Environment International xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Environment International

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

A survey of parabens in commercial pharmaceuticals from China and its implications for human exposure

Wan-Li Ma [a], Xue Zhao [a], Zhong-Yang Lin [a,b], Mohammed O.A. Mohammed [a,c], Zi-Feng Zhang [a], Li-Yan Liu [a], Wei-Wei Song [a], Yi-Fan Li [a,*]

[a] International Joint Research Center for Persistent Toxic Substances (IJRC-PTS), State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China
[b] State Nuclear Electric Power Planning Design & Research Institute, Beijing 100091,China
[c] Faculty of Public and Environmental Health, University of Khartoum, Khartoum 205, Sudan

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 13 July 2016
Accepted 24 July 2016
Available online xxxx

Keywords:
Parabens
Occurrence
Pharmaceutical
Implication
Human exposure

ABSTRACT

Parabens are widely used as antimicrobial preservatives during pharmaceutical production. However, little information is available regarding the occurrence of parabens in commercial pharmaceuticals and their implications for human exposure. In this study, six commonly used parabens were analyzed by ultra-performance liquid chromatography-tandem mass spectrometry with 100 commercial pharmaceuticals collected from China. Almost all of the pharmaceutical samples contained at least one kind of parabens with the detection frequency of 97%. The concentrations of $\Sigma_6$parabens (sum of the six parabens) ranged from below MDL to 1256 ng/g, with mean and median values of 94.8 and 119 ng/g, respectively. Methyl paraben (MeP), ethyl paraben (EtP) and propyl paraben (PrP) were the predominant compounds. Significant positive correlation was observed between concentrations of MeP and PrP, indicating their co-applications in pharmaceuticals. Levels of $\Sigma_6$parabens varied in different categories of pharmaceuticals and increased with their shelf lives. Based on the measured concentrations and daily ingestion rates of pharmaceuticals, the estimated daily intake (*EDI*) of parabens was calculated. The median values of $EDI_{pharmaceutical}$ for male adults, female adults and children were 4.05, 4.75 and 9.73 ng/kg-bw/day, respectively, which were three orders of magnitude lower than those from foodstuffs and personal care products (PCPs). It was firstly reported that the total exposure dose was 0.326 mg/kg-bw/day via foodstuffs, PCPs, and pharmaceuticals for Chinese female adults.

1. Introduction

Endocrine-disrupting compounds (EDCs) are substances that can affect human reproduction and development system (Casals-Casas and Desvergne, 2011; Crisp et al., 1998; Schug et al., 2011; WHO/UNEP, 2013). EDCs comprise a diverse group of chemicals with anthropogenic origin, including organochlorine pesticides, organotins, polychlorinated biphenyls, polybrominated diphenyl ethers, phthalates, bisphenols and alkyl esters of *p*-hydroxybenzoic (parabens) (Liao et al., 2013a; WHO/UNEP, 2013). EDCs can affect human health by interfering with body's ability to produce hormones with their properties being similar to these of estrogen. Source identification, exposure dose estimation, and health risk assessment of EDCs have being attracted more attentions recently.

Parabens are used as preservatives in cosmetics, pharmaceuticals, and foodstuffs (Andersen, 2008; Eriksson et al., 2008; Soni et al., 2005), including methyl- (MeP), ethyl- (EtP), propyl- (PrP), butyl- (BuP), and benzyl-parabens (BzP). Parabens are commonly applied due to their odorlessness, lower dosage, cost-effectiveness and safety (Andersen, 2008; Soni et al., 2005). For example, fifty years ago US Food and Drug Administration allowed usage of parabens as preservatives in foodstuffs and cosmetics. However, parabens were reported with potential characteristics of EDCs recently, and their safety in our daily products has become a public concern (Boberg et al., 2010). Studies have showed that parabens can pose a weak estrogenic activity in vitro and vivo studies (Boberg et al., 2010; Byford et al., 2002; Darbre et al., 2002; Darbre et al., 2003; Lemini et al., 1997; Lemini et al., 2003; Okubo et al., 2001). For general population, the frequent occurrence of parabens in human urine (Ma et al., 2013; Wang et al., 2013; Ye et al., 2006), and adipose tissue was also reported worldwide (Darbre et al., 2004; Harvey and Everett, 2004; Ye et al., 2008), indicating their residue in human body. Therefore, the comprehensive exposure characterization of parabens is necessary to study their health risk to human and to control their pollution in our daily life.

* Corresponding author at: International Joint Research Center for Persistent Toxic Substances (IJRC-PTS), State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, 202 Haihe Road, Nangang District, Harbin 150090, Heilongjiang, China.
E-mail address: ijrc_pts_paper@yahoo.com (Y.-F. Li).

http://dx.doi.org/10.1016/j.envint.2016.07.013
0160-4120/

Parabens are extensively used in our daily products, implying frequent exposure to these compounds with human. Generally, human are exposed to parabens via ingestion with foodstuffs and pharmaceuticals, and dermal absorption with personal care products (PCPs) (El Hussein et al., 2007; Guo and Kannan, 2013; Liao et al., 2013a; Wang et al., 2012). According to literature, occurrence of parabens in foodstuffs and PCPs have been well studied (Baranowska et al., 2014; Guo et al., 2014; Guo and Kannan, 2013; Liao et al., 2013a; Liao et al., 2013b). In a newly published paper, it was found that pharmaceuticals may be an important exposure source of parabens (Dodge et al., 2015). However, little information is available regarding their occurrence in commercial pharmaceuticals (Baranowska et al., 2014; Jaworska et al., 2005; Moreta et al., 2015), and exposure of parabens via pharmaceuticals ingestion in particular.

In this study, six commonly used parabens were analyzed in 100 commercial pharmaceutical samples collected from China in 2015, with the objectives of determining their concentrations in pharmaceuticals and estimating the exposure doses via pharmaceutical ingestion. Furthermore, a comprehensive exposure estimation to parabens via pharmaceuticals, foodstuffs and PCPs was conducted for the first time in order to draw the whole picture for human exposure to parabens.

2. Materials and methods

2.1. Chemicals and reagents

Mixed native standard solution (100 μg/mL, 99% purity), containing MeP, EtP, PrP, BuP, BzP and heptyl-paraben (HepP), was purchased from AccuStandard (New Haven, CT, USA). The molecular structure and basic information for the six parabens can be found in our previous study (Ma et al., 2013). Internal standard solution (100 μg/mL, 99% purity), containing $^{13}C_6$-MeP, $^{13}C_6$-EtP, $^{13}C_6$-PrP and $^{13}C_6$-BuP, was purchased from AccuStandard as well. Formic acid (ACS grade) was purchased from Sigma-Aldrich. All the organic solvents, including methyl *tert* butyl ether (MTBE), methanol and acetonitrile, were LC-MS grade and purchased from J.T. Baker. Ultrapure water was prepared with a Milli-Q ultrapure system. All standards and stock solutions were stored at −20 °C.

2.2. Sample collection

A totally of 100 pharmaceutical samples were collected in Harbin City, China in January 2015, which are all commonly used commercial pharmaceuticals in China (Table S1 in Supporting information). Over-the-counter (OTC) pharmaceuticals were purchased from local pharmaceutical stores, and prescription pharmaceuticals were donated from volunteers, which were purchased from local hospitals. According to their physical states, the pharmaceutical samples were divided into three categories: tablet pharmaceutical ($n = 54$), particle pharmaceutical ($n = 40$) and oral liquid pharmaceutical ($n = 6$) (Table S1). Pill and cream pharmaceuticals were not collected in this study. Samples were also categorized according to their functions & indications (i.e. anti-inflammatory medicine, cold medicine, health protection medicine, intestines and stomach medicine, throat medicine, and others), suitable population groups (adults and children), and their shelf lives for comprehensive comparison in the following sections. The following basic information was summarized in Table S2, including ingestion frequency, ingestion dose, weight per unit and shelf life. Pharmaceutical samples were sealed with aluminum foil and kept in dark in 4 °C before treatment.

2.3. Sample treatment

Solid-liquid extraction and liquid-liquid extraction were applied for solid pharmaceutical samples (tablet and particle) and liquid pharmaceutical samples (oral liquid), respectively. Each solid sample was grounded into powder, and 0.2 g sample was placed in a 12 mL glass tube, and spiked with the internal standards ($^{13}C_6$-MeP, $^{13}C_6$-EtP, $^{13}C_6$-PrP and $^{13}C_6$-BuP: 50 ng each), then equilibrated for 30 min at room temperature. Thereafter, 5 mL MTBE was added to each glass tube. Extraction was performed by a mechanical shaker for 60 min. Subsequently, each extracted sample was centrifuged at 4000 ×*g* for 20 min and the supernatant was transferred into a new glass tube. The same extraction procedure was repeated with an additional 5 mL MTBE. The combined supernatant was concentrated to 200 μL under a gentle nitrogen stream, then 200 μL mixture solvent of methanol and water (V:V, 1:1) was added. Finally, the volume was concentrated to 200 μL under a gentle nitrogen stream again. The final volume of the sample was adjusted to 1.0 mL with mixture solvent of methanol and water (V:V, 1:1) in a 1.5 mL brown sample injection vial. For liquid sample, 2 g (~2 mL) sample was used for liquid-liquid extraction, and the extraction procedure was the same to that for solid sample.

2.4. Instrumental analysis

Analysis of the six parabens was carried out using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS, Waters, Milford, MA, USA). The liquid chromatography system was the ACQUITY UPLC system (Waters, Milford, MA, USA). For the MS/MS detection, a Quattro Premier™ XE Mass Spectrometer system was employed (Waters, Milford, MA, USA). The data was acquired and analyzed with the MassLynx V4.1 Software. The negative ion multiple reaction monitoring (MRM) mode was used, and the transitions were set at 151 > 92 for MeP, 165 > 92 for EtP, 179 > 92 for PrP, 193 > 92 for BuP, 227 > 92 for BzP, 235 > 92 for HepP, 157 > 98 for $^{13}C_6$-MeP, 171 > 98 for $^{13}C_6$-EtP, 185 > 98 for $^{13}C_6$-PrP, and 199 > 98 for $^{13}C_6$-BuP. The chromatographic separation was accomplished by an ACQUITY UPLC BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm particle size). An amount of 10 μL of sample was injected. The flow rate of mobile phase was set at 200 μL/min. The mobile phases were acetonitrile with 0.1% formic acid (phase A) and Milli-Q water with 0.1% formic acid (phase B). The mobile phase composition started at 40% of phase A, and then increased linearly from 40% of phase A to 75% of phase A in 1 min, and maintained for 2 min at 75% of phase A. Thereafter, the mobile phase composition was returned to the initial condition with 40% of phase A in 1 min. All the other parameters with UPLC-MS/MS were optimized for good performance before analyzing the real samples.

2.5. Quality assurance/quality control

In order to check the interferences and/or contaminations arising from the treatment process, three method blanks were processed for each batch of real samples ($n = 20$). The results showed that the six parabens were below detection limits in method blanks. Recoveries of individual parabens through the entire procedure were determined by spiking the six parabens into real samples ($n = 3$ for each batch). The average recoveries of the six parabens were in the range of 67.2% ~78.2% (mean: 71.8% for MeP, 67.2% for EtP, 69.9% for PrP, 74.8% for BuP, 74.6% for BzP, and 78.2% for HepP). All the real samples were spiked with internal standards before extraction for checking recovery. The average recoveries of the four internal standards were 74.2% for $^{13}C_6$-MeP, 70.0% for $^{13}C_6$-EtP, 73.4% for $^{13}C_6$-PrP and 67.6% for $^{13}C_6$-BuP. The reported concentrations were corrected with recoveries of internal standards.

In order to check the repeatability performance of the extraction method, one real sample for each batch was selected for duplication testing. The results indicated that the relative standard deviation was <10%. Samples were extracted for the third time to evaluate the extraction efficiency. The result showed that concentrations of the target compounds and internal standards in the third extraction were all below detection limits, suggesting that two times extraction is sufficient for reasonably high recovery of target compounds. Limit of quantifications

Please cite this article as: Ma, W.-L., et al., A survey of parabens in commercial pharmaceuticals from China and its implications for human exposure, Environ Int (2016), http://dx.doi.org/10.1016/j.envint.2016.07.013