Temporal trends in bisphenol exposures and associated health risk among Japanese women living in the Kyoto area from 1993–2016

Zhaoqing Lyu[a], Kouji H. Harada[*,a], Sungmin Kim[b], Tomoko Fujitani[a], Toshiaki Hitomi[c], Rui Pan[a,d], Nayoun Park[b], Yukiko Fujii[e], Younglim Kho[b], Kyungho Choi[f]

[a]Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida, Kyoto 606-8501, Japan

[b]Department of Health, Environment & Safety, Eulji University, Seongnam 13135, Korea

[c]Department of Preventive Medicine, St. Marianna University School of Medicine, Kawasaki 216-8511, Japan

[d]Department of Global Environmental Health, Graduate School of Medicine, University of Tokyo, Tokyo 113-0033, Japan

[e]Department of Pharmaceutical Sciences, Daiichi University of Pharmacy, Fukuoka 815-8511, Japan

[f]Department of Environmental Health Sciences, School of Public Health, Seoul National University, Seoul 08826, Korea

*Corresponding author: Kouji H. Harada

Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Yoshida Konoe, Sakyo, Kyoto 606-8501, Japan

Tel: +81-75-753-4490; Fax: +81-75-753-4490

E-mail: kharada-hes@umin.ac.jp

Abstract

Background: Bisphenols, and especially bisphenol A, are widely used as components of epoxy resins and polycarbonate. Widespread detection and potential health risks have led to bisphenol A being replaced by other alternatives, including structurally similar bisphenol analogs. Several bisphenol analogs are suspected to have similar adverse health consequences. This study examined the temporal trends in bisphenol exposure among a group of Japanese women from 1993 to 2016, and assessed the associated health risks.

Methods: We used archived single spot urine samples of healthy Japanese women living in the Kyoto area (n = 133) collected in 1993, 2000, 2003, 2009, 2011, and 2016. We measured the concentrations of 10 bisphenols in these samples.

Results: A sharp increase in the detection rates of bisphenol F was observed after 2000. There was a distinct downward trend in urinary bisphenol A concentrations and an upward trend in bisphenol E concentrations after 2009. While the hazard index for all measured bisphenols was below 1 in all subjects, BPF was determined as the most important risk driver after 2000, rather than bisphenol A.

Discussion: Trends of decreasing bisphenol A and increasing bisphenol E exposure especially after 2011, along with no significant change in the sum of all bisphenol analogs in urine, provide clear evidence that bisphenol A has been replaced by other bisphenols in the study population. We found no significant change in the total exposure to BP during the study period. Bisphenol F might become the most important bisphenol in terms of risk, while cumulative risks due to all bisphenol exposure were deemed insignificant. Considering the accumulating evidence indicating adverse effects at lower exposure levels, further studies are warranted to assess exposure and risk from bisphenol A analogs.

Keywords: bisphenols; exposure; urine biomonitoring; temporal trend; cumulative risk; Japanese women

1. Introduction

Bisphenols (BPs) are a group of chemical compounds widely used in the manufacturing of products, including polycarbonate plastics, epoxy resins, thermal paper, and water pipes (Catenza et al., 2021). It has been reported that 16 bisphenol analogs were used for industrial production as of 2016, with bisphenol A (BPA) being one of the most commonly produced chemicals (Chen et al., 2016). BPs can leach from products, causing contamination to the environment, and are ingested by people mainly through dietary intake (EFSA, 2015; Catenza et al., 2021). In recent decades, numerous studies have demonstrated that BPA is an endocrine disruptor chemical, which can potentially affect the human reproductive and metabolic systems, and disrupt development (Rochester, 2013; Colorado-Yohar et al., 2021). Laboratory evidence has shown that BPA could cause endocrine-related diseases as well as carcinogenesis and mutagenesis (EFSA, 2015). Data from epidemiological studies have suggested possible associations between a higher level of BPA exposure and outcomes such as infertility, breast cancer, cardiovascular disease, diabetes, and obesity (Ma et al., 2019).

For these reasons, the use of BPA has been restricted. In 2002, the European Commission (EC) permitted the use of BPA in plastic objects and any materials intended to come into contact with foodstuffs with a limit of specific migration (SML) of 3 mg/kg of food (EC, 2002). In 2011, the EC proposed an SML of 0.6 mg/kg of food for BPA, and banned its use in baby bottles (no. 10/2011) (EC, 2011). The European Food Safety Authority (EFSA) also re-evaluated BPA exposure and toxicity in 2015 and reduced the tolerable daily intake (TDI) from 50 to 4 μg/kg/bw (EFSA, 2015). Restrictions on BPA use in various products have also been established in both the United States and Canada during the past two decades (Flint et al., 2012). In Japan, baby bottles and some other products containing BPA were phased out after 2000

because of general concern among consumers (Gys et al., 2020).

Manufacturers have therefore increasingly substituted other chemicals for BPA, including bisphenol analogs, such as bisphenol F (BPF) and bisphenol S (BPS). However, animal models and in vitro experiments suggest these alternative chemicals may also have similar adverse effects on human health (Eladak et al., 2015; Rochester and Bolden, 2015; Chen et al., 2016; Qiu et al., 2019). BPA exposure has been found to be ubiquitous in the last decade (Zhang et al., 2011; Colorado-Yohar et al., 2021; Tschersich et al., 2021). However, biomonitoring studies in various countries have shown a downward trend in BPA concentration in human biological samples (Lakind and Naiman, 2015; Pollock et al., 2021; Tschersich et al., 2021). A study among Japanese children found that urinary BPA concentrations decreased on average by 6.5% per year between 2012 and 2017 (Gys et al., 2020). Nevertheless, some other BPs, notably BPF and BPS, have been frequently detected in human urine samples (Liao et al., 2012a; Lehmler et al., 2018).

Both regulatory and industrial development may have affected Japanese people's exposure to BPs over the last few decades. To date, few studies have investigated long-term temporal trends of BP exposure in Japan, and relevant information is not available. However, the unclear health effects of BP analogs other than BPA mean that monitoring of exposure to BPs is required. Our study aimed to assess the temporal trends of multiple BP analogs and to identify the major BPs of potential health concern, using archived urine samples collected from Japanese women living in the Kyoto area dating back to 30 years ago.

2. Materials and methods

2.1 Sample collection

105 Single spot urine samples were provided by 133 healthy Japanese women aged
106 25 to 80 years (59.3 ± 12.3, mean ± SD; average age in each year was 49–65) when
107 they attended a cross-sectional healthcare checkup program in Kyoto and the
108 surrounding regions in Japan. The samples were collected in 1993, 2000, 2003, 2009,
109 2011, and 2016. The participants were not the same individuals in each collection period.
110 First morning urine samples were collected in paper cups, transferred to polypropylene
111 tubes, and were stored in the home refrigerator until they were taken to the health
112 checkup centers. Urine samples were then transferred to and stored at –30 °C in the
113 Kyoto University Human Specimen Bank (Koizumi et al., 2009) until analysis. During
114 sampling and storage, no polyvinyl chloride (PVC) devices were employed. This study
115 was approved by the Ethics Committee of the Kyoto University Graduate School of
116 Medicine (approval number R1478). Before the study, each participant gave their
117 informed consent, either verbally (before 2000) or in writing.
118
119 2.2 Determination of BPs in urine samples
120 A total of 10 BPs were measured: BPA, bisphenol AF (BPAF), bisphenol AP
121 (BPAP), bisphenol B (BPB), BPF, bisphenol P (BPP), BPS, bisphenol Z (BPZ),
122 tetrabromobisphenol-A (TBBPA), and bisphenol E (BPE). Compounds were obtained
123 from the Cambridge Isotope Laboratory (Andover, MA, USA).
124 Detailed experimental procedures for the measurement of BPs are described
125 elsewhere (Mok et al., 2021). After internal standard spiking, 500 μL of a urine sample,
126 200 μL of 1 M ammonium acetate buffer (pH = 5), and 10 μL of β-
127 glucuronidase/arylsulfatase (*Escherichia coli*) were added into glass vials and mixed.
128 The samples were incubated at 37 °C for 4 h for deconjugation. After adding 400 μL of
129 0.1% acetic acid in water, the samples were loaded into a solid phase extraction (SPE)

cartridge (Sep-Pak 1 cc 100 mg) conditioned with 3 mL of acetonitrile and 3 mL of water. The SPE cartridge was washed with 1 mL of 5% MeOH and dried under gentle nitrogen gas for 2 h. The dried cartridge was eluted with 1 mL of acetonitrile for analysis.

The analyses for measuring urinary BPs used a Shimadzu CBM-20A liquid chromatography system (Shimadzu Corporation, Kyoto, Japan) with an AB SCIEX API 4500 tandem mass spectrometer (AB SCIEX, Ontario, Canada). Chromatographic separation was carried out by an ACQUITY UPLC BEH C18 column (100 mm × 21 mm, 1.7 um). The column temperature was 40 °C, the injection volume was 5 μL, and the flow rate was 300 μL/min. The mobile phases were 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). The gradient condition is shown in Table S1. Eluents from the analytical column were subjected to tandem mass spectrometry. Electrospray ionization in negative ion mode and multiple reaction monitoring mode were used to determine the BP concentrations.

The calibration range was from 0.05 ppb to 100 ppb. The limit of detection (LOD) value was defined as the concept and method of the limit of detection stipulated in the verification of the analytical procedure developed by the United States Food and Drug Administration (U.S. FDA, 1997). The method validation experiment was conducted using urine samples spiked at three concentration levels (1, 5, and 20 ng/mL; n = 6 for each concentration level). The LODs and the method validation results are presented in Table S2.

2.3 Determination of creatinine concentrations in urine samples

High-performance liquid chromatography (HPLC) with a UV detector was used to analyze the concentration of urinary creatinine. Urine samples (10 μL) were diluted with 990 μL of water, and 2, 4, and 6 mg/dL standard creatinine solutions were prepared

155 in HPLC vials for measurement.

156

157 2.4 Determination of hazard quotient (HQ) and hazard index (HI) values

158 The estimated daily intake (EDI) of BPs were calculated from the BP
159 concentrations in urine samples using the equation below (U.S. EPA, 1986; Reyes and
160 Price, 2018):

161 $$EDI = UC \times CE_{smoothed} \times \frac{1}{F_{UE}}$$

162 in which EDI (μg/kg/day) is the estimated daily intake dose for BP, UC is the urinary
163 creatinine-adjusted concentrations of BPs (μg/g creatinine) and $CE_{smoothed}$ (g/kg/day) is
164 the creatinine excretion per day, which was calculated as (Mage et al., 2008):

165

166 $$CE_{smoothed}(\mu g/kg/d) = 0.993 \times 1.64[140 - age](Wt^{1.5}Ht^{0.5})/Wt$$

167

168 considering the age, sex, and race of the participants. Data for weight and height were
169 the means of the weights and heights of Japanese women in a specific age group. The
170 data were reported by the Ministry of Health, Labour and Welfare of Japan in the
171 National Health and Nutrition Surveys of 1993, 2000, 2003, 2009, 2011, and 2016
172 (Ministry of Health of Japan, 1995; Ministry of Health of Japan, 2001; Ministry of
173 Health, Labour and Welfare of Japan, 2005; Ministry of Health, Labour and Welfare of
174 Japan, 2010; Ministry of Health, Labour and Welfare of Japan, 2012; Ministry of Health,
175 Labour and Welfare of Japan, 2017). F_{UE} is the molar fraction of urinary excretion to
176 the total amount of ingested BP. Pharmacokinetic studies in humans have shown that
177 the F_{UE} for BPA is close to 100% (Thayer et al., 2015). To date, no pharmacokinetic
178 study has provided the F_{UE} for BPF and BPE in humans, and the F_{UE} of BPF and BPE
179 was therefore set at 100% given their similar chemical structures to BPA.

Hazard quotients (HQs) were calculated to assess the potential health risks of three selected BPs: BPA, BPF, and BPE. HQ is defined as EDI divided by TDI. HQ_M is the maximum HQ of the three selected BPs for each subject. The hazard index (HI) is the cumulative summation of HQs for each BP. HI > 1 suggests potentially adverse health risks posed by current exposure level to BPs.

$$HQ = EDI/TDI$$

$$HQ_M = \max HQ$$

$$HI_{BP} = HQ_{BPA} + HQ_{BPF} + HQ_{BPE}$$

The EFSA has set a tolerable daily intake (TDI) of BPA of 4 μg/kg body weight per day (EFSA, 2015). The TDI values of BPF and BPE were not established, and we therefore used 3.5 and 3.8 μg/kg/d. These were set as the amounts of the same molar levels as the TDI value for BPA, calculated as below:

$$TDI_{converted} = TDI_{BPA} \times MW_{BP\ analogue}/MW_{BPA}$$

2.5 Statistical analysis

Two-tailed p-values less than 0.05 were considered statistically significant. One-way ANOVA was used to examine the differences between sampling years. The Cochran-Armitage trend test was used to test for a temporal trend in the detection rate of BPs. When analyzing data on BP urinary concentrations, creatinine adjustment was used to reduce the urine dilution effect in spot samples. When comparing urinary concentration levels, concentrations below the LODs were set to LOD/√2. A summary metric for BPs (ΣBP) was calculated by summing the molar concentrations of the measured BPs. BPAF, BPAP, BPP, BPS, BPZ, and TBBPA were excluded from the analysis due to their low detection frequency (< 10%). A partial correlation test was performed using log-transformed data of urinary BP concentrations, in which age was

set as a control variable. A two-sample Student's t-test was performed to examine the difference in urinary BP concentrations between sampling years. We used JMP Pro Statistical Software (Version 16) for data analysis.

3. Results and discussion

Table 1 shows the demographic data of the participants. Their ages varied by sampling years ($p < 0.05$). Since creatinine excretion can be different among ages, the age was set as an adjustment variable for comparison of BP urinary concentrations between the sampling years.

Table 1. Demographic data of the study participants

	Years of sample collection							
	1993	2000	2003	2009	2011	2016	*F*	*p*
No. of subjects	10	25	25	26	22	25		
Age (years)	52.7±4.0	49.4±9.4	65.7±4.9	59.0±14.4	65.5±12.5	60.2±12.7	8.0	<0.0001
Urinary creatinine (g/L)	0.8±0.5	0.8±0.4	0.7±0.4	0.8±0.6	1.0±0.8	0.9±0.5	1.0	0.373

Data are the mean and standard deviation. ANOVA was used to test differences between years and urinary creatinine concentrations.

220 Detection rates of the BPs are shown in Table S3. Among the 10 BPs, BPF was
221 detected most frequently (76.7%) of all samples, followed by BPE (66.2%) and BPA
222 (65.4%). BPAF, BPAP, BPP, BPS, and BPZ were detected in fewer than 2% of samples,
223 and TBBPA was not detected. The distribution of the urinary concentrations and
224 creatinine-adjusted concentrations of BPs is summarized in Tables S4 and S5.
225
226 3.1 Urinary concentrations and temporal changes of BPs
227 Figure 1 shows the temporal changes for the three most frequently detected BPs, i.e.,
228 BPA, BPF, and BPE. The detection rates of BPA reduced significantly after 2009, and
229 a downward trend in the detection rates was observed during 1993–2016 (the Cochran-
230 Armitage trend test, p for trend < 0.001). However, there was an increasing trend in the
231 detection rates of BPF and BPE during the study period (p for trend = 0.001 and 0.002,
232 respectively). The detection rates for BPF increased from 10% in 1993 to 84% in 2000,
233 ranging from 68% to 96% during 2000–2016. For BPE, the detection rates increased
234 from 30% to over 90% after 2009.

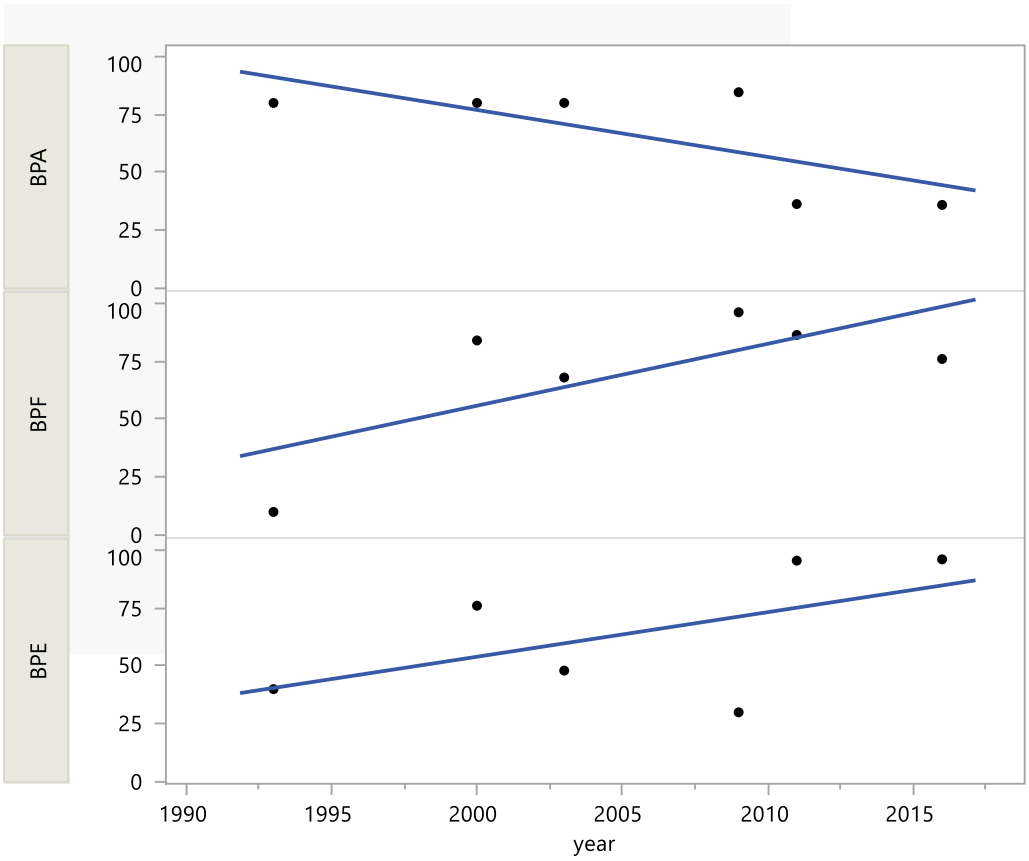
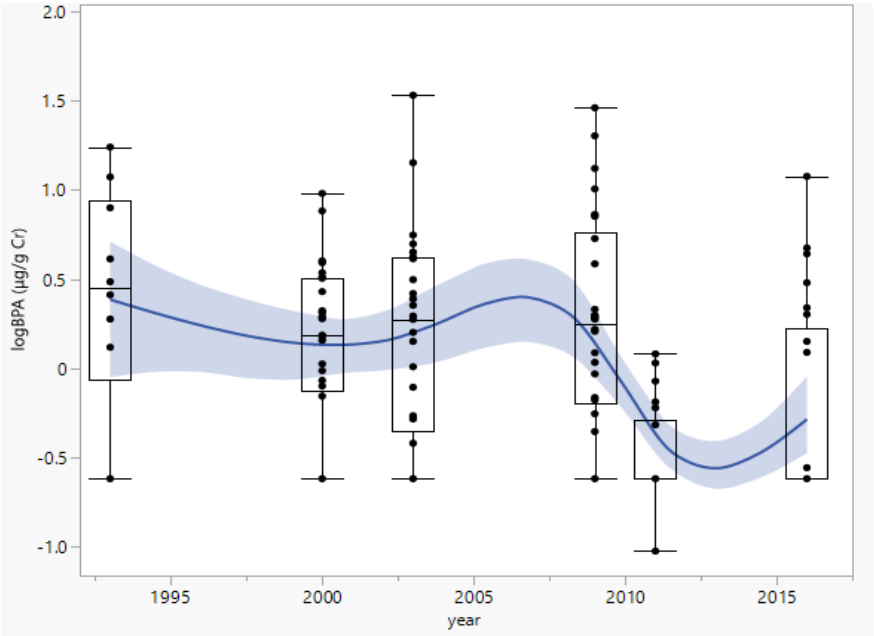


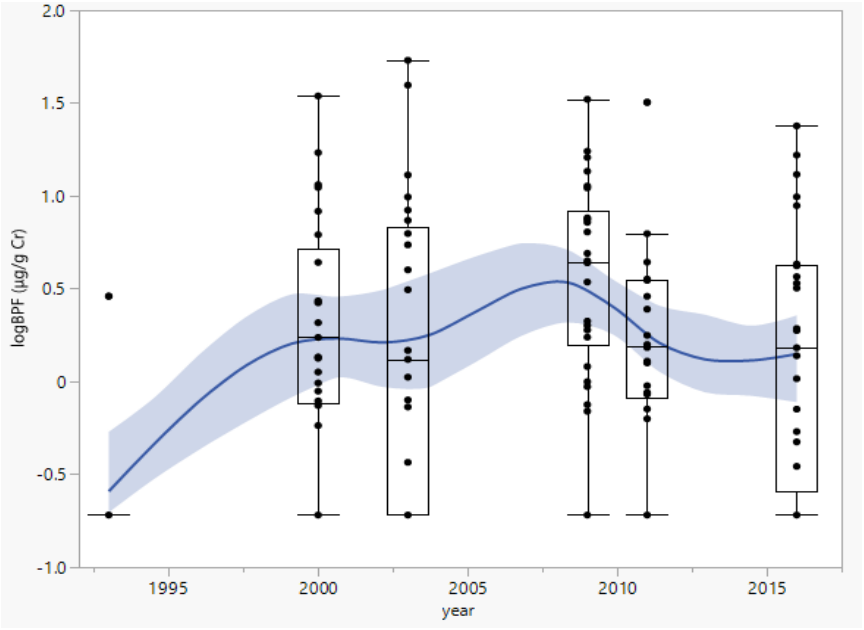
Figure 1. Detection rates (%) of urinary BPA, BPF, and BPE between 1993 and 2016.

The temporal changes in log-transformed urinary concentration levels of BPA, BPF, BPE, and ΣBP are shown in Figure 2. The sum of the median concentrations of each BP and the average proportion of the concentration of each BP in each participant are shown in Figure 3. The creatinine-adjusted concentrations of BPA showed a slight descending trend during the study period (r = -0.195, p = 0.025) (Figure 2A). The median levels of BPA concentrations ranged from 1.53 to 2.82 μg/g during 1993–2009, but the median decreased to 'not detectable' in 2011 and 2016 (Figure 3). There was an obvious decrease in the exposure level of BPA between 2009 and 2011 ($p < 0.0001$, Table S6). There was a dramatic increase in the exposure frequency and level of BPF between 1993 and 2000 (p = 0.0004, Table S6). The median creatinine-adjusted concentration of BPF was not detectable in 1993, but median levels ranged from 1.73

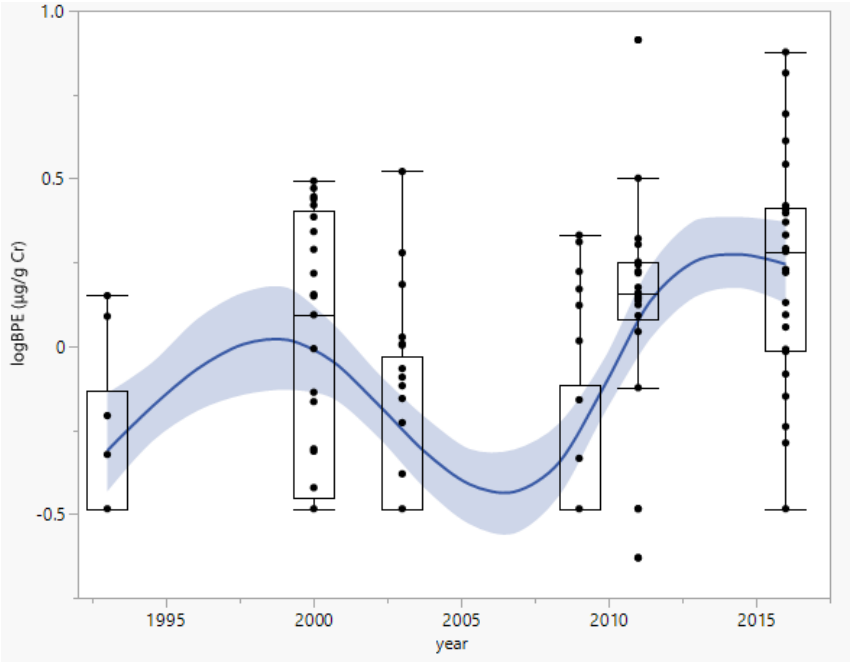
to 4.42 μg/g Cr during 2000–2016, with no observable consistent temporal trend in the concentration levels after 2000 (Figure 3). There was also a significant upward trend in the BPE urinary concentrations throughout this study period (r = 0.276, p = 0.001) (Figure 2). The median levels of BPE concentrations were not detectable in 1993, 2003, and 2009, but the median increased to 1.43 and 1.92 μg/g in 2011 and 2016 (Figure 3). The proportions of BPA decreased (49.8% to 12.5%) but BPF and BPE increased (6.8% to 29.4%, 11.1% to 26.3%, respectively) between 1993 and 2016 (Figure 3). The urinary concentrations of ΣBP remained unchanged over the observation period (r = 0.0096, p = 0.9127 for ΣBP) (Figure 2).



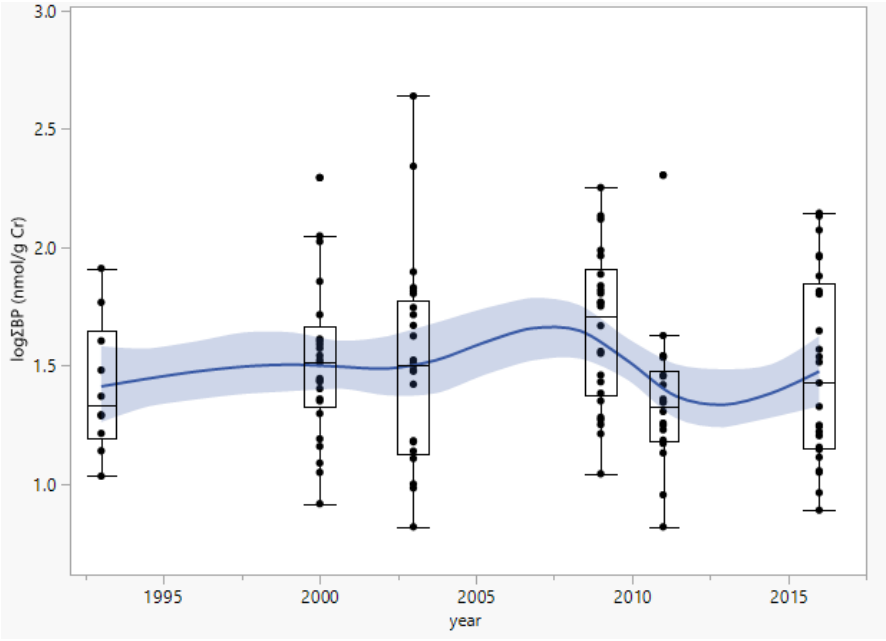
(A)


2.0
1.5
1.0
0.5
0
-0.5
-1.0
logBPF (µg/g Cr)
1995
2000
2005
2010
2015
year

(B)

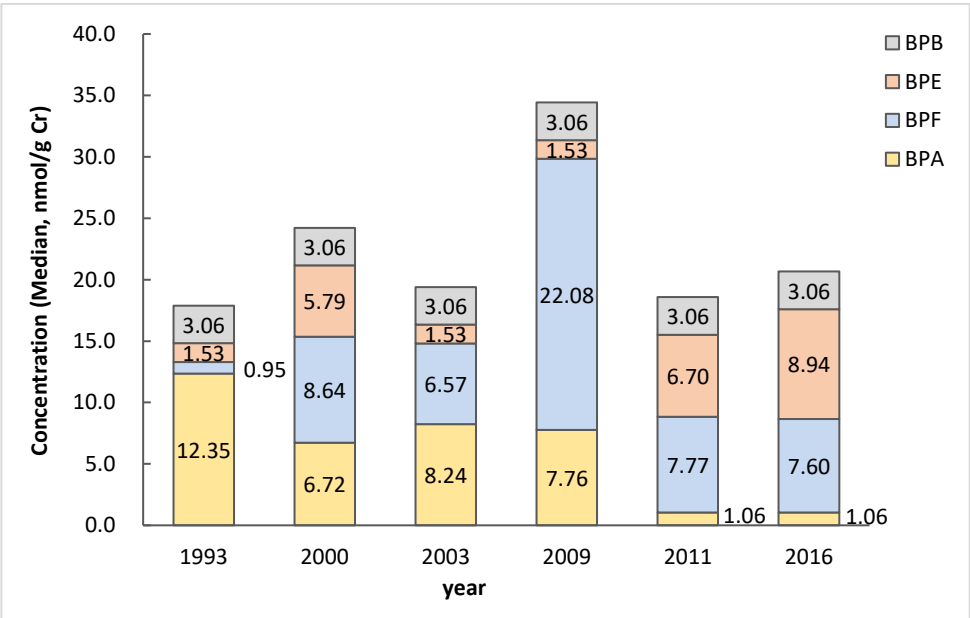

1.0
0.5
0
-0.5
logBPE (µg/g Cr)
1995
2000
2005
2010
2015
year

(C)

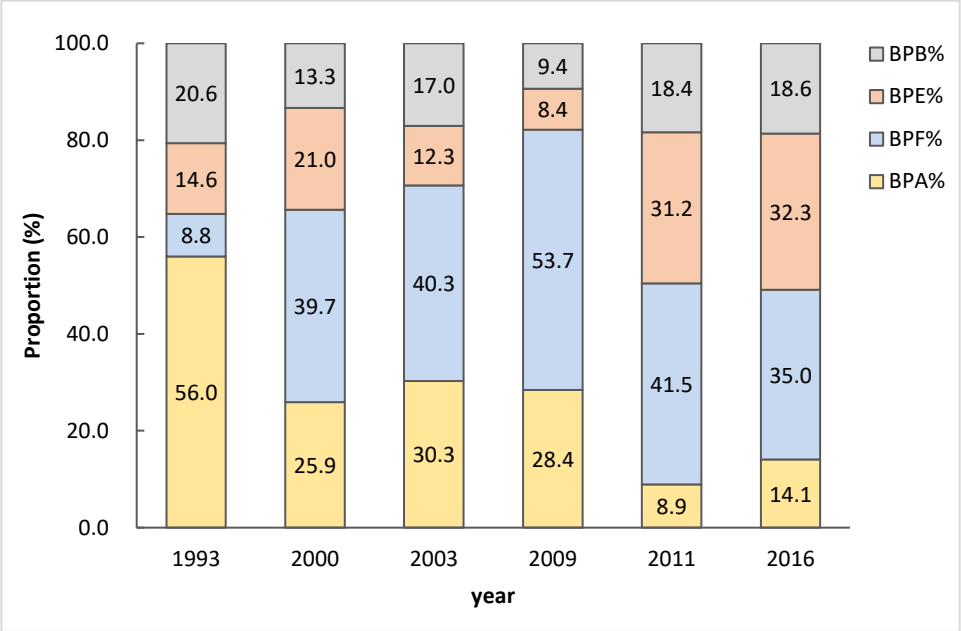


(D)

Figure 2. Boxplots of log-transformed creatinine-corrected urinary concentrations of BPA (A), BPF (B), BPE (C) (μg/g creatinine), and ΣBP (D) (nmol/g creatinine). The boxes show the interquartile ranges, and the upper and lower whiskers show the 95th and 5th percentiles. The blue curves indicate fitted smoothing splines (lambda = 0.1), and the bands show the 95% confidence intervals.



(A)



(B)

Figure 3. The sum of the median concentrations of each BP (nmol/g creatinine) (A) and average proportion (%) of the creatinine-corrected molar concentration of each BP in each participant (B). Concentrations below the limits of detection (LODs) were set to LOD/√2.

3.2 Comparison with other studies

This study found a decline in BPA exposure and an increase in BPF and BPE exposure among the study population. We compared the result to previous studies investigating various BPs, especially for current levels and temporal trends. A detailed comparison of the results is shown in Table S7.

For BPA, a meta-analysis including data from 12 studies reported a pooled estimate of urinary concentration of 1.91 μg/L (95% prediction interval: 0–3.97), ranging between 0.81 μg/L and 3.50 μg/L in adults from the general population (Colorado-Yohar et al., 2021). This was consistent with the urinary BPA levels found in our study.

For the temporal changes in BPA exposure, one study found a decline in BPA urinary concentrations in Japanese school children from 2012 to 2017 of an average of 6.5% per year (Gys et al., 2020), which is again similar to our result. Studies conducted in the United States, Canada, and some European countries also showed a decreasing trend in BPA exposure among adults and children over the last two decades (Lakind and Naiman, 2015; Ye et al., 2015; Gyllenhammar et al., 2017; Pollock et al., 2021; Tschersich et al., 2021). The findings of the Korean National Environmental Health Survey, however, showed higher urinary BPA in 2012–2017 than 2009–2011 (Choi et al., 2017, Jung et al., 2022).

To our knowledge, few studies have investigated exposure to BPs other than BPA. For BPF, the study among Japanese children suggested that urinary concentration levels were unchanged during 2012–2017 (0.05–0.07 ng/mL) (Gys et al., 2020). A U.S. study showed lower urinary concentrations of BPF than BPA among adults, with no obvious trend during 2000–2014 (0.2–0.4 ng/mL) (Ye et al., 2015). The median concentrations of BPF in our study were over 1 ng/mL in each sampling year during 2000–2016, higher than the median concentrations reported in several previous studies in multiple countries, which all reported values less than 0.5 ng/mL (Ye et al., 2015; Gyllenhammar et al., 2017; Lehmler et al., 2018; Zhang et al., 2018; Liu et al., 2019; Gys et al., 2020; Cui et al., 2021; Jung et al., 2022; Runkel et al., 2022). Further research is required to clarify the exposure pathways of BPF to Japanese adult populations.

The results for exposure to BPS varied considerably among studies. In our study, BPS was only detected in 1.5% of all samples (LOD = 0.73 ng/mL). However, Liao et al. (2012a) reported a geometric mean of 1.18 ng/mL of BPS in urine samples collected in Japan among the general population during 2010–2011. The study by Gys et al. (2020) indicated a possible slight increasing trend in BPS urinary concentrations (median

values from 0.09 to 0.12 ng/mL, $p < 0.05$) in Japanese school children during 2012–2017. Ye et al. (2015) found a significant increasing change in BPS exposure among U.S. adults during 2000–2014 (from n.d. to 0.2 ng/mL). In Canada, BPS was the most frequently detected BPA substitute among mothers during 2010–2012 (Liu et al., 2018). Cross-sectional studies in China in 2016, however, found lower urinary BPS concentrations of which median values were reported to be below the detection limit (Liu et al., 2019; Cui et al., 2021). Studies show BPS has replaced BPA in thermal paper products in several countries including Japan (Liao et al., 2012b). However, our result suggested a low level of exposure to BPS among the study population.

Previous investigations have found that BPA, BPF, and BPS were the most ubiquitous BPs present in the environment (Catenza et al., 2021). However, we also found a significant increasing trend in urinary concentration levels of BPE. There are few data on BPE exposure or any temporal changes in the general population. Several studies evaluated BPAF concentrations in urine samples, but the exposure levels were found to be lower than BPF and BPS (Ye et al., 2015; Liu et al., 2019; Gys et al., 2020; Cui et al., 2021; Mok et al., 2021). Similarly, BPAF was detected in only 1.5% of the total samples in our study.

Studies have suggested that some other BPA alternatives, such as BPAF, may also have potential endocrine-disrupting effects on humans (Zhao et al., 2019; Catenza et al., 2021). We believe further biomonitoring on exposure to BPA alternatives is still needed.

3.3 Cumulative risks for the investigated BPs

Cumulative risks from exposure to the BPs were evaluated by calculating the HQ and HI values. Figure 4 shows the temporal changes in HQ and HI values for BPA,

BPF and BPE. The HQ values for BPA showed an overall descending trend ($r = -0.354$, $p < 0.0001$), and decreased sharply between 2009 and 2011. The HQs for both BPF and BPE showed an increasing trend ($r = 0.205$, $p = 0.0185$, $r = 0.314$, $p = 0.0002$). The HQs for BPF increased dramatically between 1993 and 2000 and remained constant during 2000–2016, and the increase in HQs for BPE mainly occurred between 2009 and 2011. The HI values did not show any temporal change, and none of the HI values exceeded 1. The highest HI value was 0.26, generated by a sample collected in 2003.

The maximum HQ (HQ_M) is the HQ that contributed the most to each participant's HI value among the HQs of the three BPs. The BPs which produced HQ_M were counted in each study year, and the contribution proportion was obtained (Figure 5) (Reyes and Price, 2018; Lyu et al., 2022). The HQ_M value was produced mainly by BPA in 1993, but BPF became dominant after 2000. The proportions of BPE increased but BPA decreased between 2009 and 2011, which is consistent with the temporal trends in HQ values (Figure 4).

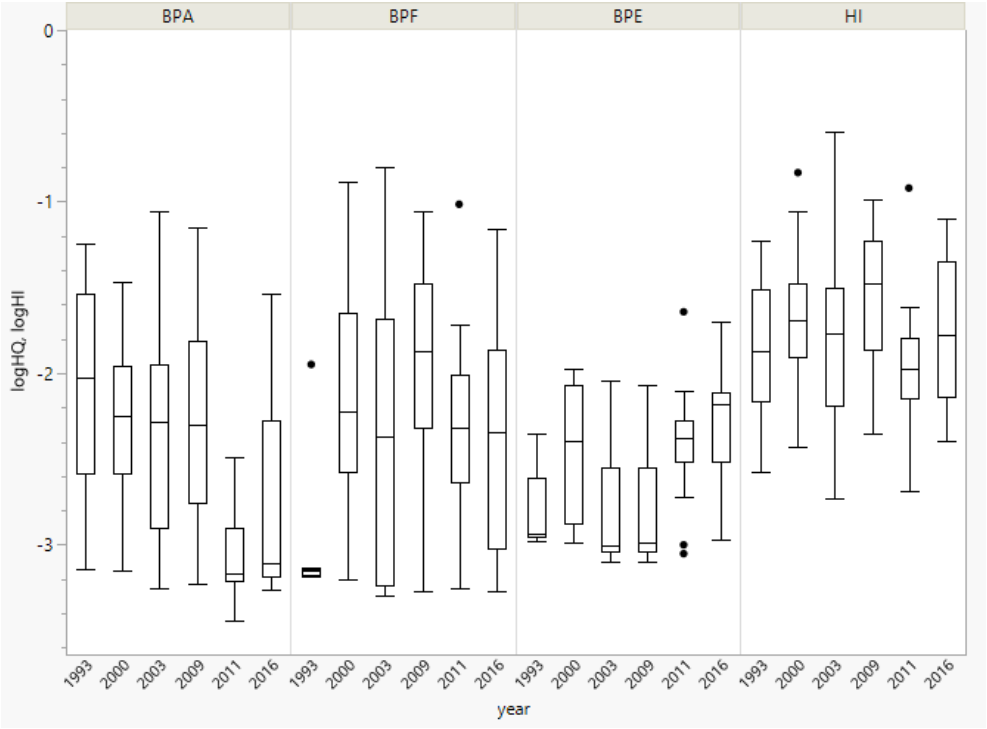


Figure 4. Outlier boxplots of log-transformed hazard quotient (HQ) values for BPA, BPF, BPE, and hazard index (HI) values for the investigated population between 1993 and 2016. The boxes show the interquartile ranges, the center lines in the boxes show the medians, the upper and lower whiskers show the maxima and minima except for outliers, and the dots indicate outliers.

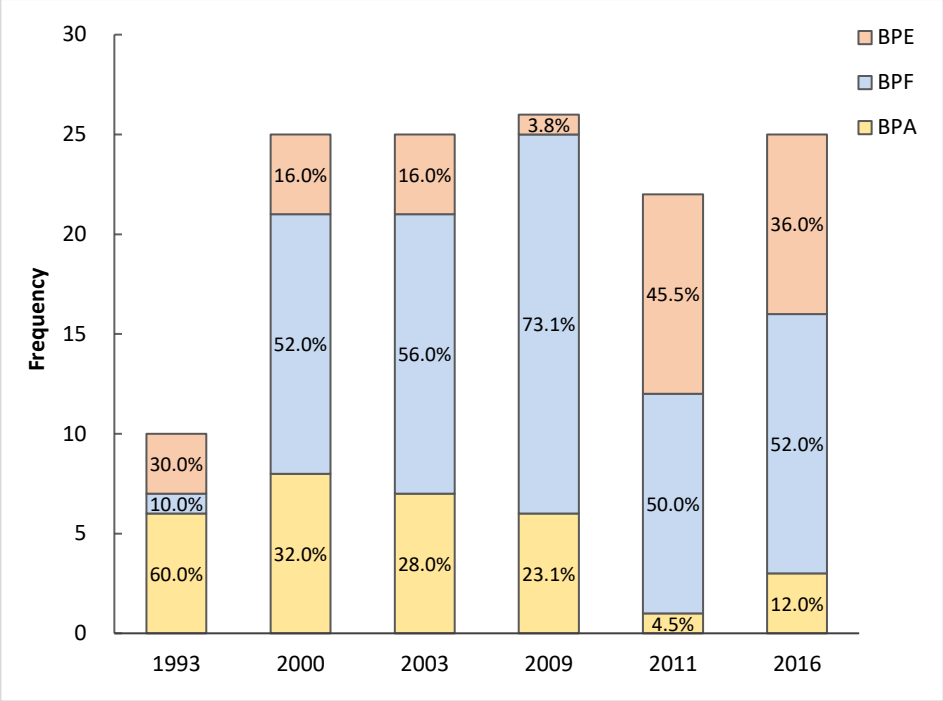


Figure 5. Proportion (%) of the BPs that produced the maximum hazard quotient (HQ_M) in each study year (n = 133). The BP that contributed most to the HI value for each participant was counted in each study year.

Previous studies also evaluated the potential health risks of BP exposure by calculating EDI values or HQ and HI values using measured urinary concentrations. A comparison of the EDIs obtained in each study is shown in Table S8. For BPA, several studies reported medians or geometric means of EDI ranging between 0.007 and 0.1 μg/kg body weight/day in various Asian countries including China, India, Japan, Korea, Kuwait, Malaysia, and Vietnam during 2006–2016 (Zhang et al., 2011; Park et al., 2016; Zhang et al., 2018; Mok et al., 2021). In our study, the medium EDI of BPA ranged between 0.020 and 0.038 μg/kg body weight/day during 1993–2009; however, it decreased to 0.003 μg/kg body weight/day during 2011–2016. Some studies reported medians or geometric means of EDI for BPS of less than 0.01 μg/kg body weight/day in Asian countries (Liao et al., 2012a; Cui et al., 2021; Mok et al., 2021). One study

reported an EDI for BPS of 1.7 μg/day in Japan (n = 36) (Liao et al., 2012a), or approximately 0.03 μg/kg body weight/day. All of those studies reported EDIs much lower than the TDI set by the EFSA (4 μg/kg/bw for BPA) (EFSA, 2015). Several studies also reported HQ and HI values for cumulative risk assessment, and the results suggest that there are unlikely to be any adverse health risks for BP exposure (Liu et al., 2019; Cui et al., 2021; Mok et al., 2021). This was consistent with our result. Nevertheless, according to our findings, BPF might become the dominant risk driver since 2000 in the studied population. Other studies in Korea and China showed that BPA was still the predominant BP in comparison with BPF, BPAF, or BPS after 2010 (Liu et al., 2019; Cui et al., 2021; Mok et al., 2021).

For the risk assessment, we used surrogate TDIs for BPs for which TDIs were not available. The hormonal activity of BPF was reported to be in the same order of magnitude and had similar effects as BPA in vitro and in vivo, according to a systematic review (Rochester and Bolden, 2015). In vitro studies showed that BPE was the most potent androgen receptor antagonist among the BPs (Rosenmai et al., 2014; Kojima et al., 2019). Another systematic review showed that the vascular toxicity and oxidative stress potency of BPF was greater than that of BPA (Ji et al., 2022). When calculating EDIs, the F_{UE} of BPF and BPE was also set at 100% as was BPA in our study. These assumptions, therefore, might have under- or over-estimated the potential health risk posed by BP alternatives.

4. Strengths and limitations

To our knowledge, this is the first study to examine long-term temporal changes in exposure to various BPs including cumulative risk assessment in a Japanese population. The participants in this study were women recruited at community health checkup

centers, meaning that the socio-economic backgrounds of the population did not change much over time.

However, this study had several limitations. First, the statistical power was limited, as there were only 10 to 26 participants in each sampling year. The study population was limited to women living in the same geographic area, but no socio-economic data were investigated. The small research population and focused study location also mean that the study results cannot be generalized to the whole Japanese population. Second, single spot urine samples were used in this study, and the concentration of BPs may vary with the timing of the sample collection. Third, creatinine adjustment was used, but potential biases could be possible and inter-day variations in exposure may occur. Fourth, the samples were stored in freezers, and degradation of target analytes might have occurred during storage. Data has shown that urine samples have a high stability for creatinine (> 10 years) at −22 °C (r = 0.99 between baseline and second measurement) (Remer et al. 2014). However, as far as we know, there is no available data for the stability of bisphenol compounds in urine samples stored for a long time. It should be noted that the exposure calculated from older samples may have been underestimated.

5. Conclusion

This study analyzed the urinary concentrations of 10 BPs among a female Japanese population residing in a single geographic area during 1993–2016. This study is the first to report the temporal trends in exposure to various BPs in the Japanese population over 20 years. The results show that exposure to BPA has decreased over the last two decades, especially after 2011, but BPE exposure increased significantly after 2011. BPF has been the most important risk driver since 2000. HI values suggested that there

was unlikely to be any significant health risk posed by BP exposure. To date, few studies have shown long-term temporal trends of exposure to BPA alternatives in Japan. Further estimations are warranted to explore several BPA alternatives, notably BPF and BPE.

Declaration of competing interest

The authors declare that they have no known competing financial interests.

Acknowledgements

The authors express their sincere thanks to Akio Koizumi, professor emeritus of Kyoto University, founder of the Kyoto University Human Specimen Bank, and to the many contributors who assisted with the bank. We also thank Melissa Leffler, MBA, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Funding

This study was supported by a Grant-in-Aid for JSPS bilateral joint research project/seminar (120188819) and Environment Research and Technology Development Fund (20219070) of the Environmental Restoration and Conservation Agency of Japan.

References

Catenza, C.J., Farooq, A., Shubear, N.S., Donkor, K.K., 2021. A targeted review on fate, occurrence, risk and health implications of bisphenol analogues. Chemosphere 268, 129273.

Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., Widelka, M., 2016. Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity—A Review. Environ. Sci. Technol. 50, 5438–5453.

Choi, W., Kim, S., Baek, Y.-W., Choi, K., Lee, K., Kim, S., Yu, S.D., Choi, K., 2017. Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012–2014). Int. J. Hyg. Environ. Health 220, 29–35.

Colorado-Yohar, S.M., Castillo-González, A.C., Sánchez-Meca, J., Rubio-Aparicio, M., Sánchez-Rodríguez, D., Salamanca-Fernández, E., Ardanaz, E., Amiano, P., Fernández, M.F., Mendiola, J., Navarro-Mateu, F., Chirlaque, M.D., 2021. Concentrations of bisphenol-A in adults from the general population: A systematic review and meta-analysis. Sci. Total Environ. 775, 145755.

Cui, F.-P., Yang, P., Liu, C., Chen, P.-P., Deng, Y.-L., Miao, Y., Luo, Q., Zhang, M., Lu, W.-Q., Zeng, Q., 2021. Urinary bisphenol A and its alternatives among pregnant women: Predictors and risk assessment. Sci. Total Environ. 784, 147184.

EC, 2002. Commission Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with foodstuffs.

EC, 2011. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13, 3978.

Eladak, S., Grisin, T., Moison, D., Guerquin, M.J., N'Tumba-Byn, T., Pozzi-Gaudin, S., Benachi, A., Livera, G., Rouiller-Fabre, V., Habert, R., 2015. A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertil. Steril. 103, 11–21.

Flint, S., Markle, T., Thompson, S., Wallace, E., 2012. Bisphenol A exposure, effects, and policy: A wildlife perspective. J. Environ. Manage. 104, 19–34.

Gyllenhammar, I., Glynn, A., Jönsson, B.A.G., Lindh, C.H., Darnerud, P.O., Svensson, K., Lignell, S., 2017. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? Environ. Res. 153, 48–54.

Gys, C., Ait Bamai, Y., Araki, A., Bastiaensen, M., Caballero-Casero, N., Kishi, R., Covaci, A., 2020. Biomonitoring and temporal trends of bisphenols exposure in Japanese school children. Environ. Res. 191, 110172.

Ji, G., Gu, J., Guo, M., Zhou, L., Wang, Z., Shi, L., Gu, A., 2022. A systematic comparison of the developmental vascular toxicity of bisphenol A and its alternatives in vivo and in vitro. Chemosphere 291, 132936.

Jung, S.K., Choi, W., Kim, S.Y., Hong, S., Jeon, H.L., Joo, Y., Lee, C., Choi, K., Kim, S., Lee, K.J., Yoo, J., 2022. Profile of Environmental Chemicals in the Korean Population-Results of the Korean National Environmental Health Survey (KoNEHS) Cycle 3, 2015-2017. Int. J. Environ. Res. Public Health 19, 626.

Koizumi, A., Harada, K.H., Inoue, K., Hitomi, T., Yang, H.R., Moon, C.S., Wang, P., Hung, N.N., Watanabe, T., Shimbo, S., Ikeda, M., 2009. Past, present, and future of environmental specimen banks. Environ. Health Prev. Med. 14, 307–318.

Kojima, H., Takeuchi, S., Sanoh, S., Okuda, K., Kitamura, S., Uramaru, N., Sugihara, K., Yoshinari, K., 2019. Profiling of bisphenol A and eight of its analogues on

transcriptional activity via human nuclear receptors. Toxicology 413, 48–55.

LaKind, J.S., Naiman, D.Q., 2015. Temporal trends in bisphenol A exposure in the United States from 2003–2012 and factors associated with BPA exposure: Spot samples and urine dilution complicate data interpretation. Environ. Res. 142, 84–95.

Lehmler, H.-J., Liu, B., Gadogbe, M., Bao, W., 2018. Exposure to bisphenol A, bisphenol F, and bisphenol S in U.S. adults and children: The National Health and Nutrition Examination Survey 2013–2014. ACS Omega 3, 6523–6532.

Liao, C., Liu, F., Alomirah, H., Loi, V.D., Mohd, M.A., Moon, H.-B., Nakata, H., Kannan, K., 2012a. Bisphenol S in urine from the United States and seven Asian countries: Occurrence and human exposures. Environ. Sci. Technol. 46, 6860–6866.

Liao, C., Liu, F., Kannan, K., 2012b. Bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol A residues. Environ. Sci. Technol. 46, 6515–6522.

Liu, J., Wattar, N., Field, C.J., Dinu, I., Dewey, D., Martin, J.W., 2018. Exposure and dietary sources of bisphenol A (BPA) and BPA-alternatives among mothers in the APrON cohort study. Environ. Int. 119, 319–326.

Liu, Y., Yan, Z., Zhang, Q., Song, N., Cheng, J., Torres, O.L., Chen, J., Zhang, S., Guo, R., 2019. Urinary levels, composition profile and cumulative risk of bisphenols in preschool-aged children from Nanjing suburb, China. Ecotoxicol. Environ. Saf. 172, 444–450.

Lyu, Z., Harada, K.H., Kim, S., Fujitani, T., Cao, Y., Hitomi, T., Fujii, Y., Kho, Y., Choi, K., 2022. Exposure to phthalate esters in Japanese females in Kyoto, Japan from 1993 to 2016: Temporal trends and associated health risks. Environ. Int. 165,

107288.

Ma, Y., Liu, H., Wu, J., Yuan, L., Wang, Y., Du, X., Wang, R., Marwa, P.W., Petlulu, P., Chen, X., Zhang, H., 2019. The adverse health effects of bisphenol A and related toxicity mechanisms. Environ. Res. 176, 108575.

Mage, D.T., Allen, R.H., Kodali, A., 2008. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. J. Expo. Sci. Environ. Epidemiol. 18, 360–368.

Ministry of Health, Labour and Welfare of Japan. (2005). The 2003 National Health and Nutrition Survey. https://www.mhlw.go.jp/bunya/kenkou/eiyou-chosa2-01/index.html (accessed 24 May 2022).

Ministry of Health, Labour and Welfare of Japan. (2010). The 2009 National Health and Nutrition Survey. https://www.mhlw.go.jp/stf/houdou/2r9852000000xtwq.html (accessed 24 May 2022).

Ministry of Health, Labour and Welfare of Japan. (2012). The 2011 National Health and Nutrition Survey. https://www.mhlw.go.jp/stf/houdou/2r9852000002q1st.html (accessed 24 May 2022).

Ministry of Health, Labour and Welfare of Japan. (2017). The 2016 National Health and Nutrition Survey. https://www.mhlw.go.jp/stf/houdou/0000177189.html (accessed 24 May 2022).

Ministry of Health of Japan. (1995). FY 1995 Current status of national nutrition: FY1993 National nutrition survey results.

Ministry of Health of Japan. (2001). Current status of national nutrition: FY2000

549 National nutrition survey results.
550 https://www.mhlw.go.jp/toukei/kouhyo/indexkk_14_7.html (accessed 24 May
551 2022).
552 Mok, S., Jeong, Y., Park, M., Kim, S., Lee, I., Park, J., Kim, S., Choi, K., Moon, H.-B.,
553 2021. Exposure to phthalates and bisphenol analogues among childbearing-
554 aged women in Korea: Influencing factors and potential health risks.
555 Chemosphere 264, 128425.
556 Park, J.-H., Hwang, M.-S., Ko, A., Jeong, D.-H., Lee, J.-M., Moon, G., Lee, K.-S., Kho,
557 Y.-H., Shin, M.-K., Lee, H.-S., Kang, H.-S., Suh, J.-H., Hwang, I.-G., 2016.
558 Risk assessment based on urinary bisphenol A levels in the general Korean
559 population. Environ. Res. 150, 606–615.
560 Pollock, T., Karthikeyan, S., Walker, M., Werry, K., St-Amand, A., 2021. Trends in
561 environmental chemical concentrations in the Canadian population:
562 Biomonitoring data from the Canadian Health Measures Survey 2007–2017.
563 Environ. Int. 155, 106678.
564 Qiu, W., Zhan, H., Hu, J., Zhang, T., Xu, H., Wong, M., Xu, B., Zheng, C., 2019. The
565 occurrence, potential toxicity, and toxicity mechanism of bisphenol S, a
566 substitute of bisphenol A: A critical review of recent progress. Ecotoxicol
567 Environ Saf 173, 192–202.
568 Remer, T., Montenegro-Bethancourt, G., Shi, L., 2014. Long-term urine biobanking:
569 Storage stability of clinical chemical parameters under moderate freezing
570 conditions without use of preservatives. Clin. Biochem. 47, 307–311.
571 Reyes, J.M., Price, P.S., 2018. Temporal trends in exposures to six phthalates from
572 biomonitoring data: Implications for cumulative risk. Environ. Sci. Technol. 52,
573 12475–12483.

574 Rochester, J.R., 2013. Bisphenol A and human health: A review of the literature. Reprod.
575 Toxicol. 42, 132–155.
576 Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A systematic review and
577 comparison of the hormonal activity of bisphenol A substitutes. Environ. Health
578 Perspect. 123, 643–650.
579 Rosenmai, A.K., Dybdahl, M., Pedersen, M., Alice van Vugt-Lussenburg, B.M.,
580 Wedebye, E.B., Taxvig, C., Vinggaard, A.M., 2014. Are structural analogues to
581 bisphenol a safe alternatives? Toxicol. Sci. 139, 35–47.
582 Runkel, A.A., Mazej, D., Snoj Tratnik, J., Tkalec, Ž., Kosjek, T., Horvat, M., 2022.
583 Exposure of men and lactating women to environmental phenols, phthalates,
584 and DINCH. Chemosphere 286, 131858.
585 Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell,
586 M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum,
587 L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral
588 administration. Environ. Int. 83, 107–115.
589 Tschersich, C., Murawski, A., Schwedler, G., Rucic, E., Moos, R.K., Kasper-
590 Sonnenberg, M., Koch, H.M., Brüning, T., Kolossa-Gehring, M., 2021.
591 Bisphenol A and six other environmental phenols in urine of children and
592 adolescents in Germany – human biomonitoring results of the German
593 Environmental Survey 2014–2017 (GerES V). Sci. Total Environ. 763, 144615.
594 U.S. EPA, 1986. Guidelines for the Health Risk Assessment of Chemical Mixtures
595 (Final Report, 1986).
596 U.S. FDA, 1997. Validation of Analytical Procedures: Methodology. Guidance for
597 Industry.
598 Ye, X., Wong, L.-Y., Kramer, J., Zhou, X., Jia, T., Calafat, A.M., 2015. Urinary

599 concentrations of bisphenol A and three other bisphenols in convenience
600 samples of U.S. adults during 2000–2014. Environ. Sci. Technol. 49, 11834–
601 11839.
602 Zhang, X.-M., Lou, X.-Y., Wu, L.-H., Huang, C., Chen, D., Guo, Y., 2018. Urinary
603 phthalate metabolites and environmental phenols in university students in South
604 China. Environ. Res. 165, 32–39.
605 Zhang, Z., Alomirah, H., Cho, H.-S., Li, Y.-F., Liao, C., Minh, T.B., Mohd, M.A.,
606 Nakata, H., Ren, N., Kannan, K., 2011. Urinary bisphenol A concentrations and
607 their implications for human exposure in several Asian countries. Environ. Sci.
608 Technol. 45, 7044–7050.
609 Zhao, Q., Howard, E.W., Parris, A.B., Ma, Z., Xing, Y., Yang, X., 2019. Bisphenol AF
610 promotes estrogen receptor-positive breast cancer cell proliferation through
611 amphiregulin-mediated crosstalk with receptor tyrosine kinase signaling. PLoS
612 One 14, e0216469.
613