STUDIES IN EXPERIMENTAL ANIMALS

CONTAM Opinion on PCDD/Fs and DL-PCBs in food and feed

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Chair WG Dioxins in food

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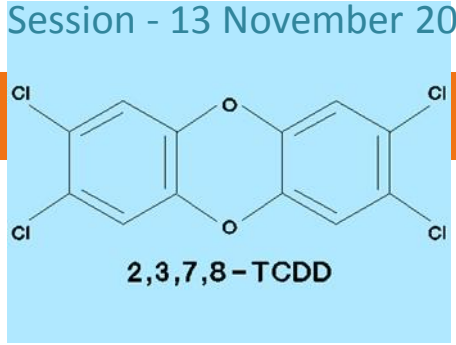
efsa
European Food Safety Authority

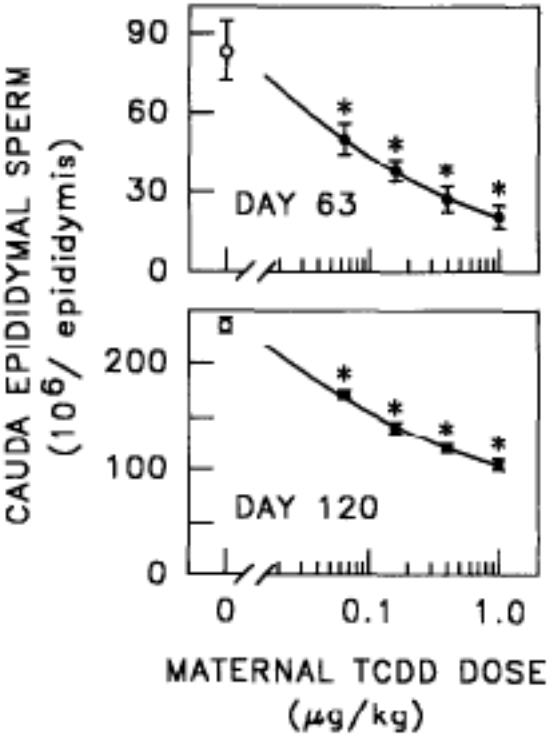
WHO (1998)

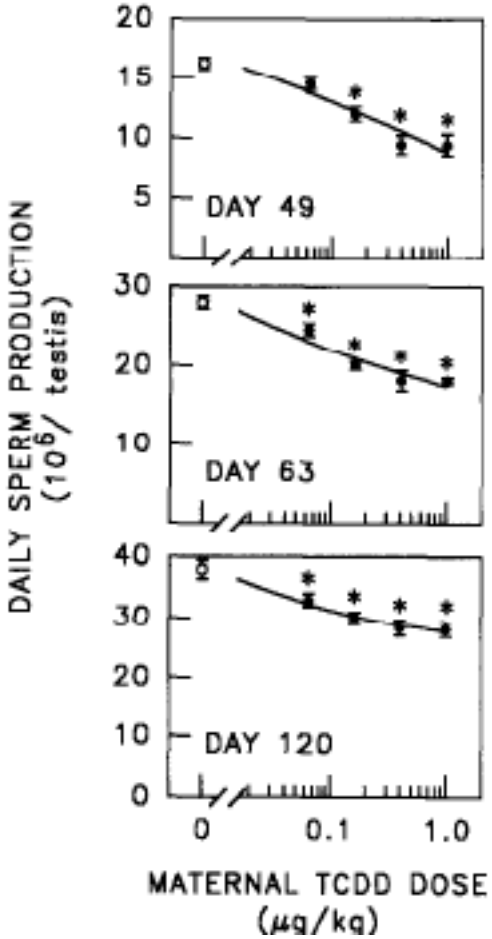


- Focus on effects TCDD in animals
  - Applying body burden concept
  - Evaluate at which body burden adverse effects occur
- Effects observed at low dose
  - Endometriosis in monkeys
  - Neurobehavioral effects in monkeys
  - Immune suppression in offspring rats
  - Decreased sperm count in male offspring of rats
- Effects in animals at body burdens of 30-70 ng/kg bw
- **TDI of 1-4 pg/kg bw/day**

Effects in male offspring (Mably et al. 1992)

- Reduced sperm production in male offspring of treated dams (single dose GD 15)
- Also decreased weights testis, epididymis and cauda epididymis
- Results imply prenatal or early postnatal effects





SCF (2001) Selected studies

Study	Endpoint	NOAEL	LOAEL	Estimated maternal steady state body burden[a] (ng/kg bw)	Associated EHDI [d] (pg/kg bw per day)
Mably et al. (1992a)	Holzman rats: Decreased sperm count in male offspring	-	64 ng/kg bw single bolus dose by gavage	100 [b]	50
Gray et al. (1997a)	Long Evans rats: Accelerated eye opening and decreased sperm count in male offspring	-	50 ng/kg bw single bolus dose by gavage	80 [b]	40
Faqi et al. (1998)	Wistar rats: Decreased sperm production and altered sexual behaviour in male offspring	-	Maintenance of 25 ng/kg bw by s.c. injections	40 [b]	20*
Ohsako et al. (2001)	Holzman rats: Decreased anogenital distance in male offspring	12.5 ng/kg bw single bolus dose by gavage	-	20 [c]	10
		-	50 ng/kg bw single bolus dose by gavage	80 [c]	40

SCF (2001)

- Also applying body burden concept
- Two studies selected
  - Decreased anogenital distance in male offspring, implying effects on external genital organs (Ohsako et al., 2001)
  - Decreased sperm concentrations (Faqi et al. 1998)
- TWI of 14 pg/kg bw/week based on EDHI of 20 pg/kg bw/week and UFs of 3 x 3.2
- JECFA (2001): TMI of 70 pg/kg bw/month
  - Same critical studies

EFSA 2018: selection of new animal studies

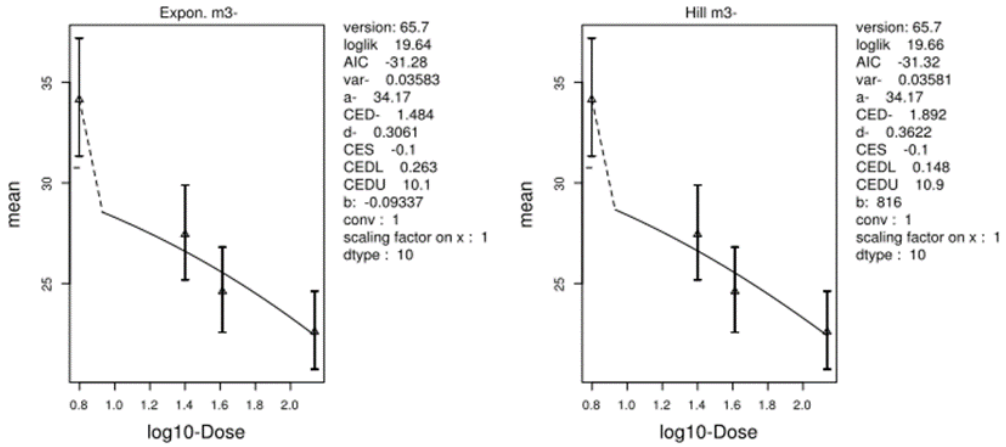
- Comprehensive review of animal studies
- Focus on studies with effects at TCDD body burdens lower than used by SCF (2001)
  - Body burden estimated or preferentially measured
  - Initially selection of studies including BB <100 ng/kg bw
  - Estimate LOAEL/NOAEL BB; if lower than Faqi/Ohsako:
  - Appraise study
- At later stage decision to focus on TCDD only
  - Effect at lower TEQ for other congener would question TEF
- Most sensitive endpoint: sperm effects observed by Faqi et al. (1998)

Faqi et al. 1998 (critical study SCF, 2001)

- Mother dams injected s.c. with:
  - 0, 25, 60 or 300 ng TCDD/kg bw, 2 weeks prior to mating
  - followed by weekly maintenance bolus doses of, respectively, 5, 12 or 60 ng/kg bw
  - Estimated BB mothers: 0, 25, 41 and 137 ng/kg bw

- Effects on sperm in offspring at PND 70 and 170
  - Lowest dose shows effect: LOAEL BB 25 ng/kg bw
  - BMD modelling not successful

Faqi et al. (1998) BMD modelling (Appendix C)

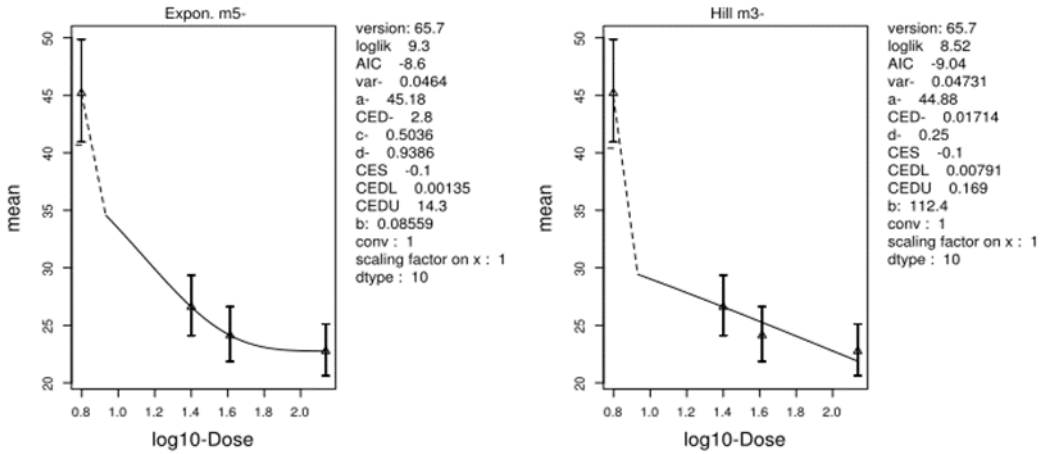


PND 70

subgroup	bmdl.lowest	bmdu.highest
all	0.148	10.9

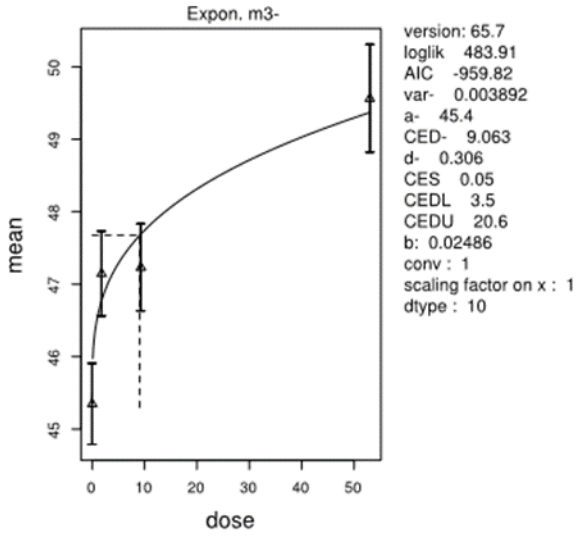
PND 170

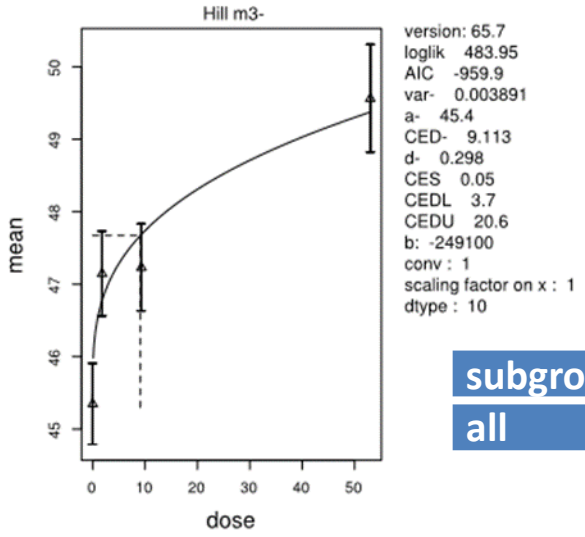
subgroup	bmdl.lowest	bmdu.highest
all	0.00135	14.3



Bell et al. (2007)

- Sperm effects not observed by Bell et al. (2007), but delayed balano-preputial separation (implying delayed puberty onset)
- $BMDL_5$ of 3.5 ng/kg bw/d on GD16/21, corresponding to BBs of 51/61 ng/kg bw



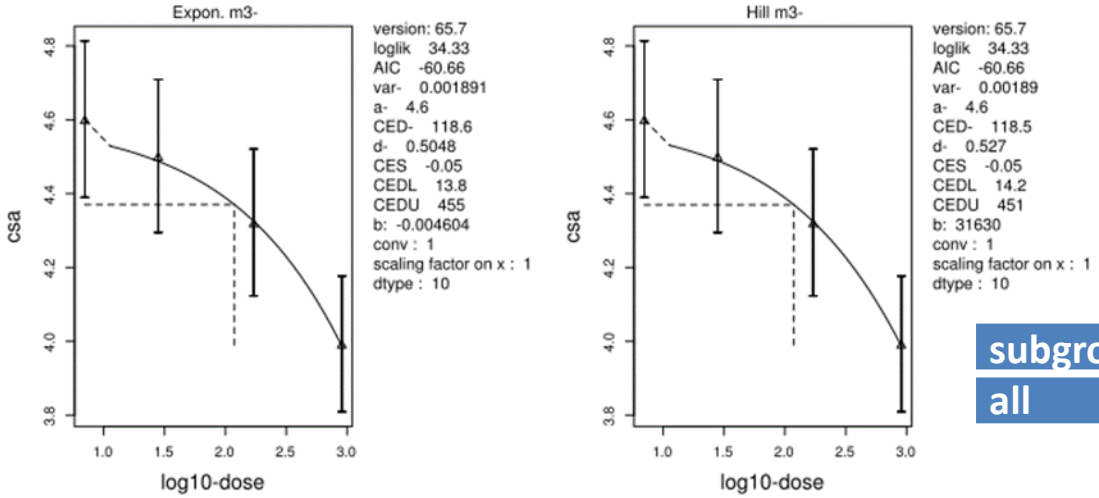


subgroup	bmdl.lowest	bmdu.highest
all	3.5	20.6

Bone effects (Jämsä et al. 2001)

- Female Long-Evans rats, treated for 20 weeks with loading and maintenance dose
  - BB of 0, 28, 171 and 904 ng/kg bw
  - Various effects on bones

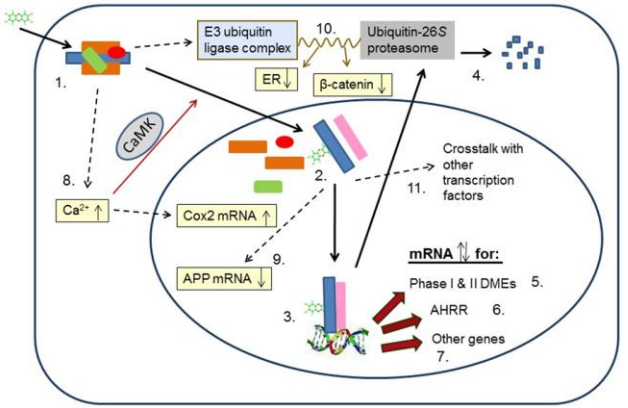
- Most sensitive effect: tibial cross-sectional area of cortex (CSA): $BMDL_5$ of 13.8 ng/kg bw



subgroup	bmdl.lowest	bmdu.highest
all	13.8	455

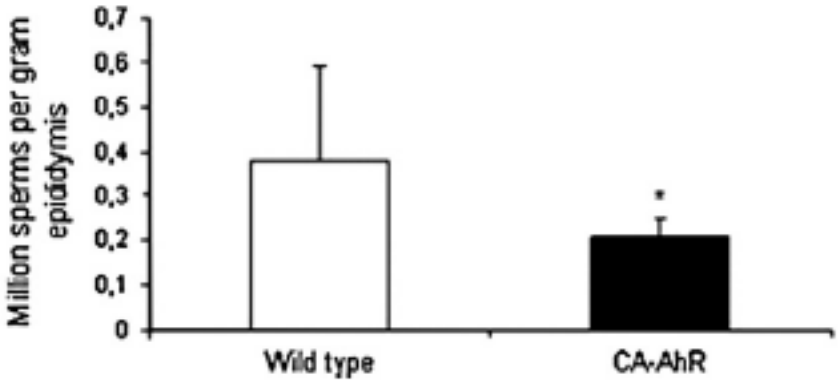
MODE OF ACTION

- The molecular initiating event behind most if not all effects of PCDD/Fs and DL-PCBs considered to be the activation of the arylhydrocarbon receptor (AHR) pathway
- This is followed by the expression of a selected number of genes (AHR battery of genes), many involved in the metabolism of endogenous compounds but also xenobiotics
- Less clear how this evolves into the various effects observed in animals and humans
- Natural ligand (hormone) not yet identified



Effects in mice constitutively expressing AHR

- Mouse show effects like:
  - Hepatomegaly
  - thymus atrophy
  - decreased bone stiffness in female mice
  - altered size of B lymphocyte subpopulations
- But also:
  - Reduced weight of testis and ventral prostate (by 12% and 33%)
  - Reduced epididymal sperm count, by 45% (Brunberg et al. 2011)



CONCLUSIONS

- Various effects in animals
  - Effects on sperm, delayed puberty onset (balano-preputial separation), bone development
- Most sensitive effect on sperm concentrations,
  - Faqi et al. (1998) with LOAEL BB of 25 ng/kg bw
  - Supports effects observed in humans