Environmental Exposures Impact Sperm Message

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Recent epidemiologic evidence linking environmental exposures with impacts on sperm health

A set of examples will be used to motivate the presentation

Epidemiologic study designs

Identifying study populations and exposures

1. High-exposure sub-populations
1. Susceptible sub-populations
1. Chemicals/agents with widespread human exposure

High exposure sub-populations

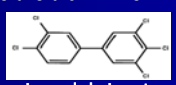
- Occupational exposure
  - Phthalate exposure: manicurists, PVC plastic production
  - Pesticide exposure: farmers and pesticide formulators
  - Lead exposure: battery manufacturers, lead smelter workers
- Communities with high ambient exposures
  - Air pollution exposure: neighborhoods near power plants and cities with meteorological/geographical characteristics that contribute to high air pollution levels (e.g., Mexico City, Los Angeles)

High exposure sub-populations

- Individuals undergoing medical treatments
  - Phthalate exposure from medical products/devices (medical devices used in neonatal intensive care units) and from medications (used on pill coatings)
- Accidental high contaminant exposure
  - Dioxin: chemical plant explosion in Seveso, Italy
  - Polychlorinated biphenyls (PCBs)/Furans: food contamination (rice oil contamination during processing in Japan and Taiwan)

Polychlorinated Biphenyls (PCBs)

- Introduced in 1929 for use in electrical transformers and capacitors (production ceased in US in 1977)
- 209 possible congeners with different toxicity and potencies (various Cl substitution patterns): Some congeners are estrogenic, others are dioxin-like.
- Persistent and bio-accumulate



- Uses: paints, plastics (PVC), adhesives, lubricants, sealants (ex. grain silos), carbonless copy paper.
- Current route of exposure is primarily through the diet (fish, meat, dairy products, fats and oils).

High PCB exposure Groups

- Yusho (Japan;1968)
- Yu-Cheng (Taiwan;1979)
  - Oil Disease
  - Consumption of contaminated rice oil with PCBs used in piping of heat exchangers. Furans were also formed due to high temperatures.
  - Approximately 2000 people poisoned
  - Chloracne, hyperpigmentation of skin, liver cancer, neurodevelopmental disorders in children.

PCBs and human semen quality

- PCBs and semen quality
  - High-exposure sub-population studies
    - Taiwan (rice oil contamination)
      - Poorer sperm morphology and lower sperm counts (motility not measured)
  - General population studies
    - U.S., Sweden, India, Netherlands
      - Consistent inverse association between PCBs and sperm motility
  - In utero exposure studies
    - Taiwan (rice oil contamination)
      - Poorer sperm morphology and lower sperm motility
- Conclusion: suggestive association of some PCB congeners with decreased sperm motility

Chemicals with widespread exposure

- Low-dose (background) exposure among the majority of the general population
  - Ambient/background exposures include:
    - Air pollution (outdoor and indoor)
    - Pesticides (dietary and residential application)
    - PCBs and dioxins (meat, fish, foods)
    - Metals (lead and cadmium)
  - Chemicals in consumer products
    - Bisphenol A (inner lining of food cans, polycarbonate plastics)
    - Fluorinated compounds (PFOA and PFOS, stain/water resistant)
    - Parabens (preservative in sunscreens, deodorant)
    - Synthetic hormones in meats
    - Phthalates (personal care products, food packaging and processing materials, building materials)

Phthalates: A Family of Chemicals

- Phthalates are known developmental and reproductive toxicants in laboratory animals
  - Produces a syndrome of effects on reproductive tract development that parallels the testicular dysgenesis syndrome ("Phthalate syndrome")
  - Reproductive health effects include altered testicular function and poor semen quality
- Widespread human exposure
  - CDC, NHANES 2001-2002 (>75% prevalence of exposure, approaches 100% for some phthalates)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Under the Microscope

From an Ingredient In Cosmetics, Toys, A Safety Concern

Male Reproductive Development Is Issue With Phthalates, Used in Host of Products

Europe, Japan Restrict Them

By Peter Waldman

In the 12th week of a human pregnancy, the momentous event of gender formation begins, as X and Y chromosomes trigger biochemical reactions that shape male or female organs. Estrogens carry the process forward in girls, while in boys, male hormones called androgens do.

Now scientists have indications the process may be influenced from beyond the womb, raising a fresh debate over industrial chemicals and safety. In recent experiments, common chemicals called phthalates, used in wide variety of products from toys to cosmetics to pills, can block the action of fetal androgens. The re-

TOXIC TRACES
New Questions About Old

Public awareness of the potential health risks of exposure to phthalates has increased.

How are we exposed?






Experimental Evidence of Reproductive Effects

- Some phthalate esters produce testicular toxicity in pubertal and adult rodents
  - Testicular atrophy
  - Reduced sperm counts
  - Altered Leydig cell structure and function
  - Sertoli cell toxicity
- inhibition of FSH-mediated cAMP production
  - Increased germ cell apoptosis
- via Fas signaling system

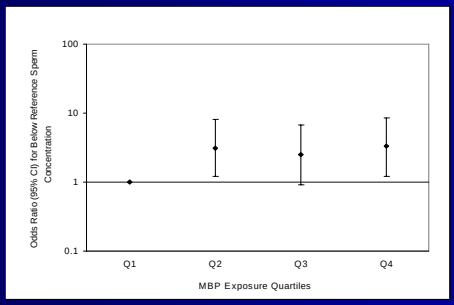
Phthalate Characteristics

- Monoester metabolites, as opposed to the parent diesters, are the biologically active toxicant
- Biological half-lives are measured in hours
- Structure-activity differences in toxicity
  - MBP, MBzP, MEHP are testicular toxicants
- Species differences in toxicity and response
  - Rats and guinea pigs > mice>>hamster (relatively resistant)
- Age-dependent sensitivity
  - Fetal > Neonatal > Pubertal > Adults

Phthalates and human semen quality

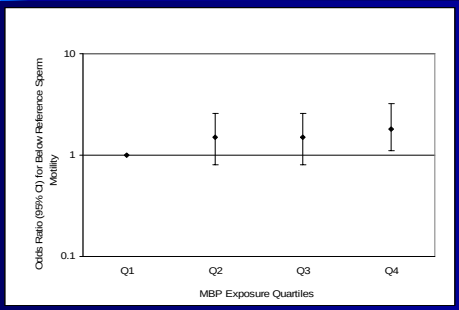
- Two studies with inconsistent results:
  - U.S. infertility clinic study (Duty et al., Epidemiology 2003; Hauser et al., Epidemiology 2006)
    - Dose response relationship between lower sperm concentration and monobutyl phthalate and monobenzyl phthalate
    - Dose-response relationship between lower 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

Figure: U.S. Study - adjusted Odds Ratio (95% CI) for below WHO reference sperm concentration in relation to monobutyl phthalate (MBP) exposure



p-value for trend 0.02

Figure: U.S. study - adjusted Odds Ratio (95%CI) for below WHO reference sperm motility in relation to monobutyl phthalate (MBP) exposure



p-value for trend 0.02

Phthalates and human semen quality

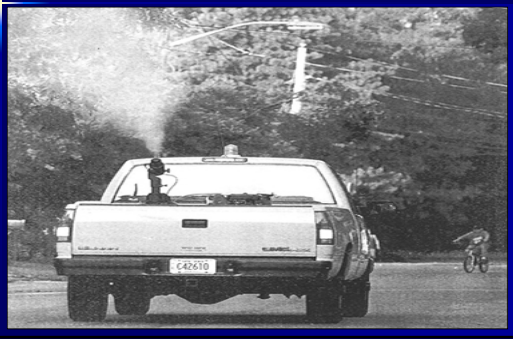
- Differences between U.S. and Swedish study
  - Study population
    - Age difference (mean of 35 yrs vs. 18-20 yrs)
    - Infertility clinic patients vs military recruits
      - May impart differences in susceptibility to environmental challenges?
  - Population differences in phthalate exposure sources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

Pesticide Characteristics

- Class of chemicals manufactured to kill target pests
- Multiple targets, modes of action, metabolism pathways
- Current movement away from persistent compounds (e.g., DDT) toward non persistents (e.g., organophosphates) in developed countries
- Need to focus on compound specific properties and effects

DDT Spraying, New Jersey, 1945

Pyrethrin Spraying, New York, 2001



Exposure simulation using fluorescent dye

DDT and Metabolites

- Insecticide (introduced in the 1940s, use peaked in 1950s, banned in 1972)
- Still used in countries for malaria control
- Parent compound (DDT)
  - Estrogenic
- Metabolites (DDE, DDD)
  - Anti-androgenic

Contemporary use pesticides

Experimental evidence of testicular toxicity

- Pyrethroids
  - inferior morphology, motility and epididymal sperm counts, reduction in testes weight, accumulation of connective tissue surrounding seminiferous tubules
- Organophosphates
  - cellular degeneration and tubular atrophy, decreases in meiotic figures and elongating spermatids, epithelial cell loss

Review of Pesticide Exposure on Human Sperm

(Perry, Human Reproduction Update 2008)

- 20 semen quality studies, 13 positive
- 6 DNA damage studies, 3 positive
- 6 sperm aneuploidy/diploidy studies, 4 positive

Studies on occupational / environmental pesticide exposure and sperm quality

Multiple Herbicides, Insecticides, Fungicides

First Author (year)	Subjects	Pesticides	Effects Assessed
Swan (2003)	50 low semen parameters; 36 within normal limits	Assorted	Associations with concentration & motility, not morphology
Sanchez-Pena (2004)	33 men selected from 227 workers	Assorted	No association with semen quality
Pant (2004)	31 pesticide sprayers; 80 unexposed	OPs	Associations with volume, motility, morphology

Studies on occupational / environmental pesticide exposure and sperm quality

Single Pesticides

First Author (Year)	Subjects	Pesticides	Effects Assessed
Meeker (2004)	272 recruited from an infertility Clinic	Carbaryl, Chlorpyrifos	Sperm concentration & motility
Lifeng (2006)	32 pesticide plant workers; 68 non-exposed controls	Fenvalerate	Sperm count significantly lower in exposed group

Studies on occupational / environmental pesticide exposure and sperm quality

DDT and Metabolites

First Author (year)	Subjects	Pesticides	Effects Assessed
Hauser (2003)	212 partners of sub fertile couples	PCBs, *p,p'*-DDE	Association for PCBs, limited association with *p,p'*-DDE
Dalvie (2004)	27 unexposed; 27 highly-exposed	DDT	Serum *p,p'*-DDT negatively associated with sperm count
Pant (2004)	45 fertile & 45 infertile men	HCH & DDT	High levels in semen of infertile men
Rignell-Hydbom (2004)	195 Swedish fishermen	*p,p'*-DDE, CB-153	Non significant association serum levels of CB-153 & sperm motility
Magnusdottir (2005)	25 with subfertility; 47 normal fertility	PCBs, *p,p'*-DDE	No difference in levels of OCs between the groups

Studies on occupational / environmental pesticide exposure and sperm DNA damage

Author (year) country Study	Study Design	Pesticides	Association
Multiple Herbicides, Insecticides and/or Fungicides			
Larsen *et al.* (1998) Denmark	Cross-sectional; Sperm chromatin structure with SCSA	Assorted	Suggestive
Sanchez-Pena *et al.* (2004) Mexico	Cross-sectional	Assorted	No association

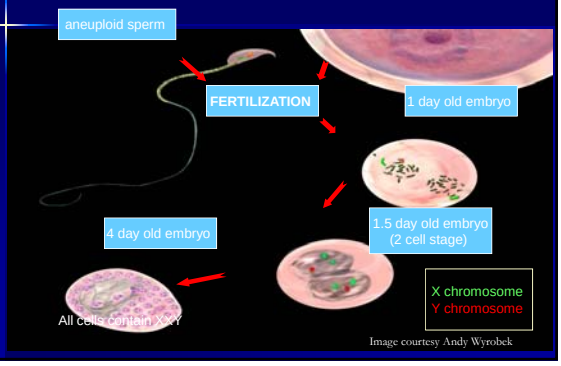
Studies on occupational / environmental pesticide exposure and sperm DNA damage

Author (year) country Study	Study Design	Pesticides	Association
	Single Pesticides		
Bian *et al.* (2004) China	Cross-sectional; Sperm DNA integrity measured by comet and TUNEL assays	Fenvalerate	Significant
Meeker *et al.* (2004) USA	Cross-sectional; Sperm DNA integrity measured by neutral comet assay	Carbaryl and Chlorpyrifos	Significant

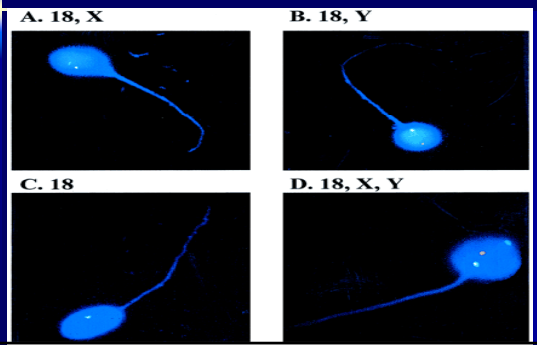
Studies on occupational / environmental pesticide exposure and sperm DNA damage

Author (year) country Study	Study Design	Pesticides	Association
	DDT and Metabolites		
Rignell-Hydbom et al. (2004) Sweden	Cohort; Serum analysis: FSH, LH and E analyzed by IFMA; CASA	*p,p'*-DDE CB-153	Not significant
Spano *et al.* (2005) Greenland, Sweden, Poland, Ukraine	Cross-sectional; Sperm-chromatin structure with SCSA	CB-153; *p,p'*-DDE	No association for *p,p'*-DDE

Aneuploidy is passed to offspring as illustrated in mouse reproduction



Spermatozoa with chromosome 18, X, and Y probes



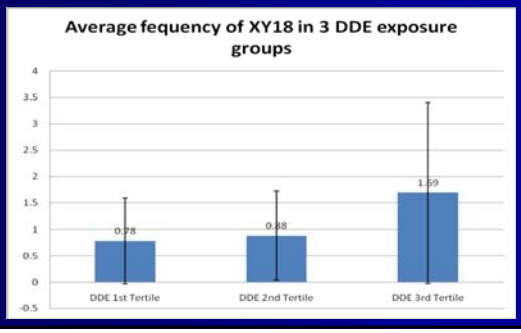
Prior pesticide-sex chromosome aneuploidy studies; one used exposure monitoring

Author, year, country	Pesticides	Effects/ Chromosomes	Association
Padungtod 1999 China n=29	OPs	Aneuploidy X ,Y, 18	Positive
Recio 2001 Mexico n=9	OPs	Aneuploidy X, Y, 18	Positive
Smith 2004 USA n=40	Assorted	Diploidy X, Y, 13, 21	Null
Xia 2004 China n=24	Pyrethroids	Disomy X ,Y, 18	Positive

Summary of studies to date

- Evidence for effects on concentration and motility
- Inconclusive evidence for DNA damage
- Suggestive evidence for aneuploidy; too few studies
- Need comparability across exposures and outcomes

Preliminary results of DDE effects on XY18 disomy (n=46)



Critical Research Directions

High exposure population studies are needed:

Phthalates (anti-androgenic)

- Neonates in intensive care units
  - What are the implications for pubertal development and adult reproductive function (fertility)?
- Medications
  - What are the implications for pregnant women (her fetus) taking these medications? For young children taking medications with phthalates?

Bisphenol A (estrogenic)

- We need to identify high exposure sub-populations
  - BPA is found in dental sealants, polycarbonate plastics, resin lining of food cans
  - Young children with dental sealants that contain BPA?

Critical Research Directions

Studies of chemicals with widespread exposure are needed:

- There is a critical need for epidemiologic studies on Bisphenol A
- How can we best study exposure to mixtures of chemicals with similar mechanisms of actions (e.g., estrogenic, anti-androgenic)?
- Pyrethroids, which have replaced some organophosphates (chlorpyrifos and diazinon), currently enjoy widespread general population use. There is little human data on potential impact on fertility. (Perry et al., Reprod Tox 2007)
- Metal (mercury, cadmium) exposure among members of the general population

Critical Research Directions

Studies among susceptible sub-populations are needed:

- Pre-natal and neonatal exposure
  - Sensitive window which imparts increased risk to reproductive tract development and function as an adult.
  - Neonates have undeveloped metabolic systems and thus altered metabolism/excretion of chemicals that may increase risk of exposure
- Childhood/pubertal exposure
  - Sensitive developmental window for testicular maturation and initiation of spermatogenesis
- Genetic susceptibility
  - Metabolic polymorphisms may impart altered susceptibility by decreasing metabolism/excretion of contaminant, increasing conversion to active metabolite
- Men with sub-fertility (E.g., older age, genetics)
  - Men with sub-optimal fertility may be more susceptible to environmental exposures which further decrease fertility

Implications for Public Health and Policy

- The contemporary use chemicals, such as phthalates and bisphenol A, are widely used and it is difficult to advise clinicians and individuals on how to avoid personal exposure.
  - Policy makers need to learn the facts to make informed decisions regarding the need (or lack thereof) for regulation and the need (or lack thereof) to lower human exposure
  - Regulation needs to be at the governmental level
  - Regulation needs to be informed by the fact that mixtures of chemicals may act synergistically. Data on this is sparse.