



# Cocktail effects in experimental animals exposed to endocrine disruptors during development

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"Kemikalie-cocktails i mad - hvordan griber vi det an i fremtiden?", 19. marts 2015

# Endocrine disrupters

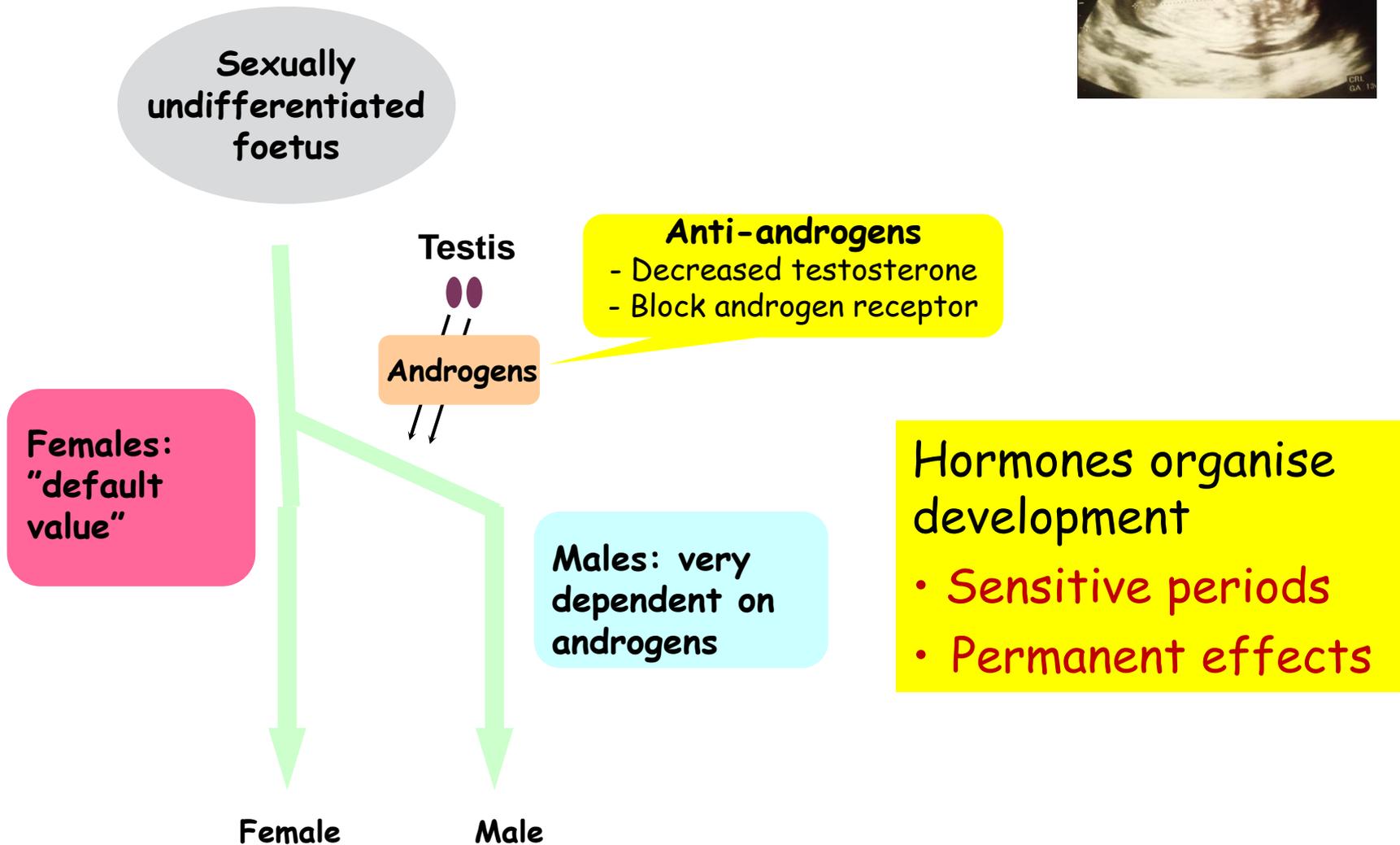
WHO definition: "An endocrine disrupter is an exogenous substance or mixture that **alters function(s) of the endocrine system** and consequently causes **adverse health effects** in an intact organism, or its progeny, or (sub)populations. "

## Concern

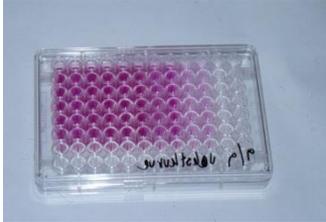
- Increasing incidence of reproductive malformations in newborn boys
- Poor semen quality in men
- More men with testicular cancer
- Experimental animals: Similar effects of anti-androgens **during development**



# Sexual differentiation of foetuses



# How to detect endocrine disruptors?



*In vitro*, QSAR  
Mechanisms



Animals, mechanisms and  
adverse effects



Humans



Mode of action  
Prioritise...

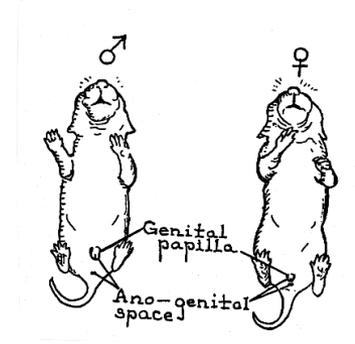


Relevant for humans  
Basis for EU's chemical regulation

# Sensitive endpoints for anti-androgenic effect

Anogenital distance, AGD, PND 1

Male ~ 2 x female



Nipple retention, PND 12/13

Male ~ 0; female = 12



Blindly to exposure group and the same technician

# Protection of humans - Risk assessment

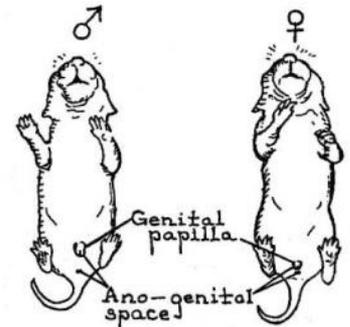
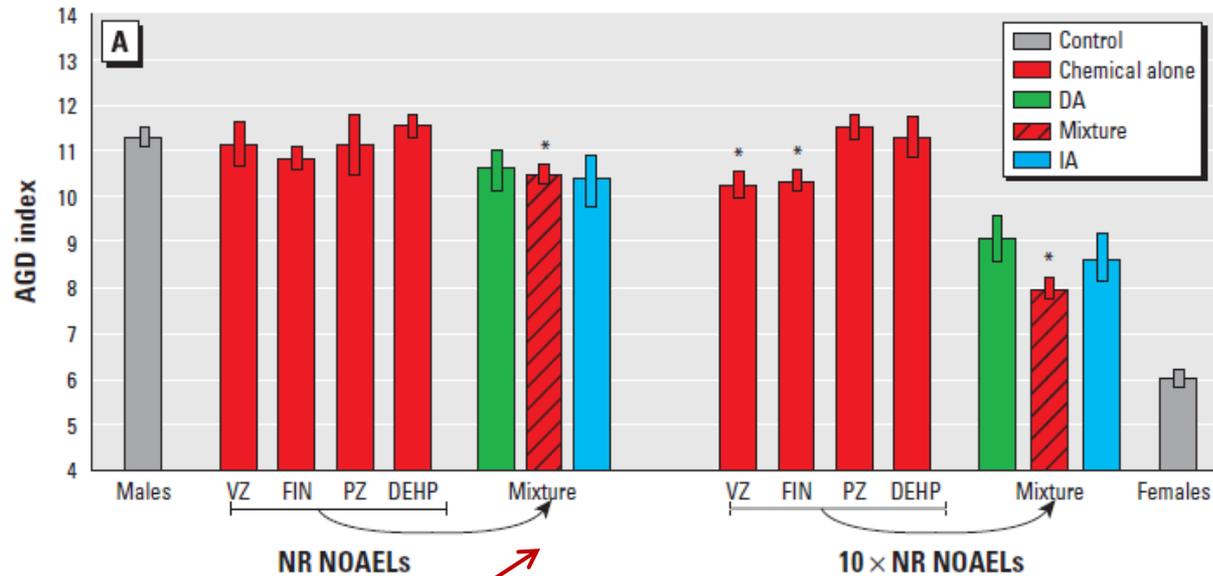
- No Observed Adverse Effect Level (NOAEL)
- Assessment factors (10 × 10)
- ADI, TDI or DNEL ~ safe level
- Compared to human exposure
- Normally one chemical at a time
- BUT...
- Cocktail effects at NOAELs for single endocrine disruptors?
- Risk for humans?



# Four anti-androgens, dissimilar mechanisms

Vinclozolin, AR-antagonist; DEHP, decreased testosterone;

Finasterid, alfa-reductase inhibitor; Prochloraz, multiple mechanisms

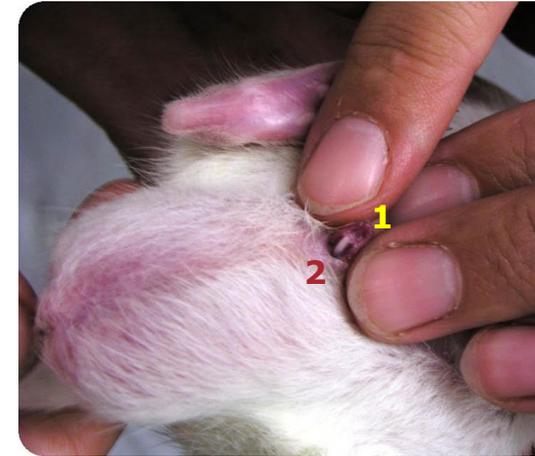
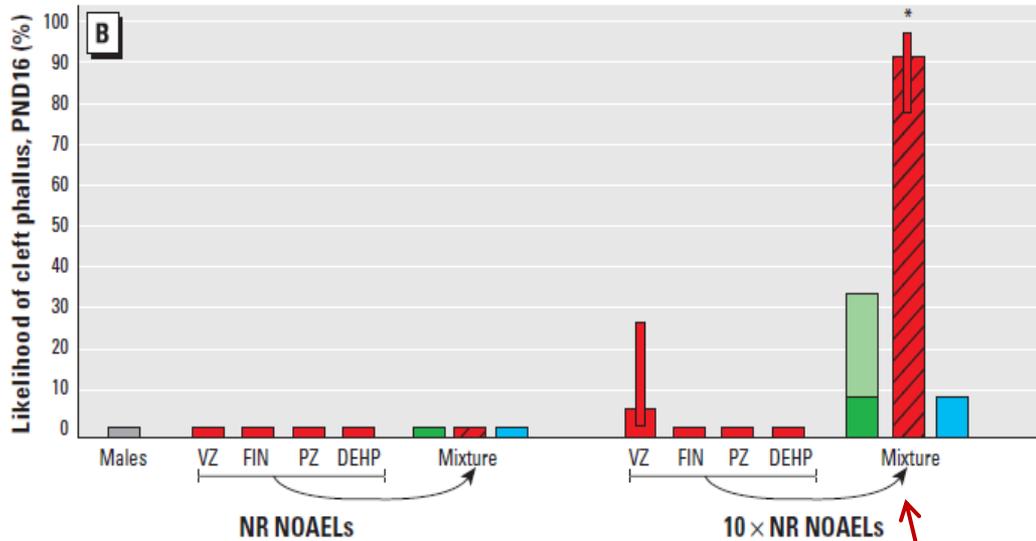


Decreased AGD at NOAEL

Cocktail effect

Predicted by dosis-addition

# Genital malformations, split penis/hypospadi

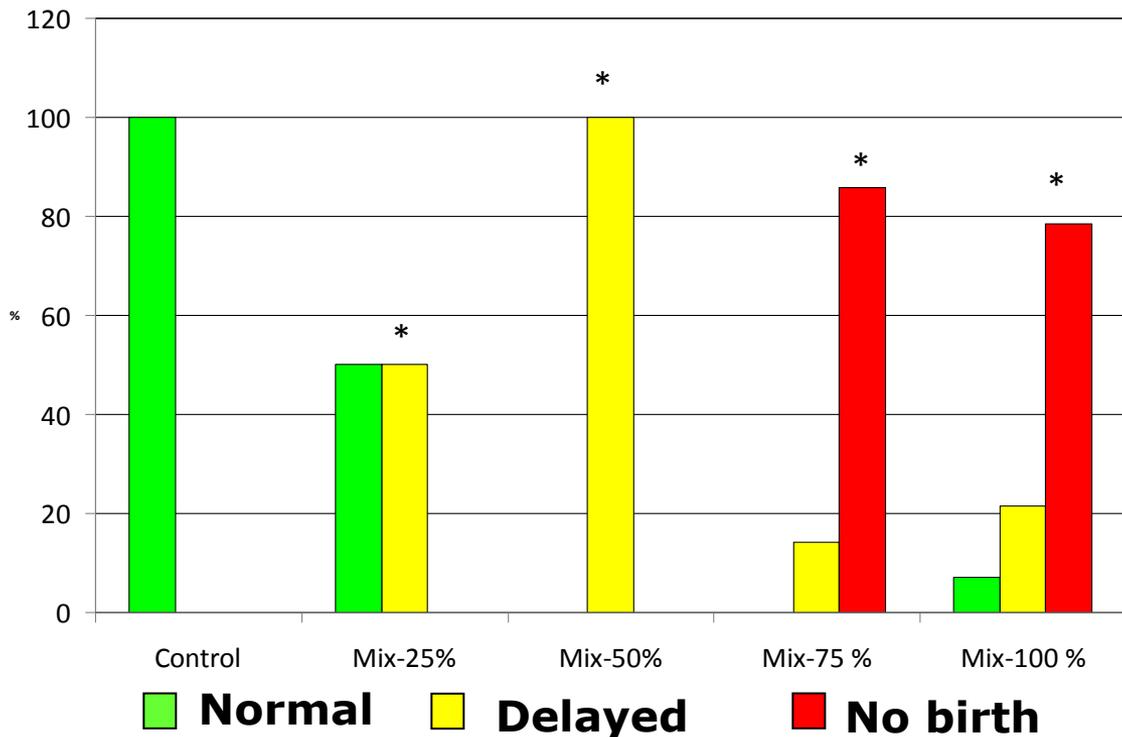


- 1) Split penis, visible os penis
- 2) Blind vaginal opening

Cocktail effects: 90% of male pups with genital malformations at 10 x NOAEL

# Mixture of 5 endocrine disrupting pesticides

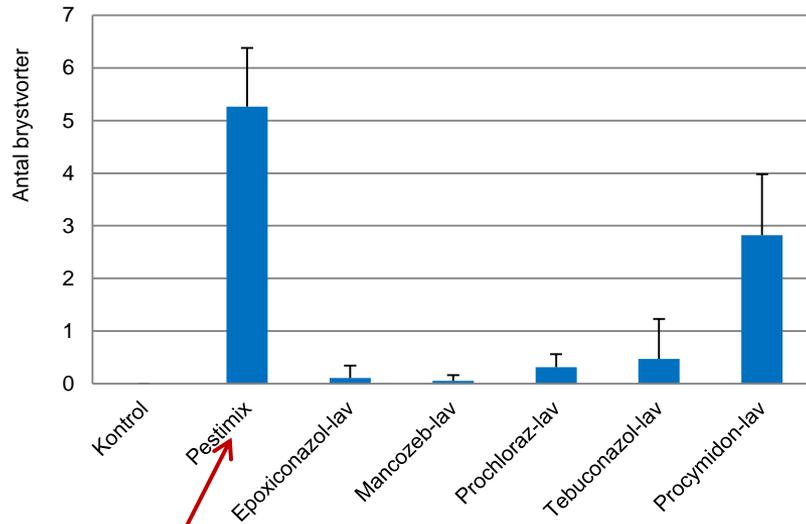
Procymidone; Mancozeb; Epoxyconazole; Tebuconazole and Prochloraz



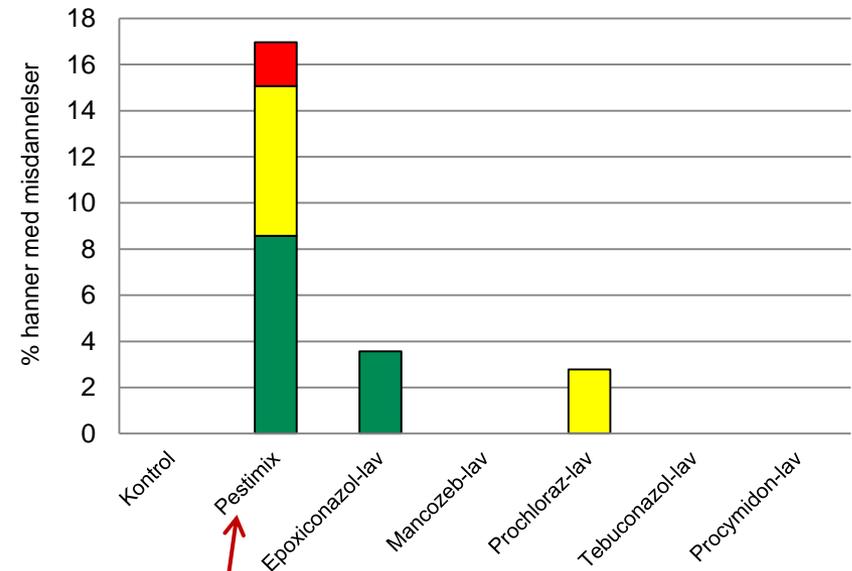
Mixture ratio:  
NOAEL for effect  
on gestation length

Severe cocktail effect on birth at 75% of NOAELs  
Predicted by dose-addition

# Anti-androgenic effects in male offspring



**Increased nipple retention**  
 Cocktail effect  
 Dosis-addition: Good prediction, at low doses



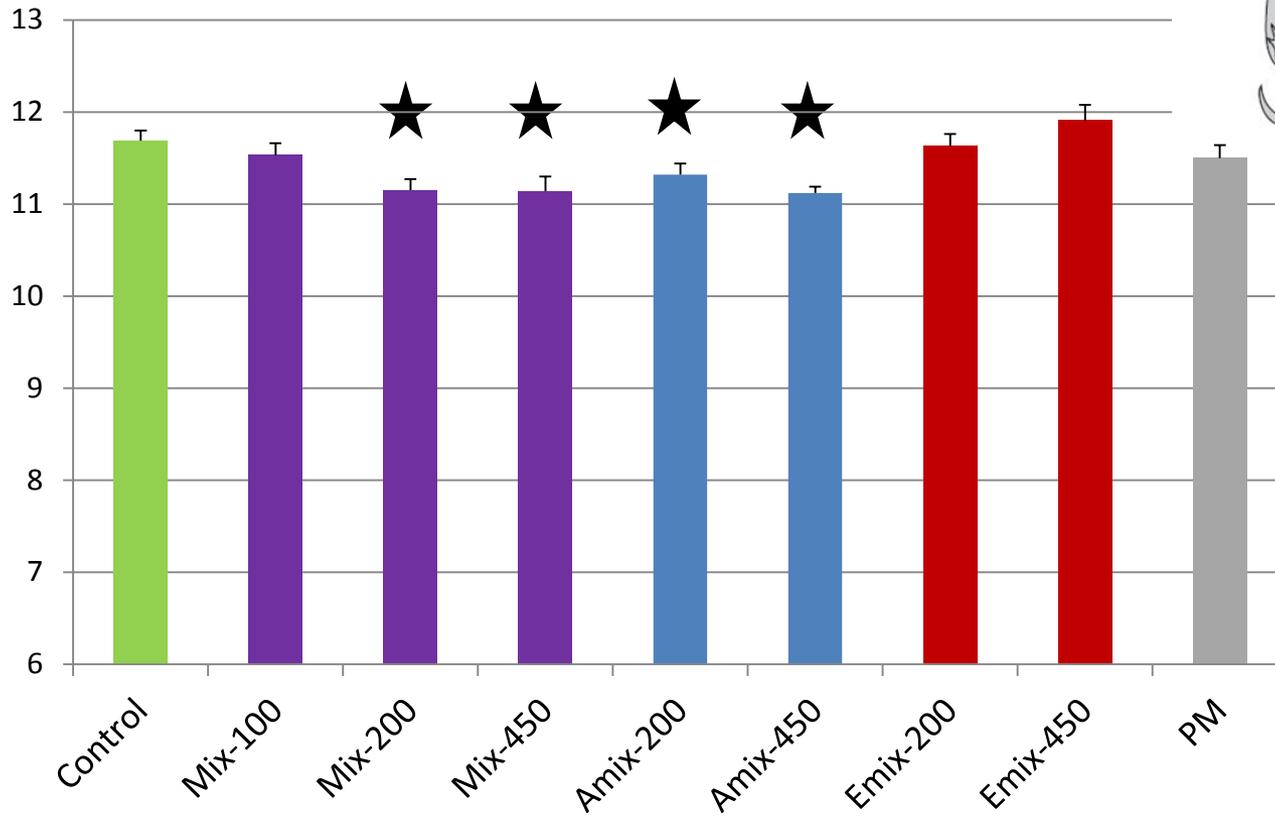
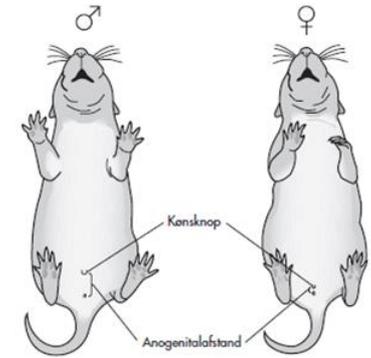
**Genital malformations**  
 Higher frequency and more severe  
 Green = mild; yellow = severe; red = very severe

# Contamed: Environmentally relevant mixture

- Anti-androgens and oestrogens
- Mixture ratio = high end human exposure
- Doses: 100, 150, 200 and 450 times human exposure
- Margin of safety > 100 ?
- 2 large studies

Chemical	Ratio
DBP	0,01
DEHP	0,02
Vinclozolin	0,009
Prochloraz	0,014
Procymidone	0,015
Linuron	0,0006
Epoxiconazole	0,01
ppDDE	0,001
Bisphenol A	0,0015
Butyl paraben	0,06
OMC	0,12
4-MBC	0,06
Paracetamol	0,8

# AGD index, male offspring, mean+sem

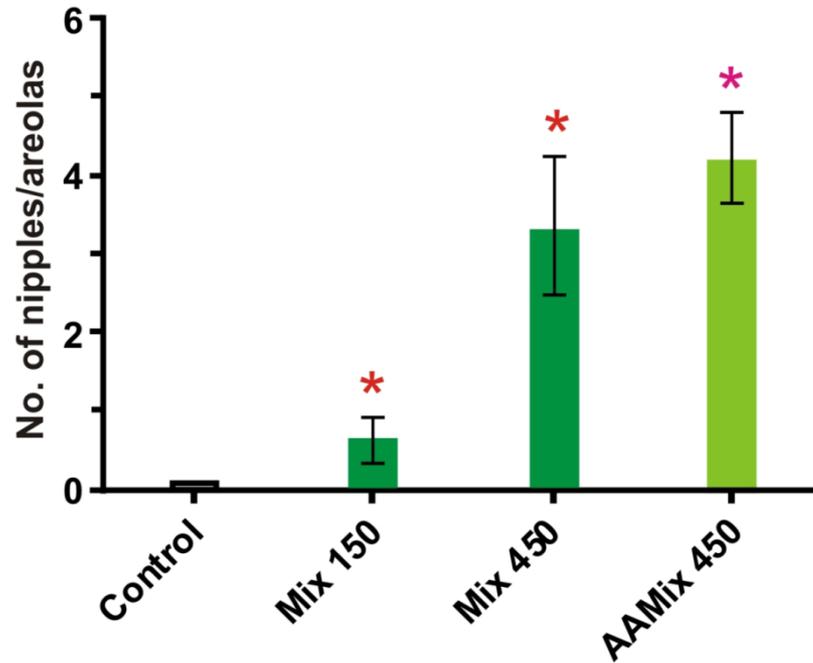


★ p < 0.05

Decreased AGD from 200 x human exposure

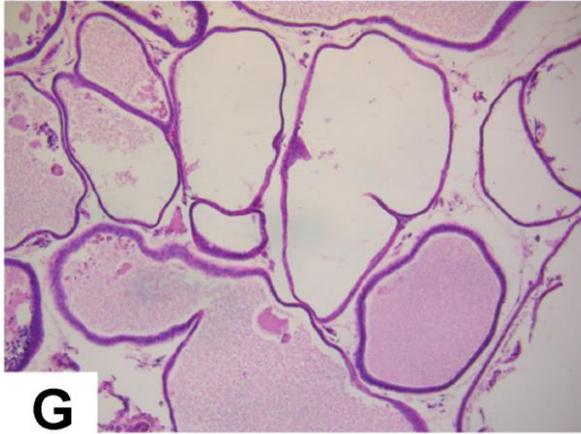
# Nipple retention, males, pup day 13

## Study 1

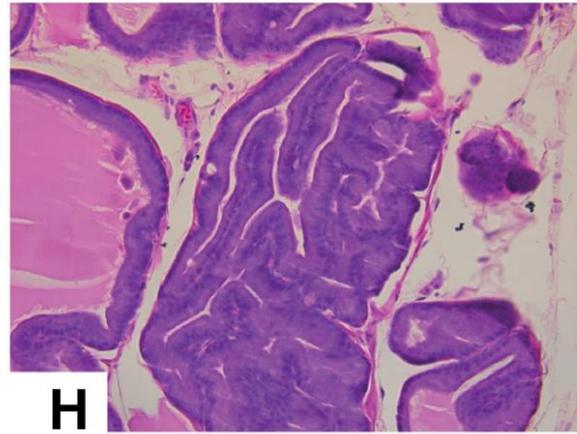


Effect at 150 times human exposure  
No NOAEL

# Late effects, prostate histology, 10 months



**G**  
Control



**H**  
Anti-androgen mixture

- Indicate a shift from the normal age-dependent epithelial atrophy towards hyperplasia
- Similarities to pre-cancerous lesions in humans

# Early and late effects of developmental exposure

This cocktail also caused:

- Decreased prostate weight and lower sperm count in male offspring
- Increased mammary outgrowth, earlier menopause and decreased ovary weight
- More results to come .....
- **Some effects late in life at 100 times human exposure => Margin of safety is not > 100**

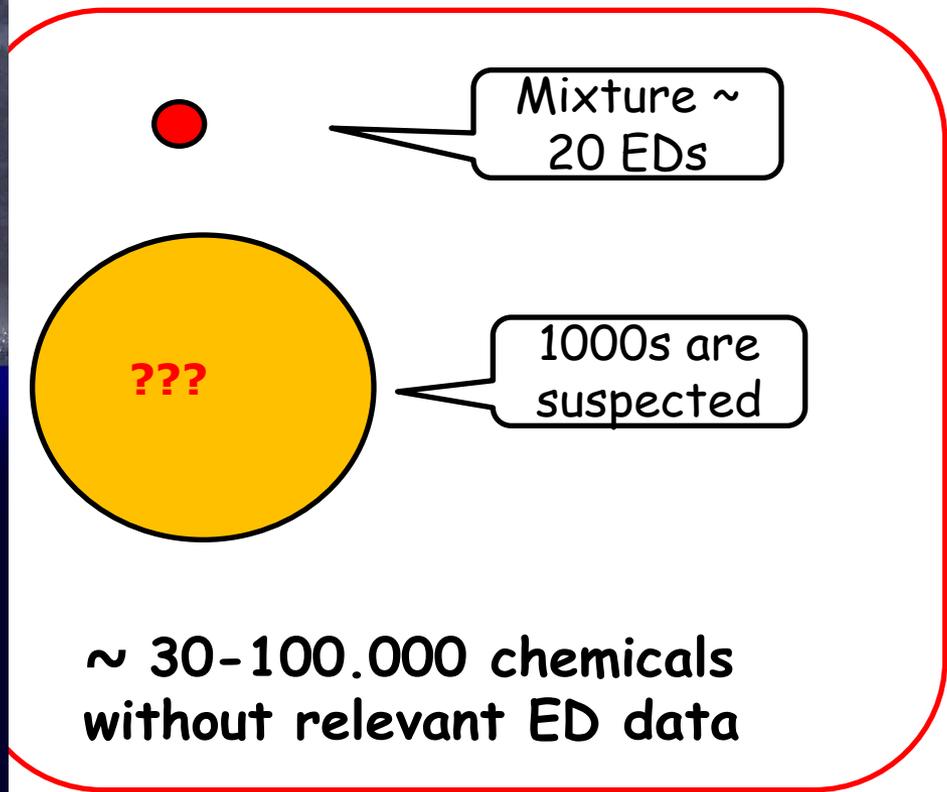
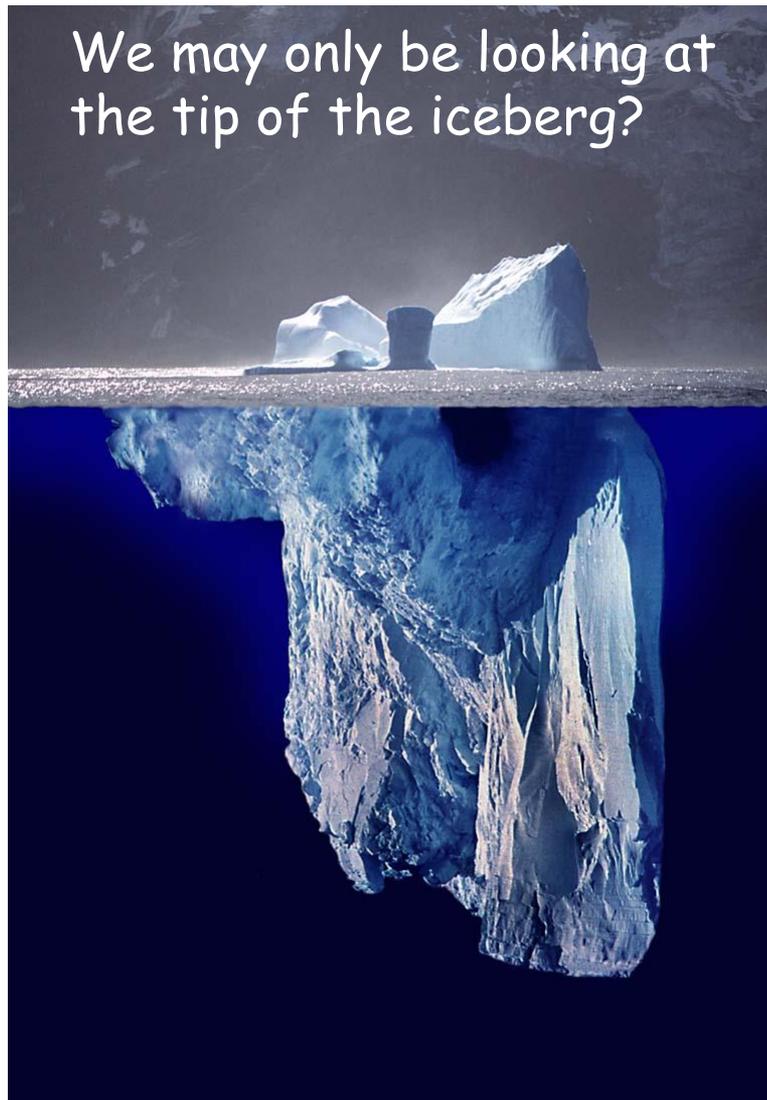


Rats: 3 months -2 years

Humans: 20-80 years



# Environmentally relevant mixture?



# Conclusions - endocrine disruptors

- Severe cocktail effects at NOAELs for single endocrine disruptors
- One chemical at a time underestimate the risk
- Cocktail effects can be predicted by dose-addition
- Highly exposed women may not be sufficiently protected
- Risk assessment of cocktails: needed and feasible





THE STRAW THAT BROKE THE CAMELS BACK!

Thank you for your attention!



Thanks to our animal assistants & the rats...  
Thanks to colleagues and sponsors