

Phthalates: Recent epidemiologic data and new research directions

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Outline

- Phthalates: what are they and how are we exposed
- Epidemiologic Evidence
 - Phthalates and biomarkers of male fertility
 - Semen quality
 - Sperm DNA damage
 - Phthalates and hormonal profile
 - Male infants with background exposure
 - Men from the general population and infertility clinic
 - Male PVC workers with high occupational exposure
- Current Regulations regarding the use of phthalates

Phthalates: A Family of Chemicals

- Some phthalates are developmental and reproductive toxicants in laboratory animals
- Widespread human exposure
 - CDC, NHANES 2001-2002 (>75% prevalence of exposure, approaches 100% for some phthalates)

There is public awareness of the potential health risks of exposure to phthalates

How are we exposed to phthalates?



Phthalate Characteristics

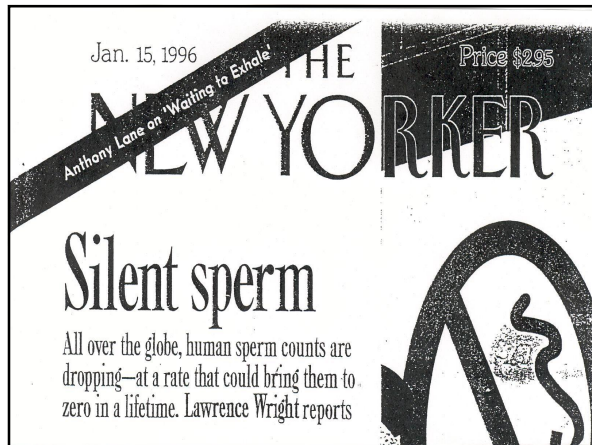
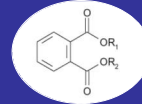
- Biological half-lives are measured in hours
- Monoester metabolites, as opposed to the parent diesters, are the biologically active toxicant
- Structure-activity differences in toxicity
 - MBP, MBzP, MEHP, but not MEP, are testicular toxicants
- Species differences in toxicity and response
 - Rats and guinea pigs > mice >> hamster (relatively resistant)
- Age-dependent sensitivity
 - Fetal > Neonatal > Pubertal > Adult

Mechanisms and manifestations of post-natal phthalate exposure in rats

- Altered Leydig cell structure and function
- Sertoli cell toxicity
 - inhibition of FSH-mediated cAMP production
- Increased germ cell apoptosis
 - via Fas signaling system
- Testicular atrophy
- Reduced semen quality

Biomarkers of Phthalate Exposure

- | <u>Diesters</u> | → | <u>Urinary levels of Monoesters</u> |
|--------------------|---|-------------------------------------|
| • Diethyl | → | • Monoethyl [MEP] |
| • Di(2-ethylhexyl) | → | • Mono(2-ethylhexyl) [MEHP] |
| • Dibutyl | → | • Monobutyl [MBP] |
| • Butyl Benzyl | → | • Monobenzyl [MBzP] and [MBP] |



Phthalates and Human Semen Quality

- **Two studies with inconsistent results:**
 - U.S. infertility clinic study (Duty et al., Epidemiology 2003; Hauser et al., Epidemiology 2006)
 - Dose-response relationship between lower sperm concentration and monobutyl phthalate and monobenzyl phthalate
 - Dose-response relationship between lower sperm motility and monobutyl phthalate
 - Swedish conscripts study (Jonsson et al., Epidemiology 2005)
 - No associations between monobutyl or monobenzyl phthalate monoester and semen quality
 - Association between monoethyl phthalate and reduced sperm motility

Male reproductive health

- US study subjects: 468 men (Epidemiology 2006)
 - Demographics
 - 96% Caucasian; mean (SD) age was 36.3 years (5.5).
 - 72% never smokers, 9% current smokers
- Semen parameter categorization
 - < 20 million sperm/ml (WHO, 1999)
 - < 50% motile sperm (WHO, 1999)
 - < 4% normally shaped sperm (Kruger Strict Criteria)
 - Comparison group defined as those at or above WHO reference values in all 3 categories
- Phthalate concentrations modeled as quartiles
- Covariates: age, smoking status, abstinence time

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Epidemiology • Volume 17, Number 6, November 2006

ORIGINAL ARTICLE

Altered Semen Quality in Relation to Urinary Concentrations of Phthalate Monoester and Oxidative Metabolites

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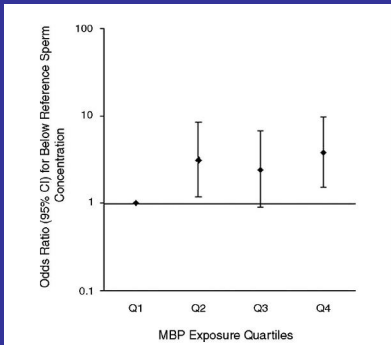


FIGURE 1. Adjusted odds ratios (95% confidence intervals) for below reference sperm concentration associated with quartiles of SG-adjusted monobutyl phthalate urinary concentration (adjusted for age, abstinence time, and smoking status).

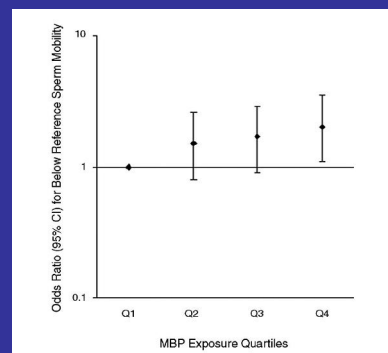
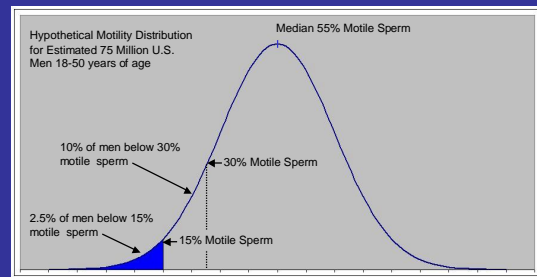


FIGURE 2. Adjusted odds ratios (95% confidence intervals) for below reference sperm motility associated with quartiles of SG-adjusted monobutyl phthalate urinary concentration (adjusted for age, abstinence time, and smoking status).

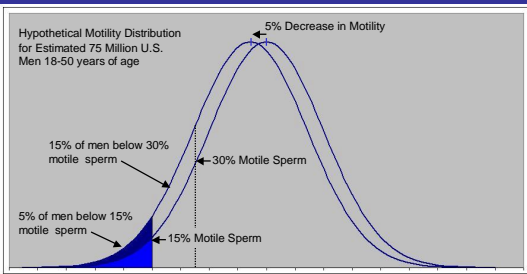
Phthalates and Human Semen Quality

- **Differences between U.S. and Swedish study**
 - Study population
 - Age difference (mean of 35 yrs vs. 18-20 yrs)
 - Infertility clinic patients vs. military recruits
 - May impart differences in susceptibility to environmental challenges?
 - Population differences in phthalate exposure sources and levels
 - Does pattern of exposure impact risk?
 - Interactions with other environmental chemicals?

Public Health Significance of Altered Semen Quality



Public Health Significance of Altered Semen Quality

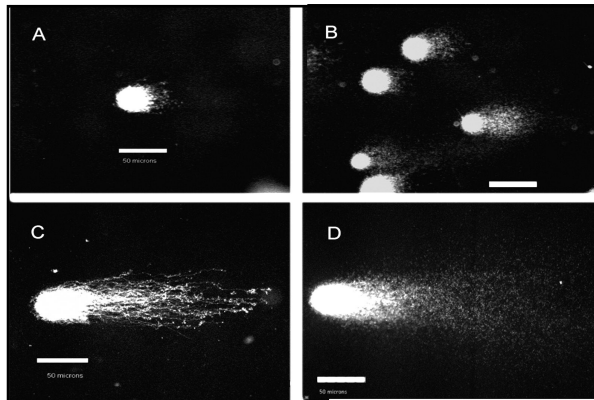
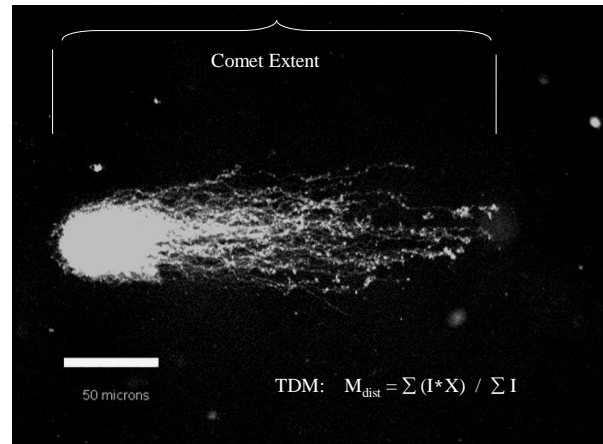


Phthalates and Sperm DNA Damage

- DNA damage can occur at multiple stages in spermatogenesis (condensed spermatids and spermatozoa lack repair capacity)
- Neutral single-cell gel electrophoresis (comet) assay quantitatively measures DNA damage
- Detergents and enzymes lyse sperm and open the highly compacted chromatin
- Quantify DNA migration in an electric field which indicates amount of double strand DNA breaks

Sperm DNA damage: Potential adverse outcomes

- Impaired embryo development that can lead to early or late pregnancy loss
- Birth defects (structural and/or functional)
- Increased risk of paternally derived cancers in children



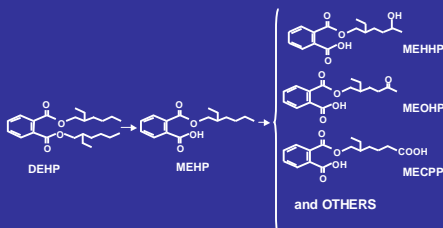
Panels a-c demonstrate the heterogeneity of comet tail lengths within an individual, while panel d depicts the comet cell referred to as 'long cell', a cell which was too long to measure with image analysis software.

Sperm DNA damage

- Recent studies:
 - U.S. study on 379 men from an infertility clinic (Duty et al., 2003. Environ Health Persp; Hauser et al., 2006. Human Reproduction)
 - confirmed earlier finding of association of monoethyl phthalate (MEP) with increased sperm DNA damage measured with the neutral comet assay
 - measured DEHP monoester metabolites and oxidative metabolites and found associations with sperm DNA damage
 - Swedish study on 234 military conscripts (Jonsson et al., 2005. Epidemiology)
 - did not find an association of any phthalate monoester metabolite with sperm DNA damage measured with the sperm chromatin structure assay

Selected metabolites of di(2-ethylhexyl) phthalate (DEHP):

mono(2-ethylhexyl) phthalate (MEHP),
mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP),
mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and
mono(2-ethyl-5-carboxypentyl) phthalate (MECPP).



Perplexing results for DEHP metabolites

- MEHP was associated with a 12% increase in sperm DNA damage
- MEHHP and MEOHP (oxidative metabolites) were associated with an 8% DECREASE in sperm DNA damage
 - Although MEHHP/MEOHP are positively correlated with MEHP, their association with sperm DNA damage was in the opposite direction
 - Could further metabolism of MEHP to oxidative metabolites be 'protective' for sperm DNA damage?

Hypothesis:

MEHP is the bioactive toxicant and its oxidative metabolism to MEHHP and MEOHP lowers internal burden of MEHP and thus lower risk of DNA damage in sperm

Conclusions

- Evidence of associations between urinary concentrations of some phthalate metabolites and decreased semen quality and increased sperm DNA damage
- Evidence that metabolism of MEHP to oxidative metabolites imparts a decreased risk of sperm DNA damage

Phthalates and serum hormones: Evidence from several population sub-groups

Serum testosterone and occupational exposure to DEHP and DBP among men

- Workers manufacturing PVC flooring compared to 'unexposed' construction workers (Pan et al., EHP 2006)
 - High exposure to DEHP and DBP
 - Median MEHP among exposed was 100 times higher than unexposed and 200 times higher than NHANES general population levels
 - Median MBP among exposed was almost 5 times higher than unexposed and 30 times higher than NHANES general population levels
 - Significantly lower free testosterone in exposed workers compared to unexposed workers
 - MEHP and MBP inversely correlated with free testosterone in exposed workers

Serum testosterone and phthalate exposure among men from an infertility clinic and the general population

- Men from an infertility clinic (Duty et al., 2005 Human Reprod)
 - Weak relationship between MEHP and decreased serum testosterone
- Military conscripts (Jonsson et al., 2005. Epidemiology)
 - No relationship between MEHP and testosterone or LH

Serum testosterone among infants

- Serum hormones measured among 3-month old infants from Denmark and Finland (Main et al., 2006 EHP)
- Phthalates measured in breast milk
 - Monobutyl and monoethyl phthalate associated with increased LH:free testosterone ratio and negatively correlated with free testosterone.
 - Monoethylhexyl phthalate associated with increased LH:free testosterone ratio and negatively correlated with free testosterone (associations were not statistically significant).
- Data suggested that Leydig cell function was affected

Summary: Evidence of inverse association between phthalates and testosterone

- Infant males from general population
 - Associations between MEHP, MBP and MEP with increased LH:free testosterone ratio and decreased free testosterone
- Adult men
 - Weak inverse association between MEHP and serum testosterone among men from an infertility clinic
 - No association between any phthalate metabolite and serum testosterone among young military conscripts
- Adult men with occupational exposure
 - Inverse association between MEHP and MBP and serum free testosterone

E.U. and Japanese Phthalate Regulations

- E.U.
 - Cosmetics
 - Banned two phthalates (DBP and DEHP) from cosmetics
 - Children/Baby toys (up to 40% phthalates by weight)
 - 2005, For all toys sold in the EU, there is a complete ban on three phthalates (DEHP, DBP, BBzP) and a ban on DINP, DIDP and DnOP in toys and childcare products that can be placed in the mouth of a child
 - European Council for Plasticizers said ban was unnecessary and that it ignored risk assessments
 - Food
 - Scientific Panel on food additives, flavorings, processing aids and materials in contact with food is evaluating the risk of exposure to DBP, BBzP, DEHP, DINP, DIDP
- Japan
 - 2003, banned certain phthalates in food-handling equipment
 - 2003, banned phthalates from all objects intended for the mouths of young children

U.S. Phthalate Regulations

- U.S. FDA has regulatory authority over cosmetics, food packaging, and medications
 - Apart from a recent CA ban (passed in 2007, effective in 2009) on phthalate use in baby/children's toys, the U.S. has not banned phthalate use in products
 - Cosmetics: A few companies abide by EU standards for products sold in the US (Proctor & Gamble Co. no longer uses phthalates in nail polish. Unilever, Revlon Inc. and L'Oreal SA's American unit agreed to eliminate all chemicals banned in EU products from the same items in the US)
 - Medical Products: FDA published a safety assessment report on 'DEHP release from medical devices'.
 - FDA published a Public Health Notification on DEHP.
 - Braun, Baxter International Inc and Abbott Laboratories are developing and selling phthalate free PVC products and PVC free products. Individual hospitals/groups of hospitals are buying phthalate free products.

Proctor & Gamble Statement

- Proctor & Gamble spokesperson was quoted as saying that his company's decision to stop using DBP "was not based on any concern about the safety of the chemical. We and other outside groups have done numerous risk assessments on phthalates. There are no health hazards associated with their use in cosmetics."

Final Phthoughts on Phthalates

- The human data is limited but growing
- Timing of exposure relative to life stage needs to be considered in designing and interpreting epidemiologic studies
- Understanding how metabolites may interact (additively or antagonistically) is needed
- Identifying relevant human endpoints is critical
- Identifying high exposure populations to study is needed

Acknowledgements

- **Collaborators** (listed alphabetically)
 - **Harvard School Public Health:** Ramace Dadd, Susan Duty, Linda Godfrey-Bailey, Christopher Lewis, John Meeker, Louise Ryan and Ana Trisini
 - **Massachusetts General Hospital Fertility Clinic:** Drs. Zuying Chen, John Petrozza, Isaac Schiff, Thomas Toth, Study Subjects, and participating physicians at MGH
 - **Centers for Disease Control and Prevention:** Dana Barr, John Brock, Antonia Calafat, Manori Silva.
 - **University of Washington:** Narendra Singh.
- Grant sponsors: NIEHS and NIOSH.
- Special thanks to Peter Hengel for donating Viscomet Image Analysis Software.