Science Question 1

- **Liver effects, including spongiosis hepatis.**

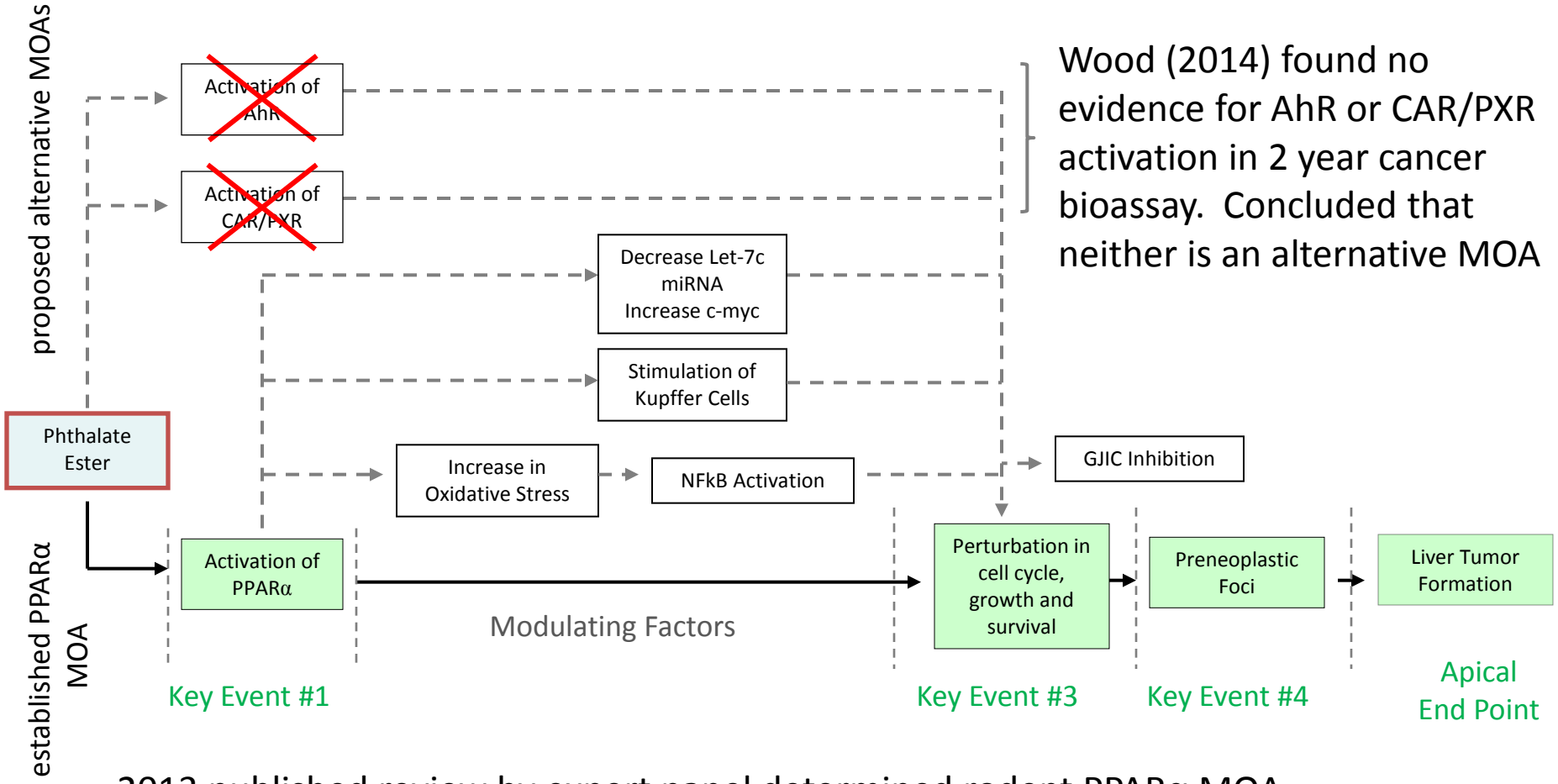
PPARα agonists induce liver tumors via a rodent-specific mode of action (MOA)

Proposed alternative MoA's are modulation factors of PPARα MoA, not separate processes

		Key events								
			KE#3							Apical endpoint
		KE#1	Perturbation of cell growth and survival		Associative event		Modulating factors			
Species	Chemical	PPARα activation	Increases in transient acute cell proliferation	Decreases in acute apoptosis	Hypolipidemic effect (decreased serum or VLDL triglycerides)	Increases in liver to body weight	Oxidative stress	NF-κB activation	Alterations in gap junctions	Hepatic tumors
Rats	See Table 5	+	+	+	+[48]	+[47]	+	+	+	+
Mice	See Table 6	+	+	+	+[48]	+[46]	+	+	+	+
Syrian hamster	Nafenopin	+[1]	−[2]	+[3]		+[4]				−[4]
	WY-14 643	+[5]	−[6]	−	+[8]	+[7]		−[9]		−[4]
	DEHP	(+)[10]	(+)[11]			+[10]				−[43]
	Methyl clofenapate	+[12]	(+)[13]	+[45]	+[8]	+[14]			+[44]	
	Ciprofibrate	+[15]	+[16], −[12]			+[15]				
	Bezafibrate	+[17]	−[12]			−[17]				
Guinea pig	Methylclofenapate	−[18]	−[19]	−[20]	+[21]	−[12]				
	Ciprofibrate	+[22], −[23]	−[12]			−[15]				
	WY-14 643	+[24], −[12]	−[12]		+[21]	−[12]				
	Nafenopin	+[25], −[26]	−[27]	+[3]		−[28]			49	
	Fenofibrate	−[29]								
	Perfluorodecanoic acid	−[30]				+[31], −[32]				
	Bezafibrate	−[17]				−[17]				
Cynomolgus monkey	DEHP	−[33]	−[33]			−[33]			−[50]	
	Diisononyl Phthalate		−[33]			−[33]			−[50]	
	Clofibrate	−[33]	−[33]			−[33]			−[50]	
	Fenofibrate	+[34]	−[34]		−[34]	−[34]	−[34]			
	Ciprofibrate	+[35] (+)[35]	−[34]			+[34]	−[36]			
Humans	See footnotes for compound used	+[37], −[38]	−[39]	−[40]	+[42]	−[41]			−[51]	

Corton, et al. (2013) Critical Reviews in Toxicology pp 1-49.

Data Confirm that PPARα is the MoA for DINP- induced rodent liver tumors and not relevant to humans

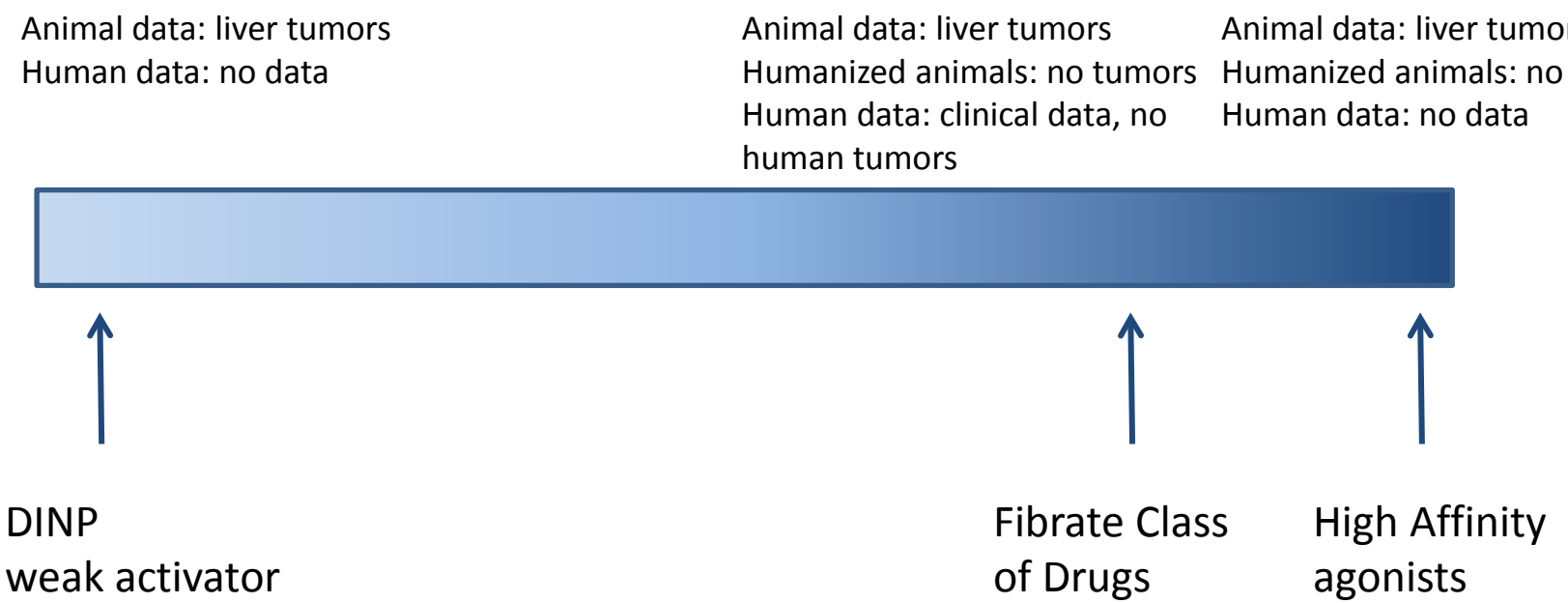


Wood (2014) found no evidence for AhR or CAR/PXR activation in 2 year cancer bioassay. Concluded that neither is an alternative MOA

2013 published review by expert panel determined rodent PPARα MOA ‘‘not relevant to humans’’ or ‘‘unlikely to be relevant to humans’’ – Corton et al 2013

Corton, et al. (2013) Critical Reviews in Toxicology pp 1-49
Wood, et al. (2014) Toxicological Sciences 139: 21-34.

DINP is a very weak activator of PPARα



Indicators of PPAR MOA Not Observed in Primates

Only effects were secondary to diarrhea caused by the high doses administered

- Marmosets
  - 13 Weeks (90 days)
  - Gavage: 0, 100, 500, 2500 mg/kg/day DINP
  - No toxicologically significant effect

- Cynomologous Monkeys
  - 14 days
  - Gavage: 0, 500 mg/kg/day DINP
  - No increase in replicative DNA synthesis, an important mechanism by which phthalates likely promote liver tumor formation

- Oral administration of DINP at dosages of up to 2500 mg/kg/day did not produce evidence of significant toxicity
  - No indication that DINP acted as a peroxisome proliferator at levels that would induce peroxisome proliferation in rodents (150 mg/kg/day in 14 day studies)
  - Minor, non-toxicologically significant changes were evident at 2500 mg/kg/day and the NOEL for DINP was considered to be 500 mg/kg/day

Science Question 4

- **Human relevance of mononuclear cell leukemia**

MNCL is a spontaneous aging lesion occurring at high frequency in F-344 rats

- Spontaneous incidence ranges from 32-74%
  - Tumor data in DINP studies similar to historical averages [22]

Incidence of MNCL from Haseman, [22] Lington, [4] and Moore [5]

	Range of MNCL in controls for NTP feeding studies (Haseman)	Highest incidence of MNCL	
		Lington	Moore
Male	32 – 74%	63.8% (51/80 rats)	49.2% (32/65 rats)
Female	14 – 52%	53.8% (43/80 rats)	46.2% (30/65 rats)

- Many factors affecting tumor frequency unrelated to treatment
  - e.g., dosing methods, caging, diet, vehicle, testing laboratory, etc.
- Species and strain specific
  - Not found in chronic studies in SD rats or in mice

Example of a treatment related vs spontaneous tumor type

Summary for DINP Studies

Organ ▼	Tumor Type ▼	Investigator(s) ▶	Bio\dynamics (1986)		Lington et al (1997)		Moore (1998)		Moore (1998)	
		Strain & Species ▶	S-D Rats		Fischer 344 Rats		Fischer 344 Rats		B6C3F1 Mice	
			male	female	male	female	male	female	male	female
Liver	Combined Adenoma + Carcinoma		NS	S	NS	NS	S	S	S	S
Blood	Mononuclear Cell Leukemia		NR	NR	S	S	S	S	NR	NR

Key:

S	Significant
NS	No Significant Trend
NR	Not Reported / Relevant

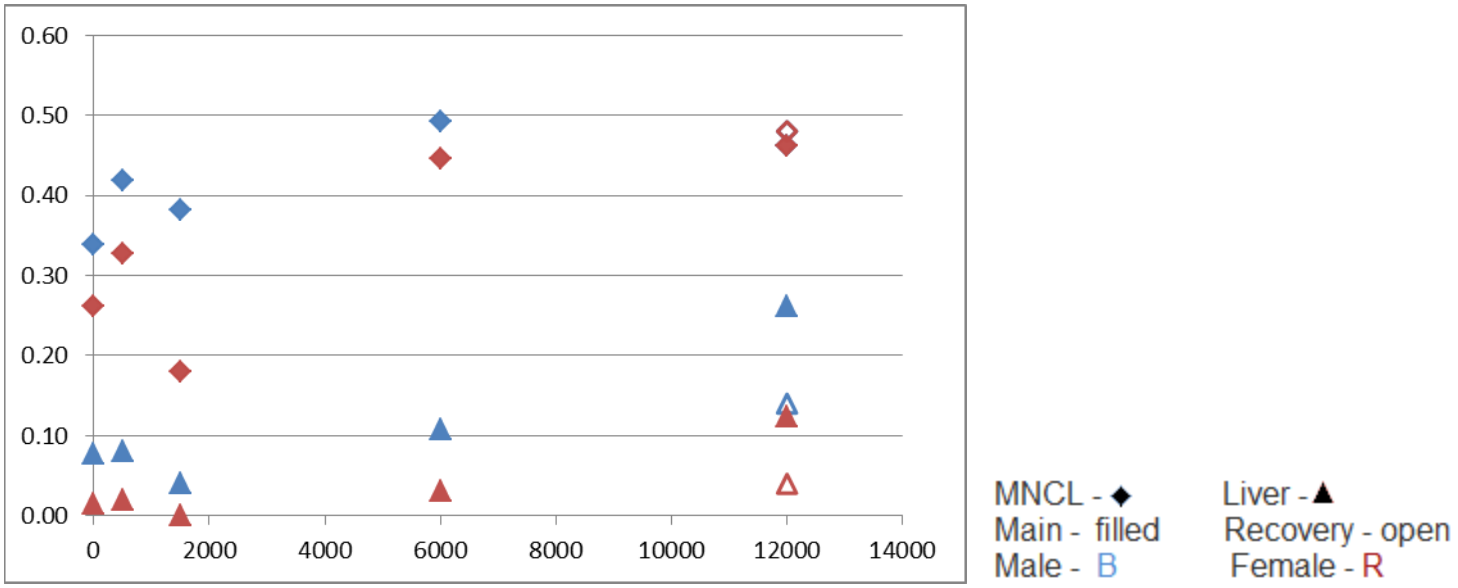
- Liver tumors
  - treatment related
  - consistent across species and strains of rodents
  - defined Mode of Action
- MNCL
  - high spontaneous background incidence
  - species and strain dependent
  - incidence influenced by non treatment factors
  - factor in recommendation that F344-N be discontinued for use by NTP

	Range of MNCL in controls for NTP feeding studies (Haseman)
Male	32 – 74%
Female	14 – 52%

No change in incidence of MNCL in recovery animals

Animals were treated at 500, 1500, 6000, & 12000 ppm in the diet, a recovery group treated at 12000 for 78 wks. then untreated for 24 wks. before sacrifice was included.

Tumor Incidence Moore - Rat



- Liver
  - Decreased incidence in recovery group
  - Consistent with treatment related hypothesis. PPARα-mediated effects would reverse with cessation of treatment
- MNCL
  - equivalent incidence in recovery group
  - Consistent with the hypothesis that these are spontaneous and age-related

The liver tumor data can be modeled but MNCL cannot (i.e., not dose-related)

Summary for DINP Studies

Organ ▼	Tumor Type ▼	Investigator(s) ▶	Bio\dynamics (1986)		Lington et al (1997)		Moore (1998)		Moore (1998)		Lington + Moore	
		Strain & Species ▶	S-D Rats		Fischer 344 Rats		Fischer 344 Rats		B6C3F1 Mice		Fischer 344 Rats	
			male	female	male	female	male	female	male	female	male	female
Liver	Combined Adenoma + Carcinoma		NS	S	NS	NS	S	S	S	S	M	M
Blood	Mononuclear Cell Leukemia		NR	NR	S	S	S	S	NR	NR	**NAM**	**NAM**

Key:

S	Significant
NS	No Significant Trend
NR	Not Reported / Relevant
M	Acceptable Model
NAM	No Acceptable Model

- Liver
  - combined data set can be modeled for BMD
- MNCL
  - combined dataset cannot be modeled for BMD

MNCL is a high frequency aging lesion occurring spontaneously in F-344 rats

- MNCL in the Fischer rat is believed to reflect a high level of spontaneous DNA damage
  - biological plausibility that DINP would act by this mechanism is low
    - uniformly non-genotoxic in both in vitro and in vivo mutagenicity studies including unscheduled DNA repair

- MNCL tumor data from DiNP studies are not treatment specific
  - no change of incidence in recovery animals
  - combined dataset cannot be modeled

- Questionable relevance to humans

Scientific Question 5

- **Transparency and utility of mechanistic data.**

Utility of mechanistic data

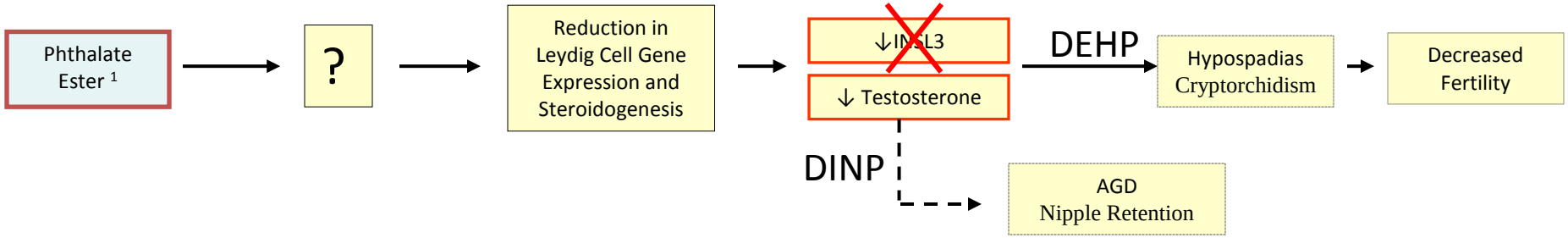
- Mechanistic data can be useful to:
  - Define precursor events
  - Evaluate species differences in susceptibility

- As an example consider the use of mechanistic data in the evaluation of male reproductive data

Testosterone is necessary but not sufficient

Summary of Mechanism of Action Studies									
Chemical	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
DEHP	↓	↓	↓	↓	↓	↓	↓	↓	↓
DINP	↓	↑	↓	↓	↑			↑	

1 = Testosterone
2 = insl3 (Insulin-like factor 3)
3 = CYP11A (Rate-limiting enzyme responsible for the conversion of cholesterol to pregnenolone)
4 = StAR = Steroidogenic Acute Regulated Protein, involved in mitochondrial cholesterol uptake
5 = LH = Lutenizing Hormone
6 = SR-B1 = Scavenger Receptor B-1, responsible for cholesterol uptake by Leydig cells
7 = PBR = Peripheral Benzodiazepene Receptor, involved in mitochondrial cholesterol uptake
8 = CYP450scc = Cytochrome P450 side chain cleavage enzyme, steroid converting enzyme
9 = SF-1 = Nuclear Receptor Steroidogenic Factor-1, regulates expression of genes involved in steroidogenesis



Evaluating species difference in susceptibility

Human fetal testis xenografts are resistant to phthalate-induced reductions in testosterone

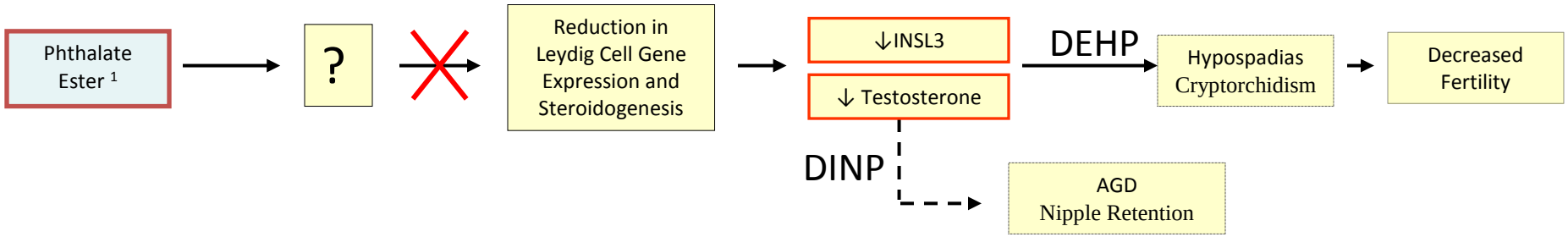
Human and rat fetal testis xenografts (Sharpe et al., 2012)

- Humans: No changes in testosterone production, testes weights of pathology
- Rats: Testosterone reductions, reduction in organ weights, gene expression, pathological changes
- "Exposure of human fetal testes to DBP is unlikely to impair testosterone production as it does in rats"

Human, mouse, and rat fetal testis xenografts (Boekelhide et al., 2012)

- Testosterone production reduced in rat xenografts but not in humans
- human fetal testis response more like a mouse (which is resistant *in vivo)* than a rat

Hypothesized MOA in Rats unlikely to be Relevant to Humans



DiNP does not cause adverse effects via endocrine-related processes.

- No effects on fertility
- No reproducible pathological changes in male reproductive organs
- In utero exposure causes testosterone reduction in rats but effects (AGD, areola retention) are reversible
  - Neonatal differences in nipple retention, AGD are reversed by sexual maturity; no toxicological consequences
  - Effects seem species specific
    - Mice less affected than rats
    - No effects in human xenografts
    - No effects in primates
- In summary, effects observed in rats related to a common process (testosterone reduction) but not relevant to humans
  - mechanistic studies assist in understanding MOA, species differences