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Exposure to phthalate esters in Japanese females in Kyoto, Japan from 1993 to 2016: Temporal trends and associated health risks

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ABSTRACT

Phthalates are used as plasticizers in many products used in daily life worldwide. Due to industrial and economic developments, exposure among general population to phthalates may vary geographically and temporally. However, studies are lacking for investigating temporal changes in phthalate exposure in the Japanese population. In the present study, the temporal trends in exposure to various phthalates were assessed among a group of Japanese adult female population over 1993–2016 and derived associated risks. For this purpose, urine samples of healthy Japanese females in Kyoto, Japan (N = 132) collected in 1993, 2000, 2003, 2009, 2011, and 2016, were employed and measured for the concentrations of 18 phthalate metabolites. Over this period, the detection rates of mono(3-carboxypropyl) phthalate (MCPP) and monoisobutyl phthalate (MiBP) decreased, and the geometric means of the urinary concentrations of mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) showed a significant decreasing trend. Cumulative risk due to exposure to dibutyl phthalate (DBP), diisobutyl phthalate (DiBP), butyl benzyl phthalate (BBP), and di-2-ethylhexyl phthalate (DEHP) showed a dramatic decrease only between 1993 and 2000. The maximum hazard quotient (HQ_M) was attributed to DEHP in most subjects regardless of sampling year. This study showed the temporal trend of the exposure of Japanese females to several phthalate esters over two decades. As of the late 2010's, DEHP was still the predominant component of phthalate ester exposure in the population. The HI value, however, indicates that direct risk due to phthalate exposure was unlikely among the studied population.

1. Introduction

Phthalates are used worldwide as plasticizers for many consumer and household products, including polyvinyl chloride (PVC), personal care products, flooring, and wall coverings (U.S. EPA, 2013). Phthalates are not chemically bound to the host polymers, so are easily released from the products. Thus, the general population can be exposed to phthalates in daily life, through various routes of ingestion of contaminated food, inhalation of indoor air and house dust, as well as dermal application of personal care products (Benjamin et al., 2017; Koch et al., 2013).

Some phthalates are considered endocrine disrupting chemicals (EDCs), affecting endocrine systems through androgen antagonism and decreasing thyroid hormones (Benjamin et al., 2017). According to animal studies, several phthalates, notably di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), and benzyl butyl phthalate (BBP), are found as anti-androgens, which can result in male reproductive abnormalities such as shortened anogenital distance (Swan, 2008). An epidemiological study has shown the potential adverse effects of several different phthalates on development and reproduction functions (Tranfo et al., 2012). Other epidemiological evidence has also revealed associations of phthalate exposure with thyroid hormone disruption, metabolic diseases in pregnancy, birth defects, and allergic diseases (Kim et al., 2019; Wittassek et al., 2011). European Food Safety Authority published the updated risk assessment for five phthalates and group tolerable daily intake of 50 μg/kg bw/day for DBP, BBP, DEHP, and diisononyl phthalate (DiNP) was retained (EFSA, 2019).

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In the past few decades, the design and formulation of phthalate in products have changed because of recent regulatory actions and related industrial practices. For instance, DEHP, which was the most commonly used phthalate for many years, mainly used as PVC plasticizer, has been gradually substituted with other high-molecular-weight phthalates such as DiNP and diisodecyl phthalate (DiDP) (Wittassek et al., 2011). The Consumer Product Safety Improvement Act of 2008 of the USA banned the use of DEHP, DBP, and BBP in toys and other articles for child use at concentrations greater than 0.1%, and the United States Consumer Product Safety Commission expanded the restriction list to total 8 phthalates in 2017, including DiNP and diisobutyl phthalate (DiBP) (CPSC, 2017). In 1998, Environment Agency of Japan published list of 67 potential EDCs and DEHP, BBP, DBP, dicyclohexyl phthalate (DCP) and diethyl phthalate (DEP) were suggested (Environment Agency of Japan, 1998). DEHP and DiNP have also been banned for use in toys in Japan in the last two decades (Ministry of Health, Labour and Welfare of Japan, 2002). More recently, in 2010, Japan revised the phthalate regulations for toys, which included new restrictions on the use of DBP, BBP, DiDP, and di-n-octyl phthalate (DnOP), as well as DEHP and DiNP (Ministry of Health, Labour and Welfare of Japan, 2010).

The aforementioned regulatory development in Japan may influence the pattern of phthalate exposure in Japanese people over the last decades. However, few studies have revealed long-term temporal changes of exposure and potential health risk to phthalate in the Japanese population. The object of our study was to evaluate the temporal trends in exposure to various phthalates over the last decades and to assess the health risks using the archived urine samples of Japanese females which were collected between 1993 and 2016. Understanding temporal changes in the exposure and the cumulative risk of phthalates in the general population will help identify priority phthalates and develop appropriate risk management measures.

2. Materials and methods

2.1. Study subjects

Urine samples were obtained from the Kyoto University Human Specimen Bank (Koizumi et al., 2009) donated by 132 healthy Japanese women aged from 25 to 80 years old (59.3 ± 12.4, mean ± SD, the average ages in each year were 49–65) living in Kyoto and the surrounding areas (Kyoto city and Uji city), Japan. Original studies focused on associations between chemical exposures and biomarkers of health outcomes, and then middle-aged or older females were mainly recruited. The spot urine specimens were collected in 1993, 2000, 2003, 2009, 2011, and 2016, when the women attended cross-sectional healthcare checkup programs. First morning urine samples were collected in paper cups and transferred to polypropylene tubes. Samples were kept in refrigerator in their home until they visited health checkup centers. Urine samples were stored at −30 °C until analysis in the Kyoto University Human Specimen Bank. The study protocol was approved by the Ethics Committee of the Kyoto University Graduate School of Medicine (approval number R1478). Informed consent in verbal (before 2000) or written form was obtained from every participant before she participated in the study.

Demographic data of participants are shown in Table 1. All the participants were female. The ages of the participants were significantly different by the sampling year ($p < 0.05$). Therefore, the age was consequently set as an adjustment factor in the models for comparing phthalate metabolite concentrations between groups.

2.2. Determination of phthalate metabolites in urine samples

Eighteen metabolites of phthalate esters were analyzed in this study. The list of name and abbreviation of phthalate metabolites and their surrogate internal standards were shown in Table S1. All compounds used for standards and internal standards were purchased from Cambridge Isotope Laboratory (Andover, MA, USA) as 100 μg/mL stock solution (chemical purity > 98%).

Table 1
Demographic characteristics of participating subjects.

	years of sample collection						*p*
	1993	2000	2003	2009	2011	2016	
No. of subjects	10	25	24	26	22	25	
age (years)	52.7 ± 4.0	49.4 ± 9.4	65.9 ± 4.9	59.0 ± 14.4	65.5 ± 12.5	60.2 ± 12.7	<0.001
urinary creatinine (g/L)	0.8 ± 0.4	0.8 ± 0.4	0.7 ± 0.4	0.8 ± 0.6	1.0 ± 0.8	0.9 ± 0.5	0.373

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

Measurements of the phthalate metabolites were performed according to previous studies (Jeong et al., 2011; Lee et al., 2019). Analysis batch was a group of study year and order of analysis batch was random. Briefly, 480 μL of a urine sample, 20 μL of each internal standard of 500 ppb, 10 μL of β-glucuronidase/arylsulfatase (*Escherichia coli*) (Sigma Aldrich, St. Louis, MO, USA), and 130 μL of 1 M ammonium acetate buffer (pH = 5) were added into glass vials and mixed by a vortex mixer for 10 s. The samples were incubated at 37 °C for 2 h for hydrolysis, then mixed again for 10 s. The deglucuronidation processes were performed for the phthalate metabolites are excreted through urine as glucuronides. After sonication for 10 min, the samples were mixed by a vortex mixer for 10 s, then neutralized with a pre-prepared mixture of 1% acetic acid and acetonitrile (50:300 μL) in 1.5-mL polypropylene tubes, mixed for 30 s. Next, the samples were centrifuged at 5000 rpm for 10 min. The supernatant was injected into a column for column-switching liquid chromatography with tandem mass spectrometry (LC-MS/MS) for analysis.

The analyses for measuring urinary phthalate metabolites were performed using a Shiseido Nanospace II liquid chromatography system (Shiseido, Tokyo, Japan) with an AB SCIEX API 4500 tandem mass spectrometer (AB SCIEX, Ontario, Canada). On-line separation of phthalate monoesters was accomplished by the switching-column technique with an on-line solid phase extraction (SPE) column (Water Oasis HLB online column, 2.1 i.d. × 20 mm length, 5 μm particle) and analytical column (Cadenza CD-C18, 150 × 2.0 mm, 3 μm). The column temperature was 40 °C, the injection volume was 10 μL, and the flow rates were 200 μL/min for the pretreatment and 200–400 μL/min for the analytical column. The mobile phases were 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). In column switching, two pumps were switched to each other for washing, equilibration, and analysis. Switching modes for analyzing phthalate metabolites are shown in Fig. S1, and Tables S2-S3. Eluents from the analytical column were subject to MS/MS. Electrospray ionization in negative ion mode and multiple reaction monitoring mode were applied for determination of phthalate metabolites.

The limit of detection (LOD) value was obtained according to the concept and method of the limit of detection stipulated in the verification of the analytical procedure defined by the Food and Drug Administration (FDA) (U.S. FDA, 1998). The LODs are shown in Table S4. The method validation study was performed at same period in the previous study (Lee et al., 2019), and the results (recovery and inter-day variation) were presented in Table S5. Blank sample storages showed no detectable parent phthalates.

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 H_2O, and 2, 4, and 6 mg/dL

standard creatinine solutions were prepared in HPLC vials for measurement.

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

The daily intake (DI) doses of phthalates were calculated from the metabolite concentrations in urine samples using the equation below (Reyes, 2018):

$$DI_{i,j,k} = \left(\left[\left(Met_{i,k}/Cr_i\right)*CE_i\right]/\left[1000*F_{UE,i,k}\right]\right) \times \left(MW_{i,j}/MW_{i,j,k}\right)$$

in which $DI_{i,j,k}$ (μg/kg/d) is the DI dose for metabolite k of participant i, $Met_{i,k}$ (ng/mL) is the urinary metabolite concentration of metabolite k, Cr_i (g/L) is the urinary creatinine concentration, and CE_i (mg/kg/d) is the creatinine excretion per day, calculated by the equation considering age, gender (all of the participants were female), and race (B = 0 if not black) of the participants (Mage, 2008).

$$CE_i = 0.993 \times 1.64[140 - age](Wt^{1.5}Ht^{0.5})(1 + 0.18B)/1000/Wt$$

The averages of the weights and heights of Japanese women in each age group were used. The data was reported by the Ministry of Health, Labour and Welfare of Japan in the National Health and Nutrition Surveys of 1993, 2000, 2003, 2009, 2011, and 2016 (Ministry of Health and Labor and Welfare of Japan, 2004; Ministry of Health and Labor and Welfare of Japan, 2010; Ministry of Health and Labor and Welfare of Japan, 2012; Ministry of Health and Labor and Welfare of Japan, 2017; Ministry of Health of Japan, 1995; Ministry of Health of Japan, 2000b).

$F_{ue,i,k}$ (unitless) is the molar fraction of metabolite excreted per its parent phthalate ingested. $MW_{i,j}$ (g/mol) and $MW_{i,j,k}$ (g/mol) are the respective molecular weights of the parent phthalate j and its metabolite k (Table S6).

DI dose of a given phthalate for participant j was calculated by taking a weighted mean of the DI of all metabolites k using $F_{ue,i,k}$ with the following equation (U.S. EPA, 1986; Reyes, 2018):

$$DI_{i,j} = \sum_{k=1}^{n_k}\left(DI_{i,j,k} \times \frac{F_{UE,i,k}}{\sum_{l=1}^{n_k} F_{UE,i,l}}\right)$$

$$HQ_{i,j} = DI_{i,j}/TDI_j$$

$$HQ_{M,i} = \max_{j\in\{i,\ldots,N\}} HQ_{i,j}$$

$$HI_i = \sum_{j=1}^{N} HQ_{i,j}$$

HQ is defined as DI divided by TDI. HQM is the maximum HQ among all the phthalates analyzed (N = 4). HI is the sum of HQ for all analyzed phthalates. HI > 1 indicates potential health risk generated by the exposure to investigated phthalates.

2.5. Statistical analysis

Data analysis was performed using JMP Pro Statistical Software, Version 15 (Cary, NC, USA), and a 2-sided p-value < 0.05 was considered statistically significant. One-way ANOVA was applied to test the difference of urinary phthalate metabolite levels by sampling year. The Cochran-Armitage trend test was performed to test for a temporal trend in the detection rate of phthalate metabolites. For urinary concentrations of the metabolites, creatinine adjustment was applied to reduce the effect of urine dilution on exposure biomarkers determined in spot samples. A partial correlation test was performed using log-transformed data of urinary metabolite concentrations, and age was set as a control variable. Metabolite concentrations below the LODs were set to $LOD/\sqrt{2}$ when calculating geometric means (GM), HQ and HI (Jeong et al., 2011; Lee et al., 2019). A summary metric for DEHP metabolites (ΣDEHP metabolites) was calculated by summing the molecular concentrations of MEHP, MECPP, MEHHP, and MEOHP. When calculating HQ and HI, only the phthalates whose detection rates for primary metabolite > 50% in each year were included.

3. Results and discussion

The results of analysis of urine samples between 1993 and 2016 were shown and discussed in following order: (1) the distribution and temporal changes in the exposure, (2) comparison with other studies in Japan, (3) international comparisons, and (4) cumulative risks for the investigated phthalates.

3.1. Levels and temporal trends of phthalate metabolites

MEP, MCMHP, and MEOHP were detected in all of the samples, MECPP was detected in all samples in 1993, 2003, 2009, and 2010 and MEHHP was detected in all samples in 1993, 2003, 2010, and 2016 (Table S7). On the other hand, MnPP and MHxP were not detected in all urine samples. Trends in detection rates were summarized in Table S7 and Fig. S2. The detection rates of MCPP and MiBP were decreasing while those of MCHP were increasing.

Fig. 1, Table S8 and Table S9 show the distribution of the urinary concentrations of phthalate metabolites. Creatinine-adjusted and -unadjusted levels showed similar trends in metabolites, and then analyses were based on creatinine-adjusted concentrations. For MEP, while the samples obtained between 1993 and 2009 showed a rather increasing trend, the samples collected from 2009 to 2011 exhibited a sharp decreasing trend (Fig. 1). The GM values of the MCPP concentrations were detected at the highest GM levels in the samples collected in 1993, and the lowest in those of 2011 (Fig. 1A). Temporal changes in MiBP concentrations were not clear, for the GM of the concentration was the highest in 1993 and the lowest in 2016, but in 2011, MiBP was not detected in any samples (Fig. 1B). This observation may be related to the changes in the use pattern for this phthalate in Japan.

Linear relationship between urinary phthalate metabolites and study years was shown in Fig. 2. Only MCPP and MEOHP concentrations showed statistically significant declining trends (Pearson's correlation coefficient r = −0.879, p = 0.021 for MCPP, r = −0.831, p = 0.041 for MEOHP) (Fig. 2). Results of a multiple linear regression analysis following an adjustment of age indicated weak but significant negative correlations of the urinary concentrations of MCPP, MiBP, MECPP, and MEOHP over time (r = −0.245, p = 0.005 for MCPP, r = −0.226, p = 0.009 for MiBP, r = −0.211, p = 0.016 for MECPP, and r = −0.299, p = 0.001 for MEOHP).

For ΣDEHP metabolites, a significant negative correlation with the year of sampling was also found (r = −0.261, p = 0.003); however, a clear decrease was observed only between 1993 and 2000 (Fig. 1F). The concentrations of MECPP and MEOHP, both metabolites of DEHP, did not monotonically decrease over time, but the highest GMs of the concentrations occurred in 1993 (Fig. 1D and E), which was consistent with the estimated trend for ΣDEHP metabolites. This decline could be due to the regulatory action on PVC gloves was adopted in 2000 (Ministry of Health of Japan, 2000a), in addition to nomination of phthalates as EDCs (Environment Agency of Japan, 1998). This result suggests that DEHP is still the most commonly used phthalate in Japan.

3.2. Comparison with other studies in Japan

A previous study conducted on a small group of Japanese adults (n = 36) showed that DEHP exposure had decreased from 1998 to 2001 (Itoh et al., 2005), as observed in this study. A possible reason for the decline can be attributable to a regulation on DEHP use in PVC gloves adopted in 2000 by the Ministry of Health of Japan (Ministry of Health of Japan, 2000a). It was reported that DEHP concentration in food decreased and average daily intake of DEHP decreased sharply during 1999–2001

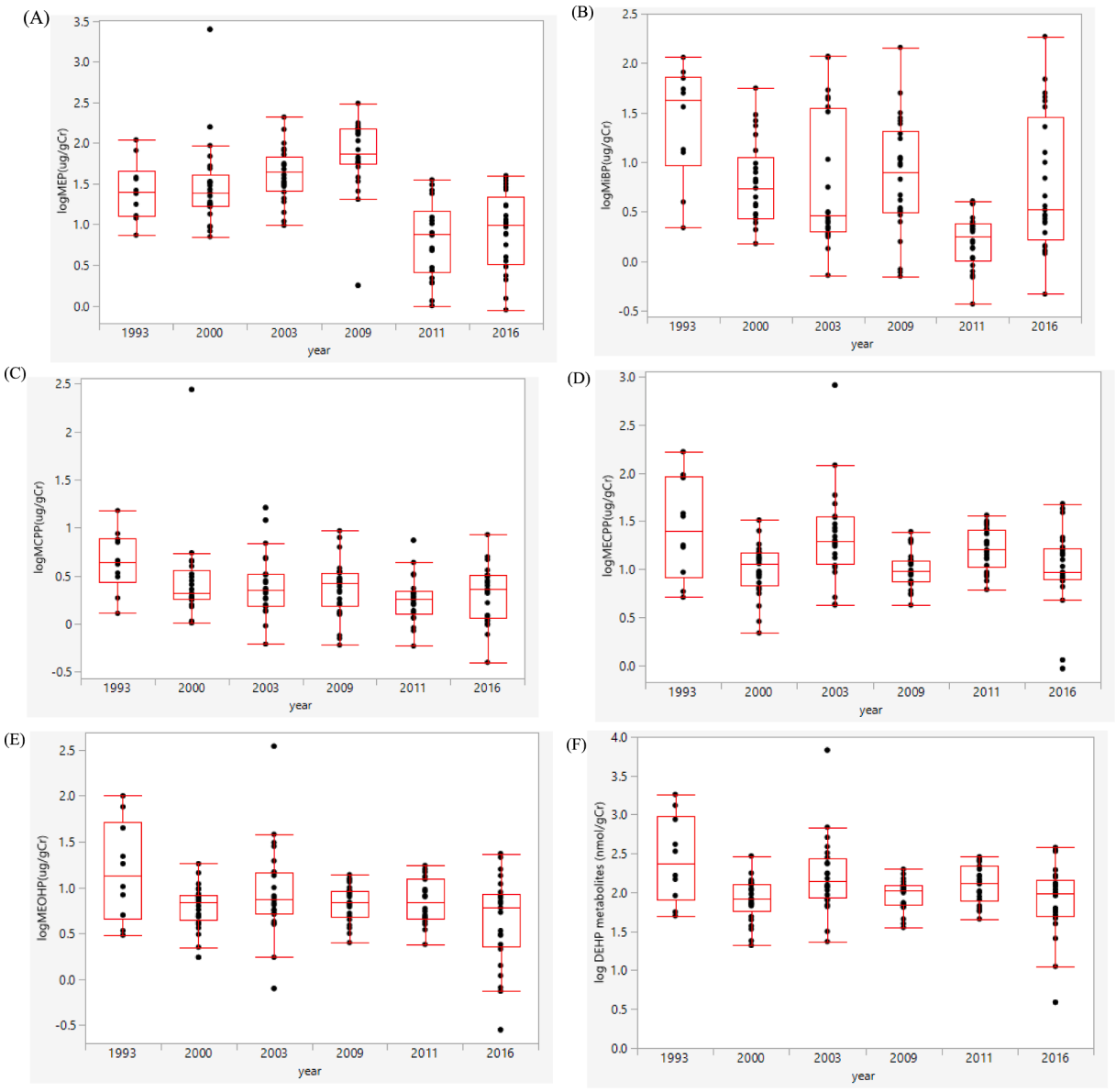

Fig. 1. Boxplots of log-transformed creatinine-corrected urinary concentrations of MEP (A), MiBP (B), MCPP (C), MECPP (D), and MEOHP (E) (μg/g creatinine), and DEHP metabolites (F) (nmol/g creatinine). The boxes show the interquartile ranges, the center lines in the boxes show the medians, the upper and lower whiskers show the 95th and 5th percentiles.

(Suzuki et al., 2009; Tsumura et al., 2003). Recently, another study on the children of Hokkaido, Japan showed that the DEHP metabolites in urine did not change significantly during 2012–2017, supporting the observations of the present study, i.e., no changes in DEHP metabolites after 2000 (Ketema et al., 2021).

3.3. Comparison with studies in other countries

In the USA, the urinary concentrations of MnBP and MBzP decreased, whereas the urinary concentrations of MiBP and mono-carboxyisooctyl phthalate (MCOP) increased dramatically, between 2001 and 2010 (Zota, et al. 2014) (Table S9). A similar trend was also observed in some European countries for DEHP, DnBP and BBzP metabolites. A study conducted in Germany showed a decreasing trend for urinary DEHP, DnBP, and BBzP metabolite concentrations during the period between 1988 and 2015: the levels observed in 2015 are approximately 10-fold lower than those detected in the late 1980s/early 1990s (Koch et al., 2017). In Germany, the urinary concentrations of MEP were also found to be decreasing after 2009 (Koch et al., 2017). Similarly, the Canadian Health Measures Survey found a 75% decline for DEHP metabolite, a 64% decline for MEP, a 42% decline for MnBP, a 62% decline for MBzP, and a 45% decline for MCPP levels in urine samples during 2007–2017 (Pollock et al., 2021). Decreases in the urinary concentrations of MEP, MnBP, and MBzP were also observed in Italy from 2011 to 2016 (Tranfo et al., 2018). From 2009 to 2017, the levels of DiBP, DnBP, BBzP, and DEHP metabolites were found reducing by more than half among Danish young men (Frederiksen et al., 2020). Another study conducted in Swedish pregnant women also found DEHP metabolites levels in urine samples decreased during 2007–2010, while DiNP metabolites increased in the period (Shu et al., 2018). However, in our study, urinary MiNP was not detected in most of the samples from all of the 6 years, with no observable trends in concentration. Although a sharp decrease in MnBP, MBzP, and MiBP concentrations was observed between 1993 and 2000, no significant temporal change was observed after 2000, and the concentrations remained consistently at lower levels compared with those in European countries (Gyllenhammar et al., 2017; Tranfo et al.,

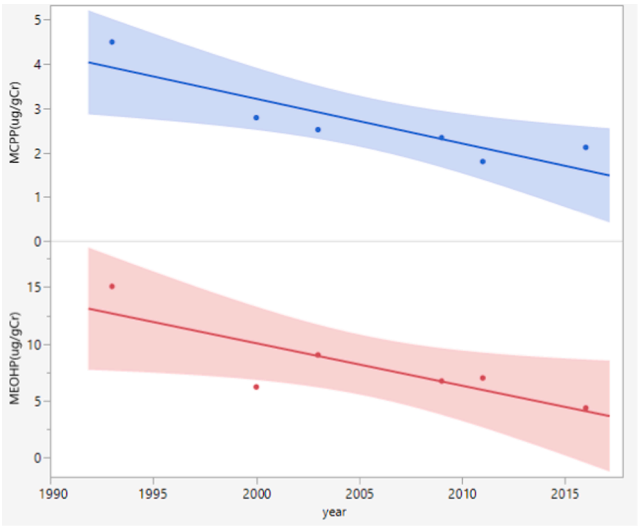

Fig. 2. Trend of creatinine-corrected concentrations (μg/g creatinine) of MCPP and MEOHP in urine collected between 1993 and 2016. The dots indicate GM and the bands show the 95% confidence intervals.

2018) and the USA (Koch et al., 2017; Zota et al., 2014). Moreover, the median concentrations of urinary MnBP and MiBP measured in our urine samples collected in the last decade, were found to be much lower than those reported in studies from the USA, several European countries (Fillol et al., 2021, Gyllenhammar et al., 2017; Koch et al., 2017; Tranfo et al., 2018), and other Asian countries, such as China (Guo et al., 2011a; Guo et al., 2011b; Zhang et al., 2018), indicating that the levels of exposure to DnBP and DiBP in Japan were relatively lower than other countries. A further comparison of the results in different studies was shown in Table S10 and Table S11.

3.4. Risk assessment

Based on the temporal trends in two decades in this study, changes in risks from exposures to phthalates were assessed. Fig. 3 shows the temporal trends in HQ and HI values. The highest HQ values of all four phthalates were found in the samples collected in the 1990s. For other phthalates such as DiNP, the detection rates in all sampling years did not exceed 50% so that they were not included in the calculation. The HQ_M value was found mainly with DEHP and DBP. DEHP was dominant in exposures among the target population during 1993–2016, similar to findings in the USA (Reyes, 2018) during 2005–2008, whereas an overall decline in the exposure to DEHP and an increase in the exposure to DiNP were observed in the USA during 2005–2014.

In only one participant showed an HI value > 1 among the total population. This subject was recruited in 1993. The HI values showed a sharp decrease between 1993 and 2000 (mean HI value from 0.21 to 0.048) and remained almost constant from 2000 until 2016. In addition, the HI values showed a quite similar trend as the HQ values of DEHP, as DEHP contributed most to the overall HI value during the whole study period (Fig. 4).

3.5. Strengths and limitations of this study

The major strength of this study is that the sampling design in this study focused on adult females living in Kyoto and the vicinity. They were recruited at community health checkup centers in same manner through the periods and their backgrounds of population would not change. In addition, all of the samples were analyzed at approximately the same time using archived urine samples, allowing us to observe the long-term temporal trends of exposures for over two decades.

This study has several limitations. First, because of the limited number of study population and study area, the present observations hence cannot be generalized to other areas in Japan. Number of samples was 10–26 in each study year and statistical power was limited. However, we applied power analysis to estimate the detectable effect size of this study (Table S12). For most of the analytes, the result indicates that

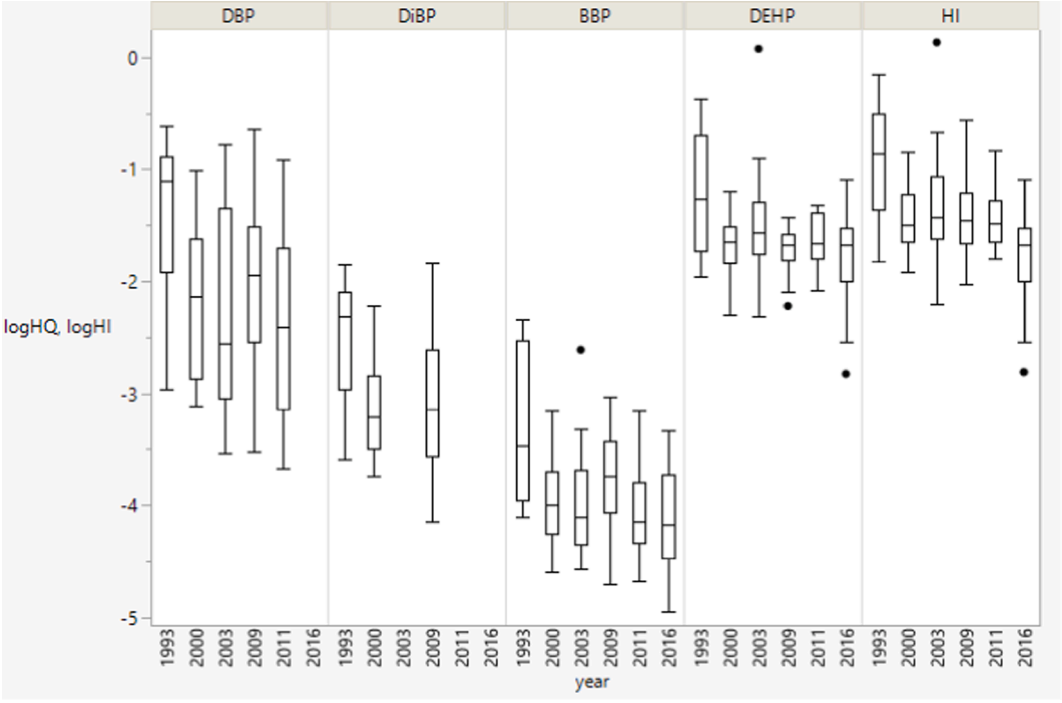

Fig. 3. Distribution of HQs of DBP, DiBP, BBP, and DEHP and their HI values determined for the Japanese women recruited between 1993 and 2016. The boxes show the interquartile ranges, the center lines in the boxes show the medians, the upper and lower whiskers show the maximums and minimums except for outliers, and the dots indicate outliers.

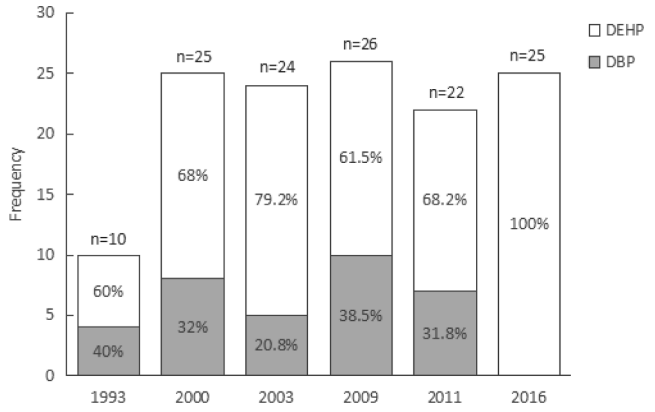

Fig. 4. Proportion (%) of the phthalates that produced the maximum hazard quotient (HQ_M) in each study year (N = 132). Phthalate which contributed most to the HI value for each subject was counted in each study year. Difference in the proportion among study years were tested by Fisher's exact test ($p = 0.0043$).

temporal changes can be detected if there would be 2 to 5 times change between years (e.g., MECPP and MEOHP). For MiBP and MnBP, the estimated effect sizes were larger, while drastic changes in exposure matrices may still be detected. It was noted that the study population was middle aged females in a community, and details of socio-economic factors were not investigated in this study. Hence, it is not necessarily extrapolated to entire population of Japan. Second, spot urine samples were used, and the concentration of metabolites may vary according to the timing of the sample collection. Third, creatinine adjustment was conducted, but potential biases could be possible and also inter-day variations in exposure may occur. Fourth, the samples have been archived in freezers, and during storage, possible degradation of target analytes might have occurred. Thus, the exposure calculated from older samples could be underestimated. Fifth, our study did not include some other emerging phthalates, such as di (2-propylheptyl) phthalate (DPHP), or alternative plasticizers such as 1,2-cyclohexane dicarboxylic acid diisononyl ester. It has been reported that the exposure to some alternative plasticizers increased in the past decade in various regions (Calafat et al., 2015; Frederiksen et al., 2020; Schwedler et al., 2020; Shu et al., 2018). Some studies conducted in European countries reported human exposure to DPHP in general populations (Porras et al., 2020a; Schwedler et al., 2020). While the DPHP exposure was much lower than exposure to most other phthalates (Porras et al., 2020b; Schmidtkunz et al., 2019), the substitution of DEHP will cause changes in exposure components. In Japan, limited information was published on the alternatives and comprehensive analysis of plasticizers is required in future studies.

4. Conclusion

The present study analyzed the urinary concentrations of 18 phthalate metabolites among a Japanese female population during 1993–2016. The results showed the temporal trend in the exposure to several phthalates among Japanese females over the two decades. As of the late 2010's, DEHP was still the predominant component of phthalate exposure in the population. The HI value indicates however that direct risk due to phthalate exposure was unlikely among the Japanese adult females. To date, limited studies have revealed long-term temporal trends of various phthalate exposure in Japan. Further estimations are also warranted regarding alternative plasticizers.

CRediT authorship contribution statement

Zhaoqing Lyu: Formal analysis, Investigation, Writing – original draft. **Kouji H. Harada:** Conceptualization, Writing – original draft, Supervision, Project administration, Funding acquisition. **Sungmin Kim:** Formal analysis, Investigation. **Tomoko Fujitani:** Formal analysis. **Yang Cao:** Writing – review & editing. **Toshiaki Hitomi:** Writing – review & editing. **Yukiko Fujii:** Formal analysis, Writing – review & editing. **Younglim Kho:** Formal analysis, Investigation, Writing – review & editing. **Kyungho Choi:** Conceptualization, 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.

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Appendix A. Supplementary material

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