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

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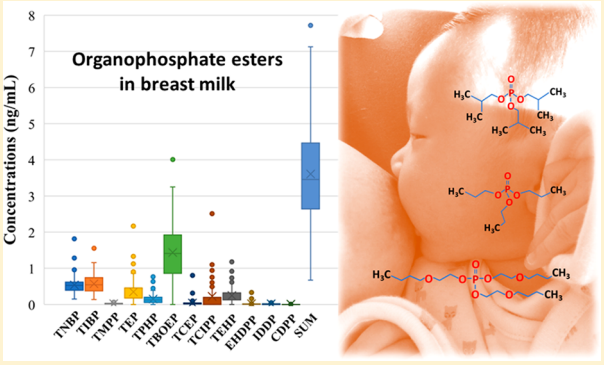
Supporting Information

ABSTRACT: Organophosphate esters (OPEs) are used in consumer products as flame retardants and plasticizers. Little is known, however, about the occurrence and profiles of OPEs in human milk. In this study, 14 OPEs were measured in 100 breast milk samples collected from the United States during the period of 2009−2012, using high-performance liquid chromatography and tandem mass spectrometry. The sum concentrations of 14 OPEs in human milk ranged from 0.670 to 7.83 ng/mL, with a mean value of 3.61 ng/mL. The highest mean concentration was found for tris-2-butoxyethyl phosphate (TBOEP, 1.44 ± 0.789 ng/mL), followed by tri-iso-butyl phosphate (TIBP, 0.569 ± 0.272 ng/mL) and tri-*n*-butyl phosphate (TNBP, 0.539 ± 0.265 ng/mL), which were the dominant OPEs found in breast milk at detection frequencies of >80%. No significant differences were observed between various maternal/infant characteristics and OPE concentrations ($p > 0.05$), except for TBOEP, for which the median concentrations in Hispanic mothers (0.765 ng/mL) were 2 times lower than those in non-Hispanic mothers (1.48 ng/mL) ($p < 0.05$). On the basis of the recommended daily milk ingestion rate, the average and the highest daily intakes of total OPEs were calculated to be in the range of 300−542 and 504−911 ng (kg of body weight)$^{-1}$ day^{-1}, respectively. The estimated daily intakes of OPEs did not exceed the current reference doses. Our study establishes baseline data for OPE exposure in breast-fed American children.



INTRODUCTION

It is challenging to assess the health risks of infant exposure to environmental chemicals present in breast milk, which is a complex and dynamic mixture of endogenous (i.e., fats, water, proteins, carbohydrates, vitamins, and antibodies) and exogenous (i.e., xenobiotics from foods, pharmaceuticals, and the environment) substances delivered to infants through breastfeeding.[1,2] Newborns have immature metabolic and immune systems and are prone to exposure to high burdens of exogenous chemicals relative to their body size.[3] Breast milk is a major source of exposure to xenobiotics in newborns. For this reason, breast milk has been recognized as one of the important matrices for monitoring organic chemicals, such as polychlorinated biphenyls (PCBs), dioxin/furans, perfluoroalkyl substances (PFASs), polybrominated diphenyl ethers (PBDEs), and bisphenol A (BPA), by the United Nations Environment Program.[4−10] Whereas studies have provided evidence of the presence of legacy contaminants in human milk, little is known about the occurrence of organophosphorus flame retardants and plasticizers in human milk from the United States. A few studies from Europe and Asia have reported the presence of organophosphorus flame retardants and plasticizers in breast milk.[11−14]

Organophosphate esters (OPEs) are widely and increasingly used in consumer products. There are concerns, however, with regard to human exposure to and potential health effects associated with these chemicals. Laboratory animal studies have shown that certain OPEs are neurotoxic, carcinogenic, and reproductive toxicants as well as endocrine disruptors.[15−18] Tris(2-ethylhexyl) phosphate (TEHP), tris-2-butoxyethyl phosphate (TBOEP), and tri-*n*-butyl phosphate (TNBP) were reported to elicit pregnane X receptor agonist activity.[19] Triphenyl phosphate (TPHP), TNBP, TBOEP, and tris(2-chloroethyl) phosphate (TCEP) were developmental neurotoxicants.[20] Tris-2-chloroisopropyl phosphate (TCIPP) and TCEP were found to affect genes involved in immune responses and steroid hormone biosynthesis and affect xenobiotic metabolic pathways.[21] Tris(1,3-dichloroisopropyl)

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Table 1. Distribution of Concentrations of Organophosphate Flame Retardants (nanograms per milliliter) in Breast Milk (n = 100) from the United States

	procedural blank	LOQ[a]	% DF[b] (>LOQ)	AM[c,f]	SD[d,f]	25th	50th	75th	95th	range
TNBP	0.184	0.301	88	0.539 (0.488−0.591)	0.265	0.390	0.525	0.620	1.01	<LOQ to 1.82
TIBP	0.176	0.275	88	0.569 (0.515−0.625)	0.272	0.373	0.545	0.735	1.09	<LOQ to 1.55
TMPP	0.002	0.010	85	0.021 (0.019−0.024)	0.012	0.010	0.020	0.030	0.050	ND[e] to 0.060
TEP	0.154	0.354	32	0.350 (0.287−0.418)	0.333	[g]	[g]	[g]	[g]	ND to 2.17
TPHP	0.032	0.109	55	0.149 (0.119−0.178)	0.146	[g]	[g]	[g]	[g]	ND to 0.760
TPP	ND	0.023	0	ND	[g]	[g]	[g]	[g]	[g]	ND
TBOEP	0.012	0.114	97	1.44 (1.29−1.58)	0.789	0.855	1.41	1.92	2.95	ND to 4.01
TCEP	0.002	0.026	37	0.036 (0.022−0.055)	0.086	[g]	[g]	[g]	[g]	ND to 0.800
TCIPP	0.128	0.390	20	0.221 (0.162−0.289)	0.326	[g]	[g]	[g]	[g]	ND to 2.51
TEHP	0.058	0.256	35	0.245 (0.197−0.291)	0.244	[g]	[g]	[g]	[g]	ND to 1.23
EHDPP	0.002	0.010	25	0.022 (0.012−0.034)	0.058	[g]	[g]	[g]	[g]	ND to 0.340
IDDP	0.005	0.023	19	0.013 (0.011−0.016)	0.014	[g]	[g]	[g]	[g]	ND to 0.070
CDPP	0.001	0.005	12	0.005 (0.002−0.008)	0.016	[g]	[g]	[g]	[g]	ND to 0.080
TMP	ND	0.166	0	ND	[g]	[g]	[g]	[g]	[g]	ND
∑OPEs[f]				3.61 (3.35−3.88)	1.40	2.64	3.46	4.47	6.07	0.670−7.83

[a]LOQ, limit of quantification. [b]DF, detection frequency. [c]AM, arithmetic mean (99% confidence intervals). [d]SD, standard deviation. [e]ND, not detected. [f]Values below LOQ were estimated using LOQ/2 and nondetects were set to zero for statistical analysis. [g]Percentiles are not given because of their low DF (<80%).

phosphate (TDCIPP) is listed as a known carcinogen by the Consumer Product Safety Commission.[22] TCEP has been reported to exert adverse effects on key biologic receptors and genes of vertebrate animals.[23] Aryl-OPEs were reported as developmental toxicants,[24] and TPHP was reported to disturb homeostasis of sex steroid hormones in human adrenal cells (H295R)[16] and to be associated with increased serum thyroxine levels, especially in women.[17]

Environmental agencies are assessing the risks of OPEs to promulgate regulatory decisions.[25] Nevertheless, production and use of OPEs, which are high-production volume chemicals,[26] have increased sharply worldwide, from 296000 tons in 2004 to 500000 tons in 2011. OPEs are present ubiquitously in various environmental matrices, including water, air, house dust, and foodstuffs.[27−32] The exposure of humans, especially infants and children, to OPEs is a great concern. Accurate determination of exposure levels and profiles of OPEs in infants is necessary to determine potential health risks.[33]

In this study, 100 human milk samples collected from the United States were analyzed for 14 OPEs. The demographic information was used to determine if they were predictive of or associated with OPE concentrations in human milk. The daily intake of OPEs through the ingestion of breast milk was estimated to assess potential health risks in breast-fed infants.

MATERIALS AND METHODS

Sample Collection. Breast milk samples were obtained from the archives and sample repository of the Vanguard phase of the U.S. National Children's Study (NCS). In brief, a total of 100 breast milk samples were collected from the United States in Tennessee (n < 10), Wisconsin (n = 21), South Dakota (n = 29), Maine (n < 10), New York (n = 12), Pennsylvania (n = 17), North Carolina (n < 10), and California (n = 11) between 2009 and 2012. Further details of the samples were reported previously.[8] Mothers were 19−40 years of age (mean of 29.8 years) and in good health, with no documented occupational exposure to OPEs. Demographic information about mothers and children was obtained from the NCS archives and is given in Table S1. The Institutional Review Board of the New York State Department of Health reviewed and approved the analysis of samples. The NCS obtained informed consent from all participants. The samples were selected randomly from the NCS repository, stored in cryogenic vials (Nalgene, Thermo Fisher Scientific, Roskilde, Denmark), and kept on dry ice during transportation to Wadsworth Center. The breast milk samples were stored at −20 °C until further analysis.

Analytical Methods. Fourteen triester OPEs were analyzed: trimethyl phosphate (TMP), triethyl phosphate (TEP), tripropyl phosphate (TPP), TNBP, tri-iso-butyl phosphate (TIBP), TEHP, TBOEP, TCEP, TCIPP, TPHP, trimethylphenyl phosphate (TMPP), cresyl diphenyl phosphate (CDPP), isodecyl diphenyl phosphate (IDDP), and 2-ethylhexyl diphenyl phosphate (EHDPP). The method for the extraction of OPEs in breast milk samples was similar to that described previously.[27] Briefly, 0.5 mL of breast milk was liquid−liquid extracted with 5 mL of 0.5% formic acid in acetonitrile and then purified by dispersive solid phase extraction (d-SPE) sorbents. Detailed information about the extraction and instrumental analysis by high-performance liquid chromatography and tandem mass spectrometry (HPLC−MS/MS) is provided in Table S2.

Quality Assurance/Quality Control. To reduce the background levels of OPEs, all experimental steps were performed in a clean fume hood. Polypropylene (PP) tubes and centrifuge tube filters were precleaned with HPLC-grade n-hexane, methanol, and acetonitrile, in that order, prior to use. $MgSO_4$ was weighed in clean glass tubes and baked in a muffle furnace at 450 °C for 6 h. Four procedural blanks were analyzed in parallel with every batch of 20 samples to check for background levels of contamination. Twelve compounds, except TPP and TMP, were found in procedural blanks at concentrations that ranged from 0.001 to 0.18 ng/mL. Matrix spikes were prepared by fortifying two different concentrations of target analytes (1 and 5 ng of each) into cow milk purchased from a local supermarket (cow milk was used as a surrogate for breast milk due to its similar composition)[4] and passed through the entire analytical procedure. The matrix spike recoveries ranged from 51% to 112% for individual

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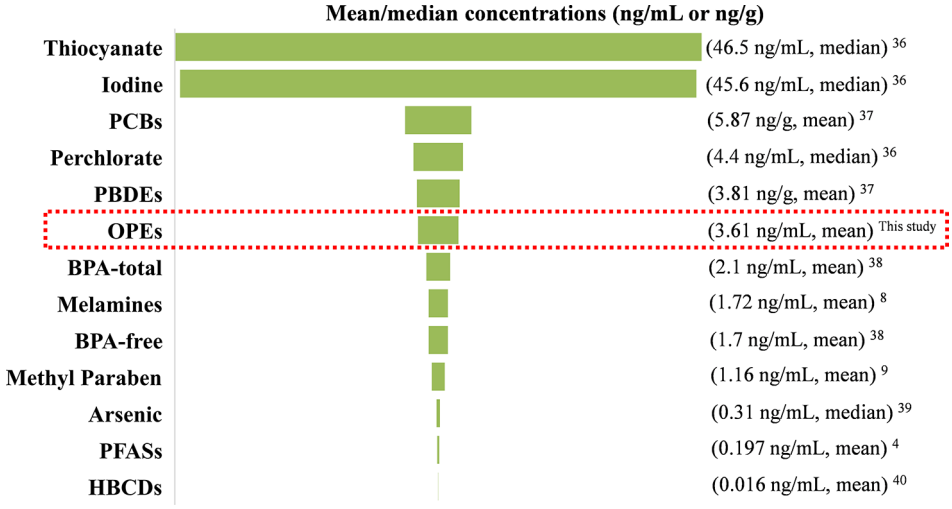

Figure 1. Comparison of mean/median concentrations of organophosphate esters (OPEs) measured in this study with those of other chemicals reported previously in human milk from the United States. Abbreviations: PCBs, polychlorinated biphenyls; PBDEs, polybrominated diphenyl ethers; BPA, bisphenol A; PFASs, perfluoroalkyl substances; HBCDs, hexabromocyclododecanes.

compounds. Recoveries of the eight labeled internal standards (TMP-d_9, TEP-d_{15}, TPP-d_{21}, TNBP-d_{27}, TEHP-d_{51}, TCEP-d_{12}, TCIPP-d_{18}, and TPHP-d_{15}), which were spiked into all samples prior to extraction, ranged from 42% to 70%, except for that of TEHP-d_{51}, which was 10%. A relatively low recovery of TEHP-d_{51} was due to the matrix effect, which was accounted for by the isotope dilution method of quantification. A 10-point calibration curve was constructed with standard solutions of the target analytes in a concentration range of 0.05−50 ng/mL, with a regression coefficient of >0.99. Duplicate injections of samples and midpoint calibration standards were performed after every 20 samples to ensure precision and accuracy of each analytical run. The limit of quantification (LOQ) was calculated according to Armbruster[34] when the target compounds, which ranged from 0.005 to 0.390 ng/mL, were present in blanks; the LOQ was set at 10 times the signal-to-noise ratio of the instrument when the compounds were not present in blanks, which were 0.023 and 0.166 ng/mL for TPP and TMP, respectively. Further details of the method and QA/QC data are listed in Table S3.

Data Analysis. The mean concentrations of target analytes found in procedural blanks were subtracted from the sample values. Concentrations below the LOQ were assigned one-half the value of the LOQ, and nondetects (NDs) were set to zero for basic statistical analysis. Percentiles were calculated when >80% of the samples had concentrations above the LOQ. Because the data were not normally distributed, nonparametric statistical tests were applied to assess the statistical significance. Spearman's correlation analysis was used to examine compound-specific associations. The Mann−Whitney U test and Kruskal−Wallis H test were used to compare differences in individual OPE concentrations with demographic variables: maternal age, parity, education, prepregnancy body mass index (BMI), family income, ethnicity, race, residential geographic area, infant gender, prematurity, birth weight, and birth season. Statistical significance was set at $p < 0.05$ level. All statistical analyses were performed using SPSS software (version 22.0, SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

OPE Concentrations and Profiles in Breast Milk. Mean concentrations and detection frequencies of OPEs in breast milk are summarized in Table 1. Twelve target OPEs, except TPP and TMP, were found in breast milk, with detection frequencies that ranged from 12% to 97%, corroborating the previous findings of the widespread occurrence of OPEs or their metabolites in human urine, breast milk, placenta, plasma, hair, and nails.[12,13,35−37] TBOEP, TNBP, TIBP, TMPP, and TPHP were quantified in 55−97% of the samples, whereas other compounds were found only in 12−37% of the samples. The highest mean concentration was found for TBOEP (1.44 ± 0.789 ng/mL), followed by TIBP (0.569 ± 0.272 ng/mL), TNBP (0.539 ± 0.265 ng/mL), TEP (0.350 ± 0.333 ng/mL), TEHP (0.245 ± 0.244 ng/mL), TCIPP (0.221 ± 0.326 ng/mL), and TPHP (0.149 ± 0.146 ng/mL), in that decreasing order. The sum concentrations of 12 OPEs (∑OPEs) ranged from 0.670 to 7.83 ng/mL, with a mean value of 3.61 ± 1.40 ng/mL, which was comparable to that reported in pooled human milk samples from Sweden (median of 3.37 ng/g, converted from the average lipid content)[11] and was >2 times higher than that reported in cow milk (median of 1.55 ng/g) collected from the United States.[27] A significant correlation ($0.341 < r < 0.762$; $p < 0.01$) was found among the concentrations of TNBP, TIBP, TMPP, and TBOEP in breast milk samples (Table S4), suggesting a common source for these four compounds.

A wide range of environmental chemicals have been reported to occur in human milk from the United States previously, and the reported mean/median concentrations of each of those chemicals are summarized in Figure 1. The mean concentrations of OPEs (3.61 ng/mL) in breast milk were lower than those of thiocyanate (median of 46.5 ng/mL),[38] iodine (median of 45.6 ng/mL),[38] PCBs (mean of 5.87 ng/g, converted from the average lipid content),[39] perchlorate (median of 4.4 ng/mL),[38] and PBDEs (mean of 3.81 ng/g, converted from the average lipid content)[39] but were ∼2 times higher than the sum concentrations of melamine and its derivatives (mean of 1.72 ng/mL),[8] BPA (mean of 2.1 ng/mL),[40] and methyl paraben (mean of 1.16 ng/mL).[9] Moreover, the mean concentrations of OPEs in breast milk

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Table 2. Estimated Daily Intakes (EDI; nanograms per kilogram of body weight per day) of Organophosphorus Esters (OPEs) through Breastfeeding in U.S. Infants

		from birth to <1 month [150 mL (kg of bw)$^{-1}$ day^{-1}][c]		from 1 to 3 months of age [140 mL (kg of bw)$^{-1}$ day^{-1}][c]		from >3 to 6 months of age [110 mL (kg of bw)$^{-1}$ day^{-1}][c]		from >6 to 12 months of age [83 mL (kg of bw)$^{-1}$ day^{-1}][c]	
	RfD[a] [ng (kg of bw)$^{-1}$ day^{-1}]	mean	max	mean	max	mean	max	mean	max
TNBP	2.40×10^3	80.9	273	75.5	255	59.3	200	44.7	151
TIBP	[b]	85.4	233	79.7	217	62.6	171	47.2	129
TMPP	1.30×10^3	3.15	9.00	2.94	8.40	2.31	6.60	1.74	4.98
TEP	1.25×10^5	52.5	326	49.0	304	38.5	239	29.1	180
TPHP	7.00×10^3	22.4	114	20.9	106	16.4	83.6	12.4	63.1
TPP	[b]	0	0	0	0	0	0	0	0
TBOEP	1.50×10^3	216	602	202	561	158	441	120	333
TCEP	2.20×10^3	5.40	120	5.04	112	3.96	88.0	2.99	66.4
TCIPP	3.60×10^3	33.2	377	30.9	351	24.3	276	18.3	208
TEHP	3.50×10^4	36.8	185	34.3	172	27.0	135	20.3	102
EHDPP	6.00×10^2	3.30	51.0	3.08	47.6	2.42	37.4	1.83	28.2
IDDP	[b]	1.95	10.5	1.82	9.80	1.43	7.70	1.08	5.81
CDPP	[b]	0.750	12.0	0.700	11.2	0.550	8.80	0.420	6.64
TMP	[b]	0	0	0	0	0	0	0	0
∑OPEs	[b]	542	911	505	850	397	668	300	504

[a]RfD, reference doses from refs 56 and 55. [b]Data not available. [c]The average daily breast milk consumption rates (milliliters per day) per infant body weight (kilograms) were from ref 54.

were 1−2 orders of magnitude higher than those found for arsenic,[41] PFASs,[4] and hexabromocyclododecanes (HBCDs)[42] (mean/median, range of 0.31−0.016 ng/mL). These results further support the significance of OPFR exposure in humans.

To the best of our knowledge, OPE concentrations in breast milk were previously reported from Sweden,[11] Australia,[43] Spain,[13] Japan, the Philippines, and Vietnam.[12] The same OPE analogues determined in earlier studies with those of ours were included for comparison (Figure S2). Among the 12 OPEs reported in our study, TBOEP was the predominant compound (40%), which was followed in abundance by TIBP (16%) and TNBP (15%). The composition of OPEs, however, varied widely in breast milk samples from various countries. For example, TMP accounted for 26% of the total OPE concentrations in breast milk from Spain (n = 20), followed by TBOEP (20%), TCIPP (17%), and TPHP (17%). In contrast, TMP was not detected in our samples. TCIPP was predominant (55%) in human milk from Sweden (n = 6), whereas TCIPP accounted for only 6% of the total OPE concentrations, with a low detection frequency in our study. TCEP (65%) and TPHP (29%) were the major OPEs found in human breast milk from the Philippines (n = 41), and TNBP accounted for 65% and 68% of the total OPE concentrations in human milk from Japan (n = 20) and Vietnam (n = 26), respectively. The concentrations of nine OPEs analyzed in breast milk from Australia (n = 3) were below the method limits of detection. Differences in the composition of OPEs in human breast milk samples from various countries may be related to the usage patterns of OPEs in these countries.[36] Nevertheless, our comparison of OPE profiles in breast milk across various countries is tempered by the differences in the number of OPE analogues measured in each of the studies and the sample size. The average annual production volume of TBOEP in the United States was between 454 and 4540 tons in 2012 (Table S5).[44] The widespread use of TBOEP in the United States is reflected in its occurrence in freshwater fish from Lake Ontario and herring gull eggs.[45−47]

Demographic Characteristics and OPE Concentrations in Breast Milk. We examined the differences in the concentrations of four major OPEs, namely, TNBP, TIBP, TMPP, and TBOEP, with various demographic features (Table S6). No significant differences were observed between various maternal/infant characteristics and OPE concentrations (p > 0.05), except for TBOEP, for which the median concentration in Hispanic mothers (0.765 ng/mL) was 2-fold lower than that in non-Hispanic mothers (1.48 ng/mL) (Mann−Whitney U test; p = 0.041). Several studies have reported significant associations between urinary total or individual OPE metabolites and demographic characteristics, such as age, gender, smoking status, and BMI.[36,43,48−52] In addition, studies have shown that body burdens of persistent organic contaminants decline in women with a history of breastfeeding.[4,53] The concentrations of OPEs in breast milk were significantly lower in multiparas than in primiparas in Vietnam, whereas an opposite trend existed for Japanese mothers.[12] We did not find significant associations between OPE concentrations in breast milk and maternal characteristics (age or BMI) or temporal factors (season), nor did we find significant differences in OPE concentrations between parity, which may be attributed to the short half-life of OPEs in human bodies and the fact that the concentrations in breast milk reflect daily exposures.[8] It is worth noting that small sample sizes analyzed in studies are subject to certain biases.

Daily Intake of OPEs via Breast Milk. An infant's daily consumption of breast milk can vary, depending on the infant's age and solid food intake.[4] The U.S. Environmental Protection Agency (EPA) estimated an average daily breast milk consumption rate for infants by age.[54] Estimated daily intakes (EDIs) of OPEs were calculated on the basis of the measured concentrations and the consumption rates of breast milk by infants between the ages of 0 and 12 months.

The respective mean and the highest EDIs of ∑OPEs were 542 and 911 ng [kg of body weight (bw)]$^{-1}$ day^{-1} for infants less than 1 month of age, 505 and 850 ng (kg of bw)$^{-1}$ day^{-1}

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for infants from 1 to 3 months of age, 397 and 668 ng (kg of bw)$^{-1}$ day^{-1} for infants from >3 to 6 months of age, and 300 and 504 ng (kg of bw)$^{-1}$ day^{-1} for infants from >6 to 12 months of age, respectively (Table 2). The EDI decreased with an increase in age, which can be explained by increasing body weight and decreasing milk ingestion level with age.

The reference doses (RfD) reported by the U.S. EPA have been used to describe noncarcinogenic risks of environmental toxicants.[55] The EDIs were compared with the corresponding RfD values reported for OPEs.[55,56] The average and highest EDIs of TNBP and TBOEP were 1−2 orders of magnitude lower than the RfDs; the average and highest EDIs of TPHP, TCEP, TCIPP, EHDPP, TEHP, and TMPP were 2−3 orders of magnitude lower than the RfDs. The average and highest hazard quotient (HQ; ratio of EDI to RfD) values of the target OPEs were up to 5 orders of magnitude below 1 (HQ ranged from 7.01×10^{-5} to $4.01 \times 10^{-1} < 1$). These results are similar to those reported previously for six pooled Swedish breast milk samples and 87 Asian breast milk samples.[11,12] These results should be interpreted with caution, however, due to the lack of a consensus RfD value for OPEs. Moreover, the concentrations of OPEs are expected to fluctuate in breast milk throughout the feeding period due to the rapid metabolism and elimination of OPEs.[1] Nevertheless, breastfeeding has been widely recommended due to a wide range of health benefits for nursing infants, including protection against infection, an improvement in cognitive development, enhancement of the immune system, and provision of balanced and adequate nutrients. Our results establish baseline data on infant exposure to OPEs through breast milk.

This is the first study of the occurrence of OPEs in human milk from the United States. OPE concentrations in the breast milk of U.S. mothers were similar to those reported for PBDEs but 2-fold higher than the concentrations of melamine measured in the same samples. Our OPE concentrations in breast milk were similar to those reported previously for Swedish mothers and were >2-fold higher than those reported in cow milk from the U.S. markets. The composition of OPEs varied widely in breast milk from various countries, in which TBOEP, TNBP, and TIBP were the predominant compounds found in U.S. breast milk. The concentrations of TBOEP in Hispanic mothers were 2-fold lower than those in non-Hispanic mothers in the United States. The EDIs of OPEs through breast milk did not exceed the current RfDs. In addition to triester OPEs, the metabolites of OPEs should be investigated in further studies to assess the risks of OPEs in breast-fed infants.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.9b00394.

Demographic characteristics of study participants (Table S1), chemical properties and MS/MS parameters of target OPE compounds (Table S2), information about instrument performance (Table S3), correlation analysis (Table S4), production volumes of OPEs (Table S5), and statistical comparison of select OPE compounds with demographic features (Table S6) and profiles of OPEs in milk samples from different countries (Figure S1) (PDF)

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Notes

The authors declare no competing financial interest.

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