Report to the
U.S. Consumer Product Safety Commission
by the
CHRONIC HAZARD ADVISORY PANEL ON PHTHALATES
AND PHTHALATE ALTERNATIVES

July 2014

APPENDIX D

CUMULATIVE RISK

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS*

3β-HSD	3β-hydroxysteroid dehydrogenase
AA	antiandrogenicity; antiandrogenic
ADHD	attention deficit hyperactivity disorder
ADI	acceptable daily intake
AGD	anogenital distance
AGI	anogenital index
ASD	Autistic Spectrum Disorders
CRA	cumulative risk assessment
ASTDR	Agency for Toxic Substances and Disease Registry
ATBC	acetyl tributyl citrate
BASC-PRS	Behavior Assessment System for Children-Parent Rating Scales
BBP	butylbenzyl phthalate
BIBRA	British Industrial Biological Research Association
BMDL	benchmark dose (lower confidence limit)
BNBA	Brazelton Neonatal Behavioral Assessment
BRIEF	Behavior Rating Inventory of Executive Function
BSI	behavioral symptoms index
CBCL	Child Behavior Check List
CDC	Centers for Disease Control and Prevention, U.S.
CERHR	Center for the Evaluation of Risks to Human Reproduction
CHAP	Chronic Hazard Advisory Panel
CHO	Chinese hamster ovary
CNS	central nervous system
CPSC	Consumer Product Safety Commission, U.S.
CPSIA	Consumer Product Safety Improvement Act of 2008
CSL	cranial suspensory ligament
cx-MIDP	mono(carboxy-isononyl) phthalate (also, CNP, MCNP)
cx-MINP	mono(carboxy-isooctyl) phthalate (also COP, MCOP)
DBP	dibutyl phthalate
DCHP	dicyclohexyl phthalate
DEHA	di(2-ethylhexyl) adipate
DEHP	di(2-ethylhexyl) phthalate
DEHT	di(2-ethylhexyl) terephthalate
DEP	diethyl phthalate
DHEPP	di-n-heptyl phthalate
DHEXP	di-n-hexyl phthalate
DHT	dihydrotestosterone
DI	daily intake
DIBP	diisobutyl phthalate
DIDP	diisodecyl phthalate

* List applies to main report and all appendices.

DIHEPP	diisoheptyl phthalate
DIHEXP	diisohexyl phthalate
DINP	diisononyl phthalate
DINCH®	1,2-cyclohexanedicarboxylic acid, diisononyl ester
DINX	1,2-cyclohexanedicarboxylic acid, diisononyl ester
DIOP	diisooctyl phthalate
DMP	dimethyl phthalate
DNHEXP	di-n-hexyl phthalate
DNOP	di-n-octyl phthalate
DPENP	di-n-pentyl phthalate
DPHP	di(2-propylheptyl) phthalate
DPS	delayed preputial separation
DSP	decrease spermatocytes and spermatids
DVO	delayed vaginal opening
ECHA	European Chemicals Agency
ECMO	extracorporeal membrane oxygenation
ED50	median effective dose
EPA	Environmental Protection Agency, U.S.
EPW	epididymal weight
FDA	Food and Drug Administration, U.S.
fue	urinary excretion factor
GD	gestational day
GGT	gamma-glutamyl transferase
GLP	good laboratory practices
grn	granulin
HBM	human biomonitoring
hCG	human chorionic gonadotrophin
HI	hazard index
HMW	high molecular weight
HQ	hazard quotient
IARC	International Agency for Research on Cancer
ICH	International Conference on Harmonisation
insl3	insulin-like factor 3
IP	intraperitoneally
LD	lactation day
LH	luteinizing hormone
LMW	low molecular weight
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantitation
MBP	monobutyl phthalate
MBZP	monobenzyl phthalate
MCPP	mono(3-carboxypropyl) phthalate
MDI	mental development index
MECPP	mono(2-ethyl-5-carboxypentyl) phthalate

MEHP	mono(2-ethylhexyl) phthalate
MEHHP	mono(2-ethyl-5-hydroxyhexyl) phthalate
MEOHP	mono(2-ethyl-5-oxohexyl) phthalate
MEP	monoethyl phthalate
MIBP	monoisobutyl phthalate
MINP	mono(isononyl) phthalate
MIS	Mullerian inhibiting substance
MMP	monomethyl phthalate
MNG	multinucleated gonocyte
MNOP	mono-n-octyl phthalate
MOE	margin of exposure
MSSM	Mount Sinai School of Medicine
MW	molecular weight
NA	not available
NAE	no antiandrogenic effects observed
NHANES	National Health and Nutritional Examination Survey
NNNS	NICU Network Neurobehavioral Scale
NOAEL	no observed adverse effect level
NOEL	no observed effect level
NR	nipple retention
NRC	National Research Council, U.S.
NTP	National Toxicology Program, U.S.
OECD	Organisation for Economic Cooperation and Development
OH-MIDP	mono(hydroxy-isodecyl) phthalate
OH-MINP	mono(hydroxy-isononyl) phthalate
OR	odds ratio
oxo-MIDP	mono(oxo-isodecyl) phthalate
oxo-MINP	mono(oxo-isononyl) phthalate
PBR	peripheral benzodiazepine receptor
PDI	psychomotor developmental index
PE	phthalate ester
PEAA	potency estimates for antiandrogenicity
PND	postnatal day
PNW	postnatal week
POD	point of departure
PODI	point of departure index
PPARα	peroxisome proliferator-activated receptor alpha
PPS	probability proportional to a measure of size
PSU	primary sampling unit
PVC	polyvinyl chloride
RfD	reference dose
RTM	reproductive tract malformation
SD	Sprague-Dawley
SDN-POA	sexually dimorphic nucleus of the preoptic area
SFF	Study for Future Families

SHBG	sex-hormone binding globulin
SR-B1	scavenger receptor class B1
SRS	social responsiveness scale
StAR	steroidogenic acute regulatory protein
SVW	seminal vesicle weight
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TDI	tolerable daily intake
TDS	testicular dysgenesis syndrome
TEF	toxicity equivalency factors
TOTM	tris(2-ethylhexyl) trimellitate
TPIB	2,2,4-trimethyl-1,3 pentanediol diisobutyrate
T PROD	testosterone production
TXIB®	2,2,4-trimethyl-1,3 pentanediol diisobutyrate
UF	uncertainty factor

1 Estimated Exposure of Phthalates Using Biomonitoring Data and Cumulative Risk Evaluation Using the Hazard Index

Biomonitoring data have provided evidence of complex human exposures to mixtures of phthalates and other antiandrogens. In the case of phthalates, urinary concentrations of phthalates monoesters (metabolites of the parent diesters) are measured through biomonitoring. These monoesters demonstrate exposure to multiple phthalates. Through calculations based on human metabolism studies, estimates of daily intake from the parent phthalate diesters can be estimated. However, the source(s) and route(s) of the exposure are impossible to determine from biomonitoring data alone.

The first objective of this appendix is to use biomonitoring data to estimate daily intake values for multiple phthalates in adult men and women of reproductive age (15–45 yrs). These are produced for comparison to the estimates from data from pregnant women and infants to estimate daily exposure to phthalates and compare these estimates to those determined through exposure assessment modeling (Chronic Health Advisory Panel [CHAP] report, Section 2.6). Two data sources were used to evaluate exposures in adults and pregnant women:

> (1) the National Health and Nutrition Examination Surveys (NHANES, 2005–2006, CDC, 2012b), and
>
> (2) the Study for Future Families (SFF; Sathyanarayana *et al.*, 2008a; 2008b) with prenatal and postnatal measurements in women.

The SFF data also include concentrations from infants (age: 2–36 months).

We included in our analyses the six phthalates under consideration by the Consumer Product Safety Improvement Act (CPSIA):

- di(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), and butylbenzyl phthalate (BBP): banned chemicals; and
- diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di-*n*-octyl phthalate (DNOP): chemicals with interim prohibition on their use.

Because diisobutyl phthalate (DIBP) is also known to be antiandrogenic (comparable to DBP), we included it in the analysis. However, exposure estimates for DNOP were not available in the SFF data and were generally not detectable in NHANES. Thus, DNOP was dropped from further consideration.

Although pregnant women and infants are exposed to DIDP, diethyl phthalate (DEP), and dimethyl phthalate (DMP) as evidenced from biomonitoring studies, evidence of endocrine disruption in experimental animal studies has not been found for these three chemicals. Thus, these three phthalates were not considered in the cumulative risk evaluation.

We used a novel approach for cumulative risk evaluation of these phthalates by calculating the hazard index (HI) per individual (*i.e.,* pregnant woman and infant) based on their urinary concentrations of mixtures of phthalates. This is in contrast to the standard HI method of using population percentiles from exposure studies on a per chemical basis. The HI is used in cumulative risk assessment of chemical mixtures based on the concept of dose-addition (Teuschler and Hertzberg, 1995).

It is the sum of hazard quotients (HQs) defined as the ratio of exposures (*e.g.,* estimate of daily intake [DI]) to intakes deemed acceptable for a specific chemical for the same period of time (*e.g.,* daily). In practical applications of the HI approach, acceptable daily intakes (ADI) and other values used in a regulatory context have been used as the denominator of HQs. Sometimes, ADIs derived from different critical toxicities were used to calculate HI for combinations of substances.

However, in adapting the HI approach for cumulative risk assessments for phthalates, the CHAP faced the following difficulties: Having defined male developmental and reproductive toxicity via an antiandrogenic mode of action as the critical effect, the CHAP deemed it as important to use such responses as the basis for cumulative risk assessments. However, ADIs or reference doses (RfDs) of similar quality based on antiandrogenicity do not exist for all phthalates of interest. Some key toxicological studies that characterized these effects were not intended to derive points of departure (POD, *i.e.,* no observed adverse effect levels [NOAELs] or benchmark dose [BMDLs]), which can form the basis for ADIs. To deal with this difficulty, the CHAP used established health benchmarks (*e.g.,* the RfDs of the U.S. EPA; ADIs of the Consumer Product Safety Commission [CPSC]) as input values for the denominator of HQs. In certain cases it was necessary to fall back on NOAELs for antiandrogenicity endpoints in *in vivo* studies. These were then combined with uncertainty factors to obtain the required input values, here termed potency estimates for antiandrogenicity (PEAA) for the mathematical expression of the HI approach:

$$Hazard\ Quotient\ (HQ_j) = \frac{DI_j\ (\mu g / kg - day)}{PEAA_j\ (\mu g / kg - day)} \qquad (1)$$

and

$$\text{Hazard Index (HI)} = \sum_{j=1}^{c} HQ_j \qquad (2)$$

where: j is the number of chemicals in the index.

The HI offers flexibility in applying different uncertainty factors when defining PEAA values for the individual substances. For the purposes of this analysis, the requirement was made to consider only endpoints with relevance to antiandrogenicity when defining PEAA values. The CHAP wishes to emphasize that the PEAA values used for the HI approach should not be confused with RfDs or ADIs that are used in a regulatory context. The PEAA values have a

purpose solely in cumulative risk assessment. They do not indicate "bright lines" that distinguish risk from absence of risk.

We include three cases for comparison of the impact of assumptions in calculating the HI:

Case 1: using PEAA values as published in Kortenkamp and Faust (2010);

Case 2: using PEAA values derived from data provided by Hannas *et al*., (2011a; 2011b); and

Case 3: using PEAA values from *de novo* analysis of individual phthalates conducted by CHAP (Section 2.3.2).

The PEAA values in these cases were derived from *in vivo* evidence of reproductive or developmental effects in pregnant animals. Less is known about the PODs for infants. However, there is evidence that the most sensitive time of exposure is *in utero*, so PEAAs associated with reproductive or developmental effects in pregnant women should be protective for infants.

2 Estimating Exposure from Biomonitoring Data in Pregnant Women and Infants

2.1 Methods

2.1.1 Calculation of Daily Intake

Following Koch *et al.* (2007), we calculated the daily intake of each parent chemical separately per adult and child. The model for daily intake includes the creatinine-related metabolite concentrations together with reference values for the creatinine excretion (David, 2000) in the following form:

$$DI(\mu g/kg_{bw}/day) = \frac{UE_{sum}(\mu mole/g_{crt}) \times CE(mg_{crt}/kg/day)}{F_{UE} \times (1000 mg_{crt}/g_{crt})} \times MW_{parent}(g/mole) \qquad (3)$$

where:

- UE_{sum} is the molar urinary excretion of the respective metabolite(s) as described.
- CE is the creatinine excretion rate normalized by body weight, which was calculated based on equations using gender, age, height, and race (Mage *et al*., 2008).[2] In the SFF data, height was not measured for prenatal and postnatal women; for these women, a fixed value of CE was used based on the following logic:

[2]When height was outside the tabulated range for gender and age categories or when weight was missing, CE was considered missing.

- A rate of 18 mg/kg-d for women is used in the general population (Harper *et al.,* 1977; Kohn *et al.,* 2000).
- Creatinine excretion on average increases by 30% during pregnancy (Beckmann *et al.,* 2010). Thus, we set CE to 23 mg/kg-d for these SFF women, a 30% increase from 18.

- The molar fraction F_{ue} describes the molar ratio between the amount of metabolite(s) excreted in urine and the amount of parent compound taken up. Values for these fractions are given in Table D-1.

- The molecular weights for each parent compound and metabolite(s) are also given in Table D-1.

2.1.2 Inference from NHANES Data to U.S. Population: Use of Survey Sampling Weights (CDC, 2012a; CDC, 2012b)

NHANES data are *not* obtained using a simple random sample. Rather, a complex, multistage, probability sampling design is used to select participants representative of the civilian, non-institutionalized U.S. population. The sample does not include persons residing in nursing homes, members of the armed forces, institutionalized persons, or U.S. nationals living abroad.

The NHANES sampling procedure consists of four stages.

- Stage 1: Primary sampling units (PSUs) are selected (*e.g.,* 15 PSUs per year) from a sampling frame that includes all counties in the United States. These are mostly single counties or, in a few cases, groups of contiguous counties with probability proportional to a measure of size (PPS).

- Stage 2: The PSUs are divided up into segments (generally city blocks or their equivalent). As with each PSU, sample segments are selected with PPS.

- Stage 3: Households within each segment are listed, and a sample is randomly drawn. In geographic areas where the proportion of age, ethnic, or income groups selected for oversampling is high, the probability of selection for those groups is greater than in other areas.

- Stage 4: Individuals are chosen to participate in NHANES from a list of all persons residing in selected households. Individuals are drawn at random within designated age-sex-race/ethnicity screening subdomains. On average, 1.6 persons are selected per household.

Based on this complex sampling design, a sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person in NHANES, reflecting the unequal probability of selection, nonresponse adjustment, and adjustment to independent population controls. The recommended and most reliable approach for estimating summary statistics for resulting data from NHANES is to use survey procedures that account for the strata (*i.e.,* PSUs) and the clusters (*i.e.,* households selected within each strata) in

addition to the weight on each subject (*e.g.,* ProcSurvey Means in SAS). Alternative approaches that only weight individuals based on their sample weight provide rough approximate estimates of summary statistics, but not their standard errors. Based on software constraints, the population percentiles presented herein in tabular form have been generated using survey procedures that account for the complex design. Summary statistics included as insets, box plots, and histograms provide rough approximations to the percentiles and distributions.

Table D-1 Molecular weights for parent compounds and metabolites. Excretion fractions (F_{ue}) of parent metabolite(s) in human urine related to the ingested amount of the parent compound determined 24 hours after oral application (adapted from Wittassek *et al.*, 2007; Anderson *et al.*, 2011).

Phthalate Diesters	Abbreviation (as denoted in NHANES when different)	Molecular weight	Comment	
a) Dimethyl phthalate	DMP	194		
b) Diethyl phthalate	DEP	222		
c) Diisobutyl phthalate	DIBP	278		
d) Di-*n*-butyl phthalate	DBP	278	BANNED	
e) Butylbenzyl phthalate	BBP	312		
f) Di(2-ethylhexyl) phthalate	DEHP	391		
g) Di-*n*-octyl phthalate	DNOP	391	INTERIM BANNED	
h) Diisononyl phthalate	DINP	419		
i) Diisodecyl phthalate	DIDP	447		
Phthalate Monoesters (%>LOD in U.S. population; NHANES, 2005–06)	**Abbreviation (as denoted in NHANES when different)**	**Molecular weight**	**Excretion Factor (F_{ue})**	
a) Mono *n*-methyl phthalate (41%)	MNM	180	69%[a]	
b) Monoethyl phthalate (>99%)	MEP	194	69%[a]	
c) Mono-iso-butyl phthalate (98%)	MIBP (MIB)	222	69%	
d) Mono-n-butyl phthalate (>99%)	MBP	222	69%	
e) Monobenzyl phthalate (98%)	MBZP (MZP)	256	73%	
f) Mono(2-ethylhexyl) phthalate (67%)	MEHP (MHP)	278	6.2%	45.2%
Mono(2-ethyl-5-hydroxyhexyl) phthalate (>99%)	MEHHP (MHH)	294	14.9%	
Mono(2-ethyl-5-oxohexyl) phthalate (99%)	MEOHP (MOH)	292	10.9%	
Mono(2-ethyl-5-carboxypentyl) phthalate (>99%)	MECPP (ECP)	308	13.2%	
g) Mono-*n*-octyl phthalate (1%)	MOP	278	omitted	
h) Mono-(carboxyisooctyl) phthalate (95%)	cx-MINP (COP)	322	9.9%	

Phthalate Diesters	Abbreviation (as denoted in NHANES when different)	Molecular weight	Comment
i) Mono-(carboxyisononyl) phthalate (90%)	cx-MIDP (CNP)	336	4%

[a] Set to 69% to be similar to DBP and MBP.

2.1.3 Analysis of Biomonitoring Data from Adults (NHANES, 2005–2006)

There were 1181 men and women of reproductive age (*i.e.,* 15–45 years) in NHANES 2005–2006 in which urinary phthalate monoesters were measured with nonmissing values for height, weight, urinary creatinine, and the sampling weight variable (*i.e.,* wtsb2yr). Using the sampling weights corresponding to this subset of participants, these adults represent 124 million non-institutionalized Americans with roughly equal representation for men (50%) and women (50%). Sixty-four percent are non-Hispanic white; 13% are non-Hispanic black; 12% are Mexican American; 4% are "other" Hispanic; and 7% "other race" including multiracial.

Daily intake was estimated for the eight phthalate diesters for men and women of reproductive age (Figure D-1; approximately adjusted by survey sampling weights). Using the survey sampling weights, these percentiles are generalizable to the adult U.S. population of reproductive age (Table D-2). The median exposure estimate for DEHP was the highest, followed by DEP (Table D-2). DMP has the lowest median daily intake estimate.

Figure D-1 Box plots for daily intake for ages 15–45 yrs (NHANES, 2005–06).

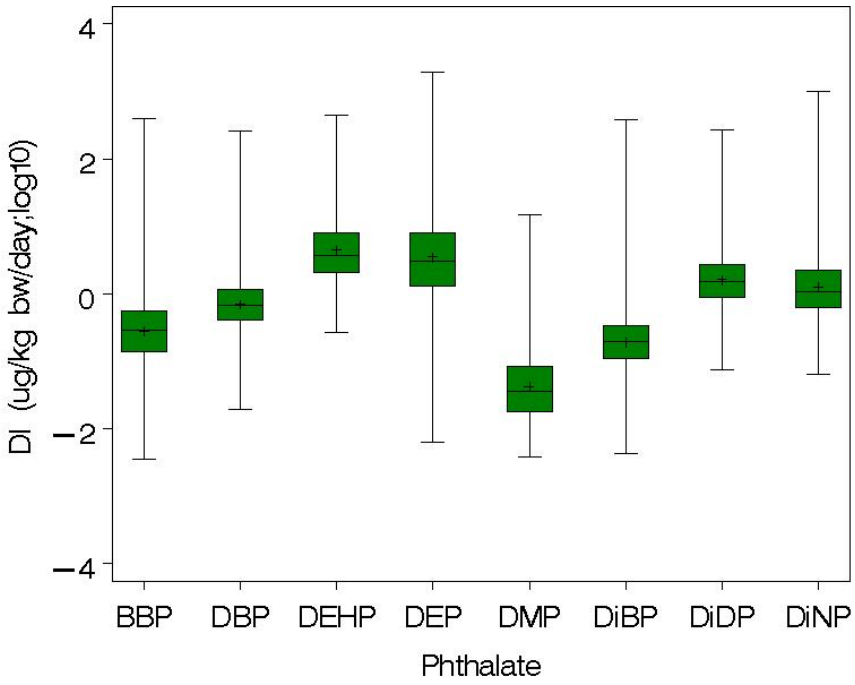


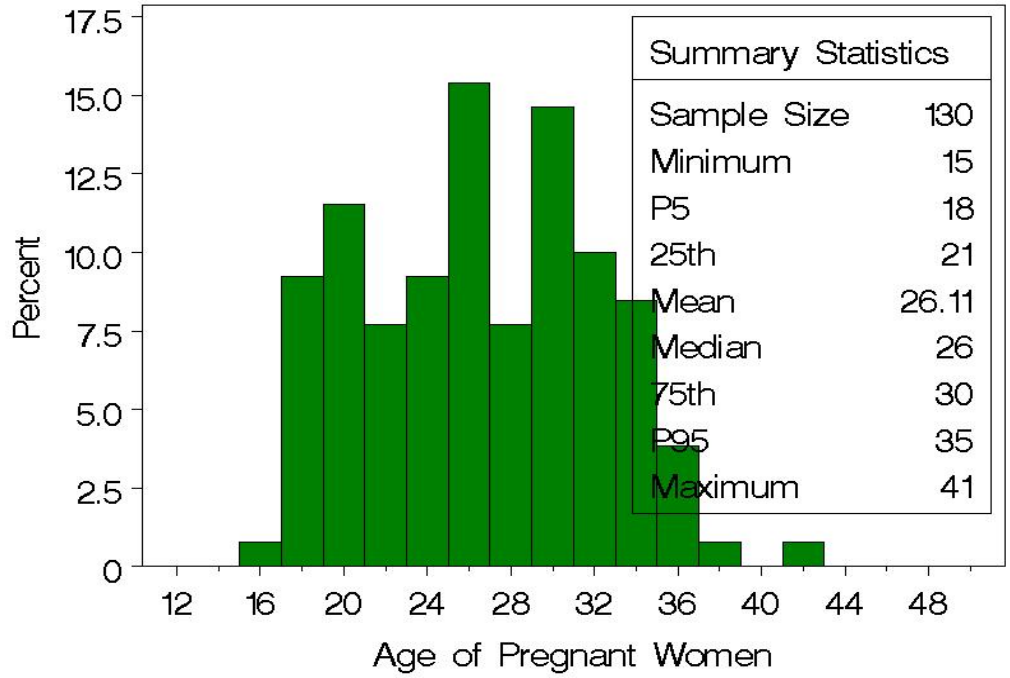
Table D-2 Summary statistics for estimated daily intake of phthalate diesters in adults of reproductive age (ages:15–45 yrs) from NHANES (2005–06) and SFF (prenatal, postnatal, and infants) biomonitoring data, estimated from exposure modeling (Wormuth *et al.*, 2006) and as given in Kortenkamp and Faust (2010).

Daily Intake Estimates (µg/kg - d)	BBP[a]	DBP	DEHP	DEP[b]	DMP	DiBP	DiDP	DiNP
Median Estimates from Biomonitoring Data (NHANES, 2005–06; 15<=Age<=45) (CDC, 2012b)								
Adults (represents 123M)	0.29	0.66	3.8	3.3	0.03	0.19	1.5	1.1
Pregnant Women (represents 5M)	0.30	0.63	3.5	3.4	0.05	0.17	1.5	1.0
99th Percentile Estimates from Biomonitoring Data (NHANES, 2005–06; 16<=Age<=45) (CDC, 2012b)								
Adults	2.5	5.5	203	118	0.80	1.9	19	35
Pregnant Women	2.7	6.4	366	357	0.68	2.0	11	27
Median Estimates from Biomonitoring Data (Sathyanarayana *et al.*, 2008a)								
Prenatal	0.51	0.88	2.9	6.6	0.06	0.15	2.3	1.1
Postnatal	0.44	0.62	2.7	3.7	0.06	0.14	1.7	0.63
Infants	1.2	1.7	5.5	4.8	0.12	0.31	6.0	3.5
99th Percentile Estimates from Biomonitoring Data (Sathyanarayana *et al.*, 2008a)								
Prenatal	4.2	5.1	69	307	0.67	1.7	28	7.6
Postnatal	4.1	4.7	45	171	0.60	1.8	68	8.1
Infants	22	13	110	217	2.1	2.9	70	24
Average Estimates from Exposure Modeling (Wormuth *et al.*, 2006)								
Adults	0.31	3.5	1.28	1.28		0.44		0.00
Women	0.28	3.5	1.40	1.40		0.42		0.004
Upper bound Estimates from Exposure Modeling (Wormuth *et al.*, 2006)								
Adults	1.8	28	58	58		1.5		0.28
Women	1.7	38	66	66		1.5		0.28
Median Intake Estimates from Kortenkamp and Faust (2010)								
German population	0.3	2	2.7			1.5		0.6
High Intake Estimates from Kortenkamp and Faust (2010)								
U.S. population	4	6	3.6			1.5		1.7

2.1.4 Analysis of Biomonitoring Data from Pregnant Women (NHANES, 2005–2006)

Pregnancy status was evaluated in females 8–59 years of age in the NHANES study. Menstruating girls 8–11 years of age and all females 12 years and over received a urine pregnancy test. If the respondent reported she was pregnant at the time of the exam, she was assumed to be pregnant regardless of the result of the urine pregnancy test.Three-hundred-eighty-two women were coded as pregnant at the time of the exam. Of these, 130 women were included in the subsample in which phthalates were evaluated with nonmissing values for height, weight, urinary creatinine, and the sampling weight. The age distribution for these women is presented in Figure D-2.

Figure D-2 Age distribution for pregnant women evaluated for phthalate exposure (NHANES, 2005–06).



Using survey-sampling weights, these 130 pregnant women are representative of 5M pregnant women in the non-institutionalized U.S. population. These are estimated to have the following characteristics:

- Marital status: 71% married, 1% divorced, 2% separated, 15% never married, 11% living with partner;
- Ethnicity/race: 27% Mexican American, 2% other Hispanic, 53% non-Hispanic white, 13% non-Hispanic black, 5% other plus multi-race; and
- Education: 5% <9th grade, 17% 9–12th grades, 15% high school graduate, 25% some college, and 38% college graduate or above.

The internal exposure for the eight phthalate diesters was estimated, and the percent from each diester per pregnant woman was calculated. The median exposure estimates for DEP and DEHP were the largest of the phthalate diesters evaluated. The mixture of phthalate diesters is different in each subject; box plots for the distributions of percentages of the mixture for each diester (calculated from the sum) per subject are provided in Figure D-3. DEP and DEHP have the largest median percentage of the mixtures. The estimated daily intakes have a complex bivariate correlation structure (Table D-3). Two clusters with significant positive correlations are (1) low molecular weight phthalates: DBP, DIBP, BBP, and (2) high molecular weight phthalates: DEHP, DINP, and DIDP.

Figure D-3 Summary statistics for the distributions of the percentage of each diester in the sum of diesters per pregnant woman (NHANES, 2005–06).

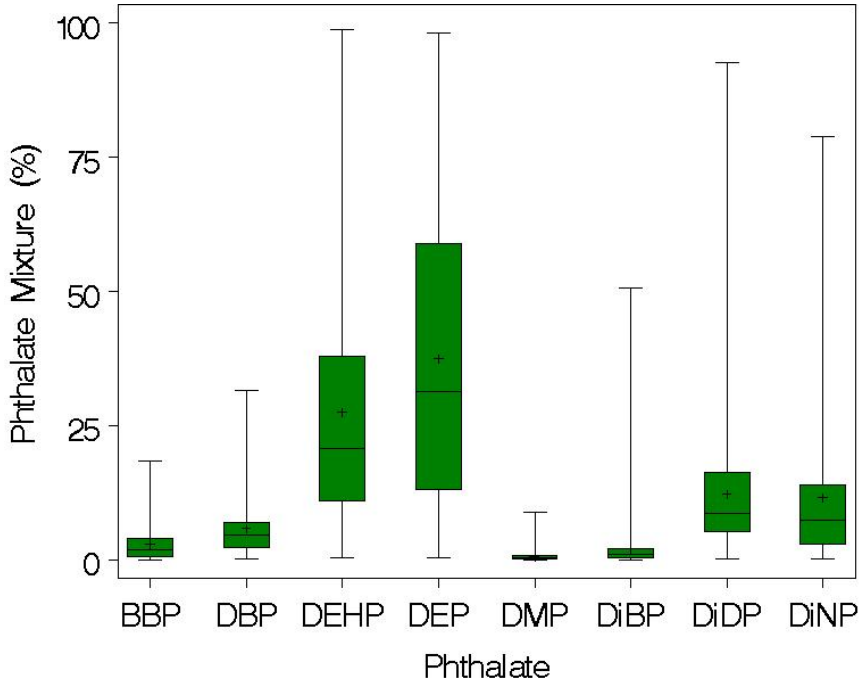


Table D-3 Pearson correlation coefficient estimates between estimated daily intakes of the eight phthalate diesters (log 10 scale) for pregnant women in NHANES (2005–06, representing 5.3 million pregnant women).

Estimate	DMP	DEP	DIBP	DBP	BBP	DEHP	DINP	DIDP
DMP	1	0.20	-0.02	-0.19	-0.05	-0.11	0.03	0.09
DEP	0.20*	1	0.12	0.12	0.04	-0.17	-0.06	0.14
DIBP	-0.02	0.12	1	0.59*	0.38*	-0.13	-0.04	0.12
DBP	-0.19	0.12	0.59*	1	0.59*	-0.05	0.17	0.15
BBP	-0.05	-0.04	0.38*	0.59*	1	-0.06	0.17	0.23*
DEHP	-0.11	-0.17	-0.13	-0.05	-0.06	1	0.40*	0.26*
DINP	0.03	-0.06	-0.04	0.17	0.17	0.40*	1	0.52*
DIDP	0.09	0.14	0.12	0.15	0.23*	0.26*	0.52*	1

* $p<0.01$; highlighted.

3 Analysis of SFF Data

Exposure data from the SFF in young children and their mothers were provided to the CHAP by Dr. Shanna Swan and are published in Sathyanarayana *et al*. (2008a). The study included prenatal and postnatal evaluation of phthalates in pregnant women and their babies. Measurements were available in four centers across the United States, including in California (n=61), Missouri (n=84), Minnesota (n=112), and Iowa (n=34). Urinary concentrations from 12 monoesters were evaluated (Table D-4) that are generally specific to 8 phthalate diesters. Although mono-3-carboxyprobyl phthalate was measured, it was considered not specific to a single phthalate; thus, a monoester specific for DNOP was not available.

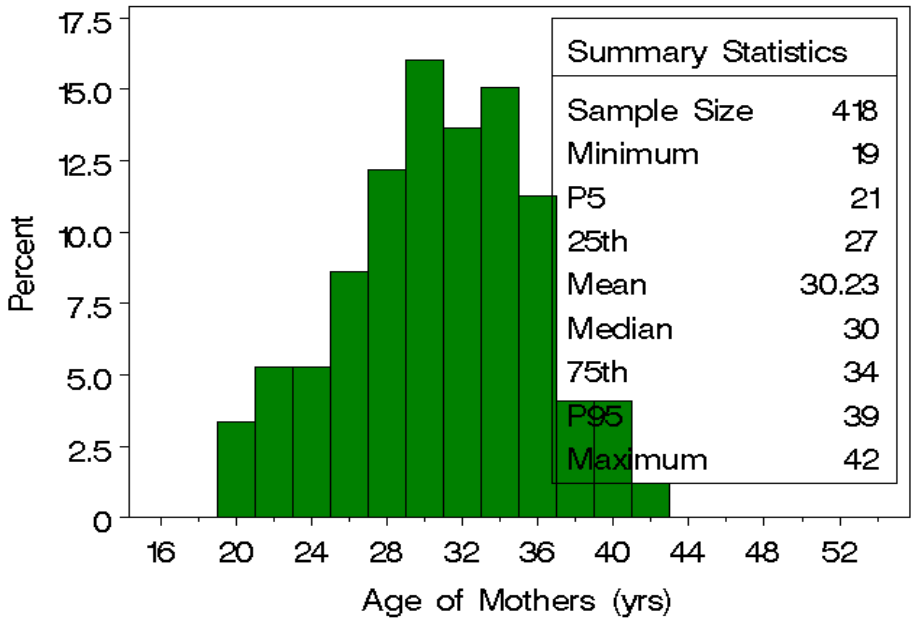
Table D-4 Phthalate monoesters evaluated by Sathyanarayana *et al*. (2008a).

Abbreviation	NHANES Variable	Monoester	Phthalate Diester(s)
mBP	urxmbp	Mono-*n*-butyl phthalate	DBP
mBzP	urxmzp	Monobenzyl phthalate	BBP
mCPP	urxmc1	Mono-3-carboxypropyl phthalate	DNOP and others
mEHHP	urxmhh	Mono(2-ethyl-5-hydroxyhexyl) phthalate	DEHP
mEHP	urxmhp	Mono(2-ethylhexyl) phthalate	DEHP
mEOHP	urxmoh	Mono(2-ethyl-5-oxohexyl) phthalate	DEHP
mECPP	urxecp	Mono(2-ethyl-5-carboxypentyl) phthalate	DEHP
mEP	urxmep	Monoethyl phthalate	DEP
mMP	urxmnm	Monomethyl phthalate	DMP
miBP	urxmib	Monoisobutyl phthalate	DIBP
mCNP	urxcnp	Mono(2,7-dimethyl-7-carboxyheptyl) phthalate	DIDP
mCOP	urxcop	Mono(2,6-dimethyl-6-carboxyhexyl) phthalate	DINP

3.1 Analysis of Prenatal and Postnatal Measurements in Women

Either or both prenatal and postnatal measurements were made in 418 pregnant women; 340 women had prenatal measurements and 335 had postnatal measurements. The median age for the mothers was 30 years, and their ages ranged between 19 and 42 (Figure D-4).

Figure D-4 Histogram for age of pregnant women with either prenatal or postnatal measurements (Sathyanarayana *et al.*, 2008a).



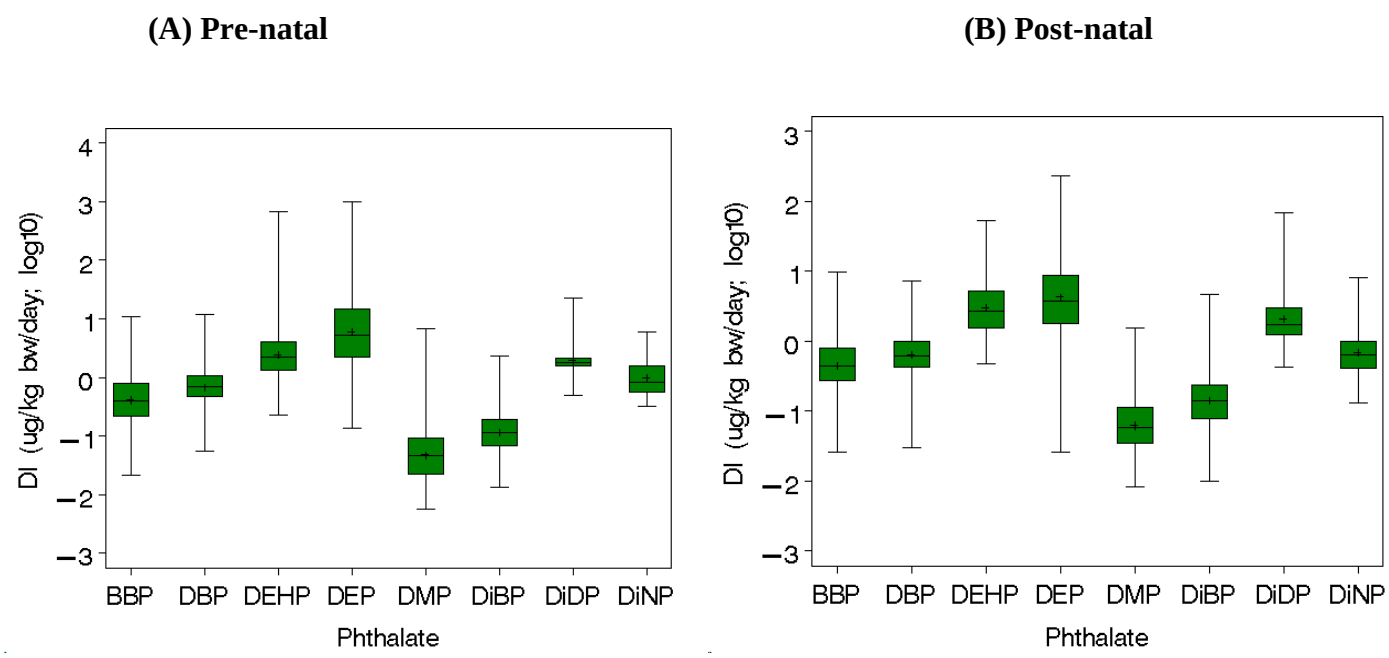
From the phthalate monoester measurements, diester values were calculated using the method of David (2000) and Koch *et al*. (2007). Box plots across the phthalates for prenatal and postnatal estimates are provided in Figure D-5. DEP and DEHP have the highest median estimates for both cases. Table D-2 provides 50th and 99th percentiles for each diester across the three measurements (*i.e.,* NHANES; SFF prenatal; SFF postnatal). The exposure distributions are generally quite similar. The SFF prenatal estimate for DEHP is slightly lower than the other two, and the distribution for DIDP in NHANES is slightly lower compared to the SFF data. However, these possible shifts are within the interquartile ranges of the comparison groups. Bivariate correlations for these estimates are provided in Table D-5. Significant correlations between prenatal and postnatal measurements of the estimated daily intake were detected for DBP, DIBP, BBP, and DIDP.

Table D-5 Pearson correlation estimates (*p<0.05 and highlighted) for estimated daily intake values (log 10 scale) for prenatal and postnatal values from N=258 women except for DINP and DIDP where N=18. There were no postnatal DMP or DEP estimates with prenatal values.

Pre\ Post	DMP	DEP	DIBP	DBP	BBP	DEHP	DINP*	DIDP*
DMP			0.12	0.09	0.06	0.04		
DEP			0.02	0.05	0.03	-0.06	0.51*	0.22
DIBP			0.15	0.06	0.05	0.06	0.28	0.13
DBP			0.07	0.13*	0.13*	0.00	0.31	0.06
BBP			-0.10	-0.05	0.29*	0.08	0.23	-0.08
DEHP			-0.03	0.01	0.02	0.11	0.40	0.51*
DINP*			0.41	0.31	0.07	0.08	0.11	0.42
DIDP*			0.44	0.40	0.11	0.02	0.13	0.66*

Significant associations are highlighted in yellow.

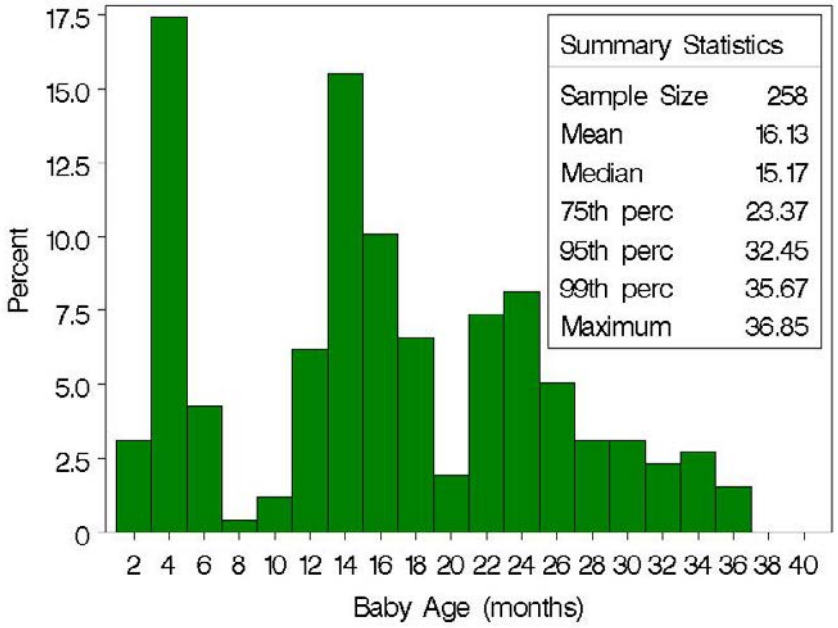
Figure D-5 Box plots across estimates of daily intake for (A) prenatal and (B) postnatal estimates.



3.2 Analysis of Infant Data

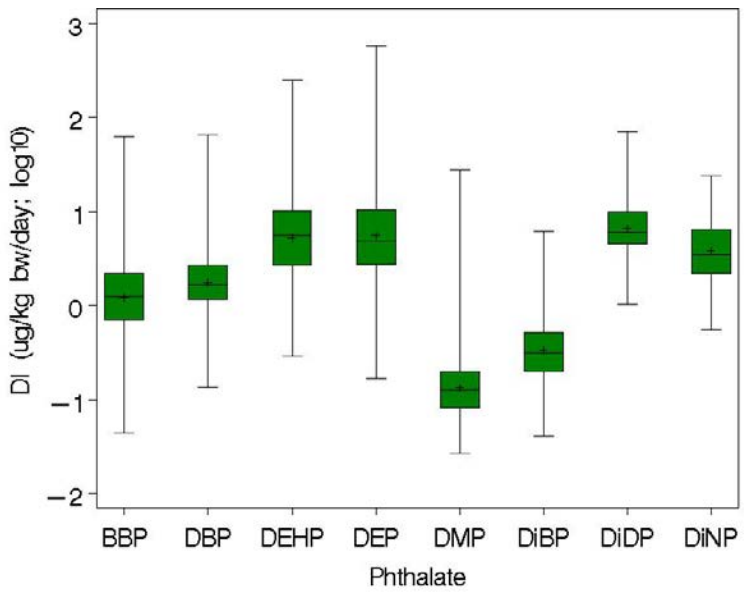
Phthalate monoesters were evaluated in 258 infants, ages 0–37 months (Figure D-6) in which daily intake can be estimated; 49% (n=127) of the babies were boys. At least one of the monoesters was detected in all babies, and seven monoesters were detected in at least 95% of the babies (Table D-6). To estimate the internal exposure for the phthalate diesters, the creatinine excretion rate was calculated using equations from Mage *et al*. (2008) based on age, gender, height, and race.

Figure D-6 Age distribution for infants evaluated by Sathyanarayana *et al*. (2008a).



Using the urinary concentrations from the 11 monoesters, the internal exposure to DBP, BBP, DEHP, DIBP, DIDP, DINP, DEP, and DMP were estimated in these infants (Table D-2). The median estimate for DEP was the highest of the eight evaluated followed by DEHP (Figure D-7).

Figure D-7 Box plots for daily intake estimates for infants from the SFF study.



Pearson correlation estimates between baby estimates for daily intake and those from the prenatal and postnatal estimates in the mothers are provided in Table D-7. The prenatal estimates for daily intake of BBP and DEP are positively correlated with that measured in the babies, with a correlation estimate of 0.31 ($p<0.001$) and 0.15 ($p=0.044$), respectively. The correlations between postnatal and baby daily intake estimates are positive and significant for DEP (0.35; $p=0.005$), DIBP (0.43; $p<0.001$), BBP (0.35; $p<0.001$), DEHP (0.35; $p<0.001$), DINP (0.26; $p=0.043$), and DIDP (0.43; $p<0.001$).

Table D-6 Percent above the limit of detection (LOD) in samples from the babies.

Abbreviation	% >LOD
MBP	99%
MBzP	96%
MEHHP	94%
MEHP	67%
MEOHP	96%
MECPP	100%
MEP	99%
MMP	64%
MiBP	88%
MCNP	96%
MCOP	96%

Table D-7 Pearson correlation estimates (* p<0.05; highlighted) for estimated daily intake values (log 10 scale) for prenatal and postnatal values with daily intake values estimated in their babies. In the prenatal values, N=191 except for DINP and DIDP where N=0; in the postnatal values N=251 except for DINP and DIDP where N=62, DEP where N=62, and DMP where N=181.

	DMP (p value)	DEP (p value)	DIBP (p value)	DBP (p value)	BBP (p value)	DEHP (p value)	DINP (p value)	DIDP (p value)
PRE \ BABY								
DMP	-0.09	-0.10	-0.11	-0.01	-0.05	0.14*		
DEP	0.03	0.15*	0.01	-0.09	-0.04	-0.10		
DIBP	-0.15*	-0.06	0.06	-0.10	0.00	0.03		
DBP	-0.04	0.05	0.07	-0.05	0.01	-0.02		
BBP	-0.06	0.05	-0.02	-0.03	0.31*	0.07		
DEHP	-0.09	-0.07	-0.09	-0.15*	-0.04	-0.03		
DINP								
DIDP								
POST \ BABY								
DMP								
DEP		0.35*	-0.05	0.00	-0.08	-0.04	-0.10	-0.15
DIBP	-0.06	0.06	0.43*	0.06	-0.09	0.08	0.02	0.02
DBP	-0.06	0.17*	0.10	0.12	-0.03	0.09	0.19	0.22
BBP	0.03	0.13*	-0.03	0.01	0.35*	-0.06	0.16	0.13
DEHP	-0.03	0.06	0.02	0.03	0.05	0.35*	0.18	0.27*
DINP		0.02	0.01	0.06	0.03	0.15	0.26*	0.26*
DIDP		-0.13	0.00	0.02	-0.09	0.15	0.28*	0.43*

4 Cumulative Risk Evaluation Using the Hazard Index

Evaluation of cumulative risk using the HI is a comparison of human exposure estimates to PODs estimates using toxicology data. The PODs are changed to so-called PEAAs with adjustments due to extrapolations using uncertainty factors. The selection of PEAAs is based on *in vivo* data with relevant endpoints. Here, the RfDs for pregnant women are based on reproductive and developmental endpoints in animal studies. Our selection of PEAAs for infants was based on the following logic: Rodents are most sensitive to the antiandrogenic effects of phthalates *in utero*. However, exposure at higher doses also induces testicular effects in adolescent and adult males, with adolescents being more sensitive than adults (Sjöberg *et al*., 1986; Higuchi *et al*., 2003). Thus, the PEAAs determined for *in utero* exposures should be protective for juvenile males.

Although pregnant women and infants are exposed to DIDP, DEP, and DMP as evidenced from biomonitoring studies, evidence of endocrine disruption in experimental animal studies has not been found for these three chemicals. Thus, these three diesters were not considered in the calculation of the hazard index.

4.1 Selection of Potency Estimates for Antiandrogenicity (PEAA) for Each Chemical

Case 1: Following Kortenkamp and Faust (2010), reference doses were determined using antiandrogenicity *in vivo* data to estimate the points of departure doses for which the effect levels could not be discriminated from untreated control animals). These are typically either NOAELs or the lower limits of benchmark doses (BMDL), as indicated in Table D-8. Uncertainty factors (UFs) were used to adjust the PODs to arrive at PEAAs to calculate the HI.

Case 2: A second case for evaluating the HI was undertaken so that the sensitivity of the results to some of the underlying assumptions could be assessed. The PEAA values were alternatively estimated using the following assumptions:

- DIBP, DBP, DEHP, and BBP are approximately equipotent in terms of testosterone modulated effects (Hannas *et al*., 2011b).
- The NOAEL is 5 mg/kg-d for DEHP; the other three phthalates were assumed to have equivalent values. An uncertainty factor of 100 was used, which sets the PEAA for the four chemicals at 50 µg/kg-d.
- Assuming DINP is 2.3 times less potent than DEHP, the PEAA is 115 µg/kg-d for DINP (Hannas *et al.*, 2011b).

Case 3: NOAELs associated with reproductive and developmental endpoints (and specifically, phthalate syndrome when available) were summarized in Section 2.3 based on *de novo* review by the CHAP.

The calculation of PEAA values from all three cases is illustrated in Table D-8.

Table D-8 Established *in vivo* antiandrogenic chemicals and chemicals showing limited evidence of antiandrogenicity. (Table and Case 1 are altered from Kortenkamp and Faust (2010); assumptions for Case 2 are from Hannas *et al*. (2011a); Case 3 is from NOAELs for developmental endpoints (Section 2.3, Table 2.1).

CASE 1					CASE 2				CASE 3			
Chemical	**Effect**	**Point of Departure (POD) (mg/kg-d)**	**Uncertainty Factor (UF)**	**PEAA[a] (µg/kg-d)**	**Effect**	**POD (mg/kg-d)**	**UF**	**PEAA (µg/kg-d)**	**Effect**	**POD (mg/kg-d)**	**UF**	**PEAA (µg/kg-d)**
Established *in vivo* anti-androgenic chemicals												
DBP	Suppression of fetal testosterone synthesis	20	200[b]	100	Disruption of testicular function and/or malformations in male rat offspring	5	100	50	NOAELs for develop-mental endpoints	50	100	500
BBP		66		330		5	100	50		50	100	500
DINP		750	500[c]	1500		11.5[g]	100	115		50	100	500
DIBP		40	200	200		5	100	50		125	100	1250
DEHP	Retained nipples in male offspring	3	100[d]	30		5	100	50		5	100	50
Chemicals with limited evidence of anti-androgenic activity												
BPA	Decreased testosterone levels in male offspring[e]	1.25	100[e]	12.5								
BPB	Suppression of testosterone levels, decreased epididymis weights, decreases in sperm production[f]	10	100	100								
PPB		100	100	1000								

[a] $RfD(\mu g/kg/day) = \frac{POD(mg/kg/day)}{UF} \times 1000$.

[b] PODs are BMDLs estimated by NRC (2008) based on Howdeshell *et al*. (2008) data; the study was of limited size; therefore, a UF of 200 was applied by Kortenkamp and Faust (2010).

[c] POD is from LOAELs from Gray *et al*. (2000) and Borch *et al.* (2004); NOAELs are not available; therefore, a UF of 500 was applied by Kortenkamp and Faust (2010).

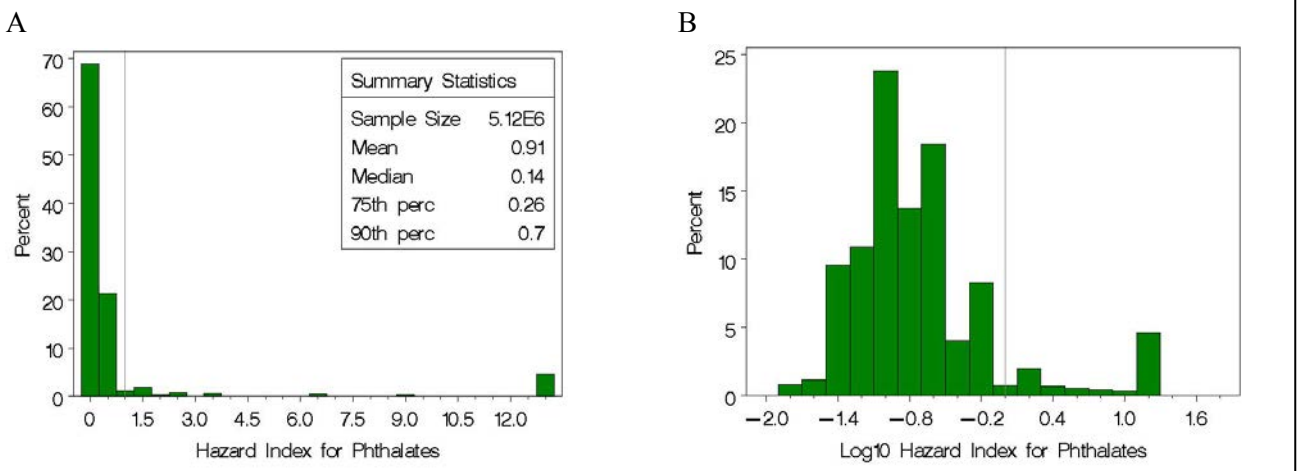
[d] POD is from NOAEL from Christiansen *et al*. (2009); standard UF applied by Kortenkamp and Faust (2010).

[e] From (Tanaka *et al.*, 2006) as applied by Kortenkamp and Faust (2010).

[f] After oral administration to post-weanling male Wistar rats (Oishi, 2001; 2002) as applied by Kortenkamp and Faust (2010).

[g] DINP is 2.3-fold less potent than DEHP (Hannas *et al.*, 2011b).

Figure D-8 Distribution of the hazard index (A,B) for five phthalates as estimated in pregnant women using daily intake estimates from urinary metabolite concentrations and Case 1 values for PEAAs. Data are from NHANES (2005–06) for the five phthalates.



5 Results of Hazard Index Evaluations

5.1 Calculation of the Hazard Index in Pregnant Women Using Case 1 PEAAs.

The hazard index was calculated per woman using the daily intake estimates for the five phthalate diesters and PEAA values as published by Kortenkamp and Faust, (2010). Figure D-8A provides a histogram for the distribution of HI for the 130 pregnant women, with the sampling weights applied so that roughly 5M pregnant women from the U.S. population are represented.[3]

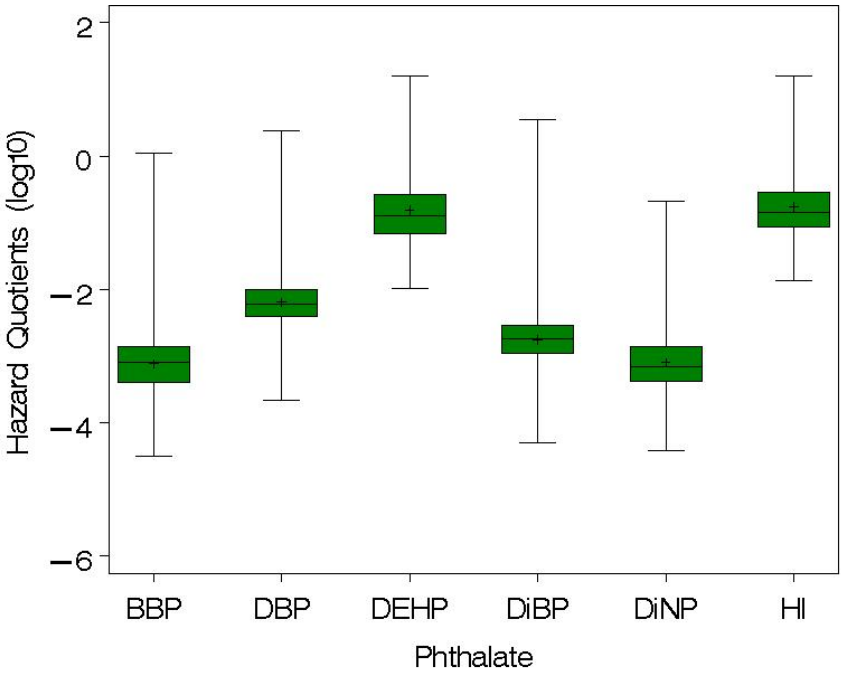
The distribution is highly skewed with a median value of 0.14 and estimated mean of 0.91. The reference value of 1 is depicted in Figure D-8A. Linearly interpolating between the 95 th percentile and the 90th percentile, roughly 10% of pregnant women in the U.S. population have estimated HIs exceeding 1.0, with PEAA values as specified in Case 1. Figure D-8B demonstrates the general bell-shaped distribution of the log of the hazard index with the exception of the upper tail; here, the reference value of 0 is shown.

Box plots for the hazard quotients for each of the five phthalates that comprise the HI are presented in Figure D-9. DEHP has the highest contribution to the HI, followed by DBP, DIBP,

[3] Percentile estimates presented in insets of histograms in this and all similar figures use positive survey sampling weights as weights in the calculations from ProcUnivariate in SAS v9.2, using a "weight" statement. This is only a rough approximation of the percentile estimates more accurately calculated using ProcSurvey Means with "strata," "cluster," and "weight" statements.

and BBP. As expected, DEHP has the highest contribution to the HI, with high exposure levels and the lowest PEAA in Case 1.

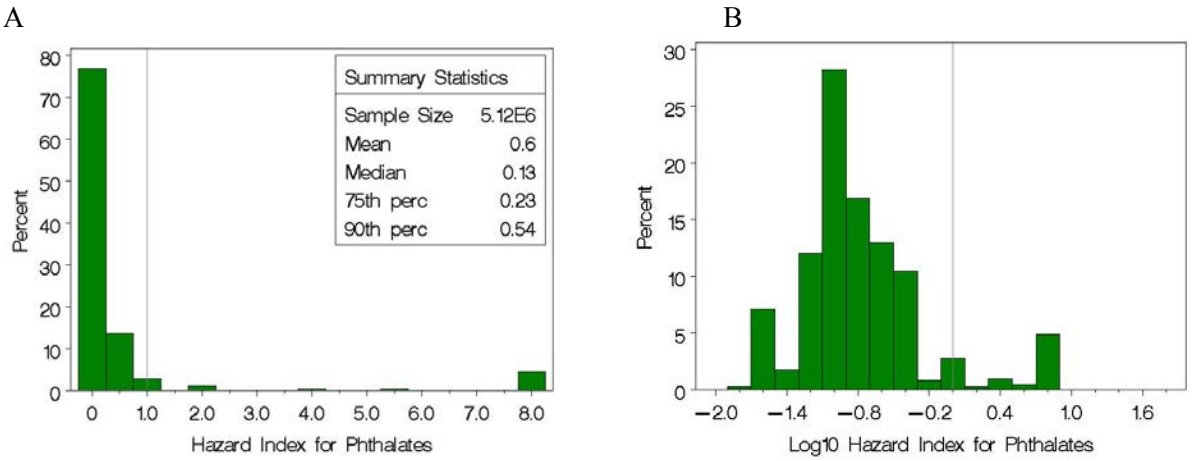
Figure D-9 Box plots for the hazard quotients that comprise the hazard index for five phthalates as estimated in pregnant women using daily intake estimates from urinary metabolite concentrations and Case 1 values for PEAAs. Data are from NHANES (2005–06).



5.2 Calculation of the Hazard Index in Pregnant Women Using Case 2 PEAAs.

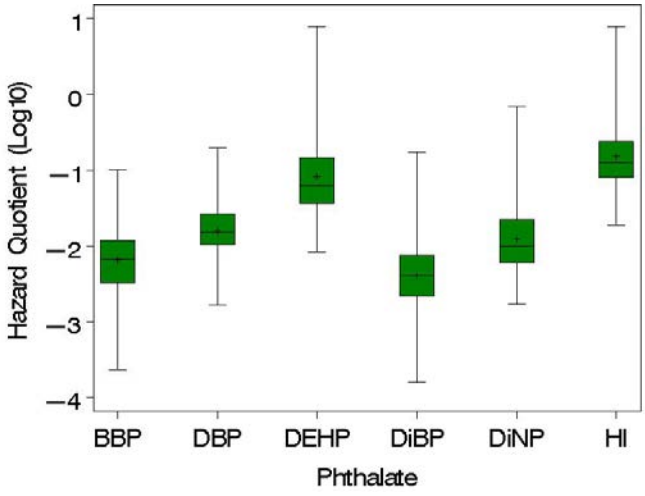
The hazard index was calculated per woman using the daily intake estimates for the five phthalate diesters, and Case 2 estimates for PEAAs (Table D-8). Figure D-10A provides a histogram for the distribution of HI for the 130 pregnant women adjusted with sampling weights to represent roughly 5.1M pregnant women in the U.S. population. The distribution is highly skewed with a median value of 0.13 and estimated mean of 0.6. The reference value of 1 is depicted in the figure. Linearly interpolating between the 95th and 90th percentiles, roughly 9% of pregnant women in the U.S. population have HI values exceeding 1.0, using Case 2 PEAAs. Figure D-10B demonstrates the general bell-shaped distribution of the log of the hazard index except with a heavy upper tail; here, the reference value of 0 is shown.

Figure D-10 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women using daily intake estimates from urinary metabolite concentrations and Case 2 values for PEAAs. Data are from NHANES (2005–06).



The contribution of each of the five phthalate diesters to the HI is presented in Figure D-11 for Case 2 PEAA values. DEHP is again the heaviest contributor to HI due to its higher exposure values. However, in this case, the PEAA values for DBP, BBP, and DIBP are the same as for DEHP, and the PEAA for DINP is about 10% of its value in Case 1. These changes in the PEAAs result in the relative contribution to the HI of these four phthalates increases compared to Case 1 (Figure D-9). However, the estimate for the percent of pregnant women with values of HI exceeding 1.0 is roughly similar.

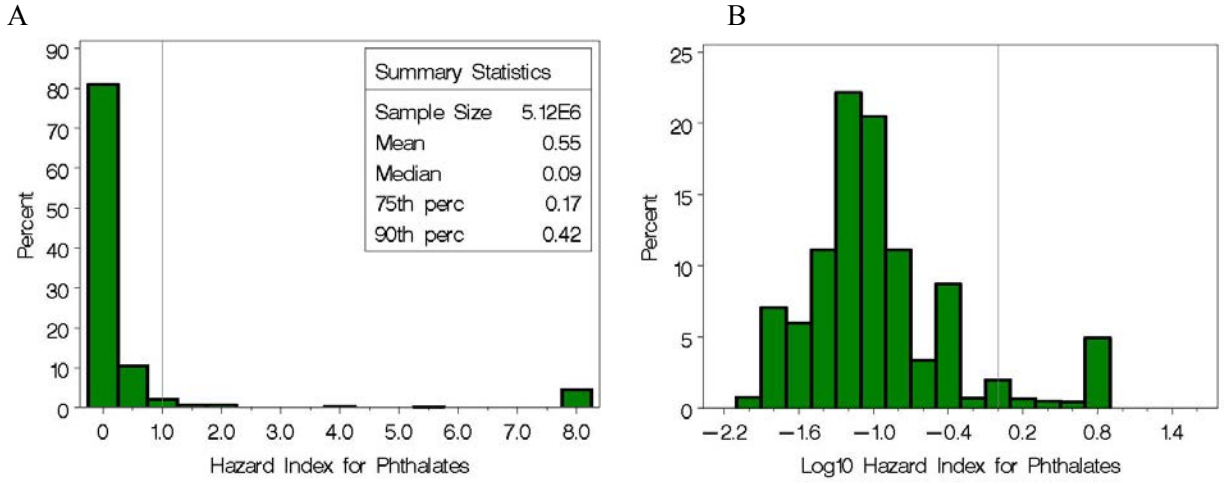
Figure D-11 Box plots for the hazard quotients that comprise the hazard index for five phthalates, as estimated in 130 pregnant women using daily intake estimates from urinary metabolite concentrations and Case 2 values for PEAAs. Data are from NHANES (2005–06).



5.3 Calculation of the Hazard Index in Pregnant Women Using Case 3 PEAAs.

The hazard index was calculated per woman using the daily intake estimates for the five phthalate diesters and Case 3 estimates for PEAAs (Table D-8). Figure D-12A provides a histogram for the distribution of HI for the 130 pregnant women, with sampling weights generalizing the analysis to 5.1M pregnant women in the U.S. population. The distribution is highly skewed with a median value of 0.09 and estimated mean of 0.55. The reference value of 1 is depicted in the figure. Interpolating between the estimate for the 95th percentile and the 90th percentile, roughly 9% of pregnant women in the U.S. population have HI values exceeding 1.0, using Case 3 PEAAs. Figure D-12B demonstrates the general bell-shaped distribution of the log of the hazard index except in the upper tail; here, the reference value of 0 is shown.

Figure D-12 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women using daily intake estimates from urinary metabolite concentrations and Case 3 values for PEAAs. Data are from NHANES (2005–06).



The contribution of each of the five phthalate diesters to the HI is presented in Figure D-13 for Case 3 PEAA values. DEHP is again the heaviest contributor to HI due to its higher exposure values and, in this case, the lowest PEAA.

The distribution of the HI is somewhat robust to the choice of PEAA values (Table D-9). In all three cases, the HI value is largely driven by the distribution of the hazard quotient for DEHP. The median and 75th percentiles are similar in cases 1, 2, and 3; and the distributions of HI based on the median, 75th, 95th, and 99th percentiles are ordered from highest to lowest with Case 1 > Case 2 > Case 3. However, the percentage of pregnant women exceeding 1.0 is similar, *i.e.,* roughly 9–10%.

Figure D-13 Box plots for the hazard quotients that comprise the hazard index for five phthalates as estimated in pregnant women using daily intake estimates from urinary metabolite concentrations and Case 3 values for PEAAs. Data are from NHANES (2005–06).

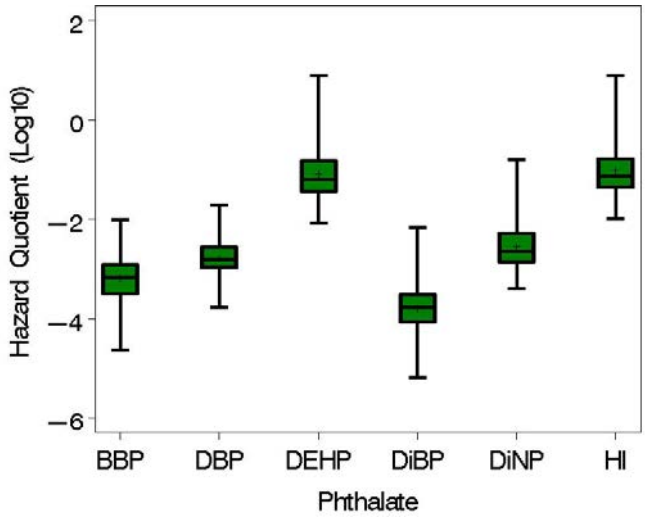


Table D-9 Summary percentiles from the hazard index distributions using five phthalates for pregnant women and children from NHANES (2005–06) and from SFF (Sathyanarayana *et al.*, 2008a). The NHANES estimates infer to 5.1 million pregnant women in the United States.

Hazard Index	AA set		PEAA Case	Percentiles			
				Median	75th	95th	99th
Pregnant Women	NHANES		1	0.14	0.26	6.1	12.2
			2	0.13	0.23	3.7	7.4
			3	0.08	0.15	3.6	7.3
	SFF	Prenatal	1	0.11	0.19	0.57	2.39
		Postnatal		0.10	0.19	0.73	1.51
		Prenatal	2	0.10	0.16	0.41	1.54
		Postnatal		0.09	0.16	0.46	0.92
		Prenatal	3	0.06	0.11	0.33	1.40
		Postnatal		0.06	0.11	0.43	0.91
Infants	SFF Infants		1	0.22	0.40	0.95	3.71
			2	0.20	0.34	0.81	2.32
			3	0.12	0.22	0.54	2.21

6 Adjusting the Hazard Index for Additional Antiandrogenic Chemicals

To focus too narrowly on phthalates when pregnant women are also exposed to other chemicals with antiandrogenicity activity may underestimate risk. We considered three other anti-androgenic (AA) chemicals available in the 2005–06 NHANES biomonitoring: BPA, BPB, and PPB. Adding these to the hazard index shifts its distribution only slightly to the right. For example, using Case 1 PEAAs, the median changes from 0.14 to 0.19. Accounting for the five phthalates and these three other AAs, 9.8% of pregnant women have HI values that exceed 1.0.

Two more extreme cases were also considered. Kortenkamp and Faust (2010) provide median and high intake values for the phthalates and other antiandrogens, including vinclozolin, prochloraz, procymidone, linuron, fenitrothion, p,p'-DDE, and BDE99. Their daily intake estimates were from German (Wittassek and Angerer, 2008), French (Menard *et al.*, 2008), and Polish (Galassi *et al.,* 2008) studies. As described in Kortenkamp and Faust (2010), estimates for the PEAAs were based on NOAELs for retained nipples for vinclozolin, prochloraz, procymidone, linuron, and p,p'-DDE, and for anogenital distance for fenitrothion and BDE99. An uncertainty factor of 100 was used for six of the seven chemicals; a value of 500 was used for linuron as a NOAEL was not available—a dose of 50 mg/kg-d induced nipple retention in male rats exposed *in utero*.

Using the median estimates for daily intake for the seven AAs (Kortenkamp and Faust, 2010) in addition to the estimated HI using biomonitoring data for the five phthalates and three AAs (BPA, PPB, and BPB) increases the HI 0.176 units (Table D-10); conservatively, the increase in the HI using the high intake estimates increases the HI 0.593 units. The most conservative case (using high intake estimates for the seven AAs) increases the distribution of HI for the 15 chemicals such that the 75th percentile is 0.88 and 21% of pregnant women have estimated HI values that exceed 1.0 (Table D-10; calculated by linearly interpolating).

Table D-10 Summary percentiles from the hazard index distributions for pregnant women with sampling weights from NHANES (2005–06) using Case 1 PEAA values.

AA Set	Percentile				
	Median	75th	90th	95 th	99th
5 phthalates	0.14	0.26	0.70	6.73	13.1
5 phthalates + 3 AAs	0.19	0.29	0.73	6.75	13.2
5 phthalates + 3 AAs + median intake of 7 other AAs	0.37	0.46	0.91	6.92	13.3
5 phthalates + 3 AAs + high intake of 7 other AAs	0.78	0.88	1.33	7.34	13.8

7 Analysis of SFF Data

7.1 Calculation of the Hazard Index in Pregnant Women Using Case 1 PEAAs.

The hazard index was calculated per woman from prenatal and postnatal values using the daily intake estimates for the five phthalate diesters. Figure D-14A provides a histogram for the distribution of HI for the 340 prenatal estimates. The distribution is highly skewed with a median HI value of 0.11, and the estimated mean was 0.30. Interpolating between the 99th and 95th percentiles, roughly 4% of the prenatal women have HI values that exceed 1.0, with one woman with an extremely high value of 29.3. Figure D-14B demonstrates the general bell-shaped distribution of the log of the hazard index.

Figure D-14 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women from **prenatal** values from the SFF data using daily intake estimates from urinary metabolite concentrations and Case 1 values for PEAAs.

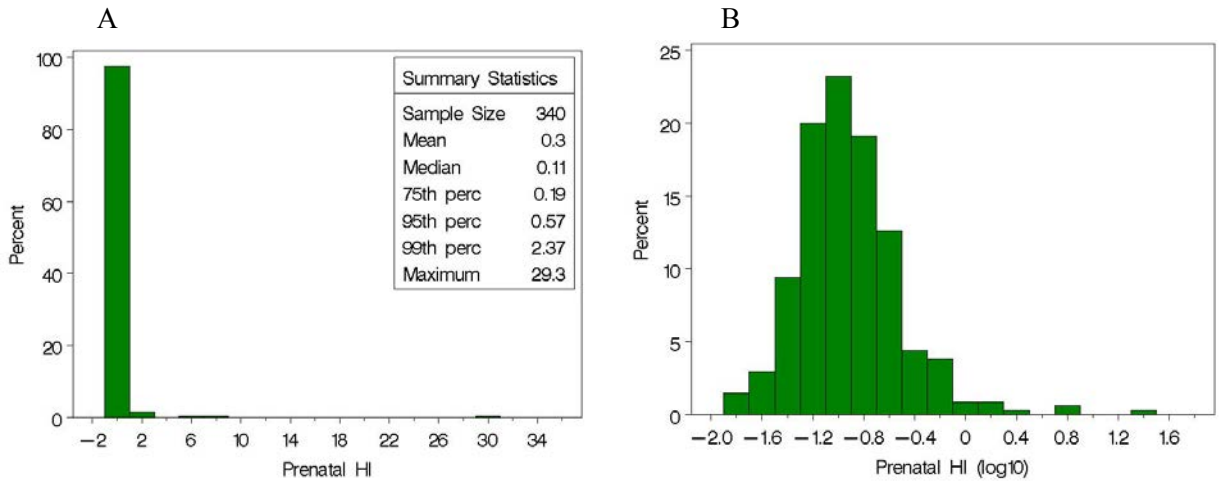
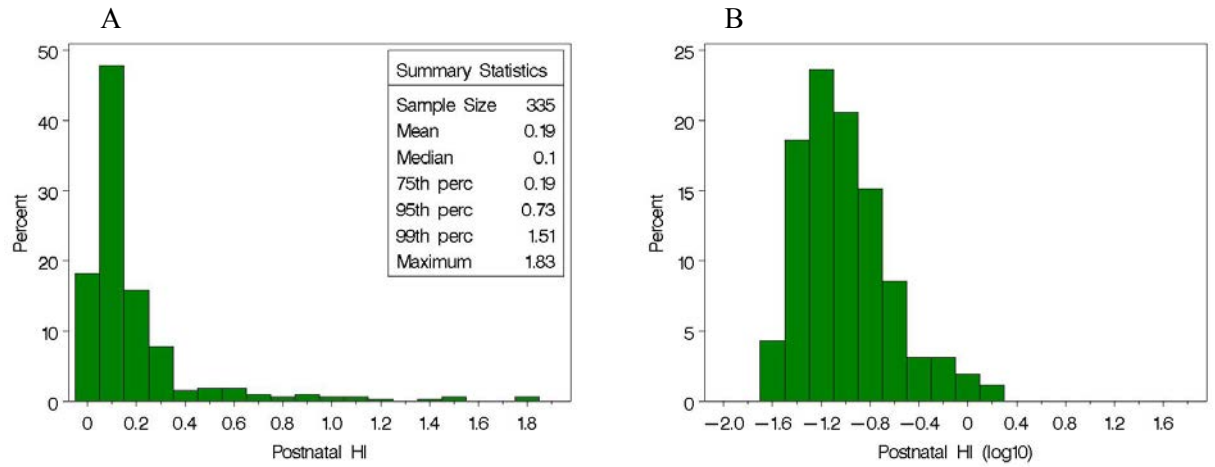


Figure D-15A provides a histogram for the distribution of HI for the postnatal estimates. The distribution is highly skewed with a median HI value of 0.10, and the estimated mean was 0.19. Interpolating between the 99th and 95th percentiles, roughly 4% of the postnatal women have values exceeding 1.0. Figure D-15B demonstrates the general bell-shaped distribution of the log of the hazard index.

Figure D-15 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women from **postnatal** values from the SFF data using daily intake estimates from urinary metabolite concentrations and Case 1 values for PEAAs.



Box plots for the hazard quotients for each of the five phthalates that comprise the HI are presented in Figure D-16. DEHP is the primary contributor to the HI for both prenatal and postnatal values using Case 1 PEAAs.

Figure D-16 Box plots for the hazard quotients for (A) prenatal and (B) postnatal hazard indices using Case 1 PEAAs.

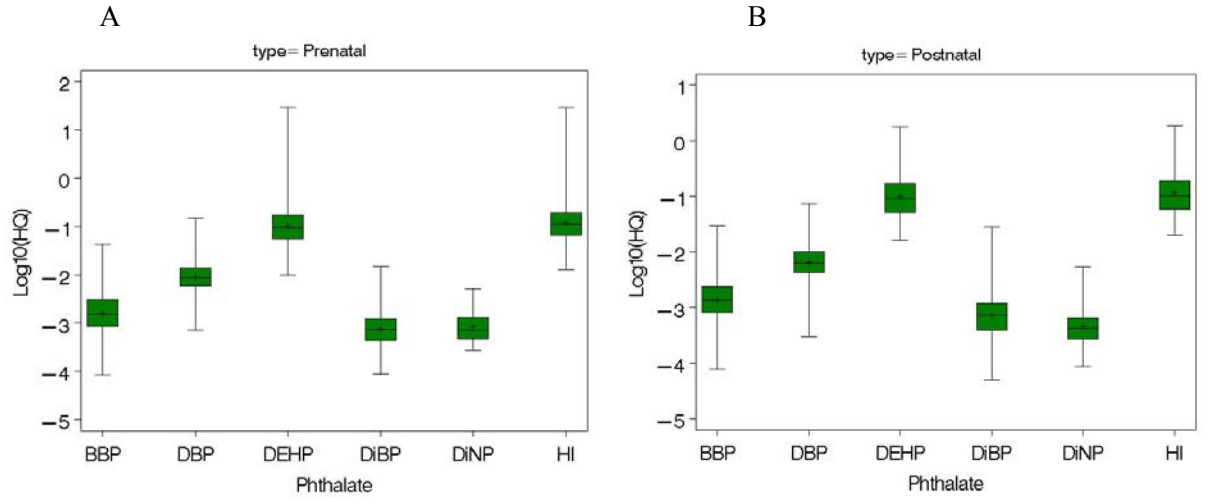
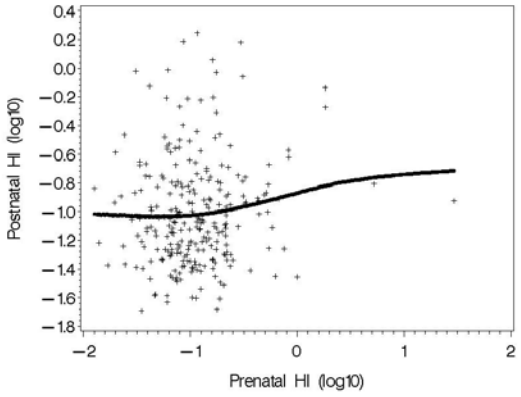
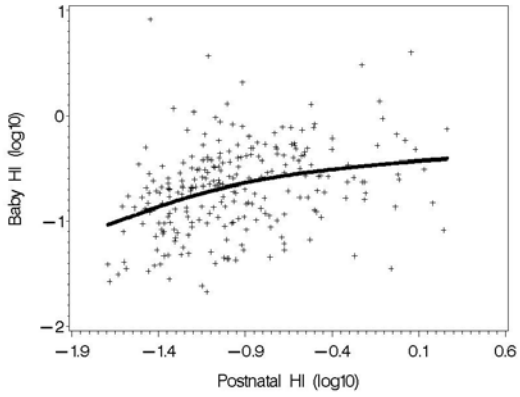


Figure D-17 Bivariate plot of (A) prenatal and postnatal and (B) postnatal and baby hazard index values from Case 1.

A



B



Although the distribution of HI from prenatal and postnatal measurements is quite similar (Table D-9), the bivariate correlation (on the log 10 scale) is not significant (p=0.120; N=258) and is estimated to be 0.10 (Figure D-17A). There is not a strong systematic relationship between prenatal and postnatal values of HI. However, there is a significant relationship between postnatal HI values and baby HI values (Figure D17B) from Case 1; the correlation estimate is 0.32 ($p<0.001$; N=251).

7.2 Calculation of the Hazard Index in Pregnant Women Using Case 2 PEAAs.

The hazard index was calculated per woman from prenatal and postnatal values using the daily intake estimates for the five phthalate diesters—or the number of nonmissing diesters. Figure D-18A provides a histogram for the distribution of HI for the 340 prenatal estimates. The distribution is highly skewed with a median HI value of 0.10, and the estimated mean was 0.22. Interpolating between the 95th and 99th percentiles, roughly 3% of the prenatal estimates for HI exceed 1.0. Figure D-18B demonstrates the general bell-shaped distribution of the log of the hazard index for prenatal values.

Figure D-18 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women from prenatal values from the SFF data using daily intake estimates from urinary metabolite concentrations and Case 2 values for PEAAs.

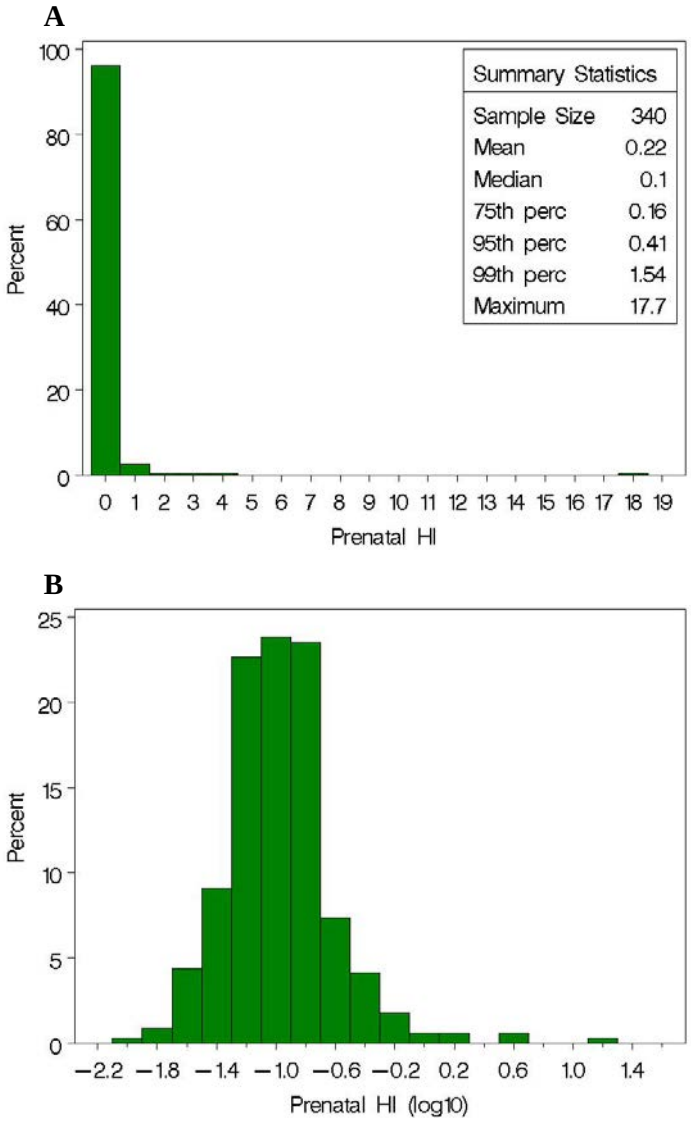
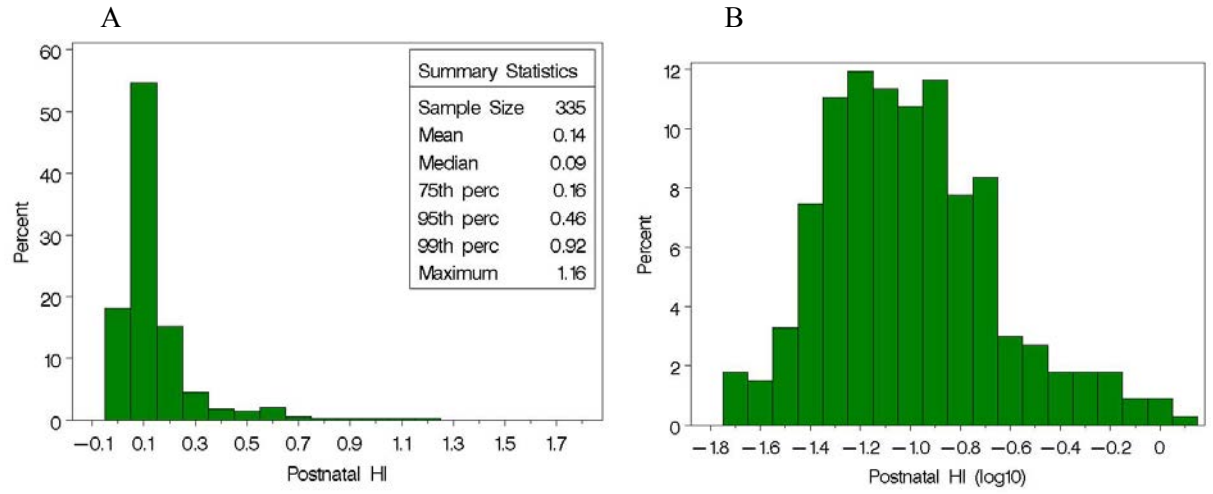


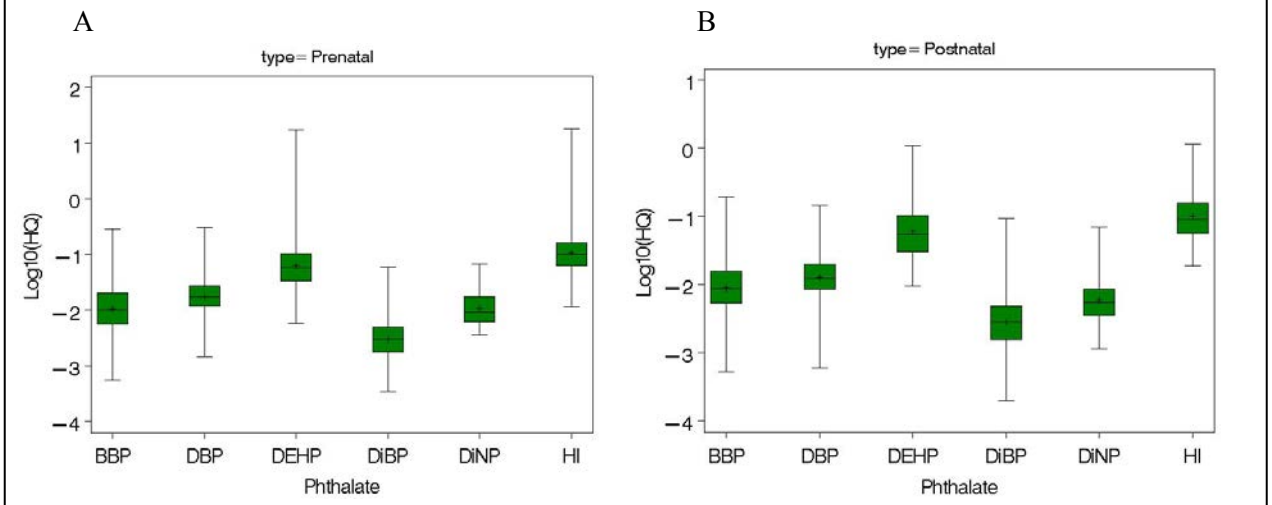
Figure D-19A provides a histogram for the distribution of HI for the 335 postnatal estimates. The distribution is highly skewed with a median HI value of 0.09, and the estimated mean was 0.14. Less than 1% of the estimates exceed 1.0. Figure D-19B demonstrates the distribution of the log of the hazard index has a heavy upper tail.

Figure D-19 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women from postnatal values from the SFF data using daily intake estimates from urinary metabolite concentrations and Case 2 values for PEAAs.



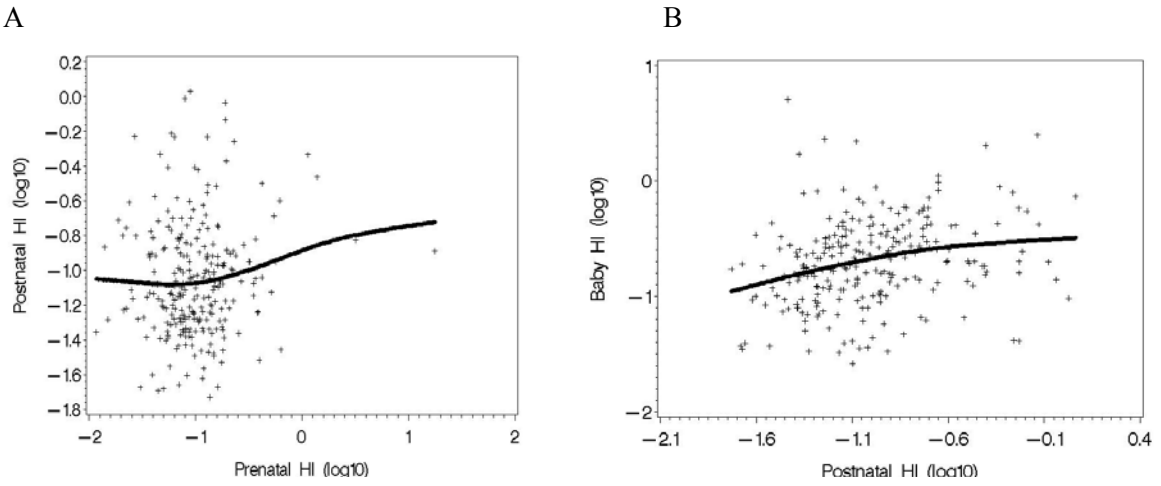
Box plots for the hazard quotients for each of the five phthalates that comprise the HI are presented in Figure D-20 for Case 2 PEAAs. DEHP is the primary contributor to the HI for both prenatal and postnatal values using Case 2 PEAAs.

Figure D-20 Box plots for the hazard quotients that comprise the hazard index for five phthalates in (A) prenatal and (B) postnatal measurements from SFF data for Case 2.



The bivariate association between the prenatal and postnatal estimates for HI is borderline significant (p=0.082; N=258) with a Pearson correlation coefficient estimate of 0.11 (Figure D-21A). Omitting the two highest prenatal HI values, the correlation estimate is 0.09 (p=0.132, N=256). However, there is a significant relationship between postnatal HI values and baby HI values with a correlation estimate of 0.26 (p<0.001, N=251; Figure D-21B).

Figure D-21 Bivariate plot of (A) prenatal and postnatal (N=258) and (B) postnatal and baby (N=251) hazard index values for Case 2.



7.3 Calculation of the Hazard Index in Pregnant Women Using Case 3 PEAAs.

The hazard index was calculated per woman from prenatal and postnatal values using the daily intake estimates for the five phthalate diesters—or the number of nonmissing diesters. Figure D-22A provides a histogram for the distribution of HI for the 340 prenatal estimates. The distribution is highly skewed with a median HI value of 0.06, and the estimated mean was 0.17. Roughly 2% of the prenatal estimates exceed 1.0, with one woman with an extremely high value of 17.6. Figure D-22B demonstrates the general bell-shaped distribution of the log of the hazard index.

Figure D-22 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women from prenatal values from the SFF using daily intake estimates from urinary metabolite concentrations and Case 3 values for PEAAs.

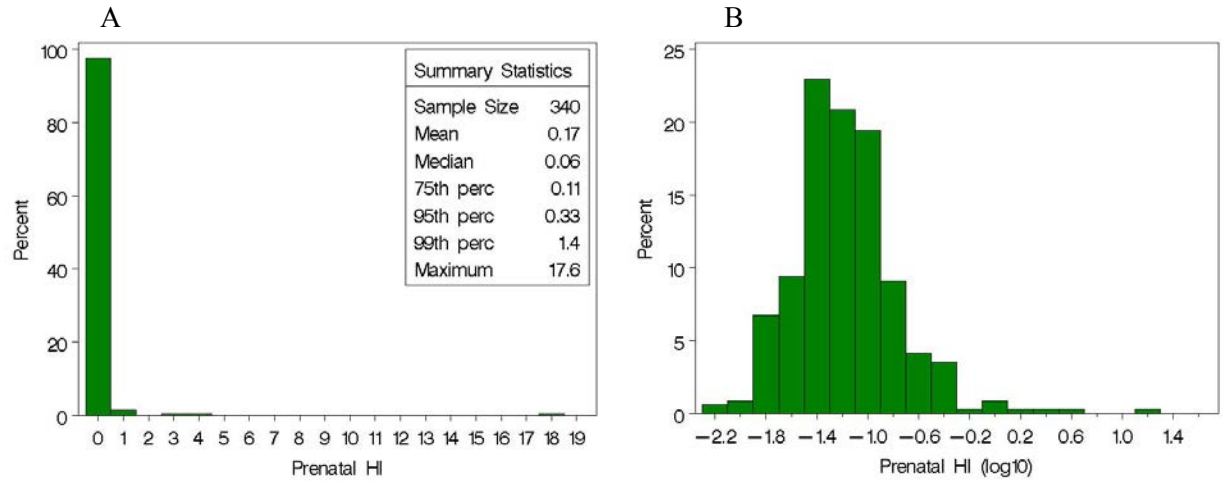


Figure D-23A provides a histogram for the distribution of HI for the 335 postnatal estimates. The distribution is highly skewed with a median HI value of 0.06, and the estimated mean was 0.11. The maximum observed value was 1.09. Figure D-23B demonstrates the general bell-shaped distribution of the log HI.

Figure D-23 Distribution of the hazard index (A,B) for five phthalates, as estimated in pregnant women from postnatal values from the SFF data using daily intake estimates from urinary metabolite concentrations and Case 3 values for PEAAs.

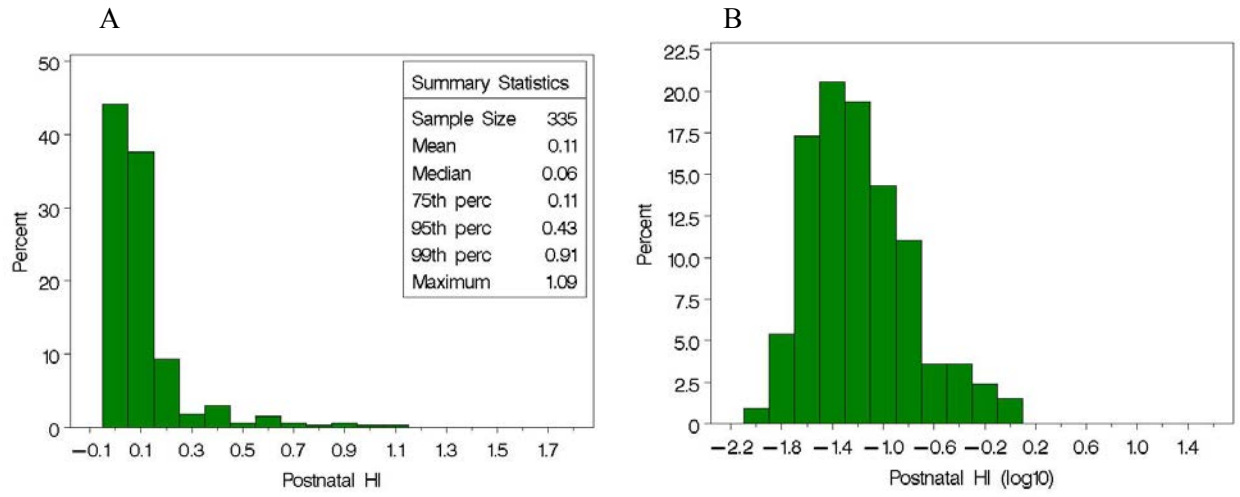
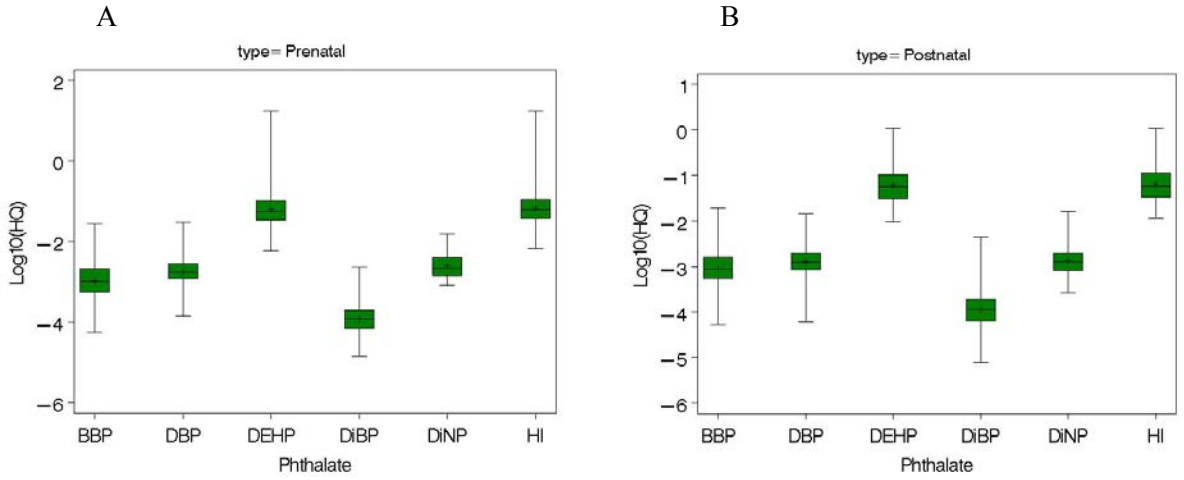


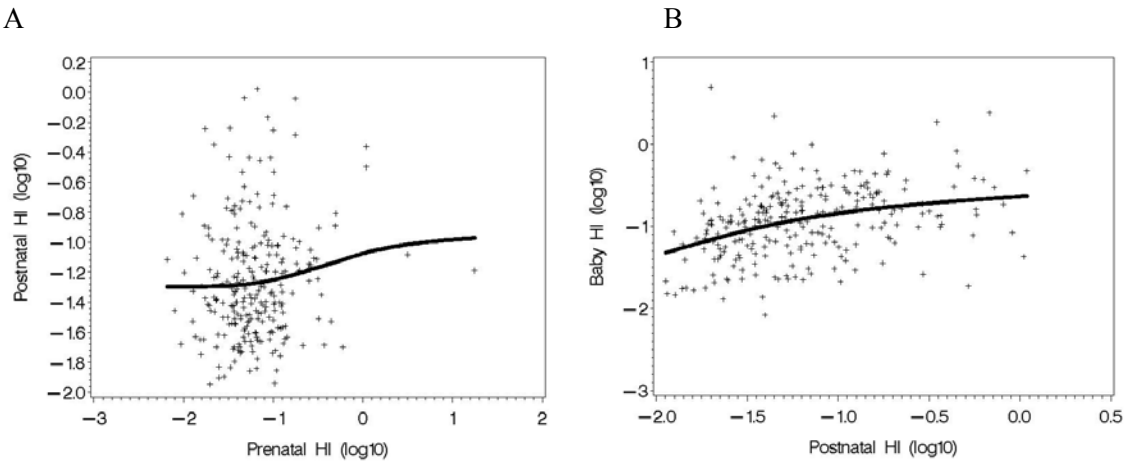
Figure D-24 provides box plots for the hazard quotients for the HI for Case 3 across the five phthalates. Again, the hazard quotient for DEHP dominates the sum for the HI.

Figure D-24 Box plots for the hazard quotients that comprise the hazard index for five phthalates in (A) prenatal and (B) postnatal measurements from SFF data for Case 3.



The bivariate association (Figure D-25) between the prenatal and postnatal HI values using Case 3 is not significant (p=0.076; N=258) with a Pearson correlation estimate of 0.11. However, there is a significant relationship between postnatal HI values and baby HI values with a correlation estimate of 0.34 (p<0.001, N=251; Figure D-25B).

Figure D-25 Bivariate plot of (A) prenatal and postnatal (N=258) and (B) postnatal and baby (N=251) hazard index values for Case 3.



8 Analysis of Infant Data

8.1 Calculation of the Hazard Index in Infants Using Case 1 PEAAs.

The hazard index was calculated per baby using the daily intake estimates for the five phthalate diesters—or the number of nonmissing diesters. Figure D-26A provides a histogram for the distribution of HI for the 258 babies. The distribution is highly skewed with a median HI value of 0.22, and the estimated mean was 0.36. Approximately 5% of the HI values from infants exceed 1.0. Figure D-26B demonstrates the general bell-shaped distribution of the log of the hazard index.

Figure D-26 Bivariate plot of (A) prenatal and postnatal (N=258); and (B) postnatal and baby (N=251) hazard index values for Case 3.

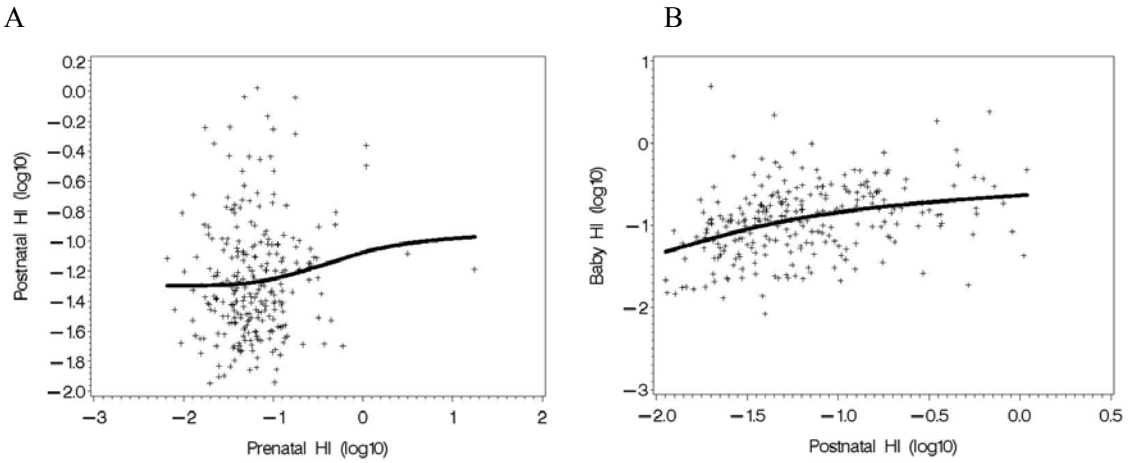
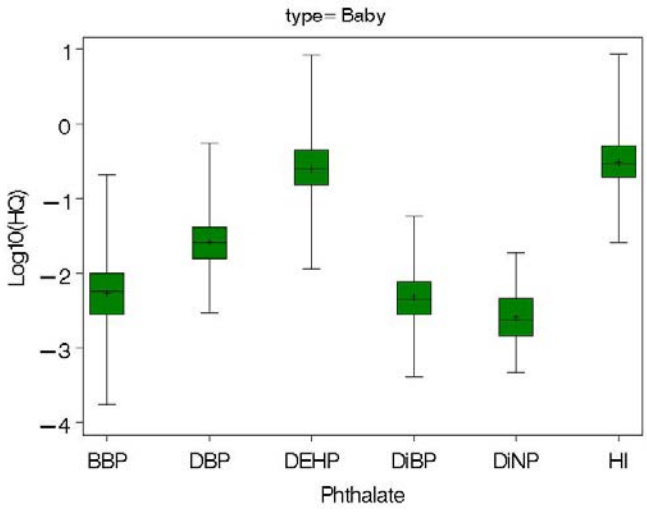


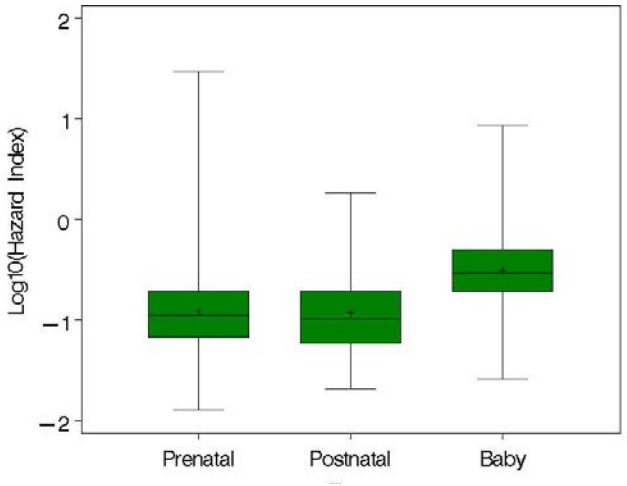
Figure D-27 provides box plots for the distributions of the hazard quotients for infants using Case 1 PEAAs. The DEHP hazard quotient dominates the HI sum.

Figure D-27 Box plots for the hazard quotients for the hazard index for infants from the SFF.



Using Case 1 values for PEAAs in calculating the HI, the distribution of the hazard index is most extreme in the infants. The median value for the infants exceeds the 75th percentiles from the prenatal and postnatal values (Figure D-28).

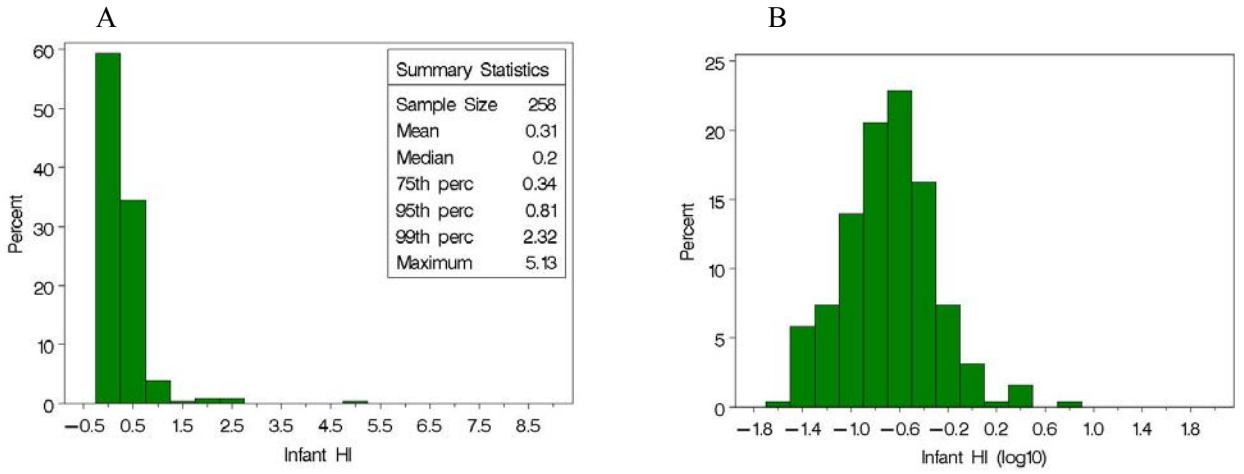
Figure D-28 Box plots comparing the distributions of the Hazard Index values using Case 1 PEAA values for prenatal and postnatal measurements, and from babies from the SFF.



8.2 Calculation of the Hazard Index in Infants Using Case 2 PEAAs.

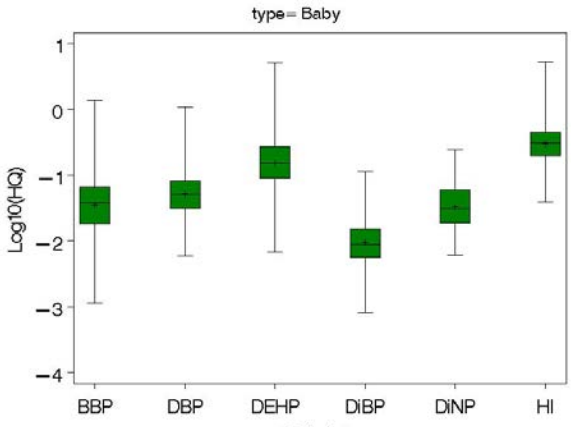
The hazard index was calculated per baby using the daily intake estimates for the five phthalate diesters—or the number of nonmissing diesters using Case 2 PEAAs. Figure D-29A provides a histogram for the distribution of HI for the 291 babies. The distribution is highly skewed with a median HI value of 0.31, and the estimated mean of 0.41. Approximately 5% of the infants have estimated HI values that exceeded 1.0. Figure D-29B demonstrates the general bell-shaped distribution of the log of the hazard index.

Figure D-29 Distribution of the (A) hazard index, and (B) log 10 hazard index using Case 2 PEAA values, as estimated in babies (0–37 months) using daily intake estimates from urinary metabolite concentrations. Data are from the SFF.



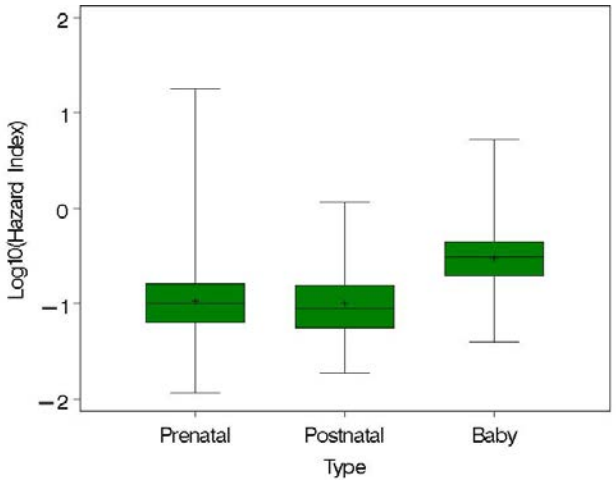
The hazard quotient for DEHP is again the dominant contributor to the HI sum (Figure D-30).

Figure D-30 Box plots for the hazard quotients for the hazard index for infants from the SFF using Case 2 PEAAs.



Using Case 2 values for PEAAs in calculating the HI, the distribution of the hazard index is most extreme in the infants. The median of HI for the infants exceeds the 75th percentiles from the prenatal and postnatal values using Case 2 PEAA values (Figure D-31).

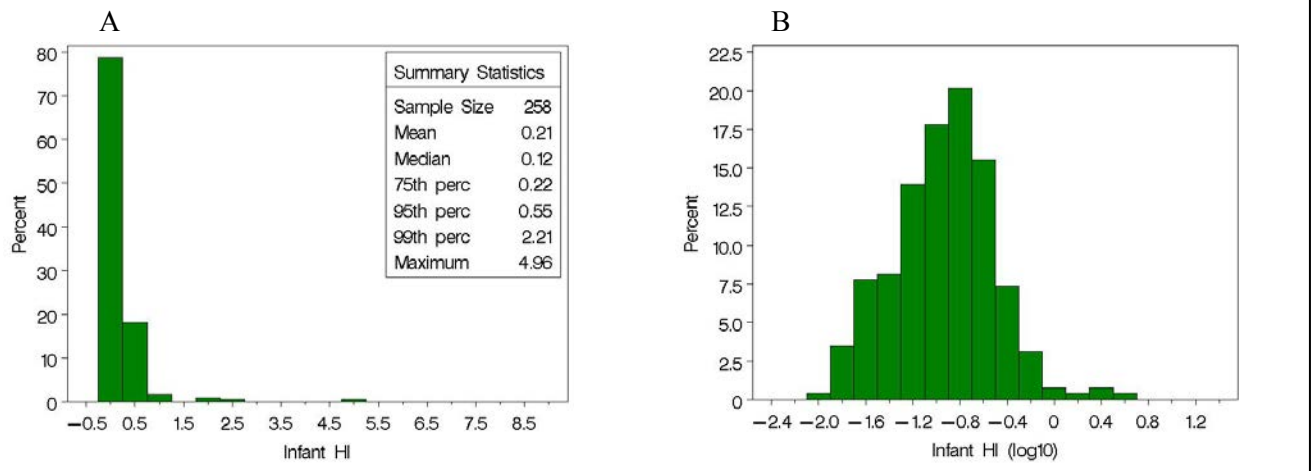
Figure D-31 Box plots comparing the distributions of the hazard index values using Case 2 PEAA values for prenatal and postnatal measurements, and from babies from the SFF data.



8.3 Calculation of the Hazard Index in Infants Using Case 3 PEAAs.

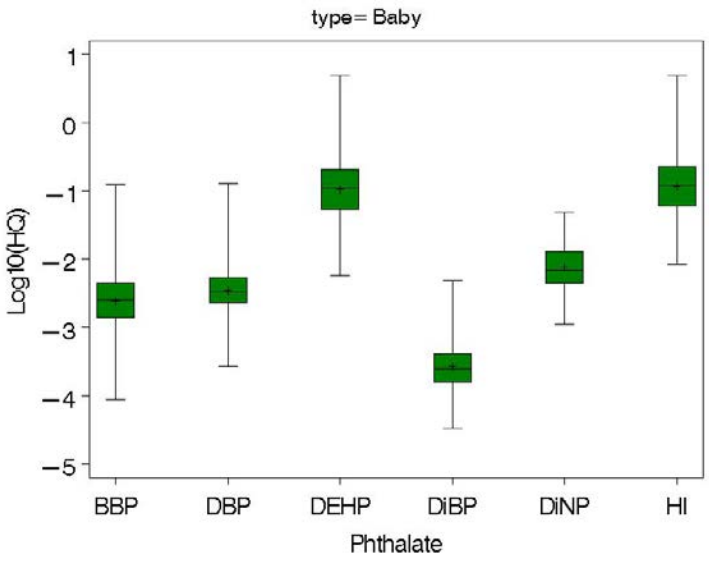
The hazard index was calculated per baby using the daily intake estimates for the five phthalate diesters—or the number of nonmissing diesters using Case 3 PEAAs. Figure D-32A provides a histogram for the distribution of HI for the 258 babies. The distribution is skewed with a median HI value of 0.12 and the estimated mean of 0.21. Roughly 4% of infants have HI estimates that exceed 1.0. Figure D-32B demonstrates the general bell-shaped distribution of the log of the hazard index.

Figure D-32 Distribution of the (A) hazard index, and (B) log 10 hazard index using Case 3 PEAA values, as estimated in babies (0–37 months) using daily intake estimates from urinary metabolite concentrations. Data are from SFF.



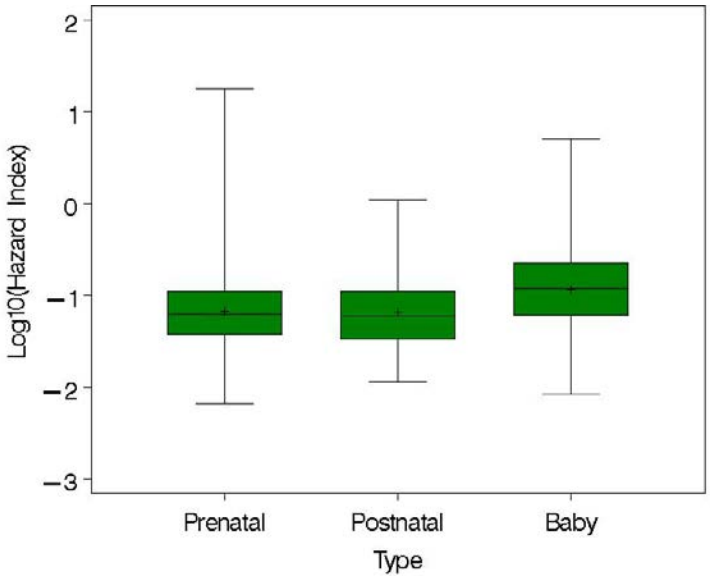
Again, the hazard quotient for DEHP dominates the HI sum using Case 3 PEAAs (Figure D-33).

Figure D-33 Box plots for the hazard quotients for the hazard index for infants from the SFF using Case 3 PEAAs.



Using Case 3 values for PEAAs in calculating the HI, the distribution of the hazard index is most extreme in the infants. As for Cases 1 and 2, the median value of HI for the infants exceeds the 75th percentiles from the prenatal and postnatal values (Figure D-34) using Case 3 PEAA values.

Figure D-34 Box plots comparing the distributions of the hazard index values using Case 3 PEAA values for prenatal and postnatal measurements, and from babies from SFF data.



9 Summary of Results

The CHAP considered three cases in calculating the HI based on different sets of PEAAs. Cases 1 and 3 were largely based on points of departures (*i.e.,* NOAELs or BMDLs) for individual chemicals. Case 2 is based on the dose-response curves and the assumptions of potencies. Four of the five phthalates (DEHP, DBP, BBP, and DIBP) were assumed to be equipotent in terms of testosterone modulated effects (Hannas *et al.*, 2011b). The potency of DINP was assumed to be 2.3 times less potent from the same set of studies.

Hazard indices for these five antiandrogens were calculated for individual pregnant women from the NHANES data (2005–06) and in prenatal and postnatal maternal concentrations from the SFF. From the NHANES data, the HI exceeds 1.0 in about 10% of pregnant women in the U.S. population. The rate was about 4–5% in the SFF data for both maternal and infant measurements.

In all three cases studied, the HI value was dominated by DEHP because it had both high exposure and a low PEAA. The smallest contributor to the HI was generally DIBP in all three cases, which was due to low exposure.

A limitation of the analyses presented here is the use of exposure data from 2005–06 for NHANES and 1999–2005 for the SFF. Since these data were collected, the Consumer Product Safety Improvement Act restricted some of the uses of the five phthalates evaluated. The impact on exposure is unknown and not accounted for in the calculation of the HI.

10 Supplement

Table S-1 Comparison of estimated percentiles for hazard quotients and hazard indices from pregnant women using survey sampling weights in NHANES 2005–06.

	Approximated as a weight (PROC UNIVARIATE)			Estimated using survey design features (strata, clusters) (PROC SURVEY MEANS)		
CASE 1	**Median**	**95th**	**99th**	**Median**	**95th**	**99th**
BBP	0.001	0.004	0.01	<0.001	0.004	0.01
DBP	0.006	0.04	0.10	0.01	0.03	0.06
DEHP	0.12	6.7	13.1	0.12	6.0	12.2
DIBP	0.001	0.005	0.01	0.001	0.005	0.01
DINP	0.001	0.01	0.02	0.001	0.01	0.02
HI	0.14	6.7	13.1	0.14	6.1	12.2
CASE 2	**Median**	**95th**	**99th**	**Median**	**95th**	**99th**
BBP	0.01	0.03	0.05	0.01	0.03	0.05
DBP	0.01	0.08	0.20	0.01	0.07	0.13
DEHP	0.07	4.0	7.9	0.07	3.6	7.3
DIBP	0.003	0.02	0.04	0.003	0.02	0.04
DINP	0.01	0.10	0.30	0.01	0.10	0.24
HI	0.13	4.1	7.9	0.13	3.7	7.4
CASE 3	**Median**	**95th**	**99th**	**Median**	**95th**	**99th**
BBP	0.001	0.003	0.005	0.001	0.003	0.005
DBP	0.001	0.008	0.02	0.001	0.007	0.01
DEHP	0.07	4.0	7.9	0.07	3.6	7.3
DIBP	<0.001	0.001	0.002	<0.001	0.001	0.002
DINP	0.002	0.02	0.07	0.002	0.02	0.05
HI	0.09	4.0	7.9	0.08	3.6	7.3

11 References

Anderson, W.A., Castle, L., Hird, S., Jeffery, J., Scotter, M.J., 2011. A twenty-volunteer study using deuterium labelling to determine the kinetics and fractional excretion of primary and secondary urinary metabolites of di-2-ethylhexylphthalate and di-iso-nonylphthalate. Food Chem Toxicol 49, 2022–2029.

Beckmann, C.R.B., Ling, F.W., Barzansky, B.M., Laube, D.W., Smith, R.P., 2010. Obstetrics and Gynecology, Sixth ed. Lippincott, Williams, and Wilkins, Baltimore, MD.

Borch, J., Ladefoged, O., Hass, U., Vinggaard, A.M., 2004. Steroidogenesis in fetal male rats is reduced by DEHP and DINP, but endocrine effects of DEHP are not modulated by DEHA in fetal, prepubertal and adult male rats. Reprod Toxicol 18, 53–61.

CDC, 2012a. Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, February 2012. Centers for Disease Control & Prevention. Atlanta, GA.

CDC, 2012b. National Health and Nutrition Examination Survey Data, National Center for Health Statistics. Department of Health and Human Services. Hyattsville, MD.

Christiansen, S., Scholze, M., Dalgaard, M., Vinggaard, A.M., Axelstad, M., Kortenkamp, A., Hass, U., 2009. Synergistic disruption of external male sex organ development by a mixture of four antiandrogens. Environ Health Perspect 117, 1839–1846.

David, R.M., 2000. Exposure to phthalate esters. Environ Health Perspect 108, A440.

Galassi, S., Bettinetti, R., Neri, M.C., Falandysz, J., Kotecka, W., King, I., Lo, S., Klingmueller, D., Schulte-Oehlmann, U., 2008. pp'DDE contamination of the blood and diet in central European populations. Sci Total Environ 390, 45–52.

Gray, L.E., Jr., Ostby, J., Furr, J., Price, M., Veeramachaneni, D.N., Parks, L., 2000. Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58, 350–365.

Hannas, B.R., Furr, J., Lambright, C.S., Wilson, V.S., Foster, P.M., Gray, L.E. Jr., 2011a. Dipentyl phthalate dosing during sexual differentiation disrupts fetal testis function and postnatal development of the male Sprague-Dawley rat with greater relative potency than other phthalates. Toxicol Sci 120, 184–193.

Hannas, B.R., Lambright, C.S., Furr, J., Howdeshell, K.L., Wilson, V.S., Gray, L.E.Jr., 2011b. Dose-response assessment of fetal testosterone production and gene expression levels in rat testes following *in utero* exposure to diethylhexyl phthalate, diisobutyl phthalate, diisoheptyl phthalate, and diisononyl phthalate. Toxicol Sci 123, 206–216.

Harper, H.A., Rodwell, V.W., Mayes, P.A., 1977. Review of Physiological Chemistry, Lange Medical Publications, Los Altos, CA.

Higuchi, T.T., Palmer, J.S., Gray, L.E., Jr., Veeramachaneni, D.N., 2003. Effects of dibutyl phthalate in male rabbits following *in utero*, adolescent, or postpubertal exposure. Toxicol Sci 72, 301–313.

Howdeshell, K.L., Wilson, V.S., Furr, J., Lambright, C.R., Rider, C.V., Blystone, C.R., Hotchkiss, A.K., Gray, L.E., Jr., 2008. A mixture of five phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner. Toxicol Sci 105, 153–165.

Koch, H.M., Becker, K., Wittassek, M., Seiwert, M., Angerer, J., Kolossa-Gehring, M., 2007. Di-n-butylphthalate and butylbenzylphthalate - urinary metabolite levels and estimated daily intakes: Pilot study for the German Environmental Survey on children. J Expo Sci Environ Epidemiol 17, 378–387.

Kohn, M.C., Parham, F., Masten, S.A., Portier, C.J., Shelby, M.D., Brock, J.W., Needham, L.L., 2000. Human exposure estimates for phthalates. Environ Health Perspect 108, A44–A442.

Kortenkamp, A., Faust, M., 2010. Combined exposures to anti-androgenic chemicals: Steps towards cumulative risk assessment. Int J Androl 33, 463–474.

Mage, D.T., Allen, R.H., Dodali, A., 2008. Creatinine corrections for estimating children's and adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine concentrations. J Expo Sci Environ Epidemiol 18, 360–368.

Menard, C., Heraud, F., Nougadere, A., Volatier, J.L., Leblanc, J.C., 2008. Relevance of integrating agricultural practices in pesticide dietary intake indicator. Food Chem Toxicol 46, 3240–3253.

NRC, 2008. Phthalates and Cumulative Risk Assessment. The Task Ahead. Committee on the Health Risks of Phthalates, National Research Council, National Academy Press, Washington, DC.

Oishi, S., 2001. Effects of butylparaben on the male reproductive system in rats. Toxicol Ind Health 17, 31–39.

Oishi, S., 2002. Effects of propyl paraben on the male reproductive system. Food Chem Toxicol 40, 1807–1813.

Sathyanarayana, S., Calafat, A.M., Liu, F., Swan, S.H., 2008a. Maternal and infant urinary phthalate metabolite concentrations: Are they related? Environ Res 108, 413–418.

Sathyanarayana, S., Karr, C.J., Lozano, P., Brown, E., Calafat, A.M., Liu, F., Swan, S.H., 2008b. Baby care products: Possible sources of infant phthalate exposure. Pediatrics 121, e260–268.

Sjöberg, P., Lindqvist, N.G., Plöen, L., 1986. Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. Environ Health Perspect 65, 237–242.

Tanaka, M., Nakaya, S., Katayama, M., Leffers, H., Nozawa, S., Nakazawa, R., Iwamoto, T., Kobayashi, S., 2006. Effect of prenatal exposure to bisphenol A on the serum testosterone concentration of rats at birth. Human Exp Toxicol 25, 369–373.

Teuschler, L.K., Hertzberg, R.C., 1995. Current and future risk assessment guidelines, policy, and methods development for chemical mixtures. Toxicology 105, 137–144.

Wittassek, M., Angerer, J., 2008. Phthalates: Metabolism and exposure. Int J Androl 31, 131–138.

Wittassek, M., Wiesmuller, G.A., Koch, H.M., Eckard, R., Dobler, L., Muller, J., Angerer, J., Schluter, C., 2007. Internal phthalate exposure over the last two decades--a retrospective human biomonitoring study. Int J Hyg Environ Health 210, 319–333.

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 Anal 26, 803–824.