Levels of phthalates and bisphenol in toys from Brazilian markets: Migration rate into children´s saliva and daily exposure

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ABSTRACT

Bisphenols (BPs) and phthalate esters (PAEs) are extensively used in toys and childcare products. Therefore, children may be exposed to these compounds, causing potential adverse effects. Despite the strict control of the levels of these contaminants in toys by some nations, routine testing in Brazil is very scarce. The present study was aimed at determining the concentrations of PAEs and BPs in toys commercialized in Brazil, employing GC-MS and LC-MS/MS, respectively. Furthermore, the migration capacity of PAEs into saliva and the daily intake (EDI) were also estimated. Di-2-ethylhexyl phthalate (DEHP) was the PAE with the highest detection rate (93%) and migration rate (0.26 µg/min). Moreover, the levels of DEHP in some samples were above the threshold values set by the European Commission and the Brazilian Institute of Metrology, Standardization, and Industrial Quality. Among the BPs analogs, BPA and BPS presented the highest positive detection rates (72% and 30%, respectively). However, their levels were below the permitted values in all analyzed samples. A daily intake of DEHP was estimated at 29.8 µg/kg bw/day, being this exposure similar to those found in other countries and below the EFSA acceptable intake limit (50 µg/kg bw/day). However, our data are referred to exposure through oral contact with the analyzed toys, while the contribution of other potential sources, such as food consumption, were not here considered. To the best of our knowledge, this is the first study estimating the exposure of Brazilian children to PAEs and BPs, considering toys as the exposure source. These preliminary data may become a valuable guide for the control of EDC levels in toys commercialized in Brazil, as well as for future studies regarding estimation of exposure to EDCs by children taking into account multiple potential sources.

Keywords: phthalate; bisphenol; endocrine-disrupting chemicals; toys; saliva migration; child exposure

1. Introduction

Endocrine-disrupting chemicals (EDCs) are emerging pollutants according to the U.S. Environmental Protection Agency (EPA) (Bila and Dezotti, 2007; Hampl et al., 2014; Hotchkiss et al., 2008; Darbre, 2019; Djordjevic et al., 2020; US.EPA, 1997; USGS, 2017). Studies reported by the EU Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 1999) have shown a relationship between EDCs and human health effects, including increasing, decreasing, or stimulating hormonal function activity at inappropriate times. After chronic exposure, human body health alterations consist of the occurrence of testicular, breast, and prostate cancer, obesity, endometriosis, declining sperm rates, deformities of the reproductive organs, and thyroid dysfunction (CSTEE, 1999; González et al., 2020a). Phthalate esters and bisphenols are important EDCs that are used as plasticizers in several commercial products and show antiandrogenic activity, making them a significant public health concern (Hotchkiss et al., 2008; Martínez et al., 2021).

Phthalate esters (PAEs) are synthetically obtained by the esterification of alcohols and are found in polyvinyl chloride (PVC) plastics. They enhance the physical flexibility of several consumer products, including building materials, household furnishings, clothing, cosmetics, pharmaceutical products, nutritional supplements, medical devices, children's toys, food packaging, cleaning materials, and insecticides (Rocha et al., 2017; Radke et al., 2019). High molecular weight PAEs, such as diisononyl phthalate (DINP) and diisodecyl phthalate (DIDP), are less toxic to human health but more common. They correspond to 80% of the PAEs that are used in Europe and are included in the *Registration, Evaluation, Authorization, and Restriction of Chemical Substances* (REACH). Moreover, low molecular weight compounds, such as dibutyl phthalate (DBP), benzyl-butyl phthalate (BBP), and di-2-ethylhexyl phthalate (DEHP), are classified as potentially dangerous to human health. DEHP is not only the most abundant PAE in food and environmental samples, but also it is highly toxic (Ventrice et al., 2013; Kondolot et al., 2016).

Bisphenol (BP) analogs are compounds used as monomers in polycarbonate plastic and epoxy resin production, and employed as plasticizers in PVC plastics (Gimeno et al., 2015; González et al., 2019; Lin et al., 2020). Bisphenol A (BPA) is of great significance concerning its use and toxicity profile, and it is one of the highest volume chemicals produced worldwide. Due to its high toxicity, several countries have reduced the production and consumption of BPA and have started its replacement by other BPs, such as bisphenol S (BPS), bisphenol F (BPF), bisphenol AF (BPAF), and bisphenol B (BPB) (Hoepner, 2019; González et al., 2020b; Lin et al., 2020; Wang et al., 2020).

Data on human exposure and toxicity profiles of these EDCs have been carried out in several countries and by researchers from Europe and the United States (Malits et al., 2018; Negev et al., 2018;

Darbre, 2019). However, these studies are scarce in most Latin American countries. Young children are a population subgroup that is vulnerable and sensitive to adverse health effects of environmental contaminants. Their exposure, even at low concentrations of EDCs, means a public health concern regarding adverse effects in children (Negev et al., 2018). Furthermore, very little is known about the interactions between phthalate esters and bisphenol analogs and other chemicals. The co-exposure to different emerging pollutants can significantly change the impact and effect on human health. Depending on the chemicals, the interactions may result in synergistic, additive, or antagonistic effects. BPA and phthalate esters may alter neural behavior, cause a metabolic disturbance, and promote oxidative damage in DNA in children. Previous studies have reported a positive association between prenatal BPA and phthalate exposure and atopic dermatitis in children. However, the lack of understanding about the safety of BPA and PAEs and their potential role as endocrine disruptors makes the characterization of the compound mixtures of high importance public health question (Kondolot et al., 2016; Rocha et al., 2017; Malits et al., 2018; Jatkowska et al., 2021; Lee et al., 2021; Mustieles et al., 2022).

The main route of exposure for children is oral exposure, highlighting sources such as breast milk, cow's milk, infant formulations, food in plastic packaging, plastic toys and food items, such as cups, and some medical devices (US.EPA, 2007). Toys, childcare articles, sleep positioners and car seats are made of various plastics that often contain EDC compounds in their composition, such as BPA and PAEs (Kirchnawy et al., 2020; Aurisano et al., 2021). Therefore, babies and young children may put these objects in their mouths, allowing chemical contact with the saliva and the intake of these environmental contaminants (Malits et al., 2018; Negev et al., 2018; Rajbux et al., 2020). Some PAEs and BPs can migrate from toys or children’s products into saliva. This is particularly relevant, for DEHP and BPA, as these EDCs are not chemically bonded to the materials (Earls et al., 2003). Moreover, although the concentrations of PAEs and BPs in toys have been regulated in many countries, their presence in these objects is still a widespread problem (Negev et al., 2018). The evaluation of the chemical migration to children's saliva is also of toxicological importance. Therefore, analytical studies of EDCs should be complemented with human exposure assessment to identify the most contributive pathways. In addition, this information remains scarce in Brazil and other Latin American countries. Taking all the above into consideration, the present study was aimed at determining the concentrations of PAEs and BPs in toy samples commercialized in Brazil, evaluating the migration rate into saliva, and estimating the daily intake of these compounds by children.

2. Materials and methods

2.1. Chemicals and reagents

The analytical standards of PAEs selected in this study included dibutyl phthalate (DBP, cas number 84-74-2, purity grade of 99 %), dimethyl phthalate (DMP, cas number 131-11-3, ≥ 99 %), bis(2-ethylhexyl)phthalate (DEHP, 117-81-7, 99 %), diethyl phthalate (DEP, 84-66-2, 99.5 %), benzyl butyl phthalate (BBP, 85-68-7, 99 %), and bis(2-ethylhexyl)phthalate-3,4,5,6-*d*4 (DEHP-*d*4, 93951-87-2, 98 atom % D), which were purchased from Sigma–Aldrich® (St. Louis, MO, USA). All stock standard solutions were prepared in hexane, stored in glass tubes at -20 °C, and protected from light. Additionally, the selected analytical BP standards were 2,2-bis(4-hydroxyphenyl)propane (BPA, 80-05-7, 99 %), 2-bis(4-hydroxyphenyl)propane-*d*16 (BPA-*d*16, 96210-87-6, 98 atom % D), 4,4'-sulfonyldiphenol (BPS, 80-09-1, 99 %), 4,4'-dihydroxydiphenylmethane (BPF, 620-92-8, 99 %), 4,4'-(hexafluoroisopropylidene)-diphenol (BPAF, 1478-61-1, 98 %), 4,4'-(1-phenylethylidene)bisphenol (BPAP, 1571-75-1, 99 %), 4,4'-(1,4-phenylenediisopropylidene)bisphenol (BPP, 2167-51-3, 99 %) and 4,4'-cyclo-hexylidenebisphenol (BPZ, 843-55-0, 98 %), which were also purchased from Sigma–Aldrich® (St. Louis, MO, USA). All stock standard solutions were prepared in methanol, stored at -20 °C, and protected from light.

HPLC-grade solvents, including methanol, acquired from JT Baker® (Phillipsburg, NJ, USA), and dichloromethane and hexane, obtained from Sigma–Aldrich® (St. Louis, MO, USA), were used during the analytical procedures. High purity deionized water (resistivity of 18.2 mΩ cm^{-1}) was obtained using a Milli-Q water purification system® (Millipore RiOs-DITM, Bedford, MA, USA) and used for cleaning and preparation of the mobile phase and synthetic saliva. Synthetic saliva was prepared using analytical-grade reagents purchased from Sigma–Aldrich® (St. Louis, MO, USA), including magnesium chloride (MgCl2.6H2O, cas number 7791-18-6, purity of 99 %), calcium chloride (CaCl2.6H2O, 7774-34-7, 98 %), dipotassium hydrogen phosphate (K2HPO4.2H2O, 16788-57-1, ≥ 99.0 %), potassium carbonate (K2CO3, 584-08-7, 99.9 %), sodium chloride (NaCl, 7647-14-5, ≥ 99.0 %), and potassium chloride (KCl, 7447-40-7, ≥ 99.9 %).

2.2. Samples

In 2014-2015, 71 samples of plastic toys were directly purchased at different local markets of Ribeirao Preto, Brazil. Samples were characterized by the predominant colors, type of toys, being classified with a numerical code (Table S1 of the Supplementary Information). In addition, samples were submitted to the *Beilstein test* to assess the presence or absence of PVC. For this, a copper wire met with a small piece of each sample followed by heating using a Bunsen burner to observe the color of the flame (Al-Natsheh et al., 2015; Stuart, 2008). The presence of PVC in the sample is confirmed

when the flame is green. The characteristics of each toy are given as Supplementary Information (Table S1).

2.3. Sample preparation for EDC determination

All samples were individually milled and homogenized with an electric ultracentrifugal mill (Retsch ZM200 with a DR100 autosampler) to obtain very small-sized and representative particles. To determine PAEs concentrations, 10 mg of each sample were placed into a clean glass tube, and the internal standard DEHP-*d4* was added. Then, 1.0 mL of hexane was transferred to each tube, followed by *vortexing* (QL-910, Biomixer) for 1 minute, ultrasonication (USC-1400, Unique®, Indaiatuba, SP, Brazil) for 10 min, and shaking on a tube shaker for 20 min. After this step, the tubes were centrifuged at 3500 rpm (80-2B-15 mL, Centribio) for 10 min. This extraction procedure was repeated three times. The organic phase was separated, joined, and evaporated using a vacuum concentrator (Martin Christ RCV 2-25 CO plus and CT 04-50 SR, Osterode am Harz, Germany). The residues were reconstituted in hexane, are then submitted to instrumental analysis employing gas chromatography coupled with mass spectrometry (GC-MS). Additionally, for the determination of BP concentration, 10 mg of each sample was placed into a clean glass tube, and the internal standard BPA-*d*16 was added. Then, 2.0 mL of dichloromethane was transferred to each tube, and they were ultrasonicated (USC-1400, Unique®, Indaiatuba, SP, Brazil) for 15 min. Afterward, 5.0 mL of methanol was added to each tube, and they were left to stand for 10 min. Then, 100 µL of the supernatant and 900 µL of a methanol: water (1:1) solution were transferred to a vial for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. After determining the PAE and BP concentrations, the samples were submitted for migration testing using synthetic saliva.

2.4. Instrumentation

2.4.1. Determination of phthalate ester concentrations employing GC-MS

Instrumental analysis for the determination of PAEs in toys and saliva samples was performed using GC-MS with a mass spectrometer from Thermo Fisher Scientific® (Waltham, Massachusetts, United States). The experimental conditions of the GC-MS were based on a related study performed by Dong et al. (2013), with modifications. The chromatographic separation of the PAEs was obtained with a TG-SQC analytical column (15 m length × 0.25 µm internal diameter, 0.25 µm particle size) from Thermo Scientific®. Helium (99.9999% purity) was used as the carrier gas at a flow rate of 1.0

mL/min. The initial oven temperature was 60 °C, which was held for 1 minute, with a subsequent rise to 220 °C at a rate of 20 °C·min^{-1}. Then, the oven temperature was increased at a rate of 15 °C·min^{-1} until reaching 280 °C, which was held for 0.5 min. The injector temperature was 230 °C. The injection volume was 2 µL performed in splitless mode. The detection system was composed of an ISQ single quadrupole mass spectrometer from Thermo Scientific® operated in electron impact ionization (EI) mode. The ion source temperature was set to 250 °C, and the transfer line temperature was 280 °C. Data were acquired using full scan mode (m/z 50 to 500), with selective ion monitoring (SIM) for each analyte and the internal standard. Data acquisition and processing were performed using Thermo Xcalibur ™ *Software*, version 2.2 (Thermo Fisher Scientific®).

2.4.2. Determination of bisphenol analogs employing LC-MS/MS

A Thermo Scientific Liquid Chromatography (LC) system, equipped with a pump (Accela 600) and an autosampler coupled with a Thermo Scientific TSQ Quantum™ Access Max electrospray triple quadrupole mass spectrometer, was used for chromatographic analysis for the determination of BPs. Chromatographic separation was performed with a Supelco Ascentis Express C18 column (75 mm length × 2.1 mm internal diameter, 2.7 µm particle size; Sigma–Aldrich®, St. Louis, MO, USA®). The column temperature was maintained at 50 °C. A mixture of methanol: water (v/v) was used as the mobile phase, and the flow rate was set to 500 µL min^{-1}. A volume of 10 µL was injected for analysis. Selective reaction monitoring (SRM) in negative ion mode was used, and two SRM transitions were chosen for each compound as the channel of quantification (^{Q}) and confirmation (^{C}). The following channels were: 227 > 212^{Q} and 227 > 133^{C} for BPA (collision energy (CE) 17 and 23); 249 > 108^{Q} and 249 > 156^{C} for BPS (CE 26 and 23); 241 > 223^{Q} and 241 > 142^{C} for BPA-d_{16} (CE 19 and 21); 199 > 93^{Q} and 199 > 105^{C} for BPF (CE 19 and 18); 345 > 330^{Q} and 345 > 315^{C} for BPP (CE 26 and 29); 267 > 173^{Q} and 267 > 197^{C} for BPZ (CE 25 and 28); 289 > 273^{Q} and 289 > 208^{C} for BPAP (CE 20) and 335 > 265^{Q} and 335 > 197^{C} for BPAF (CE 21 and 30).

2.5. Migration to saliva

The migration of EDCs into saliva was performed according to the study proposed by Earls et al. (2003), with modifications. Synthetic saliva was prepared by mixing the following in 1.0 L of deionized water 0.17 g of $MgCl_2.6H_2O$; 0.15 g of $CaCl_2.6H_2O$; 0.76 g of $K_2HPO_4.2H_2O$; 0.53 g of K_2CO_3; 0.33 g of NaCl; and 0.75 g of KCl. The final pH of this solution was set to 6.8 ± 0.1 (Osman et al., 2013).

The migration assay was performed using samples that had been previously cut into sizes of 1.0 cm^2 and washed with deionized water. Each selected sample was transferred to a glass tube (tube A), and 2.0 mL of synthetic saliva solution was added. These tubes were placed in a 37 °C shaking water bath for 60 min. After 30 min, the second portion of synthetic saliva was added to tube A. At the end of 60 min, the total mixture was transferred to another glass tube, named tube B. The synthetic saliva was added in two consecutive portions to simulate real-life conditions since the release of saliva into the mouth occurs as a function of time. The contents of tube B were subjected to the extraction procedure using 1.0 mL of hexane for PAEs and 1.0 mL of dichloromethane for BPs. All tubes were ultrasonicated (USC-1400, Unique®, Indaiatuba, SP, Brazil) for 15 min and centrifuged at 3500 rpm (80-2B-15 mL, Centribio) for 10 min. These extraction procedures were repeated three times. The organic phases were separated, combined, and evaporated using a vacuum concentrator (Christ RCV 2-25 CO plus e Christ CT 04-50 SR). The residues were reconstituted in hexane for PAEs determination by GC-MS and in methanol: water (1:1) for BP determination using LC-MS/MS.

The amounts of migrated PAEs and BPs were estimated based on the calculation described in the equation below (Eq. 1) (Earls et al., 2003). This calculation considered the compound concentration determined in the saliva ($\mu g \cdot mL^{-1}$), the toy's surface area (10 cm^2), and the shaking time of 60 min, and the results were compared with the limits established by regulatory agencies.

$$\text{Migration rate } (\mu g \text{ min}^{-1}) = (\text{EDC concentration } (\mu g \text{ mg}^{-1}) \times 10 \text{ cm}^2)/(60 \text{ min (time)} \times 1 \text{ cm}^2) \quad \textit{(Eq. 1)}$$

2.6. Method performance and quality control

For quality control of the analytical methods, duplicate matrix-spiked samples (2.0 $\mu g\ mg^{-1}$ for PAEs and 1.0 $ng\ mg^{-1}$ for BPs) were analyzed for every batch of 20 samples to evaluate the accuracy of the analysis. Hexane (GC-MS analysis) and methanol (LC-MS/MS analysis) were also injected between samples to measure the possible carryover effects of the target analyte. Standard calibration curves were prepared from blank samples spiked with the specific standards and internal standard in concentration ranges from 0.5 to 4.0 $\mu g \cdot mg^{-1}$ for PAEs and 0.15 to 2.0 $ng \cdot mg^{-1}$ for BPs. The lower limit of detection (LLOD) and the lower limit of quantification (LLOQ) of these methods were 0.15 $\mu g \cdot mg^{-1}$ and 0.5 $\mu g \cdot mg^{-1}$ for PAEs and 0.05 $ng \cdot mg^{-1}$ and 0.15 $ng \cdot mg^{-1}$ for BPs, respectively. Furthermore, the calibration curve ranged from 1.25 to 10 $\mu g \cdot mL^{-1}$ for PAEs analysis in synthetic saliva, with LLOD and LLOQ values of 0.37 $\mu g \cdot mL^{-1}$ and 1.25 $\mu g \cdot mL^{-1}$, respectively. The calibration curve ranged from 1.5 and 20 $ng \cdot mL^{-1}$ for BPs with LLOD and LLOQ values of 0.5 $ng \cdot mL^{-1}$ and 1.5

ng·mL^{-1}, respectively. The values of the samples that gave detected concentration values below the LLOD were substituted by a value equal to the LLOD divided by the square root of 2 (US. EPA, 1994). Since phthalates are widespread, some procedures were performed before the analyses to avoid interferences. All solutions and procedures were prepared and carried out using glassware. Besides, all-glass materials were submitted a specific and careful cleaning procedure. First, they were washed with detergent (Extran®) and previously heated high purity deionized water, being subsequently left in an ultrasonic bath for 15 min. They were rinsed with acetone and placed to dry in a place protected from contact with dust. Finally, all materials were wrapped in aluminum foil and stored in a clean place, separate from the rest of the glassware in the laboratory.

2.7. Estimated daily intake (EDI)

The dermal and oral routes are highly relevant during the health risk assessment when considering children's exposure to EDCs present in toys. Indeed, the oral route is crucial due to saliva contact with the toy when the child puts the toy into its mouth. Thus, it is possible to estimate the daily intake of PAEs and BPs, mainly by children under the age of 3 years old. The estimated daily intake of EDCs was determined considering a child with a body weight of 8 kg and a daily exposure of 3 h (180 min) (EU-CSTEE, 1998), with the results providing the EDC concentration and migration rate into the saliva. Thus, the estimated daily intake (EDI) was calculated according to the equation (Eq. 2) described below:

$$\text{EDI (µg/kg/day)} = \text{Migration rate (µg min}^{-1}) \times \text{EDC concentration (µg mg}^{-1}) \times 180\ \text{(min)}/8\ \text{kg (body weight)} \quad \textit{(Eq. 2)}$$

The non-carcinogenic risks associated with exposure to EDCs were also evaluated. For that purpose, the EDI of PAEs and BPs was compared with the respective References Doses (RfD) of each chemical, extracted from a recent study (Aurisano et al., 2022). RfDs for DBP, BBP, and DEHP were 100, 200, and 200 µg·kg^{-1}·day^{-1}.

3. Results and discussion

3.1. Method performance

Regarding all of the previously described methods, the calibration curves demonstrated good linearity with a correlation coefficient (*r*) greater than 0.99. The mean recoveries from toy samples spiked with PAEs and BPs were 97.6% and 90.8%, respectively. The methods used for the migration tests showed a mean recovery of 106.7% for PAEs and 98.2% for BPs.

3.2. Levels of phthalates and bisphenol analogs in toys

The determined levels of PAEs and BPs with values above the LLOD for all samples in this study were calculated and are expressed as the mean, median, minimum, and maximum values, detection rate (%), and 25th and 75th percentiles.

3.2.1. Phthalate esters

The levels of PAEs determined in all samples are summarized in Table 1. All analyzed samples had detectable and quantifiable levels of PAEs. DEHP was found in 66 out of the 71 samples, corresponding to a detection rate of 93%. On the other hand, DBP, DMP, and BBP were quantified in 30, 35, and 8 samples, respectively. Following the restrictions established by the European Commission (Commission Regulation (EU) 2018/2005) and the Brazilian Institute of Metrology, Standardization and Industrial Quality (INMETRO, 2007 and 2008), in Brazil, the PAEs DBP, BBP, and DEHP shall not be present in concentrations greater than or equal to 0.1% by weight of the plasticizer material (0.1% w/w), in particular, toys. Based on the limits established by these regulatory agencies, our data showed that the concentration of DEHP was above the allowed value in 20 samples, with an average concentration of 2.62 $\mu g \cdot mg^{-1}$ (0.26% w/w). Indeed, the highest concentration of DEHP found corresponded to 4.47 $\mu g \cdot mg^{-1}$ (0.45% of the mass of the plasticizer material). In addition to DEHP, DBP was also found in concentrations higher than the threshold in 3 samples (average concentration of 1.91 $\mu g \cdot mg^{-1}$ or 0.19% w/w). Interestingly, 40% of the analyzed samples presented phthalate levels above the limit of 0.1% w/w, with the highest concentration being 5.12 $\mu g \cdot mg^{-1}$. It is important to mention that the regulatory actions to remove some phthalate ethers from the marketplace have resulted in a decrease in PAE levels in toys. Consequently, the children's exposure to these chemicals has also significantly decreased over the years.

It must be highlighted that seven of the toys did not contain PVC (see Table 1 of the Supplementary Information). Moreover, the phthalate ester levels in these samples were low. However, the samples coded as "12" and "43" presented DEHP concentrations above the INMETRO permitted value (3.49 and 1.31 µg mg-1, respectively). Our data show the importance of controlling

PAE levels in toys and products intended for children's use in Brazil since the PAEs DEHP and DBP were found at toxicologically relevant levels.

PAE concentrations in toys and products intended for children in the present study were compared with those found in other studies from the scientific literature (Table 2). The reported studies by Al-Natsheh et al., (2015) and Kim et al. (2020) showed a higher concentration of DEHP in toys, with values corresponding to 29.6 % (w/w) in Jordan, and 28.11% (w/w) in Korea, respectively. On the other hand, the levels of PAEs in the present study were very close to those found in samples from Malaysia (Praveena et al., 2021), the United States of America (Chen and Zhang, 2013), Taiwan (Johnson et al., 2011).

3.2.2. Bisphenol analogs

The levels of BPs (BPA, BPS, BPF, BPP, BPZ, BPAF, and BPAP) determined in the 71 selected samples are summarized in Table 3. BPA and BPS were the BPs analogs with the highest detection rates (71.8% and 29.6%, respectively). On the other hand, BPF, BPZ, and BPP were detected in 7, 5, and 6 samples, respectively. Finally, BPAF and BPAP presented concentrations below the LLOD value in all the analyzed toys.

According to the restrictions established by the European Union (Prosafe – Joint Actions Best Practice, 2018) and the European Food Safety Authority (EFSA, 2015), BPA must not be present in concentrations above 0.3% by weight of the plasticizer material in toys, which corresponds to 3.0 µg mg^{-1}. Following these restrictions, our results show BPA concentrations below the permitted values in all the analyzed samples.

It is essential to highlight that limits and certain restrictions established by regulatory agencies for BPs other than BPA are still scarce. Therefore, evaluations of the BPs concentrations in toys and their migration rate into saliva were performed only for BPA. Eco-Healthy Child Care (2016) emphasized that BPA has compounds called “sisters” (e.g., BPS and BPF) that may be used as replacements for BPA in plastic materials. Therefore, plastic may be “BPA-free” but it may contain sister compounds (BP analogs).

The BP concentrations found in this study were compared with those found in other studies reported in the literature. Our results showed a mean concentration of 0.97 ng mg^{-1} for BPA. On the other hand, Negev et al. (2018) found higher concentrations with a mean value of 1.03 ng·mg^{-1} for BPA in Israel. However, in both cases, the results were below the established value permitted by the European Union (3.0 µg·mg^{-1}). More recently, de Lima et al. (2022) analyzed the content of PAEs

and BPA in 10 samples of toys and utensils for infants, when developing a method for the simultaneous determination of the EDCs. BPA levels ranged between 0.060 and 21.13 $ng \cdot mg^{-1}$.

3.3. Migration of phthalates esters and BPA into synthetic saliva

In addition to child dermal contact with toys, evaluating phthalate ester and BPA migration into saliva is highly relevant when considering oral exposure. These contaminants easily migrate from the toy's surface to the child's mouth. Table 2 of the Supplementary Information contains the maximum limits for the migration of DBP, BBP, and DEHP expressed as $\mu g/min/10\ cm^2$ (Scientific Report of the EU-Committee on Toxicity, Ecotoxicity and the Environment (EU-CSTEE, 1998)). The limit for BPA migration is expressed as $mg \cdot L^{-1}$ (Commission Directive (EU) 2017/898).

The results of phthalate ester migration into saliva are demonstrated in Figure 1. According to these results, only the phthalates DBP, BBP, and DEHP were detected and quantified in saliva after the migration test. Although the total phthalate concentrations in the samples were above the limits established by the European Union and INMETRO, in Brazil, the values obtained for the migration rate were all below the tolerable limits indicated by the European Commission CSTEE in 1998 (Table S2; Supplementary Information).

DEHP was the phthalate with the highest migration rate, with a median concentration of 0.33 $\mu g.mg^{-1}$, a range of 0.099 - 4.468 $\mu g.mg^{-1}$, and a geometric mean concentration of 0.98 $\mu g.mg^{-1}$. Our results suggest a positive correlation between the concentration and migration rate of these chemicals. Furthermore, it is essential to consider the characteristics of the samples, which can influence the speed and intensity of migration, such as surface roughness, size, type of coating, and thickness (Bouma and Schakel, 2002).

Al-Natsheh et al. (2015) found eight PAEs in toys and children's products in Jordan, and the quantified phthalate concentrations were above the allowed value (0.1% w/w) set by the Commission Regulation (EU) and INMETRO in all samples containing PVC. However, they observed values below the limits established by the CSTEE after the migration test into synthetic saliva. Osman et al. (2013) determined the levels of DBP and DEHP in toy samples obtained from Turkish markets. These PAEs were evaluated for their migration rate into artificial saliva, and the values reported were also below the limits allowed by the CSTEE. Bouma and Schakel (2002) found that DEHP and DINP were not only the predominant PAEs in toys containing PVC, but also they were found at very high concentrations (range: 30-45% w/w). Regarding the assessment of their migration from 62 toys into saliva, only six samples presented DEHP migration rates greater than the limit determined by the CSTEE ($1.67\ \mu g/min/10\ cm^2$).

Considering that the results for the migration rate of PAEs from the samples into saliva were below the previously mentioned limits, it is essential to note that many samples evaluated in this study contained more than one phthalate with total concentrations greater than 0.1% w/w. It is also worth noting that the determined PAEs, particularly those with low molecular weights such as DBP, BBP, and DEHP, are classified as dangerous and potential endocrine disruptors to animals and humans (Ventrice et al., 2013). Thus, constant exposure and the cumulative effects of these compounds can bring risks to children's health, especially those under the age of 3 years old.

Regarding the BPA migration results, its concentrations in the synthetic saliva in all samples were below the limit of quantification of the method. This fact can be justified by the low concentrations of BPA found in the toy samples in the present study, as previously mentioned. Therefore, in this study, the migration rate and the estimation of daily intake parameters were not calculated for BPA. In addition, other plasticizers and additives may be present in these samples and can contribute to health damage. Therefore, these other compounds should be also regulated and evaluated to guarantee their safety for children.

3.4. Estimated daily intake (EDI)

Based on the experimental findings from animal studies, the European Commission Scientific Committee on Health and Environmental Risks has established tolerable daily intakes (TDIs) for some PAEs. These values are related to the acute toxic effects on animal reproduction (DBP, BBP, and DEHP) and animal development (DBP and BBP). The calculation of the estimated daily intake is essential due to the high concentrations of PAEs found in toy samples, which are often above the limits established by regulatory agencies.

The daily PAE intake was calculated from sample 24, which presented the highest total PAE concentration (5.257 $\mu g \cdot mg^{-1}$); 0.229 $\mu g \cdot mg^{-1}$ for DBP, 0.420 $\mu g \cdot mg^{-1}$ for BBP, and 4,468 $\mu g \cdot mg^{-1}$ for DEHP. After the migration test, this same sample presented migration rates of 0.0086 $\mu g \cdot min^{-1}$ for DBP, 0.1552 $\mu g \cdot min^{-1}$ for BBP, and 0.2963 $\mu g \cdot min^{-1}$ for DEHP. Therefore, the estimated daily intake of these PAEs corresponded to 0.044 µg/kg body weight (bw)/day for DBP, 1.467 µg/kg bw/day for BBP, and 29.787 µg/kg bw/day for DEHP.

Considering that the tolerable daily intake is 10 µg/kg bw/day for DBP, 500 µg/kg bw/day for BBP, and 50 µg/kg bw/day for DEHP (EU-CSTEE), the estimated daily intake values found in the present study for DBP, BBP, and DEHP were below the acceptable intake limits (EU-CSTEE). However, the estimated daily intake for DEHP (29.787 µg/kg bw/day) is not far from the recommended value (50 µg/kg bw/day), particularly for children with a body weight of 8 kg.

Non-cancer risks were also assessed by determining the Hazard Quotient (HQ), which was calculated as the comparison between the children's mouthing exposure and the RfDs (100, 200, and 200 µg/kg bw/day for DBP, BBP, and DEHP, respectively). The threshold values were not exceeded for any of the PAEs, indicating that the children using the analyzed toys are not at a situation of risk. However, it must be highlighted that this investigation was only focused on a very specific pathway exposure. Children are exposed to PAEs through different routes, so eventually, an aggregated exposure should be evaluated. According to data from the scientific literature (Lioy et al., 2015), ingestion of food and beverages is the most important route of PAEs exposure for infants. However, the potential contribution of toys, via mouthing, as well as personal care products, via dermal contact, cannot be disregarded. It has been stated that mouthing might contribute up to one-fourth of young children's total PAE exposure (Lioy et al., 2015). Anyhow, the current EDI for the Brazilian children was of the same order of magnitude as that found elsewhere (Aurisano et al., 2022).

4. Conclusions

To the best of our knowledge, this study is the first to estimate the exposure of Brazilian children to PAEs and BPs considering toys as the source. Based on our results, DEHP and BPA presented the highest detection rates. BPA concentrations in all analyzed samples were below the threshold (EU-CSTEE). However, DEHP levels in 20 out of the 71 toys were above the maximum values set by the regulatory agencies (EU-CSTEE and INMETRO). Although the migration rates of phthalate esters (DBP, BBP, and DEHP) into saliva were within the tolerable values, some samples presented phthalate concentrations above the regulatory limits, which could considerably contribute to their daily intake by children. Very importantly, children could be at a potential situation of risk if other exposure pathways, such as food consumption or drinking, were also taken into account. It remarks the need to perform an aggregated exposure to chemicals, in general, and PAEs and BPs, in particular. Anyhow, these preliminary data mean a valuable guide for the control of EDC levels in toys commercialized in Brazil and for future investigations on EDC exposure assessment.

5. Declarations

5.1. Funding

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5.2. Conflicts of interest/Competing interests

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.

5.3. Availability of data and materials

All or almost data generated or analyzed during this study are included in this article [and its supplementary information files], and with the corresponding author. If necessary, the corresponding author is available for taking any question about the datasets used and/or analyzed and these can be requested by reasonable request.

5.4. Code availability

Not applicable.

5.5. Author's contributions

Juliana Maria Oliveira Souza: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft.
Marilia Cristina Oliveira Souza: Data curation, Formal analysis, Writing - original draft.
Bruno Alves Rocha: Data curation, Formal analysis, Investigation, Writing - review & editing.
Martí Nadal: Visualization, Writing - original draft.
José Luis Domingo: Writing - review & editing.

Fernando Barbosa: Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing.

5.6. Ethics approval and consent to participate

Not applicable.

5.7. Consent to participate

Not applicable.

5.8. Consent for publication

Not applicable.

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