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Use of pooled samples to assess human exposure to parabens, benzophenone-3 and triclosan in Queensland, Australia

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ABSTRACT

Parabens, benzophenone-3 and triclosan are common ingredients used as preservatives, ultraviolet radiation filters and antimicrobial agents, respectively. Human exposure occurs through consumption of processed food and use of cosmetics and consumer products. The aim of this study was to provide a preliminary characterisation of exposure to selected personal care product chemicals in the general Australian population. De-identified urine specimens stratified by age and sex were obtained from a community-based pathology laboratory and pooled ($n = 24$ pools of 100). Concentrations of free and total (sum of free plus conjugated) species of methyl, ethyl, propyl and butyl paraben, benzophenone-3 and triclosan were quantified using isotope dilution tandem mass spectrometry; with geometric means 232, 33.5, 60.6, 4.32, 61.5 and 87.7 ng/mL, respectively. Age was inversely associated with paraben concentration, and females had concentrations approximately two times higher than males. Total paraben and benzophenone-3 concentrations are significantly higher than reported worldwide, and the average triclosan concentration was more than one order of magnitude higher than in many other populations. This study provides the first data on exposure of the general Australian population to a range of common personal care product chemical ingredients, which appears to be prevalent and warrants further investigation.

1. Introduction

The type, concentration and use of chemical ingredients in personal care products are many and varied. Parabens are alkyl esters (e.g., methyl (MeP), ethyl (EtP), propyl (PrP), butyl (BuP)) of p-hydroxybenzoic acid, used widely as antimicrobial preservatives in cosmetics, pharmaceuticals and processed food products (Eriksson et al., 2008; Shen et al., 2007; Soni et al., 2005) due to their stability, high water solubility, and low cost. Exposure occurs primarily via dermal absorption (Soni et al., 2005) and after metabolism, excretion occurs largely via urine. Results from in vitro and in vivo experiments suggest estrogenic activity of MeP, EtP, PrP and BuP (Boberg et al., 2010; Darbre and Harvey, 2008), but several orders of magnitude lower than that of oestrogen. Adverse reproductive effects of BuP and PrP have been reported in some animal studies (Oishi, 2002a,b) but not others (Hoberman et al., 2008), with some association with sperm damage (Meeker et al., 2011) and altered thyroid hormones (Koeppe et al., 2013) in humans.

Benzophenone-3 is a broadband ultraviolet radiation filter used as a sunscreen and photostabiliser in various cosmetic products worldwide; dermal contact is the dominant exposure pathway (Kim and Choi, 2014; Liao and Kannan, 2014). Urinary benzophenone-3 is used as the primary biomarker of exposure (Wang and Kannan, 2013). Benzophenone-3 exhibits slight estrogenic potential, and there is evidence for influence on reproduction and sex hormone signalling in rodents (Kim and Choi, 2014), and for influence on hormone-dependent diseases and adverse birth outcomes in humans (Kunisue et al., 2012; Wolff et al., 2008).

Triclosan is a synthetic, broad spectrum antimicrobial agent used in a wide range of personal care products and other consumer items (Rodricks et al., 2010; Witorsch and Thomas, 2010), and exposure mainly occurs through dermal application or oral use of consumer products containing triclosan. Triclosan is rapidly metabolised and excreted as conjugated urinary metabolites. The endocrine disrupting potential of triclosan is under debate (Huang et al., 2014; Jung et al., 2012; Lee et al., 2014; Witorsch, 2014; Witorsch and Thomas, 2010), and recent evidence suggests liver carcinogenicity (Yueh et al., 2014). Furthermore,

Abbreviations: MeP, methyl paraben; EtP, ethyl paraben; PrP, propyl paraben; BuP, butyl paraben; CDC, Centers for Disease Control and Prevention, United States; LOD, limit of detection; ng/mL, nanograms per millilitre; GM, geometric mean; NHANES, National Health and Nutritional Examination Survey; ICC, intraclass correlation coefficient.

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concern has been raised as to the development of triclosan-resistant pathogens due to widespread use (Aiello and Larson, 2003; Levy, 2001).

Human exposure to chemicals used in personal care products occurs as a result of the frequent and complex use of such products, and biomonitoring is regarded as the gold standard for exposure assessment (Sexton et al., 2004). Biomonitoring data of the prevalence of exposures to chemicals used in personal care products exists for Northern European (Den Hond et al., 2013; Dewalque et al., 2014; Frederiksen et al., 2014; Moos et al., 2014; Pirard et al., 2012), North American (Calafat et al., 2008a,b, 2010; CDC, 2015, Health Canada, 2013; Meeker et al., 2013; Wang et al., 2013) and South East Asian (Kim et al., 2011; Ma et al., 2013; Shirai et al., 2013) populations, but the extent of Australians' exposure to personal care product chemicals is unknown. As biomonitoring is expensive pooling is an affordable alternative. The suitability of pooled biological samples for monitoring temporal and spatial trends in exposure has been demonstrated (Heffernan et al., 2013, 2014a,b). The aim of this study was to provide a preliminary characterisation of exposure to selected personal care product chemicals in the general Australian population using pooled urine specimens.

2. Materials and methods

2.1. Study population and sample collection

De-identified urine specimens were obtained from a community-based pathology laboratory (Sullivan Nicolaides Pathology, Taringa, QLD, Australia) from surplus stored urine that had been collected and analysed as part of routine testing throughout the state of Queensland, Australia. Urine specimens were collected from November 2012 to November 2013 in sterile polyethylene urine specimen containers, refrigerated for up to three days, and then frozen. As this was a pre-existing, convenience population no specific sampling protocols were employed. This work was approved by the University of Queensland ethics committee (approval number 2013000397). The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

2.2. Pooling protocol

Descriptive information about each specimen was limited to date of sample collection, sex, and date of birth of the individual. Before pooling, samples were stratified by age and sex into the following strata: 0–4, 5–14, 15–29, 30–44, 45–59, >60 years. The mean age of each pool was calculated from the average age of the individuals making up that pool. A total of 2400 individual specimens were combined into 24 pools, with 100 individual specimens contributing to each pool; there was a replicate pool for each of the 12 demographic groups. Specimens were pooled based on volume, where each individual in the pool contributed the same volume, thus the concentration measured in each pool is equivalent to the arithmetic mean of the concentration in each individual sample contributing to the pool (Caudill, 2010; Mary-Huard, 2007). During pooling, individual urine specimens were thawed, homogenised and aliquoted, after which the pooled sample was well-mixed, divided into smaller aliquots and frozen until analysis. A synthetic urine sample was included as a procedural blank (Calafat and Needham, 2009). No measures of creatinine or specific gravity were available for individual samples.

2.3. Chemical analysis

Concentrations of the free and total (sum of free and conjugated) species of MeP, EtP, PrP and BuP and benzophenone-3 and triclosan in urine were measured at the CDC (Atlanta, USA) using online solid phase extraction-high performance liquid chromatography isotope dilution tandem mass spectrometry as described previously (Ye et al., 2005,2006). Concentrations of free species were measured using the same methodology, but omitting the enzymatic hydrolysis. To monitor for accuracy and precision, each analytical run included calibration standards, reagent blanks, and quality control materials of high and low concentrations. The limits of detection (LOD) were 1 ng/mL (MeP, EtP, triclosan), 0.2 ng/mL (benzophenone-3), and 0.1 ng/mL (PrP and BuP). We did not detect the target compounds in the synthetic urine sample.

2.4. Statistical analysis

The influence of age (in years) and sex on chemical concentration was assessed via linear regression on ln-transformed urinary concentration, as follows:

$$Ln(concentration) = I + \beta_1 * Age + \beta_2 * Sex. \quad (1)$$

An interaction term between age and sex was included in the models where significant. We summed the concentrations of the four parabens to create a summary measure ($\sum$paraben). All analyses were conducted using IBM SPSS Statistics, version 22 for Windows, (IBM, New York, USA, www.ibm.com). Criteria for significance were set as $p < 0.05$. Outliers in the ln-transformed values were identified using the outlier labelling rule (Hoaglin et al., 1986; Hoberman et al., 2008).

3. Results

3.1. Parabens

Results for MeP, EtP, PrP, BuP and $\sum$paraben concentrations for samples pooled by age and sex ($n = 24$) are summarised in Table 1. MeP, EtP, PrP and BuP were detected in all samples predominantly in their conjugated form (78–100%, Table SI-1) at total concentrations ranging from 74.4–1180, 6.3–802, 10.2–530 and 0.8–227 ng/mL, respectively; geometric means (GM) were 232 ng/mL (MeP), 33.5 ng/mL (EtP), 60.6 ng/mL (PrP), and 4.3 ng/mL (BuP). $\sum$paraben total concentration ranged from 114 to 1650 ng/mL, with GM 356 ng/mL. One pooled sample (pool 10, Table 1) had relatively high total concentrations of EtP (802 ng/mL), PrP (530 ng/mL) and BuP (227 ng/mL) compared with other pools in the same age strata. These values were identified as statistical outliers for EtP and BuP, but not for PrP. Omission of the outliers yields GMs of 29.1 ng/mL and 3.64 ng/mL for EtP and BuP, respectively..

There was a small but significant inverse association between age and total concentration for MeP ($p = 0.0001$), EtP ($p = 0.008$) and PrP ($p = 0.0004$), but not BuP (Table 2). When the outlier was removed, the strength of the association increased, and age became a significant predictor of BuP concentration ($p = 0.007$) (Table SI-2). Female pools had MeP total concentrations 1.8 times higher than male pools. There were no significant differences between male and female pools for EtP and PrP. For BuP sex was significant only if the outlier was omitted, with total concentrations in the female pools being 2.3 times higher than in male pools.

3.2. Benzophenone-3 and triclosan

Benzophenone-3 was detected in all pooled samples; total concentrations ranged from 16.5 ng/mL to 312 ng/mL (GM = 61.5 ng/mL) across all strata (Table 1). There was a significant interaction between age and sex in the multivariate model ($p = 0.007$, Table SI-3), with the highest total concentrations found in older females' pools. When the male and female data were examined separately, there was a small effect of age on concentration for female pools ($p = 0.019$) but not for male pools.

Triclosan was detected in all samples at total concentrations ranging from 24.1 ng/mL to 205 ng/mL (GM = 87.7 ng/mL, Table 1). Triclosan