Dioxin toxicology

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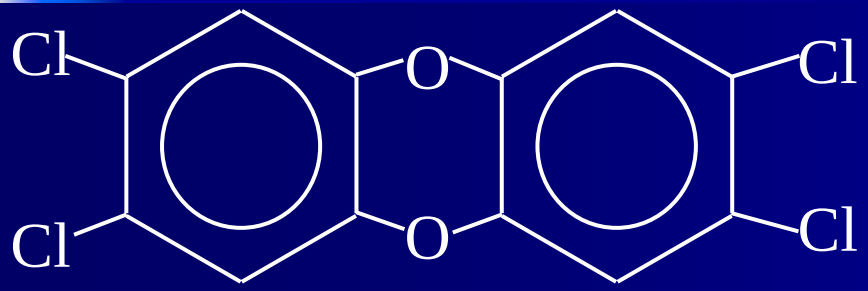
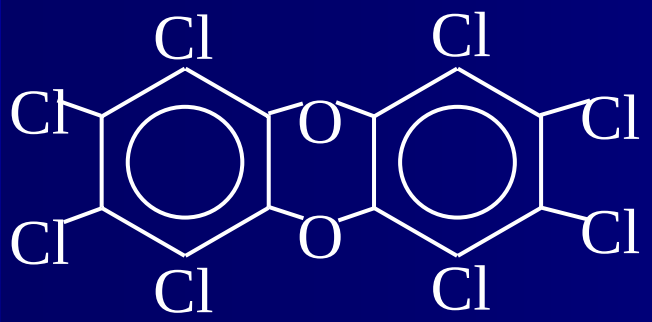
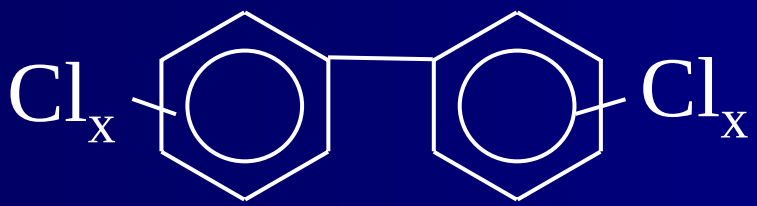
Dioxins, furans, PCBs, ...



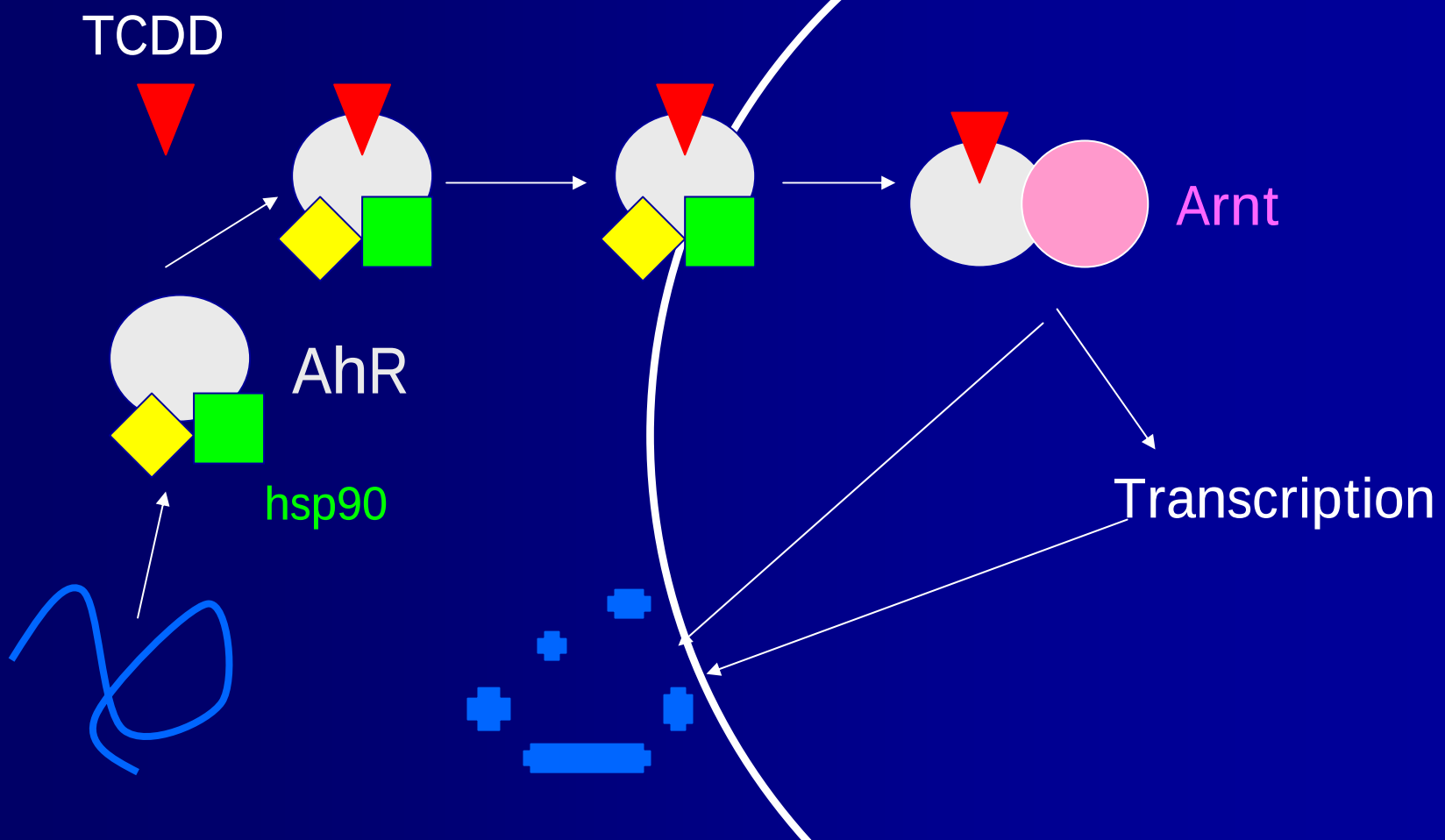


OCDD



- Highly toxic, carcinogenic, teratogenic,...
- Ubiquitous at low level
- Family of congeners which operate by binding to Ah Receptor

AhR and dioxin signalling



AhR is important

- Dioxin toxicology requires AhR
- AhR null mice are resistant to dioxin toxicity

- AhR null mice show defective development
- Dioxin can rescue development in AhR hypomorphs

PNAS 101:16677, JBC 278:17767

Biological persistence

- Polycyclic aromatic hydrocarbons
  - *e.g.* benzo(a)pyrene, 3-methylcholanthrene
    - Half-life of hours
- TCDD “half-life”
  - Human
    - ~7 years at low dose
    - 1.5-3 years at high dose (*ArchTox***76**:316)
  - Rat ~3-4 weeks

TCDD is a potent toxin

- LD_{50} values
  - 1μg/kg guinea pig
  - 20-50μg/kg rat
  - 70μg/kg monkey
  - 5000μg/kg hamster
  - Highly toxic in chicken, fish (< 1μg/kg)
- Lethality involves a “wasting syndrome”, involving many bodily organs, and taking 2-4 weeks
- Teratogenic, porphyria, cancer, *etc.*

Dioxin & human toxicity

Suspected poisoning: dose of ~1-2 mg

1998

2004

EHP109: 865, CMAJ 172:873

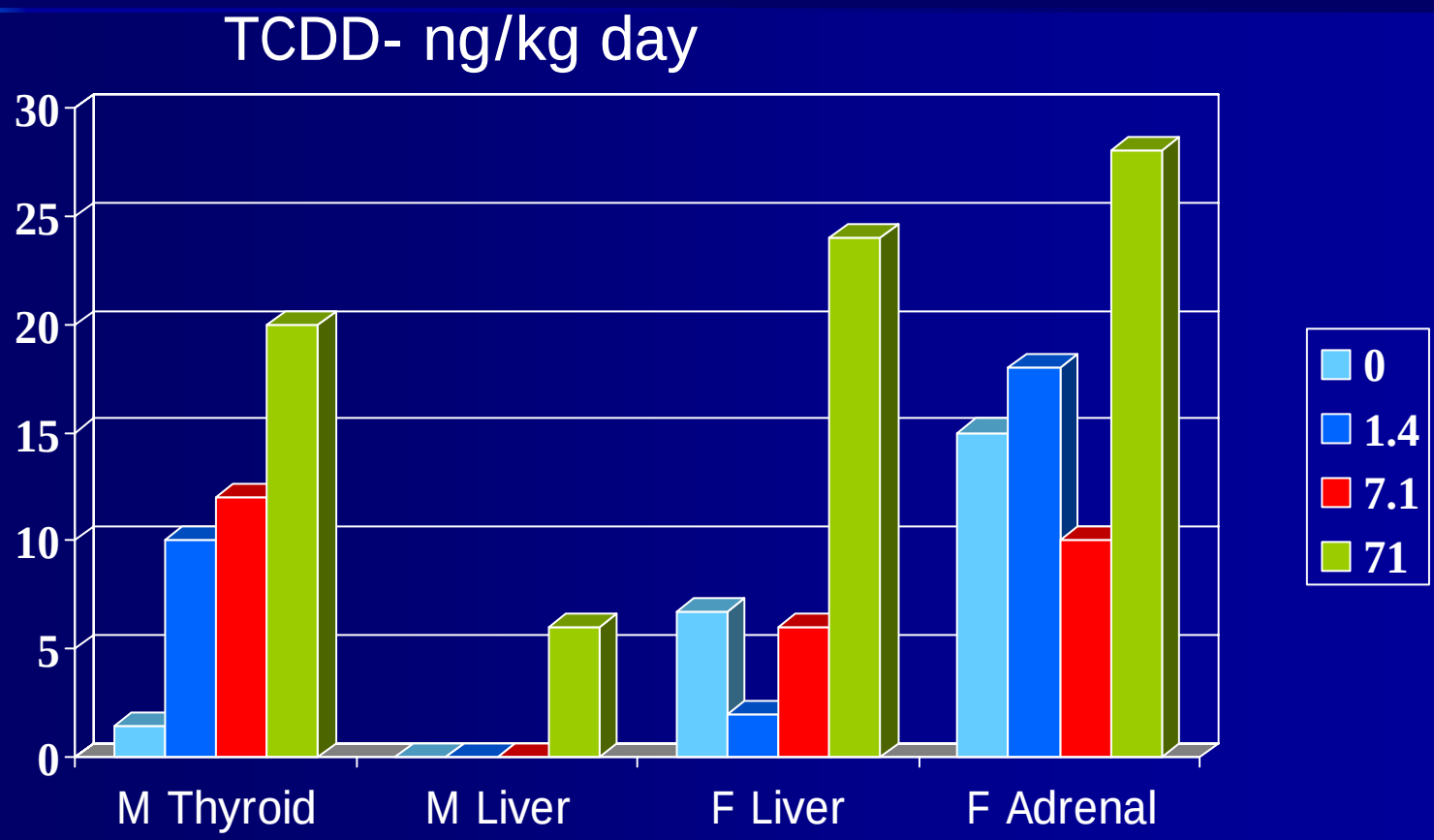
Viktor Yuschenko
Ukrainian president

26 ng/g blood fat

Normal population ~20 pg/ g blood fat

144 ng/g blood fat

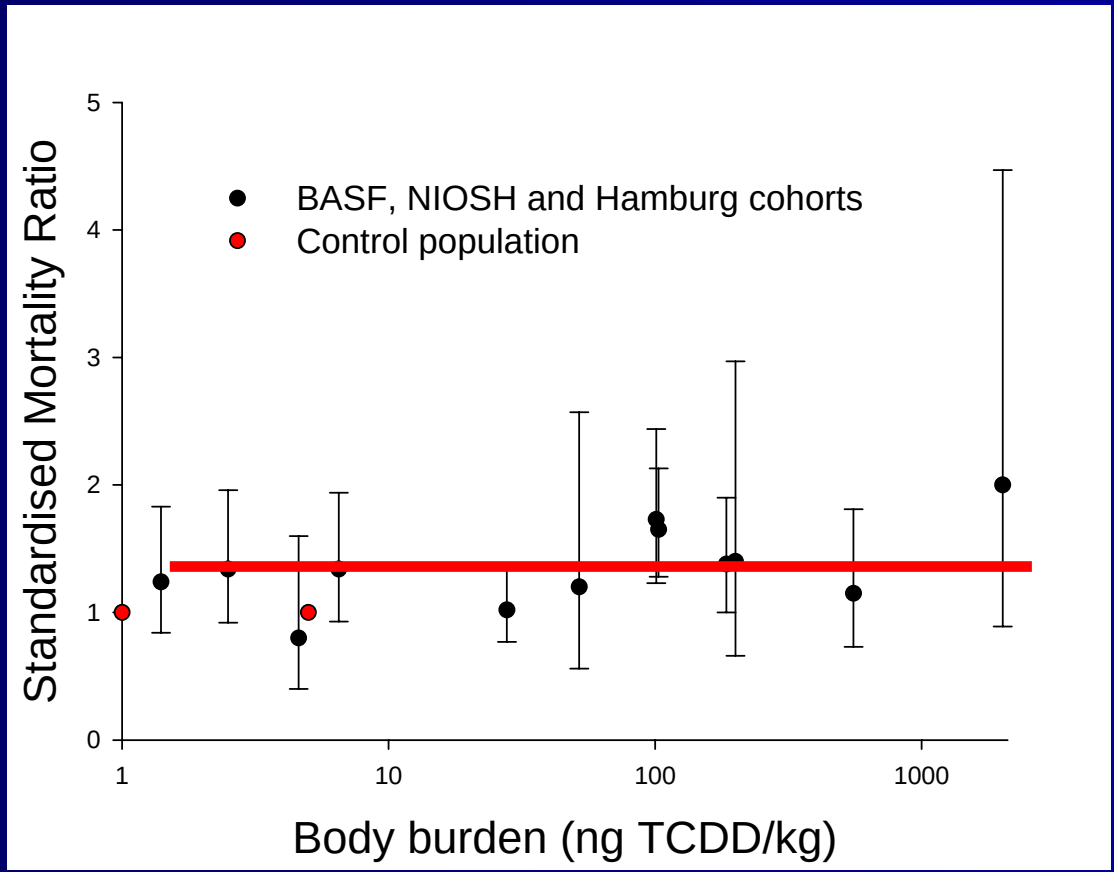
Tumour incidence in rats



Dioxin and human cancer

- Workers get chloracne
  - Reduce exposure !
- Analysis done decades after exposure
  - How to accurately estimate dose ?
  - Dose-dependent excretion
- Confounders
  - Exposure to other chemicals, smoking, etc.

Dioxin and human cancer



Dioxin and human cancer

- IARC regards dioxin as a known human carcinogen
  - Some degree of controversy !

UK Risk Assessment

- UK
  - Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT)
  - http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2001/dioxinsstate
    - Human epidemiology data inadequate for a risk assessment
  - Define most sensitive animal model of dioxin toxicity
    - Developmental effects on F_1 male sperm counts
  - Extrapolate to human on the basis of body burden of TCDD
    - Some uncertainty in rat foetal body burden of TCDD

Male Reprotox Endpoint

Dose pregnant rat

Fetus is target organ
Measure TCDD concentration

Male offspring

Day 49, ca. puberty

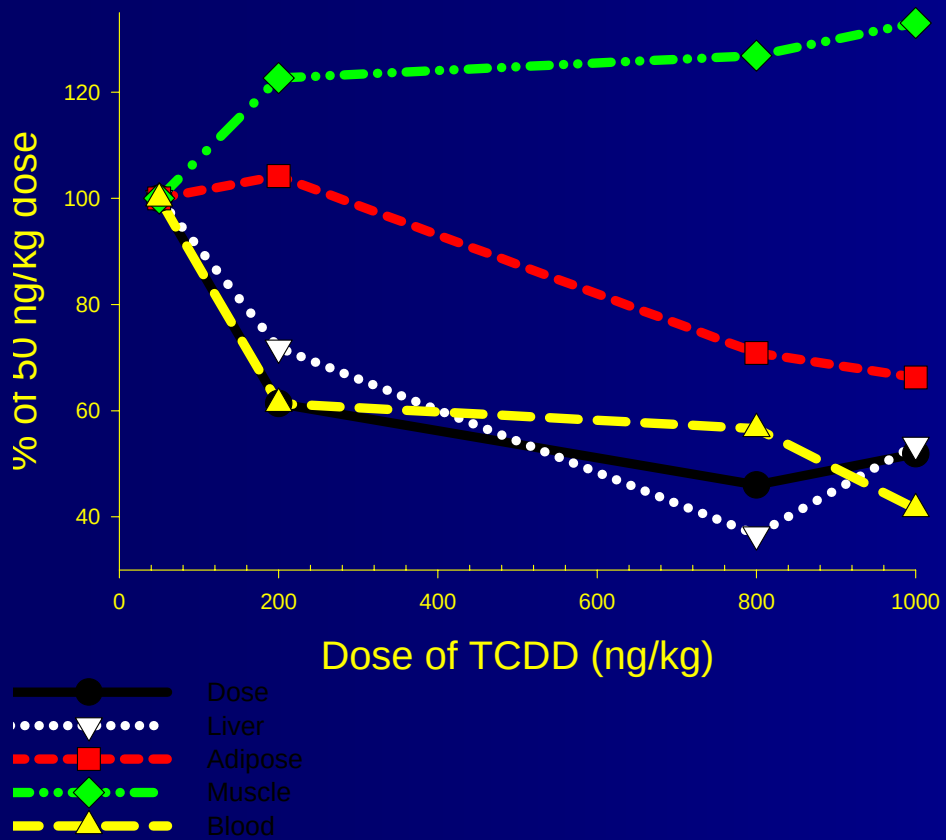
Day 63-90-120

Day 450

- Sperm production
- Epididymis size/ sperm no.
- Testis histology
- Sexual behaviour

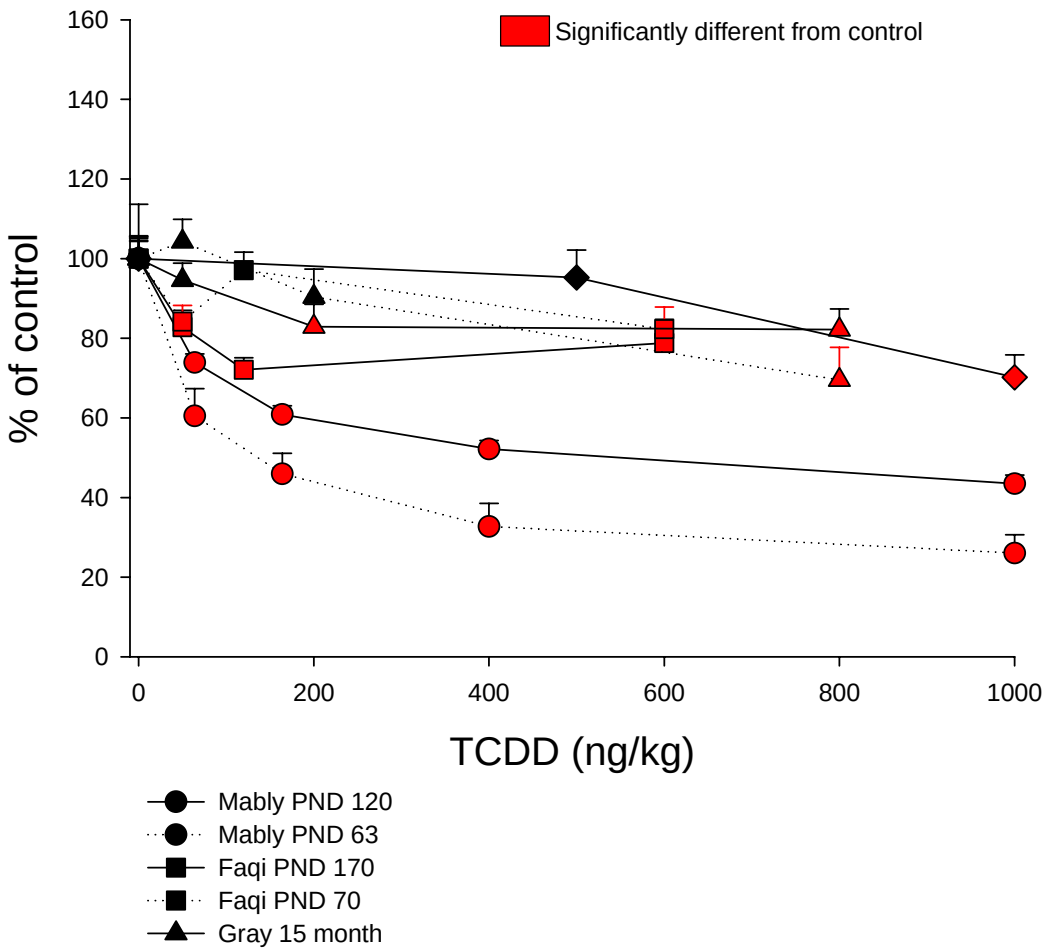
[^{3}H]-TCDD in tissue

Non-linearity of fetal TCDD dose: acute dose study



- % of TCDD measured in foetus varies as a function of dose
- [^{3}H]-TCDD is limited methodology
- 200-300% variability in foetal burden estimates

Effect of TCDD on sperm



Pre-2000 data

- Potent effect
- Low variation in sperm counts with small *n*
- Effect ~two-fold
- Flat dose-response

Rat TCDD reprotox

	Epididymal weight	Cauda Epid. Weight	Sperm prodn.	Cauda sperm	Sexual behaviour
Mably 92 64ng/kg day 40-120 Holtzman	+	+	+	+	+ 200
Faqi 98 25/5ng/kg day70/170 Wistar	-		+	+	+
Gray 97 200 ng/kg day 450 Long Evans	+	+	-	+	
Gray 97 50 ng/kg day 450 Long Evans	-	-	-	-	
Gray 97 200 ng/kg day 49-63 Long Evans	-	-	-	-	
Wilker 96 500 ng/kg day 62 SD	-		-	-	

Experimental design 1

- *In vivo* experiments to GLP
  - Experienced commercial contractor
- TCDD measurements to UKAS accreditation with HR GC-MS
- Improved and robust methodology
- Robust experimental design
  - ca. 25 dams per group
  - ca. 75 F_1 males for analysis

Experimental design II
Acute dose study

Doses of 0, 50, 200 and 1000 ng TCDD/ kg by oral gavage
Rats are Crl Wistar Han

Dose pregnant rats on GD15

GD16- **Kill**

GD21- **Kill**

Measure TCDD in
- Adipose
- Blood
- Foetus
- Liver

n=5 samples/ group

Allow ~25 rats per group to litter
Retain 5 males/ litter

Post Natal Day (PND) 70- **Kill 25 rats/ group**

Post Natal Day (PND) 120- **Kill ~60 rats/ group**

Necropsy
Seminology

TCDD analysis 1

- Sensitive and specific analysis in control tissues
- Control values ~1% of the values in the 50 ng/kg dose group at GD 16

Animal data 1

- 1000 ng TCDD kg^{-1} group
  - 4/ 20 females had total litter loss
- 1000 ng TCDD kg^{-1} represents a frankly toxic dose

Animal data 2

- Seminology at PND 120
  - Epididymal sperm counts raised at 200 and 1000 ng kg^{-1} *

Animal data 3

- 50/ 200 ng TCDD kg^{-1} groups PND120
  - 200 ng kg^{-1} group
    - Transient decrease in body weight
    - Delay in balano-preputial separation
  - Liver weights up by ~3% vs control, no dose response

Chronic study

- Administer dioxin in the food
  - Better representation of human exposure
- Three months exposure to load the animals with dioxin, before allowing animals to mate

Summary 1

- Concurrent measurement of TCDD in tissue samples
- A single oral dose of 1000 ng TCDD kg^{-1} is toxic
  - Decreased pup numbers, changed organ weights, some subtle effects
- 200 ng TCDD kg^{-1} is the LOAEL
  - Transient decreased weight, delayed BPS
- 50 ng TCDD kg^{-1} is the NOEL

Summary 2

- The developmental reproductive effects of TCDD are the basis of regulatory advice:
  - UK CoT http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2001/dioxinsstate
  - EU Scientific Committee on Food http://europa.eu.int/comm/food/fs/sc/scf/outcome_en.html
  - WHO JECFA http://www.who.int/pcs/jecfa/jecfa.htm
- Since 2000, there are 5 studies and our results which fail to show a low-dose effect of TCDD on development of male reproductive tract

Thanks

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