Supporting Information

Variability and Predictors of Urinary Concentrations of Phthalate Metabolites during Early Childhood

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Text S1. Methods for determination of phthalate metabolites in urine

We measured 11 phthalate metabolites in urine (µg/L) using online solid phase extraction coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry (SPE-HPLC-MS/MS).[1] Briefly, 100 µL of urine was spiked with the appropriate reagents and standards. This was incubated to hydrolyze the phthalate metabolites urinary conjugates, followed by online SPE-HPLC separation and detection by MS/MS. The limits of detection (LODs) varied for each metabolite, but were in the low (~0.1 to ~1) ng/mL range. The CDC laboratory is certified by the Health Care Financing Administration to comply with the requirements set forth in the 1988 Clinical Laboratory Improvement Act (CLIA) and all analytical measurements are conducted following strict quality control (QC) CLIA guidelines. Along with the study samples, each analytical run includes high- and low-concentration QC materials and reagent blanks to assure the accuracy and reliability of the data. The concentrations of the QCs, averaged to obtain one measurement of high-concentration QC and low-concentration QC for each run, are evaluated using standard statistical probability rules[2]. If these QC concentrations are outside established limits or blank values are above three times the LOD, the analytical run would be considered out of control and all of the samples in the run would be reanalyzed. In addition, at least once per year, the CDC laboratory analyzes reference urine samples fortified with several phthalate metabolites as part of the German External Quality Assessment Scheme (G-EQUAS). The program, evaluation, and certification are based on the guidelines of the German Federal Medical Council (http://www.g-equas.de/). The above procedures have been used since the early 2000s for the analyses of tens of thousands of biological specimens, including those collected as part of the ongoing US National Health and Nutrition Examination Survey (NHANES). The latest phthalate metabolites data and laboratory protocols used for 2011-2012 NHANES can be found at: http://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/PHTHTE_G_met.pdf.

Table S1. Median ΣDEHP metabolite concentrations in HOME Study children at 4 and 5 year visits with and without including MEHP in calculation

	With MEHP Median (25th, 75th percentile)	Without MEHP Median (25th, 75th percentile)
ΣDEHP (μmol/L)	0.22 (0.12, 0.39)	0.21 (0.12, 0.37)

Table S2. Number of study subjects and repeated urinary phthalate metabolite concentrations

Number of study visits where subjects provided a urine sample	Subjects with complete covariate information and ≥1 urine measure (N=296)	Number of samples from subjects (complete covariate information and ≥1 urine sample) by number of study visits
1	28	28
2	53	106
3	47	141
4	65	260
5	103	515
Total repeated urine samples		1050

Table S3. Phthalate metabolite and creatinine concentrations in HOME Study children's urine by age (median, [25th, 75th percentile]) for all subjects with at least 1 metabolite measure (n=327)

Phthalate Metabolite			Age				
	LOD	% Above LOD[a]	1 year (N = 281)	2 years (N = 235)	3 years (N = 237)	4 years (N = 172)	5 years (N = 203)
ΣDEHP[c] (μmol/L)	N/A	N/A	0.19 (0.10, 0.54)	0.25 (0.12, 0.54)	0.31 (0.15, 0.67)	0.27 (0.12, 0.55)	0.20 (0.12, 0.35)
MEHP (μg/L)	0.5	79.2	N/A	N/A	N/A	1.5 (0.6, 3.7)	1.6 (0.8, 3.3)
MEHHP (μg/L)	0.2	100	13.0 (6.4, 38.6)	20.0 (9.9, 45.6)	24.7 (11.4, 58.7)	20.9 (11.7, 41.5)	15.7 (8.5, 27.5)
MECPP (μg/L)	0.2	100	35.1 (17.9, 94.4)	43.4 (19.7, 93.3)	50.4 (26.4, 99.8)	41.7 (18.6, 79.1)	30.1 (19.3, 55.8)
MEOHP (μg/L)	0.2	100	10.2 (4.7, 27.8)	14.1 (5.8, 30.1)	16.0 (7.2, 36.8)	14.1 (7.4, 28.1)	10.6 (5.5, 18.9)
MBzP (μg/L)	0.3	99.7	10.5 (5.0, 24.2)	12.4 (5.0, 27.0)	14.9 (5.5, 38.2)	12.8 (5.8, 28.7)	9.7 (4.6, 24.0)
MCPP (μg/L)	0.2	99.6	4.2 (2.2, 8.8)	4.9 (2.1, 9.9)	6.2 (3.4, 11.0)	4.8 (2.7, 10.7)	4.2 (2.1, 8.9)
MCOP (μg/L)	0.2	100	10.0 (4.4, 21.4)	10.8 (5.3, 24.0)	13.3 (7.4, 23.3)	16.6 (8.0, 31.4)	23.8 (10.9, 47.3)
MCNP (μg/L)	0.2	100	4.5 (2.4, 9.6)	4.9 (2.2, 9.4)	4.3 (2.3, 7.3)	4.6 (2.3, 8.1)	3.8 (2.5, 6.0)
MEP (μg/L)	0.6	100	34.5 (15.6, 85.1)	30.1 (13.0, 87.9)	36.5 (15.1, 69.3)	28.3 (12.9, 70.0)	23.1 (13.0, 46.1)
MnBP (μg/L)	0.4	99.2	N/A	N/A	N/A	22.3 (11.1, 42.6)	16.1 (9.0, 31.8)
MiBP (μg/L)	0.2	99.7	N/A	N/A	N/A	11.0 (5.3, 22.3)	9.5 (5.6, 17.7)
Creatinine (mg/dL)	3.5	99.9	18.6 (11.7, 33.9)	33.2 (18.1, 60.2)	54.0 (34.8, 73.3)	61.7 (33.6, 85.4)	67.3 (45.7, 92.5)

LOD = limit of detection, N/A = not available

[c] ΣDEHP= Sum of MEHP, MEHHP, MEOHP, and MECPP

[d] MEHP, MnBP, and MiBP were measured only at visits 4 and 5.

Table S4. Unadjusted and adjusted difference in child urinary MEP concentrations at 5 years of age according to parent-reported child personal care product use in past 48 hours and (N=157)

		Unadjusted	Adjusted[a]
Product	N Using Product (%)	% Difference[b] MEP (95% CI)	% Difference[b] MEP (95% CI)
Hair spray or gel	22 (14.0)	96 (14, 238)	63 (7, 149)
Shampoo	116 (73.9)	-40 (-61, -8)	-0.2 (-33, 48)
Conditioner	60 (38.2)	5 (-29, 56)	9.9 (-22, 55)
Bar soap	81 (51.6)	138 (66, 242)	13 (-19, 59)
Liquid soap or body wash	100 (63.7)	-47 (-64, -22)	-18 (-40, 13)
Sun screen or lotion	104 (66.2)	44 (-4, 115)	-7 (-34, 29)
Hand sanitizer	100 (63.7)	17 (-22, 74)	6 (-22, 43)
Makeup	22 (14.0)	2 (-41, 79)	-20 (-49, 24)
Nail polish	10 (6.4)	8 (-51, 138)	-20 (-57, 50)

CI = confidence interval;

[a]Adjusting for sex, race of child, household income, maternal education, mean serum cotinine, urinary creatinine z-score, year of birth, and number of other personal care products used

[b] % Difference = percent difference in geometric means between those reporting product use and those not reporting product use (reference category)

Table S5. Adjusted percent difference in urinary phthalate metabolite concentrations 1-5 years of age among children born in 2005-2006 compared to children born in 2003-2004[a]

		No creatinine adjustment		Adjusted for creatinine z-score	
phthalate metabolites	Age	% Difference (95% CI)	Interaction p-value[b]	% Difference (95% CI)	Interaction p-value[b]
ΣDEHP[c]			0.02		0.24
	1	-0.9 (-21, 19)		-7.8 (-27, 12)	
	2	-11.1 (-31, 9)		-11.3 (-31, 8)	
	3	-20.3 (-40, -1)		-14.7 (-34, 5)	
	4	-28.5 (-48, -9)		-18.0 (-38, 2)	
	5	-36 (-56, -16)		-21.2 (-41, -2)	
MBzP			0.01		0.26
	1	-3.2 (-23, 16)		-11.0 (-31, 9)	
	2	-14.7 (-34, 5)		-14.9 (-35, 5)	
	3	-24.9 (-45, -5)		-18.6 (-38, 1)	
	4	-33.8 (-53, -14)		-22.1 (-42, -3)	
	5	-41.7 (-61, -22)		-25.5 (-45, -6)	
MCPP			0.08		0.96
	1	2.0 (-18, 22)		-5.2 (-25, 14)	
	2	-5.2 (-25, 14)		-5.4 (-25, 14)	
	3	-11.8 (-31, 8)		-5.5 (-25, 14)	
	4	-18.0 (-38, 2)		-5.6 (-25, 14)	
	5	-23.8 (-43, -4)		-5.8 (-25, 14)	
MCOP			0.16		<0.001
	1	-16.2 (-36, 3)		-22.2 (-42, -3)	
	2	-10.7 (-30, 9)		-10.9 (-31, 9)	
	3	-4.8 (-24, 15)		1.9 (-18, 22)	
	4	1.5 (-18, 21)		16.7 (-3, 36)	
	5	8.3 (-11, 28)		33.5 (14, 53)	
MCNP			0.15		<0.001
	1	-18.9 (-39, 1)		-24.1 (-44, -4)	
	2	-14.3 (-34, 5)		-14.5 (-34, 5)	
	3	-9.4 (-29, 10)		-3.7 (-23, 16)	
	4	-4.3 (-24, 15)		8.5 (-11, 28)	
	5	1.2 (-19, 21)		22.2 (3, 42)	
MEP			0.17		0.86
	1	-4.1 (-24, 16)		-10.6 (-30, 9)	
	2	-10.1 (-30, 10)		-10.0 (-30, 10)	
	3	-15.8 (-35, 4)		-9.5 (-29, 10)	
	4	-21.1 (-41, -1)		-8.9 (-29, 11)	
	5	-26.0 (-46, -6)		-8.3 (-28, 11)	

Table S5. (cont.)					
MnBP			0.66		0.29
	4	-35.2 (-55, -15)		-28.6 (-48, -9)	
	5	-29.4 (-49, -10)		-17.1 (-37, 3)	
MiBP			0.96		0.52
	4	-17.0 (-37, 3)		-10.4 (-30, 9)	
	5	-16.3 (-36, 3)		-2.7 (-22, 17)	

[a] Models adjusted for age and year of birth (category), and creatinine z-score when specified

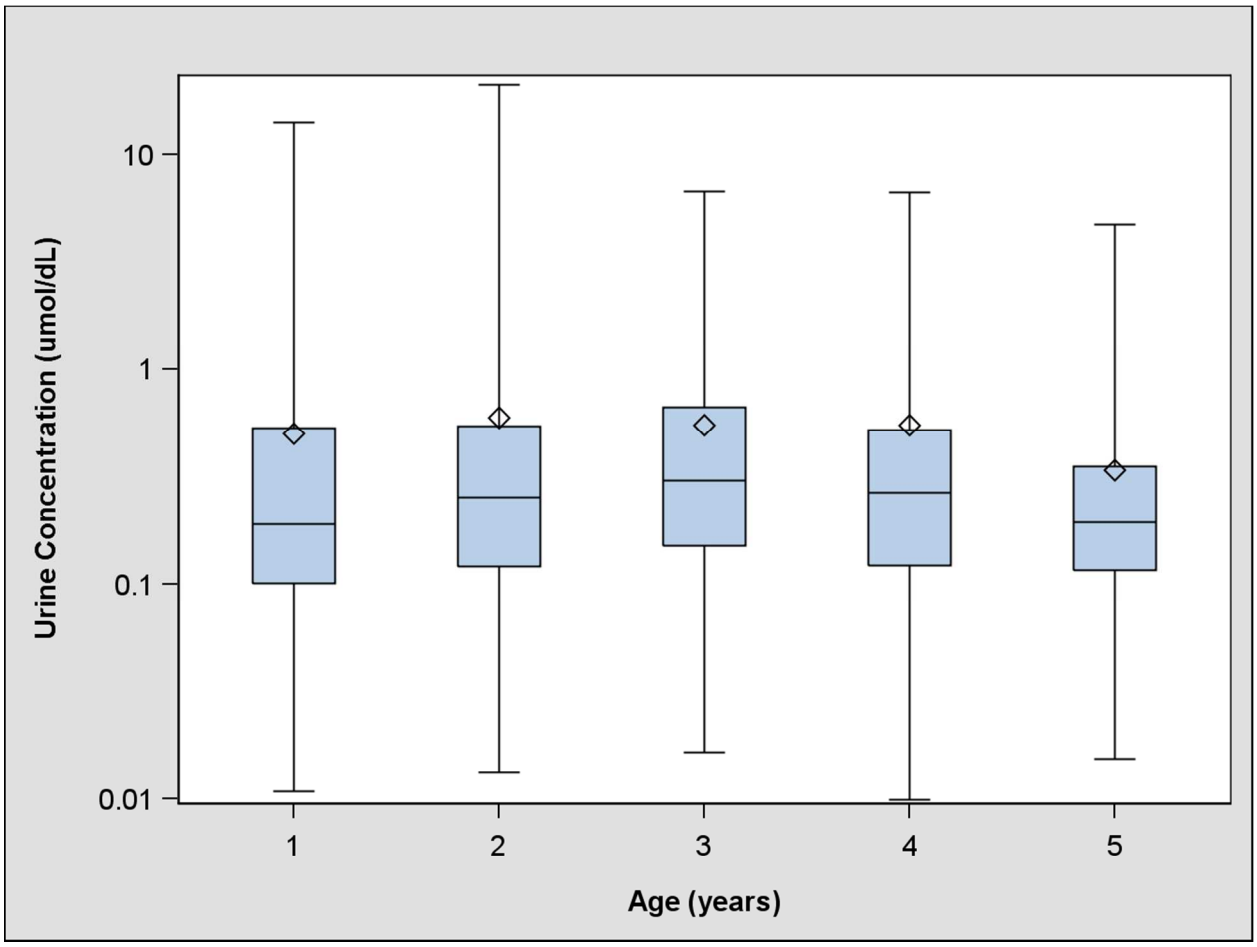
[b] % Difference = percent difference in geometric means between those born in 2005-2006 compared to those born in 2003-2004 (reference category)

[c] Interaction p-value for the cross-product interaction between continuous age in years and year of birth (2003-2004 vs. 2005-2006).

[d] ΣDEHP= Sum of MEHP, MEHHP, MEOHP, and MECPP

[e] MnBP measured only at visits 4 and 5

Figure S1. Box-and-whisker plots of HOME Study children's urinary ∑DEHP metabolite concentrations (μmol/L) at 1 to 5 years of age

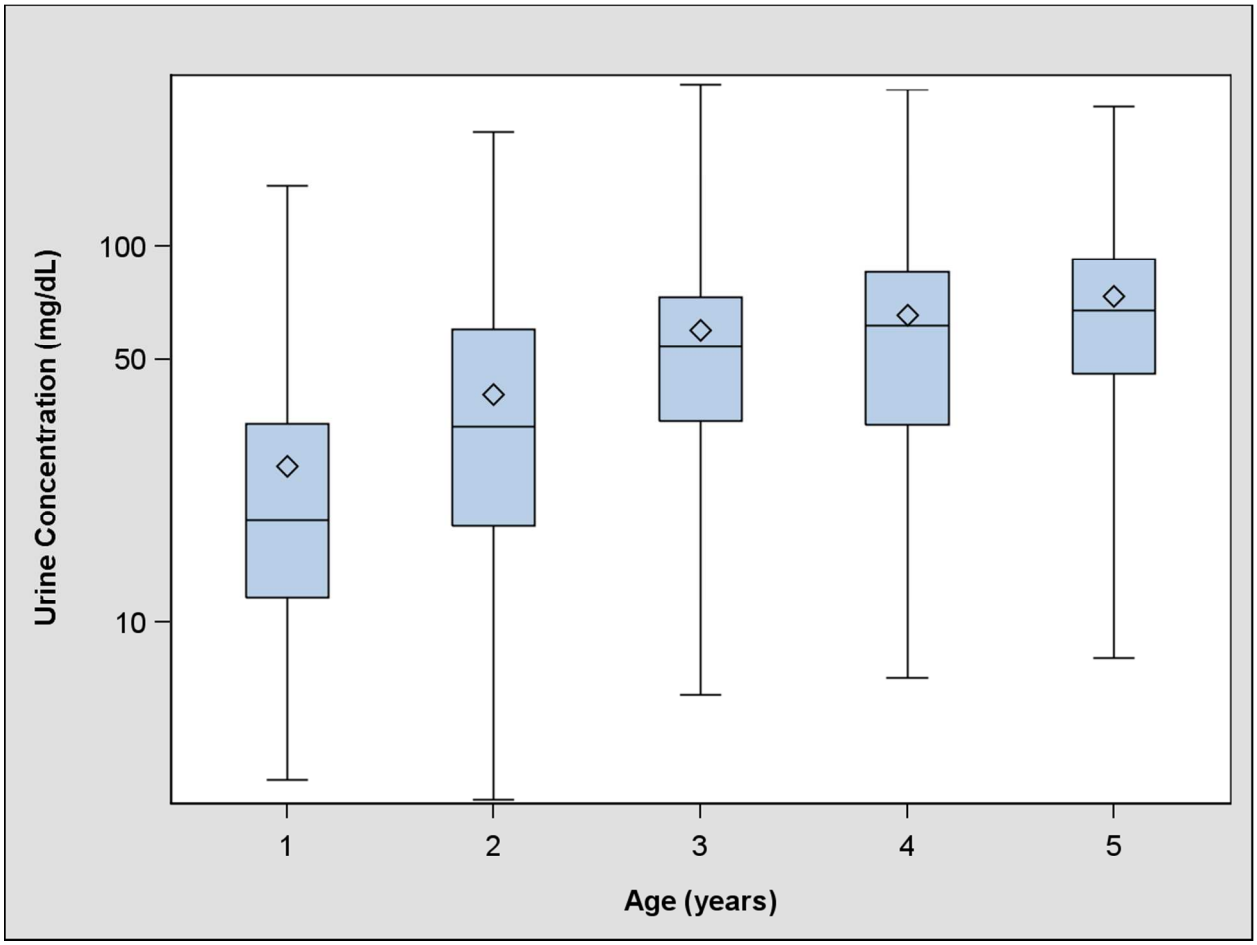


Diamond indicates arithmetic mean, whiskers indicate minimum and maximum, edges of box indicate 25th and 75th percentile, and middle line indicates median.

[c] ΣDEHP= Sum of MEHP, MEHHP, MEOHP, and MECPP

1 year n=281, 2 year n=235, 3 year n=237, 4 year n=172, 5 year n=203 (All subjects with at least 1 phthalate measurement)

Figure S2. Box-and-whisker plots of HOME Study children's urinary creatinine concentrations (mg/dL) at 1 to 5 years of age



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1 year n=281, 2 year n=235, 3 year n=237, 4 year n=172, 5 year n=203 (All subjects with at least 1 phthalate measurement)

References

[1] Silva, M. J.; Samandar, E.; Preau, J. L.; Reidy, J. A.; Needham, L. L.; Calafat, A. M., Quantification of 22 phthalate metabolites in human urine. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* **2007,** *860*, (1), 106-112.

[2] Caudill, S.P.; Schleicher, R.L.; Pirkle, J.L., Multi-rule quality control for the age-related eye disease study. *Statistics in Medicine* **2008,** 27, 4094-410