

Analysis of bisphenols and their derivatives in infant and toddler ready-to-feed milk and powdered milk by LC–MS/MS

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Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Abstract

Ready-to-feed milk and powdered milk packed in different types of packaging lined with resin are chosen as the primary or complementary diet for infants and toddlers. An HPLC–MS/MS method was established and validated for the determination of nineteen bisphenols in such samples. The recoveries were from 31 to 120%, while the values of limit of detection and quantification were in the range of 0.24-0.40 and 0.72-1.2 ng/g, respectively. The method was applied to analyse 40 samples of milk from the Polish market. Ninety-five percent of the studied samples contained a quantified concentration of bisphenols in the range of 0.53-18.51 ng/g. The gathered data were used for exposure and hazard risk assessments using hazard quotient (HQ) and hazard index (HI) values. The mean estimated daily intake (EDI) of bisphenols was determined to be 22-1162 ng/kg b.w./day. The results indicate the consumption of the daily dose of milk and one ready-to-eat product does not exceed the limits of tolerable daily intake of bisphenols. The highest HI values were found in 4-month-old female (1.8). In the average-exposure scenario, no HI values >1 were observed for any age group, whereas hazard is significantly increased taking into account EFSA (European Food Safety Authority) draft opinion.

Keywords: BPA-related compounds; endocrine-disrupting compounds; exposure assessment; liquid chromatography-tandem mass spectrometry; food analysis; food composition;



1. Introduction

Early childhood is a critical window for children's growth, proper development, and well-being. The first 1,000 days of life (from conception to two years) are believed to be one of the most important determinants of long-term human health. Disruption of the proper course of this period can have lifelong consequences because infants' and toddlers' organ systems are still developing, so they may be more sensitive to chemical exposures than adults. There is a large body of epidemiological literature on the effects of early-life exposures on widespread environmental toxicants (Braun, 2017; Dai et al., 2020; Karlsen et al., 2017; Stacy et al., 2017), pharmaceuticals (Bauer et al., 2018; Conradt et al., 2018; Kalloo et al., 2018), and nonchemical stressors (Provençal and Binder, 2015; Sage and Burgio, 2018) on behavioural problems, cognitive function, adiposity and health issues. Endocrine-disrupting compounds (EDCs) are a group of xenobiotics that have attracted the attention of researchers in this context. This is mainly due to their high structural similarity to natural hormones. It has been shown that EDCs may cause disorders in the proper functioning of the endocrine system by, inter alia, modifying the pathways of hormone synthesis and affinity to hormone receptors or causing changes in gene expression (Balaguer et al., 2019; Shanle and Xu, 2011). EDCs include a broad class of compounds such as pesticides, phthalates (PEs), bisphenols (BPs), perfluorinated compounds (PFCs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and organochlorine pesticides (OCPs) (Hu et al., 2021). One of the most widespread EDCs is bisphenol A (BPA), a synthetic compound used extensively worldwide to produce polycarbonate plastics and resins (Kawa et al., 2021). Prenatal exposure to this compound has been associated with child neurobehavioural disorders (Jiang et al., 2020), decreased birth length, increased ponderal index (Yang et al., 2021), and an increased risk of allergic diseases. Because of the health risks associated with BPA, its use has been restricted in many countries over the past few years. Therefore the consumption of BPA analogues that share the basic structure of two phenol groups, with hydroxy moieties at the para positions, and joined by a carbon or sulfur bridge such bisphenol F (BPF) and bisphenol S (BPS), bisphenol AF (BPAF), bisphenol Z (BPZ) and others has increased (Chen et al., 2016;



Liotta et al., 2022). Moreover several chemicals that are structurally similar to BPA are utilized in the manufacture of resins and plastics. Bisphenol A diglycidyl ether (BADGE) and bisphenol F ether (BFDGE) synthesized by the reaction of epichlorohydrin with BPA or BPF and are a building block of epoxy resins that coat food and beverage cans. As shown in many studies, bisphenol compounds can migrate from the inner coating of the packaging into the stored food (Errico et al., 2014; Gallo et al., 2017; Hahladakis et al., 2018; Nouredine et al., 2019).

To date, only a few reports on the negative effects of BPA-related chemicals on children's development can be found in the literature. However, *in vitro* and animal studies have suggested that bisphenol analogues may have similar health effects to BPA (Moreman et al., 2017; Russo et al., 2018; Siracusa et al., 2018). It was found that perinatal exposure to BPS may increase the risk of obesity in male mouse offspring (Ahn et al., 2020; Meng et al., 2019, 2018), whereas perinatal exposure to bisphenol AF (BPAF) was associated with impaired cognitive function of adult mouse offspring (Zhang et al., 2021). There is also evidence that early-life exposure to BPS disrupts male mammary gland morphology (Kolla et al., 2019).

Dietary intake is a major way of incorporating these compounds into humans. Infants may be more exposed to xenobiotics than adults via their diets because they consume more food per unit of body weight (Nougadère et al., 2020). In particular, since neither human milk nor infant formula is free of xenobiotics, they can contain contaminants. The occurrence of BPA and its analogues in human milk is well documented in many reports. However, there is much less information on the BPA related compounds present in infant formula samples. To fill this research gap and better assess the dietary risk of BPs, we quantified the levels of nineteen bisphenol related compounds in infants and follow-on formulas from various brands commonly consumed in Poland. In view of the high probability of the presence, (in addition to BPA analogues), of bisphenol A diglycidyl ether (BADGE) and its derivatives, which may be released from epoxy resins used in the manufacture of packaging, it was decided to extend the range of the monitored compounds also to include BADGEs for a full



assessment of exposure to bisphenols The obtained data, combined with those described in previous research, were used to evaluate the total daily intake of BPs and the health risk evaluation of children at different ages associated with consumption.

2. Materials and methods

2.1. Chemicals and materials

Standards consisted of bisphenol A (BPA), bisphenol BP (BPBP), bisphenol C (BPC), bisphenol F (BPF), bisphenol FL (BPFL), bisphenol G (BPG), bisphenol M (BPM), bisphenol S (BPS), bisphenol Z (PBZ), bisphenol A diglycidyl ether (BADGE), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H₂O), bisphenol A bis (2,3-dihydroxypropyl) ether (BADGE·2H₂O), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl), bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl) ether (BADGE·H₂O·HCl), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl), bisphenol F diglycidyl ether (BFDGE), bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE·2H₂O), bisphenol F bis (3-chloro-2-hydroxypropyl) ether (BFDGE·2HCl) (Sigma–Aldrich, St. Louis, USA) with 99% purity, while internal standards were deuterated bisphenol A diglycidyl ether (d₁₀-BADGE) and ¹³C-labelled bisphenol A (ring ¹³C₁₂) supplied by Cambridge Isotope Laboratories. Methanol (MeOH), acetone of LC–MS hypergrade purity was purchased from Merck KgaA (Darmsadt, Germany), and ammonium formate was purchased from Sigma–Aldrich. Ultrapure water was obtained with a Hydrolab HLP5 system (Straszyn, Poland) with an EDS-Pak cartridge for removal of endocrine-disrupting compounds. Membrane sheets (0.1 μm pore size, thickness 100 μm) were obtained from GVS Filter Technology (Rome, Italy). Syringe filters (nylon, 13 mm wide, 0.22 μm) were purchased from Labfil ALWSCI (Hangzhou, China).

2.2. Samples



A total of 40 milk formula samples recommended for infants and toddlers aged 0–36 months belonging to different brands, which are the most popular and the most available in the Polish market, were purchased at local supermarkets in Gdańsk, Poland, in 2021. Samples include liquid ready-to-eat (n = 11) and powder (n = 29) samples. Among them were 16 special milk samples, including milks for infants with gastrointestinal problems (n=8), lactose-free milk (n=5), premature formula (n=2) and products based on goat milk (n=3). Liquid samples were packed in polypropylene bottles (n=10) and paperboard boxes (n=1), whereas milk powders were contained in metal cans (n=12) and aluminum-plastic bags (n=17). The samples were divided into three groups based on the age feeding recommendations: group A (for infants 1 to 6 months of age), group B (over 12 months of age), and group C (over 36 months of age). Additional information on the analysed samples is included in Table S1.

All samples were stored at room temperature before analysis. The powdered formulas were prepared by dissolving them in hot Milli-Q water according to the manufacturer's recommendation, while the liquid formulas were directly subjected to the extraction process. To avoid contamination all glassware and stainless steel instruments were rinsed with prepared ultrapure water and with acetone, similar to procedure described by A. Jebara et al (Jebara et al., 2021) and by V. Lo Turco (Lo Turco et al., 2015)

2.3. Preparation of standards and calibration

Stock solutions and working solutions of all analytes and internal standards were prepared in MeOH to obtain 0.5 mg/mL. Further dilution was used for the preparation of the calibration solutions (1, 2, 5, 10, 20 and 50 ng/mLm with IS at 20 ng/mL in each) and spiked samples. Solutions were stored in a freezer (-20°C), and every week, new calibration solutions were prepared.

2.4. Sample preparation

Ultrasound-assisted solvent extraction of porous membrane-packed samples was carried out according to the elaborated procedure described in (Szczepańska et al., 2020) slight modifications. The 0.5 mL of milk (spiked or real) was added to the prepared polypropylene bag (0.75 cm x 0.75 cm) together with 100 μ L of ammonium formate buffer (20 mM, pH = 6.8 not stabilized). The bag was sealed and placed in a vial with 8 mL of methanol and sonicated for 20 min (60 W). Then, the bag was removed, and the extracted solvent was placed in the freezer (-20°C) for one hour. Afterwards, the extract solvent was centrifuged at 4000 rpm for 10 min at -4°C to separate the fat from the aqueous medium. The collected supernatant was evaporated to dryness under a gentle nitrogen stream (35°C). The remaining residue was dissolved in 1 mL of methanol and placed in an autosampler vial prior to analysis. The contact of samples with any plastic was avoided during handling at all steps to avoid contamination with possible presence of analytes. The extract volume of MeOH was kept at 8 mL and blank samples of methanol and of methanolic extracts were collected as well.

2.5. Chromatographic conditions

The analyses were performed with a Shimadzu triple quadrupole LC-MS/MS system (LCMS-8060, Shimadzu, Japan) equipped with an electrospray ionization source (ESI) working in the positive and negative multiple reaction monitoring (MRM) mode. Two methods were used to determine BADGE, BADGE·H₂O, BADGE·2H₂O, BADGE·HCl, BADGE·H₂O·HCl, BADGE·2HCl, BFDGE, BFDGE·2H₂O, BFDGE·2HCl, BPBP, BPC, BPF, BPFL, BPG, BPM, BPM, PBZ, BPA and BPS. All compounds were separated using gradient elution mode, while the last two compounds were separated using isocratic mode. All analytes were separated with the use of Phenomenex Kinetex EVO C18 (1.7 μ m, 100 Å, 100 mm x 2.1 mm). The gradient method consisted of (A) water with 0.01% v/v ammonia while (B) was MeOH (0 min – 30% B, 0.5 min – 30% B, 10 min – 70% B, 14 min – 70% B), while isocratic mode consisted of (A) water and (B) MeOH at a ratio of 55/45 v/v with a runtime of 7.5 min. The flow rate

for both methods was 0.6 mL/min, and injection volume was 1 µL. The column temperatures were 50°C and 45°C for the gradient and isocratic modes, respectively.

2.6. Infants and toddlers exposure and hazard risk assessment

2.6.1. Estimation of daily intake from milk formula feeding

To determine the amount of BPs consumed by infants and toddlers on a daily basis milk consumption, the daily intake was estimated (EDI) as previously described, according to Eq (1).

$$EDI = \frac{C \cdot IR}{BW} \quad (1)$$

where EDI is the estimated daily intake [ng/kg body weight/day], C is the concentration of the identified compound in products [ng/g], IR is the average food consumption [g/day], and BW is the average body weight [kg].

Because both the body weight of children in different months of life and the volume of milk consumed differ significantly, we estimated exposure to BPs via baby food ingestion for five different age groups: 1-month-, 4-month-, 6-month-, 12-month-, and 36-month-old infants. Additionally, taking into account the sex differences in weight, it was decided to calculate the EDI value for each sex separately. The values recommended by the manufacturer, appropriate for a specific age group of children, were adopted as the average daily consumption. The median body weight for an age group of females and males was set according to the WHO Child Growth Standards ("World Health Organization Weight-for-age," n.d.) as follows: for 1 month of age, 4.2 kg (female) and 4.5 kg (male); for 4 months of age, 6.4 kg (female) and 7 kg (male); for 6 months of age, 7.3 kg (female) and 7.9 kg (male); for 12 months of age, 8.9 kg (female) and 9.6 kg (male); and for 36 months of age, 13.9 kg

(female) and 14.3 kg (male) (“World Health Organization, Child growth standards, Weight-for-age,” n.d.).

Two different exposure scenarios—low (based on geometric mean) and high (95th percentile)—were considered for each bisphenol.

2.7. Hazard quotient and Hazard index determination

Furthermore, the hazard quotient (HQ) (eq. 2) and hazard index (HI) according to eq. 3 were calculated

$$HQ = \frac{EDI}{TDI} \quad (2)$$

$$HI = \sum_{i=k}^n THQ_i \quad (3)$$

where EDI is the estimated daily intake, TDI is the tolerable daily intake of compound and HI is the sum of individual THQ_i values obtained from equation 2.

To assess the overall dietary exposure of infants and toddlers to BPs associated with consuming baby foods, the data collected from two series of experiments were used for calculations. The EDI was calculated assuming that the daily consumption of food is one whole ready-to-eat product and the appropriate volume of milk recommended by the producer for a specific age.

EDI, HQ, and HI values were calculated taking into consideration three age groups (6, 12 and 36 months). The age group of 1 to 4 months was assumed to consume only milk, so it was omitted from these calculations. The density of milk was marginally higher than 1 (1.029-1.033 g/mL). In the step of calculating the total EDI value, the density of milk was assumed to be equal to 1 g/mL. Using this assumption, the BP content in the studied milk samples was converted from mL to g of product.

3. Results and discussion

3.1. Matrix selection and matrix influence

The main idea was to find a matrix based on natural milk or powdered natural milk. For this purpose, three different matrices were chosen: mare milk, powdered mare milk and human milk. All these matrices were prepared according to the sample preparation protocol. The obtained chromatograms of blank samples are presented in Fig. 1. In all three matrices, at least 5 compounds were detected with a signal intensity high enough to produce a repeatable signal ($n=3$); the results are presented in Table S2. Among the detected compounds, the most frequently occurring in all three matrices was BADGE·H₂O·HCl, which ranged from 2.2 to 8.2 ng/g. In the mare milk as the only matrix, BPA and BPS were detected, while the mare milk powder contained BPFL, BPM, BPG and BPP. In the case of human milk, all three compounds from the BFDGE group were detected (BFDGE, BFDGE·2H₂O and BFDGE·2HCl), which was surprising because BFDGE was banned for use in food contact materials under European Regulations (EC) No. 1895/2005. The content of these compounds might be connected with exposure to them from different sources than food including environmental sources (Lo Turco et al., 2016). Despite this, there was an attempt to spike the samples and calculate recoveries based on calibration curves. In most cases, the signal was enhanced by the sample matrix, and recoveries above 100% were obtained. Apart from the influence of the sample matrix, the detected signals from compounds disqualified these three candidates as a sample matrix for future research. It was decided to run all real samples of milk and powdered milk to determine which samples did not produce a signal above the LOD and could be used as a matrix for subsequent research. Among all samples, one candidate was chosen for subsequent research – a sample of hypoallergenic infant milk from birth. This sample was spiked at three different levels, followed by a validation procedure.

3.2. Method validation

The analytical method for the chosen matrix (powder sample of milk) was evaluated to determine the LODs, LOQs, recoveries (3 spiking levels at 5, 10 and 20ng/g, n=3) and repeatability. The results are presented in Table S3. The matrix effects were evaluated for the chosen powdered milk sample as a matrix (hypoallergic infant milk from birth) among 4 candidates, which also included fresh mare milk, a dietary supplement based on mare milk (powder) and fresh human breast milk, as described in section 3.1. The matrix-matched calibration curves were done in triplicate (n=3) and were linear in the specific range (1, 2, 5, 10, 20 and 50 ng/mL with IS at 20 ng/mL in each), while the correlation coefficients were greater than 0.9962. In most cases, the content of analytes was in the lower range, hence the need to apply a weighing factor of 1/x to increase accuracy. The values of LODs and LOQs were calculated based on calibration curve equations: $LOD=(3.3 \times S_b)/a$ and $LOQ=3 \times LOD$, where a is the slope of the obtained calibration curves and S_b is the standard deviation of the intercept of the calibration curve. The values of LODs fell within the range of 0.24-0.40 ng/g, while values for LOQs were in the range of 0.72-1.2 ng/g. All real samples were analysed on the basis of matrix matched calibration curves, while for the recovery studies, spiked samples were prepared according to the sample preparation protocol at 5, 10 and 20 ng/g in powdered milk samples (hypoallergic infant milk from birth) at three repetitions (n=3). The recoveries for all analytes were in the range of 31-120% for 5 ng/g, 38-111% for 10 ng/g and 47-111% for 20 ng/g. The RSD values for all spiked samples were from 0.3-10%.

3.3. Occurrence of BPA analogues in infant formula samples

The concentrations of BPs in the 40 ready-to-eat milk and powdered infant formula samples are summarized in Table 1.

As shown in the table, out of 40 milk samples, 38 contained a quantified concentration of BPs in the range of 0.53-18.5 ng/g. Only 2 samples of powdered milk stored in aluminium-plastic bags (sample IDs 20 and 21) were free of bisphenols.

All of the analysed bisphenols were detected in at least one sample. The total concentrations of BPs were in the range of 0.68-39.99 ng/mL. BPA was the most abundant contaminant, present in 35 milk samples (87.5%) at concentration levels ranging from 0.68 to 11.19 ng/mL, with a median value at 5.25 ng/mL. BADGE was quantified in 20 samples (50%), while BPS was quantified in 15 samples (37.5%) at concentration levels ranging from 0.73 to 12.27 ng/mL. Despite the fact that the vast majority of the samples tested positive, none of them exceeded the European Union's SML (Specific Migration Limit) for BPA, the sum of BADGE and its hydrolysed derivatives, and BADGE chlorohydrins ((EU) 2018/213, (EC) No 1895/2005) (data not shown). BPBP and BPZ were the least frequently detected compounds. These compounds were detected in only one powdered sample at the levels of 0.55 ng/mL and 0.59 ng/mL, respectively. With each sample batch the extract blanks without sample, solvent blanks and sample blanks (n=3) were run to check possible contamination. No significant signal for all analytes were recorder for blank samples.

Considering the obtained results in terms of the type of packaging in which they were stored, no significant correlation could be established. However, in terms of sample type, liquid samples had a slightly higher level of the determined compounds than powder samples. This is most likely due to the higher fat content of the liquid samples. It has been shown that bisphenols have a greater migration rate into fat-containing foodstuffs (Hahladakis et al., 2018). There is also a large variability in the content of analytes in products classified into three different age groups (Fig 2.).

The BFDGE and hydroxyl- and chlorine derivatives were quantified in some samples (10 samples). The concentration of these compounds in positive samples was in the range of 0.65-1.49 ng/mL. It can also be assumed that in this case, the presence of these compounds could be connected with contamination of infant formula/powdered milk at one of the stages of its production, such as transport, production, or storage.

Generally, variable BPA levels are reported both in ready-to-feed milk and powdered milk collected from markets in different countries of the world. The high frequency and the levels found for BPA are somewhat in line with studies on infant formula products from India. In this study, BPA was detected

in 76.47% of samples at a mean level of 4.46 ng/g (Karsauliya et al., 2021). Similar results were also obtained for milk samples from the USA and Spain. In formula samples from the USA market, BPA was detected in the range of 0.97-1.24 ng/g ww (Haffner et al., 2010) whereas the mean value of BPA in samples from the Spanish market was 3.85 ± 4.19 µg/L. In contrast, the BPA concentration reported for powdered milk and infant formula collected from the Spanish and Italian markets was much higher, in the range of 3-169 ng/g d.w. and from 70-1290 ng/g, respectively (Cirillo et al., 2015; Ferrer et al., 2011). On the other hand, there are also reports where BPA was not detected in the analysed samples. Moreover, in previous studies, BPA was not detected in any of the seventy-six infant formula powdered milk samples purchased from local supermarkets in China in 2017 and the twenty-five samples collected from markets in Brazil in 2021 (Galindo et al., 2021; Sun et al., 2017). In the case of other bisphenols, there are still few reports in the literature about their occurrence in infant formulas and powdered milk samples. However, based on the available results, it can be concluded that most of the quantified BP levels are similar to those reported by other researchers (Karsauliya et al., 2021; Li et al., 2022).

3.4. Estimated intake and risk assessment

To facilitate the daily intake and risk assessment analyses, the samples were grouped into three groups based on their intended age for consumption: 1 to 6 months of age (group A), 7 to 12 months of age (group B), and 13 to 36 months of age (group C). The contribution of each analyte to the sum of the mean concentrations of all target chemicals detected in each of the groups is presented in Fig 2.

The variability of the composition profiles of BPs between each group can be observed. However, the contributions of BPA and BADGE are the highest of all of them. Further details (mean and 95th percentile values) of analytes detected in the studied samples classified into three groups are given in Table 2. As mentioned above, due to the large differences in the body weight of infants and the volume of consumed milk, the EDI was also calculated for infants aged 1 and 4 months. The mean

and 95th percentile values obtained for group A were used for these calculations. The obtained EDI values are summarized in Table 3. The mean daily intake of the studied compounds was significantly dependent on the different feeding periods, in the range of 101-1162 ng/kg/bw/day for 1-month-old female infants and 22–353 ng/kg/bw/day at the age of 36 months. This trend could be explained by the fact that the ratio of milk intake and body weight decreases with age. The highest mean consumption for 1-month-old infants was found for BADGE·H₂O, followed by BADGE, BPA, BADGE·HCl and BPF, while the lowest was found for BPZ, BPBP and BPS. This profile was similar for 4- and 6-month-old infants, while in the case of toddlers (12 and 36 months), the highest exposure dose was found for BADGE and BPA and the lowest for BPZ, BPBP, and BADGE·2H₂O.

The calculated daily intakes are slightly higher than those reported by Karsauliya et al. (Karsauliya et al., 2021). However, in these studies, smaller volumes of consumed milk were used for the calculations, so it can be concluded that the obtained values are relatively comparable. However, the detected levels are significantly higher than the EFSA opinion from 2015, with an assessment mean exposure of 36 ng/kg bw/day for 0–6-month-old children consuming infant formula and 55–159 ng/kg bw/day for 13–36-month-old children (EFSA, 2015).

The THQ and HI are also displayed in Table 3. For each of the considered scenarios, the calculated HQ value for individual compounds was found to be less than 1, which means that BP intake was far below the TDI threshold set by the EFSA. The highest HQ values were 0.2 and 0.34 for the 95th percentile for BPA in one-month-old female infants. Taking into account that the obtained values are significantly below 1, it can be concluded that the exposure of individual bisphenols presents no apparent risk to infants and toddlers through the consumption of ready-to-feed milk and powdered formula. However, when cumulative risk is considered, there is a potential for adverse health effects. The HI value (assuming an average exposure) was found to be above 1 (1.02) only in 1-month-old female infants. However, when a high-exposure scenario was considered, values of HI >1 were obtained for 1-, 4-, and 6-month-old infants, both females and males. The highest HI value was found to be 1.9. However, taking into account that HI values between 1 and 100 indicate that there is a

possibility that adverse effects will be observed, it can be assumed that this risk associated with milk consumption is minor, although it does exist.

The obtained data, combined with those described in *Extraction and analysis of bisphenols and their derivatives in infant and toddler ready-to-feed meals by ultrasound-assisted membrane extraction followed by LC-MS/MS*, were used to evaluate the total daily intake of BPs by infants over 6 months associated with the consumption of both milk and ready-to-eat meals. The mean and 95th percentile values of the hazard quotient and total daily dietary intake of BP analogues are shown in Table 4. On the basis of the calculated values, it can be concluded that the consumption of the daily dose of milk and one ready-to-eat product does not exceed the limits of tolerable daily intake of bisphenols. The highest calculated value of the total daily BPA intake was significantly below the threshold set by EFSA (European Food Safety Authority) in 2015 (4.0 µg/kg bw/day), five times and two times in the medium case and 95th percentile, respectively. The highest HI value was found in female and male 4-month-old infants in a high-exposure scenario, 1.8 and 1.7, respectively. In the case of the average-exposure scenario, no HI values greater than 1 were noticed for any age group. Following the EFSA opinion recommending lowering the BPA EDI from 4 µg/kg/day to 0.04 ng/kg/day (EFSA, 2021), we did a simulation of the impact of the lowered BPA EDI value on determined risk levels (Table S4). Calculated HQ_{BPA} and HI values based on future scenario are significantly higher than those for the current TDI value indicating a significant health risk from BPs dietary exposure.

4. Conclusion

The results presented in this paper and in the preceding one provide a new approach for the determination of BADGE and BFDGE derivatives as well as chosen bisphenols. The sample preparation method is, in our opinion, an interesting alternative to existing methods due to its simplicity and low cost. With some modifications, sample preparation could be used for separation of these analytes from ready-to-feed meals and milk. The presented methodology is characterized by recoveries from 31 to 120%, while the LOD values are from 0.24-0.40 ng/g. We strongly believe that

this method could be suitable for the control and screening purposes of BADGE and BFDGE derivatives along with bisphenols. The limitations of this study are connected with the lack of an available matrix. Several matrices were evaluated for the presence of bisphenols and their derivatives, and only one sample was suitable for further research. The next steps should involve the composition of the matrix, which can be used for screening purposes and monitoring of these compounds.

It should be noted that several limitations are associated with our estimation of dietary exposure and hazard risk, including the small number of food samples for each age group and a lack of consideration of intake of water or other drinking products. Moreover, it should be kept in mind that due to the lack of a TDI threshold values set by the EFSA for all compounds, the assumed values for BPA and BADGE were adopted for the HQ and HI calculations. Hence, the data obtained may be underestimated or overestimated. Therefore, to carry out an accurate risk assessment, it is necessary to establish TDI values for other bisphenols.

On the basis of the obtained results, it can be stated that the health risk resulting from dietary consumption is generally in the safe range (only HI value for 1 month infant is alarming), but the younger the child is, the greater it is.

Therefore, where possible, it seems reasonable to minimize the provision of ready-made meals or milk substitutes to children. The tolerable daily intake thresholds were not exceeded in any case. However, attention must be given to the presence of many of the monitored compounds in the products studied. In most samples, several analytes were identified. Hence, the calculated health risk may be underestimated as the "cocktail effect" may occur. Additionally, if a new EFSA draft opinion concerning the reduction of the BPA TDI value reduction from 4 µg/kg bw/day to 0.04 ng/kg bw/day is issued, it is likely that the TDI will be exceeded in the future. Therefore, in the future, it seems necessary to develop monitoring programs, extend the regulations on the safety assessment of consumer products to all compounds of the bisphenol group and reduce the current SML values.

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Table 1. Real samples analysis of ready-to-drink milk and powder samples.

Sample ID	Analyte Concentration [ng/mL] ± SD																			Σ of analytes
	BADGE	BADGE·H ₂ O	BADGE·2H ₂ O	BADGE·HCl	BADGE·2HCl	BADGE·H ₂ O·HCl	BFDGE	BFDGE·2H ₂ O	BFDGE·2HCl	BPA	BPBP	BPC	BPF	BPFL	BPG	BPM	BPP	BPS	BPZ	
1	4.53±0.16	1.59±0.17	0.680±0.067	1.46±0.11	9.8±1.2	-	-	-	-	4.40±0.47	-	-	2.35±0.11	3.64±0.22	-	-	3.57±0.26	-	0.59±0.12	32.57
2	1.16±0.42	15.74±0.76	-	1.30±0.12	7.11±0.49	-	-	-	-	1.89±0.11	-	2.09±0.44	1.02±0.20	-	-	-	2.13±0.12	-	-	32.44
3	0.55±0.27	-	1.054±0.057	-	4.70±0.30	-	0.65±0.15	-	-	5.64±0.46	0.55±0.19	1.91±0.58	6.66±0.49	7.12±0.65	-	3.28±0.36	4.23±0.26	-	-	36.34
4	0.67±0.39	-	-	-	-	-	1.30±0.12	-	-	2.12±0.58	-	-	-	-	-	-	-	-	-	4.09
5	-	-	-	-	-	-	-	-	1.58±0.23	3.79±0.25	-	1.51±0.42	-	-	-	-	-	-	-	6.88
6	-	-	-	-	1.41±0.32	-	-	-	-	5.16±0.69	-	3.91±0.24	1.89±0.27	-	-	-	-	-	-	15.37
7	18.5±1.6	-	3.75±0.23	-	2.46±0.47	-	-	-	-	6.01±0.75	-	-	0.75±0.72	2.52±0.26	-	2.90±0.51	3.09±0.27	-	-	39.99
8	-	-	-	-	0.66±0.18	-	-	-	-	7.2±1.1	-	-	-	-	-	-	-	-	-	7.84
9	1.72±0.16	-	-	-	1.22±0.30	-	-	-	-	2.19±0.35	-	-	3.00±0.38	-	-	-	0.55±0.15	-	-	8.68
10	-	-	1.22±0.11	-	6.49±0.60	-	-	-	-	4.27±0.66	-	-	-	-	-	-	-	-	-	11.98
11	-	-	-	-	-	-	-	-	-	6.55±0.92	-	-	6.19±0.64	-	-	-	-	-	-	12.74
12	-	-	-	-	-	-	-	-	-	4.99±0.44	-	-	-	-	-	-	-	-	-	4.99
13	-	-	0.672±0.038	-	4.03±0.35	2.21±0.15	-	1.254±0.062	0.87±0.31	1.78±0.22	-	-	-	-	-	-	-	-	-	10.81
14	-	-	-	-	-	-	-	-	-	4.05±0.51	-	1.50±0.33	-	1.59±0.12	-	-	-	-	-	7.14
15	-	-	-	-	-	-	-	-	-	4.86±0.75	-	1.22±0.25	12.27±0.48	-	-	-	-	-	-	18.35
16	-	-	-	-	-	-	-	-	-	3.77±0.12	-	-	-	0.78±0.16	-	-	-	-	-	4.55
17	3.23±0.17	-	0.560±0.025	1.04±0.28	1.20±0.18	2.90±0.15	-	-	-	10.9±1.0	-	-	1.27±0.15	-	0.54±0.22	-	-	-	-	21.61
18	13.24±0.50	-	-	4.50±0.13	7.99±0.15	2.113±0.082	-	-	-	5.7±1.6	-	-	1.30±0.19	-	2.73±0.43	0.66±0.22	-	-	-	13.30
19	1.11±0.16	-	-	-	-	0.59±0.15	-	-	0.80±0.23	4.1±1.5	-	-	-	-	-	-	-	0.87±0.19	-	8.68
20	3.60±0.11	-	-	0.63±0.16	0.60±0.15	1.92±0.21	-	-	-	1.15±0.18	-	-	-	-	-	-	-	-	-	14.24
21	-	3.33±0.25	-	-	-	0.65±0.11	-	-	-	5.65±0.82	-	-	-	-	-	-	-	-	-	29.43
22	-	-	-	-	-	-	-	-	-	0.68±0.14	-	-	-	-	-	-	-	-	-	21.63
23	-	-	-	-	-	-	-	-	-	12.5±1.2	-	1.26±0.32	3.48±0.58	-	-	-	-	-	-	39.54
24	3.91±0.18	-	-	0.96±0.18	4.11±3.57	-	-	-	-	-	-	1.38±0.22	-	2.84±0.40	-	3.04±0.28	3.75±0.26	-	-	7.45
25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.90
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.63
27	5.15±0.27	-	-	0.81±0.19	3.35±0.38	-	1.49±0.18	-	-	6.2±1.5	-	3.12±0.30	4.53±0.12	-	-	-	-	-	-	0.68
28	-	1.73±0.27	-	-	-	-	-	-	-	5.0±1.3	-	-	1.72±0.40	-	-	-	-	-	-	17.22
29	6.86±0.11	-	-	1.15±0.29	5.20±0.16	-	-	-	-	3.63±0.53	-	-	-	-	-	-	-	-	-	19.99
30	-	-	-	-	2.52±0.22	0.82±0.15	-	-	-	-	-	-	-	1.65±0.12	-	-	0.86±0.12	-	-	-
31	-	-	-	-	1.28±0.33	0.98±0.17	-	-	-	9.2±1.4	-	-	0.73±0.12	1.15±0.21	-	-	-	-	-	-
32	-	1.85±0.16	-	-	3.80±0.29	-	1.69±0.21	-	0.56±0.25	1.18±0.30	-	-	-	-	-	-	-	-	-	24.60
33	3.90±0.17	-	-	-	2.91±0.23	-	-	-	-	6.66±0.41	-	-	-	-	-	-	-	-	-	8.48
34	19.02±0.43	-	-	1.38±0.30	7.39±0.70	-	-	-	-	1.64±0.34	-	-	-	-	-	-	-	-	-	16.84
35	2.901±0.052	-	-	1.11±0.27	-	1.07±0.18	1.43±0.20	-	-	3.51±0.93	-	9.4±1.1	-	-	1.25±0.14	-	-	-	-	20.68
36	10.61±0.30	-	-	2.46±0.13	1.65±0.11	1.13±0.18	-	-	-	7.8±1.4	-	3.63±0.27	-	-	-	-	-	0.53±0.13	-	27.85
37	4.78±0.31	-	-	1.05±0.13	-	2.08±0.22	-	-	-	11.2±1.2	-	1.22±1.1	1.19±0.22	-	1.29±0.11	-	-	-	-	22.80
38	12.59±0.63	1.44±0.13	-	2.69±0.28	2.50±0.40	1.54±0.13	-	-	-	4.08±0.41	-	5.98±1.1	-	0.54±0.10	-	-	-	-	-	31.36
39	1.13±0.23	1.49±0.13	-	-	-	0.58±0.17	-	-	0.66±0.15	-	-	-	-	-	0.59±0.14	-	-	-	-	4.45
40	-	-	-	-	-	-	-	-	-	11.5±1.9	-	-	-	-	-	-	-	-	-	11.50

not detected



Table. 2. Concentration (mean and 95th percentile) and detection frequency of bisphenols in ready-to-feed milk and powdered milk for infant and toddlers collected in Gdańsk, Poland.

group analyte	A (n=21)			B (n=13)			C (n=6)		
	mean [ng/g]	95th percentile [ng/g]	detection frequency [%]	mean [ng/g]	95th percentile [ng/g]	detection frequency [%]	mean [ng/g]	95th percentile [ng/g]	detection frequency [%]
BADGE	4.8	16	48	7.8	17	38	6.4	12	83
BADGE·H ₂ O	6.9	15	14	1.8	1.8	15	1.5	1.5	33
BADGE·2H ₂ O	1.3	3.1	29	-	-	-	-	-	-
BADGE·HCl	1.8	3.9	24	1.1	1.4	31	1.8	2.7	67
BADGE·2HCl	4.0	8.8	57	3.8	6.6	62	2.1	2.5	33
BADGE·H ₂ O·HCl	1.7	2.7	29	0.90	0.97	15	1.3	2.0	83
BFDGE	0.98	1.3	9.5	1.6	1.7	15	1.4	1.4	17
BFDGE·2H ₂ O	1.3	1.3	4.8	-	-	-	-	-	-
BFDGE·2HCl	1.2	1.5	19	0.56	0.56	7.7	0.66	0.66	17
BPA	7.2	14	100	5.7	12	69	9.0	4	83
BPBP	0.55	0.55	4.8	-	-	-	-	-	-
BPC	2.0	3.5	29	1.9	3.0	23	5.1	8.9	67
BPF	3.7	9.8	48	2.6	4.4	31	1.0	1.2	17
BPFL	3.1	6.4	24	1.9	2.7	23	0.54	0.54	17
BPG	1.6	2.6	9.5	-	-	-	1.1	1.3	50
BPM	2.3	3.2	14	3.0	3.0	7.7	-	-	-
BPP	2.7	4.1	24	1.8	3.5	23	-	-	-
BPS	0.87	0.87	4.8	-	-	-	0.53	0.53	17
BPZ	0.59	0.59	4.8	-	-	-	-	-	-

Table 3. Estimated Daily Intake (EDI), Hazard Quotient (HQ) and Hazard Index (HI) of bisphenol analogue according to the age of male (M) and female (F) infants and toddlers.

analyte	parameter	age [months]									
		1		4		6		12		36	
		F	M	F	M	F	M	F	M	F	M
BADGE	EDI [ng/kg/b.w./day] mean	850	791	611	559	459	398	499	463	363	353
	(95th percentile)	(2890)	(2695)	(1855)	(1696)	(1365)	(1246)	(1139)	(1056)	(748)	(727)
BADGE-H ₂ O	EDI [ng/kg/b.w./day] mean	1162	1085	823	753	745	688	104	97	80	78
	(95th percentile)	(2480)	(2315)	(1646)	(1505)	(1450)	(1340)	(128)	(119)	(80)	(78)
BADGE-2H ₂ O	EDI [ng/kg/b.w./day] mean	254	237	189	173	146	135	-	-	-	-
	(95th percentile)	(624)	(582)	(438)	(401)	(313)	(290)	-	-	-	-
Σ BADGE, BADGE-H ₂ O, BADGE-2H ₂ O	HQ	0.015	0.014	0.011	0.010	0.0090	0.0081	-	-	-	-
	(95th percentile)	(0.040)	(0.037)	(0.026)	(0.024)	(0.021)	(0.019)	-	-	-	-
BADGE-HCl	EDI [ng/kg/b.w./day] mean	276	258	152	139	129	119	66	61	67	65
	(95th percentile)	(564)	(527)	(180)	(165)	(161)	(149)	(93)	(86)	(99)	(96)
BADGE-2HCl	EDI [ng/kg/b.w./day] mean	711	650	497	454	395	365	247	229	170	65
	(95th percentile)	(1535)	(1433)	(1049)	(959)	(832)	(769)	(442)	(410)	(296)	(287)
BADGE-H ₂ O-HCl	EDI [ng/kg/b.w./day] mean	280	275	262	240	306	282	64	59	67	65
	(95th percentile)	(439)	(410)	(432)	(395)	(548)	(507)	(69)	(64)	(99)	(96)
Σ BADGE-HCl, BADGE- 2HCl, BADGE-H ₂ O-HCl	HQ	0.0084	0.0079	0.0061	0.0056	0.0055	0.0051	-	-	-	-
	(95th percentile)	(0.017)	(0.016)	(0.011)	(0.010)	(0.010)	(0.0095)	-	-	-	-
BFDGE	EDI [ng/kg/b.w./day] mean	201	188	143	131	109	100	88	81	59	57
	(95th percentile)	(271)	(253)	(179)	(164)	(126)	(117)	(91)	(84)	(61)	(60)
BFDGE-2H ₂ O	EDI [ng/kg/b.w./day] mean	232.14	217	195	179	180	146	-	-	-	-
	(95th percentile)	(232)	(217)	(195)	(179)	(180)	(146)	-	-	-	-
BFDGE-2HCl	EDI [ng/kg/b.w./day] mean	200	187	169	155	120	112	40	37	24	24
	(95th percentile)	(297)	(277)	(215)	(197)	(152)	(141)	(40)	(37)	(24)	(24)
Σ BFDGE, BFDGE-2H ₂ O, BADGE-2HCl	HQ	0.0042	0.0039	0.0034	0.0031	0.0027	0.0024	0.00085	0.00079	0.00055(0.00057)	0.00054
	(95th percentile)	(0.0053)	(0.0050)	(0.0039)	(0.0036)	(0.0031)	(0.0027)	(0.00087)	(0.00081)	-	(0.00055)
BPA	EDI [ng/kg/b.w./day] mean	802	749	679	628	679	627	332	308	244	237
	(95th percentile)	(1360)	(1269)	(1114)	(1019)	(1619)	(1496)	(789)	(732)	(507)	(493)
	HQ	0.20	0.19	0.17	0.16	0.17	0.16	0.083	0.077	0.061	0.059
BPBP	EDI [ng/kg/b.w./day] mean	105	98	88	94	75	70	-	-	-	-
	(95th percentile)	(105)	(98)	(88)	(94)	(75)	(70)	-	-	-	-
	HQ	0.026	0.024	0.022	0.024	0.019	0.017	-	-	-	-
BPC	EDI [ng/kg/b.w./day] mean	372	347	285	269	234	216	121	112	208	202
	(95th percentile)	(593)	(554)	(466)	(438)	(435)	(402)	(168)	(155)	(380)	(370)
	HQ	0.093	0.087	0.071	0.067	0.058	0.054	0.030	0.028	0.052	0.051
BPF	EDI [ng/kg/b.w./day] mean	666	622	560	531.23	460	425	157	146	60	58
	(95th percentile)	(1921)	(1792)	(1460)	(1403)	(1091)	(1008)	(253)	(235)	(60)	(58)

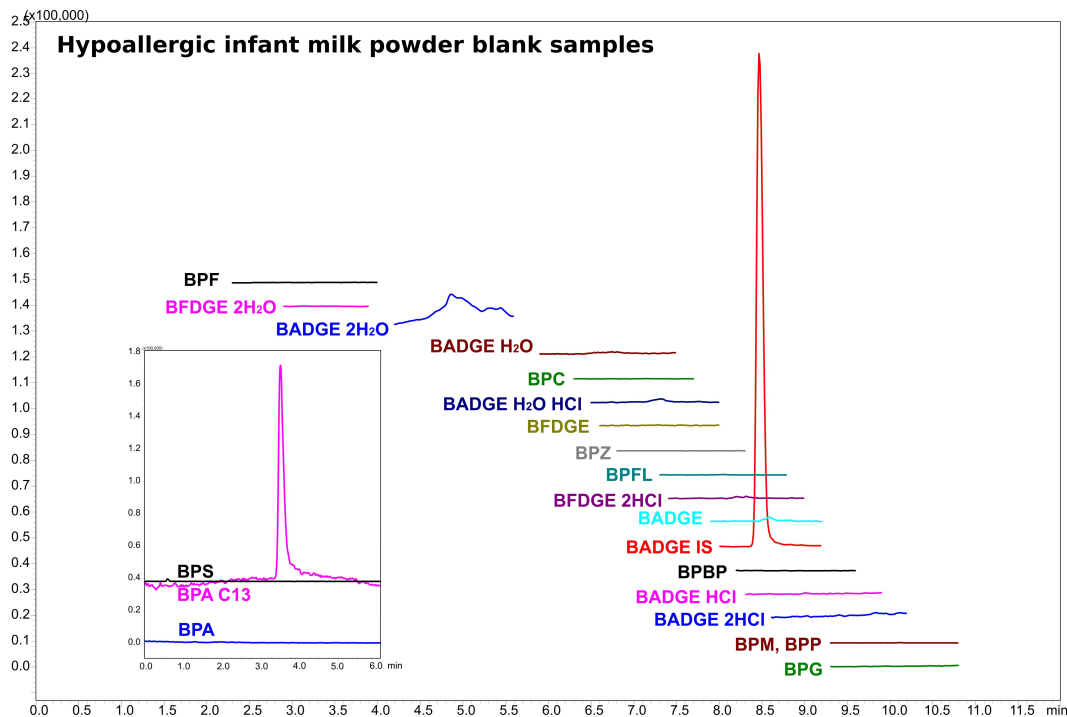
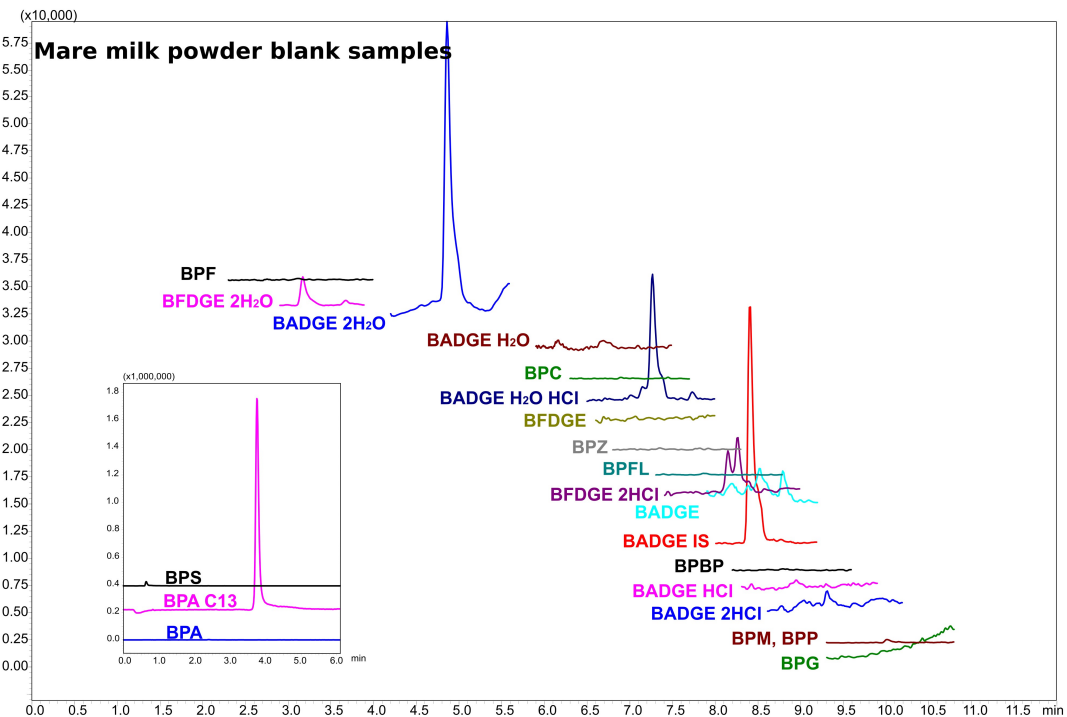
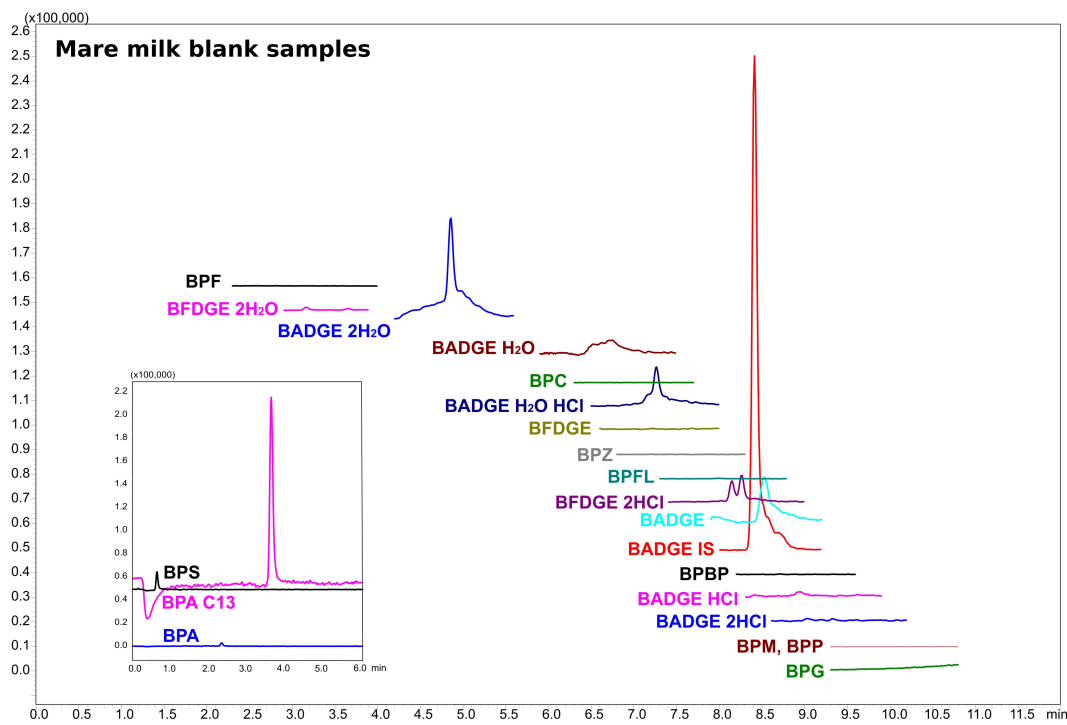
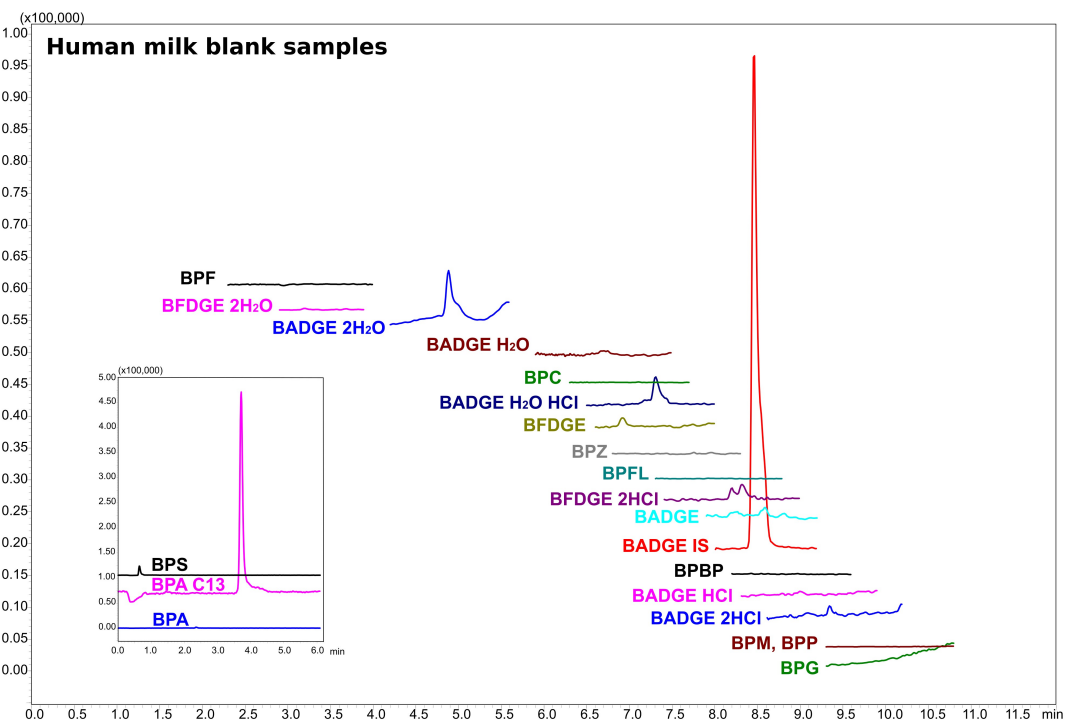
	HQ	0.17	0.16	0.14	0.13	0.11	0.11	0.039	0.036	0.015	0.015
	(95th percentile)	(0.48)	(0.45)	(0.36)	(0.35)	(0.27)	(0.25)	(0.063)	(0.059)	(0.015)	(0.015)
BPFL	EDI [ng/kg/b.w./day] mean	572	534	450	449	374	345	133	123	-	-
	(95th percentile)	(1210)	(1129)	(990)	(1051)	(852)	(787)	(193)	(179)	-	-
	HQ	0.14	0.13	0.11	0.11	0.093	0.086	0.033	0.031	-	-
	(95th percentile)	(0.30)	(0.28)	(0.25)	(0.26)	(0.021)	(0.20)	(0.048)	(0.045)	-	-
BPG	EDI [ng/kg/b.w./day] mean	237	221	84	77	78	72	-	-	60	58
	(95th percentile)	(375)	(350)	(84)	(77)	(78)	(72)	-	-	(65)	(63)
	HQ	0.059	0.055	0.021	0.019	0.019	0.018	-	-	0.015	0.015
	(95th percentile)	(0.094)	(0.087)	(0.021)	(0.019)	(0.019)	(0.018)	-	-	(0.016)	(0.016)
BPM	EDI [ng/kg/b.w./day] mean	433	404	465	468	368	340	215	200	-	-
	(95th percentile)	(620)	(579)	(517)	(553)	(441)	(408)	(215)	(200)	-	-
	HQ	0.11	0.10	0.12	0.12	0.092	0.085	0.054	0.050	-	-
	(95th percentile)	(0.16)	(0.14)	(0.13)	(0.14)	(0.11)	(0.10)	(0.054)	(0.050)	-	-
BPP	EDI [ng/kg/b.w./day] mean	514	480	377	367	300	277	127	118	33	32
	(95th percentile)	(768)	(717)	(626)	(660)	(534)	(493)	(245)	(227)	(33)	(32)
	HQ	0.13	0.12	0.094	0.092	0.075	0.069	0.032	0.029	0.0083	0.0081
	(95th percentile)	(0.19)	(0.18)	(0.16)	(0.16)	(0.13)	(0.12)	(0.061)	(0.057)	(0.0083)	(0.0081)
BPS	EDI [ng/kg/b.w./day] mean	145	135	163	149	-	-	-	-	23	22
	(95th percentile)	(145)	(135)	(163)	(149)	-	-	-	-	(23)	(22)
	HQ	0.036	0.034	0.041	0.037	-	-	-	-	0.0057	0.0056
	(95th percentile)	(0.036)	(0.034)	(0.041)	(0.037)	-	-	-	-	(0.0057)	(0.0056)
BPZ	EDI [ng/kg/b.w./day] mean	101	94	66	61	58	54	-	-	-	-
	(95th percentile)	(101)	(94)	(66)	(61)	(58)	(54)	-	-	-	-
	HQ	0.025	0.024	0.017	0.015	0.015	0.013	-	-	-	-
	(95th percentile)	(0.025)	(0.024)	(0.017)	(0.015)	(0.015)	(0.013)	-	-	-	-
HI		1.0	0.95	0.83	0.79	0.67	0.62	0.28	0.26	0.16	0.16
HI 95th		1.9	1.7	1.4	1.4	1.3	1.2	0.48	0.44	0.28	0.27



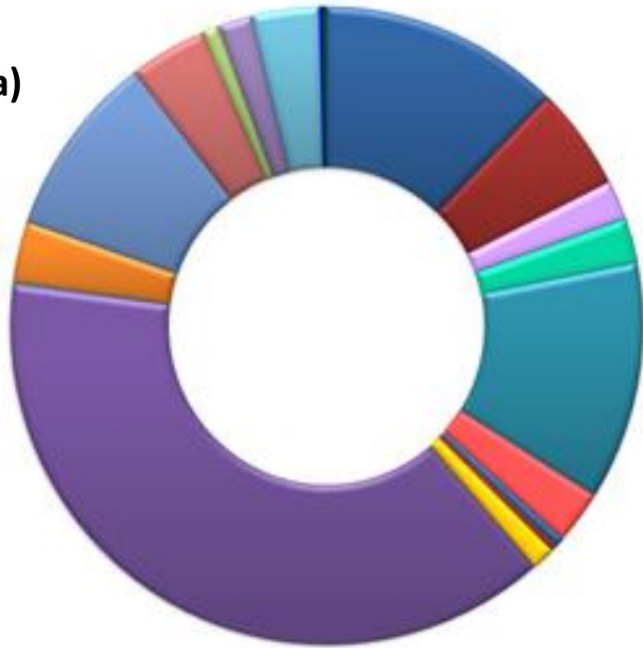
Table 4. Estimated Daily Intake (EDI), Hazard Quotient (HQ) and Hazard Index (HI) of bisphenols according to the age of male (M) and female (F) infants and toddlers connected with consumption of the daily dose of milk and one ready-to-eat product.

analyte	parameter	age [months]					
		6		12		36	
		F	M	F	M	F	M
BADGE	EDI [ng/kg/b.w./day]						
	mean	488	425	681	632	401	390
	(95th percentile)	(1411)	(1289)	(1975)	(1831)	(817)	(794)
BADGE-H ₂ O	EDI [ng/kg/b.w./day]						
	mean	772	713	157	145	184	176
	(95th percentile)	(1481)	(1369)	(269)	(250)	(269)	(261)
BADGE-2H ₂ O	EDI [ng/kg/b.w./day]						
	mean	212	197	118	109	81	78
	(95th percentile)	(520)	(481)	(302)	(280)	(120)	(117)
Σ BADGE, BADGE-H ₂ O, BADGE-2H ₂ O	HQ						
	(95th percentile)	0.010 (0.023)	0.0089 (0.021)	0.0064 (0.017)	0.0059 (0.016)	0.0044 (0.0080)	0.0043 (0.0078)
BADGE-HCl	EDI [ng/kg/b.w./day]						
	mean	129	159	105	98	114	111
	(95th percentile)	(161)	(209)	(154)	(143)	(147)	(143)
BADGE-2HCl	EDI [ng/kg/b.w./day]						
	mean	1165	1076	698	647	211	205
	(95th percentile)	(4081)	(3771)	(2051)	(1901)	(425)	(413)
BADGE-H ₂ O-HCl	EDI [ng/kg/b.w./day]						
	mean	348	320	514	477	112	109
	(95th percentile)	(623)	(576)	(1678)	(1555)	(186)	(181)
Σ BADGE-HCl, BADGE-2HCl, BADGE-H ₂ O-HCl	HQ						
	(95th percentile)	0.014 (0.037)	0.013 (0.035)	0.013 (0.037)	0.012 (0.034)	0.0056 (0.0092)	0.0054 (0.0089)
BFDGE	EDI [ng/kg/b.w./day]						
	mean	109	100	100	93	59	57
	(95th percentile)	(126)	(117)	(105)	(98)	(61)	(60)
BFDGE-2H ₂ O	EDI [ng/kg/b.w./day]						
	mean	180	146	15	14	-	-
	(95th percentile)	(180)	(146)	(16)	(15)	-	-
BFDGE-2HCl	EDI [ng/kg/b.w./day]						
	mean	143	132	62	57	41	40
	(95th percentile)	(175)	(162)	(65)	(60)	(47)	(46)
Σ BFDGE, BFDGE-2H ₂ O, BFDGE-2HCl	HQ						
	(95th percentile)	0.0029 (0.0032)	0.0025 (0.0028)	0.0012 (0.0012)	0.0011 (0.0012)	0.00606 (0.00072)	0.0065 (0.00070)
BPA	EDI [ng/kg/b.w./day]						
	mean	711	657	332	308	332	322
	(95th percentile)	(1661)	(1535)	(789)	(732)	(626)	(609)
	HQ	0.18	0.16	0.083	0.077	0.083	0.081
	(95th percentile)	(0.42)	(0.38)	(0.20)	(0.18)	(0.16)	(0.15)
BPBP	EDI [ng/kg/b.w./day]						
	mean	104	96	48	44	20	19
	(95th percentile)	(120)	(111)	(150)	(139)	(27)	(26)
	HQ	0.026	0.024	0.012	0.011	0.0049	0.0048
	(95th percentile)	(0.030)	(0.028)	(0.038)	(0.035)	(0.0067)	(0.0066)
BPC	EDI [ng/kg/b.w./day]						
	mean	394	364	234	217	237	230
	(95th percentile)	(995)	(920)	(456)	(422)	(443)	(430)
	HQ	0.10	0.091	0.058	0.054	0.059	0.058
	(95th percentile)	(0.25)	(0.23)	(0.11)	(0.11)	(0.11)	(0.11)
BPF	EDI [ng/kg/b.w./day]						
	mean	460	425	182	169	92	89
	(95th percentile)	(1091)	(1008)	(282)	(261)	(92)	(89)
	HQ	0.11	0.11	0.046	0.042	0.023	0.022
	(95th percentile)	(0.27)	(0.25)	(0.07)	(0.07)	(0.02)	(0.02)
BPFL	EDI [ng/kg/b.w./day]						
	mean	481	444	312	290	213	207
	(95th percentile)	(1082)	(1000)	(527)	(489)	(213)	(207)
	HQ	0.12	0.11	0.078	0.072	0.053	0.052
	(95th percentile)	(0.27)	(0.25)	(0.13)	(0.12)	(0.053)	(0.052)

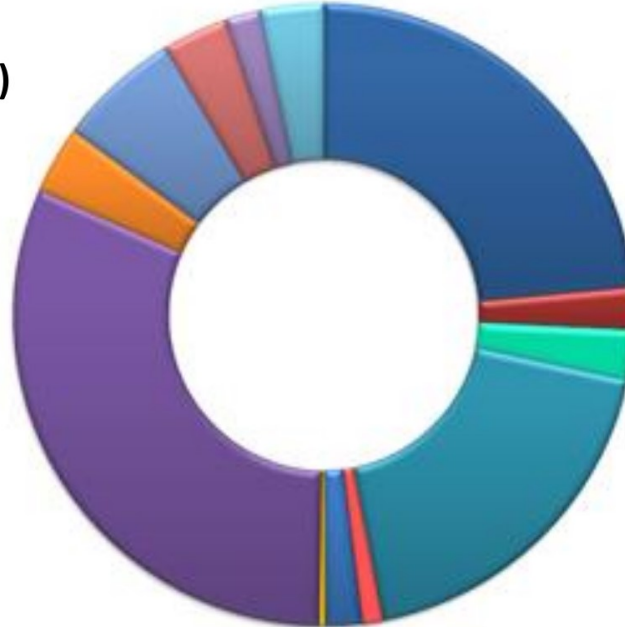
BPG	EDI [ng/kg/b.w./day]						
	mean	144	133	127	119	102	99
	(95th percentile)	(245)	(227)	(345)	(320)	(194)	(189)
	HQ	0.036	0.033	0.032	0.030	0.026	0.025
	(95th percentile)	(0.061)	(0.057)	(0.086)	(0.080)	(0.049)	(0.047)
BPM	EDI [ng/kg/b.w./day]						
	mean	368	340	235	218	9.3	9.0
	(95th percentile)	(441)	(408)	(252)	(233)	(9.3)	(9.0)
	HQ	0.092	0.085	0.059	0.054	0.0023	0.0023
	(95th percentile)	(0.11)	(0.10)	(0.063)	(0.058)	(0.0023)	(0.0023)
BPP	EDI [ng/kg/b.w./day]						
	mean	347	321	262	243	83	81
	(95th percentile)	(664)	(613)	(643)	(596)	(179)	(174)
	HQ	0.087	0.080	0.066	0.061	0.021	0.020
	(95th percentile)	(0.17)	(0.15)	(0.16)	(0.15)	(0.045)	(0.044)
BPS	EDI [ng/kg/b.w./day]						
	mean	120	111	38	35	57	56
	(95th percentile)	(426)	(393)	(72)	(66)	(57)	(56)
	HQ	0.030	0.028	0.010	0.0088	0.014	0.014
	(95th percentile)	(0.11)	(0.10)	(0.018)	(0.017)	(0.014)	(0.014)
BPZ	EDI [ng/kg/b.w./day]						
	mean	111	102	57	53	18	17
	(95th percentile)	(243)	(224)	(217)	(202)	(48)	(46)
	HQ	0.028	0.026	0.014	0.013	0.0045	0.0043
	(95th percentile)	(0.061)	(0.056)	(0.054)	(0.050)	(0.012)	(0.012)
	HI	0.83	0.77	0.47	0.44	0.30	0.29
	HI 95th	1.4	1.3	0.83	0.77	0.42	0.41



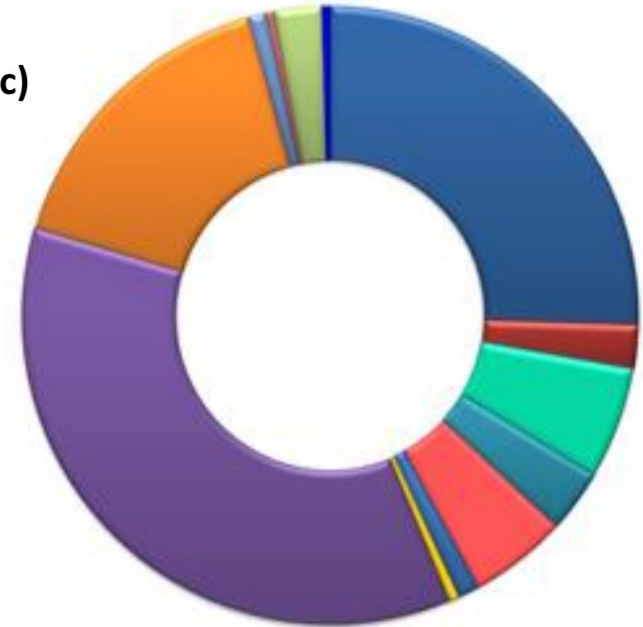
a)



b)



c)



■ BADGE

■ BADGE·H₂O·HCl

■ BPBP

■ BPM

■ BADGE·H₂O

■ BFDGE

■ BPC

■ BPP

■ BADGE·2H₂O

■ BFDGE·2H₂O

■ BPF

■ BPS

■ BADGE·HCl

■ BFDGE·2HCl

■ BPFL

■ BPZ

■ BADGE·2HCl

■ BPA

■ BPG

Analysis of bisphenols and their derivatives in infant and toddler ready-to-feed milk and powdered milk by LC–MS/MS

SUPPLEMENTARY MATERIAL

Table S1. Basic information on the commercially available ready-to-feed milk and powdered milk for infants and toddlers examined in this study.

group	Sample ID	Type of sample	Formula type	Packaging material	Macronutrients content [g/100 ml of prepared formula]			Recommended daily intake [ml]
					Fat	Sugar	Protein	
A	1	powder	infant	Metal can	3.56	7.51	1.24	1 month: 720 4 months: 720 6 months: 720
	2	powder	infant	Aluminium-plastic bag	3.4	7.3	1.3	1 month: 840 4 months: 900 6 months: 720
	3	powder	infant	Aluminium-plastic bag	3.5	0.71	1.5	1 month: 800 4 months: 1020 6 months: 1000
	4	powder	infant	Aluminium-plastic bag	3.4	7.3	1.3	1 month: 840 4 months: 900 6 months: 720
	5	powder	infant	Aluminium-plastic bag	3.4	6.1	1.5	1 month: 840 4 months: 900 6 months: 720
	6	powder	infant	Aluminium-plastic bag	3.4	6	1.4	1 month: 700 4 months: 850 6 months: 920
	7	powder	infant	Aluminium-plastic bag	3.4	3.3	1.5	1 month: 840 4 months: 900 6 months: 720
	8	powder	infant	Aluminium-plastic bag	3.6	6.9	1.25	1 month: 700 4 months: 850 6 months: 940
	9	powder	infant	Metal can	3.4	3.3	1.5	1 month: 840 4 months: 900 6 months: 720
	10	powder	infant	Metal can	3.4	1.2	1.3	1 month: 840 4 months: 900 6 months: 720
	11	powder	infant	Metal can	3.4	5	1.3	1 month: 600 4 months: 900 6 months: 1050
	12	powder	infant	Metal can	3.4	4.1	1.3	1 month: 600 4 months: 900 6 months: 1050
	13	powder	infant	Metal can	3.7	6.7	1.28	1 month: 780 4 months: 1000 6 months: 920
	14	powder	infant	Metal can	3.4	7	1.4	1 month: 700 4 months: 900 6 months: 840

	15	powder	infant	Aluminium-plastic bag	3.4	7.3	1.3	1 month: 840 4 months: 900 6 months: 720
	16	powder	infant	Metal can	3.5	7	1.4	1 month: 600 4 months: 1050 6 months: 960
	30	liquid	infant	Polypropylene bottle	3.6	7.4	1.24	1 month: 650 4 months: 1000 6 months: 1150
	31	liquid	infant	Polypropylene bottle	4	5.8	2.9	1 month:600
	32	liquid	infant	Polypropylene bottle	3.6	7	1.3	1 month: 700 4 months:1200
	33	liquid	infant	Polypropylene bottle	3.5	7	1.5	1 month: 700 4 months: 1000 6 months: 940
	34	liquid	infant	Polypropylene bottle	3.4	7.8	1.3	1 month: 650 4 months: 1000 6 months: 1150
B	17	powder	follow-up	Aluminium-plastic bag	3.2	6.3	1.4	> 7 months: 630
	18	powder	follow-up	Aluminium-plastic bag	3	6.9	1.6	> 7 months: 630
	19	powder	follow-up	Aluminium-plastic bag	3.2	8.1	1.4	> 7 months: 630
	20	powder	follow-up	Aluminium-plastic bag	3.3	6.7	1.2	>7 months: 1000 12 months: 750
	21	powder	follow-up	Aluminium-plastic bag	3.2	5.5	1.5	7 months: 540 12 months: 630
	22	powder	follow-up	Metal can	2.9	7.9	1.5	> 12 months: 500
	23	powder	follow-up	Aluminium-plastic bag	2.5	6.4	1.6	> 24 months: 400
	24	powder	follow-up	Aluminium-plastic bag	2.2	5	1.7	> 24 months: 400
	25	powder	follow-up	Metal can	3.1	6.9	1.4	> 7 months: 630
	26	powder	follow-up	Metal can	3.4	7.4	1.3	6-12 months: 630
	27	powder	follow-up	Metal can	3	8.2	1.6	> 7 months: 630
	28	powder	follow-up	Aluminium-plastic bag	3	6.5	1.4	> 7 months: 630
	29	powder	follow-up	Aluminium-plastic bag	3.2	8	1.4	> 7 months: 630
c	35	liquid	growing-up	Polypropylene bottle	8.7	4.6	9	> 3 years: 600
	36	liquid	growing-up	Polypropylene bottle	8.7	4.6	9	> 3 years: 600
	37	liquid	growing-up	Polypropylene bottle	3.4	8	9.4	> 3 years: 600
	38	liquid	growing-up	Polypropylene bottle	8.7	6	9	> 3 years: 600
	39	liquid	growing-up	Polypropylene bottle	3.5	6.5	9.4	> 3 years: 600
	40	liquid	growing-up	Paperboard box	3.9	12	7.6	> 3 years:700



Table S2. Values of recoveries together with RSDs based on different sample matrix

analyte	Mare milk (liquid)				Diet supplement based on mare milk (powder)				Human milk (liquid)			
	Recovery [%] (RSD), n=3				Recovery [%] (RSD), n=3				Recovery [%] (RSD), n=3			
	Blank [ng/g] (SD)	5 [ng/g]	10 [ng/g]	20 [ng/g]	Blank [ng/g] (SD)	5 [ng/g]	10 [ng/g]	20 [ng/g]	Blank [ng/g] (SD)	5 [ng/g]	10 [ng/g]	20 [ng/g]
BADGE·2H ₂ O	3.1 (0.13)	134 (0.8)	130 (2.1)	107 (1.2)	-	460 (2.8)	131 (2.2)	101 (13)	-	132 (0.4)	110 (1.1)	95 (1.1)
BFDGE	-	108 (4.3)	141 (2.0)	105 (0.5)	-	140 (8.7)	82 (2.7)	175 (3.7)	1.57 (0.15)	113 (5.1)	106 (2.9)	95 (2.1)
BADGE·H ₂ O	-	85 (0.1)	94 (1.8)	93 (2.2)	-	156 (6.8)	85 (1.2)	170 (6.8)	-	96 (5.4)	81 (1.4)	80 (5.3)
BFDGE·2H ₂ O	-	103 (0.5)	92 (0.4)	97 (1.1)	3.88 (0.49)	100 (2.7)	49 (1.4)	92 (3.0)	1.60 (0.22)	68 (0.3)	64 (2.1)	57 (0.3)
BADGE·2HCl	12.6 (2.3)	236 (6.6)	203 (8.1)	100 (13)	-	139 (11)	244 (4.4)	211 (4.3)	4.7 (1.1)	240 (7.7)	198 (12)	140 (4.5)
BADGE·H ₂ O·HCl	3.6 (0.40)	122 (4.2)	110 (0.7)	116 (2.4)	8.2 (0.78)	163 (8.1)	135 (0.8)	117 (6.5)	2.17 (0.35)	159 (1.1)	111 (8.8)	98 (5.9)
BFDGE·2HCl	1.56 (0.33)	152 (3.5)	138 (3.9)	113 (4)	1.8 (0.25)	209 (2.1)	181 (5.5)	111 (9.9)	0.78 (0.17)	134 (5.5)	102 (8.4)	102 (1.7)
BADGE	0.85 (0.14)	114 (3.3)	334 (3.6)	120 (0.4)	1.42 (0.14)	105 (9.7)	74 (5.4)	154 (4.2)	-	123 (1.3)	107 (1)	124 (1)
BADGE·HCl	-	106 (1.9)	165 (1.4)	109 (2.9)	-	139 (4.6)	74 (3.5)	169 (2.4)	-	111 (2.8)	102 (2.8)	95 (6)
BPF	-	25 (2.2)	75 (5.5)	100 (0.4)	-	34 (7.4)	42 (1.3)	138 (10)	-	41 (4.7)	77 (1.8)	74 (3.7)
BPC	-	119 (11)	115 (1.6)	120 (4.9)	-	156 (3.4)	86 (6.9)	179 (3.8)	-	114 (5.5)	111 (2.8)	102 (3.8)
BPFL	-	113 (3.7)	107 (0.1)	112 (1.7)	0.96 (0.18)	104 (8.1)	64 (1.5)	140 (13)	-	178 (4)	153 (1.1)	130 (1.4)
BPZ	-	117 (10)	107 (2.5)	111 (4.2)	-	134 (12)	71 (3.8)	153 (5.6)	-	122 (1.8)	111 (2.4)	88 (3.5)
BPBP	-	103 (0.8)	105 (3.5)	113 (2.5)	-	125 (11)	72 (0.7)	144 (10)	-	176 (2.1)	145 (2.4)	151 (1.7)
BPM	-	122 (7.7)	114 (1.8)	119 (1.8)	1.55 (0.11)	126 (1)	68 (6)	163 (6.2)	-	152 (4.6)	135 (7)	113 (2)
BPG	-	136 (8.6)	113 (5.3)	115 (1.7)	3.19 (0.37)	85 (18)	60 (12)	119 (5.2)	-	112 (3.5)	115 (4.6)	104 (1)
BPP	-	116 (1.0)	110 (1.1)	113 (0.8)	1.49 (0.29)	166 (1.8)	94 (4.3)	176 (1.5)	-	188 (2.7)	170 (2.5)	135 (2.8)
BPS*	5.6 (0.42)	100 (8.9)	85 (1.8)	83 (6.5)	-	89 (1.7)	67 (7.4)	69 (1)	-	35 (2.7)	42 (2.8)	51 (3.4)
BPA*	3.1 (0.47)	159 (17)	137 (11)	174 (1.5)	-	118 (2.8)	90 (10.2)	148 (4.1)	-	25 (3.4)	38 (4.1)	44 (3.6)

* based on isocratic method

- not detected



Table S3. Values of linearity with weigh applied (1/x), limits of detection (LODs), limits of quantitation (LOQs) and recoveries together with RSD based on sample matrix – hypoallergic infant milk from birth

analyte	Cal. curve equation $y=ax+b$	S_a	S_b	r	LOD [ng/g]	LOQ [ng/g]	Recovery [%] (RSD), n=3		
							5 [ng/g]	10 [ng/g]	20 [ng/g]
BADGE·2H ₂ O	$y=0.0597x+0.0924$	0.0019	0.0061	0.9963	0.34	1.0	89 (2.1)	96 (2.9)	95 (0.3)
BFDGE	$y=0.0440x+0.0040$	0.0013	0.0040	0.9974	0.30	0.90	100 (3.5)	111 (6.1)	111 (2.5)
BADGE·H ₂ O	$y=0.0474x+0.0120$	0.0011	0.0051	0.9973	0.35	1.1	94 (3.8)	105 (5.2)	101 (2.3)
BFDGE·2H ₂ O	$y=0.04204x+0.0017$	0.00083	0.0038	0.9976	0.29	0.88	70 (5.1)	77 (0.9)	77 (0.1)
BADGE·2HCl	$y=0.002425x+0.00069$	0.000062	0.00028	0.9967	0.39	1.2	107 (2.5)	92 (5.6)	92 (0.3)
BADGE·H ₂ O·HCl	$y=0.03112x+0.0074$	0.00079	0.0036	0.9968	0.38	1.1	87 (5.3)	95 (1.4)	93 (0.4)
BFDGE·2HCl	$y=0.00508x+0.00099$	0.00011	0.00050	0.9966	0.32	0.97	96 (9.2)	99 (1.8)	97 (4.4)
BADGE	$y=0.1128x+0.00533$	0.0028	0.012	0.9971	0.37	1.12	120 (7.2)	102 (1.0)	102 (0.6)
BADGE·HCl	$y=0.03199x+0.0094$	0.00087	0.0039	0.9965	0.40	1.2	107 (6.3)	107 (4.2)	104 (2.0)
BPF	$y=0.001557x+0.002934$	0.000026	0.00012	0.9987	0.25	0.75	63 (7.5)	73 (7.4)	87 (0.9)
BPC	$y=0.000474x-0.000045$	0.000012	0.000052	0.9973	0.36	1.1	93 (5.4)	80 (7.8)	85 (2.1)
BPFL	$y=0.00699x+0.000019$	0.00015	0.00069	0.9977	0.33	0.98	82 (3.5)	86 (2.5)	87 (2.2)
BPZ	$y=0.001043x-0.000011$	0.000022	0.00010	0.9980	0.31	0.94	85 (7.3)	94 (6.6)	82 (3.0)
BPBP	$y=0.001471x+0.000024$	0.000039	0.00018	0.9962	0.40	1.2	90 (5.2)	90 (7.3)	89 (4.7)
BPM	$y=0.002417x-0.00023$	0.000054	0.00024	0.9976	0.33	1.0	84 (4.4)	92 (2.2)	90 (0.7)
BPG	$y=0.0005850x+0.000012$	0.000010	0.000047	0.9978	0.26	0.79	91 (2.5)	96 (10)	99 (1.5)
BPP	$y=0.002423x-0.000016$	0.000060	0.00027	0.9970	0.37	1.1	91 (3.9)	93 (3.8)	91 (2.0)
BPS*	$y=0.3678x-0.026$	0.0059	0.27	0.9977	0.24	0.72	31 (6.6)	38 (4.7)	47 (3.2)
BPA*	$y=0.02802x-0.0013$	0.00048	0.0022	0.9982	0.26	0.77	88 (4.8)	77 (6.8)	86 (1.0)

* based on isocratic method



Table S4. Simulation on affect of the lowered BPA EDI value (EFSA draft opinion) on determined risk levels. Estimated Daily Intake (EDI), Hazard Quotient (HQ) and Hazard Index (HI) of bisphenols according to the age of male (M) and female (F) infants and toddlers connected with consumption of the daily dose of milk and one ready-to-eat product.

analyte	parameter	age [months]					
		6		12		36	
		F	M	F	M	F	M
BADGE	EDI						
	[ng/kg/b.w./day]						
	mean	488	425	681	632	401	390
	(95th percentile)	(1411)	(1289)	(1975)	(1831)	(817)	(795)
BADGE·H ₂ O	EDI						
	[ng/kg/b.w./day]						
	mean	772	713	157	145	184	179
	(95th percentile)	(1481)	(1369)	(269)	(250)	(269)	(261)
BADGE·2H ₂ O	EDI						
	[ng/kg/b.w./day]						
	mean	212	197	118	109	81	78
	(95th percentile)	(520)	(481)	(302)	(280)	(120)	(117)
Σ BADGE, BADGE· H ₂ O, BADGE· 2H ₂ O	HQ (95 th percentile)	0.010 (0.023)	0.0089 (0.021)	0.0064 (0.017)	0.0059 (0.016)	0.0044 (0.0080)	0.0043 (0.0078)
BADGE·HCl	EDI						
	[ng/kg/b.w./day]						
	mean	129	159	105	98	114	111
	(95th percentile)	(161)	(209)	(154)	(143)	(147)	(143)
BADGE·2HCl	EDI						
	[ng/kg/b.w./day]						
	mean	1165	1076	698	647	211	205
	(95th percentile)	(4080)	(3771)	(2051)	(1901)	(425)	(413)
BADGE· H ₂ O·HCl	EDI						
	[ng/kg/b.w./day]						
	mean	348	320	514	477	112	109
	(95th percentile)	(623)	(576)	(1678)	(1555)	(186)	(181)
Σ BADGE· HCl, BADGE· 2HCl, BADGE· H ₂ O·HCl	HQ (95 th percentile)	0.011 (0.032)	0.010 (0.030)	0.0088 (0.026)	0.0081 (0.024)	0.0029 (0.0051)	0.0028 (0.0049)
BFDGE	EDI						
	[ng/kg/b.w./day]						
	mean	109	100	100	93	59	57
	(95th percentile)	(126)	(117)	(105)	(98)	(61)	(60)
BFDGE· 2H ₂ O	EDI						
	[ng/kg/b.w./day]						
	mean	180	146	15	14	-	-
	(95th percentile)	(180)	(146)	(16)	(15)	-	-
BFDGE· 2HCl	EDI						
	[ng/kg/b.w./day]						
	mean	143	132	62	57	41	40
	(95th percentile)	(175)	(162)	(65)	(60)	(47)	(46)
Σ BFDGE, BFDGE· 2H ₂ O, BADGE· 2HCl	HQ (95 th percentile)	0.0029 (0.0032)	0.0025 (0.0028)	0.0012 (0.0012)	0.0011 (0.0012)	0.00066 (0.00072)	0.00065 (0.00070)
BPA	EDI						
	[ng/kg/b.w./day]						
	mean	711	657	332	308	332	322
	(95th percentile)	(1660)	(1530)	(789)	(732)	(626)	(609)
	HQ	17776	16426	8294	7689	8291	8059
(95th percentile)	(41519)	(38366)	(19735)	(18296)	(15657)	(15219)	



BPBP	EDI						
	[ng/kg/b.w./day]						
	mean	104	96	48	44	20	19
	(95th percentile)	(120)	(111)	(150)	(139)	(27)	(26)
	HQ	0.026	0.024	0.012	0.011	0.0049	0.0048
	(95th percentile)	(0.030)	(0.028)	(0.038)	(0.035)	(0.0067)	(0.0066)
BPC	EDI						
	[ng/kg/b.w./day]						
	mean	394	364	234	217	2376	230
	(95th percentile)	(995)	(920)	(456)	(422)	(443)	(430)
	HQ	0.10	0.091	0.058	0.054	0.059	0.058
	(95th percentile)	(0.25)	(0.23)	(0.11)	(0.11)	(0.11)	(0.11)
BPF	EDI						
	[ng/kg/b.w./day]						
	mean	460	425	182	169	92	89
	(95th percentile)	(1091)	(1008)	(282)	(261)	(92)	(89)
	HQ	0.11	0.11	0.046	0.042	0.023	0.022
	(95th percentile)	(0.27)	(0.25)	(0.070)	(0.065)	(0.023)	(0.022)
BPFL	EDI						
	[ng/kg/b.w./day]						
	mean	481	444	312	290	213	207
	(95th percentile)	(1082)	(1000)	(527)	(489)	(213)	(207)
	HQ	0.12	0.11	0.078	0.072	0.053	0.052
	(95th percentile)	(0.27)	(0.25)	(0.13)	(0.12)	(0.053)	(0.052)
BPG	EDI						
	[ng/kg/b.w./day]						
	mean	144	133	127	119	102	99
	(95th percentile)	(245)	(227)	(345)	(320)	(194)	(189)
	HQ	0.036	0.033	0.032	0.030	0.026	0.025
	(95th percentile)	(0.061)	(0.057)	(0.086)	(0.080)	(0.049)	(0.047)
BPM	EDI						
	[ng/kg/b.w./day]						
	mean	368	340	235	218	9.3	9.0
	(95th percentile)	(441)	(408)	(252)	(233)	(9.3)	(9.0)
	HQ	0.092	0.085	0.059	0.054	0.0023	0.0023
	(95th percentile)	(0.11)	(0.10)	(0.063)	(0.058)	(0.0023)	(0.0023)
BPP	EDI						
	[ng/kg/b.w./day]						
	mean	347	321	262	243	83	81
	(95th percentile)	(664)	(613)	(643)	(596)	(179)	(174)
	HQ	0.087	0.080	0.066	0.061	0.021	0.020
	(95th percentile)	(0.17)	(0.15)	(0.16)	(0.15)	(0.045)	(0.044)
BPS	EDI						
	[ng/kg/b.w./day]						
	mean	120	111	38	35	57	56
	(95th percentile)	(426)	(393)	(72)	(66)	(57)	(56)
	HQ	0.030	0.028	0.010	0.0088	0.014	0.014
	(95th percentile)	(0.11)	(0.10)	(0.018)	(0.017)	(0.014)	(0.014)
BPZ	EDI						
	[ng/kg/b.w./day]						
	mean	111	102	57	53	18	17
	(95th percentile)	(243)	(224)	(217)	(202)	(48)	(46)
	HQ	0.028	0.026	0.014	0.013	0.0045	0.0043
	(95th percentile)	(0.061)	(0.056)	(0.054)	(0.050)	(0.012)	(0.012)
	HI	17780	16430	8290	7689	8291	8059
	HI 95th	41520	38370	19740	18300	15660	15220

