Environmental Research 285 (2025) 122643

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Associations between chronic exposure to bisphenols and parabens and gut microbiota in children☆

Lourdes Rodrigo [a,b,c], Carlo Bressa [d,*], Mar Larrosa [e,**], Viviana Ramírez [b,c,f], Ángel Gil-Izquierdo [g], Cristóbal Sánchez-Muñoz [h], María Alba Martínez-Burgos [b,i], Alberto Zafra-Gómez [b,c,j,1], Ana Rivas [b,c,f,1]

[a] Department of Legal Medicine, Toxicology and Physical Anthropology, Faculty of Medicine, University of Granada, 18012, Granada, Spain
[b] Institute of Nutrition and Food Technology "Jose Mataix Verdú", Biomedical Research Center, Health Sciences Technological Park, University of Granada, 18016, Granada, Spain
[c] Instituto de Investigación Biosanitaria Ibs.GRANADA, 18012, Granada, Spain
[d] Facultad de Ciencias Experimentales, Universidad Francisco de Vitoria, Ctra. Pozuelo-Majadahonda km 1,800, 28223, Pozuelo de Alarcón, Madrid, Spain
[e] Department of Food Science and Nutrition. Faculty of Pharmacy. Universidad Complutense de Madrid, 28040, Spain
[f] Department of Nutrition and Food Science, Faculty of Pharmacy, University of Granada, 18071, Granada, Spain
[g] Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC, University Campus of Espinardo, 30100, Murcia, Spain
[h] Department of Physical Education and Sport, Faculty of Sport Sciences, University of Granada, 18011 Granada, Spain
[i] Department of Physiology, Faculty of Pharmacy, University of Granada, 18071, Granada, Spain
[j] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, 18071, Granada, Spain

A B S T R A C T

Bisphenols and parabens are endocrine-disrupting chemicals widely used in food packaging and personal care products. Early-life exposure to these compounds has been associated with adverse health effects, but their potential role in modulating the gut microbiota during childhood remains poorly understood. The objective of this study was to investigate the association between chronic exposure to bisphenols and parabens and gut microbiota diversity, composition, and function in children. A cross-sectional study in 97 Spanish children aged 4–12 year was conducted. Bisphenols and parabens in hair were quantified using ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS). Gut microbiota composition was assessed via 16S rRNA gene sequencing, and functional potential was inferred using PICRUSt2. Associations were explored using linear regression and random forest models, adjusting for age and sex. Total bisphenols and parabens were detected in 100 % of the children, with median concentrations of 311.33 ng/g and 1904.11 ng/g, respectively. No significant differences in overall gut microbiota diversity were observed between children with low and high exposure levels to bisphenols and parabens. However, regression models revealed associations between specific microbial genera and individual compounds. Additionally, bisphenol S was negatively associated with a predicted microbial pathway involved in methionine metabolism. Notably, *Lachnospiraceae_UCG-001* emerged as a predictive genus for propylparaben exposure. Although gut microbiota composition was similar across exposure levels, specific taxa and functional pathways were linked to chronic bisphenol and paraben exposure. These findings support the need for further research on the health implications of early-life exposure to these endocrine-disrupting chemicals.

1. Introduction

In recent decades, increasing industrialization has profoundly transformed the way we produce, consume, and store food. Alongside these changes, a wide array of chemical compounds has been introduced into the manufacturing of food packaging and utensils, primarily to enhance product durability and food safety. However, this technological progress has also led to the widespread use of synthetic chemicals, some of which pose significant risks to human health. Among these compounds are bisphenols and parabens. One of the most extensively studied compounds is bisphenol A, an organic compound widely used as a monomer in the production of polycarbonate plastics and epoxy resins.

☆ All authors declare that they have no conflicts of interest.

* Corresponding author.
** Corresponding author.
E-mail addresses: lourdesr@ugr.es (L. Rodrigo), carlo.bressa@ufv.es (C. Bressa), mlarrosa@ucm.es (M. Larrosa), vivianarl@ugr.es (V. Ramírez), angelgil@cebas.csic.es (Á. Gil-Izquierdo), csm@ugr.es (C. Sánchez-Muñoz), malbam@ugr.es (M.A. Martínez-Burgos), azafra@ugr.es (A. Zafra-Gómez), amrivas@ugr.es (A. Rivas).

[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.envres.2025.122643
Received 7 May 2025; Received in revised form 13 August 2025; Accepted 17 August 2025
Available online 21 August 2025

Bisphenol A is commonly found in water bottles, food containers, toys, and the internal linings of food and beverage cans. Its versatility and durability have made it an essential component in the manufacturing of numerous consumer products. However, due to its endocrine-disrupting properties, the use of bisphenol A has been banned or restricted in many countries. Despite increasing regulatory efforts to limit its use, such as the European Union's ban on bisphenol A in baby bottles since 2011 (European Commission, 2011a) and stricter limits in food contact materials in 2018 (European Commision, 2018) and 2024 (European Commission, 2024), bisphenol A is still used in certain food packaging materials and may migrate into food contents (Khalili Sadrabad et al., 2023; Litrenta et al., 2025), thereby exposing consumers to varying levels of this substance. In an updated safety assessment, the European Food Safety Authority (EFSA) drastically reduced the tolerable daily intake (TDI) of bisphenol A by a factor of 20,000, from 4 μg of bisphenol A per kilogram of body weight to 0.2 ng of bisphenol A per kilogram of body weight (Ramírez et al., 2023). However, recent biomonitoring evidence suggests that human exposure to bisphenol A remains widespread and often exceeds recently established safety thresholds (Acevedo et al., 2025). As an alternative to using bisphenol A, the industry has begun to utilize compounds derived from it, such as bisphenol AF, bisphenol E, bisphenol F and bisphenol S in products labeled as "bisphenol A-free." Although these substitutes share a similar structure with bisphenol A, slight differences in their molecular composition may influence their chemical behavior and, potentially, their biological effects. Indeed, some studies suggest that bisphenol A derivatives such as bisphenol F and bisphenol S may have stronger obesogenic effect than bisphenol A itself (Alharbi et al., 2022). Additionally, recent research has detected the presence of these bisphenol analogs in daily consumed products by the Spanish population, highlighting the widespread exposure to these compounds (Gálvez-Ontiveros et al., 2021).

Numerous epidemiological and experimental studies have explored the potential adverse effects of bisphenol A and its derivatives on human health, observing that these compounds can function as endocrine disruptors and have also been linked to metabolic and neurological disorders (Abouhamzeh et al., 2023; Alharbi et al., 2022; Molina-López et al., 2023). During critical stages of development, such as pregnancy and childhood, exposure to these compounds can have significant consequences. It has been demonstrated that bisphenol A crosses the placental barrier (Jiménez-Díaz et al., 2010) and is detected in breast milk (Rodríguez-Gómez et al., 2014), thereby exposing fetuses and infants during critical developmental windows when they are particularly vulnerable to hormonal disruption. In the reproductive realm, studies have suggested that prenatal exposure to bisphenol A may be associated with changes in reproductive function in offspring, including alterations in spermatogenesis and oocyte maturation (Molina-López et al., 2023). Furthermore, a possible correlation between bisphenol A and derivatives exposure and metabolic disorders such as obesity, insulin resistance, and type 2 diabetes has been found (Alharbi et al., 2022).

The precise mechanisms through which bisphenol A and its derivatives exert their biological effects are currently under investigation. These compounds appear to act as endocrine disruptors, modulating hormonal activity by binding to steroid hormone receptors, thereby affecting gene expression of adipogenesis-related genes inducing obesity (Akash et al., 2020). The evidence also suggests that exposure to bisphenol A can lead to modifications in DNA methylation and other epigenetic marks on key genes involved in metabolic regulation. These alterations may contribute to glucose and lipid dysregulation, ultimately increasing the risk of developing metabolic syndrome (Akash et al., 2020). In addition, studies in animals models have shown that exposure to bisphenol A or its analogs, bisphenol F and bisphenol S, may influence the composition and diversity of the gut microbiota, often leading to dysbiosis characterized by reductions in beneficial genera such as *Lactobacillus* and *Bifidobacterium*, and increases in potentially pathogenic taxa (Wang et al., 2021; Zheng et al., 2023). Bisphenol A-induced dysbiosis, has also been associated with impairment in cognitive and behavioral functions (Ni et al., 2021) and to increased oxidative stress and hepatotoxicity in murine models (Liu et al., 2022). While evidence from animal models is growing, studies in human populations are still emerging and are mostly based on *in vitro* and *ex vivo* approaches. In a simplified human gut microbiota model composed of eight bacterial species, exposure to a mixture of bisphenol S and bisphenol F led to increased abundances of *Anaerostipes caccae* and *Escherichia coli*, along with alterations in microbial metabolic pathways (Haange et al., 2024). In another study using fecal samples from lean and obese children, exposure to bisphenol A was associated with increased levels of *Bifidobacterium*, *Adlercreutzia*, and *Clostridium sensu stricto* (Luque et al., 2025).

On the other hand, parabens are esters of p-hydroxybenzoic acid that, due to their preservative and antimicrobial properties, have been widely used in personal care products, cosmetics, and also as additives in packaging and food (Wei et al., 2021). The most common parabens include methylparaben, ethylparaben and propylparaben. These compounds are classified as preservative additives used to prevent microbial growth and extend shelf life. Parabens can migrate from coated packaging into food, with this being the main source of food exposure, particularly to propylparaben, ethylparaben, and methylparaben (Wei et al., 2021). The EFSA has set an Acceptable Daily Intake (ADI) of 0–10 mg/kg body weight for methylparaben and ethylparaben and their sodium salts. However, this value does not apply to propylparaben that is no longer authorized as a food additive in the European Union under Regulation (EU) No 1129/2011(European Commission, 2011b).

Epidemiological and experimental studies have investigated potential connections between paraben exposure and endocrine disruption, focusing on hormonal metabolism, obesity, and other diseases, particularly during critical developmental stages like pregnancy and childhood. However, the current findings remain inconclusive. In prepubertal and pubertal children, parabens, in conjunction with bisphenols and phthalates, have shown a negative correlation with estrogen and testosterone levels (Hu et al., 2022). In a study conducted on a general population of children in the United States, urinary concentrations of parabens were inversely correlated with adiposity parameters, with more pronounced impact observed in girls (Quirós-Alcalá et al., 2018). Nevertheless, a study conducted by Kim and Chevrier (2020), did not identify any significant association between urinary concentrations of methylparaben, ethylparaben, propylparaben, or butylparaben and indicators of obesity or metabolic syndrome in children of either sex. In contrasts, among adult men, ethylparaben exposure was positively associated with a higher prevalence of metabolic syndrome (Kim and Chevrier, 2020).

Similar to bisphenols, parabens have also been suggested to potentially influence metabolism by inducting alterations in the gut microbiota. In adolescent rats, exposure to methylparaben led to a reduction in body weight, accompanied by modifications in the gut microbiota composition, specifically an increase in the abundance of the genus *Prevotella*, and a decrease in *Lactobacillus* genus. Notably, these changes were reversed in adulthood, suggesting that childhood and adolescence may represent critical windows of heightened susceptibility to microbiota-related effects of these compounds (Hu et al., 2016).

To date, only one study has examined the association between exposure to endocrine-disrupting chemicals and gut microbiota composition in children. That study evaluated the effects of perinatal exposure to phenols and poly and perfluoroalkyl substances (Davias et al., 2024a), as well as the exposure of phthalate metabolites and 2 di (isononyl) cyclohexane-1,2-dicarboxylate (DINCH) metabolites (Davias et al., 2024b) on the gut microbiota of one-year-old children. Chronic exposure in that study was assessed by collecting three urine samples per day over seven days during four separate time periods. However, the time interval between urine sampling and gut microbiome assessment may have limited the detection of relevant associations. While these findings represent an important first step, much remains to be explored regarding the potential impact of chronic exposure to endocrine-disrupting chemicals during childhood on gut microbiota

composition and function. In this context, chronic exposure refers to cumulative internal exposure over several weeks to months, as reflected by the incorporation of these compounds into hair. Addressing this gap, the objective of the present study was to investigate the association between chronic exposure to bisphenols, parabens, and their derivatives, measured in hair samples, and the composition and functional potential of the gut microbiota in a cross-sectional study in children.

2. Materials and methods

2.1. Study population and data collection

Participants enrolled in the present study were recruited from several health and educational centers in Granada, Spain, between 2020 and 2023. The study population was required to meet the following inclusion criteria: (1) children aged 3–12 years, and (2) continuous residence in the study area for at least six months. The study procedures were explained in detail to parents or legal guardians of all participants prior to obtaining written informed consent. The study protocol was approved by the Biomedical Research Ethics Committees of the Province of Granada (references: 0922-N-19; 1939-M1–22; 1742-N-23).

A total of 77 children with measurable levels of bisphenols and parabens in their hair samples were included in the present study. This analysis was part of a broader observational study, and sample size was not determined *a priori* for this specific objective. In accordance with the STROBE guidelines, a post hoc power analysis was conducted to evaluate whether the sample size was adequate to detect meaningful associations. For the analysis of potential differences between high and low exposure levels, the sample was divided into quartiles. The results showed that the comparison between Q1 and Q4 ($n = 49$) provided approximately 70 % power to detect a moderate effect ($f^2 \approx 0.08$) in the association between exposure levels and the relative abundance of *Prevotellaceae NK3B31_group*, at a significance level of 0.05. Face-to-face interviews were conducted with the parents or legal guardians of all participants by trained staff. Data were collected on sociodemographic aspects (child's age and sex, and parental or legal guardians educational level), lifestyle factors, tobacco exposure, dietary habits, and anthropometric measurements (weight, height, and body mass index (BMI)).

2.2. Assessment of chemical exposures

For this study, four bisphenols (bisphenol A, bisphenol F, bisphenol AF and bisphenol S) and five parabens (methylparaben, ethylparaben, propylparaben, isopropylparaben and butylparaben) were measured in hair samples. The analytical method for the extraction and determination of these compounds has been previously published by our research group (Rodríguez-Gómez et al., 2017). All materials employed during sample preparation, such as tubes, vials, inserts, and pipette tips, were bisphenol-free. Hair samples (3–5 cm) were obtained for each participant on the day of recruitment by cutting from the posterior vertex region, as close to the scalp as possible, to ensure a more accurate and reliable estimation of internal contaminants exposure. All the samples were stored in aluminum foil at room temperature until processing and analyzed (Rodríguez-Gómez et al., 2017). Briefly, to eliminate contaminants and surface residues, each specimen was sequentially washed twice with Milli-Q water (ultrasonicated for 5 min), followed by a 5-min wash with SDS, and finally rinsed with Milli-Q water for another 5 min. After that, samples were completely dried and pulverized using a ball mill. Subsequently, 0.05 g of each hair sample were weighed and digested with 0.5 mL of acetic acid/MeOH mixture (20:80, v/v) at 38 °C for 12 h. After cooling at room temperature, analytes extraction was performed by adding 1 mL of acetonitrile stirring for 15 min at room temperature, followed by centrifugation at 16,300×*g* for 5 min. The organic phase was transferred to a clean tube and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 250 μL of the initial mobile phase, centrifugated at 16,300×*g* and analyzed by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Quality controls and the MS/MS parameters were applied as previously described by (Rodríguez-Gómez et al., 2017).

A chromatographic system Acquity UPLC-™ H-Class (Waters, Manchester, UK), equipped with a binary solvent manager, was used for chromatographic separation. Detection was performed using a Xevo TQS triple quadrupole mass spectrometer (Waters) with an orthogonal Z-spray™ electrospray ionization (ESI) source. An Acquity UPLC® BEH C18 chromatographic column (50 mm × 2.1 mm i.d., particle size 1.7 μm) maintained at 40 °C was employed. The mobile phase consisted of water containing ammonia 0.1 % (v/v) (solvent A) and methanol (solvent B). Elution of the analytes was achieved by using the following gradient: 2.0 min at 30 % B; increased to 90 % B over 3 min; ramped to 100 % in 0.1 min, held for 1.9 min; then returned to initial conditions in 0.1 min and re-equilibrated for 2.9 min, resulting in a total run time of 10 min). The flow rate was 0.25 mL min^{-1}, and the injection volume was 10 μL. The mass spectrometer operated in selected reaction monitoring mode (SRM), with unit mass resolution set on quadrupoles Q1 and Q3. ESI was performed in negative ion mode. The MS/MS parameters were those previously described by (Rodríguez-Gómez et al., 2017).

2.3. Gut microbiota assessment

2.3.1. Fecal bacterial DNA extraction, library preparation and sequencing

Gut microbiota was analyzed using the 16S rRNA gene. Bacterial DNA was extracted from 100 mg of feces using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) and the bead homogenizer (Bullet Blender Storm, Next Advance, Averill Park, NY) following the manufacturer's instructions. DNA concentration was quantified using a Qubit fluorometer (Thermofisher, Waltham, MAS, USA). Library preparation was performed following the 16S rRNA Illumina protocol (Cod 15044223 RevB). Amplicon primers targeted the V3 and V4 hypervariable regions of the 16s rRNA gene, as described by (Klindworth et al., 2013). The resulting amplicons (459 bp) were sequenced on the Illumina HiSeq platform (Illumina, San Diego, CA).

2.3.2. Sequence processing and taxonomic assignment

Sequence analyses and data quality filtering were performed with QIIME2 v.2025.4 (Bolyen et al., 2019). Amplicon Sequence Variant (ASV) were inferred using the DADA2 plugin (Callahan et al., 2016). Taxonomic classification was obtained using classify-sklearn, a Scikit-Learn method (Pedregosa et al., 2011). The trained classifier was built using the SILVA database (version 138.1), restricted to the V3–V4 region, and the corresponding weighted data were obtained from the readytowear repository (https://github.com/BenKaehler/readytowear) (Kaehler et al., 2019). Sequences that did not match any reference in the database were excluded from the analysis.

2.3.3. Gut microbiota statistical analysis

For diversity analyses, rarefaction was applied to normalize sequencing depth across samples, and data were rarefied to a depth of 11,903 reads per sample. ASVs belonging to the same genus were merged prior to analyses. To asses differences in gut microbiota diversity and composition, participants were stratified into quartiles based on total paraben or total bisphenol content in hair samples. Alpha-diversity was evaluated using three indices: observed features, Shannon index, Pielou's evenness, and Faith's phylogenetic diversity. Statistical comparisons were performed using the Kruskal-Wallis test, with p-values adjusted using the Benjamini-Hochberg false discovery rate (BH-FDR) method (Benjamini and Hochberg, 1995). β-diversity was assessed using the Bray-Curtis dissimilarity index, Jaccard similarity index, and both unweighted and weighted Unifrac distances. Statistical significance was evaluated using the Permutation Based Analysis of Variance (PERMANOVA) with BH-FDR adjusted p-values. Differential abundance analysis was performed using Analysis of Composition of Microbiomes with Bias Correction (ANCOM-BC) (Lin and Peddada, 2020), applied without

prior data normalization. The statistical model included age and sex as covariates to control for potential confounding effects comparisons were made across quartiles of paraben and bisphenol exposure. Additionally, multivariable association analysis was conducted using Multivariable Association with Linear Models 2 (MaAsLin2) (Mallick et al., 2021) with default parameters to investigate associations between gut microbiota and individual bisphenol and paraben. Data were normalized using total sum scaling (TSS) and log-transformed. A linear model (LM) was applied, adjusting for age and sex as fixed effects along with the study variable of interest. Features with a minimum prevalence of 10 % were included, without applying a minimum abundance threshold. Multiple testing correction was performed using the BH-FDR method, with the significance threshold set at 0.25, as established in the default parameters of MaAsLin2. Functional pathway abundance was predicted with Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) (Douglas et al., 2018). Associations between functional pathways and bisphenols or parabens were evaluated using MaAsLin2 analyses applying the same parameters described above. To identify potential predictors of bisphenols and parabens exposure based on the gut microbiota composition, a supervised learning regressor analysis was performed. The dataset was divided into training and test sets in a ratio of 0.8:0.2, and the random forest algorithm was employed for estimation. All statistical analyses were conducted using QIIME2 v2025.4 (Bolyen et al., 2019) and R.4.1.5 software.

2.4. Statistical analysis

For all variables other than those related to microbiota composition, variables were assessed for normality using the Shapiro–Wilk test. When the assumption of normality was met, comparisons between two independent groups were conducted using the Student's t-test. In the absence of normal distribution, the non-parametric Wilcoxon rank-sum test was applied. Categorical variables were analyzed using the chi-square (χ^2) test to evaluate associations between groups. A two-tailed p-value of less than 0.05 was considered indicative of statistical significance. Analyses were performed using SPSS v29 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Participants' characteristics

The sociodemographic, anthropometric, and lifestyle characteristics of the 97 children included in the study (46 boys and 51 girls) are summarized in Table 1. The mean age of participants was 8.1 years (SD = 2.3), with a significant difference between sexes (p = 0.048). Median weight, height, and BMI were also comparable between boys and girls, with no statistically significant differences observed. Regarding parental education level, most participants (68 %) had at least one parent with a university degree, while 20 % had secondary education and 3.1 % only primary education. Exposure to tobacco smoke at home was reported in approximately 20 % of households, with a similar distribution between boys and girls. Additionally, 64.9 % of children reported engaging in physical activity outside of school, with no significant differences between sexes.

Table 1
General characteristics of the study population.

	Total (n = 97)	Boys (n = 46)	Girls (n = 51)	*p* value
Age (years), mean (SD)	8.1 (2.3)	7.6 (2.4)	8.5 (2.2)	0.048[a]
Weight (kg), median (IQR)	28.90 (22.10–41.80)	26.50 (19.60–41.25)	31.05 (23.50–43.22)	0.148[b]
Height (cm), mean (SD)	129.10 (118.62–139.37)	125.25 (117.25–137.88)	133.25 (120.38–140.75)	0.107[b]
BMI (kg/m^2), median (IQR)	17.09 (15.20–21.85)	16.41 (14.75–22.14)	18.04 (15.75–21.26)	0.259[b]
Parental education level, n (%)				
Up to primary	3 (3.1)	2 (4.3)	1 (2.0)	0.770[c]
Secondary	20 (20.6)	9 (19.6)	11 (21.6)	
University	66 (68.0)	33 (71.7)	33 (64.7)	
Missing data	8 (8.2)	2 (4.3)	6 (11.8)	
Smoking among family members, n (%)				
No	71 (73.2)	37 (80.4)	34 (66.7)	0.237[c]
Yes	19 (19.6)	7 (15.2)	12 (23.5)	
Missing data	7 (7.2)	2 (4.3)	5 (9.8)	
Physical activity out-of-school, n (%)				
No	27 (27.8)	14 (30.4)	13 (25.5)	0.713[c]
Yes	63 (64.9)	30 (65.2)	33 (64.7)	
Missing data	7 (7.2)	2 (4.3)	5 (9.8)	

SD: standard deviation; BMI: body mass index; IQR: interquartile range.
[a] Student's t-test.
[b] U Mann-Whitney test.
[c] Chi-square test.

3.2. Bisphenols and parabens levels in child hair

The concentrations of individual bisphenols and parabens, as well as total bisphenols and total parabens content, measured in hair samples from the full study population (n = 97), stratified by sex (74 boys and 80 girls) are shown in Table 2. For bisphenols, the most prevalent compound was bisphenol A, detected in 94.8 % of participants, with a median concentration of 242.50 ng/g. Bisphenol AF and bisphenol S were also frequently detected (44.3 % and 58.8 %, respectively), whereas bisphenol F was detected in only 24.7 % of samples. The median total bisphenol concentration was 311.33 ng/g across the population, with no significant differences between boys and girls. Regarding parabens, methylparaben was the most abundant, detected in 100 % of samples with a median concentration of 1285.94 ng/g. Ethylparaben and propylparaben were also frequently present (99.0 % and 76.3 %, respectively), while isopropylparaben and butylparaben were detected in approximately one-third of the participants. The median total paraben concentration in hair was 1904.11 ng/g, also without significant differences between sexes.

3.3. Differences in gut microbiota composition according to hair levels of bisphenols and parabens

In relation to bisphenol exposure, alpha-diversity analyses showed no statistically significant differences between quartiles for any of the alpha diversity metrics assessed (Fig. 1A–D), indicating similar microbial richness, evenness, and phylogenetic diversity regardless of exposure levels. Similarly, for paraben exposure, alpha-diversity metrics did not differ significantly between the lowest (Q1) and highest (Q4) quartiles of total paraben levels ($p > 0.05$ for all), suggesting no major variations in overall microbial diversity associated with paraben levels (Fig. 2A–D).

Beta-diversity analyses did not reveal any significant differences in microbial community structure between the lowest (Q1) and highest (Q4) quartiles of total bisphenol levels, (PERMANOVA, $p > 0.05$ for all comparisons). Principal Coordinates Analysis (PCoA) plots confirmed the absence of distinct clustering by bisphenol exposure level (Fig. 3). Similarly, beta-diversity metrics showed no significant differences in microbial community composition between Q1 and Q4 quartiles of total paraben levels (PERMANOVA, $p > 0.05$), and PCoA plots did not display

Table 2
Concentration of bisphenols and parabens in hair (ng/g).

	Total (n = 97)	Boys (n = 46)	Girls (n = 51)	*p* value[a]
BPA, median (IQR), detected (%)	242.50 (129.60–705.90), 94.8	315.30 (118.93–791.28), 91.3	229.80 (134.80–684.20), 98.0	0.963
BPF, median (IQR), detected (%)	<LOD (<LOD- < LOD), 24.7	<LOD (<LOD- < LOD), 23.9	<LOD (<LOD- < LOD), 25.5	0.908
BPS, median (IQR), detected (%)	22.60 (<LOD- 91.20), 58.8	4.65 (<LOD- 83.43), 52.2	27.50 (<LOD- 99.50), 64.7	0.298
BPAF, median (IQR), detected (%)	<LOD (<LOD-41.05), 44.3	<LOD (<LOD-29.73), 37.0	13.80 (<LOD-45.60), 51.0	0.081
Total BPs, median (IQR), detected (%)	311.33 (142.10–1006.75), 100	348.60 (124.33–927.40), 100	300.91 (171.34–1031.13), 100	0.817
MetPB, median (IQR), detected (%)	1285.94 (779.18–14150.49), 100	1669.48 (710.56–13730.13), 100	1207.26 (833.84–16401.11), 100	0.851
EthPB, median (IQR), detected (%)	102.80 (25.40–562.70), 99.0	221.40 (22.78–940.75), 97.8	81.00 (24.80–452.00), 100	0.611
PropPB, median (IQR), detected (%)	126.50 (4.35–672.00), 76.3	173.70 (23.43–776.78), 78.3	108.70 (<LOD-742.20), 74.5	0.267
iPropPB, median (IQR), detected (%)	<LOD (<LOD- < LOD), 18.6	<LOD (<LOD- < LOD), 21.7	<LOD (<LOD- < LOD), 15.7	0.409
ButPB, median (IQR), detected (%)	<LOD (<LOD- 47.35), 36.1	<LOD (<LOD-53.00), 39.1	<LOD (<LOD-32.70), 33.3	0.531
Total PBs, median (IQR), detected (%)	1904.11 (952.53–18370.97), 100	2087.51 (840.02–20847.12), 100	1781.54 (1013.68–16607.58), 100	0.977

IQR: interquartile range; LOD: limit of detection. [a]U Mann-Whitney test. BPA: bisphenol A; BPF: bisphenol F; BPS: bisphenol S; BPAF: bisphenol AF; BPs: bisphenols; MetPB: methylparaben; EthPB. ethylparaben; PropPB: propylparaben; iPropPB: isopropylparaben; ButPB: butylparaben; PBs: parabens.

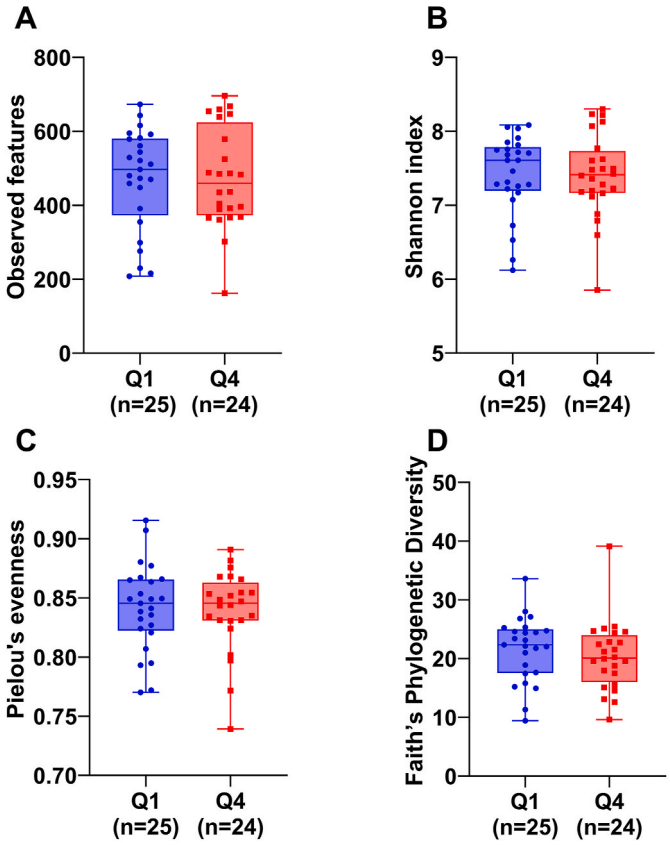

Fig. 1. Alpha diversity indices in participants with low (Q1) and high (Q4) total bisphenol levels in hair samples. Comparison of (A) Observed Features, (B) Shannon index, (C) Pielou's evenness, and (D) Faith's Phylogenetic Diversity (PD) between the first (Q1, n = 25) and fourth (Q4, n = 24) quartiles of bisphenol exposure.

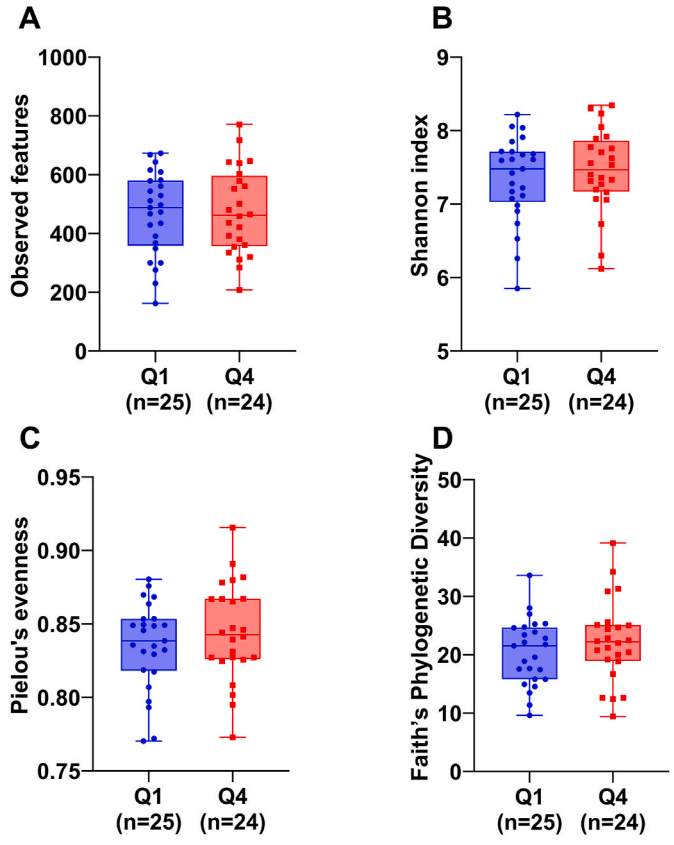

Fig. 2. Alpha diversity indices in participants with low (Q1) and high (Q4) total parabens levels in hair samples. Comparison of (A) Observed Features, (B) Shannon index, (C) Pielou's evenness, and (D) Faith's Phylogenetic Diversity (PD) between the first (Q1, n = 25) and fourth (Q4, n = 24) quartiles of parabens exposure.

any clear separation between exposure groups (Fig. 4).

Differential abundance analysis using ANCOM-BC did not identify any taxa with statistically significant differences between the lowest (Q1) and highest (Q4) quartiles of total bisphenol or paraben levels. These findings suggest that, within the range of exposures observed, neither bisphenol nor paraben content in hair samples was associated with notable shifts in the relative abundance of specific microbial taxa.

3.4. Associations between bisphenol and paraben hair levels and gut microbiota

Associations between bisphenol and paraben concentrations in hair and gut microbiota diversity and composition were assessed using multivariable models. No associations were found between hair levels of bisphenols and parabens and either alpha- or beta-diversity metrics of the gut microbiota. When associations at the genus-level taxonomic composition were examined across all bisphenols, only bisphenol S showed several associations ($q < 0.25$) with specific bacterial genera, although most did not reach statistical significance ($q < 0.05$) (Fig. 5). The genus *Izemoplasmatales* showed a positive and statistically significant association with bisphenol S (coefficient = 0.594, q = 0.047). *Streptococcus* also showed a notable negative association with BPS (coefficient = −0.475), with a q-value of 0.084, which may indicate a trend toward significance (Fig. 5). The remaining taxa, including *Anaerostipes*,

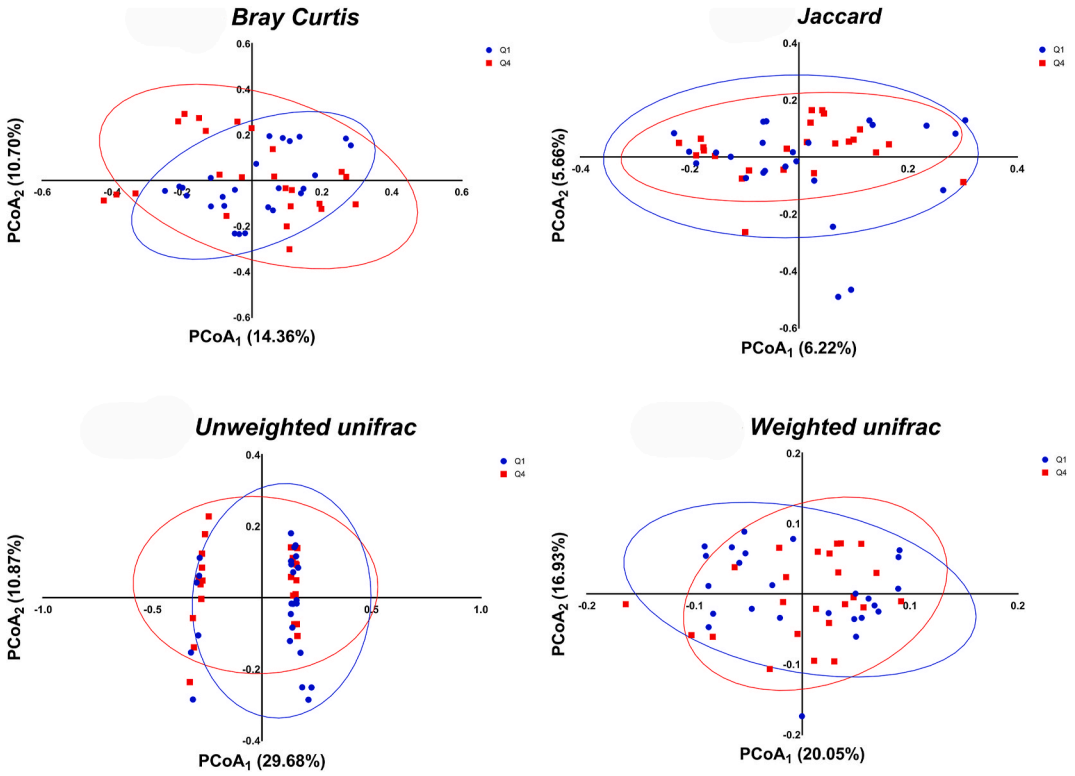

Fig. 3. Principal Coordinates Analysis (PCoA) plot based on Bray-Curtis, Jaccard index, Weighted Unifrac and Unweighted Unifrac distances representing the beta-diversity of the gut microbiota in individuals stratified by total bisphenol levels in hair. The plot shows samples from the lowest quartile (Q1, blue line, blue dots) and the highest quartile (Q4, red line, red squares).

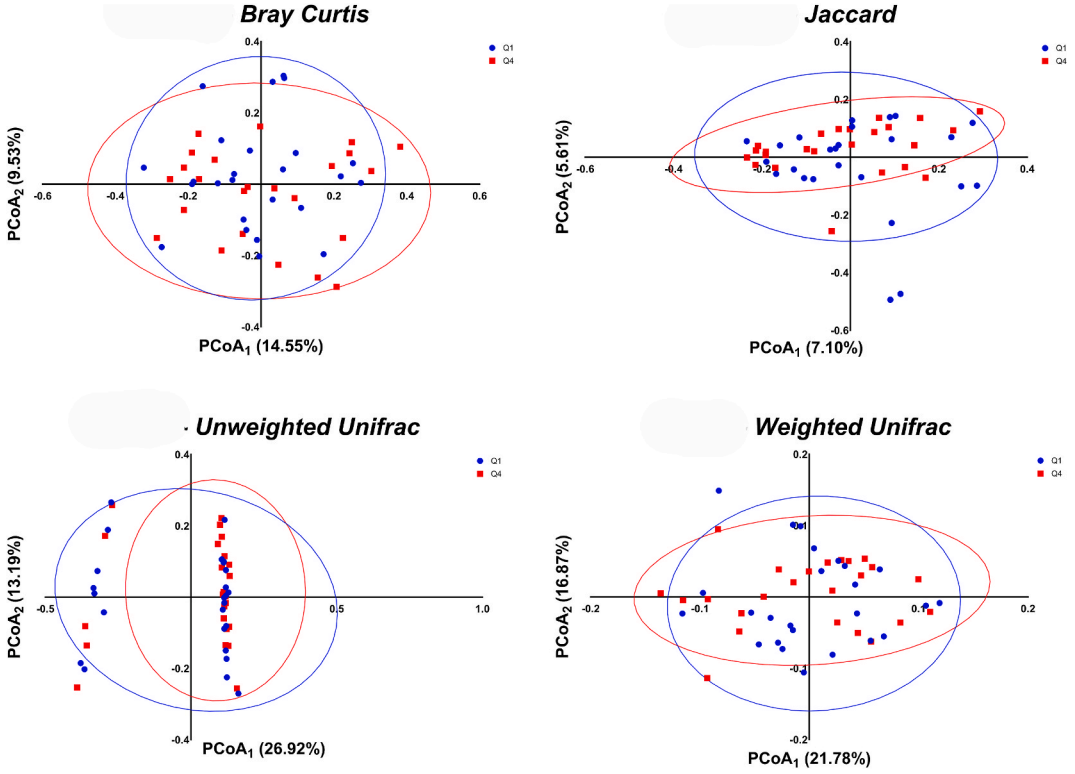

Fig. 4. Principal Coordinates Analysis (PCoA) plot based on Bray-Curtis, Jaccard index, Weighted Unifrac and Unweighted Unifrac distances representing the beta-diversity of the gut microbiota in individuals stratified by total paraben levels in hair. The plot shows samples from the lowest quartile (Q1, blue line, blue dots) and the highest quartile (Q4, red line, red squares).

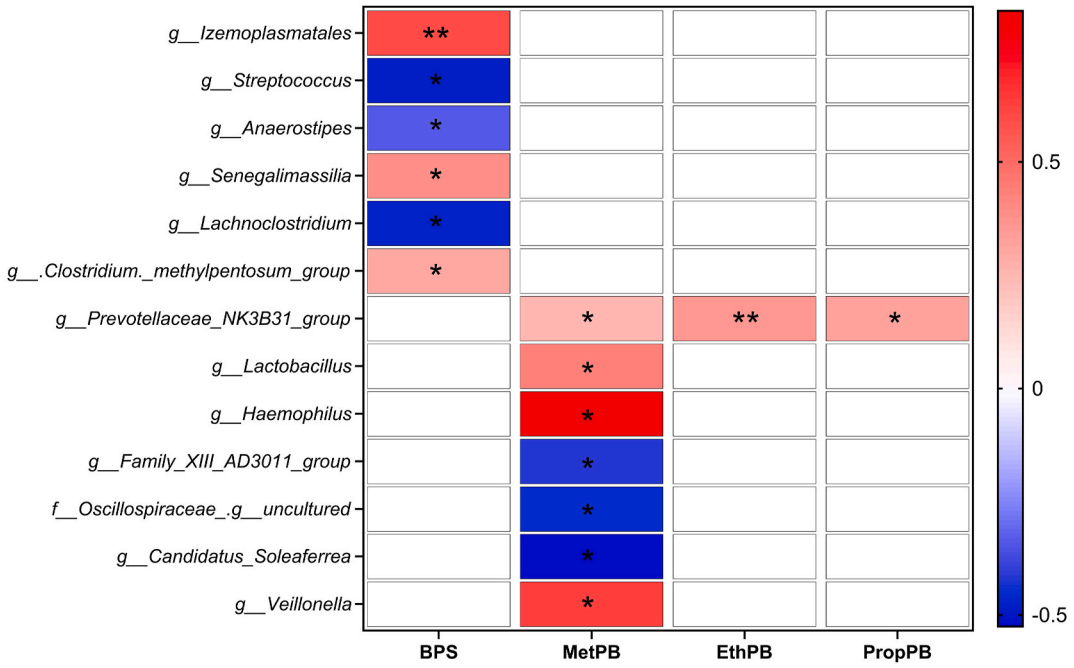

Fig. 5. Heatmap illustrating associations between microbiota genera and bisphenols and parabens, derived from MaAsLin analysis. The color intensity represents the strength and direction of associations (red for positive, blue for negative). **qval <0.05; *qval <0.25.

Senegalimassilia, *Lachnoclostridium*, and *Clostridium_methylpentosum_group*, did not reach statistical significance, showing q-values between 0.1 and 0.25.

Regarding parabens, *Prevotellaceae_NK3B31_group* was the only genus showing a statistically significant association, with a positive relationship observed with ethylparaben (coefficient = 0.365, q = 0.021) (Fig. 5). This genus also showed a positive, though non-significant, association with propylparaben (q = 0.094), and a weaker, non-significant association with methylparaben (q = 0.235) (Fig. 5). Regarding methylparaben, several bacterial genera exhibited positive or negative associations, although none reached statistical significance. *Lactobacillus* and *Haemophilus* showed positive associations (coefficients = 0.427 and 0.838, respectively), with q-values of 0.129 and 0.165. Similarly, *Veillonella* (coefficient = 0.626, q = 0.223) and *Oscillospiraceae_g_uncultured* (coefficient = −0.454, q = 0.235) did not reach significance (Fig. 5). Negative associations with methylparaben were also observed for *Family_XIII_AD3011_group* (coefficient = −0.423, q = 0.175) and *Candidatus Soleaferrea* (coefficient = −0.525, q = 0.205), though these associations were likewise not statistically significant (Fig. 5).

3.5. Differences and associations between bisphenol and paraben hair levels and gut microbiota functions

No significant differences in microbial functional profiles between quartiles of bisphenol or paraben content were found, suggesting that predicted microbial functions remained stable regardless of bisphenol or paraben exposure. However, multiple regression analysis revealed a significant negative association (q < 0.05) between the presence of bisphenol S and five microbial metabolic pathways (P4-PWY, PWY-5347, MET-SAM-PWY, HOMOSER-METSYN-PWY, and HSERMETANA-PWY), all of which are involved in methionine biosynthesis (Table 3). In the case of parabens, methylparaben was found to be significantly associated with the microbial functional pathway P163-PWY (Table 3). This suggests that higher presence of methylparaben may be linked to decreased activity in this pathway, which is responsible for the anaerobic breakdown of L-lysine into acetate and butyrate. No significant associations were identified for other bisphenols or parabens with any microbial metabolic pathways.

Table 3
Microbial metabolic pathways significantly associated with bisphenols and parabens exposure.

Pathway	Compound	Coefficient	FDR q-value
MET.SAM.PWY	BPS	−0.173	0.0142
PWY.5347	BPS	−0.157	0.0142
HOMOSER.METSYN.PWY	BPS	−0.186	0.0161
HSERMETANA.PWY	BPS	−0.192	0.0226
P4.PWY	BPS	−0.091	0.0237
P163.PWY	MetPB	−0.613	0.0149

BPS: bisphenol S; MetPB: methylparaben.

3.6. Random forest-based identification of gut microbiota features associated with bisphenol and paraben exposure

To investigate whether specific gut microbial signatures could serve as markers of exposure to bisphenols and parabens, a supervised linear regression analysis was performed. Among all compounds analyzed, only propylparaben showed a statistically significant prediction accuracy using the random forest model ($R^2 = 0.207$; $p = 0.044$), indicating a modest but significant contribution to the model. The top three genera with the highest feature importance were *Lachnospiraceae* UCG-001, an unclassified genus within the *Lachnospiraceae* family, and *Faecalibacterium* (Fig. 6).

4. Discussion

In this cross-sectional study we have investigated the potential link between chronic exposure to bisphenols and parabens and the gut microbiota in childhood. Our results did not reveal significant differences between quartiles of total bisphenols or parabens content in hair for the studied population. Additionally, when gut microbiota diversity was examined, we did not find any association between gut microbiota diversity and the quantity of bisphenols and parabens in hair. The intricate interplay between the diversity of the gut microbiota and

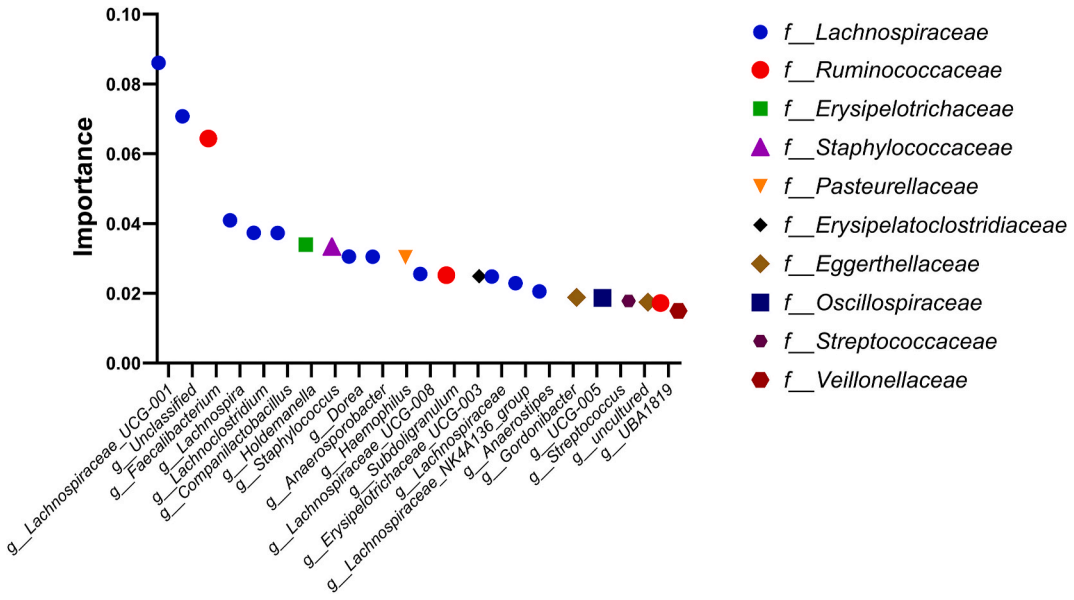

Fig. 6. Feature importance from the random forest analysis assessing the relationship between propylparaben exposure and gut microbiota composition. The plot highlights the microbial genera that contributed most to the predictive model. The symbols represent the different bacteria families.

human health is an important topic in research. A rich and diverse gut microbiota is more balanced, has higher regulatory capacity and is associated with enhanced metabolic functions, immune modulation, and protection against pathogenic invasions (Castellanos et al., 2020; Zheng et al., 2020). In animal models, exposure to bisphenols and parabens has demonstrated varied effects on the diversity of the gut microbiota. While some studies have reported modifications in the diversity of the gut microbiota (Hong et al., 2023; Hu et al., 2022; Liu et al., 2022; Wang et al., 2021), others have not observed changes (Hu et al., 2016; Javurek et al., 2016; Zheng et al., 2023), or have identified different effects depending on the sex of the individuals, or have only detected changes in a single gender (Ni et al., 2021). The impact of bisphenols and parabens on the gut microbiota may be influenced by factors such as dosage, duration of exposure, and the age at which exposure occurs. Research suggests that variations in exposure level can result in different effects on the composition of the gut microbiota (Ni et al., 2021; Wang et al., 2021). Additionally, the timing of exposure during critical developmental periods, such as early childhood, may have long-lasting consequences on the gut microbial community (Celik and Yesildemir, 2025).

In the study by (Hu et al., 2016), the impact of exposure to low doses of environmental chemicals, including methylparaben, was observed to affect the gut microbiota only in adolescent rats and not in adult rats. Furthermore, it has been reported that parental exposure to environmentally relevant concentrations of bisphenol A induced changes in the gut microbiota of unexposed offspring in mice (Javurek et al., 2016).

More detailed genus-level analyses revealed associations between specific gut microbiota genera and the presence of bisphenols and parabens. Our results show that bisphenol S levels in hair are significantly and negatively associated with the *Izemoplasmatales* genus, and a negative, although non-significant, trend also observed for the genus *Streptococcus*. To date, no direct evidence links the genus *Izemoplasmatales* to bisphenol or paraben exposure in humans. In line with our findings, a lower abundance and negative correlation of the *Streptococcus* genus has been reported in the gut microbiota of individuals with exposure to microplastics (Zhang et al., 2022). However, this is the first study to report an association between the *Streptococcus* genus and specific exposure to bisphenols in the gut microbiota. Whether the negative association of these genera reflects a sensitivity to plastic-related compounds or an indirect ecological effect remains to be determined.

Regarding parabens, we observed a significant positive association between the *Prevotellaceae_NK3B31_group* and ethylparaben, along with a positive, although non-significant, trend with propylparaben. To our knowledge, this is the first study to report such associations. No previous literature has directly linked the *Prevotellaceae_NK3B31_group* with paraben exposure. Given that parabens possess antimicrobial properties and can influence the composition of the gut microbiota (Crovetto et al., 2017; Ren et al., 2024), it is biologically plausible that certain bacterial groups may be more resilient or responsive to their presence. However, the specific role of *Prevotellaceae_NK3B31_group* in this context remains unclear, and further research is needed to elucidate the potential mechanisms underlying this relationship.

The analysis of potential functional capacities revealed that bisphenol S was associated with several metabolic pathways related to amino acid biosynthesis, particularly methionine biosynthesis. Consistent with our findings (Ni et al., 2021), reported that bisphenol A exposition alters amino acid biosynthesis pathways in mice gut microbiota. Alterations in microbial amino acids metabolism may have health consequences. For instance, it has recently been described that gut microbiota metabolism contributes to the host's methionine metabolism (Wu et al., 2022). Therefore, changes in the observed pathways may affect amino acid homeostasis, potentially influencing the production of signaling molecules and the regulation of immune responses. Notably, dietary methionine restriction in mice has been shown to induce immune alterations that promote tumor growth, and this effect is mediated by the gut microbiota (Ji et al., 2023).

Given the growing interest in the gut microbiota as a potential sensor of environmental exposures, we explored whether specific microbial signatures could serve as indirect markers of bisphenol and paraben exposure. However, the high inter- and intra-individual variability of the gut microbiota and the current lack of a universally accepted definition of a "healthy" gut microbiome (Buytaers et al., 2024), limit the robustness of such an approach. In addition, the cross-sectional design, the single time-point microbiota assessment, and the modest sample size make it difficult to disentangle exposure-specific microbial signals from other influential factors. For these reasons, the predictive analysis should be viewed as hypothesis-generating, aimed at guiding future studies rather than providing definitive evidence for microbiota-based biomarkers of environmental chemical exposure. Although the predictive capacity observed was modest, this work represents an initial step toward identifying microbiota-based patterns that may reflect

cumulative chemical exposure (Buytaers et al., 2024; De Filippis et al., 2024). The supervised linear regression analysis with random forest indicated that propylparaben showed a mild but significant contribution to the gut microbiota predictive model, suggesting its potential as a gut microbial marker of propylparaben exposure. The top three genera contributing to the predictive model were *Lachnospiraceae*_UCG-001, an unclassified genus of the *Lachnospiraceae* family, and the *Faecalibacterium* genus. The *Lachnospiraceae* family is part of the commensal gut microbiota, although its role in host physiology remains unclear. It is a family with a high enzymatic capacity for polysaccharide and fiber degradation, producing beneficial metabolites for the host, such as short-chain fatty acids (SCFA). However, its presence has also been associated with alterations in glucose and lipid metabolism, obesity, diabetes, and non-alcoholic fatty liver disease (Vacca et al., 2020). In fact, obese individuals tend to have a gut microbiota with increased SCFA-producing capacity, which may provide additional energy when the diet is rich in fiber (Ley et al., 2006; Schwiertz et al., 2010). Exposure to endocrine disruptors has been linked to obesity, and this effect may be mediated by gut microbiota alterations (Ramírez et al., 2022). Notably, *Lachnospiraceae* has also been associated with exposure to endocrine-disrupting compounds in children: perinatal exposure to methylparaben was recently linked to the abundance of *Lachnospiraceae*, although this association did not remain statistically significant after correction for multiple comparisons (Davias et al., 2024a). This finding aligns with our results, where *Lachnospiraceae* was identified as a key microbial predictor of propylparaben exposure, suggesting a potentially shared microbial response to different parabens across developmental stages. In animal models exposure to plastics has also been associated with increased abundance of the *Lachnospiraceae* family in the gut microbiota of mice (Hu et al., 2022). Additionally, in high fat diet induced obesity exposure to polystyrene microspheres was associated with increased *Lachnospiraceae* abundance (Zhai et al., 2024), and *Lachnospiraceae_NK4A136_group* has been proposed as a potential biomarker for foodborne polylactic acid micro/nanoplastics exposure (Zha et al., 2024). Whether the metabolic effects of endocrine disruptors are mediated through specific members of the *Lachnospiraceae* family remains an open question. Further research is warranted to elucidate the mechanistic pathways involved and to determine whether *Lachnospiraceae* could serve as a microbial biomarker or mediator in the context of diet- or contaminant-induced metabolic disturbances. This study presents several strengths. One of the main methodological advantages is the use of hair as a non-invasive biomonitoring matrix, which integrates exposure over several weeks to months and provides a stable and cumulative measure of internal dose. This is particularly relevant for chemicals with short biological half-lives, such as bisphenols and parabens, as hair analysis may reduce exposure misclassification and enhance the biological plausibility of observed associations with the gut microbiota. However, it is important to acknowledge that hair is not the primary route of excretion for these compounds, and some chemicals may not have been detected in certain samples due to low incorporation into hair matrix. The inclusion of a pediatric population enables the assessment of exposure during a critical developmental window. The integration of high-resolution gut microbiota profiling, functional prediction (PICRUSt2), and robust statistical methods (MaAsLin2, random forest) adds analytical depth to the study. Moreover, detailed data on lifestyle and sociodemographic variables were collected through face-to-face interviews with the participants' parents or legal guardians. Statistical models assessing the associations between gut microbiota and bisphenols/parabens exposure were adjusted for key covariates such as age, and sex, strengthening the validity of the findings. Nonetheless, several limitations should be considered when interpreting the results. First, the cross-sectional design does not allow for causal inference between bisphenols and parabens exposure and alterations in gut microbiota composition or function. The possibility of reverse causality, whereby gut microbiota characteristics may influence the metabolism of these compounds cannot be ruled out. Second, selection bias cannot be entirely excluded, as participants were recruited from educational and healthcare centers within a specific geographical area (Granada, Spain) which may limited the generalizability to other populations. Third, gut microbiota was assessed at a single time point, which may not represent long-term microbial dynamics. Additionally, although the study included a relatively large pediatric cohort, the sample size may still limit the detection of subtle associations. Finally, functional predictions based on 16S rRNA data using PICRUSt2 provide only indirect estimations of metabolic potential and should ideally be validated through shotgun metagenomics or metabolomic approaches.

5. Conclusions

In summary, no significant differences were observed in the overall gut microbiota composition of children exposed to low versus high levels of bisphenols and parabens. However, regression analyses at the genus level and functional pathways revealed associations between specific gut microbial taxa and the presence of bisphenols and parabens. These findings suggest that bisphenols and parabens may influence specific components or functions of the gut microbiota, even in the absence of global compositional shifts. Understanding the complex interplay between environmental chemical exposure, gut microbiota dynamics, and downstream systemic effects is crucial. This knowledge could support the development of targeted strategies to mitigate the adverse effects of bisphenol and paraben exposure, particularly during critical windows of development.

CRediT authorship contribution statement

Lourdes Rodrigo: Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Carlo Bressa:** Writing – review & editing, Visualization, Data curation. **Mar Larrosa:** Writing – review & editing, Writing – original draft, Validation, Methodology. **Viviana Ramírez:** Methodology, Investigation, Formal analysis, Data curation. **Ángel Gil-Izquierdo:** Validation, Investigation. **Cristóbal Sánchez-Muñoz:** Methodology, Investigation, Formal analysis, Data curation. **María Alba Martínez-Burgos:** Methodology, Investigation, Data curation. **Alberto Zafra-Gómez:** Writing – review & editing, Validation, Supervision, Formal analysis, Data curation, Conceptualization. **Ana Rivas:** Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Ethics statement

The present study has been approved by the Biomedical Research Ethics Committees of the Province of Granada (references: 0922-N-19; 1939-M1–22; 1742-N-23) and the study has been performed in accordance with the ethical standards. All subjects gave written informed consent and had parental permission to participate in this study.

Funding sources

This study has been funded by Instituto de Salud Carlos III (ISCIII) through the project "PI20/01278" and "PI23/01359" and co-funded by the European Union; and co-funded project FEDER-Consejería de Salud y Familias, Junta de Andalucía "PE-0250–2019".

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.

References

Abouhamzeh, B., Zare, Z., Mohammadi, M., Moosazadeh, M., Nourian, A., 2023. Bisphenol-S influence on oxidative stress and endocrine biomarkers of reproductive system: a systematic review and meta-analysis. Int. J. Prev. Med. 14, 37. https://doi.org/10.4103/ijpvm.ijpvm_271_21, 37.

Acevedo, J.M., Kahn, L.G., Pierce, K.A., Carrasco, A., Rosenberg, M.S., Trasande, L., 2025. Temporal and geographic variability of bisphenol levels in humans: a systematic review and meta-analysis of international biomonitoring data. Environ. Res. 264, 120341. https://doi.org/10.1016/j.envres.2024.120341.

Akash, M.S.H., Sabir, S., Rehman, K., 2020. Bisphenol A-induced metabolic disorders: from exposure to mechanism of action. Environ. Toxicol. Pharmacol. 77, 103373. https://doi.org/10.1016/j.etap.2020.103373.

Alharbi, H.F., Algonaiman, R., Alduwayghiri, R., Aljutaily, T., Algheshairy, R.M., Almutairi, A.S., Alharbi, R.M., Alfurayh, L.A., Alshahwan, A.A., Alsadun, A.F., Barakat, H., 2022. Exposure to bisphenol A substitutes, bisphenol S and bisphenol F, and its association with developing obesity and diabetes mellitus: a narrative review. Int. J. Environ. Res. Public. Health 19, 15918. https://doi.org/10.3390/ijerph192315918.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson 2nd, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Buytaers, F.E., Berger, N., Van der Heyden, J., Roosens, N.H.C., De Keersmaecker, S.C.J., 2024. The potential of including the microbiome as biomarker in population-based health studies: methods and benefits. Front. Public Health 12, 1467121. https://doi.org/10.3389/fpubh.2024.1467121.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Castellanos, N., Diez, G.G., Antúnez-Almagro, C., Bailén, M., Bressa, C., González Soltero, R., Pérez, M., Larrosa, M., 2020. A critical mutualism - competition interplay underlies the loss of microbial diversity in sedentary lifestyle. Front. Microbiol. 10, 3142. https://doi.org/10.3389/fmicb.2019.03142, 3142.

Celik, M.N., Yesildemir, O., 2025. Endocrine disruptors in child obesity and related disorders: early critical windows of exposure. Curr. Nutr. Rep. 14, 14. https://doi.org/10.1007/s13668-024-00604-1.

Crovetto, S.I., Moreno, E., Dib, A.L., Espigares, M., Espigares, E., 2017. Bacterial toxicity testing and antibacterial activity of parabens. Toxicol. Environ. Chem. 99, 858–868. https://doi.org/10.1080/02772248.2017.1300905.

Davias, A., Lyon-Caen, S., Rolland, M., Iszatt, N., Thomsen, C., Haug, L.S., Sakhi, A.K., Monot, C., Rayah, Y., Ilhan, Z.E., Jovanovic, N., Philippat, C., Eggesbo, M., Lepage, P., Slama, R., 2024a. Perinatal exposure to phenols and Poly- and perfluoroalkyl substances and gut microbiota in one-year-old children. Environ. Sci. Technol. 58, 15395–15414. https://doi.org/10.1021/acs.est.3c09927.

Davias, A., Lyon-Caen, S., Rolland, M., Iszatt, N., Thomsen, C., Sabaredzovic, A., Sakhi, A.K., Monot, C., Rayah, Y., Ilhan, Z.E., Philippat, C., Eggesbø, M., Lepage, P., Slama, R., 2024b. Associations between pre- and post-natal exposure to phthalate and DINCH metabolites and gut microbiota in one-year old children. Environ. Pollut. Barking Essex 1987 363, 125204. https://doi.org/10.1016/j.envpol.2024.125204.

De Filippis, F., Valentino, V., Sequino, G., Borriello, G., Riccardi, M.G., Pierri, B., Cerino, P., Pizzolante, A., Pasolli, E., Esposito, M., Limone, A., Ercolini, D., 2024. Exposure to environmental pollutants selects for xenobiotic-degrading functions in the human gut microbiome. Nat. Commun. 15, 4482. https://doi.org/10.1038/s41467-024-48739-7.

Douglas, G.M., Beiko, R.G., Langille, M.G.I., 2018. Predicting the functional potential of the microbiome from marker genes using PICRUSt. Methods Mol. Biol. Clifton NJ 1849, 169–177. https://doi.org/10.1007/978-1-4939-8728-3_11.

European Commision, 2018. Commission regulation (EU) 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending regulation (EU) no 10/2011 as regards the use of that substance in plastic food contact materials. Off. J. Eur. Union L41, 6–12.

European Commission, 31 December 2024. Commission regulation (EU) 2024/3190 of 19 December 2024 amending annex XVII to regulation (EC) no 1907/2006 of the european parliament and of the council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) as regards bisphenol A and other bisphenols with endocrine disrupting properties. Off. J. Eur. Union. 1–15.

European Commission, 2011a. Commission directive 2011/8/EU of 28 January 2011 amending directive 2002/72/EC as regards the restriction of use of bisphenol A in plastic infant feeding bottles. Off. J. Eur. Union L26, 11–14.

European Commission, 2011b. Commission regulation (EU) no 1129/2011 of 11 November 2011 amending annex II to regulation (EC) no 1333/2008 by establishing a union list of food additives. Off. Off. J. Eur. Union L295, 1–177.

Gálvez-Ontiveros, Y., Moscoso-Ruiz, I., Rodrigo, L., Aguilera, M., Rivas, A., Zafra-Gómez, A., 2021. Presence of parabens and bisphenols in food commonly consumed in Spain. Foods Basel Switz 10, 92. https://doi.org/10.3390/foods10010092.

Haange, S.-B., Riesbeck, S., Aldehoff, A.S., Engelmann, B., Jensen Pedersen, K., Castaneda-Monsalve, V., Rolle-Kampczyk, U., von Bergen, M., Jehmlich, N., 2024. Chemical mixture effects on the simplified human intestinal microbiota: assessing xenobiotics at environmentally realistic concentrations. J. Hazard. Mater. 474, 134683. https://doi.org/10.1016/j.jhazmat.2024.134683.

Hong, T., Zou, J., He, Y., Zhang, H., Liu, H., Mai, H., Yang, J., Cao, Z., Chen, X., Yao, J., Feng, D., 2023. Bisphenol A induced hepatic steatosis by disturbing bile acid metabolism and FXR/TGR5 signaling pathways via remodeling the gut microbiota in CD-1 mice. Sci. Total Environ. 889, 164307. https://doi.org/10.1016/j.scitotenv.2023.164307.

Hu, J., Raikhel, V., Gopalakrishnan, K., Fernandez-Hernandez, H., Lambertini, L., Manservisi, F., Falcioni, L., Bua, L., Belpoggi, F., L Teitelbaum, S., Chen, J., 2016. Effect of postnatal low-dose exposure to environmental chemicals on the gut microbiome in a rodent model. Microbiome 4, 26. https://doi.org/10.1186/s40168-016-0173-2, 26.

Hu, N., Zhang, X., Yu, Z., Du, Y., He, X., Wang, H., Pu, L., Pan, D., He, S., Liu, X., Li, J., 2022. Effects of oral exposure to leachate from boiled-water treated plastic products on gut microbiome and metabolomics. J. Hazard. Mater. 439, 129605. https://doi.org/10.1016/j.jhazmat.2022.129605.

Hu, P., Pan, C., Su, W., Vinturache, A., Hu, Y., Dong, X., Ding, G., 2022. Associations between exposure to a mixture of phenols, parabens, and phthalates and sex steroid hormones in children 6-19 years from NHANES, 2013-2016. Sci. Total Environ. 822, 153548. https://doi.org/10.1016/j.scitotenv.2022.153548.

Javurek, A.B., Spollen, W.G., Johnson, S.A., Bivens, N.J., Bromert, K.H., Givan, S.A., Rosenfeld, C.S., 2016. Effects of exposure to bisphenol A and ethinyl estradiol on the gut microbiota of parents and their offspring in a rodent model. Gut Microbes 7, 471–485. https://doi.org/10.1080/19490976.2016.1234657.

Ji, M., Xu, Xiaojiang, Xu, Q., Hsiao, Y.-C., Martin, C., Ukraintseva, S., Popov, V., Arbeev, K.G., Randall, T.A., Wu, X., Garcia-Peterson, L.M., Liu, J., Xu, Xin, Andrea Azcarate-Peril, M., Wan, Y., Yashin, A.I., Anantharaman, K., Lu, K., Li, J.-L., Shats, I., Li, X., 2023. Methionine restriction-induced sulfur deficiency impairs antitumour immunity partially through gut microbiota. Nat. Metab. 5, 1526–1543. https://doi.org/10.1038/s42255-023-00854-3.

Jiménez-Díaz, I., Zafra-Gómez, A., Ballesteros, O., Navea, N., Navalón, A., Fernández, M. F., Olea, N., Vílchez, J.L., 2010. Determination of bisphenol A and its chlorinated derivatives in placental tissue samples by liquid chromatography–tandem mass spectrometry. J. Chromatogr. B 878, 3363–3369. https://doi.org/10.1016/j.jchromb.2010.10.021.

Kaehler, B.D., Bokulich, N.A., McDonald, D., Knight, R., Caporaso, J.G., Huttley, G.A., 2019. Species abundance information improves sequence taxonomy classification accuracy. Nat. Commun. 10, 4643. https://doi.org/10.1038/s41467-019-12669-6.

Khalili Sadrabad, E., Hashemi, S.A., Nadjarzadeh, A., Askari, E., Akrami Mohajeri, F., Ramroudi, F., 2023. Bisphenol A release from food and beverage containers - a review. Food Sci. Nutr. 11, 3718–3728. https://doi.org/10.1002/fsn3.3398.

Kim, J., Chevrier, J., 2020. Exposure to parabens and prevalence of obesity and metabolic syndrome: an analysis of the Canadian health measures survey. Sci. Total Environ. 713, 135116. https://doi.org/10.1016/j.scitotenv.2019.135116.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Ley, R.E., Turnbaugh, P.J., Klein, S., Gordon, J.I., 2006. Human gut microbes associated with obesity. Nature 444, 1022–1023. https://doi.org/10.1038/4441022a.

Lin, H., Peddada, S.D., 2020. Analysis of compositions of microbiomes with bias correction. Nat. Commun. 11, 3514. https://doi.org/10.1038/s41467-020-17041-7.

Litrenta, F., Nava, V., Potortì, A.G., Lo Turco, V., Sgrò, B., Di Bella, G., 2025. Bisphenols, toxic elements, and potentially toxic elements in ready-to-eat fish and meat foods and their associated risks for human health. Toxics 13. https://doi.org/10.3390/toxics13060433.

Liu, R., Liu, B., Tian, L., Jiang, X., Li, X., Cai, D., Sun, J., Bai, W., Jin, Y., 2022. Exposure to bisphenol A caused hepatoxicity and intestinal flora disorder in rats. Int. J. Mol. Sci. 23, 8042. https://doi.org/10.3390/ijms23148042.

Luque, G., Ortiz, P., Torres-Sánchez, A., Ruiz-Rodríguez, A., López-Moreno, A., Aguilera, M., 2025. Impact of Ex vivo bisphenol A exposure on gut microbiota dysbiosis and its association with childhood obesity. J. Xenobiotics 15. https://doi.org/10.3390/jox15010014.

Mallick, H., Rahnavard, A., McIver, L.J., Ma, S., Zhang, Y., Nguyen, L.H., Tickle, T.L., Weingart, G., Ren, B., Schwager, E.H., Chatterjee, S., Thompson, K.N., Wilkinson, J. E., Subramanian, A., Lu, Y., Waldron, L., Paulson, J.N., Franzosa, E.A., Bravo, H.C., Huttenhower, C., 2021. Multivariable association discovery in population-scale meta-omics studies. PLoS Comput. Biol. 17, e1009442. https://doi.org/10.1371/journal.pcbi.1009442.

Molina-López, A.M., Bujalance-Reyes, F., Ayala-Soldado, N., Mora-Medina, R., Lora-Benítez, A., Moyano-Salvago, R., 2023. An overview of the health effects of

bisphenol A from a one health perspective. Anim. Open Access J. MDPI 13, 2439. https://doi.org/10.3390/ani13152439.

Ni, Y., Hu, L., Yang, S., Ni, L., Ma, L., Zhao, Y., Zheng, A., Jin, Y., Fu, Z., 2021. Bisphenol A impairs cognitive function and 5-HT metabolism in adult Male mice by modulating the microbiota-gut-brain axis. Chemosphere 282, 130952. https://doi.org/10.1016/j.chemosphere.2021.130952.

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., Duchesnay, E., 2011. Scikit-learn: machine learning in python. J. Mach. Learn. 12, 2825–2830.

Quirós-Alcalá, L., Buckley, J.P., Boyle, M., 2018. Parabens and measures of adiposity among adults and children from the U.S. general population: nhanes 2007-2014. Int. J. Hyg Environ. Health 221, 652–660. https://doi.org/10.1016/j.ijheh.2018.03.006.

Ramírez, V., González-Palacios, P., Baca, M.A., González-Domenech, P.J., Fernández-Cabezas, M., Álvarez-Cubero, M.J., Rodrigo, L., Rivas, A., 2022. Effect of exposure to endocrine disrupting chemicals in obesity and neurodevelopment: the genetic and microbiota link. Sci. Total Environ. 852, 158219. https://doi.org/10.1016/j.scitotenv.2022.158219.

Ramírez, V., Merkel, S., Tietz, T., Rivas, A., 2023. Risk assessment of food contact materials. EFSA J. Eur. Food Saf. Auth. 21, e211015. https://doi.org/10.2903/j.efsa.2023.e211015.

Ren, Y., Shi, X., Mu, J., Liu, S., Qian, X., Pei, W., Ni, S., Zhang, Zhengduo, Li, L., Zhang, Zhan, 2024. Chronic exposure to parabens promotes non-alcoholic fatty liver disease in association with the changes of the gut microbiota and lipid metabolism. Food Funct. 15, 1562–1574. https://doi.org/10.1039/d3fo04347a.

Rodríguez-Gómez, R., Jiménez-Díaz, I., Zafra-Gómez, A., Ballesteros, O., Navalón, A., 2014. A multiresidue method for the determination of selected endocrine disrupting chemicals in human breast milk based on a simple extraction procedure. Talanta 130, 561–570. https://doi.org/10.1016/j.talanta.2014.07.047.

Rodríguez-Gómez, R., Martín, J., Zafra-Gómez, A., Alonso, E., Vílchez, J.L., Navalón, A., 2017. Biomonitoring of 21 endocrine disrupting chemicals in human hair samples using ultra-high performance liquid chromatography-tandem mass spectrometry. Chemosphere 168, 676–684. https://doi.org/10.1016/j.chemosphere.2016.11.008.

Schwiertz, A., Taras, D., Schäfer, K., Beijer, S., Bos, N.A., Donus, C., Hardt, P.D., 2010. Microbiota and SCFA in lean and overweight healthy subjects. Obesity 18, 190–195. https://doi.org/10.1038/oby.2009.167.

Vacca, M., Celano, G., Calabrese, F.M., Portincasa, P., Gobbetti, M., De Angelis, M., 2020. The controversial role of human gut lachnospiraceae. Microorganisms 8, 573. https://doi.org/10.3390/microorganisms8040573.

Wang, Y., Wang, B., Wang, Q., Liu, Y., Liu, X., Wu, B., Lu, G., 2021. Intestinal toxicity and microbial community disorder induced by bisphenol F and bisphenol S in zebrafish. Chemosphere 280, 130711. https://doi.org/10.1016/j.chemosphere.2021.130711.

Wei, F., Mortimer, M., Cheng, H., Sang, N., Guo, L.-H., 2021. Parabens as chemicals of emerging concern in the environment and humans: a review. Sci. Total Environ. 778, 146150. https://doi.org/10.1016/j.scitotenv.2021.146150.

Wu, X., Han, Z., Liu, B., Yu, D., Sun, J., Ge, L., Tang, W., Liu, S., 2022. Gut microbiota contributes to the methionine metabolism in host. Front. Microbiol. 13, 1065668. https://doi.org/10.3389/fmicb.2022.1065668, 1065668.

Zha, H., Han, S., Tang, R., Cao, D., Chang, K., Li, L., 2024. Polylactic acid micro/nanoplastic-induced hepatotoxicity: investigating food and air sources via multi-omics. Environ. Sci. Ecotechnology 21, 100428. https://doi.org/10.1016/j.ese.2024.100428.

Zhai, Z., Yang, Y., Chen, S., Wu, Z., 2024. Long-term exposure to polystyrene microspheres and high-fat diet-induced obesity in mice: evaluating a role for microbiota dysbiosis. Environ. Health Perspect. 132, 97002. https://doi.org/10.1289/EHP13913.

Zhang, X., Wang, H., Peng, S., Kang, J., Xie, Z., Tang, R., Xing, Y., He, Y., Yuan, H., Xie, C., Liu, Y., 2022. Effect of microplastics on nasal and intestinal microbiota of the high-exposure population. Front. Public Health 10, 1005535. https://doi.org/10.3389/fpubh.2022.1005535, 1005535.

Zheng, D., Liwinski, T., Elinav, E., 2020. Interaction between microbiota and immunity in health and disease. Cell Res. 30, 492–506. https://doi.org/10.1038/s41422-020-0332-7.

Zheng, Q., Cui, L., Liao, H., Junaid, M., Li, Z., Liu, S., Gao, D., Zheng, Y., Lu, S., Qiu, J., Wang, J., 2023. Combined exposure to polystyrene nanoplastics and bisphenol A induces hepato- and intestinal-toxicity and disturbs gut microbiota in channel catfish (Ictalurus punctatus). Sci. Total Environ. 891, 164319. https://doi.org/10.1016/j.scitotenv.2023.164319.