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First Insight into Fetal Exposure to Legacy and Emerging Plasticizers Revealed by Infant Hair and Meconium: Occurrence, Biotransformation, and Accumulation

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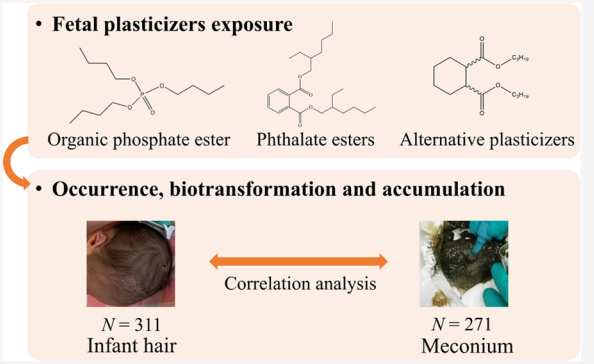
ABSTRACT: Epidemiological studies have demonstrated the embryonic and developmental toxicity of plasticizers. Thus, understanding the in utero biotransformation and accumulation of plasticizers is essential to assessing their fate and potential toxicity in early life. In the present study, 311 infant hair samples and 271 paired meconium samples were collected at birth in Guangzhou, China, to characterize fetal exposure to legacy and emerging plasticizers and their metabolites. Results showed that most of the target plasticizers were detected in infant hair, with medians of 9.30, 27.6, and 0.145 ng/g for phthalate esters (PAEs), organic phosphate ester (OPEs), and alternative plasticizers (APs), and 1.44, 0.313, and 0.066 ng/g for the metabolites of PAEs, OPEs, and APs, respectively. Positive correlations between plasticizers and their corresponding primary metabolites, as well as correlations among the oxidative metabolites of bis(2-ethylhexyl) phthalate (DEHP) and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH), were observed, indicating that infant hair retained the major phase-I metabolism of the target plasticizers. While no positive correlations were found in parent compounds or their primary metabolites between paired infant hair and meconium, significant positive correlations were observed among secondary oxidative metabolites of DEHP and DINCH in hair and meconium, suggesting that the primary metabolites in meconium come from hydrolysis of plasticizers in the fetus but most of the oxidative metabolites come from maternal−fetal transmission. The parent compound/metabolite ratios in infant hair showed a decreasing trend across pregnancy, suggesting in utero accumulation and deposition of plasticizers. To the best of our knowledge, this study is the first to report in utero exposure to both parent compounds and metabolites of plasticizers by using paired infant hair and meconium as noninvasive biomonitoring matrices and provides novel insights into the fetal biotransformation and accumulation of plasticizers across pregnancy.



KEYWORDS: *infant hair, meconium, plasticizers, fetal exposure, biotransformation*

1. INTRODUCTION

Pregnant women's exposure to pollutants from various environmental matrices can have a negative impact on the fetus due to maternal−fetal transmission. In utero exposure to environmental pollutants and their health effects have received substantial attention.[1,2] Both extensive experimental animal studies as well as epidemiological findings have demonstrated that early-life environmental factors are associated with an increased risk of developing a variety of chronic illnesses in later life.[3] Recent epidemiological and toxicological studies demonstrated that widely used plasticizers, including organic phosphate esters (OPEs) and phthalate acid esters (PAEs), are commonly present in utero and may exhibit strong embryonic and developmental toxicity early in life.[4−6] However, most environmental epidemiologic studies have focused on monitoring parental exposure, and little is known about cumulative exposure in the intrauterine environment. Further, the occurrence, biotransformation, and accumulation of plasticizers in utero remain poorly understood.

China is the world's largest producer and consumer of plasticizers, with an annual output of nearly 1 million tons.[7] OPEs and PAEs have been extensively used in furniture, household appliances, and personal care products and have high detection frequencies in ordinary indoor environments.[8] Although these chemicals are not generally persistent, they are

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ubiquitous as a result of their continuous use, and the population is at risk for persistent exposure. Given that gestation is a crucial time for the formation and development of embryonic organs, prenatal exposure to these plasticizers merits more attention. Several next-generation plasticizers, known as alternative plasticizers (APs), have recently been launched into the market. However, these new chemicals have properties, including toxicity, similar to the chemicals they were intended to replace.[9−11] Besides, there is still a lack of information concerning the possible health effects of APs, and knowledge of the in utero occurrence and fate of APs remains limited.

Measuring retroactive exposure in environmental epidemiology studies can be difficult, especially when it happens during a crucial period (e.g., in pregnancy). Maternal urine has been used in the majority of epidemiological investigations to evaluate prenatal exposure to OPEs and PAEs owing to their relatively quick excretion from the human body,[12,13] and a few studies have used maternal and umbilical cord blood.[14−21] These samples, while demonstrating that plasticizers can cause intrauterine exposure of the fetus through maternal−fetal transmission, may be biased because they indirectly characterize cumulative fetal exposure. Besides, these data are mainly instantaneous and dynamic, necessitating regular updates on exposure in long-term research.[22,23] On the contrary, infant hair and meconium analysis provide a very time-resolved way to investigate retrospective early-life exposure to toxicants, enabling noninvasive sampling and exposure estimation during critical developmental windows.[24] Infant hair begins growing after 16−17 weeks of gestation and reaches the scalp surface after approximately 3 weeks, retaining contaminants from fetal development and the amniotic fluid.[25] Meconium begins to form in weeks 12−13 of gestation from urine, intestinal secretions, and inhaled amniotic fluid. Recently, infant hair[26] and meconium[27] have been utilized for biomonitoring prenatal exposure to environmental toxic metals and drugs. Infant hair and meconium analysis may provide comprehensive data on fetal exposure levels and their chemical disposition, biotransformation, and accumulation.

In vivo and in vitro studies have mentioned that some metabolites of plasticizers are more stable than their parent compounds in the body; hence, some of the adverse effects of plasticizers may, in fact, be the result of their metabolites.[28] Simultaneous monitoring of parental plasticizers and their metabolites is useful for assessing biotransformation and accumulation in biological tissue. This knowledge may also assist in the understanding of toxicological mechanisms and clarify the bioaccessibility and exposure pathways of these compounds, as well as support the biological plausibility of environment-health correlations in population studies. However, means to determine metabolic processes in the fetus are limited. Besides, existing fetal monitoring relies largely on the properties of parent chemicals, with minimal consideration given to the products of their transformation in utero, mainly due to the poor deposition of transformation products in cord blood.[29] Our previous study found that infant hair can provide long-term exposure information for parent compounds and metabolites of persistent organic pollutants (i.e., polybrominated diphenyl ethers and organochlorine pesticides).[30] To the best of our knowledge, no investigations have used infant hair as a biomonitoring matrix to assess fetal exposure to rapidly eliminated compounds such as plasticizers and their metabolites.

In this work, we aim to demonstrate the occurrence, metabolic transformation, and accumulation of legacy and emerging plasticizers and their metabolites in utero by using infant hair and meconium as a biomonitoring matrix. The biotransformation and accumulation of plasticizers in the fetus were assessed by comparing the relationship of plasticizer concentrations to their metabolites in infant hair and meconium. Our data may assist the retroactive diagnosis and risk assessment of in utero exposure to plasticizers for early prevention and intervention.

2. MATERIALS AND METHODS

2.1. Chemicals. The target analytes included eight PAEs, 12 APs, 16 OPEs, 10 metabolites of PAEs (mPAEs), eight metabolites of APs (mAPs), and nine metabolites of OPEs (mOPEs). Table S1 contains detailed information about these analytes.

2.2. Study Population. We included mother−infant dyads who fulfilled the inclusion/exclusion criteria listed below: resided in Guangzhou City, Guangdong Province, China, throughout pregnancy without infectious diseases or malignancies. We excluded subjects with birth defects or stillbirths. At the time of recruitment, all individuals provided their informed consent. This study was approved by the Ethics Committees of the Sixth Affiliated Hospital of Sun Yat-sen University (No. E2019073). A total of 311 infant hair and 271 paired meconium samples were collected 1−2 days after delivery from January 2020 to January 2021. The infant hair was collected after the newborn's first shampoo, during which the newborn's entire body, including the hair, was wiped with mineral oil to remove dissolved fetal fat and body fluid, followed by washing with warm water. Infant hair in the occipital area was excised close to the scalp. Hair samples were wrapped in aluminum foil and stored at −20 °C. The meconium, which is the newborn's first fresh excrement, was meticulously scraped from the surface of the diaper by a metal spatula, deposited into a sterilized stool collector, and then placed at −80 °C. Refrain from collecting meconium that was close to or mixed with the diaper material.

We collected data on fetal gender, weight, length, gestational age, and head circumference at birth from the hospital's electronic medical records. Maternal covariates were collected from interviews or medical records, including parity, age, pregnancy weight gain, prepregnancy body mass index (BMI), education, occupation, and passive smoke during pregnancy. The demographic characteristics of the mothers and their infants are shown in Table 1.

2.3. Sample pretreatment and instrumental analysis. Infant hair was rinsed twice with warm Milli-Q water before freezing dry. About 0.1 g of freeze-dried infant hair was ground into powder and extracted in a 4 mL mixture of *n*-hexane, acetone, ethyl acetate, and acetonitrile (1:1:1:1, v/v/v/v) spiked with internal standards. The mixture was vortexed at 2000 rpm for 5 min, sonicated for 20 min, and centrifuged at 4000 rpm for 15 min, and then the supernatant was collected. This step was repeated three times, and supernatants were combined. The combined extract was then concentrated to 1 mL by nitrogen gas and purified by adding 20 mg of anhydrous NA_2SO_4 and 100 mg of octadecylsilane bonded silica gel (C18). The supernatant was vortexed at 1200 rpm for 10 min and then centrifuged at 3500 rpm for 15 min and then transferred to a clean glass tube, dried with nitrogen gas, and fixed with 200 mL of methanol. The dissolved sample was

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Table 1. Demographic Characteristics of Participating Mother−Infant Pairs

	mean ± SD	*N* (%)
Maternal (*n* = 298)		
age, years	30 ± 4	
<30		138 (46%)
≥30		160 (54%)
prepregnancy BMI[a], kg/m^2	20.9 ± 2.95	
<23		60 (20%)
23.0−27.5		227 (76%)
≥27.5		11 (4%)
pregnancy weight gain, kg	13.7 ± 5.32	
parity		
nulliparous		149 (50%)
multiparous		149 (50%)
education		
<college graduate		151 (51%)
≥college graduate		134 (45%)
missing		13 (4%)
occupation		
officer		75 (25%)
worker		77 (26%)
attendant		54 (18%)
others		92 (31%)
passive smoke in pregnancy		
yes		91 (31%)
no		191 (64%)
missing		16 (5%)
Infant (*n* = 311)		
gestational age (days)	272 ± 10	
infant gender		
male		167 (54%)
female		137 (44%)
missing		7 (2%)
weight, g	3100 ± 450	
length, cm	49.1 ± 2.35	
head circumference, cm	32.5 ± 1.60	

[a]BMI: Body mass index.

frozen at −20 °C for 4−6 h to remove impurities, and the supernatant was used for analysis of the target compounds. 100 μL of this solution was aliquoted and dried with nitrogen, then redissolved in 100 μL of isooctane for the analysis of PAEs and APs by GC-MS/MS. The remaining 100 μL methanol-based sample was used for the analysis of OPEs and various metabolites using LC-MS/MS.

A 25 mg sample of meconium was subjected to protein precipitation and solid−liquid extraction in a 500 μL mixture of methanol, acetonitrile, and water (2:2:1, v/v/v) spiked with internal standards. Samples were homogenized for 4 min and sonicated for 5 min in an ice−water bath. This vortex−sonication cycle was repeated three times. After being incubated for 1 h at −40 °C to remove lipids, the samples were centrifuged at 12 000 rpm for 15 min at 4 °C. The resulting supernatant was transferred to a clean glass vial for analysis. As we did not detect any parent plasticizers in the meconium samples obtained during the pretrials, we analyzed only the metabolites of plasticizers in meconium in this study.

The Supporting Information contains detailed information on the parameters of instrumental analysis.

2.4. Quality Assurance and Control. The analytical protocol for plasticizers in infant hair was validated by spiking native standards into a homogeneous hair sample at 100 ng of PAEs, APs, and OPEs and 10 ng of their metabolites. The recoveries were 82−129% for PAEs, 64−121% for APs, 73−157% for OPEs, 73−153% for mPAEs, 76−157% for mAPs, and 97−136% for mOPEs in the spiked matrices (*n* = 3). The analytical protocol for metabolites of plasticizers in meconium was validated by spiking native standards into a homogeneous meconium sample of 10 ng. The recoveries were 75−131% for mPAEs, 89−132% for mAPs, and 79−121% for mOPEs (*n* = 3). Detailed information on the recovery of each compound is shown in Table S1.

A mixture of standard solutions and solvent blanks was regularly injected to carry out instrumental quality control. To guarantee consistency in the analysis of the target compounds, standard solutions were injected after every 12 hair or meconium samples, with intra- and interdaily relative standard deviations less than 15%. During the experiment, we avoided the use of any plastic products and used stainless steel utensils and glassware. Stainless steel utensils were sequentially treated with methanol, acetone, and *n*-hexane, each for 10 min under ultrasound, followed by drying for preservation. All glassware was washed sequentially with tap water and deionized water and then dried. Subsequently, the glassware was baked at 450 °C for 4 h in a muffle oven and sealed with aluminum foil for preservation.

In order to account for any potential background contamination, procedural blank samples were examined concurrently with each batch of samples. The sample data were adjusted for the mean plasticizer levels observed in the procedure blanks. The average concentrations of target analytes in the procedure blanks plus 3 times the standard deviation were used to estimate the limits of quantification (LOQs). For compounds not detected in the procedural blanks, the LOQs were calculated as signal-to-noise ratios of 10. The LOQs for PAEs, APs, and OPEs in hair were in the range of 1.35−915, 2.35−40.9, and 0.025−12.2 ng/g, respectively. The LOQs for mPAEs, mAPs, and mOPEs in hair were in the range of 0.013−71.6, 0.007−1.58, and 0.129−71.9 ng/g, respectively. The LOQs for mPAEs, mAPs, and mOPEs in meconium were in the range of 0.007−73.5, 0.004−9.07, and 0.147−8.31 ng/g, respectively (Table S1).

2.5. Statistical Analysis. Descriptive statistics were analyzed for subject demographics and plasticizer gravimetric concentrations in infant hair and meconium samples. Plasticizer concentrations in the samples below their corresponding LODs were not included in the subsequent statistical analyses. Plasticizer concentrations were all log-transformed to satisfy the requirements for normally distributed residuals. Then, the correlations among log-transformed plasticizer levels in infant hair and paired meconium samples were estimated to assess possible sources by calculating Pearson correlation coefficients (*r*).

The molar concentrations (in nmol/g) of the plasticizers were converted by multiplying the plasticizers by their molecular weights. All further statistics were performed using the molar concentration. The coefficient, $f(x)$ for each metabolite of DEHP and DINCH was calculated for each sample as the formula "$f(x) = C_i/\sum C_i \times 100\%$".[31] Ci is the molar concentration of the individual monoesters (i: mEHP, OH-mEHHP, oxo-mEHP or cx-mEHP for DEHP, and mINCH, OH-mINCH, oxo-mINCH or cx-mINCH for DINCH). The correlations among $f(x)$ are estimated to evaluate the in utero biotransformation of DEHP and DINCH

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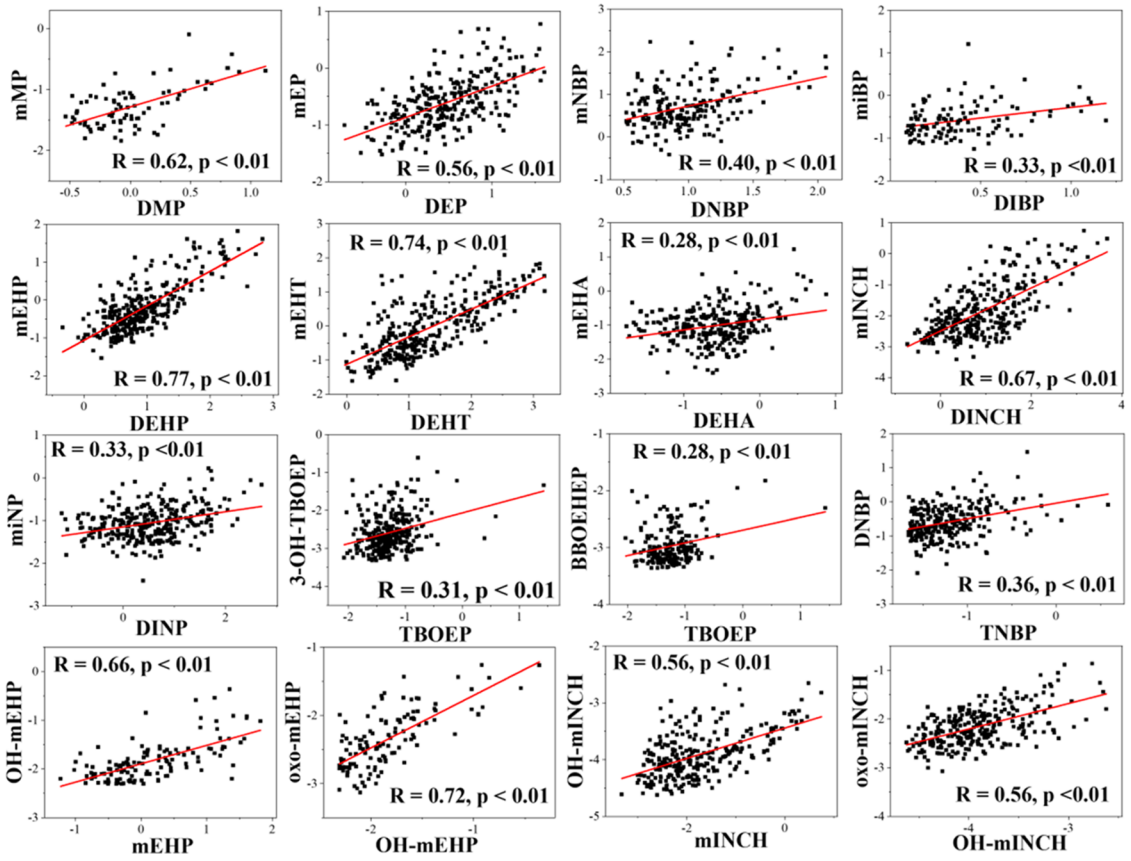

Figure 1. Correlation between logarithmic concentrations of plasticizers (*X*-axis) and their primary metabolites (*Y*-axis) in infant hair. The correlation was only calculated when the levels of the parent compound and metabolite were both >LOD in infant hair.

by calculating the Pearson correlation coefficients (r). The Mann−Whitney *U* test was used to determine differences in $f(x)$ values between infant hair and meconium. The relative metabolite/parent compound ratio, $R(x)$, for each primary metabolite was calculated for each sample using the formula: "$R(x) = C_i/C_x$".[31] Ci is the molar concentration of the individual primary metabolite (i.e., i: mEHP for x: DEHP; i: OH-mEHP for x: mEHP). To accurately estimate the biotransformation of the compounds, the parent compound and metabolite levels were only included in subsequent statistical analyses if both were >LOD in infant hair. Partial correlations were examined between demographic characteristics, including maternal age (years, continuous), parity (0, ≥1), pregnancy weight gain (kg, continuous), prepregnancy body mass index (PBMI, kg/m^2, continuous), gestational age (days, continuous), ponderal index (PI, calculated as birth weight (g)/length cm^3 × 100), and $R(x)$ or $f(x)$ values to evaluate changes of in utero biotransformation and accumulation during pregnancy. The Mann−Whitney *U* test was used to determine differences in $R(x)$ or $f(x)$ values between male and female infants. Effect sizes were defined as follows: strong (r = 0.7), medium (r = 0.5), and weak (r = 0.3).[32]

The statistical power of the above correlations was estimated by using a power analysis (Figure S1), and the results showed that the sample size in this study satisfied the pertinent statistical requirements. Specifically, when a two-tailed significant *p*-value was set at 0.05 and 90% confidence level, we can get r = 0.186, 0.226, 0.316, and 0.437 when the sample size was set at n = 300, 200, 100, and 50, respectively. All statistical analyses were performed using R software, version 4.2.3, and SPSS version 26.0. Power analysis was carried out using the R package "pwr" (version 1.3-0).

3. RESULTS AND DISCUSSION

3.1. Occurrence of Plasticizers and Their Metabolites in Infant Hair and Meconium. Detection frequencies (DFs) and gravimetric concentrations of individual compounds in infant hair and meconium are given in Tables S2 and S3, respectively. Levels of target plasticizers or their metabolites detected <LOD in all infant hair or meconium are not shown.

In infant hairs, the median of $\sum_8$PAEs, $\sum_{12}$APs and $\sum_{12}$OPEs were 9.30, 27.6 and 0.145 μg/g, respectively. The dominant compounds for PAEs were DEHP (median: 2.50 μg/g) and DnBP (median: 1.53 μg/g); for APs were DEHT (median: 5.79 μg/g), DPrHP (median: 2.98 μg/g), and DINCH (median: 2.73 μg/g); and for OPEs were TCIPP (median: 0.028 μg/g), TBOEP (median: 0.019 μg/g), and TNBP (median: 0.012 μg/g). For plasticizer metabolites, the median concentrations of $\sum_8$mPAEs, $\sum_8$mAPs, and $\sum_5$mOPEs were 1.44, 0.313, and 0.066 μg/g, respectively. mnBP (median: 1.21 μg/g), mEHT (median: 0.189 μg/g), and DNBP (median: 0.054 μg/g) showed the highest concentrations, which differed slightly from their corresponding parent plasticizers.

In meconium samples, mnBP (mean: 260 ± 249 ng/g), mEHT (median: 209 ng/g), and DNBP (median: 26.3 ng/g) emerged as the predominant compounds for mPAEs, mAPs, and mOPEs, respectively, which was consistent with their prevalence in infant hair. Notably, mBzP and cx-mINCH, which had low DFs in infant hair, were detected in 89 and

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Table 2. DEHP and DINCH Metabolite Levels and $f(x)$ in Paired Infant Hair and Meconium and Comparison to Samples from Pregnant Women, Infants, or Adult Males[a]

biological matrix	sampling time	C_{mEHP} (f)	$C_{OH\text{-}mEHP}$ (f)	$C_{OXO\text{-}mEHP}$ (f)	$C_{CX\text{-}mEHP}$ (f)	references
infant hair	within 48 h after birth	123 ng/g (98.1%)	3.33 ng/g (1.81%)	0.067 ng/g (0.79%)	1.31 ng/g (0.41%)	this study
infant hair	within 48 h after birth	188 ng/g (77.7%)	16 ng/g (6.61%)	30 ng/g (12.4%)	8 ng/g (3.31%)	Cleys et al.[39]
infant meconium	within 48 h after birth	28.1 ng/g (65.3%)	27.5 ng/g (27.2%)	1.26 ng/g (5.43%)	0.686 ng/g (2.64%)	this study
infant meconium	within 48 h after birth	10.9 ng/g (34.9%)	2.5 ng/g (8.0%)	1.3 ng/g (4.2%)	16.5 ng/g (52.9%)	Mathew et al.[48]
infant urine	within 4 days after birth	0.48 ng/mL (5.5%)	0.84 ng/mL (9.6%)	0.34 ng/mL (3.9%)	7.07 ng/mL (80.9%)	Urbancova et al.[66]
cord serum	upon delivery	2.49 ng/mL (70.2%)	<LOD (0%)	<LOD (0%)	0.28 ng/mL (29.8%)	Lin et al.[18]
cord serum	after delivery	3.74 ng/mL (94.9%)	0.12 ng/mL (3.1%)	0.08 ng/mL (2.0%)	na	Hwa et al.[17]
maternal serum	after delivery	1.82 ng/mL (95.8%)	0.05 ng/mL (2.6%)	0.03 ng/mL (1.6%)	na	
cord serum	upon delivery	12.9 ng/mL (95.3%)	<LOD (0%)	<LOD (0%)	6.65 ng/mL (4.7%)	Li et al.[19]
maternal serum	2 h before delivery	3.24 ng/mL (45.6%)	<LOD (0%)	<LOD (0%)	3.75 ng/mL (54.4%)	
amniotic fluid	upon delivery	0.832 ng/mL (86.0%)	<LOD (0%)	<LOD (0%)	1.13 ng/mL (14.0%)	
amniotic fluid	upon delivery	2.4 ng/mL (72.7%)	<LOD (0%)	<LOD (0%)	0.90 ng/mL (27.3%)	Wittassek et al.[47]
maternal hair	at the beginning of 2nd trimester	20.1 ng/g (43.7%)	25.9 ng/g (56.3%)	na	na	Katsikantami et al.[67]
male hair		44.9 ng/g (74.9%)	5.66 ng/g (9.9%)	9.17 ng/g (15.2%)	<LOD (0%)	Chang et al.[68]
male serum		6.74 ng/mL (89.3%)	0.09 ng/mL (1.2%)	0.06 ng/mL (0.8%)	0.66 ng/mL (8.7%)	Frederiksen et al.[69]
male serum		1.3 ng/mL (54.6%)	0.28 ng/mL (11.7%)	0.043 ng/mL (1.8%)	0.76 ng/mL (31.9%)	Li et al.[70]
male serum	8.3 h after oral DEHP dose of 48.1 mg	0.149 ng/mL (61.8%)	0.026 ng/mL (10.8%)	0.006 ng/mL (2.5%)	0.06 ng/mL (24.9%)	Koch et al.[51,52]
		C_{mINCH} (f)	$C_{OH\text{-}mINCH}$ (f)	$C_{OXO\text{-}mINCH}$ (f)	$C_{CX\text{-}mINCH}$ (f)	
infant hair	within 48 h after birth	48.3 ng/g (53.8%)	0.059 ng/g (0.70%)	3.17 ng/g (37.5%)	0.048 ng/g (8.15%)	this study
infant meconium	within 48 h after birth	6.10 ng/g (18.3%)	0.250 ng/g (0.89%)	13.3 ng/g (35.8%)	14.7 ng/g (45.0%)	this study
infant urine	within 4 days after birth	<0.15 ng/mL (0%)	0.30 ng/mL (39.4%)	0.16 ng/mL (21.1%)	0.30 ng/mL (39.5%)	Urbancova et al.[66]

[a] $f(x)$, $f(x) = C_i/\sum C_i \times 100\%$. C_i is the molar concentration of the individual monoesters (i: mEHP, OH-mEHHP, oxo-mEHP or cx-mEHP for DEHP, and mINCH, OH-mINCH, oxo-mINCH or cx-mINCH for DINCH); LOD, limit of detection; na, not available.

100% of the meconium samples, respectively, indicating the fetal biodegradation and excretion of BBzP and mINCH.

Plasticizers in infant hair showed weak (DEHT and mEHT, $r = 0.28$, $p < 0.05$) to strong (DEHP and mEHP, $r = 0.77$, $p < 0.05$) positive correlations with their corresponding primary metabolites (Figure 1), suggesting that these compounds came from the same source. This result clarifies our previous findings,[30] which pointed out that the lack of a positive correlation between plasticizer concentrations in paired maternal and infant hair limits the determination of the source of plasticizers in infant hair and whether they were contaminated by sampling. The significant positive correlations in the present study indicate that parent plasticizers in infant hair did originate from in utero exposure, and the interference of external exposure during sampling on the analysis of infant hair plasticizers was negligible. It is noteworthy that DPHP was a metabolite of multiple OPEs, such as EHDPP, RDP, and TPHP.[33−35] Moderate positive correlations were found between DPHP and TPHP ($r = 0.54$, $p < 0.01$) (Figure S1), which suggests that TPHP is a potential nonspecific precursor of DPHP in infant hair.

In general, our results indicate in utero cumulative exposure to phthalates in a newborn cohort from South China. These phthalates are ubiquitous as a result of their continuous use. DEHP, DNBP, TCIPP, and DEHT have been detected as the predominant PAEs, OPEs, and APs among indoor dust or air samples from Guangzhou City,[8,36] which explains their high level of deposition in infant hair. The cumulative exposure level of OPEs was lower than those of PAEs, which was consistent with previous findings in cord blood.[15,16,37,38] The levels of DEHT and DINCH, alternatives to DEHP, were comparable to those of DEHP in infant hair. A recent study using infant hair to assess perinatal plasticizer exposure also found that mEHT exposure levels were in line with those of mEHP in a general population of newborns.[39] These findings raise concerns about the potential fetal harm of APs, which have been found to have developmental toxicity and endocrine-disrupting effects.[11,40]

3.2. Relationship of Plasticizers and Their Metabolites between Paired Infant Hair and Meconium. Infant hair plasticizers likely originate from placental transfer, steady exchange with amniotic fluid, and biotransformation in the

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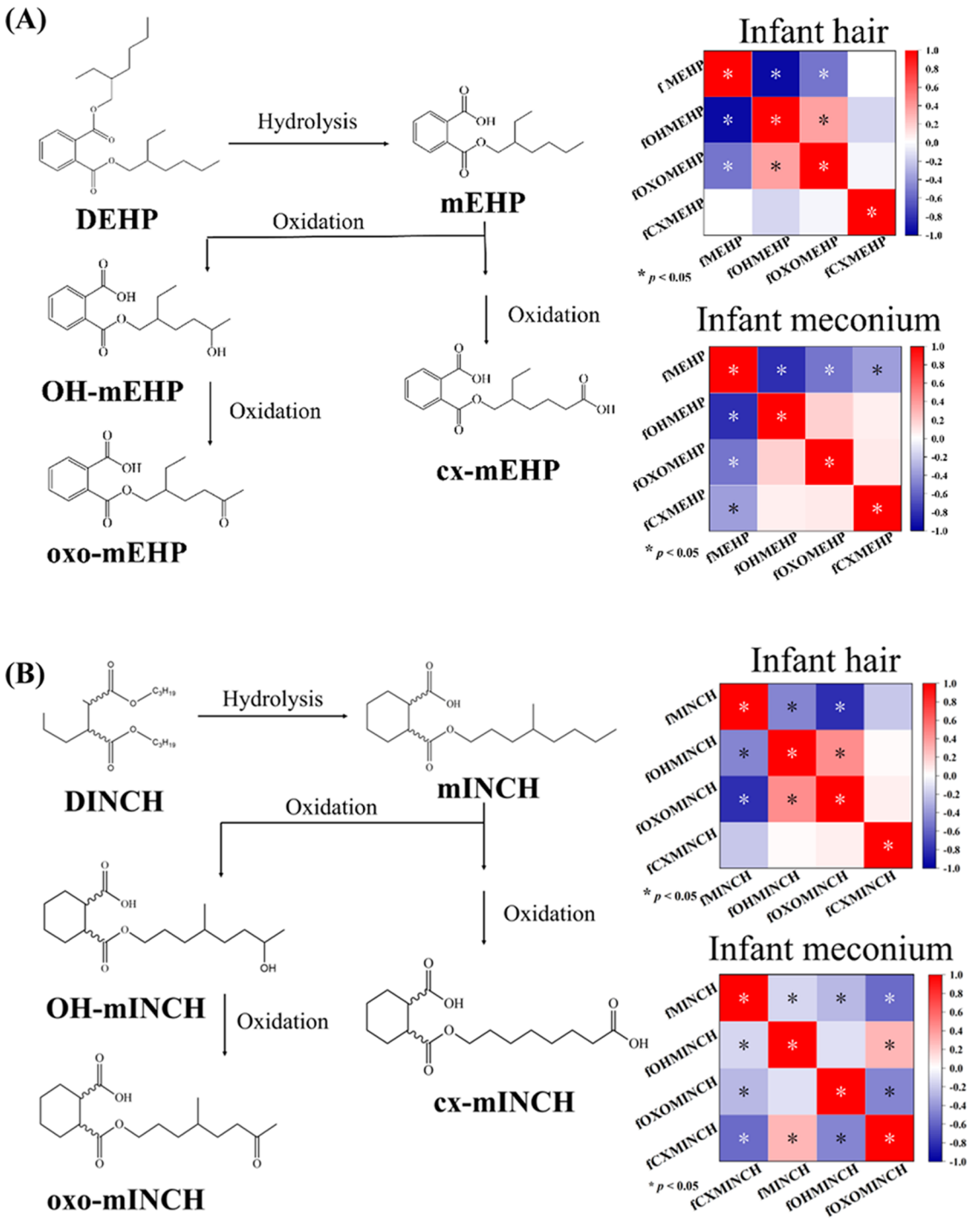

Figure 2. Metabolic pathways and correlation among metabolites of DEHP (A) and DINCH (B) in the phase-I reaction.

fetus.[30] The hydrolysis of plasticizers can occur in fetal tissues, and plasticizers and their hydrolysates in the blood can be delivered through hair follicle cells and deposited in infant hair. The source of plasticizers in amniotic fluid is complex since it is generated from both maternal plasma and cord blood and mixes with fetal urine and feces in the second and third trimesters.[41] In addition, the fetus is repeatedly exposed to plasticizers from the amniotic fluid by both oral and dermal absorption. Taken together, infant hair is a comprehensive terminal biological sample that encompasses maternal contributions and the in utero accumulation of plasticizers. The meconium typically stays in the intestines until birth and becomes the first defecation of the baby, which can potentially serve as a "sink" for environmental chemicals.[42] Since meconium is a unique stool specimen that forms a compartment within the fetus, it may have limited recycling back into fetal circulation, resulting in the isolation of plasticizers in the meconium. Thus, it is possible that the biotransformation and accumulation of plasticizers in the fetus can be assessed by comparing the relationship between plasticizer concentrations and their metabolic profiles between infant hair and meconium. We found no positive correlations between parent plasticizers in infant hair and their corresponding primary metabolites in paired meconium or between primary

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metabolites in paired infant hair and meconium (detailed correlation coefficients are shown in Table S4). However, there were significant positive correlations among the oxidative metabolites of DEHP (Table S5) in paired infant hair and meconium. Specifically, oxidative metabolites of DEHP (OH-mEHP, oxo-mEHP, and cx-mEHP) in meconium showed weak to moderate positive associations with DEHP and its metabolites in infant hair, except for oxo-mEHP in meconium with OH-mEHP or oxo-mEHP in infant hair (Table S5). With respect to DINCH, we observed weak or no positive correlations among oxidative metabolites of DINCH (mINCH, OH-mINCH, oxo-mINCH, and cx-mINCH) in meconium and DINCH and its metabolites in infant hair (Table S6).

Short-chain PAEs and APs are first hydrolyzed by esterases or lipases into their corresponding monoesters in the intestinal epithelium, liver, blood, and other tissues.[43] OPEs can be metabolized in human liver fractions by the oxidative enzymes cytochrome P450s (CYP450s) to produce diesters and hydroxylated metabolites.[44] These metabolites are eliminated in urine (major pathway) or feces (minor pathway) within 48 h.[43,44] The lack of correlation between paired infant hair and meconium among parent compounds and their primary metabolites may be explained by the enzymatic hydrolysis of plasticizers in the fetal gut. Long-chain PAE (i.e., DEHP) and AP (i.e., DINCH) monoesters can be further oxidized by CYP450s in the liver and at a lower rate in the intestine, producing secondary metabolites with hydroxy, oxo, and carboxy functional groups.[43,45] Our findings suggest that most of the oxidative metabolites in meconium come from fetal circulation since CYP450s in the fetal liver are expressed at low levels.[46] A possible explanation for the weak correlation of DINCH oxidative metabolites between paired infant hair and meconium is the further transformation of mINCH in the fetus.

3.3. Major Phase-I Metabolism of DEHP and DINCH.

To further study phase-I metabolism of DEHP and DINCH, the coefficient $f(x)$ for each monoester metabolite was calculated, and we found a significant difference in the $f(x)$ values of infant hair and meconium (Table S7, M−W test $p < 0.001$). We also compared these results to the metabolite profiles of maternal and infant samples reported in the literature (Table 2).

The pattern of DEHP metabolites (f_{mEHP} = 98.1%) in infant hair was close to that of infant hair (f_{MEHP} = 77.7%[39]), cord blood (f_{MEHP} = 70.2,[18] 94.9,[17] and 95.3%[19]), and amniotic fluid (f_{MEHP} = 72.7[47] and 86.0%[19]) reported in the literature. The distribution of DEHP metabolites in meconium was of the same order as that in infant hair, in the following descending order: mEHP (65.3%), OH-mEHP (27.2%), oxo-mEHP (5.43%), and cx-mEHP (2.64%), which was slightly different from that reported in Mathew et al.,[48] in which cx-mEHP (52.9%) and mEHP (65.3%) dominated. Among four monoester metabolites of DINCH in infant hair, mINCH was the most abundant monoester (53.8%), which was similar to levels reported in Cleys et al. with f_{mINCH} = 50.5%.[39] The metabolite profile in meconium differed substantially from infant hair, with the following descending order: cx-mINCH (45.0%), oxo-mINCH (35.8%), mINCH (18.3%), and OH-mINCH (0.89%).

Overall, our study found that the primary metabolite proportions of DEHP and DINCH metabolites in infant hair were similar across different regions, indicating the potential for using infant hair as a robust measure of in utero exposure to plasticizers. However, the fetal oxidative metabolic patterns of plasticizers showed greater variability, indicating that the fetal oxidative capacity may be significantly affected by individual and regional characteristics. Few studies have evaluated intrauterine plasticizer exposure using infant hair or meconium as exposure matrices, and more data are needed to further support these findings. Our study subjects may not be representative of the entire neonatal population, which could limit generalization to more diverse populations. However, the use of a relatively homogeneous population combined with paired infant hair and meconium can minimize the effects of intra- and interindividual variability, allowing us to obtain more accurate data on the in utero transformation and accumulation of plasticizers in this specific population.

The metabolism of mEHP and mINCH was described previously. OH-mEHP/OH-mINCH is the ω-1-hydroxylation product of mEHP/mINCH, oxo-mEHP/oxo-mINCH is the oxidation product of OH-mEHP/OH-mINCH, and cx-mEHP/cx-mINCH is the carboxy oxidation product of the ω-hydroxylation product of mEHP/mINCH[49−52] (Figure 2). The correlations among $f(x)$ of DEHP and DINCH in infant hair and meconium were investigated to further estimate the extent of the two oxidation process.[31]

In infant hair, we observed a moderate to strong negative correlation between f(mEHP) and f(OH-mEHP) ($r = -0.93$, $p < 0.01$) or f(oxo-mEHP) ($r = -0.61$, $p < 0.01$), and a moderate positive correlation between these two ω-1-oxidation products ($r = 0.67$, $p < 0.01$) (Figure 2A). Similar to mEHP, f(mINCH) was negatively associated with f(OH-mINCH) ($r = -0.44$, $p < 0.01$) and f(oxo-mINCH) ($r = -0.78$, $p < 0.01$), while f(OH-mINCH) was positively associated with f(oxo-mINCH) ($r = 0.43$, $p < 0.01$) (Figure 2B). Previous research has shown that hair is a more effective storage site for hydrophobic monoesters (such as mEHP) compared to their oxidized metabolites.[39] However, our findings further confirm the fact that infant hair retains the ω-1-oxidation products of mEHP and mINCH.

In meconium, f(mEHP) and f(mINCH) were not only negatively correlated with their corresponding two ω-1-oxidation products (Figure 2, $p < 0.05$), which was consistent with results in infant hair but also negatively correlated with the ω-oxidative metabolite (with f(cx-mEHP): $r = -0.37$, $p < 0.05$; with f(cx-mINCH): $r = -0.58$, $p < 0.05$), suggesting that the fetus may have ω-oxidative capability. Furthermore, the correlations among oxidative metabolites in meconium were different than those in infant hair. For DEHP, there was a negative correlation between f(cx-mEHP) and f(mEHP) ($r = -0.37$, $p < 0.05$), and no obvious correlation between f(OH-mEHP) and f(oxo-mEHP) ($r = 0.19$, $p > 0.05$) (Figure 2A). For DINCH, there was no obvious correlation between f(OH-mINCH) and f(oxo-mINCH) ($r = -0.12$, $p > 0.05$). Negative correlations were observed between f(cx-mINCH) and f(mINCH) ($r = -0.58$, $p < 0.05$), and f(cx-mINCH) and f(oxo-mINCH) ($r = -0.47$, $p < 0.05$). A positive correlation was observed between f(cx-mINCH) and f(OH-mINCH) ($r = 0.29$, $p < 0.05$) (Figure 2B). There are two possible explanations for differences in infant hair. Meconium is not a homogeneous sample. 80% of meconium accumulates after 38 weeks of pregnancy,[53] resulting in the nonhomogeneous distribution of compounds in meconium and an exposure window that does not correspond to infant hair.[54] On the other hand, comparing the weak correlation of DINCH oxidative metabolites between paired infant hair and

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meconium (Table S6), the fetal oxidative capacity to mINCH may be stronger than mEHP.

3.4. Factors Influencing In Utero Biotransformation of Plasticizers. The primary metabolite/parent compound ratios, $R(x)$, were used to compare differences in the biotransformation of the parent compound and the relative persistence of its metabolites.[31] In this study, several metabolites, including oxo-mINCH (median $R_{OH\text{-}mINCH}$: 48.5), DPHP (median R_{TPHP}: 3.55, median R_{EHDPP}: 1.06), BDCIPP (median R_{TCDIPP}: 4.60), and DNBP (median R_{TNBP}: 3.90) exhibited higher molar concentrations than their corresponding parent plasticizers in infant hair (Table S8).

The influence of demographic characteristics was analyzed, and we found no significant correlation between the $R(x)$ or $f(x)$ values of the target plasticizers and maternal age, prepregnancy BMI, pregnancy weight gain, or infant PI (data not shown). No significant gender differences were found in the $R(x)$ or $f(x)$ values of the target plasticizers, with the exception of f(cx-mEHP) (0.43 ± 0.12 vs. 0.36 ± 0.11, p = 0.044) and R(TPHP) (8.96 ± 7.34 vs. 4.46 ± 2.91, p = 0.024) in infant hair, for which the values in males were higher than those in females, which may be explained by gender differences in placental function,[55,56] fetal hormone levels,[57] and pharmacokinetics.[58] Although the time range of our study was narrow, spanning gestational weeks 34−41, we found the percentages of hydrolyzed and hydroxylated metabolites in meconium decreased, while the percentages of oxo- and carboxy-metabolites in meconium increased, with gestational age in days (Figure S3). The gut and serum contain esterases that can catalyze the conversion of monoesters to their primary hydroxylated monoesters.[59] The fetal environment is relatively enclosed due to its low exchange capacity, and a long residence period permits long-chain PAEs and APs to undergo multistage metabolism.[60] Combining the finding that oxidative metabolites are more dominant in meconium than in infant hair (Table 2) with the oxidation characteristics of mEHP and mINCH in meconium, the fetus may have some degree of primary metabolism of plasticizers. Thus, the detectable oxidative metabolites in infant hair may come partly from fetal metabolism.

In infant hair, f(mEHP) decreased, while f(OH-mEHP) and f(oxo-mEHP) increased, and metabolites of DINCH were the opposite, with increased f(mINCH) and decreased f(oxo-mINCH) and f(cx-mINCH) across gestation (Figure S4). This result further confirms the more efficient fetal elimination of DINCH over DEHP. So far, there are few studies on maternal and fetal exposure to APs and their metabolism during pregnancy.[61−63] The results in the present study suggest that DINCH has a similar in utero metabolic process but different metabolite accumulation compared to DEHP. The different metabolic effects of CYP450s on DEHP and DINCH in humans may explain the differences in their metabolite patterns.[43] In human blood, almost all DEHP (100%) and mEHP (97%) are bound to plasma proteins,[64] which may also explain the inefficient metabolism of DEHP and mEHP. Recent research has found that the oxidation of mEHP in pregnant women was more efficient in late pregnancy compared to early and middle pregnancy,[31] which may explain the decrease of f(mEHP) in infant hair. Further in vivo and in vitro data are needed to investigate the effects of maternal and fetal metabolic capacities on the metabolism and residues of pollutants in utero to clarify these differences. The diminished biotransformation of the bioactive mEHP into less toxic secondary metabolites, which are more water-soluble and excreted more rapidly, may pose an increased risk to fetal health.

Negative correlations were also found between gestational age and the values of R_{TBOEP}, R_{DBP}, and R_{DOP}, respectively (p < 0.05, Figure S4). These results suggest ineffective fetal elimination and cumulative exposure to these plasticizers during pregnancy (Figure S4). In the fetal liver, CYP450s are expressed at low levels. Thus, plasticizer metabolism in the fetal liver is probably limited in comparison to adults, possibly leading to accumulation in fetal tissue.[46] Physiological variation in the mother during pregnancy may also be an influencing factor. It has been shown that CYP450 activity is decreased during pregnancy, which may lead to the accumulation of parent plasticizers in pregnant women.[65] The in utero accumulation of plasticizers and their metabolites should alert people to their potential developmental toxicity in early life.

To the best of our knowledge, this is the first investigation of in utero exposure to plasticizers and their metabolites using paired infant hair and meconium as a biomonitoring matrix. We found that infant hair retained the major phase-I metabolism of the target plasticizers by correlation analysis among the target plasticizers and their metabolites. From the observed associations between plasticizers and their metabolites in paired infant hair and meconium, we suggest that the fetus has the ability to hydrolyze plasticizers into primary metabolites but has limited ability for further oxidation. Most of the oxidative metabolites of plasticizers in the meconium came from the mother through the placental barrier and subsequent partitioning into fetal tissues, body fluids, and meconium. The in utero accumulation of plasticizers and their metabolites was confirmed with correlations between the ratios of plasticizers/metabolites and gestational age. The obtained results are meaningful for assessing the health risks posed by plasticizers and their metabolites on the fetus.

It is worth noting that the phase-I metabolites of plasticizers identified in this study can be further transformed into sulfate and glucuronide conjugates via phase-II biotransformation.[43,44] These biotransformations may have led to the underestimation of plasticizer concentrations in the present study due to the absence of measurement of such phase-II metabolites. In addition, some plasticizers and metabolites were not examined because their levels were below the LOD. However, infant hair and meconium are easily obtainable, and our results provide insights into chronic exposure to typical plasticizers during intrauterine life, which can be routinely employed in neonatology wards to make an early diagnosis of prenatal plasticizer exposure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.3c11032.

Detailed information on the standards, instrumental analysis for target plasticizers, overview of quality assurance and control of plasticizer parent compounds and their respective metabolites considered in this study (Table S1), summary of plasticizer and their metabolite concentrations in infant hair samples (Table S2), concentrations of metabolites of plasticizer in paired meconium samples (Table S3), Pearson correlation of

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log-transformed plasticizer concentrations between paired infant hair and meconium samples (Table S4), Pearson correlation of log-transformed DEHP concentrations between paired infant hair and meconium samples (Table S5), Pearson correlation of log-transformed DINCH concentrations between paired infant hair and meconium samples (Table S6), results of $f(x)$ in infant hairs and paired meconium samples (Table S7), results of $R(x)$ in infant hairs (Table S8), power analysis of the sample size required to detect significant correlations at different power (Figure S1), correlation between logarithmic concentrations of DPHP and its three potential nonspecific precursors (TPHP, EHDPP and RDP) in infant hair (Figure S2), partial correlation of gestational age and $f(x)$ values in meconium samples (Figure S3), and partial correlation of gestational age and $f(x)$ values or $R(x)$ values of infant hairs (Figure S4). (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Weng, X.; Zhu, Q.; Liao, C.; Jiang, G. Cumulative Exposure to Phthalates and Their Alternatives and Associated Female Reproductive Health: Body Burdens, Adverse Outcomes, and Underlying Mechanisms. *Environ. Sci. Technol.* **2023**, *57* (22), 8189−8212.

(2) Li, Y.; Wang, X.; Zhu, Q.; Xu, Y.; Fu, Q.; Wang, T.; Liao, C.; Jiang, G. Organophosphate Flame Retardants in Pregnant Women: Sources, Occurrence, and Potential Risks to Pregnancy Outcomes. *Environ. Sci. Technol.* **2023**, *57* (18), 7109−7128.

(3) Maitre, L.; de Bont, J.; Casas, M.; Robinson, O.; Aasvang, G. M.; Agier, L.; Andrušaitytė, S.; Ballester, F.; Basagaña, X.; Borràs, E.; Brochot, C.; Bustamante, M.; Carracedo, A.; de Castro, M.; Dedele, A.; Donaire-Gonzalez, D.; Estivill, X.; Evandt, J.; Fossati, S.; Giorgis-Allemand, L.; Jun, R. G.; Granum, B.; Grazuleviciene, R.; Bjerve Gützkow, K.; Småstuen Haug, L.; Hernandez-Ferrer, C.; Heude, B.; Ibarluzea, J.; Julvez, J.; Karachaliou, M.; Keun, H. C.; Krog, N. H.; Lau, C. E.; Leventakou, V.; Lyon-Caen, S.; Manzano, C.; Mason, D.; McEachan, R.; Meltzer, H. M.; Petraviciene, I.; Quentin, J.; Roumeliotaki, T.; Sabido, E.; Saulnier, P. J.; Siskos, A. P.; Siroux, V.; Sunyer, J.; Tamayo, I.; Urquiza, J.; Vafeiadi, M.; van Gent, D.;

Vives-Usano, M.; Waiblinger, D.; Warembourg, C.; Chatzi, L.; Coen, M.; van den Hazel, P.; Nieuwenhuijsen, M. J.; Slama, R.; Thomsen, C.; Wright, J.; Vrijheid, M. Human Early Life Exposome (HELIX) study: a European population-based exposome cohort. *BMJ Open* **2018**, *8* (9), No. e021311.
(4) Qian, Y.; Shao, H.; Ying, X.; Huang, W.; Hua, Y. The Endocrine Disruption of Prenatal Phthalate Exposure in Mother and Offspring. *Front. Public Health* **2020**, *8*, 366.
(5) Du, J.; Li, H.; Xu, S.; Zhou, Q.; Jin, M.; Tang, J. A review of organophosphorus flame retardants (OPFRs): occurrence, bioaccumulation, toxicity, and organism exposure. *Environ. Sci. Pollut Res.* **2019**, *26* (22), 22126−22136.
(6) Kay, V. R.; Bloom, M. S.; Foster, W. G. Reproductive and developmental effects of phthalate diesters in males. *Crit. Rev. Toxicol.* **2014**, *44* (6), 467−498.
(7) Gao, C.-J.; Wang, F.; Shen, H.-M.; Kannan, K.; Guo, Y. Feminine Hygiene Products—A Neglected Source of Phthalate Exposure in Women. *Environ. Sci. Technol.* **2020**, *54* (2), 930−937.
(8) Tang, B.; Christia, C.; Malarvannan, G.; Liu, Y.-E.; Luo, X.-J.; Covaci, A.; Mai, B.-X.; Poma, G. Legacy and emerging organophosphorus flame retardants and plasticizers in indoor microenvironments from Guangzhou, South China. *Environ. Int.* **2020**, *143*, No. 105972.
(9) Qadeer, A.; Kirsten, K. L.; Ajmal, Z.; Jiang, X.; Zhao, X. Alternative Plasticizers As Emerging Global Environmental and Health Threat: Another Regrettable Substitution? *Environ. Sci. Technol.* **2022**, *56* (3), 1482−1488.
(10) Sheikh, I. A.; Beg, M. A. Structural characterization of potential endocrine disrupting activity of alternate plasticizers di-(2-ethylhexyl) adipate (DEHA), acetyl tributyl citrate (ATBC) and 2,2,4-trimethyl 1,3-pentanediol diisobutyrate (TPIB) with human sex hormone-binding globulin. *Reprod. Toxicol.* **2019**, *83*, 46−53.
(11) Zughaibi, T. A.; Sheikh, I. A.; Beg, M. A. Insights into the Endocrine Disrupting Activity of Emerging Non-Phthalate Alternate Plasticizers against Thyroid Hormone Receptor: A Structural Perspective. *Toxics* **2022**, *10* (5), 263.
(12) Marie, C.; Vendittelli, F.; Sauvant-Rochat, M.-P. Obstetrical outcomes and biomarkers to assess exposure to phthalates: A review. *Environ. Int.* **2015**, *83*, 116−136.
(13) Vorkamp, K.; Castaño, A.; Antignac, J.-P.; Boada, L. D.; Cequier, E.; Covaci, A.; Esteban López, M.; Haug, L. S.; Kasper-Sonnenberg, M.; Koch, H. M.; Pérez Luzardo, O.; Osite, A.; Rambaud, L.; Pinorini, M.-T.; Sabbioni, G.; Thomsen, C. Biomarkers, matrices and analytical methods targeting human exposure to chemicals selected for a European human biomonitoring initiative. *Environ. Int.* **2021**, *146*, No. 106082.
(14) Zhang, Y.; Lin, L.; Cao, Y.; Chen, B.; Zheng, L.; Ge, R.-S. Phthalate Levels and Low Birth Weight: A Nested Case-Control Study of Chinese Newborns. *J. Pediatrics* **2009**, *155* (4), 500−504.
(15) Huang, Y.; Li, J.; Garcia, J. M.; Lin, H.; Wang, Y.; Yan, P.; Wang, L.; Tan, Y.; Luo, J.; Qiu, Z.; Chen, J.-a.; Shu, W. Phthalate Levels in Cord Blood Are Associated with Preterm Delivery and Fetal Growth Parameters in Chinese Women. *PLoS One* **2014**, *9* (2), No. e87430.
(16) Wang, X.; Chen, P.; Zhao, L.; Zhu, L.; Wu, F. Transplacental Behaviors of Organophosphate Tri- and Diesters Based on Paired Human Maternal and Cord Whole Blood: Efficiencies and Impact Factors. *Environ. Sci. Technol.* **2021**, *55* (5), 3091−3100.
(17) Hwa, H.-L.; Peng, F.-S.; Ting, T.-T.; Chen, H.-W.; Chan, H.-Y.; Yang, D.-P.; Chen, P.-C.; Kuo, Y.-N.; Chen, P.-S. Monitoring Phthalates in Maternal and Cord Blood: Implications for Prenatal Exposure and Birth Outcomes. *Environ. Toxicol. Chem.* **2022**, *41* (3), 715−725.
(18) Lin, S.; Ku, H.-Y.; Su, P.-H.; Chen, J.-W.; Huang, P.-C.; Angerer, J.; Wang, S.-L. Phthalate exposure in pregnant women and their children in central Taiwan. *Chemosphere* **2011**, *82* (7), 947−955.
(19) Li, X.; Sun, H.; Yao, Y.; Zhao, Z.; Qin, X.; Duan, Y.; Wang, L. Distribution of Phthalate Metabolites between Paired Maternal−Fetal Samples. *Environ. Sci. Technol.* **2018**, *52* (11), 6626−6635.
(20) Ye, Y.; Yan, C.; Meiling, Y.; Xi, C.; Na, G.; Yaqin, J.; Zeng-Rong, S. J. Determination of five phthalate metabolites in cord serum by solid phase extraction high performance liquidchromatography-mass spectrometry. *J. Environ. Health* **2012**, *29*, 1039−1042.
(21) Latini, G.; De Felice, C.; Presta, G.; Del Vecchio, A.; Paris, I.; Ruggieri, F.; Mazzeo, P. In utero exposure to di-(2-ethylhexyl)-phthalate and duration of human pregnancy. *Environ. Health Perspect.* **2003**, *111* (14), 1783−1785.
(22) Percy, Z.; Vuong, A. M.; Ospina, M.; Calafat, A. M.; La Guardia, M. J.; Xu, Y.; Hale, R. C.; Dietrich, K. N.; Xie, C.; Lanphear, B. P.; Braun, J. M.; Cecil, K. M.; Yolton, K.; Chen, A. Organophosphate esters in a cohort of pregnant women: Variability and predictors of exposure. *Environ. Res.* **2020**, *184*, No. 109255.
(23) Luo, D.; Liu, W.; Wu, W.; Tao, Y.; Hu, L.; Wang, L.; Yu, M.; Zhou, A.; Covaci, A.; Xia, W.; Xu, S.; Li, Y.; Mei, S. Trimester-specific effects of maternal exposure to organophosphate flame retardants on offspring size at birth: A prospective cohort study in China. *J. Hazard. Mater.* **2021**, *406*, No. 124754.
(24) Appenzeller, B. M. R.; Tsatsakis, A. M. Hair analysis for biomonitoring of environmental and occupational exposure to organic pollutants: State of the art, critical review and future needs. *Toxicol. Lett.* **2012**, *210* (2), 119−140.
(25) Gareri, J.; Koren, G. Prenatal hair development: Implications for drug exposure determination. *Forensic Sci. Int.* **2010**, *196* (1), 27−31.
(26) Bose-O'Reilly, S.; Lettmeier, B.; Shoko, D.; Roider, G.; Drasch, G.; Siebert, U. Infants and mothers levels of mercury in breast milk, urine and hair, data from an artisanal and small-scale gold mining area in Kadoma/Zimbabwe. *Environ. Res.* **2020**, *184*, No. 109266.
(27) Trdin, A.; Falnoga, I.; Fajon, V.; Živković, I.; Snoj Tratnik, J.; Prpić, I.; Špirić, Z.; Horvat, M. Mercury speciation in meconium and associated factors. *Environ. Res.* **2019**, *179*, No. 108724.
(28) van der Veen, I.; de Boer, J. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* **2012**, *88* (10), 1119−1153.
(29) Fernández-Cruz, T.; Álvarez-Silvares, E.; Domínguez-Vigo, P.; Simal-Gándara, J.; Martínez-Carballo, E. Prenatal exposure to organic pollutants in northwestern Spain using non-invasive matrices (placenta and meconium). *Sci. Total Environ.* **2020**, *731*, No. 138341.
(30) Cai, F.-S.; Tang, B.; Zheng, J.; Yan, X.; Luo, W.-K.; He, M.; Luo, X.-J.; Ren, M.-Z.; Yu, Y.-J.; Mai, B.-X. Fetal exposure to organic contaminants revealed by infant hair: A preliminary study in south China. *Environ. Pollut.* **2023**, *316*, No. 120536.
(31) Zhao, H.; Li, J.; Zhou, Y.; Zhu, L.; Zheng, Y.; Xia, W.; Li, Y.; Xiang, L.; Chen, W.; Xu, S.; Cai, Z. Investigation on Metabolism of Di(2-Ethylhexyl) Phthalate in Different Trimesters of Pregnant Women. *Environ. Sci. Technol.* **2018**, *52* (21), 12851−12858.
(32) Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*; Lawrence Erlbaum Associates, 1988; pp 8−12.
(33) Ballesteros-Gómez, A.; Van den Eede, N.; Covaci, A. In Vitro Human Metabolism of the Flame Retardant Resorcinol Bis-(diphenylphosphate) (RDP). *Environ. Sci. Technol.* **2015**, *49* (6), 3897−3904.
(34) Ballesteros-Gómez, A.; Erratico, C. A.; Van den Eede, N.; Ionas, A. C.; Leonards, P. E. G.; Covaci, A. In vitro metabolism of 2-ethylhexyldiphenyl phosphate (EHDPHP) by human liver microsomes. *Toxicol. Lett.* **2015**, *232* (1), 203−212.
(35) Van den Eede, N.; Maho, W.; Erratico, C.; Neels, H.; Covaci, A. First insights in the metabolism of phosphate flame retardants and plasticizers using human liver fractions. *Toxicol. Lett.* **2013**, *223* (1), 9−15.
(36) Hou, Y.; Tang, B.; Cai, F.-s.; Yan, X.; Zheng, J.; Wang, J.-l.; Zhang, H. Legacy and novel flame retardants and plasticizers in indoor dust from residents' homes in Guangzhou: Pollution status and human exposure assessment. *Acta Sci. Circumstantiae* **2022**, *42* (7), 106−122.
(37) Fang, H.; Wang, H.; Zeng, C.; Fu, H.; Zhao, B.; Liu, A.; Yan, J. A preliminary cumulative risk assessment of Diethylhexyl phthalate and Dibutyl phthalate based on the inhibition of embryonic

https://doi.org/10.1021/acs.est.3c11032
Environ. Sci. Technol. 2024, 58, 5739−5749

development via the PPARγ pathway. *Toxicol. In Vitro* **2022**, *84*, No. 105430.
(38) Luo, W.; Liu, Y.; Yang, X.; Aamir, M.; Bai, X.; Liu, W. Prenatal exposure to emerging and traditional organophosphate flame retardants: Regional comparison, transplacental transfer, and birth outcomes. *Environ. Pollut.* **2023**, *336*, No. 122463.
(39) Cleys, P.; Panneel, L.; Bombeke, J.; Dumitrascu, C.; Malarvannan, G.; Poma, G.; Mulder, A.; Jorens, P. G.; Covaci, A. Hair as an alternative matrix to assess exposure of premature neonates to phthalate and alternative plasticizers in the neonatal intensive care unit. *Environ. Res.* **2023**, *236*, No. 116712.
(40) Kambia, N. K.; Séverin, I.; Farce, A.; Moreau, E.; Dahbi, L.; Duval, C.; Dine, T.; Sautou, V.; Chagnon, M.-C. In vitro and in silico hormonal activity studies of di-(2-ethylhexyl)terephthalate, a di-(2-ethylhexyl)phthalate substitute used in medical devices, and its metabolites. *J. Appl. Toxicol.* **2019**, *39* (7), 1043−1056.
(41) Underwood, M. A.; Gilbert, W. M.; Sherman, M. P. Amniotic Fluid: Not Just Fetal Urine Anymore. *J. Perinatol.* **2005**, *25* (5), 341−348.
(42) Barr, D. B.; Bishop, A.; Needham, L. L. Concentrations of xenobiotic chemicals in the maternal-fetal unit. *Reprod. Toxicol.* **2007**, *23* (3), 260−266.
(43) Stajnko, A.; Runkel, A. A.; Kosjek, T.; Snoj Tratnik, J.; Mazej, D.; Falnoga, I.; Horvat, M. Assessment of susceptibility to phthalate and DINCH exposure through CYP and UGT single nucleotide polymorphisms. *Environ. Int.* **2022**, *159*, No. 107046.
(44) Hou, R.; Xu, Y.; Wang, Z. Review of OPFRs in animals and humans: Absorption, bioaccumulation, metabolism, and internal exposure research. *Chemosphere* **2016**, *153*, 78−90.
(45) Choi, K.; Joo, H.; Campbell, J. L.; Clewell, R. A.; Andersen, M. E.; Clewell, H. J. In vitro metabolism of di(2-ethylhexyl) phthalate (DEHP) by various tissues and cytochrome P450s of human and rat. *Toxicol. In Vitro* **2012**, *26* (2), 315−322.
(46) Robinson, J. F.; Hamilton, E. G.; Lam, J.; Chen, H.; Woodruff, T. J. Differences in cytochrome p450 enzyme expression and activity in fetal and adult tissues. *Placenta* **2020**, *100*, 35−44.
(47) Wittassek, M.; Angerer, J.; Kolossa-Gehring, M.; Schäfer, S. D.; Klockenbusch, W.; Dobler, L.; Günsel, A. K.; Müller, A.; Wiesmüller, G. A. Fetal exposure to phthalates − a pilot study. *Int. J. Hyg. Environ. Health* **2009**, *212* (5), 492−498.
(48) Mathew, L.; Snyder, N. W.; Lyall, K.; Lee, B. K.; McClure, L. A.; Elliott, A. J.; Newschaffer, C. J. The associations between prenatal phthalate exposure measured in child meconium and cognitive functioning of 12-month-old children in two cohorts at elevated risk for adverse neurodevelopment. *Environ. Res.* **2022**, *214*, No. 113928.
(49) Völkel, W.; Kiranoglu, M.; Dettbarn, G.; John, A.; Jessel, S.; Seidel, A.; Fromme, H. Urinary toxicokinetics of di-(isononyl)-cyclohexane-1,2-dicarboxylate (DINCH) in humans following single oral administration. *Toxicol. Lett.* **2016**, *248*, 16−24.
(50) Koch, H. M.; Schütze, A.; Pälmke, C.; Angerer, J.; Brüning, T. Metabolism of the plasticizer and phthalate substitute diisononyl-cyclohexane-1,2-dicarboxylate (DINCH) in humans after single oral doses. *Arch. Toxicol.* **2013**, *87* (5), 799−806.
(51) Koch, H. M.; Bolt, H. M.; Angerer, J. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch. Toxicol.* **2004**, *78* (3), 123−130.
(52) Koch, H. M.; Bolt, H. M.; Preuss, R.; Angerer, J. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. *Arch. Toxicol.* **2005**, *79* (7), 367−376.
(53) de Campos, E. G.; da Costa, B. R. B.; dos Santos, F. S.; Monedeiro, F.; Alves, M. N. R.; Santos Junior, W. J. R.; De Martinis, B. S. Alternative matrices in forensic toxicology: a critical review. *Forensic Toxicol.* **2022**, *40* (1), 1−18.
(54) Park, B. Y.; Lee, B. K. Use of meconium in perinatal epidemiology: potential benefits and pitfalls. *Ann. Epidemiol.* **2014**, *24* (12), 878−881.
(55) Rosenfeld, C. S. Sex-Specific Placental Responses in Fetal Development. *Endocrinology* **2015**, *156* (10), 3422−3434.
(56) Widnes, C.; Flo, K.; Acharya, G. Exploring sexual dimorphism in placental circulation at 22−24 weeks of gestation: A cross-sectional observational study. *Placenta* **2017**, *49*, 16−22.
(57) Kur, P.; Kolasa-Wołosiuk, A.; Misiakiewicz-Has, K.; Wiszniewska, B. Sex Hormone-Dependent Physiology and Diseases of Liver. *Int. J. Environ. Res. Public Health* **2020**, *17* (8), 2620.
(58) Zafeiri, A.; Fowler, P. A. Expression Patterns of Analgesic Metabolising Machinery in 1st and 2nd Trimester Human Fetal Liver and Gonads. *J. Endocr. Soc.* **2021**, *5*, A488.
(59) Henriksen, L. S.; Mathiesen, B. K.; Assens, M.; Krause, M.; Skakkebæk, N. E.; Juul, A.; Andersson, A.-M.; Hart, R. J.; Newnham, J. P.; Keelan, J. A.; Pennell, C.; Main, K. M.; Frederiksen, H. Use of stored serum in the study of time trends and geographical differences in exposure of pregnant women to phthalates. *Environ. Res.* **2020**, *184*, No. 109231.
(60) Li, X.; Sun, H.; Yao, Y.; Zhao, Z.; Qin, X.; Duan, Y.; Wang, L. Distribution of Phthalate Metabolites between Paired Maternal-Fetal Samples. *Environ. Sci. Technol.* **2018**, *52* (11), 6626−6635.
(61) Rodríguez-Carmona, Y.; Ashrap, P.; Calafat, A. M.; Ye, X.; Rosario, Z.; Bedrosian, L. D.; Huerta-Montanez, G.; Vélez-Vega, C. M.; Alshawabkeh, A.; Cordero, J. F.; Meeker, J. D.; Watkins, D. Determinants and characterization of exposure to phthalates, DEHTP and DINCH among pregnant women in the PROTECT birth cohort in Puerto Rico. *J. Exposure Sci. Environ. Epidemiol.* **2020**, *30* (1), 56−69.
(62) Pacyga, D. C.; Haggerty, D. K.; Nicol, M.; Henning, M.; Calafat, A. M.; Braun, J. M.; Schantz, S. L.; Strakovsky, R. S. Identification of profiles and determinants of maternal pregnancy urinary biomarkers of phthalates and replacements in the Illinois Kids Development Study. *Environ. Int.* **2022**, *162*, No. 107150.
(63) Shu, H.; Jönsson, B. A.; Gennings, C.; Svensson, Å.; Nånberg, E.; Lindh, C. H.; Knutz, M.; Takaro, T. K.; Bornehag, C. G. Temporal trends of phthalate exposures during 2007−2010 in Swedish pregnant women. *J. Exposure Sci. Environ. Epidemiol.* **2018**, *28* (5), 437−447.
(64) Domínguez-Romero, E.; Scheringer, M. A review of phthalate pharmacokinetics in human and rat: what factors drive phthalate distribution and partitioning? *Drug Metab. Rev.* **2019**, *51* (3), 314−329.
(65) Tracy, T. S.; Venkataramanan, R.; Glover, D. D.; Caritis, S. N. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *Am. J. Obstet. Gynecol.* **2005**, *192* (2), 633−639.
(66) Urbancova, K.; Sram, R. J.; Hajslova, J.; Pulkrabova, J. Concentrations of Phthalate and DINCH Metabolites in Urine Samples from Czech Mothers and Newborns. *Exposure Health* **2022**, *14* (1), 17−27.
(67) Katsikantami, I.; Tzatzarakis, M. N.; Karzi, V.; Stavroulaki, A.; Xezonaki, P.; Vakonaki, E.; Alegakis, A. K.; Sifakis, S.; Rizos, A. K.; Tsatsakis, A. M. Biomonitoring of bisphenols A and S and phthalate metabolites in hair from pregnant women in Crete. *Sci. Total Environ.* **2020**, *712*, No. 135651.
(68) Chang, Y.-J.; Lin, K.-L.; Chang, Y.-Z. Determination of Di-(2-ethylhexyl)phthalate (DEHP) metabolites in human hair using liquid chromatography−tandem mass spectrometry. *Clin. Chim. Acta* **2013**, *420*, 155−159.
(69) Frederiksen, H.; Jørgensen, N.; Andersson, A.-M. Correlations between phthalate metabolites in urine, serum, and seminal plasma from young Danish men determined by isotope dilution liquid chromatography tandem mass spectrometry. *J. Anal. Toxicol.* **2010**, *34* (7), 400−410.
(70) Li, Z.; Wu, D.; Guo, Y.; Mao, W.; Zhao, N.; Zhao, M.; Jin, H. Phthalate metabolites in paired human serum and whole blood. *Sci. Total Environ.* **2022**, *824*, No. 153792.

https://doi.org/10.1021/acs.est.3c11032
Environ. Sci. Technol. 2024, 58, 5739−5749