.1021/acs.est.3c04492.