



Exposure scenario and risk assessment of infants and newborns to bisphenols and their derivatives from diapers

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ABSTRACT

Newborns and infants are more sensitive to harmful compounds such as bisphenols and their derivatives because of their not fully developed detoxification mechanism. Exposure to these substances can lead to developmental problems and health consequences in adulthood. Since disposable baby diapers are used from the first days of life and remain in contact with the baby skin, it seems important to monitor the levels of endocrine disrupting chemicals (EDCs) in such products. Ultrasound assisted solvent microextraction of porous membrane-packed solid sample (UASE-PMSS) was used in sample preparation. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used at determination step. Bisphenol A was quantified with the highest frequency at 81 % of samples tested, ranging from 5.0 to 520 ng/g. BADGE-2HCl was also quantified in high concentrations (from 6.8 to 530 ng/g), but was found in only 15 % of the tested samples. The daily exposure dose (DED) of bisphenols was calculated. In addition health risk assessment was conducted using previous (4 µg/kg BW) and actual (0.2 ng/kg BW) values of tolerable daily intake (TDI) of bisphenol A recommended by European Food Safety Authority (EFSA).

1. Introduction

The disposable diaper is certainly one of the most groundbreaking inventions for infants. It is now considered a basic product that contributes to the hygiene of the child. Due to the frequent use of disposable diapers, these products should be made only from non-toxic and natural ingredients (Makoś-Chelstowska et al., 2021). Unfortunately, the manufacturers of diapers do not specify the exact chemical composition of

these goods, invoking their trade secrets. Nevertheless, one can read in the literature that disposable diapers of well-known brands (even these so-called organic origin products) may contain a number of toxic compounds (DeVito and Schecter, 2002; Gifford, 2021; Sathyanarayana et al., 2008). However, it is also important to note, that there are very few studies that address the chemical composition of these personal care products that are important for infants and children.

There are numerous reports in the literature on the release of

Abbreviations: AC, Absorbent core; AHP, Absorbent hygiene products; BADGE, Bisphenol A diglycidyl ether; BADGE-d₁₀, d₁₀-labelled BADGE; BADGE-2H₂O, Bisphenol A bis(2,3-dihydroxypropyl) ether; BADGE-2HCl, Bisphenol A bis(3-chloro-2-hydroxypropyl) ether; BADGE-H₂O, Bisphenol A (2,3-dihydroxypropyl) glycidyl ether; BADGE-H₂O-HCl, Bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl) ether; BADGE-HCl, Bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether; BFDGE, Bisphenol F diglycidyl ether; BFDGE-2 H₂O, Bisphenol F bis(2,3-dihydroxypropyl) ether; BFDGE-2HCl, Bisphenol F bis (3-chloro-2-hydroxypropyl) ether; BPA, Bisphenol A; BPA-¹³C, ¹³C-labelled Bisphenol A; BPBP, Bisphenol BP; BPC, Bisphenol C; BPF, Bisphenol F; BPFL, Bisphenol FL; BPG, Bisphenol G; BPM, Bisphenol M; BPP, Bisphenol P; BPS, Bisphenol S; BPs, Bisphenols; BPZ, Bisphenol Z; BW, Body weight; CE, Collision energy; CRM, Certified reference material; CV, Coefficient of variation; DED, Daily exposure dose; EDCs, Endocrine disrupting chemicals; EFSA, European Food Safety Authority; ESI, Electrospray ionization; HI, Hazard index; HQ, Hazard quotient; IS, Internal standards; KCl, Potassium chloride; LDPE, Low density polyethylene; LOD, Limit of detection; LOQ, Limit of quantification; MeOH, Methanol; MRM, Multiple reaction monitoring; Na₂CO₃, Sodium carbonate; NaCl, Sodium chloride; NH₄HCO₂, Ammonium formate; NH₄OH, Ammonia solution; PCPs, Personal care products; PE, Polyethylene; PET, Polyethylene terephthalate; PP, Polypropylene; RSD, Relative standard deviation; SAP, Superabsorbent polymer; SD, Standard deviation; SS, Stainless steel; SW, Supporting wings; TDI, Tolerable daily intake; UASE-PMSS, Ultrasound assisted solvent microextraction of porous membrane-packed solid sample; UPLC-MS/MS, Ultra-performance liquid chromatography coupled with a tandem mass spectrometry.

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contaminants, including those belonging to the group of endocrine disrupting chemicals (EDCs), from polymeric materials (Pironti et al., 2021). For example, bisphenols can migrate from polyester or epoxy resins (Daelemans et al., 2015), while pesticides used to protect the substrates used for their production can migrate from polyamide fibers (Makoś-Cheistowska et al., 2021). The production of disposable baby diapers uses polymers that undergo various processes (heat sealing, laminating, pressing), so monomers of unreacted polymers and their transformation products may occur, such as isopropenylphenol and ortho-para-isomer of BPA (Krämer et al., 2022). Furthermore the raw polymers could be contaminated with bisphenol A (BPA) during synthesis/production. It is likely that BPA is present in baby diapers, so it may transfer to the body upon skin contact. Another possibility for the release of compounds from baby diapers could be migration from the polymer through contact with urine. However, there are few studies that confirm the content of harmful substances in products for children. Since both polyester and polyamide are used in the production of disposable diapers, it is suspected that they could be one of the sources of exposure of the youngest to harmful substances, including EDCs. Since EDCs interfere with metabolism and the endocrine system in most cases at very sensitive stages of human development and growth, knowledge of various aspects related to this group of compounds is of great importance.

Since disposable baby diapers have to be used daily, it seems very important to monitor compounds that can migrate from the diaper into the child's body. A large group of EDCs are bisphenols and their derivatives such as bisphenol A diglycidyl ether analogs (BADGE) or bisphenol F diglycidyl ether analogs (BFDGE). These chemicals trigger endocrine disruption, reproductive toxicity and genotoxicity (Xue et al., 2022). However, compared to bisphenol A (BPA), studies on the environmental occurrence, toxicokinetics and fate, as well as analytical and monitoring methods of the above compounds are limited. Therefore, great attention should be paid to these issues, especially with regard to products intended for children.

As mentioned above, there are only a few studies dealing with the determination of bisphenols and their derivatives in disposable baby diapers. For this reason, the main objective of this research was to develop a procedure, that would allow the analysis of selected bisphenol A and bisphenol F derivatives in disposable baby diaper samples. Ultrasound assisted solvent microextraction of solid samples contained in a porous membrane (UASE-PMSS) was used in the sample preparation phase. The determination of the analytes were performed by ultra-performance liquid chromatography coupled with a tandem mass spectrometry (UPLC-MS/MS). In addition, an estimate of the daily exposure doses of BPs and their derivatives via dermal absorption was calculated.

The publication is a response to the lack of a harmonized analytical method for the determination of BPs and their derivatives in disposable care products for babies and children. It also provides valuable information on the content of selected analytes (which have an impact on human health) in disposable diapers, a product that children come into contact with almost 24 h a day. It should also be noted that a used diaper ends up in a landfill, where bisphenols are released into the environment. To our knowledge, this is the first report on the application of ultrasound assisted solvent microextraction of porous membrane-packed solid samples coupled with UHPLC-MS/MS to baby diapers and the assessment of exposure to BPs derived from these baby care products.

2. Material and methods

2.1. Standards, materials and reagents

Analytical standards of bisphenol A (BPA, CAS 80-05-7), bisphenol S (BPS, CAS 80-09-1), bisphenol F (BPF, CAS 620-92-8), bisphenol C (BPC, CAS 79-97-0), bisphenol FL (BPFL, CAS 3236-71-3), bisphenol Z (BPZ,

CAS 843-55-0), bisphenol BP (BPP, CAS 1844-01-5), bisphenol M (BPM, CAS 13595-25-0), bisphenol G (BPG, CAS 127-54-8), bisphenol P (BPP, CAS 2167-51-3), bisphenol A diglycidyl ether (BADGE, CAS 1675-54-3), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE-H₂O, CAS 76002-91-0), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE-2H₂O, CAS 5581-32-8), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE-HCl, CAS 13836-48-1), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE-2HCl, CAS 4809-35-2), bisphenol A (3-chloro-2-hydroxypropyl)(2,3-dihydroxypropyl) ether (BADGE-H₂O-HCl, CAS 227947-06-0), racemic mixture of bisphenol F diglycidyl ether (BFDGE, CAS 2095-03-06), bisphenol F bis(2,3-dihydroxypropyl) ether (BFDGE-2 H₂O, CAS 72406-26-9), bisphenol F bis(3-chloro-2-hydroxypropyl) ether (BADGE-2HCl, CAS 4809-35-2) were purchased from Merck KGaA (Darmstadt, Germany). Bisphenol A ¹³C-labelled (CAS 263261-65-0) and d¹⁰-labelled BADGE (CAS 1675-54-3) from Cambridge Isotope Laboratories Inc. (Cambridge, UK) were used as internal standards (ISs) in the procedure. Potassium chloride (KCl, CAS 7447-40-7) was obtained from Avantor (Gliwice, Poland). High-purity methanol (MeOH, CAS 67-56-1) was purchased from Merck KGaA (Darmstadt, Germany). Ammonia solution 25 % (NH₄OH, CAS 1336-21-6) was purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was prepared using the HPL5 system (Hydrolab, Straszyn, Poland) equipped with an EDS-Pak cartridge from Merck KGaA (Darmstadt, Germany). Polypropylene (PP) membrane sheets were purchased from GVS Filter Technology (Rome, Italy). Nylon syringe filters (pore size 0.2 µm) were purchased from Thermo Fisher Scientific (Warsaw, Poland). The chromatographic column (Kinetex® 1.7 µm EVO C18 100 Å, 100 mm × 2.1 mm) and UHPLC precolumn were purchased from Phenomenex Inc. (Aschaffenburg, Germany).

2.2. Preparation of stock, working and calibration solutions

All stock solutions were prepared by dissolving weighted amounts of analytical standards in methanol to obtain a concentration of 100 µg/mL, further diluted to 10 µg/mL to serve as the working solution. In addition to the working solution, two internal standards (ISs) with a concentration of 10 µg/mL (¹³C-labelled bisphenol A and d₁₀-labelled BADGE) were used. The solutions for the calibration curve were obtained by diluting the stock solution also in methanol to 0.5, 1, 2, 5, 10, 20, 50 ng/mL while the concentration of IS was kept at 10 ng/mL. All solutions were stored in the freezer at -20 °C.

2.3. Real samples

Twenty three different samples of disposable baby diapers were purchased in local stores in Gdańsk, Poland. Five of them could be classified as organic origin baby diapers, and another 18 were conventional, composite materials. Each sample was divided into two groups: absorbent core (AC) and supporting wings (SW). After homogenization (machine cutting) two groups of samples were placed in polypropylene bags. They were all kept sealed in a dry place at room temperature.

2.4. Blanks and spiked samples

Blank samples were prepared to exclude possible contamination from PP bags and the stainless steel net. An appropriate amount of 20 mM KCl solution and ISs' solutions were added to the prepared PP bag. Then the bag was sealed using an impulse heat sealer and placed in a 15 mL vial. The membrane was immobilized with the stainless steel net and the extraction was performed as for the real samples. During the preliminary studies a diaper sample that was free of analytes, was selected as the matrix blank. This was done due to the fact that, to the authors' knowledge, there is no suitable reference material and it was very difficult to prepare a suitable matrix from cotton fibers and absorbent material. The matrix blank (unspiked blank) was prepared analogically to the procedural blank sample with the addition of selected analyte-free

diaper sample. The preparation of the spiked samples was similar to that of the unspiked samples. The difference was the addition of the appropriate amount of analytes to the spiked samples. The spiked blank sample was necessary to verify the repeatability and intermediate precision of developed method.

2.5. Extraction procedure

The extraction method was based on ultrasound assisted solvent microextraction through a porous membrane (UASE-PMSS) of packed diaper samples, which was adapted and heavily modified from other research (Szczepańska et al., 2020). The PP bags were made by sealing 3 of the edges of the cut membrane sheet. As the PP bags tend to float on top of the methanol, the stainless steel net was inserted into each vial to ensure that the bag was fully immersed in the extraction solvent. The stainless steel net was previously washed with ultrapure water, isopropanol and MeOH in an ultrasonic bath and then left to dry.

The homogenized sample was placed in the PP bag in weighed amount approximately 0.1 ± 0.005 g. The ISs and 20 mM KCl solution were added to the porous-membrane bag in appropriate amount, then the bag was sealed with impulse heat sealer. As the extraction solvent 7 mL of MeOH was used. The sample bags were held in the center of a 15 mL vials using the stainless steel net (showed in Fig. 1). The vials containing PP bag, net and MeOH were then closed and placed in the ultrasonic bath. The extraction process lasted for 20 min at 25 °C. After extraction, PP bag and net were removed and the extract was transferred into test tubes. The MeOH extract was evaporated under a stream of nitrogen at 45 °C. It is known that analyzed compounds with higher MeOH content are more stable (Szczepańska et al., 2019). Therefore, the dry residue was dissolved in 1 mL of MeOH. The obtained solution was vortexed, filtered with nylon syringe filters and immediately analyzed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

2.6. Ultra-performance liquid chromatography coupled with tandem mass spectrometry

All analyses were carried out using a liquid chromatograph (Schimadzu Nexera X2, Japan) coupled to tandem mass spectrometer (LC-MS-8060, Schimadzu, Japan). The electrospray ionization (ESI) was used to ionize the analytes in negative and positive mode depending on their response. All analytes were detected and quantified using of multiple reaction monitoring (MRM) mode. The values of precursor ions, fragment ions and collision energy (CE) were based on similar analysis (Jatkowska and Kubica, 2023). Separation of analytes was performed with a chromatographic column (Kinetex® 1.7 μ m EVO C18 100 Å, 100 mm \times 2.1 mm) using two chromatographic methods. Water (component A) and methanol (component B) were used as mobile phases to determine BPA, BPS together with the corresponding IS (BPA-C¹³) in isocratic mode (55 % of component A and 45 % of component B). The flow rate was kept at 0.5 mL/min, the temperature of separation was 45 °C and the injection volume was 1 μ L. For the determination of diglycidyl ethers with other bisphenols 0.01 % ammonia solution in water (component A) and methanol (component B) were used at a flow rate 0.6 mL/min. Separation of other bisphenols and diglycidyl ethers was performed by gradient elution at 50 °C oven temperature and the injection volume of 1 μ L. Gradient elution program was as follows: 0 min (30 % component B), 10 min (70 % component B), 14 min - equilibration (30 % component B), 20 min - end of analysis.

2.7. Estimation of daily exposure doses of BPs via dermal absorption

Based on the geometric mean, and 95th percentile of BPs concentrations measured in diaper samples, the daily exposure dose was determined. The daily exposure dose (DED) of BPs to which infants were exposed by wearing diapers was calculated for each diaper analyzed according to Eq. (1) (Gao and Kannan, 2020). To facilitate subsequent statistical analysis, samples were divided into seven categories (0, 1, 2, 3, 4, 5 and 6) based on the diaper size.

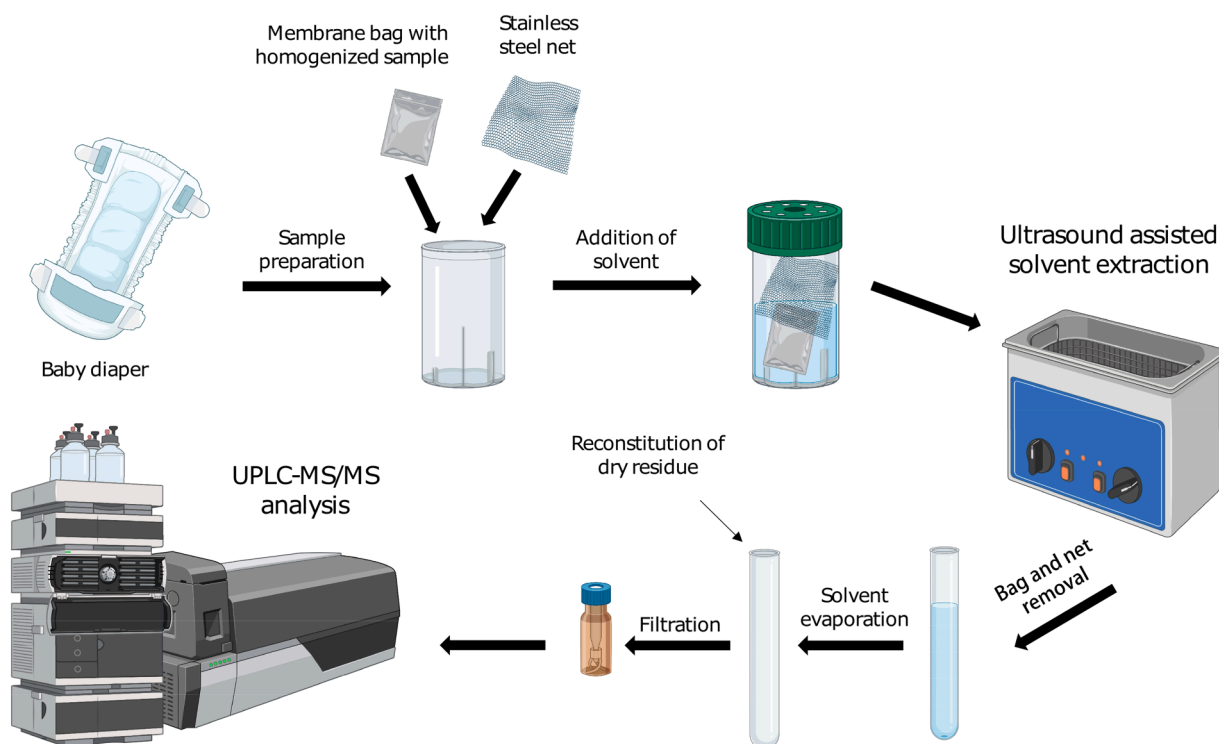


Fig. 1. Schematic representation of the elaborated extraction procedure.

$$DED = \frac{C \cdot M \cdot N \cdot A}{BW} \quad (1)$$

where:

- DED - daily exposure dose [$\mu\text{g}/\text{kg bw}/\text{day}$];
- C - concentration of identified compound in diaper [ng/g];
- M - weight of top sheet [g];
- N - number of diapers used per day;
- A - transdermal absorption rate;
- BW - average body weight [kg].

The number of diapers used per day in this study was assumed to be as follows based on information published by diaper manufacturers according to diaper size: newborn – 12, size 1–10, size 2–9, size 3–7, size 4–5. In order to better estimate the daily dose, calculations were made taking into account the minimum and maximum body weight of the infant for a given diaper size as reported by the manufacturer (Table S1). The weights of the top sheets are also given in the Table S1. Due to the scarcity of information on the dermal absorption rate of bisphenols the value determined by A. Laure Demierre and colleagues (Demierre et al., 2012) for the transdermal absorption rate of BPA ($A = 8.6\%$) was used. Considering the similar physicochemical properties of other analogues, the dermal absorption rate of all compounds was assumed to be 8.6%. As these data concerned penetration through the skin of an adult human, two additional high exposure scenarios with absorption rates of 20% and 50% were considered to better adapt the calculations to the skin of children (which is thinner than that of an adult).

The cumulative risk assessment of BPs was estimated using the hazard index (HI), which is the sum of the hazard quotient (HQ) of the individual bisphenols, with the following equations:

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

$$HI = HQ_1 + HQ_2 + HQ_3 + \dots + HQ_n \quad (3)$$

In these equations, DED is the daily exposure dose resulting from equation no. 1, and TDI is the tolerable daily intake [$\text{ng}/\text{kg bw}/\text{day}$]. In the absence of information on TDI values for other BPA and BADGE analogues, it was assumed that the TDI value established for BPA ($4 \mu\text{g}/\text{kg body weight}/\text{day}$) would be applied to BPA analogues such as BPPB, BPC, BPF, BPFL, BPG, BPM, and BPZ. Similarly, the value established for BADGE ($150 \mu\text{g}/\text{kg body weight}/\text{day}$) is adopted for other diglycidyl ethers. In the health risk assessment, it was assumed that HI below 1 indicates no health risk, while HI values above 1 indicate an exposure that may be considered of concern.

2.8. Intern-laboratory method validation

Linear calibration equations were obtained based on the peak area ratio of each analyzed compound with the corresponding IS peak area at each concentration in weighted linear regression. The weigh factor $1/x$ was applied to ensure better accuracy at the lower concentrations. The limit of detection (LOD) was calculated using the equation $LOD = 3.3 \times S_b/a$, where a is the slope of the calibration curve and S_b is the standard deviation (SD) of the y-axis intercepts of the regression lines. The limit of quantification (LOQ) was determined by multiplying LOD three times, $LOQ = 3 \times LOD$ (Konieczka and Namieśnik, 2018). Spiked samples at 3 concentration levels (20 ng/g, 50 ng/g and 100 ng/g) were processed and analyzed as described in Section 2.4 and were used to obtain recovery values. Repeatability was determined using the coefficient of variation (CV) [%] values for the data based on recoveries of 3 concentration levels within the same day, as indicated in the recovery determination. The intermediate precision was determined analogously to the repeatability, on the consecutive 3 days.

3. Results

3.1. Method development

To the Authors' knowledge there is no suitable certified reference material (CRM) compatible with baby diaper composition. For this reason, one of homogenized disposable baby diaper sample with the most complicated composition was selected to perform all optimization experiments. Series of analyses were done using $0.1 \pm 0.005 \text{ g}$ of the disposable baby diaper sample spiked with 200 ng/g. All experiments were performed in MeOH as the extraction solvent, since the proven efficiency of it for extraction of bisphenols and their diglycidyl ethers (Szczepańska et al., 2020).

3.1.1. Counteraction against the flotation

The first problem observed was the floating of the sample packed with a PP porous membrane on the extraction solvent. To eliminate this complication, some solutions were applied such as increasing the weight of the sample by adding solid salt, clean pieces of glass or salt solution. However, none of these ideas worked, so another approach was explored. To ensure complete immersion of the bag sample in the extractant, a clean SS (stainless steel) net was used in two ways: first, as an obstacle to prevent the displacement of the packed membrane bag above the extractant, and second, as a trap surrounding the tested membrane bag and increasing its weight. The SS net, which was used as an obstacle, allowed the best immersion in the extraction medium and repeatable results. This approach also allowed to reduce the volume of extraction solvent used from 10 mL to 7 mL. The use of a minimal amount of solvent was an objective of the green chemistry approach, while the sample was fully immersed in the solvent.

3.1.2. Salt addition

In order to increase the ionic strength and improve the efficiency of the extraction, some nontoxic salt solutions were studied as an addition to the sample packed with PP porous membrane. First, the type of salt was investigated: sodium chloride (NaCl), potassium chloride (KCl), sodium carbonate (Na_2CO_3) and ammonium formate (NH_4HCO_2). Each salt was used in a volume of 180 μL and a concentration of 50 mM. KCl proved to be the best solution, which was further investigated at different concentration levels: 20, 50, 100 and 200 mM, but in the same added volume. The lowest concentration level studied allowed the best extraction efficiency to be obtained without reducing repeatability and was therefore used as the final ionic strength enhancer.

3.1.3. Extraction time

The extraction time of the experiment was performed for 10, 15, 20 and 30 min. The best results were obtained with extraction of 20 and 30 min, with 20 min giving similar results compared to 30 min, so the shorter extraction time with was selected as the best with comparable efficiency.

For the optimal extraction conditions examples of obtained chromatogram are presented in Fig. S1-S4.

3.2. Intern-laboratory method validation

All obtained calibration curves were linear in the tested concentration range, with correlation coefficient greater than 0.9933 for all analyzed compounds. The collected data for the values of LOD and LOQ, the correlation coefficients and the recoveries are shown in Table 1. The recoveries obtained ranged from 45.8% to 115%, while the RSDs ranged from 1.3% to 20%.

The value of CVs obtained for BPFL repeatability deviated from the adjusted acceptance criterion ($CV \leq 35\%$) (Barbosa et al., 2019) due to the of heterogeneity of the data series and was therefore excluded for further investigation. The same criterion was applied to exclude BPS based on the results of intermediate precision determination. The results

Table 1
Parameters of weighted regression, LOD, LOQ and recoveries at 3 concentration levels.

Analyte	calibration curve equation y-ax+b	Sa	Sb	r	LOD [ng/g]	LOQ [ng/g]	Recovery [%], (RSD, n = 9)					
							20 [ng/g]	50 [ng/g]	100 [ng/g]			
BFDGE-2H ₂ O	y = 0.05255x + 0.0124	0.00024	0.0012	0.9996	0.71	2.1	87.8	(4.1)	77.5	(6.6)	85.0	(7.3)
BADGE-2H ₂ O	y = 0.07478x + 1.3493	0.00068	0.0032	0.9992	1.4	4.2	99	(14)	81.8	(7.3)	84.6	(7.5)
BADGE-H ₂ O	y = 0.05415x + 0.01673	0.00020	0.00097	0.9998	0.58	1.7	93.2	(3.8)	86.0	(9.6)	87.8	(1.5)
BADGE-2HCl	y = 0.004048x + 0.003677	0.000011	0.000053	0.9987	0.43	1.3	80.8	(5.9)	75	(10)	83.7	(6.7)
BFDGE	y = 0.04814x + 0.00392	0.00011	0.00051	0.9999	0.35	1.0	107.7	(3.6)	91.7	(1.3)	91.4	(5.7)
BADGE-H ₂ O-HCl	y = 0.03294x + 0.06268	0.00016	0.00079	0.9997	0.79	2.4	105.1	(6.1)	93.2	(7.8)	92.8	(3.8)
BFDGE-2HCl	y = 0.02598x + 0.01042	0.00012	0.00057	0.9996	0.70	2.1	106.0	(2.7)	87.2	(2.4)	90.0	(5.7)
BADGE	y = 0.10232x + 0.0323	0.00035	0.0017	0.9998	0.53	1.6	106.5	(5.2)	90.0	(3.4)	85.6	(7.5)
BADGE-HCl	y = 0.038057x + 0.00182	0.000086	0.00041	0.9999	0.36	1.1	102.6	(9.5)	85.1	(5.7)	86.8	(4)
BPF	y = 0.002831x + 0.001144	0.000018	0.000088	0.9995	1.0	3.0	64	(20)	72.6	(9.6)	81.5	(6.9)
BPC	y = 0.0013883x + 0.000806	0.0000034	0.000016	0.9992	0.38	1.2	111	(13)	69.0	(3.6)	71.4	(7.5)
BPFL	y = 0.01013x + 0.0607	0.00045	0.0021	0.9933	8.2	25	70	(13)	70.9	(8.9)	78.5	(7.9)
BPZ	y = 0.004991x + 0.001233	0.000020	0.000096	0.9997	0.63	1.9	89	(14)	73.3	(8.8)	71	(19)
BPBP	y = 0.002485x + 0.00169	0.000022	0.00010	0.9989	1.3	4.0	102	(13)	66	(10)	60	(13)
BPM	y = 0.003364x + 0.000895	0.000013	0.000064	0.9995	0.62	1.8	115	(14)	103.7	(4.2)	106.0	(4.6)
BPG	y = 0.002052x + 0.00196	0.000033	0.00010	0.9946	1.5	4.5	103.3	(7.2)	87.5	(2.1)	90.8	(7)
BPP	y = 0.002852x + 0.00107	0.000023	0.00011	0.9992	1.2	3.7	109.8	(7.6)	105	(10)	100.7	(2.2)
BPS	y = 0.04192x - 0.0084	0.00037	0.0018	0.9990	1.4	4.1	45.8	(8.2)	52	(12)	58.6	(7.6)
BPA	y = 0.002317x + 0.00085	0.000023	0.00011	0.9985	1.5	4.4	88.9	(5.5)	60.6	(4.7)	60	(12)

of repeatability and intermediate precision determination are shown in Table 2.

3.3. Real sample analysis

The real samples were analyzed using the procedure described in Section 2. The values obtained are presented in the Fig. 2. The analyzed EDCs were present in 53 % of the tested samples, both in the AC and SW. BPZ, BPBP and BPG were identified only in the AC samples, while BPC was present only in the SW sample.

The median concentration of analyzed compounds in BFDGE, BADGE, BADGE-HCl and BPA was higher in the AC samples than in SW. However, the highest concentration of quantified BPA was 520 ng/g in SW, while it was equal 200 ng/g in the AC. BADGE-2HCl was quantified with similar frequency in the samples of AC and SW, with the value of median 15 ng/g for AC and 21 ng/g for SW. The maximum concentration of this compound was 69 ng/g in SW and 530 ng/g in AC. The

highest concentration of BADGE-2HCl in the AC was the highest value recorded during the analysis. In the case of BADGE-2 H₂O, the detected concentration is significantly different from that in BADGE-2HCl. The frequency of detection of this compound is three times higher in the SW than in the AC. Furthermore, the highest quantified concentration was 79 ng/g in the SW, while only 15 ng/g were quantified in the AC. BFDGE was detected twice as often in the SW as in the AC, and the highest concentration detected was significantly higher in the SW (220 ng/g) than in the AC (66 ng/g).

Lower concentrations of the analyzed compounds were detected more frequently than higher levels in the majority of sample tested. The data series of BPA concentrations in AC and SW are differ significantly, which is shown in the Fig. 2, with a large number of outliers (16 for SW and 7 for AC). BPA was detected in 81 % of the samples tested (75 % of AC and 87 % of SW), and the range of detected concentrations was between 5.0 ng/g and 520 ng/g. Due to the great diversity of the detected concentrations, which means that not only low but also high

Table 2
Values of coefficient of variation (CV %) for repeatability and intermediate precision.

	Repeatability (within the same day, n=6**)			Intermediate precision (on the consecutive 3 days, n = 6**)		
	CV [%] for recovery of 20 ng/g	CV [%] for recovery of 50 ng/g	CV [%] for recovery of 100 ng/g	CV [%] for recovery of 20 ng/g	CV [%] for recovery of 50 ng/g	CV [%] for recovery of 100 ng/g
BFDGE-2H ₂ O	4.0	9.1	11	4.9	9.6	10
BADGE-2H ₂ O	7.2	7.2	4.9	11	9.2	8.2
BADGE-H ₂ O	2.5	4.9	8.1	3.5	11	6.0
BADGE-2HCl	9.9	10	4.7	13	13	8.0
BFDGE	6.6	7.1	4.9	5.5	5.5	5.8
BADGE-H ₂ O-HCl	5.9	6.6	14	5.2	10	10
BFDGE-2HCl	1.2	6.5	2.3	4.2	5.5	6.4
BADGE	6.9	2.1	7.6	7.0	3.9	8.0
BADGE-HCl	2.2	16	3.5	11	20	7.2
BPF	23	13	5.2	28	13	7.3
BPC	22	11	14	24	14	13
BPFL*	73	25	18	56	38	32
BPZ	11	8.6	12	21	11	21
BPBP	14	7.2	4.7	15	14	16
BPM	4.2	5.4	3.8	10	9.0	4.6
BPG	14	7.7	7.0	14	13	20
BPP	3.4	10	4.4	13	28	7.7
BPS*	11	22	28	55	45	42
BPA	9.9	5.8	9.1	8.8	7.8	19

** 3 samples with the same amount of analytes in two injections each.

* Excluded from further investigation.

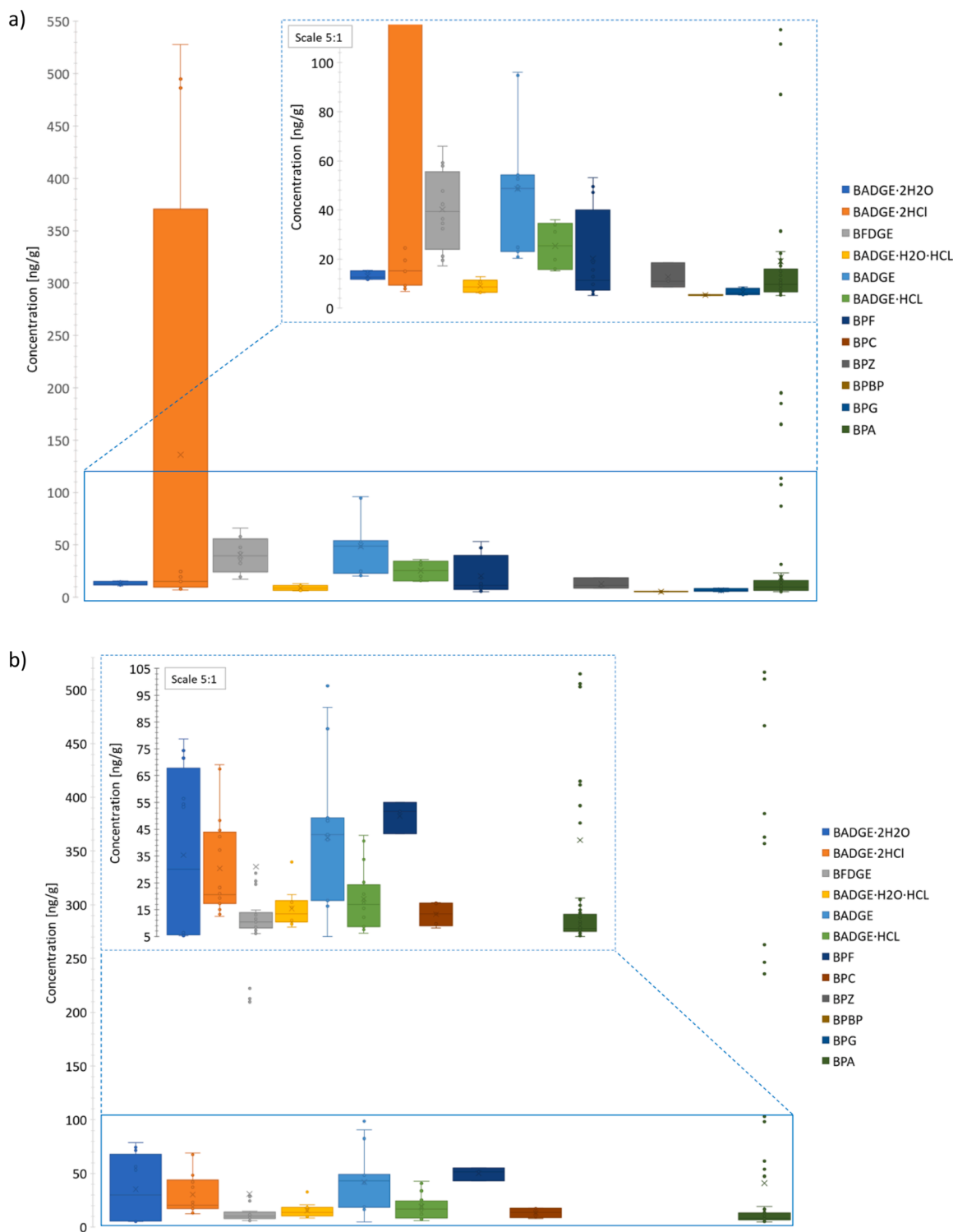


Fig. 2. Distribution of concentration ranges in a) absorbent core and b) supporting wings.

concentrations of analytes were found with a different frequencies, we believe that it is not possible to describe the characterization of each data set using only the average values. Therefore, further discussion of the results will be compared on the basis of the minimum, maximum and median values of the concentrations found.

3.4. Risk characterization

The estimated daily dermal exposure doses of BPs to infants from

direct skin contact with diapers are shown in Table 3. For this calculation, the average and 95th percentile concentrations for groups of children were used to represent average- and high-exposure scenarios, respectively. The calculated daily dermal exposure doses for infants and toddlers from BPs ranged from 0 to 412 ng/kg bw/day. The highest dose was found for BADGE-2HCl (mean 217 and 412 ng/kg bw/day for the 95th percentile) followed by BPA (95 and 172 ng/kg bw/day) associated with the use of size 6 diapers. From the analysis of the BPA data, it appears that the daily dose decreases with increasing diaper size (with

Table 3
Estimated daily dermal exposure doses of detected BPs to infants through direct skin contact with diapers.

Analyte	Parameter	Diaper size no.													
		0		1		2		3		4		5		6	
		Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight
BADGE	DED [ng/kg bw/ day]														
	mean					21	8.0	21	7.6			46	32	23	19
	95th percentile					86	32	40	15			87	61	44	37
BADGE-2 H₂O	DED [ng/kg bw/ day]														
	mean							19	8.4						
	95th percentile							36	16						
Σ BADGE															
BADGE-H₂O	HQ					0.00014	0.000053	0.00027	0.00011			0.00031	0.00021	0.00015	0.00013
	95 th percentile					0.00057	0.00021	0.00051	0.00021			0.00058	0.00041	0.00029	0.00025
BADGE HCl	DED [ng/kg bw/ day]														
	mean											11	7.6	15	12
	95th percentile											20	14	28	23
BADGE-2HCl	DED [ng/kg bw/ day]														
	mean					4.2	1.9					8.2	5.8	217	181
	95th percentile					17	7.5					16	11	412	343
BADGE-H₂O-HCl	DED [ng/kg bw/ day]														
	mean											5,7	4,0		
	95th percentile											11	7.6		
Σ BADGE-HCl															
BADGE-2HCl	HQ					0.000028	0.000013					0.00017	0.00012	0.0015	0.0013
	95 th percentile					0.0011	0.000050					0.00031	0.00022	0.0029	0.0024
BFDGE	DED [ng/kg bw/ day]														
	mean					20	7.5	21	13					26	22
	95th percentile					80	30	40	24					50	42
Σ BFDGE															
BADGE-2HCl	HQ					0.00013	0.000050	0.00014	0.000087					0.00017	0.00015
	95 th percentile					0.00053	0.00020	0.00027	0.00016					0.00033	0.00028
BFDGE-2 H₂O	HQ														
	95 th percentile														
BPA	DED														

(continued on next page)

Table 3 (continued)

Analyte	Parameter	Diaper size no.													
		0		1		2		3		4		5		6	
		Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight	Min weight	Max weight
BPF	[ng/kg bw/day]														
	mean	60	40	74	27	23	11	19	9.5	15	8.2	95	67	11	9.0
	95th percentile	60	40	104	41	55	28	24	11	35	20	172	121	16	13
	HQ	0.015	0.010	0.018	0.0068	0.0058	0.0028	0.0048	0.0024	0.0037	0.0021	0.024	0.017	0.0028	0.0023
	95 th percentile	0.015	0.010	0.026	0.010	0.014	0.0070	0.0060	0.0028	0.0088	0.0050	0.043	0.030	0.0040	0.0033
	DED														
	[ng/kg bw/day]														
	mean			63	16			20	9.0	3.2	2.0				
	95th percentile			214	53			38	17	13	8.2				
	HQ			0.016	0.0040			0.0050	0.0023	0.00080	0.00050				
95 th percentile			0.054	0.013			0.0095	0.0043	0.0032	0.0020					
BPG	DED														
	[ng/kg bw/day]														
	mean					6.2	2.3							3.2	2.6
	95th percentile					25	9.3							6.0	5.0
	HQ					0.0015	0.00058							0.00080	0.00065
	95 th percentile					0.0062	0.0023							0.0015	0.0013
	DED														
	[ng/kg bw/day]														
	mean			16	4.0										
	95th percentile			54	14										
HQ			0.0040	0.0010											
(95 th percentile)			0.014	0.0034											
HI	0.015	0.010	0.038	0.012	0.0076	0.0034	0.010	0.0050	0.0048	0.0026	0.024	0.017	0.0054	0.0045	
HI 95 th	0.015	0.010	0.093	0.027	0.022	0.0098	0.016	0.0074	0.012	0.0071	0.044	0.031	0.0091	0.0075	



Table 4
BPs and their derivatives quantified in real samples.

Material	Value	BADGE-2H ₂ O	BADGE-2HCl	BFDGE	BADGE·H ₂ O·HCl	BADGE	BADGE-HCl	BPF	BPC	BPZ	BPBP	BPG	BPA	BPS	BPP	BFDGE-2H ₂ O	Source
Infant clothing	MIN [ng/g]	1.5	NA	1.5	1.5	0.74	NA	15	NA	NA	NA	NA	2.2	0.74	0.74	3.7	(Xue et al., 2017)
	MEDIAN [ng/g]	0.82	NA	2.4	2.9	0.040	NA	0.32	NA	NA	NA	NA	11	1.0	0.0040	NA	
	MAX [ng/g]	13	NA	130	63	4.4	NA	190	NA	NA	NA	NA	13,000	390	8.0	79	
Pads	Frequency %	22	NA	29	16	7.8	NA	5.2	NA	NA	NA	NA	82	53	5.2	1.3	(Gao and Kannan, 2020)
	MIN [ng/g]	NA	NA	NA	NA	NA	NA	<LOD	NA	ND	NA	NA	<LOD	ND	ND	NA	
	MEDIAN [ng/g]	NA	NA	NA	NA	NA	NA	NA	<LOD	NA	ND	NA	NA	2.8	ND	ND	
Panty liners	MAX [ng/g]	NA	NA	NA	NA	NA	NA	3.8	NA	ND	NA	NA	56	ND	ND	NA	this study
	Frequency %	NA	NA	NA	NA	NA	NA	22	NA	ND	NA	NA	72	ND	ND	NA	
	MIN [ng/g]	NA	NA	NA	NA	NA	NA	<LOD	NA	ND	NA	NA	<LOD	<LOD	ND	NA	
Tampons	MEDIAN [ng/g]	NA	NA	NA	NA	NA	NA	8.4	NA	ND	NA	NA	5.1	<LOD	ND	NA	this study
	MAX [ng/g]	NA	NA	NA	NA	NA	NA	90	NA	ND	NA	NA	160	1.3	ND	NA	
	Frequency %	NA	NA	NA	NA	NA	NA	69	NA	ND	NA	NA	69	15	ND	NA	
Absorbent core	MIN [ng/g]	NA	NA	NA	NA	NA	NA	<LOD	NA	ND	NA	NA	<LOD	<LOD	ND	NA	this study
	MEDIAN [ng/g]	NA	NA	NA	NA	NA	NA	4.8	NA	ND	NA	NA	0.70	<LOD	ND	NA	
	MAX [ng/g]	NA	NA	NA	NA	NA	NA	15	NA	ND	NA	NA	2.5	0.22	ND	NA	
Supporting wings	Frequency %	NA	NA	NA	NA	NA	NA	92	NA	ND	NA	NA	92	8.0	ND	NA	this study
	MIN [ng/g]	12	6.8	17	6.3	20	15	5.2	ND	8.6	5.2	5.5	5.1	NA	ND	ND	
	MEDIAN [ng/g]	13	15	39	8.6	49	25	11	ND	11	5.4	6.1	9.7	NA	ND	ND	
	MAX [ng/g]	15	530	66	13	96	36	53	ND	18	5.6	8.6	200	NA	ND	ND	
	Frequency %	4.2	13	13	4.2	17	8.3	13	ND	4.2	4.2	8.3	75	NA	ND	ND	
MIN [ng/g]	5.3	12	6.1	8.5	4.9	6.2	43	8.2	ND	ND	ND	5.0	NA	ND	ND	this study	
MEDIAN [ng/g]	30	21	10	13	43	17	52	13	ND	ND	ND	8.0	NA	ND	ND		
MAX [ng/g]	79	69	220	33	98	43	55	18	ND	ND	ND	520	NA	ND	ND		
Frequency %	13	17	26	8.7	17	13	4.3	4.3	ND	ND	ND	87	NA	ND	ND		

Note:ND – not detected, NA – not analyzed

the only exception of the data calculated for diaper size 5). This trend is consistent with other reported findings (Niu et al., 2021; Cirillo et al., 2015) and may be due to the higher body weight of older children compared to the younger ones. BPA exposure doses ranged from 8.2 to 172 ng/kg bw/day, which is in consistent with the levels reported for sweaty clothing (Wang et al., 2019) and approximately 30- to 100-fold higher than the levels reported for infants exposure from wearing textiles and infant clothing (Xue et al., 2017). The HQ and HI values calculated for each compound are also presented in Table 3. The highest HQ values were for BPA (0.024 and 0.043 for the 95th percentile) and BPF (0.016 and 0.053 for the 95th percentile) for size 1 diapers. The median and HI values for the high-exposure scenario were both well below the safe level (HI = 1), indicating that exposure to these compounds from diaper use does not pose an obvious risk to infants and children. In case of high exposure scenarios with absorption rates bisphenols of 20 % and 50 % both the daily dermal exposure doses as well as the HQ and HI values were significant higher. The DED for 20 % and 50 % absorption rate of analytes ranged from 4.4 to 958 ng/kg bw/day and from 11 to 2394 ng/kg bw/day respectively. The HQs and HIs estimated under the two different scenarios are summarized in Table S2 and S3. However, despite higher DED values, all the HIs values were far below 1, and this clearly indicates no significant health risk for children. Moreover, following the EFSA re-evaluation of the risks related to the presence of BPA and the reduction of the TDI value from 4 µg/kg BW/day to 0.2 ng/kg BW/day (Lambré et al., 2023) we decided to perform a simulation of the impact of the lowered value on the determined risk level (Table S4). As can be seen, the reduction of TDI of BPA resulted in a significant increase in HQ. The HQ values vary in range from 41 to 475 for mean and from 55 to 860 for high risk scenario. Considering this scenario, it can be concluded that there is a possibility that adverse health effects could occur.

4. Discussion and comparison with another reports

Typical baby diapers are composed of different layers, mainly polymeric materials, cellulose pulp, and superabsorbents often referred to as the diaper core (Rai et al., 2009). The top layer (sheet), which is in close contact with the skin, is usually made of PP. In other cases, it is also made of polyethylene (PE), polyester or bioplastic (Makoś-Chelstowska et al., 2021). Between the top layer and diaper core, there is usually an acquisition layer that allows urine to pass into the diaper core. It may be made of polyethylene terephthalate (PET) blended with *aloe barbadensis* extract. The core of the diaper is filled with cellulose pulp mixed with polyacrylate granules (SAP, superabsorbent polymer). This minimizes the contact of SAP with the baby's skin. The task of the outer layer is to retain absorbed urine in the middle of the diaper. This layer is usually made of low density polyethylene (LDPE), polypropylene or a bioplastic film.

Table 4 shows the data comparing the content of BPs, BADGEs and BFDGEs in the analyzed samples in this and other studies. The median level of BPA in SW was determined to be 8.0 ng/g, while in AC it was 9.7 ng/g. The median BPA content in textiles for infants (Xue et al., 2017) was 11 ng/g of the sample studied, while in the feminine hygiene products it ranged from 0.70 to 5.1 ng/g. BPA was found in 75 % of the analyzed AC and in 87 % of SW. Analyses of children's clothing revealed a frequency of BPA detection of 82 % (Xue et al., 2017), while the ratio in feminine hygiene products such as sanitary pads, panty liners and tampons ranged from 69 % to 92 % (Gao and Kannan, 2020). The highest BPA concentration was 2.5 ng/g, 56 ng/g and 160 ng/g in tampons, pads and panty liners, respectively. In baby diapers, the highest quantified concentration was 200 ng/g in AC and 520 ng/g in SW. Since baby diapers and other named absorbent hygiene products (AHP) share structural similarities, it is likely that the detected levels of BPA occur. BPA was the most frequently detected compound (69–92 %), not only in baby diapers but also in baby clothing and personal care products (PCPs). The highest concentration detected in the analysis

(530 ng/g) was BADGE-2HCl present in one of the AC. Its presence could be due to the use of biocomposites with some reactive modifiers containing epoxy groups in their production. Their function is to improve some material properties, such as increasing water absorption capacity. For this reason, they can be used in disposable products such as breathable and waterproof layers in diaper backsheet or femcare products (Formela et al., 2017). BPP and BFDGE-2 H₂O were not detected in disposable diapers and PCPs, but were detected in textile samples. BPZ, BPBP, and BPG were detected only in AC, while BPC was only detected in the SW of the baby diaper. Only in this research BPZ, BPBP and BPG were tested together with other bisphenols. From the data in Table 4 it can be seen that the presented method allows to determine the largest amount of compounds from the group of bisphenols.

Although the calculated median and HI values for both the high exposure scenarios and the two absorption ratios were well below the safe level (the highest HI was 0.54), indicating that exposure to these compounds through diaper use does not pose an obvious risk to infants and children. This does not change the fact that monitoring BPs level is an important issue as they are directly related to child and environmental health. However, taking into account the reduced TDI value for BPA a health risk assessment is very alarming. Therefore, it is also necessary to look for new materials and improve production processes to minimize the occurrence of bisphenols in baby diapers.

4.1. Limitation

Although the method presented makes it possible to extract 19 analytes and separate 17 analytes at the same time with a single extraction agent, it is important to think of its limitations as well. One of them is a two-step separation necessary due to the accumulation of BPA in the chromatographic column (Wilczewska et al., 2016). In addition the stability of analyzed compound varies depending on solvents type (Szczepańska et al., 2019). Moreover, the single extraction approach was introduced, which has impact to the efficiency of extraction of BPS because of its different polarity (logP=1.9) in comparison to another BPs (ex. BPA logP=3.3, BPC logP=5.0). Due to the above and the complexity of analyzed matrix it is important to further improve extraction procedure which will increase efficiency for BPs such as BPS. Moreover, several limitations are connected with estimation of daily exposure dose and hazard risk evaluation, including: (i) adoption of numerous assumptions for calculations (such as: TDI threshold for other bisphenols, the transdermal absorption rate value, the number of diapers used per day, or the baby's body weight); (ii) high value of coefficients of variations. It is known that children are more vulnerable to contaminants than adults, however, the TDI values used for calculation for investigated compounds set by EFSA refer to adults, not toddlers and infants. Therefore, to carry out an accurate risk assessment, it is necessary to conduct more broadly research.

5. Conclusion

BPs analogs may be present in diapers and may negatively affect babies' health, hence it is necessary to control the content of individual compounds in diapers. The new method proposed in this article is a response to the lack of a harmonized analytical protocol for the determination of BPs and some of their derivatives in disposable care products for babies and children in the territory of the European Union. For the first time, ultrasound assisted solvent extraction of porous membrane-packed solid sample with quantification by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UASE-PMSS-UPLC-MS/MS) was used for the disposable baby diapers. Moreover, two groups of analytes, bisphenols and their diglycidyl ethers, were determined within a single run. In addition, the methodology is characterized by a short analysis time and a low sample volume and waste generated.

Due to the complexity of the analyzed matrix, it is important to direct

future research to finding a procedure that will give the opportunity to analyze more BPs in one step of extraction. Additionally, in order to more accurately assess health risk, it seems necessary to establish the TDI values for other xenobiotics as well as elaborate guidelines and risk assessment procedures dedicated to children, not only adults.

CRedit authorship contribution statement

J. Plotka-Wasyłka: Conceptualization, Bibliographic research, Writing – original draft, Writing – review & editing, Supervision. **A. Chabowska:** Bibliographic research, Writing – original draft. **N. Jat-kowska:** Bibliographic research, Writing – original draft, Writing – review & editing. **P. Kubica:** Bibliographic research, Writing – review & editing.

Declaration of Competing Interest

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

Data Availability

No data was used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2023.115351](https://doi.org/10.1016/j.ecoenv.2023.115351).

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