Exposure trends to the non-phthalate plasticizers DEHTP, DINCH, and DEHA in children from 2012 to 2017: the Hokkaido study

Rahel Mesfin Ketema [a,b], Monika Kasper-Sonnenberg [c], Yu Ait Bamai [a,d], Chihiro Miyashita [a], Holger M. Koch [c], Claudia Pälmke [c], Reiko Kishi [a], Atsuko Ikeda [a,b*]

[a] Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan

[b] Faculty of Health Sciences, Hokkaido University, Sapporo, Japan

[c] Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

[d] Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610, Wilrijk, Belgium

*Corresponding author: Atsuko Ikeda, PhD

Hokkaido University, Faculty of Health Sciences

Kita 12, Nishi 5, Kita ku, Sapporo 060-0812, Japan

Phone: +81-11-706-3325

Email: AAraki@cehs.hokudai.ac.jp

Abstract

Phthalates owing to their endocrine-disrupting effects are regulated in certain products leading to their replacement with substitutions such as di-2-ethylhexyl terephthalate (DEHTP), 1,2-cyclohexane dicarboxylic acid di(isononyl) ester (DINCH), and di(2-ethylhexyl) adipate (DEHA). However, information on human exposure to these substitutes, especially in susceptible subpopulations such as children, is limited. Thus, we examined levels and exposure trends of DEHTP-, DINCH-, and DEHA-metabolites in 7 year-old Japanese school children. In total, 180 urine samples collected from 2012 to 2017 were used to quantify ten DEHTP-, DINCH-, and DEHA-metabolites via isotope dilution liquid chromatography with tandem mass spectrometry. DEHTP- and DINCH-metabolites were detected in 95.6% and 92.2% of the children, respectively, and DEHA was not detected. This study, annually conducted between 2012 and 2017, revealed a significant ($p < 0.05$) 5-fold increase in DEHTP metabolites and a 2-fold increase in DINCH metabolites. However, the maximum estimated internal exposures were still below health-based guidance and toxicological reference values. Exposure levels to DEHTP and DINCH have increased considerably in Japanese school children. DEHA is less relevant. Future studies are warranted to closely monitor the increasing trend in different aged and larger populations and identify potential health effects and sources contributing to increasing exposure and intervene if necessary.

Keywords: DEHTP, DINCH, DEHA, Human biomonitoring, Urine, Children, Daily Intake

Synopsis

This is the first report documenting the increasing level of exposure to the phthalate plasticizer substitutes DEHTP and DINCH in Japanese children from 2012 to 2017. Exposure detection to the substitute DEHA is still low.

1. Introduction

Due to the association of some phthalates with endocrine disruption, legacy phthalates such as di-2-ethylhexyl phthalate (DEHP) and di(isononyl) phthalate (DINP) have been subjected to strong regulation.[1–3] Consequently, phthalate substitutes such as di-2-ethylhexyl terephthalate (DEHTP), di(isononyl) cyclohexane-1,2-dicarboxylate (DINCH), and di(2-ethylhexyl) adipate (DEHA) have been emerging as replacements in different industrial goods, packaging, and personal care products.[4–9] Similar to phthalate plasticizers, these substitutes are not chemically bound to the products and can migrate out to the environment in food, air, and dust. Humans can be exposed through inhalation, skin contact, ingestion from air, dust and food,[10] or by leaching and migrating from medical devices.[11,12]. Although data on the health effects of DEHTP and DINCH, particularly in children are currently lacking, certain in vivo and vitro studies suggested exposure to these substitutes has potential associations with reproductive toxicity,[13] inducing oxidative stress leading to adverse respiratory burst[14] and lipid metabolism.[15] Considering the comparable characteristics of regulated phthalates and their substitutes[16] it is anticipated that the substitutes may potentially pose similar health effects as the phthalates in humans which warrants biomonitoring of these substitutes.

Human biomonitoring studies allow us to estimate the total internal exposure levels, capturing all sources and pathways, and provide a useful indicator of plasticizer substitution market change after phthalates regulation.[5,17,18] Once DEHTP, DINCH, and DEHA enter the human body, they are metabolized into their respective hydrolytic/oxidative

metabolites.[19,20,9] These metabolites are excreted in urine and can be used as specific biomarkers of exposure. Previous human biomonitoring studies have reported rapid increases in exposures to DINCH and DEHTP in Europe,[21,22,5,23–26] America,[27–30,18] and parts of Asia.[31,32] Owing to the 2010 revised regulation of legacy phthalates in children's toys and fatty food packaging materials in Japan,[1] we hypothesized that exposure to the phthalate substitutes would increase in the Japanese population. Thus, this study aimed to assess the exposure levels of DEHTP, DINCH, and DEHA in Japanese children and to investigate trends from 2012 to 2017.

2. Materials and methods

2.1 Study population

The Hokkaido cohort from the Hokkaido Study on Environment and Children's Health is a prospective birth cohort study aiming to investigate the health effects of environmental chemical exposure in children starting from the prenatal stage. The study design and participant recruitment have been described in detail.[33–36] Briefly, 20,926 pregnant women were enrolled in the cohort from February 2003 to March 2012. After excluding those with spontaneous abortion, stillbirth, loss to follow-up, and voluntary withdrawal, 15,757 children could be followed up. Between 2012 and 2017, a follow-up questionnaire and a request for a urine sample were sent to 10,655 participants majority (>95%) still residing in Hokkaido when the children reached the age of 7. Among the 6,218 respondents, 2,541 responded to the complete questionnaire and also provided urine samples. This study population was originally selected to ensure the representation of our cohort data for a nested case-cohort

study to assess the association between asthma and allergies with phthalate metabolites level in 7 years old children.[37] To achieve a statistical power of 80% and a significance level of 0.05 calculation, comprising 100 cases and 136 sub-cohort was determined. Due to the overlap of cases and sub-cohort, we added 50 sub-cohort, resulting in 180 sub-cohort for measurements. Considering the representativeness of the cohort and our budget limit, for this study, we randomly selected urine samples representing 30 children as a sub-cohort from each study year between 2012 to 2017, totaling 180 to conduct the trend study urine samples. The questionnaire included personal characteristics such as sex, weight, annual household income in million yen (<3, ≥3-<5, ≥5-<8, and ≥8), and building characteristics, including types of home (detached or apartment), home structure (wood or concrete), floor material (polyvinyl chloride (PVC) floor sheet or No PVC (tatami, compressed wood, carpet, and tiles) and wall material (PVC wallpaper or no), mechanical ventilation system (yes or no), vacuum cleaning frequency per week (≤3 times or 4–7 times), window opening duration per day (<1 hour or ≥1 hour), the proximity of main road from house (<50 meters, no main road, or ≥50 meters), and building age. The dampness index (1–5) of the houses was computed by summing the yes response as 1 point to dampness indicators condensation (yes/no), moldy odor (yes/no), visible mold (yes/no), humidity in the bathroom (yes/no), and water leakage (yes/no).

2.2 DEHTP-, DINCH-, and DEHA-metabolite analysis

Details of urine sample collection have been reported elsewhere.[38,37] The parents collected the morning void urine samples and sent them to Hokkaido University, Center for

Environmental and Health Sciences, using a cool delivery service. The four DEHTP metabolites mono(2-ethyl-5-carboxypentyl) terephthalate (5cx-MEPTP), mono(2-ethyl-5-hydroxyhexyl) terephthalate (5OH-MEHTP), 1-mono-(2-ethyl-5-oxo-hexyl) benzene-1,4-dicarboxylate (5oxo-MEHTP), and 1-mono-(2-carboxyl-methyl-hexyl) benzene-1,4-dicarboxylate (2cx-MMHTP), three DINCH metabolites cyclohexane-1,2-dicarboxylic acid monohydroxy isononyl ester (OH-MINCH), cyclohexane-1,2-dicarboxylic acid monooxo isononyl ester (oxo-MINCH), and cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester (cx-MINCH), and three DEHA-metabolites 1-mono-(2-ethyl-5-hydroxyhexyl) adipate (5OH-MEHA), 1-mono-(2-ethyl-5-oxohexyl) adipate (5oxo-MEHA), and 1-mono-(2-ethyl-5-carboxylpentyl) adipate (5cx-MEPA) were measured in the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA). The urine samples were sent for analysis in a polypropylene tube at −20°C.

According to the previously published methods,[39–41] internal isotope-labeled standards and ammonium acetate buffer were added to the thawed urine samples. Following enzymatic hydrolysis with an arylsulfatase-free β-glucuronidase (*E. coli* K12), samples were injected and chromatographically resolved in a 2-column high-performance liquid chromatography assembly with detection and quantification on a triple quadrupole tandem mass spectrometer in negative electrospray ionization mode. The limits of quantification (LOQs) were 0.05 µg/L for 5cx-MEPA and 5OH-MEHA, 0.1 µg/L for 5oxo-MEHA, 0.2 µg/L for 5cx-MEPTP, 5OH-MEHTP, and 5oxo-MEHTP, 0.4 µg/L for 2cx-MMHTP, and 0.05 µg/L for OH-MINCH, cx-MINCH, and oxo-MINCH. For the DINCH metabolites OH-MINCH and cx-MINCH, the

analyzing laboratory successfully obtained certificates from the external quality assurance round robin of HBM4EU.[42,43] No external quality assessment scheme had been offered for the other metabolites at the time of measurement. Urinary creatinine was determined using a Cobas c702 autoanalyzer (Roche Diagnostics, Rotkreuz ZG, Switzerland) applying the Jaffe static method with a working range of 0.05–5.00 g creatinine/L.

2.3 Daily intake estimation

We calculated the estimated daily intake (EDI) in µg/kg bw/d for DEHTP and DINCH based on the individual creatinine-adjusted levels of their respective metabolites, similar to our previous approach[44] for nonylphenol exposure in the same study population:

EDI (µg/kg bw/d) = *C* (µmol/g creatinine) × CE smoothed × MW_P/FUE × bw (kg)

Herein, *C* represents the creatinine corrected concentration of metabolites used to calculate the estimation of DI. For DEHTP we selected the major metabolite 5cx-MEPTP, as indicated in[45]. For DINCH, the HBM-I values[45] and the HBM-GV_{GenPop}[46] are based on the two metabolites cx-MINCH and OH-MINCH. Therefore, we used metabolite 5cx-MEPTP for DEHTP[20] and metabolites OH-MINCH and cx-MINCH for DINCH[45] to calculate their EDIs. Creatinine excretion (CE) smoothed is calculated by using a formula for 6-8 year-old children using individual age and height in nmol/kg/day to maintain metabolic creatine production.[47] MW_P is the molecular weight of the alternative parent plasticizer in g/mol.

The FUE is the urinary excretion percentage of DEHTP metabolite (5cx-MEPTP = 12.95%) as reported[20] and the sum of DINCH metabolites (OH-MINCH = 10.73% and cx-MINCH =

2.03%) as reported[9]. BW is the individual body weight of the children at age 7 that participated in this study used to estimate the daily intake. For risk assessment and to evaluate potential adverse effects of the alternative plasticizers we calculated risk quotients (RQs). The RQ-HBM-I was calculated as a ratio to compare individual DEHTP and DINCH metabolite concentrations with human biomonitoring (HBM-I) values. The German HBM-I values for children are 1800 µg/L for 5cx-MEPTP (DEHTP) and 3000 µg/L for the sum of OH-MINCH + cx-MINCH (DINCH).[45]

RQ-HBM-I = Individual metabolites concentration $_{(\mu g/L)}$ / HBM-I $_{(DEHTP/DINCH\ (\mu g/L)}$[22]

The RQ-TDI was calculated as a ratio to compare the EDI with the TDI reference values of DEHTP and DINCH. The TDI-like reference value for DEHTP is 540 µg/kg bw/day recommended by the German HBM Commission[45] and for DINCH is 1000 µg/kg bw/day recommended by the European Food and Safety Authority (EFSA)[48]:

RQ-TDI =EDI $_{(\mu g/kg\ bw/day)}$ /TDI $_{(\mu g/kg\ bw/day)}$[45]

Ethical Approval and Informed Consent

This study was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environment and Health Sciences. All participants' mothers were informed about the study and signed a written consent at the time of enrollment.

2.4 Statistical analysis

For statistical calculations, the LOQ/2 values were assigned for metabolite concentrations below the LOQ. The DEHTP metabolite 2cx-MMHTP with a detection frequency of only 3.3% was excluded from further analyses. Moreover, the DEHA-metabolite detection frequency was only 1.1% (5cx-MEPA) and was omitted from further statistical analysis. The chi-square test was used for categorical variables and the Spearman correlation was used for continuous variables to compute the association of metabolite concentrations with personal and building characteristics. To compute the association between DEHTP and DINCH metabolites, bivariate Spearman's rho correlation was used. General linear regression was used to examine the exposure trend from 2012 to 2017 with natural logarithmically transformed and creatine-corrected metabolite concentrations. To compute the yearly percentage change in DI, we used a formula $[\exp(\beta) - 1] \times 100\%$ and 95% CI as $[\exp(\beta \pm 1.97 \times SE) - 1] \times 100\%$, where β is the beta estimate of the regression coefficient, and SE is the standard error of regression coefficient. The statistical analysis in this study was performed with JMP Pro 16.1.0. In addition, we tested the exposure trend of metabolites throughout the study period with the Jonckheere–Terpstra tests using SPSS version 27 (IBM Corp., Armonk, NY).

3. Results

3.1. Characteristics of the participants

The demographics and home characteristics of the study are described in Table 1. This study included 180 Japanese children, all at the age of 7. Among the participants, 47.2% were boys. In this study, 41.6% of the children resided in households with a middle annual income

ranging from ≥5 to <8 million yen. Additionally, 16.1% of the children lived in houses with PVC wallpaper and 70.0% without mechanical ventilation system. The average age of the home buildings was 12 years, and the dampness index was 2.

Table 1. Demographic and building characteristics of participants

Characteristics		N (%) or Median (range)
Sex	Male	85 (47.2)
	Female	95 (52.8)
Weight	Continuous	22 (20.1,24.7)
BMI (kg/m^2)	Continuous	15.4 (14.8,16.7)
Annual household income in million yen	<3	23 (12.8)
	≥3-<5	49 (27.2)
	≥5-<8	74 (41.1)
	≥8	25 (13.9)
	Missing	9 (5.0)
Type of home	Detached	132 (73.3)
	Apartment	48 (26.7)
Home structure	Wood	130 (72.2)
	Concrete	48 (26.7)
Floor material	PVC floor sheet	29 (16.1)
	No PVC	151 (83.9)
Wall material	PVC wallpaper	149 (82.8)
	No	31 (17.2)
Mechanical ventilation system	Yes	54 (30.0)
	No	126 (70.0)
Vacuum cleaning per week	≤3 time	86 (51.2)
	4-7 times	82 (48.8)
Window opening duration	<1hr	66 (36.7)
	≥1hr	114 (63.3)
Main road	<50 meter	37 (20.6)
	≥50 meter	143 (79.4)
Building age (years)	≤10	73 (40.7)
	11-20	55 (30.7)
	21-30	29 (16.2)
	31-40	18 (10.0)
	41-50	4 (2.2)
Dampness index (0-5)	Continuous	2 (0,5)

3.2. DEHTP-, DINCH-, and DEHA-metabolite levels and their relationship with the characteristics of the participants

The metabolite concentrations of DEHTP, DINCH, and DEHA in children's urine stratified by year from 2012 to 2017 are summarized in Table 2. The highest detection frequency and median concentration was observed for the DEHTP metabolite 5cx-MEPTP with 95.5% detection and a 1.36 µg/L median. The DINCH metabolite OH-MINCH was detected in 92.2% of the children with a median of 0.2 µg/L. Considering DEHA, only 5cx-MEPA was detected in two children (Supplemental Table 1). The concentration of metabolites associated with personal and building characteristics is depicted in Supplemental Table 2. The result showed no significant ($p < 0.05$) metabolite concentration differences in sex. Children living in a house with a mechanical ventilation system have slightly higher oxo-MINCH than those without ventilation (0.16 µg/L vs. 0.10 µg/L). Significant higher levels of DEHTP metabolites 5cx-MEPTP ($p < 0.05$) and 5OH-MEHTP ($p < 0.01$) were observed in children living in newer buildings (≤10 years) than in older buildings (≥11 years). On the contrary, a decreasing and significant ($p < 0.05$) relationship with Spearman's correlation was observed in the dampness index with 5cx-MEPTP (−0.187), 5OH-MEHTP (−0.182), and oxo-MINCH (−0.152).

Table 2. The yearly concentration of DEHTP and DINCH metabolites in 180 children (μg/L)

DEHTP metabolites

	5cx-MEPTP					5-OH-MEHTP					5-oxo-MEHTP					2cx-MMHTP				
Year	DF%	Min	Median (IQR)	95%	Max	DF%	Min	Median (IQR)	95%	Max	DF%	Min	Median (IQR)	95%	Max	DF%	Min	Median (IQR)	95%	Max
2012	86.7	<LOQ	0.54 (0.34,1.23)	4.77	4.81	16.7	<LOQ	<LOQ	0.74	1.03	13.3	< LOQ	< LOQ	0.74	1.16	0.00	< LOQ	< LOQ	< LOQ	< LOQ
2013	93.3	<LOQ	0.84 (0.41,2.07)	4.69	5.22	16.7	<LOQ	<LOQ	0.42	0.54	10.0	< LOQ	< LOQ	0.26	0.34	0.00	< LOQ	< LOQ	< LOQ	< LOQ
2014	96.7	<LOQ	1.05 (0.57,1.70)	12.2	21.6	16.7	< LOQ	< LOQ	1.21	2.07	10.0	< LOQ	< LOQ	0.53	0.78	3.30	< LOQ	< LOQ	0.30	0.43
2015	96.7	<LOQ	1.84 (0.77,5.28)	14.9	16.4	36.7	< LOQ	< LOQ (<LOQ,0.56)	2.47	3.62	33.3	< LOQ	< LOQ (<LOQ,0.35)	1.11	1.32	10.0	< LOQ	< LOQ	0.53	0.59
2016	100	0.29	1.66 (0.86,3.36)	12.6	15.0	23.3	< LOQ	< LOQ (<LOD,0.17)	2.98	3.28	36.7	< LOQ	< LOQ (<LOQ,0.33)	2.03	2.25	0.00	< LOQ	< LOQ	< LOQ	< LOQ
2017	100	0.22	2.72 (1.67,5.39)	33.3	46.8	30.0	< LOQ	< LOQ (<LOQ,0.44)	2.01	3.13	46.7	< LOQ	< LOQ (<LOQ,0.28)	1.64	2.51	6.70	< LOQ	< LOQ	0.74	1.01
All sampling years	95.5	<LOQ	1.36 (0.56,2.54)	10.5	46.8	23.3	<LOQ	<LOQ	1.20	3.62	25.0	<LOQ	<LOQ (<LOQ,0.17)	0.92	2.51	3.3	< LOQ	< LOQ	< LOQ	1.01

DINCH metabolites

	OH-MINCH					cx-MINCH					oxo-MINCH					∑OH-MINCH + cx-MINCH			
Year	DF%	Min	Median (IQR)	95%	Max	DF%	Min	Median (IQR)	95%	Max	DF%	Min	Median (IQR)	95%	Max	Min	Median (IQR)	95%	Max
2012	80.0	<LOQ	0.15 (0.06,0.22)	0.56	0.60	40.0	<LOQ	<LOQ (<LOD,0.08)	0.31	0.34	60.0	<LOQ	0.08 (<LOQ,0.17)	0.40	0.41	<LOQ	0.17 (0.09,0.34)	0.78	0.88
2013	86.7	<LOQ	0.15 (0.06,0.25)	1.53	2.32	50.0	<LOQ	<LOQ (<LOQ,0.12)	0.80	1.14	53.3	<LOQ	0.06 (<LOQ,0.17)	1.38	1.44	<LOQ	0.21 (0.08,0.38)	2.33	3.46
2014	93.3	<LOQ	0.15 (0.09,0.34)	9.13	18.8	56.7	<LOQ	0.06 (<LOQ,0.11)	12.2	26.2	70.0	<LOQ	0.14 (<LOQ,0.26)	4.88	7.74	<LOQ	0.21 (0.12,0.49)	21.3	45.1
2015	96.7	<LOQ	0.26 (0.12,0.47)	1.93	2.90	73.3	<LOQ	0.07 (<LOQ,0.20)	1.59	2.67	83.3	<LOQ	0.14 (0.06,0.31)	0.96	1.44	<LOQ	0.48 (0.17,0.67)	3.52	5.57
2016	100	<LOQ	0.22 (0.14,0.62)	3.55	5.31	83.3	<LOQ	0.12 (<LOQ,0.23)	2.63	4.13	80.0	<LOQ	0.11 (0.06,0.39)	1.48	2.27	<LOQ	0.33 (0.19,0.82)	5.93	9.44
2017	96.7	<LOQ	0.27 (0.14,0.43)	2.55	2.93	80.0	<LOQ	0.13 (0.06,0.21)	1.36	1.40	86.7	<LOQ	0.20 (0.12,0.32)	1.40	1.72	<LOQ	0.45 (0.21,0.62)	3.92	4.26
All sampling years	92.2	<LOQ	0.20 (0.09,0.35)	1.63	18.8	63.9	<LOQ	0.07 (<LOQ,0.18)	1.13	26.2	72.2	<LOQ	0.07 (<LOQ,0.18)	1.13	7.74	<LOQ	0.27 (0.12,0.51)	2.96	45.1

Abbreviations: DF: detection frequency, Min: minimum, Max: maximum, DEHTP: di-2-ethylhexyl terephthalate, DINCH: 1,2-cyclohexane dicarboxylic acid diisononyl ester, 5cx-MEPTP: mono(2-ethyl-5-carboxypentyl) terephthalate, 5OH-MEHTP: mono(2-ethyl-5-hydroxyhexyl) terephthalate, 5oxo-MEHTP: mono(2-ethyl-5-oxohexyl) terephthalate, 2cx-MMHTP: mono[2-(carboxymethyl)hexyl] terephthalate, cx-MINCH: cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester, OH-MINCH: cyclohexane-1,2-dicarboxylic acid mono hydroxy isononyl ester and oxo-MINCH: cyclohexane-1,2-dicarboxylic acid monooxo isononyl ester; LOQ: limit of quantification for 5cx-MEPTP, 5OH-MEHTP, 5oxo-MMHTP was 0.20 µg/L, for 2cx-MMHTP was 0.40 µg/L, for -OH-MINCH, cx-MINCH, and oxo-MINCH was 0.05 µg/L

3.3. DEHTP and DINCH exposure and trends

The individual urinary metabolite levels and calculated DIs of DEHTP and DINCH over time from 2012 to 2017 are presented in Figures 1A and B. All metabolites of DEHTP and DINCH except for 2cx-MMHTP showed statistically significant increasing secular trends with $p < 0.05$. The concentration and detection frequency of DEHTP and DINCH stratified by year are presented in Table 2. The maximum median concentration was observed in the DEHTP metabolite 5cx-MEPTP with 2.72 μg/L. All metabolites showed an increasing concentration and detection frequency from 2012 to 2017. The DI of DEHTP and DINCH increased significantly ($p < 0.001$) throughout the study period. Except for 5OH-MEHTP, the increasing trend of all metabolites' exposure and DI of DEHTP and DINCH were confirmed with Jonckheere–Terpstra tests with $p < 0.01$ from 2012 to 2017.

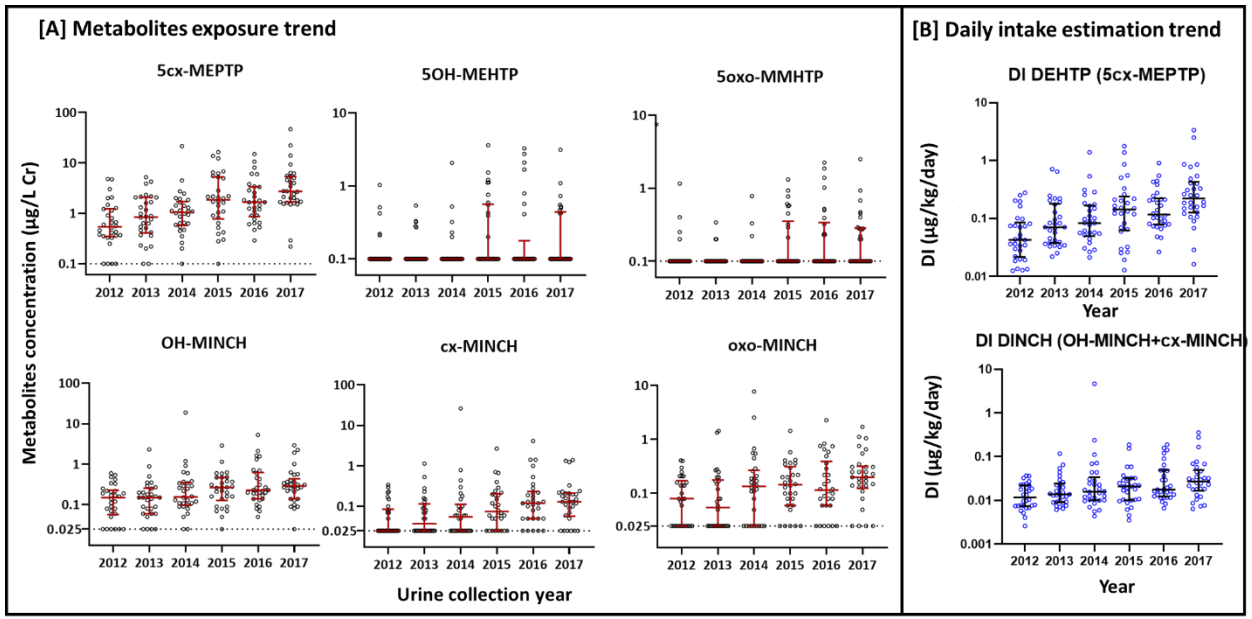

Figure 1: [A] Exposure trend of urinary metabolites concentration from 2012 to 2017, 5cx-MEPTP: mono(2-ethyl-5-carboxypentyl) terephthalate, 5OH-MEHTP: mono(2-ethyl-5-hydroxyhexyl) terephthalate, 5oxo-MEHTP: mono(2-ethyl-5-oxohexyl) terephthalate, cx-MINCH: cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester, OH-MINCH: cyclohexane-1,2-dicarboxylic acid mono hydroxy isononyl ester and oxo-MINCH: cyclohexane-1,2-dicarboxylic acid mono-oxo isononyl ester, red horizontal lines represent the median and the whiskers represent the 25th and 75th percentile. Black points represent children's metabolite concentration. Points on dotted lines showed samples with concentrations below the limit of quantification. [B] Daily intakes (DI) estimation trend in µg/kg bw/day in sampling years 2012-2017. DEHTP: di-2-ethylhexyl terephthalate and DINCH: 1,2-cyclohexane dicarboxylic acid diisononyl ester, black horizontal lines represent the median and the whiskers represent the 25th and 75th percentile, blue points represent children's DI estimation.

3.4. Risk assessments

Table 3 presents risk assessments based on HBM and TDI-like references. The reference values for DEHTP (1800 µg/L) and DINCH (3000 µg/L) for children are derived from the HBM-I values.[45] The TDI-like value for DEHTP is 540 µg/kg bw/day recommended by HBM-I[45] and DINCH is 1000 µg/kg bw/day recommended by European Food Safety Authority (EFSA).[48] The overall results revealed that none of the children exceeded HBM-I and TDI reference values. Based on a direct assessment of the metabolite concentrations via the HBM-I values, the maximum 5cx-MEPTP concentration of 46.81 µg/L was far below the HBM-I value. Likewise, the sum of the OH-MINCH + cx-MINCH concentration of 45.10 µg/L was far below the HBM-I value. Based on the EDI to TDI comparison, the maximum-estimated DI of DEHTP 3.47 µg/kg bw/day was observed in 2017, and for DINCH it was 3.58 µg/kg bw/day in 2014. Both values were below their respective TDI values by a factor of ~150 for DEHTP and ~300 for DINCH. The average yearly increase in DEHTP intake was a percentage change (95% CI): 6.6% (3.4%, 9.9%) and for DINCH: 0.4% (−1.8%, 2.8%).

Table 3 Risk quotients of DEHTP and DINCH at 50% (median), 95% and max concentrations based on HBM-I (µg/L) and daily intakes based on the TDI (µg/kg bw/day) in sampling years 2012-2017

Risk quotient (RQ) based on HBM-I values	RQ[a] DEHTP 1,800 µg/L			RQ[b] DINCH 3,000 µg/L		
Sampling year	50%	95%	Max	50%	95%	Max
2012	<0.001	0.003	0.003	<0.001	<0.001	<0.001
2013	<0.001	0.003	0.003	<0.001	0.001	0.001
2014	0.001	0.007	0.012	<0.001	0.007	0.015
2015	0.001	0.008	0.009	<0.001	0.001	0.002
2016	0.001	0.007	0.008	<0.001	0.002	0.003
2017	0.002	0.019	0.026	<0.001	0.001	0.001
Study period (2012-2017)	0.001	0.006	0.026	<0.001	0.001	0.015
RQ based on TDI	RQ[c] DEHTP 540 µg/kg bw/d			RQ [c] DINCH 1,000 µg/kg bw/d		
Sampling year	50%	95%	Max	50%	95%	Max
2012	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2013	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2014	<0.001	0.001	0.002	0.000	0.002	0.004
2015	<0.001	0.002	0.002	<0.001	<0.001	<0.001
2016	<0.001	0.002	0.002	<0.001	<0.001	<0.001
2017	<0.001	0.004	0.006	<0.001	<0.001	<0.001
Study period (2012-2017)	<0.001	0.002	0.006	<0.001	<0.001	0.004

Abbreviations: RQ: risk quotient, TDI: tolerable daily intake, HBM-I: human biomonitoring value, DEHTP: di-2-ethylhexyl terephthalate and DINCH: 1,2-cyclohexane dicarboxylic acid diisononyl ester, 5cx-MEPTP: mono(2-ethyl-5-carboxypentyl) terephthalate, OH-MINCH: cyclohexane-1,2-dicarboxylic acid mono hydroxy isononyl ester, cx-MINCH: cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester, RQ [a]: for DEHTP with 5cx-MEPTP based on HBM-I reference value of 1,800 µg/L[45], RQ [b]: for DINCH with sum of OH-MINCH and cx-MINCH based on HBM-I reference value of 3,000 µg/L[45,49], RQ [c]: based on TDI reference value of 540 µg/kg bw/day for DEHTP[45] and 1,000 µg/kg bw/day for DINCH[48]

4. Discussion

This study examined the urinary concentration of alternative plasticizers metabolites DEHTP, DINCH, and DEHA in Japanese children's urine collected from 2012 to 2017. An increasing trend of DEHTP and DINCH metabolites was observed in terms of concentration and detection frequency along with DI during the study period. DEHA exposure showed very low detection. To our knowledge, we herein present the first biomonitoring study of the phthalate substituting alternative plasticizers DEHTP, DINCH, and DEHA in Japanese children.

4.1. Exposure levels and secular trends of DINCH and DEHTP

Regarding concentration, detection frequencies, and exposure trends, among the DEHTP and DINCH metabolites, 5cx-MEPTP and OH-MINCH, respectively, were dominant in this study. These mainly detected metabolites were also strongly correlated with Spearman's rho ranging from 0.548 to ≥ 0.847, with their respective metabolites of the same parent compound ($p < 0.001$) (Supplemental Figure 1). Furthermore, in terms of metabolite excretion, the concentration of 5OH-MEHTP was approximately one-tenth of the concentration of 5cx-MEPTP. This observation aligns with previous research on human metabolism and urinary excretion, where 5cx-MEPTP has been identified as the primary metabolite excreted in urine.[19,20]

Although our Japanese children had the lowest exposure to DEHTP and DINCH in all investigated populations, specific DEHTP and DINCH metabolites were still detectable in >90% of the analyzed urine samples. A five-fold increase in DEHTP and a two-fold increase

in DINCH were observed when we compared the median exposure level in 2012 with that of 2017. This result indicates an average yearly increase in the estimated intake of DEHTP and DINCH of 6.6% and 0.4%, respectively. Such increases in DEHTP and DINCH exposure levels have recently been shown in young German adults[23,26] and Danish young men[5], summarized by Vogel et al.[21] More importantly, all three previously mentioned publications reported on European adults aged between 18 and 30 as their study participants. Hence, when comparing their findings with our study involving children, it is crucial to consider potential regional and age-related variations. As pointed out above, these changes are results of national regulatory measures limiting the use and production of some "legacy" phthalates, in turn increasing the demand for and use of alternatives.[1,2,50,51] In our previous biomonitoring study on the same Japanese children, we found rather stable levels of the legacy phthalate metabolites di-isobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), butylbenzyl phthalate (BBzP), DEHP, and DINP,[52] with less pronounced declines in phthalate exposure than those observed in other countries and especially DEHP exposures still higher than in other populations. Based on these results of stable/unchanged exposure to regulated phthalates (DBP, BBzP, DEHP and DINP)[52] and increasing DEHTP and DINCH, it seems that in Japan, the exposure of legacy phthalates is occurring at a steady pace compared to the increasing trend of their substitutes. In Japan, while there is regulation on the legacy phthalates (DBP, BBzP, DEHP, DIDP, DINP and DnOP) in children's toys and fatty food packaging materials,[1] there is no regulation regarding the alternative plasticizers (DEHTP, DINCH, or DEHA). Regarding plasticizer exposure levels and trends in Asian countries, considerable complexity in this region was reported by Lee et al. in 2021 for both substitutes and phthalates.

Furthermore, Dominguez-Romero et al. in 2022 reported a decreasing exposure trend to the legacy phthalate DEHP in South Korea and Taiwan, whereas a reverse increasing trend was observed in China.[27] It is important to note that Asia is the major consumer of plasticizers.[53] Moreover, regulation of these chemicals varies across the region. Therefore, more biomonitoring exposure trend studies of phthalates and their alternatives across this region are warranted.

Comparison of urinary DEHTP-, DINCH-, and DEHA-metabolite levels in similar aged children from different countries is shown in Table 4. Japanese children have much lower exposure levels than children in Indonesia, Thailand, Saudi Arabia, the USA, Germany, and Portugal.[54–59,17,60,18] Among the above-mentioned countries, DEHTP and DINCH metabolites were the highest in Saudi children.[56] For instance, the median of 5cx-MEPTP in Saudi children with 128 μg/L was more than 90 times higher than this study's Japanese children with 1.36 μg/L,[56] but also compared to the USA[55,59] DEHTP exposures are approximately 40 times lower in Japan. Likewise, for DINCH, exposures in our Japanese children are at the lower end of internationally observed metabolite levels. This time, the spread of exposures seems a bit lower, with Saudi Arabia, the USA, Germany, and Portugal reporting approximately 10 times higher median urinary concentrations,[56,58,59,17] and Japan in the low exposure group together with Thailand and Indonesia with similar median levels of OH-MINCH (0.2 μg/L).[56] The observed levels of exposure to DEHTP and DINCH metabolites vary across different countries and regions. These differences could be an indication of diverse utilization of DEHTP/DINCH/DEHA in various applications such as toy manufacturing, food packaging, personal care products, or polyvinyl chloride (PVC)-

made interior materials like PVC floor covering sheet.[61] Additionally, differences may also be influenced by variations in regulations of legacy phthalates and the pace at which substitution of alternative plasticizers. This study's significant increase in DEHTP metabolites in children living in newer buildings (Supplemental Table 2) could indicate DEHTP usage in buildings or interior materials in Japan, which warrants future studies to explore exposure sources. The detection frequency of DEHA-metabolite was only 1.1% for 5cx-MEPA (Supplemental Table 1), which suggests that exposure to DEHA is still neglectable among Japanese children. There is only one comparable study in Saudi Arabia in which median levels were 0.1 µg/L with a detection frequency of 56%.[56] Due to DEHA's structural similarity to DEHP, it has been used in various medical products, food package wrapping materials, adhesives, and plastic materials such as PVC[62] including in Japan.[63] In addition, DEHA was found in Japanese house dust with detection frequency of >80%, so children can be exposed to DEHA through dust inhalation and ingestion[64,65]. The reason for the detection of DEHA in house dust but not in urine samples is unknown. However, one possible explanation could be that house dust is not a relevant source of DEHA exposure or that DEHA HBM is not very sensitive to trace level DEHA exposure. The major DEHA metabolite 5cx-MEPA has a very low excretion fraction of only 0.20%.[66] This low excretion fraction is one explanation of the low detection in the current or previous study.[56] Most part of incorporated DEHA is broken down and excreted as adipic acid, which however is not specific to DEHA but has many other sources. Nevertheless, urinary 5cx-MEPA is sensitive enough to detect DEHA exposures of at least a factor of 25 below the TDI for DEHA (0.3 mg/kg/d)[66] thus protecting from potential toxic effects.

Table 4: Comparison of alternative phthalates metabolites levels in children from different countries in median (µg/L)

					DEHTP			DINCH			DEHA
References	Country	Study period	Age group	Sample size	5cx-MEPTP	5OH-MEHTP	5oxo-MEHTP	OH-MINCH	cx-MINCH	oxo-MINCH	5cx-MEPA
This study	Japan	2012-2017	7 years	180	1.36	< LOQ (0.20)	<LOQ (0.20)	0.20	0.06	<LOQ (0.05)	<LOQ (0.05)
Lee, 2021[56]	Thailand	2018	6-10 years	104	8.00	0.80	0.40	0.20	0.10		-
	Indonesia	2018	5-11 years	89	3.60	0.30	0.30	0.20	0.10		-
	Saudi Arabia	2017	3-9 years	108	128	13.8	7.40	2.90	1.50		0.10
Hammel, 2019 [59]*	USA	Sept 2014-April 2016	3-6 years	108	65.0	8.70		2.60	1.50		
Schwedler, 2020 [57,58]	Germany	Jan 2015-June 2017	3-17 years	2112	7.20	0.50	0.53	2.20	1.08	0.96	
Lessman, 2017[17]	Portugal	2014/2015	4-17 years	107	4.19	0.45	0.27				
Correia-Sá, 2017[60]	Portugal	2014/2015	4–18 years	112	-	-	-	1.83	1.03	0.89	
Vogel et al. 2023[67]	Europe	2014-2021	6-12 years	2579	-	-	-	2.34	1.25	-	
NHANES,CDC 2022[55]	USA	2011/12	6-11 years	396	-	-	-	-	-		
		2013/14	6-11 years	409	-	-	-	0.60	-		
		2015/16	6-11 years	415	38.1	7.30	-	1.10	0.80		
		2017/18	6-11 years	330	54.6	10.8	-	1.30	0.80		

*SG corrected concentrations

Abbreviations: DEHTP: di-2-ethylhexyl terephthalate, DINCH: 1,2-cyclohexane dicarboxylic acid diisononyl ester, MEPTP: mono(2-ethyl-5-carboxypentyl) terephthalate, 5OH-MEHTP: mono(2-ethyl-5-hydroxyhexyl) terephthalate, 5oxo-MEHTP: mono(2-ethyl-5-oxohexyl) terephthalate, cx-MINCH: cyclohexane-1,2-dicarboxylic acid monocarboxy isooctyl ester, OH-MINCH: cyclohexane-1,2-dicarboxylic acid mono hydroxy isononyl ester and oxo-MINCH: cyclohexane-1,2-dicarboxylic acid monooxo isononyl ester; LOQ: limit of quantification

4.2. Risk assessment

The maximum EDI of DEHTP was 150 times lower than the TDI value of 540 µg/kg bw/d [45] based on renal toxicity. Similarly, the maximum EDI of DINCH was 279 times lower than TDI value of 1000 µg/kg bw/d also based on renal toxicity.[48] The HBM-I approach for children consider the children's excretion volume of urine and reference values such as TDIs or comparable reference values which provide more accurate assessment of the internal exposure of DEHTP and DINCH in children.[45] Considering the risk assessment conducted based on the HBM-I values, the median urinary level of 5cx-MEPTP 1.36 µg/L for DEHTP was 1,323 times lower than the reference value of 1,800 µg/L.[45] DINCH with the median urinary level of ∑OH-MINCH and cx-MINCH 0.27 µg/L was 11,000 times lower than the HBM-I reference value of 3,000 µg/L.[46,45] Although these values are well below the guidelines of TDI and HBM-I, the highest EDI and urinary concentrations were observed in 2017 for DEHTP and in 2014 for DINCH. Furthermore, there was a steady increasing trend through the study period from 2012 to 2017. The risk assessments for the Japanese children in this study are well below the thresholds set by TDI and HBM-I indicate no critical health concern for now. It's crucial to notice that the HBM-I and TDI reference values are established using the upper bound of the quantile for a precautionary approach.[45] Moreover, due to children's still developing body, higher exposure to chemicals and differences in absorption, it's important to consider the specific vulnerability of children as they may have higher health effect from chemicals compared to adults.[68] Consequently, to ensure the safety of susceptible sub-population like children, the reference values could be set higher than necessary.[48] This could explain that even the maximum RQ in this study is still below 0.03.

Nevertheless, it is crucial to closely monitor the observed increasing trend to ensure prompt intervention if needed.

5. Strengths and limitations

A clear strength of the study is the state-of-the-art exposure assessment to the alternative plasticizers DEHTP, DINCH, and DEHA via well-established urinary exposure biomarkers with DINCH metabolites obtaining external validation. Robust risk assessments were performed using health-based guidance values such as the German HBM-I values and EFSA's TDIs which are widely accepted standardized risk assessment approaches and allowed to estimate the body burden and potential health risks of these compounds. Thus, we were able to provide the first exposure trend data on DEHTP and DINCH while DEHA was not detected in Japanese children. These data can serve as the baseline for future biomonitoring studies on exposure changes of DEHTP, DINCH, and DEHA in the Japanese population. All participants in this study were 7-year-old children, which allowed for an objective evaluation comparing exposure levels while eliminating age as a confounding variable. Some of the limitations of this study should be mentioned. However, as our participants are only 7-year-old children, it may limit the generalizability of the presented data to represent other age groups in Japan. Another limitation is that DEHTP, DINCH, and DEHA are chemicals with a short half-life, and using single urine samples could result in an under/overestimation of the variations. However, biomonitoring using morning void urine enabled a better comparison and improved the reliability of the children's DEHTP and DINCH exposure profiles. Moreover, there is evidence about better intraclass correlation

coefficients for morning void urine compared to spot urine, especially for DEHTP metabolites.[69] The urinary measurement of metabolites for exposure and risk assessment is a widely accepted tool both for adults and children. It has been used in various large-scale human biomonitoring studies both in children and adults (e.g. the Canadian CHMS, the US NHANES, the German GerES, the European HBM4EU, the Korean KoNHES etc). As long as no metabolism data is available for children, conversion factors derived from adult humans are deemed the best alternative, certainly better than metabolic data from animals. To account for known differences in urinary excretion of children with effect on dose back calculation, we applied corrections from[47]. However, this is still one of our limitations as age-dependent, regional, and genetic variations affecting urinary excretion might exist. The findings of our study indicate increasing DEHTP and DINCH exposure, which are alternatives of regulated phthalates such as DEHP. This highlights the need for ongoing biomonitoring studies to closely monitor the increasing trends, investigate any potential health impacts, and enable prompt response if necessary.

Supporting Information

- Concentration of DEHA metabolites (Table S1); relationship of DEHTP and DINCH metabolites with demographic and building characteristics of participants (Table S2); correlation coefficient among DEHTP and DINCH metabolites (Figure S1)

Author Information

Corresponding Author

Atsuko Ikeda - Faculty of Health Sciences and Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan; orcid.org/0000-0002-3065-262X; Email: AAraki@cehs.hokudai.ac.jp

Authors

Rahel Mesfin Ketema - Faculty of Health Sciences and Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan; orcid.org/0000-0003-2059-9498

Monika Kasper-Sonnenberg - Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany; orcid.org/0000-0002-9435-1391

Yu Ait Bamai - Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan and Toxicological Center, University of Antwerp, Belgium; orcid.org/0000-0003-2510-4757

Chihiro Miyashita - Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan; orcid.org/0000-0003-0996-3631

Holger M. Koch - Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany; orcid.org/0000-0002-8328-2837

Claudia Pälmke - Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

Reiko Kishi - Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan; orcid.org/0000-0001-8173-9269

Declaration of competing interest

The authors declare no conflict of interest to report.

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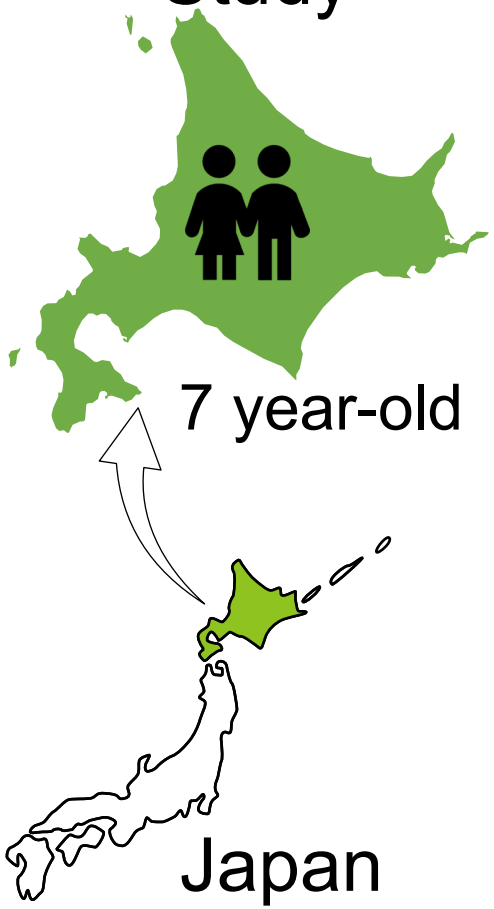
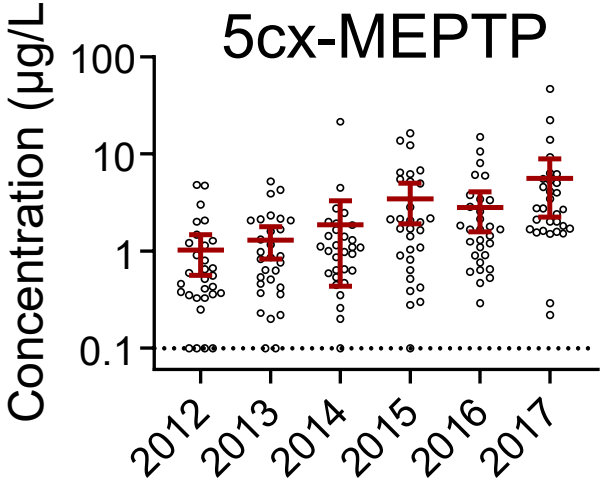
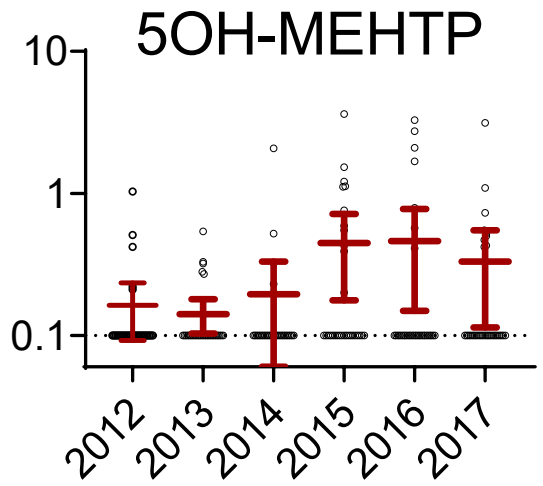
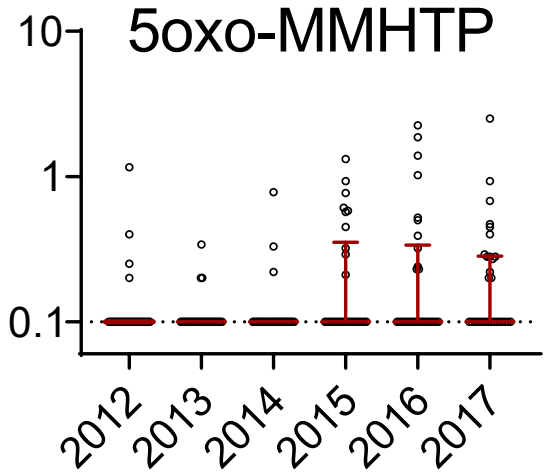
Hokkaido Study



DEHTP metabolites







DINCH metabolites