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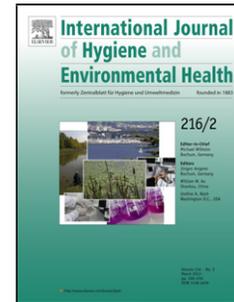
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Quantitative analysis of organophosphate insecticide metabolites in urine

extracted from disposable diapers of toddlers in Japan

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ABSTRACT

Background and aim: Epidemiological studies linking insecticide exposure to childhood neurodevelopment have been gaining global attention. Despite the rapid development of the central nervous system in early childhood, studies regarding the biological monitoring of insecticide exposure in diapered children are limited. In this study, we aimed to clarify the concentrations of organophosphate (OP) insecticide metabolites in toddler urine extracted from disposable diapers in Japan.

Methods: We recruited diapered children from the Aichi regional subcohort participants of the Japan Environment and Children's Study (JECS) at the time of their 18-month checkup. A total of 116 children wore designated disposable diapers overnight, which were then sent as refrigerated cargo. The urine was extracted from the diapers using acetone and analyzed by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) to determine the concentrations of six dialkyl phosphates (DAPs) (i.e., dimethyl phosphate [DMP], dimethyl thiophosphate [DMTP], dimethyl dithiophosphate [DMDTP], diethyl phosphate [DEP], diethyl thiophosphate [DETP], and diethyl dithiophosphate [DEDTP]). DAP absorption into the diapers was quantified to calculate the urinary DAP concentrations.

Results: The DAP recovery using the developed method yielded between 54.2%

(DEDTP) and 101.4% (DEP). Within-run precision expressed as the relative standard deviation was between 2.4% and 14.7%, and the between-run precision was between 3.1% and 8.5%. A Bland-Altman analysis confirmed the agreement between the results obtained by the developed method and by the measurements for the corresponding urine without diaper absorption. The geometric means (GM) of urinary DMP, DMTP, DMDTP, DEP, DETP, and total DAPs (ΣDAP) were 3.6, 3.9, 0.9, 6.0, 0.6 µg/L, and 137.6 nmol/L, respectively. The GM of DEDTP was not calculated due to its low detection rate.

Conclusions: We successfully established a method to measure the DAP concentrations in urine extracted from diapers and this is the first report of these pesticide concentrations in diapered children in Japan.

Keywords: organophosphate insecticide; disposable diaper; child; liquid chromatography-tandem mass spectrometry; urine; dialkyl phosphates

Introduction

Recently, epidemiological studies linking insecticide exposure to neurodevelopment in children have attracted increased global interest. Insecticides are environmental chemicals widely used in agricultural and public health settings, as well as individual households. Organophosphate (OP) insecticides are the most commonly used insecticides for the protection of agricultural crops and dwelling environment. The amount of OP insecticides used in the United States was estimated at 15 million kg, which accounted for 36% of the total insecticide use in 2007 (U.S. Environmental Protection Agency, 2011).

Inhibition of acetylcholinesterase in the nervous system by OP insecticides is the major cause of OP-related toxic effects. However, some studies have suggested an association between low-level chronic OP exposure (which does not cause detectable acetylcholinesterase inhibition) and potential neurotoxicological outcomes, such as poor mental development (Eskenazi et al., 2007; Koureas et al., 2012; Rauh et al., 2006), attention-deficit/hyperactivity disorder (ADHD) (Bouchard et al., 2010; Marks et al., 2010), and low intelligence quotient (IQ) scores (Bouchard et al., 2011; Engel et al., 2011; Rauh et al., 2011). Since the developing brain is more susceptible to neurotoxicants, and the pesticide exposure dose per body weight is likely higher in

4

children (Weiss, 2000), exposure measurements during the infant and toddler period is indispensable for epidemiological studies investigating the relationship between OP exposure and pediatric neurodevelopment.

Biological monitoring (or biomonitoring) is a primary component of pesticide exposure assessment, and urine is frequently used as a relevant biomonitoring sample due to the feasibility of its collection compared to that of other biological materials. However, epidemiological studies on the biological monitoring of OP exposure during the infant and toddler period are limited due to the difficulty in collecting urine from a large number of diapered children. Collection bags devised primarily for clinical purposes make it possible to collect intact urine from non-toilet-trained children; however, they are not ideal research tools due to inconveniences, such as possible urine leakage and skin irritation caused by the adhesive used to attach the collection bag to the child. Therefore, a special arrangement is needed.

One potential approach to overcome these drawbacks is the extraction of urine from diapers for the measurement of urinary OP metabolites, which requires minimal parental effort for urine collection compared to the urine bags. In our previous report, metabolites of pyrethroid insecticides were measured in the urine extracted from disposable diapers (Saito et al., 2014). However, no studies have yet determined OP

metabolites using this approach.

Therefore, the aims of this study were to: 1) establish a method for the measurement of six dialkyl phosphates (DAPs) as common OP metabolites, including dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), dimethyl dithiophosphate (DMDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), and diethyl dithiophosphate (DEDTP), in the urine extracted from used disposable diapers; and 2) examine OP exposure levels in diapered children in Japan.

Materials and Methods

Ethics

This study was conducted as an adjunct study of the Japan Environment and Children's Study (JECS; a population-based birth cohort study) and was outlined in the published JECS protocol (Kawamoto et al., 2014). The ethics committee of the Nagoya City University Graduate School of Medical Sciences approved the study protocol. The JECS study protocol was also approved by the Ministry of the Environment, Japan.

Reagents

DMP tetramethylammonium salt (purity 99.9%), DMTP ammonium salt (purity 98.0%),

DEP (purity 98.2%), DETP ammonium salt (purity 99.6%), DMP sodium salt-deuteride

(d) 6 (purity 98.7%), DMTP potassium salt-d6 (purity 99.0%), DEP ammonium salt-d10

(purity 99.2%), and DETP potassium salt-d10 (purity 98.2%) were purchased from

Hayashi Pure Chemical Industries (Osaka, Japan). DMDTP sodium salt and DEDTP

ammonium salt (purity 95.0%) were obtained from Cerilliant (Round Rock, TX, USA).

DMDTP ammonium salt-d6 (purity 98.8%) and DEDTP ammonium salt-d10 (purity

95.5%) were acquired from Toronto Research Chemicals (Toronto, Canada).

Creatinine (purity 98.0%) and creatinine-d3 (purity 99.9%) were obtained from Sigma

Aldrich (St. Louis, MO, USA) and C/D/N isotopes (Pointe-Claire, Canada),

respectively.

Study population

Children participating in the Aichi regional subcohort of JECS, which comprised 43%

of the children in that age group in the study area, were recruited at the time of their

18-month checkup provided by the local government. Their guardians as legally

acceptable representatives were asked to take part in the study, and informed consent for

this study was obtained. The overall participation rate was 86.3%. Participants wore

designated disposable diapers during the night, which were distributed in advance and

7

collected the next day after their use as refrigerated cargoes. Used diapers from 116

children (18–21 months of age; 59 males and 57 females) were collected between June

22 and July 31, 2015. Characteristics of the study participants (i.e., sex, age, maternal

age at delivery, annual household income, height, and weight) are presented in Table 1.

The study region was an urban location, and as a general population, the subjects were

likely to be exposed to OP primarily via food.

Urinary OP metabolite and creatinine analyses

Urine was extracted according to our previously reported method (Saito et al., 2014)

with minor modifications. In brief, the urine absorber was removed from the diaper, put

into a 10-mL syringe, and weighed to determine the wet weight. This syringe and a 20

mL syringe containing acetone were connected head-to-head, and the acetone was

manually reciprocated between the syringes five times to extract the urine from the

absorber. The eluate was then poured into a test tube, and the remaining absorber in the

syringe was dried in a vacuum for approximately 2 h and weighed to determine the dry

weight. The volume of urine in the absorber was caluculated as the difference between

the wet and dry weight of the absorber. The eluate volume was then adjusted to the

determined urine volume by evaporation at 40°C on a heat block with a gentle nitrogen

8

stream to reduce the volume to less than the determined urine volume. The sample was then diluted to the urine volume with distilled water. The urine samples were stored at −80°C until analysis.

The concentration of DAPs in the urine samples were measured according to the established method using high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Ueyama et al., 2014) with slight modifications as described below. During the procedure, DMTP, DMDTP, DETP, and DEDTP were eluted by 2 mL of 2.5% NH_3/water including 50% acetonitrile at 35°C, which yielded better recovery for solid-phase extraction (SPE). Oasis WAX SPE column 60 mg (Waters Corp., MA, USA) was used as an SPE cartridge. Acquity H-Class ultra-high performance liquid chromatographic system and Xevo TQ-S tandem mass spectrometry (UPLC-MS/MS, Waters Corp., MA, USA) were used to perform measurements. The chromatographic separation was achieved using a Scherzo SM-C18 (Imtakt, Kyoto, Japan), 100×2 mm i.d, 3 μm silica. Mobile phase A and B consisted of 1 mmol/L formic acid and acetonitrile (20:80, v/v) and 10 mmol/L formic acid solution containing 10 mmol/L ammonium formate and acetonitrile mixture (20:80, v/v), respectively. Furthermore, a gradient profile of the mobile phase composition was optimized for UPLC. Table 2 lists the optimized multiple reaction monitoring (MRM) parameters and retention times for

9

OP metabolites and their internal standards.

Urinary creatinine concentrations were measured by UPLC-MS/MS as described elsewhere (Fraselle et al., 2015).

Method validation for quantitative analyses

DAP measurement was validated in our previous report (Ueyama et al., 2014). In the present study, the within- and between-run precisions of our developed method were evaluated. To determine the absolute recoveries, we spiked DAPs at two different stages in the DAP measurement procedure (i.e., at the beginning of the extraction procedure [urine sample] and before the injection procedure into UPLC-MS/MS).

To assess the level of DAP absorption into the urine absorbent of the diaper, cross-validation tests were performed using the concentrations measured from urine samples spiked with calibration standards (direct method) and those from the same urine samples which were poured on and subsequently extracted from the diaper absorbent (diaper method). Urine samples used as calibration standard samples were prepared by mixing urine collected from Japanese adults ($n = 566$) who were considered to be exposed to OPs at normal environmental levels. Ten levels of calibration concentrations and concentration intervals were determined based on DAP

concentration ranges found in three-year-old Japanese children (Osaka et al., 2016). The

maximum concentrations for the calibration standard urine were set to 866.9 µg/L for

DMP, 296.0 µg/L for DMTP, 46.9 µg/L for DMDTP, 261.0 µg/L for DEP, 18.7 µg/L for

DETP, and 43.3 µg/L for DEDTP. The spiked urine samples were assigned

concentration values to ensure the comparability of the results between the present and

our past studies. Relationships of the measured concentrations between the direct and

diaper methods were analyzed as described in the statistical analysis methods below. To

check the potential effects of matrix density, the same cross-validation tests were

performed using a different calibration standard set with eight selected concentration

points for each DAP (7.7- 346.8 µg/L for DMP, 3.6 – 118.4 µg/L for DMTP, 0.2 – 18.8

µg/L for DMDTP, 1.3 – 104.4 µg/L for DEP, 0.5 – 7.5 µg/L for DETP, and 0.04 – 17.3

µg/L for DEDTP), which were prepared by diluting the spiked urine standards 2.5 times

with distilled water.

Statistical analysis

  Urinary DAP concentrations with a log-normal distribution were presented as

geometric means (GMs) and percentiles. A Bland-Altman analysis was performed to

assess the agreement between the concentrations obtained by the diaper method

with/without adjustment for the diaper absorption of DAPs and those by the direct

method. Regression analyses of the measured concentrations were performed between

the direct and diaper methods to obtain regression equations to calculate DAP

concentrations in the intact urine before absorption into the diapers. Each urinary

concentration was log-transformed and then substituted into the regression equation,

and the obtained value was converted into an antilogarithm. To estimate the overall

exposure to OPs, we calculated the total concentration of six DAPs (ΣDAP; sum of

DMP, DMTP, DMDTP, DEP, DETP, and DEDTP concentrations) after converting units

(μg/L or μg/g creatinine) to molar concentrations (nmol/L or nmol/g creatinine). Using

the same method, we also calculated the total concentration of dimethyl DAPs

(ΣDMAP; the sum of DMP, DMTP, and DMDTP) (Berman et al., 2013) and diethyl

DAPs (ΣDEAP; the sum of DEP, DETP, and DEDTP) (Oulhote and Bouchard, 2013).

 The limit of detection (LOD) and the limit of quantitation (LOQ) were determined as

concentrations with signal-to-noise ratios of 3 and 10, respectively. For concentrations

below the LODs, a value equal to the LOD divided by the square root of 2 was used

(Hornung and Reed, 1990).

Results

Method validation for quantitative analyses

The DAP recoveries using the diaper method yielded 91.4% ± 9.8 (SD) for DMP

(spiked at 21.1µg/L), 72.6% ± 7.0 for DMTP (9.2 µg/L), 92.8% ± 3.8 for DMDTP (0.7

µg/L), 101.4% ± 5.9 for DEP (3.8 µg/L), 96.7% ± 13.0 for DETP (1.4 µg/L), and 54.2%

± 4.0 for DEDTP (0.6 µg/L), respectively. For the within-run precision, the percent of

relative standard deviation (%RSD) ranged from 2.4% (12.5 µg/L of DMTP) to 14.7%

(8.0 µg/L of DEP). For the between-run precision, the %RSD was between 3.1% (21.8

µg/L of DMDTP) and 8.5% (93.8 µg/L of DEP). The LODs and LOQs achieved in the

present study are presented in Table 3.

The OP metabolite levels in calibration standard urine samples were assayed via both

the diaper and direct methods. Fig. 1A shows the Bland-Altman plots for both the diaper

and direct methods. The plots presenting non-diluted (cross mark) and diluted urine

(circles) samples demonstrated that the average of the differences were all negative

except for DMDTP. Proportional bias was observed for measurements of DEP. In

addition, it was suggested that DAP absorption into the diaper was not affected by the

matrix density since the biases of non-diluted and diluted urine were not significantly

different (detailed data not shown). Based on these findings, DAP concentrations in the

extracted urine were corrected using the regression formulae obtained by linear

regression analyses for the data regardless of urine dilution conditions: $y = 0.9823x + 0.1052$ for DMP; $y = 1.0026x + 0.0431$ for DMTP; $y = 1.0245x - 0.01$ for DMDTP; $y = 0.7899x + 0.5085$ for DEP; $y = 0.9725x + 0.0383$ for DETP; and $y = 0.9094x + 0.1345$

for DEDTP. An R^2 above 0.98 was associated with all analyses except for DEP (0.93)

(Fig. 2). A Bland-Altman plot was reapplied to confirm the agreement between the

results obtained by the diaper method after the correction and those by the direct method

(Fig. 1B). Any systematic difference disappeared: 95% confidence interval of the

agreement ranged between: −0.04 to 0.04 for DMP; −0.02 to 0.02 for DMTP; −0.06 to

0.06 for DMDTP; −0.09 to 0.09 for DEP; −0.03 to 0.03 for DETP; and −0.06 to 0.06 for

DEDTP. Thus, the urinary concentrations of all DAPs in the studied children were

expressed as adjusted values using these regression equations.

OP metabolite concentrations in the urine of toddlers extracted from used diapers

The distribution of urinary DAP and the creatinine-corrected DAP concentrations in

diapered children are summarized in Tables 3 and 4, respectively. GMs (ranges) and

detection rates of urinary DAPs were: 3.6 µg/L (LOD–100.8) and 94.0% for DMP; 3.9

µg/L (0.1–130.3) and 100% for DMTP; 0.9 µg/L (LOD–6.2) and 99.1% for DMDTP;

6.0 µg/L (LOD–83.6) and 84.5% for DEP; and 0.6 µg/L (0.1–55.9) and 100% for DETP,

respectively. The GM of DEDTP was not calculated due to the low detection rate

(49.1%). The maximum between-child difference in ΣDAP was approximately 62-fold

(min: 19.7 nmol/L; max: 1217.6 nmol/L).

Discussion

To our knowledge, this is the first study to examine the OP exposure levels in diapered

children in Japan using the urine extracted from used disposable diapers. Few studies

have reported environmental chemical exposure levels determined using this approach

and were limited to pyrethroid insecticides, phthalate plasticizers, and heavy metals (Hu

et al., 2004; Liu et al., 2012; Saito et al., 2014; Sathyanarayana et al., 2008; Soden et al.,

2007). Urine collection using disposable diapers is a less demanding procedure for

children's guardians and enabled us to develop an analytical method to assess OP

exposure. The method used in the present study was characterized by using 10 mL and

20 mL syringes with a head-to-head connection, and reciprocating acetone between the

syringes. This process enabled an easy extraction of large urine volumes (approximately

5 mL) at once. Moreover, with the current method, absorption of the metabolites into

the diaper absorbent was considered by using regression equations between the diaper

15

and direct methods. A Bland-Altman analysis confirmed that the means of the differences between the corrected values and those obtained by the direct method were nearly 0 in all DAPs. This is the strength of the present study, which enables us to compare the urinary DAP levels in the present study with those measured using the direct method in different age groups from other studies. Furthermore, collecting nocturnal urine from diapers also has a second advantage. In another study analyzing the urinary OP metabolites from non-toilet-trained children, DAPs were measured using spot urine samples (Eskenazi et al., 2003) in which DAP concentrations could be affected by the time elapsed from the last meal to the time of urine sampling, as well as by variations in the foods consumed during the day. Kissel *et al*. (2005) reported that the first-morning void samples displayed less variance from the creatinine-adjusted and weighted average metabolite concentrations than those collected at three other times during the day (i.e., after lunch, before dinner, and before bed). In the present study, the entire quantity of the urine ejected throughout the night was collected; thus, the urine samples analyzed in this study were similar to first morning samples in toilet-trained children. Moreover, the urinary OP metabolite levels were less likely to be affected by intraday variation. Thus, the method of OP metabolite measurement in the urine

extracted from disposable diapers described in this study is a useful approach that can also be applied to longitudinal studies.

  Several epidemiological studies have suggested associations between chronic OP exposure and neurodevelopmental outcomes (Gonzalez-Alzaga et al., 2014; Hernandez et al., 2016; Jurewicz et al., 2013). Children between 8 and 15 years of age with DMTP levels higher than the median of detectable concentrations had twice the risk of ADHD, compared to children with undetectable levels (Bouchard et al., 2010, n=1139). Additionally, significant associations have been found between the increased prenatal and postnatal (6–24 months) DAP levels and increased pervasive developmental disorders at 24 months (Eskenazi et al., 2007, n=396). In contrast, several studies have reported that no associations were found between the postnatal exposure to OP pesticides and neurodevelopmental outcomes in children (Guodong et al., 2012, n=301; Lizardi et al., 2008, n=48). Thus, further studies to assess exposure during critical windows of neurodevelopment after birth are warranted; yet, few studies have been conducted in children at this age. We previously reported urinary DMP, DMTP, DEP, and DETP levels in three-year-old children living in the same prefecture, but not the same area as the children included in the present study (Osaka et al., 2016). All percentile concentrations were found to be higher for DMP in three-year-old children

(2.43, 9.78, 14.32, 22.21, and 59.38 µg/L for 5, 25, 50, 75, and 95 percentile values,

respectively), and were likely higher for DMTP (0.43, 1.96, 5.45, 14.05, and 65.51 µg/L

for 5, 25, 50, 75, and 95 percentile values, respectively) (Osaka et al., 2016). In contrast,

the ranges for DEP and DETP were similar between the two age groups (data not

shown). Since the sampling season can affect urinary concentrations (Osaka et al.,

2016) and differed between the studies, possible differences in the urinary

concentrations between the two age groups should be thoroughly analyzed in future

studies.

 The inter-individual variance between children (i.e., %RSD, in ΣDAP) was

approximately 97% in the present study. Diet is a potential source of OP exposure

among children. A study in urban preschool-aged children found that 18 children who

consumed primarily organic fruits, vegetables, and juice exhibited lower concentrations

of urinary DETP and DMTP compared to 21 children who consumed conventionally

grown produce (Curl et al., 2003). The differences remained even after excluding

families that reported some use of OP in the household or garden, suggesting that the

variability between children determined in the present study was primarily due to the

consumption of different foods for dinner and/or lunch. This aspect, as well as the

18

socioeconomic status could be possible contributing factors for the differences in

urinary DAP concentrations, and will be explored in future studies.

  The present study had several limitations. First, the analytical method for DEP

measurement requires improvement, as the DEP concentrations were lower than the

LOD value of 1.8 µg/L in 15% of the children; a unique value of 1.3 µg/L was assigned

as the DEP concentration in these children and led to a difficult comparison of low-level

concentrations between the studies. However, the R^2 value of 0.93 in the regression

equation was sufficiently high to assess the original urinary concentrations. Second, the

recovery of DAPs through the SPE procedure tended to be low at around the maximum

concentrations of the calibration curves, which might have resulted from the absorption

of the metabolites into the SPE columns. Therefore, whether similar results can be

obtained from the same samples after dilution should be ascertained. However, this is

not likely a critical issue as respective deuterated internal standards were used for all

metabolites.

Conclusions

In this study, we successfully established a biological monitoring method for six DAPs in the urine extracted from disposable diapers. The determination of DAP concentrations in intact urine before the absorption into the diapers was possible since R^2 of the regression equations between the direct and diaper methods were satisfactorily high. The distribution of urinary DAP concentrations in diapered children was addressed for the first time in Japan.

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21

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Figure Legends

Fig. 1. A Bland-Altman plot of dialkyl phosphate (DAP) concentrations obtained by the diaper and the direct methods. Six DAPs (DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate; DEP, diethyl phosphate; DETP, diethyl thiophosphate; and DEDTP, diethyl dithiophosphate) were measured for the non-diluted calibration standard urine in a series of 10 concentrations (cross marks) and urine diluted 2.5 times with distilled water in a series of eight selected concentrations (circles). Horizontal and vertical axes represent the average of log-transformed concentrations and the difference between the log-transformed two paired measurements, respectively. For DMDTP, DEP, and DEDTP, concentrations below the limit of quantitation were excluded (one, six, and five spots, respectively). The solid and dotted lines indicate the mean of the bias and bias $\pm$ 1.96 SD, respectively. (A) The agreement between the measured concentrations by the diaper method without adjustment for diaper absorption and by the direct method. (B) The agreement between the concentrations corrected by the regression equations obtained from Fig. 2 and the concentrations of the corresponding urine without diaper absorption.

Fig. 2. DAP regressions in the urine between the diaper method and the direct method.

26

Linear regression equations were obtained from all plotted data regardless of the urine

dilution conditions (solid lines). The dotted line represents a regression of non-diluted

calibration standard urine and the dashed line represents that of urine samples diluted

2.5 times with distilled water. See notes for Fig. 1 regarding the details of the plotted

data.

Fig.1

A

B
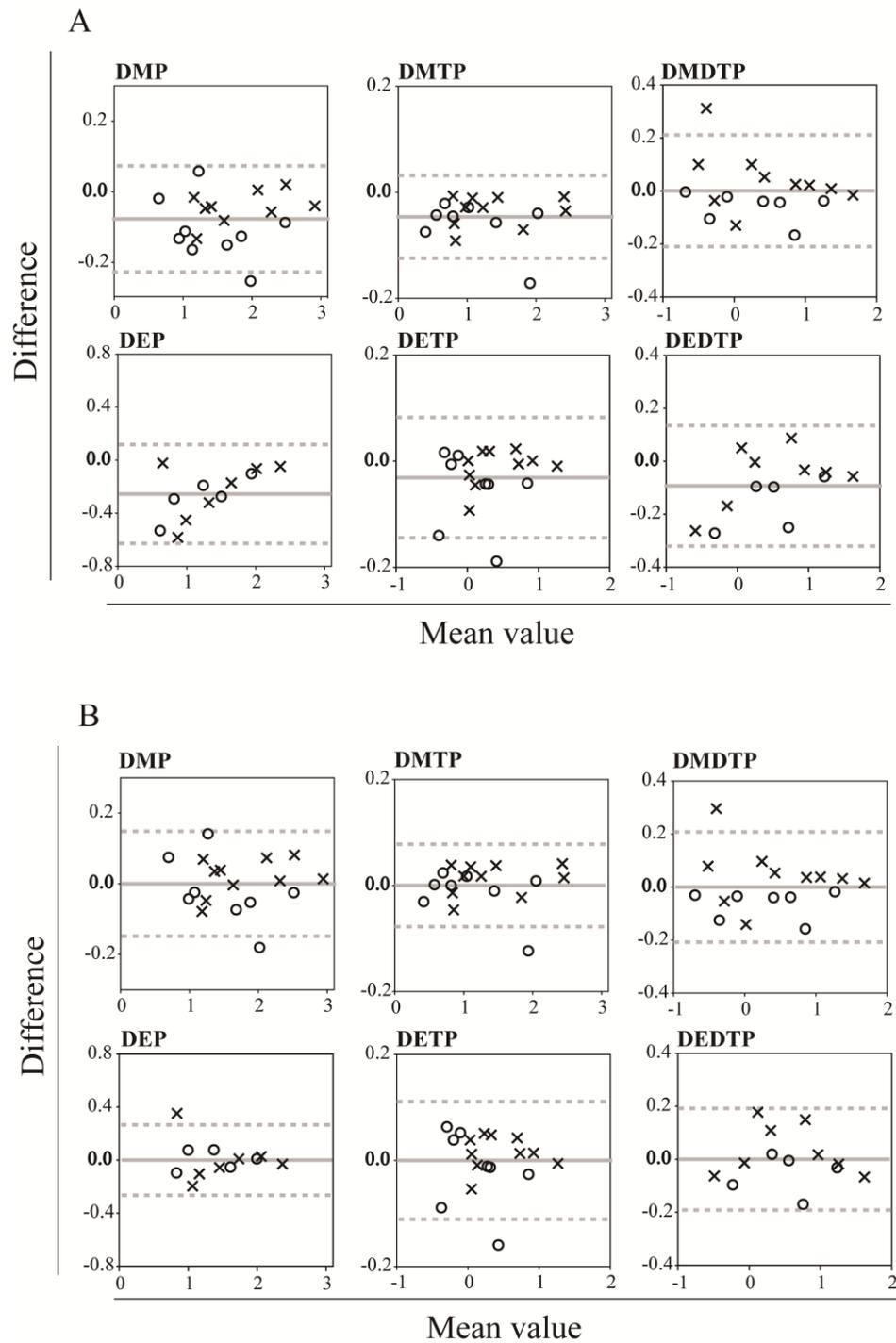
Fig.2
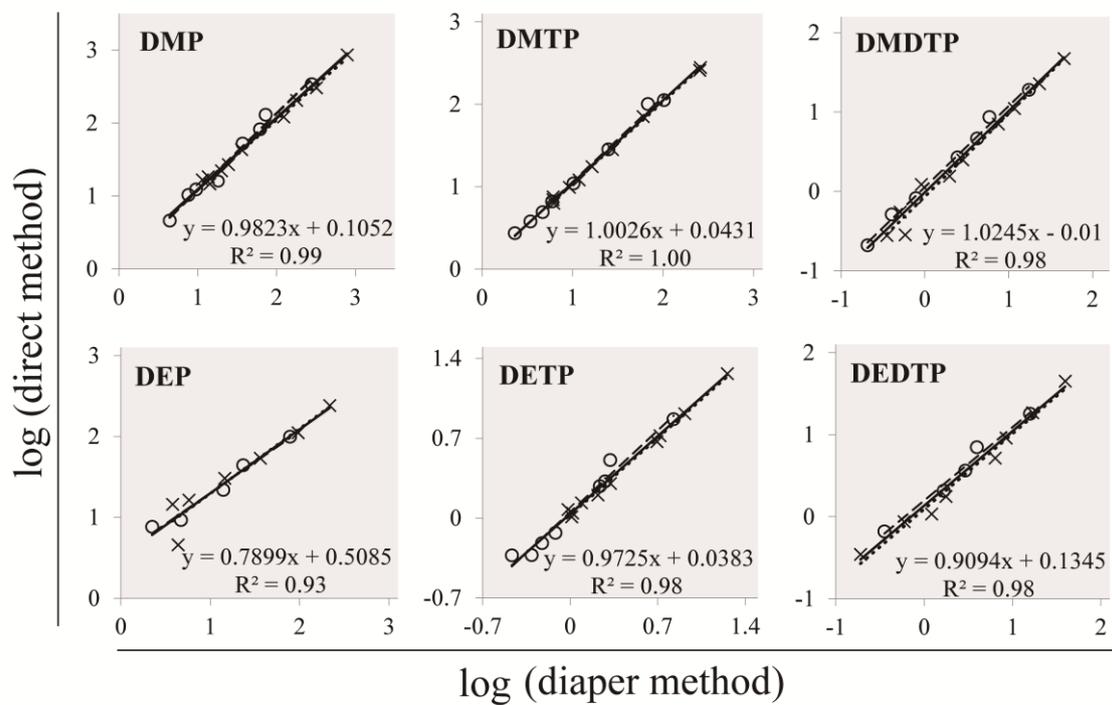
Table 1. Characteristics of the study population.

	Total (N = 116)		Male (N = 59)		Female (N = 57)	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age (months)	18.7	0.7	18.7	0.7	18.8	0.7
Maternal age at delivery (years)	32.4	4.5	32.0	4.6	32.8	4.3
Height# (cm)	78.6	3.2	79.4	3.4	77.8	2.8
Weight# (kg)	10.3	1.1	10.6	1.3	10.0	0.9
	N	*%*	*N*	*%*	*N*	*%*
Household income (million Japanese Yen)						
4 to <6†	38	32.8	20	33.9	18	31.6

#Data measured between 16- and 18-months-old toddlers (N = 91 for height and 95 for weight).

†The mode of the household income ranges in the studied population.

Table 2. Compound-specific mass spectrometer settings.

Compounds	Fragmentor (V)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Polarity	Retention time (min)
DMP	16	125	63 (Q)	20	Negative	7.50
			79 (C)	18		
DMP-d6	58	131	63	16	Negative	7.50
DMTP	28	141	126 (Q)	14	Negative	7.24
			96 (C)	20		
DMTP-d6	2	147	97	20	Negative	7.24
DMDTP	2	157	142 (Q)	14	Negative	6.80
			112 (C)	20		
DMDTP-d6	2	163	113	22	Negative	7.79
DEP	2	153	125 (Q)	12	Negative	8.00
			79 (C)	18		
DEP-d10	4	163	131	12	Negative	8.00
DETP	2	169	95 (Q)	16	Negative	7.72
			141 (C)	12		
DETP-d10	2	179	147	14	Negative	7.69
DEDTP	2	185	157 (Q)	14	Negative	7.09
			111 (C)	20		
DEDTP-d10	2	195	111	24	Negative	7.07

DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate;

DEP, diethyl phosphate; DETP, diethyl thiophosphate; DEDTP, diethyl dithiophosphate; Q,

quantification ion; C, confirmation ion.

Table 3. Limit of detection, limit of quantitation, detection rates, geometric means, and percentile

values of urinary dialkyl phosphate concentrations (μg/L or nmol/L) among 1.5-year-old diapered

children in Japan.

Compounds	LOD	>LOD (%)	LOQ	GM		Selected percentile						Max.
						5th	25th	50th	75th	95th		
DMP (μg/L)	0.47	94.0	1.53	3.6		<LOD	1.5	3.4	11.1	29.8		100.8
DMTP (μg/L)	0.03	100.0	0.09	3.9		0.3	1.3	4.0	8.0	38.1		130.3
DMDTP (μg/L)	0.04	99.1	0.14	0.9		0.2	0.5	1.0	1.4	3.7		6.2
ΣDMAP (nmol/L)	-	100.0	-	74.6		12.4	26.7	76.7	188.0	530.5		1150.7
DEP (μg/L)	1.77	84.5	4.58	6.0		<LOD	2.7	6.7	12.0	24.7		83.6
DETP (μg/L)	0.02	100.0	0.08	0.6		0.1	0.2	0.6	1.3	6.2		55.9
DEDTP (μg/L)	0.07	49.1	0.21	NC[#]		<LOD	<LOD	<LOD	0.3	0.8		1.4
ΣDEAP (nmol/L)	-	100.0	-	46.2		9.8	22.1	49.6	92.1	184.0		585.4
ΣDAP (nmol/L)	-	100.0	-	137.6		27.4	61.2	150.4	288.4	680.4		1217.6

DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate;

DEP, diethyl phosphate; DETP, diethyl thiophosphate; DEDTP, diethyl dithiophosphate; LOD,

limit of detection; LOQ, limit of quantitation; GM, geometric means; NC, not calculated.

#GM was not calculated due to low detection rates (less than 60% of the samples).

Table 4. Creatinine-corrected concentrations (μg/g creatinine or nmol/g creatinine) of geometric

means and percentile values of urinary dialkyl phosphates among 1.5-year-old diapered children

in Japan.

Compounds	GM		Selected percentile						Max.
			5th	25th	50th	75th	95th		
DMP (μg/g creatinine)	7.6		<LOD	3.6	7.8	16.1	39.6		147.0
DMTP (μg/g creatinine)	8.3		0.9	3.3	8.3	24.0	70.7		183.6
DMDTP (μg/g creatinine)	1.9		0.7	1.3	1.9	2.6	6.1		31.6
ΣDMAP (nmol/g creatinine)	159.6		28.3	78.6	159.6	332.8	805.2		1762.5
DEP (μg/g creatinine)	12.9		<LOD	7.6	13.5	22.1	45.2		124.5
DETP (μg/g creatinine)	1.3		0.3	0.5	1.2	3.0	8.5		117.7
DEDTP (μg/g creatinine)	NC[#]		<LOD	<LOD	< LOD	0.5	1.5		2.0
ΣDEAP (nmol/g creatinine)	98.8		27.5	60.1	104.1	167.0	348.1		871.1
ΣDAP (nmol/g creatinine)	294.3		81.9	155.0	294.3	478.6	1035.2		1975.3

DMP, dimethyl phosphate; DMTP, dimethyl thiophosphate; DMDTP, dimethyl dithiophosphate;

DEP, diethyl phosphate; DETP, diethyl thiophosphate; DEDTP, diethyl dithiophosphate; LOD,

limit of detection; GM, geometric means; NC, not calculated.

#GM was not calculated due to low detection rates (less than 60% of the samples).