Studies of Phthalates in Humans

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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
- Bustamente 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
  - ≥18 years, pregnancy conceived naturally
  - Questionnaires, serum and urine samples

- **SFFII:** Children born 2000-2003, whose mothers stored a urine sample
Measured in boys

Mid-pregnancy urine sample for phthalate metabolites

Measure testicular descent

Measure penile width

Measure AGD

Measure scrotal size
AGD measures in male infants
DEHP exposure was associated with multiple endpoints in boys but not girls.

- **Reduced penile width**
- **Reduced AGD**
- **Reduced scrotal size**
- **Decreased testicular descent**
## Male AGD and Phthalates

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

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Medium:low*</th>
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<th>High:low*</th>
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</thead>
<tbody>
<tr>
<td>MBP</td>
<td>5.7 (1.2, 27.3)</td>
<td>9.2 (1.8, 46.2)</td>
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<tr>
<td>MEHP</td>
<td>1.7 (0.5, 5.2)</td>
<td>3.2 (0.9, 11.5)</td>
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<tr>
<td>MEOHP</td>
<td>10.2 (1.3, 82.5)</td>
<td>29.1 (3.4, 245.6)</td>
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<tr>
<td>MEHHP</td>
<td>4.8 (1.0, 22.9)</td>
<td>13.0 (2.6, 66.4)</td>
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<tr>
<td>MEP</td>
<td>4.6 (1.0, 21.6)</td>
<td>7.9 (1.5, 41.3)</td>
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</table>

*Low < 25\(^{th}\) %, High >=75\(^{th}\)%, Medium, other

Swan 2008, Environmental Research
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)
Bustamente 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
SFFIIII 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 3rd 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 3rd trimester urine samples associated with significant shortening of gestation

• Increase in metabolite concentration from 25th to 75th 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)
- 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)

Percentage of Infants

Number of Urine Phthalate Metabolites

Sathyanarayana 2008
Phthalates in breast milk and boys serum hormones: Main 2006

<table>
<thead>
<tr>
<th>Metabolite in breast milk</th>
<th>Association with serum hormone</th>
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<tbody>
<tr>
<td>MBP</td>
<td>↓ Free testosterone</td>
</tr>
<tr>
<td>MEP, MBP</td>
<td>↑ SHBG</td>
</tr>
<tr>
<td>MEP, MBP, MMP</td>
<td>↑ LH/free SHBG</td>
</tr>
<tr>
<td>MiNP</td>
<td>↑ LH</td>
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</tbody>
</table>
Premature breast development


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-isoocyt phthalate and DEHP, respectively.
- The largest differences between cases and controls were for DEHP
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 <=0.013)
• MBP, MBzP MEP associated with increased insulin (all p-values<=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.