Supporting Information

Temporal Trends in Exposures to Six Phthalates from Biomonitoring Data: Implications for Cumulative Risk

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Materials and Methods: Daily Intake Dose and Maximum Cumulative Ratio

In brief, as presented in Christensen et al.[1] the Daily Intake (DI) was calculated through adjusting metabolite concentrations of phthalates by creatinine concentrations while incorporating other variables such as daily creatinine excretion rates, the molar fraction of a given metabolite that was excreted, and information about the molecular weights of the metabolites and their parent phthalates. Under the assumption of steady state exposures, the DI for each participant i and metabolite k originating from parent phthalate j was calculated using the following equation:

$$DI_{i,j,k} = \left(\left[100 * \left(Met_{i,k}/Cr_i\right) * CE_i\right]/\left[F_{UE,i,k} * 1000\right]\right) \times \left(MW_{i,j}/MW_{i,j,k}\right) \quad \text{(S1)}$$

where $DI_{i,j,k}$ ($\mu g/kg/d$) in urine is the DI dose for metabolite k, 100 is a unit conversion, $Met_{i,k}$ (ng/mL) is the metabolite concentration as given in the NHANES data set, Cr_i (mg/dL) is the creatinine concentration in urine as given in the NHANES data set, CE_i ($mg/kg/d$) is the creatinine excretion per day as calculated by Mage et al.[2] using information about a participant's age, race/ethnicity, gender, weight, and height, $F_{UE,i,k}$ (unitless) is the molar fraction of metabolite excreted, 1000 is a unit conversion, $MW_{i,j}$ (mg/mol) is the molecular weight of the parent phthalate, and $MW_{i,j,k}$ (mg/mol) is the molecular weight of the metabolite (Table S3). Among the phthalates that have multiple metabolites (i.e. DEHP and DINP), within an individual i, the value of $DI_{i,j}$ was calculated by taking a weighted mean of the values of $DI_{i,j,k}$ estimated from each metabolite k using $F_{UE,i,k}$.[1,3] The weighted mean was determined using the following equation:

$$DI_{i,j} = \sum_{k=1}^{n_k}\left(DI_{i,j,k} \times \frac{F_{UE,i,k}}{\sum_{l=1}^{n_k} F_{UE,i,l}}\right), \quad \text{(S2)}$$

where $DI_{i,j}$ is the DI dose for phthalate j and n_k is the number of metabolites for a given parent phthalate. In this work, $n_k \in \{1,2,4\}$.

We used the NHANES convention of setting metabolite concentrations below the Limit of Detection (LOD) to $LOD/\sqrt{2}$, which is subject to change as different analytical methods are used to determine metabolites concentrations for different NHANES cycles (Table S6).

The Maximum Cumulative Ratio (MCR) will vary across individuals in an exposed population ranging from one to N (i.e. $MCR_i \in [1, N]$), where N is the number of chemicals considered in the assessment. A value close to one indicates that one chemical was responsible for nearly all of the individual's cumulative risk and a value of N indicates that the individual receives an equitoxic dose from all chemicals.

This approach requires information on height and weight of the surveyed participants. A small number of participants were excluded for missing either height or weight information (ranging from 18 to 35 per cycle) or for missing metabolite information (ranging from 6 to 38 per cycle). After removing these participants, there is a total of 12,951 participants over five cycles of NHANES (Table S2).

Materials and Methods: Sensitivity Analysis

The equation used to estimate the DI of a phthalate using Urinary Flow Rate (UFR) is:

$$DI_{i,j,k} = \left(\left[60 * 24 * Met_{i,k} * UFR_i\right] / \left[BW_i * F_{UE,i,k} * 1000\right]\right) \times \left(MW_{i,j} / MW_{i,j,k}\right) \quad \text{(S3)}$$

where, UFR_i is the urinary flow rate in ml/min for the participant i as reported by NHANES. UFRs are only available for the three most recent cycles of NHANES.

The methodology used to calculate the relative potency-weighted dose has been described elsewhere.[4]. Benchmark Doses (BMDs) due to androgen disruption for DBP, DIBP, BBP, and DEHP were obtained from the literature.[5] The BMD for DINP was obtained from Varshavsky et al.,[4] who assumed that DINP was 2.3 less potent than DBP (Table S9).[6] DIDP is not a known androgen disruptor. Therefore, it was assumed to be ten times less potent than the least potent phthalate.

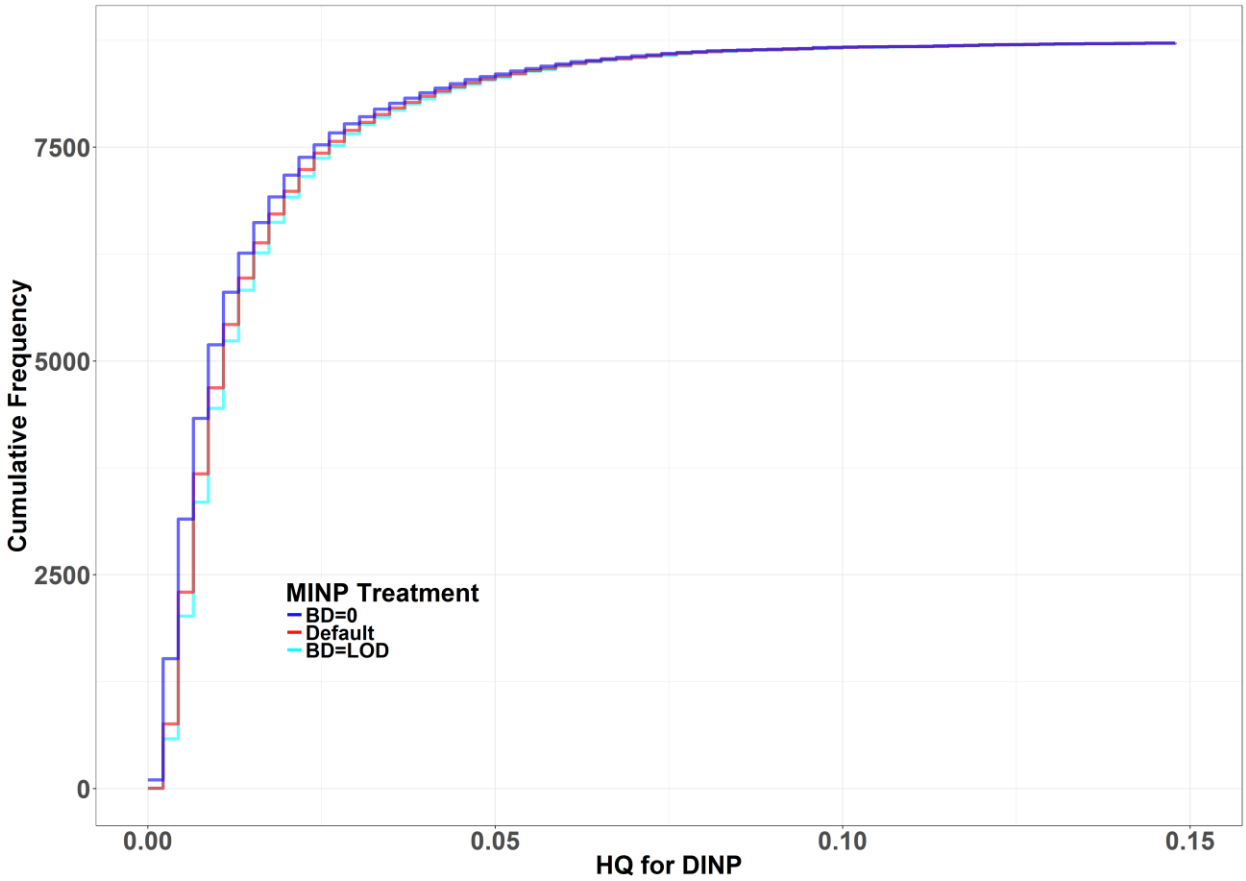

Figure S1: Cumulative frequency of different treatments of below-detects for the MINP metabolite and its corresponding influence on the Hazard Quotient (HQ) of its parent phthalate DINP from NHANES spanning from 2005-2014.

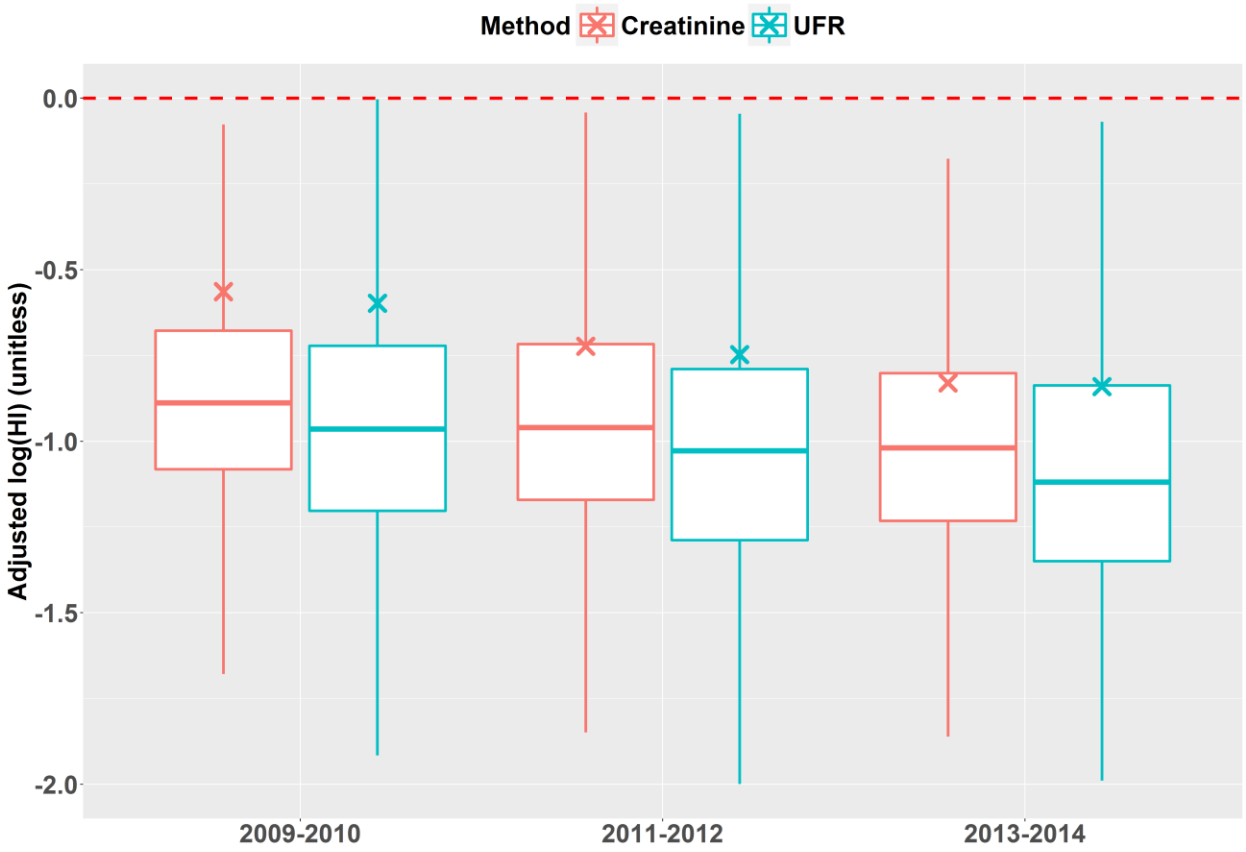

Figure S2. Population-wide boxplots of the log transform of the Hazard Index (HI) calculated by a creatinine correction approach and the Urinary Flow Rate (UFR) approach of the surveyed NHANES population presented by NHANES cycle spanning from 2009-2014 adjusted for the NHANES survey weighting factors. The boxplot is marked by the median, first and third quartile, and $\pm 1.5 * IQR$ (Inter-Quartile Range). The logarithm of the arithmetic means is identified with an "X". The dashed, horizontal, red line indicates $HI = 1$ (i.e. $log(HI) = 0$).

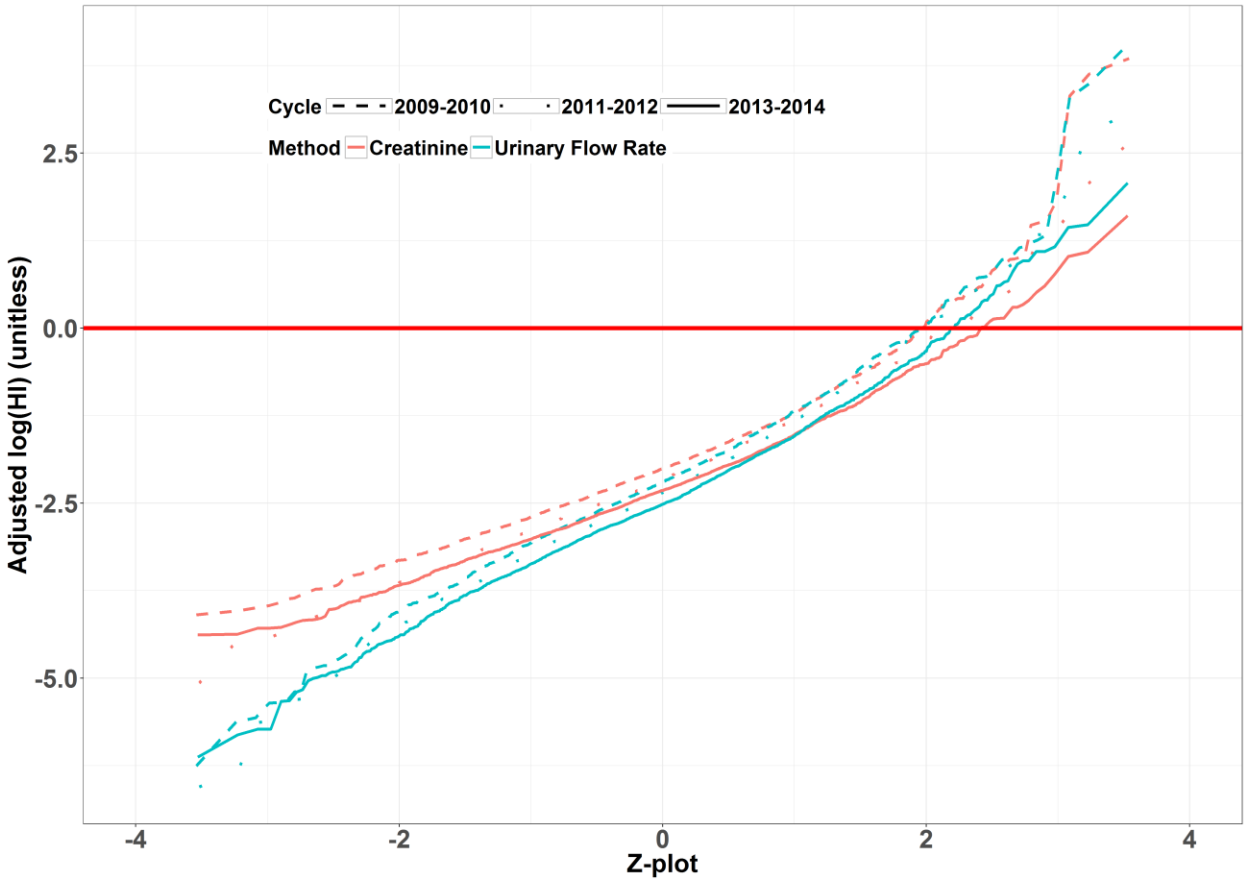

Figure S3. Z-plot of Hazard Indices (HIs) for the creatinine correction approach and the Urinary Flow Rate (UFR) adjusted by the NHANES survey weighting factors.

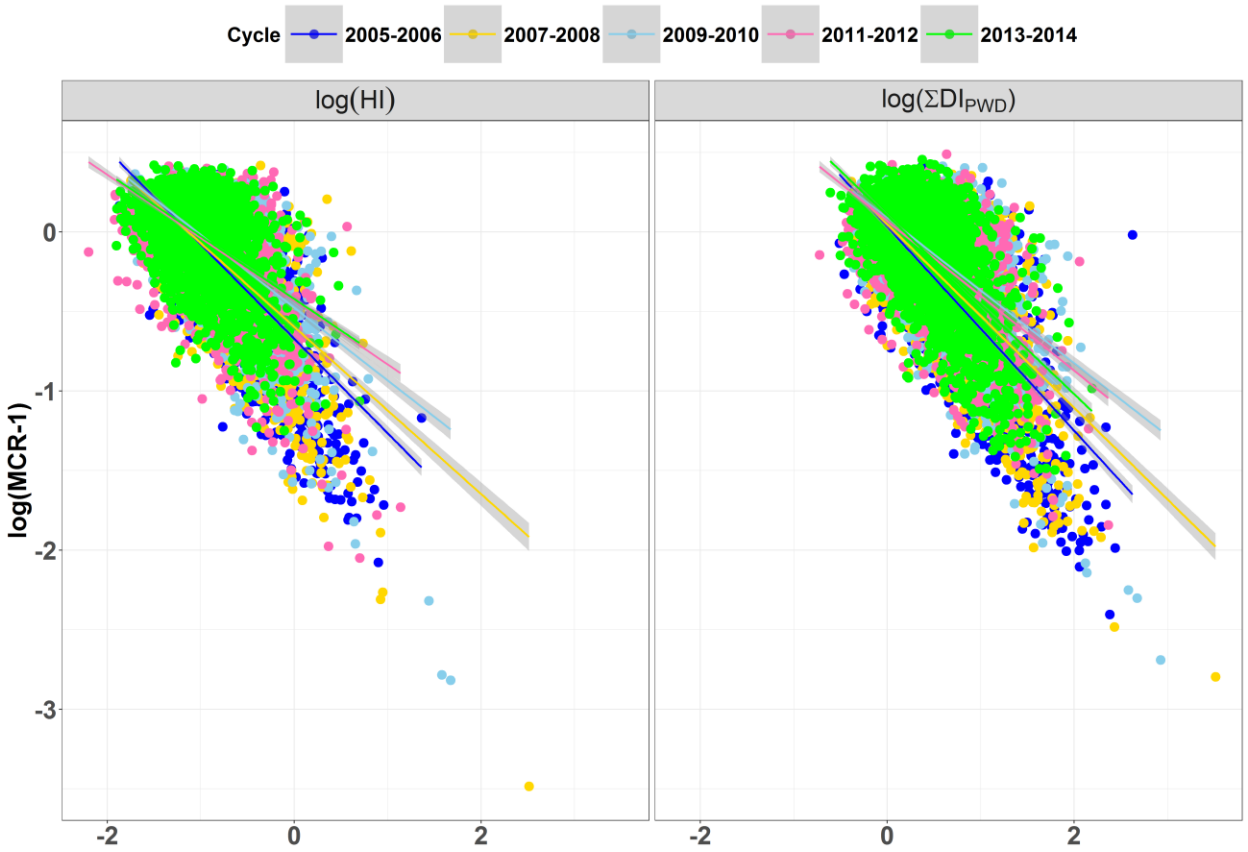

Figure S4. Plot of log Hazard Index (HI) versus $\log(MCR - 1)$ of six phthalates for all participants from 2005-2014 identified by corresponding NHANES cycle of the participant using the Tolerable Daily Intakes (left panel) and Potency-Weighted Dose (PWD) (right panel). Regression lines are presented by NHANES cycle. (See Table S10 for regression estimates.)

Table S1. Frequency at which each of the six phthalates produced the maximum Hazard Quotient (HQ_M) by Group among the NHANES participants across the cycles spanning from 2005-2014.

	HQ_M	2005-2006	2007-2008	2009-2010	2011-2012	2013-2014
Group I	DBP	8	13	14	7	0
	DEHP	103	62	33	8	3
	DIDP	0	1	0	1	1
	DINP	2	0	5	6	8
	BBP	0	0	0	0	0
	DIBP	0	0	0	0	0
	Total	**113**	**76**	**52**	**22**	**12**
Group II	DBP	944	1028	1174	743	958
	DEHP	1394	1373	1105	886	712
	DIDP	18	23	34	29	25
	DINP	31	52	343	757	947
	BBP	0	0	0	0	0
	DIBP	0	0	0	0	0
	Total	**2387**	**2476**	**2656**	**2415**	**2642**
Group IIIA	DBP	6	4	0	4	0
	DEHP	20	18	12	0	1
	DIDP	0	1	2	2	0
	DINP	4	1	2	10	5
	BBP	0	0	0	0	0
	DIBP	0	0	0	0	0
	Total	**30**	**24**	**16**	**16**	**6**
Group IIIB	DBP	0	2	0	0	1
	DEHP	0	1	0	1	1
	DINP	0	0	1	0	1
	BBP	0	0	0	0	0
	DIBP	0	0	0	0	0
	Total	**0**	**3**	**1**	**1**	**3**

Table S2. Number of participants in the NHANES data set presented by Cycle.

Cycle	Total participants with at least one phthalate metabolite measured	Participants missing height and/or weight	Additional participants missing at least one phthalate metabolite	Total number of participants used in the analysis
2005-2006	2565	18	17	2530
2007-2008	2623	25	19	2579
2009-2010	2755	24	6	2725
2011-2012	2527	35	38	2454
2013-2014	2691	21	7	2663

Table S3. Data on parent phthalates, metabolites, Tolerable Daily Intakes (TDIs), molecular weights of phthalates and their metabolites, and percentages of urinary excretions for NHANES.

Phthalate (Parent)	TDI (μg/kg-d)	MW_p (g/mole)	Metabolite	MW_m (g/mole)	daily F_{UE} (%)
di-n-butyl phthalate (DBP)	10[7]	278.34	monobutyl phthalate (MBP)[1,3]	222.24	84
diisobutyl phthalate (DIBP)	1250[8]	278.34	monoisobutyl phthalate (MIBP)[1,3]	222.24	70.3
butyl benzyl phthalate (BBP)	500[9]	312.36	monobenzyl phthalate (MBZP)[1,3]	256.25	73
di(2-ethylhexyl) phthalate (DEHP)	50[10]	390.56	mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)[1,3]	308.33	13.2
			mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)[1,3]	292.33	10.9
			mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)[1,3]	294.35	14.9
			mono(2-ethylhexyl) phthalate (MEHP)[1,3]	278.34	6.2
diisononyl phthalate (DINP)	150[11]	418.61	monoisononyl phthalate (MINP)[3]	292.37	3
			mono(carboxyoctyl) phthalate (MCOP)[1,3]	322.36	9.9
diisodecyl phthalate (DIDP)	130[12]	446.68	mono(carboxynonyl) phthalate (MCNP)[3]	336.38	9.9

Table S4. Group names, definition, and descriptions from the Hazard Index (HI), maximum Hazard Quotient (HQ_M), and Maximum Cumulative Ratio (MCR) values for each participant (reproduced from Table 2 of Vallotton and Price[13]).

Group	Total Hazard	Individual Chemical Hazard	MCR	Description
I	$HI > 1$	$HQ_M > 1$	---	The mixture presents a potential risk already based on individual components
II	$HI \leq 1$	$HQ_M \leq 1$	---	The assessment does not identify a concern
IIIA	$HI > 1$	$HQ_M \leq 1$	$MCR < 2$	The majority of the risk offered by the mixture is driven by one substance
IIIB	$HI > 1$	$HQ_M \leq 1$	$MCR \geq 2$	The potential risk is driven by multiple components

Table S5. Regressions used for the regression analysis[14] by NHANES cycle presented overall and by Age, Race/Ethnicity, and Gender. The following variables are categorical: Cycle (i.e. 2005-2006, 2007-2008, 2009-2010, 2011-2012, and 2013-2014), Age (i.e. 6-11, 12-19, and 20+), Gender (i.e. female and male), Race/Ethnicity (i.e. Mexican American, non-Hispanic White, and non-Hispanic Black), and Poverty Income Ratio (PIR) (i.e. <1, 1-3, and >3). Fasting is the number of whole hours since the participant last ate or drank anything other than water. Years is the participant's age in whole years.

Presentation	**Regression**
Overall	ln(HI)~Cycle
Age	ln(HI)~Cycle+Gender+Race/Ethnicity+PIR+Fasting+Cycle:Age
Race/Ethnicity	ln(HI)~Cycle+Years+Gender+PIR+Race/Ethnicity+Fasting+Cycle:Race/Ethnicity
Gender	ln(HI)~Cycle+Years+Race/Ethnicity+PIR+Fasting+Cycle:Gender

Table S6. Limits of detection and number (%) of samples below the limit of detection presented by cycle for each of the 10 metabolites.

	Limit of detection (ng/mL)					Number of samples (%) below the limit of detection				
Metabolite	2005-2006	2007-2008	2009-2010	2011-2012	2013-2014	2005-2006	2007-2008	2009-2010	2011-2012	2013-2014
MBZP (BBP)	0.216	0.216	0.216	0.3	0.3	29 (1.14)	46 (1.78)	13 (0.47)	44 (1.79)	63 (2.36)
MBP (DBP)	0.6	0.6	0.4	0.4	0.4	7 (0.27)	19 (0.73)	12 (0.44)	136 (5.54)	43 (1.61)
MEHP (DEHP)	1.2	1.1	0.5	0.5	0.8	789 (31.1)	850 (32.9)	615 (22.5)	568 (23.1)	1003 (37.6)
MEOHP (DEHP)	0.7	0.6	0.2	0.2	0.2	26 (1.02)	44 (1.70)	8 (0.29)	9 (0.36)	13 (0.48)
MECPP (DEHP)	0.6	0.5	0.2	0.2	0.4	1 (0.03)	2 (0.07)	1 (0.03)	7 (0.28)	6 (0.22)
MEHHP (DEHP)	0.7	0.7	0.2	0.2	0.4	10 (0.39)	20 (0.77)	2 (0.07)	6 (0.24)	19 (0.71)
MIBP (DIBP)	0.3	0.3	0.2	0.2	0.8	52 (2.05)	47 (1.82)	4 (0.14)	23 (0.93)	72 (2.70)
MCNP (DIDP)	0.6	0.5	0.2	0.2	0.2	225 (8.89)	247 (9.57)	30 (1.10)	18 (0.73)	32 (1.20)
MINP (DINP)	1.323	1.232	0.77	0.5	0.9	2181 (86.2)	2297 (89.0)	1672 (61.3)	1013 (41.2)	1586 (59.5)
MCOP (DINP)	0.7	0.7	0.2	0.2	0.3	101 (3.99)	94 (3.64)	5 (0.18)	0 (0)	3 (0.11)

Table S7. Pairwise Bonferroni comparison test of the Least Squares Geometric Mean (LSGM) of the Hazard Index (HI) within demographics across fixed NHANES cycles.

Demographic	Contrast	2005-2006	2007-2008	2009-2010	2011-2012	2013-2014
Gender	Male - Female	0.034	-0.107[b]	0.069	0.048	-0.049
Race/Ethnicity	Mexican American - Non-Hispanic White	-0.151[a]	-0.027	-0.042	-0.086	-0.061
	Mexican American - Non-Hispanic Black	-0.232[b]	-0.077	0.001	-0.154	-0.039
	Non-Hispanic White - Non-Hispanic Black	-0.081	-0.049	0.042	-0.068	0.021
Age	6-11 years - 12-19 years	0.298[b]	0.356[c]	0.357[c]	0.271[b]	0.433[c]
	6-11 years - 20+ years	0.430[c]	0.490[c]	0.466[c]	0.357[c]	0.585[c]
	12-19 years - 20+ years	0.132	0.134	0.109	0.086	0.152[a]

[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$

Table S8. Comparison of the frequency (%) of participants in Groups, Hazards Indices greater than one ($HI > 1$), and slope between Maximum Cumulative Ratio (MCR) and HI between the creatinine correction method with the Urinary Flow Rate (UFR) method from the last three NHANES Cycles.

	2009-2010		2011-2012		2013-2014	
	Creatinine	UFR	Creatinine	UFR	Creatinine	UFR
	Count (%) of participants in each Group					
Group I	47 (1.88)	38 (1.52)	21 (0.91)	30 (1.3)	10 (0.42)	20 (0.83)
Group II	2438 (97.52)	2439 (97.56)	2275 (98.48)	2255 (97.62)	2377 (99.21)	2361 (98.54)
Group IIIA	14 (0.56)	18 (0.72)	13 (0.56)	16 (0.69)	6 (0.25)	9 (0.38)
Group IIIB	1 (0.04)	5 (0.20)	1 (0.04)	9 (0.39)	3 (0.13)	6 (0.25)
Groups I and III (HI>1)	62 (2.48)	61 (2.44)	35 (1.52)	55 (2.38)	19 (0.79)	35 (1.46)
	Consistency of determination of HI>1					
Both HI>1	37 (1.48)		21 (0.91)		13 (0.54)	
Both HI<1	2414 (96.56)		2241 (97.01)		2355 (98.29)	
$\mathrm{HI_C} > 1, \mathrm{HI_{UFR}} \leq 1$	25 (1.00)		14 (0.61)		6 (0.25)	
$\mathrm{HI_C} \leq 1, \mathrm{HI_{UFR}} > 1$	24 (0.96)		34 (1.47)		22 (0.92)	
	log(MCR-1)/log(HI) Slope					
	-0.453	-0.306	-0.410	-0.262	-0.404	-0.259

Table S9. Summary of benchmark doses and relative potency factors needed to calculate a DBP potency-weighted daily intake dose.

Phthalate	Benchmark Dose	Relative Potency Factor
di-n-butyl phthalate (DBP)	30[a]	1.00[b]
diisobutyl phthalate (DIBP)	126[a]	0.24[b]
butyl benzyl phthalate (BBP)	116[a]	0.26[b]
di(2-ethylhexyl) phthalate (DEHP)	49[a]	0.61[b]
diisononyl phthalate (DINP)	---	0.26[b,c]
diisodecyl phthalate (DIDP)	---	0.024[d]

[a]NRC; [b]Vashavksy et al.; [c]Hannas et al.; [d]One tenth of lowest Relative Potency Factor

Table S10. Intercept and slope using a simple linear regression between log(HI) or log($\sum DI_{PWD}$) and log(MCR-1) and mean Maximum Cumulative Ratio (MCR) from either 1) a Hazard Index (HI) calculated by a Tolerable Daily Intake (TDI) value or 2) a dose summation using a potency-weighted approach presented by NHANES Cycle. All estimates are highly significant (i.e. $p < 0.001$).

	Intercept		**Slope**		**Mean MCR**	
Cycle	TDI	Potency-Weighted	TDI	Potency-Weighted	TDI	Potency-Weighted
2005-2006	-0.671	0.033	-0.594	-0.641	1.711	1.563
2007-2008	-0.596	0.048	-0.526	-0.576	1.767	1.636
2009-2010	-0.476	0.088	-0.457	-0.457	1.962	1.837
2011-2012	-0.435	0.070	-0.397	-0.471	1.996	1.800
2013-2014	-0.421	0.103	-0.395	-0.561	2.077	1.874

Additional File

"Reyes_EST_Phthalates_US_SI_data.xlsx" contains information in the following tabs:

1. "**data**" contains the following:
    - Data on the surveyed individuals from the original NHANES data files that includes
        - Identifying information for the individual;
        - Demographic information used in defining the creatinine levels and in investigating sub populations based on age, gender, race/ethnicity;
        - Concentration of phthalate metabolites and creatinine in urine.
    - Calculated values for each individual;
        - Daily intake of each of the six phthalates;
        - Hazard Quotients associated with each phthalate's daily intake;
        - Values of Hazard Index and the Maximum Cumulative Ratio.
2. "**definitions**" contains the definitions and units of the fields in "**data**".
3. "**metabolites**" contain information about the National Health and Nutrition Examination Survey (NHANES) metabolite code names along with Tolerable Daily Intake (TDI), molecular weight of the parent phthalate, molecular weight of the metabolite, and names and abbreviations of the corresponding metabolites and phthalates.

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