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Suspect-Screening Analysis of Environmental Chemicals in Paired Human Cerebrospinal Fluid and Serum Samples

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Introduction

Studies have reported that numerous environmental contaminants may cross the blood–brain barrier into the central nervous system (CNS),[1] some of which may adversely affect neural development and fucntions.[2] Human cerebrospinal fluid (CSF) is an integral CNS component emerging in parallel with the developing CNS. Detection of exogenous chemicals in CSF raises concern for potential neurological effects.[3] Despite reports of select environmental chemicals in CSF, none, to our knowledge, has investigated CSF exposure to a large array of environmental chemicals, particularly those with potential neurotoxic effects. Therefore, the present study aimed to develop a suspect screening-based strategy to identify environmental chemicals with potential neurotoxic effects in human CSF.

Methods

We recruited 180 outpatients ($n = 95$ female) 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. Participants were 39 ± 19 years of age and all participants completed an informed consent form. CSF and serum samples were collected the same day by doctors or nurses. Field blanks were prepared with high-performance liquid chromatography (HPLC)–grade water using the same procedures. The CSF and serum samples were pretreated following the method described by Huang et al., with minor modifications.[4] Briefly, 0.2 mL of serum/CSF was spiked with 21 internal standards (5 ng each) and extracted with a mixture of ethyl acetate and hexane (3 mL; 3:2, vol/vol, 0.6% formic acid) in three cycles.[4] The supernatants were collected after each cycle and combined, concentrated to 0.5 mL, frozen overnight, and centrifuged to collect the supernatant for analysis. It is noted that the modified methodology has not previously been reported to test CSF, to our knowledge. A single pooled extract was prepared by combining 5 μL from each of the 180 CSF or serum extracts. The study was approved by the Human Studies Committee of Sun Yat-sen University.

A suspect screening approach was established to detect environmental chemicals in human CSF and serum. First, a suspect list was developed by exploratory searches in the US Environmental Protection Agency's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) and scientific literature for chemicals with potential neurotoxic effects. Second, the CSF/serum pools were analyzed using ultra-HPLC (UHPLC) coupled to an Orbitrap Exploris 240 mass spectrometer (MS; Thermo Fisher Scientific). The suspect list was imported into the Mass List of Compound Discoverer 3.2. After identifying potential candidates with precursors mass matched with the suspect list (Δmass <5 ppm), their MS/MS spectra were compared with the spectra in the Mass Bank database or predicted by MetFrag. Further positive confirmation constituted a final list of 28 substances for quantitative analysis. Third, a quantitative method was established based on using an LC-30A UHPLC coupled to a Triple Quad 7500 MS (SCIEX).[4] Quality assurance/control (QA/QC) tests revealed that the analyte recoveries from spiking tests ranged from 72% to 128%, matrix effects 74% to 119%, interbatch coefficients of variation <20% (50 samples per batch), and absence of background contamination in field and procedural blanks (1 blank processed with 10 samples) for target analytes, except for tributyl phosphate (TBP), triphenyl phosphate (TPHP), tris(2-chloroisopropyl) phosphate (TCIPP), methyl paraben (MeP), mono-(iso)butyl phthalate [M(i)BP], and 2,6-di-*tert*-butyl-4-hydroxytoluene (BHT). Detailed information on quantitative and qualitative analysis and QA/QC procedures are available on GitHub (https://github.com/ZL1695/EHP-SI). For analytes with detection frequency (DF) ≥60%, measurements below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2 for statistical analysis. The LOD of a chemical without blank contamination was defined as the analyte response three times the standard deviation (SD) of the noise of a standard solution. For chemicals with blank contamination, the LOD was defined as the average concentration in blanks plus three times the SD of blank contamination. Relationships between serum and CSF concentrations were determined using Spearman correlation analysis (SPSS Statistics 26; IBM); we further assessed correlation between physiochemical properties [molecular weight and volume, logarithmic octanol–water partition coefficient (log K_{ow}), acid dissociation constant (pK_a), and biodegradation half-life] and the CSF/serum ratio ($R_{CSF/serum}$) to explore how these properties influence the CSF levels of target compounds. Only chemicals with detectable values in >40 pairs of CSF and serum samples were included in the analysis. The significance level was set as $\alpha = 0.05$ (two-tailed).

Results and Discussion

The 28 environmental chemicals included exhibited a range of median concentrations from <LOD to 10.5 ng/mL in serum and <LOD to 1.2 ng/mL in CSF (Table 1). They belong to the groups of per- and polyfluoroalkyl substances (PFAS; $n = 13$), organophosphate esters ($n = 3$), personal care products ($n = 4$), photoinitiators ($n = 2$), bisphenols ($n = 3$), phthalate ester metabolites ($n = 2$), and antioxidants ($n = 1$). Four PFAS compounds were detected in

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Table 1. Summary of environmental chemicals detected in human serum and cerebrospinal fluid (CSF) pairs from 180 adults in Guangzhou, China, 2022–2023.

		Serum (ng/mL)				CSF (ng/mL)			
Chemical	LOD	DF	Min	Median (IQR)	Max	DF	Min	Median (IQR)	Max
Per- and polyfluoroalkyl substances									
PFBA	0.02	68	<LOD	0.17 (<LOD to 0.27)	0.70	35	<LOD	<LOD (<LOD to 0.075)	0.32
PFHxA	0.007	57	<LOD	0.14 (<LOD to 0.57)	6.90	35	<LOD	<LOD (<LOD to 0.016)	0.18
PFHpA	0.004	97	<LOD	0.25 (0.18–0.37)	1.82	47	<LOD	<LOD (<LOD to 0.020)	0.80
PFOA	0.003	100	0.74	7.2 (4.7–11.7)	91.3	97	<LOD	0.042 (0.022–0.099)	0.87
PFNA	0.010	100	0.15	1.1 (0.79–2.0)	6.91	22	<LOD	<LOD (<LOD to <LOD)	0.41
PFDA	0.002	100	0.13	0.89 (0.47–1.6)	7.26	31	<LOD	<LOD (<LOD to 0.003)	0.13
PFUdA	0.002	100	0.053	0.81 (0.41–1.9)	12.5	46	<LOD	<LOD (<LOD to 0.005)	0.14
PFDoA	0.002	100	0.004	0.092 (0.047–0.18)	0.88	18	<LOD	<LOD (<LOD to <LOD)	0.03
PFBS	0.003	88	<LOD	0.022 (0.011–0.040)	0.36	64	<LOD	0.004 (<LOD to 0.009)	0.03
PFHxS	0.002	100	0.14	0.92 (0.51–1.6)	9.98	86	<LOD	0.006 (0.003–0.012)	0.19
PFHpS	0.004	98	<LOD	0.10 (0.059–0.20)	0.78	47	<LOD	<LOD (<LOD to 0.007)	0.04
PFOS	0.02	100	0.731	10.5 (6.6–20.8)	70.2	47	<LOD	<LOD (<LOD to 0.043)	2.03
6:2 Cl-PFESA	0.001	100	0.075	2.0 (0.99–3.8)	22.2	78	<LOD	0.001 (<LOD to 0.002)	0.38
Organophosphate esters									
TBP	0.02	78	<LOD	0.26 (0.024–0.60)	25.2	83	<LOD	0.16 (0.040–0.31)	6.97
TPHP	0.02	85	<LOD	0.10 (0.037–0.23)	4.04	68	<LOD	0.029 (<LOD to 0.11)	0.46
TCIPP	0.34	67	<LOD	1.7 (<LOD to 5.4)	38.6	52	<LOD	0.54 (<LOD to 2.6)	67.2
Personal care products									
MeP	0.01	100	0.062	0.61 (0.34–1.3)	19.4	60	<LOD	0.015 (<LOD to 0.072)	1.81
EtP	0.02	100	0.030	0.55 (0.32–1.1)	256	71	<LOD	0.072 (<LOD to 2.5)	6.91
BuP	0.01	68	<LOD	0.014 (<LOD to 0.027)	1.07	15	<LOD	<LOD (<LOD to <LOD)	0.02
TCS	0.01	89	<LOD	0.094 (0.041–0.23)	15.3	83	<LOD	0.085 (0.021–0.18)	14.0
Photoinitiators									
MK	0.01	83	<LOD	0.23 (0.076–0.46)	56	71	<LOD	0.035 (<LOD to 0.097)	6.66
PI-651	0.01	69	<LOD	0.29 (<LOD to 0.85)	275	63	<LOD	0.022 (<LOD to 0.072)	0.78
Bisphenols									
BPA	0.31	27	<LOD	<LOD (<LOD to 0.40)	37.5	41	<LOD	<LOD (<LOD to 0.42)	4.04
BPF	0.03	27	<LOD	<LOD (<LOD to 0.27)	44.0	19	<LOD	<LOD (<LOD to <LOD)	7.67
BPS	0.01	54	<LOD	0.012 (<LOD to 0.038)	2.08	48	<LOD	<LOD (<LOD to 0.072)	0.95
Phthalate ester metabolites									
MMP	0.26	100	0.27	2.4 (1.5–4.9)	80.5	55	<LOD	0.42 (<LOD to 1.1)	16.5
M(i)BP	0.08	76	<LOD	1.4 (0.10–3.1)	104	73	<LOD	1.2 (<LOD to 3.1)	41.1
Antioxidants									
BHT	0.30	78	<LOD	6.4 (<LOD to 19.0)	84.6	38	<LOD	<LOD (<LOD to 3.2)	48.8

Note: Quantitative analysis was conducted on an LC-30A ultra-high performance liquid chromatograph interfaced with a Triple Quad 7500 mass spectrometer (SCIEX).[4] Detailed information on instrumental analysis is available on GitHub (https://github.com/ZL1695/EHP-SI). 6:2 Cl-PFESA, 6:2 chlorinated perfluoroalkyl ether sulfonic acid; BHT, 2,6-di-*tert*-butyl-4-hydroxytoluene; BPA, 2,2-bis(4-hydroxyphenyl)propane; BPF, 4,4′-dihydroxydiphenylmethane; BPS, 4,4′-dihydroxydiphenylsulfone; BuP, butyl paraben; DF, detection frequency; EtP, ethyl paraben; IQR, interquartile range; LOD, limit of detection; max, maximum; MeP, methyl paraben; M(i)BP, mono-(iso)butyl phthalate; min, minimum; MK, 4,4′-bis(dimethylamino)benzophenone; MMP, monomethyl phthalate; PFBA, perfluorobutanoic acid; PFBS, perfluorobutanesulfonic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFUdA, perfluoroundecanoic acid; PI-651, 2,2-dimethoxyphenyl acetophenone; TBP, tributyl phosphate; TCIPP, tris(2-chloroisopropyl) phosphate; TCS, triclosan; TPHP, triphenyl phosphate.

≥60% of CSF samples ($n \geq 108$), including perfluorooctanoic acid (PFOA), perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), and 6:2 chlorinated polyfluoroalkyl ether sulfonate (6:2 Cl-PFESA) at median levels ranging from 0.001 to 0.042 ng/mL. Similar or higher concentrations were measured in CSF for M(i)BP (1.2 ng/mL), TBP (0.16 ng/mL), TPHP (0.029 ng/mL), 2,2 -dimethoxyphenyl acetophenone (PI-651; 0.022 ng/mL), triclosan (0.085 ng/mL), and methyl and ethyl parabens (0.015 and 0.072 ng/mL). To the best of our knowledge, only TBP, TPHP, and PFAS have been reported in human CSF prior to this study.

Some chemicals exhibited significant correlations between serum and CSF concentrations, whereas others did not (Table 1). The chemical-specific relationships between serum and CSF may be affected by several factors, including a chemical's physicochemical properties, pathways through the blood–cerebrospinal fluid barrier (BCSFB), and compromised barrier function.[5,6] The $R_{CSF/serum}$ of a compound appeared to be significantly and negatively correlated with its molecular weight (rho = –0.67; $p < 0.001$) or molecular volume (rho = –0.50; $p = 0.01$; Table 2), indicating that smaller molecules tend to diffuse across the BCSFB. A negative correlation was also observed between $R_{CSF/serum}$ and lipophilicity (represented by log K_{ow}; rho = –0.50; $p = 0.058$), suggesting that binding with lipids and proteins may restrict passive diffusion. The pK_a was also correlated with $R_{CSF/serum}$ (rho = –0.45; $p = 0.056$); however, no significant relationship was observed for biodegradation half-life and $R_{CSF/serum}$ (rho = –0.34; $p = 0.13$). Our findings align with those of a previous study that reported molecular size and lipophilicity could largely influence the ability of organophosphate esters to penetrate brain barriers.[5] Other than free passive diffusion, the penetration of exogenous chemicals into the CNS may be limited by efflux transporters distributed between the apical and basolateral membrane domains of the choroidal epithelial cells.[7] However, barrier functions may be compromised by neurological diseases, infections, trauma, and other factors, subsequently affecting chemical penetration.[6,7] These various factors may collectively impact CSF exposure to environmental chemicals and influence chemical-specific relationships between serum and CSF levels.

Our exploratory study documents a large number and breadth of potentially neurotoxic substances detected in human CSF. Chemical-specific relationships between serum and CSF concentrations raise the need for further elucidation of factors influencing the cross-BCSFB transfer of environmental chemicals. Our study highlights that efficient and accurate prediction of environmental chemicals' distribution in CSF is important to the exploration of their potential impact on neurological health, considering

Table 2. Summary of the correlations between cerebrospinal fluid (CSF) and serum concentrations of target environmental chemicals, concentration ratios of a chemical in CSF to that in paired serum samples ($R_{CSF/serum}$), and the correlations between $R_{CSF/serum}$ and selected physicochemical properties of chemicals.

Chemical[a]	N[b]	MW[c]	S_v[d]	log K_{ow}[e]	pK_a[f]	Biodeg. half-life[g]	rho[h]	p-Value[i]	$R_{CSF/serum}$[j] (IQR)
PFBA	41	214.0	135.2	2.14	−0.21	4.47	0.051	0.750	0.33 (0.26–0.44)
PFHpA	82	364.0	223.5	4.15	0.06	4.47	−0.41	<0.001	0.072 (0.039–0.26)
PFOA	174	414.0	252.9	4.81	0.34	4.90	0.31	<0.001	0.005 (0.003–0.011)
PFNA	40	464.0	282.3	5.48	0.23	4.90	−0.071	0.644	0.030 (0.015–0.048)
PFDA	56	514.0	311.7	6.15	0.4	4.90	0.006	0.965	0.007 (0.004–0.015)
PFUdA	83	564.0	341.2	6.82	0.54	4.90	0.037	0.741	0.007 (0.002–0.015)
PFDoA	47	614.0	370.6	7.49	0.54	5.50	−0.013	0.945	0.025 (0.013–0.061)
PFBS	107	300.0	177.2	1.82	−1.61	4.47	0.33	<0.001	0.26 (0.14–0.57)
PFHxS	155	399.9	236.1	3.16	−1.64	4.47	0.57	<0.001	0.007 (0.005–0.012)
PFHpS	84	449.9	265.5	3.82	−1.81	4.47	0.47	<0.001	0.052 (0.03–0.084)
PFOS	85	499.9	295.0	4.49	−1.64	4.90	0.50	<0.001	0.003 (0.002–0.005)
6:2 Cl-PFESA	140	569.9	312.9	3.10	1.58	4.68	0.45	<0.001	0.001 (0.0004–0.002)
TBP	124	266.2	271.1	3.82	NA	3.72	0.057	0.531	0.59 (0.19–1.01)
TPHP	103	326.1	281.4	4.7	NA	6.17	−0.29	0.003	0.54 (0.12–1.56)
TCIPP	140	326.0	264.8	2.89	NA	3.72	0.21	0.093	2.04 (0.65–4.82)
MeP	108	152.0	139.5	2.00	8.44	3.55	0.20	0.043	0.07 (0.021–0.18)
EtP	128	166.1	156.8	2.49	9.24	3.55	−0.074	0.409	0.24 (0.036–7.38)
TCS	138	288.0	217.0	4.66	8.66	4.47	0.35	<0.001	0.77 (0.23–1.52)
MK	114	268.2	268.4	3.50	3.04	5.5	0.19	0.046	0.17 (0.071–0.39)
PI-651	71	256.1	246.7	2.95	NA	4.9	−0.26	0.031	0.094 (0.019–0.41)
MMP	99	180.0	162.9	1.37	5.31	3.55	0.29	0.004	0.46 (0.25–0.85)
M(i)BP	128	222.1	214.8	2.77	4.66	3.55	0.28	0.003	1.33 (0.75–2.44)
BHT	54	220.2	245.6	5.03	11.8	14.8	0.06	0.664	0.32 (0.11–1.05)
rho (p-value)[k]	—	−0.67 (<0.001)	−0.50 (0.01)	−0.40 (0.058)	0.45 (0.056)	−0.34 (0.13)	—	—	—

Note: Paired samples are from 180 adults in Guangzhou, China, 2022–2023. —, Not applicable; 6:2 Cl-PFESA, 6:2 chlorinated perfluoroalkyl ether sulfonic acid; BHT, 2,6-di-*tert*-butyl-4-hydroxytoluene; biodeg, biodegradation; EtP, ethyl paraben; IQR, interquartile range; LOD, limit of detection; MeP, methyl paraben; M(i)BP, mono-(iso)butyl phthalate; MK, 4,4′-bis(dimethylamino)benzophenone; MMP, monomethyl phthalate; NA, not available; PFBA, perfluorobutanoic acid; PFBS, perfluorobutanesulfonic acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonic acid; PFUdA, perfluoroundecanoic acid; PI-651, 2,2-dimethoxyphenyl acetophenone; TBP, tributyl phosphate; TCIPP, tris(2-chloroisopropyl) phosphate; TCS, triclosan; TPHP, triphenyl phosphate.

[a]Only chemicals with measurements >LOD in >40 pairs of serum and CSF samples were included for correlation analysis.

[b]N represents the pairs of CSF and serum samples with measurements >LOD.

[c]MW: molecular weight (g/mol).

[d]S_v (Å3/molecule): van der Waals volume calculated with a model provided by Zhao et al., used to represent the molecular volume.[8]

[e]log K_{ow}: log-transformed octanol–water partition coefficient, predicted with the US Environmental Protection Agency Estimation Program Interface, version 4.11.

[f]pK_a represents the negative logarithm of the acid dissociation constant (K_a) of a solution, predicted with the Open (Quantitative) Structure-activity/property Relationship APP (OPERA) version 2.6.[9]

[g]Biodegradation half-life (day), predicted with OPERA 2.6.

[h]Spearman correlation coefficient (rho) between paired serum and CSF samples (SPSS Statistics 26; IBM).

[i]p-Values for the Spearman correlation between paired serum and CSF samples, with a two-sided $p < 0.05$ considered statistically significant.

[j]$R_{CSF/serum}$ represents the concentration ratio of a chemical in CSF to that in paired serum samples, reported as median and IQR values.

[k]Spearman correlation coefficient (rho) and p-value (in parenthesis) between $R_{CSF/serum}$ and a chemical's physicochemical properties.

the difficulty in obtaining CSF samples for clinical and epidemiological studies.

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References

1. Brown-Leung JM, Cannon JR. 2022. Neurotransmission targets of per- and polyfluoroalkyl substance neurotoxicity: mechanisms and potential implications for adverse neurological outcomes. Chem Res Toxicol 35(8):1312–1333, PMID: 35921496, https://doi.org/10.1021/acs.chemrestox.2c00072.
2. Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368(9553):2167–2178, PMID: 17174709, https://doi.org/10.1016/S0140-6736(06)69665-7.
3. Liu Y, Zhou X, Wu Y, Yang X, Wang Y, Li S, et al. 2022. Exposure and blood–cerebrospinal fluid barrier permeability of PFASs in neonates. Environ Sci Technol Lett 9(1):64–70, https://doi.org/10.1021/acs.estlett.1c00862.
4. Huang W, Pan XF, Tang S, Sun F, Wu P, Yuan J, et al. 2023. Target exposome for characterizing early gestational exposure to contaminants of emerging concern and association with gestational diabetes mellitus. Environ Sci Technol 57(36):13408–13418, PMID: 37651547, https://doi.org/10.1021/acs.est.3c04492.
5. Hou M, Zhang B, Fu S, Cai Y, Shi Y. 2022. Penetration of organophosphate triesters and diesters across the blood–cerebrospinal fluid barrier: efficiencies, impact factors, and mechanisms. Environ Sci Technol 56(12):8221–8230, PMID: 35658413, https://doi.org/10.1021/acs.est.2c01850.
6. Wang J, Pan Y, Cui Q, Yao B, Wang J, Dai J. 2018. Penetration of PFASs across the blood cerebrospinal fluid barrier and its determinants in humans. Environ Sci Technol 52(22):13553–13561, PMID: 30362723, https://doi.org/10.1021/acs.est.8b04550.
7. Ghersi-Egea JF, Strazielle N, Catala M, Silva-Vargas V, Doetsch F, Engelhardt B. 2018. Molecular anatomy and functions of the choroidal blood-cerebrospinal fluid barrier in health and disease. Acta Neuropathol 135(3):337–361, PMID: 29368213, https://doi.org/10.1007/s00401-018-1807-1.
8. Zhao YH, Abraham MH, Zissimos AM. 2003. Fast calculation of van der Waals volume as a sum of atomic and bond contributions and its application to drug compounds. J Org Chem 68(19):7368–7373, PMID: 12968888, https://doi.org/10.1021/jo034808o.
9. Mansouri K, Grulke CM, Judson RS, Williams AJ. 2018. OPERA models for predicting physicochemical properties and environmental fate end points. J Cheminform 10(1):10, PMID: 29520515, https://doi.org/10.1186/s13321-018-0263-1.