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

How?

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

\[ f(\text{MoA}) = \text{toxicity} \]
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>stereodogenesis</th>
<th>oestrus cycle</th>
<th>Male reproductive tract</th>
<th>Sperm parameters</th>
<th>Decreased AGD</th>
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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

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

- family comprises the types $\alpha$, $\gamma$ and $\beta/\delta$
- 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>
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<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>
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<tr>
<td>MEHP</td>
<td>+</td>
<td>+</td>
<td>Inhibition studies</td>
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<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>
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<tr>
<td>Bisphenol A</td>
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<td>+</td>
<td>Increased expression PPARγ</td>
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<tr>
<td>Butylparaben</td>
<td>-</td>
<td>+</td>
<td>Increased expression PPARγ</td>
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</tbody>
</table>
Altered steroidogenic pathway

Chemical initiator

PPAR binding & activation

StAR

cholesterol

TSPO

P450scc

pregnenolone

3β-HSD-III

progesterone

androstenedione

17β-HSD IV

estrone

estradiol

testosterone

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>DEHP</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>BBP</td>
<td>+ (Howdeshell et al., 2008)</td>
<td>+ (Gray et al., 2000) (Nagao et al., 2000)</td>
<td>+ (Gray et al., 2000)</td>
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<tr>
<td>DBP</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>
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<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**
- PPAR activation

**KE**
- Cholesterol transport to mitochondria → Hormone synthesis → Hormone levels → Reproductive toxicity

**Adverse Outcome**
- 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
- 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
      - Altered estrus cycle
    - Testicular toxicity
PPARα activation leading to reproductive tract malformations in males upon *in utero* exposure
### 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</td>
<td>Moderate</td>
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<td>PPARα transactivation by DEHP/MEHP, BBP, DBP, butylparaben</td>
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<td>Experiments with PPARα-null mice indicate involvement of the receptor in reproductive toxicity of phthalates</td>
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<td>Key Event: Impaired steroidogenesis</td>
<td>Impaired transport of cholesterol to mitochondria</td>
<td>Moderate</td>
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<td>decreased gene expression of SR-B1, TSPO (PBR), StAR</td>
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<td>decreased gene expression of P450scc, 3β-HSD, 17β-HSD</td>
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<td>Key Event: Decreased testosterone levels</td>
<td>Decreased testosterone levels measured in plasma</td>
<td>Strong</td>
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<td></td>
<td>Decreased testosterone production measured ex-vivo</td>
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<tr>
<td>Adverse Outcomes: Reproductive tract malformations</td>
<td>DEHP, DBP,BBP, butylparaben, decreased AGD</td>
<td>Strong</td>
</tr>
<tr>
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<td>DEHP, DBP,BBP, Hypospadias</td>
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</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

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

Thank you

for coming questions