Toxicology Letters 231 (2014) 161–168

Contents lists available at ScienceDirect

Toxicology Letters

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

Estimated daily intake and cumulative risk assessment of phthalate diesters in a Belgian general population

Lucas Dewalque [a,b,*], Corinne Charlier [a,b], Catherine Pirard [a,b]

[a] Laboratory of Clinical, Forensic and Environmental Toxicology, University of Liege (ULg), CHU (B35), Liege 4000, Belgium
[b] Center for Interdisciplinary Research on Medicines (C.I.R.M.), University of Liege (ULg), CHU (B35), Liege 4000, Belgium

HIGHLIGHTS

- Phthalate daily intakes were estimated for 261 Belgian participants.
- Cumulative risk assessment was performed for 4 phthalates in a Belgian population.
- 13 children out of the 52 were exceeding hazard index of 1.
- The dietary intake seemed to be the major route for DEHP exposure.

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 4 June 2014
Accepted 18 June 2014
Available online 23 June 2014

Keywords:
Phthalate
Daily intake
Urine
Belgium
Hazard index

ABSTRACT

The daily intakes (DI) were estimated in a Belgian general population for 5 phthalates, namely diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP), butylbenzyl phthalate (BBzP) and di-2-ethylhexyl phthalate (DEHP), based on the urinary measurements of their corresponding metabolites. DI values ranged between <LOD and 59.65 μg/kg bw/day depending on the congener, and were globally higher for children than adults. They were compared to acceptable levels of exposure (tolerable daily intakes) to evaluate the hazard quotients (HQ), which highlight an intake above the dose considered as safe for values greater than 1. If very few of our Belgian participants exceeded this threshold for phthalates considered individually, 6.2% of the adults and 25% of the children showed an excessive hazard index (HI) which took into account the cumulative risk of adverse anti-androgenic effects. These results are of concern since these HI were based on only 3 phthalates (DEHP, DiBP and DnBP), and showed a median of 0.55 and 0.29 for children and adults respectively. The comparison with previously determined dietary intakes demonstrated that for DEHP, food intake was nearly the only route of exposure while other pathways occurred mainly for the other studied phthalates.

1. Introduction

Diesters of 1,2-benzenedicarboxylic acid, or phthalates, have been highly produced for more than 50 years to be mainly used as plasticizer, especially in polyvinyl chloride (PVC). Phthalates are therefore present in a large range of everyday life products including construction materials, adhesives, toys, food packaging, house furnishing, clothes, medical materials, drugs and personal care products (Wittassek et al., 2011). Because phthalates can be released from polymers in which they are incorporated, they have been measured in numerous environmental matrices, such as indoor air, house dust, surface water, soils but also in food products and a wide range of cosmetics (Bekö et al., 2013; Chen et al., 2012; Sioen et al., 2012; Fromme et al., 2013a,b; Wittassek et al., 2011). Human exposure can therefore occur through oral, dermal and inhalation pathways.

The increase knowledge of phthalate toxicokinetic have allowed scientists to assess the human exposure measuring specific metabolites in urine, mainly the corresponding monoesters or oxidized monoesters (Wittassek et al., 2011). These phthalate metabolites have been measured worldwide (Frederiksen et al., 2013a; Fromme et al., 2013a; Song et al., 2013; Zota et al., 2014) demonstrating that the general population is largely exposed to

* Corresponding author at: Laboratory of Clinical, Forensic and Environmental Toxicology, BC+3, CHU (B35), Liege 4000, Belgium. Tel.: +32 4 366 80 95; fax: +32 4 366 88 89.
E-mail addresses: lucas.dewalque@ulg.ac.be (L. Dewalque), c.charlier@chu.ulg.ac.be (C. Charlier), c.pirard@chu.ulg.ac.be (C. Pirard).

http://dx.doi.org/10.1016/j.toxlet.2014.06.028
0378-4274/

these compounds. This biomonitoring approach has relevant advantages, integrating all routes and sources of exposure, and avoiding the external contamination due to the widespread presence of the phthalate diesters in the lab environment (Koch et al., 2011). Nevertheless, the phthalate urinary levels do not provide detailed information concerning exposure pathways. Moreover, the rate of absorption and metabolization might change according to the route of exposure, for instance avoiding first-pass metabolism after dermal resorption. Food consumption has been considered as the most important phthalate exposure pathway, especially for long-chain phthalates such as di-2-ethylhexyl phthalate (DEHP), but recently some studies suggested that other routes might be significantly involved for the short-chain compounds such as diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP) or benzylbutyl phthalate (BBzP) (Wormuth et al., 2006; Fromme et al., 2013a,b; Bekö et al., 2013).

This study presents an estimation of the daily intake (DI) for 5 phthalates in a Belgian general population, based on biomonitoring of their urinary metabolites in 261 participants aged between 1 and 85 years old (Dewalque et al., 2014a). This estimation, representing the total intake, was compared to Belgian dietary intakes estimated by Sioen et al. (2012); in order to explore the sources of phthalate exposure and therefore identify the non-diet contribution.

DnBP, DiBP, BBzP and DEHP are known to exhibit endocrine disrupting properties, especially anti-androgenic effects, inhibiting fetal testosterone synthesis (NRC, 2008; Howdeshell et al., 2008) and leading to impairment in reproductive system development (Gray et al., 2000). Moreover, their exposure has been associated in epidemiological studies with some health outcomes such as reduced anogenital distance (Swan et al., 2005), increase insulin resistance and abdominal obesity (Stahlhut et al., 2007), reduce sperm quality (Hauser et al., 2006, 2007) and neurobehavioral development impairment (Engel et al., 2010; Swan et al., 2010). For public health safety purpose, the European Food Safety Authorities (EFSA) (EFSA, 2005a,b,c) established some tolerable daily intake (TDI) values based on anti-androgenic outcomes in animal models, which aim to represent the phthalate levels of exposure considered as safe over a lifetime for humans. More recently, Kortenkamp and Faust (2010) also developed a reference dose for anti-androgenicity (RfD AA) based on the inhibition of the fetal testosterone synthesis. Risk assessment is commonly carried out by comparing the estimated chemical intakes of individual's to such acceptable level of exposure and, to a large extent is still focusing on single chemical. Nevertheless, since phthalate isomers have been demonstrated to exhibit similar toxicological actions, additive effects should be expected (Howdeshell et al., 2008). In this context, hazard index (HI) was recently introduced in the assessment of the phthalate cumulative risk of exposure (Kortenkamp and Faust, 2010; Koch et al., 2011; Søeborg et al., 2012; Kranich et al., 2014; Benson, 2009). Taking into account the cumulative adverse health effects of several phthalates based on similar toxicological endpoints, this index was evaluated by adding the ratios between DI and reference limits (TDI or RfD AA) for the different compounds. The aims of this study were (1) to estimate, in a Belgian general population, the DI of some phthalates based on their urinary measurement, (2) to investigate the diet contribution to the total exposure, (3) to assess the risk of exposure to phthalates by comparing their intake to well-recognized reference values, (4) to assess the risk of cumulative exposure based on anti-androgenic endpoints to several phthalate compounds and (5) finally to compare the risk assessment results in adults and children. Actually, several studies demonstrated that children are more exposed than adults to these environmental pollutants (Dewalque et al., 2014a; Song et al., 2013; Frederiksen et al., 2013a) consisting in a matter of concern regarding the potential vulnerability of this sub-population to developmental and endocrine toxicity exerted by phthalates (Gray et al., 2000).

2. Material and methods

2.1. Study subjects and sampling

The study population and the sampling process were already described elsewhere (Dewalque et al., 2014a). Briefly, informed and consenting subjects provided a spot urine sample and filled in a short questionnaire including data about age, sex, weight, size, smoking habits and residence localization. The study population consisted in 138 females and 123 males living in Liege or in the surrounding areas (Belgium). The participants were between 1 and 85 years old, with average body mass index of 21.9 kg/m^2 and a minority (5.8%) of smokers. Directly after the urine collection carried out in the first trimester of 2013, the samples were aliquoted and frozen at −20 °C until analysis. In this paper, the participants were categorized in 2 groups, the children aged from 1 to 12 years old (n = 52) and the adults aged between 13 and 85 years old (n = 209).

2.2. Urine analysis

The urinary concentrations of 7 phthalate metabolites, namely monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (5-OH-MEHP) and mono-2-ethyl-5-oxohexyl phthalate (5-oxo-MEHP), were measured in the spot urine collected from 261 Belgian participants using a solid phase extraction (SPE) and ultra high pressure liquid chromatography tandem mass spectrometry (UHPLC–MSMS) method previously described (Dewalque et al., 2014b). Briefly, 3 mL of urine previously fortified with the corresponding $^{13}C_4$-labeled compounds (excepted for MiBP which was quantified using $^{13}C_4$-MnBP), were added to 0.75 mL sodium acetate buffer and 25 μL of a Helix pomatia glucuronidase solution, incubated overnight at 37 °C. Samples were then acidified with 200 μL of formic acid before being loaded on Bond Elut Certify LRC cartridges previously conditioned. After a wash step with 0.5% of acetic acid, target compounds were eluated with acetonitrile, evaporated until dryness at 40 °C under a gentle stream of nitrogen and reconstituted in 70 μL of mobile phases (water and acetonitrile 0.1% acetic acid both) to be injected (5 μL) on UHPLC–MSMS. The whole procedure was validated according to the total error approach, and yielded to limits of detection (LOD) ranging between 0.13 and 0.37 μg/L depending on the phthalate metabolite. The quantification was performed by isotope dilution and the determination of unknown samples was carried out using calibration curves ranging from 0.5 to 200 μg/L. Each sequence included a blank and two levels of home-made internal quality controls (QC) (10 and 100 μg/L). The variations of the measured concentrations were below 6.2% and 7.2% respectively for the low and high-level QC for each target compound.

2.3. Daily intake estimation

The estimation of the DI of the phthalate diesters was carried out for each individual using the volumetric model developed by Koch et al. (2003) with the following relation:

$$\mathrm{DI}[\mu\gamma/\mathrm{kg\ bw/day}] = \frac{\mathrm{UC_m}[\mu\gamma/\mathrm{L}] \times \mathrm{UV}[\mathrm{L/day}] \times \mathrm{MW_d}[\mu\gamma/\mu\mu\mathrm{o}\lambda]}{\mathrm{F_{UE}} \times \mathrm{bw}[\mathrm{kg}] \times \mathrm{MW_m}[\mu\gamma/\mu\mu\mathrm{o}\lambda]}$$