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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.

1. 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 children (Weiss, 2000), exposure measurements during the infant and toddler

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Table 1
Characteristics of the study population.

	Total (N = 116)		Male (N = 59)		Female (N = 57)	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*
Age (months)	18.8	0.7	18.8	0.7	18.8	0.8
Maternal age at delivery (years)	32.4	4.5	32.0	4.6	32.8	4.3
Height[a] (cm)	78.6	3.2	79.4	3.4	77.8	2.8
Weight[a] (kg)	10.3	1.1	10.6	1.3	10.0	0.9
	N	*%*	*N*	*%*	*N*	*%*
Household income (million Japanese Yen)						
4 to <6[b]	38	32.8	20	33.9	18	31.6

[a] Data measured between 16- and 18-months-old toddlers (N = 91 for height and 95 for weight).
[b] 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) 79 (C)	20 18	Negative	7.50
DMP-d6	58	131	63	16	Negative	7.50
DMTP	28	141	126 (Q) 96 (C)	14 20	Negative	7.24
DMTP-d6	2	147	97	20	Negative	7.24
DMDTP	2	157	142 (Q) 112 (C)	14 20	Negative	6.80
DMDTP-d6	2	163	113	22	Negative	7.79
DEP	2	153	125 (Q) 79 (C)	12 18	Negative	8.00
DEP-d10	4	163	131	12	Negative	8.00
DETP	2	169	95 (Q) 141 (C)	16 12	Negative	7.72
DETP-d10	2	179	147	14	Negative	7.69
DEDTP	2	185	157 (Q) 111 (C)	14 20	Negative	7.09
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	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[a]	<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.
[a] GM was not calculated due to low detection rates (less than 60% of the samples).

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,