Urinary DEHP Metabolites and Food Fasting Time in NHANES

September 8, 2010

Prepared for:

Phthalate Esters Panel
American Chemistry Council
700 Second Street, NE
Washington, DC 20002

Prepared by:

Lesa L. Aylward
Sean M. Hays
Chris R. Kirman
Summit Toxicology, LLP
6343 Carolyn Drive
Falls Church, VA 22044

Introduction and Background

This report presents an initial exploratory analysis of the data on urinary metabolites of di-2-ethylhexyl phthalate (DEHP) from the 2005-2006 National Health and Nutrition Examination Survey (NHANES). Specifically, this analysis examines the potential relationship between DEHP metabolite levels and reported fasting time. Any such relationship could be relevant to interpretation of the measured NHANES urinary concentrations in the context of existing exposure guidance values and risk assessments. The potential of "correcting" measured DEHP levels to account for food fasting time has been raised during consultations by the Consumer Product Safety Commission. This prospect was raised under two assumptions: first, that DEHP exposure comes primarily through the food supply, and second, that urinary samples analyzed in the NHANES program were collected under fasting conditions (i.e., 12 hours or more since last food consumption).

DEHP is rapidly metabolized and eliminated following exposure (Koch et al. 2004; 2005). Urinary excretion of the four predominant urinary metabolites follows a two-phase pattern, with an initial urinary excretion half-life of about 2 hours, and a second phase with half-lives of about 5 hours. This rapid excretion raises the potential for urinary samples to "miss" elevated concentrations of DEHP metabolites if the sampling time is sufficiently long after ingestion of a dose of DEHP. Exposure to DEHP appears to occur primarily through the food supply (Wormuth et al. 2006). Thus, a sample of urine collected after a 12-hour food fast might contain substantially lower levels of DEHP metabolites than in a sample collected closer in time to the ingestion.

This report examines the following:

- Distribution of session of examination and reported fasting times among NHANES 2005-2006 participants with measured urinary concentrations of DEHP metabolites;
- Distribution of reported DEHP urinary metabolites concentrations in the NHANES 2005-2006 participants;
- Associations between measured DEHP urinary metabolite concentrations and food fasting times.

These analyses can illuminate whether a) the assumption that NHANES samples are collected under a fasting condition are true, and b) the practicality and necessity of considering fasting time in the interpretation of measured urinary DEHP metabolite concentrations in the NHANES program.

Methods

Information on food fast time and session of collection of samples is provided in the "Phlebotomy Fasting Questionnaire" data set in NHANES. Urinary DEHP metabolite concentrations are also available in the laboratory data sets. The data analyzed are from the 2005-2006 data collection cycle (available at http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/lab05_06.htm).

Concentrations of four urinary metabolites of DEHP were reported in the NHANES data set:

- Mono-2-ethylhexyl phthalate (MEHP)
- Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)
- Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)
- Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)

For most of the analyses presented here, the concentrations of the four metabolites were summed (with non-detects replaced by the limit of detection (LOD)/sqrt(2)) for each participating individual to produce a summed DEHP metabolite variable. Data were analyzed in Stata (Release 10, StataCorp LP, College Station, Texas). Population weighted percentiles were generated with application of the sample weights reported in variable wtsb2yr. Associations between DEHP metabolite concentrations and fasting times were explored using the procedure *regress*, again with application of the appropriate sample weights. Comparisons of means among categories were made using procedure *ttest*. All statistical evaluations were conducted on ln-transformed data.

Results

Session of Sample Collection

Figure 1 presents the distribution among examination session of the participants in the NHANES 2005-2006 survey. Of the total of 2548 participants with measurements of urinary DEHP metabolites, 1202 (47.2%) of the participants were examined in the morning sessions at the Mobile Examination Centers. Approximately 35% were examined in the afternoon session, and 17.8% were examined in the evening session. These examinations included the collection of blood and spot urine samples.

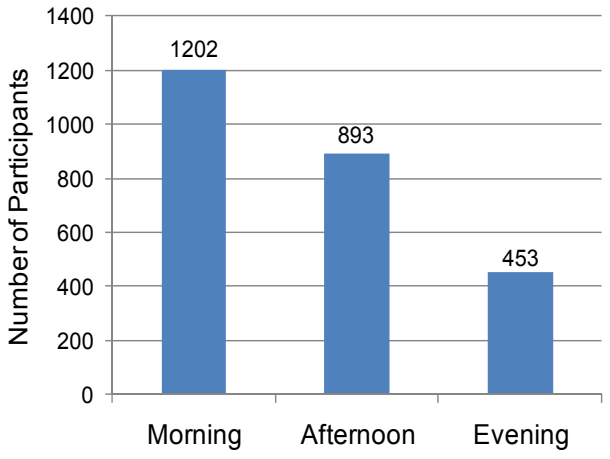

Figure 1: Distribution of session of examination for participants with measured DEHP urinary metabolites.

Food Fast Time

Figure 2 shows the cumulative frequency distribution in reported food fasting time among the participants with measured DEHP urinary metabolites. Approximately 50% of the participants reported fasting times of 6 hours or less; approximately 25% of the participants reported fasting times longer than 12 hours. A few individuals reported fasting times longer than 24 hours (n=13), with the longest reported fasting time of 61.6 hours (data not shown).

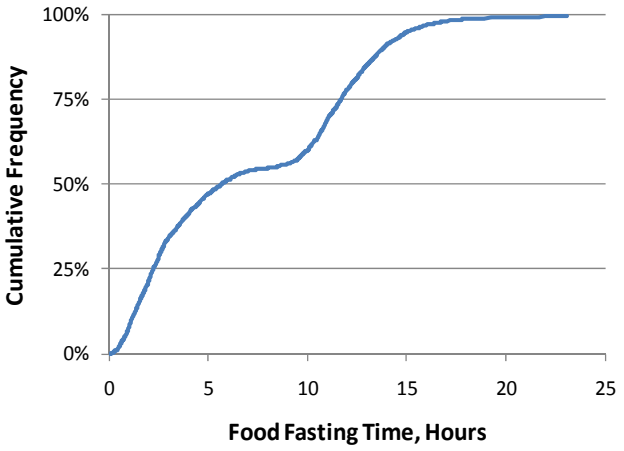

Figure 2: Cumulative frequency of reported food fasting time for 2548 participants with measured DEHP urinary metabolites.

The distribution of reported fasting times was bimodal. Figure 3 shows why: for participants in the morning session, some reported very short fasting times (presumably, they ate breakfast) and most reported relatively long fasting times, reflecting an overnight fast. For participants in the other two sessions, fasting times presumably reflect typical time since their previous meal that day (lunch or breakfast for the afternoon participants, and dinner or lunch for the evening participants). Thus, fasting times are either relatively short (less than eight hours) or fairly long (reflecting an overnight fast).

Urinary DEHP Metabolite Concentrations

Selected population-weighted percentiles for the measured urinary DEHP metabolites and the sum of four metabolites are presented in Table 1. With the exception of MEHP, the DEHP metabolites were detected in greater than 99% of the participants.

Table 1: Selected population-weighted percentiles of urinary DEHP metabolites and for the individual-by-individual sum of the four measured metabolites in 2548 participants with measured DEHP metabolites in the NHANES 2005-2006 data collection cycle.

		Percentiles, µg/L				
Metabolite	**% Detects**	**5th**	**25th**	**50th**	**75th**	**95th**
MEHP	68.8%	<LOD (1.2)	<LOD (1.2)	2.5	6.3	39.7
MEHHP	99.5%	1.3	10.7	23.8	55.1	305.9
MEOHP	99.0%	1.9	6.8	15.1	35.5	183.1
MECPP	99.9%	5.1	16.7	35.6	79.7	385.6
Sum of Four[a]		11.35	36.9	78.3	175.6	929.5

[a] Summed measure of each of the four metabolites by individual, with non-detects replaced by LOD/sqrt(2).

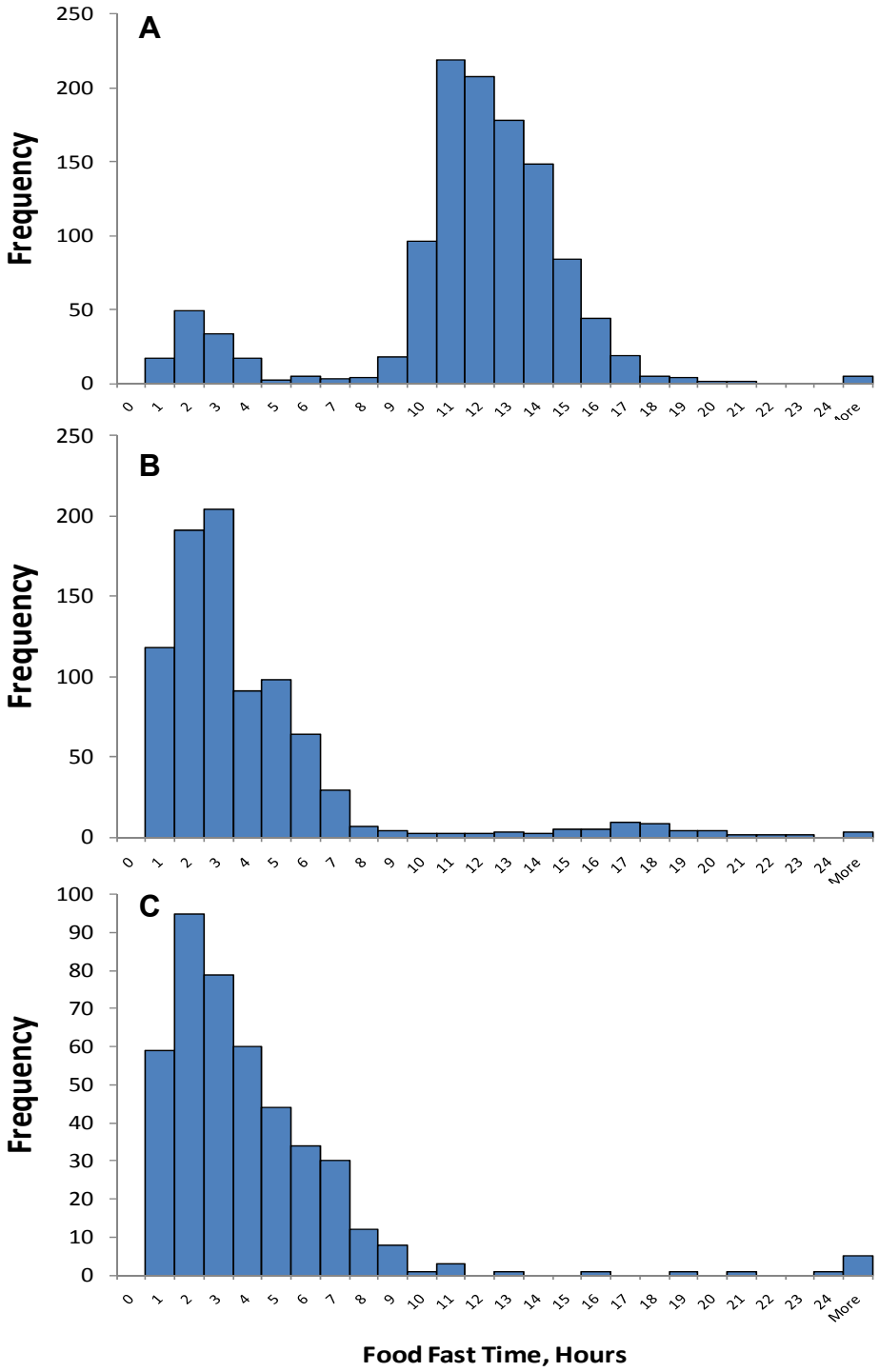

Figure 3: Distribution of reported food fast time, in hours, for participants examined in the A) morning session, B) afternoon session, and C) evening session.

Association Between Urinary DEHP Metabolites and Fasting Time

The sum of the concentrations of four metabolites versus the reported fasting time is presented in Figure 4. The bimodal distribution of reported fasting times is clearly visible in the two clusters of data points. The regression lines in the two clusters are the linear regressions for fasting times less than and greater than 8 hours (Table 2). The positive slope for the less than 8 hour fasting times is statistically significant, while the negative slope for the greater than 8 hour fasting times is not. However, the significant positive slope is of a small magnitude, and fasting time explains very little of the variability in the observed urinary concentrations (<1%). Comparison of the distribution of values between persons with short and long fasting times did not demonstrate any statistically significant differences in group geometric means (Figure 5).

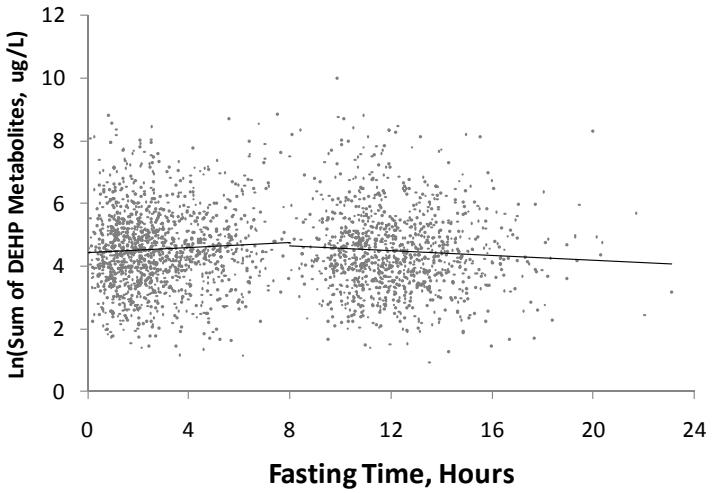

Figure 4: Scatter plot of the ln of the sum of DEHP metabolites versus fasting time in hours. Lines are linear regressions for fasting times less than or equal to 8 hours or greater than 8 hours and less than 24 hours. Participants with reported fasting times >24 hours (n=13) were omitted from this graph.

Table 2: Regression results for ln of sum of DEHP metabolites versus food fasting time in hours, for fasting times less than or equal to 8 hours, or fasting times greater than 8 hours (with and without omission of observations with food fasting times greater than 24 hours). Analysis omits 94 participants with measured urinary DEHP metabolites but no data reported on fasting times.

Group	Slope ln(sum DEHP metabolites, ug/L) per hour of food fast	95% C.I.	p-value	Adjusted R^2
Fasting time <= 8hrs (n=1346)	0.058	0.018-0.098	0.0045	0.0053
Fasting time >8 hrs and <24 hrs (n=1095)	-0.033	-0.073-0.006	0.0989	0.0016
Fasting time >8 hours (n=1108)	-0.019	-0.047-0.009	0.1809	0.0007

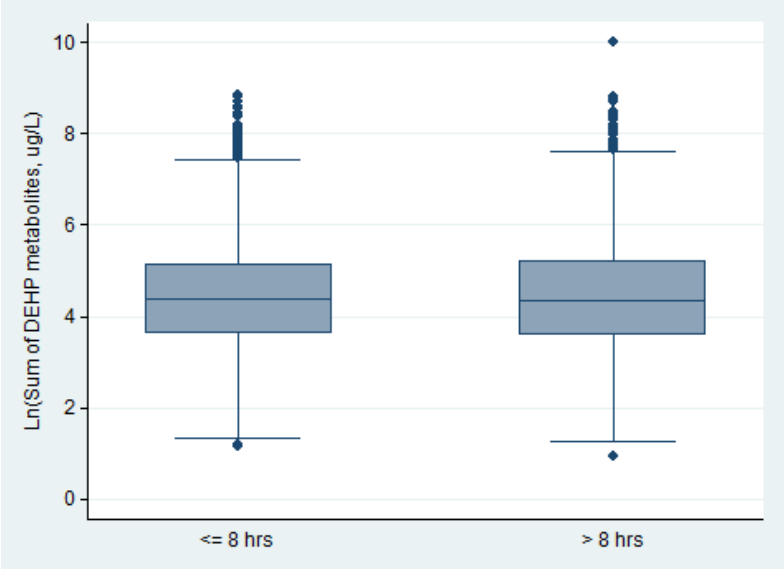

Figure 5: Box plots of the ln of the sum of DEHP metabolites by fasting time. Shaded boxes represent the interquartile range (25th to 75th percentiles); whiskers extend to 1.5 times the interquartile range. The geometric means were not statistically significantly different between the two groups (93.7 vs. 90.0, p=0.36).

DEHP metabolites have somewhat different terminal urinary half-lives. MEHP has a very short half-life (~5 hours), while MECPP has a somewhat longer half-life (~12 hours); the other two measured metabolites have half-lives of approximately 10 hours (Koch et al. 2005). Thus, MEHP might display more variation in response to fasting times than the other metabolites. Figure 6 shows the relationship between urinary MEHP levels and fasting time. Again, urinary samples collected within 8 hours of food consumption show rising levels with increasing fast time, while those collected with longer fasting times show decreasing levels. The regression coefficients (0.059 and -0.047 for less than and greater than 8 hour fasts, respectively) are similar in magnitude to those demonstrated for the sum of DEHP metabolites, and both are statistically significant ($p<0.05$). The group means were significantly different (geometric means of 3.53 vs. 2.77 µg/L for less than vs. greater than 8 hour fast, $p<0.0001$). Again, however, very little of the variation in measured MEHP levels is explained by the fasting time.

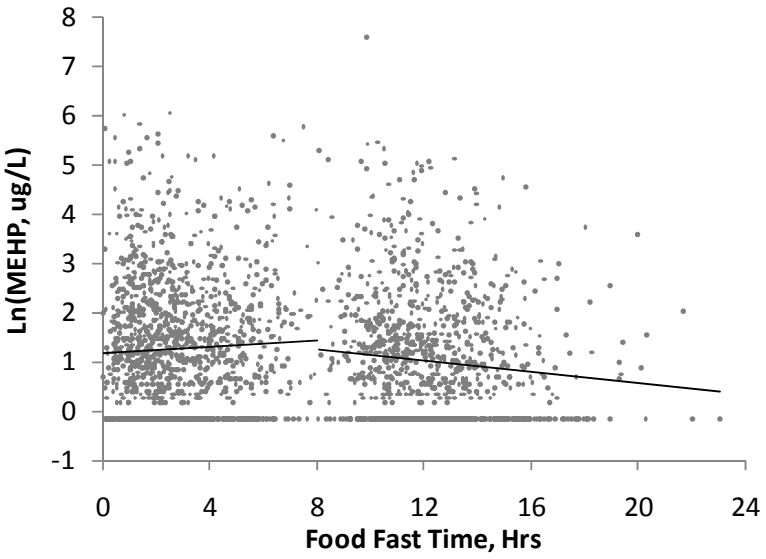

Figure 6: Urinary MEHP levels as a function of reported fasting time. Linear regression lines are presented for fasting times of less than or equal to 8 hours and greater than 8 hours; both are significant (see text).

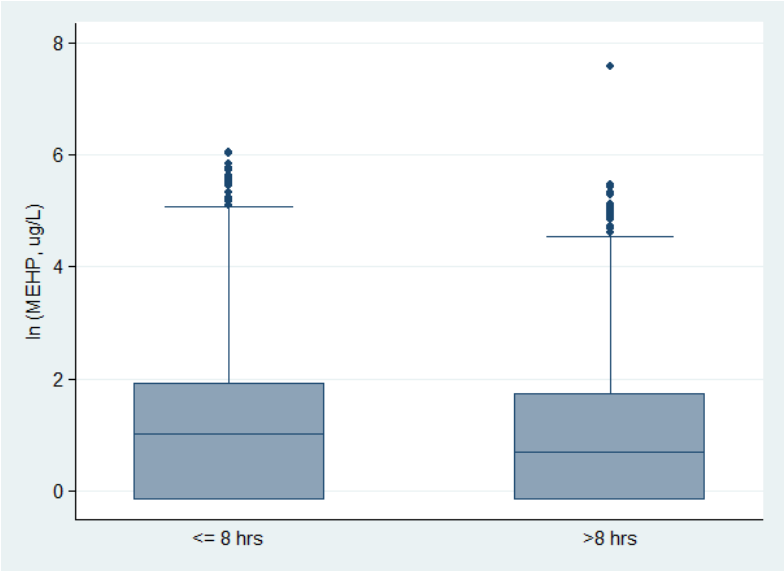

Figure 7: Box plots of the ln of MEHP by fasting time. Shaded boxes represent the interquartile range (25th to 75th percentiles); whiskers extend to 1.5 times the interquartile range. The geometric means were statistically significantly different (3.5 vs. 2.8, p<0.0001).

Discussion

The NHANES program includes community-based physical examinations in the Mobile Examination Centers, with participants being examined and giving blood and urine samples at one of three sessions: morning, afternoon, and evening. As a result, fasting times varied among participants, and only about 25% of the participants with measured DEHP concentrations exhibited fasts of 12 hours or more, which is required for a true "fasting" sample of blood or urine.

Urinary DEHP metabolite levels were related to fasting times in the NHANES 2005-2006 data set in a complex fashion, with urinary concentrations increasing with increasing fasting time up to approximately 8 hours, and decreasing with longer fasting times. This pattern does not support application of a simplistic "correction" factor related to fasting time in order to account for the rapid elimination of these compounds in interpretation of urinary biomonitoring data. Further, the distributions of summed metabolite levels were not statistically significantly different between individuals reporting short and long fasting times. Levels of MEHP, which is more transient than the other DEHP metabolites, do demonstrate a statistically significant difference by category of fasting time (<= 8 vs. >8 hours). However, MEHP represents a very small fraction of the total excretion of DEHP metabolites. Reliance on the summed metabolites, including those with longer urinary excretion half-lives, provides a more stable indicator of exposure level.

Factors other than time between dietary exposure and urinary sampling will impact the observed levels. In particular, the number and timing of urinary voids in the time between exposure and measured sample will influence the measured level (Aylward et al. 2009; Lorber et al. 2009). Because the bladder acts as a collection reservoir for excreted metabolites, a urinary sample collected 12 hours after last exposure might exhibit:

- Relatively high concentrations of metabolites, if there had been none or only one intervening urinary void, relatively close in time to exposure;
- Medium urinary concentrations, if a urinary void had occurred midway through the fast period or if the last urinary void was 6 to 8 hours prior to the measured sample collection; or
- Low urinary concentrations, if there had been several urinary voids in the intervening period, with one just an hour or so before the sample collected for analysis for DEHP metabolites.

While the NHANES program collects information on food fasting time, they do not collect information on time since last urinary void or on pattern of urinary voids in the hours preceding collection of the analyzed urine sample. Thus, no information is available to analyze this issue in relationship to the reported DEHP concentrations in the NHANES database.

Other factors such as age or sex may also influence metabolism and elimination of DEHP. Exploration of these factors is outside the scope of this report, but could be evaluated using the NHANES dataset.

The data available from the NHANES program include samples collected at various times of day and from individuals in various states of fasting. This preliminary analysis does not suggest that correction for fasting time is appropriate or necessary for the interpretation of the measured NHANES DEHP urinary metabolite levels.

References

Aylward, L.L., S.M. Hays, M. Gagné, K. Krishnan. 2009. Derivation of Biomonitoring Equivalents for di(2-ethylhexyl)phthalate (CAS No. 117-81-7). Regul. Toxicol. Pharmacol. 55(3):249-58.

Koch HM, Bolt HM, Angerer J. 2004. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch Toxicol 78(3):123-130.

Koch HM, Bolt HM, Preuss R, Angerer J. 2005. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch Toxicol 79(7):367-376.

Lorber M, Angerer J, Koch HM. 2009. A simple pharmacokinetic model to characterize exposure of Americans to Di-2-ethylhexyl phthalate. J Expo Sci Environ Epidemiol.

Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Analysis 26:803-824