Synthetic phenolic antioxidants and their major metabolites in human fingernail

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ABSTRACT
Synthetic phenolic antioxidants (SPAs) have been widely used in foods, polymers, and cosmetics, but very limited information is available about their occurrence in human tissues. In this study, five SPAs, namely 2,6-di-tert-butyl-4-methylphenol (BHT), 2-tert-butyl-4-hydroxyanisole (BHA), propyl-, octyl-, and dodecyl-gallate (PG, OG, and DG), and four major metabolites of BHT, including 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO), 2,6-di-tert-butyl-4-(hydroxymethyl) phenol (BHT-OH), 3,5-di-tertbutyl-4-hydroxybenzoic acid (BHT-COOH), and 2,6-di-tert-butyl-1,4-benzoquinone (BHT-Q), were determined in human fingernail samples collected from Nanjing, China. Total concentrations of the nine target analytes (∑9SPAs) were 523–14,000ng/g. BHT was the predominant SPA compound and detected in all samples at a range of 309–11,400ng/g. The ∑9SPAs was negatively correlated with age of fingernail donors (p < 0.05). In addition, indoor dust samples from the living places of the fingernail providers were collected with aim to better understand the SPA exposure pathways. A positive correlation (p < 0.05) was found only for DG concentrations between paired fingernail and dust samples, while not for other SPAs, suggesting that SPAs accumulated in fingernails may not be mainly from indoor dust. SPAs were measured for the first time in human fingernail, and the elevated concentrations in fingernail suggest that the health risk of SPAs should be paid more attention due to their bioaccumulation potential in human body. Further studies are warranted about exposure pathway, distribution and metabolism of SPAs in human body.

1. Introduction
As one of the main types of antioxidants, synthetic phenolic anti-oxidants (SPAs) have been widely added in rubbers, plastics, cosmetics, and even foods (Rodil et al., 2010; Lanigan and Yamarik, 2002). Commonly used SPAs include 2,6-di-tert-butyl-hydroxytoluene (BHT), 2-tert-butyl-4-hydroxyanisole (BHA), propyl-, octyl-, and dodecyl-gallate (PG, OG, and DG), among which BHT is the dominant compound (Nieva-Echevarria et al., 2015; Wang et al., 2016). For example, the world production capacity of BHT in 2000 was about 62,000t/a, including USA (7000 t/a), Japan (15,000 t/a), Western Europe (25,000 t/a), Russia (5000 t/a), India (1000 t/a), and China (9000 t/a) (OECD, 2002). BHT is ready to be metabolized to 3,5-di-tert-butyl-4-hydroxybenzaldehyde (BHT-CHO), 2,6-di-tert-butyl-4-(hydroxymethyl) phenol (BHT-OH), and 3,5-di-tert-butyl-4-hydroxybenzoic acid (BHT-COOH) via oxidation of alkyl group or to 2,6-di-tert-butyl-1,4-benzoquinone (BHT-Q) through oxidation of aromatic ring in both abiotic (Liu et al., 2017; Fries and Püttmann, 2004) and biotic systems (Matsuo et al., 1984; Wang and Kannan, 2018). BHA is easily to be metabolized to tert-butyl hydroquinone (TBHQ) by oxidation reaction (Rodil et al., 2012). For three gallates, the available data demonstrated that DG and OG are more resistant to be hydrolyzed to propyl alcohol and gallic acid as compared to PG (EFSA, 2015a, 2015b). The widespread usage and detection of SPAs have drawn increasing concerns about their potential health risk. Although SPAs are generally recognized as safe at authorized levels (Lanigan and Yamarik, 2002),
their toxicological effect has been observed in both in vitro and in vivo studies. For example, a recent study by Yang et al. (2017) revealed that BHA can trigger endocrine disorder via increasing E2 (17β-estradiol) secretion in H295R cells (in vitro) and zebrafish (in vivo) at a low concentration (1 μM). Considering the recommended safe level for BHA alone or in combination with other SPAs in foodstuffs (200 mg/kg) (EFSA, 2011), the perturbation in steroidogenesis at the low concentration highlights the necessity of toxic studies for BHA and probably other SPAs. BHT toxicity is controversial and may mainly be attributed to its metabolites. For instance, DNA cleavage caused by BHT-Q through superoxide radical generation at quite low concentration of 10−6 M has been reported in vitro studies (Oikawa et al., 1998; Nagai et al., 1993).

Although there were reports about occurrence of SPAs in environmental matrices and relevant exposure risk assessments (Wang et al., 2016; Liu et al., 2017), very limited information is available on SPA concentrations in human bodies (Collings and Sharratt, 1970; Conacher et al., 1986; Liu and Mabury, 2018). So far, only couples of literature has reported that SPAs were detected in human adipose and serum samples (Collings and Sharratt, 1970; Liu and Mabury, 2018), mainly focusing on those destructive biomarkers to which most researchers have very limited access. While due to their sampling advantages (e.g., cost reduction, less storage, and sample stability), some noninvasive biomarkers, such as fingernail, have become a popular alternative of biological samples for assessing human exposure to contaminants. As far as we know, SPA levels in human fingernail have not been reported yet. Actually, this information is very important for human biomonitoring of SPAs, since the bioaccumulation potential of SPAs in fingernail is the precondition for next step that if fingernail can be used as non-invasive biomarker.

Therefore, there is a strong rationale to study the occurrence and profile of SPAs in human fingernail. As one of the most important sinks for many organic contaminants, elevated levels of SPAs in indoor dust have been reported (Wang et al., 2016). Paired fingernail and indoor dust samples were collected to better understand the SPA exposure pathway and human risk. In this study, 50 pairs of fingernail and indoor dust samples were collected from Nanjing, China. Concentrations and profiles of SPAs and their major metabolites in these samples were measured. To our best knowledge, this is the first study to report the levels of SPAs in human fingernail.

2. Materials and methods

2.1. Chemicals and reagents

Standards of BHT, BHA, BHT-COOH, BHT-OH, BHT-Q, and DG (purity: > 97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of OG, PG, and BHT-CHO (purity: 98%) were purchased from TCI America (Portland, OR, USA). Internal standard BHT-d21 (purity: 99%) was purchased from CDN isotopes (Quebec, Canada).

Stock solutions for each analyte was individually prepared in methanol at a concentration of 100 μg/mL, and a mixed standard solution was prepared from individual stock solutions to achieve a concentration of 10 μg/mL through appropriate methanol dilution. Organic solvents including n-hexane, dichloromethane, and methanol were of HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ, USA). Milli-Q water was generated by Millipore ultrapure water system (Dubuque, IA, USA).

2.2. Paired fingernail clipping and dust sample collection

Totally 50 apparently healthy participants, voluntarily participating in this study during June to September 2016, were from resident houses (27 samples) and dormitories (23 samples) in Nanjing, China for fingernail clipping collection, and only one recruited from each dwelling provided fingernail clippings (ten finger). Participants included 20 males and 30 females (nail polish has not been used in recent one year) (aged 4–62), and were informed in advance about the aim of this study. We collected fingernail clippings and asked for personal information when they agreed with it or got permission by the legal guardian. The relevant personal information (such as age, weight, and height) was collected by questionnaires when collecting their fingernail clippings (Details can be found in Table S1 of Supporting information). Generally, fingernail clippings from 5 children (4 < age < 11), 6 teenagers (age = 16), and 39 adults (21 < age < 62) were collected from ten fingers with nail clipper precleaned with ethyl alcohol. In addition, dust samples from floor of living rooms and bedrooms of each participant were collected at the same time by vacuum cleaner. The vacuum cleaner was precleaned before each sampling. About 1–2 g dust samples were obtained from vacuum cleaner bags for each sampling site. The fingernail and dust samples were packed in clean aluminum foil and sent to laboratory immediately after sampling. Dust samples were homogenized, sieved through 150 μm stainless sieve, and stored at −20 °C until analysis.

2.3. Sample extraction

It is still controversial that if washing before extraction can remove external contaminants attached to fingernails. A number of studies have indicated that the removal efficiency of washing is compound-dependent, and also variable with washing reagents (Alves et al., 2014; Sukumar, 2005). In order to examine the effect of washing, we randomly collected fingernail samples from 3 individuals (out of the 50 samples used in the current study). Half of the fingernail samples were washed by Milli-Q water in sonicator for 20 min before extraction, while the other half was directly extracted without washing. The removal efficiencies of $E_S$ for the 3 samples were $−8.92%, −8.80%, and 16.2%$, respectively. Although the removal efficiencies varied among SPAs and their major metabolites, there was no pronounced difference between washing and non-washing treatments (Table S2). Therefore, fingernail samples in the current study were not washed before extraction due to the variability of washing performance among samples and compounds. The fingernail samples were cut into tiny pieces by stainless steel scissors prewashed by methanol according to Alves et al. (Alves et al., 2016). Although no significant difference was found between powdered and whole nail extraction efficiencies, extraction with powdered nails showed less variation among replicates (Alves et al., 2016). The powdered fingernail and dust samples were extracted following the similar procedure described by Wang et al. (2016). Briefly, about 50 mg of fingernail or 100 mg of dust was weighed and transferred to a 15-mL glass tube. After being spiked with BHT-d21, the sample was extracted with 5 mL of mixture of dichloromethane/n-hexane (3:1, v/v). Prior to ultrasound assisted extraction, samples were soaked in solvent for overnight to improve the extraction efficiency. The mixture was then sonicated for 30 min in a sonication bath and centrifuged at 3000 rpm for 10 min. The supernatant was transferred into another new glass tube, and the extraction was repeated twice with the same procedure. The extracts were combined, concentrated and solvent exchanged to 500 μL n-hexane, half of which was solvent changed to methanol for analysis on ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), and the remaining was analyzed by gas chromatography-mass spectrometry (GC-MS).

2.4. Instrumental analysis

Three analytes, including BHT, BHT-CHO, and BHT-Q were quantified using a GC (Agilent Technologies 7890A; Santa Clara, CA, USA) coupled to a MS (Agilent Technologies 5977A) in the selected ion monitoring (SIM) mode, which have been established in our previous work by Zhang et al. (2018). Analytes were separated using HP-5 capillary column (0.25 mm × 30 m × 0.25 μm; J&K Scientific, USA). Helium (99.9999%) was used as carrier gas at a flow rate of 1.0 mL/min.
The temperature of the injector and ion source were 290°C and 280°C, respectively. The oven temperature was set initially at 45°C for 1 min, then ramped up to 200°C at 20°C/min, and finally ramped up to 270°C at 10°C/min. Ions m/z were 177, 205 and 220 for BHT and BHT-Q, and 191.1, 219 and 234 for BHT-CHO, respectively. The rest six analytes were analyzed by an A-30 Altus UPLC coupled with a QSight 210 electrospray triple quadrupole mass spectrometer (UPLC-MS/MS; PerkinElmer, USA) in the negative ion multiple reaction monitoring (MRM) mode. Target analytes were separated on a Brownlee SPP C18 analytical column (2.1 mm × 100 mm × 2.7 µm; PerkinElmer) and the column temperature was kept at 40°C. The mobile phase was comprised of Milli-Q water (A) and methanol (B), and used at a flow rate of 0.4 mL/min with a gradient program as follows: 0 min, 70% A; 0–4 min, 70–10% A; 4–5.5 min, 10% A; 5.5–6 min, 10–70% A; 6–8 min, 70% A. The optimized parameters for UPLC-MS/MS analysis are listed in Table S3.

2.5. Quality assurance/quality control

To minimize cross contamination, only glassware was used for sample storage, extraction, and analysis. Before use, all the glassware was cleaned in ultrasonic bath and heated at 500°C for 4 h in muffle. Procedural recoveries were determined by spiking mixed SPAs (50 ng each) into sodium sulfate (surrogate material for fingernail and dust samples) with the recovery efficiency of 89–105%. Matrix recoveries were also performed by spiking individual SPAs (50 ng each) into fingernail and dust to check matrix effect on extraction efficiency. The matrix recoveries of SPAs in fingernail and dust samples were from 66.0 ± 0.40% for PG to 109 ± 2.90% for BHT-d21, and 63.0 ± 4.50% for PG to 101 ± 8.70% for BHT-OH, respectively (Table S4). The limits of detection (LODs) and limits of quantification (LOQs) were 0.01–0.30 ng/g and 0.04–1.20 ng/g, respectively, which were calculated based on signal/noise (S/N) ratio of 3:1 and 10:1 (Table S4). One sample randomly selected from each batch of 10 samples was re-analyzed, and the coefficient of variation between two concentrations was < 13%. Methanol blank, procedural blank, and mid-concentration (25 ppb) were injected for each batch of 20 samples as a carry-over check of analytes between samples, baseline drift, and instrumental sensitivity, respectively. Trace levels of DG (0.36–0.59 ng/g) were measured in procedural blank, and final concentrations were corrected by subtracting mean value of background (0.41 ng/g). Nine working calibration standards (range: 0.1–100 ng/L) were prepared to quantify SPAs in samples, and the regression coefficient (r²) was > 0.99 for all target analytes. For samples with concentrations above the highest calibration standard point, samples were diluted with n-hexane or methanol and determined again.

2.6. Statistical analysis

Statistical analysis was processed using SigmaPlot (version 12.5) and SPSS (version 17.0) software. For data processing, concentrations < LOQ were assigned a value of LOQ/2 for calculating geometric mean (GM). One-way ANOVA analysis was used to compare SPA levels for different sexual and age groups. A value of significant difference was set at $p < 0.05$.

3. Results and discussion

3.1. Concentrations of SPAs in fingernails

Despite their widespread use in consumer products, the accumulation of SPAs in human has been rarely reported. To our best knowledge, this is the first study investigating the occurrence of SPAs in human fingernail. The total concentrations of five SPAs and their four metabolites (totally 9 target compounds, $\Sigma_{9}$SPAs) in fingernail samples were in a range of 523–14,000 ng/g (mean: 5560, geometric median (GM):
The percentage of BHT composition (i.e., 75.7%) here is well consistent with the fact that BHT usage accounts for about 80% of the total SPA production in China (Du and Guo, 2009). BHT was detected in all the fingernail samples with a concentration range of 309–11,400 ng/g (mean: 4280, GM: 3540 ng/g) (Table 1).

The concentrations of BHT in fingernails were comparable to those reported for human adipose tissue (e.g., up to 3190 ng/g in an US female resident) (Collings and Sharratt, 1970), and 3 orders of magnitude higher than those reported in human serum recently. For example, BHT was detected in 50 individual serum samples with GM concentration of 3.37 μg/L (Liu and Mabury, 2018). The higher accumulation of BHT in fingernail and adipose (microgram per gram) levels than serum (nanogram per milliliter) may be explained by the hydrophobicity of BHT (log Kow = 5.10). The high accumulation of BHT in fingernail has never been reported, but the bioaccumulation potential in fingernail has been studied for other chemicals (Alves et al., 2016; Sukumar, 2005). Slow clearance from fingernails and negligible distributing back to plasma have been observed for some hydrophobic antibiotics. For example, maximum concentration of itraconazole in fingernails was 950 ng/g four months after cessation of pulse therapy (Felton et al., 2014). Therefore, the positive detection of BHT in fingernail here together with previous work mentioned above suggest that BHT can be accumulated in human fingernail, and fingernail has the potential to serve as a non-destructive biomarker reflecting human exposure to BHT only after validation with some well-established biomarkers, such as serum.

When compared with BHT, the rest SPAs were infrequently detected (< 34%, except DG with detection frequency (DF) of 100%) at much lower levels. Sum concentrations of the three gallates (i.e., DG, OG, and PG) 0.246–23.7 ng/g) were 2–5 orders of magnitude lower than those of BHT (309–11,400 ng/g) in fingernail samples (Table 1). In foodstuff, PG is the most used antioxidant, while DG and OG are rarely detected in food (André et al., 2010). In our fingernail samples, the DF values of the three gallates were 100%, 34%, and 0 for DG, OG, and PG, respectively. This may suggest that DG and OG detected here were not from food consumption. The non-detection of PG can be explained by its in vivo extensive hydrolysis to propyl alcohol and gallic acid (EFSA, 2015). BHA was detected in 22% of the fingernail samples with a concentration range of < LOQ-42.8 ng/g (mean: 1.79, GM: 0.257 ng/g) (Table 1). Positive correlations were observed between BHT and BHT metabolite concentrations in fingernail samples (r² = 0.38–0.39, p < 0.0001), except for BHT-COOH concentration (r² = 0.028, p = 0.25) (Fig. S1). This suggests the existence of a common source for BHT and their metabolites. The ratio of ∑MTs to BHT concentration was averaged at 34.8%, which is higher than previous finding that the ratios of ∑MTs to BHT were about 22% in dust samples and 20% in municipal sewage sludge samples (Liu et al., 2017, 2015), but comparable to the ratio of 29% recently reported in 50 human serum samples (Liu and Mabury, 2018). This implies that the metabolism potential may differ between in vivo and in vitro process, but the hypothesis needs to be further examined. Comparison with environmental samples was further conducted. It has been reported that BHT-Q was the most dominant

BHA in human fingernail observed here together with its toxicity emphasize that more strict regulations for BHA usage in food or consumer products is necessary in term of human health.

3.2. Concentrations of SPA major metabolites in fingernail

All the four BHT metabolites were measured in fingernail samples with total concentrations (∑MTs) varying from 211 to 3980 ng/g. The metabolite concentrations in fingernails were declined in the order of BHT-CHO (range: 85.3–2650, mean: 635 ng/g), followed by BHT-Q (31.7–1470, 591 ng/g), BHT-OH (< LOQ-164, 34.3 ng/g), and BHT-COOH (3.37–52.4, 16.5 ng/g) (Table 1). BHT-CHO, BHT-Q, BHT-OH, and BHT-COOH accounted for 49.1%, 45.7%, 3.28%, and 1.86% of the ∑MTs on average, respectively. Fingernail is metabolite inactive tissue, and the occurrence of BHT metabolites here suggests the bioaccumulation potential of these chemicals in fingernail. The high DF and levels of BHT metabolites accumulated in human fingernail are worth of our attention due to the adverse health effects of BHT metabolites (Oikawa et al., 1998). For examples, BHT-Q can cleave DNA at low concentration of 10⁻⁶ M (Nagai et al., 1993). In the current study, BHT-Q levels in 92% of the 50 samples were higher than 10⁻⁶ M (220 μg/L), suggesting the potential health concerns induced by BHT metabolites. Actually, there may be a higher chance for human exposed to BHT metabolites than the parent compound. For example, almost 90% of BHT in water can be removed through activated sludge system of wastewater treatment plants, while large portion of BHT-quinol and BHT-Q can be generated during the treatment (Liu et al., 2015).

Positive correlations were observed between BHT and BHT metabolite concentrations in fingernail samples (r² = 0.38–0.39, p < 0.0001), except for BHT-COOH concentration (r² = 0.028, p = 0.25) (Fig. S1). This suggests the existence of a common source for BHT and their metabolites. The ratio of ∑MTs to BHT concentration was averaged at 34.8%, which is higher than previous finding that the ratios of ∑MTs to BHT were about 22% in dust samples and 20% in municipal sewage sludge samples (Liu et al., 2017, 2015), but comparable to the ratio of 29% recently reported in 50 human serum samples (Liu and Mabury, 2018). This implies that the metabolism potential may differ between in vivo and in vitro process, but the hypothesis needs to be further examined. Comparison with environmental samples was further conducted. It has been reported that BHT-Q was the most dominant

4710 ng/g; Table 1). BHT was the most dominant compound in fingernail samples and accounted for 33.4–94.9% (averaged at 75.7%) of ∑SPAs (Fig. 1).
BHT metabolite in dust and sludge samples with median concentrations 4–5 times higher than those of BHT-CHO (Liu et al., 2017, 2015). While comparable mean concentrations of BHT-Q (591 ng/g) and BHT-CHO (635 ng/g) were measured in fingernail samples here, no significant difference ($p = 0.1$) was found for the ratios of BHT-Q/2MTs (46.3%) on average and BHT-CHO/2MTs (49.7%). This similar level of BHT-Q and BHT-CHO is consistent with that measured for serum samples (Liu and Mabury, 2018). Since BHT-Q is the product of BHT metabolism through aromatic ring oxidation, and BHT-CHO is from BHT through oxidation of alkyl substituent (Matsuo et al., 1984), it further suggests that BHT metabolism pathway may be different between environmental matrices (such as indoor dust) and in vivo process (human body).

### 3.3. Factors influencing the SPA levels in fingernail

The effect of fingernail providers’ age and gender on the SPA levels was investigated. There was no significant difference in SPAs concentrations between genders. However, age-distribution of SPAs in fingernails showed higher mean concentrations (9760 ng/g) in children than that in teenagers (4310 ng/g) and adults (5150 ng/g) (Fig. S2). It should be noted that there were only 5 fingernail samples collected from children and the small sample size may make it bias to draw a conclusion that children suffer from more SPA exposure. The total concentrations of nine target analytes ($\sum$SPAs), SPAs ($\sum$5SPAs), and their metabolites ($\sum$MTs) were significantly ($p < 0.05$) negatively correlated with ages of fingernail donors. For individual compounds, BHT, BHT-Q, BHT-CHO concentrations showed significantly ($p < 0.05$) negative association with ages of donors (Fig. 2). As antioxidant additives, SPAs (e.g., gallates) have been widely added to plastic products (Xin et al., 2014), including various children's toys. Children have more opportunity to access plastic toys, resulting in exposure to various hazardous substances contained in plastic toys. Moreover, as children have higher hand-to-mouth activity and ventilation rate, and relative less body mass, they absorb xenobiotics usually more rapidly and efficiently than adults (Mercier et al., 2011; Wu et al., 2018). Therefore, as compared to adults, children suffer greater exposure to environmental chemicals, including SPAs, through non-nutritive ingestion and inhalation, such as dust ingestion and inhalation.

This may explain the negative association of SPAs concentrations with ages of donors. Faster metabolism of xenobiotics can be expected for children than adults (Miller et al., 2002), which may explain the fact that relatively higher level of SPAs metabolites in younger donors. Actually, more significant correlation was observed for MTs ($p = 0.01$) than the parent compound (i.e., BHT, $p = 0.04$, Fig. 2) with ages of donors. It should be noted that the sample size was rather small, i.e., 11 for children and teenager (4–16 years old) vs 39 for adult (21–62 years old), and the trend of concentration of target chemicals with age may be more pronounced with larger size of samples. Nevertheless, our finding suggests that young people (such as toddlers and infants) may need more attention for SPA biomonitoring.

### 3.4. Concentrations of SPAs and their major metabolites in indoor dust

Except for PG, all the SPAs and their metabolites can be positively identified in some or all dust samples with total concentrations ($\sum$SPAs) ranging from 1620 to 65,900 ng/g (mean: 7990, GM: 5350 ng/g) (Table 1). Similar to that found for fingernail samples, BHT was the most dominant SPA compound with 100% detection frequency at a concentration range of 968–49,200 ng/g (mean: 5960, GM: 3830 ng/g) (Table 1), which is in agreement with previous findings that BHT concentrations varied from 130 to 47,400 ng/g in house dust collected from different Chinese cities (Wang et al., 2016; Liu et al., 2017; Zhang et al., 2018). In addition to dust, BHT has also been found in various environmental matrices including water, soil, and sediment as well as foodstuff (Hernandez et al., 2012; Rodil et al., 2010; Zhang et al., 2018; Kim et al., 2016). For example, BHT has been reported to occur in river water (275–871 ng/L) and sediment (90–6930 ng/g) (Rodil et al., 2010; Zhang et al., 2018). Elevated concentration of BHT has been found in edible oil at concentration of up to 61,800 ng/g (Kim et al., 2016). DG was detected in 92% of dust samples at concentrations varying from < LOQ to 57.9 ng/g, followed by OG (DF: 72%, range:< LOQ-15.4ng/g), whereas PG was not found in any dust samples. This is in accordance with previous finding that the frequencies and concentrations of DG, OG, and PG declined successively in dust samples from Saudi Arabia, Japan, and Korea, respectively (Wang et al., 2016). This trend is consistent to their Log $K_{ow}$ values (i.e.,...
DG = 6.21, OG = 3.66, and PG = 1.8), suggesting that hydrophobic compound is inclined to be sorbed by dust particles. BHA was detected in only one dust sample with low concentration of 3.33 ng/g. The four major metabolites of BHT, including BHT-Q, BHT-COOH, BHT-OH, and BHT-CHO, were all found in dust samples, and the total concentrations (ΣMTs) varied from 150 to 16,700 ng/g, with a GM value of 1190 ng/g (Table 1). Concentrations of the four metabolites were in the order of BHT-Q (range: 74.0–8550, mean: 1040 ng/g) > BHT-CHO (63.3–7560, 769 ng/g) > BHT-COOH (< LOQ-373, 39.4 ng/g) > BHT-OH (< LOQ-405, 20.9 ng/g).

3.5. Comparison of SPA levels between fingernail and indoor dust samples

The SPA levels were comparable or at the same order of magnitude between the two matrices, e.g., the GM values of BHT were 3540 and 3830 ng/g for fingernail and dust samples, respectively. The profiles of metabolites were further compared and percentage values of BHT metabolites to ΣMTs are listed in Table S5. Significant difference was found for all the metabolite percentages between fingernail and dust samples, indicating again that the metabolism process may vary between in vivo and in vitro process. Correlation analyses of SPAs concentrations between fingernail and dust samples were performed, and no correlations were found for BHT, BHT metabolites, and ΣMTs concentrations, which suggests that indoor dust was not the major source for SPAs detected in fingernail. We further estimated the daily intake (EDI) of SPAs via dust exposure (i.e., ingestion, inhalation, and dermal adsorption) for different age groups (Wang et al., 2012). The worst case scenario was assessed for SPAs and their major metabolites based on the 95th percentile concentrations determined in dust (Details can be seen in Supporting information Sections S1, Tables S6 and S7). There are no data about SPAs exposure reported for the Chinese people, and it was reported that EDI values of SPAs through diet for the U.S. population were 0.39 (mean) and 0.78 (high exposure) mg/kg/d (Wang et al., 2016). The EDI of BHT from dust here was minor (71.1 ng/kg/d maximum, Table S7) when compared with the reported dietary EDIs. Although no acute toxicity to human and lack of evidence linking SPAs intake to disease, SPAs may cause contact dermatitis reaction to some extent (EFSA, 2015b). Toxicological effects have been reported for exposure to SPAs. For example, BHA can cause cytotoxicity, DNA fragmentation and endocrine disrupting effects (Yang et al., 2017). Animal experiments showed that BHT, BHA and PG exerted endocrine disrupting effects and PG significantly affected endometrial epithelium thickness (Pop et al., 2013). Given SPAs are moderately toxic, it should be noted that continuous exposure to SPAs will threaten human health. The widespread occurrence and bioaccumulation potential of SPAs warrant further studies on adverse effects of SPAs on human body.

However, positive correlation can be found for DG concentrations between fingernail and dust samples (R = 0.76, p < 0.05) (Fig. 3). As mentioned above, PG is one of the most used antioxidants for food, while DG is seldom detected in food (André et al., 2010). DG is widely used as antiaging agent or stabilizer in plastic materials or polymers (Xin et al., 2014; Masek et al., 2014; Belbakra et al., 2017), and it can migrate into the indoor environment from consumer applications and be adsorbed by dust particles due to its strong hydrophobicity (Log Kow of DG = 6.21). The positive association observed for DG concentrations between fingernail and dust samples indicates that the DG accumulated in fingernail may be from the indoor dust. We further did the correlation analysis for samples from young providers (age ≤ 16), who has relatively higher exposure to chemicals through dust ingestion and inhalation due to their higher frequency of hand-to-mouth behavior and larger surface area to weight ratio (Mercier et al., 2011; Wu et al., 2018; Miller et al., 2002). It was found that the correlation for DG concentrations between fingernails from young providers and dusts was better (R = 0.85) than that for the whole sample pool (R = 0.76, Fig. 3), again suggesting that indoor dust made important contribution to DG detected in fingernails.

3.6. Environmental Implications

As a nondestructive biomarker, fingernail has its unique advantages. Assessing the exposure by urine is in very short time scale due to the rapid metabolic rate and consequent excretion of contaminants from urine, while for fingernails, the time window is approximately six months (Alves et al., 2014). Therefore, fingernail can generally indicate long-term exposure in human body. In addition, due to sampling advantages (e.g., cost reduction, less storage, and sample stability), fingernail is considered as a promising matrix for human biomonitoring (Alves et al., 2016; Sukumar, 2005).

In this study, most of the targeted SPAs and their major metabolites were positively detected in human fingernail, suggesting that fingernail may be used as a useful noninvasive biomarker for biomonitoring of SPAs, but further investigations are needed. For example, validation with blood or serum is highly necessary. In addition, there is currently a lack of information about SPA pharmacokinetics in human body. Metabolism of these compounds after their incorporation in the fingernail plate, either from deposition at external exposure or via blood/serum needs to be comprehensively investigated.

4. Conclusions

SPAs and their metabolites were found in human fingernail samples collected from Nanjing, China, and the total concentrations were on the order of several hundreds to a few tens of thousands of nanograms per gram, on a dry-weight basis, which were generally one order of magnitude lower than the total concentrations in indoor dust samples. BHT was the most dominant compound, accounting for 75.7% of total concentrations. No correlations were found for SPAs (except for DG) concentrations between fingernail and dust samples, suggesting that SPAs accumulated in fingernails may not be mainly from indoor dust. To our knowledge, this is the first study to report occurrence of SPAs and their metabolites in human fingernails. Our results suggest that human fingernail can be used as non-destructive biomarker for biomonitoring of SPAs.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2018.11.020.

References


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