Intra-Individual Variability in Phthalate Spot Samples: Impact on Daily Dose Estimation

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Abstract

Background: This report presents an analysis of the urinary phthalate spot sample data from Preau et al. (2010) to compare estimates of daily intake doses of DEHP based on spot sample concentrations to the actual mass of di-(2-ethylhexyl) phthalate (DEHP) metabolized each day by the participants in the study. The Preau et al. (2010) dataset includes measurements of the void volume and urinary concentration of mono-(ethylhydroxy) phthalate (MEHHP, a major metabolite of DEHP) for each urinary void over the course of a week for eight volunteers.

Methods: The actual daily intake dose of DEHP for each participant was estimated based on 24-hour summed excretion of MEHHP in combination with urinary excretion fraction data from Koch et al. (2005). Estimates of daily DEHP intake based on the individual spot sample concentrations for each individual for each day were compared to the actual dose estimated from the 24-hour summed MEHHP excretion.

Results and Conclusions: Intake dose estimates derived from spot samples varied widely within each day for each individual. The lower and upper tails of the distributions of estimated intakes based on spot samples within each day significantly under- and over-estimated actual dose based on the 24-hour MEHHP excretion for that day, respectively. The upper end of the distribution of daily intake estimates based on spot sample concentrations overestimated actual daily intake dose based on total 24-hour MEHHP excretion on average by a factor of approximately 3, while the lower end of the distribution of daily intake estimates based on spot samples underestimated actual daily DEHP dose on average by a factor of 5. Published data from a controlled dosing experiment were evaluated in the same way and provide consistent results. Caution should be used when interpreting the tails of the distribution of population-based biomonitoring data based on spot samples for biomarkers of biologically transient compounds such as DEHP and other similarly transient phthalate compounds.

Introduction

Population-based biomonitoring data provide powerful information that can be used to assess the prevalence and magnitude of exposures to chemicals. Reverse dosimetry approaches have been applied to urinary concentration data from spot samples for phthalates and other biologically transient compounds (Kohn et al. 2000; Koch et al. 2007; Calafat and McKee 2006). However, substantial intra-individual variation in urinary spot sample concentrations, both within and across days, can occur for biologically transient compounds. Recent data from the Centers for

Disease Control and Prevention (CDCP) allow for an assessment of the impact of intra-individual variability on the accuracy of estimated intake rates for DEHP based on spot sample concentrations.

Methods

This analysis relies principally upon a data set collected by the CDCP and initially published by Preau et al. (2010), with a supporting analysis based on data from a controlled administration study by Koch et al. (2005).

The CDCP data set were generated from a week-long urine collection effort. Briefly, eight volunteers (4 male, 4 female) collected each urinary void over the course of a week, recording the time and volume of each void before preserving an aliquot for analysis. Concentrations of monoethyl phthalate (MEP, the monoester metabolite of diethyl phthalate, DEP), mono-2(ethylhexyl) phthalate (MEHP, the primary monoester metabolite of di-2(ethylhexyl) phthalate), and mono-(ethylhydroxy) phthalate (MEHHP, one of the major secondary oxidative metabolites of DEHP) were measured in each sample aliquot. The creatinine concentration in each sample was also measured. Bodyweights and heights were not recorded and are not available. We obtained the dataset from CDCP via a Freedom of Information Act request.

Reconstruction of Daily DEHP Dose from Mass of MEHHP Eliminated

For each subject, the daily mass of MEHHP (M_{MEHHP}, μg/d) excreted was calculated as the sum of the products of the concentration in each spot sample (C_i) and the corresponding urinary void volume (V_i) for that day, for all voids within a day from 1 to n:

$$M_{MEHHP} = \sum_{i=1}^{n} C_{MEHHP\_i} * V_i \qquad (1)$$

Based on data from Koch et al. (2005), the urinary excretion fraction (F_{UE_molar}) of MEHHP is 0.23; that is, 0.23 moles of MEHHP are excreted in 24 hours for each mole of DEHP consumed. On a mass basis, the urinary excretion fraction is:

$$F_{UE\_mass} = F_{UE\_molar} \frac{MW_{MEHHP}}{MW_{DEHP}} \qquad (2)$$

The molecular weight of MEHHP (MW_{MEHHP}) is 294; the molecular weight of DEHP (MW_{DEHP} is 391. Based on this, the F_{UE_mass} for MEHHP is 0.17. Using this mass excretion fraction, the estimated daily intake mass of DEHP (μg/d) corresponding to the daily elimination mass of MEHHP in urine can be calculated:

$$M_{DEHP} = \frac{M_{MEHHP}}{F_{UE\_mass}} \qquad (3)$$

Because bodyweights were not measured or reported in the CDCP dataset, estimated intakes in this analysis have not been normalized to bodyweight, but rather remain in total mass per day units.

Reconstruction of Daily DEHP Intake Based on Spot Sample Concentrations

Estimated daily intakes of DEHP have often been calculated using urinary phthalate concentrations measured in spot samples (Koch et al. 2007; Kohn et al. 2000) using an equation relating the daily intake to spot sample concentration, urinary excretion fraction, and an assumption regarding daily urinary volume or daily creatinine excretion rate. The equation for estimating daily intake of DEHHP based on spot sample concentrations of MEHHP and daily urinary volume (V_{24}) is given here:

$$DI(ug) = \frac{C_{MEHHP}(\frac{ug}{L}) * V_{24}(L)}{F_{UE\_mass}} \qquad (4)$$

Daily urinary volume for adults is variable. For this effort, an assumption of approximately 2 L/d of urinary volume was used, although other values could be justified (Perucca et al. 2007). For each individual in the dataset, multiple estimates of daily intake for each of the seven days of the experiment were calculated using equation 4 based on each measured spot sample concentration during each day. This procedure is consistent with that used by various authors to estimate intake rates based on spot samples collected in population biomonitoring programs such as NHANES and the German Environmental Survey (Koch et al. 2007; Calafat and McKee 2006).

Example: Data from Controlled Dosing Experiment

Koch et al. (2005) reported data from a controlled dosing experiment using deuterium-labelled DEHP. A volunteer ingested 48.5 mg of labeled DEHP, and concentrations of DEHP metabolites in each urinary void over 48 hours following a controlled dose were plotted as a function of time. The serial urinary concentrations were digitized from Figure 3 in that publication. Urinary concentration data for mono(2-ethyl-5-carboxypropyl)phthalate (MECPP), a major metabolite with a molar urinary excretion fraction of 0.21 (0.17 on a mass basis), were used in equation 4 above to estimate apparent daily intake at each urinary void time over the course of the experiment to investigate the time course and variation in predicted daily intakes in comparison with the actual intake reported in the experiment.

Results

Table 1 provides a summary of the demographic characteristics of the CDCP study participants, as well as a summary of the number of voids collected and missed. Voids were missed on a total of 12 subject-days, reducing the total sample size from 56 subject-days to 44 subject-days with

complete sample collection. Only the subject-days with complete void collection were used in this analysis.

Table 2 presents the calculated daily intakes of DEHP based on the total mass of MEHHP excreted daily for each participant (equation 3). Calculations are presented only for subject-days with complete collection of sample voids. Paired with each daily calculated mass of DEHP intake in Table 2 are summary statistics of the minimum, average, and maximum estimated DEHP intakes calculated based on spot sample concentrations for the same days (equation 4), as well as the ratios of the minimum, average, and maximum estimates (based on spot samples) to the estimate based on total daily MEHHP excretion. Figure 1 illustrates these daily DEHP dose estimates based on daily total MEHHP mass excreted and based on spot MEHHP concentrations for each subject.

The average ratios of the minimum, average, and maximum intake estimates each day based on spot samples to the actual DEHP intake calculated from total MEHHP excretion are 0.19, 1.12, and 3.12, respectively. Thus, reliance on the lower end of the distribution of spot sample concentrations for calculation of DEHP intake will on average underestimate actual intake by a factor of approximately 5 (for the minimum spot samples), while reliance on the upper end of the distribution of spot sample concentrations will overestimate the actual intake on average by a factor of approximately 3. Average spot sample concentrations from the distribution provide a reasonably accurate estimate of the true daily intake (as noted above, the average ratio of average spot sample concentration to true daily intake is 1.12, see Table 2).

Serial urinary concentrations of MECPP, another major urinary metabolite of DEHP, over 24 hours following a controlled dosing experiment were digitized from Figure 3 from Koch et al. (2005). Using equation 4, the estimated DEHP intake dose corresponding to each measured urinary concentration of MECPP was estimated. Figure 2A shows the profile of urinary MECPP concentration over 24 hours following administration of the controlled dose, and Figure 2B presents the corresponding estimated daily DEHP intake rate derived from each spot sample concentration calculated using equation 4. The estimated 24-hour average urinary concentration and actual administered dose from the controlled dosing experiment are also presented.

Intake estimated based on the peak spot sample urinary concentration (~4 hours after exposure) overestimates actual intake by a factor of approximately 2, while reliance on urinary concentrations at 12-24 hours post-exposure underestimates actual intake by a factor of approximately 4-5. This is roughly consistent with the conclusions based on the MEHHP-derived estimates in the CDCP dataset, particularly given that MECPP is a more slowly-eliminated compound (urinary elimination half-life of a approximately 15 hours for MECPP vs. 10 to 12 hours for MEHHP, based on data from Koch et al. 2005). Because of the somewhat slower elimination kinetics for this metabolite, MECPP will likely demonstrate a lower degree of intra-day variability in urinary concentrations than MEHHP.

Figure 2 shows that actual intake dose will be either over- or under-estimated from spot sample concentrations, depending on the time since exposure. Spot samples collected immediately after exposure will not reflect recent intakes; those collected from approximately 2 to 9 hours following intake will overestimate intake; and those collected more than about 9 hours after exposure will likely underestimate actual intake. This is consistent with the data from the CDCP experiment, which demonstrates that the tails of the distribution of estimated intakes derived from spot samples will not accurately reflect actual intakes.

Discussion

Equation 4 and related calculations based on estimated 24-hour creatinine excretion rates and creatinine-corrected urinary concentrations have been widely used to back-estimate intake rates for phthalates and other biologically transient compounds based on spot urinary biomarker measurements (Koch et al. 2007; Kohn et al. 2000). Although not often discussed explicitly, this equation and approach relies upon an assumption that the measured spot sample concentration is an accurate surrogate for 24-hour average urinary concentration (either on a volume or creatinine-corrected basis). In the case of highly transient compounds such as phthalates, this assumption will introduce substantial error in estimated intake doses when relying on the tails of the distributions of observed spot sample concentrations.

Because assessments of population exposures often focus on the upper ends of the distribution of such estimates in order to identify the potential for excess exposures in the context of risk assessments, an awareness of the degree of intra-individual variability in spot urinary concentrations as well as the impact on estimated intakes based on such samples is important. Caution in the interpretation of the tails of the distribution of spot sample concentrations for biologically transient compounds is warranted.

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Table 1: Description of CDCP study participants. No information on bodyweight or height was collected or available.

Subject ID	Age	Gender	# Samples Collected	# Samples Missed	Day(s) of Missed Samples
S1	31	F	63	0	-
S2	25	F	74	14	Day 2 (3 missed) Day 3 (1 missed) Day 4 (1 missed) Day 5 (2 missed) Day 6 (3 missed) Day 7 (4 missed)
S3	58.8	F	61	1	Day 4 (1 missed)
S4	27	F	62	5	Day 1 (2 missed) Day 7 (3 missed)
S5	34	M	64	1	Day 1 (1 missed)
S6	26	M	27	0	-
S7	32	M	37	2	Day 4 (1 missed) Day 5 (1 missed)
S8	32	M	41	0	-
		Totals:	**429**	**23**	

Table 2: Comparison of estimated daily intake doses of DEHP based on total 24-hour excretion of MEHHP (using equation 3) and based on spot samples (using equation 4) for seven days for eight participants. Estimates are presented only when all urinary voids were collected for that day.

Subject and day	Sum of MEHHP excreted, µg/d	Daily DEHP Intake Estimate Based on Sum of MEHHP excreted, µg/d[a]	Daily DEHP Intake Estimates Based on Spot Samples, µg/d[b]			Ratio of estimates based on spot samples to estimate from total MEHHP excretion		
			Minimum	Average	Maximum	Minimum	Average	Maximum
S1								
Day 1	342	2013	109	2941	8761	0.05	1.46	4.35
Day 2	202	1189	490	2097	4317	0.41	1.76	3.63
Day 3	204	1198	114	1884	10495	0.10	1.57	8.76
Day 4	336	1976	224	1794	6728	0.11	0.91	3.40
Day 5	86	507	75	290	965	0.15	0.57	1.90
Day 6	31	184	7	155	444	0.04	0.84	2.41
Day 7	57	333	27	206	680	0.08	0.62	2.04
S2								
Day 1	22	129	22	120	379	0.17	0.93	2.94
Day 2	†							
Day 3	†							
Day 4	†							
Day 5	†							
Day 6	†							
Day 7	†							
S3								
Day 1	81	479	29	525	1712	0.06	1.10	3.57
Day 2	229	1350	49	1279	4000	0.04	0.95	2.96
Day 3	25	146	20	307	662	0.14	2.11	4.54
Day 4	†							
Day 5	154	905	438	1454	3706	0.48	1.61	4.10
Day 6	315	1854	190	1986	5096	0.10	1.07	2.75
Day 7	363	2133	160	4660	16296	0.08	2.18	7.64

Subject and day	Sum of MEHHP excreted, µg/d	Daily DEHP Intake Estimate Based on Sum of MEHHP excreted, µg/d[a]	Daily DEHP Intake Estimates Based on Spot Samples, µg/d[b]			Ratio of estimates based on spot samples to estimate from total MEHHP excretion		
			Minimum	Average	Maximum	Minimum	Average	Maximum
S4								
Day 1	†							
Day 2	43	254	16	130	365	0.06	0.51	1.44
Day 3	28	165	6	114	439	0.04	0.69	2.67
Day 4	24	143	16	105	323	0.11	0.74	2.27
Day 5	23	133	8	54	120	0.06	0.41	0.90
Day 6	10	56	10	62	191	0.18	1.10	3.40
Day 7	†							
S5								
Day 1	†							
Day 2	50	295	7	207	960	0.02	0.70	3.25
Day 3	71	420	49	417	1152	0.12	0.99	2.75
Day 4	75	439	15	357	1323	0.03	0.81	3.01
Day 5	40	236	16	187	657	0.07	0.79	2.79
Day 6	42	250	32	199	439	0.13	0.79	1.76
Day 7	39	231	2	141	650	0.01	0.61	2.82
S6								
Day 1	131	768	276	1281	2370	0.36	1.67	3.09
Day 2	72	426	226	460	669	0.53	1.08	1.57
Day 3	70	409	148	460	1087	0.36	1.12	2.66
Day 4	32	185	104	272	406	0.56	1.47	2.19
Day 5	29	169	166	227	328	0.98	1.34	1.94
Day 6	19	113	12	157	274	0.11	1.38	2.42
Day 7	39	227	45	301	716	0.20	1.33	3.16
S7								
Day 1	178	1049	23	631	1544	0.02	0.60	1.47
Day 2	50	295	19	331	904	0.06	1.12	3.06

Subject and day	Sum of MEHHP excreted, µg/d	Daily DEHP Intake Estimate Based on Sum of MEHHP excreted, µg/d[a]	Daily DEHP Intake Estimates Based on Spot Samples, µg/d[b]			Ratio of estimates based on spot samples to estimate from total MEHHP excretion		
			Minimum	Average	Maximum	Minimum	Average	Maximum
Day 3	25	150	37	127	402	0.25	0.85	2.68
Day 4	†							
Day 5	†							
Day 6	195	1145	138	706	2575	0.12	0.62	2.25
Day 7	93	548	7	436	1218	0.01	0.80	2.22
S8								
Day 1	13	79	6	76	295	0.08	0.96	3.74
Day 2	162	953	94	1187	3918	0.10	1.25	4.11
Day 3	418	2457	834	3886	8718	0.34	1.58	3.55
Day 4	262	1541	1136	4097	11738	0.74	2.66	7.62
Day 5	326	1919	87	1624	2976	0.05	0.85	1.55
Day 6	99	585	258	861	2105	0.44	1.47	3.60
Day 7	23	135	50	150	308	0.37	1.10	2.28
					Average ratios:	0.19	1.12	3.12

[a] Estimated using equation 3.
[b] Estimated using equation 4.
† One or more voids were not collected on this day for this subject, so the data from this day are omitted from the full analysis.

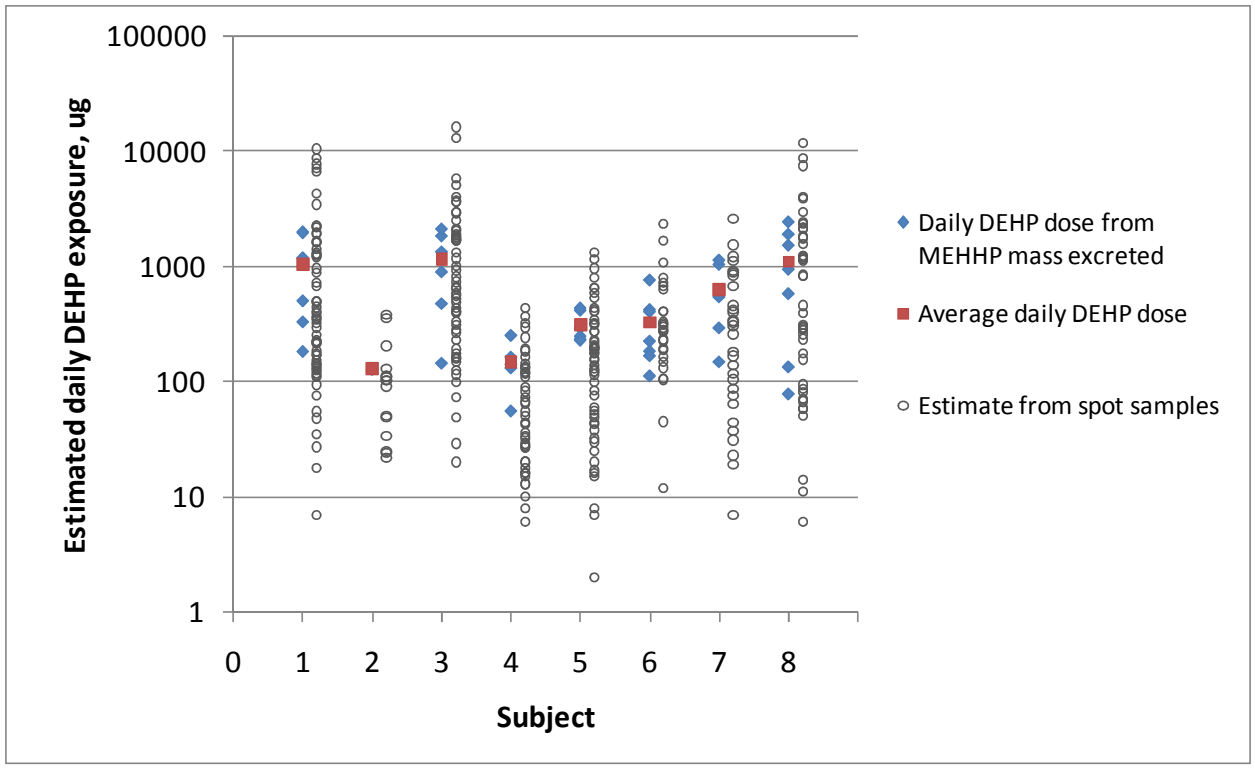

Figure 1: Estimates of daily DEHP exposure for eight subjects in CDCP dataset. Estimates are presented only for days when a subject reported complete collection of urine voids. Estimated DEHP intakes were calculated based on total MEHHP excreted per day (equation 3, filled diamonds) or based on concentrations of MEHHP in spot samples (equation 4, open circles). Subject-specific arithmetic averages of the estimated daily doses based on total MEHHP excretion for all days included in the analysis (those with complete void collection) are also presented (filled squares).

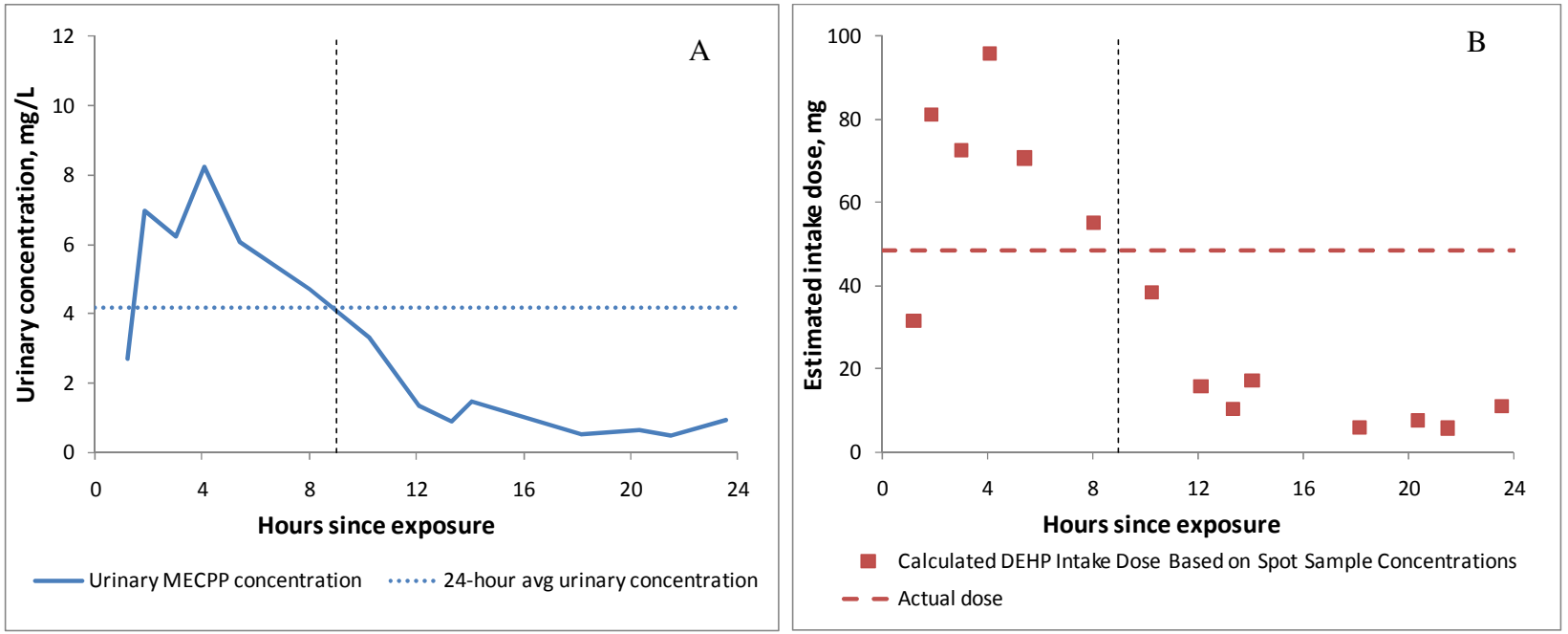

Figure 2A: Measured urinary concentrations of MECPP following a controlled dosing experiment described in Koch et al. (2005). Figure 2B: Corresponding calculated DEHP intake doses using equation 4 are presented based on the MECPP concentration from each spot sample. The spot urinary concentration corresponds to the 24-hour average urinary concentration approximately 9 hours following exposure; the estimated DEHP intake dose based on spot sample concentrations likewise corresponds to the actual administered dose approximately 9 hours following exposure.