

Report to the  
U.S. Consumer Product Safety Commission  
by the

**CHRONIC HAZARD ADVISORY PANEL  
ON PHTHALATES AND PHTHALATE  
ALTERNATIVES**

July 2014

U.S. Consumer Product Safety Commission  
Directorate for Health Sciences  
Bethesda, MD 20814



## **Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives**

Chris Gennings, Ph.D.  
Virginia Commonwealth University  
Richmond, VA

Russ Hauser, M.D., Sc.D., M.P.H.  
Harvard School of Public Health  
Boston, MA

Holger M. Koch, Ph.D.  
Ruhr University  
Bochum, Germany

Andreas Kortenkamp, Ph.D.  
Brunel University  
London, United Kingdom

Paul J. Liroy, Ph.D.  
Robert Wood Johnson Medical School  
Piscataway, NJ

Philip E. Mirkes, Ph.D.  
University of Washington (retired)  
Seattle, WA

Bernard A. Schwetz, D.V.M., Ph.D.  
Department of Health and Human Services (retired)  
Washington, DC



## TABLE OF CONTENTS

<b>LIST OF TABLES .....</b>	<b>iv</b>
<b>LIST OF FIGURES .....</b>	<b>vi</b>
<b>ABBREVIATIONS.....</b>	<b>vii</b>
<b>1 Executive Summary .....</b>	<b>1</b>
<b>2 Background and Strategy.....</b>	<b>11</b>
2.1 Introduction and Strategy Definition .....	11
2.2 Selection of Toxicity Endpoints and Life Cycle Stages .....	13
2.2.1 The Rat Phthalate Syndrome.....	15
2.2.2 The Phthalate Syndrome in Other Species (excluding humans) .....	16
2.2.3 Mechanism of Phthalate Action .....	18
2.3 Toxicology Data.....	19
2.3.1 Use of Animal Data to Assess Hazard and Risk .....	19
2.3.2 Developmental Toxicity of Phthalates in Rats .....	22
2.3.3 Reproductive and Other Toxicity Data .....	25
2.3.4 Cumulative Exposure Considerations .....	26
2.4 Epidemiology .....	27
2.4.1 Phthalates and Male Reproductive Tract Developmental Outcomes .....	28
2.4.2 Phthalates and Neurodevelopmental Outcomes .....	29
2.5 Human Biomonitoring .....	34
2.5.1 Introduction.....	34
2.5.2 Objectives.....	34
2.5.3 Methodology .....	35
2.5.4 Results .....	36
2.5.5 Conclusion .....	37
2.6 Scenario-Based Exposure Assessment .....	49
2.6.1 Introduction.....	49
2.6.2 Objectives.....	49
2.6.3 Methodology .....	50
2.6.4 Results .....	50
2.6.5 Phthalate Substitutes .....	51
2.6.6 Summary of Design.....	52
2.6.7 Conclusions.....	52
2.6.8 General Conclusion and Comment .....	53
2.7 Cumulative Risk Assessment.....	61
2.7.1 Choice of Approach for Cumulative Risk Assessment .....	61
2.7.2 Summary Description of Methods Used .....	63
2.7.3 Summary Results .....	64

<b>3</b>	<b>Phthalate Risk Assessment.....</b>	<b>68</b>
<b>4</b>	<b>Discussion.....</b>	<b>71</b>
4.1	Variability and Uncertainty.....	71
4.1.1	Developmental/Reproductive Toxicity Data.....	71
4.1.2	Exposure Scenarios .....	72
4.1.3	HBM Data, Daily Intake Calculations, Hazard Index Calculations.....	73
4.2	Species Differences in Metabolism, Sensitivity, and Mechanism.....	75
<b>5</b>	<b>Recommendations .....</b>	<b>79</b>
5.1	Criteria for Recommendations.....	79
5.2	Recommendations on Permanently Banned Phthalates .....	82
5.2.1	Di-n-butyl Phthalate (DBP) (84-74-2) .....	82
5.2.2	Butylbenzyl Phthalate (BBP) (85-68-7).....	85
5.2.3	Di(2-ethylhexyl) Phthalate (DEHP) (117-81-7).....	88
5.3	Recommendations on Interim Banned Phthalates .....	91
5.3.1	Di- <i>n</i> -octyl Phthalate (DNOP) (117-84-0) .....	91
5.3.2	Diisononyl Phthalate (DINP) (28553-12-0 and 68515-48-0) .....	95
5.3.3	Diisodecyl Phthalate (DIDP) (26761-40-0 and 68515-49-1).....	100
5.4	Recommendations on Phthalates Not Banned .....	105
5.4.1	Dimethyl Phthalate (DMP) (131-11-3) .....	105
5.4.2	Diethyl Phthalate (DEP) (84-66-2) .....	107
5.4.3	Diisobutyl Phthalate (DIBP) (84-69-5).....	110
5.4.4	Di- <i>n</i> -pentyl Phthalate (DPENP) (131-18-0).....	112
5.4.5	Di- <i>n</i> -hexyl Phthalate (DHEXP) (84-75-3) .....	114
5.4.6	Dicyclohexyl Phthalate (DCHP) (84-61-7).....	116
5.4.7	Diisooctyl Phthalate (DIOP) (27554-26-3).....	118
5.4.8	Di(2-propylheptyl) Phthalate (DPHP) CAS 53306-54-0 .....	120
5.5	Recommendations on Phthalate Substitutes .....	121
5.5.1	2,2,4-trimethyl-1,3 pentanediol diisobutyrate (TPIB) (6846-50-0) .....	121
5.5.2	Di(2-ethylhexyl) adipate (DEHA) CAS 103-23-1 .....	125
5.5.3	Di(2-ethylhexyl) terephthalate (DEHT) CAS 6422-86-2.....	129
5.5.4	Acetyl Tributyl Citrate (ATBC) CAS 77-90-7 .....	133
5.5.5	Diisononyl hexahydrophthalate (DINX) CAS 166412-78-8.....	135
5.5.6	Tris(2-ethylhexyl) trimellitate (TOTM) CAS 3319-31-1.....	139
<b>6</b>	<b>References .....</b>	<b>143</b>

## 7 Appendices

A	Developmental Toxicity.....	A-1
B	Reproductive Toxicity .....	B-1
C	Epidemiology.....	C-1
D	Hazard Index Approach .....	D-1
E	Scenario-Based Exposure Assessment	
E1	Phthalates.....	E1-1
E2	Phthalate Substitutes.....	E2-1
E3	Phthalate Exposure from Diet.....	E3-1

## LIST OF TABLES

<b>Table 2.1 Summary of NOAELs (mg/kg-d) for developmental endpoints affecting male reproductive development.....</b>	<b>24</b>
<b>Table 2.2 Phthalates and reproductive tract development. ....</b>	<b>29</b>
<b>Table 2.3 Phthalates and neurological outcomes in newborns, infants, and children.....</b>	<b>31</b>
<b>Table 2.4 Molar urinary excretion fractions (<math>f_{ue}</math>) of phthalate metabolites related to the ingested dose of the parent phthalate determined in human metabolism studies within 24 hours after oral application.....</b>	<b>38</b>
<b>Table 2.5 Median (95th percentile)<sup>a</sup> concentrations (in <math>\mu\text{g/L}</math>) of DEHP and DINP metabolites in various study populations.....</b>	<b>39</b>
<b>Table 2.6 Median (95th percentile)<sup>a</sup> concentrations (in <math>\mu\text{g/L}</math>) of DMP, DEP, DBP, DIBP, BBP, DNOP, and DIDP metabolites in various study populations. ....</b>	<b>42</b>
<b>Table 2.7 Daily phthalate intake (median, in <math>\mu\text{g/kg bw/day}</math>) of selected populations back-calculated from urinary metabolite levels. ....</b>	<b>45</b>
<b>Table 2.8 Pearson correlation coefficient estimates between estimated daily intakes (DI) of the eight phthalate diesters (log 10 scale) for pregnant women in NHANES 2005–2006 (estimated using survey weights). Highlighted values indicate clusters of low molecular weight diesters and high molecular weight diesters.....</b>	<b>47</b>
<b>Table 2.9 Pearson correlation estimates (* <math>p &lt; 0.05</math>) for estimated daily intake (DI) values (log 10 scale) for postnatal values with DI values estimated in their babies in the SFF study. N=251, except for *DINP and DIDP, where N=62.....</b>	<b>48</b>
<b>Table 2.10 Sources of exposure to PEs included by exposure route. ....</b>	<b>54</b>
<b>Table 2.11 Estimated mean and 95<sup>th</sup> percentile total phthalate ester exposure (<math>\mu\text{g/kg-d}</math>) by subpopulation.....</b>	<b>55</b>
<b>Table 2.12 Estimated oral exposure (<math>\mu\text{g/kg-d}</math>) from mouthing soft plastic objects except pacifiers.<sup>a</sup> .....</b>	<b>56</b>
<b>Table 2.13 Comparison of modeled estimates of total phthalate ester exposure (<math>\mu\text{g/kg-d}</math>). 57</b>	<b>57</b>
<b>Table 2.14 Comparison of modeled exposure estimates of total phthalate ester (PE) exposure (<math>\mu\text{g/kg-d}</math>) with estimates from biomonitoring studies. ....</b>	<b>58</b>
<b>Table 2.15 Points of Departure (PODs; mg/kg-day), UFs and potency estimates for antiandrogenicity (PEAAs; <math>\mu\text{g/kg-day}</math>) in the three cases for the five phthalates considered in the cumulative risk assessment.....</b>	<b>66</b>



**Table 2.16 Summary statistics (median, 95<sup>th</sup>, 99<sup>th</sup> percentiles) for HQs and HIs calculated from biomonitoring data from pregnant women (NHANES 2005–2006; CDC, 2012b) (SFF; Sathyanarayana *et al.*, 2008a; 2008b) and infants (SFF; Sathyanarayana *et al.*, 2008a; 2008b). NHANES values include sampling weights and thus infer to 5.3 million pregnant women in the U.S. population. SFF sample sizes range: Prenatal, N=340 (except N=18 for DINP); Postnatal, N=335 (except N=95 for DINP); Baby, N=258 (except N=67 for DINP); HI values are the sum of nonmissing hazard quotients..... 67**

**Table 5.1 Margin of exposure (MOE) estimates for pregnant women (NHANES) and infants (SFF) using median and 95<sup>th</sup> percentile (0.95) daily intake estimates from biomonitoring data using the range of PODs across the three cases. .... 80**

## LIST OF FIGURES

<b>Figure 2.1 Sources of phthalate ester exposure.....</b>	<b>59</b>
<b>Figure 2.2 Estimated phthalate ester exposure (µg/kg-d) for eight phthalates and four subpopulations.....</b>	<b>60</b>

## ABBREVIATIONS\*

3 $\beta$ -HSD	3 $\beta$ -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
DIHEPP	diisoheptyl phthalate
DIHEXP	diisoheptyl phthalate

---

\* List applies to main report and all appendices.

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
PPAR $\alpha$	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 Executive Summary

The Consumer Product Safety Improvement Act of 2008 (CPSIA) directed the U.S. Consumer Product Safety Commission (CPSC) to convene a Chronic Hazard Advisory Panel (CHAP) “to study the effects of all phthalates and phthalate alternatives as used in children’s toys and child care articles.” Specifically, Section 108(b)(2) of the CPSIA requires the CHAP to:

*“complete an examination of the full range of phthalates that are used in products for children and shall—*

- (i) examine all of the potential health effects (including endocrine disrupting effects) of the full range of phthalates;*
- (ii) consider the potential health effects of each of these phthalates both in isolation and in combination with other phthalates;*
- (iii) examine the likely levels of children’s, pregnant women’s, and others’ exposure to phthalates, based on a reasonable estimation of normal and foreseeable use and abuse of such products;*
- (iv) consider the cumulative effect of total exposure to phthalates, both from children’s products and from other sources, such as personal care products;*
- (v) review all relevant data, including the most recent, best-available, peer-reviewed, scientific studies of these phthalates and phthalate alternatives that employ objective data collection practices or employ other objective methods;*
- (vi) consider the health effects of phthalates not only from ingestion but also as a result of dermal, hand-to-mouth, or other exposure;*
- (vii) consider the level at which there is a reasonable certainty of no harm to children, pregnant women, or other susceptible individuals and their offspring, considering the best available science, and using sufficient safety factors to account for uncertainties regarding exposure and susceptibility of children, pregnant women, and other potentially susceptible individuals; and*
- (viii) consider possible similar health effects of phthalate alternatives used in children’s toys and child care articles.*

In addition, the CHAP will recommend to the Commission whether any “*phthalates (or combinations of phthalates)*” other than those permanently banned, including the phthalates covered by the interim ban, or phthalate alternatives should be prohibited.\* Based on the CHAP’s recommendations, the Commission must determine whether to continue the interim prohibition of diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di-*n*-octyl phthalate (DNOP) “*in order to ensure a reasonable certainty of no harm to children, pregnant women, or other susceptible individuals with an adequate margin of safety.*”

### Health Effects in Animals

Although phthalates cause a wide range of toxicities, the most sensitive and most extensively studied is male developmental toxicity in the rat. Specifically, exposing pregnant dams to certain phthalates causes a syndrome indicative of androgen deficiency, referred to as the “phthalate

---

\* CPSIA §108(b)(2)(C).

syndrome” in rats. Exposure results in abnormalities of the developing male reproductive tract structures, the severity and prevalence of which depends on the dose. The phthalate syndrome is characterized by malformations of the epididymis, vas deferens, seminal vesicles, prostate, external genitalia (hypospadias), and by cryptorchidism (undescended testes) as well as by retention of nipples/areolae (sexually dimorphic structures in rodents) and demasculinization of the perineum, resulting in reduced anogenital distance (AGD). The highest incidence of reproductive tract malformations is observed at higher phthalate dose levels whereas, changes in AGD and nipple/areolae retention are frequently observed at lower phthalate dose levels. Furthermore, phthalates produce this developmental toxicity in male rodents with an age-dependent sensitivity, *i.e.*, with fetuses being more sensitive than neonates, which are, in turn, more sensitive than pubertal and adult animals.

The ability to produce the rat phthalate syndrome is restricted to phthalates with three to seven (or eight) carbon atoms in the backbone of the alkyl side chain. Thus, the set of “active” phthalates includes di-*n*-pentyl (DPENP) (diamyl phthalate), butylbenzyl (BBP), dibutyl (DBP), diisobutyl (DIBP), dihexyl (DHEXP), di(2-ethylhexyl) (DEHP), dicyclohexyl (DCHP), and diisononyl (DINP) phthalates. DPENP is the most potent, while DINP is the least potent, among the “active” phthalates.

Most humans are exposed to multiple phthalates. Studies in rats have shown that mixtures of multiple phthalates act in an additive fashion in causing effects associated with the phthalate syndrome. This opens the possibility of dealing with the issue of cumulative exposure to phthalates by adopting appropriate modeling approaches. Unfortunately, phthalate mixtures have not generally been studied with respect to other health effects.

## **Health Effects in Humans**

The phthalate syndrome in rats bears a resemblance to the “testicular dysgenesis syndrome” (TDS) in humans, which includes poor semen quality, testis cancer, cryptorchidism, and hypospadias, and which is hypothesized to have its origins during fetal life. There is a rapidly growing body of epidemiological studies on the association of exposure to phthalates with human health. Most studies primarily focus on the association of maternal phthalate exposure with male reproductive tract developmental endpoints and neurodevelopmental outcomes. Two of three cohort studies found reduced AGD in male infants in relation to higher maternal urinary concentrations of phthalate metabolites. Other studies reported associations between reduced AGD and hypospadias, poor sperm quality, or reduced fertility. Seven prospective pregnancy cohort studies and two cross-sectional studies investigated associations of urinary phthalate metabolites with neurological measures in infants and children. Interestingly, although each publication utilized different neurological tests at different childhood ages, poorer test scores were generally, but not always, associated with higher urinary levels of some phthalates. Other studies found associations between reduced sperm quality and some phthalates in adult males.

Overall, the epidemiological literature suggests that phthalate exposure during gestation may contribute to reduced AGD and neurobehavioral effects in male infants or children. Other limited studies suggest that adult phthalate exposure may be associated with poor sperm quality. The AGD effects are consistent with the phthalate syndrome in rats. However, it is important to note



that the phthalates for which associations were reported were not always consistent and differed across publications. In some cases, adverse effects in humans were associated with diethyl phthalate exposure, although diethyl phthalate does not cause the phthalate syndrome in rats. None of these studies was designed to provide information on the specific sources of phthalate exposure or on the proportional contribution of exposure sources to body burden.

## **Human Exposure to Phthalates**

The CHAP used two different approaches to assess human phthalate exposure. The first was human biomonitoring studies (HBM), which provided estimates of exposure in a population by measuring phthalate metabolites in urine. Thus, HBM represents an integrated measure of exposure from multiple sources and routes but does not provide information on the contributions of individual exposure sources and routes. Biomonitoring data from the National Health and Nutrition Examination Surveys (NHANES; 2005–2006 data) were used to estimate exposure to pregnant women and women of reproductive age. NHANES is a national, statistically representative sample of the U.S. population. However, it does not include children under six years old. Thus, biomonitoring data from the Study for Future Families (SFF) was used to estimate exposure to children from 2 to 36 months old, as well as to estimate prenatal and postnatal measurements of their mothers.

The U.S. population (as the worldwide population) is co-exposed to many phthalates simultaneously. Pregnant women in the United States have similar exposures compared to women of reproductive age. Distributions are highly skewed, indicating high exposures in some women and children. Furthermore, data suggest that exposures in infants might be higher than in their mothers.

The second approach was via scenario-based exposure assessment estimates. The scenario-based exposure assessment estimates of phthalate exposure were made for individual sources such as toys, personal care products, and household products. Exposure is estimated from information on phthalate concentrations in products and environmental media, frequency and duration of contact with products and environmental media, and physiological information.

Overall, food, beverages, and drugs via direct ingestion, and *not children's toys and their personal care products*, constituted the highest phthalate exposures to all subpopulations, with the highest exposure being dependent upon the phthalate and the products that contain it. DINP had the maximum potential for exposure to infants, toddlers, and older children. However, DINP exposures were primarily from food, but also from mouthing teethingers and toys, and from dermal contact with child care articles and home furnishings. The findings of this study were more or less consistent with other phthalate exposure assessments, including studies that use the biomonitoring (direct) approach, as well as those that utilize the scenario-based (indirect) approach. The estimated aggregate exposures were typically higher than some of the other estimates, and this could be because of some of the worst-case assumptions that were carried out for this study. Nevertheless, the results are within an order of magnitude of other findings, and they provide the CPSC the ability to eliminate certain products and phthalates for further consideration in the completion of a cumulative risk assessment across products and across the populations considered at risk in this analysis because of exposures to phthalates. In addition,

modeled (scenario-based) exposure estimates are in general agreement with exposure estimates developed by the CHAP from biomonitoring data.

## **Risk Assessment**

*Cumulative Risk.* Experimental data on combination effects of phthalates from multiple studies provide strong evidence that dose addition can produce good approximations of mixture effects when the effects of all components are known. Thus, the CHAP concludes the assumption of dose addition is adequate for mixtures of phthalates to provide the foundation of a cumulative risk assessment (CRA). The hazard index (HI) is an application of the dose addition principle and is widely used in cumulative risk assessments of chemical mixtures. The HI is the sum of hazard quotients (HQs), defined as the ratio of exposure (*e.g.*, estimate of daily intake [DI]) to an acceptable exposure level for a specific chemical, such as a potency estimate or a reference dose (RfD). An HI (or HQ) greater than unity indicates that the exposure exceeds the acceptable exposure (*e.g.*, RfD) for the mixture (or for individual phthalates).

For the purposes of this analysis, the requirement was made to consider endpoints only of relevance to antiandrogenicity (*i.e.*, phthalate syndrome effects). Thus, points of departure (PODs) for antiandrogenic endpoints were combined with uncertainty factors (UFs) to obtain the required input values, here termed potency estimates for antiandrogenicity (PEAA) for the hazard index approach. Three different sources for PEAA (referred to as cases) were applied. Case 1 includes values used in a published CRA for mixtures of phthalates, case 2 includes values derived from recently published and highly reliable relative potency comparisons across phthalates from the same study, and case 3 includes values from the *de novo* literature review conducted by the CHAP. We considered these three cases to determine the sensitivity of the results to the assumptions for PEAA and the total impact on the HI approach.

Estimates of daily intake were made from the NHANES (2005–2006) and SFF studies (see above). Each individual in these studies was exposed to a unique combination of phthalates. Thus, HIs were calculated for each individual.

Roughly 10% of pregnant women in NHANES had HIs exceeding unity. In the SFF, roughly 5% of mothers and their infants in the United States had HIs greater than one. Thus, the most highly exposed individuals in the relevant subpopulations exceeded the acceptable exposure level. The results were roughly similar for all three cases (sets of PEAA) considered. In all three cases, the HI value was dominated by DEHP because it has both high exposure and a low PEAA. Three phthalates (DBP, BBP, and DINP) were roughly similar in their HQ values, while diisobutyl phthalate (DIBP) had the smallest HQs (due to low exposure).

*Compounds in Isolation.* A margin of exposure (MOE) approach was applied to characterize the risks for phthalates and phthalate alternatives in isolation. No observed adverse effect levels (NOAELs) from experimental studies with animals were compared with DI estimates from either the biomonitoring or scenario-based approach. The MOE is the ratio of the NOAEL to DI. The numerical value of these MOEs was then taken into account in arriving at recommendations for specific phthalates. Typically, MOEs exceeding 100 to 1000 are considered adequate for protecting public health, for compounds in isolation. The risks from antiandrogenic phthalates

were characterized by both the MOE approach (for phthalates in isolation) and the hazard index approach (cumulative risk). The risks from non-antiandrogenic phthalates and phthalate alternatives were characterized by only the MOE approach.

## **Uncertainty**

*Toxicity Data.* Many of the developmental toxicity studies reviewed were designed to derive mechanistic information and not NOAELs and therefore used too few dose groups, often only one, or the number of animals per dose group was less than recommended. In some studies in which multiple doses and sufficient animals per dose were used, effects were seen at the lowest dose tested, and therefore a NOAEL could not be derived. For some of the phthalate alternatives, or substitutes, peer-reviewed data were lacking, or only non-peer-reviewed industry data were available. In cases in which peer-reviewed data were not available, the CHAP made decisions on a case-by-case basis as to whether non-peer-reviewed data would be used in making recommendations to the CPSC.

*Exposure Scenarios.* The overall level of uncertainty in the analyses the CHAP conducted for the phthalates, and the phthalate alternatives, varied for each compound. For some compounds, there was a lack of information for assessing either the hazard or the exposure, or both. Further complicating the analyses was the charge to the CHAP to conduct a cumulative risk analysis. This led to additional uncertainties because data on the exposures associated with all routes of entry into the body were not consistent for each potential source of one or more compounds. In addition, the toxicological data were normally obtained via oral exposure, whereas human exposure occurs by multiple routes.

The lack of exposure information for the current CHAP phthalate analysis leaves large uncertainties, especially for some of the items deemed critical to the completion of the CHAP's tasks. Further information is required on the use and release rates of the phthalates from the products during use. Without such information, it is difficult to properly employ exposure modeling tools to complete a thorough exposure characterization for risk assessment.

*Biomonitoring.* Published urinary metabolite conversion factors for DEHP and DINP were from a study of 10 male and 10 female volunteers. As can be seen from the variability of the published conversion factors, the average conversion factors could over- or underestimate exposure to individuals by a factor of 1.2. The variability of the conversion factors for the other metabolites is probably in the same region.

Several studies have shown that although the day-to-day and month-to-month variability in each individual's urinary phthalate metabolite levels can be substantial, a single urine sample was moderately predictive of each subject's exposure over three months. In general, a single urine sample has been shown to be more reliable in predicting exposure over a certain time span for the low molecular weight phthalates (dimethyl [DMP], diethyl [DEP], dibutyl, and diisobutyl [DIBP]) than for the high molecular weight phthalates (DEHP, DINP, DIDP). However, because the biomonitoring approach is population based, we can assume that the NHANES and SFF data accurately reflect the variability of exposure relevant for the investigated population subset.

For DEHP metabolites, the geometric mean concentrations of samples collected in the evening were greater than those of samples collected in the morning or in the afternoon. Because neither NHANES nor SFF samples have been collected in the evening (representing exposure events that took place in the afternoon), there are indications that both NHANES and SFF samples might underestimate exposure to DEHP and other food-borne high molecular weight phthalates. This would indicate a factor of 1.5 for underestimation of exposure for high molecular weight phthalates such as DEHP, DINP, and DIDP. Furthermore, most of the morning urine samples in NHANES (but not SFF) were collected after a fasting period; NHANES also measures lipid and glucose levels. Fasting will certainly have an impact on food-borne phthalates, resulting in an underestimation, probably less than two-fold.

Overall, the uncertainties regarding HBM data and dose extrapolations based on HBM data are within one order of magnitude, and certain factors for the possibility of overestimation of daily intake and HIs seem to be balanced by factors for their underestimation. Human biomonitoring data therefore provide reasonable estimates of the overall phthalate exposure and resulting risk.

*Species Differences.* The majority of studies examining the effects of phthalates have been conducted in the rat. *In utero* exposure to phthalates in mice (as in rats) leads to disruptions in seminiferous cord formation, the appearance of multinucleated gonocytes, and suppression of insulin-like factor 3 (insl3). Unlike in rats, these effects in mice were not accompanied by suppression of fetal testosterone synthesis or by reduced expression of genes important in steroid synthesis. However, a recent study reported that phthalates suppress testosterone synthesis in prepubertal mice (Moody *et al.*, 2013).

A primate species, the marmoset, was investigated in two studies. In the first study, neonatal marmosets were exposed to monobutyl phthalate (MBP), the major metabolite of dibutyl phthalate. The monoester induced suppressions of serum testosterone levels shortly after administration. In the second study, marmosets were exposed to MBP during fetal development and studied at birth. Effects on testosterone production were not seen, but any reductions in testosterone synthesis experienced in fetal life are likely to have disappeared by birth.

The effects of phthalate metabolites on human fetal testis explants obtained during the first or second trimester of pregnancy were investigated. In humans, the most sensitive period is thought to be late in the first trimester. In these studies, fetal testosterone production was not suppressed in rat or human fetal tissue, but reductions in the number of germ cells and inhibition of Mullerian inhibiting substance were noted. These studies are technically very challenging, and there is considerable variation in androgen production by different explants, which compromises statistical power and may obscure effects. In contrast to the observations with fetal cultures, DEHP and mono(2-ethylhexyl) phthalate (MEHP) were able to induce significant reductions of testosterone synthesis in explants of adult testes.

Very recently, the results of two experimental studies with human fetal testes grafted onto male mice were published. In one study, monobutyl phthalate suppressed serum testosterone levels by approximately 50%, but the effect did not reach statistical significance due to high experimental variation and a small number of repeats. In the second of these studies, DBP exposure did not affect the expression of genes involved in steroidogenesis. However, several issues, confounding

factors, and disparities with other reports (discussed by the authors) must be considered before firm conclusions can be drawn.

Firstly, in both studies the human fetal material was obtained at ages at which the male programming of the testes had already occurred. This raises the possibility that any effect on testosterone synthesis was missed due to the age of the explants.

Secondly, the outcome of the testosterone assay was highly variable, a result of inherent biological variability and the technical difficulties of these studies. The obvious way of dealing with experimental variability by including larger numbers of replications cannot be readily pursued with human fetal material due to technical, practical, and ethical considerations. For these reasons, results that did not reach statistical significance have to be interpreted with great caution. At this stage, the outcome of these studies has to be regarded as inconclusive.

Thirdly, the observations of associations between phthalate exposure in fetal life and anogenital distance are difficult to reconcile with the results of the xenograft and human fetal explant experiments. Changes in anogenital distance are a robust read-out of diminished androgen action *in utero*, and these observations give strong indications that phthalates are capable of driving down fetal androgen synthesis in humans.

## **Recommendations**

The CHAP was charged with making recommendations on whether the use of additional phthalates or phthalate alternatives in children's toys and child care articles should be restricted. The CHAP assessed the risks of 14 phthalates and 6 phthalate alternatives. Generally, the risk of individual compounds (risk in isolation) was considered for all 20 chemicals, while cumulative risks were considered for antiandrogenic phthalates only. The CHAP's recommendations are divided into four categories: 1) phthalates permanently banned by the CPSIA, 2) phthalates subject to an interim ban, 3) phthalates not regulated by the CPSIA, and 4) phthalate alternatives.

*Permanently Banned Phthalates.* The CHAP recommends no further action by CPSC on dibutyl phthalate (DBP), butylbenzyl phthalate (BBP), or di(2-ethylhexyl) phthalate (DEHP) at this time because they are already permanently banned in children's toys and child care articles at levels greater than 0.1%. However, the CHAP recommends that U.S. agencies responsible for dealing with DBP, BBP, and DEHP exposures from food and other products conduct the necessary risk assessments with a view to supporting risk management steps.

*Interim Banned Phthalates.* The CHAP recommends that the interim ban on the use of diisononyl phthalate (DINP) in children's toys and child care articles at levels greater than 0.1% be made permanent. This recommendation is made because DINP does induce antiandrogenic effects in animals, although with lesser potency than other active phthalates, and therefore can contribute to the cumulative risk from other antiandrogenic phthalates. Moreover, the CHAP recommends that U.S. agencies responsible for dealing with DINP exposures from food and other products conduct the necessary risk assessments with a view to supporting risk management steps.

On the other hand, di-n-octyl phthalate (DNOP) and diisodecyl phthalate (DIDP) do not appear to possess antiandrogenic potential; nonetheless, the CHAP is aware that both are potential developmental toxicants (causing supernumerary ribs in laboratory animals) and potential systemic toxicants (causing adverse effects on the liver and kidney in laboratory animals). However, because the MOEs in humans are likely to be very high for these compounds individually, the CHAP does not find compelling data to justify maintaining the current interim bans on the use of DNOP or DIDP in children's toys and child care articles. Therefore, the CHAP recommends that the current bans on DNOP and DIDP be lifted but that U.S. agencies responsible for dealing with DNOP and DIDP exposures from food and child care products conduct the necessary risk assessments with a view to supporting risk management steps.

*Phthalates Not Banned.* The CHAP recommends no action on dimethyl phthalate (DMP) or diethyl phthalate (DEP). However, the CHAP recommends that U.S. agencies responsible for dealing with DEP exposures from food, pharmaceuticals, and personal care products conduct the necessary risk assessments with a view to supporting risk management steps.

CPSC has recently detected di(2-propylheptyl) phthalate (DPHP) in some children's toys. Given the general lack of publically available information on DPHP, the CHAP is unable to recommend any action regarding the potential use of DPHP in children's toys or child care articles at this time. However, the CHAP encourages the appropriate U.S. agencies to obtain the necessary toxicological and exposure data to assess any potential risk from DPHP.

Current exposures to diisobutyl phthalate (DIBP), di-n-pentyl phthalate (DPENP), di-n-hexyl phthalate (DHEXP), and dicyclohexyl phthalate (DCHP) individually do not indicate a high level of concern. Although DIBP is not widely used in toys or child care articles, CPSC has recently detected DIBP in some children's toys. Furthermore, the toxicological profiles of DIBP, DPENP, DHEXP, and DCHP are very similar to other antiandrogenic phthalates, including DBP and DEHP. Therefore, exposure to DIBP, DPENP, DHEXP, or DCHP contributes to the cumulative risk from other antiandrogenic phthalates. The CHAP recommends that DIBP, DPENP, DHEXP, and DCHP should be permanently banned from use in children's toys and child care articles at levels greater than 0.1%.

Toxicity data are limited for diisooctyl phthalate (DIOP), but structure-activity relationships suggest that antiandrogenic effects are possible. The CHAP recommends that DIOP be subject to an interim ban from use in children's toys and child care articles at levels greater than 0.1% until sufficient toxicity and exposure data are available to assess the potential risks.

*Phthalate Alternatives.* Although data on most phthalate alternatives are limited, there is no evidence that any of the alternatives considered by the CHAP presents a hazard to infants or toddlers from mouthing toys or child care articles. Therefore, the CHAP recommends no action at this time. However, the CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure and hazard data to estimate total exposure to the phthalate alternatives and assess the potential health risks. Specifically, the CHAP recommends:

- 2,2,4-trimethyl-1,3 pentanediol diisobutyrate (TPIB). The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure and hazard data to estimate total exposure to TPIB and assess the potential health risks.
- Di(2-ethylhexyl) adipate (DEHA). Data on exposure from toys and child care articles are not available. The CHAP recommends that the appropriate U.S. agencies obtain the necessary data to estimate DEHA exposure from diet and children's articles, and assess the potential health risks.
- Di(2-ethylhexyl) terephthalate (DEHT). Information on total exposure to DEHT is not available. The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure data to estimate total exposure to DEHT and assess the potential health risks.
- Acetyl tributyl citrate (ATBC). Data on ATBC are somewhat limited. The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure and hazard data to estimate total exposure to ATBC and assess the potential health risks.
- Diisononyl hexahydrophthalate (1,2-cyclohexanedicarboxylic acid, diisononyl ester) (DINX). Given the lack of publically available information on DINX, the CHAP strongly encourages the appropriate U.S. agencies to obtain the necessary toxicological and exposure data to assess any potential risk from DINX.
- Tris(2-ethylhexyl) trimellitate (TOTM). The CHAP strongly recommends that appropriate exposure information be obtained before TOTM is used in toys and child care products.

This page intentionally left blank



## 2 Background and Strategy

### 2.1 Introduction and Strategy Definition

The Consumer Product Safety Improvement Act of 2008 (CPSIA) directs the U.S. Consumer Product Safety Commission (CPSC) to convene a Chronic Hazard Advisory Panel (CHAP) “to study the effects of all phthalates and phthalate alternatives as used in children’s toys and child care articles.” The CHAP will recommend to the Commission whether any phthalates or phthalate alternatives other than those permanently banned should be declared banned hazardous substances. Specifically, Section 108(b)(2) of the CPSIA requires the CHAP to:

*“complete an examination of the full range of phthalates that are used in products for children and shall—*

- (i) examine all of the potential health effects (including endocrine disrupting effects) of the full range of phthalates;*
- (ii) consider the potential health effects of each of these phthalates both in isolation and in combination with other phthalates;*
- (iii) examine the likely levels of children’s, pregnant women’s, and others’ exposure to phthalates, based on a reasonable estimation of normal and foreseeable use and abuse of such products;*
- (iv) consider the cumulative effect of total exposure to phthalates, both from children’s products and from other sources, such as personal care products;*
- (v) review all relevant data, including the most recent, best-available, peer-reviewed, scientific studies of these phthalates and phthalate alternatives that employ objective data collection practices or employ other objective methods;*
- (vi) consider the health effects of phthalates not only from ingestion but also as a result of dermal, hand-to-mouth, or other exposure;*
- (vii) consider the level at which there is a reasonable certainty of no harm to children, pregnant women, or other susceptible individuals and their offspring, considering the best available science, and using sufficient safety factors to account for uncertainties regarding exposure and susceptibility of children, pregnant women, and other potentially susceptible individuals; and*
- (viii) consider possible similar health effects of phthalate alternatives used in children’s toys and child care articles.*

*The panel’s examinations pursuant to this paragraph shall be conducted de novo. The findings and conclusions of any previous Chronic Hazard Advisory Panel on this issue and other studies conducted by the Commission shall be reviewed by the panel but shall not be considered determinative.”*

In addition, the CHAP will recommend to the Commission whether any “*phthalates (or combinations of phthalates)*” other than those permanently banned, including the phthalates covered by the interim ban, or phthalate alternatives should be prohibited.\* Based on the CHAP’s recommendations, the Commission must determine whether to continue the interim

---

\* CPSIA §108(b)(2)(C).

prohibition of diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di-*n*-octyl phthalate (DNOP) “*in order to ensure a reasonable certainty of no harm to children, pregnant women, or other susceptible individuals with an adequate margin of safety.*” (Section 108 (b)(3)(A) of the CPSIA) The Commission also must determine whether to prohibit the use of children’s products containing any other phthalates or phthalate alternatives, or substitutes, “*as the Commission determines necessary to protect the health of children.*” (Section 108 (b)(3)(B) of the CPSIA)

In an effort to complete its assignment within a reasonable time frame, the CHAP drew some boundaries around the task regarding the number of chemicals to be reviewed, the identification of the most sensitive subpopulations, and the endpoints of toxicity of greatest concern. Based on toxicity and exposure data, the phthalate esters (PEs) of primary concern in this report are listed in Table 2.1 (p. 24) and discussed in Appendix A. Phthalates cause a wide range of toxicities in experimental animals but the one considered of greatest concern for purposes of this report is a syndrome indicative of androgen insufficiency in fetal life, what is referred to in rats as the phthalate syndrome, caused by exposure of pregnant dams to certain phthalates. Exposure results in abnormalities of the developing male reproductive tract structures. Therefore, the subpopulations of greatest concern are fetuses, neonates, and children. In order to protect fetuses, risk reduction measures must consider women of reproductive age, especially pregnant women.

The literature review performed by the CHAP covered all aspects of risk assessment. Thus, information and studies derived from toxicological experiments, exposure characterization, and human studies were targeted by the CHAP. Initially, these efforts were based upon previously published criteria documents, literature reviews, and reports.<sup>\*</sup> These were then augmented by subsequently published or publicly available data, studies, and risk assessments. The CHAP considered the systematic review process (Guyatt *et al.*, 2011; Higgins *et al.*, 2011; Woodruff and Sutton, 2011). Because of the nature of the subject matter and the charge questions, which involve different streams of evidence and information, the CHAP concluded that its review was not amenable to the systematic review methodology. To avoid bias, the CHAP obtained new information and opinions about the availability of other information through public comment and presentations. The stopping point for CHAP analysis and interpretation was information available by the end of 2012.

In an effort to determine whether specific phthalates or phthalate substitutes were associated with the induction of the phthalate syndrome, members of the CHAP reviewed the toxicology literature to identify the toxicological findings and toxic dose levels from relevant studies. Dose response relationships were reviewed, and no observed adverse effect levels (NOAELs) were determined. In evaluating toxicological studies, the CHAP was guided by criteria for quality assessments, such as those developed by Klimisch *et al.* (1997) in which studies are assigned reliability criteria based on adherence to good laboratory practice (GLP). However, the focus on GLP eliminates most scientific studies emanating from academic research. The CHAP believed that exclusion of scientific studies not compliant with GLP would have unduly skewed the outcome of the assessment, and for that reason, all studies available in the public domain were

---

<sup>\*</sup> These include, but are not limited to, reports from the Agency for Toxic Substances and Disease Registry (ATSDR); European Chemicals Agency (ECHA); International Agency for Research on Cancer (IARC); Center for the Evaluation of Research on Human Reproduction (CERHR), National Toxicology Program (NTP); and the National Research Council (NRC). All references are cited in the text.

analyzed. To assess their quality, CHAP was guided by the criteria of reliability, relevance, and adequacy as laid down by the Organisation for Economic Cooperation and Development (OECD, 2007). “Reliability” refers to evaluating the inherent quality of a test report or publication relating to preferably standardized methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. “Relevance” covers the extent to which data and tests are appropriate for a particular hazard identification or risk characterization. “Adequacy” means the usefulness of data for hazard/risk assessment purposes.

Similarly, studies in humans were reviewed to assess endpoints of toxicity and parameters of exposure, when known, as well as the identities of phthalates and their metabolites, and levels of exposure. Human and environmental exposure data were evaluated. Human biomonitoring data were analyzed to correlate no observed adverse effect levels with exposure data. Sources of exposure were reviewed to determine whether source information might allow targeted recommendations about efforts to minimize human exposure.

Recommendations to CPSC for regulatory actions were then derived from a combination of input on the basis of toxicity findings in animals and humans, together with hazard index (HI)<sup>\*</sup> calculations to help address concerns about vulnerable subpopulations and specific sources of exposure to individual chemicals or combinations of chemicals.

## **2.2 Selection of Toxicity Endpoints and Life Cycle Stages**

The charge to the CHAP is to “examine all of the potential health effects (including endocrine disrupting effects) of the full range of phthalates.”

Some phthalates are capable of producing carcinogenic effects, but these effects have been dismissed as not relevant to humans. In its evaluation of di(2-ethylhexyl) phthalate (DEHP), the International Agency for Research on Cancer (IARC) considered that the induction of liver tumors in rodents by DEHP was mediated by peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ), a mechanism regarded as not relevant for humans (IARC, 2000a). However, more recent evidence of induction of hepatocellular tumors in PPAR $\alpha$  knock-out mice (Ito *et al.*, 2007) suggests that a PPAR $\alpha$ -independent mechanism may also be relevant for DEHP. DEHP also produced testicular Leydig cell tumors (Voss *et al.*, 2005) and pancreatic tumors (David *et al.*, 2000) in the rat, and neither of these effects has been linked to PPAR $\alpha$ . Furthermore, Leydig cell tumors have been detected after *in utero* exposure of rats to dibutyl phthalate (DBP) (Mylchreest *et al.*, 1999; Barlow and Foster, 2003). The CHAP therefore does not rule out that carcinogenicity may be relevant for certain phthalates. However, there are considerable knowledge gaps regarding the potential carcinogenicity of other phthalates and the relevance of the underlying modes of action for human risk assessment. The most sensitive and most extensively studied endpoint is male developmental toxicity in the rat, and therefore the CHAP focused on this toxicity endpoint, consistent with the stance taken in earlier assessments by other bodies (National Research Council [NRC, 2008]).

---

<sup>\*</sup> The hazard index is the ratio of the daily intake to the reference dose.

As discussed in more detail subsequently, exposure to phthalates during the latter stages of gestation in the rat has been shown to disrupt testicular development leading to subsequent reproductive tract dysgenesis. In addition, phthalates produce this developmental toxicity in male rodents with an age-dependent sensitivity, *i.e.*, fetal animals being more sensitive than neonates, which are, in turn, more sensitive than pubertal and adult animals (Foster *et al.*, 2006). Cognizant of this age-dependent sensitivity of phthalate-induced male developmental toxicity, the CHAP decided to focus its analysis on adverse developmental effects as the phthalate toxicity endpoints and the fetus and neonate as the life cycle stages of major interest in its efforts to complete its assigned task. To complete its charge, CHAP systematically reviewed the phthalate developmental and reproductive toxicology literature, focusing on dose levels that induced phthalate toxicity endpoints related to the rat phthalate syndrome, defined subsequently.

Because much is known about the mechanisms by which phthalates induce the phthalate syndrome, CHAP also focused on a variety of molecular endpoints in the pathway leading to reproductive tract dysgenesis. Together, morphological, histopathological, and molecular toxicity endpoints were used to select NOAELs from specific studies, and these NOAELs, in turn, were used in one of the three case studies in the HI-based cumulative assessment described in Section 2.7.

Because the developmental toxicity studies reviewed in Appendix A relate to various aspects of male sexual differentiation, a brief introduction to this subject, taken directly from the 2008 NRC publication *Phthalates and Cumulative Risk Assessment: The Tasks Ahead*, is provided below (2008). This is followed by a discussion of the rat phthalate syndrome, the phthalate syndrome in other species (excluding humans), and concludes with a section on the mechanisms of phthalate action, all of which are from NRC (2008).

### ***Male Sexual Differentiation in Mammals***

*“Sexual differentiation in males follows complex interconnected pathways during embryo and fetal development that has been reviewed extensively elsewhere (Capel, 2000; Hughes, 2000a; 2000b; 2001; Tilmann and Capel, 2002; Brennan and Capel, 2004) Critical to the development of male mammals is the development of the testis in embryonic life from a bipotential gonad (a tissue that could develop into a testis or an ovary). The “selection” is genetically controlled in most mammals by a gene on the Y chromosome. The sex-determining gene (sry in mice and SRY in humans) acts as a switch to control multiple downstream pathways that lead to the male phenotype. Male differentiation after gonad determination is exclusively hormone-dependent and requires the presence at the correct time and tissue location of specific concentrations of fetal testis hormones—Mullerian inhibiting substances (MIS), insulin-like factors, and androgens. Although a female phenotype is produced independently of the presence of an ovary, the male phenotype depends greatly on development of the testis. Under the influence of hormones and cell products from the early testis, the Mullerian duct regresses and the mesonephric duct (or Wolffian duct) gives rise to the epididymis and vas deferens. In the absence of MIS and testosterone, the Mullerian ductal system develops further into the oviduct, uterus, and upper vagina, and the Wolffian duct system regresses. Those early events occur before establishment of a hypothalamic-pituitary-gonadal axis and depend on local control and production of hormones (that is, the*

*process is gonadotropin-independent). Normal development and differentiation of the prostate from the urogenital sinus and of the external genitalia from the genital tubercle are also under androgen control. More recent studies of conditional knockout mice that have alterations of the luteinizing-hormone receptor have shown normal differentiation of the genitalia, although they are significantly smaller.”*

*“Testis descent appears to require androgens and the hormone insulin-like factor 3 (insl3) (Adham et al., 2000) to proceed normally. The testis in early fetal life is near the kidney and attached to the abdominal wall by the cranial suspensory ligament (CSL) and gubernaculum. The gubernaculum contracts, thickens, and develops a bulbous outgrowth; this results in the location of the testis in the lower abdomen (transabdominal descent). The CSL regresses through an androgen-dependent process. In the female, the CSL is retained with a thin gubernaculum to maintain ovarian position. Descent of the testes through the inguinal ring into the scrotum (inguinoscrotal descent) is under androgen control.”*

*“Because the majority of studies discussed below were conducted in rats, it is helpful to compare the rat and human developmental periods for male sexual differentiation. Production of fetal testosterone occurs over a broader window in humans (gestation weeks 8–37) than in rats (gestation days [GD] 15–21). The critical period for sexual differentiation in humans is late in the first trimester of pregnancy, and differentiation is essentially complete by 16 weeks after conception (Hiort and Holterhus, 2000). The critical period in rats occurs in later gestation, as indicated by the production of testosterone in the latter part of the gestational period, and some sexual development occurs postnatally in rats. For example, descent of the testes into the scrotum occurs in gestation weeks 27–35 in humans and in the third postnatal week in rats. Generally, the early postnatal period in rats corresponds to the third trimester in humans.”*

As the authors of the 2008 NRC report conclude:

*“...it is clear that normal differentiation of the male phenotype has specific requirements for fetal testicular hormones, including androgens, and therefore can be particularly sensitive to the action of environmental agents that can alter the endocrine milieu of the fetal testis during the critical periods of development.”*

### **2.2.1 The Rat Phthalate Syndrome**

Studies conducted over the past 20 plus years have shown that phthalates produce a syndrome of reproductive abnormalities in male offspring when administered to pregnant rats during the later stages of pregnancy, *e.g.*, gestation days (GD) 15–20 (reviewed in Foster, 2006). This group of interrelated abnormalities, known as the rat phthalate syndrome, is characterized by malformations of the epididymis, vas deferens, seminal vesicles, prostate, external genitalia (hypospadias), and by cryptorchidism (undescended testes) as well as by retention of nipples/areolae (sexually dimorphic structures in rodents) and demasculinization of the perineum, resulting in reduced anogenital distance (AGD) (Mylchreest *et al.*, 1998; 1999). The highest incidence of reproductive tract malformations is observed at higher phthalate dose levels, whereas changes in AGD and nipple/areolae retention are frequently observed at lower phthalate dose levels (Mylchreest *et al.*, 2000). It is important to note that not all phthalates produce all of

the abnormalities of the rat phthalate syndrome under any one exposure scenario (Foster *et al.*, 1980; Gray *et al.*, 2000). The endocrine disrupting potency of the phthalates (producing the rat phthalate syndrome and based on the reduction of fetal testicular testosterone) seems to be restricted to phthalates with three to seven (or eight) carbon atoms in the backbone of the alkyl side chain, with the highest potency centering around five carbon atoms in the backbone (di-*n*-pentyl phthalate [DPENP]) (Gray *et al.*, 2000). “Active” phthalates start with diisobutyl phthalate (DIBP, with three carbon atoms in the alkyl backbone) and end with DINP (with~seven or eight carbons in the alky chain backbone).



Mechanistically, phthalate exposure can be linked to the observed phthalate syndrome abnormalities by an early phthalate-related disturbance of normal fetal testicular Leydig function and/or development (Foster, 2006). This disturbance is characterized by Leydig cell hyperplasia (Barlow and Foster, 2003) or the formation of large aggregates of Leydig cells at GD 21 in the developing testis. These morphological changes are preceded by a significant reduction in fetal testosterone production (Parks *et al.*, 2000), which likely results in the failure of the Wolffian duct system to develop normally, thereby contributing to the abnormalities observed in the vas deferens, epididymis, and seminal vesicles. Reduced testosterone levels also disturb the dihydrotestosterone (DHT)-induced development of the prostate and external genitalia by reducing the amount of DHT that can be produced from testosterone by 5 $\alpha$ -reductase. Because DHT is required for the normal apoptosis of nipple anlage<sup>†</sup> in males and also for growth of the perineum to produce the normal male AGD, changes in AGD and nipple retention are consistent with phthalate-induced reductions in testosterone levels. Although testicular descent also requires normal testosterone levels, insulin like factor 3 (insl3), another Leydig cell product, also plays a role (Wilson *et al.*, 2004). Phthalate exposure has been shown to decrease insl3 gene expression, and mice in which the insl3 gene has been deleted show complete cryptorchidism.

### 2.2.2 The Phthalate Syndrome in Other Species (excluding humans)

Although the literature is replete with information about the phthalate syndrome in rats, there is, interestingly, a relative dearth of information about the phthalate syndrome in other species. In an early study, Gray *et al.* (1982) found that DBP produced uniformly severe seminiferous tubular atrophy in rats and guinea pigs, only focal atrophy in mice, and no changes in hamsters. Hamsters were insensitive to other phthalates (DEHP and DPENP) as well. A study by Higuchi *et al.* (2003), using rabbits exposed orally to DBP, reported that the most pronounced effects observed were decreased testes and accessory gland weights as well as abnormal semen characteristics, *e.g.*, decreased sperm concentration/total sperm/normal sperm and an increase in acrosome-nuclear defects. In a study by Gaido *et al.* (2007), mice exposed to DBP showed significantly increased seminiferous cord diameter, the number of multinucleated gonocytes per cord, and the number of nuclei per multinucleated gonocyte. In a separate set of experiments, dosing with high levels of DBP did not significantly affect fetal testicular testosterone concentration even though the plasma concentrations of the DBP metabolite monobutyl phthalate (MBP) in mice were equal to or greater than the concentrations in maternal and fetal rats. In a

\* BBP, butylbenzyl phthalate; DBP, di-*n*-butyl phthalate; DIHEXP, diisohexyl phthalate; DEHP, di(2-ethylhexyl phthalate); DCHP, dicyclohexyl phthalate. A complete list of abbreviations begins on page .

<sup>†</sup> Precursor tissue.

third set of experiments, *in utero* exposure to DBP in mice led to the rapid induction of immediate early genes, as in the rat; however, unlike in the rat, expression of genes involved in cholesterol homeostasis and steroidogenesis were not decreased. In another study, reported only in abstract form, Marsman (1995) observed no treatment-related gross lesions at necropsy and no histopathological lesions associated with treatment in male or female mice.

Two studies have been published on the toxicity of phthalates (specifically DBP/MBP) in nonhuman primates. In one study by Hallmark *et al.* (2007), four-day-old marmosets were administered 500 mg/kg-day MBP for 14 days. In a second acute study, nine males, two to seven days of age, were administered a single oral dose of 500 mg/kg-day. Results showed that MBP did suppress testosterone production after an acute exposure; however, this suppression of testosterone production was not observed when measurements were taken 14 days after the beginning of exposure to MBP. The authors speculate that the initial MBP-induced inhibition of steroidogenesis in the neonatal marmoset leads to a “reduced negative feedback and hence a compensatory increase in luteinizing hormone (LH) secretion to restore steroid production to normal levels.” In a follow-up study, McKinnell *et al.* (2009) exposed pregnant marmosets from ~7 to 15 weeks gestation with 500 mg/kg-day MBP, and male offspring were studied at birth (1–5 days; n= 6). Fetal exposure did not affect gross testicular morphology, reproductive tract development, testosterone levels, germ cell number and proliferation, Sertoli cell number, or germ:Sertoli cell ratio.

Although limited in number, and the timing of exposure is often outside the known window of susceptibility, the studies cited above clearly show that most animals tested are more resistant to phthalates than rats. This has led some to question whether the rat is a suitable model for assessing phthalate effects in humans and stimulated the studies with nonhuman primates (marmosets). Unfortunately, the number of animals exposed is small, only one phthalate has been tested and at only one dose, and a limited number of time points have been assessed. In addition, the available data, although largely negative, is equivocal in that DBP did appear to suppress testosterone production when administered in the early neonatal period (Hallmark *et al.*, 2007). In presentations at CHAP meetings, the CHAP also became aware of unpublished studies by Richard Sharpe that appear to show that human testes, which were implanted into nude rats that are then exposed to phthalates, did not respond to DBP. Since those presentations, the studies from Dr. Sharpe’s laboratory have been published (Mitchell *et al.*, 2012). Results of these studies showed that the weight and the testosterone production of 14–20-week human fetal testis grafted under the skin of nude mice were not statistically significantly affected by DBP or MBP, although an approximately 50% reduction of testosterone levels was observed. Due to high experimental variation and the small number of repetitions, this reduction did not reach statistical significance. In contrast, exposure of rat fetal xenografts to DBP significantly reduced seminal vesicle weight and testosterone production. While these results were of interest to the CHAP, these studies do have limitations. The major limitation is that most of the human testes that were transplanted into the rat were beyond 14 weeks of gestation, which would put them beyond the critical window for the development of the reproductive tract normally under androgen control. (For further discussion of this issue, see Section 4.2.)

The CHAP agreed that additional nonhuman primate studies as well as *ex vivo* studies are needed to determine whether the rat is a good model for the human; however, the CHAP also agreed that studies in rats currently offer the best available data for assessing human risk.

### 2.2.3 Mechanism of Phthalate Action

Although the majority of animal studies have focused on the morphological and histopathological effects of exposure to phthalates relative to the male reproductive system, considerable effort has also been focused on the mechanisms by which phthalates produce their adverse effects. Initial mechanistic studies centered on phthalates acting as environmental estrogens or antiandrogens; however, data from various estrogenic and antiandrogenic screening assays clearly showed that while the parent phthalate could bind to steroid receptors, the developmentally toxic monoesters exhibited little or no affinity for the estrogen or androgen receptors (David, 2006). Another potential mechanism of phthalate developmental toxicity is through the PPAR $\alpha$ . Support for this hypothesis comes from data showing that circulating testosterone levels in PPAR $\alpha$ -null mice were increased following treatment with DEHP compared with a decrease in wild-type mice, suggesting that PPAR $\alpha$  plays a role in postnatal testicular toxicity (Ward *et al.*, 1998). PPAR $\alpha$  activation may play some role in the developmental toxicity of nonreproductive organs (Lampen *et al.*, 2003); however, data linking PPAR $\alpha$  activation to the developmental toxicity of reproductive organs are lacking.

Because other studies had shown that normal male rat sexual differentiation is dependent upon three hormones produced by the fetal testis (*i.e.*, an anti-Mullerian hormone produced by the Sertoli cells, testosterone produced by the fetal Leydig cells, and insl3) several laboratories conducted studies to determine whether or not the administration of specific phthalates to pregnant dams during fetal sexual differentiation that caused demasculinization of the male rat offspring would also affect testicular testosterone production and insl3 expression. Studies by Wilson *et al.* (2004), Borch *et al.* (2006b), and Howdeshell *et al.* (2007) reported significant decreases in testosterone production and insl3 expression after exposure to DEHP, DBP, butylbenzyl phthalate (BBP), and to DEHP + DBP (each at one-half of its effective dose). The study by Wilson *et al.* (2004) also showed that exposure to DEHP (and similarly to DBP and BBP) altered Leydig cell maturation, resulting in reduced production of testosterone and insl3, from which they further proposed that the reduced testosterone levels result in malformations such as hypospadias, whereas reduced insl3 mRNA levels lead to lower levels of this peptide hormone and abnormalities of the gubernacular ligament (agenesis or elongated and filamentous) or freely moving testes (no cranial suspensory or gubernacular ligaments). Together, these studies identify a plausible link between inhibition of steroidogenesis in fetal rat testes and alterations in male reproductive development. Other phthalates that do not alter testicular testosterone synthesis (diethyl phthalate [DEP]; Gazouli *et al.*, 2002) and gene expression for steroidogenesis (DEP and dimethyl phthalate [DMP]; Liu *et al.*, 2005) also do not produce the phthalate syndrome malformations produced by phthalates that do alter testicular testosterone synthesis and gene expression for steroidogenesis (Gray *et al.*, 2000; Liu *et al.*, 2005).

Complementary studies have also shown that exposure to DBP *in utero* leads to a coordinated decrease in expression of genes involved in cholesterol transport (peripheral benzodiazepine receptor [PBR], steroidogenic acute regulatory [StAR] protein, scavenger receptor class B1 [SR-B1]) and steroidogenesis (cytochrome P450 side chain cleavage [P450scc], cytochrome P450c17



[P450c17], 3 $\beta$ -hydroxysteroid dehydrogenase [3 $\beta$ -HSD]). This leads to a reduction in testosterone production in the fetal testis (Shultz *et al.*, 2001; Barlow and Foster, 2003; Lehmann *et al.*, 2004; Hannas *et al.*, 2011b). Interestingly, Lehmann *et al.*, (2004) further showed that DBP induced significant reductions in SR-B1, 3 $\beta$ -HSD, and c-Kit (a stem cell factor produced by Sertoli cells that is essential for normal gonocyte proliferation and survival) mRNA levels at doses (0.1 or 1.0 mg/kg-day) that approach maximal human exposure levels. The biological significance of these data is not known, given that no statistically significant observable adverse effects on male reproductive tract development have been identified at DBP dose <100 mg/kg-day and given that fetal testicular testosterone is reduced only at dose levels equal to or greater than 50 mg/kg-day.

Thus, current evidence suggests that once the phthalate monoester crosses the placenta and reaches the fetus, it alters gene expression for cholesterol transport and steroidogenesis in Leydig cells. This, in turn, leads to decreased cholesterol transport and decreased testosterone synthesis. As a consequence, androgen-dependent tissue differentiation is adversely affected, culminating in hypospadias and other features of the phthalate syndrome. In addition, phthalates (DEHP and DBP) also alter the expression of *insl3*, leading to decreased expression. Decreased levels of *insl3* result in malformations of the gubernacular ligament, which is necessary for testicular descent into the scrotal sac.

## **2.3 Toxicology Data**

### **2.3.1 Use of Animal Data to Assess Hazard and Risk**

The published literature on the toxicity of phthalates is extensive and varies widely in its usefulness for assessment of risks to humans. This section introduces the approach taken by the CHAP to evaluate such a broad and varied literature, and draws conclusions about potential risks to humans from individual chemicals or mixtures of chemicals.

What is the basis for selecting key studies that provide a basis for assessment of risk for humans? What is the threshold for determining that studies in humans or animals are either helpful for assessment of risk or not? For example, the results of a pilot study in a small number of lab animals are usually not suitable for risk assessment. The study was designed to select the appropriate dose levels for a more definitive study. Similarly, case histories on individual persons are not a sufficient basis for a risk assessment because the individual case may not be representative of the population. For the same reason, reports of cluster effects of small numbers of humans are often difficult to extrapolate beyond the cluster. The most desired data are from appropriately designed studies in humans or animals that account for confounders and have reasonable power to detect an effect (*e.g.*, 80% at 0.95 probability), with results replicated in another study of similar design and purpose.

As an example of another threshold for acceptance of data, the CHAP's goal was to use data from studies that were published in peer-reviewed journals. There were times when the only available information was from a source other than published literature, for example, the results of a study submitted to a public docket of a regulatory agency as part of a data call-in or the results of a recently completed study that had not been submitted for review by a journal. In such

cases, the CHAP has considered the data but has noted in its review that the results from the study on this particular chemical have not been published in the literature.

In its assessment of risks of human exposure to phthalates and phthalate substitutes, the CHAP focused on the charge as specified in Section 108 of the Consumer Product Safety Improvement Act of 2008. The hazard of greatest concern was considered as the potential hazard for some of the members of these chemical groups to cause structural and functional alterations to the developing reproductive organs and tissues of male offspring exposed during late gestation and the early postnatal period. These findings are most prominent in rats although inconclusive studies in humans suggest that similar effects may be seen in humans.

As the CHAP reviewed the available literature in humans and animals, we considered a number of factors to reach our conclusions. In the absence of good human data, it is prudent to rely on the results of animal studies. The distinction between hazard and risk is important to understand to predict risk to humans based on animal data. The first step in risk assessment is determination of hazard (NRC, 1983). What are the effects seen in animal tests—cancer; genotoxicity; liver, kidney, or other organ toxicity; reproductive or developmental toxicity? This step is independent of dose response. What are the targets of effect, and what effect is seen at what dose level in animals?

The second step is to assess risk for humans. This involves several considerations. What is the dose response? The response should become more severe with increasing dose, and a larger percent of the exposed population should show the response if it is really related to exposure to the test article. Knowing the dose response in animals allows one to define a level of exposure that is not associated with an observed response (*i.e.*, NOAEL) in animal studies.

Risk is a function of hazard and exposure (the probability of harm to humans). Comparison of the NOAEL in animal studies to the known or anticipated level of human exposure is the basis for calculating a margin of safety as an estimate of risk for humans. What is an acceptable margin of exposure (MOE) depends on the substance and the toxic response. It may be about 10 for a life-saving drug but for a chemical in the environment or in food, the acceptable MOE may be 100–1000 (U.S. Environmental Protection Agency [EPA], 1993). Generally, the level of concern is considered low when the MOE is greater than the net uncertainty factor for a given chemical.

Animal data, then, can be a useful basis for determining risks to humans. As with human data, animal data exist over a wide range of usefulness, depending on experimental design, power, confounders, appropriateness of the animal model for the question being asked, consistency of data between studies, replication of results, etc. National and international guidelines (*e.g.*, U.S. Food and Drug Administration [FDA], U.S. EPA, International Conference on Harmonisation [ICH], Organisation for Economic Cooperation and Development [OECD]) define standards for protocols for animal studies. Protocols designed according to these guidelines are useful for risk assessment.

What should be done when confronted with conflicting results of animal studies? Consider the quality and relevance of the studies, experimental design in the context of standard protocols,

route of exposure, power, and confounders. The conservative approach is to rely on the study reporting adverse effects unless there are compelling reasons to exclude the study, *i.e.*, considerations such as quality, design, execution or interpretation.

How should one use *in vitro* test results and data from mechanistic studies and pharmacokinetic studies? *In vitro* studies usually do not have dose response data that allow results to be used directly in risk assessment in the same sense that *in vivo* test results are used for that purpose. However, the results of *in vitro* and mechanistic studies can help to reinforce or modulate the level of concern upwards or downwards. The results of metabolic and pharmacokinetic or pharmacodynamic studies can help to determine the relevance of animal data for humans and may allow selection of laboratory animal species that are most relevant for assessment of risk for humans.

It is often difficult to determine that animal data definitely predict risk for humans. However, the results of *in vitro*, mechanistic, and metabolic/pharmacokinetic studies can help to decide whether or not the results of animal tests should be assumed to be relevant for evaluation of human risk. For example, if the ultimate toxicant is determined by animal tests to be a metabolite of a chemical that is not formed in humans, the adverse effects seen in that species of animal are not considered relevant for prediction of risk to humans who do not form that particular metabolite. It must also be remembered that some chemicals have been found to be toxic to humans when the animal studies did not predict such an effect. For example, the sedative thalidomide was found to be teratogenic in humans but did not cause effects in a majority of animal species tested by conventional methodology at the time (the 1950s). Likewise, adverse effects are sometimes discovered in humans that were not seen in a previous study with fewer human subjects.

There are also other considerations for interpreting animal data and integrating animal findings with data from humans. Data from human studies of reasonable quality generally are a stronger signal of risk to humans than findings in animal studies. However, in the absence of other data, findings in animals should be assumed to be relevant for prediction of risk to humans.

Observations in multiple animal species are a stronger signal than a finding in a single species. Studies in certain species, *e.g.*, nonhuman primates, are often stronger signals of risk to humans than study results from other species.

The dose levels at which effects are seen in animal studies must be considered along with the presence or absence of confounding toxicity to nonreproductive organs.

Animal or human studies that are negative must be examined closely for adequacy of experimental design, sufficient power, and presence of confounders that may have masked a possible effect of the test article.

Animal or human studies that are positive must be examined closely for appropriateness of experimental design and presence of confounders that may have contributed to the effects reported.

In summary, this section has presented the approach used by the CHAP to evaluate the available toxicity literature on the phthalates and phthalate substitutes under the purview of the CHAP. The reviews of studies on individual chemicals are found in Appendix A (Developmental Toxicity) and Appendix B (Reproductive and Other Toxicity) of this report.

### 2.3.2 Developmental Toxicity of Phthalates in Rats

As directed by the Consumer Product Safety Improvement Act of 2008 (CPSIA, 2008), the CHAP was also charged to: *“i) examine all of the potential health effects (including endocrine disrupting effects) of the full range of phthalates, ii) consider the potential health effects of each of these phthalates both in isolation and in combination with other phthalates and iv) consider the cumulative effect of total exposure to phthalates, both from children’s products and from other sources, such as personal care products.”*(Section 108(b)(2)(B) of 15 U.S.C. § 2077)

To complete the charge of examining the full range of phthalates, the CHAP decided after careful consideration to limit its review to 14 phthalates. Included were the 3 permanently banned phthalates (DBP, BBP, and DEHP), the 3 phthalates currently on an interim ban (DNOP, DINP, and DIDP), and 8 other phthalates (DMP, DEP,DPENP, DIBP, dicyclohexyl phthalate [DCHP], di-*n*-hexyl phthalate [DHEXP], diisooctyl phthalate [DIOP], and di(2-propylheptyl) phthalate [DPHP]). Because the first six of these phthalates were extensively reviewed by a phthalates expert panel in a series of reports from the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) in 2002, our review of these phthalates begins with a brief summary of these NTP reports. It was followed by a review of the literature since those reports (see Appendix A). For the eight other phthalates that were not reviewed by the NTP panel, the CHAP review covers all the relevant studies available to the committee. From the available literature for each of these 14 phthalates, we then identified the most sensitive developmentally toxic endpoint in a particular study as well as the highest dose that did not elicit that endpoint (NOAEL). Finally, we evaluated the “adequacy” of particular studies to select the most appropriate NOAEL for deriving a reference dose (RfD) or similar toxicological benchmark. Our criteria for an adequate study from which a NOAEL could be derived were the following: 1) at least three dose levels and a concurrent control should be used; 2) the highest dose should induce some developmental and/or maternal toxicity, and the lowest dose level should not produce either maternal or developmental toxicity; 3) each test and control group should have a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy; and 4) pregnant animals need to be exposed during the appropriate period of gestation. In addition, studies should follow the EPA guideline OPPTS 870.3700 and the OECD Guideline for the Testing of Chemicals (OECD 414, adopted 22 January 2001).

We also evaluated the potential developmental toxicity of phthalate substitutes. The phthalate substitutes include acetyl tributyl citrate (ATBC), di(2-ethylhexyl) adipate (DEHA), diisononyl 1,2-dicarboxycyclohexane (DINCH<sup>®</sup>, DINX<sup>\*</sup>), di(2-ethylhexyl) terephthalate (DEHT), trioctyl

---

\* DINCH<sup>®</sup> is a registered trademark of BASF. Although DINCH<sup>®</sup> is the commonly used abbreviation, the alternate abbreviation DINX is used here to represent the generic chemical.

trimellitate (TOTM), and 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate (TXIB<sup>®</sup>, TPIB<sup>\*</sup>). These compounds were selected from the many possible phthalate substitutes because they are already in use (ATBC, DEHT, DINX, TPIB; Dreyfus, 2010) or are considered likely to be used (DEHA, TOTM; Versar/SRC, 2010) in toys and child care articles. The same criteria were used to evaluate the “adequacy” of studies describing the developmental toxicity of phthalate substitutes as were used for phthalates. However, because of the paucity of data for many of the phthalate substitutes, studies that did not meet the listed criteria were cited. In these instances, we indicated the limitations associated with these studies.

The systematic evaluation of the developmental toxicity literature for the 14 phthalates and 6 phthalate substitutes, and the rationale for selecting a specific NOAEL for each chemical, are provided in Appendix A. A list of NOAELs is provided in Table 2.1.

To fulfill the CHAP’s charge to consider the health effects of phthalates in isolation and in combination with other phthalates, and to consider the cumulative effect of total exposure to phthalates, the CHAP relied upon its review of the following: a. the toxicology literature of phthalates and phthalate substitutes, exposure data (sources and levels), and b. data obtained from the HI approach for cumulative risk assessment (see Section 2.7.1. for details). The HI is essentially the sum of the ratios of the daily intake (DI) of each individual phthalate divided by its RfD. This approach uses NOAELs from animal studies as points of departure (PODs), which are then adjusted with uncertainty factors to yield RfDs, and biomonitoring data for DI input. Because of limitations in the biomonitoring datasets (National Health and Nutrition Evaluation Surveys, [NHANES]; Centers for Disease Control [CDC, 2012b]; and Study for Future Families [SFF], Sathyanarayana *et al.*, 2008a; 2008b)), only five phthalates (DBP, DIBP, BBP, DEHP, and DINP) were analyzed by the HI approach. . Case 3<sup>†</sup> in the HI analysis uses NOAELs generated from the available literature on the developmental toxicity of these five phthalates. To provide NOAELs, when possible, for these five phthalates, the CHAP systematically reviewed the published, peer-reviewed literature that reported information concerning the effects of *in utero* exposure of phthalates in pregnant rats.

---

\* TXIB<sup>®</sup> is a registered trademark of Eastman Chemical Co. Although TXIB<sup>®</sup> is the commonly used abbreviation, the alternate abbreviation TPIB is used here to represent the generic chemical.

† As discussed in Section 2.7.2.2., the CHAP considered three sets of reference doses (three cases) to calculate the hazard index.

**Table 2.1 Summary of NOAELs (mg/kg-d) for developmental endpoints affecting male reproductive development.**

CHEMICAL	NOAEL	ENDPOINT	REFERENCE
<b><i>Permanently Banned</i></b>			
Dibutyl phthalate (DBP)	50	↑NR; ↓AGD	Mylchreest <i>et al.</i> (2000), Zhang <i>et al.</i> (2004)
Butylbenzyl phthalate (BBP)	50	↑NR; ↓AGD	Tyl <i>et al.</i> (2004)
Di(2-ethylhexyl) phthalate (DEHP)	5	RTM; DVO; DSP	Andrade <i>et al.</i> (2006b), Grande <i>et al.</i> (2006), Blystone <i>et al.</i> (2010)
<b><i>Interim Banned</i></b>			
Di- <i>n</i> -octyl phthalate (DNOP)	NA	NA	
Diisononyl phthalate (DINP)	50	↑NR	Boberg <i>et al.</i> (2011)
Diisodecyl phthalate (DIDP)	≥600	NAE	Hushka <i>et al.</i> (2001)
<b><i>Phthalates Not Banned</i></b>			
Dimethyl phthalate (DMP)	≥750	NAE	Gray <i>et al.</i> (2000)
Diethyl phthalate (DEP)	≥750	NAE	Gray <i>et al.</i> (2000)
Diisobutyl phthalate (DIBP)	125	↓AGD	Saillenfait <i>et al.</i> (2008)
Di- <i>n</i> -pentyl phthalate (DPENP)	11	↓T PROD	Hannas <i>et al.</i> (2011a)
Di- <i>n</i> -hexyl phthalate (DHEXP)	≤50	↓AGD	Saillenfait <i>et al.</i> (2009b)
Dicyclohexyl phthalate (DCHP)	16	↓AGD	Hoshino <i>et al.</i> (2005)
Diisooctyl phthalate (DIOP)	NA	NA	
Di(2-propylheptyl) phthalate (DPHP)	NA	NA	
<b><i>Phthalate Substitutes</i></b>			
2,2,4-trimethyl-1,3 pentanediol diisobutyrate (TPIB)	≥1125	NAE	Eastman (2007)
Di(2-ethylhexyl) adipate (DEHA)	≥800	NAE	Dalgaard <i>et al.</i> (2003)
Di (2-ethylhexyl)terephthalate (DEHT)	≥750	NAE	Gray <i>et al.</i> (2000), Faber <i>et al.</i> (2007b)
Acetyl tri- <i>n</i> -butyl citrate (ATBC)	≥1000	NAE	Robins (1994), Chase and Willoughby (2002)
Cyclohexanedicarboxylic acid, dinonyl ester (DINX)	≥1000	NAE	SCENIHR (2007)
Trioctyltrimellitate (TOTM)	100	DSP	JMHW (1998)

AGD = Anogenital Distance; DSP; =Decreased Spermatocytes and Spermatids; DVO = Delayed Vaginal Opening; NA not available; NAE = No Antiandrogenic Effects Observed; NR = Nipple Retention; RTM = Reproductive Tract Malformation; T PROD = Testosterone Production

## **2.3.3 Reproductive and Other Toxicity Data**

### **2.3.3.1 Interpretation of Reproductive Toxicity Data**

#### **2.3.3.1.1 General Toxicity Studies**

These studies range in duration from acute to chronic and may have been conducted in mice, rats, dogs, or sometimes in nonhuman primates. Their purpose does not include collection of reproductive performance data, but other data may be relevant to reproductive toxicity.

- Histopathology of organs. Effects of dose, duration of treatment, sex, and recovery from exposure can all be examined.
- Organ weights. Weight of organs at time of necropsy can be very useful, especially organs from males. Weights of seminal vesicles, prostate, testis, and epididymis are often biologically significant if greater than 10% increases or decreases are seen compared to control weights. Weight changes of the ovaries and uterus of females are harder to interpret because of cyclicity.
- Hormone levels may be helpful but are often not available.
- Synchronicity of organs, particularly uterus, ovary, and vaginal epithelium, is helpful to assess appropriate integration of reproductive functionality.

Pharmacokinetic and pharmacodynamic studies may identify species or sex-related differences in absorption, metabolism, distribution, and elimination as well as differences in pathophysiology that are important in their relationship to reproductive toxicities.

#### **2.3.3.1.2 Reproductive Studies**

These studies may be nongenerational (fertility only) or single or multiple generation in design. They may involve treated males or females, or both, and they are usually conducted in rats.

- Fertility studies.
  - In females, vaginal smears are made during the dosage period. Mating is confirmed by examination for vaginal plugs. At a predetermined day of gestation, the females are sacrificed, the number of live and dead implants is counted as are the number of corpora lutea in the ovary.
  - In male fertility studies, animals are dosed for 4–10 weeks before mating with untreated females. Females are examined daily for evidence of mating (vaginal plugs). After a predetermined number of days of cohabitation, the females are sacrificed and the same data are collected as in the female fertility trial. Males are necropsied and sperm counts are conducted (low sperm counts in rodents may not be accompanied by low fertility). Organs are weighed and saved for histopathology examinations.
- Single or multigenerational reproductive study. Treated males and females are mated and percent pregnancy is calculated from the number of litters. Pups are counted and weighed to assess survival and growth. In a multigenerational study, pups are saved for parenting the next generation. Remaining pups and adults are killed for necropsy findings, organ

weights, and histopathology. The reproductive measures are repeated through successive generations.

#### **2.3.4 Cumulative Exposure Considerations**

Human subjects come into contact not with one individual phthalate, but with large numbers of these substances. In addition, there is exposure to other chemicals that may affect humans in ways similar to phthalates.

The combined effects of phthalates have been studied in experimental models with endpoints relevant to the disruption of male sexual differentiation. Combination effects of phthalates on other toxicological endpoints have not been evaluated.

Several experimental studies have shown that multicomponent mixtures of phthalates can suppress fetal androgen synthesis in male rats after administration during critical windows of susceptibility. In these studies, the effects of all individual phthalates in the mixtures were assessed by dose-response analyses. This information was then utilized to anticipate the joint effects of the combinations, by assuming that each phthalate would exert its effects without interfering with the action of the other phthalates in the mixture (the additivity assumption). In all studies published thus far, the experimentally observed effects were in good agreement with those anticipated on the basis of the dose-response relationships of the individual phthalates in the mixture (see the review in NRC, 2008, and Howdeshell *et al.*, 2007; 2008). Of note is a very recent paper in which the effects of mixtures of nine phthalates (DEHP, diisooheptyl phthalate [DIHEPP], DBP, DCHP, BBP, DPENP, DIBP, di-*n*-heptyl phthalate [DHEPP], and diisohexyl phthalate [DHEXP]) were investigated and shown to act in an additive fashion in terms of suppression of fetal androgen synthesis in rats (Hannas *et al.*, 2012). The objective of all these studies was not to investigate the effect of phthalate combinations at realistic exposures in the range of those experienced by humans. Rather, their merit is in demonstrating that mixture effects of these substances can be predicted quite accurately when the potency of individual phthalates in the mixture is known. This opens the possibility of dealing with the issue of cumulative exposure to phthalates by adopting modeling approaches.

Additional studies have shown convincingly that phthalates can also act in concert with other chemicals capable of disrupting male sexual differentiation through mechanisms different from those induced by phthalates. Of relevance are chemicals that diminish androgen action in fetal life by blocking the androgen receptor, or by interfering with androgen-metabolizing enzymes, such as various carboximide and azole pesticides.

The first study to examine the combined effects of a phthalate, BBP, and an antiandrogen, the pesticide linuron, showed that the combination induced decreased testosterone production and caused alterations of androgen-organized tissues and malformations of external genitalia. The two substances together always produced effects stronger than each chemical on its own (Hotchkiss *et al.*, 2004).

The results of a much larger a developmental toxicity mixture experiment with rats that involved mixtures of the three phthalates, BBP, DBP, and DEHP, and the antiandrogens vinclozolin, procymidone, linuron, and prochloraz in were reported by Rider *et al.* (2008; 2009). The mixture



was able to disrupt landmarks of male sexual differentiation in a way well predictable on the basis of the potency of the individual components. For other effects, such as genital malformations (hypospadias), the observed responses exceeded those expected, indicating weak synergisms. Similar results were obtained with a mixture composed of 10 antiandrogens, including the phthalates BBP, DBP, DEHP, DIBP, DPENP, and DIHEXP, and the pesticides vinclozolin, procymidone, prochloraz, and linuron (Rider *et al.*, 2010).

Christiansen *et al.* (2009) evaluated a mixture composed of DEHP and vinclozolin, finasteride, and prochloraz. Strikingly, the effect of combined exposure to the selected chemicals on malformations of external sex organs was synergistic, and the observed responses were greater than would be predicted from the toxicities of the individual chemicals. A dose of the mixture predicted to elicit only marginal incidences of malformations produced effects in nearly all the animals. With other landmarks of male sexual differentiation, the effect of this mixture was additive.

Unexpected interactions between 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and DBP in terms of epididymal and testicular malformations were reported by Rider *et al.* (2010). Although TCDD on its own did not produce these effects, there was a significant exacerbation of the responses provoked by DBP.

Of particular relevance to risk assessment is to examine whether phthalates exhibit combination effects at doses that do not induce observable effects when they are administered individually. This is important both for phthalate mixtures and for combinations of phthalates with other antiandrogenic (AA) agents. Unfortunately, most of the combination effect studies with the phthalates and other antiandrogens were not carried out with the intention of addressing this issue directly. That gap has been bridged in the NRC report (2008) on cumulative risk assessment for phthalates by re-analyzing published papers. The experiment by Howdeshell *et al.* (2008) on suppression of testosterone synthesis after developmental exposure to five phthalates indicates that phthalates are able to work together at low, individually ineffective doses. The re-analysis by NRC (2008) has shown that each phthalate was not expected to produce statistically significant effects at the doses at which they were present in the mixture tested by Howdeshell *et al.* (2008). Yet, the five phthalates jointly produced significant suppressions of testosterone synthesis. The study by Rider *et al.* (2008) also provides some indications for combination effects of phthalates and androgen-receptor antagonists at low doses.

In all experimental studies conducted with phthalates thus far, and with phthalates in combination with other chemicals, the effects of the mixture were stronger than the effect of the most potent component of the combination. This highlights that the traditional approach to risk assessment with its focus on single chemicals one by one may inadequately address the health risks that might arise from combined exposures to multiple chemicals.

## 2.4 Epidemiology

There is a rapidly growing body of epidemiological studies on the potential association of exposure to phthalates with human health. Most studies primarily focus on the association of maternal phthalate exposure with male reproductive tract developmental endpoints and neurodevelopmental outcomes. Briefly summarized below is the epidemiologic literature on

phthalates and these two primary health endpoints; additional details are provided in Appendix C. All of the studies used urinary measures of phthalate metabolites as a biomarker of exposure during gestation or early childhood. Although amniotic fluid measurements of phthalate metabolites would provide the best estimate of internal dose for the fetus, access to this matrix is highly limited. There are few published studies on human amniotic fluid levels of phthalate metabolites (Silva *et al.*, 2004; Calafat *et al.*, 2006; Wittassek *et al.*, 2009).

It is important to note that none of the epidemiological studies reviewed below were designed to provide information on the specific sources of phthalate exposure or on the proportional contribution of exposure sources to body burden. In Section 2.6, the contribution of children's toys to children and women's exposure is described.

#### **2.4.1 Phthalates and Male Reproductive Tract Developmental Effects**

The association of gestational exposure to phthalates and reproductive tract development was explored in three study cohorts (Table 2.2) (Swan, 2005; Swan, 2008; Huang *et al.*, 2009; Suzuki *et al.*, 2012). Although the results of these studies were not entirely consistent, they represent some of the first human data to assess potential risks of developmental exposure to phthalates. The Swan (2005; 2008) and Suzuki *et al.* (2012) publications reported reduced AGD in male infants in relation to higher maternal urinary concentrations of DEHP metabolites, whereas the Swan study also found similar associations of monoethyl phthalate (MEP) and MBP with reduced AGD. The Huang study (2009) did not find associations of any phthalate metabolite with reduced AGD in boys, but did in girls.

It is well known that in rodent studies some phthalates cause the phthalate syndrome, consisting of, among other endpoints, reduced AGD, increased prevalence of reproductive tract anomalies and poor semen quality (see Section 2.2 for further details). Although it is uncertain whether the phthalate syndrome occurs in humans, the data on phthalates and AGD are suggestive (Swan *et al.*, 2005; Swan, 2008; Suzuki *et al.*, 2012) and human data suggest that AGD is a relevant marker for reproductive health outcomes. Hsieh *et al.* (2008) reported that boys with hypospadias had shorter AGD than boys with normal genitals. Mendiola (2011) showed that shorter AGD was associated with poorer semen quality (*i.e.*, lower sperm concentration and motility, and poorer morphology), while Eisenberg (2011) found shorter AGD among infertile men as compared to fertile men. These human studies demonstrated that shortened AGD is associated with reproductive conditions that are similar to those observed in rats with the phthalate syndrome. This observation supports the use of human AGD as a relevant measure to assess the antiandrogenic mode of action of phthalates during fetal development.

In conclusion, these studies provide the first human data linking prenatal phthalate exposure (specifically DEP, DBP and DEHP) with antiandrogenic effects in male offspring. These results have important relevance to the hypothesized testicular dysgenesis syndrome (TDS) in humans. Skakkebaek *et al.* (2001) hypothesized that poor semen quality, testis cancer, cryptorchidism, and hypospadias were symptoms of an underlying entity referred to as TDS, which had its origins during fetal life. They further hypothesized that environmental chemicals, specifically endocrine disruptors, played an important role in the etiology of TDS through disruption of embryonal programming and gonadal development during fetal life. Currently, in humans, the

evidence on the potential effects of phthalates during fetal development is limited to shortened AGD.

Based on the human data on gestational exposure and reduced AGD, exposure to DEP, DBP and DEHP metabolites should be reduced. Further studies are needed to determine whether fetal exposure to phthalates is associated with other endpoints (*i.e.*, reproductive tract malformations and altered semen quality).

**Table 2.2 Phthalates and male reproductive tract development.**

Author, Year	Design/Sample Size	Exposure	Outcomes	Results	Comments
Suzuki <i>et al.</i> (2012)	Prospective cohort (111 mother-son pairs)	Urine concentrations of phthalate metabolites	AGD and AGI (weight-normalized index of AGD)	MEHP associated with reduced AGI, suggestive association of sum of DEHP metabolites with reduced AGI. No association of MMP, MEP, MBP, MBZP, MEHHP or MEOHP with AGI.	Small study, urine sample collected late in pregnancy, multiple examiners
Huang <i>et al.</i> (2009)	Prospective cohort (65 mother-infant pairs)	Amniotic fluid and urine concentrations of phthalate metabolites	AGD, birth length and weight, gestational length	In girls, decreased AGD in relation to amniotic fluid levels of MBP and MEHP. No associations found in boys.	Small study, no associations with male AGD
Swan <i>et al.</i> (2005)	Prospective cohort (85 mother-son pairs)	Urine concentrations of phthalate metabolites	AGD and AGI (weight-normalized index of AGD)	Decreased AGI associated with higher urinary concentrations of MBP, MIBP, MEP, and MBZP.	Small study, urine sample collected late in pregnancy
Swan (2008; extension of the 2005 study)	Prospective cohort (106 mother-son pairs)	Urine concentrations of phthalate metabolites	AGD (adjusted for weight percentiles)	Decreased AGD, adjusted for weight percentiles, associated with higher urinary concentrations of MEP, MBP, MEHP, MEHHP, and MEOHP.	Small study, urine sample collected late in pregnancy

AGD = Anogenital Distance; AGI = Anogenital Index; MEHP = mono(2-ethylhexyl) phthalate; DEHP = di(2-ethylhexyl phthalate); MMP = monomethyl phthalate; MEP = monoethyl phthalate; MBP = monobutyl phthalate; MBZP = monobenzyl phthalate; MEHHP = mono(2-ethyl-5-hydrohexyl) phthalate; MEOHP = mono(2-ethyl-5-oxohexyl) phthalate; MIBP = monoisobutyl phthalate

#### 2.4.2 Phthalates and Neurodevelopmental Outcomes

Seven prospective pregnancy cohort studies and two cross-sectional studies investigated associations of urinary phthalate metabolites with neurological measures in infants and children (Table 2.3). Synthesizing the results across studies is difficult because they used different study designs, different sets of phthalate metabolites were measured at different times during pregnancy and their concentrations differed across studies, and, most importantly, the studies

assessed different neurological outcomes at different ages using different tests. Despite this heterogeneity, there were several conclusions. More weight should be given to the results from the seven prospective cohort studies, in which urinary phthalates were measured during pregnancy and related to outcomes in infancy or childhood. Cross-sectional studies in which urinary phthalate metabolite concentrations were measured concurrent with outcome assessment are difficult to interpret because the exposure measure reflects only recent exposure (past several hours), which is likely not within the etiologically relevant exposure window.

Interestingly, although each publication utilized different neurological tests at different childhood ages, poorer test scores were generally, but not always, associated with higher urinary levels of some phthalates. However, the phthalates for which associations were reported were not always consistent and differed across publications. For instance, in the Mount Sinai School of Medicine (MSSM) study, Engel *et al.* (2009) found a significant decline in girls in the adjusted mean Orientation score and Quality of Alertness score (assessed with the Brazelton Neonatal Behavioral Assessment (BNBA) scale within five days of delivery) with increasing urinary concentrations of high molecular weight phthalates, largely driven by DEHP metabolites. In Engel's second publication (Engel *et al.*, 2010) on the same cohort, children were examined between ages four and nine. The authors found an association of higher urinary concentrations of low molecular weight (LMW) phthalates, largely driven by MEP, with poorer scores on the Behavioral Assessment System for Children Parent Rating Scale (BASC-PRS) for aggression, conduct problems, attention problems, and depression clinical scales, as well externalizing problems and behavioral symptoms index. LMW phthalates were also associated with poorer scores on the global executive composite index and the emotional control scale of the Behavior Rating Inventory of Executive Function (BRIEF). In the third MSSM publication (Miodovnik *et al.*, 2011), higher urinary concentrations of LMW phthalates were associated with higher social responsiveness scale (SRS) scores and positively with poorer scores on social cognition, social communication, and social awareness.

Both the Kim *et al.* (2011) and Whyatt *et al.* (2011) studies explored associations of gestational urinary phthalate metabolite concentrations with the mental developmental index (MDI) and psychomotor developmental index (PDI) assessed with the Bayley Scales of Infant Development at six months and three years of age, respectively. Whyatt found associations of MBP (DBP metabolite) and monoisobutyl phthalate (MIBP; DIBP metabolite) with decreased PDI scores, and in girls, MBP was associated with decreased MDI. On the other hand, Kim reported a negative association of mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP),\* mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) and MBP with PDI, whereas MEHHP was negatively associated with MDI. In boys, MEHHP, MEOHP, and MBP were negatively associated with MDI and PDI. No associations were found in girls. Therefore, there was some consistency across studies in the association of MBP with decreased MDI and PDI, but not with respect to DEHP metabolites. Sex-specific associations also varied across studies.

Based on the human data on gestational phthalate exposure and associations with poorer neurodevelopmental test scores, human exposure to DEHP, DBP, and DEP metabolites should be reduced.

---

\* MEHHP and MEOHP are secondary metabolites of DEHP; see Section 2.5.

**Table 2.3 Phthalates and neurological outcomes in newborns, infants, and children.**

Author, Year	Design/Sample Size	Exposure	Outcome	Results	Comments
Kim <i>et al.</i> (2009)	Cross-sectional (261 children)	Urine concentrations of MEHP, MEOHP, and MBP measured when child was 8 to 11 years old	Teacher assessed attention deficit hyperactivity disorder (ADHD) symptoms and neuropsychological dysfunction measured when child was 8 to 11 years old	DEHP metabolites associated with ADHD scores	cross-sectional design
Cho <i>et al.</i> (2010)	Cross-sectional (621 children)	Urine concentrations of MEHP, MEOHP, and MBP measured when child was 8 to 11 years old	Full Scale IQ, Verbal IQ, Vocabulary, and Block design scores measured when child was 8 to 11 years old	After adjusting for maternal IQ, only DEHP metabolites associated with reduced Vocabulary score	cross-sectional design
Whyatt <i>et al.</i> (2011)	Prospective cohort (319 mother-child pairs)	Urinary concentrations of MBP, MBZP, and MIBP, and four DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) measured during the third trimester	Mental developmental index (MDI) and psychomotor developmental index (PDI) using Bayley Scales of Infant Development II, behavioral problems assessed by maternal report on child behavior checklist. Assessed at three years of age.	MBP and MIBP associated with a decreased PDI score and with increased odds of motor delay. In girls, MBP associated with decreased MDI. MBP and MBZP associated with increased odds of clinically withdrawn behavior. MBZP associated with increased odds for clinically internalizing behavior.	single spot urine sample late in pregnancy
Kim <i>et al.</i> (2011)	Prospective cohort (460 mother-infant pairs)	Urinary concentrations of MEHHP, MEOHP, and MBP measured during third trimester	Mental (MDI) and psychomotor (PDI) development indices of Bayley Scales of Infant Development measured at age six months	After adjusting for maternal IQ, MEHHP was negatively associated with MDI, whereas MEHHP, MEOHP, and MBP were negatively associated with PDI. In males, MEHHP, MEOHP, and MBP were negatively associated with MDI and PDI. No associations for females.	single spot urine sample late in pregnancy
Swan <i>et al.</i> (2010)	Prospective cohort (145 mother-child pairs)	Urine concentrations of phthalate metabolites measured during third trimester	Mother assessed play behavior (preschool activities inventory questionnaire)	Among boys, inverse association of MBP, MIBP, and DEHP metabolites (MEOHP, MEHHP, and sum of DEHP metabolites) with less masculine composite scores. No associations among girls.	single spot urine sample late in pregnancy, mother reported play behavior

Author, Year	Design/Sample Size	Exposure	Outcome	Results	Comments
Engel <i>et al.</i> (2009)	Prospective cohort (295 mother-infant pairs)	Urine concentrations of phthalate metabolites measured during third trimester	Brazelton Neonatal Behavioral Assessment scale assessed within first five days of delivery	Sex-specific effects. Among girls, decline in orientation score and quality of alertness score with increased high molecular weight phthalate concentrations. Boys had improved motor performance with increased low molecular weight phthalate concentrations.	single spot urine sample late in pregnancy
Engel <i>et al.</i> (2010)	Prospective cohort (188 mother-child pairs)	Urine concentrations of phthalate metabolites measured during third trimester	Behavioral Rating Inventory executive Function (BRIEF) and Behavioral Assessment System for Children Parent Rating Scale (BASC-PRS). Assessed up to three times between ages four and nine.	Higher concentrations of low molecular weight phthalates were associated with poorer BASC scores for aggression, conduct problems, attention problems, and depression scales, as well as externalizing problems and behavioral symptoms index. Low molecular weight phthalates were associated with poorer scores on global executive composite index and the emotional control scale of the BRIEF. MBP associated with aggression and externalizing problems, and poorer scores on working memory.	single spot urine sample late in pregnancy
Miodovnik <i>et al.</i> (2011)	Prospective cohort (137 mother-child pairs)	Urine concentrations of phthalate metabolites measured during third trimester	Social responsiveness scale (SRS), assessed between ages seven and nine	Higher urinary concentrations of low molecular weight phthalates were associated with higher SRS scores, poorer scores on social cognition, social communication, and social awareness. Associations were significant for MEP and in the same direction for MBP and MMP. High molecular weight phthalate concentrations were associated with nonsignificantly poorer SRS scores (smaller magnitudes)	single spot urine sample late in pregnancy

Author, Year	Design/Sample Size	Exposure	Outcome	Results	Comments
Yolton <i>et al.</i> (2011)	Prospective cohort (350 mother-infant pairs)	Urine concentrations of phthalate metabolites measured at 16 and 26 weeks gestation	Infant neurobehavior, assessed with the NICU (neonatal intensive care unit) Network Neurobehavioral Scale (NNNS), measured at five weeks after delivery	Higher total DBP metabolites (MBP and MIBP) at 26 weeks (but not at 16 weeks) gestation were associated with improved behavioral organization as evidenced by lower levels of arousal, higher self-regulation, less handling required and improved movement quality, as well as a borderline association with movement quality. In males, higher total DEHP metabolites at 26 weeks were associated with more non-optimal reflexes	two spot urine samples at 16 and 26 weeks

## 2.5 Human Biomonitoring

### 2.5.1 Introduction

Human biomonitoring (HBM) determines internal exposures (*i.e.*, body burdens) by measuring the respective chemicals or their metabolites in human specimens (*e.g.*, urine or blood). Thus, HBM represents an integral measure of exposure from multiple sources and routes (Angerer *et al.*, 2006; NRC, 2006; Needham *et al.*, 2007) and permits an integrated exposure assessment even when the quantity and quality of external exposures are unknown and/or if the significance of the contribution of different routes of exposure is ambiguous.

Urine is the ideal matrix to determine internal phthalate exposure and urinary phthalate metabolites are measured in an increasing number of HBM studies. The extent of oxidative modification increases with the alkyl chain length of the phthalate monoester. Therefore, short-chain phthalates (*e.g.*, DMP, DEP, DIBP, or DBP) mostly metabolize only to their simple monoesters and not further. The urinary excretion of their monoesters represents approximately 70% of the oral dose. By contrast, long-chain phthalates (eight or more carbons in the alkyl chain, *e.g.*, DEHP, DINP, or DIDP) are further metabolized to oxidative side chain products (alcohols, ketones, and carboxylic acids). These secondary, oxidized metabolites are the main metabolites of the long-chain phthalates excreted in human urine.

HBM data can be used to quantify overall phthalate exposures and to compare exposures of the general population with special subpopulations (*e.g.*, children or pregnant women) and with toxicological animal data. For risk assessment, biomonitoring/biomarker measurements can be used to reliably extrapolate to daily doses of the respective phthalate(s) taken up, which can then be compared to health or toxicological benchmarks (*e.g.*, NOAEL, tolerable daily intake [TDI], and RfD) normally obtained from animal studies. HBM data can also be used in epidemiological studies to correlate actual internal exposures with observed (health) effects.

### 2.5.2 Objectives

The objectives of this section are to illustrate and quantify the omnipresence of phthalate exposure in the general population (both U.S. and worldwide) and to focus on the phthalate exposure in specific U.S. subpopulations (pregnant women, NHANES, 2005–2006; women and infants, SFF, women and infants) that are the focus of CHAP's task. HBM-derived DI calculations (performed *de novo* by the CHAP's task for these subpopulations) prepare the ground for the HI approach of Section 2.7.

We also compare daily intakes calculated from HBM data (of the above datasets) to DI estimates from the aggregate external exposure approach/scenario-based exposure estimation approach of Section 2.6. With this approach, we can reveal the presence of exposures that are possibly not reflected in the scenario-based approach (the HBM DI estimation is higher than the scenario-based DI estimation). Thus, indicating that there are pathways/routes/sources of exposure not included in the scenario-based approach; or we can reveal the presence of possible external exposures that are not reflected in the HBM approach (scenario-based DI estimation higher than HBM DI estimation), thus indicating *worst-case* exposure scenarios that are not present in the HBM approach of the subpopulations investigated.



### 2.5.3 Methodology

We performed a full literature review on HBM data on phthalates (and possible phthalate substitutes). We compiled and compared worldwide HBM data and paid special attention to pregnant women (NHANES 2005–2006; SFF women) and infants (SFF infants) in our further deliberations.

The biomonitoring data from the National Health and Nutrition Examination Surveys (2005–2006 data; CDC, 2012b) \* and the biomonitoring data from the SFF (Sathyanarayana *et al.*, 2008a; 2008b), and prenatal and postnatal measurements in women and measurements in infants (ages: 2–36 months) are the focus of this investigation. This was done because of the CHAP's task to investigate the likely levels of children's, pregnant women's, and others' exposure to phthalates and to consider the cumulative effect of total exposure to phthalates both from children's products and other sources.

Based on HBM-derived daily intake estimates in conjunction with health benchmarks for individual phthalates (hazard quotients [HQs]), we evaluated the presence or absence of risk associated with each individual phthalate, and we compared the risks associated with each phthalate with the risk associated with other phthalates (and thus identified key phthalates in terms of risk). In the last step, we evaluated the risk associated with the cumulative phthalate exposure (by adding up the individual *hazard quotients*) as expressed in the *hazard index*. See Section 2.7.

- Analysis of HBM data from pregnant women (NHANES, 2005–2006 data; CDC, 2012b): 15 phthalate metabolites are measured in the NHANES 2005–2006 dataset. Of these 15 metabolites, we used 12 to determine the exposure to nine parent phthalates: DMP, DEP, DIBP, DBP, BBP, DEHP, DINP, DIDP/DPHP, and DNOP.
- Analysis of HBM data from SFF: Exposure data from the SFF in young children and their mothers were provided to the CHAP by Dr. Shanna Swan and are published in part in Sathyanarayana *et al.*, (2008a; 2008b). Urinary concentrations from 12 monoesters were measured, of which we used 11 to determine exposure to 8 parent phthalates: DMP, DEP, DIBP, DBP, BBP, DEHP, DINP, and DIDP/DPHP. DNOP exposure was not reported in this study, due to a low detection frequency.

Dose extrapolations/DI calculations based on HBM data: We calculated the daily intake of each parent chemical separately per adult and child from urinary concentrations (David, 2000; Kohn *et al.*, 2000; Koch *et al.*, 2003a; Wittassek *et al.*, 2011). The model for DI includes the creatinine-related metabolite concentrations together with reference values for the creatinine excretion in the following form:

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

---

\* This cycle of NHANES was the most recent version in which phthalate data were available at the time of our analyses. Previous cycles were not combined with the 2005–2006 data due to study design changes associated with fasting requirements.

Where:  $UE_{\text{sum}}$  is the molar urinary excretion of the respective metabolite(s) and 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).<sup>\*</sup> 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:

- A rate of 18 mg/kg-day for women and 23 mg/kg-day for men 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-day for these SFF women, a 30% increase from 18.

The molar urinary excretion fraction  $F_{\text{ue}}$  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 2.4.

## 2.5.4 Results

Worldwide HBM data (urinary phthalate metabolites, in  $\mu\text{g/L}$ ) is compiled in Tables 2.5 and 2.6 (using sampling weights for the calculations from NHANES; see Appendix D, Section 2.1.2). Specific HBM data estimated by the CHAP are highlighted in green. The general population and the subpopulations that are the focus of the CHAP's assessment, are exposed to all of the phthalates investigated (nearly 100% positive detects). The spectrum of exposure to the various phthalates is rather similar over all populations investigated and is dominated by some phthalates (*e.g.*, DEHP and DEP).

Intake estimates (DI) for phthalates (in  $\mu\text{g/kg bw/day}$ ) are compiled in Table 2.7. Specific HBM intake data generated within this CHAP (concerning the target populations within NHANES [CDC, 2012b] and SFF [Sathyanarayana *et al.*, 2008a; 2008b]) are highlighted in green. Daily phthalate intakes in the target populations are dominated by DEP and DEHP, followed by DINP, DIDP and DBP.

In NHANES 2005–2006, comparing pregnant women to nonpregnant women in this age range, the exposures were not found to be significantly different. In the upper percentiles, as well as with weighted analyses, there are indications that exposures might be higher in pregnant women than in women in general or in the rest of the NHANES population. DIs calculated from NHANES 2005–2006 (women 15–45 years old) are generally comparable to DIs calculated from SFF women (prenatal). The SFF prenatal estimates for DEHP are slightly lower than the other two, and the distribution of DIDP in NHANES is slightly lower compared to the SFF data. However, these possible shifts are within the interquartile ranges of the comparison groups.

---

<sup>\*</sup> When height was outside the tabulated range for gender and age categories or when weight was missing, CE was considered missing.

- **Infant Data (SFF):** Inspection of the SFF data reveals that the infants might have significantly higher intakes (related to their body weights) compared to their mothers (see Figure 2.2).
- **Correlations:** Correlation coefficient estimates between estimated DI of the nine phthalate diesters (log 10 scale) for pregnant women in NHANES 2005–2006 (using survey weights) reveal two clusters with significant positive correlations: (1) low molecular weight phthalates: DBP, DIBP, BBP; and (2) high molecular weight phthalates: DEHP, DINP, and DIDP (see Table 2.8). Similar clusters of correlations can be observed in the SFF dataset (see Table 2.9).

This suggests common uses and/or common sources of exposure within the set of low molecular weight phthalates and within the set of high molecular weight phthalates, respectively. Furthermore, this means that an individual exposed to elevated amounts of one of the high molecular weight phthalates is likely exposed to elevated amounts of the other high molecular weight phthalates, too. However, the correlations are low to moderate (in agreement with other human biomonitoring data), which indicates that the variability of each phthalate (metabolite) in urine is influenced by more than just one exposure source and that exposures are similar. To understand peak relationships better, more than one spot or single urine sample is required to determine when the highest intakes occur over space and time, and among the individuals tested. Thus, there will always be intrinsic uncertainty associated with the use of single urine samples for each subject in the cumulative risk assessment.

### 2.5.5 Conclusions

The following conclusions can be drawn from phthalate HBM data:

Exposure to phthalates in the United States (as worldwide) is omnipresent. The U.S. population is co-exposed to many phthalates simultaneously. HBM data (urinary phthalate metabolite levels) can be used to reliably extrapolate to the daily intakes of the respective parent phthalate (and compared with health benchmarks for the individual phthalates as well as on a cumulative basis [see HI approach Section 2.7]).

Pregnant women in the United States (NHANES 2005–2006; CDC, 2012b)(NHANES 2005–2006) have similar exposures compared to women of reproductive age (and other NHANES subpopulations). Distributions are highly skewed, indicating high exposures in some women. The same is true for infants and children (SFF; Sathyanarayana *et al.*, 2008a; 2008b); furthermore, exposures in infants might be higher than in their mothers.

Within the same individuals, there are correlations among the high molecular weight phthalates and among the low molecular weight phthalates, and comparing mothers with children, there are indications of similar correlations. This suggests that sources and routes of exposure are similar among high molecular weight phthalates and among low molecular weight phthalates. Therefore, we assume it highly likely that the substitution of one phthalate will lead to increased exposure to another (similar) phthalate.

**Table 2.4 Molar urinary excretion fractions ( $f_{ue}$ ) of phthalate metabolites related to the ingested dose of the parent phthalate determined in human metabolism studies within 24 hours after oral application.**

Phthalate	Metabolite	$f_{ue}$	Reference
DMP	MMP	0.69*	-
DEP	MEP	0.69*	-
DBP	MBP	0.69	Anderson <i>et al.</i> , (2001)
DIBP	MIBP	0.69*	-
BBP	MBZP	0.73	Anderson <i>et al.</i> , (2001)
DEHP	MEHP	0.062	sum: 0.452 Anderson <i>et al.</i> (2011)
	MEHHP	0.149	
	MEOHP	0.109	
	MECPP	0.132	
DINP	cx-MINP	0.099	sum: 0.305 Anderson <i>et al.</i> (2011)
	OH-MINP	0.114	
	oxo-MINP	0.063	
	MINP	0.03	
DIDP/DPHP	cx-MIDP	0.04	sum: 0.34 Wittassek <i>et al.</i> (2007b); Wittassek and Angerer (2008)
	OH-MIDP	NA	
	oxo-MIDP	NA	
DNOP	MNOP		

\* $f_{ue}$  taken in analogy to DBP/MBP.

DMP = dimethyl phthalate; MMP = monomethyl phthalate; DEP = diethyl phthalate; MEP = monoethyl phthalate; DBP = dibutyl phthalate; MBP = monobutyl phthalate; DIBP = diisobutyl phthalate; MIBP = monoisobutyl phthalate; BBP = butylbenzyl phthalate; MBZP = monobenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; MEHP = mono(2-ethylhexyl) phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono(2-ethyl-5-oxohexyl) phthalate; MECPP = mono(2-ethyl-5-carboxypentyl) phthalate; DINP = diisononyl phthalate; cx-MINP = mono(carboxy-isononyl) phthalate; OH-MINP = mono(hydroxy-isononyl) phthalate; oxo-MINP = mono(oxo-isononyl) phthalate; MINP = mono(isononyl) phthalate; DIDP = diisodecyl phthalate; DPHP = di(2-propylheptyl) phthalate; cx-MIDP = mono(carboxy-isononyl) phthalate; OH-MIDP = mono(hydroxy-isodecyl) phthalate; oxo-MIDP = mono(oxo-isodecyl) phthalate; DNOP = di-*n*-octyl phthalate; MNOP = mono-*n*-octyl phthalate

**Table 2.5 Median (95th percentile)<sup>a</sup> concentrations (in µg/L) of DEHP and DINP metabolites in various study populations.**

Reference	Sampling Year	N (Age)	DEHP				cx-MINP <sup>a</sup>	DINP OH-MiNP <sup>a</sup>	oxo-MiNP <sup>a</sup>
			MECHP <sup>a</sup>	MEHHP <sup>a</sup>	MEOHP <sup>a</sup>	MEHP <sup>a</sup>			
<b>USA</b>									
Blount <i>et al.</i> (2000)	1988–1994	298 (20–60)	-	-	-	2.7 (21.5)	-	-	-
Silva <i>et al.</i> (2004)	1999–2000	2541 (>6)	-	-	-	3.2 (23.8)	-	-	-
Marsee <i>et al.</i> (2006)	1999–2002	214 pregnant women	-	10.8 (76.4)	9.8 (65.0)	4.3 (38.6)	-	-	-
Duty <i>et al.</i> (2005b)	1999–2003	295 men (18–54)	-	-	-	5.0 (131)	-	-	-
Adibi <i>et al.</i> (2008)	1999–2005	246 pregnant women	37.1 (232.2)	19.9 (149.6)	17.5 (107.6)	4.8 (46.8)	-	-	-
Meeker <i>et al.</i> (2009)	1999–2005	242 women (pre/post)	-	11.3 (44.9) 20.4 (83.1)	10.2 (42.6) 16.0 (61.7)	4.0 (21.0) 7.15 (23.6)	-	-	-
Brock <i>et al.</i> (2002)	2000	19 (1–3)	-	-	-	4.6	-	-	-
Duty <i>et al.</i> (2005a)	2000–2003	406 men (20–54)	-	-	-	5.2 (135)	-	-	-
Adibi <i>et al.</i> (2009)	2000–2004	283 pregnant women	-	11.2 (99.4)	9.9 (68.4)	3.5 (40.2)	-	-	-
CDC	2001–2002	2782 (>6)	-	20.1 (192)	14.0 (120)	4.1 (38.9)	-	-	-
CDC	2003–2004	2605 (>6)	33.0 (339)	21.2 (266)	14.4 (157)	1.9 (31.0)	-	-	-
Silva <i>et al.</i> (2006a; 2006b)	2003–2004	129 adults	15.6 (159.3)	15.3 (120.8)	7.1 (62.4)	3.1 (17.0)	8.4 (46.2)	13.2 (43.7)	1.2 (6.6)
CDC (internet)	2005–2006	2548 (>6)	35.6 (386)	23.8 (306)	15.1 (183)	2.50 (39.7)	5.10 (54.4)	-	-
CDC (internet)	2007–2008	2604 (>6)	31.3 (308)	20.7 (238)	11.4 (130)	2.20 (27.8)	6.40 (63.0)	-	-
<b>CHAP/NHANES</b>	2005–2006	1181 (15–45) (weighted)	37.2 (434)	25.5 (399)	16.2 (245)	3.3 (49.4)	5.1 (47.2)		
<b>CHAP/NHANES</b>	2005–2006	130 pregnant women (weighted)	19.9 (754)	13.3 (680)	10.0 (534)	2.4 (168)	2.7 (23.8)		
<b>CHAP/SFF</b>	1999–2005	343 women prenatal	22.9 (129.6)	13.7 (86.5)	12.7 (79.6)	4.4 (37.1)	3.6 (14.1)		
<b>CHAP/SFF</b>	1999–2005	345 women postnatal	35.7 (209.5)	20.9 (149.4)	14.9 (106.4)	6.0 (42.4)			
<b>CHAP/SFF</b>	1999–2005	291 infants (0–37 months)	156.2 (388.6)	65.6 (246.1)	49.9 (174.5)	10.4 (58.4)	17.0 (97.5)		

Reference	Sampling Year	N (Age)	DEHP				cx-MiNP <sup>a</sup>	DINP OH-MiNP <sup>a</sup>	oxo-MiNP <sup>a</sup>
			MECHP <sup>a</sup>	MEHHP <sup>a</sup>	MEOHP <sup>a</sup>	MEHP <sup>a</sup>			
Germany									
Becker <i>et al.</i> (2004)	2001–2002	254 (3–14)	-	52.1 (188)	41.4 (139)	7.2 (29.7)	-	-	-
Wittassek <i>et al.</i> (2007a)	2001–2003	120 (20–29)	19.5 (68.6)	14.6 (58.6)	13.4 (42.3)	5.0 (28.6)	-	2.2 (13.5)	1.3 (5.7)
Koch <i>et al.</i> (2003b)	2002	85 (7–63)	-	46.8 (224)	36.5 (156)	10.3 (37.9)	-	-	-
Koch <i>et al.</i> (2004b)	2003	19 (2–6) 36 (20–59)	-	49.6 (107) 32.1 (64.0)	33.8 (71.0) 19.6 (36.7)	9.0 (29.0) 6.6 (14.6)	-	-	-
Becker <i>et al.</i> (2009)	2003–2006	599 (3–14)	61.4 (209)	46.0 (164)	36.3 (123)	6.7 (25.1)	12.7 (195)	11.0 (198)	5.4 (86.7)
Fromme <i>et al.</i> (2007)	2005	399 (14–60)	24.9	19.5	14.6	4.6	-	5.5	3.0
Göen <i>et al.</i> (2011)	2002–2008	240 (19–29)	14.5 (49.7)	14.4 (42.2)	9.6 (36)	4.7 (16.6)	3.7 (22.4)	3.1 (16.5)	2.2 (11.2)
Koch & Calafat (2009)	2007	45 adults	13.9 (42.9)	11.5 (35.0)	8.2 (21.5)	1.8 (8.5)	5.3 (15.5)	4.7 (16.8)	1.7 (6.7)
Denmark									
Boas <i>et al.</i> (2010)	2006–2007	845 (4–9)	m: 30 f: 27	m: 37 f: 31	m: 19 f: 16	m: 4.5 f: 3.6	m: 7.2 f: 6.5	m: 6.6 f: 4.9	m: 3.4 f: 2.7
Frederiksen <i>et al.</i> (2011)		129 (6–21)							
Israel									
Berman <i>et al.</i> (2009)	2006	19 pregnant women	26.7	21.5	17.5	6.8	3.0	-	-
Netherlands									
Ye <i>et al.</i> (2008)	2004–2006	99 pregnant women	18.4 (31.5)	14.0 (30.0)	14.5 (27.4)	6.9 (82.8)	-	2.5 (38.3)	2.2 (30.0)
Japan									
Itoh <i>et al.</i> (2007)	2004	36 (4–70)	-	-	-	5.1	-	-	-
Suzuki <i>et al.</i> (2009)	2005–2006	50 pregnant women	-	10.6	11.0	3.96	-	-	-
China									
Guo <i>et al.</i> (2011)	2010	183	30.0	11.3	7.0	2.1	-	-	-
Taiwan									
Huang <i>et al.</i> (2007)	2005–2006	76 pregnant women	-	-	-	20.6 (273)	-	-	-
Sweden									
Jönsson <i>et al.</i> (2005)	2000	234 men (18–21)	-	-	-	<LD (54)	-	-	-

Note: Specific HBM calculations performed by the CHAP for this study are highlighted in green.

<sup>a</sup> 95<sup>th</sup> percentile values are in parentheses when available.

LD = limit of detection; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate; MEHP = mono(2-ethylhexyl) phthalate; MEHHP = mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono(2-ethyl-5-oxohexyl) phthalate; MECPHP = mono(2-ethyl-5-carboxypentyl) phthalate; cx-MINP = mono(carboxy-isooctyl) phthalate; OH-MINP = mono(hydroxy-isononyl) phthalate; oxo-MINP = mono(oxo-isononyl) phthalate

**Table 2.6 Median (95th percentile)<sup>a</sup> concentrations (in µg/L) of DMP, DEP, DBP, DIBP, BBP, DNOP, and DIDP metabolites in various study populations.**

Reference	Sampling Year	N (Age)	DMP MMP	DEP MEP	DBP MBP	DIBP MIBP	BBP MBzP	DNOP MNOP	cx- MIDP	DIDP OH- MIDP	oxo- MIDP
<b>USA</b>											
Blount <i>et al.</i> (2000)	1988–1994	298 (20–60)	-	305 (3750)	41.0 (294)	-	21.2 (137)	<LD (2.3)	-	-	-
Silva <i>et al.</i> (2004)	1999–2000	2541 (>6)	-	164 (2840)	26.0 (149)	-	17.0 (103)	<LD (2.9)	-	-	-
Marsee <i>et al.</i> (2006)	1999–2002	214 pregnant women	-	117 (3199)	16.2 (64.5)	2.5 (13.1)	9.3 (57.8)	-	-	-	-
Duty <i>et al.</i> (2005b)	1999–2003	295 men (18–54)	4.6 (32.1)	149 (1953)	14.3 (75.4)	-	6.9 (37.1)	-	-	-	-
Adibi <i>et al.</i> (2008)	1999–2005	246 pregnant women	-	202 (2753)	35.3 (174.9)	10.2 (36.1)	17.2 (146.8)	-	-	-	-
Meeker <i>et al.</i> (2009)	1999–2005	242 women (pre/post)*	0.71 (5.3) 2.1 (5.9)	131 (1340) 133 (873)	17.2 (51.8) 19.4 (68.7)	2.65 (9.0) 3.6 (14.0)	9.95 (45.8) 14.8 (64.1)	-	-	-	-
Brock <i>et al.</i> (2002)	2000	19 (1–3)	-	184.1	22.0 (203)	-	20.2 (118)	-	-	-	-
Duty <i>et al.</i> (2005a)	2000–2003	406 men (20–54)	4.5 (31.3)	145 (1953)	14.5 (75.1)	-	6.8 (41.3)	-	-	-	-
CDC	2001–2002	2782 (>6)	1.5 (9.8)	169 (2500)	20.4 (108)	2.6 (17.9)	15.7 (122)	<LD	-	-	-
CDC	2003–2004	2605 (>6)	1.3 (16.3)	174 (2700)	23.2 (122)	4.2 (21.3)	14.3 (101)	<LD	-	-	-
Silva <i>et al.</i> (2006a; 2006b)	2003–2004	129 adults	-	-	-	-	-	-	4.4 (104.4)	4.9 (70.6)	1.2 (15.0)
CDC (internet)	2005–2006	2548 (>6)	<LQ (12.4)	155 (2140)	20.6 (107)	5.8 (31.6)	12.4 (93.2)	<LQ	2.70 (17.5)	-	-
CDC (internet)	2007–2008	2604 (>6)	<LQ (11.3)	124 (1790)	20.0 (110)	8.0 (39.1)	11.7 (81.4)	<LQ	2.40 (16.1)	-	-
<b>CHAP/NHANES</b>	2005–2006	1161 (15–45) (weighted)			22.1 (106)	6.7 (32.2)	10.3 (63.7)		2.5 (15.8)		



Reference	Sampling Year	N (Age)	DMP MMP	DEP MEP	DBP MBP	DIBP MIBP	BBP MBzP	DNOP MNOP	cx-MIDP	DIDP OH-MIDP	oxo-MIDP
<b>CHAP/NHANES</b>	2005–2006	130 pregnant women (weighted)			16.0 (91.2)	3.2 (26.2)	8.4 (38.2)		1.5 (6.6)		
<b>CHAP/SFF</b>	1999–2005	343 women prenatal	1.7 (9.0)	175 (2,270)	21.0 (60.1)	3.6 (13.5)	13.4 (71.3)		3.0 (8.2)		
<b>CHAP/SFF</b>	1999–2005	344 women postnatal	2.1 (9.6)	129 (1,283)	18.9 (71.0)	4.3 (20.3)	14.7 (64.1)		2.9 (23.6)		
<b>CHAP/SFF</b>	1999–2005	304 Infants (0–37 months)	7.3 (25.2)	2735 (1,890)	82.0 (301)	15.0 (60.4)	65.8 (315)		13.2 (57.9)		
<b>Germany</b>											
Koch <i>et al.</i> (2007)	2001–2002	254 (3–14)	-	-	166 (624)	-	18.7 (123)	-	-	-	-
Wittassek <i>et al.</i> (2007a)	2001–2003	120 (20–29)	-	-	57.4 (338)	31.9 (132)	5.6 (25.0)	-	-	-	-
Koch <i>et al.</i> (2003b)	2002	85 (7–63)	-	90.2 (560)	181 (248)	-	21 (146)	<LQ	-	-	-
Fromme <i>et al.</i> (2007)	2005	399 (14–60)	-	-	49.6 (171.5)	44.9 (183)	7.2 (45.6)	-	-	-	-
Becker <i>et al.</i> (2009)	2003–2006	599 (3–14)	-	-	93.4 (310)	88.1 (308)	18.1 (76.2)	-	-	-	-
Göen <i>et al.</i> (2011)	2002–2008	240 (19–29)	-	-	32.8 (132.4)	28.3 (108)	5.0 (21.2)	-	-	-	-
Koch and Calafat (2009)	2007	45 adults	<LQ (17.2)	77.5 (396)	12.6 (43.5)	13.8 (62.4)	2.5 (8.4)	<LQ	0.7 (2.6)	1.0 (4.0)	0.2 (1.1)
<b>Denmark</b>											
Boas <i>et al.</i> (2010)	2006–2007	845 (4–9)	-	m: 21 f: 21	m: 130 f: 121	-	m: 17 f: 12	<LQ			
Frederiksen <i>et al.</i> (2011)		129 (6–21)									
<b>Israel</b>											
Berman <i>et al.</i> (2009)	2006	19 pregnant women	-	165	30.8	15.6	5.3	-	1.5	-	-
<b>Netherlands</b>											
Ye <i>et al.</i> (2008)	2004–2006	99 pregnant women	<LQ (20.1)	117 (1150)	42.7 (197)	42.1 (249)	7.5 (95.8)	<LD	-	-	-

Reference	Sampling Year	N (Age)	DMP MMP	DEP MEP	DBP MBP	DIBP MIBP	BBP MBzP	DNOP MNOP	cx- MIDP	DIDP OH- MIDP	oxo- MIDP
<b>Japan</b>											
Itoh <i>et al.</i> (2007)	2004	36 (4–70)	-	-	43	-	-	-	-	-	-
Suzuki <i>et al.</i> (2009)	2005–2006	50 pregnant women	6.61	7.83	57.9	-	3.74	<LQ	-	-	-
<b>China</b>											
Guo <i>et al.</i> (2011)	2010	183	12.0	21.5	61.2	56.7	0.6	-	-	-	-
<b>Taiwan</b>											
Huang <i>et al.</i> (2007)	2005–2006	76 pregnant women	4.3 (87.7)	27.7 (2346)	81.1 (368)		0.9 (33.4)	-	-	-	-
<b>Sweden</b>											
Jönsson <i>et al.</i> (2005)	2000	234 men (18–21)	-	240 (4400)	78 (330)	-	16 (74)	-	-	-	-

Note: Specific HBM calculations performed by the CHAP for this study are highlighted in green.

<sup>a</sup> 95<sup>th</sup> percentile values are in parentheses when available.

LD = limit of detection; LQ = limit of quantification; DMP = dimethyl phthalate; MMP = monomethyl phthalate; DEP = diethyl phthalate; MEP = monoethyl phthalate; DBP = dibutyl phthalate; MBP = monobutyl phthalate; DIBP = diisobutyl phthalate; MIBP = monoisobutyl phthalate; BBP = butylbenzyl phthalate; MBZP = monobenzyl phthalate; DNOP = di-*n*-octyl phthalate; MNOP = mono-*n*-octyl phthalate; DIDP = diisodecyl phthalate; cx-MIDP = mono(carboxy-isononyl) phthalate; OH-MIDP = mono(hydroxy-isodecyl) phthalate; oxo-MIDP = mono(oxo-isodecyl) phthalate;

**Table 2.7 Daily phthalate intake (median, in µg/kg bw/day) of selected populations back-calculated from urinary metabolite levels.**

Reference	Sampling Year	N (Age)	DEP		DBP		DIBP		BBP		DEHP		DINP	
			Median	95th P (max)	Median	95th P (max)	Median	95th P (max)	Median	95th P (max)	Median	95th P (max)	Median	95th P (max)
USA														
David (2000)	1988–1994	289 (20–60)	12.3 <sup>a</sup>	93.3 (243)	1.6 <sup>a, b</sup>	6.9 <sup>b</sup> (117)	-	-	0.73 <sup>a</sup>	3.3 (19.8)	0.60 <sup>a, c</sup>	3.1 <sup>c</sup> (38.5)	0.21 <sup>a, m</sup>	1.1 <sup>m</sup> (14.4)
Kohn <i>et al.</i> (2000)	1988–1994	289 (20–60)	12	110 (320)	1.5 <sup>b</sup>	7.2 <sup>b</sup> (110)	-		0.88	4.0 (29)	0.71 <sup>c</sup>	3.6 <sup>c</sup> (46)	<LD	1.7 <sup>m</sup> (22)
Calafat & McKee (2006)	2001–2002	2772 (6 >20)	5.5 <sup>a</sup>	61.7	-	-	-	-	-	-	0.9 <sup>a, c</sup> 2.1 <sup>a, e</sup> 2.2 <sup>a, f</sup>	7.1 <sup>c</sup> 16.8 <sup>e</sup> 15.6 <sup>f</sup>	-	-
Marsee <i>et al.</i> (2006)	1999–2002	214 pregnant women	6.6	112 (1263)	0.84	2.3 (5.9)	0.12	0.41 (2.9)	0.50	2.5 (15.5)	1.3 <sup>g</sup>	9.3 <sup>g</sup> (41.1)	-	-
CHAP/NHANES	2005–2006	1161 (15–45)	3.3	37.6	0.66	2.6	0.19	0.78	0.29	1.3	3.8	45.2	1.1	9.7
CHAP/NHANES	2005–2006	130 pregnant women (weighted)	3.4	74.8	0.64	3.5	0.17	1.0	0.30	1.3	3.5	181	1.0	11.1
CHAP/SFF	1999–2005	340 women prenatal			0.88	2.5	0.15	0.57	0.51	2.8	2.9	16.6	1.1 n=18	7.6 n=18
CHAP/SFF	1999–2005	335 women postnatal			0.62	2.2	0.14	0.68	0.44	1.9	2.7	21.6	0.64 n=95	3.2 n=95
CHAP/SFF	1999–2005	258 infants (0–37 months)			2.6	10.4	0.44	2.1	1.9	8.5	7.6	28.7	3.6 n=67	18.0 n=67
Germany														
Wittassek <i>et al.</i> (2007a)	1988/1989	120 (21–29)	-	-	7.5	21.7 (70.1)	1.1	3.6 (12.9)	0.28	0.78 (6.6)	3.9 <sup>1</sup>	9.9 <sup>1</sup> (39.8)	0.21 <sup>n</sup>	1.4 <sup>n</sup> (12.9)
Koch <i>et al.</i> (2003b)	2002	85 (7–63)	2.3	22.1 (69.3)	5.2	16.2 (22.6)	-	-	0.6	2.5 (4.5)	[13.8] <sup>i</sup> 4.6 <sup>g</sup>	[52.1 (166)] <sup>i</sup> 17.0 <sup>g</sup> (58.2)	-	-

Reference	Sampling Year	N (Age)	DEP		DBP		DIBP		BBP		DEHP		DINP	
			Median	95th P (max)	Median	95th P (max)	Median	95th P (max)	Median	95th P (max)	Median	95th P (max)	Median	95th P (max)
Koch <i>et al.</i> (2007) Wittassek <i>et al.</i> (2007b)	2001/2002	239 (2-14)	-	-	4.1 <sup>j</sup> 7.6 <sup>k</sup>	14.9 <sup>j</sup> (76.4) 30.5 <sup>k</sup> (110)	-	-	0.42 <sup>j</sup> 0.77 <sup>k</sup>	2.57 <sup>j</sup> (13.9) 4.48 <sup>k</sup> (31.3)	4.3 <sup>g,j</sup> 7.8 <sup>g,k</sup>	15.2 <sup>g,j</sup> (140) 25.2 <sup>g,k</sup> (409)	-	-
Wittassek <i>et al.</i> (2007a)	2001/2003	119 (20-29)	-	-	2.2	7.3 (116)	1.5	4.2 (12.6)	0.22	0.75 (1.7)	2.7 <sup>l</sup>	6.4 <sup>l</sup> (20.1)	0.37 <sup>n</sup>	1.5 <sup>n</sup> (4.4)
Fromme <i>et al.</i> (2007b)	2005	50 (14-60)			1.7	4.2	1.7	5.2	0.2	1.2	2.2 <sup>l</sup>	7.0 <sup>l</sup>	0.7 <sup>n</sup>	3.5 <sup>n</sup>
<b>China</b>														
Guo <i>et al.</i> (2011)	2010	183	1.1	-	8.5	-	-	-	-	-	3.4	-	-	-
<b>Japan</b>														
Itoh <i>et al.</i> (2007)	2004	35 (20-70)	-	-	1.3	(4.5)	-	-	-	-	1.8 <sup>d</sup>	(7.3) <sup>d</sup>	-	-
Suzuki <i>et al.</i> (2009)	2005-2006	50 pregnant women	0.28	(42.6)	2.18	(6.91)	-	-	0.132	(3.2)	1.73 <sup>o</sup>	(24.6) <sup>o</sup>	0.06 <sup>m</sup>	(4.38) <sup>m</sup>

DEP = diethyl phthalate; DBP = dibutyl phthalate; DIBP = diisobutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate

Note: Specific HBM calculations performed by the CHAP for this study are highlighted in green.

<sup>a</sup> Geometric mean

<sup>b</sup> No differentiation between DBP and DIBP

<sup>c</sup> Based on  $f_{uc}$  of MEHP determined by Anderson *et al.* (2001)

<sup>d</sup> Based on  $f_{uc}$  of MEHP determined by Koch *et al.* (2004a; 2005)

<sup>e</sup> Based on  $f_{uc}$  of OH-MEHP determined by Koch *et al.* (2004a; 2005)

<sup>f</sup> Based on  $f_{uc}$  of oxo-MEHP determined by Koch *et al.* (2004a; 2005)

<sup>g</sup> Based on  $f_{uc}$ s for MEHP, OH-MEHP and oxo-MEHP determined by Koch *et al.* (2004a; 2005)

<sup>h</sup> 634 persons, urine samples collected between 1988 and 2003

<sup>i</sup> Based on  $f_{uc}$ s for MEHP, OH-MEHP and oxo-MEHP determined by Schmid and Schlatter (1985)

<sup>j</sup> Creatinine-based calculation model

<sup>k</sup> Volume based calculation model

<sup>l</sup> Based on  $f_{uc}$ s of five DEHP metabolites determined by Koch *et al.* (2004a; 2005)

<sup>m</sup> Based on urine levels of mono(isononyl) phthalate (MINP)

<sup>n</sup> Based on urine levels of mono(hydroxyl-isononyl) phthalate (OH-MINP), mono(oxo-isononyl) phthalate (MINP), and mono(carboxy-isoocetyl) phthalate (cx-MINP)

**Table 2.8 Pearson correlation coefficient estimates (\*  $p < 0.05$ ) between estimated daily intakes (DI) of the eight phthalate diesters (log 10 scale) for pregnant women in NHANES 2005–2006 (estimated using survey weights). Highlighted values indicate clusters of low molecular weight diesters and high molecular weight diesters.**

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

DMP = dimethyl phthalate; DEP = diethyl phthalate; DIBP = disobutyl phthalate; DBP = dibutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate; DIDP = diisodecyl phthalate

**Table 2.9 Pearson correlation estimates (\*  $p < 0.05$ ) for estimated daily intake (DI) values (log 10 scale) for postnatal values with DI values estimated in their babies in the SFF study. N=251, except for \*DINP and DIDP, where N=62.**

Estimated P value	DEP	DIBP	DBP	BBP	DEHP	DINP	DIDP
<b>DEP</b>		-0.05	-0.003	-0.08	-0.04	-0.10	-0.15
<b>DIBP</b>	0.06		0.06		0.08	0.02	0.02
<b>DBP</b>	0.17*	0.10	0.12	-0.04	0.09	0.19	0.22
<b>BBP</b>		-0.03	0.01		-0.06	0.16	0.13
<b>DEHP</b>	0.06	0.02	0.03	0.05		0.18	
<b>DINP</b>	0.02	0.01	0.06	0.03	0.15		
<b>DIDP</b>	-0.13	0.004	0.02	-0.09	0.15		

DEP = diethyl phthalate; DIBP = disobutyl phthalate; DBP = dibutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate; DIDP = diisodecyl phthalate

## 2.6 Scenario-Based Exposure Assessment

### 2.6.1 Introduction

There are a multitude of home care products, toys, and other personal products, and each can yield varying durations, intensities, and frequencies of contact with individual and multiple phthalates over the course of a year. These contacts can lead to acute or chronic exposures among the users of individual products. Similarly, women who are pregnant or are of reproductive age will also contact products that contain phthalates. For children, the subject of the CHAP, we need to focus not only on the prenatal exposures but also on the exposures that occur during infancy and childhood, and most directly on toys and other products that are associated with children, *e.g.*, teethingers. The types of products will be different for a woman of reproductive age than for a child and the significance of the exposure on the unborn child can be related to when the exposures occur during a pregnancy.

The range of contacts with phthalates can be large in terms of number of products, duration and frequency of contact, and the ages during which the contacts will occur among young children and a woman of reproductive age. The nature of the contacts can be repetitive or periodic in character. For instance, personal care products for adults and children will be used regularly, but the use of toys can be periodic, based upon level of interest and/or the time of the year. Having such a variety of potential contacts will lead to variability in the levels detected in the urine. But there should be a baseline level that is derived from the types of products that are used routinely by an individual, and that level will be built upon the baseline that is associated with phthalates that are ingested because of their presence in foods and food packaging. In each case, the exposures to specific phthalates may not be the same because the phthalates used may be different in individual products and because there may be varying degrees of actual contact with each for each subgroup of concern.

### 2.6.2 Objectives

Given the complex nature of human exposures to phthalates from a multitude of sources and media, a comprehensive analysis based on sound scientific principles was conducted to assess phthalate human exposures. This assessment used the indirect method of assessing phthalate exposures to various human subpopulations that included pregnant women/women of reproductive age (age 15 to 44), infants (age 0 to <1), toddlers (age 1 to <3), and children (age 3 to 12). The specific objectives included estimating aggregate human exposures to eight phthalates (BBP, DBP, DEP, DEHP, DIBP, DIDP, DINP, and DNOP) by estimating human exposures to a variety of environmental sources, consumer products, household media, and food products. The exposure routes investigated included inhalation, direct and indirect ingestion, and dermal contact. Our goal was to determine the significance of exposure to phthalates in toys as a major part of our risk assessment and for comparison to biomonitoring data. In addition, to meet part of the CHAP's charge, we estimated exposure to toddlers and infants for all soft plastic articles except pacifiers\*. These compounds included the phthalates DINP and DEHP, and the phthalate substitutes TPIB, DINX, ATBC, and DEHT. Although certain phthalates are currently

---

\* Pacifiers do not contain phthalates.

banned in toys and child care articles, we estimated exposures that would hypothetically occur if phthalates were allowed in these products.

### **2.6.3 Methodology**

Phthalate concentrations in various sources and media, and associated with specific human activities, were used to predict the exposure distributions within each subpopulation. Thus, the approach focused on the phthalate concentrations associated with sources rather than within the receptors (humans) and encompassed all the complex interactions between humans and the phthalate-containing products and sources via specific routes of exposure (Table 2.10). Figure 2.1 shows seven important routes and pathways of human exposure to phthalates. It also shows how each exposure route is associated with products and sources containing phthalates and for which subpopulations are targeted by specific exposure routes, and product/source combinations.

For the nonphthalate materials we only had data that could estimate exposure caused by mouthing, which would be called nondietary ingestion.

A step-by-step approach was used to estimate scenario-based aggregate human exposures to phthalates and phthalate alternatives, and is provided in Appendices E1 to E3. This approach includes: 1) a compilation of concentrations, 2) a compilation of human exposure factors, 3) an estimation of route-specific exposures, and 4) an estimation of aggregate exposures.

### **2.6.4 Results**

#### **2.6.4.1 Pregnant Women/Women of Reproductive Age**

The daily exposures (both mean and 95<sup>th</sup> percentile) for each of the eight phthalates for the seven separate exposure sources (including diet, prescription drugs, personal care products, toys, child care articles, indoor environment, and outdoor environment) for all subpopulations are provided in Appendix E1 (Table E1-19). Tables E1-3 through E1-22 in Appendix E1 tabulate the mean and 95<sup>th</sup> percentile concentrations, exposure factors, and daily exposures for pregnant women. The aggregate daily exposures (mean and 95<sup>th</sup> percentile) for each of the four subpopulations for each of the eight phthalates are reported in Table 2.11. These exposures constitute the total daily exposure from all sources and media, and all exposure routes for a particular phthalate.

The information in Table 2.11 indicates that the highest estimated exposures to women were from DEP, DINP, DIDP, and DEHP. Exposures from DBP, DIBP, BBP, and DNOP were negligible ( $\leq 1$   $\mu\text{g}/\text{kg}\cdot\text{d}$ ). The contributions for the aggregate exposures for each of the eight phthalates for women from various exposure routes are shown in Figure 2.1. The main source of phthalate exposure to pregnant women/women of reproductive age was from food, beverages and drugs via direct ingestion. In addition to ingestion, pregnant women were also exposed to DEP from personal care products and to DEHP and DINP from the indoor environment. Upper bound exposures of women for different phthalates are shown in Table 2.11.

#### **2.6.4.2 Infants**

Tables E1-3 through E1-22 in Appendix E1 provide the mean and 95<sup>th</sup> percentile concentrations, exposure factors, and daily exposures for infants. The aggregate daily exposures (mean and 95<sup>th</sup> percentile) for infants for each phthalate are provided in Table 2.11. Infants were exposed to



primarily DINP, DEHP, DIDP, DNOP, DEP, and BBP, with DINP, DEHP, and DIDP being the highest contributors. The exposure to DINP was the highest in infants primarily from diet but also due to the presence of DINP in teethingers and toys through mouthing (Figure 2.2). DINP is currently subject to an interim ban; thus, exposures from mouthing are hypothetical. It can also be seen in Figure 2.2 that the main source of phthalate exposures to infants, as to pregnant women, was from ingestion of food and beverages. In addition to food, the other main contributors were teethingers and toys (via mouthing), and personal care products such as lotions, creams, oils, soaps, and shampoos via dermal contact. Upper bound daily exposures for infants across phthalates are shown Table 2.11.

#### **2.6.4.3 Toddlers**

Tables E1-3 through E1-22 in Appendix E1 provide the mean and 95<sup>th</sup> percentile concentrations, exposure factors, and daily exposures for toddlers. The aggregate daily exposures (both mean and 95<sup>th</sup> percentile) of toddlers for each of the eight phthalates are tabulated in Table 2.11. Toddlers were primarily exposed to DINP, DIDP, and DEHP. The contributions to exposure from DNOP, BBP, and DEP were moderate. Estimated DBP and DIBP exposures were less than 1 µg/kg-d. Exposure to toddlers from DIDP, DIBP, and DINP was primarily from food and beverages (Figure 2.1). It should be noted that the toddler exposures to phthalates via ingestion were the highest among all subpopulations. This was because they consume almost all the food products that are consumed by adults, and because they have much lower body weights, their daily exposures on a body weight basis resulted in being the highest. Similar to infants, toddlers too were exposed to DINP via mouthing of teethingers and toys. However, their exposures from mouthing were much lower than that estimated for infants. Toddlers were also exposed to DNOP, DEHP, and DINP by dermal contact with child care articles.

#### **2.6.4.4 Children**

Tables E1-3 through E1-22 in Appendix E1 provide the mean and 95<sup>th</sup> percentile concentrations, exposure factors, and daily phthalate exposures for children. The aggregate daily exposures (mean and 95<sup>th</sup> percentile) for children for each of the eight phthalates are tabulated in Table 2.11. Children were primarily exposed to DINP, BBP, and DIDP. Exposure to DNOP, DEP, and DEHP were moderate. Exposures to children from DIDP and DNOP were from food and beverages (Figure 2.1). DEP exposure was from personal care products, drugs, and the indoor environment. The indoor environment (mainly household dust) was an important source of DEHP exposure to children.

#### **2.6.5 Phthalate Substitutes**

A summary of the major results for the exposure assessment of phthalate substitutes is presented in Table 2.12. We demonstrate that all exposures in µg/kg-d for each compound are within one order of magnitude of each other for means and 95<sup>th</sup> percentiles. Daily exposures range from 0.4 to 7.2 µg/kg-d. These were derived from migration rates measured during laboratory experiments, in combination with mouthing durations from a study of children's mouthing behavior. The mouthing durations are for all soft plastic articles except pacifiers. Pacifiers are made from natural rubber or silicone. Additional details are found in Appendix E2.

### 2.6.6 Summary of Design

The overall goal was to obtain phthalate-related data from the United States published in the last ten years and to use the data to estimate inhalation, ingestion, and dermal exposures to phthalates from contact with children's toys and other sources/products. Given the multitude of complex human behavioral patterns and their interactions with various phthalate-containing products, and the lack of major field studies, it was also necessary to use data from other countries within North America and Europe, and data prior to the year 2000. Finally, in cases for which data were not available, professional judgment was used to estimate some of the parameters. These estimates were usually performed assuming worst-case scenarios that resulted in high exposures. Thus, the results obtained from this analysis can provide only order of magnitude estimates of the potential exposure. More data are needed to refine these estimates.

The estimates apply to activities during which one is in contact with a specific phthalate. Thus, results are indicative of nonhomogeneous exposures to the individual phthalates from a particular subpopulation. The selection of specific scenarios for the exposure assessment completed for this report is designed to replicate the meaningful components of a day or year in the life of an infant, toddler, child, or woman. For nonphthalate exposures, again, we can address only a specific scenario (mouthing soft plastic articles).

### 2.6.7 Conclusions

1. The highest estimated phthalate exposures to women were associated with DEP, DINP, DIDP, and DEHP. The main sources of phthalate exposure for pregnant women/women of reproductive age were from food, beverages, and drugs via direct ingestion. In addition, pregnant women were also exposed to DEP from personal care products and to DINP, DIDP, and DEHP via incidental ingestion of household dust and dermal contact with gloves and home furnishings.
2. Infants were primarily exposed to DINP, DEHP, DIDP, DEP, DNOP, DEP, and BBP, with DINP, DEHP, and DIDP being the highest contributors. The exposure to DINP was the highest in infants primarily from diet but also due to the presence of DINP in teethingers and toys through mouthing (prior to the interim ban). The other important contributors to exposures for each phthalate besides DINP were teethingers and toys (via mouthing) and personal care products such as lotions, creams, oils, soaps, and shampoos via dermal contact.
3. Toddlers were primarily exposed to DINP, DIDP, and DEHP. The contributions from DNOP, BBP, and DEP were moderate. Exposure to toddlers from DIDP, DIBP, and DINP was via food and beverages. The above notwithstanding, we determined that the toddler exposures to phthalates via ingestion were the highest among all other subpopulations (Figure 2.2). Like infants, toddlers were also exposed to DINP via mouthing of teethingers and toys. However, their estimated exposures for mouthing behavior were much lower than those of infants.
4. Older children were primarily exposed to DINP, BBP, and DIDP. Exposure to DNOP, DEP, and DEHP were moderate. Exposure to children from DIDP and DNOP was from food and beverages (Figure 2.1). DEP exposure was from personal care products, drugs,

and the indoor environment. The indoor environment (mainly household dust) was an important source of DEHP exposure to children.

5. The results concerning phthalate substitutes are limited because we have little information on all routes of exposure. However, Table 2.12 shows that, of the substitutes, ATBC yielded the highest overall average estimates of mouthing soft objects exposures, and these are equivalent to DINP exposures for the same sources. Due to the limited data available, no conclusions can be drawn other than the need to immediately complete well-designed exposure studies for all routes and sources because phthalate substitutes are being used in consumer products. Furthermore, these compounds need to be added to biomonitoring studies in the future. These data are necessary for exposure assessments associated with aggregate risk from individual compounds and cumulative risk from multiple compounds.

### **2.6.8 General Conclusion and Comment**

Overall, food, beverages, and drugs via direct ingestion, and *not children's toys and their personal care products*, constituted the highest phthalate exposures to all subpopulations, with the highest exposure (Figure 2.1; Table 2.10) being dependent upon the phthalate and the products that contain it. DINP had the maximum potential of exposure for infants, toddlers, and older children (Figure 2.2). DINP exposures were primarily from food but also from mouthing teethingers and toys, and from dermal contact with child care articles and home furnishings (Figure 2.1). The findings of this study were more or less in compliance with other phthalate exposure assessments; studies that use the direct approach (biomonitoring studies) as well as those that utilize the indirect approach (Table 2.13) (Wormuth *et al.*, 2006; Clark *et al.*, 2011). The estimated aggregate exposures were typically higher than some of the other estimates, and this could be because of some of the worst-case assumptions that were carried out for this study. Nevertheless, the results are within an order of magnitude of other findings, and they provide the CPSC the ability to eliminate certain products and phthalates for further consideration in the completion of a cumulative risk assessment across products and across the populations considered at risk because of exposures to phthalates. In addition, modeled exposure estimates are in general agreement with exposure estimates developed by the CHAP from biomonitoring data (Table 2.14).

**Table 2.10 Sources of exposure to PEs included by exposure route.**

Source	Target Population (age range)			
	Women (15 to 44) <sup>a</sup>	Infants (0 to <1)	Toddlers (1 to <3)	Children (3 to 12)
<b>Children's Products</b>				
teethers and toys	D <sup>b</sup>	O, D	O, D	D
changing pad	-	D	D	-
play pen	-	D	D	-
<b>Household Products</b>				
air freshener, aerosol	I (direct) <sup>c</sup>	I (indirect) <sup>d</sup>	I (indirect)	I (indirect)
air freshener, liquid	I (indirect)	I (indirect)	I (indirect)	I (indirect)
vinyl upholstery	D	-	D	D
gloves, vinyl	D	-	-	-
adhesive, general purpose	D	-	-	-
paint, aerosol	I, D	-	I (indirect) <sup>d</sup>	I (indirect) <sup>d</sup>
adult toys	Internal	-	-	-
<b>Personal Care Products</b>				
soap/body wash	D	D	D	D
shampoo	D	D	D	D
skin lotion/cream	D	D	D	D
deodorant, aerosol	D, I (direct)	I (indirect)	I (indirect)	D, I (direct) <sup>e</sup>
perfume, aerosol	D, I (direct)	I (indirect)	I (indirect)	D, I (direct) <sup>e</sup>
hair spray, aerosol	D, I (direct)	I (indirect)	I (indirect)	D, I (direct) <sup>e</sup>
nail polish	D	-	-	D
<b>Environmental Media</b>				
outdoor air	I	I	I	I
indoor air	I	I	I	I
dust	O	O	O	O
soil	O	O	O	O
<b>Diet</b>				
food	O	O	O	O
water	O	O	O	O
beverages	O	O	O	O
<b>Prescription Drugs</b>	O	--	O	O

<sup>a</sup> Age range, years.

<sup>b</sup> D, dermal; O, oral; I, inhalation.

<sup>c</sup> Includes direct exposure from product use.

<sup>d</sup> Includes indirect exposure from product use by others in the home.

<sup>e</sup> Females only.

**Table 2.11 Estimated mean and 95<sup>th</sup> percentile total phthalate ester exposure (µg/kg-d) by subpopulation.**

Phthalate	Women		Infants		Toddler		Children	
	(15 to <45)		(0 to <1)		(1 to <3)		(3 to 12)	
	<i>Mean</i>	<i>0.95</i>	<i>Mean</i>	<i>0.95</i>	<i>Mean</i>	<i>0.95</i>	<i>Mean</i>	<i>0.95</i>
DEP	18.1	398	3.1	14.9	2.8	2187.8	2.8	1149
DBP	0.29	5.7	0.51	1.2	0.69	1.6	0.55	7.4
DIBP	0.15	0.50	0.48	1.5	0.86	3.0	0.45	1.6
BBP	1.1	2.6	1.8	4.0	2.4	5.8	1.1	2.4
DNOP	0.17	21.0	4.4	9.6	5.4	16.0	0.525	15.45
DEHP	1.6	5.6	12.2	33.8	15.7	46.7	5.4	16.5
DINP	5.1	32.5	20.7	57.4	30.8	93.3	14.3	55.1
DIDP	3.2	12.2	10.0	26.4	16.6	47.6	9.1	28.1

DEP = diethyl phthalate; DBP = dibutyl phthalate; DIBP = diisobutyl phthalate; BBP = butylbenzyl phthalate; DNOP = di-*n*-octyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate; DIDP = diisodecyl phthalate

**Table 2.12 Estimated oral exposure (µg/kg-d) from mouthing soft plastic objects except pacifiers.<sup>a</sup>**

Plasticizer	Age Range								
	3 to <12 months			12 to <24 months			24 to <36 months		
	<i>Mean<sup>b</sup></i>	<i>R(0.95)</i>	<i>T(0.95)</i>	<i>Mean</i>	<i>R(0.95)</i>	<i>T(0.95)</i>	<i>Mean</i>	<i>R(0.95)</i>	<i>T(0.95)</i>
ATBC	2.3	7.2	5.1	1.5	4.7	2.8	1.4	4.3	3.4
DINX	1.4	3.6	5.4	0.89	2.3	3.1	0.82	2.1	3.6
DEHT	0.69	1.8	2.8	0.45	1.2	1.5	0.41	1.1	1.8
TPIB	0.92	5.8	3.8	0.60	3.8	2.0	0.55	3.4	2.4

ATBC = acetyl tributyl citrate; DINX = 1,2-cyclohexanedicarboxylic acid, diisononyl ester; DEHT = di(2-ethylhexyl) terephthalate; TPIB = 2,2,4-trimethyl-1,3 pentanediol diisobutyrate

<sup>a</sup> Results rounded to two significant figures.

<sup>b</sup> Mean, calculated with the mean migration rate and mean mouthing duration; R(0.95), calculated with the 95th percentile migration rate and mean mouthing duration; T(0.95), calculated with the mean migration rate and 95<sup>th</sup> percentile mouthing duration.

**Table 2.13 Comparison of modeled estimates of total phthalate ester exposure (µg/kg-d).**

Phthalate	Study	Adult female		Infants		Toddlers		Children	
		Ave. <sup>a</sup>	U.B.	Ave.	U.B.	Ave.	U.B.	Ave.	U.B.
DEP	Wormuth <sup>b</sup>	1.4	65.7	3.5	19.4	1.5	8.1	0.7	4.6
	Clark <sup>c</sup>	-	-	0.3	1.2	1.2	3.8	0.9	2.8
	CHAP <sup>d</sup>	18.1	398	3.1	14.9	2.8	2188	2.8	1149
DBP	Wormuth	3.5	38.4	7.6	43.0	2.7	24.9	1.2	17.7
	Clark	-	-	1.5	5.7	3.4	12.0	2.4	8.1
	CHAP	0.3	5.7	0.5	1.2	0.7	1.6	0.5	7.4
DIBP	Wormuth	0.4	1.5	1.6	5.7	0.7	2.7	0.3	1.2
	Clark	-	-	1.3	5.5	2.6	6.2	2.1	4.8
	CHAP	0.1	0.5	0.5	1.5	0.9	3.0	0.5	1.6
BBP	Wormuth	0.3	1.7	0.8	7.9	0.3	3.7	0.0	1.1
	Clark	-	-	0.5	6.1	1.5	6.1	1.0	4.0
	CHAP	1.1	2.6	1.8	4.0	2.4	5.8	1.1	2.4
DEHP	Wormuth	1.4	65.7	3.5	19.4	1.5	8.1	0.7	4.6
	Clark	-	-	5.0	27.0	30.0	124	20.0	81.0
	CHAP	1.6	5.6	12.2	33.8	15.7	46.7	5.4	16.5
DINP	Wormuth	0.004	0.3	21.7	139.7	7.1	66.3	0.2	5.4
	Clark	-	-	0.8	9.9	2.1	8.7	1.3	5.5
	CHAP	5.1	32.5	20.7	57.4	30.8	93.3	14.3	55.1

<sup>a</sup> Ave. = average; U.B. = upper bound; DEP = diethyl phthalate; DBP = dibutyl phthalate; DIBP = diisobutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate

<sup>b</sup> (Wormuth *et al.*, 2006). Mean and maximum exposure estimates. Women (female adults; 18 to 80 years); infants (0 to 12 months); toddlers (1 to 3 years); children (4 to 10 years).

<sup>c</sup> (Clark *et al.*, 2011). Median and 95<sup>th</sup> percentile exposure estimates. Combined male and female adults (20 to 70 years; not shown here); infants (neonates; 0 to 6 months); toddlers (0.5 to 4 years); children (5 to 11 years).

<sup>d</sup> This study. Mean and 95<sup>th</sup> percentile exposure estimates. Women (women of reproductive age; 15 to 44 years); infants (0 to <1 year); toddlers (1 to <3 years); children (3 to 12 years).

**Table 2.14 Comparison of modeled exposure estimates of total phthalate ester (PE) exposure (µg/kg-d) with estimates from biomonitoring studies.**

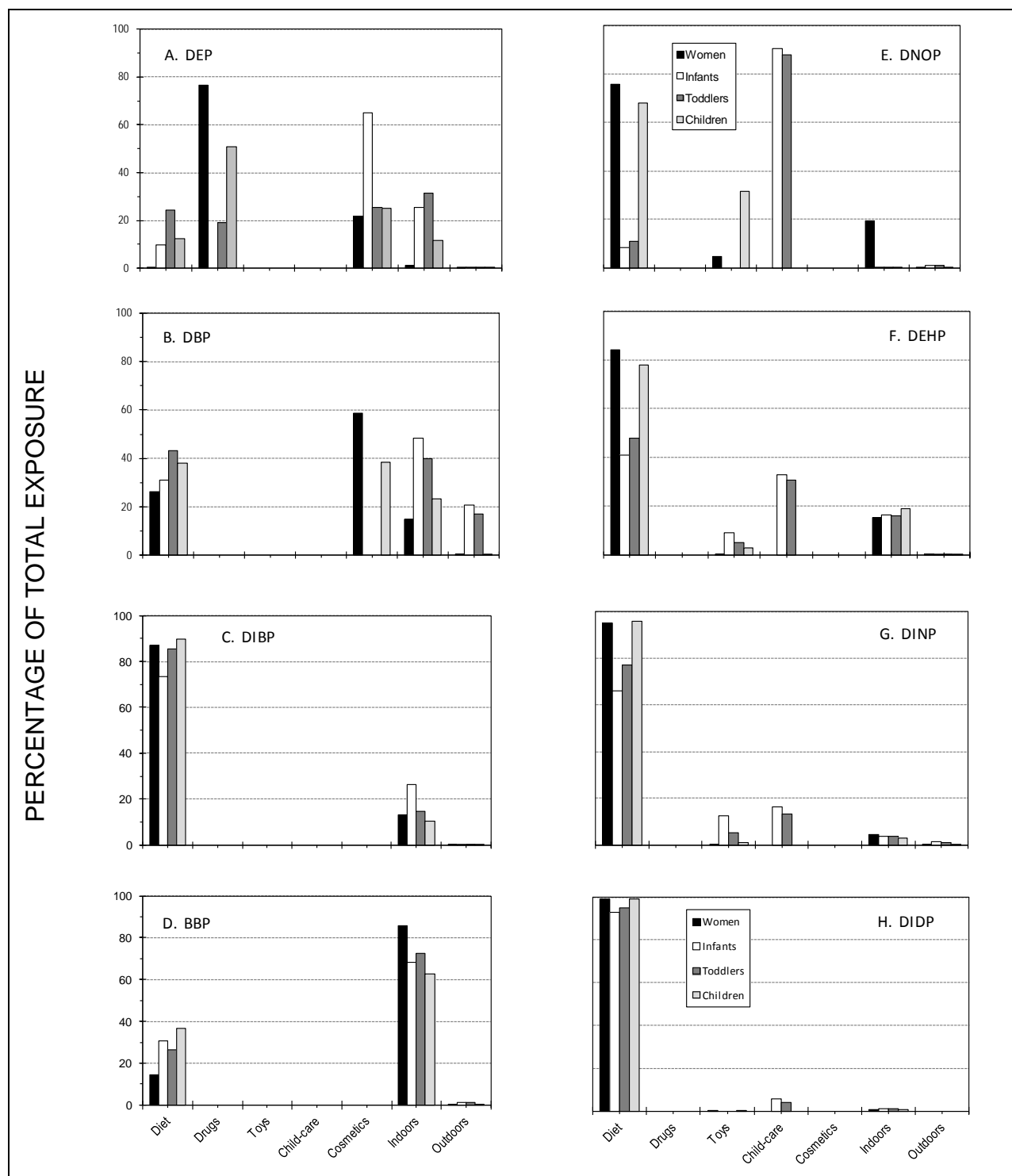
Phthalate	Method <sup>a</sup>	Women		Infants	
		Ave. <sup>b</sup>	0.95	Ave.	0.95
<b>DEP</b>	<i>Modeled</i>	18.1	398.0	3.1	14.9
	<i>SFF</i> <sup>c</sup>	NR	NR	NR	NR
	<i>NHANES</i>	3.4	74.8	NR	NR
<b>DBP</b>	<i>Modeled</i>	0.3	5.7	0.5	1.2
	<i>SFF</i>	0.8	2.4	1.7	7.0
	<i>NHANES</i>	0.6	3.5	NR	NR
<b>DIBP</b>	<i>Modeled</i>	0.1	0.5	0.5	1.5
	<i>SFF</i>	0.1	0.6	0.3	1.4
	<i>NHANES</i>	0.2	1.0	NR	NR
<b>BBP</b>	<i>Modeled</i>	1.1	2.6	1.8	4.0
	<i>SFF</i>	0.5	2.4	1.2	6.5
	<i>NHANES</i>	0.3	1.3	NR	NR
<b>DEHP</b>	<i>Modeled</i>	1.6	5.6	12.2	33.8
	<i>SFF</i>	2.8	19.1	5.5	25.8
	<i>NHANES</i>	3.5	181	NR	NR
<b>DINP</b>	<i>Modeled</i>	5.1	32.5	20.7	57.4
	<i>SFF</i>	0.7	5.4	3.5	16.5
	<i>NHANES</i>	1.1	11.1	NR	NR
<b>DIDP</b>	<i>Modeled</i>	3.2	12.2	10.0	26.4
	<i>SFF</i>	1.9	21.3	6.0	25.6
	<i>NHANES</i>	1.7	5.7	NR	NR
<b>r</b>	<i>SFF</i>	0.28	--	0.52	--
	<i>NHANES</i>	0.93	--	--	--

<sup>a</sup> Biomonitoring results from Section 2.5, based on data from NHANES (pregnant women; 2005–2006) and the Study for Future Families (Sathyanarayana *et al.*, 2008a; 2008b), Section 2.5. Modeling results from this section (2.6).

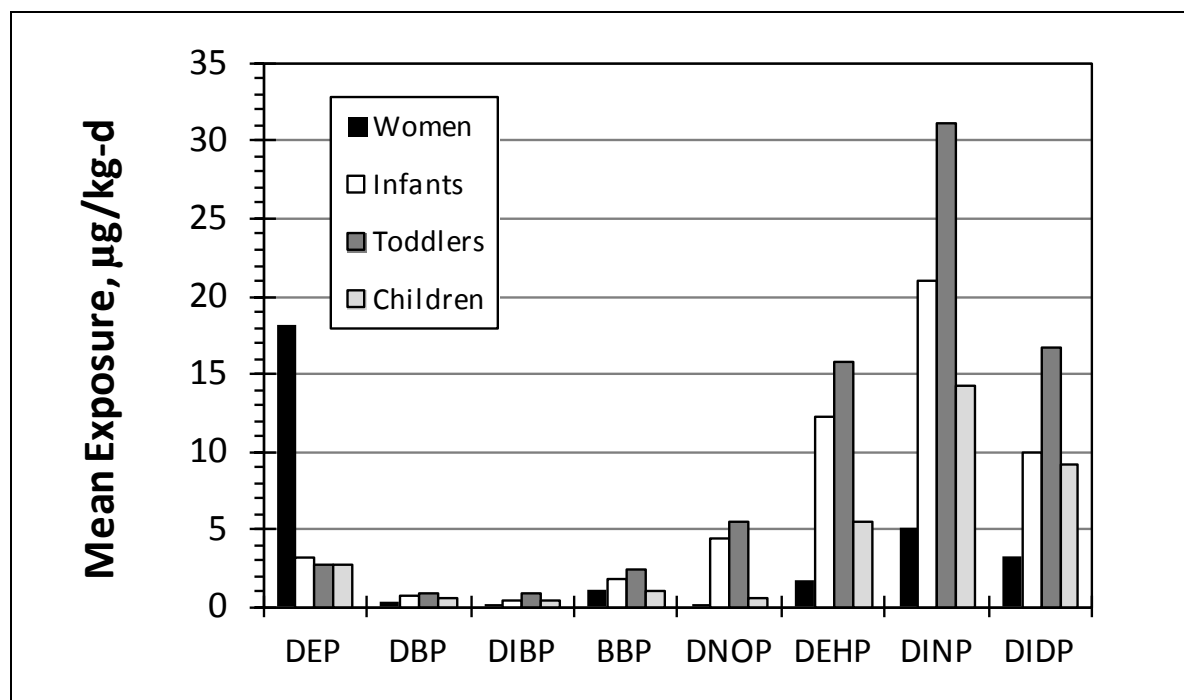
<sup>b</sup> Ave. = average, mean (modeled), or median (NHANES and SFF); 0.95, 95<sup>th</sup> percentile; NR = not reported; r, is the correlation coefficient for this study compared to either NHANES or SFF (average exposures); DEP = diethyl phthalate; DBP = dibutyl phthalate; DIBP = diisobutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate; DIDP = diisodecyl phthalate; SFF = Study for Future Families; NHANES = National Health and Nutritional Examination Survey.

<sup>c</sup> Data for SFF women are the average of prenatal and postnatal values.





**Figure 2.1** Sources of phthalate ester exposure. Percentage of total exposure for seven sources: (1) diet, (2) prescription drugs, (3) toys, (4) child care articles, (5) personal care products, (6) indoor sources, and (7) outdoor sources. Solid black bars, women; white bars, infants; dark gray bars, toddlers; and light gray bars, children. See Appendix E1 for additional details.



**Figure 2.2** Estimated phthalate ester exposure ( $\mu\text{g/kg-d}$ ) for eight phthalates and four subpopulations.

## 2.7 Cumulative Risk Assessment

### 2.7.1 Choice of Approach for Cumulative Risk Assessment

As described previously (Section 2.3; NRC, 2008), some phthalates—such as DBP, DIBP, BBP, DEHP, and DINP—are able to disrupt male sexual differentiation; this culminates in what has been described as the phthalate syndrome or more generally as the androgen-insufficiency syndrome. The NRC (2008) monograph on phthalates addressed the question of whether a cumulative risk assessment for phthalates should be conducted, and if so, to identify approaches that could be used. The report concluded that the risks associated with phthalates should be evaluated by taking account of combined exposures.

*Dose addition* and *independent action* are two concepts that allow quantitative assessments of cumulative effects by formulating the expected (additive) effects of mixtures. Experimental data on combination effects of phthalates from multiple studies (*e.g.*, Howdeshell *et al.*, 2008) provide strong evidence that dose addition can produce good approximations of mixture effects when the effects of all components are known. The NRC (2008) phthalates panel concluded that independent action often yielded similar quantitative predictions but in some cases led to substantial underestimations of combined effects. Following the work of this panel, the CHAP could not identify a case in which independent action predicted combined effects that were in agreement with experimentally observed responses and at the same time were larger than the effects anticipated by using dose addition. Thus, the CHAP concludes the assumption of dose addition is adequate for mixtures of phthalates and other antiandrogens for the foundation of a cumulative risk assessment.

The concept of *dose addition* forms the basis for a number of cumulative risk assessment methods. The hazard index (HI), the point of departure index (PODI) or toxicity equivalency factors (TEF) are examples of cumulative risk assessment approaches derived from *dose addition*.

The HI is widely used in cumulative risk assessment of chemical mixtures (Teuschler and Hertzberg, 1995). It is the sum of hazard quotients, (HQs) defined as the ratio of exposure (*e.g.*, estimate of daily intake) 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 (ADIs), RfDs 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 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 (*i.e.*, NOAELs or benchmark dose [lower confidence limit] [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 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:

$$\text{Hazard Quotient (HQ)}_j = \frac{DI_j (\mu\text{g} / \text{kg} - \text{day})}{PEAA_j (\mu\text{g} / \text{kg} - \text{day})}$$

and

$$\text{Hazard Index (HI)} = \sum_{j=1}^c \text{HQ}_j$$

where:  $c$  is the number of chemicals in the index.

The HI approach 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 RfD or ADI, which 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.

The CHAP considered utilizing the PODI (Wilkinson *et al.*, 2000) as an alternative to the HI. The PODI shows similarities to the HI method, but instead of relating estimates of daily intake to PEAA, PODs (NOAELs or BMDLs) are used. In this way, uncertainty factors of differing numerical values that may be included in the PEAA values for building the HI are removed from the calculation. An overall uncertainty factor for the mixture is used instead. However, in cumulative risk assessment for phthalates, it was necessary to deal with toxicological data of differing quality. This meant that different uncertainty factors had to be used for defining PEAA. The PODI approach cannot provide the flexibility needed in dealing with differing data quality. For this reason, the HI approach was given preference here.

Three different sources for PEAA were applied in the HI approach (three cases). Case 1 includes published values used in a cumulative risk assessment (CRA) for mixtures of phthalates (Kortenkamp and Faust, 2010), case 2 includes values derived from recently published and highly reliable relative potency comparisons across chemicals from the same study (Hannas *et al.*, 2011b), and case 3 includes values from the CHAP’s *de novo* literature review of reproductive and developmental endpoints focused on reliable NOAELs and PODs (Table 2.1). We considered these three cases to determine the sensitivity of the results to the assumptions for PEAA and the total impact on the HI approach.

To estimate daily intakes of mixtures of phthalates in pregnant women, we used human biomonitoring data (see Section 2.4). Human biomonitoring determines internal exposures (*i.e.*, body burden) to phthalates by measuring specific phthalate metabolites in urine. Thus,

biomonitoring represents an integral measure of exposure from multiple sources and routes (Angerer *et al.*, 2006; Needham *et al.*, 2007). Biomonitoring data provide evidence of exposure to mixtures of phthalates on an individual subject basis.

The CHAP has used a novel approach to calculate the HI by calculating it for each individual based on his or her urinary concentrations of mixtures of phthalates (in our case, for each pregnant woman and infant). This is in contrast to the standard HI approach of using population percentiles from exposure studies on a per chemical basis.

We applied data from two biomonitoring studies:

- National Health and Nutrition Evaluation Surveys (NHANES; 2005–2006)
- 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 (ages 2–36 months).

## **2.7.2 Summary Description of Methods Used**

Details of the analysis of the NHANES and SFF data are provided in Appendix D. Summary methods and results are presented here.

### **2.7.2.1 Chemicals**

We initially included in our analyses six phthalates described in the Consumer Product Safety Improvement Act:

- DEHP, DBP, and BBP: banned chemicals; and
- DINP, DIDP, and DNOP: chemicals with interim prohibition on their use.

Because 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 (Sathyanarayana *et al.*, 2008a; 2008b) data and were generally not detectable in NHANES. Thus, DNOP was dropped from further consideration of cumulative risk. A discussion of exposure estimates for these six phthalates is included in Sections 2.5 and 2.6.

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 chemicals. However, despite human studies reporting associations of MEP with reproductive human health outcomes, these phthalates were not considered in the calculation of the HI.

### **2.7.2.2 Potency Estimates for Antiandrogenicity: Three Cases**

The endpoints of phthalate toxicity regarded as most relevant are characteristic of disturbance of androgen action, 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.

We considered three cases for the calculation of HQs and the HI. These were chosen to evaluate the impact of assumptions in calculating the HI. The cases are discussed below.

**Case 1:** Case 1 is based upon recently published values used in a CRA for antiandrogens, including phthalates. The PEEA values for DBP, BBP, DINP, and DEHP were set as published in Kortenkamp and Faust (2010). We further assumed DIBP to be similar in potency to DBP. Although other authors have addressed CRAs for phthalates (Benson, 2009), we used the values from Kortenkamp and Faust due to their focus on *in vivo* antiandrogenicity.

**Case 2:** Case 2 is based on relative potency assumptions across phthalates. DEHP was selected as an index chemical with known *in vivo* evidence of antiandrogenicity in experimental animals and a NOAEL of 5 mg/kg-day. Three other phthalates (DIBP, DBP, and BBP) were assumed to be equipotent to DEHP, and DINP was assumed to be 2.3 times less potent (Hannas *et al.*, 2011b). An overall uncertainty factor of 100 was selected to account for inter-species extrapolation (factor of 10) and inter-individual variation (factor of 10).

**Case 3:** Case 3 is based on the *de novo* analysis of individual phthalates conducted by the CHAP. The NOAELs provided in Table 2.15 were combined with uncertainty factors of 100 to derive PEEA values. Table 2.15 provides the PODs, uncertainty factors, and RfDs for the five phthalates in the three cases considered.

### **2.7.2.3 Calculating the Hazard Index**

Using the individual daily intake estimates for each of the phthalates and relating these DI values to the respective PEAAs, the HQs and HI were calculated for each pregnant woman and infant in the NHANES and SFF (Sathyanarayana *et al.*, 2008a; 2008b) data.

Distributions of the HQs and HIs were generated for all three cases, with sampling weights used from the NHANES data to accommodate the prediction for pregnant women in the U.S. population.

## **2.7.3 Summary Results**

### **2.7.3.1 Calculation of Hazard Quotients and the Hazard Index from Biomonitoring Data**

The HI was calculated per woman and infant using the daily intake estimates for the phthalate diesters and the three cases for PEAAs. In all three cases and for both NHANES and SFF data, the distribution of the HI was highly skewed (histograms for each analysis are provided in Appendix D).

In the NHANES data, roughly 10% of pregnant women in the U.S. population (after adjustment with survey sampling weights) have HI values that exceed 1.0.\* The estimates are reduced in the SFF data in women from prenatal and postnatal measurements; 4–5% of infants have HI values that exceed 1.0 (Table 2.16).

The primary contributor(s) to the HI can be identified by evaluating the hazard quotients that comprise the HI. Clearly, the hazard quotient for DEHP dominates the calculation of the HI, as expected, with high exposure levels and one of the lowest PEAAAs. The rank contribution of the five phthalates to risk was calculated using the median 95<sup>th</sup> percentile across the cases for pregnant women in NHANES and SFF (Sathyanarayana *et al.*, 2008a; 2008b) women (prenatal and postnatal combined) and infants:

NHANES women (2005–2006): DEHP > DBP > DINP ~DIBP > BBP

SFF women: DEHP > BBP > DBP > DIBP > DINP

SFF infants: DEHP > DBP > BBP > DINP ~DIBP

In all cases, DEHP and DBP contributed strongly to the HI while DIBP and DINP contributed considerably less.

### 2.7.3.2 Summary

From biomonitoring studies there is clear evidence that both pregnant women and infants are exposed to mixtures of phthalates. Comparison of daily intake estimates to three different sets of PEAA derived from *in vivo* antiandrogenicity demonstrated a highly skewed distribution of the calculated HI in all three cases. Values of HI that exceed 1.0 are considered to signal some concern. Here, it is estimated that roughly 10% of pregnant women in the United States have HI values that exceed 1.0—a similar percentage was found in all three cases. The percentage was reduced in the SFF data but was similar for both prenatal and postnatal measurements—again, similar in all three cases with the exception of cases 2 and 3 in the postnatal percentages. Roughly 5% of infants in the SFF had HI values exceeding 1.0—and were similar across the three cases.

In all three cases studied, the HI value was dominated by DEHP because it has both high exposure and a low PEAA. DEHP had the highest HQs. Three phthalates (DBP, BBP, and DINP) were similar in their HQ values. DIBP had the smallest HQs.

---

\* When the HI >1.0, there may be a concern for adverse health effects in the exposed population.

**Table 2.15 Points of Departure (PODs; mg/kg-day), UFs and potency estimates for antiandrogenicity (PEAAs; µg/kg-day) in the three cases for the five phthalates considered in the cumulative risk assessment.**

Phthalate Diester	Case 1			Case 2			Case 3		
	POD	UF	PEAA	POD	UF	PEAA	POD	UF	PEAA
<b>DIBP</b>	40	200	200	5	100	50	125	100	1250
<b>DBP</b>	20	200	100	5	100	50	50	100	500
<b>BBP</b>	66	200	330	5	100	50	50	100	500
<b>DEHP</b>	3	100	30	5	100	50	5	100	50
<b>DINP</b>	750	500	1500	11.5	100	115	50	100	500

UF = uncertainty factor; PEAA = potency estimates for antiandrogenicity; POD = point of departure; DIBP = diisobutyl phthalate; DBP = dibutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate



**Table 2.16 Summary statistics (median, 95<sup>th</sup>, 99<sup>th</sup> percentiles) for HQs and HIs calculated from biomonitoring data from pregnant women (NHANES 2005–2006; CDC, 2012b) (SFF; Sathyanarayana *et al.*, 2008a; 2008b) and infants (SFF; Sathyanarayana *et al.*, 2008a; 2008b). NHANES values include sampling weights and thus infer to 5.3 million pregnant women in the U.S. population. SFF sample sizes range: Prenatal, N=340 (except N=18 for DINP); Postnatal, N=335 (except N=95 for DINP); Baby, N=258 (except N=67 for DINP); HI values are the sum of nonmissing hazard quotients.**

PEAA Case	NHANES Pregnant Women in U.S. Population			SFF Pregnant Women (Pre- and Postnatal)						SFF Infants		
	1	2	3	1	2	3	1	2	3	1	2	3
				Pre	Post	Pre	Post	Pre	Post			
<b>DIBP</b>	0.001	0.003	<0.001	0.001	0.001	0.003	0.003	<0.001	<0.001	0.002	0.01	<0.001
	0.01	0.02	0.001	0.003	0.003	0.01	0.01	<0.001	0.001	0.01	0.03	0.001
	0.01	0.04	0.002	0.01	0.01	0.03	0.04	0.001	0.001	0.01	0.06	0.004
<b>DBP</b>	0.01	0.01	0.001	0.01	0.01	0.02	0.01	0.002	0.001	0.02	0.03	0.003
	0.03	0.07	0.007	0.03	0.02	0.05	0.04	0.01	0.004	0.07	0.14	0.01
	0.06	0.13	0.01	0.05	0.05	0.10	0.09	0.01	0.01	0.13	0.25	0.03
<b>BBP</b>	0.001	0.01	0.001	0.002	0.001	0.01	0.01	0.001	0.001	0.04	0.02	0.003
	0.004	0.03	0.003	0.01	0.006	0.06	0.04	0.01	0.004	0.02	0.13	0.01
	0.01	0.05	0.01	0.01	0.01	0.08	0.08	0.01	0.01	0.07	0.45	0.04
<b>DEHP</b>	0.12	0.07	0.07	0.10	0.09	0.06	0.05	0.06	0.05	0.18	0.11	0.11
	6.0	3.6	3.6	0.55	0.72	0.33	0.43	0.33	0.43	0.86	0.52	0.52
	12.2	7.3	7.3	2.3	1.5	1.4	0.91	1.4	0.91	3.7	2.2	2.2
<b>DINP</b>	0.001	0.01	0.002	0.001	<0.001	0.01	0.01	0.002	0.001	0.002	0.03	0.01
	0.01	0.10	0.02	0.005	0.002	0.07	0.03	0.02	0.01	0.01	0.14	0.03
	0.02	0.24	0.05	0.005	0.01	0.07	0.07	0.02	0.02	0.02	0.21	0.05
<b>HI</b>	0.14	0.13	0.09	0.11	0.10	0.10	0.09	0.06	0.06	0.22	0.20	0.12
	6.1	3.7	3.6	0.57	0.73	0.41	0.46	0.33	0.43	0.96	0.82	0.55
	12.2	7.4	7.3	2.4	1.5	1.5	0.92	1.4	0.91	3.7	2.3	2.2
<b>% with HI&gt;1.0</b>	10	9	9	4	4	3	<1	2	<1	5	5	4

PEAA = potency estimates for antiandrogenicity; HI = hazard index; DIBP = diisobutyl phthalate; DBP = dibutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DINP = diisononyl phthalate; NHANES = National Health and Nutritional Examination Survey; SFF = Study for Future Families

This page intentionally left blank

### 3 Phthalate Risk Assessment

To arrive at transparent recommendations about restricting (or otherwise) the use of phthalates in children's toys and care products, the CHAP has employed a risk assessment approach that first analyzed the epidemiological evidence of associations between phthalate exposures and risk to human health. Such data give valuable answers to questions about whether phthalates as a group of chemicals might be linked to human disorders. However, only in rare cases is it possible to pinpoint specific chemicals as associated with health effects, and no such case is currently available for phthalates. At present, quantitative estimates of the magnitude of risks that stem from phthalate exposures cannot be derived directly from epidemiological data. For this reason, the CHAP had to rely primarily on evidence from tests with animals to underpin phthalate risk assessment.

As discussed in Science and Decisions ("The Silverbook," NRC, 2009), quantitative statements about "safe," "tolerable," or "acceptable" exposures are often inappropriately taken as "bright line" estimates that clearly demarcate "harm" from "safety," without accounting for inherent variabilities in response and the uncertainties associated with such estimates. The report advocated approaches in which the level of detail of the analysis is appropriate to the issue to be decided in risk assessment.

Accordingly, the CHAP took an approach appropriate to the charge and the richness of the available data to make recommendations about the use of phthalates in certain children's toys and care products. The CHAP made an effort to consider phthalate exposures to the developing fetus, the most vulnerable target of toxicity for phthalates, from all sources. Practically, this meant that subpopulations of interest were women of reproductive age, neonates, and toddlers.

In a hazard assessment step, the CHAP examined the toxicological profile of all relevant phthalates and phthalate substitution products, with an emphasis on endpoints related to antiandrogenic effects on male reproductive development in rodents (*i.e.*, the phthalate syndrome). The CPSIA requires the CHAP to consider the health risks from phthalates both in isolation and combination. To characterize the cumulative risks (risk in combination), the CHAP applied a hazard index approach for the antiandrogenic phthalates only: DBP, DIBP, BBP, DEHP, and DINP (Section 2.7). However, the CHAP also points out, that other antiandrogens can be added to the hazard index approach, increasing the HI (Appendix D).

To characterize the risks for compounds in isolation, quantitative estimates of PODs (NOAELs or BMDLs) were derived from experimental studies with animals, and in a risk characterization step, these estimates were compared with exposures by calculating MOEs. The numerical value of these MOEs was then taken into account in arriving at recommendations for specific phthalates. Typically, MOEs exceeding 100–1000 are considered adequate for protecting public health, for compounds in isolation. In taking this approach, it was possible to avoid misunderstandings that might have occurred had CHAP used points of departure and combined them with uncertainty factors to arrive at "tolerable exposures" or reference doses. These would have all too readily been taken as "bright lines," separating "risk" from "no risk." Considering the uncertainties inherent in extrapolating animal data to the human, this would have been inappropriate. In contrast, the MOE approach offers a level of flexibility commensurate with the task at hand. It does not imply that the points of departure used in risk characterization clearly

demarcate effect from absence of effects, and no absolute claims are made in terms of “safe” exposures that are not associated with harm or are without concern.

The risks from antiandrogenic phthalates were characterized by both the MOE approach (for phthalates in isolation) and the HI approach (cumulative risk). The risks from non-antiandrogenic phthalates and phthalate alternatives were characterized by the MOE approach.

## 4 Discussion

### 4.1 Variability and Uncertainty

#### 4.1.1 Developmental/Reproductive Toxicity Data

To fulfill the charges to consider the health effects of phthalates in isolation and in combination with other phthalates, and to consider the cumulative effect of total exposure to phthalates, the CHAP relied upon its review of the toxicology literature of phthalates and phthalate substitutes, exposure data (sources and levels), and data obtained from the HI approach for cumulative risk assessment (see Section 2.7.1 for details). Because of limitations in the biomonitoring datasets (NHANES and SFF), only five phthalates were analyzed using the HI approach: DEHP, DBP, BBP, DINP, and DIBP. Case 3<sup>\*</sup> in the HI analysis uses NOAELs generated from the available literature on the developmental toxicity of these five phthalates. To provide NOAELs, when possible, for these five phthalates, the CHAP systematically reviewed the published, peer-reviewed literature that reported information concerning the effects of *in utero* exposure of phthalates in pregnant rats.

The systematic evaluation of the developmental toxicity literature for the 14 phthalates and 6 phthalate substitutes, and the rationale for selecting a specific NOAEL for each chemical, are provided in Appendix A. Our criteria for an adequate study from which a NOAEL could be derived are: 1) at least three dose levels and a concurrent control should be used, 2) the highest dose should induce some developmental and/or maternal toxicity and the lowest dose level should not produce either maternal or developmental toxicity, 3) each test and control group should have a sufficient number of females to result in approximately 20 female animals with implantation sites at necropsy, and 4) pregnant animals need to be exposed during the appropriate period of gestation. In addition, studies should follow the EPA guideline OPPTS 870.3700 and the OECD guideline for the Testing of Chemicals (OECD 414, adopted 22 January 2001). The CHAP also gave added weight to data derived from studies replicated in different laboratories.

Although the CHAP developed the above criteria to evaluate published developmental toxicity studies and thereby derive reliable NOAELs for the nine phthalates and six phthalate substitutes, the final NOAELs used in the HI analysis are limited by the following: Many of the developmental toxicity studies reviewed were designed to derive mechanistic information and not NOAELs, and therefore used too few dose groups, often only one (*e.g.*, Gray *et al.*, 2000). Many studies did use multiple dose groups; however, the number of animals per dose group was less than recommended (*e.g.*, Howdeshell *et al.*, 2008) or it was unclear how many dose groups were used (*e.g.*, Kim *et al.*, 2010). In some studies in which multiple doses and sufficient animals per dose were used, the lowest dose used was also an effective dose, so a NOAEL could not be derived (*e.g.*, Saillenfait *et al.*, 2009a). In other studies, the exposure period used, *e.g.*, GD 7–13, did not cover the sensitive period for the disruption of male fetal sexual development (GD 15–21), which was the major endpoint of phthalate toxicity monitored. For some phthalates, *e.g.*, DIOP, only one peer-reviewed developmental toxicity study was located. The lack of replication

---

<sup>\*</sup> As discussed in Section 2.7.1., the CHAP considered three sets of reference doses (three cases) to calculate the hazard index.

introduces some level of uncertainty. For other phthalates, *e.g.*, DPHP, an insufficient number of animal data or poorly described methodologies limited the usefulness of available data. Finally, for some of the phthalate substitutes, *e.g.*, ATBC, DINX, and TPIB, peer-reviewed data were lacking, and only industry (for DINX and TPIB) or government (for TOTM) data were available. In cases in which peer-reviewed data were not available, the CHAP made executive decisions on a case-by-case basis as to whether non-peer-reviewed data would be used in making their recommendations to the CPSC.

Another level of uncertainty derives from the fact that the NOAELs used in the HI analysis and risk assessment were derived entirely from studies conducted in one species, the rat. Although some of the phthalates have been tested in mice, the available data are insufficient to derive a separate set of NOAELs.

#### **4.1.2 Exposure Scenarios**

The overall level of uncertainty in the analyses the CHAP conducted for the 14 phthalates, and the 6 nonphthalate substitutes under consideration, varied for each compound. For some compounds, the toxicological, exposure, and epidemiological information had major gaps, which led to a large degree of uncertainty in the estimated risk. In other cases, the uncertainties were driven by the lack of information for assessing either the hazard or the exposure. The nature of these gaps is reflected in two ways: 1) the comments associated with recommendations for the use or ban of a compound in children's toys and other products under the jurisdiction of the CPSC and 2) the actual recommendations for an action or the lack of a recommendation for an action made by the CHAP on the use of a compound in children's toys or other products under the jurisdiction of CPSC.

Further complicating the analyses was the charge to the CHAP to conduct a cumulative risk analysis. This led to additional uncertainties because data on the exposures associated with all routes of entry into the body were not consistent for each potential source of one or more compounds. In addition, the toxicological data were normally obtained via exposures administered by one route, or there were too few studies associated with each end point.

In the future, the government agencies need to consider how to work collaboratively and efficiently to collect the information needed to allow for detailed quantitative analysis of the exposure and hazard for use in quantitatively defining the risk to phthalates or other compounds of concern. In the case of phthalates, we were dealing with consumer products and not the raw form of the material or process intermediates. Thus, the data collected from toxicological testing and exposure measurements (biomonitoring and external sources), and risk characterization procedures, must take into account both realistic hazards and exposures. In this way Congressional mandates can be achieved with higher degrees of confidence for the specific or overall recommendations.

Within this process the CPSC must be given the resources to test the products under its jurisdiction as an initial step toward obtaining the information to conduct a characterization of exposure for a source. The lack of exposure information for the current CHAP phthalate analysis leaves numerous uncertainties, especially for some of the items deemed critical to the completion of our tasks. Without information on the use and release rates of the phthalates from the products

during use, it is difficult to properly employ exposure modeling tools to complete a thorough exposure characterization for risk assessment. Further, lack of such data from the exposure characterizations completed by the CHAP for phthalates weakens the analyses that couple biomonitoring data to external exposure characterizations to define the percent contribution of children's toys etc. to cumulative risk.

#### **4.1.3 HBM Data, Daily Intake Calculations, Hazard Index Calculations**

Human biomonitoring data, daily intake calculations based on HBM data, and, therefore, also the HI approach based on HBM data are subject to several sources of uncertainty and variability that will be identified and discussed in the following paragraphs. The CHAP will also attempt to describe the numerical magnitude of the variability, as a factor, increasing or decreasing the daily intake and resulting hazard index calculations.

Analytical variability/uncertainty: The analytical variability of the phthalate measurements in urine (in both NHANES [CDC, 2012b] and SFF [Sathyanarayana *et al.*, 2008a; 2008b]) have a standard deviation of below 20%, but in most cases below 10% (Silva *et al.*, 2008). Therefore, from the analytical perspective, the maximum factor contributing to both over- or underestimating exposure (and finally the HI) would be 1.2 but probably more in the region of 1.1. Recently, the CDC issued correction factors for two of its metabolites covered in the NHANES program, *i.e.*, correction factors 0.66 for MEP and 0.72 for monobenzyl phthalate (MBZP). All NHANES calculations were redone to include the revised data, post March 2012. In general, the standard purity can be assumed to be 95% and above. Usually the purity of the analytical standard is included in the analytical result and therefore reflected in the analytical result and the standard deviation of the method.

Individual variability in metabolism: The metabolite conversion factors for the individual metabolites have been determined in human metabolism studies (usually after oral dosing different doses of the labeled parent phthalate to human volunteers). For DEHP and DINP, Koch *et al.* (2004a; 2007a) published urinary metabolite conversion factors of 64.9% for DEHP (4 metabolites) and 43.61% for DINP (3 metabolites) based on one volunteer. Anderson *et al.* (2011) published conversion factors based on 20 individuals (10 male, 10 female) and two dose levels, and found conversion factors of  $47.1 \pm 8.5\%$  (4 DEHP metabolites) and  $32.9 \pm 6.4\%$  (3 DINP metabolites) over all volunteers (males and females) and over two different concentrations. The mean factors of Anderson *et al.* (2011) were used for our DI and HI calculations. As can be seen from the variability of the Anderson results, these mean excretion factors could over- or underestimate exposure by a factor of 1.2. The variability of the conversion factors for the other metabolites is probably in the same region. For example, for DBP and DIBP, a conversion factor of 69% has been used for the monoester metabolites. Assuming a hypothetical conversion factor of 100% (which is unrealistic) would mean that we would have overestimated the DI by a factor of 1.3 at the maximum; assuming a hypothetical conversion factor of less than 69% would mean that we would have underestimated the DI and consequently the HI.

Temporal variability of metabolite levels (exposure driven): Several studies have shown that although the day-to-day and month-to-month variability in each individual's urinary phthalate

metabolite levels can be substantial, a single urine sample was moderately predictive of each subject's exposure over three months. The sensitivities ranged from 0.56 to 0.74. Both the degree of between- and within-subject variance, and the predictive ability of a single urine sample, differed among phthalate metabolites. In particular, a single urine sample was most predictive for MEP and least predictive for MEHP (Hauser *et al.*, 2004). In general, a single urine sample for the low molecular weight phthalates (DMP, DEP, DBP, DIBP) has been shown to be more reliable in predicting exposure over a certain time span than for the high molecular weight (HMW) phthalates (DEHP, DINP, DIDP). Braun *et al.* (2012) state, "Surrogate analyses suggested that a single spot-urine sample may reasonably classify MEP and MBP concentrations during pregnancy, but >1 sample may be necessary for MBZP, DEHP. . . ." The variability issue has also been thoroughly investigated by Preau *et al.* (2010) on spot urine samples collected continuously over one week for eight individuals: they confirm the above statements: "Regardless of the type of void (spot, first morning, 24-hr collection), for MEP, inter-person variability in concentrations accounted for > 75% of the total variance. By contrast, for MEHHP, within-person variability was the main contributor (69–83%) of the total variance." However, because the DI calculations and the HI approach are population based, we can assume that the NHANES and SFF (Sathyanarayana *et al.*, 2008a; 2008b) data accurately reflect the variability of exposure relevant for the investigated population subset.

However, Preau *et al.* reported another interesting finding: ". . . for MEHHP, the geometric mean concentration of samples collected in the evening (33.2 µg/L) was significantly higher ( $p < 0.01$ ) than in samples collected in the morning (18.7 µg/L) or in the afternoon (18.1 µg/L)." Because neither NHANES nor SFF samples have been collected in the evening (representing exposure events that took place in the afternoon), there are indications that both NHANES and SFF samples might underestimate exposure to DEHP and other food-borne high molecular weight phthalates such as DINP and DIDP. This would indicate a factor of 1.5 for underestimation of the DI (and the HI) for the HMW phthalates.

Another indication of a possible underestimation (in NHANES samples) is mentioned in Lorber *et al.* (2011): "As much as 25% of all NHANES measurements contain metabolites whose key ratio suggests that exposure was "distant," that is, exposure occurred more than 24 hours before the sample was taken. This leads to another issue with NHANES samples:

Variability/uncertainty due to fasting: Most of the morning urine samples in NHANES are collected after a fasting period (first described by Stahlhut *et al.*, 2009). Fasting will certainly have an impact on food-borne contaminants, as some of the phthalates are. In the 2007–2008 NHANES sample, the 50<sup>th</sup> percentile of reported fasting times was approximately 8 hour (Aylward *et al.*, 2011). The authors could actually confirm the influence of fasting in the metabolites of DEHP: "Regression of the concentrations of four key DEHP metabolites vs. reported fasting times between 6 and 18 hours in adults resulted in apparent population-based urinary elimination half-lives, consistent with those previously determined in a controlled-dosing experiment, supporting the importance of the dietary pathway for DEHP." The correction factor for the influence of fasting (relevant for food-borne phthalates) may result in underestimation, but it is difficult to give a factor, probably less than 2-fold. Fasting is not an issue in the SFF samples.



Variability/uncertainty due to elimination kinetics and spot samples: Spot samples can over- or underestimate the mean daily exposure due to the fast elimination kinetics of the phthalates. Aylward *et al.* (2011) state, based on elimination kinetics, void volume, and last time of voiding, that theoretically “the potential degree of over- or underestimation is in the range of up to approximately four-fold in either direction. That is, at a short time since last exposure (2 to 4 h), estimated intakes based on spot sample concentrations may be overestimated by up to approximately four-fold. At a long time since last exposure (>14 h), the actual intakes may be underestimated by up to four-fold. They further state that the estimation of intake rates [ . . . ] in NHANES 2007–2008 spot samples [ . . . ] may be more likely to over- than underestimate actual exposures to DEHP, assuming fasting time is an appropriate surrogate for time since last exposure.” Overestimation is possible, but it is difficult to give a factor, probably less than 2-fold.

Creatinine correction model (used in the CHAP approach) versus volume-based model:

Both Koch *et al.* (2007) and Wittassek *et al.* (2007b) report that the creatinine-based daily intake calculations produce lower estimated intakes than the volume-based model. Daily intake values by the creatinine-based model were lower by a factor of two compared to the volume-based model. The creatinine-based model might therefore underestimate exposure by a factor of two.

Overall, the uncertainties regarding HBM data and dose extrapolations based on HBM data are within one order of magnitude, and certain factors for the possibility of overestimation of daily intake (and therefore the HI) seem to be balanced by factors for the underestimation of the DI/HI. Human biomonitoring data therefore provide a reliable and robust measure of estimating the overall phthalate exposure and resulting risk.

## **4.2 Species Differences in Metabolism, Sensitivity, and Mechanism**

When given to pregnant rats in controlled experimental exposures, phthalates produce a series of effects in the male offspring (phthalate syndrome) that are similar to disorders observed in humans, termed TDS (Skakkebaek *et al.*, 2001). In both cases, deficiency of androgen action in fetal life is strongly implicated, and for this reason, the rat has been regarded as the appropriate animal model for making extrapolations to phthalate risks in humans. However, recent comparative studies in mice and marmosets, and with human fetal testis explants grafted onto mice, have purportedly called this assumption into question.

The primary mechanism leading to phthalate-induced developmental and reproductive disorders in the rat is thought to be via suppression of testosterone synthesis in fetal life. Testosterone is a key driver of the normal differentiation of male reproductive tissues (Gray *et al.*, 2000; Scott *et al.*, 2009). Phthalates with ortho substitution and a side chain length of between four and six carbon atoms (Foster *et al.*, 1980) can drive down the expression of genes involved in cholesterol homeostasis (cholesterol is a precursor of androgens) and steroidogenesis genes in Leydig cells, within which androgen synthesis takes place. Phthalates with shorter side chains, such as DEP, are unable to induce these effects in the rat. The active principle is not the parent compound, but a monoester produced during hydrolytic reactions. Phthalate metabolites can also suppress expression of a key factor responsible for the first phase of testis descent (*i.e.*, *insl3*), leading to cryptorchidism (reviewed by Foster, 2005; 2006). The typical spectrum of effects observed in male rats after *in utero* phthalate exposure involves altered seminiferous cords, multinucleated

gonocytes, epididymal agenesis, retained nipples, shortened anogenital distance, cryptorchidism, and hypospadias.

The majority of studies examining the effects of phthalates have been conducted in the rat. More recently, comparative studies with other species have been undertaken, with the aim of examining whether the mechanisms and responses seen in the rat are species specific or whether they are of a more general nature.

*In utero* exposure to the phthalate DBP in mice, as in the rat, led to disruptions in seminiferous cord formation and the appearance of multinucleated gonocytes. However, unlike in the rat, these effects were not accompanied by suppressed fetal testosterone synthesis or by reduced expression of genes important in steroid synthesis (Gaido *et al.*, 2007). These observations were confirmed and extended in a mouse fetal testis explant system with the monoester of DEHP (MEHP) as the test substance. Depending on culture conditions, MEHP stimulated or inhibited androgen synthesis in testis explants, but the deleterious effects of MEHP on seminiferous cords and multinucleated gonocytes occurred independent of any effects on steroidogenesis (Lehraiki *et al.*, 2009). MEHP induced suppressions of *insl3* in this system, as it did in the rat.

The effects of phthalate metabolites on human fetal testis explants were investigated in several studies. In one study, fetal explants obtained during the second trimester of pregnancy were treated with MBP, but suppressions of androgen synthesis were not observed, independent of whether the cultures were stimulated with human chorionic gonadotrophin (hCG) or whether they were left unstimulated. (In human fetal testes, androgen synthesis depends on exposure to maternal hCG and later also on LH [Hallmark *et al.*, 2007].) In another study, human fetal testis explants from the first trimester of pregnancy were used and exposed to MEHP (Lambrot *et al.*, 2009). MEHP had no effect on testosterone synthesis, neither after stimulation of androgen synthesis by LH nor in cultures left unstimulated. There were also no effects on the expression of steroidogenic genes, and multinucleated gonocytes were not seen. However, reductions in the number of germ cells were noted. These studies are technically very challenging, and there is considerable variation in androgen production by different explants, which compromises statistical power and may obscure effects. In contrast to the observations with fetal cultures, DEHP and MEHP were able to induce significant reductions of testosterone synthesis in explants of adult testes (Desdoits-Lethimonier *et al.*, 2012).

A primate species, the marmoset, was investigated in two studies. In the first study (Hallmark *et al.*, 2007), neonatal marmosets were exposed to MBP. The monoester induced suppressions of serum testosterone levels shortly after administration. In the second study, marmosets were exposed to MBP during fetal development and studied at birth. Effects on testosterone production were not seen (McKinnell *et al.*, 2009), but any reductions in testosterone synthesis experienced in fetal life are likely to have disappeared by birth.

Very recently, the results of two experimental studies with human fetal testes grafted onto male mice and exposed to DBP were published (Heger *et al.*, 2012; Mitchell *et al.*, 2012). In one of the two studies (Mitchell *et al.*, 2012), the metabolite MBP was also investigated. It drove down serum testosterone levels by approximately 50%, but the effect did not reach statistical significance due to high experimental variation and a small number of repeats. DBP did not

affect testosterone levels. In the second of these studies (Heger *et al.*, 2012), testosterone was not measured. Instead, changes in testosterone synthesis were inferred from analyzing the expression of genes involved in testosterone production. DBP exposure did not affect any of these genes.

Both groups concluded that DBP exposure of normal functioning human fetal testes is probably without any effect on steroidogenesis. However, several issues, confounding factors, and disparities with other reports (discussed by the authors) must be considered before firm conclusions can be drawn.

Firstly, in both studies the human fetal material was obtained at ages by which the male programming of the testes had already occurred. This raises the possibility that in reality DBP may compromise testosterone synthesis but the effect was missed due to the age of the explants. The observations in cultured human fetal explants, in which effects on testosterone did not occur, independent of whether they were obtained during the first or second trimester (Hallmark *et al.*, 2007; Lambrot *et al.*, 2009), would argue against this possibility, but it cannot be excluded at present.

Secondly, the outcome of the testosterone assay in Mitchell *et al.* (2012) was highly variable, a result of inherent biological variability and the technical difficulties of these studies. The obvious way of dealing with experimental variability by including larger numbers of replications cannot be readily pursued with human fetal material due to technical, practical, and ethical considerations. For these reasons, results that did not reach statistical significance, as in Mitchell *et al.* (2012), have to be interpreted with great caution. At this stage, the outcome of these studies has to be regarded as inconclusive.

Thirdly, the observations of associations between phthalate exposure in fetal life and anogenital distance (Swan *et al.*, 2005; Swan, 2008) are difficult to reconcile with the results of the xenograft and human fetal explant experiments. Changes in anogenital distance are a robust read-out of diminished androgen action *in utero*, and these observations give strong indications that phthalates are capable of driving down fetal androgen synthesis in humans.

As proposed by Mitchell *et al.* and Heger *et al.*, more mechanistic studies are needed to resolve these issues. In view of these discrepancies, and until further evidence is available, the CHAP regards it as premature to assume that phthalate exposure in fetal life is of no concern to humans. In the species examined thus far—mouse, rat, and human—multinucleated gonocytes are a consistent feature of phthalate exposure *in utero*. These disruptions of gonocyte differentiation may have significant, although largely unexplored, implications for the development of carcinoma *in situ* (Lehraiki *et al.*, 2009). The long-term consequences of these abnormal germ cells are unknown but raise concerns. To dispel these concerns, further extensive studies are required.

The experimental findings in the rat and the marmoset show that neonatal exposure to certain phthalates suppresses testosterone synthesis in the testes. These observations are highly relevant considering the high phthalate exposures that may occur in some neonates.

This page intentionally left blank

## 5 Recommendations

### 5.1 Criteria for Recommendations

The CHAP was charged with making recommendations on specific phthalates and phthalate substitutes. At the present time, these chemicals exist in one of three categories: 1) permanent ban (permanently prohibits the sale of any “children’s toy or child care article” individually containing concentrations of more than 0.1% of DBP, BBP or DEHP); 2) interim ban (prohibits on an interim basis the sale of “any children’s toy that can be placed in a child’s mouth” or “child care article” containing concentrations of more than 0.1% of DNOP, DINP, or DIDP); and 3) currently unrestricted under Section 108 of the Consumer Product Safety Improvement Act of 2008. As part of its report, the CHAP will make recommendations on chemicals in each of these three categories. The recommendation may be to impose a permanent ban or an interim ban on a chemical or to take no regulatory action at this time. The recommendation for a ban or no action may be an extension of a current regulatory status or a new action.

The CPSIA prohibits the use of certain phthalates at levels greater than 0.1%, which is the same level used by the European Commission. When used as plasticizers for polyvinyl chloride (PVC), phthalates are typically used at levels greater than 10%. Thus, the 0.1% limit prohibits the intentional use of phthalates as plasticizers in children’s toys and child care articles but allows trace amounts of phthalates that might be present unintentionally. There is no compelling reason to apply a different limit to other phthalates that might be added to the current list of phthalates permanently prohibited from use in children’s toys and child care articles.

The recommendations are based on a review of the toxicology literature, exposure data, and other information such as a calculated hazard index. The issues relevant for making recommendations include the following:

1. What is the nature of the adverse effects reported in animal and human studies of toxicity? Did the findings include evidence of the phthalate syndrome or other evidence of reproductive or developmental toxicity?
2. What is the relevance to humans of findings in animal studies? Findings would generally be ascribed to one of three categories: a) known to be relevant, b) known to be irrelevant, or c) assumed to be relevant to humans.
3. What is the weight of the evidence? Is the experimental design of the study appropriate for the purpose of the study? Did the study have adequate power? Were confounders adequately controlled? Were findings replicated in other studies or other laboratories/populations?
4. What is the likely risk to humans, which we are going to evaluate based upon the MOEs (Table 5.1)? What are the exposures of concern—sources and levels? What are the hazards identified in animal studies? What are the dose-response data? What are the NOAELs? What is the relationship between levels of human exposure and POD (NOAEL)? What are the results of the HI calculations?
5. What is the recommendation? Permanent ban, interim ban, or no action at this time?
6. Would this recommendation, if implemented, affect exposure of children to this chemical? Yes, perhaps, unlikely, no, unknown?

**Table 5.1 Margin of exposure (MOE) estimates for pregnant women (NHANES) and infants (SFF) using median and 95<sup>th</sup> percentile (0.95) daily intake estimates from biomonitoring data using the range of PODs across the three cases.**

Chemical	Range of PODs (three cases)	Pregnant Women (NHANES)			Infants (SFF)		
		Daily Intake	Margin of Exposure <sup>a</sup>		Daily Intake	Margin of Exposure <sup>a</sup>	
	(mg/kg-d)	(μg/kg-d)	POD/Daily Intake (in same units)		(μg/kg-d)	POD/Daily Intake (in same units)	
	Median (0.95)	Range (0.95)		Median (0.95)	Range (0.95)		
Permanently Banned Phthalates							
DBP	5–50	0.6 (4)	8,000 (1,300	83,000 13,000)	3 (10)	1,600 (500	17,000 5,000)
BBP	5–66	0.3 (1)	17,000 (5,000	220,000 66,000)	2 (9)	2,500 (600	33,000 7,000)
DEHP	3–5	4 (181)	800 (17	1,300 28)	8 (29)	400 (100	600 200)
Interim Banned Phthalates							
DNOP	NA <sup>b, c</sup>	ND <sup>d</sup>	--	--	NA	--	--
DINP	11.5–750	1 (11)	12,000 (1,000	750,000 68,000)	4 (18)	2,900 (640	190,000 42,000)
DIDP	≥600 <sup>c, e</sup>	ND <sup>c</sup>	--	--	ND <sup>c</sup>	--	--
Phthalates Not Banned							
DMP	≥750 <sup>c, e</sup>	ND <sup>c</sup>	--	--	ND <sup>c</sup>	--	--
DEP	≥750 <sup>c, e</sup>	3 (75)	≥250,000 (≥10,000)	-- --	NA	--	--
DIBP	5-125	0.2 (1)	25,000 (5,000	625,000 125,000)	0.4 (2)	12,500 (2,500	300,000 60,000)
DPENP	11 <sup>e</sup>	NA	--	--	NA	--	--
DHEXP	≤250 <sup>e</sup>	NA	--	--	NA	--	--
DCHP	16 <sup>e</sup>	ND <sup>d</sup>	--	--	NA	--	--
DIOP	NA	NA	--	--	NA	--	--
DPHP	NA <sup>c</sup>	NA	--	--	NA	--	--

Chemical	Range of PODs (three cases) (mg/kg-d)	Pregnant Women (NHANES)		Infants (SFF)	
		Daily Intake	Margin of Exposure <sup>a</sup>	Daily Intake	Margin of Exposure <sup>a</sup>
		(µg/kg-d)	POD/Daily Intake (in same units)	(µg/kg-d)	POD/Daily Intake (in same units)
		Median (0.95)	Range (0.95)	Median (0.95)	Range (0.95)
<b>Phthalate Substitutes</b>					
<b>TPIB</b>	≥1,125 <sup>b,c</sup>	NA	-- --	NA	-- --
<b>DEHA</b>	≥800 <sup>c</sup>	NA	-- --	NA	-- --
<b>DEHT</b>	≥750 <sup>c</sup>	NA	-- --	NA	-- --
<b>ATBC</b>	≥1,000 <sup>c</sup>	NA	-- --	NA	-- --
<b>DINX</b>	≥1,000 <sup>c</sup>	NA	-- --	NA	-- --
<b>TOTM</b>	100 <sup>f</sup>	NA	-- --	NA	-- --

<sup>a</sup> Rounded to the nearest hundred or thousand.

<sup>b</sup> NA = not available; ND = not done; POD = point of departure; DBP = dibutyl phthalate; BBP = butylbenzyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DNOP = di-*n*-octyl phthalate; DINP = diisononyl phthalate; DIDP = diisodecyl phthalate; DMP = dimethyl phthalate; DEP = diethyl phthalate; DIBP = diisobutyl phthalate; DPENP = di-*n*-pentyl phthalate; DHEXP = di-*n*-hexyl phthalate; DCHP = dicyclohexyl phthalate; DIOP = diisooctyl phthalate; DPHP = di(2-propylheptyl) phthalate; TPIB = 2,2,4-trimethyl-1,3 pentanediol diisobutyrate; DEHA = di(2-ethylhexyl) adipate; DEHT = di(2-ethylhexyl) terephthalate; ATBC = acetyl tributyl citrate; DINX = 1,2-cyclohexanedicarboxylic acid, diisononyl ester; TOTM = tris(2-ethylhexyl) trimellitate; NHANES = National Health and Nutritional Examination Survey; SFF = Study for Future Families

<sup>c</sup> No evidence of antiandrogenicity.

<sup>d</sup> Biomonitoring data were largely nondetects.

<sup>e</sup> Case 3 only (Table 2.1).

<sup>f</sup> Limited evidence of antiandrogenicity.

## 5.2 Recommendations on Permanently Banned Phthalates

### 5.2.1 Di-n-butyl Phthalate (DBP) (84-74-2)

#### 5.2.1.1 Adverse Effects

##### 5.2.1.1.1 Animal

##### 5.2.1.1.1.1 Reproductive

- Over 20 animal studies were reviewed in the NTP-CERHR report (2000). Many studies showed similar effects at high doses (~ 2000 mg/kg-d) in rats. The panel's conclusions were that DBP could probably affect human development or reproduction and current exposures were possibly high enough to cause concern. The NTP concurred with the NTP-CERHR DBP panel. Both stated that there was minimal concern for developmental effects for pregnant women exposed to DBP levels estimated by the panel (2–10 µg/kg-day).
- Studies cited in the NTP-CERHR (2000) report have been confirmed and extended by more recent reports by Mahood *et al.* (2007), showing decreased male fertility and testicular testosterone, and increased testicular toxicity; Gray *et al.* (2006), showing a decrease in number of pregnant rats and live pups, decreased serum progesterone, and increased hemorrhagic corpora lutea; and Ryu *et al.* (2007), documenting changed steroidogenesis and spermatogenesis gene expression profiles. Recently, a study by McKinnel *et al.* (2009), using marmosets, did not show any effect on testicular development or function, even into adulthood.

##### 5.2.1.1.1.2 Developmental

- The NTP-CERHR (2000) reviewed the reproductive and developmental toxicity of DBP and concluded at the time of the report that the panel could locate “no data on the developmental or reproductive toxicity of DBP in humans.” The panel concluded, however, that, based on animal data, it “has high confidence in the available studies to characterize reproductive and developmental toxicity based upon a strong database containing studies in multiple species using conventional and investigative studies. When administered via the oral route, DBP elicits malformations of the male reproductive tract via a disturbance of the androgen status: a mode of action relevant for human development. This antiandrogenic mechanism occurs via effects on testosterone biosynthesis and not via androgen receptor antagonism. DBP is developmentally toxic to both rats and mice by the oral routes; it induces structural malformations. A confident NOAEL of 50 mg/kg-day by the oral route has been established in the rat. Data from which to confidently establish a lowest observed adverse effect level (LOAEL)/(NOAEL) in the mouse are uncertain.” These statements are made primarily on the basis of studies by Ema *et al.* (1993; 1994; 1998) and Mylchreest *et al.* (1998; 1999; 2002). Finally, studies by Saillenfait *et al.* (1998) and Imajima *et al.* (1997) indicated that the monoester metabolite of DBP is responsible for the developmental toxicity of DBP.



- Studies cited in the NTP-CERHR (2000) report have been confirmed and extended by more recent reports by Zhang *et al.* (2004), documenting effects on the epididymis, testis, and prostate; Lee *et al.* (2004), reporting reduced spermatocyte and epididymal development, decreased AGD, and increased nipple retention; Howdeshell *et al.* (2007), showing reduced AGD, increased number of areolae per male, and increased number of nipples per male, Jiang *et al.* (2007), reporting an increased incidence of cryptorchidism and hypospadias, and decreased AGD and serum testosterone; Mahood *et al.* (2007), reporting an increased incidence of cryptorchidism and multinucleated gonocytes, and decreased testosterone; Struve *et al.* (2009), documenting decreased AGD, fetal testicular testosterone, and testicular mRNA concentrations scavenger receptor class B, member1, steroidogenic acute regulatory protein, cytochrome P45011a1, and cytochrome P45017a1; and Kim *et al.* (2010), reporting an increased incidence of hypospadias and cryptorchidism, decreased testis and epididymal weights, and decreased AGD and testosterone levels.

#### **5.2.1.1.2 Human**

- Several epidemiologic studies measured urinary concentrations of MBP. Of those that did, there were associations of maternal urinary MBP concentrations with measures of male reproductive tract development (specifically, shortened AGD) (Swan *et al.*, 2005; Swan, 2008). However, other studies did not find associations of urinary MBP with shortened AGD (Huang *et al.*, 2009; Suzuki *et al.*, 2012). Several studies reported associations of MBP with poorer scores on neurodevelopment tests (Engel *et al.*, 2010; Swan *et al.*, 2010; Kim *et al.*, 2011; Miodovnik *et al.*, 2011; Whyatt *et al.*, 2011), whereas others did not (Engel *et al.*, 2009; Cho *et al.*, 2010; Kim *et al.*, 2011).

#### **5.2.1.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

#### **5.2.1.3 Weight of Evidence**

##### **5.2.1.3.1 Experimental Design**

Animal reproductive and developmental toxicology studies covered a broad range of species and methods, and clearly supported the overall conclusion that DBP has antiandrogenic properties. Although several of these studies report a specific NOAEL, not all studies were amenable to the calculation of a NOAEL. For example, the studies of Carruther and Foster (2005) and Howdeshell *et al.* (2007) were designed to obtain mechanistic data and therefore did not include multiple doses. The study by Higuchi *et al.* (2003) is interesting because it demonstrates that DBP produces effects in rabbits similar to those seen in the rat, but again, only one dose was used, thus precluding the determination of a NOAEL. Other studies (Lee *et al.*, 2004; Jiang *et al.*, 2007; Struve *et al.*, 2009), which did use at least three doses, used fewer than the recommended number of animals/dose (20/dose). The study by Kim *et al.* (2010) used multiple doses; however, it was difficult to ascertain how many animals were used per dose. The studies of Mylchreest *et al.* (2000) and Zhang *et al.* (2004), on the other hand, used multiple doses and approximately 20 animals/dose. In the absence of maternal toxicity, Mylchreest

reported an increase in nipple retention in male pups at 100 mg/kg-d, whereas Zhang *et al.* reported increased male AGD at 250 mg/kg-day. In both studies, these LOAELs correspond to a NOAEL of 50 mg/kg-day. A NOAEL of 50 mg/kg-day is supported by the study by Mahood *et al.* (2007), which reported a LOAEL of 100 mg/kg-day for decreased fetal testosterone production after exposure to DBP. Using the data of Mylchreest *et al.* (2000) and Zhang *et al.* (2004) the CHAP committee assigned a NOAEL of 50 mg/kg-day for DBP. Human correlation studies suggested that subjects with higher levels of DBP metabolites were associated with reproductive impairments. Some of these studies (e.g., Murature *et al.*, 1987), however, did not adequately consider or describe potential confounders.

#### **5.2.1.3.2 Replication**

A sufficient number of studies were replicated to confirm study findings and endpoints.

### **5.2.1.4 Risk Assessment Considerations**

#### **5.2.1.4.1 Exposure**

No quantifiable exposures associated with toys or children's personal care products were located. DBP is used in nail polish. DBP metabolites (MBP) have been detected in human urine samples in the U.S. general population (Blount *et al.*, 2000; NHANES 1999–2000, 2001–2002, 2003–2004; CDC, 2012b), New York City pregnant women (Adibi *et al.*, 2003), Japanese adults (Itoh *et al.*, 2005), and infertility clinic patients in Boston (men; Duty *et al.*, 2004; Hauser *et al.*, 2007). When compared to children 6–11 years old, urine concentrations for MBP were 50% lower in neonates and 6-fold higher in toddlers (Brock *et al.*, 2002; Weuve *et al.*, 2006). In another study, geometric mean levels of MBP in urine were significantly higher in children 6–11 years old when compared to adolescents or adults (Silva *et al.*, 2004). MBP urine levels have also been reported to differ by gender (Silva *et al.*, 2004). CHAP calculations estimate that the median/high intake (95<sup>th</sup> percentile) from NHANES biomonitoring data for DBP is 0.6/4 µg/kg-day, respectively.

#### **5.2.1.4.2 Hazard**

A relatively complete dataset suggests that exposure to DBP can cause reproductive or (nonreproductive) developmental effects. DBP can also induce other target organ effects, such as changes in body weight and liver weight.

#### **5.2.1.4.3 Risk**

Both animal and human data support maintaining the permanent ban on DBP in children's toys and child care articles. Currently, DBP is not allowed in these articles at levels greater than 0.1 %.

The MOEs from biomonitoring estimates range from 8,000 to 83,000 using median exposures and from 1300 to 13,000 using 95<sup>th</sup> percentiles. Typically, MOEs exceeding 100–1000 are considered adequate for public health; however, the cumulative risk of DBP with other antiandrogens should also be considered.

#### **5.2.1.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends no further action regarding toys and child care articles at this time because DBP is already permanently banned in children's toys and child care articles at levels greater than 0.1%.

However, CHAP recommends that U.S. agencies responsible for dealing with DBP exposures from food, pharmaceuticals, and other products conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.2.1.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DBP?**

No, because DBP is already permanently banned in children's toys and child care articles.

### **5.2.2 Butylbenzyl Phthalate (BBP) (85-68-7)**

#### **5.2.2.1 Adverse Effects**

##### ***5.2.2.1.1 Animal***

##### ***5.2.2.1.1.1 Reproductive***

- The NTP-CERHR reviewed the reproductive and developmental toxicity of BBP (NTP, 2003a). The panel's conclusions were that BBP could probably affect human development or reproduction but that current exposures were probably not high enough to cause concern. The NTP stated that there was minimal concern for developmental effects in fetuses and children, and that there was negligible concern for adverse reproductive effects in exposed men.
- Two two-generation reproductive toxicity studies in rats not reviewed in the 2003 NTP-CERHR document reported that BBP exposure led to decreased ovarian and uterine weights (F0 females); decreased mating and fertility indices (F1 males and females); decreased testicular, epididymal, seminal vesicle, coagulating gland, and prostate weights; increased reproductive tract malformations (*i.e.*, hypospadias); decreased epididymal sperm number, motility, and progressive motility; and increased histopathologic changes in the testis and epididymis (F1 males). In the F2 generation, AGD was reduced in male pups and male pups also had increased nipple/areolae retention.

##### ***5.2.2.1.1.2 Developmental***

- The NTP-CERHR (2003a) reviewed the reproductive and developmental toxicity of BBP and, as with DBP, concluded at the time of the report that the panel could locate "no data on the developmental or reproductive toxicity of BBP in humans." The panel concluded, however, that there was an adequate amount of data on rats and mice to do

an assessment of “fetal growth, lethality and teratogenicity,” but that none of the studies included a postnatal evaluation of “androgen-regulated effects (*e.g.*, nipple retention, testicular descent, or preputial separation)” and that prenatal studies with the monoesters were adequate to conclude “that both metabolites (monobutyl phthalate and monobenzyl phthalate) contribute to developmental toxicity.” These statements were based on studies by Ema *et al.* (1990; 1992; 1995), Field *et al.* (1989), and Price *et al.* (1990). Developmental NOAELs in these studies ranged from 420 to 500 mg/kg-d, and the panel caveated conclusions by saying it was not confident in the NOAELs because the studies would not detect postpubertal male reproductive effects (*i.e.*, decreased AGD, increased incidence of retained nipples, etc.).

- Several studies subsequent to the NTP-CERHR (2000) extended the reports cited in this document with studies in which exposures occurred during late gestation and into the postnatal period. Gray *et al.* (2000) reported that BBP increased the incidence of areolas/nipples, decreased testis weights, and increased the incidence of hypospadias. Nagao *et al.* (2000) reported reduced AGD, delayed preputial separation, reduced serum testosterone in male pups, and increased AGD in female pups. Piersma *et al.* (2000) reported increased frequency of developmental anomalies (increased incidence of fused ribs and reduced rib size, anophthalmia, and cleft palate) and also increased the incidence of retarded fetal testicular caudal migration. Saillenfait *et al.* (2003) reported an increase in exencephalic fetuses in rats and an increase in exencephaly, facial cleft, meningocele, spina bifida, onphalocele, and acephalostomia in mice. Ema found increased incidence of undescended testes and decreased AGD at doses of 500 mg/kg-d or greater in one study (Ema and Miyawaki, 2002) and at doses of 250 mg/kg-d or greater in a subsequent study (Ema *et al.*, 2003). Tyl *et al.* (2004) reported reduced AGD in F1 and F2 male offspring, delayed acquisition of puberty in F1 males and females, increased retention of nipples and areolae in F1 and F2 males, and increased incidence of abnormal male reproductive organs (hypospadias, missing epididymides, testes, and/or prostate). BBP significantly reduced fetal testosterone production in male pups at 300 mg/kg-d or greater in Sprague-Dawley (SD) rats (Howdeshell *et al.*, 2008).

#### **5.2.2.1.2 Human**

- Several epidemiologic studies measured urinary concentrations of the BBP metabolite MBZP. In those that did, there were no associations of maternal urinary MBZP concentrations with measures of male reproductive tract development (specifically, shortened AGD) (NTP, 2000; Swan, 2008; Huang *et al.*, 2009; Suzuki *et al.*, 2012). A few studies reported associations of MBZP with poorer scores on neurodevelopment tests (*e.g.*, Wyatt *et al.*, 2011), whereas others did not (Swan *et al.*, 2010).

#### **5.2.2.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### 5.2.2.3 Weight of Evidence

#### 5.2.2.3.1 Experimental Design

The study by Gray *et al.* (2000) could not be used to generate a NOAEL because only one dose was used, whereas the study by Saillenfait *et al.* (2003) could not be used because the sensitive period for the disruption of male fetal sexual development in the rat (GD 15–21) was not included in the study's exposure protocol (GD 7–13). The remaining studies were judged to be adequate for determining a NOAEL for BBP. The CHAP committee determined a NOAEL of 100 mg/kg-d from the Nagao *et al.* (2000) study. Piersma *et al.* (2000) calculated a benchmark dose of 95 mg/kg-d, and a NOAEL of 250 mg/kg-d was determined from the data of the Ema and Myawaki study (2002), and of 167 mg/kg-d from the data of Ema *et al.*, (2003). Tyl *et al.* (2004) determined a NOAEL of 50 mg/kg-d from data generated in their two-generation study. Thus, the NOAELs ranged from a low of 50 to a high of 250 mg/kg-d. Finally, Howdeshell *et al.* (2008) reported significantly reduced fetal testosterone production at 300 mg/kg-d or greater. The CHAP decided to take the conservative approach and to recommend a NOAEL of 50 mg/kg-d for BBP.

#### 5.2.2.3.2 Replication

A sufficient number of studies demonstrating similar adverse reproductive and developmental endpoints have been performed.

### 5.2.2.4 Risk Assessment Considerations

#### 5.2.2.4.1 Exposure

Little to no exposure derived from toys or children's personal care products is known to occur in children, toddlers, and infants. (BBP is not found in these articles at levels greater than 0.1 %.) However, BBP is found in the diet. BBP metabolites (MBZP) have been detected in human urine samples in the U.S. general population (NHANES 1999–2000, 2001–2002, 2003–2004, 2005–2006, 2007–2008; Blount *et al.*, 2000), New York City pregnant women (Adibi *et al.*, 2003), infertility clinic patients in Boston (men; Duty *et al.*, 2004; Hauser *et al.*, 2007), young Swedish men (Jönsson *et al.*, 2005), German residents (Koch *et al.*, 2003a; Wittassek *et al.*, 2007b), and women in Washington, D.C., (CDC, 2005; Hoppin *et al.*, 2004). Urine concentrations for MBZP were similar between children 6–11 years old and children younger than 2 years. In general, levels of MBZP were higher in females when compared to males, and children > adolescents > adults (Silva *et al.*, 2004). MBZP levels have decreased consistently over the survey periods for the total (geometric mean; 15.3 to 10.0 µg/L), for all age, gender, and race classes. CHAP calculations estimate that the median/high (95<sup>th</sup> percentile) intake from NHANES biomonitoring data for BBP is 0.3/1.3 µg/kg-day, respectively, in pregnant women and that MOEs for modeling and biomonitoring range from 6,800 to 147,000.

#### 5.2.2.4.2 Hazard

A relatively complete dataset suggests that exposure to BBP can cause reproductive or (nonreproductive) developmental effects. BBP can also induce other target organ effects, such as changes in body weight and liver weight.

#### **5.2.2.4.3 Risk**

Both animal and human data support maintaining the permanent ban on BBP in children's toys and child care articles.

The margin of exposure for total BBP exposure in infants (SFF; Sathyanarayana *et al.*, 2008a; 2008b) at the 95<sup>th</sup> percentile of exposure was from 770 to 10,000. MOEs were slightly higher in pregnant women, ranging from 5000 to 66,000. Typically, MOEs exceeding 100–1000 are considered adequate for public health; however, the cumulative risk of BBP with other antiandrogens should also be considered.

#### **5.2.2.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends no further action regarding toys and child care articles at this time because BBP is already permanently banned in children's toys and child care articles at levels greater than 0.1%.

However, CHAP recommends that U.S. agencies responsible for dealing with BBP exposures from food and other products conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.2.2.6 Would this recommendation, if implemented, be expected to reduce exposure of children to BBP?**

No, because BBP is already permanently banned in children's toys and child care articles.

### **5.2.3 Di(2-ethylhexyl) Phthalate (DEHP) (117-81-7)**

#### **5.2.3.1 Adverse Effects**

##### **5.2.3.1.1 Animal**

##### **5.2.3.1.1.1 Reproductive**

- The NTP-CERHR (2006) reviewed developmental and reproductive effects of DEHP. The panel's conclusions were that DEHP could probably affect human development or reproduction and that current exposures were high enough to cause concern. The NTP concurred with the panel and stated that there was serious concern over DEHP exposures during certain intensive medical treatments for male infants and that these exposures may result in levels high enough to affect development of the reproductive tract. They also concurred that there was concern over adverse effects on male reproductive tract development resulting from certain medical procedures on pregnant and breastfeeding women, that there was concern for male infants (<1 year old) reproductive tract development following exposure, that there was some concern for male children (> 1 year old) reproductive tract development following exposure, that there was some concern for male offspring reproductive tract development following exposures to pregnant women not exposed via medical procedures, and that there is

minimal concern for reproductive toxicity in adults who are exposed medically or nonmedically. Sixty-eight (predominately rodent) studies were reviewed by the NTP-CERHR panel.

#### **5.2.3.1.1.2 Developmental**

- The NTP-CERHR (2002) reviewed developmental and reproductive effects of DEHP. Forty-one animal prenatal developmental toxicity studies “were remarkably consistent” and “DEHP was found to produce malformations, as well as intrauterine death and developmental delay. The NOAEL based upon malformations in rodents was ~40 mg/kg-d, and a NOAEL of 3.7–14 mg/kg-d was identified for testicular development/effects in rodents.”
- The NTP-CERHR (2006) update on the developmental and reproductive effects of DEHP reviewed multiple human studies and concluded that there is “insufficient evidence in humans that DEHP causes developmental toxicity when exposure is prenatal . . . or when exposure is during childhood.” The panel reviewed animal studies as well and concluded that there is “sufficient evidence that DEHP exposure in rats causes developmental toxicity with dietary exposure during gestation and/or early postnatal life at 14–23 mg/kg-d as manifest by small or absent male reproductive organs” (NOAEL = 3.5 mg/kg-d).
- Three developmental toxicity reports have appeared since the 2006 NTP-CERHR study that confirmed and extended the studies already reviewed. These latest studies show that DEHP exposure delays the age of vaginal opening and first estrus in females, delays male preputial separation, increases testis weight and nipple retention, and decreases AGD (Grande *et al.*, 2006; Andrade *et al.*, 2006a; Christiansen *et al.*, 2010).

#### **5.2.3.1.1.3 Human**

- Several epidemiologic studies measured urinary concentrations of metabolites of DEHP, including MEHP, MEHHP, MEOHP, and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP). Of those that did, there were associations of maternal urinary mono(2-ethylhexyl) phthalate (MEHP), MEHHP, and MEOHP concentrations with measures of male reproductive tract development (specifically, shortened AGD) (Swan *et al.*, 2005; Swan, 2008; Suzuki *et al.*, 2012). However, one other study did not find associations of urinary MEHP with AGD (Huang *et al.*, 2009). Several studies reported associations of MEHP with poorer scores on neurodevelopment tests (Engel *et al.*, 2009; Kim *et al.*, 2009; Swan *et al.*, 2010; Kim *et al.*, 2011; Miodovnik *et al.*, 2011; Yolton *et al.*, 2011), whereas others did not (Engel *et al.*, 2010; Whyatt *et al.*, 2011).

### **5.2.3.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### 5.2.3.3 Weight of Evidence

#### 5.2.3.3.1 Experimental Design

The Gray *et al.* (2000) study could not be used to determine a NOAEL because only one dose was used. The studies by Moore *et al.* (2001), Borch *et al.* (2004), and Jarfelt *et al.* (2005) could not be used because in each case the lowest dose used produced a significant effect and therefore a NOAEL could not be determined. The studies by Grande *et al.* (2006), Andrade *et al.* (2006a), Gray *et al.* (2009), and Christian *et al.* (2010) are all well-designed studies employing multiple doses at the appropriate developmental window and using relatively large numbers of animals per dose group. Although different phthalate syndrome endpoints were used to set a NOAEL, the resulting NOAELs cluster tightly around a value of 3–11 mg/kg-d. It is noteworthy that this cluster is consistent with the NOAEL identified in the NTP study (4.8 mg/kg-d; Foster *et al.*, 2006). In contrast, using fetal testosterone production as an endpoint, Hannas *et al.* (2011b) reported a LOAEL of 300 mg/kg-d and a NOAEL of 100 mg/kg-d, a NOAEL approximately 10 times the one derived using morphological endpoints. Using a weight-of-evidence approach, the CHAP has conservatively set the NOAEL for DEHP at 5 mg/kg-d.

#### 5.2.3.3.2 Replication

A sufficient number of animal studies demonstrating similar adverse reproductive and developmental endpoints have been performed.

### 5.2.3.4 Risk Assessment Considerations

#### 5.2.3.4.1 Exposure

Currently, DEHP is not allowed in children's toys and child care products at levels greater than 0.1%. The frequency and duration of exposures have not been determined; however, metabolites of DEHP (MEHP, MEHHP, MEOHP, and MECPP) have been detected in human urine samples in the U.S. general population (NHANES 1999–2000, 2001–2002, 2003–2004; CDC, 2012b), New York City pregnant women (Adibi *et al.*, 2003), women in Washington, D.C., (Hoppin *et al.*, 2004), people in South Korea (Koo and Lee, 2005), Japanese adults (Itoh *et al.*, 2005), Swedish military recruits (Duty *et al.*, 2004; Duty *et al.*, 2005b), infertility clinic patients (men; Hauser *et al.*, 2007), plasma and platelet donors (Koch *et al.*, 2005a; Koch *et al.*, 2005b), and people in Germany (Koch *et al.*, 2003a; Becker *et al.*, 2004; Koch *et al.*, 2004b; Preuss *et al.*, 2005; Wittassek *et al.*, 2007b). Trends over time for these metabolites are unclear. CHAP calculations estimate that the median/high (95<sup>th</sup> percentile) intake from NHANES biomonitoring data for DEHP is 3.5/181 µg/kg-day.

#### 5.2.3.4.2 Hazard

A complete dataset suggests that exposure to DEHP *in utero* can induce adverse developmental changes to the male reproductive tract. Exposure to DEHP can also adversely affect many other organs such as the liver and thyroid.



#### **5.2.3.4.3 Risk**

Both animal and human data support maintaining the permanent ban on DEHP in children's toys and child care articles.

The margin of exposure for total DEHP exposure in infants (SFF; Sathyanarayana *et al.*, 2008a; 2008b) at the 95<sup>th</sup> percentile of exposure was 116–191. MOEs were similar in pregnant women, ranging from 17 to 28. The margins of exposure for total DEHP exposure are insufficient considering the severity of the effects described above. Furthermore, DEHP dominates the hazard index for cumulative exposure to antiandrogenic phthalates. Based on NHANES data (NHANES 2005–2006; CDC, 2012b), the CHAP estimates that about 10% of pregnant women exceed a cumulative hazard index of 1.0, which is largely due to DEHP exposure.

#### **5.2.3.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends no further action regarding toys and child care articles at this time because DEHP is permanently banned in children's toys and child care articles at levels greater than 0.1%.

However, CHAP recommends that U.S. agencies responsible for dealing with DEHP exposures from all sources conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.2.3.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DEHP?**

No, because DEHP is already permanently banned in children's toys and child care articles.

### **5.3 Recommendations on Interim Banned Phthalates**

#### **5.3.1 Di-*n*-octyl Phthalate (DNOP) (117-84-0)**

##### **5.3.1.1 Adverse Effects**

###### **5.3.1.1.1 Animal**

###### **5.3.1.1.1.1 Systemic**

- Hardin *et al.* (1987) reported on a developmental screening toxicity test in female CD-1 mice in which DNOP (0, 9780 mg/kg-day) was administered via gavage during GD 6–13. DNOP administration did not change the number of maternal deaths or body weight.
- Heindel *et al.* (1989) (and Morrissey *et al.*, 1989) conducted a one-generation continuous breeding reproductive toxicity test in CD-1 Swiss mice in which DNOP (0, 1800, 3600, and 7500 mg/kg-day) was administered in the diet for 7 days prior and 26 weeks following cohabitation. Treatment with DNOP did not affect body

- weight gain or food consumption, but did significantly increase liver weight (F1, LOAEL = 750 mg/kg-day) and kidney weight (female F1, LOAEL = 750 mg/kg-day).
- (Hinton *et al.*, 1986) reported on short-term toxicity testing in Wistar rats in which DNOP (0, 2%) was administered in the feed for 3, 10, or 21 days. Treatment with DNOP caused hepatomegaly, a changed liver texture and appearance, hepatic fat accumulation, peroxisome proliferation, smooth endoplasmic reticulum proliferation, a decrease in serum thyroxine (T<sub>4</sub>) and increased triiodothyronine (T<sub>3</sub>).
  - Khanna *et al.* (1990) reported on the subchronic kidney toxicity in albino rats (10 male/group) in which DNOP (0, 100, 300, 600 mg/kg) was administered via intraperitoneal injection once daily for 5 days a week for 90 days. Dose-dependent changes in kidney histopathology were noted and suggested that irreversible nephrotoxicity was occurring.
  - Lake *et al.* (1984) reported on intermediate-term toxicity in male SD rats (6/group) in which DNOP (0, 1000, 2000 mg/kg-day) was administered via gavage daily for 14 days. Exposure to DNOP significantly increased the relative liver weight and altered liver enzyme activities.
  - Lake *et al.* (1986) reported on the intermediate-term liver toxicity in male SD rats in which DNOP (0, 1000 mg/kg-day) was administered daily via gavage for 14 days. As with Lake's previous study, DNOP exposure increased relative liver weight and altered liver enzyme functions.
  - Mann *et al.* (1985) reported on short- and intermediate-term liver toxicity in male Wistar rats in which DNOP (0, 2%; ~2000 mg/kg-day) was administered via the diet for 3, 10, or 21 days. DNOP increased the relative liver weight, changed the texture and appearance of the liver, changed the liver ultrastructurally and enzymatically, and marginally increased the peroxisome number.
  - Poon *et al.* (1997) conducted a subchronic toxicity study in SD rats (10/sex/group) in which DNOP (0, 0.4/0.4, 3.5/4.1, 36.8/40.8, 350.1/402.9 mg/kg-day; M/F) was administered via the diet for 13 weeks. DNOP exposure did not alter body weight, food consumption, liver weight, kidney weight, or the number or distribution of peroxisomes but did alter liver enzyme activity and liver ultrastructure. Reduced thyroid follicle size (F, 40.8 mg/kg-day) and decreased colloid density (M/F; 3.5/40.8 mg/kg-day) were observed in dosed groups.
  - Smith *et al.* (2000) reported on the intermediate-term toxicity in male Fischer 344 rats and B6C3F1 mice in which DNOP (0, 1000, 10,000 ppm [rats], and 0, 500, 10,000 ppm [mice]) was administered via the diet for two- and four-weeks. In rats, DNOP exposure increased the relative liver weight, peroxisomal activity, and periportal hepatocellular replicative activity but didn't change gap junctional intercellular communication. In mice, only peroxisomal activity was altered following exposure to DNOP.
  - Saillenfait *et al.* (2011) conducted a prenatal developmental toxicity test in SD rats in which DNOP (0, 250, 500, and 1000 mg/kg-day) was administered via gavage once a day on GD 6–20. DNOP exposure did not affect maternal feed consumption, body weight, body weight change, or liver histopathology but did significantly increase the liver weight and liver weight normalized to body weight on GD 21 (LOAEL = 1000

mg/kg-day). DNOP also significantly increased various liver biochemical markers such as ASAT, ALAT, and cholesterol.

#### **5.3.1.1.1.2 Reproductive**

- Heindel *et al.* (1989) (and Morrissey *et al.*, 1989) conducted a one-generation continuous breeding reproductive toxicity test in CD-1 Swiss mice in which DNOP (0, 1800, 3600, and 7500 mg/kg-day) was administered in the diet for 7 days prior and 26 weeks following cohabitation. Reproductive parameters were not affected by dosing with DNOP.
- Poon *et al.* (1997) conducted a subchronic toxicity study in SD rats in which DNOP (0, 0.4/0.4, 3.5/4.1, 36.8/40.8, 350.1/402.9 mg/kg-day; M/F) was administered in the diet for 13 weeks. No reproductive parameters were affected by dosing with DNOP.
- Foster *et al.* (1980) conducted a short-term toxicity test in male SD rats in which DNOP (0, 2800 mg/kg-day) was administered via gavage once a day for four days. Changes in testis weight or pathology were not observed.

#### **5.3.1.1.1.3 Developmental**

- The NTP-CERHR reviewed the reproductive and developmental toxicity of DNOP in five animal studies (Singh *et al.*, 1972; Gulati *et al.*, 1985; Hardin *et al.*, 1987; Heindel *et al.*, 1989; Hellwig *et al.*, 1997) and concluded that “available studies do suggest a developmental toxicity response with gavage or intraperitoneal (IP) administration with very high doses.”
- Saillenfait *et al.* (2011) conducted a prenatal developmental toxicity test in SD rats in which DNOP (0, 250, 500, and 1000 mg/kg-day) was administered via gavage once a day on GD 6–20. A dose-related increase in the incidence of supernumerary ribs was noted at nonmaternally toxic doses. The authors calculated BMD<sub>05</sub> and BMDL<sub>05</sub> values for supernumerary ribs (58/19 mg/kg-day, respectively). No adverse effects on reproductive tissue were observed.

#### **5.3.1.1.2 Human**

- No published human studies.

### **5.3.1.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.3.1.3 Weight of Evidence**

#### **5.3.1.3.1 Experimental Design**

In the Heindel and Poon studies, the number of animals dosed was insufficient to have high confidence in the data (n=20 breeding pairs per dose group and n=13 animals per dose group, respectively). Further, the dosing schedule for these studies (and for the Foster *et al.*, 1980 study) did not cover the standard length of time needed to determine male reproductive effects or reproductive effects resulting from developmental issues (10 weeks of dosing pre-mating). In all but one study of the five reviewed by NTP, exposure occurred before GD 15 (rat) and GD 13 (mouse). The NTP panel noted that limited study

designs “do not provide a basis for comparing consistency of response in two species, nor do they allow meaningful assessment of dose-response relationships and determination of either LOAELs or NOAELs with any degree of certainty.” The recently published Saillenfait study was of appropriate design to have confidence in observed toxicological effects. The Khanna study utilized an exposure route (IP) that was not relevant to common human exposure scenarios.

#### **5.3.1.3.2 Replication**

No published full reproduction studies exist. Further replication is needed for the one developmental study (Saillenfait). DNOP-induced systemic adverse effects were noted in animal test subject's thyroid, immune system, kidney, and liver in two, three, three, and eight published studies, respectively. Sufficient data were available from the studies reporting DNOP-induced liver toxicity to calculate a subchronic oral ADI of 0.37 mg/kg-day (Carlson, 2010), based on a NOAEL of 37 mg/kg-d (Poon *et al.*, 1997) and an overall uncertainty factor of 100.

### **5.3.1.4 Risk Assessment Considerations**

#### **5.3.1.4.1 Exposure**

Undetermined frequency and duration of exposures but metabolites of DNOP (mono-*n*-octyl phthalate [MNOP], mono(3-carboxypropyl [MCPP] have been detected in human urine samples in the United States (NHANES 1999–2000, 2001–2002, 2003–2004; CDC, 2012b), in Washington, D.C., (Hoppin *et al.*, 2002), and in Germany (Koch *et al.*, 2003a). However, based on HBM data, exposure seems to be negligible with 99% of the samples having MNOP concentrations below the limit of quantitation (LOQ). Trends over time for these metabolites are unclear. Based upon aggregate exposure estimates for women of reproductive age and children, most DNOP exposure is from food. For infants and toddlers, child care articles are the greatest potential source of exposure. Modeled DNOP exposures for infants and toddlers range from 4.5 µg/kg-d (average, infants) to 16 µg/kg-d (upper bound, toddlers) (Table 2.11).

#### **5.3.1.4.2 Hazard**

On the one hand, a limited developmental toxicity dataset did not identify DNOP as an antiandrogen; however, with the exception of the Saillenfait study, the developmental toxicity studies making up this dataset all have major limitations. Although DNOP was not antiandrogenic in the Saillenfait study, exposure to this phthalate was associated with developmental toxicity, *i.e.*, supernumerary ribs, although developmental toxicologists are divided over whether this effect is a malformation or a minor variation. On the other hand, a systemic toxicity dataset, although incomplete, suggests that exposure to DNOP can induce adverse effects in the liver, thyroid, immune system, and kidney.

#### **5.3.1.4.3 Risk**

Based on a POD of 37 mg/kg-d (0.037 µg/kg-d) (see above), the CHAP estimates that MOE for infants and toddlers range from 2,300 to 8,200.

#### **5.3.1.5 Recommendation to CPSC regarding children's toys and child care articles**

DNOP does not appear to possess antiandrogenic potential; nonetheless, the CHAP is aware that DNOP is a potential developmental toxicant, causing supernumerary ribs, and a potential systemic toxicant, causing adverse effects on the liver, thyroid, immune system, and kidney. However, because the MOE in humans are likely to be very high, the CHAP does not find compelling data to justify maintaining the current interim ban on the use of DNOP in children's toys and child care articles. Therefore, the CHAP recommends that the current ban on DNOP be lifted but that U.S. agencies responsible for dealing with DNOP exposures from food and child care products conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.3.1.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DNOP?**

No. DNOP use would be allowed in children's toys and child care articles.

### **5.3.2 Diisononyl Phthalate (DINP) (28553-12-0 and 68515-48-0)**

#### **5.3.2.1 Adverse Effects**

##### **5.3.2.1.1 Animal**

##### **5.3.2.1.1.1 Systemic**

- DINP was tested in two chronic studies in Fischer-344 rats (Lington *et al.*, 1997; Moore, 1998b) and one in B6C3F1 mice (Moore, 1998a). Systemic effects in the liver and kidney were reported.
- Kidney effects included increased kidney weight (rats and female mice), increased urine volume, increased mineralization (male rat), and progressive nephropathy (female mice). The NOAEL for kidney effects was 88 mg/kg-d (male rat) (Moore, 1998b).
- Liver effects included hepatomegaly, hepatocellular enlargement, peroxisome proliferation, focal necrosis, and spongiosis hepatis (microcystic degeneration) (reviewed in CPSC, 2001; Babich and Osterhout, 2010). Increased levels of liver-specific enzymes were also reported. The NOAEL for liver effects was 15 mg/kg-d (Lington *et al.*, 1997).
- Peroxisome proliferation, hepatocellular adenomas, and hepatocellular and carcinomas were found in the livers of both mice and rats. For DINP the CHAP attributed the hepatocellular tumors to peroxisome proliferation, which is not expected to occur in humans (CPSC, 2001) (see also, Klaunig *et al.*, 2003).
- A low incidence of renal tubular cell carcinomas was observed in male rats only (Moore, 1998b). These tumors were shown to be the result of the accumulation of  $\alpha$ 2u-globulin (Caldwell *et al.*, 1999), a mode of action that is unique to the male rat.
- The incidence of mononuclear cell leukemia was elevated in Fischer-344 rats (Lington *et al.*, 1997; Moore, 1998b). This lesion is commonly reported in Fischer rats. The CHAP

on DINP concluded that for DINP mononuclear cell leukemia is of uncertain relevance to humans (CPSC, 2001).

- The NOAEL for noncancer effects was 15 mg/kg-d. The CHAP on DINP (CPSC, 2001) derived an ADI of 0.12 mg/kg-d, based on a benchmark dose analysis of the incidence of spongiosis hepatitis in the Lington *et al.*, (1997) study.

#### **5.3.2.1.1.2 Reproductive**

- The NTP-CERHR (2003c) panel reviewed developmental and reproductive effects of DINP. The panel's conclusions were that DINP could probably affect human development or reproduction but that current exposures were probably not high enough to cause concern. The NTP stated that there was minimal concern for DINP causing adverse effects to human reproduction or fetal development.
- Since the 2003 NTP-CERHR report, one reproductive study in Japanese medaka fish showed no effects on survival, fertility, or other factors associated with reproduction (Patyna *et al.*, 2006).

#### **5.3.2.1.1.3 Developmental**

- The 2003 summary of the NTP-CERHR report on the reproductive and developmental toxicity of DINP (NTP, 2003c) concludes that, as of its report, there were “no human data located for Expert Panel review.” The panel did review two rat studies evaluating prenatal developmental toxicity of DINP administered by gavage on GD 6–15 (Hellwig *et al.*, 1997; Waterman *et al.*, 1999), developmental toxicity of DINP in a two-generation study in rats (Waterman *et al.*, 2000), and prenatal developmental toxicity of isononyl alcohol, a primary metabolite of DINP (Hellwig and Jackh, 1997). The two rat prenatal studies showed effects on the developing skeletal system and kidney following oral exposures to DINP from GD 6–15, while in the two-generation study in rats, effects on pup growth were noted. The prenatal developmental toxicity study with isononyl alcohol provided evidence that this primary metabolite of DINP “is a developmental and maternal toxicant at high (~1000 mg/kg) oral doses in rats.” From these studies, the panel concluded that the toxicology database “is sufficient to determine that oral maternal exposure to DINP can result in developmental toxicity to the conceptus.” The panel also noted that “some endpoints of reproductive development that have been shown to be sensitive with other phthalates were not assessed.” Therefore, the panel recommended that “a perinatal developmental study in orally exposed rats that addresses landmarks of sexual maturation such as nipple retention, anogenital distance, age at testes descent, age at prepuce separation, and structure of the developing reproductive system in pubertal or adult animals exposed through development” should be considered.

The perinatal studies recommended by the NTP-CERHR panel have now been performed. Five such studies have shown that DINP exposure in rats during the perinatal period is associated with increased incidence of male pups with areolae and other malformations of androgen-dependent organs and testes (Gray *et al.*, 2000), reduced testis weights before puberty (Masutomi *et al.*, 2003), reduced AGD (Lee *et al.*, 2006), increased incidence of multinucleated gonocytes, increased nipple retention, decreased

sperm motility, decreased male AGD, and decreased testicular testosterone (Boberg *et al.*, 2011), and reduced fetal testicular testosterone production and decreased StAR and Cyp11a mRNA levels (Adamsson *et al.*, 2009; Hannas *et al.*, 2011b). Although the Hannas *et al.* (2011) study was not designed to determine a NOAEL, a crude extrapolation of their dose response data (Hannas *et al.*, 2011b, Figure 6) suggests that the NOAEL is approximately 100 mg/kg-day for reduced fetal testicular testosterone production. This NOAEL would be higher by a factor of 20 compared to the NOAEL of DEHP (for gross reproductive tract malformations (RTMs) associated with the phthalate syndrome of 5 mg/kg-d; Blystone *et al.*, 2010). In the same paper, however, Hannas *et al.* (2011), based upon their dose-response assessment of fetal testosterone production, found that DINP reduced fetal testicular testosterone production (T PROD) with an only 2.3-fold lesser potency than DEHP. This would lead to a NOAEL of 11.5 mg/kg-d for DINP extrapolated from the NOAEL of DEHP. In more recent studies, Clewell *et al.* (2013a, b) reported a no observed effect level (NOEL) of ~50 mg/kg-day for DINP-induced multinucleated gonocytes (MNGs) and a NOEL of ~250 mg/kg-day for reduced AGD. However, even in the highest dose group (750 mg/kg-d) Clewell *et al.* (2013) reported no effect on fetal testicular T production, contrary to studies by Boberg *et al.* (2011), Hannas *et al.* (2011), and Hannas *et al.* (2012).

#### **5.3.2.1.2 Human**

No epidemiologic studies measured metabolites of DINP in relation to male reproductive health or neurodevelopment endpoints.

### **5.3.2.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.3.2.3 Weight of Evidence**

#### **5.3.2.3.1 Experimental Design**

Several of the studies were judged to be inadequate for ascertaining a NOAEL for DINP. The Gray *et al.* (2000) study used only one dose, and the Masutomi *et al.* (2003), Borch *et al.* (2004), and Adamsson *et al.* (2009), studies used relatively small numbers of animals per dose group. Further, the Lee *et al.* (2006) study used the individual fetus rather than the litter as the unit of measurement, thus calling into question their conclusions. In contrast, the Boberg *et al.* (2011) study used multiple doses (four plus control), exposure occurred during the developmentally sensitive period (GD 7–postnatal day [PND] 17), and used a relatively high number of dams per dose (16). On the basis of increased nipple retention at 600 mg/kg-d, the authors report a NOAEL of 300 mg/kg-d. However, the same authors also observed a dose-dependent reduction in testicular testosterone production that was still evident in the low-dose group (300 mg/kg-d), as shown in figure 2A of Boberg *et al.* (2011). Furthermore, several of the other studies provide additional data that the CHAP considered relevant. The Hannas *et al.* (2011b) study found a LOAEL of 500 mg/kg-d, based on decreased fetal testosterone production, suggesting that the NOAEL for this endpoint is clearly below this level. Extrapolation of their dose response data (Figure 6) suggests that the NOAEL is approximately 100 mg/kg-day. In addition, data from Clewell *et al.* (2013b) show that the NOEL for DINP-

induced MNGs is approximately 50 mg/kg-day. Taken together, the data from Boberg *et al.* (2011), Hannas *et al.* (2011b), and Clewell *et al.* (2013a; 2013b) indicate that the developmental NOAEL, based upon antiandrogenic endpoints (nipple retention, fetal testosterone production, and MNGs), is between 50 and 300 mg/kg-day. Taking a conservative approach, the CHAP assigns the NOAEL for DINP at 50 mg/kg-day. However, the CHAP also wants to point out that a simple extrapolation based upon relative potencies (as described by Hannas *et al.*, 2011b) with 2.3-fold lesser potency of DINP than DEHP (in terms of fetal testicular T reduction) would lead to a NOAEL of 11.5mg/kg-d for DINP. This scenario is reflected in case 2 of the HI approach.

#### **5.3.2.3.2 Replication**

Although the developmental toxicity literature for DINP is not data rich, a number of animal studies demonstrating adverse reproductive and developmental endpoints (antiandrogenic) have been reported. NOAELs for DINP-induced antiandrogenic toxicities range from 50 mg/kg-day (MNGs) to 300 mg/kg-day (nipple retention). In addition, the CHAP is aware that DINP is a systemic toxicant, *e.g.*, inducing significant liver toxicity. CPSC has calculated an ADI of 0.12 mg/kg-day using the lowest NOAEL (12 mg/kg-day) for DINP-induced liver toxicity (Babich and Osterhout, 2010). The NOAEL for liver toxicity for DINP (12 mg/kg-day), as for DIDP, is lower than the lowest NOAEL for antiandrogenic toxicity (50 mg/kg-day for MNGs).

### **5.3.2.4 Risk Assessment Considerations**

#### **5.3.2.4.1 Exposure**

DINP has been used in children's toys and child care articles in the past. The CHAP estimates that infants' exposure to DINP from mouthing soft plastic articles may range from 2 (mean) to 9 (upper bound) µg/kg-d. The frequency and duration of exposures have not been determined; however, metabolites of DINP (cx-MINP) have been detected in human urine samples in the U.S. general population (NHANES 2005–2006, 2007–2008; CDC, 2012b). Although only two survey durations have been monitored, MCOP levels have slightly increased in the last survey period for the total (geometric mean; 5.39 to 6.78 µg/L) all age, gender, and race classes. Another urinary metabolite of DINP, mono(isononyl) phthalate (MINP), has also been detected infrequently in human urine samples in the U.S. general population (NHANES 1999–2000, 2001–2002, 2003–2004, 2005–2006, 2007–2008; CDC, 2012b). MINP was not detected in most samples. CHAP calculations estimate that the median and high intake (95<sup>th</sup> percentile) from NHANES biomonitoring data for DINP is 1.0 and 11.1 µg/kg-day, respectively.

#### **5.3.2.4.2 Hazard**

A relatively complete dataset suggests that exposure to DINP can cause reproductive or (nonreproductive) developmental effects, although it is less potent than other active phthalates, for example, DEHP.



#### **5.3.2.4.3 Risk**

##### **5.3.2.4.3.1 Male Developmental Effects**

In infants in the SFF study, the MOE for total exposure ranged from 640 to 42,000 using 95<sup>th</sup> percentile estimates of exposure. For pregnant women, the MOE for total DINP exposure ranged from 1000 to 68,000. Typically, MOEs exceeding 100–1000 are considered adequate for public health; however, the cumulative risk of DINP with other antiandrogens should also be considered.

##### **5.3.2.4.3.2 Systemic Effects (Liver)**

In infants in the SFF study, the estimated total DINP exposure ranged from 3.6 to 18.0 µg/kg-d (median and 95<sup>th</sup> percentile) (see Table 2.7). For women in NHANES (2005–2006), the estimated total exposure ranged from 1.0 to 9.4 µg/kg-d (Table 2.7). Using the NOAEL of 15 mg/kg-d for systemic toxicity, the MOE for infants ranged from 830 to 4,200. The MOE for women ranged from 1600 to 15,000. Typically, MOEs exceeding 100–1000 are considered adequate for public health.

#### **5.3.2.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends that the interim ban on the use of DINP in children's toys and child care articles at levels greater than 0.1% be made permanent. This recommendation is made because DINP does induce antiandrogenic effects in animals, although at levels below that for other active phthalates, and therefore can contribute to the cumulative risk from other antiandrogenic phthalates.

Moreover, the CHAP recommends that U.S. agencies responsible for dealing with DINP exposures from food and other products conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.3.2.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DINP?**

No, because DINP is currently subject to an interim ban on use in children's toys and child care articles at levels greater than 0.1%.

### 5.3.3 Diisodecyl Phthalate (DIDP) (26761-40-0 and 68515-49-1)

#### 5.3.3.1 Adverse Effects

##### 5.3.3.1.1 Animal

##### 5.3.3.1.1.1 Systemic

- British Industrial Biological Research Association (BIBRA) reported on a 21-day feeding study in which Fischer-344 rats (5/sex/dose) were fed 300, 1000, or 2000 mg/kg-day DIDP. The NOAEL for both sexes was 300 mg/kg-day based on increased absolute and relative liver weights, increased cyanide-insensitive palmitoyl-CoA oxidation, increases in the number and size of hepatocyte peroxisomes, a change in serum triglycerides and cholesterol, a change in hepatocyte cytoplasm staining properties, and increased relative kidney weights.
- An abstract by Lake *et al.* (1991) described a 28-day feeding study of male Fischer-344 rats (5/sex/dose) that were fed approximately 25, 57, 116, 353, and 1287 mg DIDP/kg-day. A NOEL of 57 mg/kg-day is assumed, based on a statistically significant increase in relative liver weight of 116 mg/kg-day. Liver palmitoyl-CoA oxidation activity increased at 353 mg/kg-day, as did absolute liver weights. Testicular atrophy was not observed at any dose.
- BASF fed SD rats 0, 800, 1600, 3200, and 6400 ppm DIDP (approximately 55, 100, 200, and 400 mg/kg-day for males and 60, 120, 250, and 500 mg/kg-day for females) for 90 days. Relative liver weights were significantly increased in all males; absolute liver weights were significantly increased only in males at 6400 ppm. In females, relative and absolute liver weights were significantly increased at >1600 ppm and >3200 ppm, respectively. Relative kidney weights were significantly increased at all treated doses in males. In females, relative kidney weights were significantly increased in a non-dose-dependent manner at 1600 ppm and 3200 ppm, but not at 6400 ppm. There were no observed pathological abnormalities. Peroxisome proliferation was not studied. A NOAEL of 200 mg/kg-day for males and 120 mg/kg-day for females was determined by CERHR (NTP, 2003b).
- In a three-month feeding study, 20 Charles River CD rats were given 0, 0.05, 0.3, or 1% DIDP (approximately 28, 170, and 586 mg/kg-day for males and 35, 211, and 686 mg/kg-day for females) (Hazleton, 1968a). Absolute and relative liver weights were significantly increased in both sexes at 1% DIDP (586 and 686 mg/kg-day for males and females). Relative kidney weights were significantly increased in males at 0.3% and 1% DIDP (170 and 586 mg/kg-day). There were no effects on food consumption, body weight, or clinical chemistry. There were no histological changes in liver, kidney or testis. Peroxisome proliferation was not studied. A NOAEL was reported as 170 and 211 mg/kg-day for males and females, respectively. The LOAEL was 586 and 686 mg/kg-day for males and females, respectively, for increased liver weight.
- In a 13-week diet study, beagle dogs (3/sex/group) were given approximately 0, 15, 75, and 300 mg/kg-day DIDP (Hazleton, 1968b). A NOAEL of 15 mg/kg-day was reported, based on increased liver weights and histological changes. A LOAEL was reported at 75 mg/kg-day for increased liver weight and slight to moderate swelling and vacuolation of hepatocytes.

- In a two-year oral toxicity/carcinogenicity study of DIDP, Fischer-344 rats were exposed to 0, 400, 2000, or 8000 ppm DIDP (0.85, 4.13, 17.37 mg/kg-day for males and 0.53, 3.03, 13.36 mg/kg-day for females). At the high dose, there was a significant decrease in the overall survival and body weight, with a significant increase in relative liver and kidney weights in males and females. No treatment-related neoplastic lesions were observed in internal organs, including the liver of either sex (Cho *et al.*, 2008).
- Cho *et al.* (2008) also fed 50 rats/dose 0, 400, 2000, or 8000 ppm DIDP or 12,000 ppm DEHP, as a positive control, and sacrificed them after 12 or 32 weeks. After 12 weeks the levels of catalase in the 8000 ppm DIDP group were increased compared to the controls, yet after 32 weeks there were no differences in the catalase levels or activity. In the positive DEHP-treated control animals, catalase levels and activity were increased at both 12 and 32 weeks.
- An inhalation study exposed SD rats to 505 mg/m<sup>3</sup> DIDP vapor for two weeks, six hours per day for five days per week. No systemic effects were reported (GMRL, 1981).

#### **5.3.3.1.1.2 Reproductive**

- The systemic studies summarized above (Hazleton, 1968a; Hazleton, 1968b; BIBRA, 1986; Lake *et al.*, 1991) reported no changes in the histopathology of testes. However, relative testis weights were significantly increased at 2000 mg/kg-day DIDP in a 21-day feeding study in Fischer 344 rats (BIBRA, 1986).
- In a Hershberger assay, castrated prepubertal SD Crl:CD rats (6/group) were given 0, 20, 100, or 500 mg/kg-day DIDP by gavage in combination with 0.4 mg/kg-day testosterone. Treatment with 500 mg/kg-day DIDP led to a significant decrease in ventral prostate and seminal vesicle weight compared to the testosterone-positive control, suggesting that DIDP does possess antiandrogenic activity. The NOAEL for this study was set at 100 mg/kg-day (Lee and Koo, 2007).
- One single-generation and two multigeneration animal studies were completed by Exxon Biomedical Sciences (Exxon, 1997; ExxonMobil, 2000). In the one-generation study, rats received dietary levels of 0, 0.25, 0.5, 0.75, and 1% DIDP. In the first multigenerational study, Crl:CD BR-VAF/Plus (SD) rats (30/sex/dose) were given 0, 0.2, 0.4, or 0.8% DIDP in their diet for 10 weeks prior to and during mating. Females continued to receive DIDP throughout gestation and lactation. The second multigeneration study was identical to the first except that rats received 0, 0.02, 0.06, 0.2, or 0.4% DIDP. DIDP did not appear to have effects on male reproductive tract development or function. There was a significant decrease in ovary weight (parental) and significant increases in F1 males' relative testis, epididymis, and seminal vesicle weights without accompanying changes in histology or reproductive function at 0.8%. There was a nonreproducible increase in the age at vaginal opening at doses of 0.4% and 0.8% in the first multigenerational study only. There was a non-dose-related decrease in the number of normal sperm of F0 treated males in the first study and an increase in the length of the estrous cycle in the F0 females treated with 0.8% DIDP; neither effect was observed in the F1 generation. There were no effects on mating, fertility, or gestational indices in any generation. The CERHR (NTP, 2003b)

considered the reproductive NOAEL to be the highest dose (0.8%), or 427–929 mg/kg bw/day for males and 508–927 mg/kg bw/day for females.

#### **5.3.3.1.1.3 Developmental**

- A one-generation comparative developmental screening test was performed on Wistar rats (10/dose). DIDP, at doses of 0, 40, 200, and 1000 mg/kg-day, was given by gavage 2 weeks prior to mating for a total of 29 days for males or until PND 6 for females (Hellwig *et al.*, 1997). Fetuses were examined on GD 20 for weight, external, visceral, and skeletal malformations. Maternal toxicity was observed in the high-dose group with significantly reduced feed consumption, significantly increased absolute and relative liver weights, and vaginal hemorrhage in three dams. Maternal kidney weight was unaffected. There were increases in fetal variations per litter (rudimentary cervical and/or accessory 14th ribs) reaching statistical significance at the top two doses. The Expert Panel for the Center for the Evaluation of Risks to Human Reproduction (NTP, 2003b) set the developmental NOAEL at 40 mg/kg-day and the maternal NOAEL at 200 mg/kg-day.
- SD rats (25/dose) were given DIDP by gavage at 0, 100, 500, or 1000 mg/kg-day from GD 6 to 15 (Waterman *et al.*, 1999). Maternal toxicity was seen at 1000 mg/kg-day and included weight gain and decreased food consumption. Effects on fetal weight, mortality, mean numbers of corpora lutea, total implantation sites, post-implantation loss and viable fetuses of treated animals were comparable with controls. A dose-related increase in percent fetuses with a supernumerary (7th) cervical rib and incidence of rudimentary lumbar (14th) ribs was observed and was statistically significant at 500 mg/kg-day (on a per fetus basis) and 1000 mg/kg-day (on a per litter and fetus basis). Waterman *et al.* assigned a LOAEL for maternal and developmental toxicity at 1000 mg/kg bw-day and a NOAEL of 500 mg/kg bw/day, whereas the CERHR (NTP, 2003b), using a different approach to the linearized data model, selected a developmental NOAEL of 100 mg/kg bw/day, based on the significant incidence of cervical and accessory 14th ribs.
- Two multigenerational animal studies were completed by Exxon Biomedical Sciences and were published by Hushka *et al.* (2001). In the first study (study A) CrI:CD BR-VAF/Plus (SD) rats (30/sex/dose) were given 0, 0.2, 0.4, or 0.8% DIDP in their diet for 10 weeks prior to and during mating. Females continued to receive DIDP throughout gestation and lactation. There was significantly decreased F1 pup survival at birth and on PND 4 in the 0.8% treatment group. In the F2 generation, there was a significant decrease in pup survival in all treatment groups on PND 1 and 4. This decrease in pup survival was also observed on PND 7 and at weaning in the high-dose group. Postnatal body weight gain was reduced at the high dose in F1 and F2 pups. Liver weight (mean relative) was increased in F1 male pups at 0.8% and F1 female pups at 0.4 and 0.8%. Hepatic hypertrophy and eosinophilia were seen in F1 and F2 pups at 0.4 and 0.8%. A developmental NOAEL was not established due to decreased pup survival at all doses in the F2 offspring generation. The 0.2% dose (131–152 mg/kg-day and 162–319 mg/kg-day in F0 and F1 dams during gestation and lactation, respectively, as calculated by Hushka *et al.*, [2001]) was identified as the developmental LOAEL.

- The second multigenerational Exxon Biomedical Sciences study (2000) was identical to the first except that rats received 0, 0.02, 0.06, or 0.2 or 0.4% DIDP. In the F1 pups, there were no effects on survival, body weight gain, organ weight, anogenital distance, nipple retention, preputial separation, or vaginal opening. In the F2 pups there was significantly decreased pup survival on PND 1 and 4 at 0.2 and 0.4% DIDP. In the F2 generation, significantly decreased pup body weight was observed at 0.2% and 0.4% on PND 14 (females) and PND 35 (males). There were no differences in anogenital distance or nipple retention in the F2 pups. The age of preputial separation was increased by 1.2 days in the F2 pups at 0.4% DIDP, but the difference was not statistically significant. Overall, NOAEL and LOAEL for offspring survival effects were 0.06% and 0.2%, respectively (approximately 50 mg/kg-day and 165 mg/kg-day). A developmental NOAEL was set at 0.06% by the authors (38–44 mg/kg-day and 52–114 mg/kg-day during pregnancy and lactation, respectively).
- Cross-fostering and switched-diet studies were completed to determine whether postnatal developmental effects in pups were due to lactational transfer. Twenty CRI:CDBR VAF Plus rats per group were fed 0 or 0.8% DIDP for 10 weeks prior to mating through gestation and lactation. For the cross-fostered study, pups from 10 treated dams were switched with pups from 10 control dams. After weaning, the diet of the pups continued as per dam exposure. For the diet-switch portion of the study, pups from control dams were fed the DIDP diet after weaning, and pups from the treated dams were given the control diet after weaning. Results show that control pups switched to a 0.8% DIDP fed dam had significantly lower body weight on PND 14 and 21 due to lactational exposure. Pups exposed to DIDP *in utero* but nursed by a control dam did not show body weight changes. In the switched-diet study, pups exposed to DIDP *in utero* and while nursing recovered body weight after receiving control diets after weaning (Hushka *et al.*, 2001).

#### **5.3.3.1.2 Human**

- No published human studies.

#### **5.3.3.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans. However, it should be noted that peroxisome proliferation has questionable relevance to hazard characterization in humans.

#### **5.3.3.3 Weight of Evidence**

##### **5.3.3.3.1 Experimental Design**

Some of the systemic studies and all of the reproductive studies described were conducted according to GLP standards using relevant exposure routes. Although some of the studies had small dose groups (particularly the BASF 90-day dog study and the Hellwig developmental study), results were consistent and reproducible indicating a reasonable experimental design.

#### **5.3.3.3.2 Replication**

The liver was identified as a target organ based on results that were qualitatively consistent in rats and dogs. Furthermore, NOAELs were fairly consistent for all dietary rat studies (116–264 mg/kg bw/day). From these studies CPSC calculated an ADI of 0.15 mg/kg-day using the lowest NOAEL (15 mg/kg-day) for DIDP-induced liver effects (Hazleton, 1968b). CPSC also calculated an ADI of 0.13–0.17 mg/kg-day using the lowest dose (13.36–17.37 mg/kg-day) that led to significant DIDP-induced kidney toxicity (Cho *et al.*, 2008). Similarly, the developmental studies by Waterman *et al.* (1999) and Hellwig *et al.* (1997) yielded similar effects (increases in lumbar and cervical ribs) at similar dose levels. Using these studies, the CPSC calculated an ADI of 0.4 mg/kg-day using the lowest developmental NOAEL of 40 mg/kg-day for DIDP-induced supernumerary ribs. Three well-conducted rat studies suggest that oral DIDP exposure is not associated with reproductive toxicity at the levels tested.

### **5.3.3.4 Risk Assessment Considerations**

#### **5.3.3.4.1 Exposure**

DIDP is used in the PVC used to manufacture flooring, film, and coating products. Consumers may also be exposed via food, food packaging, clothing, and children's vinyl toys. Oxidative metabolites of DIDP found in urine samples indicate exposure to this compound is prevalent. CHAP calculations estimate that the median and 95<sup>th</sup> percentile intake from NHANES biomonitoring data (pregnant women) for DIDP are 1.5 and 4.6 µg/kg-day, respectively, and that the median and 95<sup>th</sup> percentile intake from SFF biomonitoring data are 1.9 and 14.2 (women) and 6.0 and 16.5 (infants) µg/kg-day, respectively. Based upon aggregate exposure estimates, the following intakes are estimated: women median: 3.2, 95<sup>th</sup> percentile: 12.2; infants median: 10; 95<sup>th</sup> percentile: 26.4 µg/kg-day.

#### **5.3.3.4.2 Hazard**

CPSC staff has previously concluded that DIDP may be considered a “probable toxicant” in humans by the oral route, based on sufficient evidence of systemic, reproductive, and developmental effects in animals.

#### **5.3.3.4.3 Risk**

Based on the lowest POD (15 mg/kg-day) the MOEs range from 2500 to 10,000 for median intakes and from 586 to 3300 for 95<sup>th</sup> percentile intakes.

### **5.3.3.5 Recommendation to CPSC regarding children's toys and child care articles**

DIDP does not appear to possess antiandrogenic potential; nonetheless, the CHAP is aware that DIDP is a potential developmental toxicant, causing supernumerary ribs, and a potential systemic toxicant, causing adverse effects on the liver and kidney. However, because DIDP is not considered in a cumulative risk with other antiandrogens, its MOE in humans is considered likely to be relatively high. The CHAP does not find compelling data to justify maintaining the current interim ban on the use of DIDP in children's toys

and child care articles. Therefore, the CHAP recommends that the current ban on DIDP be lifted but that U.S. agencies responsible for dealing with DIDP exposures from food and child care products conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.3.3.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DIDP?**

No. DIDP use would be allowed in children's toys and child care articles.

### **5.4 Recommendations on Phthalates Not Banned**

#### **5.4.1 Dimethyl Phthalate (DMP) (131-11-3)**

##### **5.4.1.1 Adverse Effects**

###### **5.4.1.1.1 Animal**

###### **5.4.1.1.1.1 Reproductive**

- No single- or multigeneration reproductive guideline studies have been published. No reproductive effects were observed in developmental studies.

###### **5.4.1.1.1.2 Developmental**

- Although an early study (Singh *et al.*, 1972) reported a dose-dependent increase in the incidence of skeletal defects after rats were dosed IP on GD 5, 10, and 15 with DMP (0, 400, 800, 1340 mg/kg-d), other studies (Plasterer *et al.*, 1985; Hardin *et al.*, 1987; NTP, 1989; Field *et al.*, 1993) observed no developmental or reproductive abnormalities after rats and mice were dosed by gavage during GD 6–15 and 6–13, respectively. Likewise, no developmental effects were observed after rats were dosed by gavage from GD 14 to PND 3 (Gray *et al.*, 2000).

###### **5.4.1.1.2 Human**

- Only a few epidemiologic studies measured urinary concentrations of the DMP metabolite monomethyl phthalate (MMP). In those that did, there were no associations of maternal urinary MMP concentrations with measures of male reproductive tract development (specifically, shortened AGD) (Swan *et al.*, 2005; Swan, 2008; Huang *et al.*, 2009; Suzuki *et al.*, 2012). No human studies reported associations of MMP with neurodevelopment. Three publications (Engel *et al.*, 2009; Engel *et al.*, 2010; Miodovnik *et al.*, 2011) measured MMP but reported associations of neurodevelopmental tests with a summary measure of low molecular weight phthalates (including MEP, MMP, MBP, and MIBP).

##### **5.4.1.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.4.1.3 Weight of Evidence**

#### **5.4.1.3.1 Experimental Design**

No published reproductive toxicity studies exist. One full developmental study in SD rats (Field, 1993) and one study in CD-1 mice (Plasterer *et al.*, 1985) had sufficient numbers of animals (29–30 on full study, n=8 on range finder, n=43–50, respectively) and adequate experimental design to support overall conclusions. The other identified studies have lower confidence because the dosing route in one study was not relevant to anticipated human exposures (Singh *et al.*, 1972; intraperitoneal), and the number of dosed litters was low (Gray *et al.*, 2000; 4 litters treated [21 male pups]).

#### **5.4.1.3.2 Replication**

No published full reproduction studies exist. “The available [developmental] data, particularly the studies of Field *et al.*, (1993) (GD 6–15 exposure) and Gray *et al.* (2000) (GD 14–PND 3 exposure), support the conclusion that DMP is not a developmental toxicant.” The CHAP concludes that the male reproductive effect has a NOAEL = 750 mg/kg-d (Appendix A, Table 7).

### **5.4.1.4 Risk Assessment Considerations**

#### **5.4.1.4.1 Exposure**

Although the frequency and duration of exposures and the quantification of exposures from children’s toys and personal care products have not been determined, DMP metabolites (MMP) have been detected in human urine samples in the United States (NHANES 2001–2002, 2003–2004; CDC, 2012b) and in 75% of the men attending an infertility clinic in Boston (Hauser *et al.*, 2007). Adjusted concentrations of urinary MMP were higher in children 6–11 when compared to juveniles 12–19, or to adults 20+ years old. In addition, women participants had higher urinary concentrations than men (NHANES 2005–2006; CDC, 2012b). CHAP calculations estimate that the median/high (95<sup>th</sup> percentile) intake from NHANES biomonitoring data for DMP is 0.05/0.55 µg/kg-day, respectively, in pregnant women.

#### **5.4.1.4.2 Hazard**

An incomplete dataset suggests that exposure to DMP does not induce reproductive or developmental effects in animals. DMP may induce other effects, however, such as changes in body weight, liver weight, and blood composition.

#### **5.4.1.4.3 Risk**

Risks to humans are currently indeterminate due to the lack of relevant data.

### **5.4.1.5 Recommendation to CPSC regarding children’s toys and child care articles**

The CHAP recommends no action at this time.



#### **5.4.1.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DMP?**

No. However, the CHAP concludes that DMP is not a reproductive or developmental toxicant in animals or humans.

### **5.4.2 Diethyl Phthalate (DEP) (84-66-2)**

#### **5.4.2.1 Adverse Effects**

##### **5.4.2.1.1 Animal**

##### **5.4.2.1.1.1 Reproductive**

- High-dose F1 sexually mature male mice had significantly decreased sperm concentration and increased absolute and relative prostate weights after exposure to DEP in a continuous breeding study (Lamb *et al.*, 1987).
- Fujii *et al.* (2005) conducted a two-generation reproductive toxicity study in SD rats in which DEP was administered 10 weeks prior to mating and continued through mating, gestation, and lactation. A substantial dose-related increase in the number of tailless sperm was reported in the F1 generation. In F1 parental females, the high-dose group had shortened gestation lengths. Increased age at pinna detachment and decreased age at incisor eruption was seen in high-dose F0 males, and an increase in the age of vaginal opening was noted in F1 female pups. A dose-related decrease in absolute and relative uterus weight was reported for F2 weanlings.
- Oishi and Hiraga (1980) conducted a short-term study in Wistar rats in which DEP (0 and 1000 mg/kg-d) was administered in the diet for seven days. Dietary exposure to DEP significantly decreased serum testosterone, serum dihydrotestosterone, and testicular testosterone.

##### **5.4.2.1.1.2 Developmental**

- Studies by Singh (1972) and Field *et al.* (1993) reported an increased incidence of skeletal defects (rudimentary ribs) in rats after exposure to DEP (as to DMP) by gavage or through the diet during early gestation (GD 5–15). Exposure to DEP by gavage during late gestation and early postnatal periods did not significantly affect any developmental parameters in male pups (Gray *et al.*, 2000).

##### **5.4.2.1.2 Human**

- Several epidemiologic studies measured urinary concentrations of the DEP metabolite MEP. Of those that did, some reported associations of maternal urinary MEP concentrations with measures of male reproductive tract development (specifically, shortened AGD) (Swan *et al.*, 2005; Swan, 2008), whereas other studies did not find associations with AGD (Huang *et al.*, 2009; Suzuki *et al.*, 2012). Several studies reported associations of poorer scores on neurodevelopment tests with MEP

(Miodovnik *et al.*, 2011) or with a summary measure of low molecular weight phthalates that was largely explained by MEP concentrations (Engel *et al.*, 2010).

#### **5.4.2.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

#### **5.4.2.3 Weight of Evidence**

##### **5.4.2.3.1 Experimental Design**

Two reproduction studies of sufficient design (Lamb *et al.*, 1987; Fujii *et al.*, 2005) are available to support conclusions. In Oishi and Hiraga (1980), decreases in testosterone are reported after dosing with phthalates that inhibit testosterone production. Increases in testicular testosterone, however, are reported following exposure to DBP, DIBP, and DEHP, phthalates that have been reported to decrease testicular testosterone in other studies. This finding decreases confidence in conclusions regarding DEP-induced testosterone inhibition.

One full developmental study in SD rats (Field *et al.*, 1993) has sufficient numbers of animals (n=31–32) and experimental design to support overall conclusions. The other identified studies have lower confidence because the dosing route in one study was not relevant to anticipated human exposures and had low n (Singh *et al.*, 1972; intraperitoneal; five rats per dose group) and the number of dosed litters was low (Gray *et al.*, 2000; three litters treated).

Epidemiological studies have drawn conclusions from small populations of exposed humans.

##### **5.4.2.3.2 Replication**

Reproductive toxicity results are sufficiently replicated in more than one study. Only one standard developmental study is available, and replicate epidemiology studies are not available. The available [developmental] data, particularly the studies of Field *et al.* (1993) (GD 6–15 exposure) and Gray *et al.* (2000) (GD 14–PND 3 exposure), support the conclusion that DEP is not a developmental toxicant for reproductive systems. Data from two studies, however, suggest that DEP may increase the incidence of extra rudimentary ribs.

#### **5.4.2.4 Risk Assessment Considerations**

##### **5.4.2.4.1 Exposure**

Some exposure results from contact with personal care products in infants and toddlers, but mostly with personal care products in older children. DEP metabolites (MEP) have been detected in human urine samples in the U.S. general population (NHANES 1999–2000, 2001–2002, 2003–2004), New York City pregnant women (Adibi *et al.*, 2003), women in Washington, D.C., (Hoppin *et al.*, 2004), German residents (Koch *et al.*, 2003a), Swedish military recruits (Duty *et al.*, 2004), and infertility clinic patients in Boston (men; Hauser *et al.*, 2007). A small study suggested that MEP levels in children

<2 years old were about twice as high as those in children 6–11 years old (Brock *et al.*, 2002). Further, MEP concentrations in the urine increased with age, were dependent on sex and race/ethnicity, and were lower in juveniles 6–11 years old when compared to other age classes (CDC, 2012a). CHAP calculations estimate that the median/high (95<sup>th</sup> percentile) intake from NHANES biomonitoring data for DEP is 3.4/75 µg/kg-day, respectively, in pregnant women.

#### **5.4.2.4.2 Hazard**

A relatively complete dataset suggests that exposure to DEP can induce reproductive or (nonreproductive) developmental effects in humans. DEP can also induce other target organ effects, such as changes in body weight and liver weight. Changes in AGD, AGI, and sperm parameters have been correlated to MEP concentration in humans. For the most part, these have not been confirmed in animal studies.

#### **5.4.2.4.3 Risk**

There are indications from epidemiological studies that DEP exposures are associated with reproductive and developmental outcomes. These observations take precedence over findings in animal experiments for which comparable effects could not be recapitulated and suggest that harmful effects in humans have occurred at current exposure levels. There is, therefore, an urgent need to implement measures that lead to reductions in exposures, particularly for pregnant women and women of childbearing age.

#### **5.4.2.5 Recommendation to CPSC regarding children's toys and child care articles**

Because DEP exposures from articles under the jurisdiction of CPSC are currently negligible, CHAP recommends no further action.

CHAP recommends that U.S. agencies responsible for dealing with DEP exposures from food, pharmaceuticals, and personal care products conduct the necessary risk assessments with a view to supporting risk management steps.

#### **5.4.2.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DEP?**

There would be no reduction in exposure for the articles under CPSC jurisdiction. However, exposures from personal care products, diet, some pharmaceuticals, food supplements, etc., can be substantial. There is a case for other competent authorities in the United States to conduct thorough risk assessments for DEP, especially for women of reproductive age.

### 5.4.3 Diisobutyl Phthalate (DIBP) (84-69-5)

#### 5.4.3.1 Adverse Effects

##### 5.4.3.1.1 Animal

###### 5.4.3.1.1.1 Reproductive

- One short-term toxicity study showed that DIBP exposure caused a significant decrease in testis weight, an increase in apoptotic spermatogenic cells, and disorganization or reduced vimentin filaments in Sertoli cells (Zhu *et al.*, 2010), and a subchronic toxicity study showed that DIBP exposure via the diet caused reduced absolute and relative testis weights (Hodge, 1954).

###### 5.4.3.1.1.2 Developmental

- Six studies in which rats were exposed to DIBP by gavage during late gestation showed that this phthalate reduced AGD in male pups, decreased testicular testosterone production, increased nipple retention, increased the incidence of male fetuses with undescended testes, increased the incidence of hypospadias, and reduced the expression of P450scc, insl3, genes related to steroidogenesis, and StAR protein (Saillenfait *et al.*, 2006; Borch *et al.*, 2006a; Boberg *et al.*, 2008; Howdeshell *et al.*, 2008; Saillenfait *et al.*, 2008; Hannas *et al.*, 2011b).

##### 5.4.3.1.2 Human

Several epidemiologic studies measured urinary concentrations of MIBP. Of those that did, there were associations of maternal urinary MIBP concentrations with measures of male reproductive tract development (specifically, shortened AGD) (Swan *et al.*, 2005; Swan, 2008). Several studies reported associations of MBP with poorer scores on neurodevelopment tests (Engel *et al.*, 2010; Swan *et al.*, 2010; Kim *et al.*, 2011; Miodovnik *et al.*, 2011; Whyatt *et al.*, 2011), whereas another did not (Engel *et al.*, 2009).

#### 5.4.3.2 Relevance to Humans

The reported animal studies are assumed to be relevant to humans.

#### 5.4.3.3 Weight of Evidence

##### 5.4.3.3.1 Experimental Design

The Boberg *et al.*, 2008 study results could not be used to determine a NOAEL because only one dose was used. The Howdeshell *et al.* (2008) study, which used multiple doses but small numbers of animals per dose group, was designed, as the authors point out “to determine the slope and median effective dose (ED<sub>50</sub>) values of the individual phthalates and a mixture of phthalates and not to detect NOAELs or low observable adverse effect levels.” The same is true for the Hannas *et al.* (2011b) study, which also used multiple doses but small numbers of animals per dose group. The two Saillenfait studies

(Saillenfait *et al.*, 2006; 2008) both included multiple doses and exposure during the appropriate stage of gestation, and employed relatively large numbers of animals per dose. Using the more conservative of the two NOAELs from the 2008 Saillenfait study, the CHAP assigns a NOAEL of 125 mg/kg-day for DIBP.

#### **5.4.3.3.2 Replication**

No published full reproductive toxicity studies exist. At least four developmental toxicity studies (three from different labs) confirmed that DIBP has antiandrogenic properties.

### **5.4.3.4 Risk Assessment Considerations**

#### **5.4.3.4.1 Exposure**

While DIBP has not been detected frequently in toys and child care articles in the United States (Chen, 2002; Dreyfus, 2010), DIBP has been detected in some toys during routine compliance testing. No quantifiable exposures to infants, toddlers, or children from toys or children's personal care products were located. DIBP has many of the same properties as DBP, so it can be used as a substitute. In general, DIBP is too volatile to be used in PVC but is a component in nail polish, personal care products, lubricants, printing inks, and many other products. DIBP metabolites (MIBP) have been detected in human urine samples in the U.S. general population (NHANES 2001–2002, 2003–2004, 2005–2006, 2007–2008; CDC, 2012b), and in Germany (Wittassek *et al.*, 2007a). Urinary MIBP levels have increased over the past four surveys in all age groups, genders, and races, and in total. Total levels (geometric means) during the last sample duration (2007–2008; 7.16 µg/L) are two- to three-fold higher than the earliest monitoring year (2001–2002; 2.71 µg/L) at all percentiles. CHAP calculations estimate that the median/high (95<sup>th</sup> percentile) intake from NHANES biomonitoring data for DIBP is 0.17/1.0 µg/kg-day, respectively, in pregnant women.

#### **5.4.3.4.2 Hazard**

Animal and human studies suggest that exposure to DIBP can cause reproductive and developmental effects.

#### **5.4.3.4.3 Risk**

The margins of exposure (95<sup>th</sup> percentile total DIBP exposure) for pregnant women in the NHANES study ranged from 5,000 to 125,000. For infants in the SFF study, the MOE (95<sup>th</sup> percentile total DIBP exposure) ranged from 3,600 to 89,000. The values are larger using the median exposure estimates. Typically, MOEs exceeding 100–1000 are considered adequate for public health; however, the cumulative risk of DBP with other antiandrogens should also be considered.

### **5.4.3.5 Recommendation to CPSC regarding children's toys and child care articles**

Current exposures to DIBP alone do not indicate a high level of concern. DIBP is not widely used in toys and child care articles. However, CPSC has recently detected DIBP in some children's toys. Furthermore, the toxicological profile of DIBP is very similar to

that of DBP, and DIBP exposure contributes to the cumulative risk from other antiandrogenic phthalates. The CHAP recommends that DIBP should be permanently banned from use in children's toys and child care articles at levels greater than 0.1 %.

#### **5.4.3.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DIBP?**

There would be little reduction in exposure. However, the recommendation, if implemented, would prevent future exposure from this chemical in such products.

### **5.4.4 Di-*n*-pentyl Phthalate (DPENP) (131-18-0)**

#### **5.4.4.1 Adverse Effects**

##### ***5.4.4.1.1 Animal***

##### ***5.4.4.1.1.1 Reproductive***

- The CHAP has not written a summary on reproductive toxicity studies using DPENP.
- Heindel *et al.* (1989) conducted a continuous breeding toxicity test in CD-1 mice in which DPENP (0.5, 1.25, 2.5%) was administered in the diet 7 days pre- and 98 days post-cohabitation. DPENP exposure reduced fertility in a dose-related fashion (LOAEL = 0.5%), decreased testis and epididymal weights, decreased epididymal sperm concentration, and increased the incidence of seminiferous tubule atrophy.

##### ***5.4.4.1.1.2 Developmental***

- Howdeshell *et al.* (2008) and Hannas *et al.* (2011a) conducted developmental toxicity studies in pregnant SD rats in which DPENP was administered via gavage on GDs 8 to 18. DPENP exposure reduced fetal testicular testosterone production, StAR, Cyp11a, and ins13 gene expression, and increased nipple retention.

##### ***5.4.4.1.2 Human***

No published human studies.

#### **5.4.4.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

#### **5.4.4.3 Weight of Evidence**

##### ***5.4.4.3.1 Experimental Design***

No published multigenerational reproductive toxicity studies exist. There are only two studies available describing the effects of DPENP on reproductive development in rats after *in utero* exposure during late gestation. Although these studies were not designed to determine NOAELs, the data presented on the effects of DPENP on fetal testosterone production and gene expression of target genes involved in male reproductive

development revealed that reduction in testosterone production was the most sensitive endpoint, with a LOAEL of 33 mg/kg-day (Hannas *et al.*, 2011a). Thus, on the basis of this study, the CHAP assigns the NOAEL for DPENP at 11 mg/kg-day.

#### **5.4.4.3.2 Replication**

No published multigenerational reproductive toxicity studies exist. Developmental studies reported similar toxicological endpoints using similar dosing strategies. Because both developmental studies have many of the same authors, verification of these results from an independent laboratory would be beneficial.

### **5.4.4.4 Risk Assessment Considerations**

#### **5.4.4.4.1 Exposure**

DPENP is currently not found in children's toys or child care articles, and it is not widely found in the environment. DPENP is primarily used as a plasticizer in nitrocellulose. The metabolite MHPP has been proposed as an appropriate biomarker for DPENP exposure and has been detected in human urine (Silva *et al.*, 2010).

#### **5.4.4.4.2 Hazard**

DPENP is clearly among the most potent phthalates regarding developmental effects.

#### **5.4.4.4.3 Risk**

DPENP is the most potent phthalate with respect to developmental toxicity. However, it is currently not found in children's toys or child care articles, and it is not widely found in the environment. Due to low exposure, current risk levels are believed to be low.

### **5.4.4.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends that DPENP should be permanently banned from use in children's toys and child care articles at levels greater than 0.1%. The toxicological profile of DPENP is very similar to that of the other antiandrogenic phthalates, and DPENP exposure contributes to the cumulative risk.

### **5.4.4.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DPENP?**

No. However, the recommendation, if implemented, would prevent future exposure from this chemical in such products.

## 5.4.5 Di-n-hexyl Phthalate (DHEXP) (84-75-3)

### 5.4.5.1 Adverse Effects

#### 5.4.5.1.1 Animal

##### 5.4.5.1.1.1 Reproductive

- A comparative study by Foster *et al.* (1980) indicated that di-n-hexyl phthalate (DHEXP) caused the second most severe testicular atrophy (NTP, 1997) in rats, after diamyl phthalate. Following exposure to 2400 mg/kg bw/day, relative testis weights were significantly lower than those of control rats, with atrophy of the seminiferous tubule and few spermatogonia and Sertoli cells. Leydig cell morphology was normal. An accompanying increase in urinary zinc was noted, likely the result of a concomitant depression in gonadal zinc metabolism (Foster *et al.*, 1980).
- The NTP-CERHR reviewed a study of DHEXP (NTP, 2003d) in which reproductive toxicity was assessed using the fertility assessment by continuous breeding protocol in Swiss CD-1 mice (NTP, 1997). The reproductive NOAEL of the one-generation study was determined to be less than the lowest dose of ~380 mg/kg-day, based on significant decreases in the mean number of litters per pair, the number of live pups/litter, and the proportion of pups born alive, all of which occurred in the absence of an effect on postpartum dam body weights. Results of a follow-up cross-over mating experiment using control and high-dose (~1670 mg/kg-day) mice indicated that the toxicity of DHEXP to fertility was strongly but not exclusively a result of paternal exposure; both sexes were effectively infertile at this level of DHEXP exposure. Necropsy of these mice revealed lower uterine weights but no treatment-related microscopic lesions in the ovaries, uterus, or vagina. Males had lower absolute testis weights, and lower adjusted epididymis and seminal vesicle weights, as well as reduced epididymal sperm concentration and motility. The percentage of abnormal sperm was equivalent to that of controls (NTP, 1997).
- The NTP-CERHR concluded that data are sufficient to indicate that DHEXP is a reproductive toxicant in both sexes of two rodent species following oral exposure.

##### 5.4.5.1.1.2 Developmental

- The NTP-CERHR (NTP, 2003d) panel reported on DHEXP and indicated that no human developmental toxicity data were located. They reported that only one animal developmental screening test was available. In this study, mice were administered DHEXP (0, 9900 mg/kg-d) via gavage from GD 6 through 13. Pregnant dams that were treated did not give birth to any live litters. The panel concluded that “the database is insufficient to fully characterize the potential hazard. However, the limited oral developmental toxicity data available (screening level assessment in mice) are sufficient to indicate that DHEXP is a developmental toxicant at high doses (9900 mg/kg-d). These data were inadequate for determining a NOAEL or LOAEL because only one dose was tested.” Since the NTP-CERHR report, one developmental toxicity study has reported that DHEXP exposure reduced the AGD in male pups in a dose-related fashion and increased the incidence of male fetuses with undescended testes (Saillenfait *et al.*, 2009a).



- Saillenfait *et al.* observed reproductive tract malformations, including hypospadias, underdeveloped testes, and undescended testes, in young adult male rats exposed prenatally to doses of 125 mg/kg-d DHEXP or greater (Saillenfait *et al.*, 2009b). They also observed seminiferous tubule degeneration at two doses. The NOAEL in the study was 50 mg/kg-d. They concluded that prenatal exposure to DHEXP led to permanent alterations of the male rat reproductive tract, with a profile similar to that of DEHP.

#### **5.4.5.1.2 Human**

- No published human studies.

### **5.4.5.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.4.5.3 Weight of Evidence**

#### **5.4.5.3.1 Experimental Design**

The NTP (1997) continuous breeding fertility study used an established protocol with high sample sizes (20 mice/sex/dose) and a concurrent 40 pairs of controls. A NOAEL was not established because effects on fertility were observed at the lowest dose. Furthermore, the mid- and low-dose groups were not evaluated at necropsy. Therefore, the NTP-CERHR panel concluded that their confidence in the LOAEL was only moderate-to-low, although the study itself was of high quality. Based on this study, a single-dose study of male reproductive toxicity in rats, and *in vitro* evidence in rats, the panel concluded that data were sufficient to determine that DHEXP acts as a reproductive toxicant in males and females of two rodent species.

Among developmental studies, the one by Saillenfait *et al.* (2009a) is fairly robust (*i.e.*, multiple doses, number of animals per dose group [20–25], and appropriate exposure time), but a NOAEL for AGD could not be determined because the lowest dose tested was the LOAEL. The other study cited by the NTP-CERHR had only one dose and a dosing strategy (GD 6–13) that may have missed the sensitive window for antiandrogenic impairment in mice. These reasons made it less useful than the Saillenfait study for determining the developmental effects of DHEXP.

#### **5.4.5.3.2 Replication**

Verification of multigenerational reproduction and developmental studies is needed.

#### **5.4.5.4 Risk Assessment Considerations**

##### **5.4.5.4.1 Exposure**

DHEXP is currently not found in children's toys or child care products, and it is not widely found in the environment. DHEXP is primarily used in the manufacture of PVC and screen printing inks. It is also used as a partial replacement for DEHP.

##### **5.4.5.4.2 Hazard**

DHEXP is a reproductive toxicant with a profile similar to DEHP. An incomplete dataset suggests that exposure to DHEXP can induce adverse effects in reproductive organs and is a developmental toxicant.

##### **5.4.5.4.3 Risk**

DHEXP is believed to induce developmental effects similar to those induced by other active phthalates. Due to low exposure, current risk levels are believed to be low.

#### **5.4.5.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends that DHEXP should be permanently banned from use in children's toys and child care articles at levels greater than 0.1%. The toxicological profile of DHEXP is very similar to that of the other antiandrogenic phthalates, and DHEXP exposure contributes to the cumulative risk.

#### **5.4.5.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DHEXP?**

No. However, the recommendation, if implemented, would prevent future exposure from this chemical in such products.

#### **5.4.6 Dicyclohexyl Phthalate (DCHP) (84-61-7)**

##### **5.4.6.1 Adverse Effects**

###### **5.4.6.1.1 Animal**

###### **5.4.6.1.1.1 Reproductive**

- In one reproductive toxicity study, DCHP exposure increased the atrophy of the seminiferous tubules, decreased the spermatid head count in F1 males, and increased the estrus cycle length in F0 females (Hoshino *et al.*, 2005).

###### **5.4.6.1.1.2 Developmental**

- Two studies in rats exposed to DCHP by gavage during late gestation showed that this phthalate prolonged preputial separation, reduced AGD, increased nipple retention, and increased hypospadias in male offspring (Saillenfait *et al.*, 2009a;

Yamasaki *et al.*, 2009). One study in rats exposed to DCHP in the diet showed that DCHP decreased the AGD and increased nipple retention in F1 males (Hoshino *et al.*, 2005).

#### **5.4.6.1.2 Human**

- No published human studies.

#### **5.4.6.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

#### **5.4.6.3 Weight of Evidence**

##### **5.4.6.3.1 Experimental Design**

Only one multigenerational reproduction study was determined. Two of the three studies available (Hoshino *et al.*, 2005; Yamasaki *et al.*, 2009) report DCHP-induced effects on male reproductive development (decreased anogenital distance and nipple retention in males), and the third study (Saillenfait *et al.*, 2009a) reported only the former. The Saillenfait study could not be used to determine a NOAEL because the lowest dose used in their study was a LOAEL. Of the two remaining studies, the two-generation study by Hoshino *et al.* (2005) reported adverse effects on male reproductive development at a calculated dose of 80–107 mg/kg-d; NOAEL of 16–21 mg/kg-d, whereas the Yamasaki *et al.* (2009) prenatal study reported adverse effects on male reproductive development at a dose of 500 mg/kg-d; NOAEL of 100 mg/kg-d. Using the more conservative of the two NOAELs, the CHAP assigned a NOAEL of 16 mg/kg-d for DCHP.

##### **5.4.6.3.2 Replication**

Only one multigenerational reproduction study was found, and therefore, conclusions as to the reproductive toxicity of DCHP need to be verified. Similar adverse developmental effects (*i.e.*, decreased male pup AGD) were reported in three independent studies.

#### **5.4.6.4 Risk Assessment Considerations**

##### **5.4.6.4.1 Exposure**

DCHP is currently not found in children's toys or child care articles, and it is not widely found in the environment. DCHP is FDA-approved for use in the manufacture of various articles associated with food handling and contact. Studies have reported migration of DCHP from the product (food wrap, printing ink, etc.) into food substances. DCHP is also the principal component in hot melt adhesives (>60%). MCHP, the metabolite of DCHP, has been found infrequently in the urine of U.S. residents (NHANES 1999–2000, 2001–2002, and 2003–2004; CDC, 2012b).

##### **5.4.6.4.2 Hazard**

An incomplete reproductive toxicity dataset suggests that exposure to DCHP can induce adverse effects in reproductive organs and is a developmental toxicant.

#### **5.4.6.4.3 Risk**

DCHP induces developmental effects similar to other active phthalates. Due to low exposure, current risk levels are believed to be low.

#### **5.4.6.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends that DCHP should be permanently banned from use in children's toys and child care articles at levels greater than 0.1%. The toxicological profile of DCHP is very similar to that of the other antiandrogenic phthalates, and DCHP exposure contributes to the cumulative risk.

#### **5.4.6.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DCHP?**

No. However, the recommendation, if implemented, would prevent future exposure from this chemical in such products.

### **5.4.7 Diisooctyl Phthalate (DIOP) (27554-26-3)**

#### **5.4.7.1 Adverse Effects**

##### **5.4.7.1.1 Animal**

##### **5.4.7.1.1.1 Reproductive**

- No published single or multigenerational reproduction studies.

##### **5.4.7.1.1.2 Developmental**

Grasso (1981) conducted a study in which DIOP (0, 4930, 9860 mg/kg-d) was injected intraperitoneally into female rats on GD 5, 10, and 15. Both treated groups had a higher incidence of soft tissue abnormalities. (Quantitative information for this study is not available.)

##### **5.4.7.1.2 Human**

- No epidemiologic studies measured metabolites of DIOP in relation to male reproductive health or neurodevelopmental endpoints.

#### **5.4.7.2 Relevance to Humans:**

The reported animal studies are assumed to be relevant to humans.

#### **5.4.7.3 Weight of Evidence**

##### **5.4.7.3.1 Experimental Design**

The one relevant study dosed animals via a route of exposure (IP) that is not relevant to exposures from consumer products under the U.S. CPSC's jurisdiction. Further,

quantitative information was not available for the summarized results, and it is unclear whether tissue abnormalities were reproductive in nature.

#### **5.4.7.3.2 Replication**

No published full reproduction or full developmental studies exist.

### **5.4.7.4 Risk Assessment Considerations**

#### **5.4.7.4.1 Exposure**

Frequency and duration of exposures are unknown. DIOP is primarily used in the manufacture of wire insulation. It is also approved for various food-associated products by the FDA and was found in a pacifier and bottle nipple (Chen, 1998). The primary metabolite of DIOP (MIOP) may have co-eluted with MEHP in many samples (including controls) in a small human study by Anderson *et al.* (2001).

#### **5.4.7.4.2 Hazard**

The hazard from DIOP is unknown; minimal data do not demonstrate antiandrogenic hazard. However, the isomeric structure of DIOP suggests that DIOP is within the range of the structure-activity characteristics associated with antiandrogenic activity.

#### **5.4.7.4.3 Risk**

Currently, there is a lack of exposure data for DIOP. Human exposure to DIOP appears to be negligible. Toxicity data are limited, but structure-activity relationships suggest that antiandrogenic effects are possible.

### **5.4.7.5 Recommendation to CPSC regarding children's toys and child care articles**

The CHAP recommends that DIOP be subject to an interim ban from use in children's toys and child care articles at levels greater than 0.1% until sufficient toxicity and exposure data are available to assess the potential risks.

### **5.4.7.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DIOP?**

Yes. The recommendation, if implemented, would prevent exposure from DIOP in such products.

## **5.4.8 Di(2-propylheptyl) Phthalate (DPHP) CAS 53306-54-0**

### **5.4.8.1 Adverse Effects**

#### **5.4.8.1.1 Animal**

##### **5.4.8.1.1.1 Reproductive**

- One industry-conducted subchronic study in rats showed that DPHP exposure in the diet was associated with up to a 25% reduction in sperm velocity indices (Union Carbide Corporation, 1997).

##### **5.4.8.1.1.2 Developmental**

- One industry-conducted developmental toxicity study in rats showed that DPHP exposure by gavage was associated with increased incidence of soft tissue variations (dilated renal pelvis) at the maternally toxic high dose (BASF, 2003). In a screening developmental toxicity study, exposure by gavage was not associated with any maternal or fetal effects (Fabjan *et al.*, 2006).

#### **5.4.8.1.2 Human**

- No published human studies.

### **5.4.8.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.4.8.3 Weight of Evidence**

#### **5.4.8.3.1 Experimental Design**

No published full reproduction studies exist. Results in the BASF developmental study were “preliminary,” even though the number of animals used per dose (n=25) was satisfactory.

#### **5.4.8.3.2 Replication**

No published full reproduction or full developmental studies exist.

### **5.4.8.4 Risk Assessment Considerations**

#### **5.4.8.4.1 Exposure**

The CHAP is not aware of any uses of DPHP in children’s toys or child care articles. DPHP was not detected in toys or child care articles tested by CPSC (Dreyfus, 2010). Currently, there is an undetermined frequency and duration of exposures; however, analytical methods cannot differentiate DPHP metabolites from DIDP metabolites because they are closely related. DPHP has substantially replaced other linear phthalates as a plasticizer in certain PVC applications. DPHP has increased its proportion in the phthalate production marketplace dramatically between 2005 and 2008 (CEH, 2009).

DPHP is approved for use in food packaging and handling. Many uses are at high concentration (30 to 60%).

#### **5.4.8.4.2 Hazard**

The hazard from DPHP is unknown; the? minimal data available do not demonstrate antiandrogenic hazard.

#### **5.4.8.4.3 Risk**

Currently, DPHP metabolites cannot be distinguished from the metabolites of DIDP. Production levels of DPHP have increased in recent years, suggesting that human exposure may also be increasing.

#### **5.4.8.5 Recommendation to CPSC regarding children's toys and child care articles**

Given the general lack of publically available information on DPHP, the CHAP is unable to recommend to CPSC any action regarding the potential use of DPHP in children's toys or child care articles at this time. However, the CHAP encourages the appropriate U.S. agencies to obtain the necessary toxicological and exposure data to assess any potential risk from DPHP.

#### **5.4.8.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DIDP?**

No. DIDP use would be allowed in children's toys and child care articles.

### **5.5 Recommendations on Phthalate Substitutes**

#### **5.5.1 2,2,4-trimethyl-1,3 pentanediol diisobutyrate (TPIB) (6846-50-0)**

##### **5.5.1.1 Adverse Effects**

##### **5.5.1.1.1 Animal**

##### **5.5.1.1.1.1 Systemic**

- Astill *et al.* (1972) reported on a 13-week repeat-dose study of TPIB performed by Eastman Kodak Company. Four beagle dogs/sex/group received dietary doses approximately equivalent to 22, 77, and 221 mg/kg bw-day for males and 26, 92, and 264 mg/kg-day for females 6 days per week for 13 weeks. Based on extensive gross, microscopic, and histopathological analyses, there was no mortality or evidence of neurological stimulation, depression, or reflex abnormality, and no effects on growth or food consumption at any dose. No changes were observed in the hematology, clinical chemistry, histopathology, or urine analyses. Relative organ weights were similar to control animals, except for the liver and pituitary gland in the two higher-dose groups, which were increased slightly compared to controls. However, elevated pituitary gland weights were still within the normal range, and the absence of microscopic pathological findings in pituitary and liver indicates that the observed

weight change was not adverse. The NOEL for this studied was 22–26 mg/kg-day, and the NOAEL was 221 and 264 mg/kg-day, the highest doses for male and female dogs, respectively.

- Astill *et al.* (1972) also reported on a feeding study in rats. Ten albino Holtzman rats/sex/dose, received TPIB for 103 days in the diet at doses approximately equivalent to 75.5 and 772 mg/kg-day for males and 83.5 and 858.5 mg/kg-day for females. Appropriate vehicle control groups were also run. Treated and control rats were statistically similar with respect to feed consumption, weight gain, and growth, and no histological differences were observed in the liver, esophagus, small or large intestine, trachea, lung, thyroid, parathyroid, spleen, brain, heart, kidney, bladder, adrenal, gonad, or bone. Relative liver weights in both sexes<sup>\*</sup> and absolute liver weights in male rats were slightly significantly higher in high-dose rats compared with controls; however, all weights were within the normal range of values. Study authors derived a NOAEL of 772–858.5 mg/kg bw/day, the highest dose.
- Krasavage *et al.* (1972) fed SD rats (10/sex/group) diets containing 0, 147.5, or 1475 mg/kg-day TPIB continuously for 52 days (experiment I), for 99 days (experiment II), or for 52 days followed by the control diet for 47 days or they received the control diet for 52 days followed by TPIB diet for 47 days (experiment III). There was no significant treatment-related effect on mean body weight gain, group feed consumption, hematological parameters, alkaline phosphatase activity, tissue histology, or absolute organ weight in any group compared to controls. Serum glutamic oxaloacetic transaminase levels were elevated in all high-dose animals relative to controls, except for females in experiment I. However, elevated levels were still within normal ranges. The relative liver weights of high-dose rats were significantly greater than controls in all three experiments, except for experiment III rats fed TPIB first and the control diet second. Differences in other relative organ weights were not determined to be treatment related. Likewise, the only consistent finding with respect to microsomal enzymes was an increase in activity at the high-dose level, but only when the animal was consuming TPIB at the time of sacrifice (*i.e.*, not in the experiment III rats that ate a control diet in the second part of the experiment). Temporary liver weight increase and microsomal enzyme activity induction are responses frequently associated with stress. In the absence of hepatic damage, study authors interpreted them as physiological adaptations.
- Krasavage *et al.* (1972) also injected (IP) groups of six male rats seven times per day with 25 or 100 mg/kg bw TPIB or 2,2,4-trimethyl-1,3-pentanediol (TMPD), the parent glycol and a metabolite of TPIB in rats. At the higher dose, TPIB and TMPD significantly increased P-NDase levels; BG-Tase levels were unaffected. A lower level of enzyme induction by TMPD suggests that TPIB, and not its metabolic product, is the active inducer.
- Eastman Chemical (2007) carried out the combined repeated dose and reproductive/developmental toxicity screening test (OECD TG 422) using SD rats (also summarized in JMHLW, 1993; OECD, 1995). Rats (12/sex/dose) were administered gavage doses of 0, 30, 150, or 750 mg/kg-day TPIB (purity: 99.7%)

---

<sup>\*</sup> Astill *et al.* reported that relative liver weights in females were significantly higher in the high-dose group. In Eastman Chemical's 2007 summary of this study, they note that the laboratory report did not report this result as significant and that the published manuscript contained this finding in error.



starting 14 days before mating. Males continued receiving the test substance for 30 days thereafter, and females, through day three of lactation. At the high-dose level, depressed body weight gain (males) and increased food consumption (females) were observed. Rats receiving 150 or 750 mg/kg-day had higher levels of creatinine and total bilirubin, and high-dose males had higher total protein content in the blood, suggesting liver and kidney effects. Indeed, relative liver weights were higher for male rats receiving the two higher doses of TPIB, with discoloration and hepatocellular swelling and decreased fatty change at the highest dose. Absolute and relative kidney weights were elevated in high-dose males and basophilic changes in the renal tubular epithelium and degeneration of hyaline droplet were observed in male rats receiving 150 mg/kg-day or more.

In addition, necrosis and fibrosis of the proximal tubule and dilatation of the distal tubule were observed in male rats receiving 750 mg/kg-day. At the lowest dose only, there was a decrease in absolute but not relative thymus weight, which was not considered treatment related. Eastman Chemical (2007) determined a NOEL for systemic toxicity of 30 mg/kg-day for males and 150 mg/kg-day for females. The NOAEL was determined to be 150 mg/kg-day, based on the assertion that effects seen at this dose were adaptive in nature.

#### ***5.5.1.1.2 Reproductive***

- Eastman Chemical (2007) conducted a combined reproductive/developmental screening toxicity test in SD rats in which TPIB (0, 30, 150, and 750 mg/kg-day) was administered via gavage for 14 days prior to mating through 30 days post-mating (males) or lactation day (LD) 3 (females). No TPIB-related reproductive effects were observed (NOAEL<sub>repro/devel</sub> = 750 mg/kg-day). This study is unpublished.
- Eastman Chemical (2001) conducted a combined reproductive/developmental screening toxicity test (OECD GL 421) in SD rats in which TPIB (0, 91, 276, and 905 mg/kg-day in males; 0, 120, 359, and 1135 mg/kg-day in females) was administered in the diet for 14 days pre-mating, during mating, through gestation, and through PND 4–5. Changes in epididymal and testicular sperm counts were reported by the authors but considered not to be adverse. No other TPIB-related male reproductive effects were observed (NOAEL<sub>male repro/devel</sub> = 905 mg/kg-day). This study is unpublished.

#### ***5.5.1.1.3 Developmental***

- See the above Eastman Chemical studies (2001; 2007) for developmental toxicity screening results.

#### ***5.5.1.1.2 Human***

- No published human studies.

### **5.5.1.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.5.1.3 Weight of Evidence**

#### ***5.5.1.3.1 Experimental Design***

The 1972 animal studies by Astill and Krasavage had low sample sizes (4 dogs per dose, 10 rats per dose), and the rat studies used only two dose levels. Adverse, treatment-related effects were not clearly established at any dose level in these studies, with the exception of one of the Krasavage groups. Studies were published in respected journals subject to peer review.

Neither repro-developmental study was published, but they appear to have met OECD GL 421 requirements. As reported in the guideline, “This test does not provide complete information on all aspects of reproduction and development. In particular, it offers only limited means of detecting post-natal manifestations of prenatal exposure, or effects that may be induced during post-natal exposure. Due (amongst other reasons) to the relatively small numbers of animals in the dose groups, the selectivity of the end points, and the short duration of the study, this method will not provide evidence for definite claims of no effects. Although, as a consequence, negative data do not indicate absolute safety with respect to reproduction and development, this information may provide some reassurance if actual exposures were clearly less than the dose related to the NOAEL.”

#### ***5.5.1.3.2 Replication***

No published full reproduction or full developmental studies exist. As the CHAP has reported, “in neither study is there any indication of any antiandrogenic effects of TPIB when administered to females at doses as high as 1125 mg/kg-day for 14 days before mating, during mating (1–8 day), throughout gestation (21–23 days), and through PND 4–5. Thus, the developmental NOAEL for TPIB is greater than 1125 mg/kg-day.”

### **5.5.1.4 Risk Assessment Considerations**

#### ***5.5.1.4.1 Exposure***

TPIB is a secondary plasticizer used in combination with other plasticizers. While TPIB is not an HPV chemical, it is widely used in many products, including weather stripping, furniture, wallpaper, nail care products, vinyl flooring, sporting goods, vinyl gloves, inks, water-based paints, and toys. TPIB has been detected in indoor air in office buildings, schools, and residences. TPIB was found in one-quarter of the toys and child care articles tested by CPSC (Dreyfus, 2010).

Estimates of total TPIB exposure are not available. The mean and 95<sup>th</sup> percentile exposures to infants from mouthing all soft plastic objects except pacifiers are 0.92 to 5.8 µg/kg-d, respectively (Section 2.6; Appendix E2).

#### ***5.5.1.4.2 Hazard***

The database is somewhat limited. There is evidence of effects in the liver and kidneys in rats (Eastman, 2007). The NOEL for systemic effects is 30 mg/kg-d in males and 150 mg/kg-d in female rats. The study authors proposed 150 mg/kg-d as the NOAEL.

#### **5.5.1.4.3 Risk**

Assuming a point of departure of 30 mg/kg-d, the MOE's for mouthing all soft plastic objects except pacifiers by infants range from 5,200 to 33,000.

#### **5.5.1.5 Recommendation to CPSC regarding children's toys and child care articles**

Although data are somewhat limited, there is no evidence that TPIB presents a hazard to infants or toddlers from mouthing toys or child care article containing TPIB. Therefore, the CHAP recommends no action on TPIB at this time.

The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure and hazard data to estimate total exposure to TPIB and assess the potential health risks.

#### **5.5.1.6 Would this recommendation, if implemented, be expected to reduce exposure of children to TPIB?**

No.

### **5.5.2 Di(2-ethylhexyl) adipate (DEHA) CAS 103-23-1**

#### **5.5.2.1 Adverse Effects**

##### **5.5.2.1.1 Animal**

##### **5.5.2.1.1.1 Systemic**

- Effects induced by DEHA in 13-week mouse studies are consistent with those of DEHP and other hepatic peroxisome proliferators in rats and mice (Lake, 1995; Cattley *et al.*, 1998; Chevalier and Roberts, 1998; Doull *et al.*, 1999; IARC, 2000a; IARC, 2000b).
- Kang *et al.* (2006) reported a large (50%) increase in relative liver weight and a decrease in body weight in male Fischer-344 rats exposed to 1570 mg/kg-day DEHA in the diet for 4 weeks. There were no effects on serum indicators of hepatotoxicity (ALT, AST, GGT) or seen with light microscopy of the liver. No hepatic changes were observed at 318 mg/kg-day.
- Similarly, Miyata *et al.* (2006) observed significant increases in relative liver weight without accompanying serum chemistry or histopathology changes in Crj:CD (SD) rats of both sexes receiving a gavage dose of 1000 mg/kg-day DEHA, but not in those receiving 200 mg/kg-day or lower for 28 days or more.
- Dietary 13-week studies performed by NTP (1982) as dose range-finding studies for cancer bioassays in F344 rats and B6C3F1 mice (described below) showed no effects in histopathology of the liver, kidneys, or other tissues of males or females of either species exposed to DEHA concentrations as high as approximately 2500 mg/kg-day (rats) and 4700 mg/kg-day (mice). Organ weights were not measured.

- Nabae *et al.* (2006) also reported no evidence of renal histopathology, serum chemistry, or urinalysis findings indicative of renal pathology in male F344 rats exposed to 1570 mg/kg-day DEHA in the diet for 4 weeks. However, small increases in relative kidney weights were noted.
- Kidney lesions were observed by Miyata *et al.* (2006) in male, but not female, Crj:CD (SD) rats treated with 1000 mg/kg-day, but not with 200 mg/kg-day or lower, of DEHA by gavage for 28 days. The type of lesions (increased eosinophilic bodies and hyaline droplets) and gender-dependent occurrence suggest that this finding may be related to male rat-specific alpha-2u-globulin nephropathy. Small increases in relative kidney weight were also observed in treated rats. Miyata *et al.* (2006) found no effects on hematology parameters or in a functional observational battery for neurological effects in treated rats.
- NTP (1982) fed F344 rats (50/sex/dose) and B6C3F1 mice (50/sex/dose) diets containing approximately 2040 or 4250 mg/kg-day (mice), 948 or 1975 mg/kg-day (male rats), or 1104 or 2300 mg/kg-day (female rats) DEHA for 103 weeks followed by a 1–3 week observation period. High-dose rats of both sexes had reduced mean body weights compared to controls. No lesions or other compound-related adverse effects were observed in rats. For mice, mean body weights of all treated animals were lower than controls throughout the study and the decreases were dose related. Survival did not appear to be affected by DEHA, but liver tumors were induced in both sexes with the combined incidence of hepatocellular adenomas and carcinomas significantly increased in high-dose males and in all treated females. No compound-related nonneoplastic lesions were observed in the liver or other tissues.
- Hodge *et al.* (1966) briefly and inadequately reported carcinogenicity results of chronic feeding studies of DEHA in rats and dogs. No compound-related tumors were induced in rats exposed to 0, 0.1, 0.5 or 2.5% DEHA in the diet for two years or in dogs exposed to 0, 0.07, 0.15 or 0.2% DEHA in the diet for one year.
- Hodge *et al.* (1966) also exposed C3H/AnF mice (50/sex/dose) to DEHA by dermal application and subcutaneous injection. In the dermal study, a lifetime weekly application of 0.1 or 10 mg of DEHA in acetone to a clipped area of back skin under non-occlusive conditions caused no gross or histological evidence of tumor formation at the application site. In the subcutaneous study, a single 10 mg dose of DEHA caused no injection site tumors following lifetime observation.

#### **5.5.2.1.1.2 Reproductive**

- No published multigenerational reproduction studies.
- The NTP (1982) conducted subchronic and chronic studies in F344 rats and B6C3F1 mice in which DEHA was administered in the diet at up to ~2500 mg/kg-day (rats, 13 weeks), ~4700 mg/kg-day (mice, 13 weeks), ~2100 mg/kg-day (rats, 103 weeks), and ~4250 mg/kg-day (mice, 103 weeks). No adverse histopathological changes were reported in either male or female reproductive organs in any of the studies.
- Nabae *et al.* (2006) and Kang (2006) conducted an intermediate-term study in F344 rats in which DEHA was administered in the diet at 0, 318, and 1570 mg/kg-day for 4 weeks. No changes were seen in spermatogenesis, weight, or histology of the testes, epididymides, prostate, or seminal vesicles (NOAEL<sub>repro</sub> = 1570 mg/kg-day). No

DEHA-induced testicular toxicity was seen in rats pretreated with thioacetamide or folic acid (in contrast to DEHP).

- Miyata *et al.* (2006) conducted an intermediate-term study in SD rats in which DEHA was administered via oral gavage at 0, 40, 200, or 1000 mg/kg-day for 4 weeks. Increased follicular atresia and prolonged estrous cycle was seen in female rats in the high dose group (F, NOAEL<sub>repro</sub> = 200 mg/kg-day). No reproductive effects were seen in male rats (M, NOAEL<sub>repro</sub> = 1000 mg/kg-day).

#### **5.5.2.1.3 Developmental**

- Dalgaard (2002) conducted a pilot developmental study in Wistar rats in which DEHA was administered via oral gavage at 0, 800, and 1200 mg/kg-day on GD 7 through PND 17. Decreased pup weights were seen at 800 and 1200 mg/kg-day. No antiandrogenic effects were observed.
- Dalgaard (2003) conducted a developmental study in Wistar rats in which DEHA was administered via oral gavage at 0, 200, 400, and 800 mg/kg-day on GD 7 through PND 17. Postnatal deaths were higher in the 400 mg/kg-day group (NOAEL<sub>devel</sub> = 200 mg/kg-day). Increased gestation length in the high-dose group was reported. No antiandrogenic effects were seen.

### **5.5.2.2 Human**

- No published human studies.

### **5.5.2.3 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans. However, it should be noted that peroxisome proliferation has questionable relevance to hazard characterization in humans. As well, adverse effects involving alpha-2u-globulin nephropathy in rats are not predictive of renal effects in humans.

### **5.5.2.4 Weight of Evidence**

#### **5.5.2.4.1 Experimental Design**

Studies by Nabae, Kang, and Miyata each had small dose groups (6 or 10 per group). The Hodge (1966) dog and rat studies were not well reported. The chronic NTP study appears to be of sufficient design and rigor. There were no published reproductive studies. The NTP study had sufficient N per group (n=49–50 for 103 weeks) but did not include organ weight measures. The Nabae and Kang studies had only 6 rats per dose group. The Miyata study had only 10 animals per group. Antiandrogenic conclusions are, therefore, weak. The lack of antiandrogenic effects seen in these studies, however, is supported by unpublished findings from a one-generation reproduction study (ICI, 1988).

The Dalgaard (2003) full developmental study (n=20 per dose group) is of sufficient study design and rigor to support the conclusion of no antiandrogenic effects. The pilot study had only n=8 per group, however.

#### **5.5.2.4.2 Replication**

DEHA studies, similar to DEHP studies, consistently show peroxisome proliferation and its associated adverse effects. The chronic study showing increased liver tumor incidence in mice has not been replicated but is a sound study.

No published reproduction studies exist. Because of a low N, only one developmental study can reliably support antiandrogenic conclusions. The CHAP has recommended using a NOAEL of 800 mg/kg-day with an additional uncertainty factor of 10 to be used in the calculation of an RfD.

### **5.5.2.5 Risk Assessment Considerations**

#### **5.5.2.5.1 Exposure**

DEHA is a high production volume chemical. It is approved for use in food contact materials. Dietary exposures have been estimated for European (0.7 µg/kg-d) (Fromme *et al.*, 2007b); Japanese (12.5 µg/kg-d) (Tsumura *et al.*, 2003); and Canadian (137 to 259 µg/kg-d) (Page and Lacroix, 1995; Carlson and Patton, 2012) populations. DEHA is also found in adhesives, vinyl flooring, carpet backing, and coated fabrics (Versar/SRC, 2010).

DEHA has been found in some toys and child care articles in the past (Chen, 2002) but was not found in a recent study by CPSC (Dreyfus, 2010). Estimates of exposure from mouthing toys and child care articles are not available.

#### **5.5.2.5.2 Hazard**

The toxicity of DEHA has been reviewed by Versar/SRC (Versar/SRC, 2010). NTP conducted a two-year feed study in mice and rats (NTP, 1982). Liver tumors (adenomas plus carcinomas) were elevated in high-dose males and in females at all doses. The tumors may be due to peroxisome proliferation. The noncancer NOAEL in mice was 4,250 mg/kg-d, the highest dose tested.

In a subchronic gavage study in SD rats, increased follicular atresia and prolonged estrous cycle were seen in high dose females. The NOAEL was 200 mg/kg-d.

A developmental study was performed in Wistar rats by gavage (Dalgaard *et al.*, 2003). Gestational length was significantly increased at the high dose (800 mg/kg-d). The developmental NAOEL was 200 mg/kg-d, based on postnatal deaths.

#### **5.5.2.5.3 Risk**

Assuming a point of departure of 200 mg/kg-d, the margins of exposure from dietary DEHA exposure range from 770 to 290,000.

#### **5.5.2.6 Recommendation to CPSC regarding children's toys and child care articles**

Data on exposure from toys and child care articles are not available. Given the lack of exposure data on DEHA, the CHAP is unable to recommend to CPSC any action regarding the potential use of DEHA in children's toys or child care articles at this time. The CHAP recommends that the appropriate U.S. agencies obtain the necessary data to estimate DEHA exposure from diet and children's articles, and assess the potential health risks.

#### **5.5.2.7 Would this recommendation, if implemented, be expected to reduce exposure of children to DEHA?**

No.

### **5.5.3 Di(2-ethylhexyl) terephthalate (DEHT) CAS 6422-86-2**

#### **5.5.3.1 Adverse Effects**

##### **5.5.3.1.1 Animal**

##### **5.5.3.1.1.1 Systemic**

- Eastman Kodak Co. (1975) reported an intermediate-term study in male albino rats (five/group) in which DEHT (0, 0.1, 1%; 0, not reported, 890 mg/kg-day) was administered in the diet five days a week for two weeks. DEHT-treated rats were not significantly different from controls. Infection of control and treated rats confounded the interpretation of this study.
- Topping *et al.* (1987) reported an intermediate-term toxicity study in SD rats (5/sex/group) in which DEHT (0, 0.1, 0.5, 1.0, 1.2, or 2.5%; estimated doses for M: 0, 86, 431, 861, 1033, 2154 mg/kg-day; for F: 0, 98, 490, 980, 1176, 2450 mg/kg-day) was administered in the diet for three weeks. Exposure to DEHT reduced body weight gain and feed consumption (M&F: 2154, mg/kg-day), increased relative liver weight (M: 2154, F: 980, 1176, 2450 mg/kg-day), increased serum cholesterol, triglycerides, liver enzymes, and peroxisomes (M&F: 2154, 2450 mg/kg-day). The review author identified a NOAEL of 1033 (M) and 1176 (F) mg/kg-day based on decrements in body weight gain and food consumption.
- Barber and Topping (1995) reported an intermediate-term toxicity study in SD rats (20/sex/group) in which DEHT (0, 0.1, 0.5, 1%; M: 0, 54, 277, 561 mg/kg-day; F: 0, 61, 309, 617 mg/kg-day) was administered in the diet for 90 days. No changes in body weight gain or food consumption were observed. DEHT exposure significantly increased relative liver weights (males at 561 mg/kg-day and females at 617 mg/kg-day) but no other organ weights. Various hematology parameters (but not serum chemistry) were statistically different from controls. Peroxisomal proliferation was not observed in treated groups. The study authors assigned NOAELs of 277 and 309 mg/kg-day (M&F respectively), based on changes in the liver and hematology.

- Eastman Kodak Co. (1983) conducted an intermediate-term inhalation toxicity study in rats (5/group) in which DEHT (0, 46.3 mg/m<sup>3</sup>) was administered 8 hours/day, 5 days/week for 2 weeks. No significant effects were reported in hematology, serum chemistry, or pathology. The study was poorly described, limiting its interpretation.
- Deyo (2008) reported a chronic toxicity study in Fischer 344 rats (50/sex/group) in which DEHT (0, 1500, 6000, 12000 ppm; M: 0, 79, 324, 666 mg/kg-day, F: 0, 102, 418, 901 mg/kg-day) was administered in the diet for 104 weeks. Body weight gain was significantly lower in high-dose animals over the two years and lower in the mid-dose rats during the first year. Terminal body weights were significantly different from controls (F: 901 mg/kg-day). Hematology, clinical chemistry, and urinalysis were not consistently affected by DEHT treatment. DEHT increased the relative liver weights in females (significant at 901 mg/kg-day) and males (not significant at 666 mg/kg-day), and increased the incidence of portal lymphoid foci (M: 666 mg/kg-day). Changes in kidney weight were not dose related or supported by histopathology. The author attributed other organ weight changes to individual variation or as secondary to body weight changes. DEHT exposure also increased the incidence of eosinophilic inclusions in the nasal turbinates and atrophy of the outer nuclear layer of the retina (F: 418 mg/kg-day), but the study author regarded these as not toxicologically significant. Changes in the incidence of large granular cell lymphomas were not dose related.
- Faber *et al.* (2007b) reported a two-generation reproduction study in SD rats (see below). High-dose females had more mortalities than controls, and high-dose males had significant reductions in body weight gain (week 3 and 7). Absolute (F0) and relative (F0, F1) liver weights were increased in mid- and high-dose females but were not correlated to morphological changes in the liver. Maternal body weight gain through gestation, body weight on GD 20 through lactation, and feed consumption were significantly reduced in F0 and F1 dams (530 mg/kg-day). Body weight and feed consumption were also reduced during LD 7–14 in mid-dose F1 dams (316 mg/kg-day). Relative spleen and thymus weight was reduced and relative brain weight increased in various populations of rats. The study author identified a NOAEL of 158 mg/kg-day for parental systemic effects.
- Faber *et al.* (2007a) reported a developmental study in SD rats (see below). Maternal body weight gain was reduced during GD 16–20 in the DEHT high-dose group, but body weights were similar to controls during the entire treatment period. A significant increase in absolute liver weight was also reported for high-dose rats. The NOAEL was reported to be 458 mg/kg-day, based on mean and net maternal body weight decrements.
- Barber (1994) and Divincenzo *et al.* (1985) reported that reverse mutations were not induced in bacteria, forward mutations in the HGPRT locus of Chinese hamster ovary (CHO) cells, or chromosomal aberrations in CHO cells *in vitro*.

#### **5.5.3.1.1.2 Reproductive**

- Faber *et al.* (2007b) reported a two-generation reproduction study in SD rats in which DEHT was mixed in the diet at 0, 0.3, 0.6, and 1.0% (F0 males = 0, 158, 316, and 530 mg/kg-day). Males were exposed for 10 weeks prior to and during mating. Females were exposed 70 days prior to mating, during mating, and through gestation and



lactation. Weaned offspring were dosed similarly starting on PND 22. No reproductive effects were reported at any dose level for any generation (NOAEL<sub>repro</sub> = 530 mg/kg-day).

#### **5.5.3.1.1.3 Developmental**

- Gray *et al.* (2000) reported a developmental study in SD rats in which DEHT was dosed via gavage at 0 or 750 mg/kg-day on GD 14 through PND 3. No male reproductive tract malformations were observed in male pups (NOAEL<sub>devel</sub> = 750 mg/kg-day).
- Faber *et al.* (2007a) reported a developmental study in SD rats in which DEHT (0, 0.3, 0.6, and 1.0%; 0, 226, 458, and 747 mg/kg-day) was administered via the diet on GD 0 through GD 20. Adverse reproductive effects were not observed in dosed animals. A dose-related increase in the incidence of 14th rudimentary ribs was observed in treated groups (NOAEL = 458 mg/kg-day).
- Faber *et al.* (2007a) reported a developmental study in which DEHT was fed via the diet (0, 0.1, 0.3, and 0.7%; 0, 197, 592, and 1382 mg/kg-day) to pregnant ICR mice at GD 0 through GD 18. No antiandrogenic effects were observed in the study (NOAEL<sub>devel</sub> = 1382 mg/kg-day).

#### **5.5.3.1.2 Human**

No published human studies.

### **5.5.3.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.5.3.3 Weight of Evidence**

#### **5.5.3.3.1 Experimental Design**

The two generation reproduction study and the developmental studies (Faber *et al.*, 2007a; 2007b) had a sufficient number of rats per group (n=25–30) and adequate study design to support the conclusions based on their results. The Gray study had only eight pregnant rats per treatment group. The chronic and intermediate-term toxicity studies had an acceptable number of animals per dose group (50 and 20/sex/group, respectively). Other studies looking at systemic endpoints generally had lower Ns (5/group).

#### **5.5.3.3.2 Replication**

Only one reproduction study (Faber *et al.*, 2007b) has been performed with DEHT. Two full developmental studies in different species were performed by one lab (Faber *et al.*, 2007a), and a targeted developmental study was performed by a different lab (Gray *et al.*, 2000). On the basis of these two [developmental] studies and the results of the two-generation study in rats, the CHAP recommends a NOAEL for DEHT of 750 mg/kg-day. NOTE: The CHAP assessment for reproductive toxicity lists NOAEL = 530 mg/kg-day, and the developmental assessment lists NOAEL = 747 mg/kg-day for Faber *et al.*, (2007b). Systemic toxicity was described by at least two larger studies, one long-term and one intermediate-term, and by a handful of additional smaller studies. In these

studies, DEHT exposure decreased body weight gain (five studies), feed consumption (two studies), and increased liver weight (five studies), and serum cholesterol, triglycerides, liver enzymes, and peroxisomes (one study). Hepatic changes seen following exposure to DEHT paralleled those seen in rats following ortho-phthalate exposures. DEHT-induced adverse changes in nasal turbinates and the retina are not typically described for ortho phthalates.

#### **5.5.3.4 Risk Assessment Considerations**

##### **5.5.3.4.1 Exposure**

DEHT is a high production volume chemical. It was present in about one-third of the toys and child care articles tested by CPSC (Dreyfus, 2010). The exposure to infants from mouthing all soft plastic articles except pacifiers was estimated to be 0.69 µg/kg-d (mean), with an upper bound of 2.8 µg/kg-d. Information on total exposure is not available.

##### **5.5.3.4.2 Hazard**

Peer-reviewed toxicological studies on DEHT are available. The reproductive NOAEL was 158 mg/kg-d in a two-generation study in SD rats, based on parental effects (Faber *et al.*, 2007b). The developmental NOAEL was 458 mg/kg-d in rats, based on increased incidence of 14th rudimentary ribs (Faber *et al.*, 2007a). DEHT did not produce antiandrogenic effects in rats at 750 mg/kg-d (Gray *et al.*, 2000). No developmental effects were observed in mice (Faber *et al.*, 2007a).

##### **5.5.3.4.3 Risk**

Assuming a point of departure of 158 mg/kg-d, the margin of exposure for mouthing soft plastic articles is 56,000 to 230,000.

#### **5.5.3.5 Recommendation**

There is no evidence that DEHT presents a hazard to infants or toddlers from mouthing toys or child care articles containing DEHT. Therefore, the CHAP recommends no action on DEHT.

However, information on total exposure to DEHT is not available. The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure data to estimate total exposure to DEHT and assess the potential health risks.

#### **5.5.3.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DEHT?**

No.

## 5.5.4 Acetyl Tributyl Citrate (ATBC) CAS 77-90-7

### 5.5.4.1 Adverse Effects

#### 5.5.4.1.1 Animal

##### 5.5.4.1.1.1 Systemic

- Finkelstein and Gold (1959) exposed small groups of animals (four rats or two cats) to dietary ATBC for six to eight weeks. Wistar rats were fed approximately 7620 or 15,240 mg/kg-day and cats received 5250 mg/kg-day. Growth was reduced in cats and high-dose rats by 30–35%, and both had diarrhea. Treatment with ATBC had no effect on blood counts or on gross or microscopic pathology.
- SD rats (5/sex/dose) were administered ATBC (purity>98%) in the diet at doses of 0, 1000, 2700, or 5000 mg/kg-day for 14 consecutive days as part of a dose-range finding study (Jonker and Hollanders, 1990). Transient dose-related reductions in body weights were reported among all dose groups. Body weights among high-dose rats and mid-dose male rats remained slightly lower than control rats throughout the study, with food consumption in the former group also reduced. Increased cytoplasmic eosinophilia accompanied by reduced glycogen content of periportal hepatocytes was observed in the livers of 2/5 mid-dose male rats and all of the high-dose rats. No further details of this study were available.
- SD rats (20/sex/dose) were administered ATBC (purity >98%) in the diet *ad libitum* at doses of 0, 100, 300, or 1000 mg/kg-day for 13 weeks (Jonker and Hollanders, 1990). The following endpoints showed no treatment-related changes: mortality, clinical signs, appearance, behavior, motor activity, sensory activity, autonomic activity, body weight, hematology, clinical chemistry, and urinalysis. Relative liver weights were higher among mid-dose males and high-dose males and females. There was a slight increase in the relative kidney weights of high-dose male rats, but statistical significance was not reported. It is not clear whether absolute organ weights were unchanged or not reported. Gross necropsy and histopathology did not reveal any treatment-related effects in the liver, kidneys, or other organs. The high dose of 1000 mg/kg-day appears to be a NOAEL due to the absence of toxicologically significant findings.
- Soeler *et al.* (1950) fed three groups of Sherman rats (20 rats/dose) (gender not specified) a diet containing ATBC (99.4% purity) at approximately 0, 10, 100, and 1000 mg/kg-day. There was no ATBC-induced effect on growth. Mortality occurred in 20% of the treated rats (12/60) and the control rats (8/40) prior to study termination but may have been related to pulmonary infection. Lymphomas were observed in both control and treated rats, and were not considered to be related to treatment with ATBC. The NOAEL for this study is 1000 mg/kg-day.

##### 5.5.4.1.1.2 Reproductive

- Robins *et al.* (1994) conducted a two-generation reproduction study in SD rats in which ATBC was mixed in the diet at 0, 100, 300, and 1000 mg/kg-day. Males were exposed for 11 weeks and females for 3 weeks prior to mating, then during mating, gestation, and lactation. ATBC was administered to pups for 10 weeks after weaning.

No reproductive effects were reported at any dose level (NOAEL<sub>repro</sub> = 1000 mg/kg-day).

- Chase and Willoughby (2002) conducted a one-generation reproduction study in Wistar rats in which ATBC was mixed in the diet at 0, 100, 300, and 1000 mg/kg-day. F0 parents were exposed for four weeks prior to mating, then during mating, gestation, and lactation. No reproductive effects were seen at any dose level (NOAEL<sub>repro</sub> = 1000 mg/kg-day).

#### **5.5.4.1.1.3 Developmental**

- No published animal developmental studies. Developmental effects were not observed in the above reproductive studies.

#### **5.5.4.1.2 Human**

- No published human studies.

### **5.5.4.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.5.4.3 Weight of Evidence**

#### **5.5.4.3.1 Experimental Design**

Repeat dose studies described here are old, have small sample sizes, and are missing methodological and statistical details (Soeler *et al.*, 1950; Finkelstein and Gold, 1959; Jonker and Hollanders, 1990; 1991). The Soeler *et al.* (1950) study is of limited value as a cancer bioassay because group sizes were relatively small (20 per treated group and 40 in controls), 20% of animals died early from infection, lymphomas were high in control animals, and doses were inadequate (the high dose did not approach the maximum tolerated dose). Furthermore, oral metabolism studies in rats and in rat liver homogenates reveal that ATBC is extensively absorbed and rapidly metabolized and excreted (Davis, 1991; Edlund and Ostelius, 1991; Dow, 1992; CTFA, 1998). Thus, any liver, and possibly kidney, enlargement noted in some of these studies may be an adaptive change occurring as a consequence of metabolic load.

As presented, the two-generation study by Robins *et al.* (1994) seems of appropriate rigor to substantiate the lack of ATBC-induced pathologies. The one-generation study, however, does not have a sufficient duration of dosing pre-mating (a minimum of 10 weeks) to adequately assess male reproductive effects.

#### **5.5.4.3.2 Replication**

Studies did not adequately replicate the effects observed occasionally in body weight, liver, or kidney. Results from the one-generation reproduction study are not directly comparable to the two-generation reproduction study, and, therefore, conclusions need to be confirmed. The CHAP has recommended using a NOAEL of 1000 mg/kg-day with an additional uncertainty factor of 10 to be used in the calculation of an RfD.

#### **5.5.4.4 Risk Assessment Considerations**

##### **5.5.4.4.1 Exposure**

ATBC is a high production volume chemical. It is used in food packaging, food (as a flavor additive), medical devices, personal care products, adhesives, and pesticides (inert ingredient) (Versar/SRC, 2010). ATBC was found in about half of the toys and child care articles tested by CPSC (Dreyfus, 2010). The exposure to infants from mouthing all soft plastic articles except pacifiers is estimated to have a mean of 2.3 µg/kg-d and a 95<sup>th</sup> percentile of 7.2 µg/kg-d.

##### **5.5.4.4.2 Hazard**

The overall NOAEL in a 13-week study in SD rats was 1,000 mg/kg-d, based on systemic effects (Jonker and Hollanders, 1990). The NOAEL was also 1,000 mg/kg-d (the highest dose tested) in two studies: a two-generation study (Robins, 1994) and a one-generation study (Chase and Willoughby, 2002).

##### **5.5.4.4.3 Risk**

Assuming a point of departure of 1,000 mg/kg-d, the MOE for mouthing soft plastic articles by infants is estimated to be from 14,000 (upper bound exposure) to 43,000 (mean exposure).

#### **5.5.4.5 Recommendation to CPSC regarding children's toys and child care articles**

Although data are somewhat limited, there is no evidence that ATBC presents a hazard to infants or toddlers from mouthing toys or child care articles containing ATBC. Therefore, the CHAP recommends no action on ATBC by CPSC at this time.

The CHAP recommends that the appropriate U.S. agencies obtain the necessary exposure and hazard data to estimate total exposure to ATBC and assess the potential health risks.

#### **5.5.4.6 Would this recommendation, if implemented, be expected to reduce exposure of children to ATBC?**

No.

### **5.5.5 Diisononyl hexahydrophthalate (DINX) CAS 166412-78-8**

#### **5.5.5.1 Adverse Effects**

##### **5.5.5.1.1 Animal**

##### **5.5.5.1.1.1 Systemic**

- No published studies.

- SCENIHR (2007) reported a summary of a 28-day oral toxicity study in an undisclosed species (presumed to be rat at 5 rats/sex/dose) in which DINX was (presumed) to be dosed via the diet at 0, 600, 3000, and 15,000 ppm (M/F, 64/66, 318/342, 1585/1670 mg/kg-day). The highest dose of DINX resulted in increased gamma-glutamyl transferase (GGT) and degenerated epithelial cells in the urine. SCENIHR reported 3000 ppm (318/342 mg/kg-day) as the NOAEL but left open the question of whether these changes were adverse or not.
- SCENIHR (2007) reported a summary of a 90-day oral toxicity study in an undisclosed species (presumed to be rat at 10 rats/sex/dose) in which DINX was (presumed) to be dosed via the diet at 0, 1500, 4500, and 15,000 ppm (M/F, 107/128, 325/389, 1102/1311 mg/kg-day). An increase in liver and thyroid weight (absolute or relative not reported), phase I and II liver enzymes, and serum GGT, and thyroid stimulating hormone, as well as hyperplasia/hypertrophy of the thyroid follicles, was described. Relative testis weight was increased at all doses but did not have a dose-related relationship or associated histopathological changes. Blood and urinary tract transitional epithelial cells were also found in the urine (without histopathological changes in the kidney) and alpha 2u-globulin accretions in the renal tubules in the male rats. The review author considered the liver changes at which they affected thyroid follicles to be a LOAEL (but did not conclude what this LOAEL was).
- SCENIHR (2007) reported a summary (no quantitative data) of a two-generation reproduction study in an unnamed species (presumably rats at 20 rats/sex/dose) in which DINX was mixed in the diet at 0, 100, 300, and 1000 mg/kg-day. Although not detailed, it is presumed that males were exposed for at least 10 weeks prior to mating and during mating, and that weaned offspring were dosed similarly (because the study was performed under OECD TG 416). Increased liver, kidney, and thyroid weights in F0 rats were observed at 1000 mg/kg-day. Increased thyroid weight and thyroid hyperplasia/hypertrophy in F1 rats were observed at 300 mg/kg-day and higher (LOAEL = 300 mg/kg-day). Exposure to DINX also increased serum GGT and decreased total bilirubin in F0 females.
- SCENIHR (2007) also reported a summary of a prenatal developmental toxicity study in rats and rabbits that were orally administered DINX at 0, 100, 300, 1000 (1200 – rat) mg/kg-day on GD 6–19 (rat) or GD 6–29 (rabbit). Details on the methodology and results are not available, but “no effects were observed in either species,” suggesting NOAELs of 1200 (rat) and 1000 (rabbit) mg/kg-day for maternal toxicity.
- BASF (2005) reported data for a chronic toxicity/carcinogenicity study in Wistar rats (50/sex/dose) in which DINX (0, 40, 200, 1000 mg/kg-day) was administered in the feed for two years. DINX exposure increased thyroid weight, follicular cell hyperplasia, and follicular adenomas in a dose-related fashion in male and female rats ( $\geq 200$  and 1000 mg/kg-day, respectively). Urinary tract transitional epithelial cells were also reported (at an unspecified dose) but were considered to be adaptive by the SCENIHR because there was no histopathological changes in the kidney. This study identified a NOAEL (M/F 40/200 mg/kg-day) and a LOAEL (M/F, 200/1000 mg/kg-day) for nonneoplastic effects in the thyroid. Note, the SCENIHR suggested that thyroid effects (including adenomas) were not relevant in humans. This is not consistent with EPA policy (1998), which concludes that rodent noncancer/cancer

thyroid effects resulting from disruption of the thyroid-pituitary axis do represent a noncancer/cancer health hazard to humans.

- SCENIHR and BASF report that DINX does not induce mutations in bacteria or CHO cells *in vitro*. It also does not induce chromosomal aberrations in Chinese hamster V79 cells *in vitro* or micronuclei in mouse bone marrow cells *in vivo*.

#### **5.5.5.1.1.2 Reproductive**

- No published reproduction studies.
- SCENIHR (2007) reported a summary of a two-generation reproduction study in an unnamed species (presumably rats) in which DINX was mixed in the diet at 0, 100, 300, and 1000 mg/kg-day. Although not detailed, it is presumed that males were exposed for at least 10 weeks prior to mating and during mating, and that weaned offspring were dosed similarly (because the study was performed under OECD TG 416). No reproductive effects were reported at any dose level (NOAEL<sub>repro</sub> = 1000 mg/kg-day).

#### **5.5.5.1.1.3 Developmental**

- No published animal developmental studies.
- SCENIHR (2007) reported a summary of a pre- and postnatal developmental toxicity study in rats and rabbits that were orally administered DINX during gestation (at dose levels as high as 1200 mg/kg-day on gestational days 6–19 in the rat and 0, 100, 300, or 1000 mg/kg-day on gestation days 6–29 in the rabbit). Although discrete methods and data were not available in the summary, it was reported that no effects were observed in either species, suggesting apparent NOAEL<sub>s<sub>devel</sub></sub> of 1200 mg/kg-day in rats and 1000 mg/kg-day in rabbits.
- SCENIHR (2007) also reported a summary of a developmental toxicity study in rats that were orally administered DINX at 0, 750, and 1000 mg/kg-day from 3 days post-coitum to PND 20. Details on the methodology and results are not available. A 7–8% decrease in AGD in males and the AGD index in both sexes was reported at the high dose on PND 1. This was considered to be a study artifact, however, because other male reproductive parameters were not affected (NOAEL<sub>devel</sub> = 1000 mg/kg-day).
- No developmental variations or malformations were observed in the SCENIHR reproduction summary.

#### **5.5.5.1.2 Human**

- No published human studies.

### **5.5.5.2 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.5.5.3 Weight of Evidence**

#### **5.5.5.3.1 Experimental Design**

All studies were unpublished and their experimental design had to be inferred from the SCENIHR review. This reduces the confidence in the conclusions drawn by the authors.

#### **5.5.5.3.2 Replication**

No published studies exist. The available summaries of unpublished studies are brief and generally insufficient with respect to information on experimental design and results, particularly quantitative data and dose-response relationships. While DINX is entering the market as a component of consumer products such as children's articles, the insufficiency of these study summaries preclude independent evaluation of the results and reliable identification of adverse effect levels. Systemic results that are presented, however, support the conclusion that DINX increases liver weight (two studies), thyroid weight (four studies), GGT (three studies), epithelial cells in the urine (three studies), and follicular hyperplasia (two studies).

#### **5.5.5.4 Risk Assessment Considerations**

##### **5.5.5.4.1 Exposure**

Although DINX is not a high production volume chemical, its production has grown rapidly in recent years (CEH, 2009). DINX is used in food packaging and processing materials. It is a potential substitute for DEHP in medical devices. DINX was present in about one-third of the toys and child care articles tested by CPSC (Dreyfus, 2010). The estimated mean exposure from mouthing soft plastic articles except pacifiers is 1.4 µg/kg-d, with an upper bound of 5.4 µg/kg-d (Section 2.6; Appendix E2). Estimates of total exposure are not available.

##### **5.5.5.4.2 Hazard**

The available toxicity studies are proprietary; only summaries prepared by the manufacturer are available. In a two-year bioassay in Wistar rats (BASF, 2005) DINX exposure led to thyroid hypertrophy, follicular cell hyperplasia, and follicular adenomas in mid- and high-dose males and females. The noncancer NOAEL was 40 mg/kg-d (low dose); the LOAEL was 200 mg/kg-d.

Few details were available on a two-generation study (OECD TG 416). The species and number of animals were not reported (SCENIHR, 2007). The systemic NOAEL was 100 mg/kg-d. Liver, kidney, and thyroid weights were increased in F0 and F1 animals at the middle dose (300 mg/kg-d). Thyroid hyperplasia was reported in F1 animals. Increased serum GGT and decreased bilirubin were reported in F0 females. The reproductive/developmental NOAEL was 1000 mg/kg-d, the highest dose tested.

##### **5.5.5.4.3 Risk**

Assuming a point of departure of 40 mg/kg-d, the MOE for infants mouthing soft plastic articles is between 7400 (upper bound exposure) and 29000 (mean exposure).

#### **5.5.5.5 Recommendation**

Based on the limited information available, there is no evidence that DINX presents a hazard to infants or toddlers mouthing soft plastic articles. However, given the lack of publically available information on DINX, the CHAP strongly encourages the



appropriate U.S. agencies to obtain the necessary toxicological and exposure data to assess any potential risk from DINX.

#### **5.5.5.6 Would this recommendation, if implemented, be expected to reduce exposure of children to DINX?**

No.

### **5.5.6 Tris(2-ethylhexyl) trimellitate (TOTM) CAS 3319-31-1**

#### **5.5.6.1 Adverse Effects**

##### **5.5.6.1.1 Animal**

##### **5.5.6.1.1.1 Systemic**

- United Nations Environment Programme (UNEP, 2002) reported an intermediate-term toxicity study in SD rats (5/sex/group) in which TOTM (0, 100, 300, 1000 mg/kg-day) was administered daily via gavage for 28 days. TOTM exposure did not induce any adverse effects in any treatment groups (NOAEL = 1000 mg/kg-day).
- Nuodex (1983) reported an intermediate-term toxicity study in Fischer 344 albino rats (M, 5/group) in which TOTM (0, 1000 mg/kg-day) was administered via gavage for 5 days/week for 4 weeks. Triglycerides in the treated rats were significantly lower than controls, however, body and organ weights in exposed rats were similar to controls.
- CMA (1986) and Hodgson (1987) reported a short-term feeding study in which Fischer 344 rats (5/sex/group) were administered TOTM (0, 0.2, 0.67, or 2%; M:0, 184, 642, 1826 mg/kg-day, F:0, 182, 666, 1641 mg/kg-day) in the diet for 4 weeks. TOTM significantly reduced red blood cell count and hemoglobin, and increased serum albumin (not dose related). TOTM also significantly increased absolute and relative liver weights (M&F; dose-related; NOAEL = 184 and 182 mg/kg-day). Biochemically, TOTM increased cyanide-insensitive palmitoyl CoA oxidation (pCoA) and carnitine acetyl transferase activity in the liver (M&F), and catalase activity (M). High-dose rats had histopathologically reduced cytoplasmic basophilia (F) and slightly increased centrilobular and periportal peroxisomes in the liver (M&F). The review author considered liver changes of questionable relevance to humans and considered the NOAEL to be 1826 mg/kg-day.
- CMA (1986) and Hodgson (1987) reported an intermediate-term toxicity study in which Fischer 344 rats (5/sex/group) were administered TOTM (0, 200, 700, 2000 mg/kg-day) daily via gavage for 21 days. TOTM significantly increased absolute and relative liver weight (F; not dose-related). Histologically, the quantity of neutral lipids in the liver was reduced. Biochemically, pCoA activity (M&F; 2000 mg/kg-day) and lauric acid 12-hydroxylase activity (M; all doses) was increased. Hepatic peroxisomes were increased in male rats (2000 mg/kg-day). The review author considered 2000 mg/kg-day to be the NOAEL for this study.

- Japan Ministry of Health and Welfare (JMHW, 1998) conducted a one-generation reproduction study (see below). No treatment-related effects were reported for body weight or food consumption.
- Huntington Life Sciences (2002) conducted a developmental toxicity test (see below). No significant changes in maternal body weight were observed during gestation or lactation for any dose group.
- UNEP (2002), EPA (1983), CMA (1983; 1985a; 1985b), and Zeiger *et al.* (1988) reported that TOTM does not induce reverse mutations in various strains of bacteria, forward mutations in the HGPRT locus in Chinese hamster ovary cells, unscheduled DNA synthesis in primary rat hepatocytes, or chromosomal aberrations in Chinese hamster lung cells *in vitro*. TOTM was also negative for dominant lethal mutations in Swiss white mice *in vivo*.

#### **5.5.6.1.1.2 Reproductive**

- Japan Ministry of Health and Welfare (JMHW, 1998) reported a one-generation reproduction study in rats in which TOTM was administered via gavage at 0, 100, 300, and 1000 mg/kg-day for 46 days to males (including mating) and 14 days prior to mating through LD 3 in females. Mid- and high-dose males had reduced numbers of spermatocytes and spermatids in the testes (NOAEL<sub>repro</sub>=100 mg/kg-day).

#### **5.5.6.1.1.3 Developmental**

- Huntington Life Sciences (2002) reported a pre- and postnatal developmental toxicity study in SD rats dosed with TOTM (0, 100, 500 or 1050 mg/kg-day) on GD 6–19 for the prenatal assessment and GD 6 through LD 20 for the postnatal assessment. Increases in the number of fetuses (from treated dams) exhibiting displaced testes were reported, but these were within historical control ranges. A statistically significant increase was seen in the number of high-dose male offspring with retained areolar regions (on PND 13 but not PND 18; a slight developmental delay; NOAEL = 1050 mg/kg-day).

### **5.5.6.2 Human**

- No published human studies.

### **5.5.6.3 Relevance to Humans**

The reported animal studies are assumed to be relevant to humans.

### **5.5.6.4 Weight of Evidence**

#### **5.5.6.4.1 Experimental Design**

The number of animals in the Japan Ministry of Health and Welfare study (JMHW, 1998) was small (n=12) when considering standard reproduction studies. The Huntington study (2002) had sufficient number of rats per group and appropriate study design. Studies assessing systemic effects were limited to a handful of short to intermediate duration exposures. These studies primarily were of low N (5 rats/group), suggesting that conclusions made from these studies may be of lower confidence.

#### **5.5.6.4.2 Replication**

Studies verifying changes in testicular spermatocytes and spermatids, displaced testes, and areola region development have not been performed. The CHAP recommends that the conservative NOAEL of 100 mg/kg-day derived in the Japanese study be assigned for TOTM. Systemic effects included increased liver weight (two studies), increased liver enzymes (two studies), increased peroxisomes (two studies), decreased triglycerides (one study), and changes in hematology (one study). Hepatic changes seen following exposure to TOTM (as to DEHT) paralleled those seen in rats following ortho phthalate exposures.

### **5.5.6.5 Risk Assessment Considerations**

#### **5.5.6.5.1 Exposure**

TOTM is a high production volume plasticizer used in electrical cable, lubricants, medical tubing, and controlled release pesticide formulations. It is preferred for use in high-temperature applications. TOTM was not found in toys or child care articles tested by CPSC. Estimates of daily exposure from toys and child care articles are not available. However, it is expected that TOTM will have a low leaching/migration rate and low volatility because of its high molecular weight and very low vapor pressure. TOTM has a lower migration rate than DEHP when assessed in medical tubing.

#### **5.5.6.5.2 Hazard**

Several repeated-dose studies ranging from 21 to 28 days in duration have been reported. In one study in F344 rats (CMA, 1986; Hodgson, 1987), TOTM exposure significantly reduced red blood cell counts and hemoglobin, and increased serum albumin. The NOAEL for these effects was 182 mg/kg-d. Evidence of peroxisome proliferation was also reported. The reproductive NOAEL was 100 mg/kg-d in a one-generation study in rats (JMHW, 1998). The developmental NOAEL was 1,050 mg/kg-d in SD rats exposed on either GD 6–19 or GD 6 to lactational day 20 (Huntingdon Life Sciences, 2002). Effects in male offspring included displaced testes and retained areolae (PND 13). The authors reported that the incidence of displaced testes was within the range of historical controls, and the retained areolae were absent by PND 18.

#### **5.5.6.5.3 Risk**

The margin of exposure cannot be calculated because data on exposure from toys and child care articles are not available.

#### **5.5.6.6 Recommendation to CPSC regarding children's toys and child care articles**

There is insufficient information on exposure to assess the potential risks of the use of TOTM in toys and child care articles. However, the migration of TOTM from PVC products is expected to be relatively low. The CHAP recommends no action on TOTM. However, the CHAP strongly recommends that appropriate exposure information be obtained before using TOTM in toys and child care products.

#### **5.5.6.7 Would this recommendation, if implemented, be expected to reduce exposure of children to TOTM?**

No.

## 6 References

- Adamsson, A., Salonen, V., Paranko, J., Toppari, J., 2009. Effects of maternal exposure to di-isononylphthalate (DINP) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE) on steroidogenesis in the fetal rat testis and adrenal gland. *Reproductive Toxicology* (Elmsford, NY) 28, 66–74.
- Adham, I.M., Emmen, J.M., Engel, W., 2000. The role of the testicular factor INSL3 in establishing the gonadal position. *Mol Cell Endocrinol* 160, 11–16.
- Adibi, J.J., Hauser, R., Williams, P.L., Whyatt, R.M., Calafat, A.M., Nelson, H., Herrick, R., Swan, S.H., 2009. Maternal urinary metabolites of di-(2-ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. *American Journal of Epidemiology* 169.
- Adibi, J.J., Perera, F.P., Jedrychowski, W., Camann, D.E., Barr, D., Jacek, R., Whyatt, R.M., 2003. Prenatal exposures to phthalates among women in New York City and Krakow, Poland. *Environ Health Perspect* 111, 1719–1722.
- Adibi, J.J., Whyatt, R.M., Williams, P.L., Calafat, A.M., Camann, D., Herrick, R., Nelson, H., Bhat, H.K., Perera, F.P., Silva, M.J., Hauser, R., 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. *Environ Health Perspect* 116, 467–473.
- 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 and Chemical Toxicology* 49, 2022–2029.
- Anderson, W.A., Castle, L., Scotter, M.J., Massey, R.C., Springall, C., 2001. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. *Food Addit Contam* 18, 1068–1074.
- Andrade, A.J., Grande, S.W., Talsness, C.E., Gericke, C., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I., 2006b. A dose response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Reproductive effects on adult male offspring rats. *Toxicology* 228, 85–97.
- Andrade, A.J., Grande, S.W., Talsness, C.E., Grote, K., Golombiewski, A., Sterner-Kock, A., Chahoud, I., 2006a. A dose-response study following *in utero* and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): Effects on androgenic status, developmental landmarks, and testicular histology in male offspring rats. *Toxicology* 225, 64–74.
- Angerer, J., Bird, M.G., Burke, T.A., Doerr, N.G., Needham, L., Robison, S.H., Sheldon, L., Zenick, H., 2006. Strategic biomonitoring initiatives: Moving the science forward. *ToxSci* 93, 3–10.

- Astill, B.D., Terhaar, C.J., Fassett, D.W., 1972. Toxicology and fate of 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate in the rat. *Toxicol Appl Pharmacol* 22, 387–399.
- Aylward, L.L., Lorber, M., Hays, S.M., 2011. Urinary DEHP metabolites and fasting time in NHANES. *Journal of Exposure Science and Environmental Epidemiology* (2011) 21, 615–624.
- Babich, M.A., Osterhout, C.A., 2010. Toxicity Review of Diisononyl Phthalate (DINP). U.S. Consumer Product Safety Commission, Bethesda, MD. April 2010.  
<https://www.cpsc.gov/PageFiles/126539/toxicityDINP.pdf>.
- Barber, E.D., 1994. Genetic toxicology testing of di(2-ethylhexyl) terephthalate. *Environmental and Molecular Mutagenesis* 23, 228–233.
- Barber, E.D., Topping, D.C., 1995. Subchronic 90-day oral toxicology of di(2-ethylhexyl) terephthalate in the rat. *Food and Chemical Toxicology* 33, 971–978.
- Barlow, N.J., Foster, P.M., 2003. Pathogenesis of male reproductive tract lesions from gestation through adulthood following *in utero* exposure to Di(n-butyl) phthalate. *Toxicol Pathol* 31, 397–410.
- BASF, 2003. Results of a full-scale prenatal developmental toxicity study in Wistar rats with bis-(2-propylheptyl) phthalate. BASF Corporation. October 2003. 8HEQ-1003-15438.
- BASF, 2005. Summary of an unpublished 24-months combined chronic toxicity/carcinogenicity study in Wistar rats with 1,2-cyclohexanedicarboxylic acid, dinonly ester, branched and linear, CASRN 474919-59-0. BASF Corporation. EPA ID 8HEQ-0805-16146A; OTS 88050000352.
- Becker, K., Goen, T., Seiwert, M., Conrad, A., Pick-Fuss, H., Muller, J., Wittassek, M., Schulz, C., Kolossa-Gehring, M., 2009. GerES IV: phthalate metabolites and bisphenol A in urine of German children. *International Journal of Hygiene and Environmental Health* 212, 685–692.
- Becker, K., Seiwert, M., Angerer, J., Heger, W., Koch, H.M., Nagorka, R., Roßkamp, E., Schlüter, C., Seifert, B., Ullrich, D., 2004. DEHP metabolites in urine of children and DEHP in house dust. *International Journal of Hygiene and Environmental Health* 207, 409–417.
- 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.
- Benson, R., 2009. Hazard to the developing male reproductive system from cumulative exposure to phthalate esters—dibutyl phthalate, diisobutyl phthalate, butylbenzyl phthalate, diethylhexyl phthalate, dipentyl phthalate, and diisononyl phthalate. *Regul Toxicol Pharmacol* 53, 90–101.

- Berman, T., Hochner-Celnikier, D., Calafat, A.M., Needham, L.L., Amitai, Y., Wormser, U., Richter, E., 2009. Phthalate exposure among pregnant women in Jerusalem, Israel: Results of a pilot study. *Environment International* 35, 353–357.
- BIBRA, 1986. A 21-day feeding study of diisodecyl phthalate to rats: Effects on the liver and liver lipids. British Industrial Biological Research Association (BIBRA), Project No 3.0495.5, Report No 0495/5/85 submitted to the Chemical Manufacturers Association (CMA). As cited in CERHR, 2003; NICNAS, 2008.
- Blount, B.C., Silva, M.J., Caudill, S.P., Needham, L.L., Pirkle, J.L., Sampson, E.J., Lucier, G.W., Jackson, R.J., Brock, J.W., 2000. Levels of seven urinary phthalate metabolites in a human reference population. *Environ Health Perspect* 108, 979–982.
- Blystone, C.R., Kissling, G.E., Bishop, J.B., Chapin, R.E., Wolfe, G.W., Foster, P.M.D., 2010. Determination of the di-(2-ethylhexyl) phthalate NOAEL for reproductive development in the rat: Importance of the retention of extra animals to adulthood. *Toxicol Sci* 116, 640–646.
- Boas, M., Frederiksen, H., Feldt-Rasmussen, U., Skakkebaek, N.E., Hegedus, L., Hilsted, L., Juul, A., Main, K.M., 2010. Childhood exposure to phthalates: Associations with thyroid function, insulin-like growth factor I, and growth. *Environ Health Perspect* 118, 1458–1464.
- Boberg, J., Christiansen, S., Axelstad, M., Kledal, T.S., Vinggaard, A.M., Dalgaard, M., Nellemann, C., Hass, U., 2011. Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. *Reproductive Toxicology* (Elmsford, NY) 31, 200–209.
- Boberg, J., Metzдорff, S., Wortziger, R., Axelstad, M., Brokken, L., Vinggaard, A.M., Dalgaard, M., Nellemann, C., 2008. Impact of diisobutyl phthalate and other PPAR agonists on steroidogenesis and plasma insulin and leptin levels in fetal rats. *Toxicology* 250, 75–81.
- Borch, J., Axelstad, M., Vinggaard, A.M., Dalgaard, M., 2006a. Diisobutyl phthalate has comparable anti-androgenic effects to di-n-butyl phthalate in fetal rat testis. *Toxicol Lett* 163, 183–190.
- 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. *Reproductive Toxicology* (Elmsford, NY) 18, 53–61.
- Borch, J., Metzдорff, S.B., Vinggaard, A.M., Brokken, L., Dalgaard, M., 2006b. Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. *Toxicology* 223, 144–155.
- Braun, J.M., Smith, K.N., Williams, P.L., Calafat, A.M., Ehrlich, B.K., Hauser, R., 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. *Environ Health Perspect* 120, 739–745.

- Brennan, J., Capel, B., 2004. One tissue, two fates: Molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet* 5, 509–521.
- Brock, J.W., Caudill, S.P., Silva, M.J., Needham, L.L., Hilborn, E.D., 2002. Phthalate monoesters levels in the urine of young children. *Bull Environ Contam Toxicol* 68, 309–314.
- Calafat, A.M., Brock, J.W., Silva, M.J., Gray, L.E. Jr., Reidy, J.A., Barr, D.B., Needham, L.L., 2006. Urinary and amniotic fluid levels of phthalate monoesters in rats after the oral administration of di(2-ethylhexyl) phthalate and di-n-butyl phthalate. *Toxicology* 217, 22–30.
- Calafat, A.M., McKee, R.H., 2006. Integrating biomonitoring exposure data into the risk assessment process: Phthalates [diethyl phthalate and di(2-ethylhexyl) phthalate] as a case study. *Environ Health Perspect* 114, 1783–1789.
- Caldwell, D.J., Eldridge, S., Lington, A.W., McKee, R.H., 1999. Retrospective evaluation of alpha 2u-globulin accumulation in male rat kidneys following high doses of diisononyl phthalate. *ToxSci* 51, 153–160.
- Capel, B., 2000. The battle of the sexes. *Mech Dev* 92, 89–103.
- Carlson, K.R., 2010. Toxicity Review of Di-*n*-Octyl Phthalate (DnOP). U.S. Consumer Product Safety Commission, Bethesda, MD. March 2010.  
<https://www.cpsc.gov/PageFiles/126540/toxicityDNOP.pdf>.
- Carlson, K.R., Patton, L.E., 2012. U.S. CPSC staff assessment of phthalate dietary exposure using two food residue data sets and three food categorization schemes. U.S. Consumer Product Safety Commission, Bethesda, MD. February 2012.
- Carruthers, C.M., Foster, P.M.D., 2005. Critical window of male reproductive tract development in rats following gestational exposure to di-n-butyl phthalate. *Birth Defects Res B Dev Reprod Toxicol* 74, 277–285.
- Cattley, R.C., DeLuca, J., Elcombe, C., Fenner-Crisp, P., Lake, B.G., Marsman, D.S., Pastoor, T.A., Popp, J.A., Robinson, D.E., Schwetz, B., Tugwood, J., Wahli, W., 1998. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regul Toxicol Pharmacol* 27, 47–60.
- CDC, 2012a. Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, February 2012. Centers for Disease Control and 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.
- CEH, 2009. Plasticizers. Chemical Economics Handbook-SRI Consulting.



- Chase, K.R., Willoughby, C.R., 2002. Citroflex A-4 toxicity study by dietary administration to Han Wistar rats for 13 weeks with an *in utero* exposure phase followed by a 4-week recovery period. Huntingdon Life Sciences, Ltd., UK. Project No. MOX 022/013180.
- Chen, S.-B., 1998. Laboratory Sciences Report on the Migration of Diisononyl Phthalate from Polyvinyl Chloride Children's Products U.S. Consumer Product Safety Commission, Bethesda, MD 20814. November 25, 1998.
- Chen, S.-B., 2002. Screening of Toys for PVC and Phthalates Migration, Bethesda, MD. In CPSC 2002. June 20, 2002.
- Chevalier, S., Roberts, R.A., 1998. Perturbation of rodent hepatocyte growth control by nongenotoxic hepatocarcinogens: Mechanisms and lack of relevance for human health (review). *Oncol Rep.* 5, 1319–1327.
- Cho, S.C., Bhang, S.Y., Hong, Y.C., Shin, M.S., Kim, B.N., Kim, J.W., Yoo, H.J., Cho, I.H., Kim, H.W., 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. *Environ Health Perspect* 118, 1027–1032.
- Cho, W.-S., Han, B.S., Ahn, B., Nam, K.T., Choi, M., Oh, S.Y., Kim, S.H., Jeong, J., Jang, D.D., 2008. Peroxisome proliferator di-isodecyl phthalate has no carcinogenic potential in Fischer344 rats. *Toxicology Letters* 178, 110–116.
- Christiansen, S., Boberg, J., Axelstad, M., Dalgaard, M., Vinggaard, A.M., Metzdorff, S.B., Hass, U., 2010. Low-dose perinatal exposure to di(2-ethylhexyl) phthalate induces anti-androgenic effects in male rats. *Reproductive Toxicology (Elmsford, NY)* 30, 313–321.
- 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.
- Clark, K.E., David, R.M., Guinn, R., Kramarz, K.W., Lampi, M.A., Staples, C.A., 2011. Modeling human exposure to phthalate esters: A comparison of indirect and biomonitoring estimation methods. *Human and Ecological Risk Assessment* 17, 923–965.
- Clewell, R.A., Sochaski, M., Edwards, K., Creasy, D.M., Willson, G., Andersen, M.E., 2013a. Disposition of diisononyl phthalate and its effects on sexual development of the male fetus following repeated dosing in pregnant rats. *Reproductive Toxicology (Elmsford, NY)* 35, 56–69.
- Clewell, R.A., Thomas, A., Willson, G., Creasy, D.M., Andersen, M.E., 2013b. A dose response study to assess effects after dietary administration of diisononyl phthalate (DINP) in gestation and lactation on male rat sexual development. *Reproductive Toxicology (Elmsford, NY)* 35, 70–80.
- CMA, 1983. Tris(2-ethylhexyl) trimellitate: A voluntary testing program under Section 4 of the Toxic Substances Control Act (Final Revision). Chemical Manufacturers Association (CMA). OTS 0510616. Doc. ID 40-8365005.

- CMA, 1985a. Evaluation of tris(2-ethylhexyl) trimellitate in the CHO/HGPRT forward mutation assay (Final Report) with cover letter dated 062485. Chemical Manufacturers Association (CMA). OTS 0510642. Doc. ID 40-8565041.
- CMA, 1985b. Evaluation of tris(2-ethylhexyl) trimellitate in the rat primary hepatocyte unscheduled DNA synthesis assay. Chemical Manufacturers Association (CMA). Final Report. OTS0510641. Doc. ID 40-8565039.
- CMA, 1986. A 28-day toxicity study with tri(2-ethylhexyl) trimellitate in the rat and EPA acknowledgement letter. Chemical Manufacturers Association (CMA). EPA ID 8688700000425; OTS 0513174.
- CPSC, 2001. Report to the U.S. Consumer Product Safety Commission by the Chronic Hazard Advisory Panel on Diisononyl Phthalate (DINP). U.S. Consumer Product Safety Commission, Bethesda, MD. June 2001.  
<http://www.cpsc.gov/PageFiles/98260/dinp.pdf>.
- CPSIA, 2008. Consumer Product Safety Improvement Act (CPSIA) of 2008. Public Law 110-314. Consumer Product Safety Commission, Bethesda, MD.
- CTFA, 1998. Acetyl tributyl citrate dossier for evaluation. Cosmetic Toiletry and Fragrance Association (CTFA). December 4, 1998. Prepared for the ATBC Industry Group by Toxicology International.
- Dalgaard, M., Hass, U., Lam, H.R., Vinggaard, A.M., Sorensen, I.K., Jarfelt, K., Ladefoged, O., 2002. Di(2-ethylhexyl) adipate (DEHA) is foetotoxic but not anti-androgenic as di(2-ethylhexyl)phthalate (DEHP). *Reproductive Toxicology* (Elmsford, NY) 16, 408.
- Dalgaard, M., Hass, U., Vinggaard, A.M., Jarfelt, K., Lam, H.R., Sorensen, I.K., Sommer, H.M., Ladefoged, O., 2003. Di(2-ethylhexyl) adipate (DEHA)-induced developmental toxicity but not antiandrogenic effects in pre- and postnatally exposed Wistar rats. *Reproductive Toxicology* (Elmsford, NY) 17, 163–170.
- David, R.M., 2000. Exposure to phthalate esters. *Environ Health Perspect* 108, A440.
- David, R.M., 2006. Proposed mode of action for *in utero* effects of some phthalate esters on the developing male reproductive tract. *Toxicol Pathol* 34, 209–219.
- David, R.M., Moore, M.R., Finney, D.C., Guest, D., 2000. Chronic toxicity of di(2-ethylhexyl) phthalate in mice. *ToxSci* 58, 377–385.
- Davis, P., 1991. Technical report on the metabolism of acetyltributylcitrate (ATBC) and tributylcitrate (TBC) in human serum and rat liver homogenates. University of Texas, USA. As cited in U.S. EPA (2008).
- Desdoits-Lethimonier, C., Albert, O., Le Bizec, B., Perdu, E., Zalko, D., Courant, F., Lesné, L., Guillé, F., Dejucq-Rainsford, N., Jégou, B., 2012. Human testis steroidogenesis is inhibited by phthalates. *Human Reproduction* 27, 1451–1459.

- Deyo, J.A., 2008. Carcinogenicity and chronic toxicity of di-2-ethylhexyl terephthalate (DEHT) following a two-year dietary exposure in Fischer-344 rats. *Food and Chemical Toxicology* 46, 990–1005.
- Divincenzo, G.D., Hamilton, M.L., Mueller, K.R., Donish, W.H., Barber, E.D., 1985. Bacterial mutagenicity testing of urine from rats dosed with 2-ethylhexanol derived plasticizers. *Toxicology* 34, 247–259.
- Doull, J., Cattley, R., Elcombe, C., Lake, B.G., Swenberg, J., Wilkinson, C., Williams, G., van Gemert, M., 1999. A cancer risk assessment of di(2-ethylhexyl) phthalate: Application of the new U.S. EPA Risk Assessment Guidelines. *Regul Toxicol Pharmacol* 29, 327–357.
- Dow, 1992. Metabolism and disposition of acetyl tributyl citrate in male Sprague-Dawley rats. Sanitized Laboratory Report. Dow Chemical Company. As cited in U.S. EPA (2008).
- Dreyfus, M., 2010. Phthalates and Phthalate Substitutes in Children’s Toys. U.S. Consumer Product Safety Commission, Bethesda, MD. March 2010.  
<http://www.cpsc.gov/PageFiles/126545/phthallab.pdf>.
- Duty, S.M., Ackerman, R.M., Calafat, A.M., Hauser, R., 2005a. Personal care product use predicts urinary concentrations of some phthalate monoesters. *Environ Health Perspect* 113, 1530–1535.
- Duty, S.M., Calafat, A.M., Silva, M.J., Brock, J.W., Ryan, L., Chen, Z., Overstreet, J., Hauser, R., 2004. The relationship between environmental exposure to phthalates and computer-aided sperm analysis motion parameters. *J Androl* 25, 293–302.
- Duty, S.M., Calafat, A.M., Silva, M.J., Ryan, L., Hauser, R., 2005b. Phthalate exposure and reproductive hormones in adult men. *Human Reproduction* 20, 604–610.
- Eastman, 1975. Basic toxicology of bis(2-ethylhexyl)terephthalate (dioctyl terephthalate, DOTP). Eastman Kodak Company. TSCATS Fiche OTS0206571.
- Eastman, 1983. Toxicity and Health Hazard Summary with Cover Letters. Eastman Kodak Company. OTS 0206572. Doc. ID 878214436.
- Eastman, 2001. Reproduction/developmental toxicity screening test in the rat with 2,2,4-trimethyl-1,3-pentanediol diisobutyrate - Final Report w/Cover Letter Dated 082401. Eastman Chemical Company, Kingsport, TN. August 2001. Submitted to U.S. EPA. U.S. EPA/OPTS Public Files; Fiche #: OTS0560045-1; Doc#: 89010000299. TSCATS.
- Eastman, 2007. Toxicity summary for Eastman TXIB<sup>®</sup> formulation additive. Eastman Chemical Company, Kingsport, TN. November 2007.
- ECB, 2000. Substance ID: 27554-26-3. Diisooctyl phthalate. IUCLID Dataset. European Chemicals Bureau.

- Edlund, P.O., Ostelius, J., 1991. *In vitro* hydrolysis of acetyl-tributylcitrate in human serum and rat liver homogenate. Kabi Invent/Procordia OraTech for Procordia Oratec, Inc. As cited in U.S. EPA (2008).
- Eisenberg, M.L., Jensen, T.K., Walters, R.C., Skakkebaek, N.E., Lipshultz, L.I., 2011. The relationship between anogenital distance and reproductive hormone levels in adult men. *J Urol* 187, 594–598.
- Ema, M., Amano, H., Itami, T., Kawasaki, H., 1993. Teratogenic evaluation of di-n-butyl phthalate in rats. *Toxicol Lett* 69, 197–203.
- Ema, M., Amano, H., Ogawa, Y., 1994. Characterization of the developmental toxicity of di-n-butyl phthalate in rats. *Toxicology* 86, 163–174.
- Ema, M., Itami, T., Kawasaki, H., 1992. Teratogenic evaluation of phthalate in rats by gastric intubation. *Toxicol Lett* 61, 1–7.
- Ema, M., Kurosaka, R., Amano, H., Ogawa, Y., 1995. Developmental toxicity evaluation of mono-n-butyl phthalate in rats. *Toxicol Lett* 78, 101–106.
- Ema, M., Miyawaki, E., 2002. Effects on development of the reproductive system in male offspring of rats given butyl benzyl phthalate during late pregnancy. *Reproductive Toxicology* (Elmsford, NY) 16, 71–76.
- Ema, M., Miyawaki, E., Hirose, A., Kamata, E., 2003. Decreased anogenital distance and increased incidence of undescended testes in fetuses of rats given monobenzyl phthalate, a major metabolite of butyl benzyl phthalate. *Reproductive Toxicology* (Elmsford, NY) 17, 407–412.
- Ema, M., Miyawaki, E., Kawashima, K., 1998. Further evaluation of developmental toxicity of di-n-butyl phthalate following administration during late pregnancy in rats. *Toxicol Lett* 98, 87–93.
- Ema, M., Murai, T., Itami, T., Kawasaki, H., 1990. Evaluation of the teratogenic potential of the plasticizer butyl benzyl phthalate in rats. *JAT* 10, 339–343.
- Engel, S.M., Miodovnik, A., Canfield, R.L., Zhu, C., Silva, M.J., Calafat, A.M., Wolff, M.S., 2010. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. *Environ Health Perspect* 118, 565–571.
- Engel, S.M., Zhu, C., Berkowitz, G.S., Calafat, A.M., Silva, M.J., Miodovnik, A., Wolff, M.S., 2009. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. *Neurotoxicology* 30, 522–528.
- EPA, 1983. Bacterial mutagenicity test results. OTS 206016. Doc. ID 878211440.

- EPA, 1993. Reference Dose (RfD): Description and Use in Health Risk Assessments. Background Document 1A. Environmental Protection Agency. March 15, 1993. <http://www.epa.gov/iris/rfd.htm>. Accessed April 4, 2013.
- EPA, 1998. Assessment of Thyroid Follicular Cell Tumors. Risk Assessment Forum. U.S. Environmental Protection Agency, Washington, DC., EPA/630/R-97/002.
- Exxon, 1997. Two-generation reproduction toxicity study in rats with di-isodecyl phthalate (DIDP; MRD-94-775). Exxon Biomedical Sciences, Inc., East Millstone, NJ.
- ExxonMobil, 2000. Two-generation reproduction toxicity study in rats with MRD-94-775 [DIDP]. Project Number 1775355A. ExxonMobil Biomedical Sciences, Inc., East Millstone, NJ.
- Faber, W.D., Deyo, J.A., Stump, D.G., Navarro, L., Ruble, K., Knapp, J., 2007a. Developmental toxicity and uterotrophic studies with di-2-ethylhexyl terephthalate. *Birth Defects Res B Dev Reprod Toxicol* 80, 396–405.
- Faber, W.D., Deyo, J.A., Stump, D.G., Ruble, K., 2007b. Two-generation reproduction study of di-2-ethylhexyl terephthalate in Crl:CD rats. *Birth Defects Res B Dev Reprod Toxicol* 80, 69–81.
- Fabjan, E., Hulzebos, E., Mennes, W., Piersma, A.H., 2006. A category approach for reproductive effects of phthalates. *Crit Rev Toxicol* 36, 695–726.
- Field, E.A., Price, C.J., Marr, M.C., Myers, C.B., 1989. Developmental toxicity evaluation of butyl benzyl phthalate (CAS No. 85-68-7) administered in feed to CD rats on gestational days 6 to 15. National Toxicology Program. Research Triangle Park, NC. NTP Study Number: TER88025. <http://ntp.niehs.nih.gov/index.cfm?objectid=07304777-91CB-60E1-1ED36A4D76C04359>.
- Field, E.A., Price, C.J., Sleet, R.B., George, J.D., Marr, M.C., Myers, C.B., Schwetz, B.A., Morrissey, R.E., 1993. Developmental toxicity evaluation of diethyl and dimethyl phthalate in rats. *Teratology* 48, 33–44.
- Finkelstein, M., Gold, H., 1959. Toxicology of the citric acid esters: tributyl citrate, acetyl tributyl citrate, triethyl citrate, and acetyl triethyl citrate. *Toxicol Appl Pharmacol* 1, 283–298.
- Foster, P.M., 2006. Disruption of reproductive development in male rat offspring following *in utero* exposure to phthalate esters. *Int J Androl* 29, 140–147; Discussion 181–145.
- Foster, P.M., Bishop, J., Chapin, R., Kissling, G.E., Wolfe, G.W., 2006. Determination of the di-(2-ethylhexyl) phthalate (DEHP) NOAEL for reproductive development in the rat: Importance of retention of extra F1 animals. *Toxicologist* 90, 430.

- Foster, P.M., Thomas, L.V., Cook, M.W., Gangolli, S.D., 1980. Study of the testicular effects and changes in zinc excretion produced by some n-alkyl phthalates in the rat. *Toxicol Appl Pharmacol* 54, 392-398.
- Foster, P.M.D., 2005. Mode of action: Impaired fetal Leydig cell function—Effects on male reproductive development produced by certain phthalate esters. *Critical Reviews in Toxicology* 35, 713–719.
- Frederiksen, H., Aksglaede, L., Sorensen, K., Skakkebaek, N.E., Juul, A., Andersson, A.M., 2011. Urinary excretion of phthalate metabolites in 129 healthy Danish children and adolescents: Estimation of daily phthalate intake. *Environ Res* 111, 656–663.
- Fromme, H., Bolte, G., Koch, H.M., Angerer, J., Boehmer, S., Drexler, H., Mayer, R., Liebl, B., 2007. Occurrence and daily variation of phthalate metabolites in the urine of an adult population. *International Journal of Hygiene and Environmental Health* 210, 21–33.
- Fromme, H., Gruber, L., Schlummer, M., Wolz, G., Bohmer, S., Angerer, J., Mayer, R., Liebl, B., Bolte, G., 2007b. Intake of phthalates and di(2-ethylhexyl) adipate: Results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. *Environment International* 33, 1012–1020.
- Fujii, S., Yabe, K., Furukawa, M., Hirata, M., Kiguchi, M., Ikka, T., 2005. A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats. *J Toxicol Sci* 30 Spec No., 97–116.
- Gaido, K.W., Hensley, J.B., Liu, D., Wallace, D.G., Borghoff, S., Johnson, K.J., Hall, S.J., Boekelheide, K., 2007. Fetal mouse phthalate exposure shows that gonocyte multinucleation is not associated with decreased testicular testosterone. *ToxSci* 97, 491–503.
- Gazouli, M., Yao, Z.X., Boujrad, N., Corton, J.C., Culty, M., Papadopoulos, V., 2002. Effect of peroxisome proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport, and steroidogenesis: Role of the peroxisome proliferator-activator receptor alpha. *Endocrinology* 143, 2571–2583.
- GMRL, 1981. Toxicity and fate of di-iso decyl phthalate following the inhalation exposure in rats 878210881. General Motors Research Laboratories. Warren, MI. As cited in CERHR 2003.
- Goen, T., Dobler, L., Koschorreck, J., Muller, J., Wiesmuller, G.A., Drexler, H., Kolossa-Gehring, M., 2011. Trends of the internal phthalate exposure of young adults in Germany—Follow-up of a retrospective human biomonitoring study. *International Journal of Hygiene and Environmental Health* 215, 36–45.
- Grande, S.W., Andrade, A.J., Talsness, C.E., Grote, K., Chahoud, I., 2006. A dose-response study following *in utero* and lactational exposure to di(2-ethylhexyl) phthalate: Effects on female rat reproductive development. *ToxSci* 91, 247–254.

- Grasso, P., 1981. Di-2-ethylhexyl and other phthalate esters: An appraisal of the toxicological data. BP Chemicals, Ltd. CTL report I24070. (as cited in ECB, 2000).
- Gray, L.E., Jr., Barlow, N.J., Howdeshell, K.L., Ostby, J.S., Furr, J.R., Gray, C.L., 2009. Transgenerational effects of di (2-ethylhexyl) phthalate in the male CRL:CD(SD) rat: Added value of assessing multiple offspring per litter. *ToxSci* 110, 411–425.
- 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. *ToxSci* 58, 350–365.
- Gray, L.E.J., Laskey, J., Ostby, J., 2006. Chronic di-*n*-butyl phthalate exposure in rats reduces fertility and alters ovarian function during pregnancy in female Long Evans hooded rats. *ToxSci* 93, 189–195.
- Gray, T.J., Rowland, I.R., Foster, P.M., Gangolli, S.D., 1982. Species differences in the testicular toxicity of phthalate esters. *Toxicol Lett* 11, 141–147.
- Gulati, D.K., Chambers, R., Shaver, S., Sabwehrwal, P.S., Lamb, J.C.t., 1985. Di-*n*-octyl phthalate reproductive and fertility assessment in CD-1 mice when administered in feed. National Toxicology Program, Research Triangle Park, NC. April 1985. NTP report no. RACB85047.
- Guo, Y., Wu, Q., Kannan, K., 2011. Phthalate metabolites in urine from China, and implications for human exposures. *Environment International* 37, 893–898.
- Guyatt, G., Oxman, A.D., Akl, E.A., Kunz, R., Vist, G., Brozek, J., Norris, S., Falck-Ytter, Y., Glasziou, P., DeBeer, H., Jaeschke, R., Rind, D., Meerpohl, J., Dahm, P., Schunemann, H.J., 2011. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *Journal of Clinical Epidemiology* 64, 383–394.
- Hallmark, N., Walker, M., McKinnell, C., Mahood, I.K., Scott, H., Bayne, R., Coutts, S., Anderson, R.A., Greig, I., Morris, K., Sharpe, R.M., 2007. Effects of monobutyl and di(*n*-butyl) phthalate *in vitro* on steroidogenesis and Leydig cell aggregation in fetal testis explants from the rat: Comparison with effects *in vivo* in the fetal rat and neonatal marmoset and *in vitro* in the human. *Environ Health Perspect* 115, 390–396.
- 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. *ToxSci* 120, 184–193.
- Hannas, B.R., Lambright, C., Furr, J., Evans, N., Foster, P., Gray, L., Wilson, V.S., 2012. Evaluation of genomic biomarkers and relative potency of phthalate-induced male reproductive developmental toxicity using a targeted RTPCR array approach. *Toxicologist* 126, 23–38.

- 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, diisooheptyl phthalate, and diisononyl phthalate. *ToxSci* 123, 206–216.
- Hardin, B.D., Schuler, R.L., Burg, J.R., Booth, G.M., Hazelden, K.P., MacKenzie, K.M., Piccirillo, V.J., Smith, K.N., 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. *Teratog Carcinog Mutagen* 7, 29–48.
- Harper, H.A., Rodwell, V.W., Mayes, P.A., 1977. *Review of Physiological Chemistry*, Lange Medical Publications, Los Altos, CA.
- Hauser, R., Duty, S., Godfrey-Bailey, L., Calafat, A.M., 2004. Medications as a source of human exposure to phthalates. *Environ Health Perspect* 112, 751–753.
- Hauser, R., Meeker, J.D., Singh, N.P., Silva, M.J., Ryan, L., Duty, S., Calafat, A.M., 2007. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. *Hum Reprod* 22, 688–695.
- Hazleton, 1968a. Three-month dietary administration - albino rats DIDP - FDA grade (plasticizer) Hazleton Laboratories. Submitted to Dewey and Almy Chemical Division, WR Grace and Company. As cited in CERHR, 2003.
- Hazleton, 1968b. 13-week dietary administration - dogs plasticizer (DIDP) Hazleton Laboratories. Submitted to WR Grace and Company. As cited in CERHR, 2003..
- Heger, N.E., Hall, S.J., Sandrof, M.A., McDonnell, E.V., Hensley, J.B., McDowell, E.N., Martin, K.A., Gaido, K.W., Johnson, K.J., Boekelheide, K., 2012. Human fetal testis xenografts are resistant to phthalate-induced endocrine disruption. *Environ Health Perspect* 20, 1137–1143.
- Heindel, J.J., Gulati, D.K., Mounce, R.C., Russell, S.R., Lamb, J.C.t., 1989. Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. *Fundam Appl Toxicol* 12, 508–518.
- Hellwig, J., Freudenberger, H., Jackh, R., 1997. Differential prenatal toxicity of branched phthalate esters in rats. *Food and Chemical Toxicology* 35, 501–512.
- Hellwig, J., Jackh, R., 1997. Differential prenatal toxicity of one straight-chain and five branched-chain primary alcohols in rats. *Food and Chemical Toxicology* 35, 489–500.
- Higgins, J.P., Altman, D.G., Gotzsche, P.C., Juni, P., Moher, D., Oxman, A.D., Savovic, J., Schulz, K.F., Weeks, L., Sterne, J.A., 2011. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ (Clinical research ed.)* 343, d5928.
- 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. *ToxSci* 72, 301–313.



- Hinton, R.H., Mitchell, F.E., Mann, A., Chescoe, D., Price, S.C., Nunn, A., Grasso, P., Bridges, J.W., 1986. Effects of phthalic acid esters on the liver and thyroid. *Environ Health Perspect* 70, 195–210.
- Hiort, O., Holterhus, P.M., 2000. The molecular basis of male sexual differentiation. *Eur J Endocrinol* 142, 101–110.
- Hodge, H., 1954. Preliminary acute toxicity tests and short term feeding tests of rats and dogs given di-isobutyl phthalate and di-butyl phthalate. University of Rochester, Rochester, NY. Submitted under TSCA Section 8D; EPA document number 87821033. OTS 0205995.
- Hodge, H.C., Maynard, E.A., Downs, W.L., Ashton, J.K., Salerno, L.L., 1966. Tests on mice for evaluating carcinogenicity. *Toxicol Appl Pharmacol* 9, 583–596.
- Hodgson, J.R., 1987. Results of peroxisome induction studies on tri(2-ethylhexyl) trimellitate and 2-ethylhexanol. *Toxicology and Industrial Health* 3, 49.
- Hoppin, J.A., Ulmer, R., London, S.J., 2004. Phthalate exposure and pulmonary function. *Environ Health Perspect* 112, 571–574.
- Hoppin, J.F., Brock, J.W., Davis, B.J., Baird, D.D., 2002. Reproducibility of urinary phthalate metabolites in first morning urine samples. *Environ Health Perspect.* 110, 515–518.
- Hoshino, N., Iwai, M., Okazaki, Y., 2005. A two-generation reproductive toxicity study of dicyclohexyl phthalate in rats. *J Toxicol Sci* 30 Spec No., 79–96.
- Hotchkiss, A.K., Parks-Saldutti, L.G., Ostby, J.S., Lambright, C., Furr, J., Vandenberg, J.G., Gray, L.E. Jr., 2004. A mixture of the “antiandrogens” linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion. *Biol Reprod* 71, 1852–1861.
- Howdeshell, K.L., Furr, J., Lambright, C.R., Rider, C.V., Wilson, V.S., Gray, L.E. Jr., 2007. Cumulative effects of dibutyl phthalate and diethylhexyl phthalate on male rat reproductive tract development: Altered fetal steroid hormones and genes. *ToxSci* 99, 190–202.
- 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. *ToxSci* 105, 153–165.
- Hsieh, M.H., Breyer, B.N., Eisenberg, M.L., Baskin, L.S., 2008. Associations among hypospadias, cryptorchidism, anogenital distance, and endocrine disruption. *Curr Urol Rep* 9, 137–142.
- Huang, P.C., Kuo, P.L., Chou, Y.Y., Lin, S.J., Lee, C.C., 2009. Association between prenatal exposure to phthalates and the health of newborns. *Environment International* 35, 14–20.

- Huang, P.C., Kuo, P.L., Guo, Y.L., Liao, P.C., Lee, C.C., 2007. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. *Hum Reprod* 22, 2715–2722.
- Hughes, I.A., 2000a. A novel explanation for resistance to androgens. *N Engl J Med* 343, 881–882.
- Hughes, I.A., 2001. Minireview: Sex differentiation. *Endocrinology* 142, 3281–3287.
- Hughes, P.I., 2000b. How vulnerable is the developing testis to the external environment? *Arch Dis Child* 83, 281–282.
- Huntingdon Life Sciences, 2002. TEHTM study for effects on embryo-fetal and pre- and post-natal development in CD rat by oral gavage administration. Huntingdon Life Sciences, Ltd. (2002). June 2002. Sanitized Version.
- Hushka, L.J., Waterman, S.J., Keller, L.H., Trimmer, G.W., Freeman, J.J., Ambroso, J.L., Nicolich, M., McKee, R.H., 2001. Two-generation reproduction studies in rats fed diisodecyl phthalate. *Reproductive Toxicology (Elmsford, NY)* 15, 153–169.
- IARC, 2000a. Di(2-ethylhexyl) adipate. IARC Monographs on the evaluation of carcinogenic risks to humans 77, 149–175.
- IARC, 2000b. Di(2-ethylhexyl) phthalate. IARC Monographs on the evaluation of carcinogenic risks to humans 77, 41–148.
- ICI, 1988. Di-(2-ethylhexyl) adipate (DEHA) fertility study in rats. ICI Central Toxicology Laboratory, Imperial Chemical Industries (ICI). Report no CTL/P/2229.
- Imajima, T., Shono, T., Zakaria, O., Suita, S., 1997. Prenatal phthalate causes cryptorchidism postnatally by inducing transabdominal ascent of the testis in fetal rats. *J Pediatr Surg* 32, 18–21.
- Ito, Y., Yamanoshita, O., Asaeda, N., Tagawa, Y., Lee, C.H., Aoyama, T., Ichihara, G., Furuhashi, K., Kamijima, M., Gonzalez, F.J., Nakajima, T., 2007. Di(2-ethylhexyl) phthalate induces hepatic tumorigenesis through a peroxisome proliferator-activated receptor alpha-independent pathway. *Journal of Occupational Health* 49, 172–182.
- Itoh, H., Yoshida, K., Masunaga, S., 2005. Evaluation of the effect of government control of human exposure to two phthalates using a urinary biomarker approach. *International Journal of Hygiene and Environmental Health* 208, 237–245.
- Itoh, H., Yoshida, K., Masunaga, S., 2007. Quantitative identification of unknown exposure pathways of phthalates based on measuring their metabolites in human urine. *Environmental Science and Technology* 41, 4542–4547.

- Jarfelt, K., Dalgaard, M., Hass, U., Borch, J., Jacobsen, H., Ladefoged, O., 2005. Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. *Reproductive Toxicology* (Elmsford, NY) 19, 505–515.
- Jiang, J., Ma, L., Yuan, L., Wang, X., Zhang, W., 2007. Study on developmental abnormalities in hypospadiac male rats induced by maternal exposure to di-n-butyl phthalate (DBP). *Toxicology* 232, 286–293.
- JMHLW, 1993. Japan Existing Chemical Data Base (JECDB). Test report on 2,2,4-trimethyl-1,3-pentanediol diisobutyrate ( 6846-50-0). Japanese Ministry of Health, Labor, and Welfare. Abstract only.
- JMHW, 1998. Toxicity Testing Report 6: 569-578. As cited in UNEP 2002.
- Jonker, I.D., Hollanders, V.M.H., 1990. Range-finding study (14-day, dietary) with acetyl tributyl citrate (ATBC) in rats. TNO Nutrition and Food Research, the Netherlands. Report no. V 90.335. As cited in EPA 2008.
- Jonker, I.D., Hollanders, V.M.H., 1991. Subchronic (90-day) dietary toxicity study with acetyl tributyl citrate (ATBC) in rats. TNO Nutrition and Food Research, the Netherlands. Report no. V 91.255. As cited in EPA 2008.
- Jönsson, B.A., Richthoff, J., Rylander, L., Giwercman, A., Hagmar, L., 2005. Urinary phthalate metabolites and biomarkers of reproductive function in young men. *Epidemiology* 16, 487–493.
- Kang, J.S., Morimura, K., Toda, C., Wanibuchi, H., Wei, M., Kojima, N., Fukushima, S., 2006. Testicular toxicity of DEHP, but not DEHA, is elevated under conditions of thioacetamide-induced liver damage. *Reproductive Toxicology* (Elmsford, NY) 21, 253–259.
- Khanna, S., Dogra, R.K.S., Bhatnagar, M.C., Shukla, L.J., Srivastava, S.N., Shanker, R., 1990. Nephrotoxicity of dioctyl phthalate treated rats: Histological evidence. *Environmental Biology* 11, 27–34.
- Kim, B.N., Cho, S.C., Kim, Y., Shin, M.S., Yoo, H.J., Kim, J.W., Yang, Y.H., Kim, H.W., Bhang, S.Y., Hong, Y.C., 2009. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. *Biol Psychiatry* 66, 958–963.
- Kim, T.S., Jung, K.K., Kim, S.S., Kang, I.H., Baek, J.H., Nam, H.S., Hong, S.K., Lee, B.M., Hong, J.T., Oh, K.W., Kim, H.S., Han, S.Y., Kang, T.S., 2010. Effects of in utero exposure to di(n-butyl) phthalate on development of male reproductive tracts in Sprague-Dawley rats. *J Toxicol Environ Health A* 73, 1544–1559.
- Kim, Y., Ha, E.H., Kim, E.J., Park, H., Ha, M., Kim, J.H., Hong, Y.C., Chang, N., Kim, B.N., 2011. Prenatal exposure to phthalates and infant development at 6 months: Prospective Mothers and Children's Environmental Health (MOCEH) study. *Environ Health Perspect* 119, 1495–1500.

- Klaunig, J.E., Babich, M.A., Baetcke, K.P., Cook, J.C., Corton, J.C., David, R.M., DeLuca, J.G., Lai, D.Y., McKee, R.H., Peters, J.M., Roberts, R.A., Fenner-Crisp, P.A., 2003. PPAR $\alpha$  agonist-induced rodent tumors: Modes of action and human relevance. *Critical Reviews in Toxicology* 33, 655–780.
- Klimisch, H.J., Andreae, M., Tillmann, U., 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data. *Regul Toxicol Pharmacol* 25, 1–5.
- Koch, H.M., Angerer, J., 2007a. Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. *International Journal of Hygiene and Environmental Health* 210, 9–19.
- Koch, H.M., Angerer, J., Drexler, H., Eckstein, R., Weisbach, V., 2005a. Di(2-ethylhexyl) phthalate (DEHP) exposure of voluntary plasma and platelet donors. *International Journal of Hygiene and Environmental Health* 208, 489–498.
- Koch, H.M., Becker, K., Wittassek, M., Seiwert, M., Angerer, J., Kolossa-Gehring, M., 2007. Di-*n*-butyl phthalate and butylbenzyl phthalate–urinary metabolite levels and estimated daily intakes: Pilot study for the German Environmental Survey on children. *J Expo Sci Environ Epidemiol* 17, 378–387.
- Koch, H.M., Bolt, H.M., Angerer, J., 2004a. Di(2-ethylhexyl) phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. *Arch Toxicol* 78, 123–130.
- Koch, H.M., Bolt, H.M., 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, 367–376.
- Koch, H.M., Bolt, H.M., Preuss, R., Eckstein, R., Weisbach, V., Angerer, J., 2005b. Intravenous exposure to di(2-ethylhexyl) phthalate (DEHP): Metabolites of DEHP in urine after a voluntary platelet donation. *Arch Toxicol* 79, 689–693.
- Koch, H.M., Calafat, A.M., 2009. Human body burdens of chemicals used in plastic manufacture. *Philos Trans R Soc Lond B Biol Sci* 364, 2063–2078.
- Koch, H.M., Drexler, H., Angerer, J., 2003a. An estimation of the daily intake of di(2-ethylhexyl) phthalate (DEHP) and other phthalates in the general population. *International Journal of Hygiene and Environmental Health* 206, 77–83.
- Koch, H.M., Drexler, H., Angerer, J., 2004b. Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl) phthalate (DEHP). *International Journal of Hygiene and Environmental Health* 207, 15–22.
- Koch, H.M., Rossbach, B., Drexler, H., Angerer, J., 2003b. Internal exposure of the general population to DEHP and other phthalates: Determination of secondary and primary phthalate monoester metabolites in urine. *Environ Res* 93, 177–185.

- 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. *Environmental Health Perspectives* 108, A44–A442.
- Koo, H.J., Lee, B.M., 2005. Human monitoring of phthalates and risk assessment. *Journal of Toxicology and Environmental Health, Part A* 68, 1379–1392.
- Kortenkamp, A., Faust, M., 2010. Combined exposures to antiandrogenic chemicals: Steps towards cumulative risk assessment. *Int J Androl* 33, 463–474.
- Krasavage, W.J., Tischer, K.S., Roudabush, R., 1972. The reversibility of increased rat liver weights and microsomal processing enzymes after feeding high levels of 2,2,4-trimethyl-1,3-pentanediol diisobutyrate. *Toxicol Appl Pharmacol* 22, 400–408.
- Lake, B.G., 1995. Peroxisome proliferation: Current mechanisms relating to nongenotoxic carcinogenesis. *Toxicol Lett.* 82–83, 673–681.
- Lake, B.G., Cook, W.M., Worrell, N., Cunningham, M.E., Evans, J.G., Price, R.J., Young, P.J., Carpanini, F., 1991. Dose-response relationships for induction of hepatic peroxisome proliferation and testicular atrophy by phthalate esters in the rat. *Human and Experimental Toxicology* 10, 67–68.
- Lake, B.G., Gray, T.J., Gangolli, S.D., 1986. Hepatic effects of phthalate esters and related compounds—*in vivo* and *in vitro* correlations. *Environ Health Perspect* 67, 283–290.
- Lake, B.G., Rijcken, W.R., Gray, T.J., Foster, J.R., Gangolli, S.D., 1984. Comparative studies of the hepatic effects of di- and mono-*n*-octyl phthalates, di-(2-ethylhexyl) phthalate, and clofibrate in the rat. *Acta Pharmacologica et Toxicologica* 54, 167–176.
- Lamb, J.C., Chapin, R.E., Teague, J., Lawton, A.D., Reel, J.R., 1987. Reproductive effects of four phthalic acid esters in the mouse. *Toxicol Appl Pharmacol* 88, 255–269.
- Lambrot, R., Muczynski, V., Lécureuil, C., Angenard, G., Coffigny, H., Pairault, C., Moison, D., Frydman, R., Habert, R., Rouiller-Fabre, V., 2009. Phthalates impair germ cell development in the human fetal testis *in vitro* without change in testosterone production. *Environ Health Perspect* 117, 32–37.
- Lampen, A., Zimnik, S., Nau, H., 2003. Teratogenic phthalate esters and metabolites activate the nuclear receptors PPARs and induce differentiation of F9 cells. *Toxicol Appl Pharmacol* 188, 14–23.
- Lee, B.M., Koo, H.J., 2007. Hershberger assay for antiandrogenic effects of phthalates. *Journal of Toxicology and Environmental Health-Part A* 70, 1336–1370.
- Lee, H.C., Yamanouchi, K., Nishihara, M., 2006. Effects of perinatal exposure to phthalate/adipate esters on hypothalamic gene expression and sexual behavior in rats. *J Reprod Dev* 52, 343–352.

- Lee, K.Y., Shibutani, M., Takagi, H., Kato, N., Takigami, S., Uneyama, C., Hirose, M., 2004. Diverse developmental toxicity of di-n-butyl phthalate in both sexes of rat offspring after maternal exposure during the period from late gestation through lactation. *Toxicology* 203, 221–238.
- Lehmann, K.P., Phillips, S., Sar, M., Foster, P.M., Gaido, K.W., 2004. Dose-dependent alterations in gene expression and testosterone synthesis in the fetal testes of male rats exposed to di (n-butyl) phthalate. *ToxSci* 81, 60–68.
- Lehraiki, A., Racine, C., Krust, A., Habert, R., Levacher, C., 2009. Phthalates impair germ cell number in the mouse fetal testis by an androgen- and estrogen-independent mechanism. *Toxicological Sciences* 111, 372–383.
- Lington, A.W., Bird, M.G., Plutnick, R.T., Stubblefield, W.A., Scala, R.A., 1997. Chronic toxicity and carcinogenic evaluation of diisononyl phthalate in rats. *Fundamental and Applied Toxicology* 36, 79–89.
- Liu, K., Lehmann, K.P., Sar, M., Young, S.S., Gaido, K.W., 2005. Gene expression profiling following *in utero* exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis. *Biol Reprod* 73, 180–192.
- Lorber, M., Koch, H.M., Angerer, J., 2011. A critical evaluation of the creatinine correction approach: Can it underestimate intakes of phthalates? A case study with di-2-ethylhexyl phthalate. *J Expo Sci Environ Epidemiol* 21, 576–586.
- 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. *Journal of Exposure Science and Environmental Epidemiology* 18, 360–368.
- Mahood, I.K., Scott, H.M., Brown, R., Hallmark, N., Walker, M., Sharpe, R.M., 2007. *In utero* exposure to di(n-butyl) phthalate and testicular dysgenesis: comparison of fetal and adult endpoints and their dose sensitivity. *Environ Health Perspect* 115 (suppl 1), 55–61.
- Mann, A.H., Price, S.C., Mitchell, F.E., Grasso, P., Hinton, R.H., Bridges, J.W., 1985. Comparison of the short-term effects of di-(2-ethylhexyl) phthalate, di-(n-hexyl) phthalate, and di-(n-octyl) phthalate in rats. *Toxicology and Applied Pharmacology* 77, 116–132.
- Marsee, K., Woodruff, T.J., Axelrad, D.A., Calafat, A.M., Swan, S.H., 2006. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. *Environ Health Perspect* 114, 805–809.
- Marsman, D., 1995. NTP technical report on the toxicity studies of dibutyl phthalate (CAS No. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. *Toxic Rep Ser* 30, 1–G5.

- Masutomi, N., Shibutani, M., Takagi, H., Uneyama, C., Takahashi, N., Hirose, M., 2003. Impact of dietary exposure to methoxychlor, genistein, or diisononyl phthalate during the perinatal period on the development of the rat endocrine/reproductive systems in later life. *Toxicology* 192, 149–170.
- McKinnell, C., Mitchell, R.T., Walker, M., Morris, K., Kelnar, C.J., Wallace, W.H., Sharpe, R.M., 2009. Effect of fetal or neonatal exposure to monobutyl phthalate (MBP) on testicular development and function in the marmoset. *Hum Reprod* 24, 2244–2254.
- Meeker, J.D., Sathyanarayana, S., Swan, S.H., 2009. Phthalates and other additives in plastics: Human exposure and associated health outcomes. *Philos Trans R Soc Lond B Biol Sci* 364, 2097–2113.
- Mendiola, J., Stahlhut, R.W., Jorgensen, N., Liu, F., Swan, S.H., 2011. Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. *Environ Health Perspect* 119, 958–963.
- Miodovnik, A., Engel, S.M., Zhu, C., Ye, X., Soorya, L.V., Silva, M.J., Calafat, A.M., Wolff, M.S., 2011. Endocrine disruptors and childhood social impairment. *Neurotoxicology* 32, 261–267.
- Mitchell, R.T., Childs, A.J., Anderson, R.A., van den Driesche, S., Saunders, P.T., McKinnell, C., Wallace, W.H., Kelnar, C.J., Sharpe, R.M., 2012. Do phthalates affect steroidogenesis by the human fetal testis? Exposure of human fetal testis xenografts to di-n-butyl phthalate. *J Clin Endocrinol Metab* 97, E341–348.
- Miyata, K., Shiraishi, K., Houshuyama, S., Imatanaka, N., Umano, T., Minobe, Y., Yamasaki, K., 2006. Subacute oral toxicity study of di(2-ethylhexyl) adipate based on the draft protocol for the “Enhanced OECD Test Guideline no. 407.” *Arch Toxicol.* 80, 181–186.
- Moody, S., Goh, H., Bielanowicz, A., Rippon, P., Loveland, K.L., Itman, C., 2013. Prepubertal mouse testis growth and maturation and androgen production are acutely sensitive to di-n-butyl phthalate. *Endocrinology* 154, 3460–3475.
- Moore, M.R., 1998a. Oncogenicity Study in Mice with Di(isononyl)phthalate Including Ancillary Hepatocellular Proliferation and Biochemical Analyses. Covance Laboratories Inc., Vienna, VA 22182. For Aristech Chemical Corporation, Pittsburgh, PA 15230. January 29, 1998. Covance 2598–105.
- Moore, M.R., 1998b. Oncogenicity Study in Rats with Di(isononyl)phthalate Including Ancillary Hepatocellular Proliferation and Biochemical Analyses. Covance Laboratories, Inc., Vienna, VA 22182. For Aristech Chemical Corporation, Pittsburgh, PA 15230. May 13, 1998. Covance 2598-104.
- Moore, R.W., Rudy, T.A., Lin, T.M., Ko, K., Peterson, R.E., 2001. Abnormalities of sexual development in male rats with *in utero* and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. *Environ Health Perspect* 109, 229–237.

- Morrissey, R.E., Lamb, J.C., IV, Morris, R.W., Chapin, R.E., Gulati, D.K., Heindel, J.J., 1989. Results and evaluations of 48 continuous breeding reproduction studies conducted in mice. *Fundam Appl Toxicol* 13, 747–777.
- Murature, D.A., Tang, S.Y., Steinhardt, G., Dougherty, R.C., 1987. Phthalate esters and semen quality parameters. *Biomed Environ Mass Spectrom* 14, 473–477.
- Mylchreest, E., Cattley, R.C., Foster, P.M., 1998. Male reproductive tract malformations in rats following gestational and lactational exposure to di(*n*-butyl) phthalate: an antiandrogenic mechanism? *ToxSci* 43, 47–60.
- Mylchreest, E., Sar, M., Cattley, R.C., Foster, P.M., 1999. Disruption of androgen-regulated male reproductive development by di(*n*-butyl) phthalate during late gestation in rats is different from flutamide. *Toxicol Appl Pharmacol* 156, 81–95.
- Mylchreest, E., Sar, M., Wallace, D.G., Foster, P.M., 2002. Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di(*n*-butyl) phthalate. *Reproductive Toxicology* (Elmsford, NY) 16, 19–28.
- Mylchreest, E., Wallace, D.G., Cattley, R.C., Foster, P.M., 2000. Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di(*n*-butyl) phthalate during late gestation. *ToxSci* 55, 143–151.
- Nabae, K., Doi, Y., Takahashi, S., Ichihara, T., Toda, C., Ueda, K., Okamoto, Y., Kojima, N., Tamano, S., Shirai, T., 2006. Toxicity of di(2-ethylhexyl) phthalate (DEHP) and di(2-ethylhexyl) adipate (DEHA) under conditions of renal dysfunction induced with folic acid in rats: Enhancement of male reproductive toxicity of DEHP is associated with an increase of the mono-derivative. *Reproductive Toxicology* (Elmsford, NY) 22, 411–417.
- Nagao, T., Ohta, R., Marumo, H., Shindo, T., Yoshimura, S., Ono, H., 2000. Effect of butyl benzyl phthalate in Sprague-Dawley rats after gavage administration: a two-generation reproductive study. *Reproductive Toxicology* (Elmsford, NY) 14, 513–532.
- Needham, L.L., Calafat, A.M., Barr, D.B., 2007. Uses and issues of biomonitoring. *International Journal of Hygiene and Environmental Health* 210, 229–238.
- NRC, 1983. *Risk Assessment in the Federal Government: Managing the Process*. National Research Council, National Academy Press, Washington, D.C.
- NRC, 2006. *Human Biomonitoring for Environmental Chemicals*, National Academy of Sciences, Washington, DC.
- 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.



- NRC, 2009. Science and Decisions. Advancing Risk Assessment. Committee on Improving Risk Analysis Approaches used by the U.S. EPA, National Research Council, National Academy Press, Washington, DC.
- NTP, 1982. Carcinogenesis bioassay of di(2-ethylhexyl) adipate (CAS No. 103-23-1) in F344 rats and B6C3F1 mice (feed study). National Toxicology Program (NTP), Research Triangle Park, NC. NTP technical report series No. 212.  
[http://ntp.niehs.nih.gov/ntp/htdocs/LT\\_rpts/tr212.pdf](http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr212.pdf).
- NTP, 1989. Developmental Toxicity of Dimethyl Phthalate (CAS No. 131-11-3) Administered to CD Rats on Gestational Days 6 Through 15. National Toxicology Program. NTP Study: TER88066. January 9, 1989.
- NTP, 1997. Reproductive assessment by continuous breeding: Evolving study design and summaries of ninety studies. Environmental Health Perspectives 105 (Suppl 1), 199–395.
- NTP, 2000. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Butyl Phthalate (DBP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC.
- NTP, 2002. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di(2-Ethylhexyl) Phthalate (DEHP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC.
- NTP, 2003a. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. March 2003. NIH publication no. 03-4487.
- NTP, 2003b. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-Isodecyl Phthalate (DIDP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. April 2003. NIH publication no. 03-4485.
- NTP, 2003c. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-isononyl Phthalate (DINP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. March 2003. NIH publication no. 03-4484.
- NTP, 2003d. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-*n*-Hexyl Phthalate (DnHP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. March 2003. NIH publication no. 03-4489.
- NTP, 2006. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di(2-Ethylhexyl) Phthalate (DEHP). Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, Research Triangle Park, NC. November 2006. NIH publication no. 06-4476.

- Nuodex, 1983. 28-day hepatotoxicity study in rats conducted for Tenneco Chemicals Incorporated with samples Nuoplaz TOTM, Nuoplaz DOP. Tenneco Chemicals, Inc. OTS 0206575. Doc. ID 878214468.
- OECD, 1995. Screening Information Dataset (SIDS) initial assessment report for 2,2,4-trimethyl-1,3-pentanediol diisobutyrate. Organization for Economic Cooperation and Development. <http://www.inchem.org/documents/sids/sids/6846500.pdf>.
- OECD, 2007. Manual for Investigation for High Production Volume Chemicals. Organisation for Economic Co-operation and Development. Paris, France.
- Oishi, S., Hiraga, K., 1980. Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations. *Toxicol Appl Pharmacol* 53, 35–41.
- Page, B.D., Lacroix, G.M., 1995. The occurrence of phthalate ester and di-2-ethylhexyl adipate plasticizers in Canadian packaging and food sampled in 1985–1989: A survey. *Food Addit Contam* 12, 129–151.
- Parks, L.G., Ostby, J.S., Lambright, C.R., Abbott, B.D., Klinefelter, G.R., Barlow, N.J., Gray, L.E., Jr., 2000. The plasticizer diethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat. *ToxSci* 58, 339–349.
- Patyna, P.J., Brown, R.P., Davi, R.A., Letinski, D.J., Thomas, P.E., Cooper, K.R., Parkerton, T.F., 2006. Hazard evaluation of diisononyl phthalate and diisodecyl phthalate in a Japanese medaka multigenerational assay. *Ecotoxicol Environ Saf* 65, 36–47.
- Piersma, A.H., Verhoef, A., te Biesebeek, J.D., Pieters, M.N., Slob, W., 2000. Developmental toxicity of butyl benzyl phthalate in the rat using a multiple dose study design. *Reproductive Toxicology (Elmsford, NY)* 14, 417–425.
- Plasterer, M.R., Bradshaw, W.S., Booth, G.M., Carter, M.W., Schuler, R.L., Hardin, B.D., 1985. Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: naphthalene, p-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives. *J Toxicol Environ Health* 15, 25–38.
- Poon, R., Lecavalier, P., Mueller, R., Valli, V.E., Procter, B.G., Chu, I., 1997. Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. *Food and Chemical Toxicology* 35, 225–239.
- Preau, J.L., Jr., Wong, L.Y., Silva, M.J., Needham, L.L., Calafat, A.M., 2010. Variability over one week in the urinary concentrations of metabolites of diethyl phthalate and di(2-ethylhexyl) phthalate among eight adults: an observational study. *Environ Health Perspect* 118, 1748–1754.

- Preuss, R., Koch, H.M., Angerer, J., 2005. Biological monitoring of the five major metabolites of di-(2-ethylhexyl)phthalate (DEHP) in human urine using column-switching liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 816, 269–280.
- Price, C., Field, E.A., Marr, M.C., Myers, C.B., 1990. Final report on the developmental toxicity of butyl benzyl phthalate (CAS No. 85-68-7) in CD-1 Swiss mice. National Toxicology Program (NTP), Research Triangle Park, NC. NTP 90–114.
- Rider, C.V., Furr, J., Wilson, V.S., Gray, L.E., Jr. 2008. A mixture of seven antiandrogens induces reproductive malformations in rats. *Int J Androl* 31, 249–262.
- Rider, C.V., Furr, J.R., Wilson, V.S., Gray, L.E., Jr. 2010. Cumulative effects of *in utero* administration of mixtures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity. *Int J Androl* 33, 443–462.
- Rider, C.V., Wilson, V.S., Howdeshell, K.L., Hotchkiss, A.K., Furr, J.R., Lambright, C.R., Gray, L.E., Jr., 2009. Cumulative effects of *in utero* administration of mixtures of “antiandrogens” on male rat reproductive development. *Toxicol Pathol* 37, 100–113.
- Robins, M.C., 1994. A two-generation reproduction study with acetyl tributyl citrate in rats. BIBRA Toxicology International, Surrey, UK. No 1298/1/2/94.
- Ryu, J.Y., Lee, B.M., Kacew, S., Kim, H.S., 2007. Identification of differentially expressed genes in the testis of Sprague-Dawley rats treated with di(*n*-butyl) phthalate. *Toxicology* 234, 103–112.
- Saillenfait, A.M., Gallissot, F., Sabate, J.P., 2009a. Differential developmental toxicities of di-*n*-hexyl phthalate and dicyclohexyl phthalate administered orally to rats. *JAT* 29, 510–521.
- Saillenfait, A.M., Payan, J.P., Fabry, J.P., Beydon, D., Langonne, I., Gallissot, F., Sabate, J.P., 1998. Assessment of the developmental toxicity, metabolism, and placental transfer of di-*n*-butyl phthalate administered to pregnant rats. *ToxSci* 45, 212–224.
- Saillenfait, A.M., Roudot, A.C., Gallissot, F., Sabate, J.P., 2011. Prenatal developmental toxicity studies on di-*n*-heptyl and di-*n*-octyl phthalates in Sprague-Dawley rats. *Reproductive Toxicology (Elmsford, NY)* 32, 268–276.
- Saillenfait, A.M., Sabate, J.P., Gallissot, F., 2003. Comparative embryotoxicities of butyl benzyl phthalate, mono-*n*-butyl phthalate and mono-benzyl phthalate in mice and rats: *in vivo* and *in vitro* observations. *Reproductive Toxicology (Elmsford, NY)* 17, 575–583.
- Saillenfait, A.M., Sabate, J.P., Gallissot, F., 2006. Developmental toxic effects of diisobutyl phthalate, the methyl-branched analogue of di-*n*-butyl phthalate, administered by gavage to rats. *Toxicol Lett* 165, 39–46.

- Saillenfait, A.M., Sabate, J.P., Gallissot, F., 2008. Diisobutyl phthalate impairs the androgen-dependent reproductive development of the male rat. *Reproductive Toxicology* (Elmsford, NY) 26, 107–115.
- Saillenfait, A.M., Sabate, J.P., Gallissot, F., 2009b. Effects of *in utero* exposure to di-n-hexyl phthalate on the reproductive development of the male rat. *Reproductive Toxicology* (Elmsford, NY) 28, 468–476.
- 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.
- SCENIHR, 2007. Preliminary report on the safety of medical devices containing DEHP-plasticized PVC or other plasticizers on neonates and other groups possibly at risk. Scientific Committee on Emerging and Newly-Identified Health Risks (SCENIHR), European Commission, Brussels.  
[http://ec.europa.eu/health/ph\\_risk/committees/04\\_scenihr/docs/scenihr\\_o\\_014.pdf](http://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_014.pdf).
- Schmid, P., Schlatter, C., 1985. Excretion and metabolism of di(2-ethylhexyl)phthalate in man. *Xenobiotica* 15, 251–256.
- Scott, H.M., Mason, J.I., Sharpe, R.M., 2009. Steroidogenesis in the fetal testis and its susceptibility to disruption by exogenous compounds. *Endocrine Reviews* 30, 883–925.
- Shultz, V.D., Phillips, S., Sar, M., Foster, P.M., Gaido, K.W., 2001. Altered gene profiles in fetal rat testes after *in utero* exposure to di(n-butyl) phthalate. *ToxSci* 64, 233–242.
- Silva, M.J., Barr, D.B., Reidy, J.A., Malek, N.A., Hodge, C.C., Caudill, S.P., Brock, J.W., Needham, L.L., Calafat, A.M., 2004. Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999–2000. *Environ Health Perspect* 112, 331–338.
- Silva, M.J., Furr, J., Samandar, E., Preau, J.L., Jr., Gray, L.E., Needham, L.L., Calafat, A.M., 2010. Urinary and serum metabolites of di-n-pentyl phthalate in rats. *Chemosphere* 82, 431–436.
- Silva, M.J., Preau, J.L., Needham, L.L., Calafat, A.M., 2008. Cross validation and ruggedness testing of analytical methods used for the quantification of urinary metabolites. *Journal of Chromatography B* 873, 180–186.
- Silva, M.J., Reidy, J.A., Preau, J.L., Jr., Needham, L.L., Calafat, A.M., 2006a. Oxidative metabolites of diisononyl phthalate as biomarkers for human exposure assessment. *Environ Health Perspect* 114, 1158–1161.

- Silva, M.J., Reidy, J.A., Preau, J.L., Samandar, E., Needham, L.L., Calafat, A.M., 2006b. Measurement of eight urinary metabolites of di(2-ethylhexyl) phthalate as biomarkers for human exposure assessment. *Biomarkers* 11, 1–13.
- Singh, A.R., Lawrence, W.H., Autian, J., 1972. Teratogenicity of phthalate esters in rats. *J Pharm Sci* 61, 51–55.
- Sjöberg, P., Lindqvist, N.G., Plöen, L., 1986. Age-dependent response of the rat testes to di(2-ethylhexyl) phthalate. *Environmental Health Perspectives* 65, 237–242.
- Skakkebaek, N.E., Rajpert-De Meyts, E., Main, K.M., 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 16, 972–978.
- Smith, J.H., J.S., I., Pugh, G.J., Kamendulis, L.M., Ackley, D., Lington, A.W., Klaunig, J.E., 2000. Comparative *in vivo* hepatic effects of di-isononyl phthalate (DINP) and related C7–C11 dialkyl phthalates on gap junctional intercellular communication (GJIC), peroxisomal beta-oxidation (PBOX), and DNA synthesis in rat and mouse liver. *ToxSci* 54, 312–321.
- Soeler, A.O., Clinton, M., Boggs, J., Drinker, P., 1950. Experiments on the chronic toxicity of acetyl tributyl citrate. Department of Industrial Hygiene, Harvard Medical School, Boston, MA, USA..
- Stahlhut, R.W., Welshons, W.V., Swan, S.H., 2009. Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environ Health Perspect* 117, 784–789.
- Struve, M.F., Gaido, K.W., Hensley, J.B., Lehmann, K.P., Ross, S.M., Sochaski, M.A., Willson, G.A., Dorman, D.C., 2009. Reproductive toxicity and pharmacokinetics of di-n-butyl phthalate (DBP) following dietary exposure of pregnant rats. *Birth Defects Res B Dev Reprod Toxicol* 86, 345–354.
- Suzuki, Y., Niwa, M., Yoshinaga, J., Watanabe, C., Mizumoto, Y., Serizawa, S., Shiraishi, H., 2009. Exposure assessment of phthalate esters in Japanese pregnant women by using urinary metabolite analysis. *Environ Health Prev Med* 14, 180–187.
- Suzuki, Y., Yoshinaga, J., Mizumoto, Y., Serizawa, S., Shiraishi, H., 2012. Foetal exposure to phthalate esters and anogenital distance in male newborns. *Int J Androl* 35, 236–244.
- Swan, S.H., 2008. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. *Environ Res* 108, 177–184.
- Swan, S.H., Liu, F., Hines, M., Kruse, R.L., Wang, C., Redmon, J.B., Sparks, A., Weiss, B., 2010. Prenatal phthalate exposure and reduced masculine play in boys. *Int J Androl* 33, 259–269.

- Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect* 113, 1056–1061.
- Teuschler, L.K., Hertzberg, R.C., 1995. Current and future risk assessment guidelines, policy, and methods development for chemical mixtures. *Toxicology* 105, 137–144.
- Tilman, C., Capel, B., 2002. Cellular and molecular pathways regulating mammalian sex determination. *Recent Prog Horm Res* 57, 1–18.
- Topping, D.C., Ford, G.P., Evans, J.G., Lake, B.G., O'Donoghue, J.L., Lockhart, H.B., 1987. Peroxisome induction studies on di(2-ethylhexyl) terephthalate. *Toxicology and Industrial Health* 3, 63–78.
- Tsumura, Y., Ishimitsu, S.S., I., Sakai, H., Y., T., Tonogai, Y., 2003. Estimated daily intake of plasticizers in 1-week duplicate diet samples following regulation of DEHP-containing PVC gloves in Japan. *Food Additives and Contaminants* 30, 317–324.
- Tyl, R.W., Myers, C.B., Marr, M.C., Fail, P.A., Seely, J.C., Brine, D.R., Barter, R.A., Butala, J.H., 2004. Reproductive toxicity evaluation of dietary butyl benzyl phthalate (BBP) in rats. *Reproductive Toxicology (Elmsford, NY)* 18, 241–264.
- UNEP, 2002. OECD SIDS Initial Assessment Report for SIAM 14. Tris(2-ethylhexyl)benzene-1,2,3-tricarboxylate. United Nations Environment Programme (UNEP). Paris, France, 26-28 March 2002.
- Union Carbide Corporation, 1997. Letter from Union Carbide Corp to USEPA regarding: bis-2-propylheptyl phthalate subchronic feeding study in rats, dated 03/17/1997. Union Carbide Corporation. Submitted under TSCA Section FYI. EPA Document No. FYI-OTS-0397-1292. NTIS No. OTS0001292.
- Versar/SRC, 2010. Review of Exposure and Toxicity Data for Phthalate Substitutes Versar, Inc., Springfield, VA 22151. Syracuse Research Corporation, North Syracuse, NY 13212. Prepared for the U.S. Consumer Product Safety Commission, Bethesda, MD 20814. January 2010.
- Voss, C., Zerban, H., Bannasch, P., Berger, M.R., 2005. Lifelong exposure to di-(2-ethylhexyl)-phthalate induces tumors in liver and testes of Sprague-Dawley rats. *Toxicology* 206, 359–371.
- Ward, J.M., Peters, J.M., Perella, C.M., Gonzalez, F.J., 1998. Receptor and nonreceptor-mediated organ-specific toxicity of di(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice. *Toxicol Pathol* 26, 240–246.
- Waterman, S.J., Ambroso, J.L., Keller, L.H., Trimmer, G.W., Nikiforov, A.I., Harris, S.B., 1999. Developmental toxicity of di-isodecyl and di-isononyl phthalates in rats. *Reproductive Toxicology (Elmsford, NY)* 13, 131–136.

- Waterman, S.J., Keller, L.H., Trimmer, G.W., Freeman, J.J., Nikiforov, A.I., Harris, S.B., Nicolich, M.J., McKee, R.H., 2000. Two-generation reproduction study in rats given diisononyl phthalate in the diet. *Reproductive Toxicology* (Elmsford, NY) 14, 21–36.
- Weuve, J., Sánchez, B.N., Calafat, A.M., Schettler, T., Green, R.A., Hu, H., Hauser, R., 2006. Exposure to phthalates in neonatal intensive care unit infants: Urinary concentrations of monoesters and oxidative metabolites. *Environ Health Perspect* 114.
- Whyatt, R.M., Liu, X., Rauh, V.A., Calafat, A.M., Just, A.C., Hoepner, L., Diaz, D., Quinn, J., Adibi, J., Perera, F.P., Factor-Litvak, P., 2011. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. *Environ Health Perspect* 120, 290–295.
- Wilkinson, C.F., Christoph, G.R., Julien, E., Kelley, J.M., Kronenberg, J., McCarthy, J., Reiss, R., 2000. Assessing the risks of exposures to multiple chemicals with a common mechanism of toxicity: How to cumulate? *Regul Toxicol Pharmacol* 31, 30–43.
- Wilson, V.S., Lambright, C., Furr, J., Ostby, J., Wood, C., Held, G., Gray, L.E., Jr., 2004. Phthalate ester-induced gubernacular lesions are associated with reduced *insl3* gene expression in the fetal rat testis. *Toxicol Lett* 146, 207–215.
- Wittassek, M., Angerer, J., 2008. Phthalates: Metabolism and exposure. *Int J Androl* 31, 131–138.
- Wittassek, M., Angerer, J., Kolossa-Gehring, M., Schafer, S.D., Klockenbusch, W., Dobler, L., Günsel, A.K., Müller, A., Wiesmüller, G.A., 2009. Fetal exposure to phthalates—a pilot study. *International Journal of Hygiene and Environmental Health* 212, 492–498.
- Wittassek, M., Heger, W., Koch, H.M., Becker, K., Angerer, J., Kolossa-Gehring, M., 2007b. Daily intake of di(2-ethylhexyl)phthalate (DEHP) by German children: -- A comparison of two estimation models based on urinary DEHP metabolite levels. *International Journal of Hygiene and Environmental Health* 210, 35–42.
- Wittassek, M., Koch, H.M., Angerer, J., Brüning, T., 2011. Assessing exposure to phthalates--the human biomonitoring approach. *Mol Nutr Food Res* 55, 7–31.
- Wittassek, M., Wiesmüller, A., Koch, H.M., Eckard, R., Dobler, L., Müller, J., Angerer, J., Schluter, C., 2007a. Internal phthalate exposure over the last two decades:--A retrospective human biomonitoring study. *International Journal of Hygiene and Environmental Health* 210, 319–333.
- Woodruff, T.J., Sutton, P., 2011. An evidence-based medicine methodology to bridge the gap between clinical and environmental health sciences. *Health Affairs (Project Hope)* 30, 931–937.
- Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? *Risk Anal* 26, 803–824.

- Yamasaki, K., Okuda, H., Takeuchi, T., Minobe, Y., 2009. Effects of *in utero* through lactational exposure to dicyclohexyl phthalate and p,p'-DDE in Sprague-Dawley rats. *Toxicol Lett* 189, 14–20.
- Ye, X., Pierik, F.H., Hauser, R., Duty, S., Angerer, J., Park, M.M., Burdorf, A., Hofman, A., Jaddoe, V.W., Mackenbach, J.P., Steegers, E.A., Tiemeier, H., Longnecker, M.P., 2008. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: The Generation R Study. *Environ Res* 108, 260–267.
- Yolton, K., Xu, Y., Strauss, D., Altaye, M., Calafat, A.M., Khoury, J., 2011. Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. *Neurotoxicol Teratol* 33, 558–566.
- Zeiger, E.B., Anderson, S., Haworth, S., Lawlor, T., Mortelmans, K., 1988. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environmental and Molecular Mutagenesis* 11, 1–158.
- Zhang, Y., Jiang, X., Chen, B., 2004. Reproductive and developmental toxicity in F1 Sprague-Dawley male rats exposed to di-n-butyl phthalate *in utero* and during lactation and determination of its NOAEL. *Reproductive Toxicology (Elmsford, NY)* 18, 669–676.
- Zhu, X.B., Tay, T.W., Andriana, B.B., Alam, M.S., Choi, E.K., Tsunekawa, N., Kanai, Y., Kurohmaru, M., 2010. Effects of di-iso-butyl phthalate on testes of prepubertal rats and mice. *Okajimas Folia Anat Jpn* 86, 129–136.