International Journal of Hygiene and Environmental Health 217 (2014) 845–853

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Rapid determination of nine parabens and seven other environmental phenols in urine samples of German children and adults

Rebecca K. Moos[a], Jürgen Angerer[a], Jürgen Wittsiepe[b], Michael Wilhelm[b], Thomas Brüning[a], Holger M. Koch[a,*]

[a] Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of Ruhr University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, D-44789 Bochum, Germany
[b] Department of Hygiene, Social and Environmental Medicine, Ruhr University Bochum, Universitätsstrasse150, D-44780 Bochum, Germany

ARTICLE INFO

Article history:
Received 26 February 2014
Received in revised form 13 June 2014
Accepted 13 June 2014

Keywords:
Human biomonitoring
Internal exposure
Urine
Parabens
Environmental phenols

ABSTRACT

We developed a fast, selective and sensitive on-line LC/LC–MS/MS method for the simultaneous determination of nine parabens and seven environmental phenols in urine. Parabens are widely used as antimicrobial preservatives. Bisphenol A, triclosan, triclocarban, 2-phenylphenol, and benzophenones are used inter alia in disinfectants, sunscreens and in polymers. Some of these substances are suspected endocrine disruptors.

Limits of quantification and analytical quality criteria fully met the needs for determining exposure levels occurring in the general population. We analyzed 157 spot urine samples from the general German population (59 females, 39 males and 59 children). For the parabens, we found methyl, ethyl and *n*-propyl paraben with high detection rates (77–98%), followed by *n*-butyl (36%), *iso*-butyl (17%), *iso*-propyl (3%) and benzyl paraben (3%). We detected no pentyl and heptyl paraben. Urinary concentrations were highest for methyl paraben (median 24.5 μg/L; 95th percentile 379 μg/L) followed by ethyl (1.4 μg/L; 35.2 μg/L) and *n*-propyl paraben (1.2 μg/L; 68.1 μg/L). Other environmental phenols with high detection rates were BPA (95%), triclosan (45%) and benzophenone 1 and 3 (26%). For most of the parabens/environmental phenols we found higher urinary levels in females than in males or children, probably due to differences in (personal care) product use. However, high levels (in the mg/L range) were also observed in children.

Exposure to the above substances is occurring worldwide. Differences between countries do seem to exist and might be caused by different product compositions or different use habits. Human metabolism data is urgently needed to extrapolate from urinary biomarker levels to doses actually taken up.

Introduction

Because of the ubiquitous presence and the extensive use of environmental phenols and parabens in personal care and consumer products, food and beverage processing, pharmaceuticals and disinfectants humans are constantly exposed to these substances. Esters of *p*-hydroxybenzoic acid (parabens) are widely used as antimicrobial preservatives in cosmetics, pharmaceuticals and food for more than 50 years (Guo and Kannan, 2013; Soni et al., 2005; SCCS, 2010). Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; TCS) is a broad spectrum antibacterial agent used in personal care products, including toothpaste, mouthwash, deodorants (Calafat et al., 2008a; Ye et al., 2008; Dann and Hontela, 2011; SCCP, 2009). Triclocarban (1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)urea; TCC) is employed as an antimicrobial agent in personal care products including bar soap, detergents, toothpaste, deodorant, and cleansing lotions (Ye et al., 2011; SCCP, 2005). 2-phenylphenole (2-PP) is used as active ingredient in broad spectrum fungicides used in wood preservation and as a surface biocide. Furthermore, it is used for inhibition of mold growth on citrus fruits (Bomhard et al., 2002; US EPA, 2006). Bisphenol A (4,4′-(propane-2,2-diyl)diphenol); BPA) is used to manufacture polycarbonate plastics and epoxy resins. Polycarbonate is a plastic widely used in articles we are in contact trough everyday life, e.g., such as tableware, storage containers, returnable bottles and dental sealants and composites, and thermal receipt paper (Frederiksen et al., 2013a; EFSA, 2006). Benzophenone-3 (2-hydroxy-4-methoxybenzophenone; BP-3) and its metabolites (Wang and Kannan, 2013b) Benzophenone-1 (2,4-dihydroxybenzophenone; BP-1) and benzophenone-8

* Corresponding author. Tel.: +49 0234 302 4647; fax: +49 0234 302 4505.
E-mail address: koch@ipa-dguv.de (H.M. Koch).

http://dx.doi.org/10.1016/j.ijheh.2014.06.003
1438-4639/

(2,2′-dihydroxy-4-methoxybenzophenone; BP-8) are sunscreen agents used in cosmetics, including sun creams and anti-aging products, and also in plastic surface coating for food packaging (Calafat et al., 2008b; SCCP, 2006). BP-1 and BP-8 are not authorized as additives in Europe.

The above phenolic substances are currently under intensive scrutiny because of their possible endocrine disrupting potency both alone and in combination (Schlumpf et al., 2001; Krause et al., 2012; Christiansen et al., 2012; Isling et al., 2013). In regard to parabens, *in vitro* studies have shown the potential for endocrine modifying effects with estrogenic activity increasing with length of the alkyl side chain (Darbre and Harvey, 2008; Boberg et al., 2010). All parabens can be regarded as estrogenic *in vivo* due to their uterotrophic effects, with LOELs for butyl- and propylparaben of 7 and 20 mg/kg bw/day (Lemini et al., 2003), respectively. Additionally, reproductive toxicity studies in rodents have shown effects on sperm count and testosterone levels after dietary exposure to butyl- and propyl paraben (Oishi, 2001, 2002a,b). Overall, the data basis for a toxicological evaluation of the parabens is still insufficient. BPA and TCS have also been reported to have weak estrogenic effects (Dann and Hontela, 2011; Clayton et al., 2011; Henry and Fair, 2013; Jung et al., 2012; Richter et al., 2007). Several studies also report low dose effects of BPA on sexual development (Christiansen et al., 2013; Vandenberg et al., 2010). Additionally, effects of both chemicals on thyroid function have been reported (Axelstad et al., 2013; Chevrier et al., 2013). BP-3 and phenylphenols are regarded to have weak estrogenic or anti-androgenic properties (Calafat et al., 2008b; Krause et al., 2012; Li et al., 2010). TCC is suspected to have a weak androgen effect (Ahn et al., 2008; Chen et al., 2008; Duleba et al., 2011).

Because of the widespread use of these phenolic compounds, their presence in personal care products, in foodstuff (intended or unintended) and in other applications, exposures will occur in an aggregate manner and via multiple routes of uptake, i.e., through dermal absorption, ingestion, and inhalation. To determine the exposure to such chemicals via classical means of exposure assessment (measurements in environmental media, personal care products and foodstuff; collection of questionnaire data on personal lifestyle, product use and food consumption; estimations of contact times and incorporated quantities) can be cumbersome and prone to errors (due to lack of knowledge, e.g., on relevant sources and pathways). Human biomonitoring, the determination of chemicals or their metabolites in human tissues like urine as an integral measure of exposure, allows assessing exposures even when the quantity and quality of external exposures are unknown or ambiguous. Urinary concentrations of several of the above compounds (or their metabolites) have already been successfully used as biomarkers of internal exposure (Ye et al., 2006). Various studies have shown that the general population is exposed to parabens and other environmental phenols (Frederiksen et al., 2013b, 2014; Asimakopoulos et al., 2014a,b; CDC, 2013; Calafat et al., 2010; Casas et al., 2011; Li et al., 2013; Pirard et al., 2012; Philippat et al., 2012; Ye et al., 2011; Wang et al., 2013).

In this work we present a fast, robust and reliable LC/LC–MS/MS method with isotope dilution for the determination of nine parabens (methyl- (MeP), ethyl- (EtP), *n*-propyl- (*n*-PrP), *n*-butyl- (*n*-BuP), benzyl- (BeP), pentyl- (PeP) and heptyl paraben (HeP); including *iso*-butyl- (*iso*-BuP) and *iso*-propyl paraben (*iso*-PrP)) and seven environmental phenols (BPA, 2-PP, BP-1, BP-3, BP-8, TCS and TCC) in urine covering a wide concentration range. With this method we can thus assess exposures resulting from environmental background exposures, personal care product use and occupational exposures. We applied this method on urine samples of children and adults from the general German population and for the first time provide internal exposure data for this set of chemicals in Germany.

Experimental

Materials

Methyl-, ethyl-, *n*-propyl-, *n*-butyl-, *iso*-butyl- and benzyl paraben (esters of *p*-hydroxybenzoic acid), triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol; TCS), triclocarban (1-(4-chlorophenyl)-3-(3,4-dichlorophenyl)urea; TCC), 2-phenylphenol, bisphenol A (4,4′-(propane-2,2-diyl)diphenol, BPA), benzophenone-1 (2,4-dihydroxybenzophenone, BP-1), benzophenone-3 (2-hydroxy-4-methoxybenzophenone, BP-3), benzophenone-8 (2,2′-dihydroxy-4-methoxybenzophenone, BP-8) were purchased from Sigma–Aldrich (Steinheim, Germany). *Iso*-propyl- and heptyl paraben were purchased from TCI Europe (Eschborn, Germany). Pentyl paraben was purchased from VWR (Darmstadt, Germany). The deuterated standards d_4-methyl-, d_4-ethyl-, d_4-propyl-, d_4-butyl- and d_4-benzyl paraben, d_3-triclosan, d_4-triclocarban, d_{16}-bisphenol A and d_5-benzophenone-3 were purchased from C/D/N Isotopes (Dr. Ehrenstorfer GmbH, Augsburg, Germany). $^{13}C_6$-2-phenylphenol was purchased from Cambridge Isotope Laboratories (Wesel, Germany). All standards had a purity ≥95%. The deuterated compounds had no detectable impurities of the unlabeled or partially labeled compounds. Water and acetonitrile (LC/MS grade) and acetic acid (glacial, extra pure) were purchased from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). β-Glucuronidase enzyme Type HP-2 and ammonium acetate p.a. were purchased from Sigma–Aldrich (Steinheim, Germany).

Analytical method

Preparation of stock solutions

Standard stock solutions were prepared by dissolving 10 mg of the standards in 10 ml acetonitrile (1 g/L). Stock solutions were stored at −20 °C until further use. For further analysis, eight calibration standards were prepared by diluting the stock solutions to final concentrations in a range between 0.5 μg/L and 300 μg/L water. The stock solutions for the internal standards were prepared by dissolving 5 mg of each labeled standard in 5 mL acetonitrile (1 g/L). These stock solutions were used to prepare an internal standard mixture with a concentration of 0.275 mg/L acetonitrile.

Sample preparation

Urine samples were stored in 250 mL polyethylene containers at −20 °C. Before analysis all samples were equilibrated to room temperature and homogenized by shaking thoroughly. Aliquots of 300 μL urine were transferred into 1.8 mL glass screw-cap vials. Each sample was spiked with 300 μL 1 M ammonium acetate buffer at pH 5.0, 25 μL internal standard solution and 6 μL β-glucuronidase/arylsulfatase solution (≥100,000 U/mL) for the hydrolysis of the conjugated species. Thus, the total concentration (free and conjugated species) of each target analyte was measured. After incubation in a water bath at 37 °C for 3.5 h, all samples were frozen overnight to precipitate cryophobic proteins, subsequently thawed and centrifuged (3500 × *g* for 10 min). The supernatant was transferred into a second 1.8 mL screw-cap vial. A volume of 100 μL was injected into the HPLC-system.

Calibration procedure and quality control

Calibration standards (prepared in water), quality controls and blanks (Millipore water) were treated equally to the urine samples (described in Section 'Sample preparation'). Quadratic calibration curves were obtained with a $1/x$ weighting by plotting the quotients of peak areas of each analyte and the peak areas of the specific internal standard as a function of the concentration. Quality control material was prepared from two different pooled urine mixtures. The composition of the urine mixtures was chosen based upon the