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

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Adverse Outcome Pathways:
From Research to Regulation
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Serving society
Stimulating innovation
Supporting legislation

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At the beginning

- We had an AIM

To develop a strategy for building a MoA based chemical category

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

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

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

**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

AOP-linked chemical initiators

MIE                KE                        Adverse Outcome

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 PPARα-null mice indicate involvement of the receptor in reproductive toxicity of phthalates</td>
</tr>
<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>
</tr>
<tr>
<td>Butylparaben</td>
<td>-</td>
<td>+</td>
<td>Increased expression PPARγ</td>
</tr>
</tbody>
</table>
Altered steroidogenic pathway

Chemical initiator

- Chemical initiator
- PPAR binding & activation
- TSPO
- cholesterol
- StAR
- pregnenolone
- P450scc
- cholesterol
- androstenedione
- aromatase
- estradiol
- aromatase
- estrone
- progesterone
- 3β-HSD-III
- 17β-HSD IV
- testosterone
- estradiol
- aromatase

KE
<table>
<thead>
<tr>
<th>Chemical Initiator</th>
<th>Decreased testosterone levels</th>
<th>Malformation of reproductive organs</th>
<th>Testicular toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEHP</td>
<td>+ (Howdeshell et al., 2008)</td>
<td>+ (Gray et al., 2000)</td>
<td>+ (Kwack et al., 2009)</td>
</tr>
<tr>
<td>BBP</td>
<td>+ (Howdeshell et al., 2008)</td>
<td>+ (Gray et al., 2000)</td>
<td>+ (Gray et al., 2000)</td>
</tr>
<tr>
<td>DBP</td>
<td>+ (Howdeshell et al., 2008)</td>
<td>+ (Barlow et al., 2003)</td>
<td>+ (Mylchreest, 2000)</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>+ (Tanaka et al., 2006)</td>
<td>+/- (Takagi et al., 2004)</td>
<td>+ (Talsness et al., 2000)</td>
</tr>
<tr>
<td></td>
<td>(Nakamura et al., 2010)</td>
<td>(Kobayashi et al., 2002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Talsness et al., 2000)</td>
<td>(Talsness et al., 2000)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Tinwell et al. 2002)</td>
<td></td>
</tr>
<tr>
<td>Butyl paraben</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  KE  Adverse Outcome

- PPAR activation
  - cholesterol transport to mitochondria
  - Hormone synthesis
  - Hormone levels
  - Reproductive toxicity

- Malformation of reproductive organs
- Decreased AGD
- Hypospadias
- Altered oestrus cycle
- Decreased ovary weight
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
    - Estradiol synthesis
  - Testosterone synthesis
  - Estradiol synthesis
  - Hormone levels (Male)
    - Malformations Reproductive organs
  - Hormone levels (Female)
    - Altered estrus cycle
  - Hormone levels (Male)
    - Testicular toxicity
PPARα activation leading to reproductive tract malformations in males upon *in utero* exposure

**MIE**

- PPARα activation
- Cholesterol transport to mitochondria

**KE**

- Testosterone synthesis
- Testosterone levels

**Adverse Outcome**

- Malformation Reproductive organs
  - Decreased AGD
  - Hypospadias
### 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>Molecular Initiating Event: Binding to and activation to PPARα</td>
<td>DEHP/MEHP, BBP, DBP binding to PPARα in vitro, in silico&lt;br&gt;PPARα transactivation by DEHP/MEHP, BBP, DBP, butylparaben&lt;br&gt;Experiments with PPARα-null mice indicate involvement of the receptor in reproductive toxicity of phthalates</td>
<td>Moderate</td>
</tr>
<tr>
<td>Key Event: Impaired steroidogenesis</td>
<td>Impaired transport of cholesterol to mitochondria&lt;br&gt;decreased gene expression of SR-B1, TSPO (PBR), StAR&lt;br&gt;decreased gene expression of P450scc, 3β-HSD, 17β-HSD</td>
<td>Moderate</td>
</tr>
<tr>
<td>Key Event: Decreased testosterone levels</td>
<td>Decreased testosterone levels measured in plasma&lt;br&gt;Decreased testosterone production measured ex-vivo</td>
<td>Strong</td>
</tr>
<tr>
<td>Adverse Outcomes: Reproductive tract malformations</td>
<td>DEHP, DBP,BBP, butylparaben, decreased AGD&lt;br&gt;DEHP, DBP,BBP, Hypospadias</td>
<td>Strong</td>
</tr>
</tbody>
</table>
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 $\alpha$ or/and $\gamma$), 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

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Thank you

for coming questions