Peroxisome proliferator-activated receptors (PPARs) activation leading to reproductive toxicity in rodents

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Adverse Outcome Pathways: From Research to Regulation
September 3-5, 2014
At the beginning

- We had an AIM

To develop a strategy for building a MoA based chemical category

\[ f(\text{chemical structure}) = \text{toxicity} \]

\[ f(MoA) = \text{toxicity} \]

- How?
Building MoA-based chemical category for toxicity prediction

**STEP 1.** Chose endocrine active, data rich chemicals

**STEP 2.** MoA matrix display of experimental data

<table>
<thead>
<tr>
<th>Phthalates</th>
<th>ER</th>
<th>PPAR</th>
<th>AR</th>
<th>AhR</th>
<th>Sertoli cells</th>
<th>spermatogenesis</th>
<th>Leydig cells</th>
<th>Decreased testosterone</th>
<th>stereoidogenesis</th>
<th>oestrus cycle</th>
<th>Male reproductive tract</th>
<th>Sperm parameters</th>
<th>Decreased AGD</th>
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</tbody>
</table>

**STEP 3.** Mechanistic "blueprint" of phthalates

**STEP 4.** Search for mechanistic analogues (other chemicals that have similar MoA)
PPAR activation leading to reproductive toxicity in rodents

MIE  KE  Adverse Outcome

AOP-linked chemical initiators

PPAR activation  cholesterol transport  Hormone synthesis  Hormone levels  Reproductive toxicity
PPARs peroxisome proliferator-activated receptors

- family comprises the types α, γ and β/δ
- are nuclear receptor superfamily of transcription factors that respond to specific ligands
- regulate lipid and carbohydrate metabolism
- embryonic and foetal development
- cholesterol uptake and transport
- represent a potential molecular link between reproductive function and carbohydrate and lipid metabolism
## PPAR activation: evidence

<table>
<thead>
<tr>
<th>Chemical initiator</th>
<th>In vitro binding</th>
<th>in vitro transactivation</th>
<th>Knock-out/inhibition/increased expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEHP</td>
<td>-</td>
<td>+</td>
<td>Experiments with PPARa-null mice indicate involvement of the receptor in reproductive toxicity of phthalates</td>
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<tr>
<td>MEHP</td>
<td>+</td>
<td>+</td>
<td>Inhibition studies</td>
</tr>
<tr>
<td>BBP</td>
<td>+/-</td>
<td>+</td>
<td>To be verified</td>
</tr>
<tr>
<td>DBP</td>
<td>+/-</td>
<td>+</td>
<td>To be verified</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>-</td>
<td>+</td>
<td>Increased expression PPARγ</td>
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<tr>
<td>Butylparaben</td>
<td>-</td>
<td>+</td>
<td>Increased expression PPARγ</td>
</tr>
</tbody>
</table>
Altered steroidogenic pathway

Chemical initiator

PPAR
binding & activation

pregnenolone

P450scc

cholesterol

StAR

cholesterol

TSPO

3β-HSD-III

progesterone

androstenedione

aromatase

estrone

17β-HSD IV

testosterone

estradiol

aromatase
<table>
<thead>
<tr>
<th>Chemical Initiator</th>
<th>Decreased testosterone levels</th>
<th>Malformation of reproductive organs</th>
<th>Testicular toxicity</th>
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</thead>
<tbody>
<tr>
<td><strong>DEHP</strong></td>
<td>+ (Howdeshell et al., 2008)</td>
<td>+ (Gray et al., 2000) (Parks, 2000)</td>
<td>+ (Kwack et al., 2009)</td>
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<tr>
<td><strong>BBP</strong></td>
<td>+ (Howdeshell et al., 2008)</td>
<td>+ (Gray et al., 2000) (Nagao et al., 2000)</td>
<td>+ (Gray et al., 2000)</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td>+ (Howdeshell et al., 2008) (Barlow et al., 2003) (Mylchreest, 2000)</td>
<td>+ (Barlow et al., 2003) (Mylchreest, 2000)</td>
<td>+ (Mylchreest, 2000)</td>
</tr>
<tr>
<td><strong>Butyl paraben</strong></td>
<td>+ (Zhang et al., 2014)</td>
<td>+ (Zhang et al., 2014)</td>
<td>+ (Oishi et al., 2001)</td>
</tr>
</tbody>
</table>

+ effect present
/ no change
? no information
*testosterone production
PPAR activation leading to reproductive toxicity in rodents

- **MIE**
  - PPAR activation

- **KE**
  - Cholesterol transport to mitochondria
  - Hormone synthesis
  - Hormone levels

- **Adverse Outcome**
  - Reproductive toxicity
    - Altered oestrus cycle
    - Decreased ovary weight
  - Malformation of reproductive organs
    - Decreased AGD
    - Hypospadias
PPAR activation leading to reproductive toxicity in rodents

**AOP 1**
- PPAR activation
- Cholesterol transport to mitochondria
- Estradiol synthesis
- Hormone levels
- Reproductive toxicity

**AOP 2**
- PPAR activation
- Cholesterol transport to mitochondria
- Testosterone synthesis
- Hormone levels
- Reproductive malformations

**AOP 3**
- PPAR activation
- Cholesterol transport to mitochondria
- Estradiol synthesis
- Hormone levels
- Altered estrus cycle

- PPAR activation
- Cholesterol transport to mitochondria
- Testosterone synthesis
- Hormone levels
- Testicular toxicity
PPAR activation leading to reproductive toxicity in rodents

- **PPAR activation**
  - Cholesterol transport to mitochondria
  - **Testosterone** synthesis → Hormone levels → Malformations Reproductive organs
  - **Estradiol** synthesis → Hormone levels → Altered estrus cycle
  - **Testosterone** synthesis → Hormone levels → Testicular toxicity
PPARα activation leading to reproductive tract malformations in males upon *in utero* exposure

**MIE**
- PPARα activation

**KE**
- Cholesterol transport to mitochondria
- Testosterone synthesis
- Testosterone levels

**Adverse Outcome**
- Malformation Reproductive organs
- Decreased AGD
- Hypospadias

AOP-linked chemical initiators
**PPARα activation leading to reproductive tract malformations in males upon *in utero* exposure**

<table>
<thead>
<tr>
<th>Key Events</th>
<th>Experimental Support</th>
<th>Strength of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular Initiating Event:</strong> Binding to and activation to PPARα</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEHP/MEHP, BBP, DBP binding to PPARα in vitro, in silico</td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td>PPARα transactivation by DEHP/MEHP, BBP, DBP, butylparaben</td>
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<tr>
<td>Experiments with PPARα-null mice indicate involvement of the receptor in reproductive toxicity of phthalates</td>
<td></td>
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</tr>
</tbody>
</table>

| **Key Event:** Impaired steroidogenesis | | |
| | Impaired transport of cholesterol to mitochondria | Moderate |
| | decreased gene expression of SR-B1, TSPO (PBR), StAR | |
| | decreased gene expression of P450scc, 3β-HSD, 17β-HSD | |

| **Key Event:** Decreased testosterone levels | | |
| | Decreased testosterone levels measured in plasma | Strong |
| | Decreased testosterone production measured ex-vivo | |

| **Adverse Outcomes:** Reproductive tract malformations | | |
| | DEHP, DBP, BBP, butylparaben, decreased AGD | Strong |
| | DEHP, DBP, BBP, Hypospadias | |
Challenges for these AOPs

Data mining

- Literature organisation and structural capturing of the biological events
Challenges for these AOPs cd.

Data mining
- Literature organisation and structural capturing of the biological events
- Quality and quantity of data in literature
  - (PPAR α or/and γ), dose levels, more mechanisms involved

Relevance for humans
- Mode of action
  - PPAR expression
  - Steroidogenesis is conserved

- Adversity
  - TDS- Testicular Dysgenesis Syndrome in humans
Future plans

- To insert quantitative data into the OECD AOP-Knowledge Base
- To further substantiate AOP with evidence from other chemicals
- To develop other pathways interconnected with the current ones aiming at AOP network
- To further develop the database for capturing the literature and provide a template for structured data gathering
Acknowledgment

Brigitte Landesmann
Edward Carney
Sharon Munn
Andrew Worth
Julien Burton
Alfonso Lostia

Thank you

for coming questions