Supporting Information

Organophosphorus Flame Retardants and Plasticizers in Breast Milk from the United States

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The method for extraction and instrumental analysis

Fourteen triester OPEs were analyzed; triethyl phosphate (TEP), tripropyl phosphate (TPP), tri-n-butyl phosphate (TNBP), tris(2-butoxyethyl) phosphate (TBOEP), tris(2-ethylhexyl) phosphate (TEHP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), triphenyl phosphate (TPHP), trimethylphenyl phosphate (TMPP), cresyl diphenyl phosphate (CDPP), and isodecyl diphenyl phosphate (IDDP) were purchased from AccuStandard (New Haven, CT, US). Trimethyl phosphate (TMP), tri-iso-butyl phosphate (TIBP), and 2-ethylhexyl diphenyl phosphate (EHDPP) were purchased from Sigma-Aldrich (St. Louis, MO, US). TPP-d_{21}, TNBP-d_{27}, TCEP-d_{12}, TCIPP-d_{18}, and TPHP-d_{15} were purchased from Cambridge Isotope Laboratories (Tewksbury, MA, US), TMP-d_9 and TEHP-d_{51} were purchased from Toronto Research Chemicals (North York, ON, Canada), and TEP-d_{15} was purchased from Sigma-Aldrich.

Briefly, 0.5 mL of breast milk was transferred into a polypropylene (PP) tube and spiked with 5 ng each of internal standards (TMP-d_9, TEP-d_{15}, TPP-d_{21}, TNBP-d_{27}, TEHP-d_{51}, TCEP-d_{12}, TCIPP-d_{18}, TPHP-d_{15}). After the addition of 5 mL of 0.5% formic acid in acetonitrile (ACN), the sample was extracted by shaking in an orbital shaker for 2 h, followed by ultrasonication for 30 min. To the extract, 500 mg of anhydrous $MgSO_4$ was added, vortexed for 1 min, and centrifuged for 10 min at 4500 r/min. Then, the supernatant was transferred into a precleaned PP tube for purification, followed by the addition of d-Solid phase extraction (d-SPE) sorbents (150 mg $MgSO_4$, 50 mg primary-second amine (PSA), and 50 mg C_{18}). The mixture was vortexed for 1 min and then centrifuged at 4500 r/min for 5 min. The supernatant was transferred into a new PP tube for evaporation under gentle N_2 stream at 30 °C to near dryness, and reconstituted with 200 μL of water/methanol (4:6; v/v) and filtered through a 0.2 μm nylon centrifuge tube filter (Corning, Salt Lake City, UT, US) at 10000 r/min for 1 min, and then transferred into a glass vial.

The analysis of 14 OPEs was performed using a Shimazu LC-30 AD Series high performance liquid chromatography (HPLC, Shimazu Corporation, Kyoto, Japan) coupled to an API 5500 triple quadrupole mass spectrometry system (MS/MS, Applied Biosystems, Foster City, CA, US). The extract was injected onto a Betasil C_{18} column (100 mm × 2.1 mm, 5 μm; Thermo, Waltham, MA, US), connected to a Betasil C_{18} guard column (20 mm × 2.1 mm, 5 μm; Thermo). The mobile phase consisted of HPLC-grade water with 0.1% formic acid (v/v) (A) and methanol with 0.1% formic acid (v/v) (B), at a flow rate of 200 μL/min. The gradient flow started at 40:60 A/B that was held for 2 min, then decreased linearly to 1:99 A/B over 5.5 min, held for 9.5 min, before reversing to 40:60 A/B within 0.5 min, and equilibrated for 7.5 min. Multiple reaction monitoring (MRM) mode was used for quantitative determination of 14 OPEs and 8 internal standards, and the MRM transitions are shown in Table S2.

Table S1 Demographic characteristics of participants (n = 100)

		N (%)
Maternal characteristics		
Maternal age (years)	16 – 26	26 (26%)
	27 – 33	50 (50%)
	34 – 44	24 (24%)
Parity	Primipara	86 (86%)
	Multipara	14 (14%)
Maternal education (years completed)	less than high school	13 (14%)
	High school or some college	66 (72%)
	College or higher	13 (14%)
	Missing	8
Pre-pregnancy BMI (kg/m^2)	Underweight (< 18.5)	2 (2%)
	Normal (18.5 – 24.9)	52 (60%)
	Overweight (25 – 29.9)	17 (20%)
	Obese (≥ 30)	15 (18%)
	Missing	14
Family income	< $49,999	39 (44%)
	$49,999 – $99,999	28 (31%)
	> $99,999	22 (25%)
	Missing	11
Ethnicity	Hispanic	10 (10%)
	Non-Hispanic	90 (90%)
Race	White	77 (77%)
	Non-white	23 (23%)
Geographic areas	West	11 (11%)
	Middle	51 (51%)
	East	38 (38%)
Child characteristics		
Sex	Boy	50 (50%)
	Girl	50 (50%)
Preterm (< 37 weeks gestation)	No	89 (92%)
	Yes	8 (8%)
	Missing	3
Birth weight	< 3000 g	17 (21%)
	3000 – 3999 g	53 (65%)
	> 4000 g	12 (14%)
	Missing	18
Season of birth	Winter (Dec – Feb)	23 (23%)
	Spring (Mar – May)	37 (37%)
	Summer (Jun – Aug)	29 (29%)
	Fall (Sep – Nov)	11 (11%)
	Mean	± SD
Maternal age	29.8	± 4.99
Maternal BMI (kg/m^2)	25.2	± 5.74
Birth of weight (g)	3356	± 556
Birth of height (cm)	51.3	± 3.06

SD: standard deviation.
BMI: body mass index.

Table S2 Chemical properties and MS/MS parameters of target OPE compounds

Name	Abbreviation	CAS NO.	Formula	Molecular Weight	Quantitative transition		Declustering Potential	Collision Energy	Collision Cell Exit Potential	Retention Time (min)
Tri-n-butyl phosphate	TNBP	126-73-8	$C_{12}H_{27}O_4P$	266.31	267.1	99	31	22	14	8.00
Tri-iso-butyl phosphate	TIBP	126-71-6	$C_{12}H_{27}O_4P$	266.32	267.1	99	31	22	14	7.90
Trimethylphenyl phosphate	TMPP	1330-78-5	$C_{21}H_{21}O_4P$	368.36	369.1	166.1	70	50	15	8.71
Triethyl phosphate	TEP	78-40-0	$C_6H_{15}O_4P$	182.15	183	99	30	20	12	2.34
Triphenyl phosphate	TPHP	115-86-6	$C_{18}H_{15}O_4P$	326.28	327.1	77	110	46	19	7.35
Tripropyl phosphate	TPP	513-08-6	$C_9H_{21}O_4P$	224.23	225.1	99	30	15	15	6.02
Tris(2-butoxyethyl) phosphate	TBOEP	78-51-3	$C_{18}H_{39}O_7P$	398.47	399.1	199	65	16	16	8.23
Tris(2-chloroethyl) phosphate	TCEP	115-96-8	$C_6H_{12}Cl_3O_4P$	285.49	284.9	63.1	30	50	8	3.04
Tris(2-chloroisopropyl) phosphate	TCIPP	13674-84-5	$C_9H_{18}Cl_3O_4P$	327.57	327.1	99	40	35	17	6.10
Tris(2-ethylhexyl) phosphate	TEHP	78-42-2	$C_{24}H_{51}O_4P$	434.63	435.4	99	20	30	12	12.38
2-Ethylhexyl diphenyl phosphate	EHDPP	1241-94-7	$C_{20}H_{27}O_4P$	362.4	363.2	251	14	13	35	8.99
Isodecyl diphenyl phosphate	IDDP	29761-21-5	$C_{22}H_{31}O_4P$	390.4	391	250.9	16	18	37	9.56
Cresyl diphenyl phosphate	CDPP	26444-49-5	$C_{19}H_{17}O_4P$	340.3	341.1	152	35	50	18	7.84
Trimethyl phosphate	TMP	512-56-1	$C_3H_9O_4P$	140.8	141	108.9	30	25	12	1.32

Table S3 Instrumental performance, matrix spiked recoveries, procedure blanks, limit of quantitation (ng/mL)

Aalytes	IS	% IS recovery	% Matrix spike recovery (n=10)			Liner/R	Procedure blanks	LOB	LOD	LOQ
			1 ng/g	5 ng/g	Mean					
TNBP	TNBP-d_{27}	45 ± 29	114 ± 27	91 ± 5	103 ± 22	0.9994	0.1841 ± 0.0355	0.2425	0.301	0.301
TIBP	TNBP-d_{27}		109 ± 19	115 ± 8	112 ± 15	0.9990	0.1761 ± 0.0301	0.2256	0.275	0.275
TMPP	TPHP-d_{15}		81 ± 12	99 ± 15	90 ± 16	0.9999	0.0021 ± 0.0023	0.0059	0.010	0.010
TEP	TEP-d_{15}	65 ± 15	113 ± 23	107 ± 13	110 ± 18	0.9999	0.1537 ± 0.0608	0.2537	0.354	0.354
TPHP	TPHP-d_{15}	42 ± 29	107 ± 16	109 ± 13	108 ± 14	0.9996	0.0318 ± 0.0234	0.0703	0.109	0.109
TPP	TPP-d_{21}	63 ± 17	68 ± 10	69 ± 4	68 ± 8	0.9997	ND		0.007	0.023
TBOEP	TNBP-d_{27}		104 ± 35	104 ± 14	104 ± 26	0.9998	0.0119 ± 0.0311	0.0630	0.114	0.114
TCEP	TCEP-d_{12}	70 ± 16	111 ± 5	104 ± 4	108 ± 5	0.9996	0.0023 ± 0.0025	0.0063	0.010	0.026
TCIPP	TCIPP-d_{18}	57 ± 22	91 ± 23	91 ± 19	91 ± 21	0.9999	0.1276 ± 0.0796	0.2586	0.390	0.390
TEHP	TEHP-d_{51}	10 ± 25	93 ± 16	91 ± 17	92 ± 16	0.9997	0.0578 ± 0.0603	0.1570	0.256	0.256
EHDPP	TPHP-d_{15}		69 ± 6	61 ± 8	65 ± 8	0.9997	0.0023 ± 0.0025	0.0064	0.010	0.010
IDDP	TPHP-d_{15}		54 ± 6	49 ± 5	51 ± 6	0.9997	0.0047 ± 0.0056	0.0138	0.023	0.023
CDPP	TPHP-d_{15}		96 ± 11	95 ± 4	96 ± 8	0.9993	0.0005 ± 0.0015	0.0029	0.005	0.005
TMP	TMP-d_9	46 ± 22	105 ± 7	98 ± 4	101 ± 6	0.9985	ND		0.050	0.166

IS: internal standard, average recoveries ± standard deviation (SD)

LOB: limit of blank, LOB = $mean_{blank}$ + 1.645 (SD_{blank}) [1]

LOD: limit of detection, LOD = LOB + 1.645 (SD_{blank}) if compounds present in the blanks; LOD = 3 signal/noise (S/N) if compounds not present in the blanks.

LOQ: limit of quantitation, LOQ = LOD if compounds present in the blanks; LOD= 10 S/N if compounds not present in the blanks.

Table S4 Spearman's rank correlation coefficient between concentrations of TNBP, TIBP, TMPP, and TBOEP in human breast milk samples from the US

	TNBP	TIBP	TMPP	TBOEP
TNBP	1	0.762**	0.341**	0.487**
TIBP		1	0.393**	0.510**
TMPP			1	0.524**
TBOEP				1

** $p < 0.01$ (two-tailed).

Table S5 National Aggregation Production Volume* (PV, Lbs) in the United States

Chemical	Chemical ID	2015 Aggregate PV	2014 Aggregate PV	2013 Aggregate PV	2012 Aggregate PV
TNBP	126-73-8	1,000,000 – 10,000,000	10,000,000 – 50,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
TIBP	126-71-6	withheld	withheld	withheld	withheld
TMPP	1330-78-5	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
TEP	78-40-0	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
TPHP	115-86-6	1,000,000 – 10,000,000	10,000,000 – 50,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
TPP	513-08-6	–	–	–	–
TBOEP	78-51-3	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
TCEP	115-96-8	25,000 – 100,000	100,000 – 500,000	25,000 – 100,000	25,000 - 100,000
TCIPP	13674-84-5	50,000,000 – 100,000,000	50,000,000 – 100,000,000	10,000,000 – 50,000,000	10,000,000 - 50,000,000
TEHP	78-42-2	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
EHDPP	1241-94-7	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 – 10,000,000	1,000,000 - 10,000,000
IDDP	29761-21-5	withheld	withheld	withheld	withheld
CDPP	26444-49-5	100,000 – 500,000	100,000 – 500,000	100,000 – 500,000	25,000 - 100,000
TMP	512-56-1	100,000 – 500,000	100,000 – 500,000	25,000 – 100,000	25,000 - 100,000

* Production volume were cited from 2016 Chemical Data Reporting Results [2].

"–" not available.

Table S6 Comparison of concentrations of TNBP, TIBP, TMPP, and TBOEP (ng/mL) in human breast milk samples from the US with various demographic factors

	N	TNBP				TIBP				TMPP				TBOEP			
		AM[a]	Median	GM[b]	*P**	AM	Median	GM	*P*	AM	Median	GM	*P*	AM	Median	GM	*P*
Maternal characteristics																	
Maternal age (years)																	
16 – 26	26	0.528	0.536	0.465	0.656	0.532	0.557	0.456	0.452	0.023	0.019	0.020	0.918	1.255	1.128	1.073	0.289
27 – 33	50	0.562	0.537	0.492		0.613	0.583	0.532		0.022	0.022	0.000		1.557	1.501	0.000	
34 – 44	24	0.502	0.494	0.462		0.518	0.514	0.472		0.021	0.019	0.018		1.379	1.287	1.201	
Parity																	
Primipara	86	0.540	0.519	0.471	0.925	0.563	0.511	0.483	0.272	0.021	0.020	0.000	0.207	1.445	1.408	0.000	0.937
Multipara	14	0.528	0.532	0.515		0.610	0.602	0.587		0.026	0.028	0.022		1.376	1.336	1.205	
Maternal education (years completed)																	
less than high school	13	0.393	0.488	0.474	0.131	0.437	0.438	0.370	0.235	0.017	0.019	0.000	0.249	1.224	0.906	0.000	0.533
High school or some college	66	0.570	0.430	0.353		0.589	0.561	0.513		0.022	0.020	0.018		1.492	1.488	0.000	
College or higher	13	0.521	0.546	0.502		0.618	0.606	0.589		0.026	0.026	0.023		1.314	1.154	1.100	
Missing	8																
Pre-pregnancy body mass index (kg/m^2)																	
Underweight (< 18.5)	2	0.559	0.559	0.559	0.644	0.766	0.766	0.765	0.545	0.021	0.021	0.021	0.975	2.263	2.263	2.137	0.642
Normal (18.5 – 24.9)	52	0.557	0.533	0.495		0.591	0.575	0.514		0.022	0.021	0.019		1.464	1.504	0.000	
Overweight (25 – 29.9)	17	0.537	0.566	0.489		0.552	0.536	0.505		0.024	0.018	0.019		1.280	1.134	1.114	
Obese (≥ 30)	15	0.513	0.480	0.437		0.537	0.500	0.461		0.021	0.021	0.017		1.491	1.214	1.123	
Missing	14																
Family income																	
< $49,999	39	0.471	0.480	0.441	0.142	0.535	0.520	0.466	0.294	0.020	0.019	0.017	0.684	1.332	1.214	1.150	0.165
$49,999 – $99,999	28	0.600	0.552	0.529		0.645	0.601	0.567		0.024	0.022	0.021		1.756	1.708	1.431	
> $99,999	22	0.545	0.569	0.485		0.577	0.602	0.513		0.023	0.021	0.018		1.268	1.100	0.000	
Missing	11																
Ethnicity																	
Hispanic	10	0.426	0.445	0.361	0.162	0.512	0.464	0.453	0.490	0.019	0.020	0.000	0.508	0.956	0.765	0.000	***0.041***
Non-Hispanic	90	0.551	0.537	0.492		0.575	0.560	0.502		0.022	0.021	0.019		1.489	1.477	0.000	
Race																	
White	77	0.544	0.532	0.483	0.749	0.565	0.560	0.493	0.822	0.021	0.019	0.000	0.216	1.451	1.422	0.000	0.575
Non-white	23	0.522	0.504	0.459		0.582	0.522	0.508		0.024	0.024	0.020		1.384	1.122	1.104	
Geographic areas																	
West	11	0.594	0.576	0.568	0.057	0.618	0.600	0.562	0.135	0.022	0.019	0.021	0.437	1.465	1.142	1.228	0.575
Middle	51	0.574	0.544	0.509		0.606	0.598	0.525		0.023	0.022	0.019		1.476	1.518	0.000	
East	38	0.475	0.460	0.416		0.506	0.479	0.445		0.020	0.020	0.000		1.374	1.220	0.000	
Child characteristics																	
Sex																	
Boy	50	0.502	0.507	0.457	0.280	0.539	0.504	0.477	0.265	0.021	0.020	0.000	0.452	1.402	1.408	0.000	0.600
Girl	50	0.575	0.538	0.498		0.599	0.576	0.517		0.023	0.021	0.019		1.469	1.350	0.000	
Preterm (< 37 weeks gestation)																	
No	89	0.535	0.522	0.471	0.635	0.569	0.554	0.491	0.186	0.022	0.020	0.000	0.757	1.464	1.474	0.000	0.189
Yes	8	0.561	0.458	0.516		0.501	0.441	0.476		0.023	0.024	0.021		1.387	1.324	1.200	
Missing	3																
Birth weight																	
< 3000 g	17	0.526	0.544	0.460	0.866	0.603	0.650	0.529	0.776	0.021	0.018	0.018	0.659	1.320	1.214	1.086	0.749
3000 – 3999 g	53	0.546	0.542	0.482		0.553	0.532	0.479		0.023	0.021	0.000		1.454	1.394	0.000	
> 4000 g	12	0.492	0.470	0.429		0.527	0.548	0.453		0.019	0.019	0.016		1.231	1.108	0.000	
Missing	18																

Season of birth																	
Winter (Dec – Feb)	23	0.545	0.542	0.467	0.399	0.557	0.500	0.479	0.320	0.021	0.022	0.018	0.694	1.489	1.506	0.000	0.317
Spring (Mar – May)	37	0.576	0.544	0.506		0.611	0.642	0.538		0.025	0.022	0.020		1.594	1.520	1.396	
Summer (Jun – Aug)	29	0.516	0.478	0.467		0.532	0.466	0.453		0.021	0.019	0.017		1.285	1.348	1.013	
Fall (Sep – Nov)	11	0.459	0.480	0.433		0.552	0.508	0.524		0.018	0.020	0.000		1.188	1.134	0.000	

[a] AM: arithmetic mean.
[b] GM: geometric mean.
[*] *p* values were obtained from Mann-Whitney *U* test or Kruskal-Wallis H test.

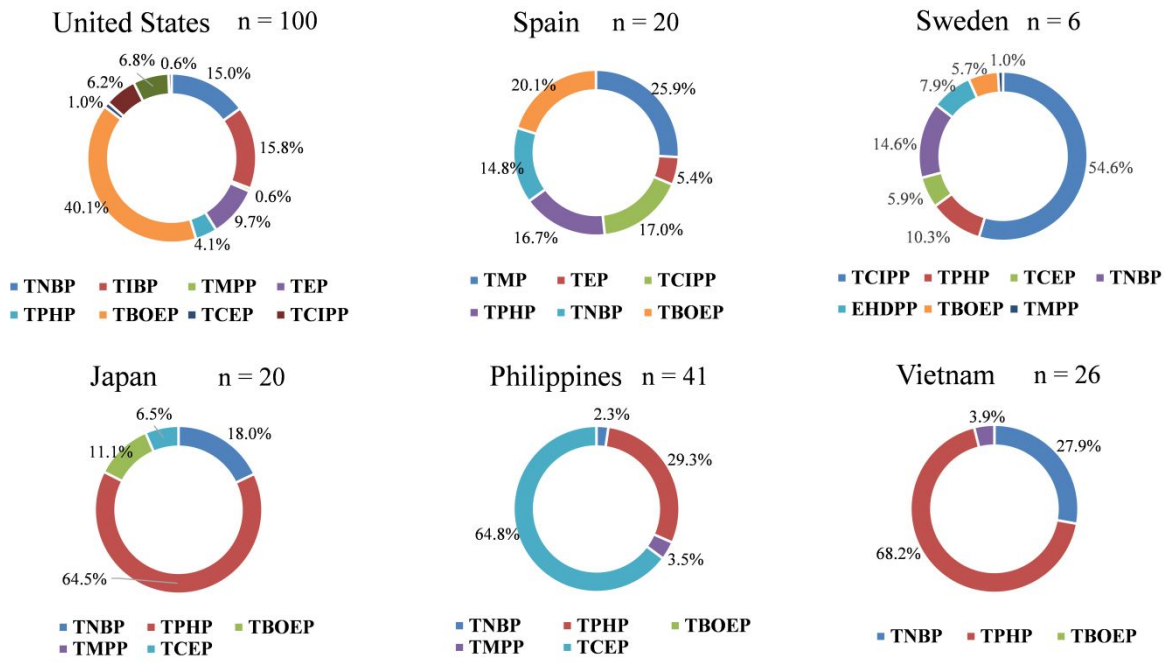

Figure S1. Composition (%) of individual organophosphorus esters (OPEs) to sum concentrations in breast milk from the United States (this study), Sweden [3], Spain [4], Japan, Philippines, and Vietnam [5]. Data for individual OPEs are not one-to-one correspondence among different countries.

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