“Safe” water – or is it?

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Outline of presentation

- background information EDCs
- multidisciplinary integration biosentinels
- exposed human/environmental populations
- challenge

US EPA

EDC definition (1998)

“........ describes an endocrine disruptor as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects -- at the level of the organism, its progeny, populations, or subpopulations of organisms...”

Rachel Carson's Silent Spring (1962)

- DDT entered the food chain, accumulated in fatty tissues of animals, including human beings, and caused cancer and genetic damage
- insecticidal, but not selective; persistent, POP
- chemical industry = commotion and controversy

Legacy of the Silent Spring

- public awareness: nature vulnerable human intervention
- conservation never raises broad public interest
- threats Carson outlined -- the contamination of the food chain, cancer, genetic damage, the deaths of entire species -- were too frightening to ignore
- need to regulate industry to protect the environment

...health and environmental threats created by man-made chemical contaminants that interfere with hormones in humans and wildlife....
Diethylstilbestrol (DES)

- synthetic estrogen
- 1938 to 1971 during pregnancy (NCI, 2011)
- adenocarcinoma vagina pubertal girls exposed to DES in utero

Cohort exposed pregnant women

- abortions
- neonatal deaths
- premature births

Diethylstilbestrol (DES)

DES-EXPOSED BOYS

- Small penis, hypospadias, small testes, UDT
- epididymal and prostatic abnormalities
- low sperm count

ANIMAL STUDIES

PRENATAL EXPOSURE DES

- mice similar structural and functional anomalies reported in humans
- in addition, testicular tumors, rete testis adenocarcinoma
- pathognomonic of estrogenic exposure

DES as growth stimulant in cattle???

Chemical Safety Regulations

75,000 existing chemicals [http://www.epa.gov]

- carcinogenicity
- mutagenicity
- teratogenicity
- inert substances not labeled

EDCs

- estrogenic/antiestrogenic effects
- antiandrogens
- thyroid
ESTROGENIC CHEMICALS

- lipophylic, stable, toxic compounds
- resistant chemical and biodegradation
- accumulate food chain

**diet**
- milk and dairy products
- meat, fish, fruit, vegetables

**industrial**

EDCs: disruptive in minute quantities

"The dose makes the poison."
Paracelsus, physician
(1493-1541)

EDC

- interactive effects: additive/synergistic
- estrogenic chemicals below no-observed-effects levels (NOELs) produce an effect
- traditional risk assessments of estrogenic chemicals - significant underestimations of risk

ESTROGENIC CHEMICALS

- man-made
  - alkylphenols (nonyl-phenol (p-NP))
  - polychlorinated biphenyls (PCB)
  - organochlorine pesticides (OC)
  - bisphenol A, phthalates
- **phyto- and mycoestrogens**

HUMAN MALE REPRODUCTIVE HEALTH

- increasing incidence of testicular Ca
- increasing frequencies UDT and hypospadias
- lower/declining semen quality
- a growing demand for AR

Testicular dysgenesis syndrome (TDS)

[Diagram showing causal factors and risk factors related to testicular dysgenesis syndrome (TDS).]

[Text: "Environmental factors incl. endocrine disruptors"
- "Reduced sperm cell function"
- "Reduced sperm cell motility"
- "Reduced sperm count"
- "Decreased genetic integrity"
- "Reduced reproductive function"
- "Reduced sperm quality"
- "Reduced sperm viability"
- "Testicular maldevelopment"

Stephanou et al. 2021]
EDCs and male laboratory animals

- in utero or perinatal exposure
  - exogenous oestrogens diethylstilboestrol, ethinyl estradiol and bisphenol A
  - anti-androgens flutamide, vinclozolin and p,p-DDE

Developed hypospadias, UDT, low sperm counts, intersex, testicular tumours


Eland testicular findings

- Reminded
  - OAT syndrome human male infertility
  - Findings in rats after p-NP exposure

Testicular dysgenesis syndrome (TDS)

CRUCIAL QUESTION

......sufficiently high levels of EDC exist in the general environment to exert adverse health effects on aquatic or terrestrial animals, or humans.

RIETVELI NATURE RESERVE (RNR)

- two dams, Marais (MD) and Rietvlei (RVD), interconnected wetland and channel
- catchment areas part of Ekurhuleni Metropolitan area
- stream effluent sewage treatment plants, industries, informal settlements, agriculture

- one of world’s largest urban nature reserves
- one of very few reserves grassland biome central South African highveld
**Chemical**

- Reporter gene (Dioxin, PCB & furans)

**Biological**

- Yeast Screen, ER-Calux ®

**Sentinel species**

- Fish
- Frogs
- Snails
- Mammals

**Health Risk Assessment**

- Lapalala Nature Reserve
- Suikerbosrand Nature Reserve
- Potchefstroom, and more...

**Reference site**

- Catfish laboratory conditions
- "EDC-free environment"
- Water negative TA
- No intersex sexually mature catfish or tilapia

**Analytical chemistry**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Detection</th>
<th>Range</th>
<th>Lower Limit</th>
</tr>
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<tbody>
<tr>
<td>BHC</td>
<td>1.15</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Lindane</td>
<td>0.58-14.91</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Endrin</td>
<td>0.57-0.65</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Endrin aldehyde</td>
<td>0.65</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>1.14</td>
<td>ND</td>
<td></td>
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<tr>
<td>p,p'-DDT</td>
<td>0.25-2.27</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td>o,p'-DDD</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDD</td>
<td>0.9-1.1</td>
<td>7.75</td>
<td>0.1-9.75</td>
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<tr>
<td>o,p'-DDE</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>0.02</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>p-NP</td>
<td>0.6-50.05</td>
<td>14.0</td>
<td>0.1-0.6</td>
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<tr>
<td>OCp</td>
<td>2.26-6.56</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>DEP</td>
<td>3.16-3.63</td>
<td>All</td>
<td>10.0-0.3</td>
</tr>
<tr>
<td>DBP</td>
<td>3.93-8.33</td>
<td>All</td>
<td>10.0-0.7</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.33-2.78</td>
<td>All</td>
<td>10.0-0.6</td>
</tr>
<tr>
<td>PCB153</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>&gt;TWQR, &gt;CEV, &gt;AEV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>&lt;TWQR</td>
<td>&gt;water</td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>&gt;TWQR, &gt;CEV, &gt;AEV</td>
<td></td>
<td>6.3-23.3</td>
</tr>
<tr>
<td>Mercury</td>
<td>&gt;AEV</td>
<td>Low/absent</td>
<td></td>
</tr>
</tbody>
</table>

ND = not detected, NT = not tested; TWQR = Target Water Quality Range; CEV = chronic effect value; AEV = acute effect value

**Bio-assays**

- Estrogenic activity
- Bioavailable EE >1ng/L → intersex fish
- 8/10 RNR samples >1ng/L **fish at risk**

**Biosentinel species**

- Catfish
- African frog
- Freshwater snail
- Striped mouse
- Eland

**Tragelaphus oryx**

- n=31
  - Vacuolization Sertoli cells
  - Sloughing germ cells
  - Adenosis rete testis
  - Atypical germ cells resembling CIS
Eland testicular findings

- 11/17 eland fat samples
  \( p\)-NP 35.0–286.0 µg/kg fat
  \( 178.2 \pm 60.8 \) µg/kg
- No OCs, PCBs

CONCLUSIONS

- adenomatous lesions rete testis eland similar to DES-induced rete adenocarcinoma laboratory animals
- indicative of possible chronic estrogenic exposure
- EDCs bioaccumulate in terrestrial mammals as in aquatic life

SUMMARY

- MD most polluted site predominantly industrial contaminants
- rely on **water treatment plant**
- any increase industrial, agrochemical or anthropogenic load water system RNR collapse aquatic ecosystem

Health Risk Assessment

- risks developing cancer
  - 5 and 4 in 10 000 RVD and MD respectively
- RVD - lindane, DDT, DEHP (agro/indust)
- DEHP in Marais Dam (industrial)

Health Risk Assessment

- untreated water both RVD and MD
  - domestic
  - agricultural
  - recreational activities
- unacceptably high human health risks
  - carcinogenic
  - toxic effects
  - endocrine disruption
Adverse health effects and EDCs in animals

So what????

The double burden of communicable and non-communicable diseases in developing countries

Malaria risk areas

Historic use of DDT

- 1945 DDT introduced for IRS, +BHC
- 1966 DDT alone
- DDT annually sprayed malaria control
- >60 years continuous use

DDT-dilemma

- Malaria killing millions of children
- Transferred to fetus and neonate
- Impact chronic, low-dose exposure (EDC) human health unknown
1,1,1-trichloro-2,2-bis-(4-chlorophenyl)ethane (DDT)

- banned international use
- Stockholm Convention SA restricted use

• applied DDT estrogenic
• persistent metabolite DDE anti-androgen

IN UTERO DDT/DDE MALE ANIMALS

- reduced penile size
- impaired reproductive capacity
- low sperm density, abnormal
- development
  - ovarian tissue
  - hypospadias
  - undescended testes

Bulger & Kupper 1983; Sharpe & Shackleford 1986; Danzo1997; Kelce et al. 1995; Guillette et al.1995

DDT exposure & general health effects

- type 2 diabetes mellitus (Rignell-Hydbom et al, 2007; Cox et al. 2007)
- thyroid hormone levels in men (Meder et al., 2000).  
- modulates immune responses humans (Cooper et al., 2004)
- in utero exposure & neurodevelopment
- breast cancer risk exposure, <age 14yrs (Cohn et al., 2007)

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Impact DDT residential exposure

- pre-term abortion, still birth, or shortened lactation (Longnecker, 2005; Damstra et al., 2004)
- Impaired semen quality non-occupational exposed to DDT used in IRS (de Jager et al., 2006; Aneck-Hahn et al., 2007)
- $p,p'$-DDE risk of both testicular cancer (McGlyn 2008)

Testicular dysgenesis syndrome (TDS)

- estrogenic
- anti-androgenic

DDT

Objective

- determine association of external urogenital birth defects (UGBDs) in newborn boys with DDT exposure from spraying in a malaria area.
Newborn boys

- Of 3310 newborns 10.8% (357) had UGBDs
- mothers in villages DDT-sprayed 1995 to 2003
  - statistically significant greater chance (33%)
    baby UGBD (OR 1.33, 95% CI 1.04–1.72)
  - being a homemaker significantly increased risk
    by 41% (OR 1.41, 95% CI 1.13–1.77).

Urogenital birth defects reported from South Africa

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>Kalafong*</th>
<th>Polokwane*</th>
<th>Tshilidzini</th>
</tr>
</thead>
<tbody>
<tr>
<td>not in malaria area</td>
<td>0.92</td>
<td>1.41</td>
<td>55.2</td>
</tr>
<tr>
<td>hypospadias</td>
<td>0.29</td>
<td>0.79</td>
<td>18.8</td>
</tr>
<tr>
<td>UDT</td>
<td>0.23</td>
<td>0.52</td>
<td>10.8</td>
</tr>
<tr>
<td>Total</td>
<td>17351</td>
<td>7816</td>
<td>7146</td>
</tr>
<tr>
<td>/1000 live births</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How does DDT get there?

ENVIRONMENTAL CONTAMINATION

- no residues in water/sediment
  - estrogenicity 0.14–0.31 ng/L ER-CatLux®
  - 57% male tilapia testicular oocytes
- tilapia
  - fat DDT 4.35 ± 2.4 µg/g
  - DDE 3.82 ± 0.34 µg/g
- aquatic
  - DDE 0.01 – 4.54 µg/g
- birds
- chicken
  - DDT 0.3 – 7.1 µg/g
  - fat DDE 3.5 – 21.2 µg/g

THEA

- exposure through various pathways including indoor air, dust, soil, food and water
- human serum, indoor air, floor dust, outside soil, potable water, leafy vegetables, and chicken samples (muscle, fat and liver)
Nonylphenol

- $p$-NP major chemical sediment (29 and 851; $155.3 \pm 87.26$) mg/kg.
- surfactants in detergents, paints, herbicides, pesticides and cosmetics
- relatively persistent, bioaccumulates lipids
- more persistent effect than natural estrogens (Tapiero et al., 2002).

$p$-NP

- diet major source of intake
- $p$-NP breast milk samples Italian mothers close to TDI (Ademollo et al., 2008)
- also cow’s milk and formula feeding (Dorea, 2009)
- fish and water dietary sources - animals raised on fishmeal or recycled animal products

MIXTURE EFFECTS:

DDT, DM, $p$-NP AND PHYTOESTROGENS (OECD 415)

Anogenital distance

Kilian et al., 2007

MIXTURE EFFECTS

- Epidemiology needs to abandon its focus on single endocrine disrupters
- embrace the reality of endocrine disrupter mixture effects by developing biomarkers that capture cumulative exposure to endocrine disrupters

Kortenkamp, 2008