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Quantitative Identification of and Exposure to Synthetic Phenolic Antioxidants, including Butylated Hydroxytoluene, in Urine

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Abstract

Synthetic phenolic antioxidants (SPAs) such as 2,6-di-*tert*-butyl-4-hydroxytoluene (butylated hydroxytoluene, BHT), are used in a wide variety of consumer products, including certain foodstuffs (e.g. fats and oils) and cosmetics. Although BHT is considered generally safe as a food preservative when used at approved concentrations, there is debate whether BHT exposure is linked to cancer, asthma, and behavioral issues in children. Little is known with regard to human exposure to SPAs and the methods to measure these chemicals in urine. In this study, six SPAs and the metabolites were analyzed in 145 urine samples collected from four Asian countries (China, India, Japan, and Saudi Arabia) and the United States. BHT was found in 88% of the urine samples at median and maximum concentrations of 1.26 and 15 ng/mL, respectively. BHT metabolites and butylated hydroxyanisole (BHA) were found in 39% to 89% of the urine samples at a concentration range of <LOQ-46 ng/mL. 3,5-Di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH), the major metabolite of BHT, is suggested as a potential urinary biomarker of exposure to BHT. The estimated median daily intake (EDI) of BHT, calculated from urinary concentrations, in children and adults were 0.38–56.6 and 0.21–31.3 μg/kg bw/day, respectively. BHT levels were high in urine samples from Japan, India, and the United States.

Graphical Abstract

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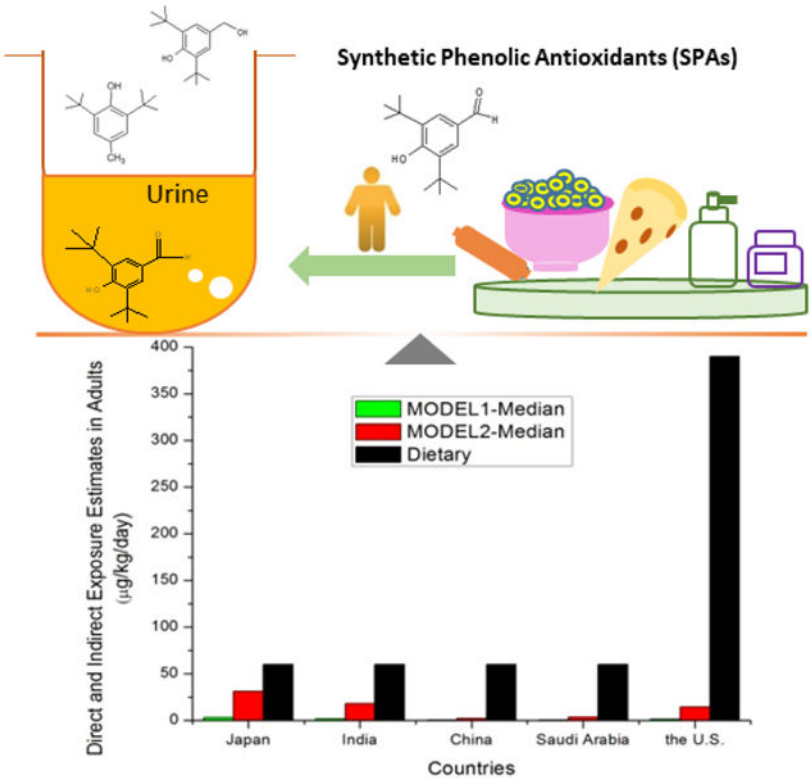
Competing interests

All authors declare that they have no competing interests.



Keywords

Synthetic phenolic antioxidant; BHT; Exposure; Biomonitoring; Biomarker; Urine

1. Introduction

Synthetic phenolic antioxidants (SPAs), such as 2,6-di-*tert*-butyl-4-hydroxytoluene (butylated hydroxytoluene, BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in certain foodstuffs, cosmetics, and plastics since the late 1940s (Wang et al., 2016). As antioxidants, BHT and BHA maintain freshness or prevent spoilage of foods and other products that are incorporated with these compounds. BHT and BHA are generally recognized as safe by the U.S. Food and Drug Administration (USFDA, 1984) when the total content does not exceed 0.02% wet weight (w/w) of the total fat or oil content of the food. Although generally recognized as safe, the widespread occurrence and existence of multiple sources of human exposure are of concern (Nieva-Echevarria et al., 2014). It has been postulated that, owing to their antioxidant and effective radical scavenging properties, BHT and BHA could modify the effects of carcinogens that act by oxidizing biomolecules or producing reactive radicals (Chung, 1999). Studies on the modulating effects of BHT and BHA on known carcinogens have yielded mixed results, depending on the timing of exposure to these antioxidants (WHO, 1986). Toxic effects, including carcinogenicity and reproductive toxicity, of BHT have been reported in animal studies (Clapp et al., 1973; Grogan, 1986; Lindenschmidt et al., 1986; Olsen et al., 1986; Shlian and Goldstone, 1986;

Inai et al., 1988; Takahashi, 1992; Rao et al., 2000; Al-Akid et al., 2001). Similarly, BHA was reported as a tumor promoter, estrogenic disruptor, and carcinogen (Grice et al., 1998; Kahl and Kappus, 1993). The metabolites of BHT have been shown to elicit cellular DNA damage, genotoxicity, and carcinogenicity in animal models (Oikawa et al., 1998). Some BHT metabolites, such as 3,5-di-*tert*-butyl-4-hydroxy-benzaldehyde (BHT-CHO) and 2,6-di-*tert*-butylcyclohexa-2,5-diene-1,4-dione (BHT-Q), were reported to be more toxic than BHT itself (Nagai et al., 1993). Timing and doses of exposure are some important factors that modulate toxicities of these chemicals (WHO, 1986), and the carcinogenic potentials of BHT and BHA are controversial and subject to debate (Hirose et al., 1981; Shirai et al., 1982; William et al., 1990; Bomhard et al., 1992; Iverson, 1995; Whysner et al., 1996; Williams et al., 1999; Botterweck et al., 2000).

Despite the widespread use of SPAs in various consumer products, including dietary products, very little is known about human exposure levels of these chemicals. Sources other than foodstuffs can contribute to BHT exposures, and, therefore, such sources should be taken into account in the assessment of cumulative risks of BHT to humans (Wang et al., 2016). SPAs are metabolized in the human body, and the biological half-lives of parent compounds of SPAs are on the order of several hours to a few days (Verhagen et al., 1989). Two studies have reported the occurrence of SPAs in human specimens (Liu and Mabury, 2018; Li et al., 2019). Prevalence of SPAs in human sera and fingernails has been documented (Liu and Mabury, 2018; Li et al., 2019). Nevertheless, it has been reported that BHT and BHA were excreted predominantly in urine (WHO, 1986). It is prudent to measure SPAs in urine to enable the estimation of total daily exposure doses. To our knowledge, no earlier studies have measured SPAs in urine. This is the first study to determine the occurrence and profiles of BHA, BHT, and four of its metabolites in urine samples collected from the general population in China, India, Japan, Saudi Arabia, and the United States, to delineate baseline exposure levels in Asian countries and the United States. The aims of this study were to describe the occurrence of SPAs in human urine, investigate the geographic distribution in concentrations and profiles of BHT, and estimate exposure doses of SPAs in humans.

2. Materials and Methods

2.1. Sample Collection

Urine samples were collected during the period of 2010–2012 from China ($n = 53$), India ($n = 36$), Japan ($n = 24$), Saudi Arabia ($n = 9$), and the United States ($n = 23$). These were spot urine samples collected in polypropylene (PP) tubes for the analysis of other environmental contaminants, as reported elsewhere (Asimakopoulos et al., 2013; Guo et al., 2011; Honda et al., 2018; Liao et al., 2012). The age of the donors from the five countries ranged from 2 to 87 years. Details with regard to sampling locations, age, sex, and collection years have been reported earlier (Table S1, supplementary information) (Asimakopoulos et al., 2013; Guo et al., 2011; Honda et al., 2018; Liao et al., 2012). All samples were stored in PP tubes at −20 °C until analysis. The New York State Department of Health Institutional Review Board approved the study for the analysis of environmental chemicals in urine.

2.2. Chemicals and Reagents

The target chemicals and their structures are shown in Table S2. BHT, BHT-d21, 2,6-di-*tert*-butyl-4-(hydroxyethyl)phenol (BHT-OH), BHT-Q, BHT-CHO, and 3,5-di-*tert*-butyl-4-hydroxybenzoic acid (BHT-COOH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Isotopically-labeled $^{13}C_{12}$-methyl paraben ($^{13}C_{12}$MeP;- RING-$^{13}C_{12}$, 99%) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). HPLC-grade methanol was supplied by J.T. Baker (Phillipsburg, NJ, USA). Ultra-pure water (18.2Ω) was generated using a Milli-Q system (Millipore, Billerica, MA, USA). β-glucuronidase (from *Helix pomatia*, containing 145,700 U/mL β-glucuronidase and 887 U/mL sulfatase) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Sample Preparation

BHT and four of its metabolites as well as BHA were analyzed in urine samples (2 mL) after enzymatic deconjugation, followed by liquid-liquid extraction. Briefly, two mL of urine sample were transferred into a glass tube, spiked with BHT-d21 (40 ng) and $^{13}C_{12}$-MeP (40 ng) as internal standards (IS), buffered with 300 μL of 1 M ammonium acetate that contained 22 U of β-glucuronidase (prepared by the spiking of 50 μL of β-glucuronidase into 100 mL of 1 M ammonium acetate solution) and incubated at 37 °C for 12 h. After deconjugation, target analytes were extracted with 4 mL of ethyl acetate twice. The mixture was shaken in an oscillator shaker for 60 min (Eberbach Corp., Ann Arbor, MI, USA) and then centrifuged at 2000 × g for 5 min (Eppendorf Centrifuge 5804, Hamburg, Germany). The combined extract was evaporated to 1 mL under nitrogen, 500 μL was taken for the analysis of BHT by gas chromatography-mass spectrometry (GC-MS), and the remaining 500 μL was solvent transferred to methanol for high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis of the total (i.e., free plus conjugated) forms of the remaining analytes.

2.4. Instrumental Analysis

BHT was analyzed, using an Agilent 6890 GC (Agilent Technologies, Inc., Santa Clara, CA, USA) coupled to an Agilent 5975 MS (Agilent Technologies, Inc.) in the selected ion monitoring (SIM) mode. A fused-silica capillary column (DB-5; 15 m × 0.25 mm i.d. × 0.25 μm film thickness) was used for chromatographic separation. The GC oven temperature program was as follows: initial temperature of 70 °C held for 4 min, first ramp at 10 °C min $^{-1}$ to 270 °C and second ramp at 25 °C min^{-1} to 290 °C (held for 10 min). Helium was used as the carrier gas at a flow of 1 mL min^{-1}. Transfer line, MS quadrupole and MS source temperatures were maintained at 290, 150 and 280 °C, respectively. Detection of BHT was carried out by electron ionization (70 eV) in selected ion monitoring (SIM) mode at m/z 220, 205 and 177. Other five SPAs were determined, using an Agilent 1260 HPLC (Agilent Technologies, Inc.) interfaced with an Applied Biosystems QTRAP 4500 mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA, USA). A Betasil® C18 column (100 × 2.1 mm, particle size 5 μm; Thermo Electron Corporation, Waltham, MA, USA) connected to a Javelin Betasil® C18 guard column (20 × 2.1 mm, particle size 5 μm) was used for chromatographic separation of the five compounds. The mobile phase comprised of Milli-Q water (A) and methanol (B), used at a flow rate of 0.2 mL/min with a gradient as follows:

0−1.5 min, 1% methanol; 1.5−3 min, 1−75% methanol; 3−5 min, 75–99% methanol; 5–14 min, 99% methanol; 14−16 min, 99−1% methanol, 16–23 min, 1% methanol. The sample injection volume was 10 μL. The negative ion multiple reaction monitoring (MRM) mode was used in the detection of target chemicals. Nitrogen was used as both a curtain and a collision gas. The MS/MS parameters were optimized by infusion of individual compounds into the MS through a flow injection system (Table S3). The MRM transitions of the target chemicals are listed in Table S4.

2.5. Quality Assurance and Quality Control (QA/QC)

With every set of 20 samples analyzed, a procedural blank, a pair of pre-extraction matrix spikes (standards fortified in samples prior to extraction), a pair of post-extraction matrix spikes (standards fortified in sample extracts after extraction), and duplicate samples were analyzed. Trace levels of BHT-OH (median: 0.01 ng/mL), BHT-CHO (median: 0.03 ng/mL) and BHT- COOH (0.02 ng/mL) were found in procedural blanks. For procedural blanks, 2 mL of water, instead of urine, were used and passed through the entire analytical procedure. A background subtraction was performed when reporting concentrations of these compounds in samples. The procedural blanks also were prepared in PP tubes to assess contamination that could arise from the use of such tubes for sample collection. Recoveries of SPAs in spiked urine matrices ranged from 97 ± 8.4% for BHT-COOH to 108 ± 7.1% for BHA (Table S3). Duplicate analysis of randomly selected samples ($n = 3$) yielded a coefficient of variation of <20% between results. Quantification of SPAs was performed by an isotope-dilution method, based on the response of BHT-d21 (for BHT) and $^{13}C_{12}$-MeP (for BHT-Q, BHA, BHT-OH, BHT-CHO, and BHT-COOH). The limits of quantification (LOQs), calculated at a signal-to-noise ratio of 10, were 0.1 ng/mL for BHA, BHT-Q, BHT-CHO, BHT-COOH, and BHT-OH and 0.2 ng/mL for BHT (Table S3). A midpoint calibration standard (in methanol) was injected to check for instrumental drift in sensitivity after every 20 samples, and a pure solvent (methanol) was injected as a check for carry-over of target chemicals between samples. Instrumental calibration was verified by the injection of 10-point calibration standards at concentrations that ranged from 0.1 to 100 ng/mL for BHA, BHT-OH, BHT-Q, BHT-CHO, BHT-COOH, and BHT in methanol. The linear regression coefficient (using a 1/x weighing factor) of the calibration curve (r^2) was >0.99 for each of the target analytes. In this study, the sum concentrations of six targeted SPAs and the sum of the four metabolites of BHT (BHT-OH, BHT-Q, BHT-CHO, and BHT-COOH) were referred to as Σ_{6SPAs} and $\Sigma_{4metabolites}$, respectively. Concentrations below the LOQ were assigned a value of zero for data analysis.

2.6. Data Analysis

Statistical analyses (correlation analysis, test for normality, and ANOVA) were performed with SPSS Version 16.0. Normality of the data was checked by a Shapiro-Wilk test. Differences between groups were compared, using a one-way ANOVA. Urinary concentrations of SPAs were not normally distributed, and therefore log-transformation was performed to meet the normality assumptions. A probability value of $p \leq 0.05$ was set for statistical significance.

3. Results and Discussion

3.1. BHA, BHT, and BHT Metabolites in Urine

BHT was the most frequently detected SPA in urine samples (detection frequency [DF%]: 88.3%; Table 1) at concentrations that ranged from <LOQ to 15 ng/mL (mean: 1.7 ng/mL, median: 1.26 ng/mL). The highest median concentrations of Σ6SPAs were found in urine samples from India (7.95 ng/mL), Japan (6.47 ng/mL), and the United States (4.24 ng/mL). BHA was present in 39% of the urine samples analyzed at concentrations that ranged from <LOQ to 4.31 ng/mL. Although studies have reported the occurrence of BHT and BHA in certain foods, indoor dust, wastewater, and consumer products (Wang et al., 2016ab; Wang and Kannan, 2017), to the best of our knowledge, this is the first study to report urinary concentrations of SPAs. BHT concentrations in urine samples from the five countries were significantly lower than those reported in fingernail samples at a concentration range of 309–11400 ng/g (mean: 4280, median: 3540 ng/g) (Li et al., 2019), but similar to those reported for sera samples from the United States (range: <LOQ-22.6, geometric mean [GM]: 3.37 ng/mL) (Liu and Mabury, 2018). Higher concentrations of BHT found in fingernails than in serum and urine suggests accumulation of BHT in keratinized tissues (log Kow = 5.10). A slow slow clearance from fingernails has also been suggested (Alves et al., 2016; Sukumar, 2005; Li et al., 2019).

The metabolites of BHT, namely BHT-OH, BHT-Q, BHT-CHO, and BHT-COOH, were detected in 48%, 60%, 60%, and 89%, respectively, of the samples analyzed. BHT-COOH was found at the highest concentrations, with a median value of 1.78 ng/mL, followed by BHT-CHO (0.45 ng/mL), BHT-Q (0.37 ng/mL), and BHT-OH (n.d.). Overall, the concentrations of $\sum_{4\text{metabolites}}$ (median 2.45 ng/mL, mean 4.70 ng/mL) were higher than those of BHT (median 1.26 ng/mL, mean 1.70 ng/mL), suggesting that BHT is transformed quickly in the human body (Wang et al., 2016ab). BHT-COOH (DF%: 80–100%) was the predominant compound found in urine samples from the five countries. Urinary BHT-COOH can be a suitable biomarker of BHT exposure due to its high detection frequency and high measurable concentrations. Further, background contamination of BHT-COOH in sample containers and the analytical procedure is minimal. Nevertheless, it should be noted that the correlation between BHT and BHT-COOH concentrations in urine was weak (Table S5). A significant correlation ($p < 0.05$) existed between BHT and its metabolites BHT-Q and BHT-OH (Table S5), although these two metabolites were found at low concentrations with low detection frequencies. The use of BHT-COOH as a biomarker of BHT exposure has an advantage in that it is a biological metabolite and unlikely to pose an issue of background contamination during collection and analysis of urine. The predominant metabolic pathway of BHT involves oxidation of the 4-methyl group yielding BHT- COOH, which is excreted as free and glucuronidated forms (WHO, 1986).

Oral exposure to BHT yielded BHT-COOH as the major metabolite in urine samples (Lanigan and Yamarik, 2002; WHO, 1986; Holder et al., 1970), and free and glucuronidated BHT-COOH were the major metabolites identified in adult human urine (Holder et al., 1970). Oxidative metabolism (Phase 1 reactions) mediated by microsomal monooxygenase system is the major route of BHT biotransformation, with oxidation as occurring in the *tert*-

butyl group (Conning and Philips, 1986). Tissue accumulation is also suggested for BHT (Conning and Philips, 1986). Because the concentrations of BHT metabolites were similar to those of the parent compound, for the assessment of exposure doses, it is important to measure the concentrations of both parent compounds and metabolites. Overall, BHT-COOH is an important biomarker of BHT exposure, and inclusion of this metabolite in biomonitoring studies will enable better understanding of BHT exposure.

3.2. Daily Exposure Dose

The concentrations of BHT metabolites measured in urine were converted into an exposure dose of the parent compound, using a simple first-order pharmacokinetic model, based on the fraction of the dose excreted as the metabolite (BHT-COOH) in relation to the ingested amount of BHT. The measured median and maximum concentrations of BHT-COOH were applied to estimate median and high BHT exposure doses (Table 2). The European Food Safety Authority (EFSA) recommended an acceptable daily intake (ADI) of 0.25 mg/kg bw/d for BHT. This ADI value was based on hepatic enzyme induction in two independent two-generation studies in rats (Olsen et al., 1986; Price, 1994; EFSA, 2012). The body weight-normalized exposure doses of BHT were estimated based on an average body weight (BW) of 70 kg for adults and 15 kg for children (US EPA, 2011). Based on the urinary concentrations of BHT-COOH and a simple steady-state exposure model, as shown in Eq. 1, human exposure doses of BHT were estimated for the five countries studied.

$$EDI = \frac{CV}{BW} \times \frac{1}{p} \quad \text{(Eq. 1)}$$

where EDI is the estimated total daily intake of BHT (μg/kg bw/day), C is the urinary BHT-COOH concentration (μg/L), V is the human daily excretion volume of urine (L/day), and p is the fraction of the dose excreted as BHT-COOH in relation to the ingested amount of BHT. For V, we used an average urine excretion volume of 0.66 and 1.7 L/d for children and adults, respectively (Remer et al., 2002; Perucca et al., 2007); for p, the values of 3% (model 1) and 0.3% (model 2) were used based on two human exposure studies (Daniel et al., 1968; Wiebe et al., 1978). Studies of the metabolism and excretion of BHT in humans showed that the percentage of the dose excreted as BHT-COOH was 3% (in MODEL 1) (Daniel et al., 1968) or 0.3% (in MODEL 2) (Wiebe et al., 1978). The EDIs of BHT for the five countries studied here were in the range of 0.38–613 μg/kg bw/day for children and 0.21–372 μg/kg bw/day for adults (Table 2). Using a p value of 3% (MODEL 1), the respective median EDI values of BHT (μg/kg bw/day) for Japan, India, China, Saudi Arabia, and the United States were 5.66, 3.29, 0.38, 0.65, and 2.61 for children and 3.12, 1.81, 0.21, 0.36, and 1.44 for adults. The highest respective EDI values of BHT (μg/kg bw/day) for Japan, India, China, Saudi Arabia, and the United States were 35.8, 35.2, 7.30, 8.27, and 67.5 for children and 19.8, 19.4, 4.03, 4.57, and 37.2 for adults. Using a p value of 0.3% (Model 2), the median EDI values of BHT for adults (2.07–31.3 μg/kg bw/day) and children (3.75–56.6 μg/kg bw/day) were below the ADI values recommended by the EFSA, whereas the highest EDI values for children in Japan (325 μg/kg bw/day), India (320 μg/kg bw/day), and the United States (613 μg/kg bw/day) were above the ADI values. The EDIs of BHT for populations in

Japan (56.6 and 31.3 μg/kg bw/day for children and adults) were the highest among the five countries, possibly due to high exposure from dietary sources and/or cosmetics. Although there are uncertainties associated with these estimates, our preliminary results suggest the need for identifying sources of exposure to BHT in Japan, India, and the United States.

3.3. Sources of BHT Exposure

A few studies have reported that dietary intake and indoor dust ingestion are important sources of BHT exposure. The mean dietary intakes of BHT for the general population in South Korea and the United States were 40 and 390 μg/kg bw/day, respectively (Nieva-Echevarria et al., 2015; JECFA, 2000). A general dietary dose of BHT was calculated based on the intake estimates reported for several countries (0.02–0.1 mg/kg bw/day for Australia, Brazil, China, Finland, France, Japan, New Zealand, Spain, the United Kingdom, and the United States), and a value of 0.06 mg/kg bw/day was derived (JECFA, 2000). Wang et al. (2016a) reported that exposure dose of BHT through indoor dust ingestion was in the range of 0.4–222 ng/kg bw/day for populations in 12 countries. The total BHT exposure dose calculated from urinary concentrations was much higher (~10^3-10^4 times) than those calculated from indoor dust ingestion but comparable to the values estimated from dietary intake (Figure 1). For countries such as India and Japan, diet and dust accounted for only half of the total exposures, suggesting the existence of other sources of BHT. Dietary exposure, however, appears to be the predominant route of BHT exposure in China, Saudi Arabia, and the United States. The cumulative exposure doses calculated from dietary sources were higher than those estimated from urinary concentrations, which suggests potential accumulation of BHT in human tissues.

It should be noted that uncertainties exist in our calculations on exposure doses, as several pharmacokinetic models and assumptions were employed in these calculations. Further, the sample size from each country studied is limited, and, therefore, measured concentrations may not represent the population in the entire country. The spot urine samples analyzed in this study may not represent cumulative exposure over time due to temporal fluctuations in BHT exposures. Potential degradation of SPAs during prolonged storage of urine may underestimate the actual exposure levels. Significance of internal standard selection, β-glucuronidase and arylsulfatase activity in the analysis of total urinary concentrations of SPAs requires further validation. Nevertheless, our study presents novel and baseline information on a class of chemicals for which no exposure information existed.

4. Conclusions

This study demonstrates universal exposure to BHT by populations across several countries. Although diet is thought to be the main contributor to BHT exposure, other pathways, such as indoor air inhalation, dust ingestion, and dermal exposure from personal care products, cannot be ruled out. BHT-COOH was the major metabolite found in 80–100% of the urine samples. Elevated exposures to BHT were noted in countries such as Japan, India, and the United States. Estimated daily intake of BHT was in the range of 0.21–372 μg/kg bw/day for adults and 0.38–613 μg/kg bw/day for children, with some of the exposure doses exceeding the ADI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Highlights

- Synthetic phenolic antioxidants were measured in human urine for the first time.
- 3,5-di-tert-butyl-4-hydroxybenzoic acid is a biomarker of BHT in urine.
- The highest BHT exposure was found in samples from Japan, India and the U.S.
- Besides diet, several other sources contribute to BHT exposure.

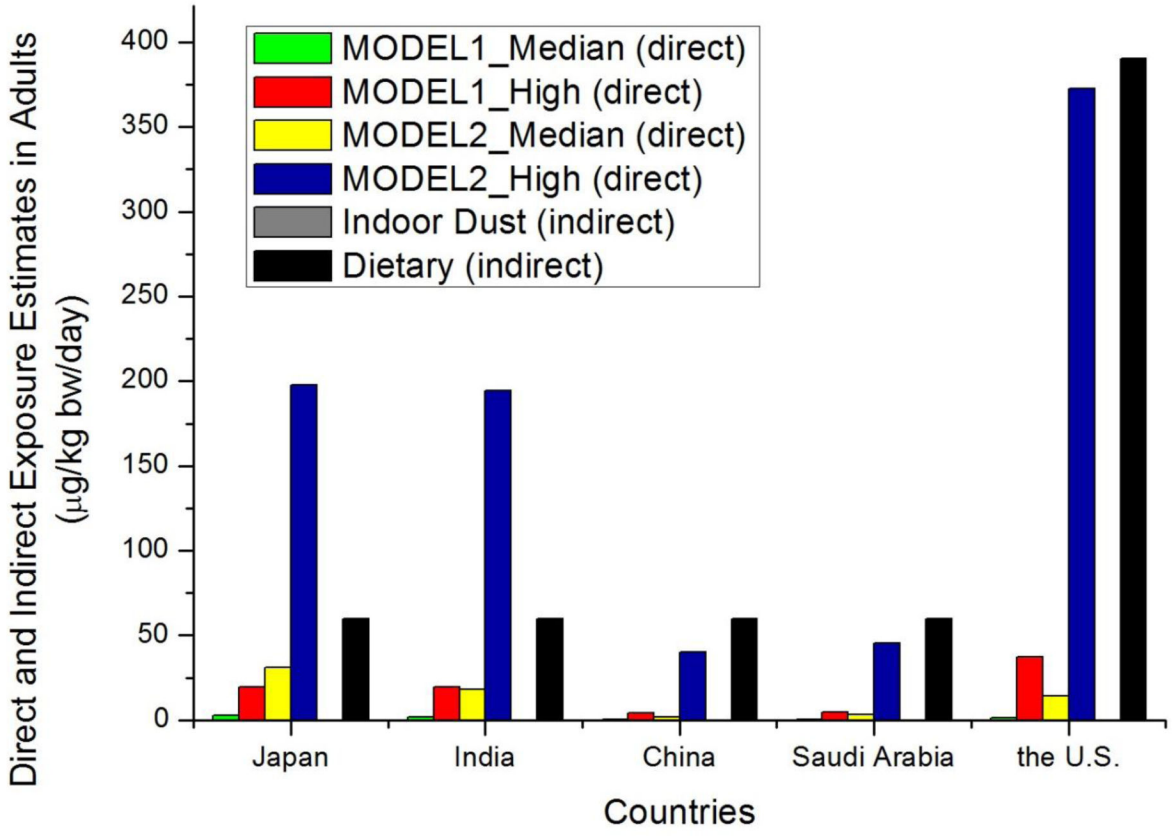

Figure 1.

Comparison of BHT exposure sources in five countries. Direct total exposure dose estimated from urinary concentrations were compared with diet (indirect) and dust (indirect) ingestion doses.

Table 1.

Concentrations of synthetic phenolic antioxidants (SPAs) and their metabolites in human urine samples from five countries (unit: ng/mL).

		BHT-OH	BHT-Q	BHT-CHO	BHA	BHT-COOH	BHT	$\sum_{4metabolites}$	$\sum_{6SPAs}$
Japan n=24	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.31
	Max	0.25	1.54	2.15	0.66	24.4	4.29	25.5	28.6
	DF%	12.5	8.3	91.7	4.2	91.7	75.0	91.7	100.0
	Mean	n.d.	n.d.	0.94	n.d.	5.49	1.47	6.55	8.06
	Median	n.d.	n.d.	0.84	n.d.	3.86	1.40	4.70	6.47
India n=36	Min	n.d.	n.d.	n.d.	n.d.	n.d.	0.03	n.d.	n.d.
	Max	3.92	2.94	1.48	3.43	24.0	15.0	27.4	36.8
	DF%	73.5	75.8	5.7	34.3	94.3	91.4	68.6	88.6
	Mean	0.63	0.66	n.d.	0.35	5.15	3.82	5.05	9.22
	Median	0.49	0.59	n.d.	n.d.	2.24	2.14	2.94	7.95
China n=53	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Max	1.31	1.85	2.51	4.31	4.98	8.42	8.17	8.15
	DF%	49.0	63.3	70.6	44.0	80.4	86.8	66.0	96.2
	Mean	0.18	0.45	0.66	0.33	0.56	1.48	1.69	3.55
	Median	n.d.	0.40	0.61	n.d.	0.26	1.26	1.82	3.73
Saudi Arabia n=9	Min	n.d.	n.d.	0.05	n.d.	0.11	0.01	0.47	0.98
	Max	1.39	1.43	1.21	0.29	5.64	1.21	5.79	6.09
	DF%	55.6	66.7	88.9	66.7	100.0	44.4	100.0	100.0
	Mean	0.26	0.30	0.39	0.14	1.46	0.40	2.42	2.95
	Median	0.11	0.16	0.29	0.15	0.44	0.20	1.98	2.67
the U.S. n=23	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.21
	Max	0.50	1.20	0.69	2.41	46.0	4.29	46.4	47.3
	DF%	39.1	82.6	73.9	59.1	95.7	95.8	95.8	100.0
	Mean	n.d.	0.39	0.24	0.47	7.44	1.33	7.77	9.53
	Median	n.d.	0.37	0.19	0.15	1.78	1.09	2.45	4.24
Total n=145	Min	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Max	3.92	2.94	2.51	4.31	46.0	15.0	46.4	47.3
	DF%	47.5	60.1	59.9	38.6	89.4	88.3	77.9	95.9
	Mean	0.23	0.38	0.56	0.26	4.02	1.70	4.70	6.66
	Median	n.d.	0.37	0.45	0.10	1.78	1.26	2.45	4.24

n.d.: not detected or below LOQ.

Table 2.

Estimated total daily exposure dose to BHT measured from urinary concentrations in five countries (μg/kgbw/day); compared with exposure doses from dietary and dust ingestion; median and high exposure scenarios were employed.

		Reference Dose[a]	unit	Japan		India		China		Saudi Arabia		the U.S.	
				Median[d]	High[e]	Median	High	Median	High	Median	High	Median	High
MODEL 1	Children	250 μg/kg/day	μg/kg/day	5.66	35.8	3.29	35.2	0.38	7.30	0.65	8.27	2.61	67.5
MODEL 1	Adults	250 μg/kg/day	μg/kg/day	3.12	19.8	1.81	19.4	0.21	4.03	0.36	4.57	1.44	37.2
MODEL 2	Children	250 μg/kg/day	μg/kg/day	56.6	325	32.9	320	3.75	66.4	6.48	75.2	26.1	613
MODEL 2	Adults	250 μg/kg/day	μg/kg/day	31.3	198	18.1	194	2.07	40.3	3.58	45.7	14.4	372
Dust[b]	Children		ng/kg/day	31.3		3.6		10.9		5.6		7.4	
Dust[b]	Adults		ng/kg/day	13.9		1.6		4.9		2.5		2.6	
Dietary[c]	Adults		μg/kg/day	60		60		60		60		390	

[a]Reference doses of EFSA (EFSA, 2012)

[b]Daily intake dose data at 95UCL (95% Upper Confidence Limits) were applied (Wang et al., 2016a)

[c]A dietary intake value of 0.06 mg/kg/day of BHT was estimated based on the dietary intake estimates reported for several countries (0.02–0.1 mg/kg/day) (JECFA, 2000), except for the dietary intake value for population in the U.S. (390 μg/kg/day) (Nieva-Echevarria et al., 2015; JECFA, 2000).

[d]Median exposure levels were estimated based on the median concentrations of BHT in the urine samples of targeted population.

[e]High exposure levels were estimated based on the maximum concentrations of BHT in the urine samples of targeted population.