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Original article

Bisphenol A Exposure On Exclusively Breastfed Infants In Lactating Women: An Observational Cross-Sectional Study

[a]Asst. Prof. Seda Çiftçi*, İzmir Democracy University Faculty of Health Sciences, Department of Nutrition and Dietetics, İzmir, Turkey; Email addresses: seda.ciftci@idu.edu.tr; ORCID ID: https://orcid.org/0000-0002-4103-1618
[b]Prof. Dr. Sıddıka Songül Yalçın, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey; Email: siyalcin@hacettepe.edu.tr (Sıddıka Songül Yalçın); ORCID ID: https://orcid.org/0000-0001-9061-4281
[c]Prof. Dr. Gülhan Samur, Hacettepe University Faculty of Health Sciences, Department of Nutrition and Dietetics, Ankara, Turkey; Email: gsamur@hacettepe.edu.tr (Gülhan Samur); ORCID ID: https://orcid.org/0000-0003-0456-4623

What is already known on this topic?
Bisphenol A is one of the endocrine distributors, and exposure to BPA throughout breastfeeding is crucial for health. It is not clear Turkish exclusively breastfed infants BPA exposure.

What this study adds?
Bisphenol A is measured in all the human milk samples collected from Turkish mothers. Our study is the first study to estimate exclusively breastfed infants' daily BPA exposure and BPA risk index. Exclusively breastfed infants' estimated BPA exposure was significantly within TDI (4 µg/kg body weight/day). There was a weak negative correlation between exclusively breastfed infants' BPA exposure and their current body weight.

Abstract
Objective: Bisphenol A exposure is crucial for lactating women and exclusively breastfed infants. Bisphenol A transfers directly by breastfeeding and may cause adverse health outcomes. We conduct this study to determine maternal human milk bisphenol A level and exclusively breastfed infants' bisphenol A exposure. We investigated the effect of exposure according to participants' nutritional habits.
Methods: We enrolled voluntarily, healthy postnatal, exclusively breastfeeding women (n=80) and collected hindmilk samples. Human milk-free bisphenol A concentration was analyzed using a competitive ELISA method. Free (unconjugated) BPA has been detected in human samples indicating that humans are internally exposed to estrogenically active BPA. Participants' demographic properties, nutritional habits were questioned with an elaborated survey face-to-face by the researcher.
Results: Human milk median free bisphenol A level is 0.63 µg/L. There was no statistically significant association between maternal body mass index, birth type, parity, infant birth week, infant birth weight, and human milk bisphenol A concentration. Nevertheless, we only found a statistically significant association between human milk bisphenol A level and fast-food, carbonated drinks consumption ($p=0.022$ and $p=0.018$, respectively). Exclusively breastfed infants' bisphenol A exposure was 0.0099±0.0079 µg/kg bw/day. There was a negative moderate statistically significant correlation between infant bisphenol A exposure and infant current body weight ($r= 0.327$, $p=0.003$).
Conclusion: Exclusively breastfed infants bisphenol A exposure was under the tolerable bisphenol A level (4 µg/kg bw/day), and infants' current dietary exposure level was safe.
Key Words: Bisphenol A, Breastfeeding, Exposure, Lactation, Maternal Exposure

Asst. Prof. Seda Çiftçi İzmir Democracy University Faculty of Health Sciences, Department of Nutrition and Dietetics, İzmir, Turkey
seda.ciftci@idu.edu.tr
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Introduction

Bisphenol A (BPA) is a human-made chemical compound and used in the production of polycarbonate (PC) plastics (food packaging materials) and epoxy resins (coating the inside of food cans and water storage tanks) all over the world (1). The primary reason for BPA exposure via food is direct contact with packages, and it penetrates foods rapidly (2). Almost all the dietary free BPA, absorbed through the gastrointestinal system, metabolized at the liver, and conjugated with glucuronide. In a healthy population, conjugated BPA (BPA-glucuronide) is mostly excreted through the urine (3,4).
Moreover, free (unconjugated) BPA (biologically active endocrine-disrupting form) was detected in human samples, especially lipid-rich biofluids such as breast milk (5,6). Because of rapid metabolism, short half-life (<6 hours), determinable amounts of excretion (completed in 24 hours), and biological accumulation in the human body, BPA ingestion should be considered while evaluating exposure to the endocrine-disrupting chemical through diet (7). BPA exposure may be an excellent concern for fetal, neonatal development. Fetal/neonatal and childhood liver has an inadequate metabolic enzyme capacity to inactivate BPA via conjugation. It leads to relatively elevated free BPA concentration urine and plasma concentrations in toddlers than adults (8,9). Fetus and neonates are sensitive to perturbation by hormone-like chemicals. Early-life exposure to low-dose BPA can alter the epigenetic mechanism. The epigenetic alterations triggered by BPA may clarify the increased risk of developing adult-onset diseases (10).

BPA is determined in a different range of biological fluids such as human blood serum, urine, saliva (11,12), human milk, and colostrum (5). European Food Safety Authority (EFSA) published its scientific opinion on the safety of BPA and assigned a new threshold value (4 µg/kg bw/day) for temporary tolerable daily intake (t-TDI) (12).
Human milk is a gold standard and contains a considerable amount of essential nutrients. Accordingly, exclusively breastfeeding is recommended by the World Health Organization (WHO) up to six months alone and up to two years of age or beyond with appropriate complementary foods (13). Human milk contains proteins, lipids, and carbohydrates, and chemical contaminants such as persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), dichlorodiphenyltrichloroethane (DDT), and dichlorodiphenyldichloroethylene (DDE) isomers. Furthermore, human milk composition varies owning to maternal diet, genetics, lactation stage, breastfeeding practices, maternal and infant health status, and environmental exposures (14). Diet is considered the primary route of BPA exposure and accounts for the highest estimated daily intake of BPA per body weight among the general population, with infants and children (12). Exclusively breastfed infants BPA exposure can be assessed by maternal human milk BPA concentration (15). In vitro studies elucidate that postnatal BPA exposure impacted sperm production and reproductive success (16), genomic damage, significant alterations in liver enzymes, and lipid profile (17). This study aims to determine maternal human milk BPA level and exclusively breastfed infants' BPA exposure with BPA exposure risk index. Furthermore, we aim to investigate exposure according to participants' baseline characteristics such as nutritional habits and preferred culinary materials and food packaged type on human milk BPA level.

Methods

Study setting and participants

We conducted this study at Hacettepe University İhsan Doğramacı Child Hospital, Social Pediatrics Department in Ankara province between August 2018-December 2018. Before we had launched the investigation, the study protocols were approved by the Ethics Boards and Commission of Hacettepe University. We enrolled healthy voluntary exclusively breastfeeding women (n=80) whose age between 19-40 years, brought her healthy infant (1-3 months) for routine health control to the hospital. All participants gave their informed consent before being included in the study. We excluded smokers and women who had multi-pregnancies, gestational diabetes, diabetes mellitus, chronic disease and used medicine and vitamin and mineral, alcohol. Whoever exposed BPA because of occupational reasons, we excluded from our study. The questionnaire was conducted face-to-face by the researcher. The first section of the questionnaire has demographic and anthropometric questions about the mother and her infant. Mothers' anthropometric measurements such as weight and height are measured, and body mass index (BMI) was calculated by dividing weight (kg) by height^2 (m^2) (18). Infants' weight and height were measured at the hospital by trained infant nurses. The second section questions are about daily used culinary tools material, nutritional habits, and purchased food packaging information. All the participants provided 5 mL mature human milk (hindmilk) samples directly from the nipple into a BPA free sterile tube. Samples remained at the freezer (-80 °C) until the analysis day.
Investigation of human milk-free-BPA levels using an Enzyme-Linked Immunosorbent Assay (ELISA) [EuroProxima, Holland] kit is unique for milk samples. The principles and method of ELISA, target antigen (or antibody) capture in samples using a specific antibody (or antigen), and target molecule detection/quantitation using an enzyme reaction with its substrate. The antibody, conjugate, and standard/sample are pipetted into the wells and incubated for 1 hour at 4ºC. After washing, the procedure ready-to-use substrate is added and incubated for 30 minutes at 20°C-25°C. When the reaction stops, the absorbance is read in a spectrophotometer at 450 nm. Then we the measured optical density into the concentration of the metabolite in the starting material. The detection limit of the ELISA kit is between 0.2 and 10 ng/ml. The BPA ELISA of EuroProxima is validated for water and milk using sample preparation procedures developed and validated in cooperation with the RIKILT (Wageningen, the Netherlands) (19).

Statistical Analysis

Exclusively breastfed infants BPA exposure was calculated by ingestion human milk average quantity due to the infants' months. Because it is hard to calculate exclusively breastfed infants' daily human milk consumption precisely, we used human milk intake average constant value. Commonly maternal milk production of an exclusively breastfeeding woman is between 1- and 6-months' averages 750 to 800 mL/day (20). Thus, we assumed that infants daily ingest approximately 800 mL of human milk (21). According to the following expression, the Risk Index (RI) was determined for human milk to estimate risks to exclusively breastfed infants' health associated with BPA intake. Moreover, we respectively used the following equations (15).

$$\text{Daily Intake (DI) (ng/kg bw/day)} = \frac{\text{Milk Consumption } \left({}^{ml}/_{day}\right) \text{x BPA consantration } \left({}^{\text{ng}}/_{ml}\right)}{\text{Infant body weight (kg)}}$$

$$\text{Risk Index (RI)} = \frac{\text{Daily Intake (DI) (ng/kg bw/day)}}{\text{Tolerable Daily BPA Intake (µg/kg bw/day)}}$$

We considered two critical points when we gave a new value to the data below the detection limit. Firstly, because the number of data remaining below the detection limit value is less than 15% of all data, human milk BPA concentrations below the limit of detection (LOD) were assigned a value equal to the LOD divided by the two. Samples were analyzed according to standards specified by the U.S. Environmental Protection Agency (USEPA). We elaborately reviewed analytical data to ensure quality control (22). Human milk BPA data were normally distributed (Kolmogorov-Smirnova test significance is p=0.200). Secondly, the different countries could use different approaches for assigning values to measurements below the limit of detection. However, Canadian Health Measures Survey and Korean National Environmental Health Survey give a value of LOD/2 for measures below the LOD. Here, we prefer to assign the value of LOD/2 for measurements below the LOD for all datasets (23). Statistical analysis was accomplished for comparing distribution among groups. We used independent Sample t-Test for parametric data while Mann-Whitney U and Kruskal-Wallis tests for non-parametric data. The correlation was performed using the Pearson coefficient test for non-parametric data and the Spearman coefficient test for parametric data. For this knowledge, Pearson correlation coefficients were

calculated to determine the association between human milk free-BPA concentration and infant birth weight, infant current body weight. And for the other correlation, we use Pearson correlation coefficients. Statistically, significance was set at $p<0.05$. Data management and analysis were performed using IBM SPSS version 23.

Results

Subjects

The baseline characteristics of the participants are presented in Table 1. The mean± standard deviation of maternal age and BMI is 28.88±5.17 and 26.41±4.28, respectively. According to their statement, they did not use a medicine, and they were healthy. All infants were born singleton at full-term with the usual neonatal outcome, and they were healthy according to parental report and had no complications during pregnancy or delivery.

Exclusively Breastfeeding Infants' BPA Exposure

The median value of free-BPA concentrations in breast milk is 0.63 ng/mL, mean±SD is 0.49±0.37 ng/mL. Exclusively breastfed infants, free-BPA exposure mean±SD is 0.0099±0.0079 µg/ kg bw/day, and the geometric mean is 0.0073. In this study, free-BPA exposure levels were in the range of 0,0008-0,489 (Table 2). Furthermore, according to infants' BPA exposure, we calculated the risk index of BPA exposure. Exclusively breastfeed infants' risk index mean±SD is 0.002±0.0019 and the geometric mean is 0.0018 (Table 2).

As seen in Table 3, we did not find a statistically significant correlation between human milk free-BPA concentration and infant birth week, birth weight, body weight. However, we found a weak negative correlation between infants' BPA exposure and infant body weight ($r=-0.327$, $p=0.003$). However, we did not find any significant correlation between infant BPA exposure and infant birth week and infant birth weight.

We computed the infants' BPA exposure risk index for identifying human milk-free BPA concentrations significance over the exclusively breastfed infants.

We identified a direct high positive correlation between exclusively breastfed infants' BPA exposure and BPA risk index ($r=0.990$; $p<0.05$).

Nutritional Habits and Human Milk BPA Level

To determine human milk BPA contaminants, we conducted a nutritional survey to participants. The table below illustrates the evaluation of culinary tool materials, dietary habits, and purchased food package information (Table 4). Culinary materials such as water heaters, water bottles, and baking molds play an essential role in our daily lives. Although we use them frequently on an ordinary day, we could not find a statistically significant association between human milk BPA level and culinary materials. We found no statistically significant differences except for fast-food consumption when we examined the association between human milk BPA level and participants' nutritional habits. Within the fast-food consumption group, once a month, fast-food consumed participants had higher BPA levels than participants who did not consume fast food ($p=0.02$). There was no statistically significant difference between human milk BPA level and the package type of drinking water and vegetable oil. However, we explored a statistically significant relationship between human milk BPA level and packaged carbonated drinks ($p=0.018$). When we examined inter binary groups, we only found that participants did not consume carbonated beverages had higher human milk BPA level than the other carbonated drink consumer who preferred glass and PET packages (respectively $p=0.042$ and $p=0.020$).

Table 5 shows human milk free-BPA parameters' distribution and comparison with other studies. Human milk free-BPA's median value is well compatible with Japan and USA populations human milk free-BPA while Spain and Canadian women's human milk free-BPA median value is not. Korean mother's human milk free-BPA level has the highest value.

Discussion

In this study, we analyzed the free-BPA level on human milk samples by using the ELISA method. Total BPA is equal to the sum of free BPA and BPA glucuronide (27).

Moreover, we calculated exclusively breastfed infants' BPA exposure value with the BPA exposure risk index. Based on the results of BPA exposure for mothers and infants, we reached a variety of conclusions and discussed them below.

We selected healthy mothers who have 1-3 months exclusively breastfed a healthy infant. Exclusively breastfed infants' approximately average breast milk utilization is 750-800 mL/day (20). To the best of our knowledge, BPA is excreted into human milk (28) and naturally transferred to the infant via breastfeeding (5). Exclusively breastfed infants BPA exposure is inevitable. The adverse outcome of BPA exposure varies according to the exposure dose and term (29).

To metabolize BPA in humans, require uridine diphosphate glucuronosyltransferases (UGTs) enzyme activity, which gradually rises at the beginning of 3-6 months to 10 years then reaches normal levels. Infants between 1-3 months have not effective and enough hepatic UGTs metabolic enzyme to metabolize BPA (30). Recently developed ELISA method kit has had high sensitivity and comparatively low cost (28,31).

Because conjugated BPA is not an active biologic form to make a detailed evaluation, conjugated and free BPA concentration should be determined together (25). Studies detect free and total BPA levels on human milk (6,28,32). Findings indicate no statistically significant association between human milk BPA level and mothers' age, BMI, birth type, and parity (Table 1). The primary BPA exposure source is dietary (12,33), so dietary habits change among the countries. The study presented here is one of the first investigations to assess the impact of BPA exposure and risk index for exclusively breastfed Turkish infants. We found a BPA exposure average value of 0.0099 µg/kg bw/day. EFSA suggested that BPA exposure first three months exclusively breastfed infants are 0.2 µg/kg bw/day (12). The difference in BPA exposure values may be stem from nutritional habits and socioeconomic status. Furthermore, we found a BPA exposure risk index value of 0.002 for exclusively breastfed infants based on EFSA's BPA TDI value. RI value is less than one so that there is no BPA exposure risk among exclusively breastfed infants (15). The correlation between human milk BPA level and infant birth week, weight, and current body weight are not statistically significant (Table 3). Casas et al. l. did not find a statistically significant association between BPA exposure during pregnancy and fetal growth parameters (34). Intriguingly, we found a negative moderate statistically significant correlation between infants' BPA exposure and infant current body weight (Table 3). This means the increase in BPA exposure has a negative effect on weight gain. Normally after delivery when lactating launches, estrogen level starts to decrease (35), but BPA's concentration may inhibit lactation. Kasper et al.

(36) demonstrated an association between maternal BPA exposure and decreased breastfeeding at one month postpartum. As presented in Figure 1, we determined a strong uphill (positive) linear relationship between infants' BPA exposure and infants' BPA risk index. Because BPA can be transferred to the infant via human milk (5), for exclusively breastfed infants, BPA exposure way is only human milk. A considerable amount of research showed that the primary source of BPA exposure is diet (37,38). That is why pregnant, and lactation women should carefully consider their diet.

According to used culinary materials, nutritional habits, and purchased packaged information, the median and percentile of human milk BPA scores are presented in Table 4 for each group. EFSA reports that (12) estimated BPA exposure caused by polycarbonate plastic water kettles ranged from 2 to 3.2 ng/kg bw/day. Moreover, plastic kettle use was found to be a critical exposure route for BPA (39). The highest values are observed in adults due to their higher consumption of coffee and tea. However, there was no significant difference in BPA exposure associated with the presently used type of water heater. This association may be explained by lower coffee and tea consumption among lactation women than in adults.

As can be seen from the data in Table 4 that the once-a-month fast food consumption group had statistically significantly higher BPA exposure than the not consume group. Still, there was not a significant difference between the other groups. A cross-sectional study among the U.S population Zota et al. (40) showed that fast-food consumption might not be a BPA exposure source. EFSA's scientific opinion of BPA presence in foodstuffs indicated that PC plastic packages pose no health risk over consumers of any age group (including unborn children, infants, and adolescents) (12). Hence, a possible explanation for the difference determined in this study might be the variation of the preferred fast-food menu concept because only hamburger's BPA value is 10.9 ng/g (41). Polycarbonate plastics, used in reusable water carboys (42). Moreover, considerable amounts of BPA (approximately 0.15 µg/L) were leached from polycarbonate bottles within the first 24 h of storage (43).

If PC carboys are stored at or under room temperature, BPA water levels could be expected normal. PC carboys are suitable containers due to PC's properties (clear and rigid plastic) (44). In our study, human milk BPA level is not change statistically significant according to preferred drinking water. According to the FDA, scientific evidence has supported the safety of BPA for the currently confirmed utilization in food containers and packaging (45).

Our study found that who consumed glass bottle carbonated drinks had statistically significant higher human milk BPA level than who did not consume carbonated beverages and consumed PET bottle carbonated drinks ($p=0.042$ and $p=0.020$, respectively). This rather intriguing result may be due to the ingested carbonated drinks type and consumption quantity and frequency.

BPA concentrations were detected in canned carbonated drinks and plastic bottled water. The BPA concentrations in canned carbonated drinks were between 83-340 ng/L. However, BPA was not detected out of two canned carbonated beverages (46).

These results are consistent with previous researches (8,47). Studies examining the human milk free-BPA level are summarized in Table 5. Although these results differ from some published studies (6,25,26), they are consistent with some other published studies (8,24). Firstly, a possible explanation for this might be that the difference could be related to the applied analysis method suggested by Yi et al. (26). The detection range of BPA was broader in the HPLC (High-Performance Liquid Chromatography) method than the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, but the BPA concentrations in the HPLC with fluorescence detector (HPLC/FLD) analysis were lower than those in the LC-MS/MS analysis. Secondly, the primary BPA exposure source is nutritional habits (12), and there are disparities in dietary patterns across the world (33). Another possible explanation for this is that dietary habits change among the countries.

In our study, we have several limitations. Firstly, we only collected human milk samples one day from mothers. Unfortunately, a one-day sample collection may have cause bias about exposure because a single administration of sample collection may not reflect BPA exposure precisely so that two or more non-consecutive sample collection should be required to estimate the normal average human milk BPA level (48). Secondly, we could use chromatographic methods instead of ELISA for determining the BPA level of samples of human milk. However, ELISA can be used for screening purposes, and it is an inexpensive method. (49) Finally, we did not consider the seasonal difference while collecting human milk samples. We collected the first sample in August and the last in November. BPA leaching into foods and beverages from packaging or storage containers, especially when heated to high temperatures (50).

Conclusions

Exposure to BPA is a concern because of the possible health effects, and it plays a role in the pathogenesis of several endocrine disorders, including obesity, asthma, and neurobehavioural disturbances. The present study extends our knowledge of BPA exposure among breastfeeding women and their infants. Exclusively breastfed infants' BPA exposure positively correlates with human milk BPA concentration. The findings reported here suggested that BPA exposure of exclusively breastfed infants was far below tolerable BPA level (4 µg/kg bw/day). According to findings, it can be assumed that the BPA exposure of exclusively breastfed infants in our country have not got exposure risk. It is thought to be current regulatory restrictions on BPA use have reduced exposure levels. However, the possible exposure of low dose BPA cannot be ruled out. Further studies, which take these variables into account, will need to be undertaken.

Declarations

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Competing Interest: The authors declare that they have no conflict of interest regarding this article's publication.

Ethical Approval: This research study was ethically approved on July 24, 2018, by the Hacettepe University Clinical Research Ethical Board with project number GO 18/715 and decision number GO 15/715-33.

Consent to Participate: All procedures performed in this study involving human participants followed the institutional and national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

Consent to Publish: All authors consent to this article to publish.

Code availability: None applicable.
Availability of data and materials: Due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.
Authors' contributions: GS designed the model and the computational framework. SC collected samples and performed analysis. SSY took lead sample collection. All authors discussed the results and contributed to the final manuscript.

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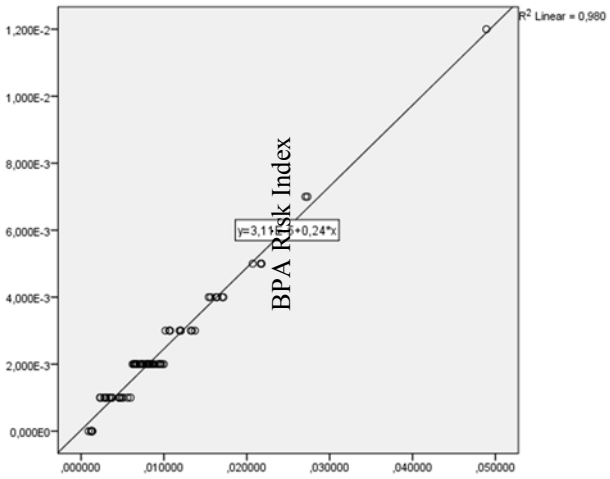

Figure 1. Association between infants BPA exposure and BPA risk index

Table 1. Characteristics of Mothers and Their Infants

Features	**N (%)**	**Human Milk BPA Level (µg/L)**	**p-Value**
Mothers		**Median (P25-P75)**	
Age (years)			
19-28	40 (50%)	0.5792 (0.3432-0.7878)	0.554*
29-40	40 (50%)	0.6457 (0.3127-0.8033)	
BMI (kg/m²)			
18.5-24.9	35 (43.75%)	0.6464 (0.4136-0.8388)	0.521***
25.0-29.9	30 (37.5%)	0.5686 (0.2779-0.7674)	
30.0-34.9	9 (11.25%)	0.6790 (0.4524-1.0234)	
35.0-39.9	6 (7.5%)	0.4684 (0.2153-0.7204)	
Birth Type			
Normal spontaneous virginal delivery	40 (50%)	0.6667 (0.3607-0.8180)	0.225*
Caesarean	40 (50%)	0.5711 (0.3044-0.7464)	
Parity			
0	38 (47.5%)	0.6857 (0.4891-0.8224)	0.095**
≥1	42 (52.5%)	0.5328 (0.2711-0.7181)	
Infants			
Birth Week (week)			
Boy	40 (50%)	0.6518 (0.3430-0.8114)	0.513**
Girl	40 (50%)	0.5738 (0.3044-0.7559)	
Total	n=80 (100%)		
Birth Weight (g)			
Boy	40 (50%)		
Girl	40 (50%)		
Total	n=80 (100%)		

P25: 25th percentile (P25) and P75: 75th percentile (P75)

Table 2. Infants BPA Exposure level and Risk Index due to Human Milk BPA concentration

	Ingested Human Milk free-BPA Level (µg/kg bw/day)					
	$\bar{X}$ (SD)	G.M	S_x	Median	Min	Max
Exclusively Breastfed Infants BPA Exposure	0.0099 (0.0079)	0.0073	0.0008	0.008	0.0008	0.489
Risk Index (RI)	0.002 (0.0019)	0.0018	0.0002	0.002	0.0002	0.0122

$\bar{X}$: Mean; SD: Standard Deviation; GM: Geometric mean; S_x: Standard Error; Min: Minimum; Max: Maximum.

Table 3. Association Between Human Milk BPA Concentration, Infant BPA exposure and Birth Week, Birth Weight, Infant Current Body Weight.

BPA	Infant Birth Week		Infant Birth Weight		Infant current body weight	
	r	p	r	p	r	p
Human Milk free-BPA concentration (µg/L)	-0.010[b]	0.932[b]	0.071[a]	0.530[a]	0.000[a]	0.997[a]
Infant BPA Exposure (µg/kg bw/day)	-0.024[b]	0.836[b]	0.035[b]	0.759[b]	-0.327[b]	**0.003[b*]**

Table 4. Association between the human Milk BPA Level (µg/L) and culinary materials, nutritional habits, and packaged information.

	Human Milk BPA Level (µg/L)		
	N	Median (P25-P75)	p-Value
Culinary Materials			
Water Heater			
Steal Teapot	50	0.6482 (0.3180-0.7951)	
Steel Kettle	18	0.6122 (0.2904-0.8392)	0.989
Plastic Kettle	12	0.5919 (0.4385-0.7038)	
Water Bottle			
Plastic	25	0.6450 (0.4136-0.7858)	
Glass	55	0.5172 (0.2766-0.8351)	0.451
Baking Molds			
Teflon	42	0.5973 (0.3359-0.7354)	
Glass (Heat Resistance)	14	0.5993 (0.3352-1.0234)	
Silicon	9	0.7078 (0.2554-0.8557)	0.937
Granit	15	0.6412 (0.2538-0.7858)	
Nutritional Habits			
Main Meal Consumption in a day			
2 times a day	25	0.615 (0.29-0.79)	0.976
3 times a day	55	0.641 (0.34-0.79)	
Fast-food Consumption			
Once a month	22	0.7536 (0.6035-0.9238)	
Twice a month	9	0.5152 (0.2716-0.8123)	**0.022**
Not Consumed	49	0.5124 (0.2603-0.7101)	
Instant- Packaged Meal Consumption			
No	58	0.6301 (0.2956-0.7726)	0.714
Yes	22	0.6103 (0.4255-0.8392)	
Canned Food Consumptions (Tuna fish, soup, corn, pea)			
No	60	0.6431 (0.3374-0.8180)	0.764
Yes	20	0.5713 (0.3044-0.7512)	
Canned Beverage Consumptions			
No	65	0.6152 (0.3195-0.7632)	0.315
Yes	15	0.6501 (0.4544-1.1269)	
Packaged Information			
Drinking-Water			
PET (polyethylene terephthalate) plastics	30	0.6631 (0.3950-0.7994)	

Carboy (Recycling Code=7)	26	0.6518 (0.3359-0.7588)	0.678
Tap	24	0.5327 (0.2683-0.9536)	
Carbonated Drinks			
PET (polyethylene terephthalate) plastics	62	0.5696 (0.2975-0.7607)	**0.018**
Canned	9	0.6450 (0.5541-0.8297)	
Glass	4	0.4414 (0.1509-0.6723)	
Not Consumed	5	1.0818 (0.8723-1.3696)	
Vegetable Oil			
PET (polyethylene terephthalate) plastics	37	0.5632 (0.3185-0.7820)	
Canned	36	0.6438 (0.3484-0.8224)	0.788
Glass	6	0.7409 (0.2544-0.7990)	
Not consumed[¥]	1	-	

[¥] *Because no consumed group consists of one data, we do not include it in a statistical test.*

Table 5. Distribution of the parameters and comparison with other studies for free BPA concentration of human milk.

			Free-BPA (ng/mL)				
Country	**(n)**	**Method**	***n*>LOD***	**X (S)**	**Median**	**Min-Max**	**Reference**
Turkey	80	ELISA	71 (%88.75)	0.49 (0.37)	0.63	<LOD*-1.9	This Study
Japan	23	HPLC	23 (%100)	-	0.61	0.28-0.97	Sun et al., (24)
USA	21	UHPLC-MS/MS	13 (%62)	3.13	0.68	<0.22-10.8	Zimmers et. al., (8)
Spain	120	HPLC-MS/MS	92 (%77.4)	0.15 (4.8)	0.10	<LOD*-41	Dudalge et. al., (6)
Canada	278	GC-MS/MS	46 (%16.5)	0.11	0.10	<0.036-2.3	Ca X-L et. al., (25)
Korea	100	LC/MS/MS	100	-	6.60	0.65-29.9	Yi B. et al., (26)

**LOD: Limit of detection (0.2-10 ng/mL)*