Report to the

U.S. Consumer Product Safety Commission

by the

CHRONIC HAZARD ADVISORY PANEL ON PHTHALATES

AND PHTHALATE ALTERNATIVES

July 2014

APPENDIX C

EPIDEMIOLOGY

TABLE OF CONTENTS

1. Phthalates and Male Reproductive Tract Development 1
1.1 Supporting Evidence for Antiandrogenic Effects of Phthalates..... 2
1.2 Maternal Occupational Exposure and Male Reproductive Tract Anomalies..... 2
2. Phthalates and Neurodevelopmental Outcomes 3
3. Pubertal Development and Gynecomastia..... 6
4. Adult Exposure and Semen Quality 8
5. References 9

LIST OF TABLES

Table C-1 Phthalates and pubertal measures. 7

ABBREVIATIONS*

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

* List applies to main report and all appendices.

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

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

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

1. Phthalates and Male Reproductive Tract Development

The association of gestational exposure to phthalates and reproductive tract development was explored in three study cohorts. Swan and colleagues (Swan *et al*., 2005; Swan, 2008) published two papers on the association of urinary phthalate metabolite concentrations and anogenital distance (AGD) in male infants from the same multi-center pregnancy cohort study. In Swan's first paper (2005), there were 85 mother-son pairs with prenatal urinary phthalate concentrations (mean 28.6 weeks of gestation) and AGD measures (mean age at examination was 12.6 months). To account for differences in body size, they defined anogenital index (AGI) as AGD/body weight, a weight-normalized index of AGD. For short AGI, the odds ratio (OR) (95% confidence interval) for high compared with medium and low concentrations of monobutyl phthalate (MBP) were 3.8 (1.2, 12.3) and 10.2 (2.5, 42.2), respectively. The corresponding OR (95% CI) for short AGI for high compared with medium and low concentrations of monobenzyl phthalate (MBZP), monoethyl phthalate (MEP), and monoisobutyl phthalate (MIBP) were 3.1 (1.002, 9.8) and 3.8 (1.03, 13.9), 2.6 (0.9, 7.8) and 4.7 (1.2, 17.4), 3.4 (1.1, 10.5) and 9.1 (2.3, 35.7), respectively. There were no associations of AGI with monomethyl phthalate (MMP) or mono(3-carboxypropyl) phthalate (MCPP) (metabolites of dimethyl phthalate [DMP] and di-*n*-octyl phthalate [DNOP], respectively).

In addition to exploring associations with individual phthalate metabolites, the authors calculated a summary phthalate score to explore associations with joint exposure to more than one phthalate. The summary phthalate score was strongly associated with short AGI. It is important to note that the summary scores were defined using the results from the analyses for the individual phthalates with AGI. Therefore, it is expected that the summary measure would have a stronger association with AGI. As a group, boys with incompletely descended testicles or a scrotum categorized as small and/or not distinct from surrounding tissue had a shorter AGI.

In 2008, Swan *et al.* published an update extending their analyses on maternal phthalate exposure and genital development to 106 mother-son pairs; 68 of the sons had AGD measured at two visits. This updated analysis included the original 85 mother-son pairs (Swan *et al.*, 2005). To further reduce confounding by the babies' weights, the authors calculated weight percentile, defined as the expected weight for age using sex-specific estimates of weight percentiles in the U.S. population. Statistical methods accounting for the repeated measures were used, controlling for age and weight percentile. There were significant associations of five phthalate metabolites (MEP, MBP, mono(2-ethylhexyl) phthalate [MEHP], mono(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP], and mono(2-ethyl-5-oxyhexyl) phthalate [MEOHP]) with shortened AGD. This differs from the earlier analysis in which di(2-ethylhexyl) phthalate (DEHP) metabolites were not significantly (MEHP) or marginally (MEOHP and MEHHP) associated with AGD. However, the direction of the associations for the DEHP metabolites with AGD were consistent in the original (Swan *et al.*, 2005) and updated analysis (Swan, 2008). MBZP, of borderline significance with AGD in the original analysis, was not associated with AGD in the updated analysis. MMP and MIBP were of borderline significance with reduced AGD. MCPP was not associated with AGD. As in the earlier paper, the summary phthalate score was more strongly associated with shorter AGD than were individual phthalate measures.

In a small study on 33 male and 32 female infants, researchers from Taiwan (Huang *et al.*, 2009) explored associations of prenatal urine and amniotic fluid levels of MEHP, MBP, MBZP, MMP,

and MEP with AGD measured at birth. AGD for female infants, after adjusting for birth weight or length, were significantly shorter among those above the median for amniotic fluid MBP or MEHP concentrations, as compared to those below the median. In female infants, urine concentrations of MBP had suggestive negative associations with AGD after adjustment for birth weight or length. Among male infants, birth weight, length, and AGD were not associated with amniotic fluid levels of MBP or MEHP.

In a study from Japan, Suzuki *et al.* (2012) explored associations of urinary phthalate metabolite concentrations with AGI (AGD normalized for body weight) among 111 mother-son pairs. Urine was collected between the 9th and 40th week of gestation (mean [SD] was 29 [9] weeks), and AGD was measured at birth. There were significant associations of MEHP with reduced AGI and suggestive associations with the sum of DEHP metabolites. There was no association of MMP, MEP, MBP, MBZP, MEHHP, or MEOHP with AGI. One primary limitation of this study was that 23 examiners performed the AGD measures on the newborns, contributing to possible measurement error and potential attenuation of associations.

1.1 Supporting Evidence for Antiandrogenic Effects of Phthalates

A Danish-Finnish study on 130 three-month-old male infants, 62 cases with cryptorchidism and 68 controls, explored the association of phthalate concentrations in breast milk with serum reproductive hormones (Main *et al.,* 2006). Breast milk phthalate concentrations were not associated with cryptorchidism, but there were associations with hormones related to Leydig cell function. MMP, MEP, and MBP were positively associated with the luteinizing hormone (LH):free testosterone ratio (a 10-fold increase in MMP, MEP, and MBP concentrations raised the LH:free testosterone ratio from 18% to 26%). There were suggestive positive associations of MEHP and mono(isononyl) phthalate (MINP) with the LH:free testosterone ratio and suggestive positive associations of MMP, MEP, MBP, and MEHP with the LH:testosterone ratio. MINP was associated with increased LH (a 10-fold increase in MINP was associated with a 97% increase in LH), and there was a suggestive association with increased testosterone. MBP was inversely associated with free testosterone, whereas MEP and MEHP showed similar directions of association but were nonsignificant. For Sertoli cell markers (*i.e.*, FSH and inhibin B), positive nonsignificant associations were found for MBzP and MEHP with inhibin B. All monoesters were negatively associated with the FSH:inhibin B ratio, which was significant for MEHP. Finally, MEP and MBP were positively associated with sex-hormone binding globulin (SHBG), and there were suggestive nonsignificant positive associations of MBZP and MINP with SHBG.

The Main *et al.* results for MEP, MBP, and MEHP suggest that human Leydig cell development and function is affected following perinatal exposure. The reduced free testosterone and the increased LH:free testosterone ratio support the associations of phthalates with reduced AGD reported in Swan *et al.* (2005). Although the changes in hormones related to Leydig cell function may or may not pose a significant health effect in a single individual, such a shift on a population basis could presumably lead to potential adverse health outcomes.

1.2 Maternal Occupational Exposure and Male Reproductive Tract Anomalies

Several epidemiological studies investigated the association of maternal occupational exposure to phthalates with male reproductive tract anomalies, including cryptorchidism and hypospadias

(Van Tongeren *et al.*, 2002; Vrijheid *et al.*, 2003; Ormond *et al.*, 2009; Morales-Suarez-Varela *et al.*, 2011). None of these studies used biological markers to assess phthalate exposure, but instead, assigned potential exposure to phthalates based on job titles or self-reported occupational histories. Therefore, these studies are only briefly described because their relevance to the report is limited by the nonspecific assessment of phthalate exposure and the lack of data for specific phthalates.

Analyzing data from the Danish National Birth Cohort, Morales-Suarez-Varela *et al.* (2011) reported an association between hypospadias and exposure to phthalates using a job exposure matrix for endocrine disruptors. In Southeast England, Ormond and coworkers (2009) reported an association between phthalate exposure, defined using job exposure matrices, and increased odds of hypospadias. Using data from the National Congenital Anomaly System in England and Wales, Vrijheid *et al.* (2003) did not find an association of phthalates with hypospadias. Overall, these studies provide limited evidence of an association of hypospadias with jobs that may have phthalate exposure. Critical study design limitations include: 1) nonspecific assessment of phthalate exposure based on job title or occupational histories, 2) lack of information on exposure to specific phthalates while at work and their potential level of exposure, and 3) inability to adjust for important co-exposures at work that may confound these associations.

2. Phthalates and Neurodevelopmental Outcomes

Swan and colleagues (2010) assessed the association of prenatal exposure to phthalates with play behavior of children from their multi-center prospective pregnancy cohort study. The child's mother completed a preschool activities inventory questionnaire that assessed her child's sexually dimorphic play behavior. The association of urinary phthalate metabolite concentrations with play behavior scores (masculine and feminine composite) was assessed separately for boys (n=74, mean age 5 years, range 3.6 to 6.4 years) and girls (n=71, mean age 4.9 years, range 3.6 to 6.0 years). Multivariate regression analyses controlling for the child's age, mother's age and education, and parental attitude toward atypical play choices were adjusted for. Among boys, there was an inverse association of urinary concentrations of MBP, MIBP, and their sum with decreased (less masculine) composite scores. In addition, DEHP metabolites, MEOHP and MEHHP, and the sum of these two metabolites with MEHP were associated with a decreased masculine score. Among boys, for the other phthalate metabolites measured, the authors did not find associations with play behavior. Among girls, there were no associations of play behavior with any of the phthalate metabolites. Study limitations include the use of a single urine sample during pregnancy to assess exposure to phthalates and self-reported play behavior by the mother. However, it is unlikely that these limitations would introduce bias away from the null, but rather would attenuate associations.

Three publications utilizing data from the Mount Sinai School of Medicine Children's Environmental Health Cohort reported on children's neurodevelopmental outcomes in relation to prenatal urinary phthalate concentrations (Engel *et al.*, 2009; Engel *et al.*, 2010; Miodovnik *et al.*, 2011). The Mount Sinai study was a prospective multiethnic birth cohort of 404 primiparous women with singleton pregnancies recruited in New York City between 1998 and 2002. In their first publication, Engel *et al.* (2009) analyzed the association of prenatal urinary phthalate concentrations with scores on the Brazelton Neonatal Behavioral Assessment Scale (BNBAS) measured in 295 children within the first 5 days after delivery. Maternal urine was collected

during the third trimester between 25 and 40 weeks' gestation (mean, 31.2 weeks). The exposure assessment approach summed 10 phthalate urinary metabolites on a molar basis into low molecular weight (LMW) (MMP, MEP, MBP, and MIBP) and high molecular weight (HMW) (MBZP, mono(2-ethyl-5-carboxypentyl) phthalate [MECPP], MEHHP, MEOHP, MEHP, and MCPP) phthalates. Of note is that MEP was the largest contributor, by a wide margin, to the LMW phthalate sum, while the DEHP metabolites were the largest contributors to the HMW sum. This should be taken into account when interpreting the molecular weight (MW) sums because the contribution of the individual metabolites is not equivalent within the sum. There were few associations of individual phthalate metabolites (data not shown) and their molar sums with most BNBAS scores. However, there were significant sex-phthalate interactions ($p<0.10$) for the Orientation and Motor domains and the overall Quality of Alertness score. Among girls, there was a significant decline in adjusted mean Orientation score and Quality of Alertness score with increasing urinary concentrations of HMW phthalates. Boys and girls showed opposite patterns of association between low and high MW phthalates and motor performance, with suggestion of improved motor performance in boys with increasing LMW concentrations. Although BNBAS domains represent general central nervous system (CNS) organization, the authors hypothesized that there may be sex-specific effects of phthalates.

The second publication from the Mount Sinai study by Engel *et al.* (2010) reported on the association of prenatal urinary phthalate concentrations with behavior and executive functioning among 188 children assessed up to three times between age 4 and 9 years. Mothers completed the parent-report forms of the Behavioral Rating Inventory of Executive Function (BRIEF) and the Behavior Assessment System for Children Parent Rating Scales (BASC-PRS). Higher urinary concentrations of LMW phthalates were associated with poorer BASC scores for aggression, conduct problems, attention problems, and depression clinical scales, as well externalizing problems and behavioral symptoms index ([BSI], the apical summary score that assessed overall level of behavioral functioning). LMW phthalates were also associated with poorer scores on the global executive composite index and the emotional control scale of the BRIEF. Although urinary MBP concentrations were significantly associated with only aggression and externalizing problems, the magnitude of the MBP associations were very similar to LMW phthalates for attention problems, adaptability and the BSI. MBP was also associated with poorer scores on working memory, and the associations for other domains were similar to the LMW associations.

The authors concluded that the profile of the parent-reported behaviors was suggestive of the behavioral profiles of children clinically diagnosed with disruptive behavior disorders, conduct disorder, or attention deficit hyperactivity disorder (ADHD). Furthermore, although few children in the study met the standard at-risk or clinically significant criteria on the BASC, the patterns across scales and the consistency of the findings across instruments suggest associations of prenatal LMW phthalate exposure with the emergence of disruptive behavior problems in children. Limitations in the Mount Sinai publications include the use of a single spot urine sample late in pregnancy to assess exposure and the use of parent self-report of behavioral and executive function. However, it is unlikely that these limitations would introduce bias away from the null, but rather would attenuate associations.

The third publication from the Mount Sinai study, by Miodovnik (2011), investigated relationships between prenatal urinary phthalate concentrations and Social Responsiveness Scale

(SRS) measurements among 137 children assessed between ages 7 and 9 years. The SRS is a quantitative scale for measuring the severity of social impairment related to Autistic Spectrum Disorders (ASD). Higher urinary concentrations of LMW phthalates were associated with higher SRS scores, positively with poorer scores on Social Cognition, Social Communication, and Social Awareness, but not with Social Motivation or Autistic Mannerisms. These associations were statistically significant for MEP and in the same direction for MBP and MMP but not significant. HMW phthalates and the sum of DEHP metabolites were nonsignificantly associated with poorer SRS scores, though of a smaller magnitude. Limitations discussed above for the Mount Sinai study also apply to this report and include the use of a single spot urine sample late in pregnancy to assess exposure and the use of a parent rating survey. It is important to note that the study did not include clinical diagnoses of ASD, but rather symptoms common to the disorder. Finally, the associations reported were modest on an individual level.

In a cross-sectional study on 621 Korean school-age children (mean age 9.05 years, range 8 to 11 years old), Cho *et al.* (2010) explored associations of urinary MEHP, MEOHP, and MBP concentrations with intelligence scores. These were the only phthalate metabolites measured in the spot urine samples. In multivariate models, there were significant associations of the DEHP metabolites with decrements in Full Scale IQ, Verbal IQ, Vocabulary and Block design scores measured using the abbreviated form of the Korean Educational Development Institute-Wechsler Intelligence Scale for Children (KEDI-WISC). Urinary concentrations of MBP were significantly associated with decrements in Vocabulary and Block design scores. However, after adjusting for maternal IQ, only the association of DEHP metabolites with Vocabulary score remained significant. A second Korean study (Kim *et al.*, 2009) explored cross-sectional associations of urine phthalate concentrations with ADHD symptoms and neuropsychological dysfunction among 261 children 8 to 11 years of age. Urine DEHP metabolites (MEHP and MEOHP), but not MBP, were associated with teacher-assessed ADHD scores. Conclusions based on these two cross-sectional studies are limited because the spot urine samples were collected concurrently with the outcome assessments.

In a third Korean study, Kim *et al.* (2011) conducted a multi-center prospective cohort study on 460 mother infant pairs, recruited during their first trimester of pregnancy. Spot urine samples, collected during weeks 35 to 41 of gestation, were analyzed for MEHHP, MEOHP, and MBP. They reported negative associations between MEHHP, MEOHP, and MBP with mental development indices (MDI) of the Bayley Scales of Infant Development assessed at six months of age. The psychomotor development indices (PDI) were negatively associated with MEHHP. In a subset analysis adjusted for maternal intelligence, there were negative associations of MEHHP with MDI, and MEHHP, MEOHP, and MBP with PDI. They reported sex-specific differences whereby in boys, MDI and PDI were negatively associated with MEHHP, MEOHP, and MBP. Coefficients were negative in girls for these associations but were not statistically significant.

Whyatt and colleagues (2011) explored the association of mental, motor, and behavioral development at age three years with urinary phthalate concentrations measured during the third trimester of pregnancy. In their prospective cohort study on 319 women-child pairs from New York (U.S.), they reported negative associations between urinary concentrations of MIBP and MBP and PDI, and among girls they found a negative association of MBP with MDI. MBP and MIBP were also associated with increased odds of psychomotor delay on the BSID-II, with no

differences based on child gender. However, there were child sex differences in the relationship between MBP and mental delay. The authors did not find associations between the sum of DEHP metabolites and measures of neurodevelopment. In the total cohort, MNBP was associated with increased somatic complaints, withdrawn behavior, and internalizing behaviors on the Child Behavior Check List (CBCL); there were no associations with child sleep problems or scales in the externalizing domains. MIBP was associated with increased emotionally reactive behavior, whereas MBZP was associated with increased withdrawn behavior and internalizing behavior. There were several differences based on the child's gender. Among boys only, MBP was associated with emotionally reactive behavior, somatic complaints, withdrawn behavior, and internalizing behaviors. Among girls only, MBZP was associated with anxious/depressed behavior, somatic complaints, withdrawn behavior, and internalizing behaviors. When scores on borderline and clinical ranges of CBCL were used, the authors found increased odds for MBP and MBZP with scores in the clinical range for withdrawn behavior, scores in the borderline range for internalizing behavior in association with MIBP and MBZP, and scores in the clinical range on internalizing behaviors for MBZP.

In the seventh prospective pregnancy cohort study, Yolton *et al.* (2011) reported on the association of early infant neurobehavior, assessed with the NICU Network Neurobehavioral Scale (NNNS), measured at 5 weeks after delivery in 350 mother-child pairs. The NNNS evaluates neurological functioning, provides a behavioral profile, and measures signs of stress in young infants. They measured maternal urinary phthalate metabolites at 16 and 26 weeks of gestation. Higher total dibutyl phthalate (DBP)/diisobutyl phthalate (DIBP) 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. There were no sex by DBP interactions. In males, higher total DEHP metabolites at 26 weeks were associated with more non-optimal reflexes.

3. Pubertal Development and Gynecomastia

Several epidemiologic studies reported on the association of measures of phthalate exposure with pubertal development or gynecomastia (Colon *et al.,* 2000; Lomenick *et al.,* 2009; Durmaz *et al.,* 2010). In a small study on pubertal gynecomastia in boys, Durmaz and colleagues (2010) measured plasma phthalate concentrations of DEHP and MEHP in 40 newly diagnosed pubertal gynecomastia cases and 21 age-matched control children without gynecomastia or other endocrinologic disorders. They reported higher concentrations of serum DEHP and MEHP in the children with pubertal gynecomastia compared to the control group. In an earlier study, Colon *et al.* (2000) reported associations between serum concentrations of DEHP with premature thelarche in a case (n= 41) control (n=35) study. In a small case control study (Lomenick *et al.,* 2009) on 28 girls with central precocious puberty and 28 age- and race-matched prepubertal girls, there were no differences in urinary phthalate metabolite concentrations between the cases and controls.

These three studies were very small, limiting their power to detect associations. In addition, each used a single spot sample (*i.e.,* blood or urine) to measure phthalate concentrations, which represents only recent exposure and may not reflect exposure during the relevant window of susceptibility, such as gestational or early childhood. Furthermore, two studies had important

limitations in methods used to assess phthalate exposure (Colon *et al.*, 2000; Durmaz *et al.*, 2010). They measured the diester in serum, raising concern with contamination, which may occur at the collection or analysis phase. Therefore, these two studies need to be interpreted very cautiously due to critical limitations.

Another study with a very limited sample size was conducted by Rais-Bahrami *et al.* (2004) on 19 children who presumably had high DEHP exposure as neonates from extracorporeal membrane oxygenation (ECMO) while in the intensive care unit. They examined and collected blood from 13 boys and 6 girls at ages 14 to 16 years. All the children (except for one with Marfan syndrome) had normal growth percentiles for age and sex, and normal values for thyroid, liver, and renal functions. Reproductive hormones (LH, FSH, and testosterone for males and estradiol of girls) were appropriate for Tanner stage of pubertal development. Although comprehensive assessments were performed on the children at ages 14 to 16 years, the very limited sample size makes comparisons with population distributions non-informative because the power to detect subtle shifts in distributions is minimal. However, the design of the study is a strength because children receiving ECMO, or other medical treatments, in neonatal intensive care units represent a population with potentially high DEHP exposure (Calafat *et al.*, 2009). Larger studies on NICU populations would be informative and should be conducted.

Table C-1 Phthalates and pubertal measures.

Author, yr	Design	Exposure Metric	Outcome	Results	Comments
Durmaz *et al.* (2010),	Case (n=40) control (n=21)	Serum concentrations of DEHP and MEHP	Pubertal gynecomastia in boys	Higher serum concentrations of DEHP and MEHP among cases	Small sample size and concern with contamination of blood
Lomenick *et al.* (2009)	Case (n=28) control (n=28)	Urine concentrations of 9 phthalate metabolites	Central precocious puberty in girls	No difference in cases or controls for any of the phthalate metabolites	Small sample size
Colon *et al.* (2000)	Case (41) control (35)	Serum concentrations of DEHP (MEHP), DBP, BBP, DMP, DOP	Premature thelarche in girls	Higher serum concentrations of DEHP among the cases	Small sample size and concern with contamination of blood
Rais-Bahrami *et al.* (2004)	Follow-up of 19 children who underwent ECMO as neonates	Presumed high DEHP exposure from ECMO as a neonate in the intensive care unit	Pubertal assessment, physical growth, reproductive hormones in boys and girls 14 to 16 years old	As compared to population norms, no differences in hormones or growth percentiles	Small sample size

ECMO = extracorporeal membrane oxygenation;

4. Adult Exposure and Semen Quality

In addition to epidemiologic studies that investigated health outcomes in relation to gestational, infant, and/or childhood exposure to phthalates, there is a growing literature on adult exposure to phthalates and semen quality, an outcome relevant to the hypothesized testicular dysgenesis syndrome. All of the semen quality studies were cross-sectional; during adulthood they measured urinary concentrations of phthalate metabolites and semen quality (Liu *et al.,* 2012; Murature *et al.,* 1987; Rozati *et al.,* 2002; Duty *et al.,* 2003; Duty *et al.,* 2004; Hauser *et al.,* 2006; Zhang *et al.,* 2006; Hauser *et al.,* 2007; Lili *et al.,* 2007; Pant *et al.,* 2008; Wirth *et al.,* 2008; Herr *et al.,* 2009; Won Han *et al.,* 2009). The evidence was inconsistent across studies, with several publications from an infertility clinic suggesting associations of reduced semen quality with urinary concentrations of MBP and MEHP, and other studies not confirming these associations. These studies are less relevant to this report because exposure was measured during adulthood and cannot be used to infer childhood or early life exposure because phthalates have short biological half-lives and exposure patterns change with life stage. Therefore, they are not discussed further.

5. References

Calafat, A.M., Weuve, J., Ye, X., Jia, L.T., Hu, H., Ringer, S., Huttner, K., Hauser, R., 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environ Health Perspect 117, 639–644.

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.

Colon, I., Caro, D., Bourdony, C.J., Rosario, O., 2000. Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development. Environ Health Perspect 108, 895–900.

Durmaz, E., Ozmert, E.N., Erkekoglu, P., Giray, B., Derman, O., Hincal, F., Yurdakok, K., 2010. Plasma phthalate levels in pubertal gynecomastia. Pediatrics 125, e122–129.

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., Silva, M.J., Barr, D.B., Brock, J.W., Ryan, L., Chen, Z., Herrick, R.F., Christiani, D.C., Hauser, R., 2003. Phthalate exposure and human semen parameters. Epidemiology 14, 269–277.

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.

Hauser, R., Meeker, J.D., Duty, S., Silva, M.J., Calafat, A.M., 2006. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology 17, 682–691.

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.

Herr, C., zur Nieden, A., Koch, H.M., Schuppe, H.C., Fieber, C., Angerer, J., Eikmann, T., Stilianakis, N.I., 2009. Urinary di(2-ethylhexyl)phthalate (DEHP): Metabolites and male human markers of reproductive function. Int J Hyg Environ Health 212, 648–653.

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. Environ Int 35, 14–20.

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, 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.

Lili, Q., Lixing, Z., Depei, C., 2007. Study on the di-n-butyl phthalate and di-2-ethylhexyl phthalate level of girl serum related with precocious puberty in Shanghai. J Hyg Res, 93–95.

Liu, L., Bao, H., Liu, F., Zhang, J., Shen, H., 2012. Phthalates exposure of Chinese reproductive age couples and its effect on male semen quality: A primary study. Environ Int 42, 78–83.

Lomenick, J.P., Calafat, A.M., Melguizo Castro, M.S., Mier, R., Stenger, P., Foster, M.B., Wintergerst, K.A., 2009. Phthalate exposure and precocious puberty in females. J Pediatr 156, 221–225.

Main, K.M., Mortensen, G.K., Kaleva, M.M., Boisen, K.A., Damgaard, I.N., Chellakooty, M., Schmidt, I.M., Suomi, A.M., Virtanen, H.E., Petersen, D.V., Andersson, A.M., Toppari, J., Skakkebaek, N.E., 2006. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ Health Perspect 114, 270–276.

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.

Morales-Suarez-Varela, M.M., Toft, G.V., Jensen, M.S., Ramlau-Hansen, C., Kaerlev, L., Thulstrup, A.M., Llopis-Gonzalez, A., Olsen, J., Bonde, J.P., 2011. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: A study in the Danish National Birth Cohort study. Environ Health 10, 3.

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.

Ormond, G., Nieuwenhuijsen, M.J., Nelson, P., Toledano, M.B., Iszatt, N., Geneletti, S., Elliott, P., 2009. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: Case-control study. Environ Health Perspect 117, 303–307.

Pant, N., Shukla, M., Kumar Patel, D., Shukla, Y., Mathur, N., Kumar Gupta, Y., Saxena, D.K., 2008. Correlation of phthalate exposures with semen quality. Toxicol Appl Pharmacol 231, 112–116.

Rais-Bahrami, K., Nunez, S., Revenis, M.E., Luban, N.L., Short, B.L., 2004. Follow-up study of adolescents exposed to di(2-ethylhexyl) phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. Environ Health Perspect 112, 1339–1340.

Rozati, R., Reddy, P.P., Reddanna, P., Mujtaba, R., 2002. Role of environmental estrogens in the deterioration of male factor fertility. Fertil Steril 78, 1187–1194.

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.

Van Tongeren, M., Nieuwenhuijsen, M.J., Gardiner, K., Armstrong, B., Vrijheid, M., Dolk, H., Botting, B., 2002. A job-exposure matrix for potential endocrine-disrupting chemicals developed for a study into the association between maternal occupational exposure and hypospadias. Ann Occup Hyg 46, 465–477.

Vrijheid, M., Armstrong, B., Dolk, H., van Tongeren, M., Botting, B., 2003. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med 60, 543–550.

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.

Wirth, J.J., Rossano, M.G., Potter, R., Puscheck, E., Daly, D.C., Paneth, N., Krawetz, S.A., Protas, B.M., Diamond, M.P., 2008. A pilot study associating urinary concentrations of phthalate metabolites and semen quality. Syst Biol Reprod Med 54, 143–154.

Won Han, S., Lee, H., Han, S.Y., Lim, D.S., Jung, K.K., Kwack, S.J., Kim, K.B., Lee, B.M., 2009. An exposure assessment of di-(2-ethylhexyl) phthalate (DEHP) and di-n-butyl phthalate (DBP) in human semen. J Toxicol Environ Health A 72, 1463–1469.

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.

Zhang, Y.H., Zheng, L.X., Chen, B.H., 2006. Phthalate exposure and human semen quality in Shanghai: A cross-sectional study. Biomed Environ Sci 19, 205–209.