



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

Małgorzata Nepelska

[www.jrc.ec.europa.eu](http://www.jrc.ec.europa.eu)

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



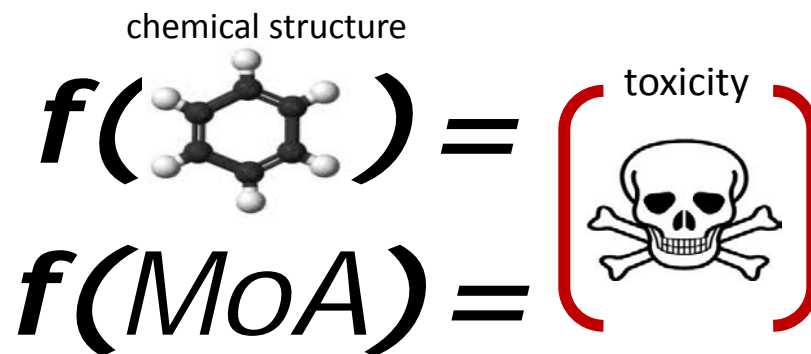
*Serving society  
Stimulating innovation  
Supporting legislation*

*DISCLAIMER: This presentation and its contents do not constitute an official position of the European Commission or any of its services. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of this presentation or its contents*

## At the beginning

⇒ We had an AIM

To develop a strategy for building a MoA based chemical category



⇒ How?

## Building MoA-based chemical category for toxicity prediction

STEP 1. Chose endocrine active, data rich chemicals

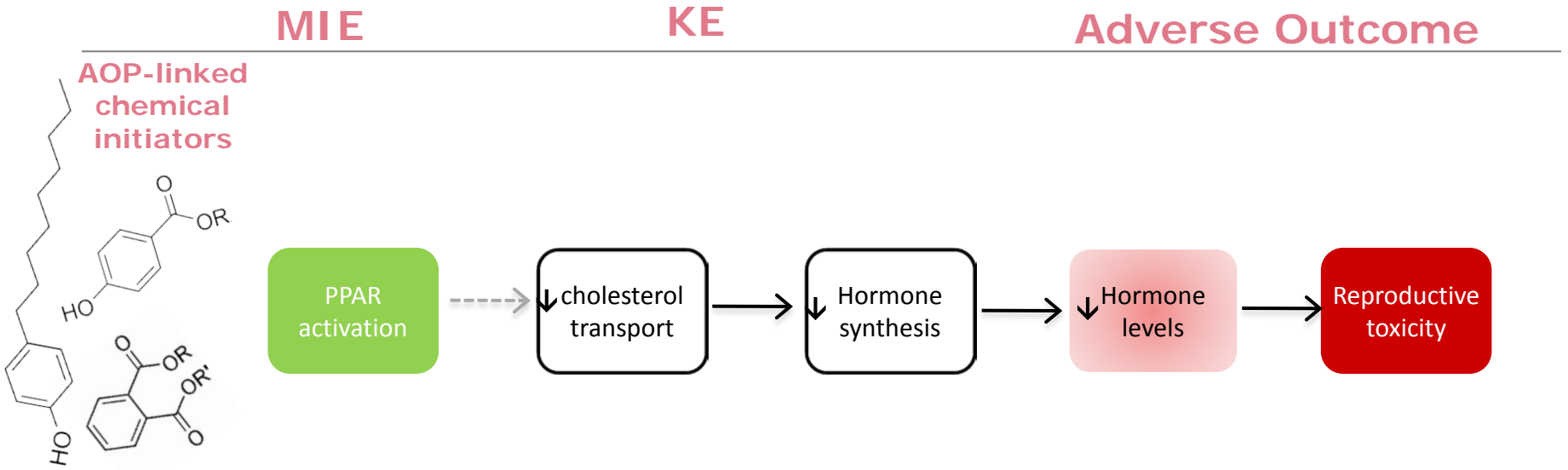
STEP 2. MoA matrix display of experimental data

Phthalates	MIE				KE					AO			
	ER	PPAR	AR	AhR	Sertoli cells	spermatogenesis	Leydig cells	Decreased testosterone	steroidogenesis	oestrus cycle	Male reproductive tract	Sperm parameters	Decreased AGD
DEP	0		1				1	1	0		0	/	0
DiBP	1	1			1	1	1	1	1		1		1
DPP	0				1	1		1	1		1	1	1
DCHP	0	0			1	1	1	1	1	1	1	1	1
DHP	0		/		1	1	1	1	1		1	1	1
DINP	0		0	0	1	1	1	1	1		0	1	/
DIDP	0		0	1				0	0		/	1	0
DnOP	0	1								0	0	/	
BBP	1	1	1	1		1	1	1	1		1	1	1
DprP											1	1	1
MEHP		1	1		1	1	1	1	1		1	1	1
DEHP	/	1	/	1	1	1	1	1		1	1	1	1

STEP 3. Mechanistic "blueprint" of phthalates

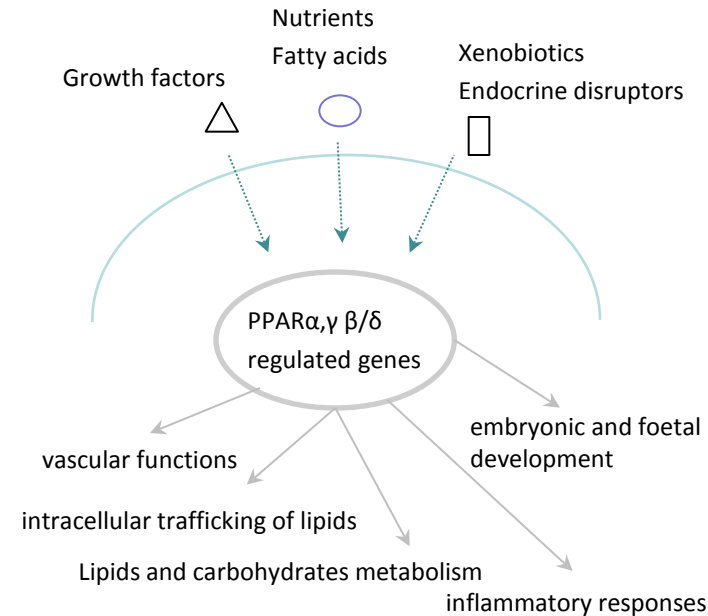
STEP 4. Search for mechanistic analogues (other chemicals that have similar MoA)

# PPAR activation leading to reproductive toxicity in rodents



## PPARs peroxisome proliferator-activated receptors

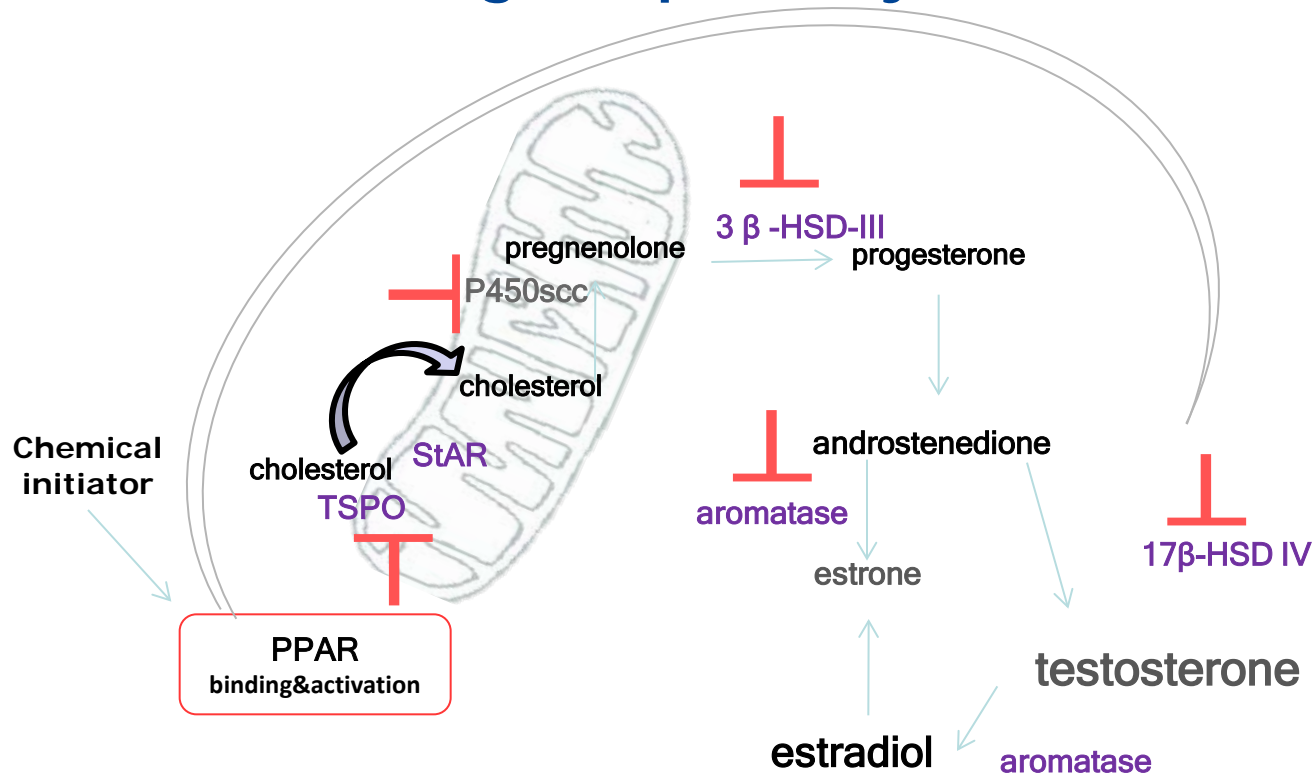
- ➔ family comprises the types  $\alpha$ ,  $\gamma$  and  $\beta/\delta$
- ➔ are nuclear receptor superfamily of transcription factors that respond to specific ligands
- ➔ regulate lipid and carbohydrate metabolism
- ➔ embryonic and foetal development
- ➔ cholesterol uptake and transport
- ➔ represent a potential molecular link between reproductive function and carbohydrate and lipid metabolism



## PPAR activation: evidence

Chemical initiator	In vitro binding	in vitro transactivation	Knock-out/inhibition/increased expression
DEHP	-	+	Experiments with PPAR $\alpha$ -null mice indicate involvement of the receptor in reproductive toxicity of phthalates
MEHP	+	+	Inhibition studies
BBP	+/-	+	To be verified
DBP	+/-	+	To be verified
Bisphenol A	-	+	Increased expression PPAR $\gamma$
Butylparaben	-	+	Increased expression PPAR $\gamma$

## Altered steroidogenic pathway



Chemical Initiator	Decreased testosterone levels	Malformation of reproductive organs	Testicular toxicity
<b>DEHP</b>	+ (Howdeshell et al., 2008)	+ (Gray et al., 2000) (Parks, 2000)	+ (Kwack et al., 2009)
<b>BBP</b>	+ (Howdeshell et al., 2008)	+ (Gray et al., 2000) (Nagao et al., 2000)	+ (Gray et al., 2000)
<b>DBP</b>	+ (Howdeshell et al., 2008) (Barlow et al., 2003) (Mylchreest, 2000)	+ (Barlow et al., 2003) (Mylchreest, 2000)	+ (Mylchreest, 2000)
<b>Bisphenol A</b>	+ (Tanaka et al., 2006) (Nakamura et al., 2010) (Talsness et al., 2000)	+/- (Takagi et al., 2004) (Kobayashi et al., 2002) (Talsness et al., 2000) (Tinwell et al. 2002)	+ (Talsness et al., 2000)
<b>Butyl paraben</b>	+ (Zhang et al., 2014)	+ (Zhang et al., 2014)	+ (Oishi et al., 2001)

+ effect present  
/ no change  
? no information  
\*testosterone production

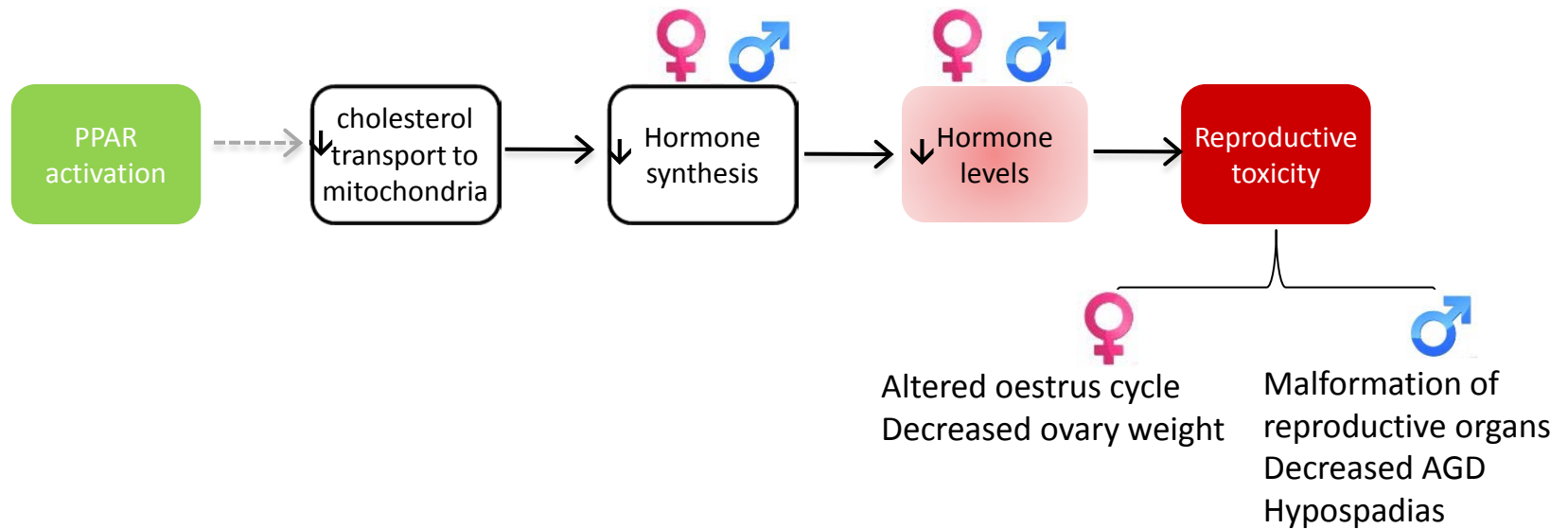


# PPAR activation leading to reproductive toxicity in rodents

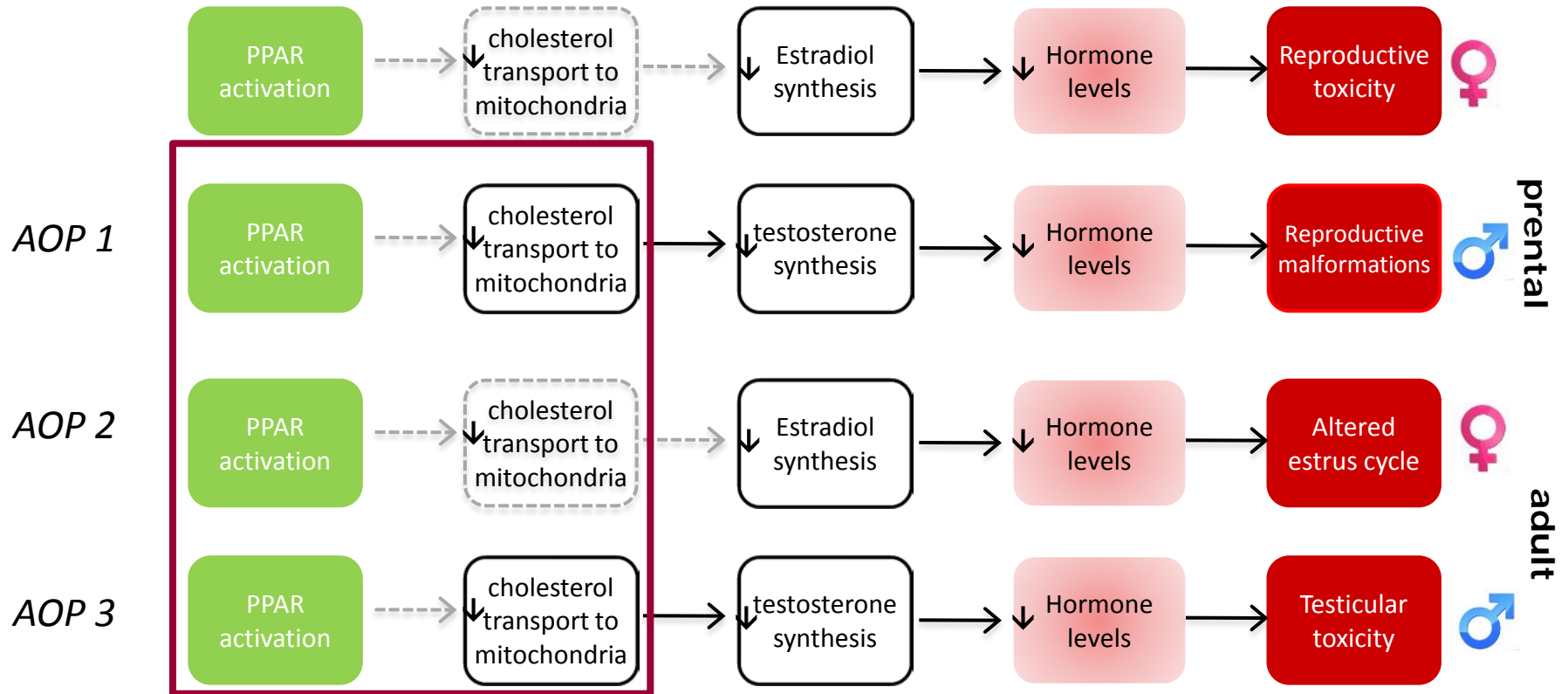
MIE

KE

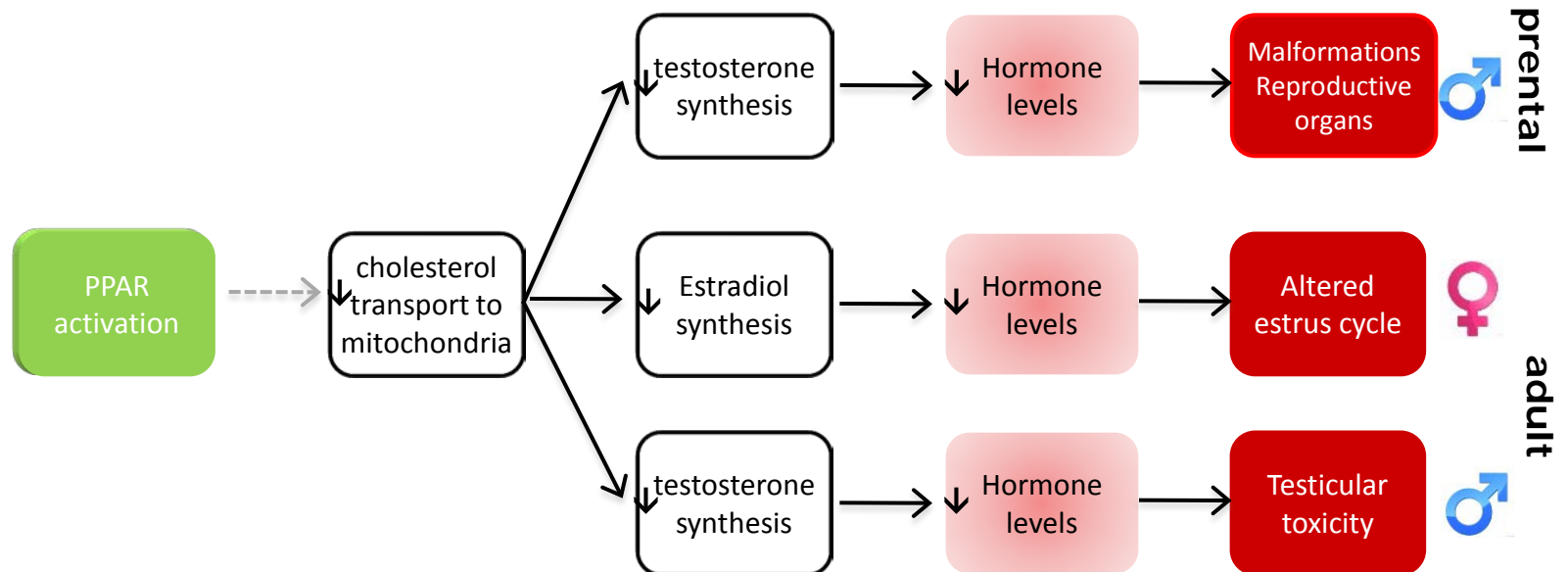
Adverse Outcome



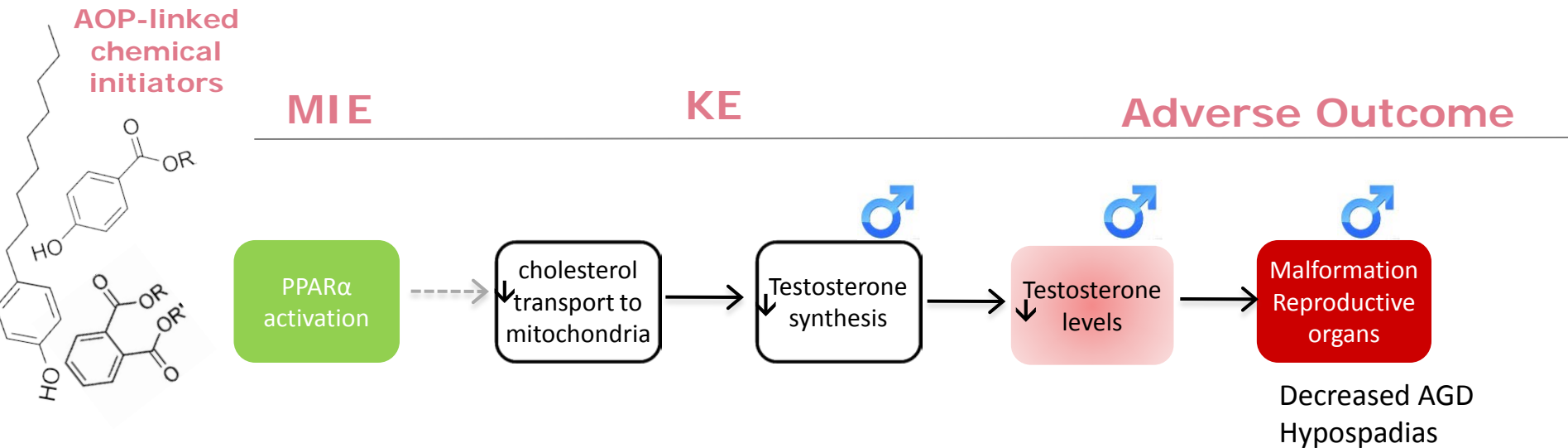
# PPAR activation leading to reproductive toxicity in rodents



# PPAR activation leading to reproductive toxicity in rodents



# PPAR $\alpha$ activation leading to reproductive tract malformations in males upon *in utero* exposure



## PPAR $\alpha$ activation leading to reproductive tract malformations in males upon *in utero* exposure

Key Events	Experimental Support	Strength of Evidence
<b>Molecular Initiating Event: Binding to and activation to PPAR<math>\alpha</math></b>	<ul style="list-style-type: none"> <li>➤ DEHP/MEHP, BBP, DBP binding to PPAR<math>\alpha</math> in vitro, in silico</li> <li>➤ PPAR<math>\alpha</math> transactivation by DEHP/MEHP, BBP, DBP, butylparaben</li> <li>➤ Experiments with PPAR<math>\alpha</math>-null mice indicate involvement of the receptor in reproductive toxicity of phthalates</li> </ul>	Moderate
<b>Key Event: Impaired steroidogenesis</b>	<ul style="list-style-type: none"> <li>➤ Impaired transport of cholesterol to mitochondria</li> <li>➤ decreased gene expression of SR-B1, TSPO (PBR), StAR</li> <li>➤ decreased gene expression of P450<sub>scc</sub>, 3<math>\beta</math>-HSD, 17<math>\beta</math>-HSD</li> </ul>	Moderate
<b>Key Event: Decreased testosterone levels</b>	<ul style="list-style-type: none"> <li>➤ Decreased testosterone levels measured in plasma</li> <li>➤ Decreased testosterone production measured ex-vivo</li> </ul>	Strong
<b>Adverse Outcomes: Reproductive tract malformations</b>	<ul style="list-style-type: none"> <li>➤ DEHP, DBP, BBP, butylparaben, decreased AGD</li> <li>➤ DEHP, DBP, BBP, Hypospadias</li> </ul>	Strong



Weak

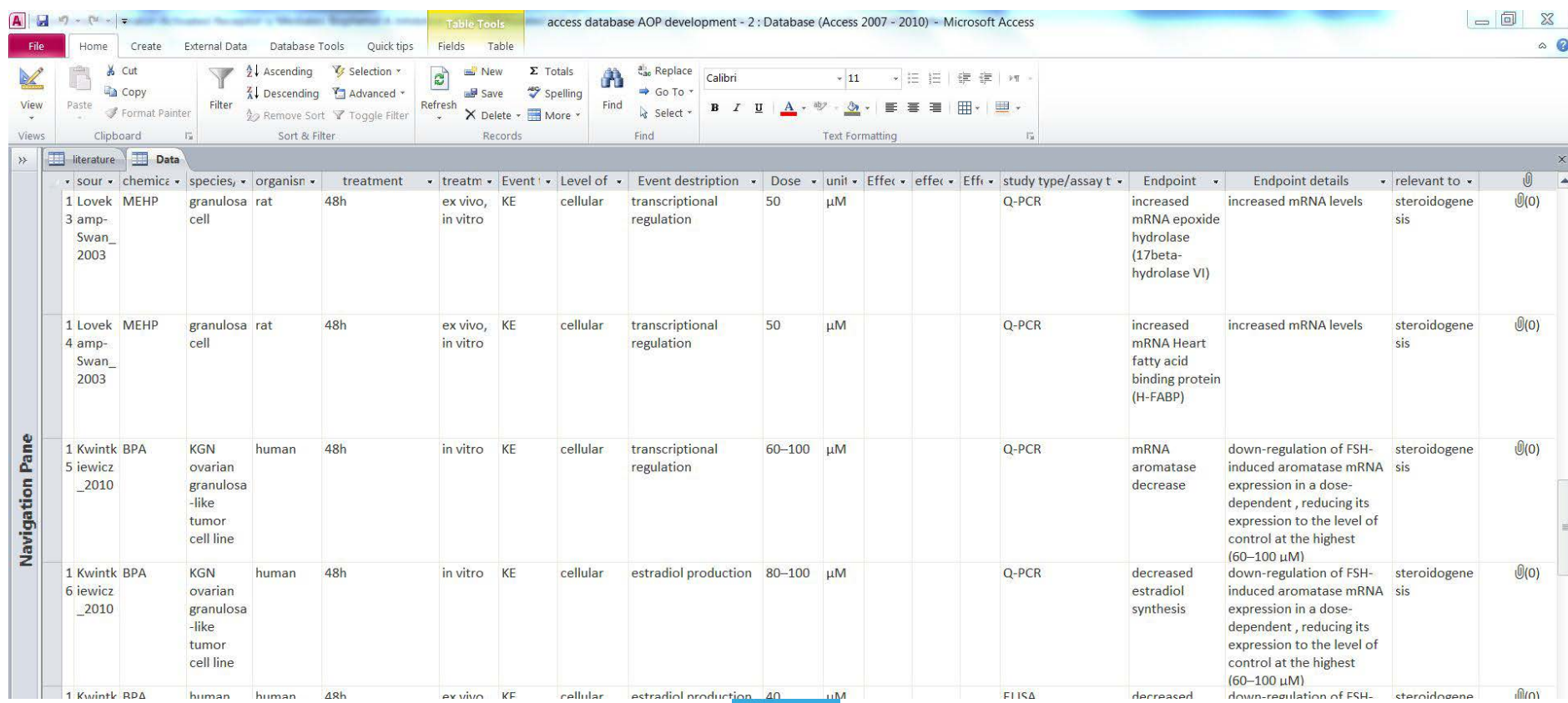
Moderate

Strong

# Challenges for these AOPs

## Data mining

### ➔ Literature organisation and structural capturing of the biological events



source	chemical	species	organism	treatment	treatm	Event	Level of	Event description	Dose	unit	Effec	effec	Effi	study type/assay t	Endpoint	Endpoint details	relevant to
1 Lovek 3 amp- Swan_ 2003	MEHP	granulosa cell	rat	48h	ex vivo, in vitro	KE	cellular	transcriptional regulation	50	µM				Q-PCR	increased mRNA epoxide hydrolase (17beta- hydrolase VI)	increased mRNA levels	steroidogene sis
1 Lovek 4 amp- Swan_ 2003	MEHP	granulosa cell	rat	48h	ex vivo, in vitro	KE	cellular	transcriptional regulation	50	µM				Q-PCR	increased mRNA Heart fatty acid binding protein (H-FABP)	increased mRNA levels	steroidogene sis
1 Kwintk 5 iewicz _2010	BPA	KGN ovarian granulosa -like tumor cell line	human	48h	in vitro	KE	cellular	transcriptional regulation	60-100	µM				Q-PCR	mRNA aromatase decrease	down-regulation of FSH- induced aromatase mRNA expression in a dose- dependent , reducing its expression to the level of control at the highest (60-100 µM)	steroidogene sis
1 Kwintk 6 iewicz _2010	BPA	KGN ovarian granulosa -like tumor cell line	human	48h	in vitro	KE	cellular	estradiol production	80-100	µM				Q-PCR	decreased estradiol synthesis	down-regulation of FSH- induced aromatase mRNA expression in a dose- dependent , reducing its expression to the level of control at the highest (60-100 µM)	steroidogene sis
1 Kwintk	BPA	human	human	48h	ex vivo	KE	cellular	estradiol production	40	µM				ELISA	decreased	down-regulation of FSH-	steroidogene

## 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