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Phthalate esters, parabens and bisphenol-A exposure among mothers and their children in Greece (Rhea cohort)

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ABSTRACT

Exposure to endocrine disruptors, used as additives, preservatives, plasticisers and solvents in numerous consumer products, might cause adverse health effects. Humans exposed to these chemicals, metabolise and excrete them mostly via urine. Urinary metabolite concentrations are used as biomarkers of exposure. We evaluated the exposure of 4-month pregnant women and their children at 2 years of age to phthalates, parabens and bisphenol-A. Concentrations of eight phthalate metabolites, six parabens and bisphenol-A were measured in 239 mother–child pairs of the "Rhea" cohort in Greece. Concentration levels in mother and children were comparable with corresponding concentrations in other countries worldwide. Low Spearman correlation coefficients (CC 0.1–0.2, p-value < 0.01) were observed for di-ethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl-benzyl phthalate (BBP) and ethyl paraben (EPB) between mothers and their children. We observed higher median daily intake (DI_u) for mothers (e.g. di-ethyl phthalate 6.9 μg d^{-1} kg^{-1}) than for their children (1.4 μg d^{-1} kg^{-1}) for all examined compounds, except for di-2-ethylhexyl phthalate (DEHP) and bisphenol-A. Principal component analysis (PCA) indicated two main sources of exposure (plastic related and personal care-hygiene products) for phthalates, parabens and bisphenol-A. Differences in DEHP metabolism were observed among mothers–children and female–male children.

1. Introduction

Endocrine disruptors are chemicals, which alter functions of the endocrine system of humans and consequently can cause adverse health effects (World Health Organization, 2012). They interfere with hormone biosynthesis, metabolism or actions resulting in a deviation from normal homeostatic control or reproduction in humans (Diamanti-Kandarakis et al., 2009). They disrupt the endocrine system by competing with naturally occurring hormones such as estradiol or by altering the synthesis and metabolism of these hormones (National Institute of Health, 2010). There is evidence of reproductive toxicity in laboratory animals and possible health effects in humans (Crinnion, 2010). Pregnant mothers (their embryos) and children are the most vulnerable populations to endocrine disruptor exposure (World Health Organization, 2012). Phthalates, with around 1 million tons annual production in Europe (AgPU, 2006) and bisphenol-a (BPA) with about 3.6 million tons annual global production (Geens et al., 2012) represent some of the world's highest production chemicals. Parabens are used in over 13,200 formulations in nearly all types of cosmetics (Elder, 1984). All the above-mentioned chemicals are well recognised endocrine disruptors (Witorsch and Thomas, 2010). Human exposure to these chemicals is occurring through the environment, food intake and the use of products containing them, through inhalation, dermal contact and ingestion (ATSDR DEHP, 2002; ATSDR DEP, 1995; ATSDR DnBP, 2001; Meeker, 2010; Soni et al., 2001).

Abbreviations: BPA, bisphenol-A; BBP, butyl-benzyl phthalate; CC, correlation coefficient; C_u, metabolite concentration, μg/L; DEHP, di-2-ethylhexyl phthalate; DEP, di-ethyl phthalate; DiBP, di-iso-butyl phthalate; DI_u, daily intake calculated using urinary metabolites; DnBP, di-n-butyl phthalate; EPB, ethyl paraben; HPLC, high performance liquid chromatography; isoBPB, iso-butyl paraben; isoPPB, iso-propyl paraben; F_{ue}, urinary excretion factor; mBzP, mono-benzyl phthalate; mCOP, mono-carboxy-octyl phthalate; mEHHP, mono-2-ethyl-5-hydroxy-hexyl phthalate; mEOHP, mono-2-ethyl-5-oxo-hexyl phthalate; mEP, mono-ethyl phthalate; mLOD, method limit of detection; mnBP, mono-n-butyl phthalate; mNP, mono-iso-nonyl phthalate; MPB, methyl paraben; MW_1, molecular weight of phthalate diester; MW_2, molecular weight of phthalate metabolite; nBPB, n-butyl-paraben; NC, not calculated; ND, not detected; nPPB, n-propyl paraben; NR, not reported; PCA, principal component analysis; RfD, reference dose; SPE, solid phase extraction; SRM, selected reaction monitoring; RMR, relative metabolic rate; RMR_1, mEHHP/mEHP molar concentrations ratio; RMR_2, mEOHP/mEHHP molar concentration ratio; TDI, tolerable daily intake; UPLC, ultra performance liquid chromatography; W, body weight.

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Phthalates (1,2-diesters of phthalic acid) have a variety of common uses. Higher molecular weight phthalates are used in plastic as softeners and lower molecular weight phthalates are used in personal care products and pharmaceuticals (Wormuth et al., 2006). Previous animal tests and epidemiological studies have associated exposure to phthalates with detrimental effects to reproductive and developmental health, as well as increased risk for cancer (ATSDR DEHP, 2002; ATSDR DEP, 1995; ATSDR DnBP, 2001). Once ingested/absorbed, lower molecular weight phthalates are hydrolysed to their monoesters, while higher molecular weight phthalates can be subsequently oxidised to several other metabolites from their primary monoesters. Primary and secondary metabolites resulting from phthalate breakdown can be further biotransformed to their glucoronide analogues before being excreted via urine (Calafat et al., 2006).

Parabens are a group of alkyl esters of p-hydroxybenzoic acid. They have low cost of production, and demonstrate chemical stability, inertness, and low acute toxicity (World Health Organization, 2012). These characteristics made them desirable as antimicrobial preservatives against mould and yeast, in cosmetics, in pharmaceuticals and in food and beverage processing (Elder, 1984). Parabens also occur naturally in food, wine, and plants (Soni et al., 2005). In vitro studies indicate that parabens induce the growth of MCF-7 human breast cancer cells and influence the expression of oestrogen dependent genes (Byford et al., 2002). In general, parabens are partially hydrolysed by esterases to p-hydroxy-benzoic acid and produce glycine/glucuronide/sulphate conjugates, with increased water solubility that are more amenable to urinary excretion than are the free species (Soni et al., 2005; Wang and James, 2006).

BPA (4,4′-(propane-2,2-diyl) diphenol) is widely used in polycarbonate and epoxy resin production. It can be found in many products like dental sealants, food packaging, beverage cans, personal care products, baby bottles, building materials, flame retardant materials, optical lenses, DVDs and household electronics (Geens et al., 2012; Staples et al., 1998). After epidemiological studies in human beings and experiments in mice, BPA exposure is suspected of causing several adverse health effects, such as cancer, obesity and disorders in endocrine, renal and reproductive systems (Rubin, 2011). BPA is excreted mainly via urine in its glucuronide conjugate form (Chapin et al., 2008).

In order to assess the exposure of phthalates, BPA and parabens in humans, measurement of their urinary concentration of free species and their conjugates is essential (Silva et al., 2003a; Ye et al., 2006). In this study, 8 phthalate metabolites, 6 parabens and BPA (Table 1) were measured in 239 mother–child pairs in Heraklion, Crete (Rhea cohort). We aimed: a) to evaluate, for the first time, the levels of exposure to phthalates, parabens and BPA in Greece, b) to investigate the potential correlation in the exposure levels between the mothers and their children, c) to estimate the daily intake (DI_u) of the phthalates, parabens and BPA, d) to compare our results with other similar studies worldwide and e) to attempt the assessment of potential exposure sources.

2. Materials and methods

2.1. Study population

The present study is part of the "Rhea" project, a pregnancy cohort which examines prospectively a population-based cohort of pregnant women and their children at the prefecture of Heraklion, Crete, Greece (Chatzi et al., 2009; Patelarou et al., 2011). Briefly, women who became pregnant during February 2007–February 2008 participated in the study. Pregnant women (N = 1600), residents of the study area, >16 years of age, completed face-to-face interviews, visiting a participating hospital or private clinic during the 10th–13th weeks of gestation. Of them, 1278 provided blood and spot urine samples. Participants were contacted again during the 14th–18th and 28th–32nd weeks of pregnancy and at birth. Of 1363 singleton live births in the Rhea study, 390 children participated at the 2 year follow-up and provided spot urine samples. Of them, 239 mother–child pairs (103 female/136 male children; 2.3 ± 0.72 years old; collection during March 2009–June 2011) were included in the present analysis. The selection criteria were, a) available samples from the 4th month of pregnancy (mother–child pairs) and b) available Bayle test results.

Table 1
Studied endocrine disruptors and their method limits of detection (mLOD).

Parent compounds	Studied metabolites	Method limit of detection (mLOD) ng/mL urine
Di-ethyl phthalate (DEP)	Mono-ethyl phthalate (mEP)	1.3
Di-n-butyl phthalate (DiBP)	Mono-n-butyl phthalate (miBP)	2.1
Di-iso-butyl phthalate (DnBP)	Mono-iso-butyl phthalate (miBP)	2.5
Di-2-ethylhexyl phthalate (DEHP)	Mono-2-ethylhexyl phthalate (mEHP)	0.8
	Mono-2-ethyl-5-hydroxy-hexyl phthalate (mEHHP)	0.9
	Mono-2-ethyl-5-oxo-hexyl phthalate (mEOHP)	1.8
Butyl-benzyl phthalate (BBP)	Mono-benzyl phthalate (mBzP)	2.2
Di-iso-nonyl phthalate (DNP)	Mono-isononyl phthalate (mNP)	2.2
Methyl paraben (MPB)		0.06
Ethyl paraben (EPB)		0.06
iso-Propyl paraben (isoPPB)		0.13
n-Propyl paraben (nPPB)		0.09
iso-Butyl paraben (isoBPB)		0.04
n-Butyl paraben (nBPB)		0.04
Bisphenol-A (BPA)		0.01

Urine samples were collected in all-purpose urine sample containers and stored at 4 °C until processing. Within 4 h, samples were aliquoted in 4 mL cryovials and stored at −80 °C. Urine containers and cryovials were made of polypropylene and checked for possible contamination. Creatinine levels were 0.50 ± 0.31 g/L (arithmetic mean ± standard deviation) for children and 1.20 ± 0.67 for mothers. Samples with creatinine values, not in the 0.3–3 g/L range for mothers (Barr et al., 2005a, 2005b) and 0.1–3.0 range for children, respectively, were excluded from analysis. We did not apply the same exclusion criteria for mothers and children, because creatinine values below 0.3 mg/L in children do not necessarily indicate excessive dilution but are indicative of lower muscle mass compared to that of adults (Koch et al., 2011). All participant mothers provided written, informed consent for themselves and their child after having received a complete description of the study, which was approved by the Ethics Committee of the University Hospital in Heraklion, Greece.

2.2. Instrumental analysis

An aliquot of each sample (1 mL) was analysed for eight phthalate metabolites and six parabens (Table 1) during February 2010–December 2012. Our primary goal was to measure only phthalate metabolites for an EU funded FP7 project (Envirogenomarkers). Therefore, we used *Escherichia coli* β-glucuronidase for the enzymatic hydrolysis of conjugated endocrine disruptors in urine. As a result, we obtained total phthalate metabolites (free and glucuronated), but not all the paraben and BPA species [sulfated metabolites demand *Helix pomatia* β-glucuronidase (Dewalque et al., 2014; Volkel et al., 2002)]. However, glucuronated BPA is practically equal to the total in urine (Chapin et al., 2008) and glucuronated paraben percentage of the total is known (Dewalque et al., 2014). Thus, we considered that parabens and BPA data should be a significant input, especially when we take into account the lack of analogous studies in Greece for BPA and in general for parabens. Treatment and clean-up of the samples were based on