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

At the beginning

➲ We had an AIM

To develop a strategy for building a MoA based chemical category

chemical structure

$f($  $) =$ toxicity

$f(MoA) =$ 

➲How?

Building MoA-based chemical category for toxicity prediction

STEP 1. Chose endocrine active, data rich chemicals

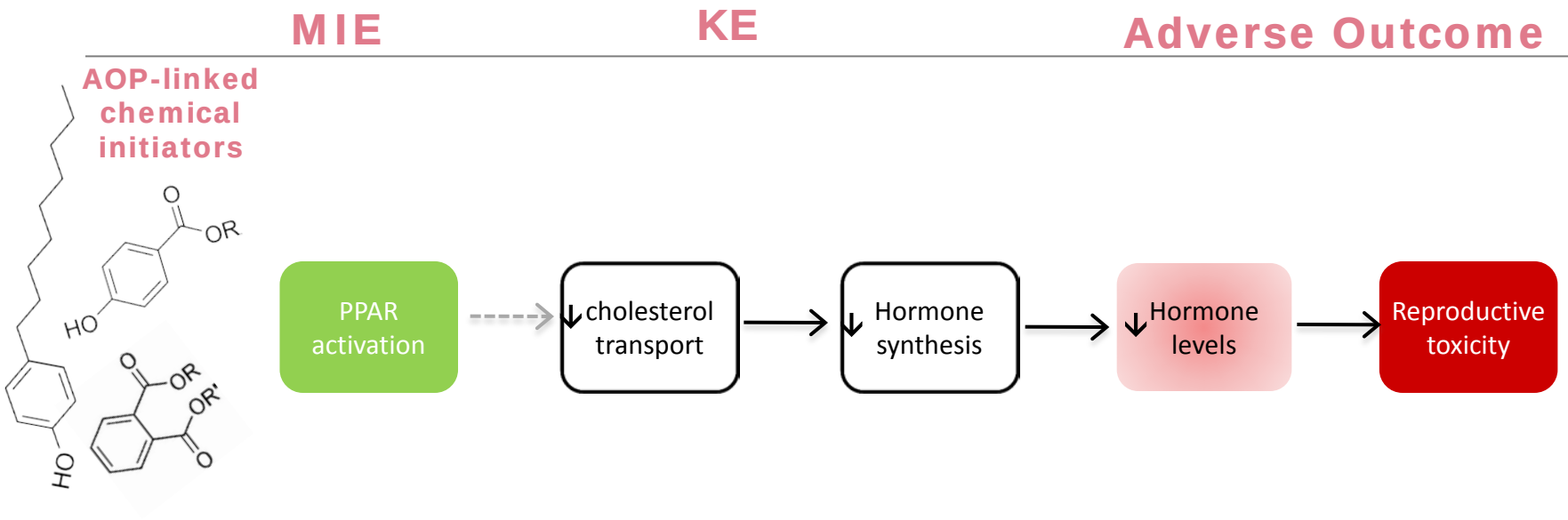
STEP 2. MoA matrix display of experimental data

	MIE				KE						AO		
Phthalates	ER	PPAR	AR	AhR	Sertoli cells	spermato genesis	Leydig cells	Decreased testosterone	stereodo genesis	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)

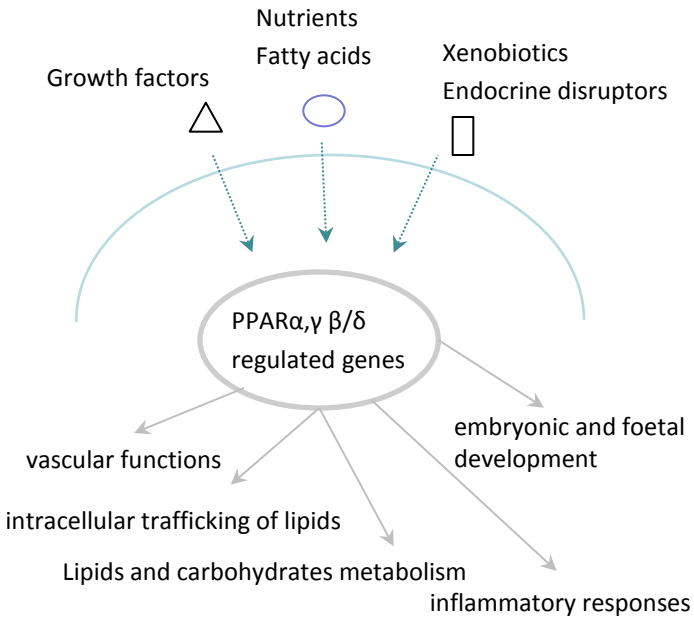
PPAR activation leading to reproductive toxicity in rodents



MIE

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



MIE

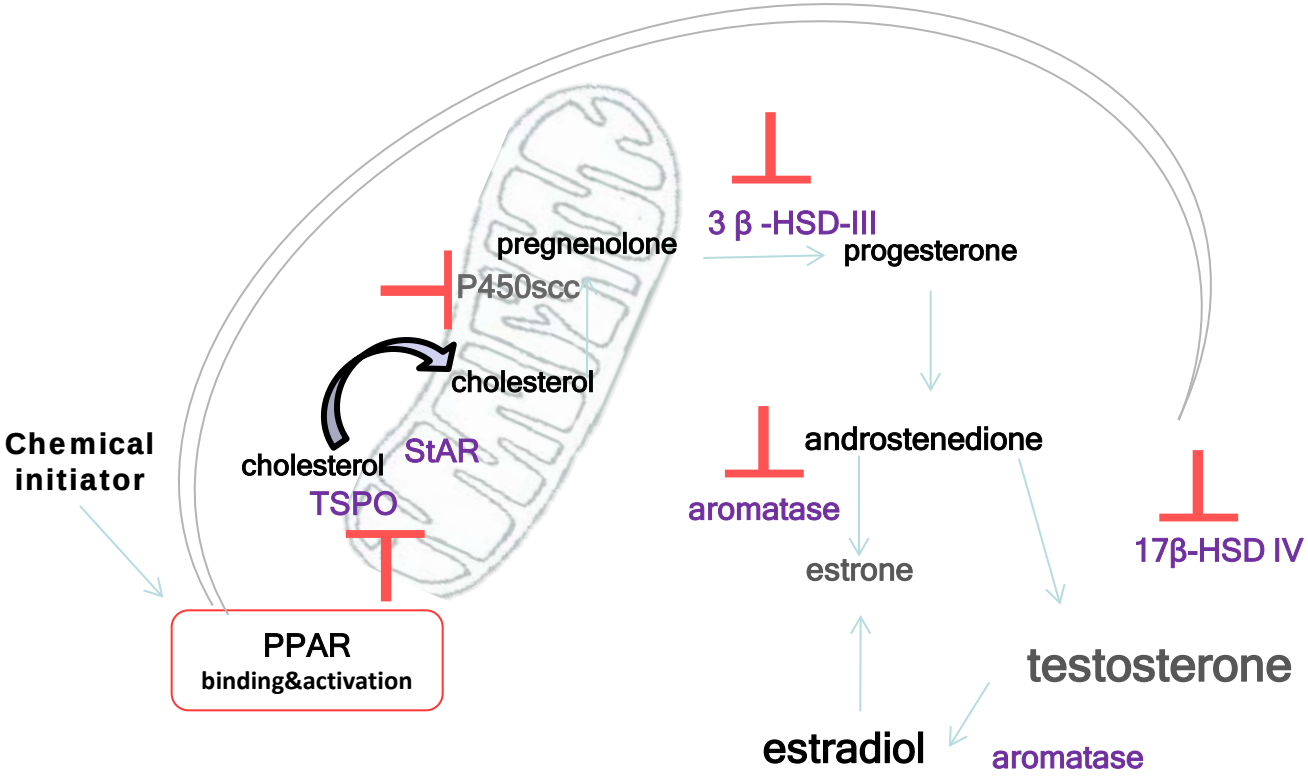
PPAR activation: evidence

Chemical initiator	In vitro binding	in vitro transactivation	Knock-out / inhibition / increased expression
DEHP	-	+	Experiments with PPARα-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 PPARγ
Butylparaben	-	+	Increased expression PPARγ

KE

European Commission

Altered steroidogenic pathway

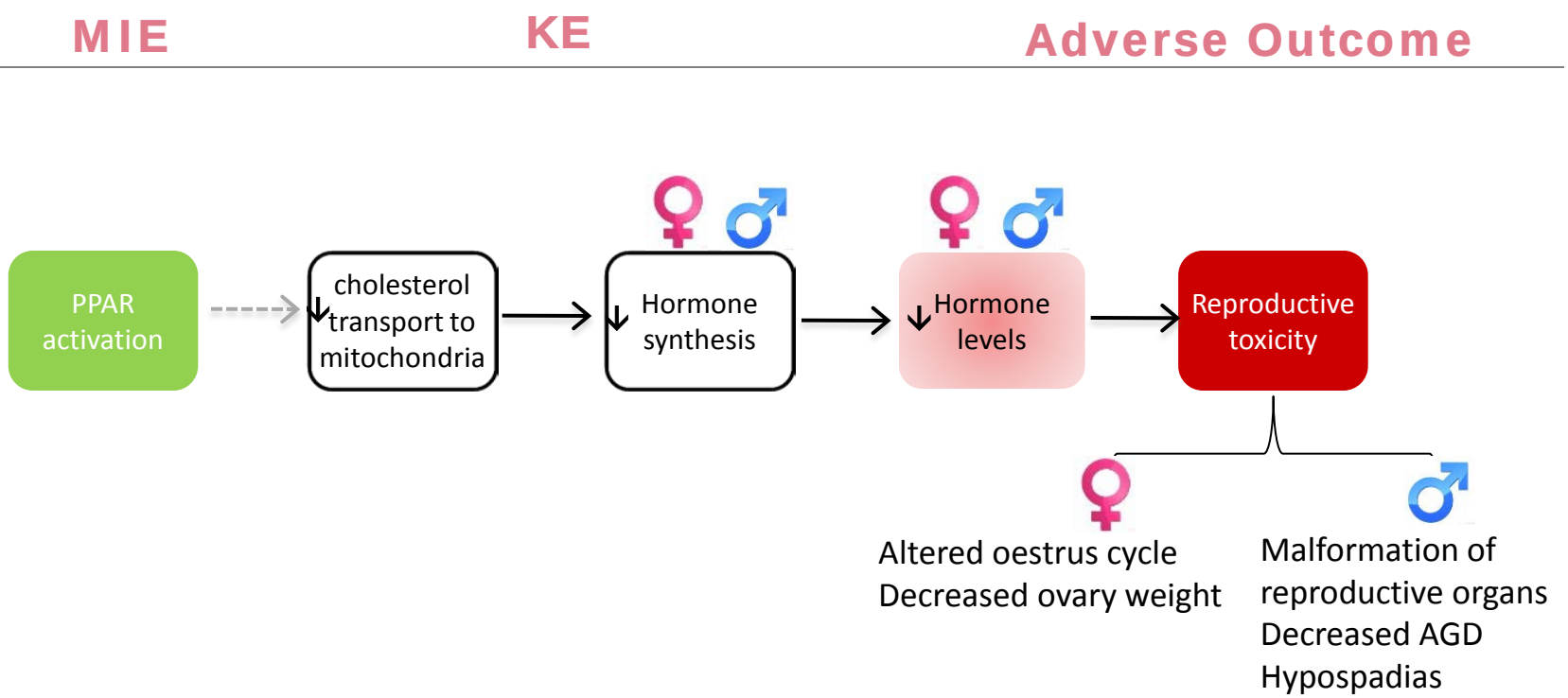


Joint Research Centre

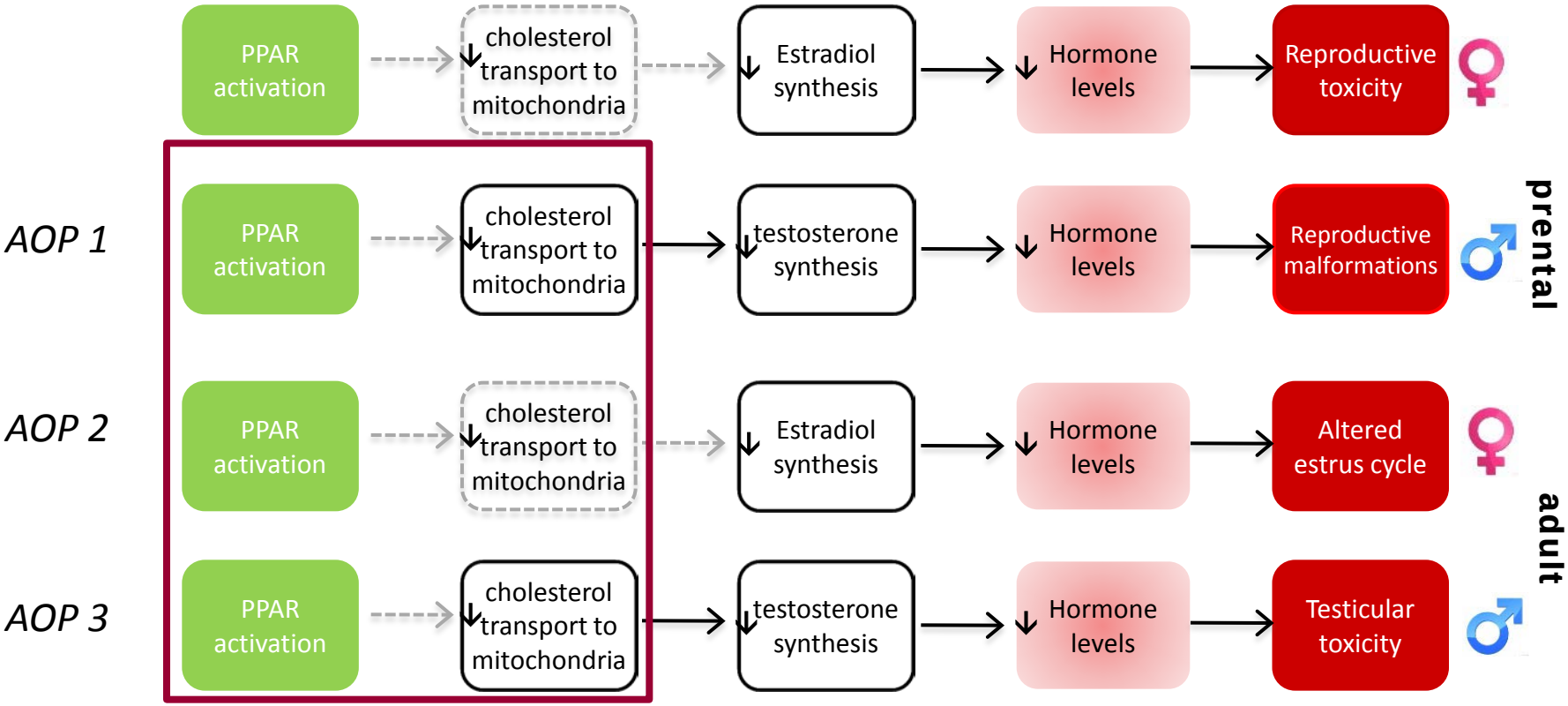
Chemical Initiator	Decreased testosterone levels	Malformation of reproductive organs	Testicular toxicity
DEHP	+ (Howdeshell et al., 2008)	+ (Gray et al., 2000) (Parks, 2000)	+ (Kwack et al., 2009)
BBP	+ (Howdeshell et al., 2008)	+ (Gray et al., 2000) (Nagao et al., 2000)	+ (Gray et al., 2000)
DBP	+ (Howdeshell et al., 2008) (Barlow et al., 2003) (Mylchreest, 2000)	+ (Barlow et al., 2003) (Mylchreest, 2000)	+ (Mylchreest, 2000)
Bisphenol A	+ (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)
Butyl paraben	+ (Zhang et al., 2014)	+ (Zhang et al., 2014)	+ (Oishi et al., 2001)

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

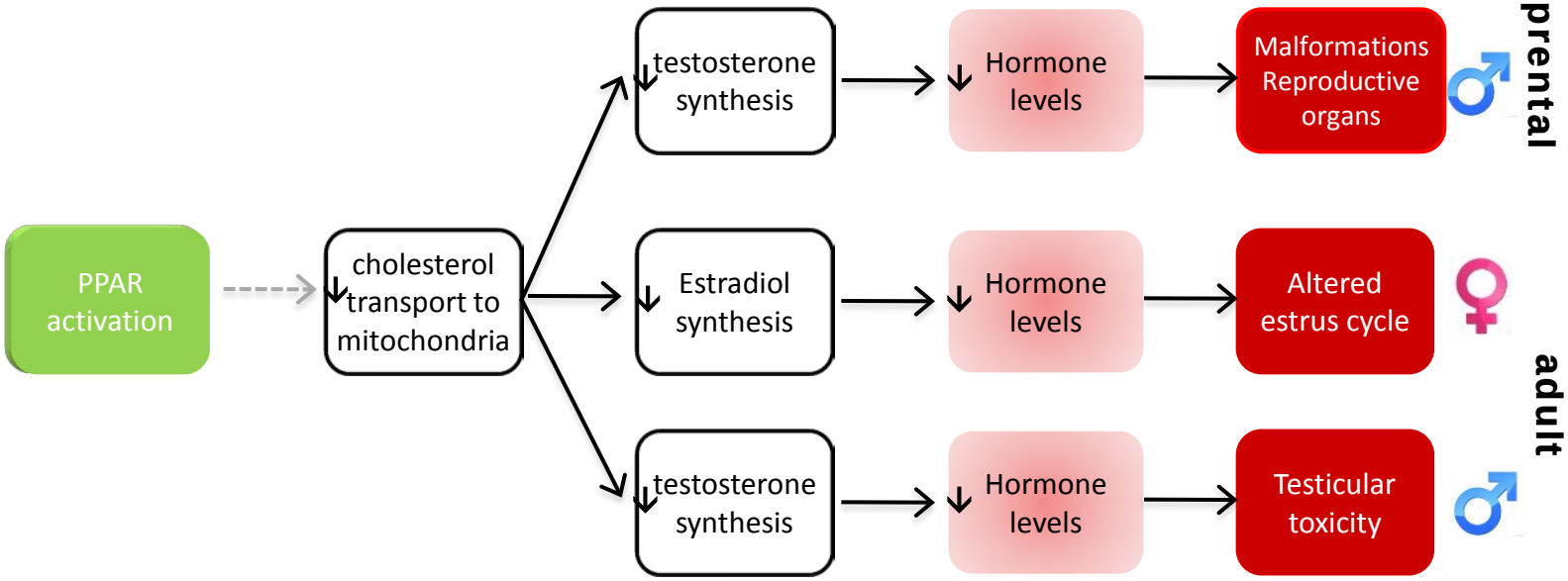
PPAR activation leading to reproductive toxicity in rodents



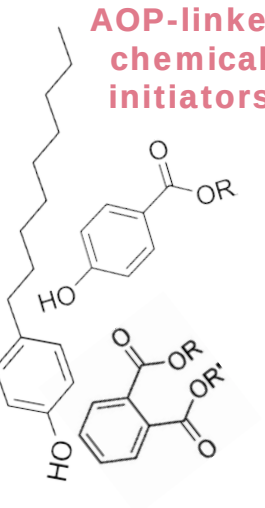
PPAR activation leading to reproductive toxicity in rodents



PPAR activation leading to reproductive toxicity in rodents



PPARα activation leading to reproductive tract malformations in males upon *in utero* exposure



MIE

KE

Adverse Outcome

PPARα activation

cholesterol transport to mitochondria

Testosterone synthesis

Testosterone levels

Malformation Reproductive organs

Decreased AGD
Hypospadias

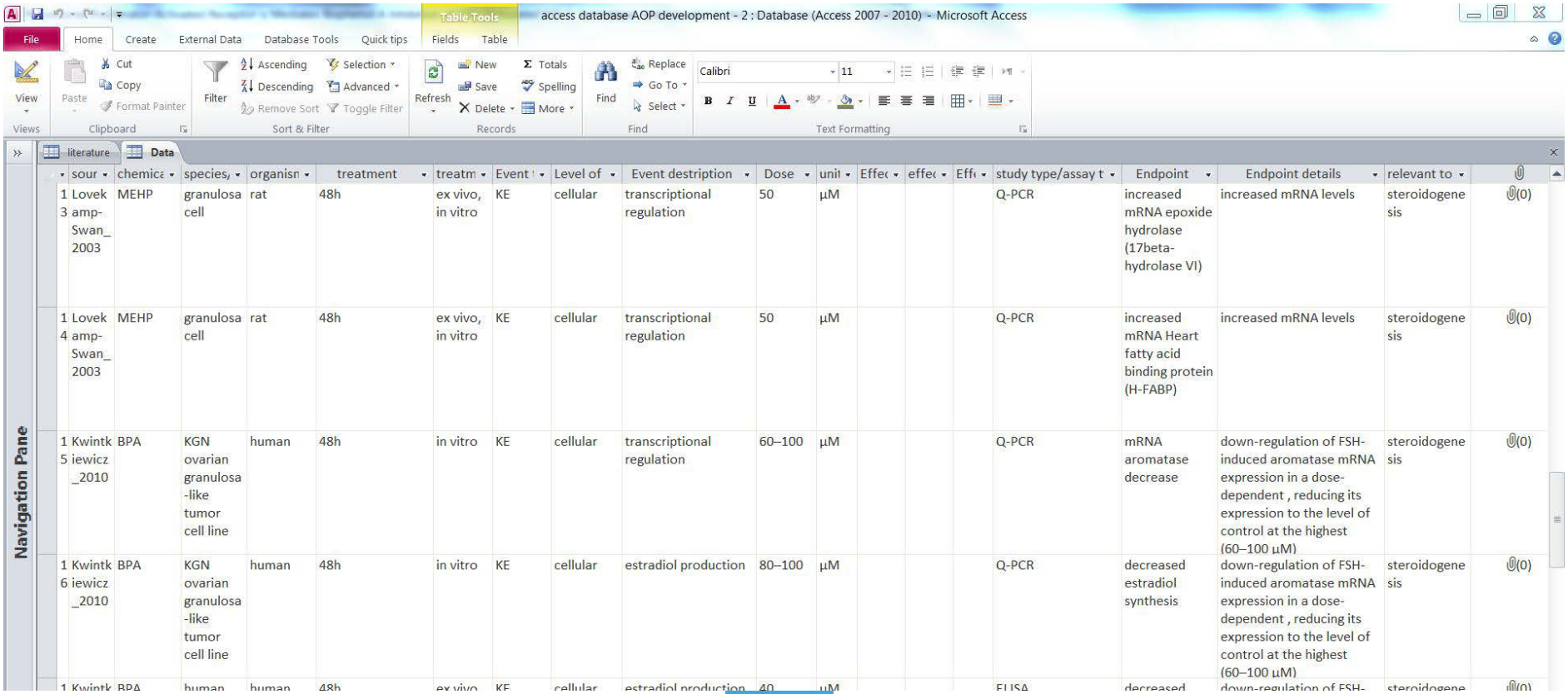
PPARα activation leading to reproductive tract malformations in males upon *in utero* exposure

Key Events	Experimental Support	Strength of Evidence	
Molecular Initiating Event: Binding to and activation to PPARα	➲ DEHP/MEHP, BBP, DBP binding to PPARα in vitro, in silico ➲ PPARα transactivation by DEHP/MEHP, BBP, DBP, butylparaben ➲ Experiments with PPARα-null mice indicate involvement of the receptor in reproductive toxicity of phthalates	Moderate	Weak
Key Event: Impaired steroidogenesis	➲ Impaired transport of cholesterol to mitochondria ➲ decreased gene expression of SR-B1, TSPO (PBR), StAR ➲ decreased gene expression of P450scc, 3β-HSD, 17β-HSD	Moderate	Moderate
Key Event: Decreased testosterone levels	➲ Decreased testosterone levels measured in plasma ➲ Decreased testosterone production measured ex-vivo	Strong	Strong
Adverse Outcomes: Reproductive tract malformations	➲ DEHP, DBP,BBP, butylparaben, decreased AGD ➲ DEHP, DBP,BBP, Hypospadias	Strong	

Challenges for these AOPs

Data mining

- **Literature organisation and structural capturing of the biological events**



	sour	chemica	species,	organism	treatment	treatm	Event	Level of	Event destription	Dose	unit	Effec	effec	Effe	study type/assay t	Endpoint	Endpoint details	relevant to	
13	Lovekamp-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	steroidogenesis	(0)
14	Lovekamp-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	steroidogenesis	(0)
15	Kwintkiewicz_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)	steroidogenesis	(0)
16	Kwintkiewicz_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)	steroidogenesis	(0)
1	Kwintk	BPA	human	human	48h	ex vivo	KE	cellular	estradiol production	40	µM				ELISA	decreased	down-regulation of FSH-	steroidogene	(0)

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

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Thank you

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