

# Studies of Phthalates in Humans

Shanna H. Swan

Chronic Hazard Advisory Panel on  
Phthalates and Phthalate Substitutes

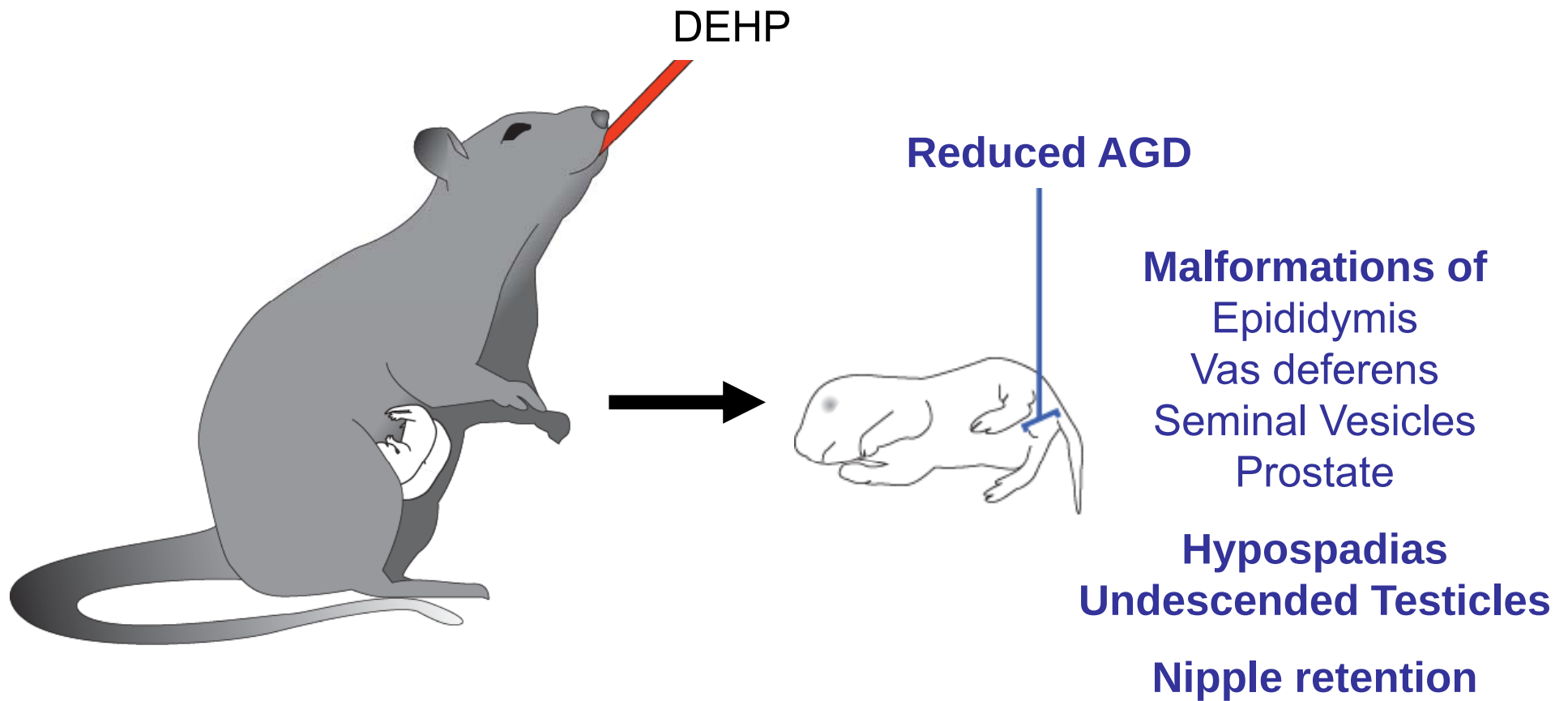
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# Endpoints assoc with ***prenatal phthalate*** exposure in humans

- Alterations of infant genitalia:
  - Anogenital distance (AGD)
  - Penile size
  - Hypospadias
- Length of gestation
- Behavior and neurodevelopment

# Alterations of infant genitalia

- Swan et al. 2005
- Swan et al. 2008
- Bustamante et al, 2008 (ISEE abstract)
- Huang et al. 2008
- Ormond et al. 2008



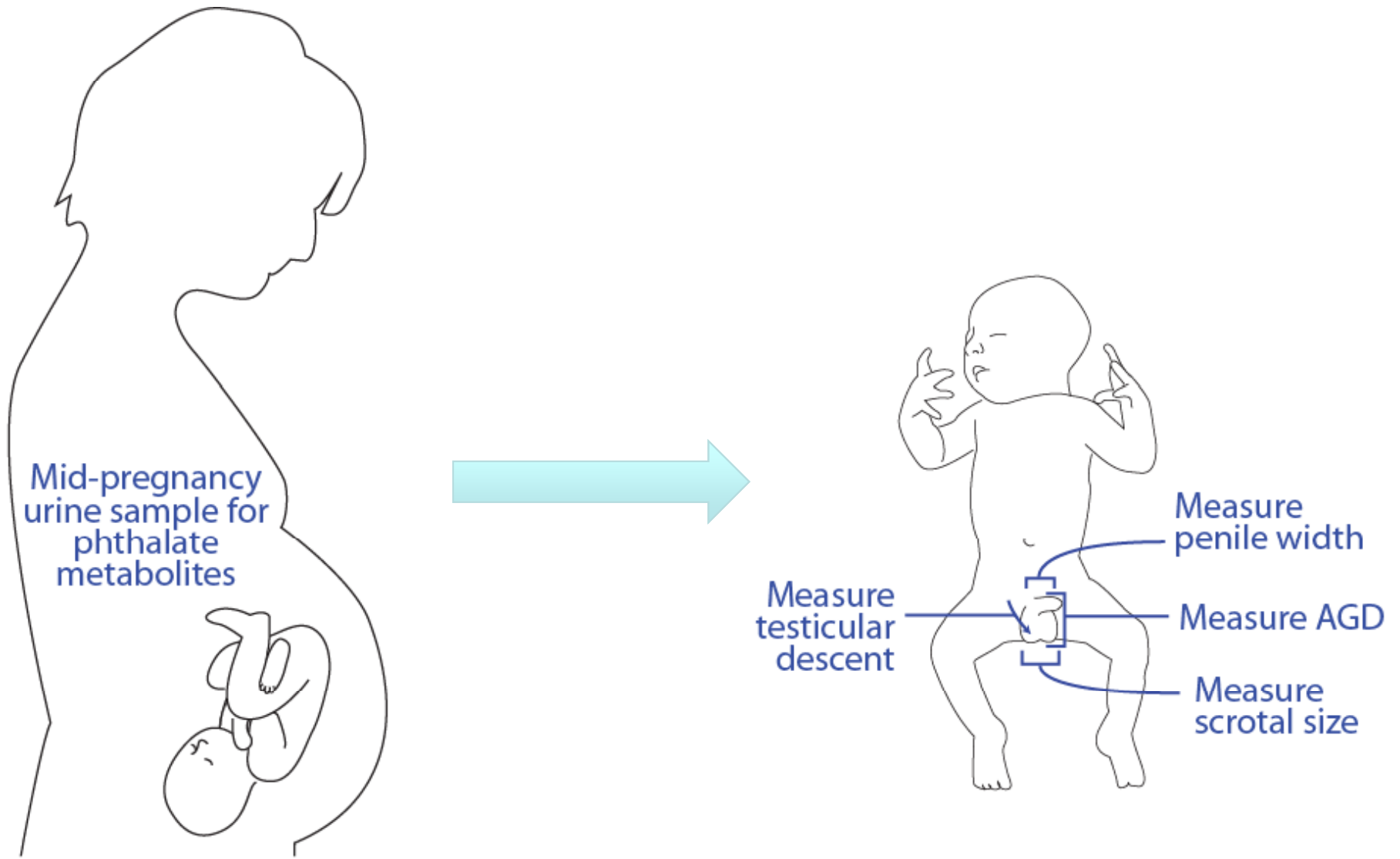
Together these make up the “phthalate syndrome”

Gray and Foster 2003, Foster 2005

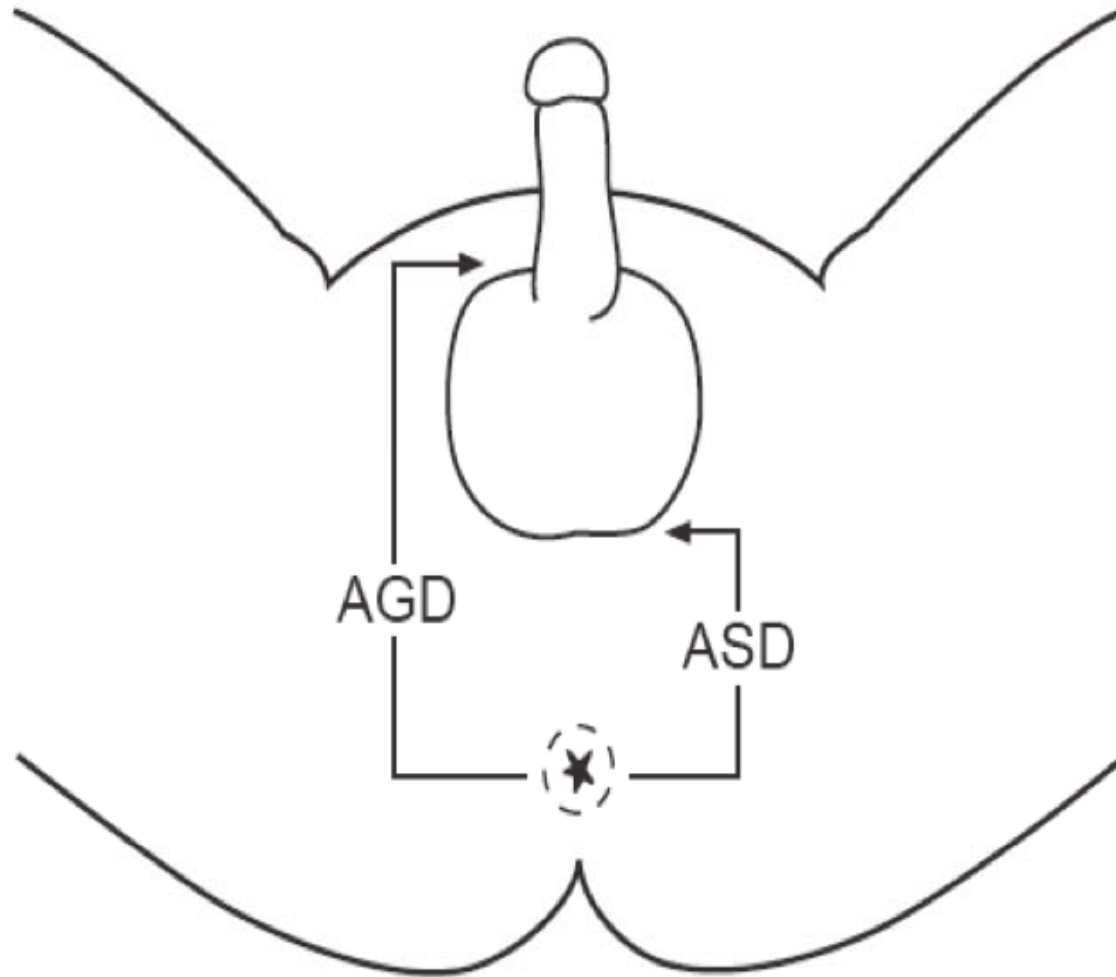
# Study for Future Families (SFF)

- SFFI: Women and partners recruited at prenatal clinics in MO, MN, CA and IA
  - $\geq 18$  years, pregnancy conceived naturally
  - Questionnaires, serum and urine samples
- SFFII: Children born 2000-2003, whose mothers stored a urine sample

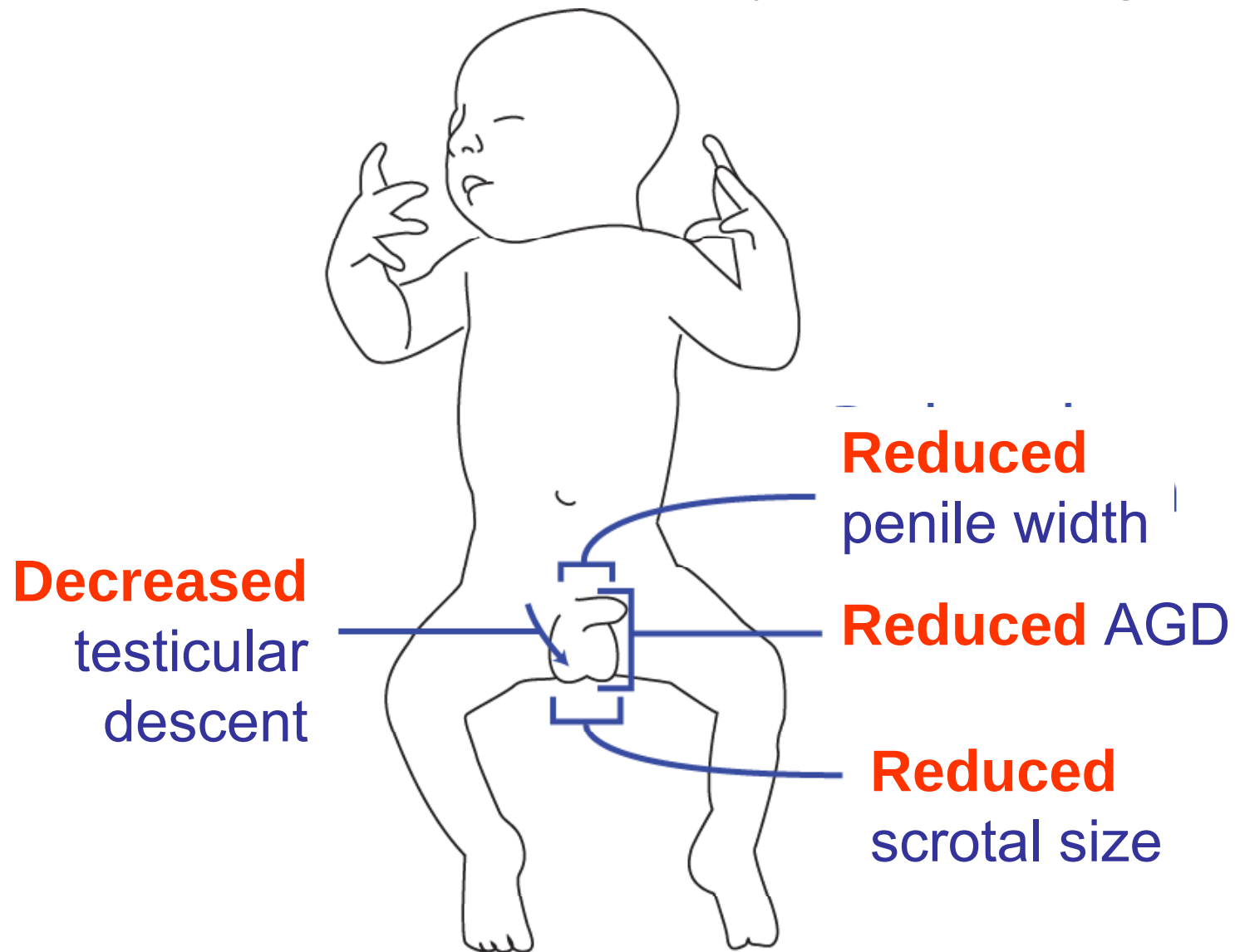
# Measured in boys



## AGD measures in male infants



DEHP exposure was associated with multiple endpoints in boys but not girls





# Male AGD and Phthalates

## Odds Ratio (95% CI) for Shorter AGD

| Exposure | Medium:low*      | High:low*         |  |  |
|----------|------------------|-------------------|--|--|
| MBP      | 5.7 (1.2, 27.3)  | 9.2 (1.8, 46.2)   |  |  |
| MEHP     | 1.7 (0.5, 5.2)   | 3.2 (0.9, 11.5)   |  |  |
| MEOHP    | 10.2 (1.3, 82.5) | 29.1 (3.4, 245.6) |  |  |
| MEHHP    | 4.8 (1.0, 22.9)  | 13.0 (2.6, 66.4)  |  |  |
| MEP      | 4.6 (1.0, 21.6)  | 7.9 (1.5, 41.3)   |  |  |

\*Low < 25<sup>th</sup> %, High ≥ 75<sup>th</sup>%, Medium, other

# Huang et al. 2008

- 65 Taiwanese women undergoing amniocentesis
- MBP, MEHP, MEP measured in amniotic fluid (AF) and urine (MEP not detected in AF)
- AGD measured to base of scrotum (ASD) in males, to fourchette in females (AFD)
- ***MBP and MEHP inversely associated with female AGD (N=32)***
- ***No associations seen in males (N=33)***

# Bustamante et al. 2008 (abstract)

- Toluca, Mexico, Mexican women (N=73)
- Phthalates measured in 3rd trimester urine
- Male genital outcomes: AGD. Penile length, penile width
- ***MBP significantly and inversely related to penile length and width*** ( $p<0.05$ ,  $p<0.01$ )
- ***MEP significantly and inversely related to two AGD measures*** ( $p<0.01$ ,  $p<0.05$ )
- ***No associations reported for MEHP.***

# Ormond et al. 2008

- Case-control study of hypospadias (UK)
- 471 cases, 490 controls
- Phthalate exposure in first trimester determined by job exposure matrix
- Exposures associated with hypospadias:
  - Phthalate (OR 3.12, 95% CI 1.04, 11.46)
  - Hairspray (OR 2.39, 95% CI 1.48, 4.82)

# Behavior and neurodevelopment

- Swan et al. 2009 (prospective)
- Kim et al. 2009 (cross-sectional)
- Engel et al. 2009 (prospective)
- Engel et al 2010 (prospective)
- Cho et al. 2010 (cross-sectional)

# SFF III: Play behavior in SFF children

- Used the maternally completed Preschool Activities Inventory (PSAI)
- The PSAI has been widely used and validated and shown associations with Congenital Adrenal Hyperplasia in girls and prenatal PCB exposure
- 24 questions yield three scores: masculine, feminine and composite (masculine adjusting for feminine items)

# SFFIII population

- Questionnaires mailed to 334 moms who
  - joined our study while pregnant in 2000-2003
  - stored a urine sample
- Many questionnaires were not deliverable
- 72% of those delivered were returned completed
- 74 boys and 71 girls with usable data

# SFFIII Hypothesis

We hypothesized:

- Less masculine scores in boys with higher prenatal DEHP and DBP exposure (most anti-androgenic phthalates)
- No phthalate-related differences in girls



# DEHP metabolites and boys' scores

- *Masculine score* significantly and inversely associated with some DEHP metabolites
  - ***MEHHP: Coefficient = -3.64 (p=0.02)***
  - ***MEOHP: Coefficient = -3.29 (p=0.03)***
  - *But not MEHP*  
Coefficient = -1.33 (p=0.40)
- Composite and feminine scores not significantly associated with DEHP

# Boys: Other phthalate metabolites

- *Masculine score borderline significant for MBzP*
  - MBzP: Coefficient = -2.27 (P=0.054)
- *Composite score and DBP metabolites:*
  - Significant for MiBP: Coefficient -4.77 (P=0.017)
  - Borderline for MBP: Coefficient -3.91 (P=0.066)
- No other metabolites associated with play behavior

# Kim et al. 2009

- Cross-sectional study of Korean children 8-11, 46% female, (N=261)
- Urinary metabolites of DEHP and DBP at time of assessment
- Teacher-Rated ADHD, Computerized Measurements of Inattention and Impulsivity and IQ assessed
- DEHP metabolites, but not DBP metabolites associated w. ADHD
- MBP associated with errors of commission and omission
- Not analyzed by sex of child

# Engel 2009

- Multiethnic NYC prenatal population
- Low molecular weight (LMW) and high molecular weight (HMW) phthalate metabolites summed
- Infants 1-5 days assessed using Brazelton Neonatal Behavioral Assessment Scale
- Among girls, Orientation and Quality of Alertness scores declined with increasing urinary concentrations of HMW phthalate metabolites ( $p = 0.02$  and  $0.01$ , respectively)

# Engel 2010

- Multiethnic NYC prenatal population
- Third trimester maternal urines analyzed for phthalate metabolites. Cognitive and behavioral development assessed in children 4 and 9 years (n = 188).
- MEP, MBP and other low-molecular weight phthalates associated with aggression, attention problems and hyperactivity, particularly in boys.

# Cho 2010

- Cross-sectional study of children (mean 9 yrs, 48% female), S. Korea (N= 621)
- IQ assessed in mother and child
- Phthalate metabolites in urine collected at time of study
- Controlling for mothers IQ:
  - MEHHP and MEOHP inversely assoc with vocabulary score
  - In text, associations only in boys (not in tables)

# Length of gestation

- Latini et al. 2003
- Wolff et al. 2008
- Adibi et al. 2009
- Whyatt et al. 2009
- Meeker et al. 2009

# Latini et al.

- Brindisi, Italy, consecutive newborns (N=84)
- MEHP and DEHP in cord blood:
  - not quantified (+/-)
- <1 day shorter gestation when MEHP present (39.4 vs. 38.6 days,  $p=0.03$ )
- No association with DEHP



# Wolff et al. 2009

- Multiple racial/ethnic, NYC, N=352
- Gestational age *increased* by 1.1 days(95% CI: 0.1–2.0 days) for each 1-logarithmic unit increase in MEHP in 3rd trimester urine
- Gestational age also increased with sum of low molecular weight phthalates

# Wolff 2008

- Multiethnic NYC population, 404 prenatal women
- 10 phthalate metabolites in 3<sup>rd</sup> T urine samples
- Low-molecular weight metabolites positively association with gestational age (0.97 day gestational age per ln-biomarker) and with head circumference.

# Adibi et al. 2009

- California, Iowa, Minnesota, and Missouri) participants ion SFF (N=284 )
- 1 log unit Increase in mid-pregnancy (28 weeks) urinary MEHP, MEOHP, and MEHHP concentrations associated with significant ***increase*** in gestational age of 1.1 to 1.3 days
- Significant assoc with unscheduled C-Section and at delivering >41 weeks

# Whyatt et al. 2009

- African-American, Dominican women in NYC (N= 311)
- Concentration of *all* DEHP metabolites in 3<sup>rd</sup> trimester urine samples associated with significant ***shortening of gestation***
- Increase in metabolite concentration from 25<sup>th</sup> to 75<sup>th</sup> quartile associated with ***5 days*** shorter gestation

# Meeker et al. 2009

- Nested case-control study, Mexico
- 30 cases of premature birth, 30 controls
- All phthalate metabolites higher in case
- After adjustment for urine dilution, strongest associations were with MBP and MCPP

# Differences across studies

- Ethnicity and socio-economic status
- Type of sample (urine and blood)
- Timing of sample
- Phthalate exposure level
- Method of control for urinary concentration  
(in Wolff, Whyatt and Adibi, 2 methods in Meeker)

# DEHP and gestational age

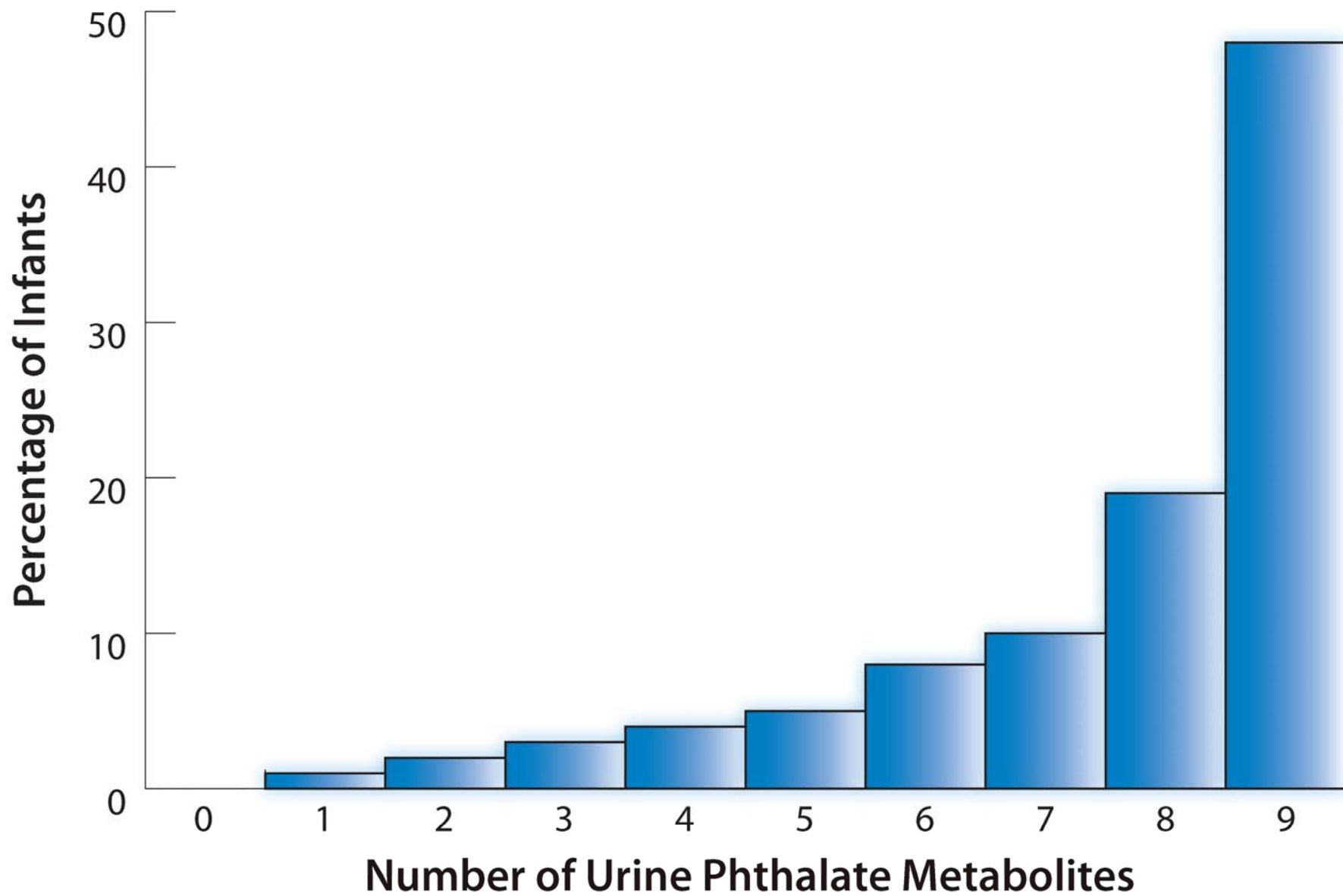
- Most (not Meeker) found associations strongest with DEHP
- Considerable geographic differences noted among and within studies
- Ethnic variability
- Need to repeat in comparable populations
- Currently available data are inconclusive

# Postnatal exposures (almost all associations in males)

- Serum hormones (Main et al. 2006)
- Premature breast development (Colon et al. 2000, Durmaz et al. 2009, Wolff 2010)
- Behavior/neurodevelopment (Swan et al. 2009, Engel et al., 2009, 2010, Cho et al. 2009, Kim et al. 2009)
- Liver function (Von Rettburg et al. 2009)
- Allergy and asthma (Bornehag 2004, Kolarik, 2008)



## Number of Phthalate Metabolites Found in Infant Urine Samples (N=163)



Sathyanarayana 2008

# Phthalates in breast milk and boys serum hormones: Main 2006

| Metabolite in breast milk | Association with serum hormone |
|---------------------------|--------------------------------|
| MBP                       | ↓ Free testosterone            |
| MEP, MBP                  | ↑ SHBG                         |
| MEP, MBP, MMP             | ↑ LH/free SHBG                 |
| MiNP                      | ↑ LH                           |

# Premature breast development

- Colon et al. 2000. Identification of phthalate esters in the serum of young Puerto Rican **girls** with premature breast development. *Environmental Health Perspectives*, 108(9), 895-900.
- Durmaz et al. 2009. Plasma phthalate levels in pubertal gynecomastia in **boys**. *Pediatrics*, 125(1), e122-129.

# Colon et al. (2000)

- Girls with premature thelarche (mean age 31 months) in Puerto Rico
- High levels of DMP, DEP, DBP, DEHP, and MEHP in serum were detected in 28 thelarche patients (68%) compared to 1 and 5 control subjects who had detectable levels of di-isooctyl phthalate and DEHP, respectively.
- The largest differences between cases and controls were for DEHP

# Durmaz et al. 2009

- Cases of male gynaecomastia (mean 13 years of age) in Turkey (N=40).
- normal controls (N=21).
- Serum DEHP and MEHP significantly higher in cases than in controls
- DEHP/MEHP not related to serum hormones

# Asthma and allergy studies

- Bornehag et al. (2004): Swedish children
  - 198 cases with persistent asthma, rhinitis, and /or eczema, 202 controls without disease
- Kolarik et al. (2008):
  - Wheezing, rhinitis and eczema associated with DEHP in house dust
- Bornehag (2010): Review article cites multiple studies associating PVC in home or bedroom with allergy/asthma

# Phthalate exposure in adults

- Semen quality (Duty et al., 2003; Jonsson et al, 2005, Hauser et al., 2006, Zhang 2006)
- Adult male serum hormones (Jonsson 2005, Duty et al. 2005, Pan et al. 2006, Meeker )

# Phthalate exposure in adults

- Pulmonary function (Hoppin et al, 2004)
- Metabolic syndrome (Stahlhut et al, 2007)
- Thyroid function (Meeker et al 2007)



# Phthalates and human semen quality

- MGH (Infertility clinic) study
  - MBP and MBzP inversely associated with sperm concentration
  - MBP inversely associated with sperm motility
- Swedish conscripts study
  - No associations between MBP or MBzP and semen parameter
  - Association between MEP and reduced sperm motility

# Phthalates and semen quality

- Published data inconsistent:
  - Harvard studies suggest adverse effects in less fertile men
  - Swedish study in young unselected men finds no associations
  - Two new, large studies to be published soon:
    - One finds no associations in fertile men
    - One finds significant adverse effects in young unselected men (in whom semen quality is poor)

# Male Serum Hormones

- MGH: 425 men seen in infertility clinics (Boston)
- SFF: 425 fertile men from MO, IA, CA and MN
- Differences in serum hormones reflecting difference in study populations
- Phthalate metabolite levels also differed (DEHP metabolites higher in MGH, other metabolites higher in SFF)

# DEHP and serum hormones

- One or more DEHP metabolites associated with decreased free testosterone (FAI and or FT) in both populations
- MEHP associated with decreased estradiol and total testosterone and MGH
- Gonadotropins (FSH and LH) not associated with any metabolites in either study

# Stahlhut et al, 2007

- Men in NHANES (1999=2002, N=1,292)
- MBzP, MEHHP, MEOHP, MEP associated with increased waist circumference (all p-values  $\leq 0.013$ )
- MBP, MBzP MEP associated with increased insulin (all p-values  $\leq 0.011$ )

# Thyroid function

- Meeker et al. 2007
- Men seen for infertility (N=400)
- T3 (triiodothyronine), T4 (thyroxine), TSH (thyroid stimulating hormone)
- Inverse association between MEHP urinary concentrations and free T4 and total T3 serum levels
- No other phthalates associated with any thyroid measure

# What is next?

- Number of studies has grown exponentially since 2000 (expect another 50 by 2015)
- Ongoing large studies: TIDES, SELMA, MIREC etc. will provide much more conclusive data
- Analysis of mixtures, more precise exposure windows and outcome measures

# Could these findings as a group be due to chance?

- Exposure misclassification is undoubtedly present (in most cases leading to underestimates of strength of association)
- Since subjects (and investigators) cannot know exposure (and chem labs do not know outcomes) these studies cannot be subject to recall and ascertainment bias



# Could these findings as a group be due to chance?

- The number of studies with findings only or predominantly in males is highly unlikely to occur by chance)
- Far more studies find associations with DEHP and/or its metabolites than would be expected by chance (metabolites of 9 phthalates routinely measured)
- While individual studies are imperfect, as a body they provide evidence of risk to multiple systems from both prenatal and postnatal exposures