Scientific Question 5

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

Utility of mechanistic data

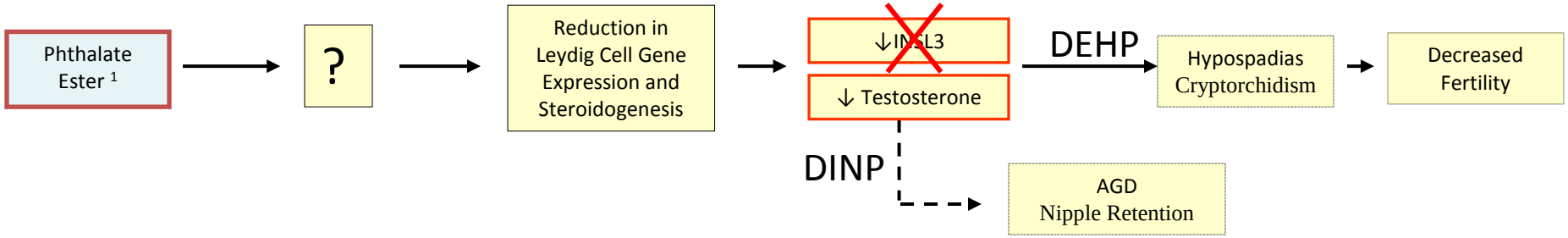
- Mechanistic data can be useful to:
  - Define precursor events
  - Evaluate species differences in susceptibility

- 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

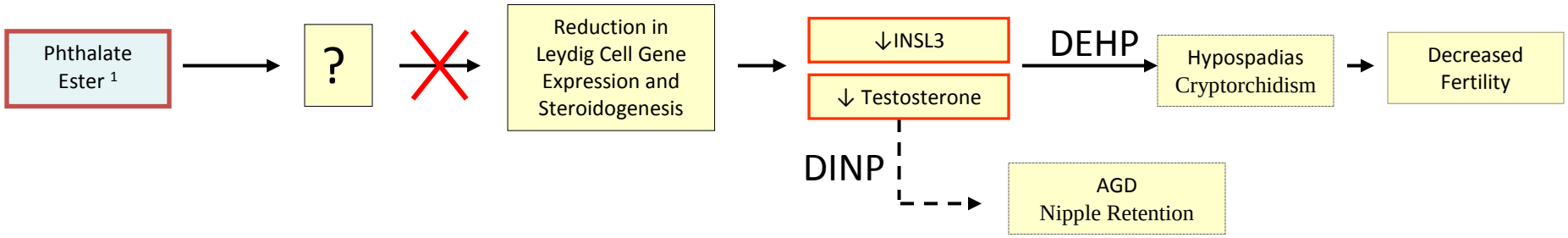
Human and rat fetal testis xenografts (Sharpe et al., 2012)

- 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