Effects on rat reproductive system after developmental exposure to mixtures of endocrine disrupter compounds

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Early exposure to endocrine disrupters may lead to disease later in life

From Bergman et al: State of the science of Endocrine Disrupting Chemicals 2012
National Food Institute, Technical University of Denmark
Environmentally EDCs
Agenda

Reproductive study on mixture of EDCs in rats

• Early effects of early exposure
  – Sexual differentiation

• Late effects of early exposures
  – Pituitary gland
  – Ovaries
  – Prostates

• Conclusions
Study design - Mixture of 13 known endocrine disrupters

Mixture ratio based on high end human exposures

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP</td>
<td>0.01</td>
</tr>
<tr>
<td>DEHP</td>
<td>0.02</td>
</tr>
<tr>
<td>Vinclozolin</td>
<td>0.009</td>
</tr>
<tr>
<td>Prochloraz</td>
<td>0.014</td>
</tr>
<tr>
<td>Procymidone</td>
<td>0.015</td>
</tr>
<tr>
<td>Linuron</td>
<td>0.0006</td>
</tr>
<tr>
<td>Epoxiconazole</td>
<td>0.01</td>
</tr>
<tr>
<td>ppDDE</td>
<td>0.001</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0.0015</td>
</tr>
<tr>
<td>Butyl paraben</td>
<td>0.06</td>
</tr>
<tr>
<td>OMC</td>
<td>0.12</td>
</tr>
<tr>
<td>4-MBC</td>
<td>0.06</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>0.8</td>
</tr>
</tbody>
</table>

- 8 Anti-androgens
- 4 Estrogens
- Paracetamol

Christiansen et al. 2012
Study design - Mixture doses of 100-450 times “high human exposure”

### Study 1

<table>
<thead>
<tr>
<th>Mixture</th>
<th>Group-dose</th>
<th>EDs in the mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mix-150</td>
<td>Anti-androgens + Estrogens + Paracetamol</td>
</tr>
<tr>
<td></td>
<td>Mix-450</td>
<td>Anti-androgens + Paracetamol</td>
</tr>
<tr>
<td></td>
<td>AMix-450 + PM</td>
<td>Anti-androgens + Paracetamol</td>
</tr>
</tbody>
</table>

14 litters per group
Offspring: 1½ years old

### Study 2

<table>
<thead>
<tr>
<th>Group-dose</th>
<th>EDs in the mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>Mix-100</td>
<td>Anti-androgens + Estrogens + Paracetamol</td>
</tr>
<tr>
<td>Mix-200</td>
<td>Anti-androgens + Estrogens + Paracetamol</td>
</tr>
<tr>
<td>Mix-450</td>
<td>Anti-androgens</td>
</tr>
<tr>
<td>Amix-200</td>
<td>Anti-androgens</td>
</tr>
<tr>
<td>Amix-450</td>
<td>Anti-androgens</td>
</tr>
<tr>
<td>Emix-200</td>
<td>Estrogens</td>
</tr>
<tr>
<td>Emix-450</td>
<td>Estrogens</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Paracetamol</td>
</tr>
</tbody>
</table>

14-20 litters per group
Offspring: 1 year old
Study design

Exposure of dam: Gestation day 7-21 and Pup Day 1 to 21

Via placenta

Birth

Via milk

Pups:
Early effects of early exposure: AGD, NR, organ weights

Adult offspring:
Late effects of early exposure: histology, cycle, organ weights.
Early and late endpoints examined in perinatally exposed rats

- Anogenital distance, Nipple retention
- Organ weights, histology
- Hormone levels
- Mammary development
- Offspring endpoints
- Estrous cyclicity
- Behaviour
- Timing of sexual maturation
- Malformations of reproductive organs
- Semen quality
Early effects of early exposure

- Ano-genital distance (AGD)
- Nipple retention (NR)
- Organ weights
Early effects – Androgen sensitive endpoints

• Decreased anogenital distance (AGD) in males on Pup Day 1
  – Mix200, Mix450, Amix200, Amix450

• Increased nipple retention (NR) in males on Pup Day 13
  – Mix150, Mix450, Amix200, Amix450, PM

• Male organ weights on Pup Day 16
  – Prostate ↓: Mix450, Amix200, Amix450
  – Levator ani/ bulbo cavernosus muscles ↓: Amix450, PM

Axelstad et al. 2014, Christiansen et al. 2012
Late effects of early exposure

- Pituitary glands
- Female reproductive organs
- Male reproductive organs
Late effects – pituitary gland

Age: 1.5 years old (study 1)
Pituitary tumors increased by anti-androgens at 18 months

Influence on mammary gland?
Influence on reproductive organs?

Pituitary gland adenomas

- male
- female

% of animals

Control | Mix150 | Mix450 | AMix450

p = 0.053

** p < 0.01

*** p < 0.001

Isling et al. 2014
Ovary weights reduced at 13 months, but not at 18 months

Study 2

Unpublished data

- Anti-androgens and paracetamol contribute more than estrogens?
Fewer corpora lutea in ovaries at 13 months, but not at 18 months
Fewer corpora lutea in ovaries at 13 months, but not at 18 months

Unpublished data

• Larger variations at 18 months, incl. cysts
Mean number of corpora lutea correlates with ovary weight at 1 year of age

Unpublished data
Estrous cyclicity

• Vaginal smears
• Irregularly cycling rats:
  – cycles of 6 days or more
  – cycles shorter than 4 days

• Study 1: No effect at 5 and 9 months
• Study 2: unpublished data

• Both studies: irregular cycles at 12 months of age

Jacobsen, PhD thesis 2013
Altered estrus cyclicity at 12 months of age
- Longer cycles, prolonged diestrus

Study 1

Study 2

Study 1+2

Unpublished data

N=13-14
*: P<0.05
Late effects in females

• Unpublished data

→ Sign of earlier reproductive senescence

Earlier menopause in humans:
• Limits the fertile period
• Menopause is associated with increased risk of diseases in
  – cardiovascular system (Chae and Derby 2011)
  – bone density (Lo, et al. 2011)
Late effects in males: prostates at 19 months

- Arrows: normal atrophy in aging prostates
- Arrows: atypical hyperplasia and cribriform pattern

Isling et al. 2014
Shift from atrophy to hyperplasia in aging prostates exposed to Amix

Epithelial atrophy

Atypical hyperplasia

Isling et al. 2014
Conclusions

• Early changes in sexual differentiation
  – Decreased AGD and increased NR

• Late effects of early exposure
  – Increased risk for pituitary tumors
  – Signs of early female reproductive senescence: Early menopause, decreased ovary weight and fewer corpora lutea
  – Increased risk for prostate tumors: prostate hyperplasia

• Late effects may be overlooked, as generation guideline studies terminate at 3 months of age

• Effects at 100-200 fold “high human exposure”: are highly exposed women protected sufficiently?
  – Safety margin of 100 not present…
Thank you for your attention!

EU project Contamed
Danish Environmental Protection Agency

National Food Institute, Technical University of Denmark
References


References continued