Supplementary Information

for

Organophosphate esters in human serum: A relatively simple and efficient liquid chromatography-mass spectrometry method

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Text S1 Extraction of serum OPEs using liquid-liquid extraction (ethyl acetate)

First, 200 μL of serum was placed in a glass tube, and then 10 μL of internal standard mix (400 ng/mL), 40 μL of formic acid, and 2 mL of ethyl acetate were added sequentially. After mixing for 1 min, the supernatant was shaken with a shaker at 300 r/min for 10 min and then centrifuged at 6000 rpm for 10 min. The supernatant was transferred to a new glass tube, the extracts were dried under a gentle nitrogen stream at 40°C (the nitrogen flow rate should not be too large, enabling slight fluctuations of the liquid level). 200 μL of aqueous acetonitrile (50:50, V:V) was added to the dried sample, which was then redissolved by ultrasonication for 5 min. Finally, the mixture was filtered through a 0.22 μm nylon needle filter into an injection vial for analysis.

Text S2 Procedure of solid phase extraction

Strata™-X-AW column: First, 200 μL of serum was added to the glass tube, then 10 μL of internal standard mix (400 ng/mL) was added and vortexed for 1 min to mix and set aside for use. The StrataX-AW column was firstly activated with 2 ml of acetonitrile and 2 ml of water. Then serum was added to the column, and the serum was allowed to pass through the column as slowly as possible. Then the column was rinsed with 2 mL of ultrapure water and vacuum pumped for 40 min. 4 mL of 5% triethylamine-acetonitrile was used for elution and the eluate was collected. The eluate was dried under a gentle nitrogen stream at 40°C (the nitrogen flow rate should not be too large, enabling slight fluctuations of the liquid level). 200 μL of aqueous acetonitrile (50:50, V:V) was added to the dried sample, which was then redissolved by ultrasonication for 5 min. Finally, the mixture was filtered through a 0.22 μm nylon needle filter into an injection vial for analysis.

Supelclean™ ENVI-18 column: First, 200 μL of serum was added to the glass tube, then 10 μL of internal standard mix (400 ng/mL) was added and vortexed for 1 min to mix and set aside for use. The ENVI-18 column was activated sequentially with 5 mL of acetonitrile and 5 mL of ultrapure water. Then serum was added to the column, and the serum was allowed to pass through the column as slowly as possible. Then the column was rinsed with 10 mL of ultrapure water and vacuum pumped for 60 min. 6 mL of acetonitrile containing 25% dichloromethane was used for elution and the eluate was collected. The eluate was dried under a gentle nitrogen stream at 40°C (the nitrogen flow rate should not be too large, enabling slight fluctuations of the liquid level). 200

μL of aqueous acetonitrile (50:50, V:V) was added to the dried sample, which was then redissolved by ultrasonication for 5 min. Finally, the mixture was filtered through a 0.22 μm nylon needle filter into an injection vial for analysis.

Oasis HLB column: First, 200 μL of serum was added to the glass tube, then 10 μL of internal standard mix (400 ng/mL) was added and vortexed for 1 min to mix and set aside for use. The HLB column was activated by 5 mL acetonitrile, 5 mL methanol and 10 mL ultrapure water. Then serum was added to the column, and the serum was allowed to pass through the column as slowly as possible. Then the column was rinsed with 10 mL of ultrapure water and vacuum pumped for 60 min. 10 mL of acetonitrile was used for elution and the eluate was collected. The eluate was dried under a gentle nitrogen stream at 40°C (the nitrogen flow rate should not be too large, enabling slight fluctuations of the liquid level). 200 μL of aqueous acetonitrile (50:50, V:V) was added to the dried sample, which was then redissolved by ultrasonication for 5 min. Finally, the mixture was filtered through a 0.22 μm nylon needle filter into an injection vial for analysis.

Sep-Pak C18 column: Consistent with the procedure of the HLB column.

Table S1 Full name, abbreviation, CAS number, molecular formula, and molecular weight of nine OPEs

Compounds	Abbreviation	CAS NO.	Formula	Molecular weight
Tri-ethyl-phosphate	TEP	78-40-0	$C_6H_{15}O_4P$	182.15
Tri-n-butyl-phosphate	TBP	126-73-8	$C_{12}H_{27}O_4P$	266.31
Tris-2-butoxy ethyl-phosphate	TBEP/TBOEP	78-51-3	$C_{18}H_{39}O_7P$	398.47
Tri-propyl-phosphate	TPrP	513-08-6	$C_9H_{21}O_4P$	224.23
Tris-2-chloroethyl-phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$	285.49
Tris-chloropropyl-phosphate	TCPP/TCIPP	1067-98-7	$C_9H_{18}Cl_3O_4P$	327.57
Tris-1,3-dichloro-2-propyl-phosphate	TDCP/TDCPP/TDCIPP	13674-87-8	$C_9H_{15}Cl_6O_4P$	430.91
Tris-2,3-dibromo propyl-phosphate	TDBP/TDBPP	126-72-7	$C_9H_{15}Br_6O_4P$	697.61
Tri-phenyl phosphate	TPhP/TPHP	115-86-6	$C_{18}H_{15}O_4P$	326.28

Table S2 The gradient elution conditions of OPEs

Time (min)	Flow Rate (mL/min)	Mobile Phase A[a] (%)	Mobile Phase B[b] (%)
0.00	0.25	80.0	20.0
1.00	0.25	80.0	20.0
3.00	0.25	10.0	90.0
6.00	0.25	10.0	90.0
6.10	0.25	80.0	20.0
9.00	0.25	80.0	20.0

[a]: Ultrapure water with 0.1% formic acid;

[b]: Acetronitrile.

Table S3 Retention time and exact mass number of OPEs in MS mode

Compound	Mean t_R	Theoretical Mass(Da)	Experimental Mass(Da)	Mass error (mDa)	Adducts
TEP	4.13	182.07080	182.0704	-0.4	+H
TBP	5.70	266.16470	266.1644	-0.3	+Na, +H
TBEP	5.83	398.24334	398.2425	-0.9	+H
TPrP	5.01	224.11775	224.1173	-0.5	+Na, +H
TCEP	4.63	283.95388	283.9537	-0.2	+H
TCIPP	5.02	326.00083	326.0022	1.4	+H, +Na
TDCPP	5.40	427.88391	427.8841	0.2	+H
TDBPP	5.53	697.57480	697.5744	-0.4	+H
TPhP	5.55	326.07080	326.0702	-0.6	+H, +Na

Table S4 Chromatographic and mass spectrometric characteristics of the analytes.

Compound	RT (min)[a]	Precursor (m/z)	CV(V)[b]	Product (m/z)	CE (V)[c]
TEP	4.13	183.0791	10	98.9854[d]	10
				127.0232[e]	5
TBP	5.70	267.1745	10	98.9874[d]	15
				155.0511[e]	10
TBEP	5.83	400.2721	30	300.1802[d]	15
				199.0850[e]	25
TPrP	5.01	225.1256	10	98.9854[d]	15
				141.0363[e]	10
TCEP	4.63	284.9617	20	98.9854[d]	25
				160.9815[e]	15
TCIPP	5.02	327.0068	30	98.9874[d]	20
				174.9934[e]	15
TDCPP	5.40	430.8889	20	98.9854[d]	30
				208.9561[e]	15
TDBPP	5.53	698.5813	20	98.9854[d]	25
				498.7246[e]	15
TPhP	5.55	327.0821	30	215.0270[d]	25
				152.0613[e]	30
TCEP-d12	4.62	296.0478	20	167.0162[d]	15
				102.0050[e]	20
TCIPP-d18	5.00	344.1421	30	102.0046[d]	25
				183.0433[e]	10
TPrP-d21	4.98	245.2824	10	102.0067[d]	20
				150.0901[e]	10
TBP-d27	5.67	294.3542	20	102.0067[d]	15
				230.2316[e]	10
TPhP-d15	5.52	342.1717	20	223.0790[d]	25
				161.1215[e]	15

[a]: Retention time

[b]: Cone voltage

[c]: Collision energy

[d]: Quantitative ion

[e]: Qualitative ion

Table S5 ME of the target OPEs (n=6)

Analyte	ME (%)	Internal standards	MEi (%)
TEP	43.5	TPrP-d21	82.7
TBP	38.0	TBP-d27	86.3
TBEP	36.9	TBP-d27	83.9
TPrP	53.2	TPrP-d21	101.0
TCEP	65.1	TCEP-d12	113.1
TCIPP	59.3	TCIPP-d18	86.4
TDBPP	47.3	TPhP-d15	99.6
TDCPP	54.1	TPhP-d15	113.9
TPhP	41.1	TPhP-d15	86.5
TBP-d27	44.0	/	/
TCEP-d12	57.6	/	/
TPrP-d21	52.6	/	/
TCIPP-d18	68.6	/	/
TPhP-d15	47.5	/	/

Table S6 Concentrations of OPEs (ng/mL) in serum samples[a] (N=269)

Chemicals	Detection frequency (%)	5th	25th	50th	75th	95th	Rang	Trimmean±Std[b]
TBP	53	<MDL[c]	<MDL	0.04	4.18	19.28	<MDL~68.00	1.69±2.91
TEP	32	<MDL	<MDL	<MDL	0.14	1.23	<MDL~21.16	0.08±0.10
TBEP	73	<MDL	<MDL	0.06	0.17	0.86	<MDL~10.43	0.10±0.09
TPrP	88	<MDL	<MDL	0.36	0.46	0.62	<MDL~0.80	0.31±0.17
TCEP	76	<MDL	0.07	0.15	0.75	2.50	<MDL~9.71	0.33±0.35
TCIPP	60	<MDL	<MDL	1.31	3.64	8.74	<MDL~455.60	1.71±1.82
TDCPP	83	<MDL	<MQL[d]	1.23	3.30	12.52	<MDL~106.80	1.77±1.87
TDBPP	85	<MDL	0.14	0.37	0.80	2.0	<MDL~52.15	0.46±0.37
TPhP	66	<MDL	<MDL	0.15	0.85	6.96	<MDL~385.80	0.48±0.66
ΣOPFRs	100	3.41	5.84	9.68	14.47	54.18	0.79~552.26	10.50±4.88

[a]: Reported values were calculated by subtracting the average blank value from the initial concentration.

[b]: The trimmed mean was calculated by removing 10% of the data from both ends to calculate the average value.

[c]: Below the method, the detection limit was defined as not detected and was not included in the calculation of the detection frequency. Values below the method detection limit were used in the statistical calculation at 1/2 the detection limit.

[d]: Range from the MDL to MQL. Values below the method quantification limit were used in the statistical calculation at 1/2 the detection limit.

Table S7 Detection of blood OPEs in populations by regions

Area	Type	Samples	Median concentration(min~max,detection frequency)									Reference
			TBP	TEP	TBEP	TPrP	TCEP	TCIPP	TDCPP	TDBPP	TPhP	
Beijing	Whole blood	57(ng/ml)	/[a]	0.43(0.146-9.88,100%)	0.16(n.d.[b]-19.5%)	n.d.(n.d.–0.126,11%)	n.d.(n.d.-1.80,14%)	n.d.(n.d.-15.2,49%)	n.d.(n.d.-6.06,25%)	/	0.366(n.d.-7.80,65%)	[1]
	Serum	57(ng/ml)	/	0.432(0.081-12.3, 74%)	0.21(n.d.-0.976,84%)	n.d.(n.d.–0.025,16%)	n.d.(n.d.-1.66,18%)	1.05(0.166-5.24,82%)	n.d.(n.d.–5.16,26%)	/	n.d.(n.d.–0.692, 32%)	
Tianjin	Serum	319(ng/ml)	/	1.52(n.d.-9.63,62.1%)	1.44(n.d.-2.72,9.09%)	/	0.33(n.d.-2.89,96.6%)	3.19(n.d.-30.3,75.2&)	0.16(n.d.-1.97,79.3%)	/	/	[2]
Shangdong	Whole blood	352(ng/ml)	/	0.14(n.d.-18.4,54%)	n.d.(n.d.-6.17,41%)	n.d.(n.d.-0.250 ,0.9%)	0.30(n.d.-5.06,56%)	0.74./0.508(n.d.-4.45,77%)	n.d.(n.d.-2.57,34%)	/	0.40(n.d.-7.52,78%)	[3]
Shangdong	Serum [c]	239(ng/ml)	108(50%)	/	n.d. (39%)	n.d. (28%)	18(74%)	n.d. (18%)	n.d. (49%)	/	/	[4]
Jiangsu	Serum/Plasma	99(ng/ml)	/	0.15(n.d.-2.8,79%)	0.05(n.d.-14.4,51%)	/	0.1(n.d.-3.1,66%)	0.05(n.d.-7.0,57%)	n.d.(n.d.-1.4,24%)	/	0.35(n.d.-14.4,80%)	[5]
Shenzhen	Whole blood	257(ng/ml)	/	0.49(n.d.-4.59,96.1%)	0.54(n.d.-16.0.98.1%)	/	0.16(n.d.-2438,63.0%)	0.71(n.d.-21.61,90.3%)	0.01(n.d.-3.41,47.1%)	/	0.43(n.d.-1.21,98.4%)	[6]
Dalian	Serum	89(ng/g lw)	/	/	/	9(n.d-65.3, 83.2%)	214(n.d.-894,76.4%)	0.61(n.d.-31.5,4.5%)	/	/	0.51(n.d.-92.6,36%)	[7]
Zhejiang	Serum	145(ng/ml)	0.27(n.d.-1.54,88%)	1.18(n.d.-9.84,95%)	0.034(n.d.-0.39,63%)	/	0.45(n.d.-2.4,95%)	0.49(n.d.-1.72,93%)	0.027(n.d.-0.85,62%)	/	0.18(n.d.-2.06,80%)	[8]
Zhejiang	plasma	96(ng/ml)	0.87(n.d.-2.85,97%)	/	0.28(n.d.-3.45,95%)	/	0.87(n.d.-2.79,88%)	0.494(n.d.-2.94,64%)	n.d.(0%)	/	1.83(n.d.-6.582,98%)	[9]

[a]: The substance was not addressed in the study;
[b]: Not detected;
[c]: Concentration range values for OPFRs were not provided in this study.

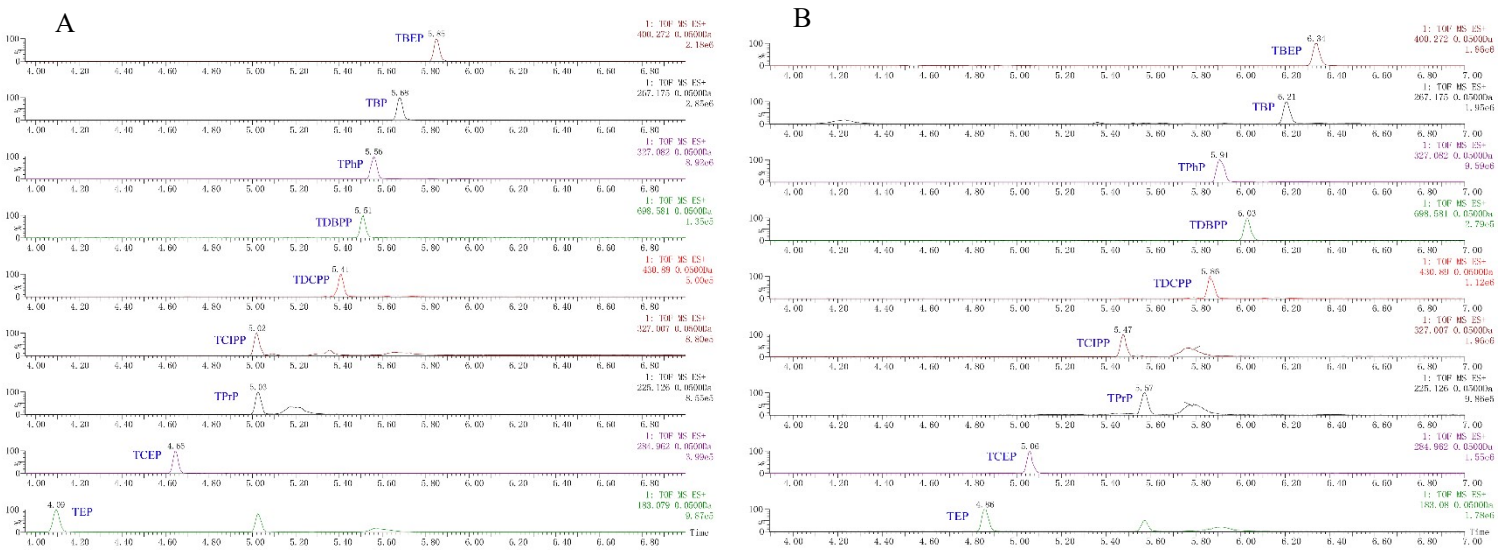

Fig. S1 Comparison of mobile phase types on peak shape and separation of OPEs. (A) The organic phase is acetonitrile ; (B) The organic phase is methanol; The concentration of OPEs is 20ng/mL in the figures.

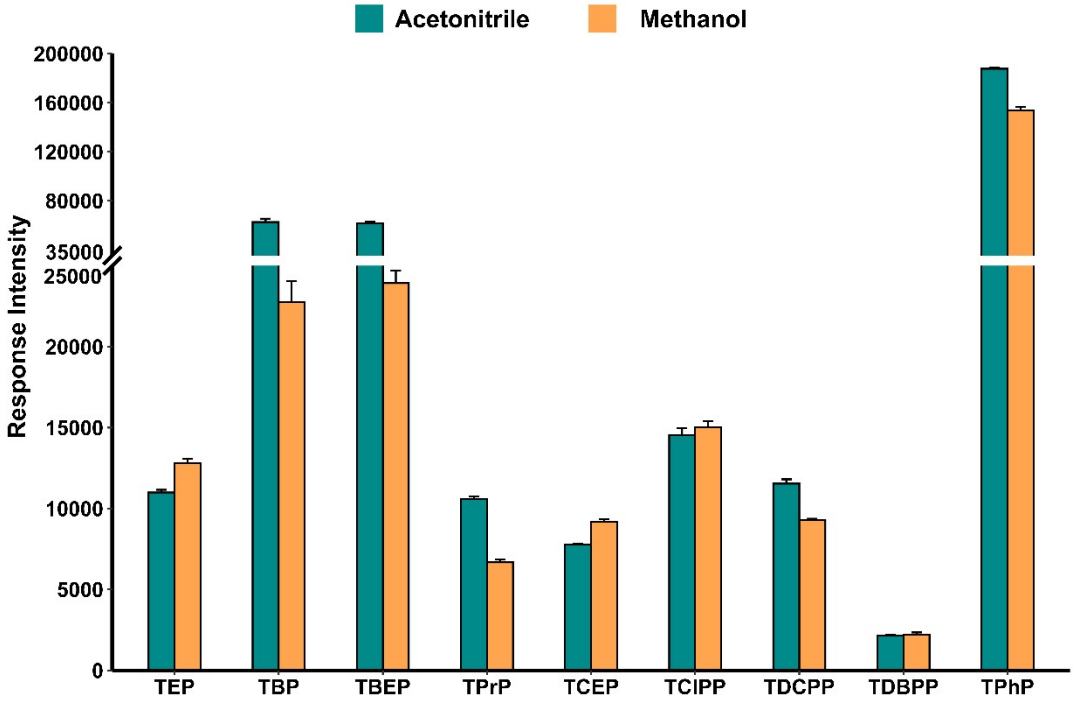

Fig. S2 Effect of mobile phase type on the response strength of OPEs. The concentration of OPEs is 20ng/mL in the figure.

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