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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

previous work (Silva et al., 2003b), but modified in several steps. Urine samples (1 mL) were spiked with internal standards and *E. coli* β-glucuronidase buffer (per sample: 10 μL *E. coli* β-glucuronidase and 250 μL ammonium acetate buffer, 1 M in aqueous solution, pH 6.5) was added. Hydrolysis was completed at 37 °C for 90 min. After enzymatic hydrolysis completion, 1 mL of ammonium hydroxide buffer (0.15% w/v $NH_4OH$ in 1:1 acetonitrile–water) was added to the samples, which were loaded onto the first solid phase extraction cartridge (Varian Nexus, 60 mg). The eluents of the first cartridge were acidified with 3 mL monosodium phosphate buffer (0.14 M $NaH_2PO_4$, aqueous solution, at pH 2) and loaded onto the second solid phase extraction cartridge (Varian Nexus, 200 mg). The eluents from the second cartridge were discarded. Both cartridges were eluted with 3 mL acetonitrile and 3 mL ethyl acetate each. The eluents of both cartridges (12 mL in total) were combined and evaporated to dryness with a rotational vacuum concentrator RVC 2-25 (Martin Christ, Germany) (60 °C, 20–45 mbar, 150 min for 18 samples). The residues were dissolved in 0.4 mL of water and transferred to a 2 mL autosampler glass vial with a 0.4 mL volume insert. Subsequently, phthalate metabolite and paraben LC–MS determination was performed. In order to enhance the BPA detection limit, aqueous ammonium hydroxide (160 μL 7% v/v) and dansyl chloride (40 μL 12.5 mg/mL) in acetone were added to the autosampler vials containing the samples (200 μL, the rest was discarded). Dansylation was completed with 0.5 h heating at 65 °C, and samples were re-analysed with LC-MS. Two new gradient elution programmes were developed a) for phthalate metabolite and paraben analysis: mobile phase was acetonitrile and water, both containing 0.1% acetic acid; flow rate at 350 μL/min; 36 min run; from 4% organic phase to 100% and back to 4%; Thermo Phenyl Betasil column, 3 μm, 100 mm × 2.1 mm, and b) mobile phase was acetonitrile and water, both containing 0.1% formic acid; flow rate at 200 μL/min; 18.5 min run; from 60% organic phase to 100% and back to 60%; MZ PerfectSil $C_8$ (3 μm, 125 mm × 2.1 mm). In order to minimise the BPA limits of detection, after LC–MS determination of parabens and phthalate metabolites, extracted samples were derivatised with dansyl chloride, and dansylated-BPA was monitored. Detection performed with a TSQ Quantum triple quadrupole (Thermo Finnigan, San Jose, USA) with ESI source operated in negative mode for phthalate metabolites/parabens and in positive mode for dansylated-BPA. Mass spectrometer was set in selected reaction monitoring (SRM).

The method's limits of detection of studied compounds are presented in Table 1. Isotopically labelled analogues of studied compounds were used as internal standards. Labelled isoPPB, isoBPB, miBP and mEHP were not commercially available at the time of analysis and labelled nPPB, nBPB, mnBP and mEOHP were used as their internal standards. Blank contamination was lower than the obtained detection limits and recovery was >59% for all studied compounds except for mEHP in which case it was 44%. Standard deviation of accuracy and repeatability tests were <13.1% and <6.2%, respectively. Linearity was observed ($R^2 > 0.99$) for the range LOD-512 ng/mL. Samples exceeding the upper limit of linearity were diluted with nanopure water and reanalysed. Two quality control samples (spiked pooled urine) and two blank samples (synthetic urine) were analysed with every forty-six (46) urine samples. A second aliquot of 0.5 mL urine was analysed for creatinine concentration using the OLYMPUS 2700 immunoassay system (Beckman Coulter, USA). All samples were analysed in duplicate. The amount of each analyte was quantified by the standard curve performed in each assay.

### 2.3. Statistical analysis

Statistical analysis was performed with the software SPSS 22.0 (IBM Corporation, U.S.A.). Measurements below mLOD (not detected) were substituted by the mLOD divided by the square root of 2 (two) (Hornung and Reed, 1990), as the most widely used way to handle non-detects in this type of studies (Ferguson et al., 2014; Song et al., 2013). Arithmetic mean, minimum, median, 95th percentile, maximum, geometric mean and 95% confidence interval of geometric mean (95% CI) values were calculated for both unadjusted/creatinine-adjusted concentrations and estimated daily intake data.

The daily intake of phthalates and parabens was estimated by adapting a commonly used toxicokinetic model to the obtained data (Eq. (1)) (Beko et al., 2013; Dirtu et al., 2013; Ma et al., 2013), where: $DI_u$ (daily intake calculated using urinary metabolites, $\mu g \times d^{-1} \times kg^{-1}$ of body weight), $C_u$ (metabolite concentration, μg/L), $F_{ue}$ (urinary excretion factor, molar ratio of parent compound intake to metabolite excreted), $MW_1$ (molecular weight of phthalate diester, g/mol), $MW_2$ (molecular weight of phthalate metabolite, g/mol) and W (body weight, kg). For parabens in particular, we included a multiplier (P) of 1.72 (for n-BPB and iso-BPB 1.04) since we measured free plus glucuronated parabens which represent 58% of the total urinary paraben concentrations (glucuronated n-BPB and iso-BPB, 96%) (Dewalque et al., 2014). The glucuronated form of BPA practically represents the total BPA excretion, so no normalisation factor was used for the sulphated form (Volkel et al., 2002), since the underestimation practically is equal to zero.

$$DI_u = \frac{C_u \times V_u \times P \times MW_1}{W \times F_{ue} \times MW_2} \quad (1)$$

$$\left(\mu g \text{ of endocrine disruptor} \times d^{-1} \times kg^{-1} \text{ of body weight}\right)$$

$F_{ue}$ values for mEHHP and mEOHP were taken from Koch et al. (2004a, 2005a), for mBzP and mnBP from Anderson et al. (2001), for parabens from Ma et al. (2013). Since for miBP and mEP $F_{ue}$ values were not available, we used the same with mnBP. $F_{ue}$ value for BPA was set equal to 1 since BPA is excreted via urine nearly 100% during a 24 h period (Volkel et al., 2002). $V_u$ considered 2 L for mothers (Guo et al., 2011) and 0.0224 L/kg body weight for children (Miller and Stapleton, 1989; Szabo and Fegyverneki, 1995). We chose to use a value for child urinary volume, which doesn't take into account the body weight when it is applied to our toxicokinetic model because children especially in the ages used in this study grow rapidly. A stable volume of urine as is used for mothers could introduce uncertainty in the estimated daily intake.

In order to investigate possible differentiations in DEHP metabolism among population groups, the relative metabolic rate (RMR) of DEHP was calculated as described in the literature (Boas et al., 2010; Song et al., 2013). Briefly, $RMR_1$ (1st step of metabolism), considered the molar concentration ratio of mEHP/mEHHP and $RMR_2$ (2nd step) the ratio of mEHHP/mEOHP. Only samples with positive detection in all three DEHP metabolites were used. For Spearman correlations, creatinine adjusted molar (μmol/g) concentrations were used. For Pearson correlations, unnormalised RMR values were used since RMR data were not skewed.

Principal component analysis (PCA) with Kaiser normalisation was applied to molar, unadjusted for creatinine, log10 transformed concentrations. Mann–Whitney *U* test (gender-based comparison) was applied to the comparison of both unadjusted (ng/mL)–creatinine adjusted (μg/g) concentration levels and RMR levels. Wilcoxon signed ranks test was applied in order to compare daily intake and RMR mother–child pairs based comparison. A *t*-test was utilised for independent samples to compare creatinine levels, since creatinine data were not skewed. For PCA and correlation studies, molar concentration levels were used and mEHHP–mEOHP concentrations summed as DEHP metabolites.

Analytes with detectability lower than 50% (isoPPB, isoBPB and nBPB) were excluded from PCA, correlation studies, gender-based comparison and geometric mean calculation (Table 2). DEHP-$DI_u$ was considered the arithmetic mean of $DI_u$ for mEHHP and mEOHP. mEHP was excluded from the above analyses (PCA, correlation studies, $DI_u$) due to its relatively lower levels in urine and shorter half-life compared to the other two measured DEHP metabolites, mEHHP and mEOHP (Frederiksen et al., 2007; Koch et al., 2005a; Silva et al., 2006a, 2006c; Wittassek and Angerer, 2008) (Table 4).

**Table 2**
Descriptive statistics for phthalate metabolite, paraben and BPA urinary concentrations.

| | Detectability % | Minimum | Median | 95% percentile | Maximum | Arithmetic mean | Geometric mean | 95% CI |
|---|---|---|---|---|---|---|---|---|
| *239 mothers, ng of analyte/mL urine (μg of analyte/g creatinine)* | | | | | | | | |
| mEP | 100.0 | 2.6 (4.8) | 133.9 (132.6) | 1462.9 (1230.9) | 4103.7 (3993.8) | 360.9 (323.2) | 141.9 (143.5) | 119.3–171.8 (122.0–169.8) |
| miBP | 98.0 | <LOD[a] | 39.2 (38.7) | 189.4 (131.9) | 616.1 (720.0) | 62.0 (54.7) | 36.7 (37.1) | 32.2–42.3 (33.2–41.6) |
| mnBP | 95.9 | <LOD | 36.1 (33.2) | 210.5 (157.1) | 94670.7 (48799.3) | 463.9 (260.2) | 32.1 (32.5) | 27.3–38.5 (28.4–37.5) |
| mEHHP | 96.4 | <LOD | 25.7 (24.4) | 125.5 (107.8) | 6267.3 (5095.4) | 66.3 (60.5) | 22.1 (22.3) | 18.9–26.1 (19.5–26.1) |
| mEOHP | 93.6 | <LOD | 17.6 (15.9) | 100.5 (84.8) | 3610.6 (2935.4) | 49.4 (42.6) | 15.5 (15.7) | 13.1–18.5 (13.7–18.5) |
| mBzP | 91.6 | <LOD | 6.0 (7.0) | 38.2 (32.1) | 199.4 (132.0) | 12.8 (11.2) | 6.9 (7.0) | 6.1–8.0 (6.2–7.9) |
| mEHP | 72.7 | <LOD | 7.6 (7.3) | 50.1 (47.1) | 3401.3 (2765.3) | 28.2 (25.3) | 7.0 (7.1) | 6.0–8.2 (6.1–8.3) |
| MPB | 99.2 | <LOD | 98.3 (121.9) | 3098.4 (3191.5) | 67461.3 (46089.3) | 1200.7 (1138.8) | 102.1 (103.2) | 79.8–132.8 (80.5–132.6) |
| EPB | 93.6 | <LOD | 2.6 (2.9) | 120.5 (77.3) | 377.5 (146.3) | 19.8 (16.0) | 3.1 (3.2) | 2.5–4.1 (2.5–4.1) |
| isoPPB | 12.0 | <LOD | <LOD | 0.97 (0.85) | 63.9 (51.1) | 0.9 (0.9) | NC[b] | NC |
| nPPB | 90.8 | <LOD | 13.4 (17.5) | 685.4 (461.4) | 28182.1 (20387.3) | 413.4 (365.7) | 11.2 (11.3) | 8.0–15.4 (8.1–15.4) |
| isoBPB | 26.5 | <LOD | <LOD | 2.6 (2.3) | 59.2 (39.2) | 0.6 (0.5) | NC | NC |
| nBPB | 38.6 | <LOD | <LOD | 28.2 (21.3) | 242.3 (148.8) | 6.2 (5.1) | NC | NC |
| BPA | 99.6 | <LOD | 1.2 (1.1) | 4.7 (5.6) | 144.0 (116.1) | 2.6 (2.4) | 1.2 (1.2) | 1.1–1.4 (1.1–1.4) |
| *239 children, ng of analyte/mL urine (μg of analyte/g creatinine)* | | | | | | | | |
| mEP | 99.6 | <LOD | 34.4 (86.6) | 230.4 (477.7) | 2460.1 (3617.8) | 79.2 (182.7) | 35.3 (88.5) | 30.2–41.0 (76.9–102.0) |
| miBP | 98.7 | <LOD | 34.4 (101.6) | 202.4 (280.6) | 886.0 (681.6) | 62.4 (123.2) | 36.0 (90.3) | 31.2–41.4 (81.0–100.8) |
| mnBP | 96.2 | <LOD | 23.9 (62.3) | 162.3 (261.0) | 1250.5 (962.0) | 47.1 (90.8) | 23.3 (58.3) | 19.9–27.0 (51.5–65.9) |
| mEHHP | 97.1 | <LOD | 30.5 (71.0) | 158.2 (246.7) | 626.3 (1204.3) | 51.2 (102.2) | 24.9 (62.4) | 20.8–29.7 (54.3–71.9) |
| mEOHP | 95.4 | <LOD | 20.0 (51.0) | 116 (171.6) | 391.1 (611.1) | 35.0 (68.4) | 16.9 (42.5) | 14.2–20.5 (37.3–48.9) |
| mBzP | 86.2 | <LOD | 6.5 (17.0) | 35.2 (73.5) | 241.9 (394.2) | 12.9 (27.8) | 6.8 (17.0) | 5.9–7.8 (15.0–19.3) |
| mEHP | 57.3 | <LOD | 2.8 (9.1) | 23.5 (78.5) | 95.01 (203.8) | 7.0 (18.2) | 3.8 (9.6) | 3.4–4.4 (8.5–11.0) |
| MPB | 100.0 | <LOD | 17.1 (42.6) | 942.9 (2710.5) | 6805.9 (17014.8) | 198.2 (512.2) | 25.0 (62.7) | 20.0–31.5 (49.5–79.7) |
| EPB | 93.3 | <LOD | 1.5 (3.7) | 96.2 (318.2) | 1116.9 (1801.5) | 22.0 (66.0) | 1.8 (4.5) | 1.4–2.4 (3.4–6.0) |
| isoPPB | 2.1 | <LOD | <LOD | <LOD | 10.8 (13.0) | 0.1 (0.4) | NC | NC |
| nPPB | 79.1 | <LOD | 0.9 (2.3) | 111.7 (328.5) | 1491.3 (3728.3) | 25.7 (73.1) | 1.3 (3.2) | 0.9–1.7 (2.4–4.4) |
| isoBPB | 6.3 | <LOD | <LOD | 0.1 (0.4) | 1.1 (1.7) | 0.1 (0.1) | NC | NC |
| nBPB | 25.9 | <LOD | <LOD | 1.3 (2.2) | 93.2 (665.9) | 1.0 (5.9) | NC | NC |
| BPA | 99.6 | <LOD | 2.1 (5.2) | 16.6 (31.0) | 68.7 (121.7) | 4.5 (9.6) | 2.0 (5.0) | 1.7–2.4 (4.3–5.8) |

[a] <LOD: lower than limit of detection.
[b] NC: not calculated.

### 2.4. Comparison with other studies worldwide

Literature search for similar studies was performed via EndNote X7 (Thompson Reuters) in PubMed database on December 04, 2014. The search criteria were the following: for phthalate metabolites/BPA, titles containing (*phthalate or bisphenol-a or bpa) and (child* or mother* or pregnan* or women) and for parabens titles containing *paraben*. The selection criteria were: for studies measuring total metabolites (free, glucuronated and sulphated) from pregnant women or children for over 200 urine samples for phthalate metabolites/BPA and 100 for parabens, with creatinine normalised median concentrations available for BPA or at least for 4 common phthalate metabolites or 4 common parabens with our study.

Initially, 796 articles were identified: 93 hits for (*phthalate* and child*), 10 for (*phthalate* and mother*), 63 for (*phthalate* and pregnan*), 46 for (*phthalate* and women), 44 for (bisphenol-a and pregnan*), 53 for (bisphenol-a and child*), 8 for (bisphenol-a and mother*), 41 for (bisphenol-a and women), 3 for (bpa and women), 1 for (bpa and mother*), 2 for (bpa and pregnan*), 4 for (bpa and child*) and 428 for *paraben*. Fifteen (15) of them fulfilled the search criteria: (Boas et al. (2010); Braun et al. (2009, 2011); Casas et al. (2013); Frederiksen et al. (2013); Harley et al. (2013); Hong et al. (2013); Kasper-Sonnenberg et al. (2014); Lee et al. (2014); Mortamais et al. (2012); Quiros-Alcala et al. (2013); Tefre de Renzy-Martin et al. (2014); Teitelbaum et al. (2012); Wang et al. (2014); Zeman et al. (2013)). References of the selected papers were also checked but no

additional articles identified. In the case of concentrations given separately for male/female children or at different time-points of pregnancy, the arithmetic mean of the given median values was used.

## 3. Results

### 3.1. Exposure levels

#### 3.1.1. Concentration levels

The metabolites mEP, mnBP, miBP, mEOHP, mEHHP, mBzP, MPB, EPB, nPPB and BPA were detected in >90.8% of mother samples and in >86.2% of children samples (239 mother–child pairs), while mEHP was detected in the 72.7% of mother and 57.3% of children samples; isoPPB, isoBPB and nPPB ranged from 12.0% to 38.6% detectability for mothers and 2.1% to 25.9% for children (Table 2). mNP was not detected in any of the analysed samples. Due to the variable density of urine spot samples (Barr et al., 2005b), creatinine normalisation was used in order to rank the concentration levels of the studied compounds.

Among the creatinine adjusted median levels of phthalate metabolites, mEP was the most abundant for mothers (133.6 μg/g) and miBP for children (101.6 μg/g); miBP concentrations were at higher levels compared to mnBP for both categories; from DEHP metabolites, mEHHP and mEOHP were at higher levels compared to mEHP also for both categories; mBzP was the less abundant detected phthalate metabolite (Table 2). Concerning creatinine adjusted median paraben levels, MPB was the most abundant for both categories (mothers: 121.9 μg/g, children: 42.6 μg/g), but for mothers, nPPB levels were higher compared to EPB and for children EPB levels were higher than nPPB; the other three parabens had median values <mLOD (Table 2). BPA levels were found at 1.1 μg/g for mothers and at 5.2 μg/g for children. When children were categorised by gender, males exhibited statistically significant (Mann–Whitney *U* test; p-value < 0.05), higher unnormalised concentrations (ng/mL) for nPPB and all phthalate metabolites except for mEHP (Table 3). The same trend was observed for creatinine adjusted levels, although the differences were not statistically significant. This fact can be attributed to the higher urinary creatinine levels in males (arithmetic mean, males: 0.53 g/L, females: 0.45 g/L; independent samples *t*-test, p-value: 0.043).

#### 3.1.2. Estimated daily intake

The estimated daily intake levels for all parabens and phthalates (median values, Table 4) were higher for mothers during pregnancy than for children at about two years of age (Wilcoxon signed ranks test, p-value < 0.001), except for DEHP (also higher in mothers but p-value was 0.055), as shown in Fig. 1. In contrast, BPA-$DI_u$ was higher in children compared to their mothers (Wilcoxon signed ranks test, p-value 0.013). DEP for mothers and DEHP for children were the two phthalates with the highest median values of $DI_u$, followed by DiBP, DnBP and BBP in decreasing order. The estimated $DI_u$ for all phthalates was higher for mothers compared to that for children, in contrast to the creatinine-normalised values (Table 5). Furthermore, DEHP $DI_u$ was the highest for children (4.0 μg $d^{-1}$ $kg^{-1}$, Table 5) and the second highest for mothers (4.4 μg $d^{-1}$ $kg^{-1}$, Table 4), in contrast to DEHP metabolites levels, which were relatively lower compared to those of other phthalate metabolites. For paraben daily intake, the same patterns were observed with the concentration values (Tables 2 and 4, Fig. 1). BPA-Diu was the lowest among the examined endocrine disruptors (median values, Table 4) for both mothers (0.03 μg $d^{-1}$ $kg^{-1}$) and children (0.05 μg $d^{-1}$ $kg^{-1}$).

**Table 3**
Gender-based comparison of children median concentration levels, ng/mL of urine (μg/g of creatinine).

| | Male | Female | p-Value |
|---|---|---|---|
| mEP | 40.3 (92.3) | 24.8 (76.0) | 0.006 (0.265) |
| miBP | 43.5 (114.5) | 28.5 (90.7) | 0.004 (0.104) |
| mnBP | 27.4 (65.0) | 21.1 (59.6) | 0.011 (0.229) |
| mEHHP | 35.6 (76.0) | 20.9 (59.5) | 0.005 (0.032) |
| mEOHP | 23.3 (53.2) | 15.4 (46.2) | 0.015 (0.070) |
| mBzP | 7.1 (19.5) | 5.1 (15.6) | 0.009 (0.143) |
| mEHP | 2.9 (8.0) | 2.5 (11.1) | 0.606 (0.122) |
| MPB | 21.5 (41.9) | 13.6 (43.2) | 0.095 (0.699) |
| EPB | 1.7 (3.7) | 1.3 (3.6) | 0.425 (0.699) |
| nPPB | 1.4 (2.3) | 0.6 (2.3) | 0.036 (0.261) |
| BPA | 2.2 (5.2) | 1.8 (5.0) | 0.256 (0.813) |

#### 3.1.3. Comparison with other studies worldwide

The overall phthalate exposure, evaluated in our study, is generally comparable with literature reports from other countries. In general, the specific phthalate exposure pattern is also similar. Comparison with other studies (Table 5) demonstrated slightly higher exposure to phthalates for mothers in Greece compared to those in Denmark (Tefre de Renzy-Martin et al., 2014) and slightly lower compared to those in France (Mortamais et al., 2012; Zeman et al., 2013). Moreover, for children there are no distinct differences for phthalate metabolite levels in general (Table 5). However, a study from Germany (Kasper-Sonnenberg et al., 2014) reported the lowest concentrations (except for miBP) levels. More specifically the comparison, presented in Table 5, shows the highest miBP levels in Greece (our study), the highest mnBP levels in Denmark (Boas et al., 2010) and the highest mEP levels in the USA (Teitelbaum et al., 2012).

Paraben concentration levels for both mothers and children in Greece appear to be clearly higher compared to those reported in a study from Denmark (Frederiksen et al., 2013). We have to underline that our data represent only free and glucuronated parabens in contrast to the above referred study, which reports total paraben concentrations. Finally, children BPA levels in our study were the second highest (5.2 μg/g), but the highest levels (Braun et al., 2011) were about three times higher compared to ours.

### 3.2. Exposure patterns

#### 3.2.1. Correlation studies

Statistically significant Spearman correlations were observed between the creatinine adjusted molar concentrations (μmol/g) of the studied compounds both for mothers and children. We present only correlations with p-values lower than 0.01 (Table 6). We thus observed positive correlations between almost all phthalate metabolites and BPA for mothers (correlation coefficient, CC: 0.170–0.488). All paraben concentration levels were positively correlated (CC: 0.468–0.841) and also correlated with mEP (CC: 0.251–0.335). Likewise, for children almost all studied metabolites correlated positively (CC: 0.167–0.809; Table 6). Furthermore, metabolites of DEP, DnBP and BBP as well as EPB showed weak (0.133–0.225) correlation between mother and children levels. Child age correlated negatively (−0.193 to −0.476) with all studied metabolite concentration levels (Table 6). No statistically significant correlations were found for the remaining studied compounds between mothers and children (Table 6).

#### 3.2.2. Principal component analysis

In order to obtain more information about exposure sources, a PCA was applied to mother and children metabolite concentration data. Four factors were retained with Eigen values >1.000 and expressed 66.2% of the variance (Table 7). Mothers were associated with factors 2 and 4 while children were associated with 1 and 3. Factors 1–2 represent combined exposure to phthalate/BPA and factors 3–4 exposure to parabens/DEP. Also, the study of Spearman correlation was conducted to the analogous results.

**Table 4**
Estimated daily intake of phthalates, parabens and BPA.

| | Minimum | Median | 95% percentile | Maximum | Arithmetic mean | Geometric mean | 95% CI |
|---|---|---|---|---|---|---|---|
| *239 mothers (μg $d^{-1}$ $kg^{-1}$)* | | | | | | | |
| DEP | 0.2 | 6.9 | 74 | 182.4 | 17.9 | 7.1 | 6.0–8.4 |
| DiBP | NC[a] | 2.1 | 11 | 30.6 | 3.4 | 2 | 1.7–2.3 |
| DnBP | NC | 1.9 | 11.4 | 4839.8 | 24 | 1.7 | 1.5–2.1 |
| DEHP | NC | 4.4 | 25.6 | 1015.0 | 12.2 | 4.1 | 3.5–4.8 |
| BBzP | NC | 0.3 | 1.8 | 9.9 | 0.6 | 0.3 | 0.3–0.4 |
| MPB | NC | 500.0 | 17076.1 | 388041.8 | 6388.1 | 532.7 | 413.6–688.8 |
| EPB | NC | 13.2 | 599.9 | 2388.4 | 109.3 | 16.4 | 12.4–21.3 |
| isoPPB | NC | NC | 4.0 | 281.2 | 4.8 | NC | NC |
| nPPB | NC | 73.3 | 3818.5 | 162105.5 | 2203.1 | 58.2 | 40.1–82.2 |
| isoBPB | NC | NC | 8.1 | 183.5 | 2.0 | NC | NC |
| nBPB | NC | NC | 82.6 | 781.4 | 20.0 | NC | NC |
| BPA | NC | 0.03 | 0.14 | 4.23 | 0.08 | 0.04 | 0.03–0.04 |
| *239 children (μg $d^{-1}$ $kg^{-1}$)* | | | | | | | |
| DEP | NC | 1.4 | 8.6 | 91.4 | 2.9 | 1.3 | 1.1–1.5 |
| DiBP | NC | 1.4 | 8.2 | 36.0 | 2.5 | 1.5 | 1.30–1.7 |
| DnBP | NC | 1.0 | 6.6 | 50.8 | 1.9 | 0.9 | 0.8–1.1 |
| DEHP | NC | 4.0 | 21.6 | 69.6 | 6.8 | 3.3 | 2.8–3.9 |
| BBzP | NC | 0.2 | 1.3 | 9.0 | 0.5 | 0.3 | 0.2–0.3 |
| MPB | NC | 66.6 | 3674.9 | 26526.7 | 772.5 | 97.5 | 77.5–123.54 |
| EPB | NC | 5.8 | 375.1 | 4353.3 | 85.9 | 7.0 | 5.4–9.1 |
| isoPPB | NC | NC | NC | 42.0 | 0.5 | NC | NC |
| nPPB | NC | 3.4 | 435.4 | 5812.6 | 100.1 | 5.0 | 3.7–6.7 |
| isoBPB | NC | NC | 0.16 | 2.6 | 0.1 | NC | NC |
| nBPB | NC | NC | 2.9 | 217.0 | 2.3 | NC | NC |
| BPA | NC | 0.05 | 0.37 | 1.54 | 0.10 | 0.04 | 0.04–0.05 |

[a] NC: not calculated.

### 3.3. DEHP metabolism

Relative metabolic rates $RMR_1$ and $RMR_2$ were calculated for mothers (N = 170) and children (total N = 136, female and male) for samples in which, all three DEHP metabolites were detected. The $RMR_1$ arithmetic mean for mothers was 3.33 and for children 8.06, and the arithmetic mean $RMR_2$ for mothers was 0.80 and for children 0.76. Furthermore, the RMR levels between mother–child pairs (where all DEHP metabolites had been detected in both mother and child, N = 107) were compared. $RMR_1$ was found to be statistically significant in higher levels in mothers (arithmetic means; 7.60 and 3.42 respectively; Wilcoxon signed ranks test; p-value < 0.001). On the other hand, $RMR_2$ was found to be statistically significant in the lower levels in mothers (arithmetic means; 0.76 and 0.81 respectively; Wilcoxon signed ranks test; p-value 0.002). Male children mean $RMR_1$ (8.83) was higher than female children (6.67), but without a statistically significant difference (Mann–Whitney *U* test, p-value 0.06). Instead, male children mean $RMR_2$ (0.72) was significantly lower (p-value 0.02) compared to female children (0.81). Comparison of the age of children with $RMR_1$ and $RMR_2$ did not show any statistically significant correlation (Spearman; p-values: 0.18 for $RMR_1$ and 0.10 for $RMR_2$).

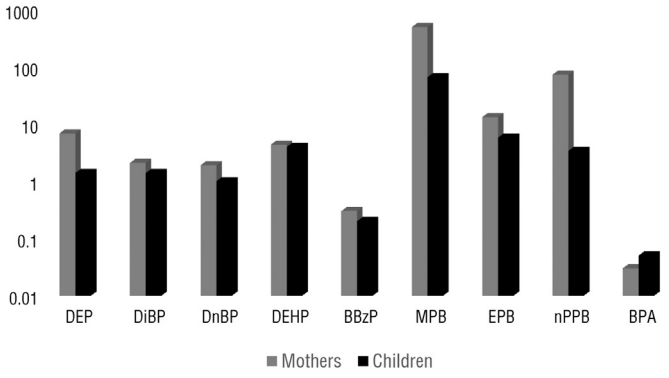


**Fig. 1.** Estimated daily intake mother–child comparison, median values, μg $d^{-1}$ $kg^{-1}$.

## 4. Discussion

### 4.1. Exposure levels

Phthalate metabolite and BPA levels in Greek mother–child populations are comparable with those reported for other countries. Parabens seem to be at higher levels compared to a report form Denmark (Frederiksen et al., 2013) for both mothers and children, especially when taking into account that our values are slightly underestimated (representing only the glucuronated plus free forms of parabens in contrast with the Danish study which monitored total paraben levels). However, it has to be mentioned that bibliographic comparisons suffer from several weaknesses. They don't take into account a) market changes of phthalates, parabens and BPA, corresponding to different time periods of sample collection, b) different ages of children and phases of pregnancy, c) different methods of analysis and analyte detection limits, and d) lack of statistical tests for inter-study comparisons, etc. Especially, for paraben levels, more data are needed in order to assess globally their exposure. mNP is not considered a proper urine biomarker for DiNP exposure. Secondary metabolites (e.g. mCOP, mono-carboxy-octyl phthalate) of this phthalate should be used as biomarkers. The same conclusions concerning mNP were also drawn in other studies (Koch et al., 2007; Silva et al., 2006b).

Taking into account that creatinine excretion is related to muscle mass (which is higher in males) (Baxmann et al., 2008), comparison of unnormalised concentration levels of the studied metabolites instead of creatinine adjusted, seems more appropriate. Higher levels of phthalate metabolites (except DEHP) and nPPB in male children indicate less effective metabolism and excretion compared to females. Previous studies did not report general differences between sexes (Boas et al., 2010; Cutanda et al., 2014; Guo et al., 2011; Langer et al., 2014; Song et al., 2013) or have shown slightly higher concentrations in males only for DEHP metabolites (Wittassek et al., 2007).

Daily intake of the studied compounds is higher in mothers compared to their children. We must take into account that there is a two and a half year gap between samplings and that except for differentiations in pregnant–child exposure, changes in the phthalate, paraben and BPA market may also affect exposure levels. DEHP-$DI_u$ was the

**Table 5**
Comparison with similar studies, creatinine adjusted median (μg/g) values.

| Country; number of samples; reference | mEP | miBP | mnBP | mEHHP | mEOHP | mBzP | mEHP | MPB | EPB | isoPPB | nPPB | isoBPB | nBPB | BPA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mothers | | | | | | | | | | | | | | |
| Greece; n = 239; this study | 132.6 | 38.7 | 33.2 | 24.4 | 15.9 | 7.0 | 7.3 | 121.9 | 2.9 | <LOD | 17.5 | <LOD | <LOD | 1.1 |
| France; n = 287; Mortamais et al. (2012) | 106.0 | 45.7 | 48.5 | – | – | 16.0 | – | – | – | – | – | – | – | – |
| Denmark; n = 200; Tefre de Renzy-Martin et al. (2014) | 18.9 | 35.3 | 13.9 | 5.7 | 3.72 | 2.3 | 1.1 | – | – | – | – | – | – | – |
| France; n = 279; Zeman et al. (2013) | 34.3 | 68.7 | 45.5 | 44.4 | 32.9 | 13.0 | 17.9 | — | — | — | — | — | — | — |
| Denmark; n = 143; Frederiksen et al. (2013) | — | — | — | — | — | — | — | 16.0 | 0.91 | <LOD | 1.8 | <LOD | <LOD | — |
| Korea; n = 757; Lee et al. (2014) | — | — | — | — | — | — | — | — | — | — | — | — | — | 1.6 |
| USA; n = 866; Quiros-Alcala et al. (2013) | — | — | — | — | — | — | — | — | — | — | — | — | — | 1.1 |
| Spain; n = 479 Casas et al. (2013) | — | — | — | — | — | — | — | — | — | — | — | — | — | 2.2 |
| USA; n = 244; Braun et al. (2011) | — | — | — | — | — | — | — | — | — | — | — | — | — | 2.2 |
| USA; n = 249; Braun et al. (2009) | — | — | — | — | — | — | — | — | — | — | — | — | — | 1.8 |
| Children | | | | | | | | | | | | | | |
| Greece; n = 239; this study | 86.6 | 101.6 | 62.3 | 71.0 | 51.0 | 17.0 | 9.1 | 42.6 | 3.7 | <LOD | 2.3 | <LOD | <LOD | 5.2 |
| Denmark; n = 845; Boas et al. (2010) | 33.5 | — | 209.0 | 52.0 | 27.0 | 23.0 | 6.8 | — | — | — | — | — | — | — |
| USA; n = 379; Teitelbaum et al. (2012) | 164.9 | 22.5 | 68.4 | 73.9 | 47.6 | 41.8 | 6.4 | — | — | — | — | — | — | — |
| Germany; n = 465; Kasper-Sonnenberg et al. (2014) | 21.4 | 41.1 | 42.3 | 20.2 | 13.5 | 6.0 | 2.23 | — | — | — | — | — | — | 1.8 |
| Denmark; n = 143; Frederiksen et al. (2013) | — | — | — | — | — | — | — | 0.9 | 0.26 | <LOD | <LOD | <LOD | <LOD | — |
| USA; n = 292; Harley et al. (2013) | — | — | — | — | — | — | — | — | — | — | — | — | — | 3.2 |
| China; n = 1089; Hong et al. (2013) | — | — | — | — | — | — | — | — | — | — | — | — | — | 1.3 |
| China; n = 666; Wang et al. (2014) | — | — | — | — | — | — | — | — | — | — | — | — | — | 2.2 |
| USA; n = 229; Braun et al. (2011) | — | — | — | — | — | — | — | — | — | — | — | — | — | 14.0 |
| USA; n = 249; Braun et al. (2009) | — | — | — | — | — | — | — | — | — | — | — | — | — | 1.9 |

highest for phthalates among children and second highest among mothers in contrast with DEHP metabolites which are in relatively lower levels. This observation is due to the extended metabolism of DEHP to many compounds (Koch et al., 2005b). The values of Reference Doses (RfD, μg $d^{-1}$ $kg^{-1}$; DEP:800, DnBP:100, BBP:200, DEHP:20) established by U.S. Environmental Protection Agency (U.S. E.P.A., 2005a, 2005b, 2005c) and of Tolerable Daily Intake (TDI, μg $d^{-1}$ $kg^{-1}$; DEP:500, DnBP:10, BBP:500, DEHP:50) set by the European Food Safety

**Table 6**
Spearman's correlation coefficients, p-values <0.01.

| | M-DnBP | M-BBzP | M-DEHP | M-MPB | M-EPB | M-nPPB | M-BPA | C-DEP | C-DiBP | C-DnBP | C-BBzP | C-DEHP | C-MPB | C-EPB | C-nPPB | C-BPA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M[a]-DEP | | | 0.17 | 0.25 | 0.34 | 0.28 | | 0.20 | | | | | | | | |
| M-DiBP | 0.44 | 0.35 | 0.42 | | | | | | | | | | | | | |
| M-DnBP | | 0.37 | 0.49 | | | | | | | 0.23 | | | | | | |
| M-BBzP | | | 0.44 | | | | 0.25 | | | | 0.18 | | | | | |
| M-MPB | | | | | 0.54 | 0.84 | | | | | | | | | | |
| M-EPB | | | | | | 0.46 | | | | | | | | 0.13 | | |
| M-BPA | | | | | | | | | | | | | | | | |
| C[b]-age (y) | | | | | | | | −0.43 | −0.43 | −0.48 | −0.37 | −0.19 | −0.38 | −0.47 | −0.38 | −0.21 |
| C-DEP | | | | | | | | | 0.52 | 0.48 | 0.38 | 0.27 | 0.36 | 0.40 | 0.37 | 0.19 |
| C-DiBP | | | | | | | | | | 0.76 | 0.44 | 0.42 | 0.32 | 0.31 | 0.34 | 0.29 |
| C-DnBP | | | | | | | | | | | 0.58 | 0.48 | 0.29 | 0.34 | 0.34 | 0.31 |
| C-BBzP | | | | | | | | | | | | 0.50 | 0.27 | 0.31 | 0.27 | 0.32 |
| C-DEHP | | | | | | | | | | | | | 0.17 | | 0.19 | 0.30 |
| C-MPB | | | | | | | | | | | | | | 0.67 | 0.81 | |
| C-EPB | | | | | | | | | | | | | | | 0.67 | 0.20 |

[a] M-: mothers.
[b] C-: children.

**Table 7**
PCA: rotated component matrix and total variance explained.

| Component | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Eigenvalue | 4.663 | 3.274 | 2.177 | 1.800 |
| % of variance | 25.9 | 18.2 | 12.1 | 10.0 |
| Cumulative % | 25.9 | 44.1 | 56.2 | 66.2 |
| C-DnBP[a] | 0.897 | | | |
| C-DiBP | 0.880 | | | |
| C-DEHP | 0.843 | | | |
| C-BBzP | 0.803 | | | |
| C-DEP | 0.662 | | 0.319 | |
| C-MPB | | | 0.899 | |
| C-EPB | | | 0.856 | |
| C-nPPB | | | 0.906 | |
| C-BPA | 0.635 | | | |
| M-DnBP[b] | | 0.752 | | |
| M-DiBP | | 0.748 | | |
| M-DEHP | | 0.844 | | |
| M-BBzP | | 0.790 | | |
| M-DEP | | 0.448 | | 0.421 |
| M-MPB | | | | 0.913 |
| M-EPB | | | | 0.740 |
| M-nPPB | | | | 0.906 |
| M-BPA | | 0.509 | | |

Coefficients <0.200 are not presented. Rotation converged in 6 iterations.
[a] C-: children.
[b] M-: mothers.

Authority (EFSA, 2005), were compared with our results. For DnBP, 5.9% of mothers revealed higher DnBP-$DI_u$ than DnBP-TDI (0.8% of subjects higher compared to DnBP-RfD). For DEHP, 6.8% of mothers' $DI_u$ exceeded RfD (0.6% exceeded DEHP-TDI). For children, 1.7% of the cases studied were found to exceed DnBP-TDI. Finally, 6.3% of children's DEHP-$DI_u$ was higher than the corresponding DEHP-RfD (1.3% of subjects exceeded TDI). The higher intake of parabens and phthalates (except from DEHP) by mothers can be interpreted by the extensive usage of cosmetics (Elder, 1984). This was not observed for DEHP, which is widely used as a plasticiser (Wormuth et al., 2006). Finally, none of the participants of this study exceeded either current or recommended BPA-TDI (current: 50 μg $d^{-1}$ $kg^{-1}$/recommended: 5 μg $d^{-1}$ $kg^{-1}$) (EFSA, 2014).

### 4.2. Exposure patterns

Correlation studies and PCA suggest exposure to mixtures of parabens, phthalates and BPA. For both mothers and children, two distinct exposure sources are implied, one containing all determined phthalates/BPA and a second with all three studied parabens and DEP (whose metabolite is mEP). The DEP/paraben relationship suggests cosmetic and personal care products as sources, while phthalate/BPA relationships indicate plastic and food packaging as a possible source (Elder, 1984; Geens et al., 2012; Wormuth et al., 2006).

Correlations between mother–child pair levels (for EPB, DEP, DnBP and BBP metabolites) indicate that, although we examined two different individuals within a time interval of ca. three years (sample collection time from women during pregnancy and from their children) implying also changes in the phthalate–paraben–BPA market, some sources of exposure common to people living together (house, car use, etc.). Analogous observations have been reported previously (Cutanda et al., 2014). The elimination half times of phthalates, parabens and BPA are low. For example, BPA and DEHP metabolite EHT values are some hours (Koch et al., 2004a, 2005a, 2005b; Volkel et al., 2002) and obviously these observations cannot be assigned to in utero exposure. Although, even the very low half-lives of these compounds, the correlations between mothers and their children point out, that our study-design (with the limitations of analysing spot samples from only a part of Rhea participants) can offer a reliable evaluation of exposure levels. Finally, the need for repeated measurements is highlighted since the correlations between mother and child levels are weak and not general.

The negative correlation of child age with concentration levels of metabolites can be assigned to a) the food exposure ratios (consumed food mass/body mass), b) the corresponding dermal exposure ratios (skin surface/body mass), and c) the floor-to-mouth behaviour decrease as child age increases (Casas et al., 2011; Wittassek et al., 2007).

### 4.3. DEHP metabolism

To sum up: I) the transformation of mEHP to mEHHP (hydroxylation) is faster in mothers compared to children, as was also reported in another study (Song et al., 2013). However, other studies (Barr et al., 2003; Becker et al., 2004; Kasper-Sonnenberg et al., 2012; Koch et al., 2004b) reported contrasting results; II) the transformation of mEHHP to mEOHP (oxidation) is faster in children compared to mothers and III) the transformation of mEHHP to mEOHP (oxidation) seems to be faster in male children, indicating that DEHP metabolism is related both to children age/gender and differentiates between mothers and children.

### 4.4. Conclusions

In our study, the first in Greece and one of the few existing in this scale globally, we observed lower phthalate (except DEHP) and paraben daily intake for children than mothers while BPA daily intake was higher in children. Children metabolite levels decrease with age increase. Some sources of exposure seem to be the same in mothers during pregnancy and afterwards. The PCA indicated possible sources of phthalates, parabens and BPA as originating from plastic and personal care-hygiene products for both mothers and children. Male children demonstrated higher concentrations of six phthalate metabolites and n-PPB compared to females. DEHP metabolism appears to be differentiated between mother–child pairs and female–male children. For DiNP exposure, mNP is not a suitable biomarker. Our results were comparable with literature reports.

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