

# Exposure to the plasticizer di(2-ethylhexyl) terephthalate (DEHTP) in Portuguese children – Urinary metabolite levels and estimated daily intakes

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## Keywords:

Di(2-ethylhexyl) terephthalate

Plasticizer

Human biomonitoring

Children

Urine

Daily intake

## A B S T R A C T

Classical ortho-phthalate plasticizers are, due to their endocrine disrupting potency and reproductive toxicity, increasingly replaced by alternative plasticizers. Di(2-ethylhexyl) terephthalate (DEHTP) is one of these substitutes. In this study, we investigated DEHTP exposure in 107 Portuguese children (4–17 years old) by analyzing specific DEHTP metabolites in their urine using a newly developed LC-MS/MS method. We could detect the major, specific DEHTP metabolite mono(2-ethyl-5-carboxypentyl) terephthalate (5cx-MEPTP) in 100% of the samples with levels above the limit of quantification in 96% of the samples (median concentration 4.19 µg/L; 95th percentile 26.4 µg/L; maximum 3400 µg/L). Other minor DEHTP metabolites (5OH-MEHTP, 5oxo-MEHTP and 2cx-MMHTP) were detected at lower rates and levels. Daily DEHTP intakes calculated from urinary 5cx-MEPTP levels were generally far below the tolerable daily intake (TDI) of 1000 µg/kg bw/d (median 0.67 µg/kg bw/d; 95th percentile 6.25 µg/kg bw/d; maximum 690 µg/kg bw/d). However, for one child the biomarker-derived health-based guidance value (HBM-I value) for 5cx-MEPTP of 1800 µg/L was exceeded by about a factor of two. Levels of 5cx-MEPTP and calculated daily DEHTP intakes were higher in normal/underweight children who nourished on their usual diet compared to overweight/obese children who received nutritional guidance with fresh and unprocessed food ( $p = 0.043$  and  $p < 0.001$  respectively). This indicates to processed and fatty foodstuff as a major source of DEHTP exposure. Additionally, we found children of lower age having higher DEHTP intakes ( $p = 0.045$ ). Again, foodstuff as a major DEHTP source, together with other child specific DEHTP sources such as mouthing of toys or ingestion of dust might be contributing factors. With the present study, we provide a first data set on the omnipresent DEHTP exposure in children. So far, general levels of DEHTP exposure seem no cause for concern. However, due to the increasing use of DEHTP as an ortho-phthalate substitute, possible increasing exposures in the future should be followed closely.

## 1. Introduction

Di(2-ethylhexyl) terephthalate (DEHTP), CAS Registry No. 6422-86-2, a structural isomer of Di(2-ethylhexyl) phthalate (DEHP), is used as an alternative plasticizer for polymers like polyvinylchloride (PVC). Lately, some “classic” PVC plasticizers such as the high molecular weight (HMW) phthalate DEHP, are under scrutiny due to their proven reproductive toxicity and anti-androgenic activity in rodents. These effects, also known as the “phthalate syndrome” are mainly caused by inhibition of fetal testicular testosterone production during sexual

differentiation leading to reproductive tract malformations, reduced fertility and/or influences on the male phenotype (shortening of the anogenital distance and areola/nipple retention) (Foster, 2006, Boberg et al., 2011, Kilcoyne et al., 2014). As a consequence, DEHP has been classified as toxic to reproduction category 1B according to the European Regulation on classification, labelling and packaging of substances (EU CLP Regulation) (European Parliament, 2008). Since 1999, DEHP, di(isononyl) phthalate (DiNP), di(isodecyl) phthalate (DiDP) and di(n-octyl) phthalate (DnOP) have been banned or restricted in sensitive applications such as toys or childcare articles

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according to Regulation (EC) No 1907/2006, Annex XVII, 51/52. From February 2015 on (REACH sunset date), DEHP must not be placed on the EU market any more, being listed in Annex XIV of the REACH regulation EC No 1907/2006 (European Commission, 2006). However, since plasticizers are still indispensable in many applications, alternative plasticizers like DEHTP, with advantageous toxicological profiles (Gray et al., 2000, Furr et al., 2014) and no use restrictions are gaining importance in the worldwide plasticizer market. In the year 2002 the Western European consumption volume of DEHTP amounted to a total of 2000 mt. Consumption rose to 100,000 mt in 2014. Predictions for the year 2019 estimate a total production of about 135,000 mt, clearly reflecting the growing importance of DEHTP as an alternative plasticizer (Malveda et al., 2015).

Typical effects associated with DEHP toxicity have not been observed for DEHTP (Gray et al., 2000). Furr et al. (2014) reported no disruption of fetal testosterone synthesis or altered testis gene expression in rats in their Fetal Phthalate Screen (FPS). Toxicological studies with DEHTP reported no or very weak peroxisome proliferating potential in rats (Barber and Topping, 1995, Topping et al., 1987) indicated by increased relative liver weight at the highest dietary DEHTP content of 2.5%. However, the authors concluded, that relative liver weight might have been increased only due to reduced feed consumption. The European Food Safety Authority (EFSA) evaluated DEHTP (EFSA, 2008) and derived a tolerable daily intake (TDI) of 1000 µg/kg bw/d based upon a 2-year combined toxicity/carcinogenicity study (Deyo, 2008); the most sensitive end points observed were effects on the retina and nasal turbinates. Recently, the German Human Biomonitoring Commission has published new HBM values for emerging chemicals, DEHTP being one of them (Apel et al., 2016). The HBM-I value for the main specific urinary metabolite mono(2-ethyl-5-carboxypentyl) terephthalate (5cx-MEPTP) in urine, above which a possible adverse health effect cannot be excluded anymore, was derived to be 1800 µg/L based on the endpoint “effects on the retina” as observed by Deyo (2008).

According to Commission Regulation (EU) Regulation No 10/2011, DEHTP is approved as an additive in food contact materials with a specific migration limit of 60 mg/kg food (European Commission, 2011). Together with the gradually increasing production of DEHTP, a widespread exposure of the general population to DEHTP has to be expected. Human biomonitoring has been proven to be an ideal tool to assess population exposure to phthalates or other plasticizers (Silva et al., 2003, Koch et al., 2004, 2005, 2013a, 2013b, Koch and Angerer, 2012, Kasper-Sonnenberg et al., 2014, Schütze et al., 2014). A pilot biomonitoring study with German adults already indicated an omnipresent exposure of non-occupationally exposed individuals to DEHTP (Lessmann et al., 2016a). More than 90% of the urine samples analyzed contained DEHTP metabolites above the limit of quantification. With this study we intend to broaden the knowledge on DEHTP exposure to Portugal, another country in the European Union. Furthermore, previous studies have reported that the plasticizer body burden of children can be higher, compared to adults (Koch and Angerer, 2007, Kasper-Sonnenberg et al., 2014, Den Hond et al., 2015, Cutanda et al., 2015, Fromme et al., 2016). Thus, investigating children in this study was of additional interest. Due to the special composition of the children population of this study (obese children under nutritional guidance vs. normal weight children on their usual diet) another aim was to investigate possible differences in DEHTP exposures among these children.

## 2. Methods

### 2.1. Subjects and urine specimens

The present study is part of an ongoing study investigating exposure of obese/overweight and regular weight children to certain environmental chemicals. The initial aim of this project was the determination of exposure to several suspected or confirmed (predominately persis-

tent) endocrine disruptors and/or obesogens. Due to the ongoing substitution process and considering new regulatory requirements, plasticizers and plasticizer substitutes like DINCH and DEHTP have subsequently been added to the list of substances of interest. The study design itself has not been created with relevance to DEHTP. Originally, 112 Portuguese children donated first morning urine voids, and their complete anthropometric data (gender, age, height, and weight) were recorded. Samples were collected in the years 2014/2015 in the pediatric appointment at Hospital de S. João, and several local schools in the regions of Oporto and Aveiro, located in the north and central region of Portugal. At the time of analysis of the present study, sample material of 107 children, aged 4–17 years, was left. The available samples were divided into two groups according to the respective children's body mass index (BMI). Group 1 consisted of 39 normal/underweight children, without any known associated diseases, on a usual diet without further nutritional guidance. Group 2 consisted of 68 overweight/obese children, without other known associated diseases, receiving specific nutritional guidance with fresh and unprocessed food (meaning fresh fruits and vegetables, whole grains, low-fat and nonfat dairy products, beans, fish, and lean meat). The children's nutritional status was assessed according to the World Health Organization growth charts (WHO, 2007). Body weight and BMI differed significantly ( $p < 0.05$ ) between the two investigated study groups, whereas age, gender, height and urinary creatinine did not ( $p > 0.05$ ). A detailed description of the study population is given in Table 1.

### 2.2. Chemical analysis

The on-line HPLC-MS/MS method used for quantification of specific urinary DEHTP metabolites has been described in detail by Lessmann et al. (2016a). In short, to each urine sample aliquot, ammonium acetate buffer and internal standard solution were added. After enzymatic hydrolysis with  $\beta$ -glucuronidase from *E. coli* K12 (arylsulfatase free), the pH was adjusted with acetic acid and samples were frozen over night to precipitate proteins. After thawing, samples were centrifuged and the supernatant was injected into an Agilent Technologies LC 1260 system (Agilent 1260 autosampler, two Agilent 1260 binary pumps) coupled to an AB Sciex 4500 triple quadrupole mass spectrometer in negative ionization mode. On-line SPE column assembly, HPLC gradient and MS/MS conditions remain as described in Lessmann et al. (2016a). The limit of quantification (LOQ) was 0.2 µg/L for mono(2-ethyl-5-carboxypentyl) terephthalate (5cx-MEPTP) and mono(2-ethyl-5-oxohexyl) terephthalate (5oxo-MEHTP), 0.3 µg/L for mono(2-ethyl-5-hydroxyhexyl) terephthalate (5OH-MEHTP), and 0.4 µg/L for mono[2-(carboxymethyl)hexyl] terephthalate (2cx-MMHTP). Urinary creatinine concentrations were determined according to a modified Jaffe method (Jaffe, 1886) with an Olympus AU5400® Chemistry Analyzer.

**Table 1**  
General characteristics of the investigated study population.

Population characteristics	Group 1 Normal/under weight, usual diet	Group 2 Overweight/obese, nutritional guidance	Total
n	39	68	107
Age (years) median	11.0	9.0	10.0
Gender (%)	44% female, 56% male	56% female, 44% male	49% female, 51% male
Height (cm) median	143	142	142
Body weight (kg) median	35	46	45
BMI (kg/m <sup>2</sup> ) median	17.1	24.7	22.3
Creatinine (g/L) median	0.87	0.96	0.93

### 2.3. Daily intake estimation

For the estimation of daily intakes based on 5cx-MEPTP levels in spot urine samples of children, we applied the approach published by Koch and Angerer (2007) and Wittassek et al. (2007) for the plasticizers Di(n-butyl) phthalate (DnBP), butyl-benzyl phthalate (BBzP) and DEHP. In short, the calculation is based on the creatinine related metabolite concentration, combined with reference values for the 24-hour creatinine excretion in children according to Remer et al. (2002). The daily intake in  $\mu\text{g/kg bw/d}$  is calculated by the following equation modified according to previous daily intake calculations (David, 2000, Kohn et al., 2000, Koch et al., 2003a, 2003b, Schütze et al., 2014):

$$\text{DI}(\mu\text{g/kg}_{\text{bw}}/\text{day}) = \frac{\text{UE}_{5\text{cx-MEPTP}}(\mu\text{mol/g}_{\text{crea}}) \times \text{CE}_{\text{smoothed}}(\text{g/day})}{\text{F}_{\text{UE}} \times \text{bw}(\text{kg}) \times \text{MW}_{\text{DEHTP}}(\text{g/mol})}$$

$\text{UE}_{5\text{cx-MEPTP}}$  is the concentration of 5cx-MEPTP in the respective urine sample in micromole per gram creatinine.  $\text{CE}_{\text{smoothed}}$  is the gender- and body height dependent 24-hour reference value for the creatinine excretion of healthy Caucasian children (aged 4–17 years) in gram creatinine per day according to Remer et al. (2002). The urinary excretion factor  $\text{F}_{\text{UE}}$  represents the percentage of excreted metabolite 5cx-MEPTP, relative to the dose of the parent compound DEHTP. We used the  $\text{F}_{\text{UE}}$  0.13 as determined in an oral human metabolism study with three healthy male volunteers (Lessmann et al., 2016b), meaning that 13% of the orally administered dose of DEHTP were recovered as 5cx-MEPTP in urine.  $\text{MW}_{\text{DEHTP}}$  is 390.56 g/mol, the molecular weight of DEHTP.

### 2.4. Statistical analysis

Statistical analysis was conducted with IBM SPSS statistics 23. Boxplots were generated with OriginPro 2016. DEHTP metabolite concentrations were calculated in  $\mu\text{g/L}$  and creatinine adjusted values in  $\mu\text{g/g}$  creatinine. Metabolite concentrations below the LOQ were substituted with  $\text{LOQ}/2$  (Hornung and Reed, 1990). For associations between metabolite levels, the bivariate correlation Spearman's rho was used. To investigate associations between metabolite levels or daily intake and gender, body weight or age, we applied the Mann-Whitney-U test. For the statistical analysis, age groups were categorized according to European guidelines for clinical studies with pediatric patients (ICH, 2000). By use of the Jonckheere-Terpstra test (Jonckheere, 1954), we investigated a possible age dependent trend in the daily intake of DEHTP. The Jonckheere-Terpstra test is a nonparametric test for independent samples, comparable to the Kruskal-Wallis test. In contrast to the Kruskal-Wallis test, the Jonckheere-Terpstra test analyzes an existence of a trend among groups.

## 3. Results and discussion

### 3.1. Metabolite levels

The main specific DEHTP metabolite 5cx-MEPTP was detectable in all 107 samples analyzed with levels above the limit of quantification in

96% of the samples. The median concentration was  $4.19 \mu\text{g/L}$ , the 95th percentile  $26.4 \mu\text{g/L}$ , and the maximum concentration  $3400 \mu\text{g/L}$ . The other metabolites were detected at lower rates and concentrations. Detailed results including creatinine adjusted concentrations are shown in Table 2. The metabolic pattern reflected in the median and 95th percentile metabolite concentrations is comparable to the pattern observed in the oral metabolism study with three male volunteers performed by our group (Lessmann et al., 2016b). 5cx-MEPTP was clearly the dominant specific urinary metabolite followed by 5OH-MEHTP and 5oxo-MEHTP at concentrations about an order of magnitude lower. 2cx-MMHTP was detected only in those urine samples with highest levels of the other DEHTP metabolites.

We observed significant correlations between all three major DEHTP metabolites (see Fig. 1). Similar correlations have also been reported for the specific, oxidized metabolites of DEHP (Koch et al., 2003a, 2003b, Barr et al., 2003) or the metabolites of the phthalate substitute DINCH (Schütze et al., 2012, 2017). Obviously, the correlation between 5cx-MEPTP and 5oxo-MEHTP was weakest among the three oxidized DEHTP metabolites. From the human metabolism study we know, that parameters of elimination kinetics differ the most between 5cx-MEPTP ( $t_{\text{max}} = 4.2 \text{ h}$ ) and 5oxo-MEHTP ( $t_{\text{max}} = 5.2 \text{ h}$ ) (Lessmann et al., 2016b). Likewise to DEHP or DINCH, correlations were strongest for those metabolites with similar behavior in elimination kinetics.

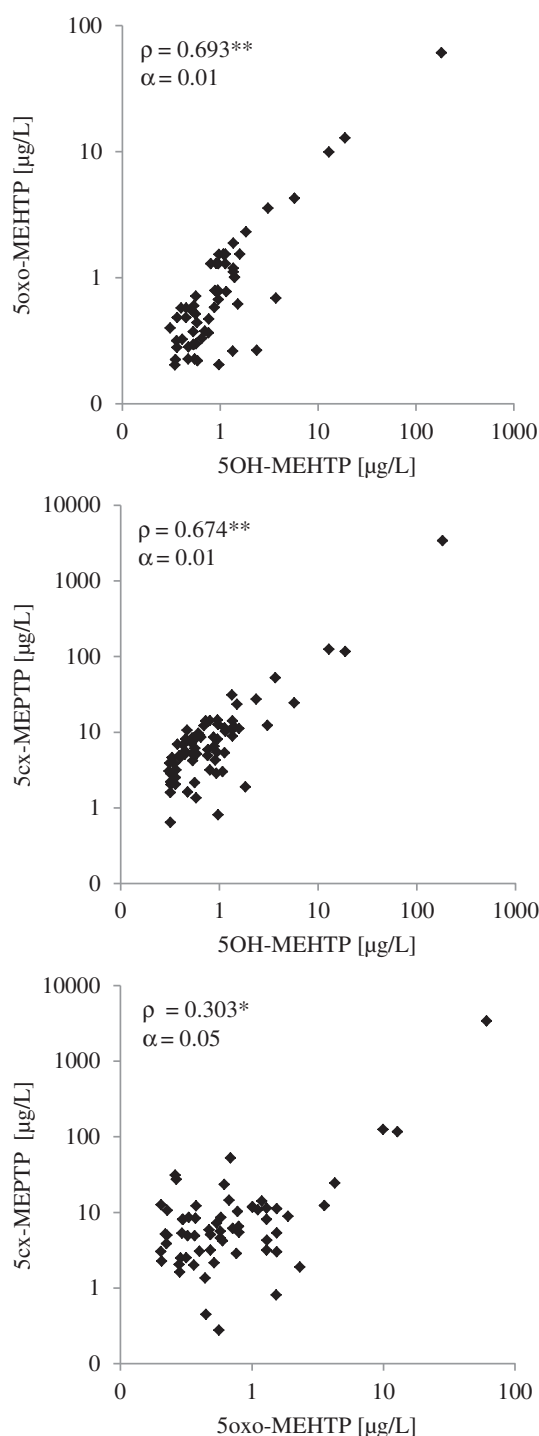
We can compare the metabolite levels of this study with the, so far, only other human biomonitoring study on DEHTP with metabolite levels in 34 German adults from the year 2014 (Lessmann et al., 2016a). Compared to the median 5cx-MEPTP level of  $0.90 \mu\text{g/L}$  in German adults, the median level in Portuguese children ( $4.19 \mu\text{g/L}$ ) is approximately 5-times higher. The maximum concentration of 5cx-MEPTP ( $38.7 \mu\text{g/L}$  vs.  $3400 \mu\text{g/L}$ ) is almost 100-times higher in the Portuguese children. For the other metabolites a comparison is difficult because of their rather low detection rates in the German adults. Previous studies have already reported children being additionally exposed to plasticizers, due to behavioral differences and an increased food uptake (Wittassek et al., 2007, Frederiksen et al., 2013, Cutanda et al., 2015, Myridakis et al., 2015, Fromme et al., 2016). In the European DEMOCOPHES study (Černá et al., 2015, Den Hond et al., 2015) children consistently excreted higher levels of DEHP metabolites compared to their mothers, both in Germany and Portugal. At the same time, DEHP metabolite excretions were generally higher in Portugal than in Germany. Similar country and age specific differences in exposure can thus be assumed also for DEHTP as a direct substitute of DEHP and explain the 5-times higher median metabolite levels in Portuguese children compared to German adults.

In a more detailed investigation of the Portuguese children we checked for possible influences of gender, study group (normal-/underweight children on usual diet vs. overweight/obese children on nutritional guidance), and age on urinary 5cx-MEPTP concentrations (both in  $\mu\text{g/L}$  and  $\mu\text{g/g}$  creatinine). The results are shown in Table 3 and depicted as boxplots in Fig. 2.

We did not evaluate the other DEHTP metabolites, due to their considerably lower urinary concentrations and detection rates. We found no influence of gender on urinary 5cx-MEPTP concentrations in  $\mu\text{g/L}$  and  $\mu\text{g/g}$  creatinine. However, we observed significantly higher

**Table 2**  
Results of the human-biomonitoring study with 107 Portuguese children, aged 4–17.

Metabolite	LOQ [ $\mu\text{g/L}$ ]	> LOQ [%]	Concentration [ $\mu\text{g/L}$ ]			Concentration [ $\mu\text{g/g}$ creatinine]		
			Median	95th P.	Max.	Median	95th P.	Max.
5OH-MEHTP	0.3	67	0.45	2.86	182	0.49	4.01	209
5oxo-MEHTP	0.2	58	0.27	2.18	60.7	0.31	3.28	69.6
5cx-MEPTP	0.2	96	4.19	26.4	3400	3.88	35.0	3900
2cx-MMHTP	0.4	7	< LOQ	0.57	67.9	< LOQ	1.07	77.9



**Fig. 1.** Spearman-correlations between urinary concentrations of the specific DEHP metabolites 5OH-MEHTP, 5cx-MEPTP and 5oxo-MEHTP; only values above LOQ are depicted.  $\rho$  = Spearman's rank correlation coefficient,  $\alpha$  = level of significance.

urinary 5cx-MEPTP concentrations in the group of normal weight children compared to the group of overweight children. This significant difference (with obese children exhibiting lower exposures to DEHP than regular weight children) stands in some contrast to previous (epidemiological) studies on phthalate plasticizers that found e.g. DEHP body burden to be associated with an increase in body weight and BMI (Trasande et al., 2013, Buser et al., 2014, Hou et al., 2015, Yaghjian et al., 2015, Kim et al., 2016). However, as part of the study design, the group of overweight/obese children received nutritional guidance with

unprocessed and fresh food, whereas the regular weight children fed on their usual diet. Several studies have already identified certain groups of food as possible sources of DEHP exposure (Sathyanarayana et al., 2013, Mervish et al., 2014). Additionally, exposure to DEHP can be reduced by dietary interventions (Rudel et al., 2011, Koch et al., 2013a, 2013b, Ackerman et al., 2014). Since DEHTP is a direct substitute for DEHP, we thus assume that the influencing factor resulting in significantly lower urinary levels of 5cx-MEPTP in the overweight children is their style of diet. In regard to age, we found elevated 5cx-MEPTP concentrations in the younger children only after creatinine adjustment and not for unadjusted levels in  $\mu\text{g/L}$ . Creatinine excretion is known to be lower in younger children (Barr et al., 2005), which in turn drives creatinine adjusted concentrations to higher values in younger children compared to older children. The significance of this effect in terms of actual exposure doses is investigated in more detail in the following daily intake section.

### 3.2. Daily intakes

We calculated the daily DEHTP intakes, based on the urinary 5cx-MEPTP concentrations, taking account of body height and gender based reference values for urinary creatinine excretion (Remer et al., 2002; Koch and Angerer, 2007). For the study population as a whole, the median daily DEHTP intake was  $0.67 \mu\text{g/kg bw/d}$  (95th percentile  $6.25 \mu\text{g/kg bw/d}$ ; maximum  $690 \mu\text{g/kg bw/d}$ ). Detailed results for the different subgroups (gender, study group, age) are shown in Table 4 and depicted as boxplots in Fig. 3.

We could not observe any gender specific differences in daily DEHTP intakes. Actually, boys and girls had very similar median daily DEHTP intakes ( $0.62$  vs.  $0.67 \mu\text{g/kg bw/d}$ ). However, the children who received specific nutritional guidance, had about 2.5 fold lower daily intakes compared to the children who fed on their usual diet ( $0.40 \mu\text{g/kg bw/d}$  vs.  $1.01 \mu\text{g/kg bw/d}$ ;  $p < 0.001$ ). In terms of absolute intakes (calculated as a product of each child's estimated daily intake and the respective body weight), findings were pointing in the same direction with children on nutritional guidance having lower absolute intakes compared to children feeding on a usual diet ( $23.1 \mu\text{g/d}$  vs.  $32.4 \mu\text{g/d}$  respectively). These findings confirm previous assumptions that the children's style of diet is an important factor influencing DEHTP exposure. Furthermore, calculated median daily DEHTP intakes of the younger children, 4–11 years of age, were about 2 fold higher compared to older children, 12–17 years of age ( $0.71$  vs.  $0.36 \mu\text{g/kg bw/d}$ ;  $p = 0.045$ ).

To further investigate age dependency of DEHTP exposure, we divided the children into six smaller age groups and applied the Jonckheere-Terpstra test. The results are depicted as boxplots in Fig. 4.

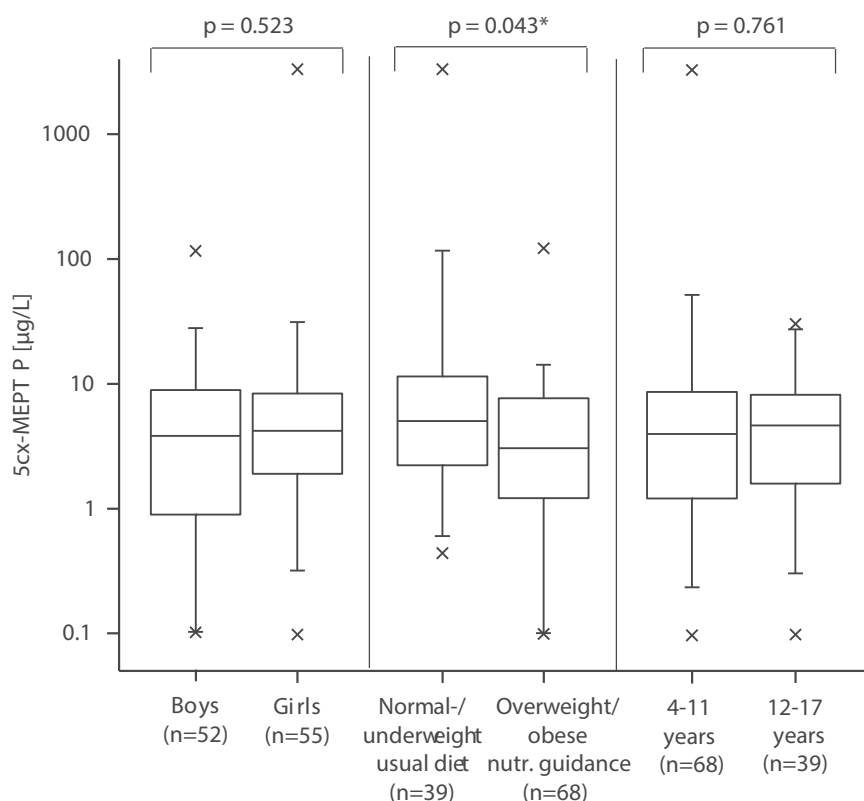
We could observe a significant downward trend for the median daily DEHTP intakes with increasing age ( $p = 0.014^*$ , level of significance  $\alpha = 0.05$ ). The youngest children (median DI  $1.7 \mu\text{g/kg bw/d}$ ) had > 4 fold higher daily DEHTP intakes compared to the older children (median DI  $0.4 \mu\text{g/kg bw/d}$ ). This, again, points to contaminated foodstuff as a major source of DEHTP exposure, because young children have an increased food intake per kg body weight. Similar findings on age dependent plasticizer exposure have already been reported for DEHP and other plasticizers (Fromme et al., 2007, 2013, Koch and Angerer, 2007, Wittassek et al., 2007).

Higher plasticizer exposures in young children might additionally be caused by other age dependent characteristics, like mouthing of toys and playing near the ground leading to increased dust intake (Moya et al., 2004, Sathyanarayana, 2008, Lee et al., 2014, Ginsberg et al., 2016). Biedermann-Brem et al. (2008) determined plasticizers in > 250 toys and could detect DEHTP in 10% of all samples analyzed. Another study investigated the mass content of alternative plasticizers in toys and childcare articles and found DEHTP to be the most frequently used plasticizer with contents of up to 25% DEHTP relatively to PVC (Xie et al., 2016). In 953 dust samples, collected in German households

**Table 3**

Median concentrations of 5cx-MEPTP in µg/L and µg/g creatinine graded by gender, study group and age; significant differences (level of significance  $\alpha = 0.05$ ) marked with an asterisk.

	Gender		Study group		Age	
	Boys (n = 52)	Girls (n = 55)	Normal-/underweight, usual diet (n = 39)	Overweight/obese, nutritional guidance (n = 68)	4–11 years (n = 68)	12–17 years (n = 39)
5cx-MEPTP median [µg/L]	3.73	4.19	4.99	3.04	4.03	4.61
p-Value (Mann-Whitney-U)	0.523		0.043*		0.761	
5cx-MEPTP Median [µg/g creatinine]	3.86	3.94	4.74	3.03	4.77	2.51
p-Value (Mann-Whitney-U)	0.509		0.029*		0.024*	



**Fig. 2.** Urinary concentrations of 5cx-MEPTP graded by gender, study group, and age. Bottom and top of the box represent the first and third quartiles. The band inside the box shows the median. The whiskers represent the 5th and the 95th percentile. The minimum and maximum values are represented by an x. p-Value from Mann-Whitney-U test. Significant differences marked with an asterisk.

between 1997 and 2009, Nagorka et al. (2011) reported a rapid increase in DEHTP detection frequencies (< 5% in the early samples, 94% in samples of the year 2009) and concentration levels (95th percentile in 2009: 24 mg/kg). Fromme et al. (2016) detected DEHTP in all dust samples from 63 German daycare centers for children collected in 2011/2012 with a median concentration of 40 mg/kg dust. However, they concluded that DEHTP intake by dust ingestion was low compared to tolerable daily intake values even under worst case assumptions.

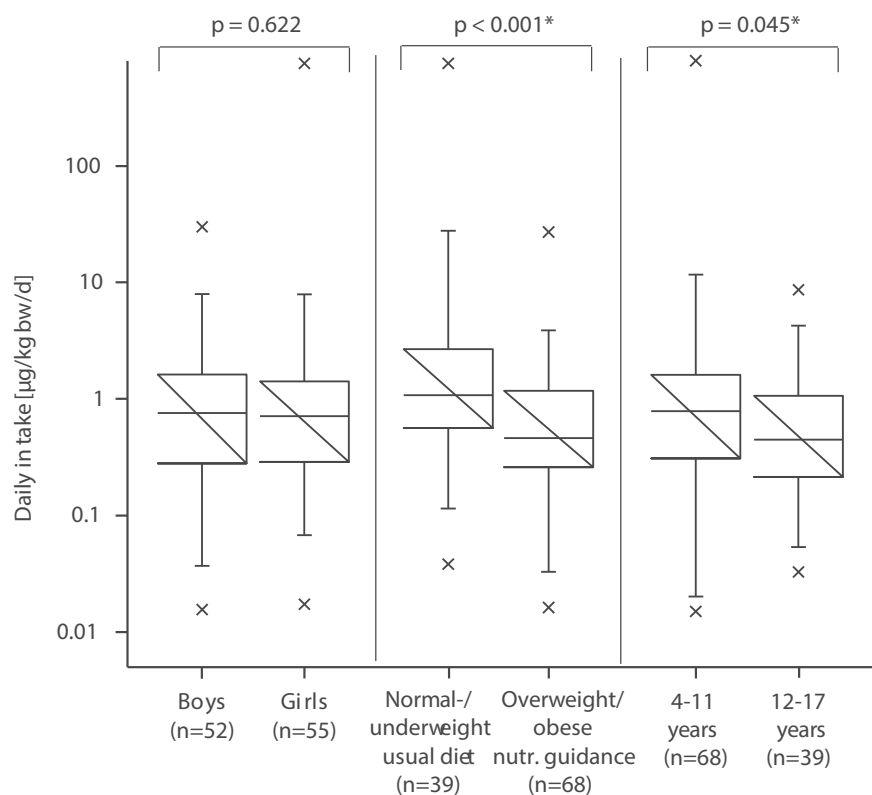
### 3.3. Risk assessment

For a risk assessment of DEHTP exposure in the Portuguese children, we can either compare urinary metabolite concentrations directly to the Human-Biomonitoring Value (HBM-I value) for DEHTP, a biomarker derived, health based guidance value of the German Human Biomonitoring Commission (Apel et al., 2016), or we can compare the calculated daily intakes to the TDI value of EFSA (2008). The HBM-I

**Table 4**

Median daily intakes of DEHTP in µg/kg bw/d graded by gender, study group, and age; significant differences (level of significance  $\alpha = 0.05$ ) marked with an asterisk.

	Gender		Study group		Age	
	Boys (n = 52)	Girls (n = 55)	Normal-/underweight, usual diet (n = 39)	Overweight/obese, nutritional guidance (n = 68)	4–11 years (n = 68)	12–17 years (n = 39)
Daily DEHTP intake [µg/kg bw/d]	0.62	0.67	1.01	0.40	0.71	0.36
p-Value (Mann-Whitney-U)	0.622		< 0.001*		0.045*	

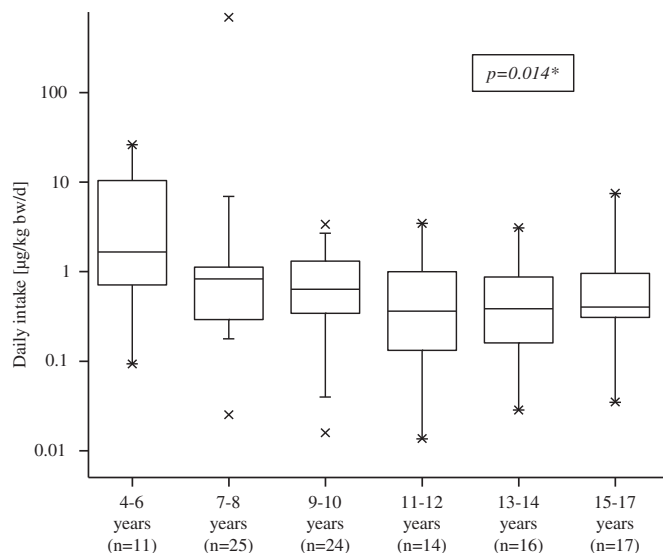


**Fig. 3.** Daily DEHTP intakes graded by gender, study group, and age. Bottom and top of the box represent the first and third quartiles. The band inside the box shows the median. The whiskers represent the 5th and the 95th percentile. The minimum and maximum values are represented by an x. p-Value from Mann-Whitney-U test. Significant differences marked with an asterisk.

value describes the concentration of a substance in the body matrix (e.g. urine) below which no adverse health effect should be expected (Angerer et al., 2011). Urinary 5cx-MEPTP concentrations in our study (median 4.19 µg/L; 95th percentile 26.4 µg/L) were considerably below the HBM-I value of 1800 µg/L indicating that detrimental health effects caused by DEHTP are unlikely. However, in one urine sample with a maximum 5cx-MEPTP concentration of 3400 µg/L the HBM-I value was clearly exceeded. The high 5cx-MEPTP concentration in this sample

was confirmed by high levels of the other DEHTP metabolites (see Fig. 1), and metabolite ratios were within the range expected from a human metabolism study. Single exceedances of HBM-I values are no reason for immediate concern but should spark repeat sampling (of the respective individual) and verification measurements. If these measurements confirm the initial result an investigation of potential sources of exposure should be undertaken. In the case of this single child, we were not able to obtain a repeat sample.

The TDI for DEHTP is 1000 µg/kg bw/d (EFSA, 2008). For comparison of the daily DEHTP intakes of our study with the TDI of EFSA we calculated the hazard quotient (HQ) defined as the ratio of a daily intake and the respective TDI. HQ values of > 1 are generally considered a cause of concern. The median daily DEHTP intake in our study for children eating usual diet (group with highest DEHTP exposure) was 1.01 µg/kg bw/d. This daily intake is a factor of 1000 below the TDI, resulting in a HQ of 0.001. At the 95th percentile of daily intake of about 10 µg/kg bw/d (children eating usual diet) the HQ was 0.01, again far from the HQ of 1. The highest calculated daily intake of 690 µg/kg bw/d was also lower than the TDI of 1000 µg/kg bw/d (HQ = 0.69). Thus, with regard to the TDI of 1000 µg/kg bw/d, for none of the investigated children the calculated DEHTP burden poses a toxicological risk. It must be noted that the daily intakes have been calculated based on metabolite levels determined in first morning urine voids as the only specimen available in the study. However, for chemicals like plasticizers with short elimination half-lives and food as a predominant exposure route, this type of sampling might lead to slight underestimations (about up to a factor of 2) since exposures during the day can be missed (Aylward et al., 2011; Lorber et al., 2011; Koch and Angerer, 2012). Another uncertainty in daily intake calculations certainly arises from the assumption of a fixed metabolic conversion factor of 0.13 for 5cx-MEPTP. This conversion factor has been derived from three adult volunteers and reflects the mean of conversion factors ranging from 0.07 to 0.20 (Lessmann et al., 2016b). While we



**Fig. 4.** Daily DEHTP intakes graded by age. Bottom and top of the box represent the first and third quartiles. The band inside the box shows the median. The whiskers represent the 5th and the 95th percentile. The minimum and maximum values are represented by an x; p value from Jonckheere-Terpstra test.

have no indications of metabolic differences between the adults of the metabolism study and the children of our study (very similar metabolite ratios), taking account of the variability observed in the metabolism study, a worst case scenario calculation with the lower end conversion factor of 0.07 would have led to a maximum daily intake of 1280 µg/kg bw/d.

#### 4. Conclusion

The results of the present study for the first time document an omnipresent DEHTP exposure in Portuguese children. Median urinary metabolite levels in this study are approximately 5 times higher than levels found in German adults. These differences are in line with findings for the related plasticizer DEHP, for which children also have higher exposures than adults and for which exposure in Portugal has been reported to be slightly higher than in Germany.

As for other high molecular weight plasticizers, diet seems to be the dominant route of exposure to DEHTP. We could show that children who received specific nutritional guidance with predominately fresh and unprocessed food had about 2.5 fold lower daily intakes than children on a usual diet. Other, child specific sources of exposure might be DEHTP containing toys or house dust. The complex nature of the original study design, however, demands caution and adds some uncertainty to these findings, because body weight status and nutritional characteristics were combined in a manner that is probably reciprocal to the real-life scenario. Unfortunately, we were not able to obtain urine samples collected before, or at the onset of the dietary intervention.

Urinary metabolite data indicate that current exposures to DEHTP both at the median and the upper bounds (95th percentile) are well below health based limit values such as the HBM-I value or the TDI (factor of 1000 at the median and factor of 100 at the 95th percentile). However, for one child we detected DEHTP exposure close to the TDI and exceeding the HBM-I value.

DEHTP is the second ortho-phthalate substitute (after DINCH) for which we could prove the omnipresent exposure of the general population by means of human biomonitoring. As already shown for DINCH (Schütze et al., 2014), we also have to expect a rise in exposure to DEHTP, with predicted consumption numbers doubling from 2012 to 2018. Future studies should therefore closely follow the time course of DEHTP exposure. Such timely exposure assessments in combination with risk assessments will enable us to verify a successful substitution of critical phthalates with alternatives of a preferred toxicological profile. Such exposure assessments will also enable us to intervene and advise regulatory measures, if exposures to the alternatives approach limit values, or if toxicological re-evaluations result in the lowering of these limit values.

#### Compliance with ethical standards

The study design was in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Approval for the study protocol was obtained from the Ethics Commission of the Faculty of Medicine of Oporto University, Portugal (ref. 163.13). Written informed consent was obtained from all parents of individual participants included in the study.

#### Conflict of interest

The authors claim, that they do not have any conflict of interest.

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

#### Acknowledgements

Luísa Correia-Sá is grateful for the doc fellowship financed by FCT (SFRH/BD/87019/2012).

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