Exposure assessment to bisphenol A (BPA) in Portuguese children by human biomonitoring

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Abstract

Exposure to bisphenol A (BPA) is known to be widespread and available data suggests that BPA can act as an endocrine disruptor. Diet is generally regarded as the dom- inant BPA exposure source, namely through leaching to food from packaging materials. The aim of this study was to eval- uate the exposure of 110 Portuguese children (4–18 years old), divided in two groups: the regular diet group ($n = 43$) com- prised healthy normal weight/underweight children with no dietary control; the healthy diet group ($n = 67$) comprised children diagnosed for obesity/overweight (without other known associated diseases) that were set on a healthy diet for weight control. First morning urine samples were collected and total urinary BPA was analyzed after enzymatic hydrolysis via on-line HPLC-MS/MS with isotope dilution quantifica- tion. Virtually, all the children were exposed to BPA, with 91% of the samples above the LOQ (limit of quantification) of 0.1 µg/L. The median (95th percentile) urinary BPA levels for non-normalized and creatinine-corrected values were 1.89 µg/L (16.0) and 1.92 µg/g creatinine (14.4), respectively. BPA levels in the regular diet group were higher than in the healthy diet group,

but differences were not significant. Calculated daily BPA intakes, however, were significantly higher in children of the regular diet group than in children of healthy diet group. Median (95th percentile) daily intakes amounted to 41.6 (467) ng/kg body weight/day in the regular diet group, and 23.2 (197) ng/kg body weight/day in the healthy diet group. Multiple logistic regression analysis re- vealed that children in the healthy diet group had 33% lower intakes than children in the regular diet group (OR 0.67; 95% CI 0.51–0.89). For both groups, however, urinary BPA levels and daily BPA intakes were within the range reported for other children's populations and were well below health guidance values such as the European Food Safety Authority (EFSA) temporary tolerable daily intake (t-TDI) of 4 µg/kg body weight/day. In addition, lower daily BPA intakes were more likely linked with the inherent dietary approach rather than with high BMI or obesity.

Keywords Bisphenol A · Exposure assessment · Human biomonitoring · Urine · Children · Diet

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Introduction

Bisphenol A (BPA) was first synthesized in the early twentieth century. However, its beneficial chemical and physical prop- erties as a plasticizer were only discovered later, with the production of epoxy resins and polycarbonate plastics (Geens et al. 2012a). Its industrial production volume is ap- proximately 2.9 million tons per year, making BPA one of the chemicals with the highest production volume worldwide (Aaroe Morck 2012). BPA is used in the production of several consumer products including food/beverage can linings, stor- age containers and packing material (such as paperboard), plastic bags, dental sealants, thermal paper, medical

equipment, tableware, and toys (Braun and Hauser 2011; Fenichel et al. 2013; Geens et al. 2012a; Hehn 2016; Rubin 2011). BPA can leach from plastic components into food and liquids (Mansilha et al. 2013), and from thermal papers, especially when exposed to high temperatures and acidic or basic conditions (Braun and Hauser 2011; Cao et al. 2011; Correia-Sá et al. 2014; Fenichel et al. 2013; Geens et al. 2010, 2012a, b; Hehn 2016; Li et al. 2013b; Lv et al. 2017; Noonan et al. 2011; Rubin 2011). Diet is generally regarded as the dominant BPA exposure source; however, not all sources and routes of exposure are thoroughly understood (Christensen et al. 2012; Geens et al. 2011, 2012a). Several studies have addressed the impact of die- tary interventions in lowering BPA exposure levels, name- ly by limiting pre-packaged foods (e.g., canned) and in- creasing fresh or organic food (Carwile et al. 2009, 2011; Hagobian et al. 2016; Rudel et al. 2011; Sathyanarayana et al. 2013).

Both free and total BPA (free + conjugated species) have been measured in a variety of human body fluids including urine, blood, saliva, amniotic fluid, and breast milk (Asimakopoulos et al. 2012; Calafat et al. 2005; Koch et al. 2012; Søeborg et al. 2014; Vandenberg et al. 2010; vom Saal and Welshons 2014). Following oral absorption, BPA undergoes rapid first pass metabolism in the intestine and liver, is then conjugated with glucuronic acid (Dekant and Völkel 2008; Teeguarden et al. 2011, 2015a; Völkel et al. 2002, 2005; Yang et al. 2015), and excreted via urine within 24 h. Thus, urine is the preferred matrix for estimating human exposure to BPA (Calafat et al. 2013, 2015; Dekant and Völkel 2008; Koch and Calafat 2009).

BPA is considered an endocrine disruptor (Correia-Sá et al. 2014; Fenichel et al. 2013; Teeguarden and Hanson-Drury 2013) and there is some concern raised by several studies, suggesting a correlation between BPA exposure and health outcomes such as diabetes (Lang et al. 2008; Tai and Chen 2016), decreased sperm quality (Meeker et al. 2010; Vitku et al. 2016), and increased body mass index/weight, obesity, or adiposity (Bhandari et al. 2013; Braun et al. 2014; Hao et al. 2017; Hoepner et al. 2016; Li et al. 2013a; Shankar et al. 2012; Trasande et al. 2012; Vafeiadi et al. 2016; Wang et al. 2012). Regarding obesity, a recent study addressing the effect of low-dose BPA exposure on adipogenesis and adipocytes has concluded that during critical stages of adi- pose tissue development, BPA may cause adipocyte meta- bolic dysfunction and inflammation, increasing the risk of developing obesity-related diseases (Ariemma et al. 2016). These human health effects from low-level exposures to BPA are currently being discussed (EFSA 2015; Hengstler et al. 2011; Rubin 2011; Teeguarden et al. 2013; Teeguarden and Hanson-Drury 2013; Vandenberg et al. 2009, 2013; Völkel 2017; vom Saal and Welshons 2014). A recent review on child health and BPA exposure classified evidence

as "insufficient" because of large inconsistencies in study results (Vrijheid et al. 2016).

The majority of biomonitoring studies on BPA showed a ubiquitous exposure: detection rates in urine, depending on the respective LOQs, are generally above 90% and median and ranges of exposure are very similar (Bushnik et al. 2010; Calafat et al. 2008; CDC 2015; Covaci et al. 2015; Frederiksen et al. 2013a, b; Geens et al. 2014; Health Canada 2015; Kasper-Sonnenberg et al. 2014, 2012; Koch et al. 2012; LaKind and Naiman 2015; Vandenberg et al. 2010). However, urinary BPA levels in younger children seem to be higher in comparison with adolescents and adults (Becker et al. 2009; Bushnik et al. 2010; Calafat et al. 2008; Frederiksen et al. 2013a). Nevertheless, there is a general lack of data for children, also for European countries and for specific sub-groups such as individuals on a weight management program with a prescribed healthy and calorie restricted/ controlled diet.

Therefore, the general aim of this study was to evaluate BPA exposure in 110 Portuguese children. Currently, in Portugal, human biomonitoring data on BPA are not available. Children were of special interest, because previous studies have reported that, e.g., plasticizer body burdens of children can be higher compared to adults due to increased food intakes in children relative to their body weight or other child related characteristics. Finally, since the study population comprised two groups, one being on a regular diet and the other being on a healthy, balanced diet (obese/overweight children in a weight management program), an additional aim was to assess possible differences in BPA exposures among these two groups and to elucidate if we could indeed observe a lowering effect of a green, healthy diet in a weight management program on BPA exposure levels.

Material and methods

Study subjects and sample collection

The present study is part of an ongoing study to assess possible differences between obese/overweight children on a healthy diet to lose/manage weight and normal weight/ underweight children without diet control or constraints in Portugal. Initially, the aim of the study was to determine the exposure to suspected or confirmed (predominantly persistent) endocrine disruptors and/or obesogens. Children were recruited from the pediatric appointment at Hospital de S. João, and several local schools, in 2014 and 2015. Children lived in Oporto and Aveiro, two Portuguese districts, in the North and Central region of the country. One hundred ten children (54 boys, 56 girls) participated in this study with an age range between 4 and 18 years old (arithmetic mean ± standard deviation 10.41 ± 3.33 years old).

The children were divided in two groups: the group "regular diet" included healthy children which were normal weight/underweight not changing their regular diet; the group "healthy diet" included children diagnosed for obesity/overweight without other known associated diseases, counseled for healthy and balanced nutrition and thus set on a prescribed diet, for at least 3 months. First morning urine samples were collected from each participating child. All the specimens were kept cool during transportation and then stored at −20 °C until analyses. The study was approved by the ethics committee of the Centro Hospitalar S. João/FMUP (Faculdade de Medicina da Universidade do Porto, ref. 163.13) and all the parents provided written consent.

Determination of urinary creatinine

Urinary creatinine concentration was measured through a modified Jaffe method (Jaffé 1986) with an Olympus AU5400® Chemistry Analyzer (Beckman-Coulter®, Porto, Portugal) at São João Hospital, Department of Clinical Pathology. Four out of the 110 children had creatinine values below 0.3 g/L. However, all urine samples were included in the statistical analysis, because creatinine concentrations below a value of 0.3 g/L (WHO 1996) (a cutoff for adult populations) in children do not necessarily indicate excessive dilution, but can be indicative of lower muscle mass in children

compared to adults (Barr et al. 2005; Koch et al. 2011).

Analysis of BPA in urine and quality control

Total BPA was analyzed after enzymatic hydrolysis via on-line HPLC-MS/MS with isotope quantification. The limit of quantification (LOQ) was 0.1 µg/L. Details of the method have been described elsewhere (Kasper-Sonnenberg et al. 2012; Koch et al. 2012). Briefly, to a 300-µL aliquots, 150 µL of 1 M ammonium acetate (at pH 5.0), 25 µL of internal standard, and 6 µL of β-glucuronidase/arylsulfatase were added. Then, the samples were gently mixed and placed in a water bath at 37 °C for 4 h for enzymatic hydrolysis. Thereafter, all samples were frozen overnight to precipitate proteins, thawed and equilibrated at room temperature and centrifuged for 10 min. The supernatant was transferred into a second 1.8-mL screw-cap vial. A volume of 25 µL was injected into an Agilent Technology LC1200 system coupled with an AB Sciex QTrap 5500 tandem mass spectrometer. We used a two column assembly for chromatographic work-up, with a RAM (Restricted Access Material) column (LiChrospher® RP-8 ADS (25 µm; 25 mm × 4 mm RAM)) for clean-up and enrichment and, after back flush, an Atlantis T3 analytical column (3.0 × 150 mm; 3 µm) for chromatographic separation. The mean accuracy for BPA, determined from spiked urine samples (spike level 10 µg/L), was 96.8% and relative standard deviations of the laboratory control material at two

concentration levels (2.9 and 11.8 µg/L) were consistently below 10%. To ensure data accuracy and precision, we included internal quality control samples at two concentration levels, standards, and reagent blanks in each batch of samples. Laboratory blank values of BPA were consistently below the limit of detection of 0.05 µg/L. Furthermore, analyses were performed also without enzymatic deconjugation to test for possible external BPA contamination in the pre-analytical phase. The analyzing laboratory has obtained proficiency testing certificates in the German External Quality Assessment Scheme (G-EQUAS) and has further served as reference laboratory within the European DEMOCOPHES project to ensure high comparability with other laboratories worldwide (Schindler et al. 2014).

Daily intake estimation

For the estimation of daily BPA intakes (DIs) from the total urinary levels, we applied a calculation approach based on the creatinine-related BPA concentrations (Koch et al. 2007; Wittassek et al. 2007), adjusted to the height/age dependent reference values for creatinine excretion (Remer et al. 2002). The daily intake in micrograms per kilogram body weight (bw) per day was calculated by the following equation:

$$DI\ (\mu g=kg\ bw=day) = \frac{UE \times CE_{\frac{1}{2}g=day}}{FUE \times BW} \times MW_{BPA}$$

UE is the molar urinary excretion of the measured urinary BPA (in micromole per gram creatinine). The smoothed creatinine excretion rates (CE smoothed) are body height and sex-based reference values for urinary creatinine excretion for healthy, Caucasian, 3–18-year-old children in gram creatinine per day (Remer et al. 2002). FUE is the mass urinary excretion fraction for the analyte (mass of analyte excreted in urine/mass of parent compound ingested); we used a FUE of 1 for BPA (Völkel et al. 2008). MW is the BPA molecular weight and BW the body weight (kg) for each child.

Statistical analysis

Basic statistical analysis was performed using SPSS 20.0 (IBM Corporation). Several statistical data is presented, such as median, geometric and arithmetic means, maximum values, and percentiles. Concentrations below the LOQ were set to ½ LOQ (Lotz et al. 2013). For the present analysis, age was divided in two groups in agreement with the European regulations for clinical studies in pediatric patients (ICH 2000): (i) children from 2 to 11 years old; (ii) adolescents from 12 to 18 years old. For the population's characterization, Mann-Whitney U tests were performed to assess possible differences across distribution between group 1 and 2 regarding age, sex, weight, height, BMI, and urinary creatinine values.

As the variables regarding urinary concentrations (creatinine-corrected and non-normalized values) and calculated daily intakes presented a non-normal distribution, non-parametric tests were applied. A Mann-Whitney U test was performed to assess possible differences across distribution between group 1 and 2, for the urinary values (µg/L and µg/g creatinine) and daily intakes. Statistical analysis for sex and age dependency regarding urinary values was also tested by Mann-Whitney U test. Moreover, the correlation between creatinine (g/L) and age was assessed by Spearman's rank correlation.

Multivariate logistic regression analysis was performed using children with regular diet and with healthy diet as dependent and BPA daily intakes (ng/kg bw/day) as independent variable, adjusted for age (years) and sex, and predicting the probability of having a healthy diet. The daily BPA intake was log-transformed by means of logarithm to the basis of 2. Therefore, its association with the dependent variable was estimated for a doubling of the daily BPA intake in the natural scale. The changes of the dependent variable by a unit change of the independent variables is presented as odds ratios (OR) together with their corresponding 95% confidence intervals (95% CI) and also as the parameter estimates β and their standard errors. This part of the analysis and the boxplot were performed with the statistical software SAS 9.4 © SAS Institute Inc., Cary, NC, USA.

For all analyses, a p value ≤ 0.05 was considered as statistical significant if not otherwise stated.

Results and discussion

Study population

BPA concentrations were investigated in 110 urine samples, from 4 to 18 years old Portuguese children. The study group was composed of 51% girls and 49% boys, with a median age of 10 years old. The majority of the children was overweight/obese and underwent a diet with nutritional guidance (healthy diet group) (61%; $n = 67$). From the 43 children of the regular diet group, 42 were normal weight and one child was underweight. More detailed information for the obese/overweight children on a healthy diet and under/normal weight children with a regular diet are given in Table 1. Although the discriminators body weight and BMI differed significantly between the two groups, age, sex, height, and urinary creatinine were evenly distributed (Table 1).

Urinary BPA levels

From human metabolism studies, we know that BPA is predominantly excreted in its conjugated form (> 99%); in population studies with stored samples, free BPA (if detectable) is usually below 15% of total BPA and shares of free BPA higher than 20% are likely to indicate contamination (Guidry et al. 2015; Koch et al. 2012; Teeguarden et al. 2015b; Völkel et al. 2002; Ye et al. 2015). In all samples from this study, shares of free BPA were below 11% of total BPA, except in two samples, where free BPA amounted to > 80% of total BPA (sample 1: total BPA 21.1 µg/L, free BPA 17.6 µg/L; sample 2: total and free BPA 230 µg/L). Consequently, these two samples were excluded from the original study population of 112 children, resulting in 110 children, as described before. In nearly all of the 110 analyzed urine samples (detection rate of 91%), BPA (total BPA after enzymatic hydrolysis) was detected. In 10 children, urinary concentrations were below the LOQ of 0.1 µg/L. Results (in µg/L and µg/g creatinine) for the entire population are shown in Table 2, including median, means, 95th percentile, and maximum. The median concentration was 1.89 µg/L, the 95th percentile 16.0 µg/L, and the maximum concentration 66.9 µg/L.

Table 1 General characteristics of the studied population

Population characteristics	Regular diet ($n = 43$)[a]			Healthy diet ($n = 67$)[a]			Total population ($n = 110$)			p value*
	Median	95th P.	Max	Median	95th P.	Max	Median	95th P.	Max	
Age (years)	11.0	17.0	18.0	9.00	15.6	17.0	10.0	16.0	18.0	0.391
Sex (%)	Female—44% Male—56%			Female—55% Male—45%			Female—51% Male—49%			0.261
Weight (kg)	34.8	64.8	75	45.5	81.9	118	44.7	77.3	118	< 0.001
Height (cm)	143	181	184	142	167	169	142	169	184	0.679
BMI (kg/m^2)	17.1	22.9	24.1	24.6	32.7	42.3	22.2	29.6	42.3	< 0.001
Creatinine (g/L)	0.88	2.55	2.64	0.98	2.47	3.81	0.94	2.50	3.81	0.857

Significant differences between the regular and healthy diet groups ($p ≤ 0.05$) are marked in italics

[a] The underweight/normal weight and obese/overweight groups were defined according to the WHO charters (WHO 2007)

*Mann-Whitney U test, two-tailed

Table 2 Results of the human biomonitoring study with 110 Portuguese children, aged 4–18 (µg/L and µg/g creatinine); 91% of samples were above the limit of quantification (LOQ; 0.1 µg/L)

BPA	Non-normalized values (µg/L)	Creatinine-corrected values (µg/g)
Number	$n = 110$	
Median	1.89	1.92
Arithmetic mean (SD)	4.11 (± 8.91)	3.60 (± 6.17)
Geometric mean (95% CI)	1.58 (1.18–2.13)	1.76 (1.38–2.41)
Percentile 95th	16.0	14.4
Maximum	66.9	46.0

SD standard deviation of the arithmetic mean, *95% CI* 95% confidence interval

Detailing the investigation further we checked whether sex, study group (regular diet vs. healthy diet) or age would influence urinary BPA concentrations (both in µg/L and µg/g creatinine). The results are depicted in Table 3.

No significant differences were found regarding sex, age, or study group for BPA urinary concentrations (both adjusted and non-adjusted values). However, the children in the regular diet group had approximately 20% higher urinary BPA concentrations than the children in the healthy diet group, both in µg/L (median 2.28 vs. 1.87 µg/L) and after creatinine adjustment (median 2.14 vs. 1.76 µg/g). Albeit not significant, the small difference between the groups may reflect differences in external BPA exposure, as children with healthy diet were being followed in a nutritional appointment. The healthy diet relied primarily on fresh food and less on processed and packaged food items. Additionally, the amount of calories was set accord- ing to their nutritional needs. Contrary to this group, chil- dren with regular diet continued their usual diet.

Table 4 Daily intake of BPA (ng/kg bw/day) in Portuguese children

BPA	Total population $n = 110$	Study group	
		Regular diet ($n = 43$)	Healthy diet ($n = 67$)
Median	31.3	41.6	23.2
Percentile 95th	221	467	197
Maximum	624	624	578
p value*	–	*0.002*	

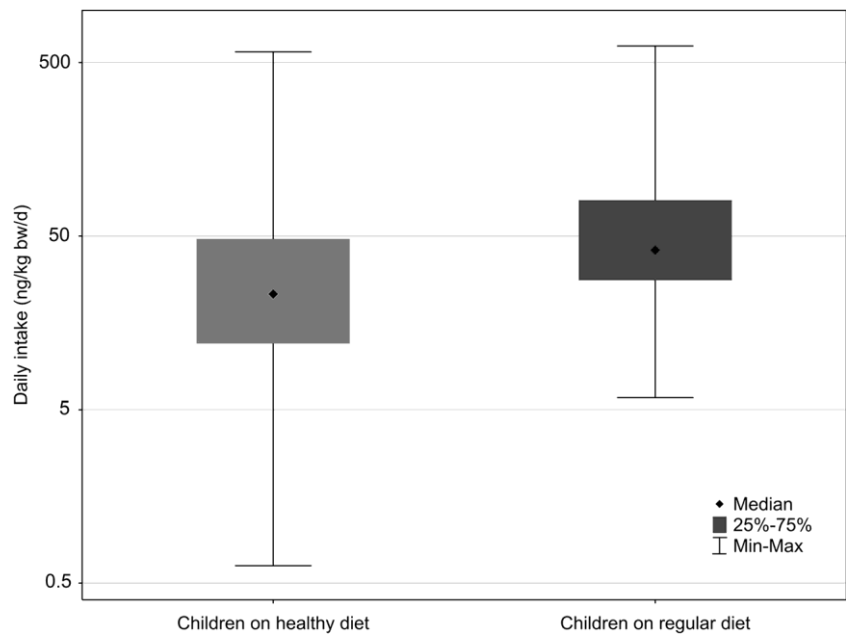Significant differences *(p ≤ 0.05)* are marked in italics
*Mann-Whitney *U* test, two-tailed

Regarding influence of age, urinary concentration values in micrograms per liter in older children (12–18 years old) were found to be higher than in younger children (4–11 years old) with median concentrations of 2.25 and 1.87 µg/L, respectively. Creatinine adjustment inverted this outcome and led to higher BPA concentrations in younger children compared to older ones, with median concentrations of 2.10 and of 1.76 µg/g creatinine, respectively. In both cases, however, differences were not statistically significant. The results are in agreement with reports from other studies (Bushnik et al. 2010; Calafat et al. 2008). The reversing effect between micrograms per liter and micrograms per gram creatinine-adjusted concentrations is probably due to the fact that creatinine excretion itself is highly correlated with children's age and development (Barr et al. 2005; Remer et al. 2002).

Daily BPA intakes

For the total study population, the median daily BPA intake was 31.3 ng/kg bw/day (95th percentile 221 ng/kg bw/day;

Table 3 Total BPA urinary levels for the Portuguese children (µg/L and µg/g creatinine) according to age ranges, sex, and study groups

BPA	Sex		Age		Study group	
	Boys ($n = 54$)	Girls ($n = 56$)	4–11 years ($n = 70$)	12–18 years ($n = 40$)	Regular diet ($n = 43$)	Healthy diet ($n = 67$)
Non-normalized values (µg/L)						
Median	2.20	1.86	1.87	2.25	2.28	1.87
Percentile 95th	20.6	16.1	16.0	16.5	29.1	16.0
Maximum	66.9	54.6	54.6	66.9	66.9	54.6
p value*	0.318		0.056		0.134	
Creatinine-corrected values (µg/g)						
Median	2.01	1.79	2.10	1.76	2.14	1.76
Percentile 95th	19.8	12.9	18.6	6.01	23.1	14.8
Maximum	31.0	46.0	46.0	25.9	31.0	46.0
p value*	0.211		0.488		0.158	

*Mann-Whitney *U* test, two-tailed

Fig. 1 Daily intakes of BPA (ng/kg bw/day) in the two study groups

maximum 624 ng/kg bw/day). Detailed results for the different study groups are shown in Table 4.

The children on a regular diet had significantly higher daily BPA intakes than the children on a healthy diet (Table 4 and Fig. 1). Median (95th percentiles) daily intakes were 41.6 (467) and 23.2 (197) ng/kg bw/day for regular and healthy diet group, respectively.

In younger children (4–11 years), the median creatinine value (0.80 g/L) was significantly lower than in older children (12–18 years; 1.37 g/L), and creatinine was positively correlated with the children's age (Spearman's correlation coefficient r 0.533; p value ≤ 0.01). The effect of rapidly changing child physiology with age, however, was accounted for in the daily intake calculations by using height/age dependent reference values for creatinine excretion.

To further investigate the influence of the diet on the BPA daily intakes, a multiple logistic regression analysis was performed. The associations between children with regular and healthy diet (dependent variable, outcome) and the DIs, adjusting for sex and age, were analyzed and the results are presented in Table 5.

The model indicated that daily BPA intakes were significantly associated with children with a healthy diet. Approximately 33% lower daily intakes were predicted for the healthy diet group compared to the regular diet group (OR 0.67; 95% CI 0.51–0.89).

As highlighted by other authors regarding chemicals with short half-lives, and food as an important exposure source, a healthy diet can have an impact by lowering exposure (Christensen et al. 2012; Hagobian et al. 2016; Rudel et al. 2011; Sathyanarayana et al. 2013). Thus, the different dietary patterns may explain some of the differences between the two groups. However, we have to point out that, next to diet, the physical characteristics of the two groups were considerably different with 61% obese and 39% normal-/underweight children. Renal clearance itself is also influenced to some extent by the physiological differences (namely BMI) between both groups (Hays et al. 2015).

Dietary sources seem to most likely constitute 2/3 of general BPA background exposure in humans, with only 1/3 of BPA exposure attributable to other sources (Christensen et al. 2012). Dust, thermal paper, dental and medical materials have

Table 5 Multiple logistic regression analyses of the diet groups (regular and healthy diet) and associations with the daily BPA intakes (ng/kg bw/day)

Model	β (SE)	Lower 95% CI	Odds ratio (OR)	Upper 95% CI
Daily intakes (ng/kg bw/day)[a]	− 0.40 (0.14)*	0.51	0.67	0.89
Age (years)	− 0.07 (0.06)	0.82	0.93	1.05
Sex	0.33 (0.42)	0.61	1.39	3.13

β estimate beta, *SE* standard error, *95% CI* 95% confidence interval

[a] Unit of exposure change = doubling the daily BPA intake (log2)

*Significance level is p ≤ 0.05

been identified as relevant non-dietary sources of BPA (Biedermann et al. 2010; Geens et al. 2012a, b; Hehn 2016). Contamination of food with BPA is possible to occur dur- ing the production process or when packaging by contact with materials containing epoxy resins and polycarbonate mate- rials. Dietary BPA exposure may depend more on food pack- aging material than the food item per se. For example, canned food has been shown to contain higher BPA levels than uncanned or unprocessed food items (Cao et al. 2009; Cunha et al. 2011, 2012; Cunha and Fernandes 2013; Larsson et al. 2014; Noonan et al. 2011). Other authors con- firmed the presence of BPA in paper and cardboard used as food containers (Lopez-Espinosa et al. 2007). Several epide- miological studies support the association between urinary BPA levels and food consumption (Cao et al. 2011; Casas et al. 2013; Christensen et al. 2012; Lakind and Naiman 2011; Quirós-Alcalá et al. 2013; Teeguarden et al. 2011).

BPA exposure has also been reported to be associated with an increase in body weight and BMI (Li et al. 2013a; Shankar et al. 2012; Trasande et al. 2012; Wang et al. 2012). Since diet is a major source of BPA exposure, reverse causality cannot be excluded, as obese children may have greater exposure to BPA due to higher dietary intakes or differently concentrated urines (Hays et al. 2015; Hoepner et al. 2016). In this study, the overweight/obese children (who were on a healthy diet for weight loss/management) had lower BPA exposures than nor- mal-/underweight weight children (with regular diet). This outcome supports Hoepner et al. 2016 suspicion that previous cross-sectional studies could be biased due to associations between BMI and higher food intake, leading to higher expo- sures to BPA from food. Since obese or overweight children were on dietary restriction, their healthy and balanced nutri- tion, based on fresh food and less packaged and processed food items, provided a plausible explanation to their lower BPA exposure.

Risk assessment

For risk assessment, both urinary BPA levels and calculated BPA daily intakes were compared with health based guidance values. The German Human Biomonitoring (HBM) Commission defined a provisional HBM value for BPA in children's urine of 1500 µg/L (HBM 2012) that was updated and lowered to 100 µg/L in 2015 (Apel et al. 2016), taking into account the BPA re-evaluation of EFSA (EFSA 2015). In our study population, the 95th percentile was 16.0 µg/L and the maximum was 66.9 µg/L (Table 2). Thus, all urinary BPA concentrations in the study were below this HBM value.

In 2015, the European tolerable daily intake (TDI) for BPA was lowered to 4 µg/kg of bw/day by EFSA (EFSA 2015). All daily BPA intakes calculated in our study were below this TDI, with a 95th percentile of 0.22 µg/kg bw/day and a max- imum of 0.62 µg/kg bw/day (Table 4).

First morning urine samples were the only bio-specimen available in this study. First morning urine samples have been previously shown to some extent to underestimate exposures to BPA (Teeguarden et al. 2011). Food related exposures dur- ing lunch or dinner (from the day before) may be under- represented in these samples due to the rapid BPA elimination kinetics. Even considering this hypothetical underestimation, daily intake levels of BPA for the Portuguese children can currently be classified as safe.

BPA daily intakes were calculated based upon urinary levels, taking into account the body height and sex based reference values for urinary creatinine excretion (Remer et al. 2002; Wittassek et al. 2007). As a note of caution, it is important to highlight that all reference values were derived from standard weight children (Remer et al. 2002) and their applicability to obese/overweight children has not been stud- ied before. Urinary flow rate and also creatinine were found to be influence by age, sex and BMI category (Barr et al. 2005; Hays et al. 2015). Finally, variations in hydration status have also to be recognized as an important source of variability in measured urinary concentrations (Hays et al. 2015).

International comparison

To our knowledge, this is the first BPA biomonitoring study performed on Portuguese children. Only one other study re- ported preliminary results for Portugal, on 20 adult volunteers (Cunha and Fernandes 2010). Worldwide, BPA in urine has been analyzed in numerous studies of the general population (or specific subpopulations) reviewed by several authors (Dekant and Völkel 2008; Vandenberg et al. 2010). A com- prehensive review of all biomonitoring data for BPA in all populations is beyond the scope of this paper. In Table 6, this study's data was compared with other studies performed in child populations.

Our data, both in terms of urinary BPA levels and calculat- ed daily intakes, is in accordance with other studies on chil- dren in Europe and other parts of the world. Median urinary BPA concentrations are within a range of 1 to 3 µg/L and median daily BPA intakes are consistently below 0.1 µg/kg bw/day.

The European DEMOCOPHES project (Covaci et al. 2015) provided BPA exposure data for 6–11 year-old children from six European countries (Belgium, Denmark, Luxembourg, Slovenia, Spain, and Sweden). The population in the present study had a very similar urinary BPA concentration (1.89 µg/L) when com- pared with overall DEMOCOPHES median (1.96 µg/L). Furthermore, exposures in Portuguese children are similar to exposures in the Spanish DEMOCOPHES children (1.91 µg/L); Spain is Portugal's sole land border in the Iberian Peninsula. Daily intake calculations in the DEMOCOPHES ap- proach are in agreement with our calculated data.

Table 6 Overview of worldwide biomonitoring studies for BPA concentrations (µg/L) in child populations

Country	Number	Population characteristics	Sampling years	Median (µg/L)	DI calculation model	Daily intake (ng/kg bw/day)	References
Portugal (all)	110	4–18 years old (obese/overweight and normal weight/underweight with or without a diet control)	2014/2015	1.89	Creatinine-based	31.3	This study
Belgium—DEMOCOPHES	125	6–11 years 6–12 years for Slovenia	2011/2012	2.27[a]	Creatinine output Fixed urinary output	39.9 46.7	(Covaci et al. 2015)
Denmark—DEMOCOPHES	142			1.71[a]	Creatinine output Fixed urinary output	40.2 37.6	
Luxembourg—DEMOCOPHES	59			1.38[a]	Creatinine output Fixed urinary output	37.8 30.0	
Slovenia—DEMOCOPHES	112			3.30[a]	Creatinine output Fixed urinary output	49.9 59.7	
Spain—DEMOCOPHES	118			1.91[a]	Creatinine output Fixed urinary output	40.2 34.9	
Sweden—DEMOCOPHES	97			1.31[a]	Creatinine output Fixed urinary output	30.6 25.0	
DEMOCOPHES all	653	6–11 years 6–12 years for Slovenia		1.96[a]	Creatinine output Fixed urinary output	38.2 37.9	
Germany	599	3–14 years	2007/2008	2.74	Volume-based Creatinine-based	GM 60.0 GM 50.0	(Becker et al. 2009)
	465	8–10 years	2009/2010	2.04	n.a.	n.a.	(Kasper-Sonnenberg et al. 2014)
	104	6–8 years	2006/2009	2.30	n.a	n.a	(Kasper-Sonnenberg et al. 2012)
Denmark	143	Urban and rural resident children 6–11 years	2011	1.70	Creatinine-based— urban Creatinine-based—rural	38.0 43.0	(Frederiksen et al. 2013b)
	49 55	6–10 years 11–16 years	2007	2.78 0.81	24-h urine	66.6 15.2	(Frederiksen et al. 2013a)
Belgium	193	14–15 years	2008/2009	2.21	n.a	n.a	(Geens et al. 2014)
Spain	130	4 years	2008/2010	3.10	n.a	n.a	(Casas et al. 2013)
Canada	1031 980	6–11 years 12–19 years	2007/2009	GM 1.30 GM 1.50	n.a n.a	n.a n.a	(Bushnik et al. 2010)
	1004 992	6–11 years 12–19 years	2012/2013	1.20 1.40	n.a n.a	n.a n.a	(Health Canada 2015)
USA	409 462	6–11 years 12–19 years	2013/2014	1.40 1.20	n.a n.a	n.a n.a	(CDC 2015)
	356	6–11 years	2005/2006	2.70		51.3	

Table 6

Country	Number	Population characteristics	Sampling years	Median (µg/L)	DI calculation model	Daily intake (ng/kg bw/day)	References
	702	12–19 years		2.40	24-h urinary output data were collected for the NHANES	45.7	(Lakind and Naiman 2011)
	90	6–8 years	2004/2005	1.80	n.a	n.a	(Wolff et al. 2007)
	35	6–10 years		3.60	n.a	n.a	(Teitelbaum et al. 2008)
Mexico	53	Boys 8–13 years	2010	1.20	n.a	n.a	(Lewis et al. 2013)
	55	Girls 8–13 years		1.10			
Australia	67	Pooled urine samples from > 0 to < 15 years, boys and girls	2012	GM 2.57	Urinary flow rate-based	GM 70.3	(Heffernan et al. 2014)
China	287	3–24 years	n.a	GM 3.00	n.a	n.a	(Li et al. 2013b)
Several Asian countries	47	≤ 19 years	Korea (2006/2007), Vietnam, Malaysia, India, Kuwait, Japan (2010)	76	Volume-based	All Asian countries: 39.0	(Zhang et al. 2011)

[a] un adjusted data, n.a not available, GM geometric mean

Some limitations of this study should be noted. The complex nature of the original study design (determination of exposure to suspected or confirmed predominantly persistent endocrine disruptor and/or obesogens) was less suitable to analyze effect-modifications with urinary BPA concentrations because of its short half-life. BPA as potential obesogen has been included after the study population was enrolled and nutritional guidance took place. Therefore, urine samples were not collected before the nutritional guidance started, nor a dietary questionnaire was applied, and thus, it was not possible to address the direct effect of the type of the diet to the exposure levels. Nevertheless, the strength of this study lies on the analysis of BPA exposures in obese/overweight children on a healthy diet compared with the other children on a regular diet. Although the initial study design was not perfectly adapted to these questions, evidence is provided that BPA intake is more likely associated with an increased intake of contaminated food rather than a direct correlation between BPA exposures and the occurrence or development of obesity in children.

Conclusion

With this study, we could confirm that BPA exposure in Portuguese children is within the range of exposures in Europe or other populations of the Globe previously investigated. We found evidence that BPA intake depends to some extent on the diet, with children on a dietary program (healthy diet) for weight loss/management revealing 33% lower daily intakes compared to children on their regular diet. Thus, contaminated food is likely to be an important but not the only source of BPA exposure for the Portuguese children studied. Notwithstanding, none of the children exceeded any of the health guidance values (HBM-I, TDI) derived for BPA.

Compliance with ethical standards The study was designed in ac- cordance 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 Faculdade de Medicina da Universidade do Porto, Portugal (ref. 163.13). Written informed consent was obtained from all parents of individual participants included in the study.

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