

Report to the  
U.S. Consumer Product Safety Commission

by the

CHRONIC HAZARD ADVISORY PANEL ON PHTHALATES  
AND PHTHALATE ALTERNATIVES

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## **APPENDIX B**

# **REPRODUCTIVE AND OTHER TOXICOLOGY**



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## 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

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\* List applies to main report and all appendices.

DIHEXP	diisohexyl phthalate
DINP	diisononyl phthalate
DINCH®	1,2-cyclohexanedicarboxylic acid, diisononyl ester
DINX	1,2-cyclohexanedicarboxylic acid, diisononyl ester
DIOP	diisooctyl phthalate
DMP	dimethyl phthalate
DNHEXP	di-n-hexyl phthalate
DNOP	di-n-octyl phthalate
DPENP	di-n-pentyl phthalate
DPHP	di(2-propylheptyl) phthalate
DPS	delayed preputial separation
DSP	decrease spermatocytes and spermatids
DVO	delayed vaginal opening
ECHA	European Chemicals Agency
ECMO	extracorporeal membrane oxygenation
ED50	median effective dose
EPA	Environmental Protection Agency, U.S.
EPW	epididymal weight
FDA	Food and Drug Administration, U.S.
fue	urinary excretion factor
GD	gestational day
GGT	gamma-glutamyl transferase
GLP	good laboratory practices
gm	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 Introduction

Dialkyl esters of *o*-phthalic acid (phthalate esters or PEs) are a chemical class consisting of a large family of chemicals, about 50 of which are commercial products, many of which are considered high production volume chemicals in the United States. Toxicology data have accumulated over several decades because of widespread human exposure and concern over additivity of effects. Studies in recent years have shown that certain PEs cause reproductive and developmental health effects in animal models. These effects, in particular, will be the primary focus of this report because of the toxicological significance of the effects and the existence of similar observations in humans that may also be related to exposure to certain PEs.

There are little or no toxicology data on many members of the large family of PEs. Most of these are chemicals of no commercial importance and do not contribute to human exposures to PEs. The **PEs banned** by the Consumer Product Safety Improvement Act of 2008 (CPSIA) are as follows:

<u>Phthalate</u>	<u>CAS number</u>
<i>Permanent ban</i>	
Dibutyl phthalate (DBP)	84-74-2
Benzyl butyl phthalate (BBP)	85-68-7
Di(2-ethylhexyl phthalate) (DEHP)	117-81-7
<i>Interim ban</i>	
Di- <i>n</i> -octyl phthalate (DNOP)	117-84-0
Diisononyl phthalate (DINP)	28553-12-0; 68515-48-0
Diisodecyl phthalate (DIDP)	267651-40-0; 68515-49-1

**PEs not banned** by the CPSIA were also reviewed by Chronic Hazard Advisory Panel (CHAP):

Dimethyl phthalate (DMP)	131-11-3
Diethyl phthalate (DEP)	84-66-2
Diisobutyl phthalate (DIBP)	84-69-5
Dicyclohexyl phthalate (DCHP)	84-61-7
Diisoheptyl phthalate (DIHEPP)	71888-89-6
Diisooctyl phthalate (DIOP)	27554-26-3
Di(2-propylheptyl) phthalate (DPHP)	53306-54-0

**PE alternatives** were also reviewed because they are widely used substitutes for phthalates or are solvents or alternative plasticizers:

Acetyl tri-n-butyl citrate (ATBC)	77-90-7
Di(2-ethylhexyl) adipate (DEHA)	103-23-1
Diisononyl 1,2-dicarboxycyclohexane (DINX, DINCH <sup>®</sup> )*	474919-59-0
Di(2-ethylhexyl) terephthalate (DEHT)	6422-86-2
Tris(2-ethylhexyl) trimellitate (TOTM)	3319-31-1
2,2,4-Trimethyl-1,3-pentanediol diisobutyrate (TPIB, TXIB <sup>®</sup> ) <sup>†</sup>	6846-50-0

## 1.1 Nonreproductive Toxicity

The family of PEs is generally characterized by low acute toxicity and lack of genotoxicity. Thus, the carcinogenicity and reproductive toxicity of certain PEs are likely related to nongenotoxic mechanisms such as peroxisome proliferation, interference with testosterone production in the fetus, or other mechanisms of action.

Absorption of PEs is more efficient from the gastrointestinal tract than it is from other routes. Absorption is less efficient through the respiratory tract and least efficient through the skin. Absorption is enhanced by hydrolysis of the diesters to a monoester. Once absorbed, the monoester continues to be metabolized into substances that are excreted in the urine (Albro and Moore, 1974). Rats are more efficient at hydrolyzing the esters to monoesters than nonhuman primates are (Rhodes *et al.*, 1986; Short *et al.*, 1987). Thus, primates have a lower systemic exposure to the metabolites of PEs than rats exposed to the same amount orally (Rhodes *et al.*, 1986). This probably accounts for the greater sensitivity of rats compared to primates, especially for higher molecular weight esters.

DEHP and DINP cause significant increases in liver tumors in two-year studies in rats and mice, while DEP, DMP, and BBP show no evidence or equivocal evidence of carcinogenicity in the same type of studies (National Toxicology Program [NTP], 1995; NTP, 1997). Because PEs are nongenotoxic, other mechanisms of carcinogenic activity are assumed, specifically peroxisome proliferation. In rodents, peroxisome proliferators stimulate enzyme activities in the liver, causing an increase in endoplasmic reticulum and an increased size and number of peroxisomes. Chronic exposure of rodents results in hypertrophy of the liver and carcinogenesis. Chronic exposure of humans to PEs is much less than levels of exposure used in most animal studies and does not cause the same response in humans as seen in rodents, leading to the conclusion that the mechanism that accounts for carcinogenesis in rodents does not exist in humans (International Agency for Research on Cancer [IARC], 2000). As a result, the potential of PEs to cause cancer in humans is not a driving force for regulatory actions compared to concerns about their potential to disturb the hormone-dependent development of young males. Therefore, the primary focus of this report is on the risk from exposure to PEs on the hormone-dependent development of young males.

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

† TXIB<sup>®</sup> is a registered trademark of Eastman Chemical Co. The abbreviation TPIB is used here to represent the generic chemical.

Among the various types of studies conducted by toxicologists to evaluate and characterize the toxicological properties of chemicals, it has been common to distinguish between effects on development (developmental toxicity, teratogenicity) and effects on reproduction (effects on adult male and female reproductive performance). However, reproduction is a total life cycle process with various windows of vulnerability that differ from one species to another or from one chemical to another. In the case of the PEs, the window of greatest vulnerability is during late gestation (day 16–19 in the rat), and permanent damage is evident during the early neonatal period. (Some recovery occurs in non-developmentally altered tissues if exposure is curtailed.). The standard protocol for assessment of developmental toxicity in the rat includes exposure from gestation day 6–15. Thus, developmental toxicity studies designed according to international regulatory requirements are usually insensitive to the effects of PEs on the development of male reproductive structures. In this report, the effects of concern of PEs are developmental effects on reproductive tissues. The relevant literature on the studies that describe these effects is included in Appendix A and Section 2.3.2 of the main report. The literature on the reproductive toxic effects of PEs is summarized in this appendix (Appendix B) and Section 2.3.3 of the main report.

## **2 Permanently Banned Phthalates**

### **2.1 Di-n-Butyl Phthalate (DBP)**

Comments from the NTP-Center for the Evaluation of Risk to Human Reproduction (CERHR) Monograph of the Potential Human Reproductive and Developmental Effects of Di-n-Butyl Phthalate (DBP), (NTP, 2000).

*Summary of NTP-CERHR panel for DBP:*

Are people exposed to DBP? Yes

Can DBP affect human development or reproduction? Probably

Are current exposures to DBP high enough to cause concern? Possibly

*NTP statements upon review of the report of the NTP-CERHR DBP panel:*

The NTP concurs with the CERHR panel that there is minimal concern for developmental effects when pregnant women are exposed to DBP levels estimated by the panel (2–10 µg/kg-d).

Based upon recently estimated DBP exposures among some women of reproductive age, the NTP has some concern for DBP causing adverse effects to human development, particularly of the male reproductive system.

The NTP concurs with the CERHR panel that there is negligible concern for reproductive toxicity in exposed adults.

#### **2.1.1 Human Data**

One study reported the effects of exposure to DBP on human reproductive measures (Murature *et al.*, 1987). Total sperm number and concentration of DBP in cellular fractions of ejaculates were measured in the semen of college students. There was a negative correlation between DBP concentration and sperm indices, but the causal relationship was unclear. Confounders were not adequately taken into account.

### **2.1.2 Animal Data**

Over 20 studies were reviewed. All studies showed similar effects at high doses (~ 2 g/kg in rats). Representative or key studies are described below.

In a study reported by Gray *et al.* (1982), adult rats, mice, guinea pigs, and hamsters were given DBP by gavage for seven or nine days at dose levels of two or three g/kg-d. Testes weights were decreased and histopathologic exams showed reduction in spermatids and spermatogonia with adverse effects in almost all tubules. The effects in rats were > mice > hamsters. The monoester had minimal effect in the hamster (only one of eight animals had more than 90% tubular atrophy of the testes).

Wine *et al.* (1997) reported the results of a continuous breeding study in Sprague-Dawley (SD) rats given doses of 0, 52, 256, or 509 mg/kg-d via the diet. They observed infertility and lighter and fewer pups. A no observed adverse effect level (NOAEL) was not established.

A multigenerational reproduction study in Long Evans rats was reported by Gray *et al.* (1999). Females were given 0, 250, or 500 mg/kg-d, and males were given 0, 250, 500, or 1000 mg/kg-d orally. The researchers observed a delay in puberty in males, decreased fertility, increased testicular atrophy, decreased sperm counts, mid-term abortions, and malformations among offspring, including abdominal testes and hypospadias.

### **2.1.3 Studies Reported Since the NTP-CERHR Report in 2000**

#### **2.1.3.1 Human Data**

Duty *et al.*, (2005) studied phthalate metabolites, including monobutyl phthalate (MBP), and reproductive hormones in the urine of adult men recruited from Massachusetts General Hospital. The authors admit that changes in hormones did not follow the expected pattern, raising the question of whether the changes were physiologically relevant or were the product of multiple statistical comparisons.

Huang *et al.* (2007) examined the association between thyroid hormones and phthalate monoesters in serum and urine from pregnant women. There was a significant positive association between estradiol and progesterone, T3 and T4, and T4 and FT4. There was a significant negative association between T4 and MBP, and FT4 and MBP.

Main *et al.* (2006) studied phthalates, including DBP, in human breast milk and their association with altered endogenous reproductive hormones in three-month-old infants. There was a significant association between MBP and sex hormone binding globulin.

Jönsson *et al.* (2005) reported human reproductive effects relative to phthalate exposure in men undergoing military examinations, including sperm concentrations, motility, integrity, semen volume, epididymal and prostate function, and serum reproductive hormones. For those who had DBP metabolites in urine, there was no association between DBP and reproductive endpoints.

Zhang *et al.* (2006) studied the relationship between phthalate levels in semen and semen measures in men from the Shanghai Institute of Planned Parenthood Research. There was no

correlation between DBP concentration in semen and sperm concentration or viability. The time for liquefaction of semen increased with increased DBP concentration. Semen quality decreased with increased DBP concentration.

Reddy (2006) studied blood from infertile women with endometriosis and those without but having other causes of infertility. The author concluded that DBP serum concentrations may be associated with increased endometriosis in women.

### **2.1.3.2 Animal Data**

Mahood *et al.*, (2007) evaluated adult and fetal toxicity in Wistar male and female rats given DBP at 0, 4, 20, 100, or 500 mg/kg-d on gestation days 13.5 to 20.5 or 21.5. There was a dose-dependent decrease in male fertility at 20 mg/kg-d and above, with the decrease being significant at 500 mg/kg-d. Testicular toxicity was increased, while testicular testosterone was decreased at 100 and 500 mg/kg-d. Fetal endpoints were the most sensitive to DBP effects. The NOAEL was 20 mg/kg-d.

The effect of DBP on female reproductive measures was reported in two studies by Gray *et al.* (2006). Long Evans hooded rats were dosed orally from lactation day 21 to gestation day 13 of a third pregnancy. DBP did not affect maturation, estrus cyclicity, or percent mating or pregnant. There was a decrease in the number of live pups from treated females in the first and second pregnancies.

In a second study, 24-day-old female rats were dosed orally with 0, 250, 500, or 1000 mg DBP/kg-d 5 days/week for 110 days, then 7 days/week until during the second pregnancy when they were killed. Pregnancies and the number of live pups were decreased at 500 and 1000 mg/kg-d. In the females at the high dose level, serum progesterone was decreased and hemorrhagic corpora lutea were observed on ovaries of females at necropsy.

Ryu *et al.* (2007) examined DNA changes in male SD rats dosed orally with 0, 250, 500, or 750 mg DBP/kg-d for 30 days. They saw changes in genes involved in xenobiotic metabolism, testis development, sperm maturation, steroidogenesis, and immune response. They also saw upregulation of peroxisome proliferation and lipid homeostasis genes. The authors concluded that DBP can affect gene expression profiles involved in steroidogenesis and spermatogenesis, thus affecting testicular growth and morphogenesis.

In a publication since the NTP-CERHR review, McKinnell *et al.* (2009) reported that MBP given to marmosets did not measurably affect testis development or function, or cause testicular dysgenesis. No effects emerged after adulthood. Effects on germ cell development were inconsistent or of uncertain significance.

Human and animal studies published since the NTP-CERHR review of DBP support the conclusion of the earlier review that DBP probably can affect human development or reproduction.

## 2.2 Butylbenzyl Phthalate (BBP)

Comments from the NTP-CERHR Monograph of the Potential Human Reproductive and Developmental Effects of Butyl Benzyl Phthalate (BBP), (NTP, 2003a).

*Summary of NTP-CERHR panel for BBP:*

Are people exposed to BBP? Yes

Can BBP affect human development or reproduction? Probably

Are current exposures to BBP high enough to cause concern? Probably not.

*NTP statements upon review of the report of the NTP-CERHR BBP panel:*

The NTP concludes that there is minimal concern for developmental effects in fetuses and children.

The NTP concurs with the CERHR panel that there is negligible concern for adverse reproductive effects in exposed men.

### 2.2.1 Human Data

No human data on BBP alone were available for review by the panel.

### 2.2.2 Animal Data

Six studies were reviewed. No study was definitive, and no multigenerational study had been published for BBP. Representative or key studies include:

A reproductive screen of BBP was published by Piersma (2000). The study design was that of the standard Organisation for Economic Cooperation and Development (OECD) screen number 421 protocol. Male and female Harlan Cpb-WU rats were gavaged with 0, 250, 500, or 1000 mg/kg-d for 14 days. Males and females were dosed for 14 days during mating. Males were killed at 29 days; dosing of the females continued to postnatal day (PND) 6 after which females were killed and necropsied. Pups were counted and examined on PND 1 and 6.

Low fertility, testicular degeneration, and interstitial cell hyperplasia were observed in the high-dose males. The NOAEL was of uncertain value because of the screen-design of the study.

A one-generation reproduction study designed according to OECD guideline number 415 protocol was conducted in Wistar rats (TNO, 1993). BBP mixed in the diet provided 0, 106, 217, or 446 mg/kg-d to males and 0, 108, 206, or 418 mg/kg-d to females. All reproductive indices were normal. Liver and reproductive organs were normal upon histopathologic examination.

A 10-week modified mating trial study was conducted by the NTP in male F344 rats (NTP, 1997). BBP mixed in the diet provided 0, 20, 200, or 2,200 mg/kg-d. After 10 weeks of dosing, the treated males were mated 1 male to 2 untreated females. Females were necropsied on gestational day (GD) 13 for examination of uterine contents. There was a decrease in the number of sperm in the epididymis at each dose level. There were no pregnancies at the high-dose level

of the males. The NOAEL was considered uncertain by the CERHR panel because there was no assessment of reproductive systems in the F1 generation.

### **2.2.3 Studies Reported Since the NTP-CERHR Report in 2003**

#### **2.2.3.1 Human Data**

No new studies were reported on BBP. However, see reviews of studies on MBP under the review of DBP.

#### **2.2.3.2 Animal Data**

Tyl *et al.* (2004) reported on a two-generation reproductive study of BBP given to CD rats in the diet at concentrations to provide 0, 50, 250, or 750 mg/kg-d for 10 weeks prior to mating and through the second generation pups. Systemic effects included reduction in body weights, increased organ weights, and in F0 females, decreased ovarian and uterine weights. There were no significant effects in F0 males.

In the F1 generation, mating and fertility indices were reduced, and the weights of testes, epididymis, seminal vesicles, coagulating glands, and prostate were reduced. Also, there were reproductive tract malformations—hypospadias, missing organs, and abnormal organ size and shape.

Findings in males included decreased epididymal sperm number, motility, progressive motility, and increased histopathologic changes in the testes and epididymis. In the females, the mating and fertility indices were reduced along with uterine implants, total and live pups, number of live pups, and ovarian weight. Uterine weights were increased.

In the F2 generation, findings were similar to those in F1 and also included decreased anogenital distance in males at 250 mg/kg-d and above, increased nipple/areola retention in males at 750 mg/kg-d.

NOAELs:	adult reproductive toxicity	250 mg/kg-d
	F1, F2 offspring reproductive toxicity	250 mg/kg-d
NOAEL:	F1, F2 decreased anogenital distance in males	50 mg/kg-d

Findings in a two-generation reproductive study reported by Aso *et al.* (2005) were in agreement with those of Tyl *et al.* (2004). The no observed effect level(NOEL)/NOAEL for the parental animals and for offspring growth and development was less than 100 mg/kg-d.

Animal studies published since the NTP-CERHR review of BBP in 2003 support the conclusions of that review that BBP can probably affect human development or reproduction.

### **2.3 Di (2-ethylhexyl) Phthalate (DEHP)**

Comments from the NTP-CERHR Monograph of the Potential Human Reproductive and Developmental Effects of Di(2-ethylhexyl) Phthalate (DEHP), (NTP, 2006)

*Summary of the NTP-CERHR panel for DEHP:*

Are people exposed to DEHP? Yes

Can DEHP affect human development or reproduction? Probably

Are current exposures to DEHP high enough to cause concern? Yes

*NTP statements upon review of the report of the NTP-CERHR DEHP panel:*

The NTP concurs with the CERHR DEHP panel that there is serious concern that certain intensive medical treatments of male infants may result in DEHP levels that affect development of the reproductive tract.

The NTP concurs with the CERHR DEHP panel that there is concern for adverse effects on the development of the reproductive tract in male offspring of pregnant and breast-feeding women undergoing certain medical procedures that may result in exposure to high levels of DEHP.

The NTP concurs with the CERHR DEHP panel that there is concern for effects of DEHP exposure on the development of the reproductive tract for infants less than one year old.

The NTP concurs with the CERHR DEHP panel that there is some concern for the effects of DEHP exposure on the development of the reproductive tract in male children older than one year.

The NTP concurs with the CERHR DEHP panel that there is some concern for adverse effects of DEHP exposure on the development of the reproductive tract in male offspring of pregnant women not medically exposed to DEHP.

The NTP concurs with the CERHR DEHP panel that there is minimal concern for reproductive toxicity in adults exposed at 1–30 µg/kg-d. This level of concern is not altered for adults medically exposed to DEHP.

### **2.3.1 Human Data (Summarized from the November 2006 CERHR Report)**

Modigh *et al.* (2002) evaluated time-to-pregnancy in the partners of men potentially exposed to DEHP occupationally. Three hundred twenty-six pregnancies from 234 men were available for analysis. Pregnancies were categorized as unexposed (n=182), low exposure (n=100), or high exposure (n=44), based on measurements of DEHP concentrations in air at the worksite.

Median time-to-pregnancy was 3.0 months in the unexposed group, 2.25 months in the low-exposure group, and 2.0 in the high-exposure group. The authors concluded that there was no evidence of a DEHP-associated prolongation in time-to-pregnancy, although they recognized that there were few highly exposed men in their sample. The mean DEHP exposure level for men in the study was less than 0.5 mg/m<sup>3</sup>.

Rozati *et al.* (2002) measured phthalate esters in the seminal plasma of 21 men with unexplained infertility. Comparison was made to seminal plasma phthalate concentrations in a control group with evidence of conception and normal semen analysis.

The mean +/- SD seminal plasma phthalate ester concentration in the infertile group was 2.03 +/- 0.214 µg/mL compared to 0.06 +/- 0.002 µg/mL in the control group (p<0.05). There was a significant inverse correlation between seminal phthalate ester concentration and normal sperm morphology, and a positive correlation between seminal phthalate ester concentration and the percent acid-denaturable sperm chromatin. There was no significant correlation between semen phthalate ester concentration and ejaculation volume, sperm concentration, progressive motility, sperm vitality, sperm osmoregulation, or sperm chromatin decondensation. The authors concluded that adverse effects of phthalate esters were consistent with published data on male reproductive toxicity of these compounds.

The CERHR panel concluded that the sample size was small and there was very little information on the selection of controls for infertile cases. There was little assessment of confounders and no evidence that exposure assessment was blind to the case/control status of participants.

The CERHR panel considered this study to be of limited usefulness in the evaluation process.

Duty *et al.* (2003a; 2003b) and Hauser *et al.* (2005) report on the results of evaluations of reproductive measures of men examined in a clinic as part of a fertility evaluation. The study population included 28 men (17%) with low sperm concentration, 74 men (44%) with < 50% motility, 77 men (46%) with more than 4% normal form and 77 men who were normal in all three domains. HPLC/MS methods were used to measure urinary levels of the PE metabolites mono(2-ethylhexyl) phthalate (MEHP) and for monoethyl, monomethyl, mono-*n*-butyl, monobenzyl, mono-*n*-octyl, monoisononyl, and monocyclohexyl phthalates. There were no significant associations between abnormal semen parameters and MEHP urine concentrations above or below the group median. The authors did not present any conclusions relative to MEHP (Duty *et al.*, 2003a).

Duty *et al.* (2004) evaluated urinary MEHP levels and sperm motion parameters in males presenting for fertility evaluation without regard to whether the male had a fertility problem. One-hundred eighty-seven of the subjects had measurements of sperm motility and urine phthalate levels. Methods for urinary phthalate measurements were similar to those reported in Duty *et al.* (2003a). The authors concluded that there was a pattern of decline (nonstatistically significant) in motility parameters. Lack of statistical significance may have reflected the relatively small sample size.

Duty *et al.* (2003b) evaluated a possible association between urinary phthalate monoester concentrations and sperm DNA damage using the neutral comet assay. Subjects were a subgroup (n=141) of Duty *et al.* (2003a). There were no significant associations between comet assay parameters and MEHP urinary concentrations.

This series of papers by Duty and Hauser were considered by the CERHR panel to be useful in the evaluation process, but use of a subfertile population was a weakness of the study design.

### **2.3.2 Animal Data (Summarized from the November 2006 CERHR Report)**

Sixty-eight studies, predominantly in rodents, were reviewed, building on the original observation that DEHP produced testicular atrophy in a subchronic toxicity study (Gray *et al.*, 1982). Most studies used high dose levels, *e.g.*, 2,000 mg/kg-d. All reported similar effects on the testes. Representative or key studies include:

A key study for quantitative assessment of the reproductive toxicity of DEHP is by Reel *et al.* (1984) and Lamb *et al.* (1987). This was a continuous breeding protocol with cross-over mating trials using CD-1 Swiss mice. DEHP was administered in the feed in concentrations to deliver 0, 14, 141, or 425 mg/kg-d. At 425 mg/kg-d, no breeding pairs delivered a litter; at 141 mg/kg-d, fertility was significantly reduced. The cross-over mating trial coupled high-dose males with untreated females and untreated males with high-dose females. The treated females had no litters; in the matings with treated males, only 4/20 had a litter. When the high-dose males were necropsied, testicular and epididymal weights were reduced and there was histologic evidence of seminiferous tubule destruction. The NOAEL was ~14 mg DEHP/kg-d.

Fisher 344 rats (Agarwal *et al.*, 1986) were given DEHP in the diet for 60 days at concentrations providing 0, 18, 69, 284, or 1,156 mg/kg-d, followed by 5 days of mating with untreated females while on control diets. There were testicular lesions at the high-dose level but not at lower-dose levels. The high-dose level was the LOAEL and 284 mg/kg-d was the NOAEL.

Rhoades *et al.* (1986) reported two studies in marmosets. One involved oral doses of DEHP to 5 males and females for 14 days at a dose level of 2,000 mg/kg-d and an IP study in which five 2-year old males were given 1 g/kg-d for 14 days. There were insufficient data in the published report to support the conclusions. More data on this study were available in an EPA docket, but confidence in the data was limited because of the single dose used as well as the procedures used for histological examination of tissues.

Schilling *et al.* (2001) reported the results of a two-generation reproduction study in Wistar rats. DEHP was given in the feed at concentrations to provide 0, 113, 340, or 1,088 mg/kg-d. The authors concluded that reproductive performance and fertility were affected only at the high dose level. Developmental toxicity noted at the top two doses included increased stillbirths and pup mortality, decreased pup body weight, decreased male anogenital distance, and increased retained nipples/areolae in males. There was a delay in sexual maturation of F1 males and female offspring at the high dose.

While the authors concluded that there were significant effects only at the high dose level, the CERHR panel concluded that there were effects at all dose levels.

### 2.3.3 Studies Reported Since the NTP-CERHR Report in 2006

#### 2.3.3.1 Human Data

Studies since the NTP-CERHR report of 2006 reinforce the conclusion that “DEHP can probably affect human reproduction and development.” DEHP-induced reproductive effects are less well described in humans than in animals. Studies associating DEHP exposure with human fertility have been informative. Sperm DNA damage has been associated with urinary MEHP concentrations (Hauser *et al.*, 2007) and a slight increase in the odds ratio (OR=1.4; CI=0.7–2.9 adjusted for age, abstinence, and smoking (Duty *et al.*, 2003a).

Human studies are not uniformly positive when relating DEHP exposures to reproductive deficiencies. While human studies were often limited by small sample sizes, confounders, and sampling methodologies, they have shown correlations between certain sperm parameters (morphology, chromatin structure, and mobility) and DEHP or MEHP exposures.

#### 2.3.3.2 Animal Data

Foster *et al.* (2006) repeated the study of DEHP in rats reported by Reel *et al.* (1984) using the continuous breeding protocol of the NTP to determine whether examination of a larger number of littermates would increase the sensitivity to detect a lower NOAEL. Increasing the cohort examined from breeding males (as done in the previous study) to a larger cohort by including nonbreeding males lowered the NOAEL from 50 mg/kg-d to 5 mg/kg-d in this study.

Gray *et al.* (2009) studied the dose response curve for phthalate syndrome effects in SD rats given DEHP by gavage at dose levels of 0, 11, 33, 100, or 300 mg/kg-d on gestation day 8 to lactation day 17. Exposure for some males continued to age 63–65 days. A significant percent of F1 males displayed one or more of the phthalate syndrome lesions at 11 mg/kg-d or greater. This confirms the NTP study (Reel *et al.*, 1984; Lamb *et al.*, 1987), which reported a NOAEL and LOAEL of 5 and 10 mg/kg-d, respectively, via the diet.

While there are many more animal studies on the effects of DEHP and its metabolites on reproductive measures than there are human studies, the experimental design of many of them is not sufficiently robust to assess components of the phthalate syndrome at low levels of exposure. Gray *et al.* (2009) commented that their study and the NTP study (Reel *et al.*, 1984; Lamb *et al.*, 1987) are the only two studies “that provide a comprehensive assessment of phthalate syndrome in a large enough number of male offspring to detect adverse reproductive effects at low dose levels.”. Considered overall, animal studies have repeatedly demonstrated that DEHP induces reproductive deficits in males of many species, including many strains of rats and mice. Female reproductive deficits have also been reported in numerous animal studies.

Andrade *et al.* (2006a) reported an extensive dose-response study following *in utero* and lactational exposure of Wistar rats to DEHP given orally by gavage at a series of dose levels ranging from 0.0015 to 405 mg/kg-d. Phthalate syndrome effects were seen in male offspring of females dosed at 405 mg/kg-d. Delayed preputial separation was seen at 15 mg/kg-d and higher. Testes weight was significantly increased at dose levels of 5, 15, 45, and 135 mg/kg-d, but not at 405. The NOAEL was 1.215 mg/kg-d.

In another study, Andrade *et al.* (2006b) reported on the reproductive effects of *in utero* and lactational exposure to DEHP in adult male rats. The experimental design duplicated Andrade *et al.* (2006a). Reduced daily sperm production and cryptorchidism were the most frequent effects seen in adult males. The NOAEL for these effects was 1.215 mg/kg-d.

### **3 Interim Ban Phthalates**

#### **3.1 Di-*n*-Octyl Phthalate(DNOP)**

Comments from the NTP-CERHR Monograph of the Potential Human Reproductive and Developmental Effects of Di-*n*-Octyl Phthalate (DnOP), (NTP, 2003d)

*Summary of NTP-CERHR panel for DnOP [DNOP]:*

Are people exposed to DnOP? Yes

Can DnOP affect human development or reproduction? Probably not

Are current exposures to DnOP high enough to cause concerns? Probably not

*NTP statement upon review of the report of the NTP-CERHR DnOP panel:*

The NTP concurs with the CERHR panel that there is negligible concern for effects on adult reproductive systems.

##### **3.1.1 Human Data**

No human data on DNOP were available for review by the panel.

##### **3.1.2 Animal Data**

One reproductive study in CD-1-Swiss mice was reported by Heindel *et al.* (1989). DNOP was mixed in the diet to provide 0, 1800, 3600, or 7500 mg DNOP/kg-d. There were no effects on the ability to produce litters, litter size, sex ratio, or pup weight, or viability over five successive litters. The last litters were mated to produce the F1 generation. There were no effects on fertility, litter size, or pup weight or viability. Sperm indices and estrus cycles were unchanged.

Poon *et al.* (1997) reported a subchronic toxicity study in SD rats given DNOP for 13 weeks at dose levels up to 350 mg/kg-d. Testes weights and histology were normal at all dose levels.

Foster *et al.* (1980) gavaged male SD rats with 2800 mg DNOP/kg-d for 4 days. No testicular lesions were observed.

##### **3.1.3 Studies Reported Since the NTP-CERHR Report in 2003**

Neither animal nor human studies have been published since the 2003 NTP-CERHR review that would change the conclusion of that review that DNOP would not be expected to affect human development or reproduction.

#### **3.2 Diisononyl Phthalate (DINP)**

Comments from the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-Isononyl Phthalate (DINP), (NTP, 2003c)

*Summary of NTP-CERHR panel for DINP:*

Are people exposed to DINP? Yes

Can DINP affect human development or reproduction? Probably

Are current exposures to DINP high enough to cause concern? Probably not

*NTP statements upon review of the report of the NTP-CERHR DINP panel:*

The NTP concurs with the conclusions of the CERHR panel and has minimal concern for DINP causing adverse effects to human reproduction or fetal development.

The NTP has minimal concern for developmental effects in children.

### **3.2.1 Human Data**

No human data on DINP were available for review by the panel.

### **3.2.2 Animal Data**

One study was reviewed that included one- and two-generation feeding studies in SD rats exposed *in-utero* during the entire duration of gestation (Waterman *et al.*, 2000). In the one-generation dose range finding study, rats were given dietary levels of 0, 0.5, 1.0, or 1.5% DINP. In the two-generation study, rats were given 0, 0.2, 0.4, or 0.8% DINP (up to 665–779 mg DINP/kg-d in males or 555–1,229 mg/kg-d in females). In the two-generation study, reproductive parameters, including mating, fertility, and testicular histology, were unaffected in both generations at the highest dose level.

### **3.2.3 Studies Reported Since the NTP-CERHR Report in 2003**

#### **3.2.3.1 Human Data**

No studies were found for review.

#### **3.2.3.2 Animal Data**

Patyna *et al.* (2006) evaluated the reproductive and developmental effects of DINP and DIDP in a three-generation study in Japanese medaka fish given 0 or 20 ppm DINP-1 in the diet (flake food). The estimated dose was 1 mg/kg-d. There were no significant effects on survival or fertility, or on the number of eggs and no evidence of endocrine-induced effects such as changes in gonad morphology or weight, sex ratio, intersex conditions, or sex reversal.

Available publications support the NTP conclusion of the CERHR review in 2003 that there is minimal concern for DINP causing adverse effects to human reproduction.

## **3.3 Diisodecyl Phthalate (DIDP)**

Comments from the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Di-Isodecyl Phthalate (DIDP), (NTP, 2003b).

*Summary of the NTP-CERHR Panel for DIDP:*

Are people exposed to DIDP? Yes

Can DIDP affect human development or reproduction? Possibly development but not reproduction

Are current exposures to DIDP high enough to cause concern? Probably not

*NTP statements upon review of the report of the NTP-CERHR DIDP panel:*

The NTP concurs with the CERHR panel that there is minimal concern for developmental effects in fetuses and children.

The NTP concurs with the CERHR panel that there is negligible concern for reproductive toxicity to exposed adults.

### **3.3.1 Human Data**

No human data on DIDP were available for review by the panel.

### **3.3.2 Animal Data**

One report was reviewed that consisted of two two-generation reproduction studies (ExxonMobil, 2000). Dose levels for the first study were selected on the basis of range finding studies. Dose levels for the second study were selected on the basis of the results of the first. All studies were in CrI:CDBR VAF rats given DIDP in the diet. Based on standard measures and procedures, no adverse reproductive effects were observed in either two-generation study at dose levels that caused decreased weight gain and increased liver and kidney weights in the adults. The highest dose level, 0.8% DIDP in the diet, resulted in the following doses of DIDP in mg/kg-d: males, F0—427–781; F1—494–929, during pre-mating; females, F0—641–1,582; F1—637–1,424 during gestation and lactation.

### **3.3.3 Studies Reported Since the NTP-CERHR Report in 2003**

Neither human nor animal studies have been published since the NTP-CERHR review in 2003 that would change the conclusion of that review that DIDP would not be expected to affect human reproduction.

## **4 Phthalates Not Banned by the CPSIA**

### **4.1 Dimethyl Phthalate (DMP)**

#### **4.1.1 Human Data**

No human studies were available for review.

#### **4.1.2 Animal Data**

No single or multiple generation reproductive studies in animals were available for review.

## 4.2 Diethyl Phthalate (DEP)

### 4.2.1 Human Data

Jönsson *et al.* (2005) examined urine, serum, and semen samples from 234 young Swedish men. The highest quartile for urinary monoethyl phthalate (MEP) had 8.8% fewer sperm, 8.9% more immotile sperm, and lower LH values compared to subjects in the lowest quartile.

Hauser *et al.* (2007) and Duty *et al.* (2003b) reported that sperm DNA damage correlated with urinary MEP levels in men who presented to a health facility for semen analyses as part of an infertility investigation.

Pant *et al.* (2008) found a significant inverse relationship between sperm concentrations and the level of DEP in semen in a group of 300 males 20–40 years of age.

### 4.2.2 Animal Data

Lamb *et al.* (1987) and NTP (1984) reported on a two-phase study in which mice were first given DEP in the diet at concentrations that provided 451, 2,255, and 4,509 mg/kg-d to males and 488, 2,439, and 4,878 mg/kg-d to females for 7 days prior to mating and for 98 days of cohabitation plus 21 days after separation. Following exposure, there were no effects on reproductive indices—number fertile pairs, pups/litter, live pups/litter, or the live pup birth weight. Offspring of these mice were subsequently given DEP in their diets (4,509 or 4,878 mg/kg-d) from weaning through seven weeks pre-mating plus the continuous breeding period. F1 parental males had 32% increased prostate weight, 30% decreased sperm concentrations, increased rates of abnormal sperm (excluding tailless sperm), 25% decreased body weight, and 14% decreased total number of live F2 pups (male and female combined) per litter at birth versus controls. F1 parental females had a nonsignificant decrease in absolute and relative uterine weight (LOAEL = 4,878 mg/kg-d).

Fujii *et al.* (2005) reported on a two-generation reproductive study in rats given DEP in the diet at concentrations to provide 1,016 mg/kg-d to males and 1,375 mg/kg-d to females for 10 weeks prior to mating, throughout mating, and during gestation and lactation. There were no effects on fertility or fecundity. Decreased serum testosterone levels in F0 males and increased tailless sperm in F1 males were considered nonsignificant.

A dose-related decrease in the absolute and relative uterine weight (F1 and F2 weanlings; LOAEL = 1,297–1,375; NOAEL = 255–267 mg/kg-d) and a decrease in the number of gestation days (F0, F1 adults; LOAEL = 1,297–1,375; NOAEL = 255–267 mg/kg-d) were reported for female rats.

Oishi and Hiraga (1980) also reported significantly decreased serum testosterone, serum dihydrotestosterone, and testicular testosterone in JCL:Wistar rats following dietary exposure. These results are questionable, however, when taken in the context of other results of the study in which increases in testosterone levels were seen after exposure to DBP, DIBP, and DEHP.

### **4.3 Diisobutyl Phthalate (DIBP)**

#### **4.3.1 Human Data**

No studies were reported in humans.

#### **4.3.2 Animal Data**

No single or multiple generation reproductive toxicology studies were reported.

Zhu *et al.* (2010) reported on testicular effects in male adolescent rats given DIBP orally once or for seven days at dose levels of 0, 100, 300, 500, 800, and 1,000 mg/kg-d and higher. In rats dosed for seven days, there was a significant decrease in testes weights, increase in apoptotic spermatogenic cells, disorganization or reduced vimentin filaments in Sertoli cells at doses of 500 mg/kg-d and higher.

Hodge *et al.* (1954) reported the effects of DIBP in a four-month subchronic study in albino rats. DIBP was mixed in the diet at concentrations of 0, 0.01, 1.0, and 5%. The estimated mg/kg-d by the authors were 0, 67, 738, and 5,960.

Absolute and relative testis weights were significantly decreased at the high dose. Thus, the NOAEL was 1.0% or 738 mg/kg-d.

### **4.4 Dicyclohexyl phthalate (DCHP)**

#### **4.4.1 Human Data**

No human studies were available for review.

#### **4.4.2 Animal Data**

Hoshino *et al.* (2005) reported on a study in SD rats given DCHP in the diet at concentrations of 0, 240, 1,200, and 6,000 ppm.

The estrus cycle length was increased in F0 females at 6,000 ppm (500–534 mg/kg-d). However, this effect is the opposite of what is reported for other phthalates and is therefore of questionable toxicological significance.

Atrophy of seminiferous tubules was increased at 1,200 and 6,000 ppm.

There was a significant decrease in spermatid head count in F1 males at 1,200 and 6,000 ppm. Prostate weight was significantly decreased at all dose levels; relative prostate weight was decreased at 6,000 ppm. However, the relevance is uncertain because other sperm parameters were normal and this finding was not reported with other phthalates.

The NOAELs stated by the authors:

- reproductive toxicity in F1 males—240 ppm or 18 mg/kg-d,
- reproductive toxicity in females—6,000 ppm or 511–534 mg/kg-d.

## **4.5 Diisooheptyl Phthalate (DIHEPP)**

### **4.5.1 Human Data**

No human studies were available for review.

### **4.5.2 Animal Data**

McKee *et al.* (2006) and ExxonMobil Chemical Co. (2003) reported a two-generation reproductive toxicity study in SD rats given DIHEPP in the diet at concentrations of 0, 1,000, 4,500, and 8,000 ppm.

Fertility was decreased at 4,500 and 8,000 ppm. Sperm concentration and sperm production were decreased at all dose levels. Weights of testis, epididymis, cauda epididymis, and ovary were decreased at 8,000 ppm. There was degeneration of seminiferous tubules in F1 males at 4,500 and 8,000 ppm. The authors concluded that some of the effects seen in F1 males could be related to clinical signs of toxicity associated with changes in the external genitalia (hypospadias, absent or undescended testes) observed in the F1 males.

Concentrations of DIHEPP in the diet of males after breeding were 4,500 ppm (227 mg/kg-d) and 1,000 ppm (50 mg/kg-d). Thus, the NOAEL in this study is 50 mg/kg-d.

## **4.6 Diisooctyl Phthalate (DIOP)**

### **4.6.1 Human Data**

No human studies were available for review.

### **4.6.2 Animal Data**

No animal studies were available for review.

### **4.6.3 Mode of Action**

While activation of peroxisome proliferator-activated receptor alpha (PPAR- $\alpha$ ) is involved in carcinogenesis in rodents, it probably does not play a significant role in the induction of developmental toxicity or testicular toxicity. Genetically modified mice (PPAR- $\alpha$  knockout mice) are susceptible to phthalate-induced developmental and testicular effects. Also, PPAR- $\alpha$  null mice have less frequent and less severe testicular lesions following exposure to DEHP (Ward *et al.*, 1998). This mouse does express PPAR- $\gamma$  in the testes (Maloney and Waxman, 1999). The roles of PPAR- $\beta$  and  $\gamma$  activation in reproductive toxicity have not been thoroughly studied.

Guinea pigs, a nonresponding species to the peroxisome proliferating effects of DBP, is susceptible to the testicular effects of this phthalate (Gray *et al.*, 1982).

Gray *et al.* (1982) investigated the reason for the lack of testicular lesions in hamsters administered DBP and the monobutyl ester (MBP) orally at doses higher than those that cause testicular lesions in rats. The levels of MBP in urine were 3–4 fold higher in the rat than in the hamster. A significantly higher level of testicular beta-glucuronidase in the rat compared to the hamster caused the authors to speculate that damage in the rat may be related to higher levels of unconjugated MBP, the putative toxicant. In addition, MEHP and di-*n*-pentyl phthalate (DPENP) did cause testicular effects in the hamster (Gray *et al.*, 1982).

All phthalates that cause testicular toxicity produce a common lesion characterized by alterations in Sertoli cell ultrastructure and function (Gray and Butterworth, 1980; Creasy *et al.*, 1983; Creasy *et al.*, 1987). More recent studies have concluded that testicular toxicity caused by some phthalates during development are related to decreased testosterone production (Mylchreest *et al.*, 1998; Parks *et al.*, 2000; 2002; Barlow and Foster, 2003).

Hannas *et al.* (2011) reported that DPENP is much more potent than other phthalates in disrupting fetal testis function and postnatal development of the male SD rat. Compared to the effect of DEHP under similar conditions of dosing, dipentyl phthalate was eight-fold more potent in reducing testosterone production and two- to three-fold more potent in inducing development of early postnatal male reproductive malformations.

## **4.7 Di(2-propylheptyl) Phthalate (DPHP)**

### **4.7.1 Human Data**

No human studies were available for review.

### **4.7.2 Animal Data**

No published animal studies were available for review. A summary of a preliminary report of a 90-day dietary subchronic study in rats was available from Union Carbide Corp (1997).

There was a significant reduction in sperm velocity indices (n=6 rats/group). Other factors associated with sperm function and concentration (total sperm, static count, percent motile, motile count, total sperm concentration, and concentration of sperm/gm of tissue) were not affected, nor was this endpoint reported in other studies. Further, males had a 23% decrease in body weight. Spermatid endpoints, therefore, are of questionable value.

## **5 Phthalate Substitutes**

### **5.1 Nonreproductive Toxicity**

The phthalate substitute chemicals reviewed here are generally low in acute toxicity by several routes of exposure. They are also generally negative in tests for genotoxic potential.

These substitutes have a different carcinogenic profile than the phthalates they have replaced. Phthalates, to varying degrees, activate PPAR- $\alpha$  receptors in rodent tissues that result in peroxisome proliferation in the liver and cancer of the liver. That is not a general property of the substitutes.

A carcinogenesis study conducted on ATBC in rats did not have an increase in tumors, but the study had low group sizes and low power to detect an effect. Two-year studies on DEHA in rats were negative, but an increased number of liver tumors were seen in both male and female mice. The increase in tumors may have been related to peroxisome proliferation. There was a significant increase in thyroid tumors in rats given DINX in the diet for two years. A carcinogenesis study of DEHT in rats was negative. No cancer studies have been done on TOTM.

Likewise, none of the substitutes caused the same kind of developmental abnormalities of male offspring caused by certain phthalates. The only substitute that caused damage to spermatogenesis in adult male rodents was TOTM, which caused a decrease in the number of spermatocytes and spermatids upon histopathologic examination of the testes of rats. Reproductive studies on other substitutes did not show the types of testicular toxicity or developmental abnormalities that are characteristic of certain phthalates.

## **5.2 Reproductive Toxicity**

### **5.2.1 2,2,4-trimethyl-1,3-pentanediol-diisobutyrate (TPIB)**

#### **5.2.1.1 Human Data**

No published data were available for review.

#### **5.2.1.2 Animal Data**

Eastman Chemical (2007) reported the results of a combined repeated dose and reproductive/developmental toxicity screening test in Sprague-Dawley rats given TPIB by gavage at dose levels of 0, 30, 150, or 750 mg/kg-d from 14 days before mating to 30 days after mating (males) or day 3 of lactation (females). The authors reported that TPIB had no significant effect on mating, fertility, the estrus cycle, delivery, or lactation period. Measures were limited to body weights on postnatal days 0 and 4 and necropsy results on day 4. No TPIB-related effects were reported at any dose level. The NOAEL for reproduction and development was 750 mg/kg-d.

Another study by Eastman Company (2001) was conducted according to OECD test guideline 421. SD rats (12/sex/dose level) were given TPIB in the diet at concentrations to give 0, 120, 359, or 1,135 mg/kg-d to females and 0, 91, 276, or 905 mg/kg-d to males for 14 days before mating, during mating (1–8 days), through gestation (21–23 days), and through postnatal day 4 or 5. Transient decreased body weight gains were noted in parents at high dose levels. There were decreases in the number of implantation sites and corpora lutea. Changes in epididymal and testicular sperm counts were not considered adverse by the authors. Other reproductive measures were not affected. The authors concluded that the NOAEL for reproduction was 276 mg/kg-d for males and 359 mg/kg-d for females, based on total litter weight and size on postnatal day 4 and the decreased number of implants and corpora lutea.

### **5.2.2 Di(2-ethylhexyl) Adipate (DEHA)**

### 5.2.2.1 Human Data

There were no published data to review.

### 5.2.2.2 Animal Data

DEHA was administered in the diet of F344 rats and B6C3F1 mice in subchronic and chronic studies reported by the NTP (1982). No histopathologic effects were observed in reproductive organs (testes, seminal vesicles, prostate, ovary, or uterus) at ~2,500 mg/kg-d in rats or 4,700 mg/kg-d in mice.

Nabae *et al.* (2006) and Kang *et al.* (2006) reported on the testicular toxicity of DEHA given to F344 rats in their diet at concentrations that gave 0, 318, or 1,570 mg/kg-d. There were no changes in body weight, spermatogenesis, relative weight, or histology of testes, epididymis, prostate, or seminal vesicles. Kang *et al.* (2006) found that DEHA caused no testicular toxicity in rats pretreated with thioacetamide to induce liver damage or folic acid to induce chronic renal dysfunction; the testicular toxicity of DEHP was enhanced with the same pretreatments.

Miyata *et al.* (2006) reported a study in Crj:CD (SD) rats given DEHA by gavage at dose levels of 0, 40, 200, or 1,000 mg/kg-d for at least 28 days. Reproductive endpoints in both sexes were measured, but there was no mating trial. The estrus cycle was prolonged in females at the high dose level. No reproductive toxicity was observed in males at any of the dose levels.

Dalgaard (2002; 2003) reported on perinatal exposure of Wistar rats by gavage at dose levels of 0, 800, or 1,200 mg/kg-d on gestation day 7 through postnatal day 17. This was a dose range finding study to examine pups for evidence of antiandrogenic effects—none were observed. Decreased pup weights were seen at both dose levels. In the main study, DEHA was given by gavage at dose levels of 0, 200, 400, and 800 mg/kg-d on gestation day 7 through postnatal day 17. No antiandrogenic effects were seen; a NOAEL of 200 mg/kg-d was based on postnatal deaths.

## 5.2.3 Di(2-ethylhexyl)terephthalate (DEHT)

### 5.2.3.1 Human Data

No published data were available for review.

### 5.2.3.2 Animal Data

Faber *et al.* (2007) reported the results of a two-generation reproduction study in SD rats given DEHT in the diet. The dietary admix was given to males and females for 70 days prior to mating plus during pregnancy and lactation. Concentrations in the diet gave 0, 158, 316, or 530 mg/kg-d to males and 0, 273, 545, or 868 mg/kg-d to females. No adverse effects on reproduction were observed in either generation at any dose level. Weight gain was decreased in F0 high-dose males. Weight gain was decreased in F1 and F2 males at the top two dose levels. The NOAEL for reproductive effects was 530 mg/kg-d; the NOAEL for parental and pup systemic toxicity was 158 mg/kg-d.

Gray *et al.* (2000) reported a study to look for antiandrogenic effects of DEHT. Pregnant SD rats were dosed by gavage with 0 or 750 mg/kg-d on gestation day 14 through postnatal day 3. No antiandrogenic effects were observed.

#### **5.2.4 Acetyl Tri-n-Butyl Citrate (ATBC)**

##### **5.2.4.1 Human Data**

There were no published data to review.

##### **5.2.4.2 Animal Data**

A two-generation reproduction study in SD rats was reported by Robbins (1994). ATBC was mixed in the diet at concentrations to give 0, 100, 300, 1,000 mg/kg-d. Males were exposed for 11 weeks and females for 3 weeks before mating, during mating, and through gestation and lactation. Male and female pups were given diets with ATBC for 10 weeks after weaning. There were no reproductive or developmental effects attributable to ATBC at any dose level.

Chase and Willoughby (2002) reported a one-generation reproduction study (summary only) in Wistar rats given ATBC in the diet at concentrations to provide 0, 100, 300, or 1,000 mg/kg-d for four weeks prior to and during mating plus during gestation and lactation. The F0 parents produced an F1 generation of litters. No systemic or reproductive effects were seen at any dose level.

#### **5.2.5 Cyclohexanedicarboxylic Acid, Dinonyl Ester (DINX)**

##### **5.2.5.1 Human Data**

No published data were available for review.

##### **5.2.5.2 Animal Data**

A two-generation reproduction study was reported by SCENIHR (2007) in summary form only. Because the study used OECD TG 416, it was likely conducted in rats. Dose levels by diet were 0, 100, 300, or 1,000 mg/kg-d. The authors reported that there were no effects on fertility or reproductive performance in F0 and F1 parents, and no developmental toxicity in F1 or F2 pups. A substudy designed to look for antiandrogenic effects reportedly showed no developmental toxicity at any dose level.

#### **5.2.6 Tris(2-ethylhexyl) Trimellitate (TOTM)**

##### **5.2.6.1 Human Data**

No published human data were available for review.

##### **5.2.6.2 Animal Data**

A one-generation reproduction study was reported in SD rats given TOTM by gavage at dose levels of 0, 100, 300, or 1,000 mg/kg-d (JMHW, 1998). Males were dosed for 46 days and females for 14 days prior to mating and during mating through lactation day 3. Histologic

examination showed a decrease in spermatocytes and spermatids at the top two dose levels. No other reproductive toxicity was seen. The NOAEL was 100 mg/kg-d.

Pre- and postnatal effects of TOTM in SD rats were reported from Huntington Life Sciences (2002). Rats were given 0, 100, 500, or 1,050 mg/kg-d by gavage on days 6–19 of pregnancy or day 3 through day 20 of lactation. There were no significant effects on developmental measures. There was a slight delay in the retention of areolar regions on postnatal day 13, but not on day 18 (not considered to be toxicologically significant).

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