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Methodological approaches for the assessment of bisphenol A exposure

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ABSTRACT

Bisphenol A (BPA) is an endocrine disruptor used in food contact materials, by the application of polycarbonate plastics and epoxy resins. The main objective of this study is to compare the estimate of daily BPA exposure at 13 years of age and in the adult Portuguese population, using different methodological approaches, and assess the associations between this exposure and sociodemographic characteristics.

Methodology: Cross-sectional data of 13-years follow-up from a population-based birth cohort Generation XXI (GXXI) (n = 2804) and from the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015–2016) (n = 3845, ≥18 years old) was used. Dietary information was collected through three food diaries for adolescents and two non-consecutive 24-hour-recalls for adults.

To estimate the daily exposure to BPA, three methodological approaches were used. "Food groups attribution" merged the food consumption data with the concentration of BPA in food groups. "Regression tree model" and "random forest" combined food consumption information with urinary BPA, measured in a subsample of 24-hour urine (in adolescents n = 216, and in adults n = 82), both used to predict BPA exposure in the remaining sample. The fit-index of the methodologies was assessed through the root mean square error (RMSE), mean absolute error (MAE) and Spearman correlation coefficient (ρ). Associations between BPA exposure and sociodemographic variables were tested by linear regression models, adjusted for sex, age groups (in adults) and educational level. Tolerable Daily Intake (TDI) of 0.2 ng/kg body weight (bw), recently proposed by the European Food Safety Authority (EFSA), was used for the risk characterization of BPA exposure.

Results: The "random forest" was found as the best methodology to estimate the daily BPA exposure (adolescents: RMSE = 0.989, MAE = 0.727, ρ = 0.168; adults: RMSE = 0.193, MAE = 0.147, ρ = 0.250). The median dietary BPA exposure, calculated by "food groups attribution", was 79.1 and 46.1 ng/kg bw/day for adolescents and adults, respectively, while "random forest" estimated a BPA exposure of 26.7 and 38.0 ng/kg bw/day. 99.9% of the Portuguese population presented a daily exposure above TDI. Male adolescents, females and higher educated adults, were those more exposed to BPA.

Conclusions: The estimated daily BPA exposure strongly depends on the methodological approach. Food groups attribution may overestimate the exposure while the random forest appears to be a better methodological approach to estimate BPA exposure. Nevertheless, for all methods, the Portuguese population presented an unsafe BPA exposure by largely exceeding the safe levels proposed by EFSA.

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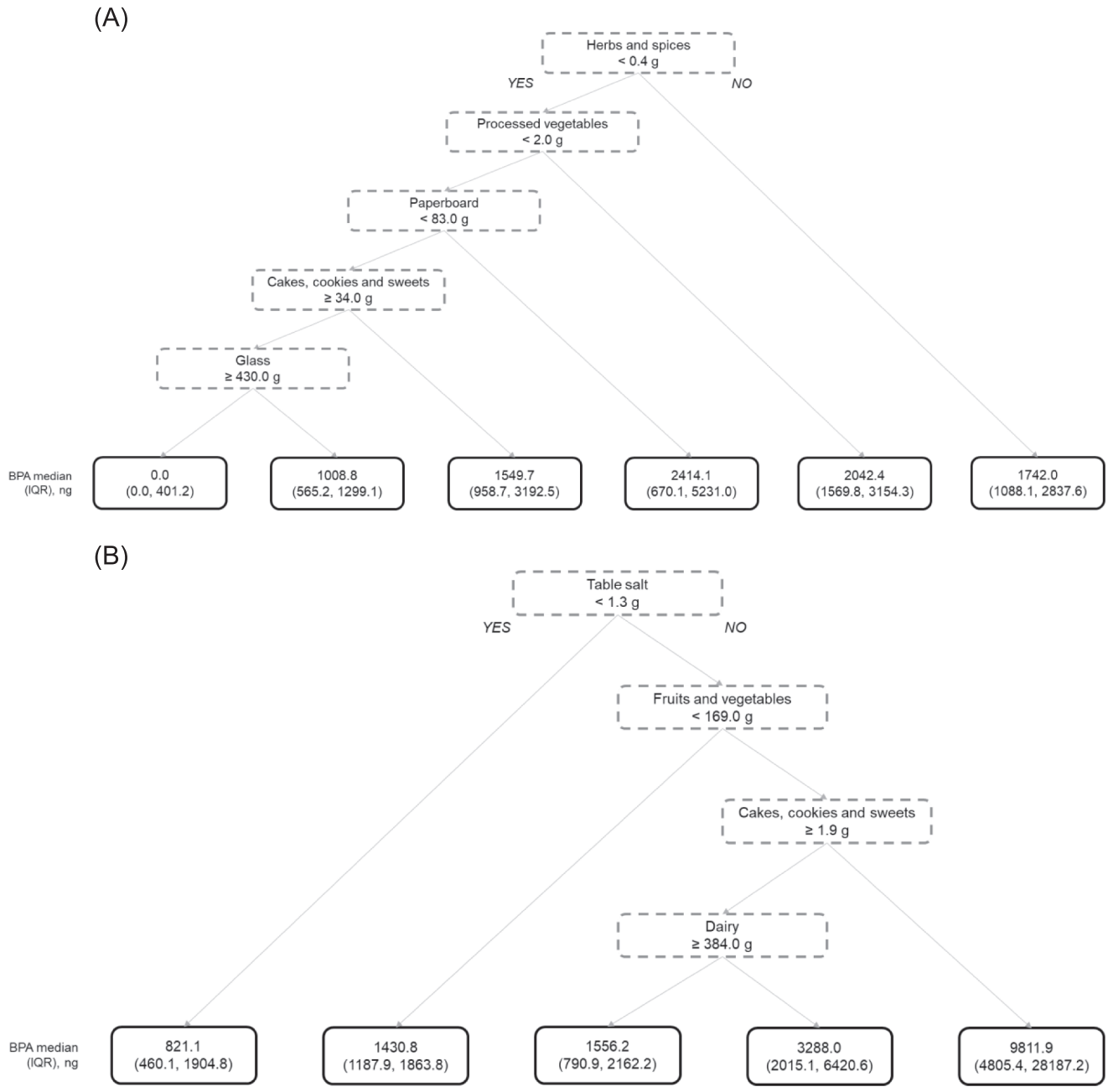


Fig. 1. Regression tree plot from the first imputation for adolescents' sample (n = 216) (A), and adults' sample (n = 82) (B). Variables of food groups and packaging materials (dashed line) used as predictors of absolute BPA, in nanograms (ng), measured in 24-hour urine samples (solid line).

1. Introduction

Bisphenol A (BPA) is a monomer widely used in the production of polycarbonate plastics and epoxy resins, as well as an additive for other polymeric materials. Due to their physical properties, polycarbonate plastics are applied to food contact materials, namely in reusable beverage bottles, infant feeding bottles, tableware, cookware, microwave ovenware, food containers and reservoirs for water dispensers (Vandenberg et al., 2007). Epoxy resins are frequently used in protective linings of food and beverage cans and as a coating for vats, storage tanks and supply systems (European Food Safety Authority, 2015). BPA has also been used to manufacture diverse non-food-related applications, such as toys, pacifiers, medical devices, thermal paper, printing inks, electronic products, and flame retardants (Kang et al., 2006; Olea et al., 1996; Pulgar et al., 2000).

Different studies concluded that individuals who consumed more food from packages containing BPA presented a higher concentration of this compound in their urine samples (Chang et al., 2019; Lakind & Naiman, 2011; Liu et al., 2018; Rudel et al., 2011). Depending on the storage conditions (temperature, pH and contact surface), the BPA can leach the components of the packaging materials and migrate into food and beverages (Cacho et al., 2012; Chang et al., 2019; Kang et al., 2006; Noureddine El Moussawi, Ouaini, et al., 2019; Noureddine El Moussawi, Cladière, Chébib, Ouaini, & Camel, 2019; Schecter et al., 2010; Sungur et al., 2014). Thus, diet is the most important source of BPA exposure across all population groups (European Food Safety Authority, 2015; Vandenberg et al., 2007; Von Goetz et al., 2010).

During the last years, a large amount of evidence has been published suggesting potential adverse health effects for humans due to BPA chronic exposure, even at low doses (Rochester, 2013; Vandenberg

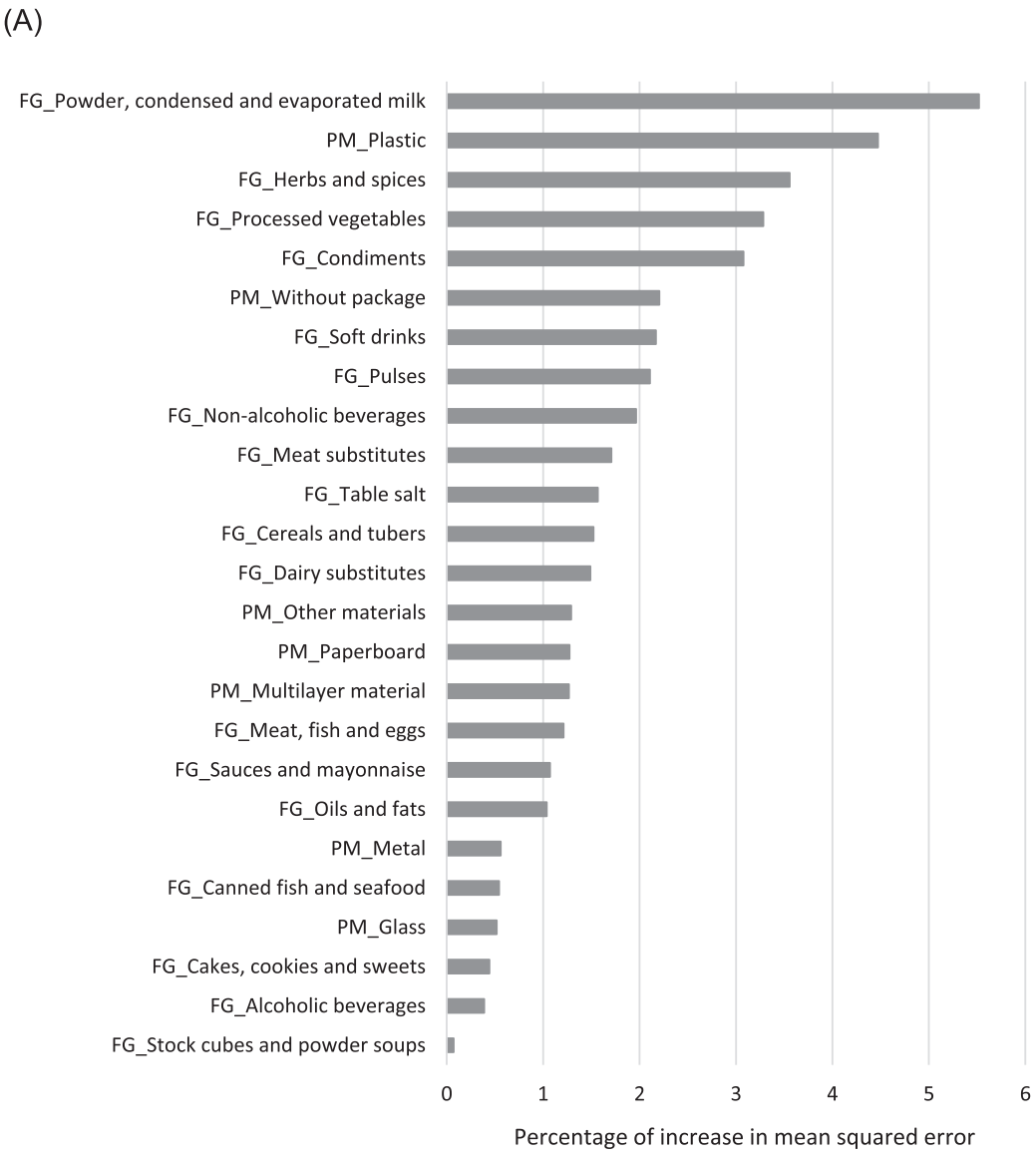


Fig. 2. Percentage of increase in mean squared error according to random forest variables importance for adolescents' sample (n = 216) (A), and for adults' sample (n = 82) (B). Plots from the first imputation.[11]

et al., 2007). This includes endocrine system deregulation, metabolic syndrome, obesity, diabetes mellitus, cardiotoxicity, hyperuricemia, immunological disabilities, reproductive complications and neuro-developmental/ neuroendocrine effects (Bao et al., 2020; Maffini et al., 2006; Rochester, 2013).

In that context, and based on the precautionary principle, the European Commission regulated the use of BPA in food contact materials (Regulation EU 10/2011 (European Commission, 2011b)), and the use of BPA in all plastic infant feeding bottles was restricted by the Regulation EU 321/2011 (European Commission, 2011a). In February 2018, the specific migration limit of BPA was updated to 0.05 mg/kg of food (Regulation EU 213/2018 (European Commission, 2018)). Moreover, in 2015, the European Food Safety Authority (EFSA) established a temporary Tolerable Daily Intake (t-TDI) of 4 μg/kg of body weight/day, pending on the outcomes of future long-term studies (European Food Safety Authority, 2015). But in 2023, EFSA has re-evaluated the risks to public health related to the presence of BPA in foodstuffs and recently proposed an updated TDI-value of 0.2 ng/kg of body weight (bw) (European Food Safety Authority, 2023; European Food Safety Authority Panel on Food Contact Materials Enzymes and Processing Aids, 2021).

Despite its hydrophobic characteristic (Hansch et al., 1995; Lide, 2005), BPA has no evidence of persisting in the environment or animal tissues (Cousins et al., 2002). After exposure, BPA is rapidly and extensively metabolized in the liver through conjugation reactions, being subsequently excreted in the urine, with a half-life of fewer than 6 h in the human body (Huang et al., 2017; Stahlhut et al., 2009; Thayer et al., 2015). BPA is completely eliminated via urine (Thayer et al., 2015), allowing the estimation of the daily exposure through daily urinary excretion (Vandenberg et al., 2007). Furthermore, different authors concluded that the level of BPA in the 24-h urine sample is similar to the level of dietary exposure to this contaminant due to the lower contribution of other exposure sources (Maffini et al., 2006; Meslin et al., 2022; Von Goetz et al., 2010).

Dietary exposure to food contaminants is usually assessed by indirect methods, such as food frequency questionnaires, 24-hour recalls and food diaries (de Fátima Poças & Hogg, 2007). Nevertheless, direct methods, namely measuring urinary biomarkers of exposure, can be used jointly with the traditional indirect approaches to better estimate

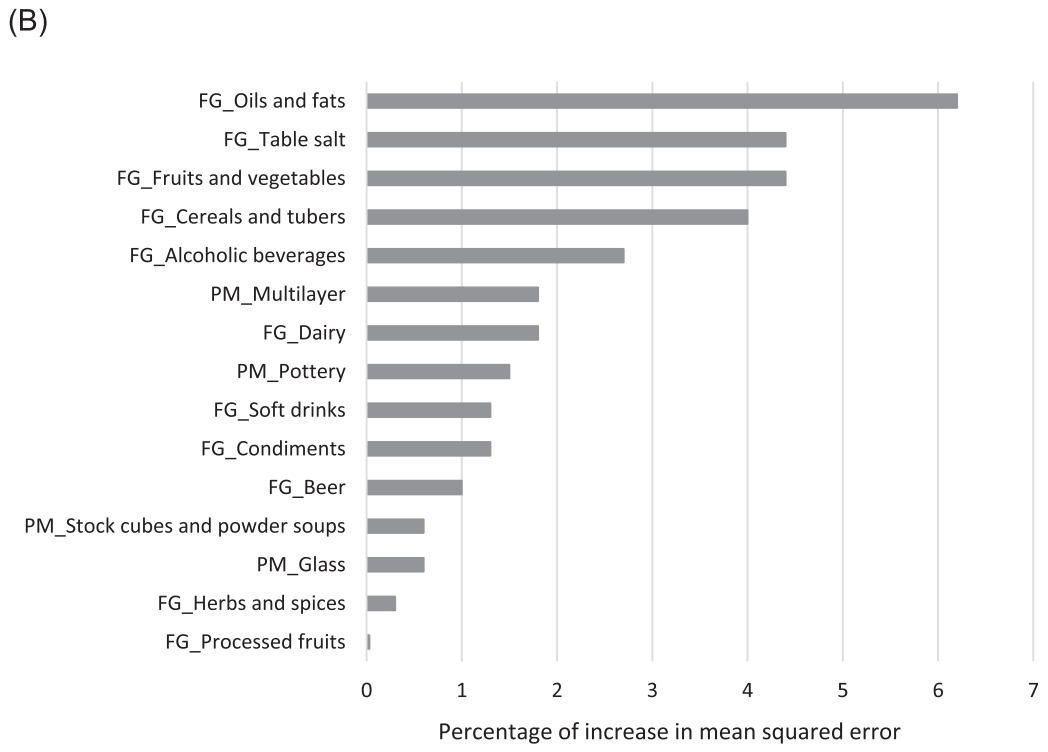


Fig. 2. (*continued*).

Table 1
Fit-index between bisphenol A measured in 24-hour urine samples, after box-cox transformation, and daily exposure to bisphenol A, using different methodological approaches for the exposure assessment.

	RMSE	MAE	ρ (95% CI)
Adolescents			
Full data			
Food groups attribution* *(n = 220)*	0.669	0.541	0.128 (-0.045, 0.294)
Regression tree** *(n = 216)*	0.909	0.684	0.482 (0.373, 0.578)
Random forest** *(n = 216)*	0.584	0.427	0.979 (0.972, 0.984)
Cross-validation 5 folds			
Regression tree** *(n = 216)*	1.044	0.790	0.093
Random forest** *(n = 216)*	0.989	0.727	0.168
Adults			
Full data			
Food groups attribution* *(n = 82)*	0.220	0.172	0.227 (-0.040, 0.464)
Regression tree** *(n = 82)*	0.152	0.118	0.632 (0.481, 0.746)
Random forest** *(n = 82)*	0.118	0.089	0.959 (0.938, 0.974)
Cross-validation 5 folds			
Regression tree** *(n = 82)*	0.205	0.154	0.250
Random forest** *(n = 82)*	0.193	0.147	0.250

Abbreviations: RMSE – root mean square error; MAE – mean absolute error; ρ – Spearman correlation coefficient; CI – confidence interval.
The random forest was the best methodology since it presented lower values for RMSE and MAE and a higher correlation coefficient compared to the regression tree and food groups attribution, for both adolescents and adults, in full data analysis and after the cross-validation.
* mean values of 5 imputations.
** values of the first imputation.

the exposure to food contaminants (Beckmann et al., 2020; Brouwer-Brolsma et al., 2017). In fact, limitations of indirect methods related to misreporting, participation bias, and identification of all sources and/or food contaminants occurrence data, can be overcome by direct methods that can provide objective estimates of xenobiotics exposure (Bingham, 2002; Brouwer-Brolsma et al., 2017; Gorecki et al., 2017).

Considering the recent EFSA scientific opinion and the new TDI value, there is a significant public health concern regarding dietary exposure to BPA for the entire population since the safety threshold was

[1] Abbreviations: FG – food group; PM – packaging material.

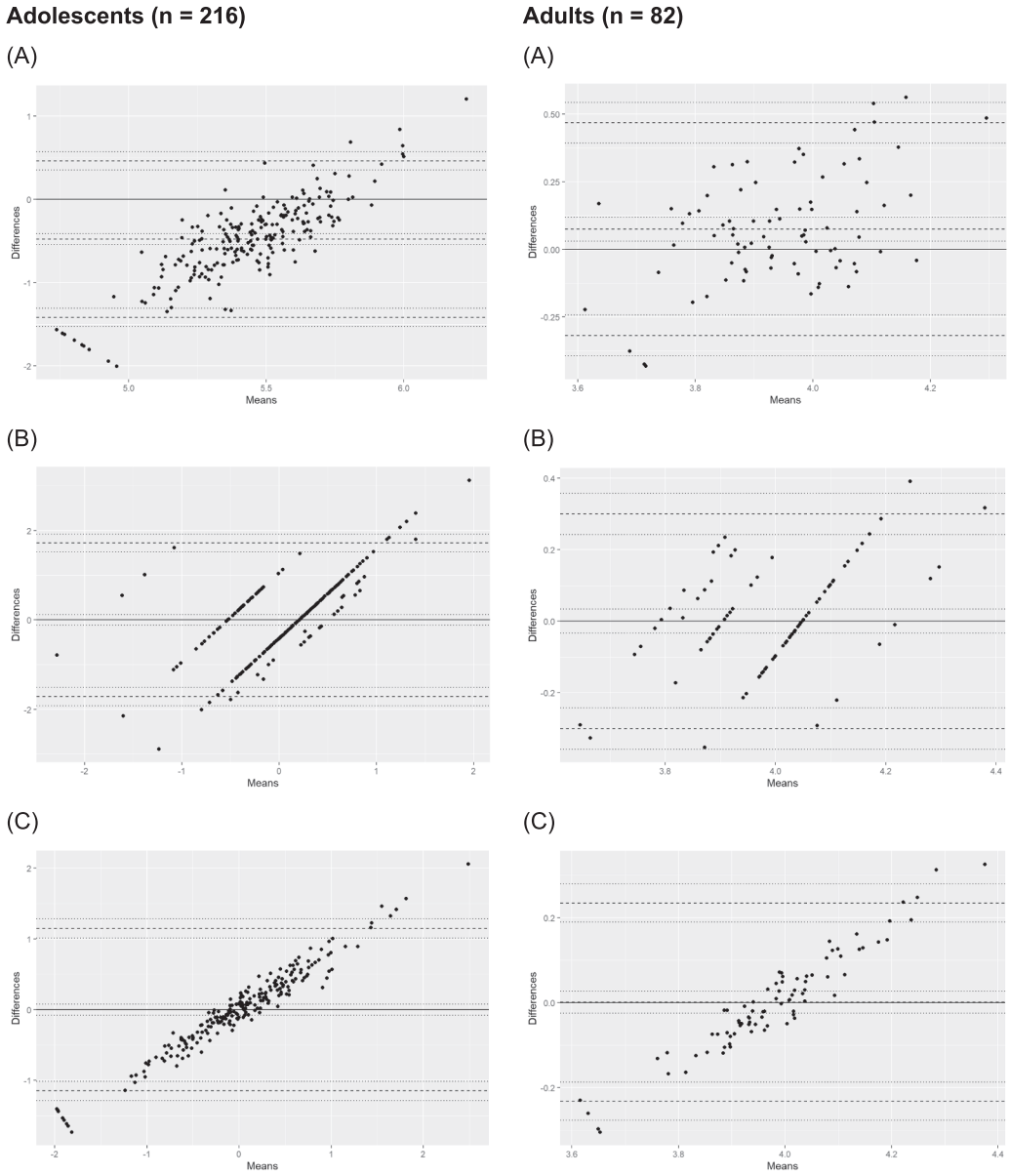


Fig. 3. Bland-Altman plots from the first imputation.[21]

largely exceeded (European Food Safety Authority, 2023; Meslin et al., 2022). However, this EFSA estimation could misrepresent the current dietary exposure (European Food Safety Authority, 2023), becoming essential to apply different methods to assess the dietary exposure to BPA further and characterize the related risk.

The present study aims to compare three different methodological approaches to estimate daily BPA exposure at 13 years of age and in the adult Portuguese population. Secondly, the best methodological approach will be used to assess the associations between BPA exposure and sociodemographic characteristics in both adolescents at 13 years of age and in the adult Portuguese population.

2. Methods

2.1. Study design and participants

This project included data from two population studies with available dietary information and urine samples: adolescents from a Portuguese population-based birth cohort and adults from a Portuguese national survey.

The adolescent sample was from Generation XXI (GXXI), an ongoing prospective population-based birth cohort, assembled between April 2005 and August 2006 at the five public units providing obstetrical and neonatal care in the metropolitan area of Porto, Portugal, as previously described (Larsen et al., 2013). In the 24 to 72 h after delivery, 91% of all invited mothers accepted to enrol in the cohort GXXI, resulting in 8,495 mothers and 8,647 children. Subsequently, all cohort participants were invited to participate in the 4-, 7-, 10- and 13-year follow-ups, with participation rates of 86%, 80%, 76% and 54%, respectively. It is important to highlight that the 13-year follow-up was interrupted in March 2020 due to issues related to the COVID-19 pandemic, reflected in the lower participation rate observed during this wave. This study used cross-sectional data from the 13-year follow-up which collected 24-hour urine (n = 240) and with complete information on food diaries (n = 2804).

Data from the National Food, Nutrition and Physical Activity Survey (IAN-AF 2015–2016) was used to assess the exposure to BPA in the adult Portuguese population. This survey included a representative sample of

Table 2
Validation of the different methodological approaches used to estimate the exposure to bisphenol A (ng/ day), by sex, educational level and body mass index categories. Differences were assessed through the Mann-Whitney test.

	Female	Male	*p-value*	≤12y of school[a]	>12y of school[a]	*p-value*	Underweight/ normal weight	Pre-obesity / obesity	*p-value*
Adolescents (n = 216)[b]									
Urine, median (IQR)	**1156 (1692)**	**1601 (2213)**	**0.002**	1481 (1901)	1456 (1794)	0.58	**1316 (1640)**	**1827 (2431)**	**0.005**
Food groups attribution, median (IQR)	**7060 (3095)**	**7770 (3222)**	**<0.001**	7439 (3127)	7255 (3382)	0.26	7392 (3222)	7390 (3236)	0.66
Regression tree, median (IQR)	1731 (948)	1731 (7)	0.60	1731 (478)	1731 (7)	0.87	1731 (7)	1731 (948)	0.13
Random forest, median (IQR)	**1311 (426)**	**1471 (523)**	**<0.001**	1359 (541)	1381 (425)	0.92	1385 (520)	1343 (569)	0.33
Adults (n = 82)									
Urine, median (IQR)	2074 (2316)	2818 (2962)	0.39	2445 (4512)	2909 (1563)	0.73	2462 (5152)	2881 (2031)	0.85
Food groups attribution, median (IQR)	**3043 (1709)**	**4739 (1699)**	**<0.001**	3889 (2260)	3999 (2695)	0.88	3766 (2244)	3909 (2449)	0.65
Regression tree, median (IQR)	4003 (2018)	4003 (2018)	0.21	4003 (2018)	4003 (2161)	0.31	4003 (2305)	4003 (2018)	0.71
Random forest, median (IQR)	2570 (1166)	3038 (1851)	0.10	2686 (1692)	2643 (720)	0.75	2585 (2305)	2794 (1208)	0.88

Abbreviations: IQR – interquartile range.
Significant values are in **bold**.
[a] Parent's educational level for adolescents.
[b] Values of the first imputation.

Table 3
The daily exposure of bisphenol A (ng/ kg of body weight/ day), estimated by applying different methodological approaches.

	Food groups attribution *median (IQR)*	Regression tree *median (IQR)*	Random forest *median (IQR)*
Adolescents (n = 2804)			
Total	79.1 (61.1, 102.2)	31.6 (22.0, 37.9)	26.7 (22.1, 31.8)
Sex			
Female	75.3 (58.1, 96.9)	31.7 (22.5, 37.4)	26.2 (22.0, 30.8)
Male	83.6 (64.5, 107.2)	31.5 (21.7, 38.4)	27.3 (22.4, 32.7)
Parents' educational level			
None, 1st and 2nd cycle	77.4 (60.2, 98.0)	30.5 (19.9, 37.0)	26.5 (20.7, 32.0)
3rd cycle and high school	78.8 (61.1, 101.9)	31.2 (20.3, 37.4)	26.6 (22.1, 31.5)
Higher education	81.0 (62.8, 103.6)	32.4 (24.7, 38.6)	27.1 (23.0, 32.0)
Adults (n = 3845)[*]			
Total	46.1 (33.1, 63.9)	41.7 (27.5, 58.9)	38.0 (32.1, 44.8)
Sex			
Female	41.4 (29.3, 55.1)	42.0 (27.1, 62.4)	39.3 (32.9, 47.0)
Male	52.3 (39.1, 72.5)	41.4 (28.2, 56.9)	37.0 (31.1, 42.2)
Age groups			
18–64 years	49.2 (36.0, 66.9)	41.3 (27.5, 57.8)	38.5 (32.5, 45.4)
65–84 years	36.4 (25.8, 49.7)	43.7 (29.3, 62.3)	36.0 (30.5, 42.5)
Educational level			
None, 1st and 2nd cycle	38.7 (27.7, 54.3)	42.1 (29.2, 60.4)	35.5 (30.1, 43.0)
3rd cycle and high school	49.6 (36.4, 67.2)	40.7 (26.8, 56.8)	38.5 (33.3, 44.9)
Higher education	50.0 (37.8, 65.0)	44.1 (27.6, 61.0)	39.3 (33.3, 46.8)

[*] Adults' results were weighted for the complex survey design.

the Portuguese population between 3 months and 84 years old, randomly selected by a multistage sampling methodology, as previously described (Lopes et al., 2017, 2018). Individual information of adult participants (≥18 years old) with two completed interviews (n = 3852) and with 24-h urine samples (n = 95) was used for the current analysis.

2.2. Anthropometrics measurements

Anthropometric measurements were performed under standard procedures, after 12 h of fasting, with subjects in light clothing and barefoot (Guerra et al., 2014). For both GXXI and IAN-AF 2015–2016 participants (Oliveira et al., 2018), the height was measured to the nearest centimetre using a wall stadiometer (SECA, Hamburg, Germany) and body weight to the nearest tenth of a kilogram using a digital scale (SECA, Columbia, USA). In the present study, 7 adult participants were excluded due to missing values of body weight measurement.

The body mass index (BMI) was calculated as weight over the squared height and adults were classified into the categories defined according to standards of the World Health Organization (WHO): underweight, normal weight, overweight and obesity. Adolescents' body mass index (BMI) was classified according to age and sex-specific BMI z-scores published by WHO (WHO Multicentre Growth Reference Study-Group., 2006).

2.3. Dietary assessment methods

Adolescents from GXXI were invited to complete a 3-day food diary before the face-to-face interview, helped by their parents/main caregiver and a set of oral and written instructions given by the research team. The participants described each food, drink and supplement consumed, including brands, packaging materials, food preparation method and consumption place. The food diaries were reviewed by trained researchers during the face-to-face interview and codified into the "eAT24" software, previously developed for the IAN-AF 2015–2016, described in detail bellowing in the text. The present study included participants that reported at least two completed days in the food diary.

[2] (A) Food groups attribution; (B) Regression tree; (C) Random forest.

Table 4
Associations between the daily exposure to bisphenol A (ng/ kg of body weight/ day), with socio-demographic variables in adolescents (n = 2804) and adults (n = 3845).

	Regression tree		**Random forest**	
	Crude model	Model 1	Crude model	Model 1
Adolescents	MD (95% CI)	MD (95% CI)	MD (95% CI)	MD (95% CI)
Sex				
Female	ref	ref	ref	ref
Male	0.74	0.72	**1.14**	**1.34**
	(-2.12, 3.60)	(-1.42, 2.84)	**(0.57, 1.70)**	**(0.62, 2.06)**
Parents' educational level				
None, 1st and 2nd cycle	ref	ref	ref	ref
3rd cycle and high school	−0.66	−0.70	0.18	0.11
	(-6.67, 5.35)	(-6.70, 5.30)	(-0.85, 1.21)	(-0.91, 1.13)
Higher education	1.10	1.08	0.73	0.70
	(-4.74, 6.94)	(-4.76, 6.91)	(-0.34, 1.81)	(-0.38, 1.77)
Adults*				
Sex				
Female	ref	ref	ref	ref
Male	0.97	0.97	**0.94**	**0.94**
	(0.93, 1.01)	(0.94, 1.01)	**(0.93, 0.95)**	**(0.93, 0.96)**
Age groups				
18–64 years	ref	ref	ref	ref
65–84 years	**1.07**	**1.08**	**0.97**	1.00
	(1.02, 1.12)	**(1.03, 1.14)**	**(0.95, 0.98)**	(0.98, 1.03)
Educational level				
None, 1st and 2nd cycle	ref	ref	ref	ref
3rd cycle and high school	**0.96**	0.98	**1.06**	**1.06**
	(0.92, 1.00)	(0.94, 1.03)	**(1.04, 1.08)**	**(1.04, 1.08)**
Higher education	**1.06**	**1.09**	**1.11**	**1.10**
	(1.01, 1.12)	**(1.04, 1.15)**	**(1.08, 1.13)**	**(1.08, 1.13)**

Abbreviations: BMI – body mass index; BPA – bisphenol A; MD – mean difference; CI – confidence interval; ref – reference category.
Crude model – without adjustment; Model 1 – adjusted for sex and parents' educational level in adolescents and adjusted for sex, age groups and educational level in adults.
Significant values are in **bold**.
* Adults' results were weighted for the complex survey design.

For the IAN-AF 2015–2016 adult participants, dietary information was collected in two face-to-face interviews, between October 2015 and September 2016, by trained interviewers in two non-consecutive 24-hour dietary recalls, with an interval of 8 to 15 days, following the EU Menu methodology proposed by EFSA (European Food Safety Authority, 2014). The collection and transformation of food data into nutrients resulted from using the validated "eAT24" software (Goios et al., 2020; Lopes et al., 2018), which integrates the Portuguese Food Composition Table and international data for missing items. All food, beverages and dietary supplements consumed by the participants in 24 h were reported, quantified and described as eaten according to the eating occasion. A complete description of all the food items reported was obtained by adopting the FoodEx2 classification system with their different facets and corresponding descriptors (European Food Safety Authority, 2011). This system allowed an extensive collection of information, namely the food packaging materials (pottery, wood, cork, glass, paper, paperboard, vegetal paper, film, plastic, textiles, multilayer materials, metal, aluminium, edible materials and other materials). Finally, foods, beverages and recipes were quantified through different methods available: weight (grams), volume (millilitres), food picture book (186 food photo series for foods/ recipes (6 portions each) and 11 photo series for household measures) (Torres et al., 2017), standard units, household measures and default portions.

2.4. Other variables

In GXXI, information regarding sociodemographic characteristics, namely sex and parents' educational level, was collected by trained interviewers at baseline and updated in each follow-up evaluation.

In the IAN-AF 2015–2016, demographic and socio-economic information, such as data on sex, age and educational level ("none, 1st and 2nd cycle", "3rd cycle and high school" and "higher education") was also collected.

2.5. Urine collection and chemical analysis

A convenience sample of 240 participants from the GXXI birth cohort was invited to collect urine for 24 h, as close as possible to the interview. Participants were selected based on the availability of complete food diary records throughout all follow-up waves, which occurred at 4, 7, 10, and 13 years. All procedures were explained, orally and by writing, to adolescents and respective parents/main caregivers, and the necessary material was made available, namely a BPA-free container.

As described previously, a sub-sample of 95 individuals from the IAN-AF 2015–2016 sampling frame was invited to participate in a validation study (Goios et al., 2020). These participants were instructed to bring a 24-hour urine sample at the second interview, corresponding to the second 24-hour dietary recall. Exclusion criteria were taking diuretics; having diabetes, kidney disease, haemophilia, or any condition requiring supplemental oxygen; donating blood or plasma during or less than 4 weeks before the study; being pregnant or lactating women; being under a dietary therapy; or having a urinary tract infection (Goios et al., 2020).

For all samples, urinary creatinine was measured by the Jaffe method (Beckman Coulter). Total BPA concentration levels were measured in urine using a dispersive liquid–liquid microextraction followed by gas chromatography coupled to mass spectrometry, as previously described (Cunha & Fernandes, 2010). The analytical method's detection and quantification limits were 0.03 ng/mL and 0.1 ng/mL, respectively (Cunha & Fernandes, 2010).

From the 240 samples from GXXI, 20 were not considered in the statistical analysis due to incomplete information regarding dietary consumption and additionally 4 for missing in the urinary output volume, ending with a sample of 216.

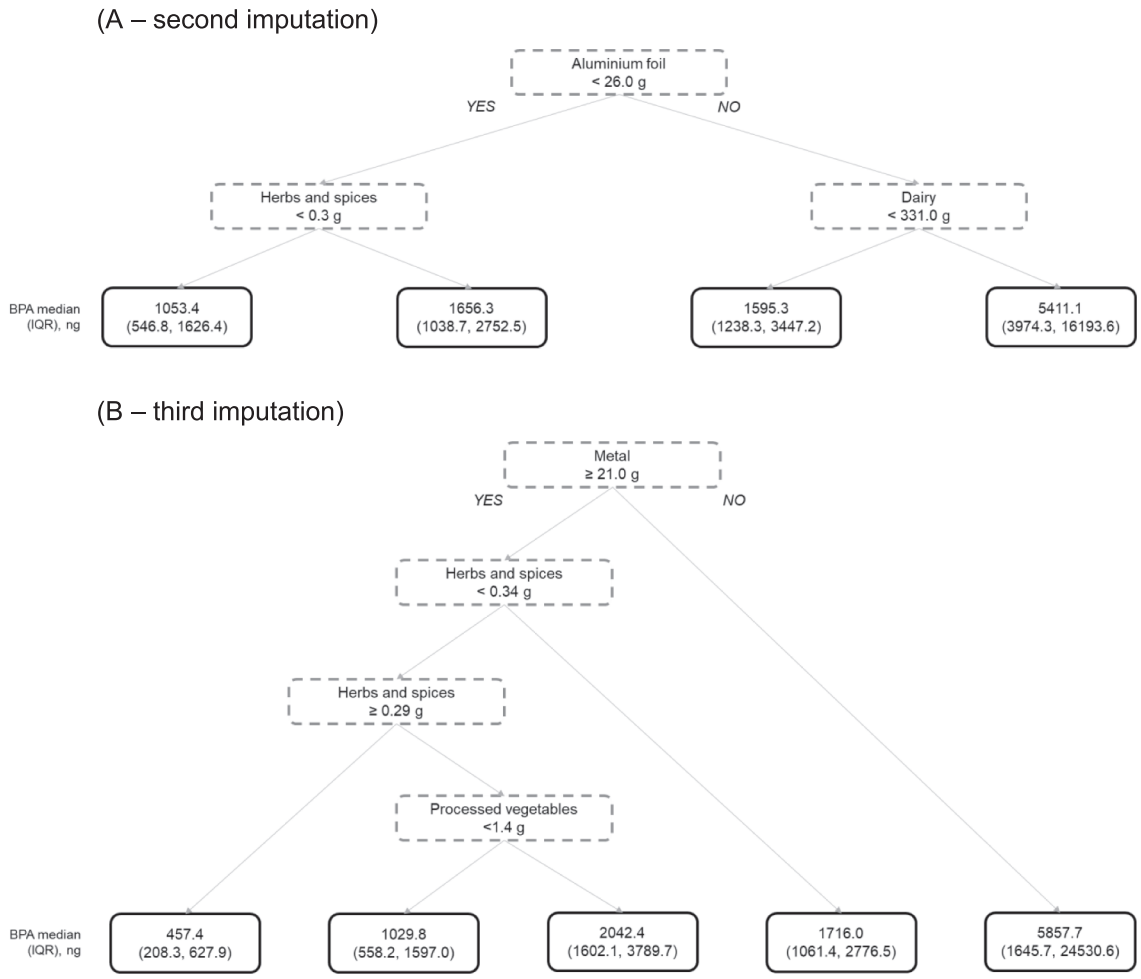


Fig. A1. Regression tree plot, from the second to the fifth imputation (A - D), for adolescents' sample (n = 216). Variables of food groups and packaging materials (dashed line) used as predictors of absolute BPA, in nanograms (ng), measured in 24-hour urine samples (solid line).

From the 95 urinary samples from IAN-AF 2015–2016, 9 were excluded due to their incomplete collection determined by the creatinine coefficient, i.e. creatinine excretion (mg/d) by body weight (kg) (if out of the parameters: 14.4 – 33.6 in males and 10.8 – 25.2 in females) (World Health Organization, 1984), and by the total volume of 24-hour urine collected (if less than 500 ml) (Wang et al., 2013). Moreover, 4 samples were excluded by the insufficient sampling volume available for laboratory analysis, leading to a final sample of 82.

2.6. Exposure assessment to BPA

To estimate the daily exposure to BPA, three methodological approaches were used. The first method, "food groups attribution", estimated the daily dietary exposure to BPA by merging the food consumption data with the concentration data of BPA in food groups, published by EFSA in 2015 (European Food Safety Authority, 2015). The EFSA scientific opinion considered BPA occurrence reported in the scientific literature, only considering studies of samples collected in Europe, and obtained through a specific EFSA calls for data. Occurrence data from foodstuffs collected in Portugal were considered, namely for canned products of "Fish and other seafood", "Non-alcoholic beverages", "Alcoholic beverages", "Food for infants and small children" and "Products for special nutritional use" food groups.

For each food group, according to the upper bound scenario, an average value of BPA concentration reported by EFSA was used to estimate the BPA exposure by adolescents from GXXI and adults from the Portuguese population. For this, a FoodEx2 code (base term and descriptor of packaging material facet, namely, canned or non-canned) was attributed to each food group reported in EFSA's publication (European Food Safety Authority, 2015). These codes were used to merge the occurrence data with the food consumption data, also codified with a base term and packaging material descriptor, according to the FoodEx2 classification system. In the end, the sum of exposure for each day by the individual was calculated, and then the daily average for the specific survey period was derived.

Additionally, two methods were developed combining the dietary information with urinary BPA: regression tree model and random forest, both used to predict the daily exposure to BPA in the remaining sample.

2.7. Risk characterization

Since BPA is rapidly metabolized, and there is a residual contribution of other exposure sources, it was assumed that the estimates gathered from regression tree model and random forest represent approximately daily dietary exposure to BPA. Thus, for the risk characterization of BPA exposure, the proportion of individuals above the TDI was calculated, for the three methodological approaches, using the recently proposed TDI value by the EFSA, 0.2 ng/kg bw (European Food Safety Authority, 2023).

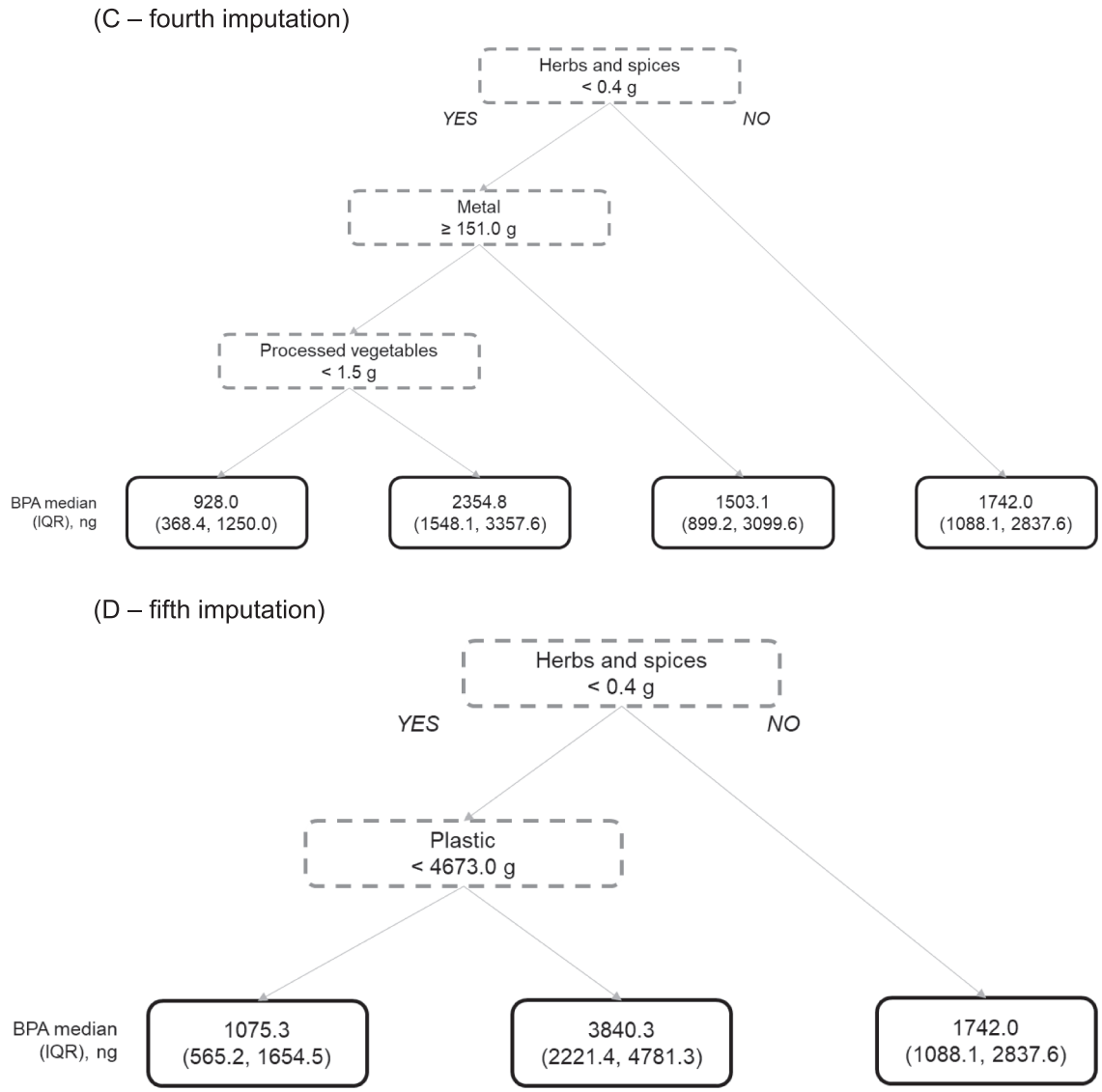


Fig. A1. (*continued*).

2.8. Ethics approval and consent to participate

The protocol of the GXXI birth cohort was approved by the Ethics Committee of São João University Hospital and registered with the Portuguese Individual Data Protection Authority. For IAN-AF 2015–2016, ethical approval was obtained from the National Commission for Data Protection, the Ethical Committee of the Institute of Public Health of the University of Porto and the Ethical Commissions of the Regional Administrations of Health. For GXXI participants, written informed consent was obtained from parents, or legal caregivers, and oral assent from adolescents. Written informed consent was obtained for adult participants from IAN-AF 2015–2016. Both studies complied with the national legislation and the Ethical Principles for Medical Research expressed in the Declaration of Helsinki. All documents with identification data were treated separately and stored in a different dataset. The databases with individual information are anonymized, allowing the non-identification of the participants. The Data Protection Officer at the Institute of Public Health of the University of Porto ensured data protection.

2.9. Statistical analysis

For GXXI and IAN-AF 2015–2016, to handle missing information on packaging material, a decision tree was used to generate a multiple imputation model (5 imputations), and combined according to Rubin's rule, as described elsewhere (Costa et al., 2021). The developed decision tree combined data from different variables such as participants' sex, age groups (in IAN-AF 2015–2016), educational level (or parents' educational level for GXXI), BMI categories, day of the dietary collection, eating place, eating occasion, food groups and packaging materials reported.

The multiple imputations did not significantly change the results for adults, so only the results of the first imputation are presented. However, slight differences were found for adolescents between the five imputations. Thus, the first imputation is presented in the manuscript, and the results of the second to the fifth imputation are in the supplementary material.

Total BPA excretion (ng/day) was calculated by multiplying the urinary BPA concentration (ng/L) and the urine volume (L/day), and compared with the BPA dietary exposure. A Box-Cox transformation was performed to approximate the distribution of the total BPA measured in urine to the normal distribution curve, applying a calculated exponent of

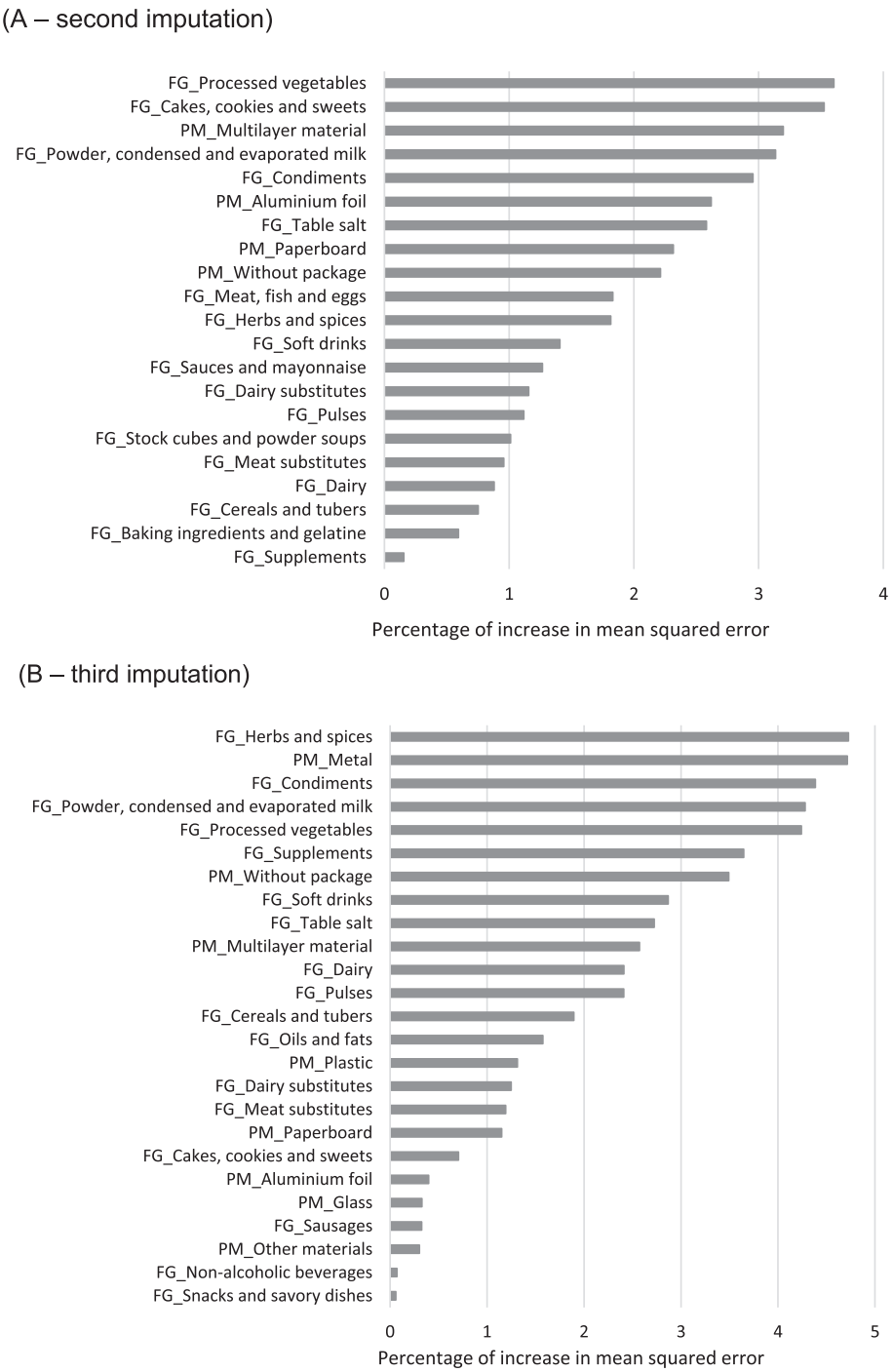


Fig. A2. Percentage of increase in mean squared error according to random forest variables importance. Plots from the second to the fifth imputation (A - D), for adolescents' sample (n = 216).[1]

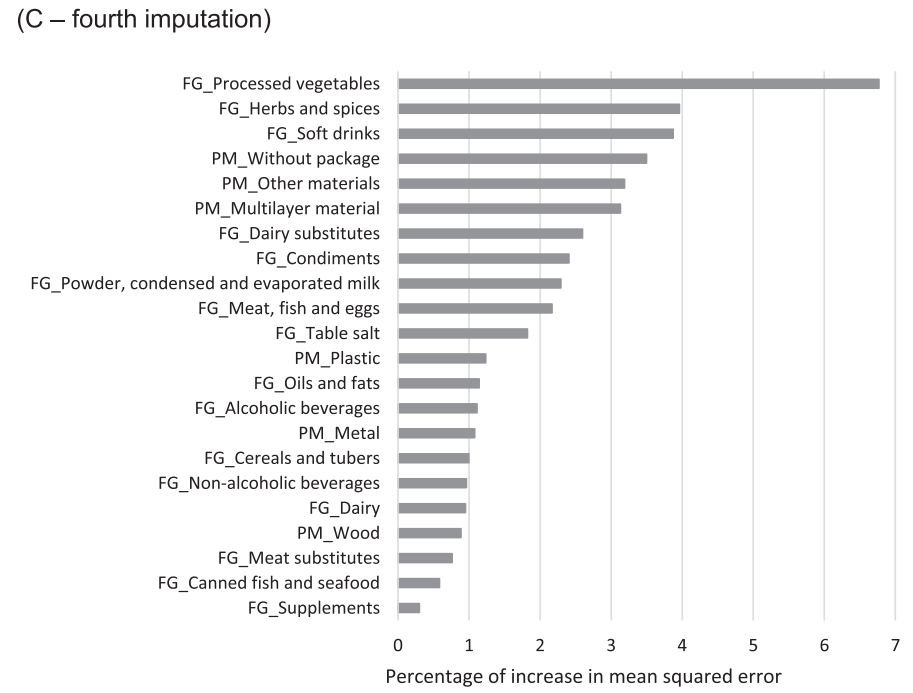
−0.2 for adults and −0.1 for adolescents.

The fit-index between urinary BPA and the daily exposure, estimated using the three methodological approaches, was assessed through the root mean square error (RMSE), mean absolute error (MAE) and Spearman correlation coefficient (ρ). In addition, to handle the possible overfitting of the models, a repeated 2-times 5-fold cross-validation was performed.

Mann-Whitney test was used to test the median differences between urinary BPA and the three estimated daily exposure to BPA. This analysis was stratified by sex, educational levels and BMI categories. The variable educational level was dichotomized: "≤ 12 years of school" and "> 12 years of school". Finally, underweight and normal weight categories were grouped, as well as pre-obesity and obesity.

Bland-Altman plots were used to illustrate the difference between the three methods against the BPA measured in urine samples.

Associations between BPA exposure and sociodemographic characteristics were tested in 13 years old adolescents using linear regression models without transformation due to the approximated normal distribution, presented by mean differences (MD) and 95% confidence interval (CI). For adults, the differences were assessed by relative mean differences (RD) and the respective 95% CI, through linear regression models, after log-transforming the outcome due to its heavy-tailed distribution. Two separate models were applied: a crude model; and a model adjusted for sex, age groups (adults) and educational level (or



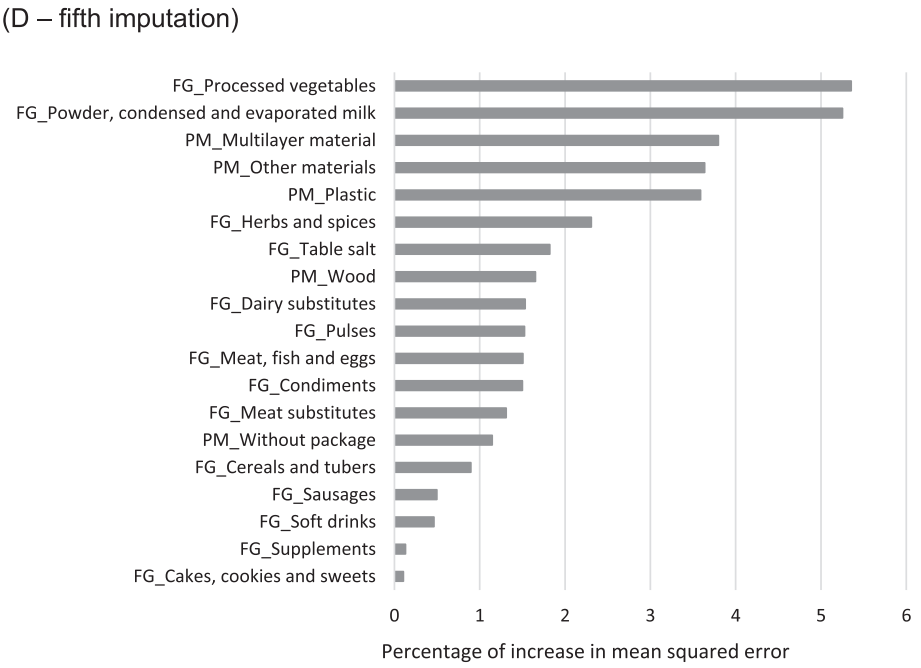


Fig. A2. (*continued*).

parent's educational level for adolescent sample) (model 1).

Analyses were performed using the R software version 4.2.1 for Windows, with a significance level of 5%.

3. Results

3.1. Development of regression tree and random forest

In this study, three methodological approaches were used to estimate the daily exposure to BPA in adolescents and adults: food groups attribution, regression tree and random forest.

Fig. 1 presents the regression tree plot of the first imputation, both for adolescents and adult samples. To estimate BPA exposure, the model used as predictors the food groups and the packaging material reported. Depending on the consumption in each decision node of the tree, participants were categorized into one terminal node of the possible branches, i.e. final output. For example, an adolescent was categorized in the lower BPA exposure group (131.1 ng/day) if consumed less than 0.4 g of herbs and spices, less than 2.0 g of processed vegetables, less than 83.0 g of food items packaged in paperboard, more or equal to 34.0 g of cakes, cookies and sweets and more or equal to 430.0 g of food items packaged in glass. But if the adolescent consumed more or equal to 0.4 g of herbs and spices the participant was categorized in the higher BPA exposure group (1732.7 ng/day) (see Fig. A1 for second to fifth imputation results, in supplementary material). The same interpretation is applied to adults; if they consumed less than 1.3 g of table salt, they were categorized in the lower BPA exposure group (821.1 ng/day). If they consumed more or equal to 1.3 g of table salt, more or equal to 169.0 g of

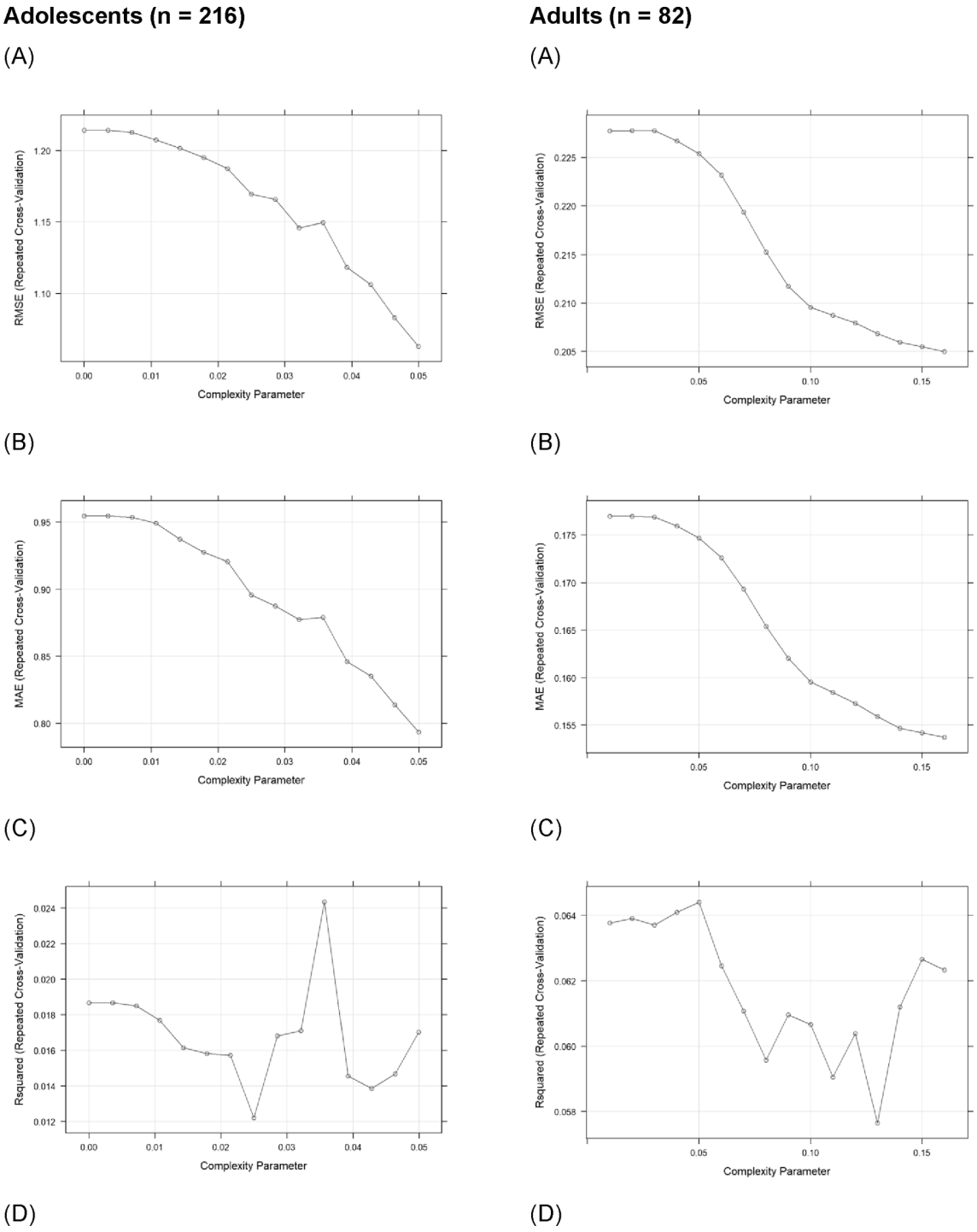


Fig. A3. Plots of fit-index parameters from the first imputation, after cross-validation (5 folds, repeated 2 times for adolescents and 50 times for adults), using different methodological approaches.[31]

fruits and vegetables and less than 1.9 g of cakes, cookies and sweets, they were categorized in the higher BPA exposure group (9811.9 ng/day).

Regarding the random forest, this methodology combines the various estimates of BPA exposure from multiple regression trees to reach a single result. In this study, 1000 uncorrelated regression trees were generated to create the random forest, using as predictors food groups and the packaging material reported. Fig. 2 shows the plot of the random forest variables' importance, in adolescents and adults, represented by the percentage of increase in the mean square error (%IncMSE) introduced in the model by changing those specific variables (see Fig. A2 for second to fifth imputation results, in supplementary material). For the first imputation, in adolescents, the consumption of powder, condensed and evaporated milk was the variable with higher importance for the estimation of BPA exposure (5.5 %IncMSE), followed by the consumption of food items packaged in plastic (4.5 %IncMSE) (Fig. 2). In adults, the most important variable was the consumption of oils and fats (6.2 % IncMSE), followed by table salt intake and the consumption of fruit and

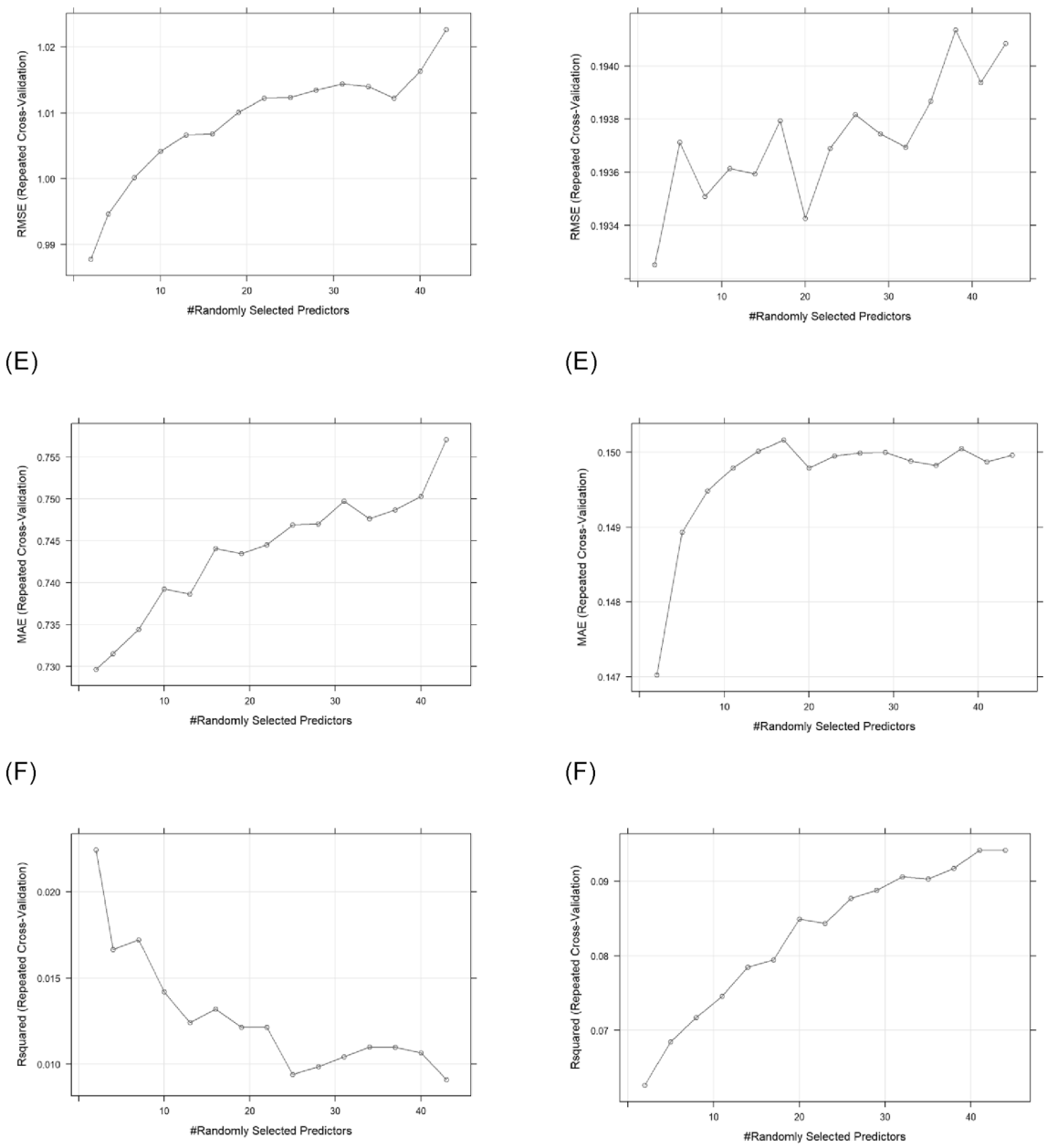


Fig. A3. (*continued*).

vegetables (4.4 %IncMSE, in both) (Fig. 2).

3.2. Fit of the exposure assessment methods

Table 1 presents the results for the fit-index between BPA measured in 24-hour urine samples, after box-cox transformation, and daily exposure to BPA, estimated by applying the three different methodological approaches. The random forest was the best methodology to estimate the daily exposure to BPA in our sample since it presented lower values for RMSE and MAE and a higher correlation coefficient compared to the regression tree and food groups attribution, for both adolescents and adults, in full data analysis and after the cross-validation (adolescents: RMSE = 0.989, MAE = 0.727, ρ = 0.168; adults: RMSE = 0.193, MAE = 0.147, ρ = 0.250). The plots of these parameters are presented in the supplementary material (figures A3 and A4). The Bland-Altman plot of the first imputation, for the three methodological approaches and full data analysis, in adolescents and adults, are presented in Fig. 3 (figure A5: second to the fifth imputation). Both for adolescents and adults, the food groups attribution plot shows a systematic bias, overestimating the exposure, and a large scatter of the differences, indicating that this approach was inaccurate for estimating BPA daily exposure (Fig. 3 - A). For the random forest (Fig. 3 – C), no systematic bias between exposure estimation and urinary excretion was observed. Nevertheless, the plot indicated possible overfitting using this model, confirming the need to perform cross-validation.

These results were corroborated by comparing BPA measured in urine samples with the value estimated by the different methodological approaches (Table 2). For adolescents and adults, the random forest was the methodology that better predict the daily exposure to BPA, presenting the closest values to the original BPA measured in urine and the

[3] (A) root mean squared error for regression tree; (B) mean absolute error for regression tree; (C) Spearman correlation coefficient for regression tree; (D) root mean squared error for random forest; (E) mean absolute error for random forest; (F) Spearman correlation coefficient for random forest

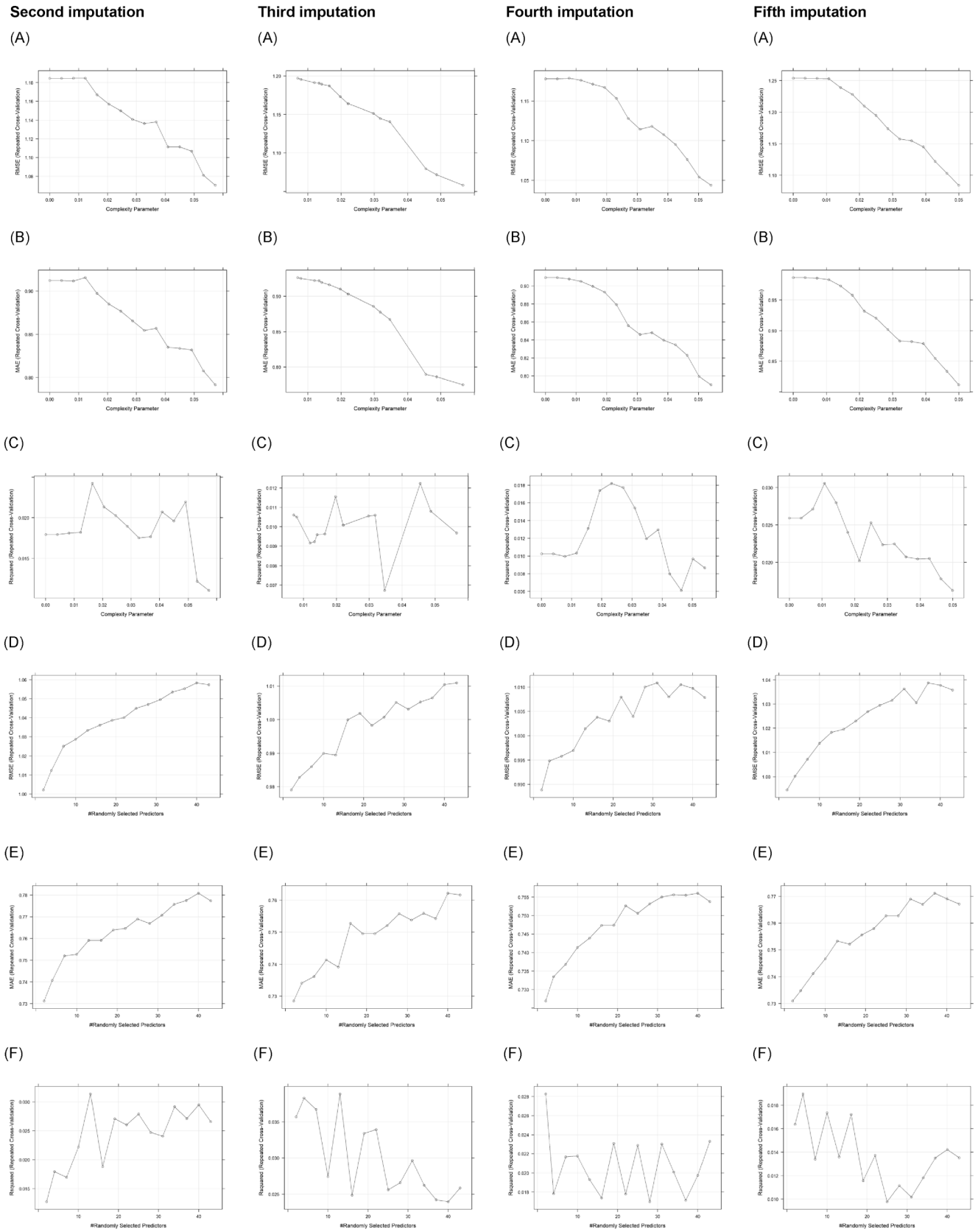


Fig. A4. Plots of fit-index parameters from the second to the fifth imputation, for adolescents' sample (n = 216), after cross-validation (5 folds, repeated 2 times), using different methodological approaches.[3]

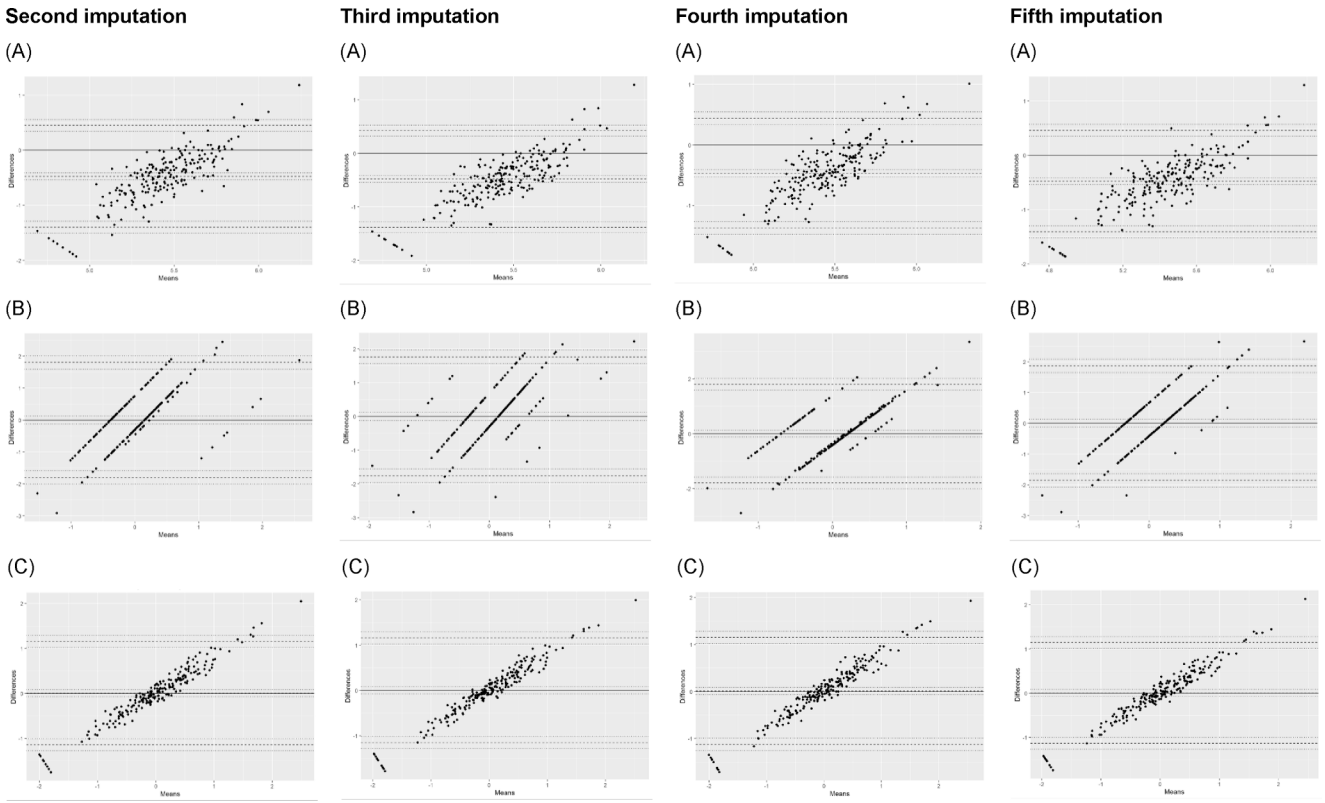


Fig. A5. Bland-Altman plots from the second to the fifth imputation, for adolescents' sample (n = 216).[2]

same tendency when the sample was stratified by sex, educational level and BMI categories. Moreover, food groups attribution was the methodology with the worst BPA daily exposure estimation, with the median values of BPA being 5–6 times higher than those measured in urine for adolescents and 1.5–2 times for adults.

3.3. BPA daily exposure assessment

Table 2 also shows that, in adolescents, males compared to females presented a significantly higher BPA value calculated from urinary samples (female = 1156 ng/day, male = 1601 ng/day, *p-value* = 0.003; random forest: female = 1311 ng/day, male = 1471 ng/day, *p-value* = less than0.001). Pre-obese and obese adolescents presented a significantly higher median exposure to BPA in urine samples, not detected in the remaining methodological approaches (female = 1316 ng/day, male = 1785 ng/day, *p-value* = 0.007).

In adults, according to food groups attribution, males consumed a significantly higher amount of BPA compared to females (female = 3043 ng/day, male = 4739 ng/day, p-value = less than0.001), not validated by urinary BPA.

Table 3 shows the exposure assessment of BPA, in ng/kg bw/day, estimated for the remaining adolescent sample and the adult population, according to "food groups attribution", regression tree and random forest. As expected by results from Table 2, compared to random forest estimates, food groups attribution overestimated the daily exposure to BPA in adolescents at 13 years of age and in adults from the Portuguese population. Using the "food groups attribution" methodological approach, the median daily dietary exposure to BPA in adolescents was 79.1 ng/kg bw/day, while 46.1 ng/kg bw/day in adults. The median value of BPA daily exposure in adolescents was 31.6 and 26.7 ng/kg bw/day for the regression tree and random forest, respectively. In the random forest approach, males were more exposed to BPA than females ($median_{female}$ = 26.2 ng/kg bw/day, $median_{male}$ = 27.3 ng/kg bw/day). In both methodologies, BPA daily exposure was higher in adolescents whose parents presented a higher educational level.

The median value of BPA daily exposure in adults was 41.7 and 38.0 ng/kg bw/day for the regression tree and random forest, respectively (Table 3). Males presented a lower exposure in the two approaches (regression tree: $median_{female}$ = 42.0 ng/kg bw/day, $median_{male}$ = 41.4 ng/kg bw/day; random forest: $median_{female}$ = 39.3 ng/kg bw/day, $median_{male}$ = 37.0 ng/kg bw/day), because of their higher body weight compared to females. Regarding age and educational level, the results differ according to the methodology. For random forest, the median of exposure to BPA decreased with age and increased in the most educated participants.

3.4. BPA risk characterization

Regarding risk characterization, in all estimates for all groups, independently of the methodological approach, 99.9% of the participants were above the TDI of 0.2 ng/kg bw, which may indicate a public health concern for these adolescents and the adults of the Portuguese population.

3.5. BPA exposure associated factors

Finally, Table 4 describes the associations between daily exposure to BPA and socio-demographic characteristics among adolescents and adults, respectively. With the random forest methodology, in adolescents, a statistically significant association between BPA exposure and sex was found. After adjustment for sex and parents' educational level (model 1), adolescent males were 0.34 ng/kg bw/day more exposed to BPA compared to females (MD = 1.34 [95%CI: 0.62, 2.06]). For adults, according to the random forest, in the adjusted model, it was observed an association between BPA exposure and sex and educational level. Males were 6% less exposed to BPA than females (RD = 0.94 [95%CI: 0.93, 0.96]). Moreover, BPA exposure was associated with higher educational levels (model 1), where individuals with 3rd cycle and high

school were 6% more exposed. Those with higher education were 10% more exposed to BPA, compared with those with lower education (RD = 1.06 [95%CI: 1.04, 1.08]; RD = 1.10 [95%CI: 1.08, 1.13], respectively).

4. Discussion

Usually, the research on human exposure to BPA focuses on food consumption estimates (with or without the combination of environmental sources) or urinary estimates rather than combining the information from direct and indirect methods. Moreover, most of the studies use urinary spot samples to assess the exposure to BPA, despite the sizeable intra-individual variability and the inconstant concentration of BPA in urine throughout the day, given the short half-life of this contaminant (Koch et al., 2014; LaKind & Naiman, 2008; Pollack et al., 2016). To the best of our knowledge, this was the first study comparing the estimates of BPA exposure using data from food consumption with 24-hour urinary excretion. The 24-hour urinary excretion of BPA is generally accepted as reflecting daily dietary exposure (15,30). Interestingly, in the regression models, only variables related to food consumption were selected.

The comparison with other studies is difficult considering the differences between the sample size, the sample representativeness, the protocol design, and the exposure assessment methodology. Regarding "food groups attribution", the median daily dietary exposure to BPA in the adolescent sample was 79.1 ng/kg bw/day, higher than the daily BPA exposure (expressed in a range for the median) estimated in 2014 and 2017 for the French adolescent population, from the Second National Individual Dietary Consumption Survey (Bemrah et al., 2014; Gorecki et al., 2017), which were, respectively, 42–46 and 39–41 ng/kg bw/day. The estimates from the present study are also 2–3 times above the daily dietary BPA exposure assessed in a non-representative sample of Korean adolescents (Park et al., 2016) and in a representative sample of Taiwanese adolescents (Chang et al., 2019). Nevertheless, in EFSA Opinion from 2015 (European Food Safety Authority, 2015), the daily average dietary exposure estimated for European adolescents was 159 ng/kg bw/day, almost two times above the BPA dietary exposure calculated in this paper. Compared to the Chinese Total Diet Study (Yao et al., 2020), the presented exposure was several times lower than the mean dietary BPA exposure calculated for this population (269.8 ng/kg bw/day and 321.1 ng/kg bw/day for females and males, respectively). However, the culture and the food patterns of Korea, Taiwan and China could be highly different from those observed in Western countries like Portugal, as previously discussed (Gerofke et al., 2023; Sakhi et al., 2014). Thus, these differences can be expected.

According to the food groups attribution methodology, the median daily dietary exposure to BPA for adults in the Portuguese population was 46.1 ng/kg bw/day. This result was in line with previous papers, namely compared to French Second National Individual Dietary Consumption Survey published in 2014 (range for the median: 29–31 ng/kg bw/day) and in 2017 (range for the median: 33–35 ng/kg bw/day) (Bemrah et al., 2014; Gorecki et al., 2017), and with Korean, Nigerian and Taiwanese studies (Adeyi & Babalola, 2019; Chang et al., 2019; Park et al., 2016), despite the differences observed in food habits between Portugal and the last countries. The Portuguese estimation was slightly above the estimate from a non-representative Belgian adult sample (mean = 15 ng/kg bw/day) (Geens et al., 2010). Nevertheless, as observed for the adolescent sample, the Chinese Total Diet Study (Yao et al., 2020) and EFSA Scientific Opinion (European Food Safety Authority, 2015) presented a dietary BPA exposure several times above the present results. The mean dietary exposure estimated for Chinese adults was 201.0 ng/kg bw/day (Yao et al., 2020). In Europe, the daily dietary BPA exposure of females, aged between 18 and 45 years old, was 132 ng/kg bw/day, while for males the exposure was 126 ng/kg bw/day (European Food Safety Authority, 2015).

Concerning the estimates calculated from urinary BPA applying random forest methodology, the results for the adolescent sample (26.7 ng/kg bw/day) and the adult Portuguese population (38.0 ng/kg bw/day) were in accordance with previous research. Most of the studies have made a direct backward calculation with urinary BPA concentration, not considering dietary variables and not adding uncertainty to the method, as it was done in this study using random forest and cross-validation, which limits a linear comparison of the results.

In 2017 a study of BPA estimates from global urinary concentration data was published, using information from 30 countries, including Portugal (Huang et al., 2017). The worldwide estimated BPA daily intake was 30.8 ng/kg bw/day in adults and 60.1 ng/kg bw/day in children. That publication used data from 20 adult participants from Portugal, estimating a BPA daily intake of approximately 36.0 ng/kg bw/day, very close to the one presented here. In a non-representative sample of children from Turkey, the geometric mean of BPA daily exposure was 35.0 ng/kg bw/day (Çok et al., 2020). In a non-representative sample of the general population from Korea, the BPA daily exposure was estimated as 23.0 ng/kg bw/day (Park et al., 2016). Finally, in a representative study from the United States, the median estimate of BPA daily intake using 2003–2004 NHANES urinary data was slightly higher than the estimates in this study: 50.5 ng/kg bw/day for the general population, 77.3 ng/kg bw/day for adolescents, 56.3 and 41.5 ng/kg bw/day for adults aged between 20 and 39 and 40 – 59 years, respectively (LaKind & Naiman, 2008).

Therefore, in our study, the random forest was the best methodological approach to estimate daily exposure to BPA. Recently, researchers also demonstrated that random forest performed better than linear regression in predicting serum vitamin D levels using intake data from food frequency questionnaires and lifestyle habits of Southern Europeans (Valer-Martinez et al., 2023). On the other hand, the validity of the food groups attribution is questionable. While the estimates align with some previous studies, this method may overestimate daily dietary exposure compared to urinary BPA levels. Additionally, all estimates of the three methodological approaches were above the TDI of 0.2 ng/kg body weight, which may indicate a public health concern for these adolescents and the adults of the Portuguese population.

The toxicological concern of BPA relies on its affinity to estrogenic receptors and, therefore, can interfere with the endocrine system's normal function, mimicking the effects of estrogenic hormones and modifying different signalling pathways (Gould et al., 1998; Maffini et al., 2006). In addition, the plausible mode of action of BPA, studied essentially in animal and *in vitro* models, indicated that this contaminant assigned in the development of metabolic syndrome, obesity, diabetes mellitus, cardiotoxicity, hyperuricemia, immunological disabilities, reproductive complications and neurodevelopmental/ neuroendocrine effects (Ballegaard & Bøgh, 2023; Bao et al., 2020; Fu et al., 2022; Maffini et al., 2006; Rochester, 2013; Shi et al., 2022; Yoo et al., 2020). Nevertheless, there are some uncertainties regarding the effects of BPA in humans that cannot be discarded due to the lack of epidemiological studies, especially with longitudinal designs (European Food Safety Authority, 2015; Vandenberg et al., 2007).

Another concern is the use of BPA analogues in their place due to the food legislation for this food contaminant (Meslin et al., 2022), which seems to have a similar mode of action to BPA once inside the organism (Liu et al., 2018; Rochester & Bolden, 2015).

The following steps should answer the uncertainties raised in the most recent authority's scientific opinions, longitudinally assessing the exposure of BPA, and their analogues, from childhood to adult life, and their association with health outcomes. This knowledge will help public health professionals and policymakers develop new and relevant health policies.

Some limitations of the present work must be considered in this discussion. First, the relatively small sample size of 24-h urine collection, especially in adults. However, this limitation was overcome by the type of biological samples collected, i.e. 24-h instead of a single spot (Gerofke et al., 2023; LaKind & Naiman, 2008; Meslin et al., 2022). Second, the possible bias associated with the collection of dietary

information, namely the participation bias in adolescents of 13 years or the possibility of misreporting in adults (Magalhães et al., 2020). Third, in adolescents, the food diary did not always correspond to the urine sample collected. Nevertheless, several non-consecutive days of dietary information may ameliorate this limitation. Fourth, the uncertainty in the data collected by adolescents regarding food packaging material may contribute to the difference between the results estimated by direct and indirect methods. Lastly, the interruption of the 13-year follow-up due to the COVID-19 pandemic affected the participation rate observed during this wave.

This study's main strength was applying direct and indirect methods to estimate the exposure to BPA, developing three different methodological approaches and identifying the most appropriate. This study proved that by applying the random forest, even with a limited number of urine samples, it was possible to estimate BPA daily exposure for the remaining sample/population. This methodological approach can now be used to estimate the daily exposure to other food contaminants, possibly testing the combined effect of different xenobiotic compounds. Another relevant strength was the availability of data on food consumption and BPA measured in urine samples from a cross-sectional sample of a prospective population-based birth cohort and a representative sample of the adult Portuguese population. The detail of the collected information was achieved by following standardized European methodology (European Food Safety Authority, 2014), especially related to the packaging material. Lastly, the daily dietary exposure to BPA was weighted for the complex survey design assuring national representativeness for adults.

The present study highlights the need to reduce dietary exposure to BPA, since chronic exposure safe levels are exceeded and adverse health effects are probable. For that, consumers should reduce the consumption of canned food and beverages and/or choose less contaminated food items, for example, fresh and unpackaged products.

5. Conclusion

The estimated daily exposure to BPA strongly changed depending on the methodological approach. According to the concentration of BPA measured in urine samples, the food groups attribution may overestimate the daily exposure to this food contaminant while the random forest was the most suitable methodology to estimate the daily exposure to BPA. Nevertheless, whatever the method used, adolescents at 13 years old and the adult Portuguese population presented an unsafe level of daily exposure to BPA, largely exceeding the safe levels recently proposed by EFSA.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Appendices.

See Figs. A1-A5.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113251.

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