Environmental Science & Technology Letters

pubs.acs.org/journal/estlcu

Letter

Co-occurrence of and Infant Exposure to Multiple Common and Unusual Phenolic Antioxidants in Human Breast Milk

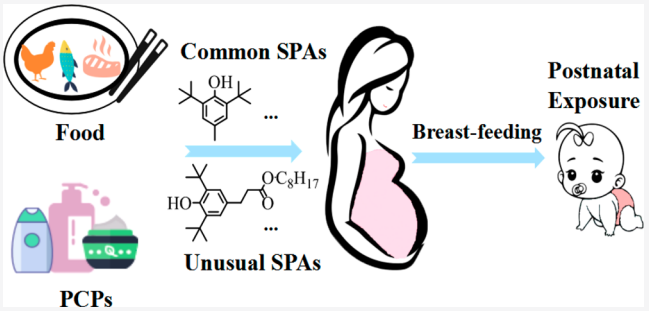
Yun Zhang, Bibai Du, Jiali Ge, Liangying Liu, Mingshan Zhu, Juan Li, and Lixi Zeng*

Cite This: *Environ. Sci. Technol. Lett.* 2020, 7, 206−212

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: In this study, eight common and two unusual synthetic phenolic antioxidants (SPAs), as well as four transformation products (TPs), were comprehensively analyzed in 80 human breast milk samples collected from lactating women in South China. All 10 SPAs and 4 TPs were detected in the breast milk samples. 2,4-Di-*tert*-butylphenol (DBP) was detected at high concentrations (mean of 16.9 ng/mL), followed by 2,6-di-*tert*-butyl-hydroxytoluene (BHT) (mean of 1.52 ng/mL). DBP and BHT collectively contributed to 91.9% of the mean ∑SPAs (sum concentrations of all 10 detected SPAs). The lipid content was identified as an important endogenous factor that influenced the levels of DBP and BHT in breast milk. The concentrations of BHT, DBP, and ∑TPs (sum concentrations of all four detected TPs) in the breast milk were significantly associated with maternal education level, eating habits, and personal care product use ($p < 0.05$). Furthermore, the concentrations of BHT and its TPs in the breast milk were also found to be significantly associated with newborn birth outcomes, including prematurity and birth length ($p < 0.05$). This is the first study to report significant associations of the concentrations of some SPAs in breast milk with some important demographic characteristics and lifestyle factors.



INTRODUCTION

Synthetic phenolic antioxidants (SPAs) are some of the anthropogenic antioxidants most frequently used in foodstuffs, food packaging materials, personal care products (PCPs), and various industrial products to inhibit oxidative degradation and increase product stability.[1,2] In general, the commonly used SPAs are low-molecular weight (MW) compounds, including 2,6-di-*tert*-butyl-hydroxytoluene (BHT), 2-*tert*-butyl-4-hydroxyanisole (BHA), 2,4-di-*tert*-butylphenol (DBP), and 2,2′-methylenebis(4-methyl-6-*tert*-butylphenol) (AO 2246), among others.[3] Among these common SPAs, BHT and DBP have been listed as high-production volume (HPV) chemicals[4,5] and have been identified as the most frequently used SPAs in food packaging materials and various PCPs, including cosmetics.[6,7] Previous studies[8−10] have confirmed that BHT can be degraded to more persistent and toxic transformation products (TPs), namely, 2,6-di-*tert*-butyl-4-(hydroxymethyl) phenol (BHT-OH), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde (BHT-CHO), 2,6-di-*tert*-butyl-1,4-benzoquinone (BHT-Q), and 2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadienone (BHT-quinol), through biotic or abiotic transformation. In recent years, the common SPAs, together with their TPs, have been detected in various environmental matrices, including air,[11] water,[12,13] sediment,[14,15] sludge,[3,16,17] dust,[2,5,18] foodstuffs,[19−22] biota,[23] and human specimens (urine and blood).[4,24,25] In addition to the common SPAs, some unusual macromolecular SPAs with a large sterically hindered hydrophobic group, such as octyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (Irganox 1135) and octadecyl-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-propionate (Irganox 1076), have been newly identified in natural and artificial environments,[26] but there is no information about these unusual SPAs in biota or humans.

Aside from their widespread occurrence, growing concern regarding SPAs is due to their potential multiple toxicities. A growing number of animal studies have shown that BHT and BHA are carcinogenic, reproductive and developmental toxicants, and endocrine disruptors.[27−33] BHT-Q and BHT-CHO can cause cleavage of DNA and apoptosis at the low concentration of 1 μM.[34,35] In addition, DBP, AO 2246, and 4-*tert*-octylphenol (4-*t*OP) have also been shown to be endocrine disruptors.[32,36−38] Therefore, human exposure to SPAs and related TPs should be given more attention, especially in pregnant women and infants.

The exposure to exogenous toxic substances during key periods of development is of particular concern for fetuses and

Received: February 11, 2020
Revised: February 26, 2020
Accepted: February 26, 2020
Published: February 26, 2020

Table 1. Distribution of Lipids, Proteins, and Lactose and Concentrations of Multiple Common and Unusual SPAs and TPs (nanograms per milliliter) in Breast Milk Samples (n = 80) from Mothers in South China

	MQL	% DF[a] (>MQL)	GM[b]	mean	median	95th[c]	range	abundance (% mean)
lipids (%)			2.96	2.96	2.96	3.26	2.65−3.41	
proteins (%)			3.16	3.45	3.37	5.51	0.52−7.81	
lactose (%)			4.42	4.42	4.43	4.83	3.97−4.96	
Synthetic Phenolic Antioxidants (SPAs)								
BHT	0.13	100	1.16	1.52	1.13	4.48	0.17−6.31	7.6
DBP	1.20	85	9.94	16.9	14.2	39.0	<MQL−61.6	84.3
AO 246	0.061	59	0.074	0.085	0.079	0.20	<MQL−0.25	0.4
DTBSBP	0.021	88	0.085	0.12	0.093	0.31	<MQL−0.58	0.6
BHA	0.023	55	0.033	0.051	0.037	0.18	<MQL−0.76	0.3
D*t*AP	0.031	78	0.056	0.066	0.068	0.13	<MQL−0.17	0.3
4-*t*OP	0.043	56	0.045	0.049	0.045	0.10	<MQL−0.15	0.2
AO 2246	0.006	83	0.020	0.18	0.016	2.18	<MQL−3.14	0.9
Irganox 1076	0.79	34	<MQL	1.06	<MQL	2.05	<MQL−18.5	5.3
Irganox 1135	0.008	38	0.012	0.021	<MQL	0.071	<MQL−0.13	0.1
∑SPAs[d]		100	14.7	20.1	16.7	44.0	1.96−64.9	100
Transformation Products (TPs) of BHT								
BHT-OH	0.018	99	0.10	0.14	0.095	0.37	<MQL−0.42	6.4
BHT-CHO	0.070	90	0.44	0.67	0.51	2.24	<MQL−2.72	30.5
BHT-Q	0.097	96	0.58	0.72	0.58	1.56	<MQL−1.83	32.7
BHT-quinol	0.093	95	0.53	0.67	0.58	1.40	<MQL−2.20	30.5
∑TPs[e]		100	1.83	2.21	2.07	5.00	0.21−5.55	100

[a]Detection frequency (%). [b]Geometric mean. [c]The 95th percentile. [d]Total concentrations of 10 SPAs. [e]Total concentrations of 4 TPs of BHT.

newborns owing to the potential adverse influence of toxicants on human development and growth.[39] Our previous investigations have shown that newborns can be prenatally exposed to multiple common SPAs through transplacental transfer,[9] and they are likely to be further exposed postnatally through breastfeeding. Breast milk is the major dietary source for newborns; therefore, the presence of SPAs in breast milk may pose a substantial exposure risk to newborns given their immature metabolic and immune functions. However, information about the identification of SPAs except DBP in human breast milk is not available,[40] and thus, there is a currently lack of understanding of the postnatal exposure of newborns to SPAs in breast milk.

Our study continues from our previous investigation[9] of prenatal exposure to SPAs and extends the investigation period to include postnatal exposure to a broader range of SPAs to obtain a comprehensive understanding of human exposure to SPAs in the early life stages of newborns. A total of 80 breast milk samples were collected from lactating women and simultaneously analyzed for multiple common and unusual SPAs. The associations of SPA concentrations with the maternal/newborn characteristics and SPA concentrations with mother lifestyles were explored to determine the main influencing factors. The daily intake of SPAs through breastfeeding was estimated to assess potential risk in breastfed newborns.

MATERIALS AND METHODS

Materials. Eight commonly used SPAs [BHT, DBP, BHA, AO 2246, 4-*t*OP, 2,4,6-tri-*tert*-butylphenol (AO 246), 2,6-di-*tert*-butyl-4-*s*-butylphenol (DTBSBP), and 2,4-di-*tert*-amylphenol (D*t*AP)] and two unusual SPAs (Irganox 1076 and Irganox 1135), as well as four TPs (BHT-OH, BHT-CHO, BHT-Q, and BHT-quinol), were included in this study (Table S1 and Figure S1). Two isotope-labeled standards, 2,6-di(*tert*-butyl-d_9)-4-methyl(phenol-3,5,*O*-d_3) (BHT-d_{21}) and [$^{13}C_{12}$]butyl paraben ([$^{13}C_{12}$]BuP), were used as the internal standards. Details about the standards and reagents are provided in the Supporting Information.

Study Population and Sample Collection. Eighty postpartum women were randomly recruited from the Zhujiang Hospital of Southern Medical University in South China between 2018 and 2019. All of the participants were healthy mothers who gave birth to an infant without congenital abnormalities. Each mother was informed of the purpose of this research and completed a questionnaire. The detailed demographic information about the maternal/infant characteristics, together with the information about mothers' lifestyles, was obtained from the questionnaires in combination with the medical records regarding the delivery. A total of 80 breast milk samples (one from each mother) were collected by a breast pump and then transferred to precleaned glass centrifuge tubes. The breast pump was rinsed with ultrapure water at least three times before sample collection to exclude potential contamination. All of the samples were stored at −20 °C until analysis.

Sample Preparation and Instrumental Analysis. An efficient procedure of ultrasound-assisted liquid−liquid extraction followed by solid-phase extraction was developed to extract, concentrate, and purify the target SPAs and TPs from the breast milk samples. The instrumental analysis used to determine common SPAs and TPs has been described in detail in our previous work.[9] The quantitative analysis of the two unusual SPAs was developed using UPLC-MS/MS with electrospray ionization (ESI) in negative-ion multiple-reaction monitoring (MRM) mode. The details about the sample preparation and instrumental analysis are provided in the Supporting Information text and Table S2. Representative chromatograms are shown in Figures S2 and S3.

Quality Assurance and Quality Control. To reduce potential SPA contamination, all glass centrifuge tubes were rinsed with HPLC-grade solvent before use, and the sample

https://dx.doi.org/10.1021/acs.estlett.0c00104
Environ. Sci. Technol. Lett. 2020, 7, 206−212

preparation was conducted in a clean fume hood. Two procedural blank controls were included in every batch of 10 breast milk samples. Only stable and low levels of BHT, DBP, and Irganox 1076 were found in the procedural blanks, and thus, the quantitative concentrations of these three SPAs were blank-subtracted. The matrix spike recoveries of the target analytes were evaluated by fortifying known concentrations (1 and 5 ng/mL of each) into a breast milk sample, and the values for the individual analytes ranged from 58% to 95%, with relative standard deviations (RSDs) of <11% (n = 3). The matrix effects of the target analytes were observed to be between 81% and 102%, indicating no significant ionization suppression/enhancement. The method quantification limits (MQLs) for BHT, DBP, and Irganox 1076 were defined as being 10 times the standard deviation of the procedural blanks. For the other analytes that were not detected in the procedural blanks, the MQLs were defined as the concentrations in the sample matrix with a signal-to-noise ratio of 10. An eight-point calibration curve was established within a concentration range of 0.01−50 ng/mL and a regression coefficient (r^2) of >0.99. The detailed QA/QC results are listed in Table 1 and Table S3.

Data Analysis. Data analysis was performed using SPSS software (version 22.0). The concentrations below the MQL were reported as the MQL divided by the square root of 2 for statistical analysis. Spearman's analysis was used to examine the correlation among the analyte concentrations and the associations of the analyte concentrations with the lipid, protein, and lactose contents in the breast milk. The normality of the measured concentration data was tested by the Shapiro−Wilk test. Logarithmically ($\log_{10}$) transformed concentrations better approximated the normal distribution in most cases, and therefore, a t test and a one-way analysis of variance (ANOVA) test were used to compare differences in $\log_{10}$-transformed concentrations stratified by various demographic/lifestyle categories. Linear regression was performed between SPA concentrations and an individual predictor variable, and then, multivariable linear regression and binary logistic regression were used to adjust for confounding factors. The $p < 0.05$ level was considered to be statistically significant.

RESULTS AND DISCUSSION

Concentrations of Multiple Common and Unusual Antioxidants in Human Breast Milk. The detection frequencies (DFs) and measured concentrations of SPAs and TPs in the breast milk samples from South China are summarized in Table 1. All eight common and two unusual SPAs were detected in the breast milk samples. The ∑SPAs (sum concentrations of all of the detected SPAs) ranged from 1.96 to 64.9 ng/mL, with a geometric mean (GM) of 14.7 ng/mL. The ∑SPA levels in the breast milk from lactating women were similar to the plasma (GM of 12.7 ng/mL) levels from pregnant women in our previous report.[9] BHT, a frequently used SPA, was detected in all of the breast milk samples with a concentration range of 0.17−6.31 ng/mL (GM of 1.16 ng/mL). The levels of BHT in the breast milk were severalfold lower than in the plasma (GM of 7.32 ng/mL) from pregnant women in the same sampling area.[9] In previous studies,[4,24] BHT levels were much lower in urine samples than in serum samples in U.S. adults. These comparative results indicated that BHT shows a preferential partitioning to human blood compared to human breast milk and urine. BHT had the second highest concentration among the 10 target SPAs in the breast milk samples. The relatively high concentrations and DF of BHT in breast milk may reflect the ongoing exposure of lactating women to various foods, food packaging materials, and PCPs in which BHT has been reported to be widely added as a main antioxidant.

DBP was detected in 85% of the breast milk samples, with concentrations (GM of 9.94 ng/mL) that were unexpectedly much higher than the concentrations of BHT and the other SPAs. In contrast to BHT, the levels of DBP in breast milk were severalfold higher than those previously reported in plasma from pregnant women (GM of 1.84 ng/mL).[9] In a recent study,[24] extremely high concentrations of DBP were found in the urine of U.S. adults, rather than in their serum. These results indicated that DBP has a pharmacokinetic behavior that is different from that of BHT, and it is prone to excretion from the human body rather than accumulation in the human body. AO 2246 was detected in 83% of the breast milk samples; however, it was found at low concentrations (GM of 0.02 ng/mL). These findings were not in agreement with the high concentrations (GM of 1.36 ng/mL) previously found in plasma from pregnant women,[9] which indicates that AO 2246 preferentially partitions to human blood like BHT does. The other five common SPAs (AO 246, DTBSBP, BHA, DtAP, and 4-tOP) were detected in 55−88% of the breast milk samples but all at low levels. Two unusual SPAs, Irganox 1076 and Irganox 1135, were detected in 34% and 38% of the samples, with maximum concentrations of 18.5 and 0.13 ng/mL, respectively. This is the first detection of unusual macromolecular SPAs in the human specimens. Overall, DBP contributed to 84.3% of the mean concentration of ∑SPAs, followed by BHT (7.6%) and Irganox 1076 (5.3%). The composition profile of the SPAs in the breast milk was significantly different from that in the plasma of pregnant women ($p < 0.001$),[9] suggesting different pharmacokinetic behaviors of these SPAs. In previous studies,[24,25] only two or three SPAs were detected in human urine samples. However, the co-occurrence of multiple common and unusual SPAs in human breast milk should raise concern about human exposure to these compounds.

All four TPs were detected in >90% of the breast milk samples. The ∑TPs (sum concentrations of the four detected SPAs) ranged from 0.21 to 5.55 ng/mL (GM of 1.83 ng/mL). The concentrations of BHT-CHO, BHT-Q, and BHT-quinol in breast milk were similar, with individual contributions of 30.5−32.7% to the ∑TPs, which were significantly higher than that of BHT-OH ($p < 0.001$). The individual TP and ∑TP concentrations in the breast milk of lactating women showed no significant differences compared to those in the plasma of pregnant women.[9] However, the TP composition profile in breast milk was slightly different from the profile in plasma from pregnant women in which BHT-Q accounted for a greater abundance.[9] Significant correlations were found among the four TPs ($p < 0.01$) and between BHT and individual TPs [$p < 0.05$ (Table S4)], which indicated that the TPs have a common source and that the dominant source may be from the biotransformation of BHT in the human body. In a previous study,[26] Irganox 1076 and Irganox 1135 were reported to be transformed into BHT-Q under light irradiation conditions. We therefore infer that, in addition to BHT, other SPAs would also contribute to the occurrence of TPs in human specimens via enzyme-mediated biotransformation.

The concentrations of the two predominant SPAs, DBP and BHT, showed a significant correlation in the breast milk

https://dx.doi.org/10.1021/acs.estlett.0c00104
Environ. Sci. Technol. Lett. 2020, 7, 206−212

Table 2. Log_{10}-Transformed Concentrations (mean, nanograms per milliliter) of BHT, DBP, and ∑TPs in Breast Milk Samples Stratified by Demographic Characteristics and Lifestyles of the Study Population (n = 80)

		BHT		DBP		∑TPs	
demographic characteristics and lifestyles	n	mean	p value[a]	mean	p value	mean	p value
Mothers' Characteristics							
maternal age (years)							
20–25	12	−0.05	0.253	1.17	0.403	0.30	0.905
26–30	47	0.11		1.00		0.26	
31–36	21	0.04		0.90		0.26	
parity							
1 (primipara)	51	0.06	0.706	1.02	0.640	0.26	0.962
≥2 (multipara)	29	0.09		0.96		0.27	
maternal education							
less than high school	8	−0.17	**0.016**[c]	0.76	**0.037**	0.02	**0.024**
high school	21	0.01		0.82		0.28	
college or higher	51	0.14		1.12		0.30	
passive smoking							
yes	24	0.12	0.340	0.94	0.552	0.23	0.488
no	56	0.04		1.02		0.28	
Mothers' Lifestyles							
eating habit							
mainly fresh foods	36	−0.10	**<0.001**	0.63	**<0.001**	0.11	**<0.001**
mainly prepackaged foods	15	0.42		1.41		0.54	
both	29	0.08		1.23		0.31	
use of PCPs[b]							
<6 PCPs used daily	40	−0.05	**0.001**	0.76	**<0.001**	0.18	**0.009**
≥6 PCPs used daily	40	0.18		1.23		0.35	
Newborn Characteristics							
gender							
boy	43	0.05	0.646	1.00	0.911	0.29	0.389
girl	37	0.08		0.99		0.23	
prematurity							
no (≥37 weeks)	64	0.02	**0.013**	1.04	0.242	0.26	0.703
yes (<37 weeks)	16	0.24		0.86		0.29	
birth weight							
<3 kg	23	0.14	0.208	0.96	0.711	0.31	0.394
≥3 kg	57	0.04		1.01		0.25	
birth length							
<50 cm	24	0.22	**0.002**	1.10	0.227	0.37	**0.014**
≥50 cm	56	−0.01		0.94		0.21	

[a]p values were obtained from a t test or a one-way ANOVA test. [b]PCPs are personal care products, including face creams, hand creams, body lotions, shampoos, hair conditioners, toothpastes, mouth washes, shower gels, hand sanitizers, facial cleansers, soap, and laundry detergents. [c]The bold indicates $p < 0.05$.

samples [$p < 0.05$ (Table S4)], implying their common usage as antioxidants in products and their similar sources. Furthermore, we found that the concentrations of both DBP and BHT were significantly correlated with lipid content [$p < 0.05$ (Table S4)] but not with the protein or lactose content of breast milk. This finding indicated that lipid content is an important endogenous factor influencing the levels of DBP and BHT in breast milk.

The excretion of chemicals in human breast milk is mainly governed by passive diffusion, and several key physiochemical properties of the chemicals, including the molecular weight (MW), lipid solubility (K_{ow}), ionization status (pK_a), and biotransformation half-life (HLB), play an important role in affecting their transfer to breast milk in passive diffusion.[41,42] Generally non-ionized chemicals with a lower MW (<200), a higher lipid solubility, and a longer HLB can more readily diffuse into breast milk.[41,42] The predicted physiochemical properties of target SPAs are summarized in Table S1. All SPAs except 4-tOP have a high pK_a of >11, indicating that these phenols are nearly exclusively present in the non-ionized form in human blood and have a favorable potential to be excreted in breast milk. BHT and DBP are the two most commonly used SPAs, and both were identified as the dominant SPAs in human blood[9] and breast milk. However, different from BHT and DBP in human blood,[9] higher concentrations of DBP than BHT in breast milk may be due to the fact that DBP, relative to BHT, has a lower MW (206), a higher lipophilicity (log K_{ow} of 5.33), slower biotransformation (HLB of 2.00 days), and a lower bioaccumulation potential (bioconcentration factor of 1,870), which facilitated efficient transfer of DBP to breast milk. Moreover, AO 2246 was detected at relatively high concentrations in human blood[9] but at low levels in breast milk, which may be mainly attributed to its rapid biotransformation (HLB of 0.47 days) and high MW (340) that restricted the transfer to breast milk. In addition to the physiochemical properties of the chemicals, other factors, such

https://dx.doi.org/10.1021/acs.estlett.0c00104
Environ. Sci. Technol. Lett. 2020, 7, 206−212

Table 3. EDIs (nanograms per kilogram of bw per day) of BHT, DBP, ∑SPAs, and ∑TPs for Infants through Breastfeeding

		<1 month [150 mL (kg of bw)$^{-1}$ day^{-1}]		1−3 months [140 mL (kg of bw)$^{-1}$ day^{-1}]		3−6 months [110 mL (kg of bw)$^{-1}$ day^{-1}]		6−12 months [83 mL (kg of bw)$^{-1}$ day^{-1}]	
	TDI[a]	MES[b]	HES[c]	MES	HES	MES	HES	MES	HES
BHT	250000	170	672	158	627	124	493	94	372
DBP	NA[d]	2130	5850	1990	5460	1560	4290	1180	3240
∑SPAs		2500	6600	2340	6160	1840	4840	1390	3650
∑TPs		311	750	290	700	228	550	172	415

[a]TDI, tolerable daily intake (nanograms per kilogram of bw per day). [b]MES, median exposure scenario. [c]HES, high-end exposure scenario. [d]NA, not available.

as plasma protein binding and environmental exposure dose, may also be important determinants of SPA concentrations in breast milk,[41] which warrants further study.

Association of Concentrations with the Mothers' Demographic and Lifestyle Characteristics and Newborn Birth Outcomes. The associations of the mothers' demographic and lifestyle characteristics (age, parity, education level, passive smoking, eating habit, and PCP use) and the newborn birth outcomes (gender, prematurity, birth weight, and birth length) with the concentrations of the two dominant SPAs (BHT and DBP) and the ∑TPs in breast milk were examined by dividing the mothers and newborns into stratified groups based on the demographic and lifestyle information. The differences in the $\log_{10}$-transformed concentrations between or among the stratified groups were evaluated using the t test or the one-way ANOVA test. As shown in Table 2, the BHT, DBP, and ∑TP concentrations showed significant association with the mothers' education level, eating habit, and PCP use ($p < 0.05$). To eliminate the potential influence of confounding factors, multivariate linear regression models were established to explore the association between $\log_{10}$-transformed concentrations in breast milk and the mothers' eating habit, PCP use, and education level. The models adjusted for potential confounders, including maternal age, parity, and passive smoking status. As shown in Table S5, the multivariate linear regression coefficients [adjusted β (95% confidence intervals)] in the adjusted models were statistically significant, as well ($p < 0.05$). These results indicated that mothers with higher education levels had higher concentrations of BHT, DBP, and ∑TPs in their breast milk. In addition, mothers who ate more prepackaged foods in their daily life had significantly higher concentrations of BHT, DBP, and ∑TPs in their breast milk compared with mothers who ate more fresh foods or both fresh and prepackaged foods ($p < 0.001$); meanwhile, mothers who used more PCPs daily (≥6 kinds per day) had significantly higher concentrations of BHT, DBP, and ∑TPs in their breast milk compared to those who used fewer PCPs daily (<6 kinds per day) ($p < 0.05$).

As reported in previous studies, BHT can be added as a preservative to various packaged foods.[43,44] Although DBP is not used as a food additive, it was reported to be a natural antioxidant that is present in some foods.[45,46] In addition, BHT and DBP are present in food packaging materials and may be transferred into packaged foods.[7,47,48] A recent study reported that BHT and DBP are two major SPAs that are widely detected in PCPs.[6] Thus, it can be expected that the increased use of multiple PCPs will lead to greater human exposure to BHT and DBP via dermal absorption. Our work provides the first evidence that packaged food intake and multiple PCP use are two major exposure pathways for lactating women.

Moreover, we also found the BHT and ∑TP concentrations in breast milk were significantly associated with the newborn birth outcomes, including prematurity and/or birth length [$p < 0.05$ (Table 2)]. To eliminate potential effluence of confounders, binary logistic regression models were established to explore the associations between birth outcomes (prematurity, birth weight, and birth length) and SPA concentrations (low, middle, and high levels). The models adjusted for covariates, including maternal age, parity, passive smoking status, newborn gender, etc. The binary logistic regression coefficients [adjusted OR (95% confidence intervals)] with a statistical significance test are listed in Table S6. The results congruously indicated that mothers with higher levels of BHT and ∑TPs in breast milk were statistically more likely to give birth to a premature infant with a shorter birth length. However, no significant correlations between birth outcomes and DBP concentrations in breast milk were observed [$p > 0.05$ (Table 2 and Table S6)]. This is the first report on the associations of SPA concentrations with the mothers' demographic and lifestyle characteristics and newborn birth outcomes.

Daily Infant Exposure to Multiple Antioxidants via Breastfeeding. The estimated daily intake (EDI) values of SPAs and TPs for various age groups of infants via breastfeeding were evaluated on the basis of the measured concentrations (C) and daily milk ingestion rate [MIR, milliliters per kilogram of body weight (bw) per day] using the equation EDI = $C \times$ MIR.[49,50] The median and 95th percentile concentrations were used to represent the median exposure scenario (MES) and high-end exposure scenario (HES), respectively. As BHT and DBP were the dominant congeners, only the EDIs of BHT, DBP, ∑SPAs, and ∑TPs are listed in Table 3. The EDIs under both exposure scenarios decreased with an increase in infant age due to decreasing MIR with increasing infant body weight. The <1-month-old infant group had the highest exposure risk, and their EDIs of BHT, DBP, ∑SPAs, and ∑TPs under the high-end exposure scenario were up to 672, 5850, 6600, and 750 ng (kg of bw)$^{-1}$ day^{-1}, respectively. The potential health risks to infants via breastfeeding were further assessed using the hazard quotient (HQ) and by comparing the EDI values with the tolerable daily intake (TDI) values using the equation HQ = EDI/TDI. To date, BHT is the only SPA with an available TDI. The European Food Safety Authority recommended a TDI for BHT of 250000 ng (kg of bw)$^{-1}$ day^{-1}.[44] If the highest EDI of BHT for infant groups was 2−3 orders of magnitude lower than the reference dose, then there would be little health risk posed by BHT to infants via breastfeeding according to the TDI. The TDIs of other SPAs are currently not available, precluding further risk assessment of most of the SPAs detected in breast milk; however, our results provide valuable

https://dx.doi.org/10.1021/acs.estlett.0c00104
Environ. Sci. Technol. Lett. 2020, 7, 206−212

baseline data on infant exposure to SPAs and TPs via breastfeeding. Considering our present findings on the associations of the levels of some SPAs in breast milk with neonatal birth outcomes, official threshold values for SPA individual and combined exposure are urgently needed to establish accurate risk assessment of their adverse human health effects.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.0c00104.

Physicochemical properties of target chemicals (Table S1), instrument parameters (Table S2), quality assurance/quality control data (Table S3), correlation analysis (Table S4), adjusted multivariable linear regression coefficients (Table S5), and binary logistic regression coefficients (Table S6) with a statistical significance test, chemical structures (Figure S1), and representative chromatograms (Figures S2 and S3) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Lixi Zeng − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China;* orcid.org/0000-0002-4815-3431; Phone: +(86)-20-37336629; Email: lxzeng@jnu.edu.cn; Fax: +(86)-20-37336629

Authors

Yun Zhang − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China*

Bibai Du − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China*

Jiali Ge − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China*

Liangying Liu − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China*

Mingshan Zhu − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China;* orcid.org/0000-0002-5926-5383

Juan Li − *Guangdong Key Laboratory of Environmental Pollution and Health, School of Environment, Jinan University, Guangzhou 511443, China;* orcid.org/0000-0003-3940-4777

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.estlett.0c00104

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (21876063 and 21707045) and the Guangdong (China) Innovative and Entrepreneurial Research Team Program (2016ZT06N258).

■ REFERENCES

(1) Rodil, R.; Benito Quintana, J.; Basaglia, G.; Pietrogrande, M. C.; Cela, R. Determination of synthetic phenolic antioxidants and their metabolites in water samples by downscaled solid-phase extraction, silylation and gas chromatography-mass spectrometry. *J. Chromatogr. A* **2010**, *1217*, 6428−6435.

(2) Wang, W.; Asimakopoulos, A. G.; Abualnaja, K. O.; Covaci, A.; Gevao, B.; Johnson-Restrepo, B.; Kumosani, T. A.; Malarvannan, G.; Minh, T. B.; Moon, H. B.; Nakata, H.; Sinha, R. K.; Kannan, K. Synthetic Phenolic Antioxidants and Their Metabolites in Indoor Dust from Homes and Microenvironments. *Environ. Sci. Technol.* **2016**, *50*, 428−434.

(3) Liu, R. Z.; Song, S. J.; Lin, Y. F.; Ruan, T.; Jiang, G. B. Occurrence of Synthetic Phenolic Antioxidants and Major Metabolites in Municipal Sewage Sludge in China. *Environ. Sci. Technol.* **2015**, *49*, 2073−2080.

(4) Liu, R.; Mabury, S. A. Synthetic Phenolic Antioxidants and Transformation Products in Human Sera from United States Donors. *Environ. Sci. Technol. Lett.* **2018**, *5*, 419−423.

(5) Liu, R.; Mabury, S. A. Synthetic phenolic antioxidants and transformation products in dust from different indoor environments in Toronto. *Sci. Total Environ.* **2019**, *672*, 23−29.

(6) Liu, R.; Mabury, S. A. Synthetic Phenolic Antioxidants in Personal Care Products in Toronto, Canada: Occurrence, Human Exposure, and Discharge via Greywater. *Environ. Sci. Technol.* **2019**, *53*, 13440−13448.

(7) Xia, Y.; Rubino, M. Effect of cut edge area on the migration of BHT from polypropylene film into a food simulant. *Polym. Test.* **2016**, *51*, 190−194.

(8) Matsuo, M.; Mihara, K.; Okuno, M.; Ohkawa, H.; Miyamoto, J. Comparative metabolism of 3,5-di-tert-butyl-4-hydroxytoluene (BHT) in mice and rats. *Food Chem. Toxicol.* **1984**, *22*, 345−54.

(9) Du, B. B.; Zhang, Y.; Lam, J. C. W.; Pan, S. L.; Huang, Y. X.; Chen, B. W.; Lan, S. Y.; Li, J.; Luo, D.; Zeng, L. X. Prevalence, Biotransformation, and Maternal Transfer of Synthetic Phenolic Antioxidants in Pregnant Women from South China. *Environ. Sci. Technol.* **2019**, *53*, 13959−13969.

(10) Fernandez-Alvarez, M.; Lores, M.; Jover, E.; Garcia-Jares, C.; Bayona, J. M.; Llompart, M. Photo-solid-phase microextraction of selected indoor air pollutants from office buildings. Identification of their photolysis intermediates. *J. Chromatogr. A* **2009**, *1216*, 8969−8978.

(11) Weschler, C. J.; Nazaroff, W. W. Dermal Uptake of Organic Vapors Commonly Found in Indoor Air. *Environ. Sci. Technol.* **2014**, *48*, 1230−1237.

(12) Liu, R.; Ruan, T.; Song, S.; Lin, Y.; Jiang, G. Determination of synthetic phenolic antioxidants and relative metabolites in sewage treatment plant and recipient river by high performance liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. A* **2015**, *1381*, 13−21.

(13) Fries, E.; Puttmann, W. Monitoring of the antioxidant BHT and its metabolite BHT-CHO in German river water and ground water. *Sci. Total Environ.* **2004**, *319*, 269−282.

(14) Zhang, R.; Li, C.; Li, Y.; Cui, X.; Ma, L. Q. Determination of 2,6-di-tert-butyl-hydroxytoluene and its transformation products in indoor dust and sediment by gas chromatography-mass spectrometry coupled with precolumn derivatization. *Sci. Total Environ.* **2018**, *619*, 552−558.

(15) Wang, X.; Hou, X.; Zhou, Q.; Liao, C.; Jiang, G. Synthetic Phenolic Antioxidants and Their Metabolites in Sediments from the Coastal Area of Northern China: Spatial and Vertical Distributions. *Environ. Sci. Technol.* **2018**, *52*, 13690−13697.

(16) Wang, W.; Kannan, K. Inventory, loading and discharge of synthetic phenolic antioxidants and their metabolites in wastewater treatment plants. *Water Res.* **2018**, *129*, 413−418.

(17) Lu, Z.; Smyth, S. A.; De Silva, A. O. Distribution and fate of synthetic phenolic antioxidants in various wastewater treatment processes in Canada. *Chemosphere* **2019**, *219*, 826−835.

(18) Liu, R.; Lin, Y.; Ruan, T.; Jiang, G. Occurrence of synthetic phenolic antioxidants and transformation products in urban and rural indoor dust. *Environ. Pollut.* **2017**, *221*, 227−233.
(19) Noel Robledo, S.; Alicia Zon, M.; Daniel Ceballos, C.; Fernandez, H. Qualitative and quantitative electroanalysis of synthetic phenolic antioxidant mixtures in edible oils based on their acid-base properties. *Food Chem.* **2011**, *127*, 1361−1369.
(20) Perrin, C.; Meyer, L. Quantification of synthetic phenolic antioxidants in dry foods by reversed-phase HPLC with photodiode array detection. *Food Chem.* **2002**, *77*, 93−100.
(21) Saad, B.; Sing, Y. Y.; Nawi, M. A.; Hashim, N.; Mohamedali, A.; Saleh, M. I.; Sulaiman, S. F.; Talib, K. M.; Ahmad, K. Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chem.* **2007**, *105*, 389−394.
(22) Kim, J.-M.; Choi, S.-H.; Shin, G.-H.; Lee, J.-H.; Kang, S.-R.; Lee, K.-Y.; Lim, H.-S.; Kang, T. S.; Lee, O.-H. Method validation and measurement uncertainty for the simultaneous determination of synthetic phenolic antioxidants in edible oils commonly consumed in Korea. *Food Chem.* **2016**, *213*, 19−25.
(23) Wang, X.; Hou, X.; Hu, Y.; Zhou, Q.; Liao, C.; Jiang, G. Synthetic Phenolic Antioxidants and Their Metabolites in Mollusks from the Chinese Bohai Sea: Occurrence, Temporal Trend, and Human Exposure. *Environ. Sci. Technol.* **2018**, *52*, 10124−10133.
(24) Liu, R.; Mabury, S. A. Unexpectedly high concentrations of 2,4-di-tert-butylphenol in human urine. *Environ. Pollut.* **2019**, *252*, 1423−1428.
(25) Wang, W.; Kannan, K. Quantitative identification of and exposure to synthetic phenolic antioxidants, including butylated hydroxytoluene, in urine. *Environ. Int.* **2019**, *128*, 24−29.
(26) Wu, Y.; Venier, M.; Hites, R. A. Identification of Unusual Antioxidants in the Natural and Built Environments. *Environ. Sci. Technol. Lett.* **2019**, *6*, 443−447.
(27) Yang, X.; Sun, Z.; Wang, W.; Zhou, Q.; Shi, G.; Wei, F.; Jiang, G. Developmental toxicity of synthetic phenolic antioxidants to the early life stage of zebrafish. *Sci. Total Environ.* **2018**, *643*, 559−568.
(28) Olsen, P.; Meyer, O.; Bille, N.; Wurtzen, G. Carcinogenicity study on butylated hydroxytoluene (BHT) in Wistar rats exposed in utero. *Food Chem. Toxicol.* **1986**, *24*, 1−12.
(29) Lindenschmidt, R. C.; Tryka, A. F.; Goad, M. E.; Witschi, H. P. The effects of dietary butylated hydroxytoluene on liver and colon tumor development in mice. *Toxicology* **1986**, *38*, 151−60.
(30) Kahl, R.; Kappus, H. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Z. Lebensm.-Unters. Forsch.* **1993**, *196*, 329−38.
(31) Jobling, S.; Reynolds, T.; White, R.; Parker, M. G.; Sumpter, J. P. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. *Environ. Health Perspect.* **1995**, *103*, 582−7.
(32) Yang, X.; Song, W.; Liu, N.; Sun, Z.; Liu, R.; Liu, Q. S.; Zhou, Q.; Jiang, G. Synthetic Phenolic Antioxidants Cause Perturbation in Steroidogenesis in Vitro and in Vivo. *Environ. Sci. Technol.* **2018**, *52*, 850−858.
(33) Le Gal, K.; Ibrahim, M. X.; Wiel, C.; Sayin, V. I.; Akula, M. K.; Karlsson, C.; Dalin, M. G.; Akyurek, L. M.; Lindahl, P.; Nilsson, J.; Bergo, M. O. Antioxidants can increase melanoma metastasis in mice. *Sci. Transl. Med.* **2015**, *7*, 308re8.
(34) Nagai, F.; Ushiyama, K.; Kano, I. DNA cleavage by metabolites of butylated hydroxytoluene. *Arch. Toxicol.* **1993**, *67*, 552−557.
(35) Oikawa, S.; Nishino, K.; Oikawa, S.; Inoue, S.; Mizutani, T.; Kawanishi, S. Oxidative DNA damage and apoptosis induced by metabolites of butylated hydroxytoluene. *Biochem. Pharmacol.* **1998**, *56*, 361−370.
(36) Pedersen, S. N.; Christiansen, L. B.; Pedersen, K. L.; Korsgaard, B.; Bjerregaard, P. In vivo estrogenic activity of branched and linear alkylphenols in rainbow trout (Oncorhynchus mykiss). *Sci. Total Environ.* **1999**, *233*, 89−96.
(37) Kotula-Balak, M.; Chojnacka, K.; Hejmej, A.; Galas, J.; Satola, M.; Bilinska, B. Does 4-tert-octylphenol affect estrogen signaling pathways in bank vole Leydig cells and tumor mouse Leydig cells in vitro? *Reprod. Toxicol.* **2013**, *39*, 6−16.
(38) Creusot, N.; Budzinski, H.; Balaguer, P.; Kinani, S.; Porcher, J.-M.; Ait-Aissa, S. Effect-directed analysis of endocrine-disrupting compounds in multi-contaminated sediment: identification of novel ligands of estrogen and pregnane X receptors. *Anal. Bioanal. Chem.* **2013**, *405*, 2553−2566.
(39) Vreugdenhil, H. J. I.; Slijper, F. M. E.; Mulder, P. G. H.; Weisglas-Kuperus, N. Effects of perinatal exposure to PCBs and dioxins on play behavior in dutch children at school age. *Environ. Health Perspect.* **2002**, *110*, A593−A598.
(40) Tran, C. D.; Dodder, N. G.; Quintana, P. J. E.; Watanabe, K.; Kim, J. H.; Hovell, M. F.; Chambers, C. D.; Hoh, E. Organic contaminants in human breast milk identified by non-targeted analysis. *Chemosphere* **2020**, *238*, 124677.
(41) Wilson, J. T.; Brown, R. D.; Cherek, D. R.; Dailey, J. W.; Hilman, B.; Jobe, P. C.; Manno, B. R.; Manno, J. E.; Redetzki, H. M.; Stewart, J. J. Drug excretion in human breast milk: principles, pharmacokinetics and projected consequences. *Clin. Pharmacokinet.* **1980**, *5*, 1−66.
(42) Agatonovic-Kustrin, S.; Ling, L. H.; Tham, S. Y.; Alany, R. G. Molecular descriptors that influence the amount of drugs transfer into human breast milk. *J. Pharm. Biomed. Anal.* **2002**, *29*, 103−119.
(43) Nieva-Echevarria, B.; Manzanos, M. J.; Goicoechea, E.; Guillen, M. D. 2,6-Di-Tert-Butyl-Hydroxytoluene and Its Metabolites in Foods. *Compr. Rev. Food Sci. Food Saf.* **2015**, *14*, 67−80.
(44) Lanigan, R. S.; Yamarik, T. A. Final report on the safety assessment of BHT. *Int. J. Toxicol.* **2002**, *21*, 19−94.
(45) Choi, S. J.; Kim, J. K.; Kim, H. K.; Harris, K.; Kim, C.-J.; Park, G. G.; Park, C.-S.; Shin, D.-H. 2,4-Di-tert-butylphenol from Sweet Potato Protects Against Oxidative Stress in PC12 Cells and in Mice. *J. Med. Food* **2013**, *16*, 977−983.
(46) Lee, J.; Lee, Y.; Ha, J.; Yoo, M.; Jang, H. W. Simultaneous determination of four bioactive compounds in Korean rice wine (makgeolli) by solvent extraction coupled with gas chromatography-mass spectrometry. *Int. J. Food Prop.* **2018**, *21*, 139−146.
(47) Yang, Y.; Hu, C.; Zhong, H.; Chen, X.; Chen, R.; Yam, K. L. Effects of Ultraviolet (UV) on Degradation of Irgafos 168 and Migration of Its Degradation Products from Polypropylene Films. *J. Agric. Food Chem.* **2016**, *64*, 7866−7873.
(48) Gao, Y.; Gu, Y.; Wei, Y. Determination of Polymer Additives-Antioxidants and Ultraviolet (UV) Absorbers by High-Performance Liquid Chromatography Coupled with UV Photodiode Array Detection in Food Simulants. *J. Agric. Food Chem.* **2011**, *59*, 12982−12989.
(49) Ma, J.; Zhu, H.; Kannan, K. Organophosphorus Flame Retardants and Plasticizers in Breast Milk from the United States. *Environ. Sci. Technol. Lett.* **2019**, *6*, 525−531.
(50) Zhu, H.; Kannan, K. Occurrence of Melamine and Its Derivatives in Breast Milk from the United States and Its Implications for Exposure in Infants. *Environ. Sci. Technol.* **2019**, *53*, 7859−7865.

https://dx.doi.org/10.1021/acs.estlett.0c00104
Environ. Sci. Technol. Lett. 2020, 7, 206−212