Organophosphate ester flame retardants and plasticizers in a Chinese population : significance of hydroxylated metabolites and implication for human exposure

Organophosphate flame retardants in a Chinese population: Significance of hydroxylated metabolites and implication for human exposure

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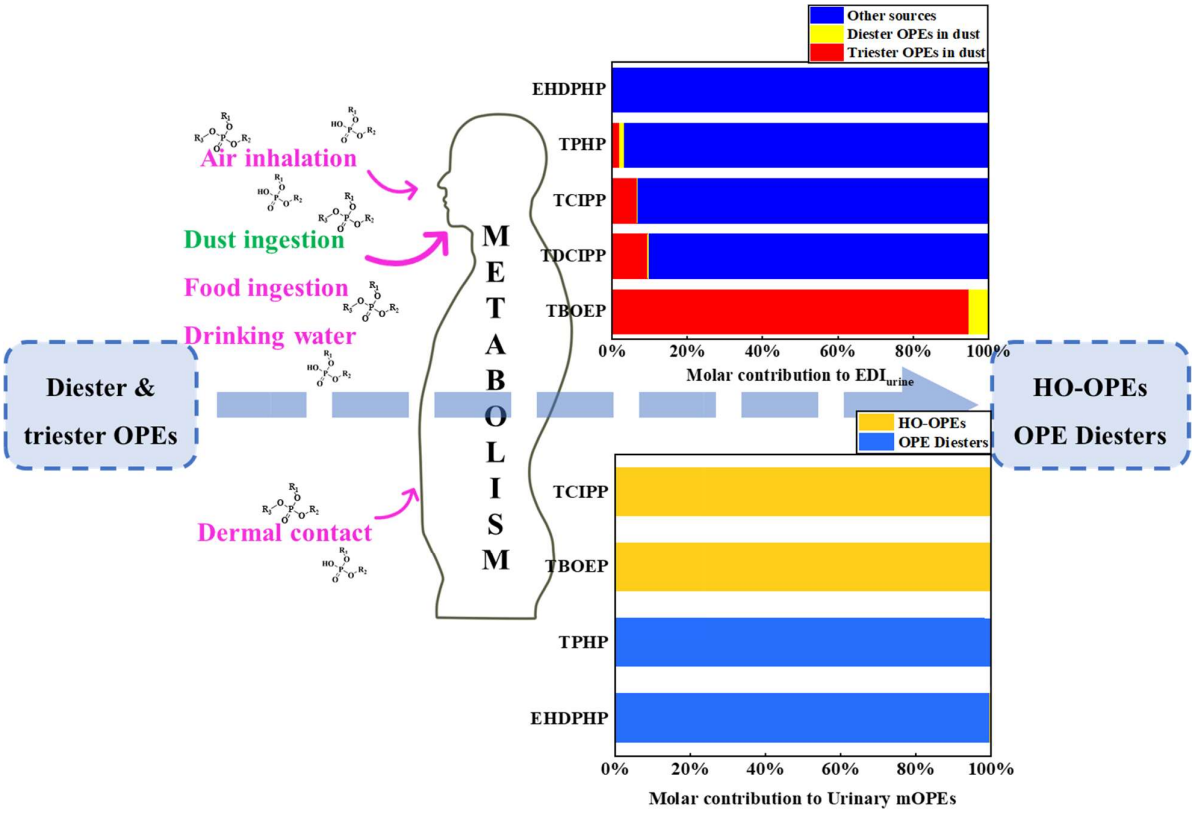
GRAPHIC ABSTRACT



HIGHLIGHTS

- HO-OPEs were ubiquitously detected in urine samples.
- HO-OPEs are important biomarkers in addition to OPE diesters in human biomonitoring.
- OPEs in indoor dust explained <30% of internal exposure levels except for TBOEP.
- Exposure to OPE diesters in indoor dust accounted for up to 50% of internal exposure levels.
- The proportion of HO-OPEs in OPE metabolites are associated with lifestyle behaviors.

Keywords:

Organophosphate flame retardants (OPEs); Urine; Hydroxylated OPE metabolites; Diester exposure; Estimated daily intake

ABSTRACT

Organophosphate esters (OPEs) are widely used as flame retardants, plasticizers and defoamers and have been reported to cause a number of adverse effects in humans. In this study, tris(chloroethyl) phosphate and ten OPE metabolites including hydroxylated OPEs (HO-OPEs) were analyzed in 46 urine samples, collected from 8 provinces located across different regions in China. The detection frequencies of HO-OPEs, such as 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP) and 2-hydroxyethyl bis(2-butoxyethyl) phosphate, were 89.1% and 54.3%, respectively, which were all higher than the corresponding OPE diesters. BCIPHIPP, with a median concentration of 0.68 ng/mL (range: n.d. – 8.61 ng/mL), was found as a major metabolite of tris(2-chloroisopropyl) phosphate (TCIPP). As suggested from the correlation analysis between paired indoor dust and urine samples, direct exposure to OPE diesters may occur via dust ingestion. Estimated daily intake values derived from urinary levels (EDI_{urine}) in this study were generally higher than those calculated from dust levels (EDI_{dust}), which only accounted for <30% of EDI_{urine}, except for tris(2-butoxyethyl) phosphate (TBOEP). The ratio of EDI_{dust} to EDI_{urine} increased by no more than 50% when considering contribution of OPE diesters in dust. HO-OPEs contributed >36% of the EDI_{urine} for TBOEP, TCIPP, and triphenyl phosphate. Overall, our results suggested a significant role in monitoring HO-OPEs for internal exposure levels. Direct exposure to OPE diesters via different sources may substantially contribute to the internal human exposure levels of OPE metabolites.

1. Introduction

Organophosphate esters (OPEs) are a class of triphosphate esters with side chains substituted by alkyl, aryl or chloroalkyl groups. Chlorinated OPEs (Cl-OPEs) are commonly applied in flexible and rigid polyurethane foams, while alkyl and aromatic OPEs are frequently used as plasticizers and defoamers in plastics, textiles, and personal care products (van der Veen and de Boer 2012, Wei et al. 2015). In China, the production of OPEs was estimated to be 100,000 tons in 2011 and with an annual projected increase rate of 15% (Wang et al. 2015). The applied OPEs are not chemically bound to the polymers, which facilitates their release into the environment via volatilization, abrasion, and dissolution (van der Veen and de Boer 2012, Wang et al. 2015). This leads to a ubiquitous occurrence of OPEs in various indoor environmental matrices, such as drinking water, indoor air and dust, which brings human exposure through ingestion, inhalation, and dermal absorption (Bacaloni et al. 2007, Fromme et al. 2014).

Toxicological studies on animals showed that OPEs can be associated with a number of adverse health effects (Qi et al. 2019, Rhyu et al. 2019). The evidences from toxicological and epidemiological studies based on human subjects also suggested a positive link between OPE exposure and certain endocrine disruptive effects (Carignan et al. 2018, Deziel et al. 2018, Dishaw et al. 2011, Hoffman et al. 2018, Ingle et al. 2018, Kojima et al. 2013, Lu et al. 2017, Sun et al. 2018, van der Veen and de Boer 2012). Therefore, the biomonitoring of internal exposure levels of OPEs is prerequisite for risk assessment, but their associations with external exposure sources and species remain unclear.

In vivo and in vitro studies have revealed general metabolic pathways of OPEs including O-dealkylation, hydroxylation, carboxylation, and oxidative dehalogenation (only for Cl-OPEs), which result in corresponding phosphate diesters and hydroxylated (HO-OPEs) and carboxylated metabolic isomers (Hou et al. 2016). These hydrophilic metabolites are more easily eliminated and excreted (Cooper et al. 2011, Van den Eede et al. 2013). As the major metabolites, OPE diesters have been frequently reported in human biomonitoring studies (Chen et al. 2018, He et al. 2018a, Van den Eede et al. 2015). More often, OPE diesters are usually

the only species representing internal exposure levels of OPEs in occupational workers and the general population. For instance, bis(chloroethyl) phosphate (BCEP, geometric mean (GM): 0.72 ng/mL) and diphenyl phosphate (DPHP, GM:0.55 ng/mL) were the most abundant OPE diesters in e-waste recycling workers (Lu et al. 2017). Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and DPHP were the most frequently detected OPE diesters in the urine samples of children (Sugeng et al. 2017, Zhang et al. 2018a), mothers (Hoffman et al. 2014, Hoffman et al. 2017c, Romano et al. 2017), and other general populations (Hoffman et al. 2017a, Van den Eede et al. 2015).

Indoor dust have been frequently investigated and found as an important exposure source of OPEs in indoor environment. Significant correlations were particularly observed between tris(chloroethyl) phosphate (TCEP) and tris(2-butoxyethyl) phosphate (TBOEP) in indoor dust or air samples and the respective urinary OPE diesters of children, who attend daycare centers in Germany, but not for other OPEs (Fromme et al. 2014). An extremely weak correlation was also proposed for triphenyl phosphate (TPHP) in dust samples and DPHP in prenatal urine samples of women from California, the United States, while tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) was not correlated with BDCIPP (Castorina et al. 2017). The inconsistent correlations indicate that the external exposure profiles may differ between countries and other sources of exposure may occur.

Although OPE diesters are acknowledged as the most frequently detected biomarkers (Castorina et al. 2017, Chen et al. 2018, Fromme et al. 2014, Hoffman et al. 2017b, Wang et al. 2019, Zhang et al. 2018b), the estimated exposure levels of OPEs may be biased based on OPE diesters only. In vitro tests showed that HO-OPEs of 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP), 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP) and 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP) rather than their diesters were the major OPE metabolites transformed from TBOEP, TPHP and tris(2-chloroisopropyl) phosphate (TCIPP) (Van den Eede et al. 2013). More recently, TBOEP and tri-n-butyl phosphate (TNBP) were found primarily metabolized into BBOEHEP and dibutyl-3-hydroxybutyl phosphate (3-HO-TNBP), respectively (Hou et al. 2018). Mono-hydroxylated metabolites were also found as

101 the major metabolites of 2-ethylhexyldiphenyl phosphate (EHDPHP) in human liver
102 microsomes culture (Ballesteros-Gomez et al. 2015). These HO-OPEs are potential conjugation
103 blocks for phase II metabolism and can be released when undergo enzymatic hydrolysis in
104 sample pretreatment.
105 Nevertheless, only a limited number of studies reported the concentrations of HO-OPEs in
106 urine samples from adults and children (Ait Bamai et al. 2019, Bastiaensen et al. 2019, He et
107 al. 2018a, Phillips et al. 2018, Su et al. 2016, Van den Eede et al. 2015, Voelkel et al. 2018, Xu
108 et al. 2019). The urinary levels of BCIPHIPP in general population from Australia were found
109 at 0.37 – 9.43 ng/mL, which were comparable to those of BCIPP (Van den Eede et al. 2015).
110 BBOEHEP, bis(2-butoxyethyl) 3'-hydroxy-2-butoxyethyl phosphate (3-HO-TBOEP), and
111 BCIPHIPP were also frequently detected at mean urinary levels of 0.075 ng/mL, 0.029 ng/mL,
112 and 0.43 ng/mL in infants and young children from Australia, respectively (He et al. 2018a).
113 Even so, the estimated daily intakes (EDIs) have not been well considered for urinary HO-OPEs.
114 So far, only one study investigated urinary levels of HO-OPEs in Chinese population and
115 proposed that HO-OPEs are reliable biomarkers for OPE internal exposure (Zhao et al. 2019a).
116 Therefore, urinary levels of HO-OPEs is an essential supplement to biomonitoring of internal
117 exposure to OPEs and their associations with their external exposure profiles are yet to be clarify.
118 In this study, 4 HO-OPEs were analyzed along with 6 OPE diesters and a trimester of TCEP
119 in 46 urine samples collected from 8 provinces of China. EDIs based on urinary levels were
120 calculated and compared with the available reference doses (RfD). The biomonitoring data were
121 also correlated with their external exposure to both OPEs and OPE diesters in paired indoor
122 dust samples as reported in our previous study (Wang et al., 2019). The purpose of this study
123 was to evaluate the significance of HO-OPEs as biomarkers of OPE exposure in a Chinese
124 population and the contribution of OPE diesters in dust to their urinary exposure levels.
125
126 **2. Materials and methods**
127 *2.1. Standards and reagents*

The abbreviations and structures of OPE metabolites and their parent compounds are shown in Table S1 and Figure S1 in supporting information (SI). Standards of 4-HO-TPHP, 4-HO-DPHP, 3-HO-TBOEP, BBOEP, BBOEHEP, BCIPP, BCIPHIPP, BCEP, BDCIPP, EHPHP, and 5-HO-EHDPHP, and mass-labeled standards of TCEP-D_{12}, BBOEHEP-D_4, BBOEP-D_4, BCEP-D_8, BDCIPP-D_{10}, and TBOEP-D_6 were synthesized by Dr. Vladimir Belov (Max Planck Institute, Göttingen, Germany). TCEP was obtained from Chiron AS (Trondheim, Norway). DNBP, DPHP, DPHP-D_{10}, and TPHP-D_{15} were supplied by Sigma-Aldrich (Bornem, Belgium). The purities of all target analytes were >98%. Stock solutions of both native and internal standards (IS) were prepared in acetonitrile (ACN) at 2 and 10 ng/μL, respectively. Liquid chromatography-grade (LC-grade) ACN and methanol (MeOH) were obtained from Merck (LiChrosolv, Merk, Darmstadt, Germany). Formic acid (99–100%) and ammonium acetate (NH_4AC) were of LC-grade and purchased from Sigma-Aldrich (Bornem, Belgium). Milli-Q water was generated by a PURELAB Flex system (ρ = 18.2 MΩ/cm, Elga Veolia, Tienen, Belgium). β-Glucuronidase enzyme solution was purchased from Sigma-Aldrich (lyophilized powder from *E.* coli, >10,000,000 unit/g). Solid-phase extraction (SPE) was performed using a Visiprep SPE vacuum manifold with 24 ports (Sigma-Aldrich).

2.2. Study area and sample collection

This study was approved by the Institutional Review Board of Nankai University, Tianjin, China. All participants and their parent/guardians provided informed consent and completed questionnaire surveys with information regarding age, gender, place of residence and so on (Table S2). The sampling locations are shown in Figure S2. First morning void urine samples (total: n = 46) were collected from local residents living in Provinces of Anhui (AH, n = 7), Shandong (SD, n = 7), Hebei (HeB, n = 7), Shanxi (SXJ, n = 10), Liaoning (LN, n = 4), Gansu (GS, n = 2), Henan (HeN, n = 5), and Chongqing municipality (CQ, n = 4) during August – September 2017. Geographically, SD, HeB, SXJ, LN and GS provinces are categorized to the north and AH, HeN and CQ provinces are to the south. All 46 participants were comprised of 23 males and 23 females with the age of donors ranging from 3 to 72 years (yrs) (Table S2).

All participants were healthy without any infectious diseases. None of the female participants were pregnant or menstruating during the sampling period. All collected urine samples were immediately stored in polypropylene (PP) centrifuge tubes, which were precleaned with MeOH. The samples were stored at -20°C until analysis at the Toxicological Center, University of Antwerp, Belgium.

2.3. Sample preparation and instrumental analysis

The established procedure for extraction of OPE diesters and HO-OPEs in urine has been described in detail elsewhere (Bastiaensen et al. 2019). A mixture of labeled IS (5 ng for each IS) was added to 2 mL of urine followed by 1.5 mL of phosphate buffer (1 M, pH 6) and 100 µL of deconjugation enzyme (β-glucuronidase from *E*. coli, 2 mg/mL in phosphate buffer). After gently vortex for 10 s, samples were incubated for 2 h at 37°C and enzymatic deconjugation was terminated by adding 100 µL of formic acid. Prepacked Bond-Elut C18 SPE cartridges (3 mL, 200 mg, Agilent Technologies, Santa Clara, CA, USA) were conditioned using 3 mL of MeOH followed by 2 mL of Milli-Q water. After loading the urine sample, the tubes were rinsed with 1 mL of Milli-Q water, which was then loaded onto the cartridge. After extraction, the cartridges were washed with 1.5 mL Milli-Q water and eluted with 3 mL of MeOH. The eluent was concentrated under a gentle stream of ultrapure nitrogen after addition of 50 µL of Milli-Q water as a keeper solvent. The residues were reconstituted in 100 µL of Milli-Q water: MeOH (50/50 v/v), filtered through a 0.2 µm centrifugal filter membrane (nylon membrane, VWR International, Leuven, Belgium), and transferred to amber glass vials for instrumental analysis.

Instrumental analysis of urine extracts was carried out according to previously reported method (Bastiaensen et al. 2019). Specifically, the separation and quantification of target analytes was performed on an Agilent 1290 infinity liquid chromatography system coupled to an Agilent 6460 triple quadrupole mass spectrometer (LC-MS/MS, Santa Clara, CA, USA) with an electrospray ionization source. A Phenomenex Kinetex biphenyl reversed-phase column (2.1 × 100 mm, 2.6 µm; Torrance, CA, USA) was used for analyte separation at a flow rate of 0.35

184 mL/min and the injection volume was 5 μL. The mobile phases were A: H_2O (2% MeOH and
185 5 mM NH_4AC) and B: MeOH (2% H_2O and 5 mM NH_4AC). Samples were quantified using an
186 8-point calibration curve ranging from 0.04 to 10 ng/mL, except for BCIPP and 4-HO-DPHP,
187 which ranged from 0.2 to 50 ng/mL in neat solvents. Urinary creatinine levels were also
188 measured by LC-MS/MS and the details of the method are described in SI. The details of quality
189 assurance and quality control are also given in SI.
190
191 *2.4. Data analysis*
192 The arithmetic mean, concentration range, and detection frequency (DF) were used to
193 describe the levels of OPE metabolites in urine samples. For some tests, the sum of urinary
194 OPE metabolites was also considered. Molar concentrations (nM) were calculated for ΣOPE
195 diesters (sum of BCIPP, DNBP, DPHP, BDCIPP, BBOEP and EHPHP in urine) and for ΣHO-
196 OPEs (sum of 4-HO-DPHP, BCIPHIPP, BBOEHEP and 5-HO-EHDPHP in urine). Spearman
197 correlation analysis and Mann-Whitney U test were performed using SPSS V19.0. Only the
198 results for compounds with DF >50% were displayed. The levels of OPE metabolites in urine
199 samples were incorporated into correlation analysis with OPEs and their diesters in paired
200 indoor dust samples as reported in another study (Wang et al., 2019). Concentrations below
201 method detection limits (MDLs) were substituted with MDL/√2 for statistical analysis
202 (Hornung and Reed 1990).
203 To evaluate human exposure risks to OPEs, their EDIs were estimated based on urinary
204 concentrations of OPE metabolites and excreted molar fraction (F_{UE}) of the metabolite with
205 respect to its parent compound (Lynn et al. 1981, Suzuki et al. 1984, Van den Eede et al. 2013)
206 (Table S3). Although urinary excretion rates of DNBP and BDCIPP metabolites in orally
207 exposed rats have been reported (Lynn et al. 1981, Suzuki et al. 1984), data on the kinetics or
208 metabolism of OPEs in humans are still very limited and only in vitro results are available (Van
209 den Eede et al. 2013). The equation used for the calculation of EDIs was previously employed
210 in other studies (Chen et al. 2018, Fromme et al. 2014, Hoffman et al. 2017b, Zhang et al. 2018b)
211 and is shown in Eq 1:

$$\mathrm{EDI} = \left(\frac{C_m \times V_{urine}}{F_{UE} \times m}\right) \times \frac{MW_p}{MW_m} \quad (1)$$

where EDI (ng/kg·bw) represents the estimated total daily intake of individual OPE metabolites; C_m (ng/mL) is the urinary concentration of OPE metabolites; V_{urine} (mL/day) is the reference values for daily urinary excretion (Zhang et al. 2018b) (Table S4, V_{urine} data from the International Commission on Radiological Protection. Basic Anatomical and Physiological Data for Use in Radiological Protection: Reference Values); F_{UE} is the excreted urinary molar fraction of metabolite with respect to its parent compound. Due to limited data available from both in vitro and in vivo models and lack of experimental data on human subjects, reasonable F_{UE} values of 0.1 and 0.9 were assumed for high and low exposure scenarios, respectively, and thus a range of EDI values were calculated for TCIPP, TPHP, TDCIPP, TBOEP, and EHDPHP, whose metabolites were detected at frequencies >50% ; m (kg) is the body weight; MW_p and MW_m are the molecular weights of the parent OPE and its metabolite (g/mol), respectively (Table S3).

The hazard quotients (HQs) were defined as the ratio of EDIs of OPEs to the corresponding RfDs. The RfD values for TCIPP, TPHP, TDCIPP, and TBOEP (DFs of metabolites >50%) were 10000, 70000, 20000, and 15000 ng/kg bw/day, respectively. Although these baseline data can introduce some uncertainties, the primary estimates may reveal the potential risks of OPEs under current exposure scenarios. The equation used to estimate exposure risks of OPEs is shown in eq 2 (Wang and Kannan 2018):

$$\mathrm{HQ} = \frac{EDI}{RfD} \quad (2)$$

where RfD is the reference dose of OPEs (ng/kg bw/day), proposed by the US EPA and described in a previous study (Li et al. 2018). The risk potential is considered to be significant when the HQ value is ≥1. The hazard index (HI) was calculated by summing up HQs of OPEs in concern. If HI < 1, no immediate risk is considered from OPE exposure. If HI > 1, a significant risk is of concern for OPE exposure.

3. Results and discussion

3.1.Concentrations of OPE metabolites in urine samples

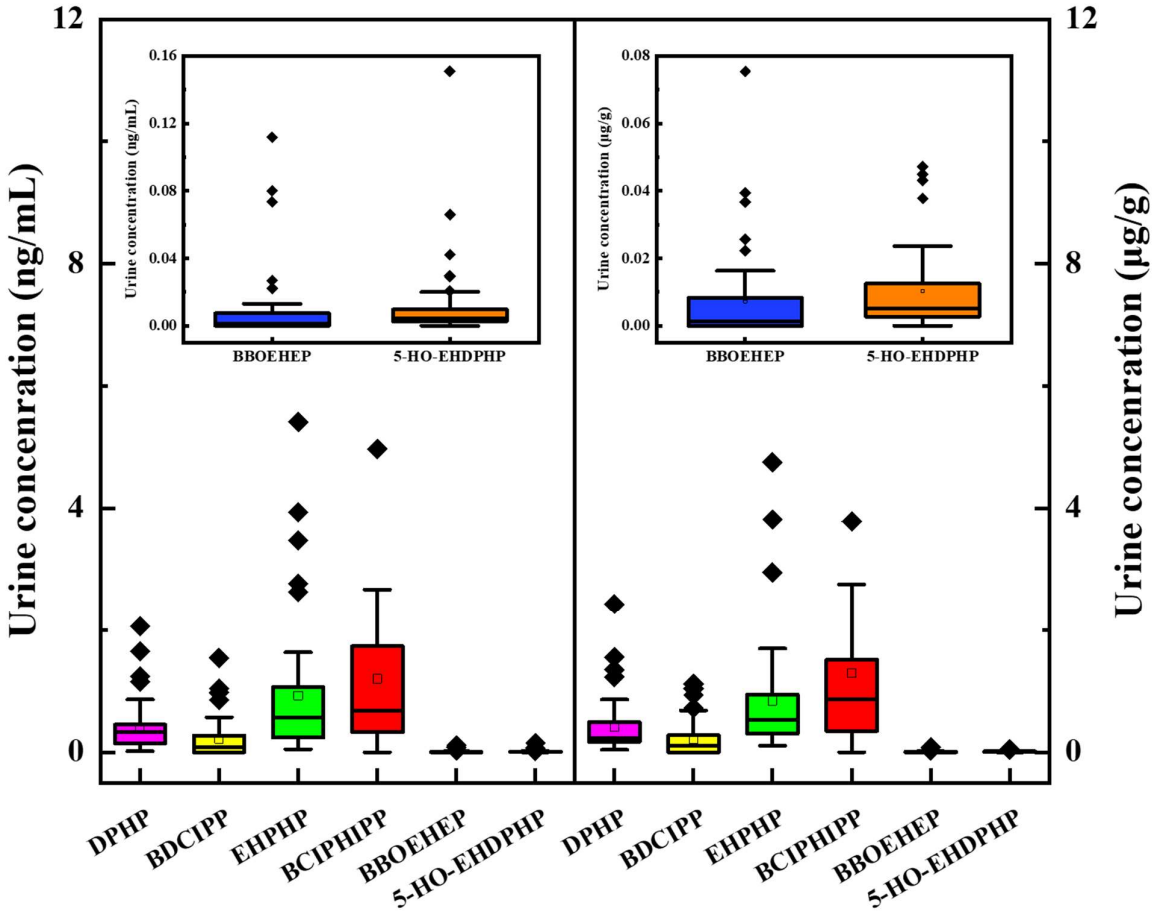


Fig. 1 The unadjusted and creatinine-adjusted levels of OPE metabolites (DF > 50%) in urine samples (n = 46) from 8 provinces of China.

Among ten OPE metabolites, DPHP (100%), EHPHP (100%), BCIPHIPP (89.1%), 5-HO-EHDPHP (82.6%), BDCIPP (63%), and BBOEHEP (54.3%) were detected at frequencies >50% in urine samples (n = 46) collected from 8 provinces of China and their unadjusted and creatinine-adjusted concentrations are shown in Fig. 1. The DFs were 30.4% for 4-HO-DPHP and below 10% for DBP, BCIPP, and BBOEP, while BCEP, HO-TBOEP, and 4-HO-TPHP were not detected (Table S5). In terms of median levels, BCIPHIPP was detected at 0.68 ng/mL, followed by EHPHP at 0.57 ng/mL, DPHP at 0.33 ng/mL and BDCIPP at 0.08 ng/mL. BBOEHEP and 5-HO-EHDPHP were consistently at levels < 0.2 ng/mL. As a parent compound, TCEP was also occasionally detected (26.1%) at a maximum level of 6.94 ng/mL indicating its immediate excretion before being metabolized. In vitro liver metabolism studies suggested a

low hepatic clearance of TCEP due to its high water solubility, thus a high renal clearance as compared to other parent OPEs (Dodson et al. 2014, Van den Eede et al. 2013).

EHPHP was detected in all samples at 0.05 – 5.41 ng/mL, while 5-HO-EHDPHP was detected in 82.6% of the urine samples but with a lower level of n.d. – 0.15 ng/mL. Their parent compound EHDPHP is applied in food packaging and prevalently detected in food samples, which may account for the substantial internal exposure (Poma et al. 2017, Xu et al. 2017). Two phenyl substituent groups and one long-chain alkyl group render EHDPHP a low solubility. The ubiquitous occurrence of 5-HO-EHDPHP indicated that hydroxylation was a common reaction in human metabolism of EHDPHP besides hydrolysis that produces EHPHP or DPHP.

DPHP was detected in all samples at levels of 0.02 – 2.06 ng/mL (mean: 0.4 ng/mL). 4-HO-DPHP was detected at a lower frequency of 30.4%, but with a higher maximum concentration of 8.30 ng/mL. It should be noted that DPHP is a non-specific biomarker for TPHP exposure because it can be derived from multiple parent OPEs, including resorcinol bis-diphenyl phosphate (RDP), and EHDPHP (Ballesteros-Gomez et al. 2015, Dodson et al. 2014) as well as metabolized from several other aryl-OPEs (Butt et al. 2014, Phillips et al. 2018, van der Veen and de Boer 2012, Wang et al. 2019). Meanwhile, DPHP is directly used as a plasticizer, although with significantly lower production than that of TPHP (Carignan et al. 2017, Makiguchi et al. 2011). This raises concerns for the direct exposure to DPHP as they are recently found ubiquitous in food (He et al. 2018b) and indoor dust (Tan et al. 2019). Therefore, HO-TPHPs are likely specific biomarkers of TPHP exposure, but HO-TPHPs were hardly detectable in this study and others (Ait Bamai et al. 2019, Bastiaensen et al. 2019). This brings a higher uncertainty to evaluate TPHP exposure based on urinary DPHP. Biomarkers other than DPHP are yet to be validated in human biomonitoring.

For the three metabolites of TBOEP, BBOEP was detected in only one sample at 0.47 ng/mL and 3-HO-TBOEP was not detected, while BBOEHEP was detected with frequencies over 50% at low concentrations (<0.11 ng/mL). In another study, although BBOEP was detected at higher frequencies of 79% than corresponding hydroxylated metabolites in urine samples from Japanese children, the mean concentrations of BBOEHEP were found comparatively higher

than those of BBOEP (0.64 ng/mL vs. 0.34 ng/mL) (Bastiaensen et al. 2019). This indicated a prevalence of HO-OPEs potentially for possible phase II reactions. In median molar concentration, ΣHO-OPEs (4.65 nM, range: 0.02 to 33.2 nM) was even higher than ΣOPE diesters (3.84 nM, range: 0.42 to 21.7 nM) (Table S5).

Most of the OPE metabolites were detected at higher levels in the provinces from the north. Specifically, the urinary levels of DPHP ($p < 0.01$), 5-HO-EHDPHP ($p < 0.01$) and ∑OPE diesters ($p < 0.05$) from the north were significantly higher than those from the south (Table S6). The significance remains for the creatinine-adjusted data, and BDCIPP showed significantly higher levels in the north after the adjustment ($p < 0.05$). Interestingly, the investigated people from the north were found with a significantly lower annual income (<80,000 RMB, $p < 0.01$) and longer indoor residential time (>12 h, $p < 0.05$). This suggested that the indoor environment is a major exposure source of OPEs and the residential time can be an effective predictor on a population level.

3.2. Correlation analysis

As shown in Table S7, the urinary OPE metabolites were found frequently correlated with each other, which was consistent with the results in Japanese urine samples (Ait Bamai et al. 2019, Bastiaensen et al. 2019). DPHP, EHPHP, and 5-HO-EHDPHP were strongly correlated (r=0.566 – 0.650, $p < 0.01$) as they may share common sources. BBOEHEP was also strongly correlated with DPHP and EHPHP (r=0.587 – 0.652, $p < 0.01$). BCIPHIPP was weakly correlated with EHPHP and BBOEHEP (r=0.375 – 0.442, $p < 0.05$). As a whole, ∑OPE diesters was strongly correlated with BBOEHEP and 5-HO-EHDPHP (r=0.626 – 0.688, $p < 0.01$). These results suggested that hydroxylated OPE metabolites were generally interrelated with their OPE diesters. Although DPHP can be derived from multiple chemicals, its strong correlations with EHPHP and 5-HO-EHDPHP may indicate a substantial contribution of EHDPHP. EHDPHP has been frequently found as a dominant OPE in foodstuffs from China and Europe, and it was particularly enriched in proceeded food such as cereals, eggs, and sweets, which can lead to a substantial diet exposure (Poma et al. 2018, Zhao et al. 2019b). EHDPHP

was also frequently found abundant in building and decoration materials from China (Wang et al. 2017). Consequently, the indoor dust/air exposure of EHDPHP may collaboratively add to internal levels of DPHP in urine.

Table 1 Spearman's correlation coefficient between molar concentrations of OPE metabolites in urine (creatinine-adjusted, nmol/g) and OPE triesters and diesters in paired indoor dust (ng/g, n = 44). Only compounds with DF > 50% were used. Data were ln-transformed before statistical analysis. The detail data of indoor dust samples are available in Table S8 (Wang et al., 2019).

	OPE triesters and diesters in indoor dust															
		TNBP	TCIPP	TPHP	TBOEP	TDCIPP	TCEP	EHDPHP	DNBP	BCIPP	DPHP	BBOEP	BDCIPP	BCEP	∑tri-OPEs[a]	∑di-OPEs[b]	∑OPEs[c]
OPE metabolites in urine	DPHP	0.117	0.129	0.045	-0.248	0.080	0.008	0.100	-0.025	-0.235	0.096	-0.270	0.063	0.040	0.127	0.058	0.114
	BDCIPP	0.306	-0.114	0.088	0.003	0.446*	0.271	0.262	0.221	0.172	0.013	-0.050	0.379*	0.503**	0.200	0.165	0.200
	EHPHP	0.155	-0.153	0.018	0.099	0.098	-0.079	0.052	0.085	-0.133	-0.043	-0.214	0.057	0.063	0.002	-0.014	-0.019
	BCIPHIPP	-0.062	0.413**	-0.117	-0.067	0.071	0.007	-0.148	0.149	-0.065	-0.001	0.126	-0.276	0.077	0.173	0.024	0.170
	BBOEHEP	0.057	-0.103	-0.368	0.286	0.267	-0.342	-0.330	-0.238	-0.184	-0.372	0.081	-0.213	-0.303	-0.245	-0.447*	-0.287
	5-HO-EHDPHP	-0.087	0.019	0.060	-0.003	0.028	-0.063	0.307	-0.042	-0.002	0.021	0.119	-0.042	-0.187	0.016	-0.082	0.009
	∑di-OPEs	0.144	-0.036	-0.053	-0.024	0.266	-0.043	0.133	0.030	-0.134	-0.073	-0.186	0.097	0.170	0.037	-0.052	0.013
	∑HO-OPEs	-0.080	0.495**	-0.130	-0.083	-0.033	-0.014	-0.215	0.104	0.029	-0.004	0.232	-0.334*	0.026	0.117	0.034	0.121
	∑OPE metabolites[d]	0.023	0.255	-0.111	-0.138	0.108	-0.077	-0.017	0.092	-0.165	-0.002	0.000	-0.083	0.154	0.115	0.043	0.098

[a]∑tri-OPEs: the sum of molar concentrations of OPE triesters.

[b]∑di-OPEs: the sum of molar concentrations of OPE diesters.

[c]∑OPEs: the sum of molar concentrations of tri-OPEs and di-OPEs.

[d]∑OPE metabolites: The sum of molar concentrations of di-OPEs and HO-OPEs.

**signifies the level of 0.01 (two-tailed).

*signifies the level of 0.05 (two-tailed).

As shown in Table 1, a significant correlation was found between dust TCIPP and urinary BCIPHIPP (r=0.413, $p < 0.01$), which were also found moderately correlated using the paired data from Japan (Bastiaensen et al. 2019). In this study, dust TDCIPP and urinary BDCIPP were also significantly correlated (r=0.446, $p < 0.05$), while no other urinary metabolites were found correlated with their parent compounds. It is noting that weak or insignificant correlations were commonly found between dust OPE triesters and urinary OPE metabolites, however the direct exposure to OPE diesters in house dust was not considered (Bastiaensen et al. 2019, Castorina et al. 2017, Cequier et al. 2015, Hoffman et al. 2015, Meeker et al. 2013, Phillips et al. 2018). In fact, OPE diesters were recently found ubiquitously occurring in indoor dust (Tan et al. 2019). With the paired data, BDCIPP was found moderately correlated with itself in dust (r=0.379, $p < 0.05$) as well as more significantly with dust BCEP (r=0.503, $p < 0.01$) (Table 1). Although no other dust OPE diesters were correlated urinary OPE metabolite, this strongly suggested that the direct exposure to dust OPE diesters may contribute to their internal levels. Indirect photolysis of OPEs initiated by sunlight radiation was observed in natural surface water, where TBOEP and TPHP were the most actively degraded (Cristale et al. 2017, Regnery and Püttmann 2010). Therefore, OPE diesters in the environment are likely derived from triesters via abiotic processes apart from direct application and impurity in products. It is also conceivable that heterogeneous photolysis of OPEs in indoor and outdoor dust can possibly be a source to OPE diesters. Together with diet exposure, it raises increasing concerns that the exposure to OPE diesters directly may add up to the exposure risk of their parent compounds, which merits further study.

3.3. Exposure assessment

3.3.1. Risk assessment

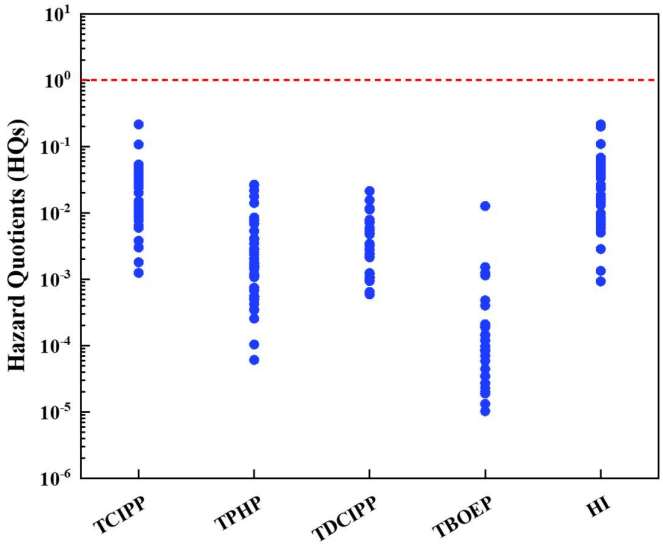


Fig. 2. Hazard quotients (HQs) calculated for four OPEs in the high exposure scenario (F_{UE} = 0.1, n = 46). The dashed line represents the HQ of 1. Hazard index (HI) is the summation of HQs of four OPEs.

The median EDIs for EHDPHP, TPHP, and TCIPP were 273 ng/kg·bw (range: 31.7 – 1750 ng/kg·bw), 199 ng/kg·bw (range: 17.9 – 1870 ng/kg·bw) and 146 ng/kg·bw (range: 2.76 – 2150 ng/kg·bw) in the high exposure scenario (F_{UE} = 0.1), respectively. These values were significantly higher than those of TDCIPP (21.5 ng/kg·bw, range: 6.12 – 427 ng/kg·bw) and TBOEP (0.52 ng/kg·bw, range: 0.38 – 191 ng/kg·bw) (Table S9). Nevertheless, as shown in Fig. 2, TCIPP shows the highest HQ values up to 0.215 and HQ values of TDCIPP were up to 0.021. Although lower in median EDI, Cl-OPEs may have a higher risk potential to induce adverse health effects. Cl-OPEs are suspected carcinogens with observed tumor growth not only in kidney, liver and thyroid for TCEP and TCIPP, but also in brains and testes for TDCIPP (van der Veen and de Boer 2012, Wei et al. 2015). Both TCIPP and TDCIPP showed a concentration dependent neurotoxicity (Dishaw et al. 2011). TDCIPP displayed pregnane X receptor agonistic activity (Kojima et al. 2013) and may be associated with altered hormone levels and decreased sperm concentration (Meeker and Stapleton 2010). In addition, TPHP

(median: 0.003) shows comparatively lower HQ values than Cl-OPEs, but significantly higher than TBOEP (median: 3.45×10^{-5}) (Mann-Whitney, $p < 0.01$) (Fig. 2). The ubiquitous detection of DPHP indicated a co-exposure to EHDPHP, TPHP, and other parent OPEs of DPHP. This may bring additional risks to current estimation. For instance, the EDIs of EHDPHP were estimated up to 1750 ng/kg·bw ($F_{UE} = 0.1$, Table S9), but was not taken into evaluation due to a lack of RfD values. Apart from this, the highest HI of TCIPP, TPHP, TDCIPP, and TBOEP was 0.216 ($F_{UE} = 0.1$), which indicated no immediate cumulative adverse effects.

3.3.2. Literature comparison

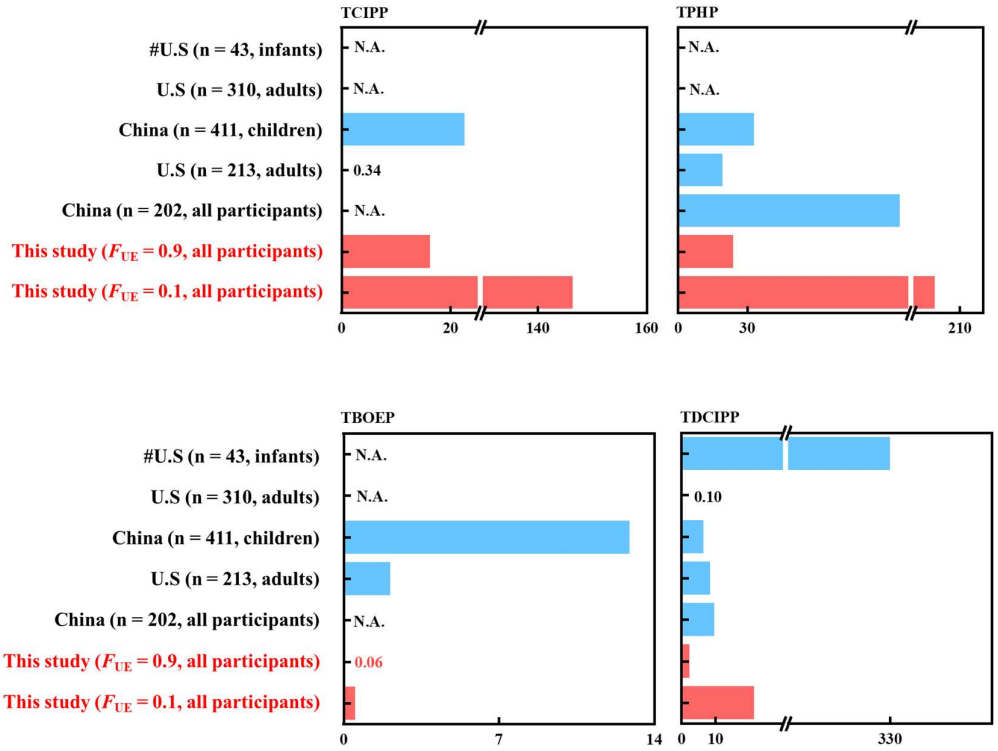


Fig. 3. Comparison of median EDIs (ng/kg·bw) of OPEs (DF > 50%) derived from urinary levels with those reported in various countries worldwide. "N.A." represents not analyzed; "#" represents GM values; EDIs estimated from F_{UE} values of 0.9 and 0.1 were displayed in this study (both adults and children, n = 46); other data were cited from previous studies (Castorina et al. 2017, Chen et al. 2018, Hoffman et al. 2017b, Wang et al. 2019, Zhang et al. 2018b).

Only a few studies were available to report EDIs derived from internal exposure as summarized in Fig. 3. In comparison, the EDIs of TCIPP in this study were comparable to those reported on children urines from South China (22.6 ng/kg·bw) (Chen et al. 2018) and much higher than those on adult urines from New York, the United States (0.34 ng/kg·bw) (Wang et al. 2019). The median EDI of TPHP was up to 199 ng/kg·bw in the high exposure scenario (F_{UE} = 0.1) while the low-exposure-scenario median EDI was 23.9 ng/kg·bw, which was comparable to those from South China (33.1 – 96.4 ng/kg·bw) (Chen et al. 2018, Zhang et al. 2018b) and the United States (19.4 ng/kg·bw) (Wang et al. 2019). The median EDI of TBOEP was 0.52 ng/kg·bw at most (F_{UE} = 0.1), which was lower than those from the United States (2.10 ng/kg·bw) (Wang et al. 2019) and considerably lower than those from South China (12.9 ng/kg·bw) (Chen et al. 2018). The median EDIs of TDCIPP was 2.39 – 21.5 ng/kg·bw, which were substantially lower than those reported on infant urines from the United States (330 ng/kg·bw) (Hoffman et al. 2017b). In general, the exposure risk to TCIPP is typically high in China. As compared to adults, children and infants are under high exposure risk to OPEs. In addition, the median EDIs of EHDPHP were calculated with urinary EHPHP, DPHP, and 5-HO-EHDPHP in this study (30.3 – 273 ng/kg·bw, Table S9). As DPHP was more often assumed to completely metabolized from TPHP in such estimation, no other study has reported the EDI of EHDPHP based on urinary estimation. This can be critical in risk assessment since the external exposure to EHDPHP was frequently found at high levels.

3.3.3. Contribution of dust exposure to OPEs and OPE diesters

Table 2 Comparison between EDI of OPEs in indoor dust with EDI calculated from metabolites in urine by a conservative estimation (only adults, n = 40, F_{UE} = 0.9). In indoor dust estimation, the median concentrations for an average exposure scenario and the 95th percentile for a high exposure scenario were considered in this calculation. In urinary estimation, the median concentrations of OPE diesters and HO-OPEs for an average exposure scenario.

EDI (pmol/kg·bw) [e]			EHDPHP	TCIPP	TDCIPP	TPHP	TBOEP	ΣOPEs
Indoor dust estimation (Wang et al., 2019)	OPEs	Average	0.025	0.492	0.065	0.064	0.018	0.663
		High	0.243	3.33	0.485	0.766	0.723	5.54
				BCIPP	BDCIPP	DPHP	BBOEP	ΣOPE diesters
	OPE diesters	Average		3.16E-04	3.66E-05	4.40E-02	1.23E-03	0.046
		High		0.044	0.016	0.464	0.040	0.563
	OPEs + OPE diesters	Average		0.492	0.065	0.108	0.019	0.709
		High		3.37	0.501	1.23	0.763	6.11
Urinary estimation (this study)	OPE diesters+HO-OPEs	Average	69.6	49.7	5.10	37.9	0.08	162
Dust to urine Ratio (%)	OPEs	Average[a]	0.04%	0.99%	1.27%	0.17%	22.5%	0.41%
		High[b]	0.35%	6.70%	9.51%	2.02%	904%	3.42%
	OPEs + OPE diesters	Average[c]	-	0.99%	1.27%	0.28%	23.8%	0.44%
		High[d]	-	6.78%	9.82%	3.25%	954%	3.77%

[a]Ratio of the EDI_{dust} of OPEs to the EDI_{urine} of OPE diesters and HO-OPEs in an average exposure scenario.

[b]Ratio of the EDI_{dust} of OPEs in a high exposure scenario to the EDI_{urine} of OPE diesters and HO-OPEs in an average exposure scenario.

[c] Ratio of the EDI_{dust} of OPEs and OPE diesters to the EDI_{urine} of OPE diesters and HO-OPEs in an average exposure scenario.

[d] Ratio of the EDI_{dust} of OPEs and OPE diesters in a high exposure scenario to the EDI_{urine} of OPE diesters and HO-OPEs in an average exposure scenario.

[e] The EDI_{dust} of the OPEs and OPE diesters and the EDI_{urine} of OPE diesters and HO-OPEs are calculated by the molar concentration.

In comparison, EDI_{urine} values derived from urinary levels in this study were generally higher than EDI_{dust} in the average-scenario dust exposure and EDI_{dust} only accounted for <22.5% of EDI_{urine}. In the high-scenario dust exposure, the ratios of EDI_{dust} to EDI_{urine} remain <9.51% for most OPEs, however the ratios of TBOEP were up to 904%, which suggests that indoor dust is a major exposure route of TBOEP for adults.

Assuming a 100% excretion of OPE diesters, the contribution of direct exposure to OPE diesters was estimated. Taking in to account the EDI_{dust} of OPE diesters, the ratios increased to <9.82% and <23.8% in the high and average scenarios, respectively. These results indicated that a significant contribution of OPE diesters from other exposure sources may as well occur, such as drinking water and food consumption. The EDIs of BDCIPP and DPHP from diet were found to be higher than those of their parent OPEs, as OPE diesters occurred in a variety of foods from Australia (He et al. 2018c). Furthermore, several studies found OPEs in foods and drinking water in China and calculated the EDI of OPEs via these two pathways (Ding et al. 2018, Ding et al. 2015, Li et al. 2014, Zhang et al. 2016). Nine OPEs have been investigated in five types of drinking water from Eastern China and the EDI of OPEs ranged from 0.14 to 7.07 ng/kg·bw (Ding et al. 2015). These values accounted for <4.36% of the EDI_{urine} (F_{UE} = 0.9) in this study (Table 2). TPHP and TCIPP were the most commonly found components in tap water (Li et al. 2014), which is in accordance with our results that EDI_{TPHP} and EDI_{TCIPP} contributed predominantly to the total daily intake of OPEs (the sum of EDIs). In addition, the EDIs of ten OPEs were estimated at 55.0 ng/kg·bw for dietary exposure to various food matrices from a city in Eastern China including chicken, pork, fishes, vegetables, tofu, eggs, milk and cereals (Ding et al. 2018). This value accounted for 34.0% of the EDI_{urine} (F_{UE} = 0.9) in this study and were higher than exposure via drinking water. Cereals were identified as the major contributor accounting for 26% of the total OPEs among different types of foodstuffs (Ding et al. 2018). Rice ingestion was considered a potential major pathway for human exposure to OPEs in China and the mean EDI of OPEs via rice were proposed to be 539 – 601 ng/kg·bw (Zhang et al. 2016), which were much higher than the EDI_{urine} (F_{UE} = 0.9) in this study. Comparatively, the EDIs of OPEs from indoor dust ingestion (Wang et al., 2019) and drinking water (Ding et al. 2015, Li et al. 2014) were lower than that EDIs from dietary intake (Ding et al. 2018, Zhang et al. 2016). The results suggested that dietary exposure can possibly explain the deficits occurring between EDI_{urine} and EDI_{dust} of most OPEs in this study, while dust TBOEP exposure can be a significant source. Additionally, the direct exposure to OPE diesters is of great importance to be accounted for by further field studies.

449

450 *3.3.4. Significance of HO-OPEs*

451 The molar contributions of HO-OPEs for OPE metabolites exposure estimation are displayed
452 in Figure S3. 4-HO-DPHP contributed 36.6% – 97.0% to the metabolites of TPHP and
453 EHDPHP in 30% of the urine samples and 5-HO-EHDPHP contributed 0.06% – 1.91% in all
454 the samples. For metabolites of TCIPP and TBOEP, BCIPHIPP, and BBOEHEP contributed
455 100% in most samples. The molar concentrations of HO-OPEs in OPEs metabolites (F_{UE} = 0.9)
456 categorized by study population characteristics was summarized in Table S10. However, the
457 proportion of 5-HO-EHDPHP in metabolites of EHDPHP was detected higher in smokers than
458 non-smokers (mean: 1.07% vs. 0.53%, $p < 0.05$). It was also found that drinkers had higher
459 proportions of 4-HO-DPHP in metabolites of TPHP than non-drinkers (mean: 40.1% vs. 14.9%,
460 $p < 0.05$). Nevertheless, no significant difference was observed between other stratified groups,
461 such as BMI and gender etc. Alcohol consumption may significantly enhanced the microsomal
462 hydroxylation of fatty acid and thus alter hepatic function for metabolic processes (Ma et al.
463 1993). The metabolism of nicotine also involves hydroxylation of cotinine, which followed by
464 phase II metabolism (Tricker 2003). Therefore, alcohol intake and smoking may inductively
465 enhance hepatic hydroxylase, which possibly results in more hydroxylated forms of xenobiotics
466 like OPEs. These results suggest that physical state and lifestyle may influence metabolic
467 functions in relations with different enzymes for dealkylation or hydroxylation, and that the
468 internal exposure ratios of HO-OPEs to OPE diesters may provide a significant indicator for
469 human biomonitoring and epidemiological studies. This further emphasizes the need to
470 simultaneously determine OPE diesters and HO-OPEs when evaluating the exposure levels of
471 OPEs.

472

473 *3.4. Limitations*

474 The present study has a few limitations. The size of the paired dataset were still relatively
475 small and the DFs were <50% for some OPE metabolites in concerns, which restrained the
476 statistical power for exploring the associations between OPE exposure and lifestyle parameters.

Therefore, the results needed to be treated and extrapolated with caution. Nevertheless, the data emphasized on an extended exposure profiles in Chinese population and further studies on a larger scale are warranted.

4. Conclusions

In summary, this is the first study to report four urinary HO-OPEs levels in Chinese residents. Our results demonstrated that HO-OPEs were the predominant metabolites in urine samples from a general population. The high DF of OPE metabolites in urine suggest that the investigated Chinese population is widely exposed to OPEs across different sampling locations. The results indicated that dust ingestion is a major exposure route of TBOEP while dietary and drinking water intake may account for a large portion of the internal levels of OPE metabolites. The direct contribution of OPE diesters to human exposure is of great importance to be accounted for by further field studies. The ratios of HO-OPEs to OPE metabolites were found associated with population behaviors thus may be a significant indicator for future human biomonitoring and epidemiological studies. These results emphasized on the necessity of simultaneous determination of HO-OPEs and OPE diesters when evaluating human exposure risks to OPEs, although the HQs suggested that no immediate exposure risk occurred for the investigated Chinese residents but their long-term chronic effects remain worth further concerns.

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