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Trends of the internal phthalate exposure of young adults in Germany—Follow-up of a retrospective human biomonitoring study

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ABSTRACT

The exposure of the general population to phthalates is of increasing public health concern. Variations in the internal exposure of the population are likely, because the amounts, distribution and application characters of the phthalate use change over time. Estimating the chronological sequences of the phthalate exposure, we performed a retrospective human biomonitoring study by investigating the metabolites of the five most prominent phthalates in urine. Therefore, 24 h-urine samples from the German Environmental Specimen Bank (ESB) collected from 240 subjects (predominantly students, age range 19–29 years, 120 females, 120 males) in the years 2002, 2004, 2006 and 2008 (60 individuals each), were analysed for the concentrations of mono-n-butyl phthalate (MnBP) as metabolite of di-n-butyl phthalate (DnBP), mono-iso-butyl phthalate (MiBP) as metabolite of di-iso-butyl phthalate (DiBP), monobenzyl phthalate (MBzP) as metabolite of butylbenzyl phthalate (BBzP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (5OH-MEHP), mono-(2-ethyl-5-oxohexyl) phthalate (5oxo-MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) and mono-(2-carboxymethyl hexyl) phthalate (2cx-MMHxP) as metabolites of di(2-ethylhexyl) phthalate (DEHP), monohydroxylated (OH-MiNP), monooxidated (oxo-MiNP) and monocarboxylated (cx-MiNP) mono-iso-nonylphthalates as metabolites of di-iso-nonyl phthalates (DiNP). Based on the urinary metabolite excretion, together with results of a previous study, which covered the years 1988–2003, we investigated the chronological sequences of the phthalate exposure over two decades. In more than 98% of the urine samples metabolites of all five phthalates were detectable indicating a ubiquitous exposure of people living in Germany to all five phthalates throughout the period investigated. The medians in samples from the different years investigated are 65.4 (2002), 38.5 (2004), 29.3 (2006) and 19.6 μg/l (2008) for MnBP, 31.4 (2002), 25.4 (2004), 31.8 (2006) and 25.5 μg/l (2008) for MiBP, 7.8 (2002), 6.3 (2004), 3.6 (2006) and 3.8 μg/l (2008) for MBzP, 7.0 (2002), 5.6 (2004), 4.1 (2006) and 3.3 μg/l (2008) for MEHP, 19.6 (2002), 16.2 (2004), 13.2 (2006) and 9.6 μg/l (2008) for 5OH-MEHP, 13.9 (2002), 11.8 (2004), 8.3 (2006) and 6.4 μg/l (2008) for 5oxo-MEHP, 18.7 (2002), 16.5 (2004), 13.8 (2006) and 10.2 μg/l (2008) for 5cx-MEPP, 7.2 (2002), 6.5 (2004), 5.1 (2006) and 4.6 μg/l (2008) for 2cx-MMHxP, 3.3 (2002), 2.8 (2004), 3.5 (2006) and 3.6 μg/l (2008) for OH-MiNP, 2.1 (2002), 2.1 (2004), 2.2 (2006) and 2.3 μg/l (2008) for oxo-MiNP and 4.1 (2002), 3.2 (2004), 4.1 (2006) and 3.6 μg/l (2008) for cx-MiNP. The investigation of the time series 1988–2008 indicates a decrease of the internal exposure to DnBP by the factor of 7–8 and to DEHP and BzBP by the factor of 2–3. In contrast, an increase of the internal exposure by the factor of 4 was observed for DiNP over the study period. The exposure to DiBP was found to be stable. In summary, we found decreases of the internal human exposure for legally restricted phthalates whereas the exposure to their substitutes increased. Future investigations should verify these trends. This is of increasing importance since the European Commission decided to require ban or authorization from 1.1.2015 for DEHP, DnBP, DiBP and BzBP according to REACh Annex XIV.

Introduction

Phthalate diesters (phthalates) are frequently used plasticizers in polymers, particularly in soft-PVC. Therefore, phthalates are part of many consumer products, e.g. children toys,

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cleaning materials, clothing, cosmetics and personal care products, but also automobiles, building materials, food packaging, furnishings, lubricants, medical devices and pharmaceuticals (ECHA, 2009a,b,c; Hermández-Diaz et al., 2009; Schettler, 2006; Wormuth et al., 2006). In Western Europe about 1 million tons of phthalates are produced annually (ECHA, 2009a,b,c). Over the last two decades, the marketing pattern of phthalate esters has changed in the European Union: The production and use of one of the most prominent phthalates di(2-ethylhexyl) phthalate (DEHP) declined. In contrast, marketing shares increased for di-iso-nonyl phthalate (DiNP) and di-iso-decyl phthalate (DiDP), two homologous chemicals of DEHP (ECHA, 2009a,b,c; ECPI, 2010; PlasticsEurope, 2006). Several studies revealed that the intensive use of phthalate in PVC results in ubiquitous exposures of the population and its environment (Fromme et al., 2002; Kohn et al., 2000; Wormuth et al., 2006; Becker et al., 2009).

Phthalates are considered to be of low acute toxicity but exhibit chronic effects in long term studies, e.g. hepatic peroxisome proliferation and cancer, and changes in kidney and thyroid (Kavlock et al., 2006). The most prominent adverse effects found in animal studies were reproductive and developmental effects (Fabjan et al., 2006; Lyche et al., 2009). These effects were observed in rodent studies for di-n-butyl phthalate (DnBP) (Barlow and Foster, 2003; Foster et al., 2000; Lee et al., 2004; Mylchreest et al., 2000), di-isobutyl phthalate (DiBP) (Borch et al., 2006; Saillenfait et al., 2006), benzyl butyl phthalate (BzBP) (Nagao et al., 2000; Tyl et al., 2004), DEHP (Blystone et al., 2010; Gray et al., 2000; Parks et al., 2000) and also for DiNP (Borch et al., 2004; Gray et al., 2000). In vivo studies revealed that phthalates do not only act as separate substances, they cause cumulative adverse effects (Rider et al., 2010; U.S. CHR, 2008; National Research Council, 2008). By a similar mechanism of action, mixtures of individual phthalates have direct additive effects on the foetal testosterone production and the course of pregnancy (Howdeshell et al., 2007, 2008). There is a direct relationship between chemical structure and activity: Obviously, the potential for reproductive and developmental toxicant effects is to be attributed to phthalate diesters with at least four carbon atoms in the side chain in ortho position (Fabjan et al., 2006, Howdeshell et al., 2008; Lyche et al., 2009).

Based on the toxicological data and taking acceptable daily intake into account, the German Human Biomonitoring Commission derived a Human Biomonitoring Value I indicating at which exposure level of DEHP adverse effects on humans can no longer be excluded (Kommission Human-Biomonitoring, 2007a,b,c). Estimation of intake from metabolite levels in urine indicated an exceedance of TDI in children as well as in adults (Wittassek et al., 2007a,b).

Several studies indicate that phthalates act as endocrine disrupters in humans, too (Engel et al., 2010; Hauser et al., 2006; Meeker et al., 2009; Swan et al., 2005, 2010; Zhang et al., 2009). Moreover, phthalates appear to modify several physiological processes such as thyroid signaling, immune functions and metabolic homeostasis (Desvergne et al., 2009; Huang et al., 2007; Meeker et al., 2007; Stahlhut et al., 2007).

Since 2000 analytical methods have been available for quantifying specific phthalate metabolites in urine. These techniques are applied in human biomonitoring studies to investigate the exposure of human populations to phthalates (Blount et al., 2000; Koch et al., 2003a; Silva et al., 2004; Becker et al., 2009). One of the outcomes was an ubiquitous high exposure of the population in industrial countries to, e.g. DnBP, DiBP, BzBP and DEHP. In consequence, particularly EU agencies regulated the use of phthalates in consumer products (EC, 1999; EU, 2005). If these risk management measures are successful, then not only the marketing volumes of regulating chemicals will change but also the internal concentration of the population which is exposed to these chemicals.

We applied a retrospective approach on the basis of samples provided by the German Environmental Specimen Bank (ESB) to investigate whether the regulatory use restriction, issued for individual phthalates, is successful, or not. The ESB is a cryogenic archive of human and environmental specimens which are collected in a standardised manner and stored for future analysis. The human samples are collected annually from young adults, particularly students, at four selected locations in Germany. Following sampling the material is portioned, biologically and chemically characterised, processed and finally stored under cryogenic conditions (Wiesmüller et al., 2007). This study reports concentration of phthalate metabolites in human urine samples of the ESB that were collected between 2002 and 2008. Wittassek et al. (2007a,b) analysed the same selection of phthalates in samples from the same ESB population covering the years 1988–2003. The samples from both studies cover a time period when particularly DEHP was not regulated, then was regulated and has now been regulated for some years.

Materials and methods

Subjects and urine specimens

In this study we analysed 240 urine samples of the ESB. The samples were 24 h-urine samples, which were collected at the University of Münster (Germany) in the years 2002, 2004, 2006 and 2008 from 240 volunteers, predominantly students (age range 19–29 years). The sampling years, the sample size per sampling year, the age of the individuals, the sex distribution, the body-mass-index (BMI), the volume of the 24 h-urine samples and the creatinine concentration in the urine samples of the study population are given in Table 1. 60 individual samples (uniformly distributed for gender) were investigated from each sampling year. Ten percent of the study group were smokers. The constitution of the individuals ranged from a BMI of 15 up to 30 kg/m^2, but did not differ between sampling years. The daily excretion of urine ranged from 350 to 5460 ml and the urinary creatinine ranged from 0.12 to 2.37 g/l. Creatinine concentration below 0.30 g/l was found in ten samples (4%).

The samples taken from the participants were aliquoted to several portions in polypropylene tubes and were stored under cryogenic conditions in the gaseous phase above liquid nitrogen at a temperature below −150 °C immediately after sample collection. The cryogenic conditions minimize chemical and/or biological changes in the samples over long time periods. All samples were blinded after they had been retrieved from the archive and before they were analysed in the laboratories of the Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine at the University of Erlangen-Nuremberg.

The results of this study were evaluated and discussed in connection of the previous study published by Wittassek et al. (2007a,b). The study of Wittassek et al. (2007a,b) was performed under the same conditions like in this study: individuals were selected from students of the university of Münster; urine sampling took place over 24 h; sampling numbers in each year were 30 female and 30 male individuals at least.

Chemical analysis

The analytical procedure applied is based on a multidimensional liquid chromatography tandem mass spectrometry method for the simultaneous determination of several phthalate metabolites in human urine (Koch et al., 2003a,b; Koch and Angerer, 2007; Preuss et al., 2005). The method allows the determination of several metabolites of DnBP, DiBP, BBzP, DEHP and DiNP within a