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Organophosphate flame retardants and diesters in the urine of e-waste dismantling workers: associations with indoor dust and implications for urinary biomonitoring†

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Indoor dust ingestion is one of the main pathways for human exposure to organophosphate flame retardants (PFRs). The urinary concentrations of diesters (DAPs) are usually used as biomarkers to assess human exposure to PFRs. In this study, the PFR and DAP levels were measured in morning and evening urine samples of 30 workers from an e-waste dismantling site in southern China. The indoor dust samples were also collected from workshops and houses for analyzing associations between PFR and DAP levels in urine and dust. Tris(1-chloro-2-propyl) phosphate (TCIPP) and triphenyl phosphate (TPHP) were the dominant PFRs in dust, while bis(2-chloroethyl) phosphate (BCEP) and diphenyl phosphate (DPHP) were the major DAPs in dust. A significant positive correlation was observed between TPHP and DPHP concentrations in dust ($p < 0.001$), suggesting their potentially same source and the degradation of TPHP to form DPHP. TCIPP and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) were the predominant PFRs, and BCEP, bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), and DPHP were the main DAPs in both the morning and evening urine samples. The DPHP levels in evening urine samples were significantly correlated with TPHP and DPHP levels ($p < 0.01$) in dust. A similar correlation was found for the BCEP levels in the evening urine samples and the TCEP and BCEP levels ($p < 0.01$) in dust. These results indicated that in addition to being biotransformed from their respective parent PFRs, direct ingestion from indoor dust could also be the potential source for urinary DPHP and BCEP. Since relatively low detection frequencies were observed for bis(1-chloro-2-propyl) phosphate (BCIPP) and bis(butoxyethyl) phosphate (BBOEP) in urine, they may not be the major metabolites of TCIPP and tris(2-butoxyethyl) phosphate (TBOEP), respectively, in the human body. However, BDCIPP can be considered a useful biomarker because it is a unique metabolite of TDCIPP and has high detection frequencies in urine samples. The results of this study indicated the limitations of solely using urinary DAPs as biomarkers for the evaluation of human exposure to PFRs, and certain PFRs as well as hydroxylated PFRs (OH-PFRs) should also be considered for urinary biomonitoring in future studies.

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Environmental significance

In this study, organophosphate flame retardant (PFR) and diester (DAP) levels were measured in morning and evening urine samples of 30 workers from an e-waste dismantling site in southern China. The indoor dust samples were also collected from workshops and houses for analyzing associations between PFR and DAP levels in urine and dust. The results indicated the limitations of solely using urinary DAPs as biomarkers for the evaluation of human exposure to PFRs, and certain PFRs as well as hydroxylated PFRs (OH-PFRs) should also be considered for urinary biomonitoring in future studies.

1. Introduction

Flame retardants (FRs) are frequently used as additives in various commercial products such as furniture, textiles, and electronic devices to reduce their flammability and to meet fire safety standards.[1] With global restrictions and the phase-out of brominated FRs such as polybrominated diphenyl esters due to their persistence, bioaccumulation, and toxicity, the production

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and usage of organophosphate FRs (PFRs) as a primary alternative have increased significantly in recent years.[2,3] Besides, some non-chlorinated PFRs are also used as plasticizers, floor polishes, and in engine oils.[2,3] Since PFRs are used as additives instead of being chemically bonded to carrier materials, they are readily released into the ambient environment during production, use, and disposal of commercial products.[2–4] As a result, PFRs have been demonstrated to be ubiquitous in the environment.[3] Additionally, some PFRs, such as tris(2-chloroethyl) phosphate (TCEP), triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), and tris(2-chloroisopropyl) phosphate (TCIPP), have been reported to show different toxic effects, including endocrine disruption, neurotoxicity, carcinogenicity, and reproductive toxicity,[2,3,5] which has raised increasing concerns regarding human health risks due to PFR exposure.

PFRs are not persistent chemicals, though sometimes also considered as pseudo-persistent chemicals due to their ubiquitous presence in the environments, and could be efficiently biotransformed to their diesters (dialkyl- and diaryl-phosphates, DAPs), and/or other metabolites in the human body, and the PFR half-lives in human blood were reported to be on the order of hours.[6] Therefore, urinary DAPs have been considered useful biomarkers to evaluate human exposure to PFRs in previous studies.[7–11] Moreover, some DAPs have been demonstrated to be more toxic than their parent PFRs.[12]

Indoor dust ingestion is one of the main pathways of human exposure to PFRs, and numerous studies have reported high PFR levels in indoor dust worldwide.[3,13–15] Furthermore, significant associations have been observed between the PFR levels (*e.g.*, TPHP, TCIPP, and TDCIPP) in indoor dust and the levels of their respective DAPs in human urine,[16–18] suggesting vital contributions of external indoor exposure to the overall human exposure to PFRs. However, several recent studies have reported the wide co-occurrence of PFRs and their DAPs in indoor dust collected in China and the Midwestern USA,[14,19,20] indicating that DAPs are not only human PFR metabolites but can be part of the PFR contaminant mixture in indoor environments. Diphenyl phosphate (DPHP) was also frequently detected in indoor dust samples collected in Spain and the Netherlands and was significantly positively correlated with TPHP in dust.[21] The DAPs in the environmental matrix are likely to be present in PFR mixtures and consumer products as impurities or degradation products of parent PFRs in the environment.[21] Certain DAPs such as DPHP, bis(2-ethylhexyl) phosphate (BEHP), and dibutyl phosphate (DBP) are also directly used for commercial applications.[22] Consequently, the co-existence of the PFRs and DAPs found in dust has raised concerns about the reliability of using urinary DAPs as biomarkers for the assessment of human exposure to PFRs.[21,23] Additionally, a DAP in urine could be biotransformed from several possible parent PFRs in the human body. For instance, DPHP is the main metabolite of TPHP; however, other PFRs such as 2-ethylhexyl diphenyl phosphate (EHDPHP), bisphenol A bis(diphenyl phosphate) (BDP), and resorcinol bis(diphenyl phosphate) (RDP) can also be biotransformed to form DPHP.[21] Therefore, simultaneous monitoring of PFRs and DAPs in both human urine and the indoor dust from living and/or workplace environments can provide valuable information about PFR accumulation and biotransformation in the human body, as well as a more accurate assessment of human exposure to PFRs by using the appropriate urinary biomarkers.

High PFR concentrations have been reported in indoor dust collected from workshops and houses in e-waste recycling areas in South China,[13,24] where e-waste is manually dismantled by family-run workshops located in the backyards of the workers' homes. This results in heavy occupational exposure to pollutants including PFRs through dust ingestion, dermal contact, air inhalation, *etc.* High concentrations of DAPs, especially BCEP and DBP, were observed in the urine samples of the e-waste dismantling workers from these areas in a previous study.[25] However, the association between the PFR and DAP levels in the urine of the e-waste dismantling workers and those in the corresponding indoor dust samples remains unclear.

In the present study, morning and evening urine samples of e-waste dismantling workers and corresponding dust samples from their indoor workshops and houses were collected from an e-waste site in South China. The PFR and DAP levels in both the urine and dust samples were analyzed. The primary objectives of this study were (i) to investigate the potential associations between PFR and DAP concentrations in urine and the corresponding indoor dust samples and (ii) to evaluate the suitability of utilizing urinary DAPs as biomarkers for the assessment of human exposure to PFRs.

2 Materials and methods

2.1 Chemicals and materials

Standards of eight PFRs (TPHP, tris-(2-ethylhexyl) phosphate (TEHP), tributyl phosphate (TNBP), TCEP, tris(2-butoxyethyl) phosphate (TBOEP), TDCIPP, EHDPHP, and TCIPP) were purchased from AccuStandard, Inc. (New Haven, CT, USA). Five internal standards (ISs), d_{15}-TPHP, d_{27}-TNBP, d_{12}-TCEP, d_{18}-TCIPP, and d_{15}-TDCIPP, were obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). The DAP standards bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), DPHP, bis(butoxyethyl) phosphate (BBOEP), bis(1-chloro-2-propyl) phosphate (BCIPP), and bis(2-chloroethyl) phosphate (BCEP) and the ISs for the DAPs (d_{10}-DPHP, d_8-BBOEP, d_8-BCEP, d_{18}-DBP, d_{12}-BCIPP and d_{10}-BDCIPP) were purchased from Toronto Research Chemicals (North York, ON, Canada). The DBP standard was obtained from the Max Planck Institute for Biophysical Chemistry (Göttingen, Germany). Additional information on PFR and DAP standards is provided in Table S1 in the ESI,† and detailed descriptions of the chemicals and materials used in this study are reported in the ESI† as well.

2.2 Sample collection

Urine samples were collected from 30 full-time e-waste dismantling workers (including 16 males and 14 females; Table S2†) living in a village located in South China in September 2018. Detailed information on the e-waste recycling area has been provided in previous studies.[25,26] The experiments

of this study were performed in compliance with "The Regulations of Ethical Reviews of Biomedical Research Involving Human Subjects" issued by the National Health and Family Planning Commission of the People's Republic of China. This work has received approval for research ethics from the Ethics Committee of the School of Life Science, Sun Yat-sen University. Informed consent was obtained from the participants after they were clearly informed of the study's objectives, and then a short questionnaire was administered to obtain general information about participants, including age, gender, body mass index (BMI), and occupational history (Table S2†). The participants' ages ranged between 18 and 72, and their occupational durations ranged between 6.5 and 20 years.

Fresh urine samples were collected using quality certified 50 mL brown glass containers. Morning urine samples were collected at approximately 7:00 a.m. and evening urine samples were collected after one day of work, at approximately 7:00 p.m. of the same day, as it has been revealed that the variations of DAPs in the morning urine or evening urine of e-waste workers among different sampling days can be neglected.[27] Due to the ready biotransformation properties of PFRs, the PFR metabolite levels in the morning and evening urine samples likely reflect the workers' pre- and post-working exposure to PFRs, respectively. Thirty morning and thirty evening urine samples were obtained, transported on ice to the laboratory, stored at −20 °C, and then thawed and shaken prior to experimental analysis.

Indoor dust samples (n = 30) were collected from the surfaces of furniture, windowsills, and floors of the workshops and workers' homes using woolen brushes, which were pre-cleaned with ethanol according to the methodology described in a previous study.[13] Since the e-waste dismantling activities are generally conducted in the workers' backyards or even in their living rooms in these sites, the workplace dust and home dust were combined for individual homes, respectively. The dust samples (2–5 g for individual samples) were then wrapped in clean aluminum foil, sealed in plastic bags, transported to the laboratory, and stored at −20 °C until analysis. The dust samples were sieved with a stainless-steel sieve (<500 μm) prior to extraction.

2.3 Sample preparation

The urine samples were prepared according to the methodology described in our previous study.[28] Briefly, approximately 2 mL urine was mixed with sodium acetate buffer (pH = 5, 1 M) in a 50 mL Teflon tube, and a mixture of IS solution for PFRs and DAPs (100 ng each) was added. Then, the mixture was extracted three times using a mixture of 5 mL methyl *tert*-butyl ether (MTBE) and 5 mL solution of dichloromethane (DCM) and *n*-hexane (HEX) (4 : 1, v/v) by vortexing for 10 min followed by centrifugation at 5000 rpm for 15 min. The supernatant (organic phase, containing PFRs) was evaporated to near dryness, solvent-exchanged to 1 mL HEX, and then loaded on a Florisil ENVI (500 mg, 3 mL) cartridge pre-conditioned with 6 mL ethyl acetate (EtAC) followed by 6 mL HEX. The target PFRs were eluted with 6 mL EtAC and the eluate was evaporated to near dryness and solubilized in a 100 μL solution of Milli-Q water and acetonitrile (1 : 1, v/v) prior to instrumental analysis for PFRs. The aqueous phase of the urine (containing DAPs) was loaded onto a Strata-X-AW (60 mg, 3 mL) cartridge pre-conditioned with 6 mL 5% triethylamine in ACN followed by 6 mL Milli-Q water, and the DAPs were eluted using 10 mL 5% triethylamine in ACN. The eluate was concentrated under a gentle stream of nitrogen and re-dissolved in 100 μL solution of Milli-Q water and ACN (1 : 1, v/v) in an injection vial prior to analysis for DAPs.

The extraction and clean-up for dust samples were conducted according to the methodology described in a previous study[21] with minor modifications. Briefly, 50 mg of dust was weighed in a 15 mL centrifuge tube and spiked with a mixture of IS solution (100 ng for each IS) for PFRs and DAPs. Then the dust sample was extracted three times with a 6 mL ammonium acetate solution (3 M) : acetonitrile (ACN) (1 : 1, v/v) by vortexing for 5 min, followed by centrifugation at 5000 rpm for 5 min. The extracts were collected in a pre-cleaned glass tube, concentrated to 1.5 mL under nitrogen stream, and transferred to a 2 mL microcentrifuge tube containing 75 mg $MgSO_4$, 25 mg PSA, 25 mg C_{18}, 25 mg GCB, and 75 μL triethylamine for dispersive SPE clean-up. After purification by vortexing and centrifugation, the supernatant was concentrated to near dryness, solubilized in a 1 mL solution of Milli-Q water and ACN (1 : 1, v/v), and then filtered with a 0.22 μm nylon filter prior to instrumental analysis.

2.4 Instrumental analysis

The analysis of target chemicals was performed by using an Agilent 1200 liquid chromatograph (Santa Clara, CA, USA) coupled with an AB SCIEX API4000+ MS/MS (Applied Biosystems, Foster City, CA, USA) with an electrospray ionization (ESI) source which was operated in positive mode for PFRs and negative mode for DAPs, respectively. PFRs were separated using a Phenomenex Kinetex EVO-C18 100A column (2.1 × 100 mm, 5 μm; Torrance, CA, USA). The mobile phases were (A) ammonium acetate (10 mM) and (B) methanol. The gradient elution program was set as follows: 35% B (0–0.1 min), 35–95% B (0.1–9 min), 95–100% B (9–13 min), 100% B (13–14 min), 100–35% B (14–15.1 min), and 35% B (15.1–20 min). The flow rate was 0.25 mL min^{-1}, and the column temperature was set at 40 °C. A volume of 5 μL of extract was injected for each sample, and the multiple reaction monitoring (MRM) mode was used for PFR quantification. The ionization voltage was 4000 V, and the source temperature was 350 °C. Additional optimized mass spectrometry parameters are shown in Table S3.† The DAPs were separated using a Poroshell 120 EC-C18 column (4.6 × 50 mm, 2.7 μm, Agilent, USA). The LC-MS/MS parameters utilized are defined in a previous study,[25] and the mass spectrometry parameters are presented in Table S3.† The chromatograms of the target analytes are shown in Fig. S1.†

2.5 Quality assurance and quality control

The quality control check was performed by regular analysis of the procedural blanks and spiked urine or dust samples (20 ng for each PFR and DAP). Only trace amounts of target chemicals

Table 1 Concentrations of PFRs and DAPs in dust samples collected from an e-waste dismantling site in South China (ng g^{-1})[a]

Analytes	LOQs	Related parent compound	DFs (%)	25th	75th	Median	Range
TCEP	0.59	Parent	83	nd	207	37.2	nd to 408
TCIPP	0.09	Parent	100	286	1110	758	51.7–15 150
TDCIPP	7.9	Parent	80	nd	167	40.9	nd to 381
TBOEP	0.02	Parent	70	nd	8.54	5.29	nd to 127
TEHP	0.02	Parent	100	10.1	21.9	18.9	3.46–115
TPHP	0.21	Parent	97	155	2630	526	nd to 16 790
EHDPHP	6.50	Parent	57	nd	21.9	8.24	nd to 73.7
TNBP	11.0	Parent	0	nd	nd	nd	nd
$\sum_7$PFRs			**100**	**839**	**4375**	**1780**	**244–18 010**
BCEP	1.11	TCEP	73	274	906	680	nd to 1940
BCIPP	0.80	TCIPP	10	nd	nd	nd	nd to 558
DBP	0.27	TNBP	7	nd	nd	nd	nd to 133
DPHP	0.80	TPHP/EHDPHP	73	nd	1830	349	nd to 13 130
BDCIPP	2.00	TDCIPP	0	—	—	—	—
BBOEP	1.43	TBOEP	0	—	—	—	—
$\sum_4$DAPs			**97**	**760**	**3010**	**1360**	**26.0–13 390**

[a] nd, not detected.

were observed in the procedural blanks (Tables S4 and S5†), and the average analyte concentrations in the procedural blanks were subtracted from the values detected in the urine and dust samples, respectively. The target analyte recoveries in the spiked dust samples were 92–128% for PFRs and 93–119% for DAPs, with a relative standard deviation (RSD) less than 15% (Table S6†). The PFR and DAP recoveries in the spiked urine samples were 87–104% and 93–120%, respectively, with RSD < 15% (Table S7†). Limits of quantification (LOQs) were defined as the average concentrations in the blanks plus three times the standard deviations. For analytes not present in the blanks, LOQs were calculated as a signal-to-noise ratio of 10. The LOQs for target analytes were 0.02–11.0 ng g^{-1} and 0.01–0.28 ng mL^{-1} in dust and urine samples, respectively, and details are presented in Tables 1 and 2, respectively.

2.6 Statistical analysis

Statistical analyses were performed for chemicals with detection frequencies (DFs) greater than 60% using SPSS 20 software (SPSS, Inc., Chicago, IL, USA). The concentrations of target analytes were log-transformed to follow a normal distribution, and concentrations below the LOQs were substituted as 1/2 × LOQ for statistical analysis. Spearman rank correlation was used for the analysis of the correlation among the concentrations of diverse analytes in the dust and urine samples. Disparities among concentrations in different sample groups were identified using independent sample *t*-tests. The level of significance was set at $p = 0.05$ throughout the study.

3. Results and discussion

3.1 PFRs and DAPs in dust

3.1.1 Concentrations and patterns of PFRs and DAPs in dust. Seven of the eight target PFRs were detected in the indoor dust samples (DF = 57–100%), while TNBP was not detected in any dust sample, probably due to its high LOQ in this study (Table 1). The concentrations of the $\sum_7$PFRs in dust were 434–18 010 ng g^{-1}, with a median of 1760 ng g^{-1} (Table 1). The $\sum_7$PFR levels measured in this study were in the range of those (median values: 2180, 5560, and 6750 ng g^{-1}) in indoor dust collected from the same e-waste site in 2013.[13] The composition profiles of PFRs in indoor dust are presented in Fig. S2.† TPHP and TCIPP were the most abundant PFRs, accounting for 48% and 39%, respectively, of the total PFR concentrations, which is consistent with the results of a previous study which investigated indoor dust samples from the same area,[13] as well as in office and road dust collected from southern China.[29] Additionally, relatively high contributions of TCIPP and TPHP were also observed in living room dust from Japan, UK, Belgium, Germany, and Norway, as reported in previous studies.[18,30–32]

Of the six target DAPs, BDCIPP and BBOEP were not detected in any dust sample (Table 1), and the DFs for DPHP, BCEP, BCIPP, and DBP were 73%, 73%, 10%, and 7%, respectively. The median concentrations of DPHP, BCEP, and $\sum_4$DAP were 349, 680, and 1360 ng g^{-1}, respectively. In contrast to the extensive number of studies on PFR contaminants, data on DAPs in indoor dust are still limited. DPHP was measured in indoor dust samples collected from Spain and the Netherlands with concentrations ranging from 106 to 79 661 ng g^{-1}.[21] In a study by Wang *et al.*, indoor dust samples were collected across mainland China, and the results showed that DPHP was observed in all the dust samples (DF = 100%) with concentrations of 0.33–2810 ng g^{-1} (median: 47.5 ng g^{-1}).[19] Tan *et al.* reported the DAP levels in indoor house dust from southern China and the Midwestern USA, and the DPHP and BBOEP concentrations in dust were 31.24–4070 ng g^{-1} and <LOQ to 12 880 ng g^{-1} from South China, respectively, and 903–27 460 ng g^{-1} and 96.0–85 950 ng g^{-1} from the USA, respectively.[14] In a recent study by Du *et al.*, ten DAPs were detected in indoor dust collected from workshops and adjacent residential homes of a mega e-waste recycling industrial park in South China, and

Table 2 Urinary concentrations of PFRs and DAPs in e-waste dismantling workers (ng mL^{-1})[a]

		Morning urine (n = 30)					Evening urine (n = 30)				
Analytes	LOQs	DFs (%)	25th	75th	Median	Range	DFs (%)	25th	75th	Median	Range
TCEP	0.10	13	nd	nd	nd	nd to 0.86	50	nd	0.17	0.50	nd to 1.08
TCIPP	0.05	70	nd	0.13	0.07	nd to 0.36	67	nd	0.10	0.07	nd to 0.40
TNBP	0.10	40	nd	0.28	nd	nd to 0.74	60	nd	0.64	0.16	nd to 5.19
TDCIPP	0.03	53	nd	0.09	0.04	nd to 1.36	43	nd	0.06	nd	nd to 0.32
TPHP	0.02	17	nd	nd	nd	nd to 0.24	27	nd	0.03	nd	nd to 0.37
$\sum_5$PFRs		87	0.07	0.71	0.16	nd to 1.76	90	0.13	1.21	0.39	nd to 5.27
BCEP	0.12	60	nd	3.98	1.08	nd to 20.6	77	0.15	9.51	6.08	nd to 41.8
BCIPP	0.25	27	nd	0.28	nd	nd to 4.13	30	nd	0.31	nd	nd to 1.41
DBP	0.09	53	nd	0.23	0.15	nd to 2.25	43	nd	0.12	nd	nd to 0.25
BDCIPP	0.20	80	0.35	0.89	0.58	nd to 18.5	93	0.36	0.66	0.53	nd to 20.4
DPHP	0.17	77	0.23	0.66	0.31	nd to 3.28	97	0.29	0.96	0.51	nd to 3.98
BBOEP	0.20	0	nd	nd	nd	nd	0	nd	nd	nd	nd
$\sum_5$DAPs		**93**	**1.10**	**7.35**	**2.85**	**nd to 24.4**	**97**	**1.37**	**12.1**	**7.79**	**nd to 43.6**

[a] nd, not detected. Concentrations of PFRs and DAPs measured in individual urine samples are presented in Tables S10 and S11, respectively, in the ESI.

DPHP was identified as the most abundant DAP.[20] These results were slightly different from those of this study, as BCEP and DPHP were the most abundant DAPs found in the dust samples from the e-waste site (Fig. S2†). In general, the wide occurrence of DAPs in dust indicates the possibility of PFR degradation in the environments.

3.1.2 Correlation between PFRs and DAPs in dust. The correlations between the levels of three pairs of PFRs and their DAPs (*i.e.*, TPHP–DPHP, EHDPHP–DPHP, and TCEP–BCEP) in indoor dust were investigated, due to the relatively high DFs for these compounds. A significant positive correlation was found between TPHP and DPHP levels (R^2 = 0.949, p < 0.001; Fig. 1 and Table S8†), while there was no correlation between EHDPHP and DPHP levels. The molar concentration ratios ($R_{DAP/PFR}$) of DPHP to TPHP were calculated as 0.41–1.35 (median: 0.93) (Fig. 1). These results suggest that the majority of the DPHP in dust had similar emission sources to the TPHP at the e-waste site; therefore, DPHP could be the degradation product of TPHP or added to the same consumer products with TPHP. Similar results have also been reported by Tan *et al.*[14] No significant correlation was found between the TCEP and BCEP levels (p > 0.05; Table S8†); however, high $R_{DAP/PFR}$ values were observed for BCEP to TCEP levels in dust (median: 4.89, range: 1.46–9.20). Based on these results, TCEP degradation is unlikely to be the major BCEP source associated with house dust. The direct use of BCEP as a commercial chemical for certain products was speculated as the potential main BCEP source in indoor dust, although the presence of BCEP as an impurity of commercial TCEP formulas cannot be excluded; however, information on the industrial and commercial use of BCEP is limited. Nevertheless, these results indicate that more attention should be given to e-waste dismantling workers' exposure to DAPs, especially DPHP and BCEP. Further investigations on the commercial use of

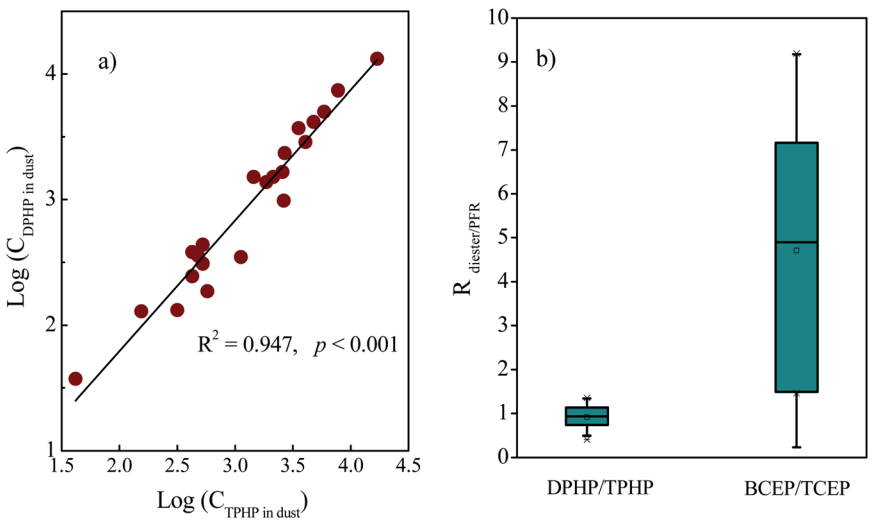

Fig. 1 Correlation between concentrations of TPHP with DPHP (a) and the molar concentration ratios of DPHP/TPHP and BCEP/TCEP in indoor dust (b) (the black horizontal line inside each box represents the median, the boxes represent the 25th and 75th percentiles, whiskers represent a value of 1.5 standard deviation and the dots represent outliers).

DAPs and the environmental degradation of PFRs are crucial to elucidate the DAP sources in indoor environments.

3.2 PFRs and DAPs in urine

3.2.1 PFRs in urine. As shown in Table 2, five out of eight parent PFRs (TPHP, TCIPP, TDCIPP, TCEP, and TNBP) were detected in the morning and evening urine samples in the present study, with DFs ranging between 13 and 70%. TCIPP had the highest DF (70% and 67% in morning and evening urine, respectively), followed by TDCIPP (53% and 43% in morning and evening urine, respectively) and TNBP (40% and 60% in morning and evening urine, respectively). TCEP had a relatively high DF (50%) in evening urine, but a low DF (13%) in morning urine. Low DFs were found for TPHP in both morning (17%) and evening (27%) urine, while TEHP, TBOEP, and EHDPHP were not detected in any urine samples. The $\sum_5$PFR concentrations in morning and evening urine were nd (not detected) to 1.76 ng mL^{-1} (median: 0.16 ng mL^{-1}) and nd to 5.27 ng mL^{-1} (median: 0.39 ng mL^{-1}), respectively. The $\sum_5$PFR levels in evening urine were significantly higher than those in morning urine ($p < 0.05$), which could be related to the dismantling activities performed by the workers during the daytime.[27]

While PFR metabolites have frequently been measured in human urine samples, only a few studies have reported parent PFRs in urine. He *et al.*[11] observed eight PFRs in urine samples collected from Australian children, with total PFR concentrations similar to those found in the evening urine samples in the present study, and TDCIPP (DF = 63%), TBOEP (DF = 55%), and TCIPP (DF = 35%) were the most frequently detected PFRs in urine. Nine PFRs were observed in urine samples of college students from Beijing[33] with TCIPP and TDCIPP being present in all the urine samples (DFs = 100%), and the total PFR concentrations (median: 0.55 ng mL^{-1}, range 0.07–5.66 ng mL^{-1}) were comparable to those in evening urine samples in this study, but higher than those in the morning urine samples in the present study. TCIPP and TDCIPP were the most frequently detected PFRs in urine samples from young children in Queensland, Australia,[11] and from university students in Beijing, China,[33] probably owing to their wide use in commercial products and relatively low log K_{OW} values (2.59 and 3.8 for TCIPP and TDCIPP, respectively), which results in them being prone to be soluble in urine.[2,3,33] Additionally, the variable biotransformation efficiencies and speeds among individual PFRs could also be responsible for their different DFs in human urine, as results of *in vitro* experiments using human hepatic microsomes suggest a low clearance for TCIPP (33%) and TDCIPP (46%) after a 24 h incubation period.[34]

3.2.2 DAPs in urine. Five out of six DAPs, *i.e.*, DPHP, BDCIPP, BCEP, DBP, and BCIPP, were detected in 77%, 80%, 60%, 63%, and 27% of the morning urine samples, respectively, and in 97%, 93%, 77%, 43%, and 30% of evening urine samples, respectively (Table 2). This indicates the ubiquitous presence of PFRs in the workers' tissues. Generally, the DFs for the DAPs, with the exception of DBP, were higher in the evening urine than in the morning urine (Table 2); however, BBOEP was not detected in any of the urine samples in the present study. As previously mentioned, TBOEP (the corresponding parent PFR for BBOEP) was not detected in any urine sample in the present study; hence, the low DFs for BBOEP in urine are probably due to other major metabolites rather than BBOEP being formed from TBOEP and eliminated from the human body. Therefore, urinary BBOEP is not considered a useful TBOEP biomarker for future urinary biomonitoring studies. Recently, bis(2-butoxyethyl) hydroxyethyl phosphate (BBOEHEP) and bis(2-butoxyethyl) hydroxyl-2-butoxyethyl phosphate (HO-TBOEP), which are hydroxylated metabolites of TBOEP, have been identified as the main metabolites in an *in vitro* experiment on human liver microsomes,[35] and a recent study suggested that urinary BBOEHEP should be considered as the most appropriate biomarker for human exposure to TBOEP.[36]

DPHP, BDCIPP, and BCEP were the most frequently detected DAPs in the urine samples, with median concentrations of 0.31, 0.58, and 1.08 ng mL^{-1} in morning urine, respectively, and 0.51, 0.53, and 6.08 ng mL^{-1} in evening urine, respectively (Table 2). The only significant correlation observed in the morning and evening urine samples was between the DPHP and BDCIPP concentrations ($p < 0.01$) (Table S9†). Although the toxicokinetics data (*e.g.*, elimination rate and half-life) for PFRs in human remains have not been studied thus far, PFRs were demonstrated to be readily biotransformed by human hepatic enzymes.[34,35] Therefore, in addition to being affected by the environmental PFR exposure sources (*i.e.*, the e-waste dismantling activities) in the studied area, the varying PFR elimination rates in the human body would also contribute to poor correlations among the urinary PFR metabolite levels in this study.[25]

The total $\sum_5$DAP concentrations were nd to 24.4 ng mL^{-1} (median: 2.85 ng mL^{-1}) and nd to 43.6 ng mL^{-1} (median: 7.79 ng mL^{-1}) in morning and evening urine, respectively. The median levels of urinary DPHP, BDCIPP, BCEP and $\sum_5$DAPs in this study were comparable to those observed in the urine samples of e-waste dismantling workers from the same site[27] and in the same DAP level range reported in previous studies.[7,8,23,37] In accordance with the results found in a previous study,[27] $\sum_5$DAP levels in evening urine were significantly higher than those in morning urine ($p < 0.05$; Fig. 2). Similarly, significantly higher DPHP and BCEP levels were observed in evening urine than in morning urine ($p < 0.05$; Fig. 2). These results were probably because workers were occupationally

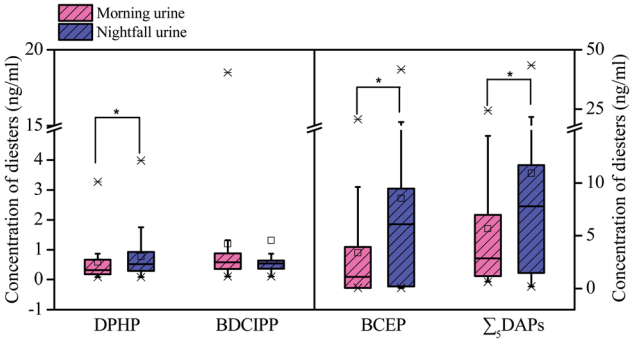

Fig. 2 Urinary concentrations of DAPs (DFs > 60%) for the workers. Note: *$p < 0.05$.

exposed to PFRs during the e-waste dismantling activities in the daytime.[27]

The composition patterns of DAPs were similar in morning and evening urine samples (Fig. S3†). BCEP was the predominant DAP in urine samples, accounting for 44% and 58% in morning and evening urine, respectively, followed by BDCIPP and DPHP, with contributions of 30% and 16% in the morning urine, respectively, and 17% and 21% in the evening urine, respectively (Fig. S3†). DBP and BCIPP together contributed only 10% and 4% of the total DAPs in the morning and evening urine, respectively (Fig. S3†). Similar to the results of this study, BCEP has also been reported as the most abundant DAP in urine samples collected from e-waste dismantling workers and non-occupational populations in China in previous studies.[8,38,39] However, DPHP and BDCIPP were found to be the most abundant PFR metabolites in urine samples collected from other locations in previous studies.[7,18,37] These results indicate the different urinary DAP composition patterns between populations in China and other locations such as Australia,[7] Europe,[7] and the USA.[18] Residents in China had a relatively high potential exposure to TCEP and BCEP, because high BCEP levels were also found in the dust samples. Likewise, since high TPHP and DPHP levels were observed in the dust samples, the higher DPHP percentages observed in the urine samples of the workers might be caused by biotransformation of PFRs in the human body or because it was directly ingested from the environment. TCIPP accounted for 39% of the total PFRs in the dust samples, but its diester metabolite (BCIPP) contributed only 2–3% of the total DAPs in the urine samples. The discrepancy between TCIPP in dust and BCIPP in urine could be explained by the existence of other biotransformation pathways and metabolites for TCIPP in the human body, as other studies have found high DFs for 1-hydroxy-2-propylbis (1-chloro-2-propyl) phosphate (BCIPHIPP), a hydroxylated metabolite of TCIPP, in adult urine samples from Norway (DF > 98%)[36] and Australia (DF = 100%).[7]

3.2.3 Influence of gender, age, and exposure time on DAPs in urine. The DAP levels in both morning and evening urine were similar between male and female workers, and no gender difference ($p > 0.05$) was found in the present study. This result is consistent with the result from a previous study where the gender difference for urinary DAP levels was insignificant for workers from the same e-waste site.[25]

The correlations between DAP levels and workers' age and occupational time were analyzed to investigate their influences on urinary DAPs. Significant positive correlations were observed between the workers' age and DPHP levels in both morning ($R = 0.419$, $p < 0.05$) and evening ($R = 0.398$, $p < 0.05$) urine (Table S9†). However, significant negative associations between the DAP levels and the workers' age were generally reported in previous studies.[7,8,38] These opposite results could be due to the age differences among the participants. In previous studies, participants generally included children and adults,[7,8,38] and compared to adults, the distinctive breathing zone and behavior of children contributed to higher contamination levels in the children's urine. However, in the present study, all participants were adult workers; therefore, the DAP levels in urine might be due to the different metabolic capacities of the PFRs for workers at different ages. Moreover, the BCEP levels in both the morning and evening urine samples were significantly correlated with the occupational exposure time for the workers ($R = 0.416$, $p < 0.05$, and $R = 0.523$, $p < 0.05$, respectively) (Table S9†). This result is different from the findings in a previous study, where the urinary BDCIPP levels were significantly negatively correlated with the occupational exposure time of workers from the same e-waste site.[25] However, the cause of this discrepancy is still unclear because the PFRs could undergo biotransformation *in vivo*, allowing them to be eliminated from the human body efficiently.

3.3 Correlation between urine and dust levels: implications for urinary biomonitoring

PFRs have been used in electronic products for decades, and studies have reported that crude e-waste dismantling activities can release PFRs into the ambient environments,[13,24] which results in high PFR exposure to the e-waste recycling workers through dust ingestion and/or other pathways such as air inhalation, as relatively high levels of PFRs (775–13 823 pg m^{-3}, median 3321 pg m^{-3}) were also observed in outdoor fine particulate matter (PM2.5) from this e-waste site.[40]

The DAP levels in the morning urine samples were not significantly correlated to the levels of DAPs or their respective parent PFRs in the dust samples ($p > 0.05$). This result was due to the less nighttime activity level of the workers, implying that dust ingestion might not be the primary pathway for PFR exposure at night. On the other hand, statistically significant correlations of moderate strength were observed between the DPHP levels in the evening urine samples and the TPHP ($R^2 = 0.455$, $p < 0.01$) and DPHP ($R^2 = 0.478$, $p < 0.01$) levels in the dust samples (Fig. 3). However, no significant correlation was found between the EHDPHP level in dust samples and the DPHP levels in urine or dust in the present study ($p > 0.05$). The median TPHP and DPHP concentrations in dust were one order of magnitude higher than that of EHDPHP, suggesting that EHDPHP was not the primary PFR in the studied area. In addition to being formed by the biotransformation of TPHP, DPHP could also be directly ingested, though the biotransformation of other aryl-PFRs (excluding EHDPHP) cannot be eliminated. Similarly, BCEP levels in evening urine were also moderately but significantly correlated to TCEP ($R^2 = 0.479$, $p < 0.01$) and BCEP ($R^2 = 0.427$, $p < 0.01$) levels in dust (Fig. 3). However, no significant association was observed for BDCIPP in urine and TDCIPP in dust ($p > 0.05$), which was consistent with the result of a previous study.[36] This discrepancy is probably attributable to other possible exposure pathways such as air inhalation, food intake, or dermal absorption, which can also contribute to the increase of DAPs in human urine.

Collectively, the results of this study indicated the limitations of solely using urinary DAPs as biomarkers for the evaluation of human exposure to PFRs, especially for TCIPP and TBOEP, because BCIPP and BBOEP could not be their major metabolites in the human body, respectively, and hydroxylated metabolites should be considered in future studies.[36] Due to the multiple potential precursors of DPHP in the human body and

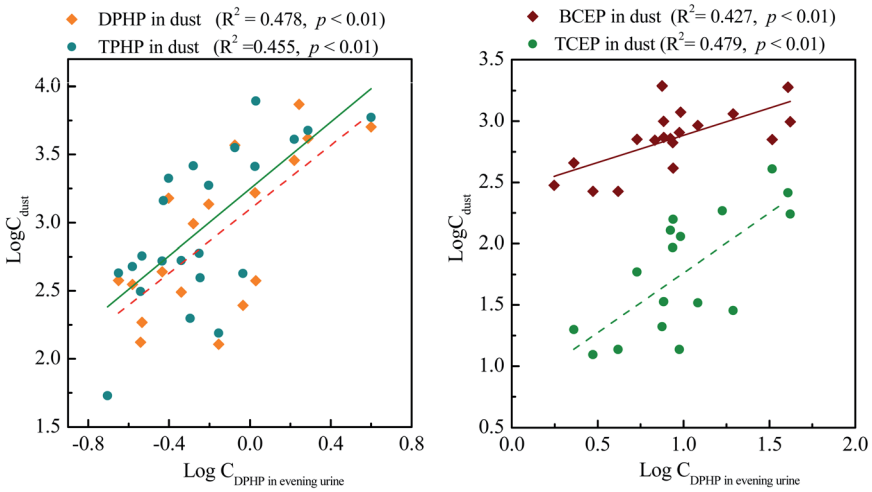

Fig. 3 Correlations between DAP levels in evening urine (x) and DAP or PFR levels in dust (y).

the direct exposure to DPHP from the environment, it cannot be used as a good TPHP biomarker. 3-Hydroxyphenyl diphenyl phosphate (3-HO-TPHP) and 4-hydroxyphenyl diphenyl phosphate (4-HO-TPHP) were proposed as specific metabolites for TPHP, and 2-ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-EHDPHP) and ethylhexyl phenyl phosphate were proposed for EHDPHP;[41] however, only a few studies have reported their presence in human urine.[36,42,43] Additional studies are warranted to examine the appropriateness of using them as biomarkers for TPHP and EHDPHP, respectively. Likewise, significantly higher BCEP levels than TCEP levels were observed in the indoor dust, and an *in vitro* study has indicated that only a small proportion (7%) of TCEP can be converted to BCEP using human liver microsomes.[34] Therefore, the urinary BCEP levels might reflect the exposure pathway of direct ingestion of BCEP rather than TCEP, its parent PFR. Given the relatively high DFs for TCEP in evening urine in the present study, both TCEP and BCEP should be considered for urinary biomonitoring in future studies.[23] DBP was detected in nearly half of the urine samples (53% and 43% for morning and evening urine, respectively). A previous study reported mono-*n*-butyl phosphate (MBP) as a major TNBP metabolite in urine, which was detected at levels two orders of magnitude higher than those of DBP.[44] However, as this study only detected DBP as a biomarker for TNBP, a recommendation for other metabolites as biomarkers for TNBP cannot be made. BDCIPP is the major metabolite of TDCIPP, as suggested by previous studies[23,45] and supported by an *in vitro* study.[34] BDCIPP was detected in 80% and 94% of the morning and evening urine samples, respectively, and it should be considered as an appropriate biomarker because it is unique to TDCIPP and no other PFR is transformed to BDCIPP in the human body.

4. Conclusions

In summary, both PFRs and DAPs were measured in the morning and evening urine of e-waste dismantling workers, as well as in indoor dust samples from their workplaces and homes. The significant positive correlation between TPHP and DPHP levels in the dust suggests that they potentially have the same emission source. TCIPP and TDCIPP were the most abundant PFRs, and BCEP, BDCIPP, and DPHP were the predominant DAPs in both the morning and evening urine samples. However, the PFR and DAP concentrations in the evening urine were significantly higher than those in the morning urine samples, indicating higher PFR exposure for the workers during the daytime. The frequent detection of certain DAPs along with their parent compounds in dust implies direct ingestion as a potential pathway for human exposure to these DAPs. BDCIPP can be considered as a useful biomarker for TDCIPP in urine samples, while urinary BCIPP, BBOEP, DPHP, and BCEP may not be appropriate biomarkers for PFRs. In addition to DAPs, PFRs and hydroxylated PFRs (OH-PFRs) should also be considered for urinary biomonitoring in future studies. However, it should be noted that several limitations exist in the present study: (1) the sample size (n = 30) of this study is relatively small, (2) the urine samples of the workers were collected in one single day, and (3) other metabolites (*e.g.*, HO-PFRs) were not considered for analysis. More samples and more sampling points, as well as a control group (non-professional exposure populations), are still necessary to elucidate the time variability and the most appropriate biomarkers for urinary biomonitoring of human exposure to PFRs.

Conflicts of interest

There are no conflicts to declare.

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