National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Developmental effects versus negligible exposure

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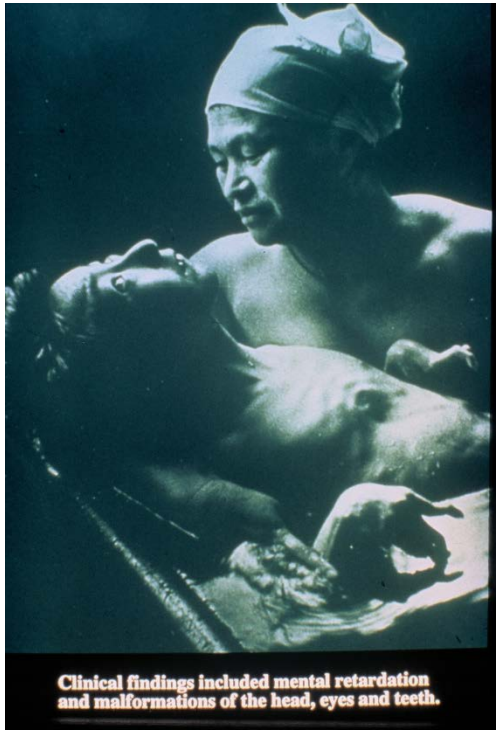
Berlin Workshop 140514

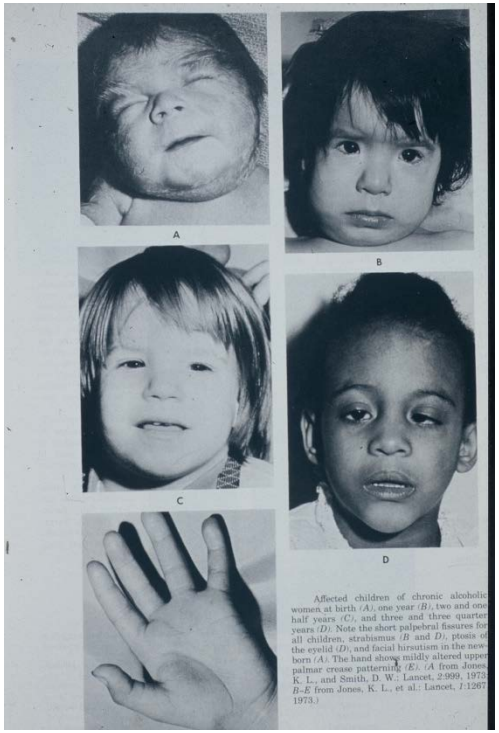
Dedicated to the Prevention of Birth Defects

methyl mercury



thalidomide

alcohol



ELSEVIER

Food and Chemical Toxicology 42 (2004) 65–83

www.elsevier.com/locate/foodchemtox

Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet

R. Kroes[a], A.G. Renwick[b], M. Cheeseman[c], J. Kleiner[d,*], I. Mangelsdorf[e], A. Piersma[f], B. Schilter[g], J. Schlatter[h], F. van Schothorst[e], J.G. Vos[f], G Würtzen[i]

- The NOELs for teratogenicity in most cases were considerably greater than 3 mg/kg. Therefore, the existing TTCs would be lower than any threshold related to teratogenicity. On the basis of a NOAEL analysis of 50 compounds the Expert Group decided that consideration of a separate class of teratogens would not be necessary.

Kroes et al., 2004: consideration of endocrine disrupting chemicals

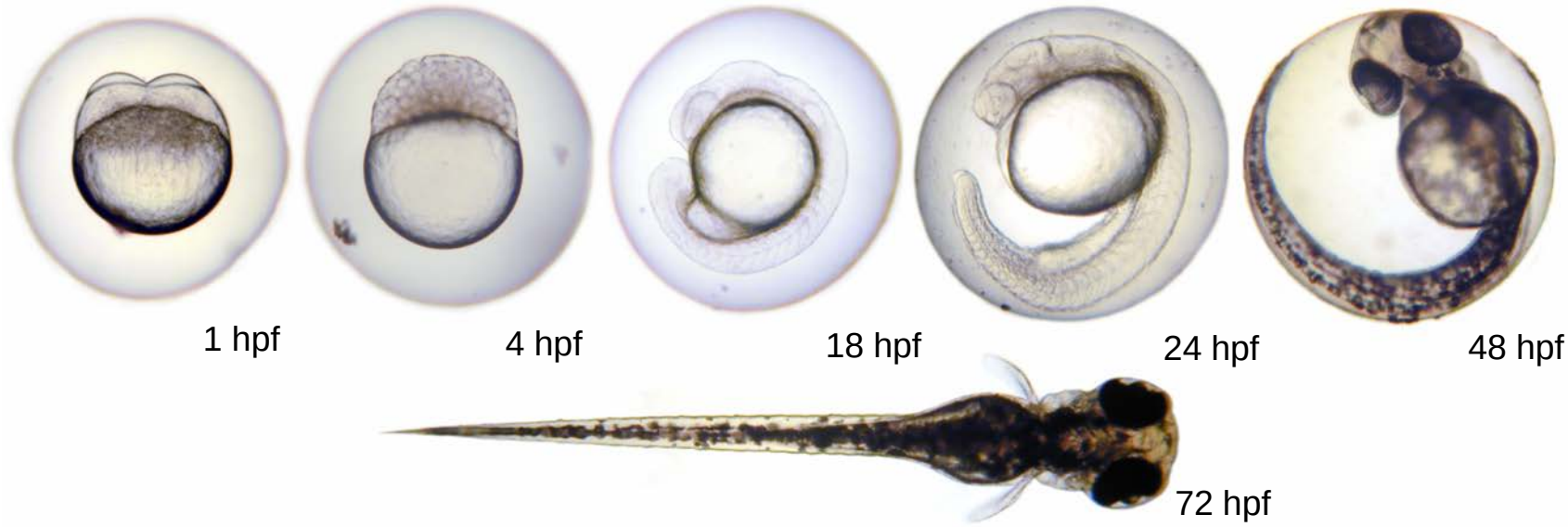
- Low-dose effects were demonstrated in laboratory animals exposed to certain endocrine active agents but the effects were dependent on the compound studied and the endpoint measured. In some cases where low-dose effects have been reported, the findings have not been replicated. The validity and toxicological significance of many of these latter observations has not been determined.
- The Low-Dose Peer Review Panel recommended additional research to replicate previously reported key low-dose findings, to characterise target tissue dosimetry during critical periods of development, to identify sensitive molecular markers that would be useful in understanding mechanistic events associated with low-dose effects, and to determine the long-term health consequences of low-dose effects of endocrine active agents.
- The findings of the panel indicate that the current testing paradigm used for assessments of reproductive and developmental toxicity should be revisited to see if changes are needed regarding dose selection, animal model selection, age when animals are evaluated, and the endpoints being measured following exposure to endocrine active agents.

Some observations - 1

- Classical regulatory toxicology is largely based on effects that are generally considered adverse.

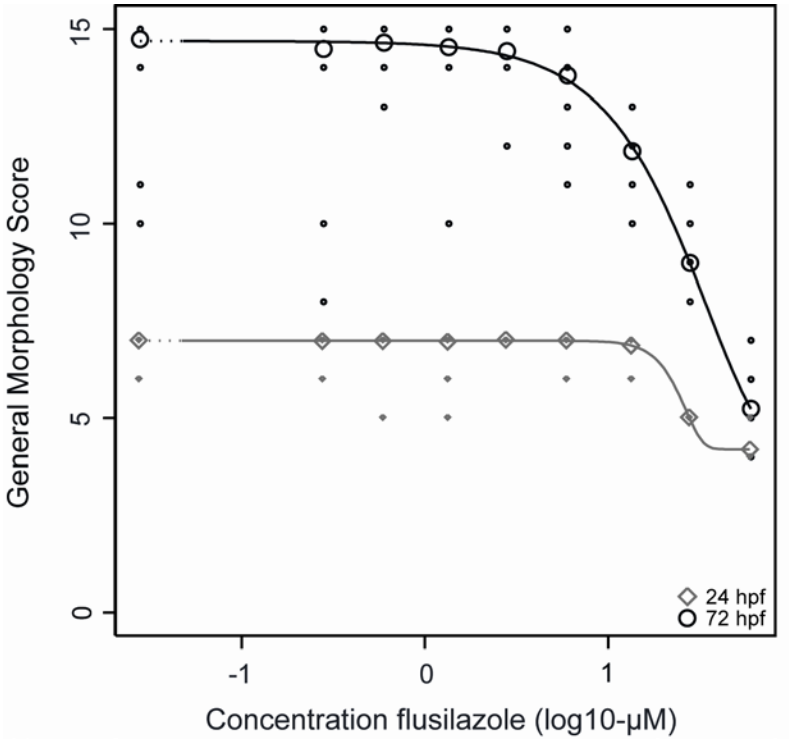
  – Death, body and organ weight, clinical signs etc.
- We are now able to detect a large variety of subtle changes in gene expression and biochemical parameters that may be adaptive or may indicate an adverse effect.
- How do we discriminate adaptive physiological changes from adverse health effects?

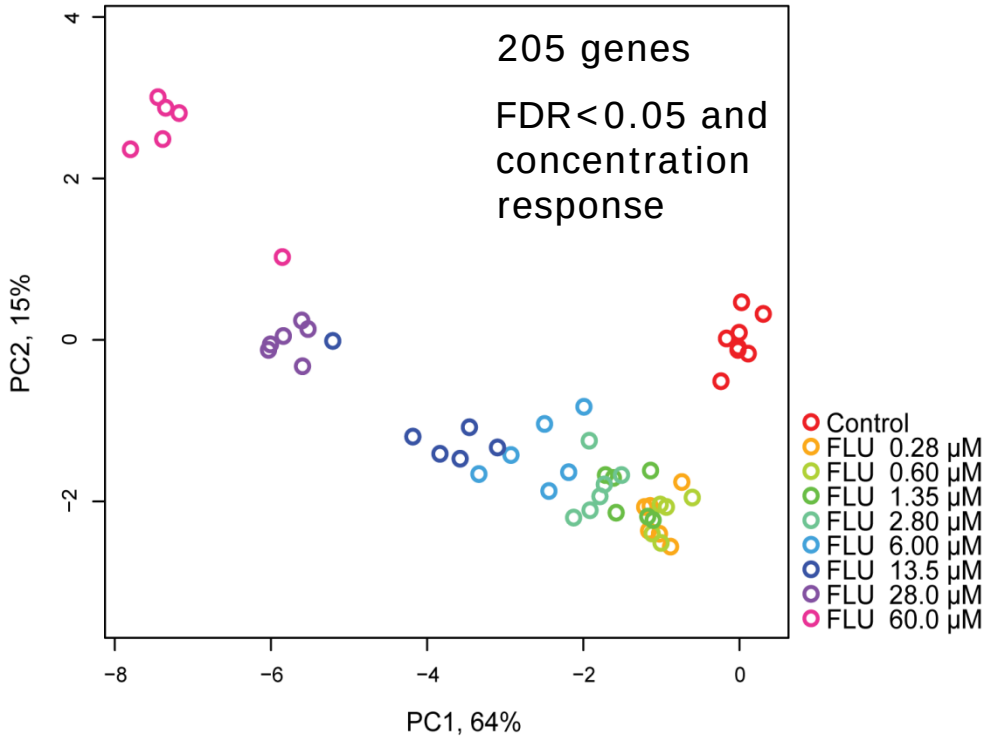
Zebrafish Embryotoxicity Test (ZET)



Hermsen et al., 2011

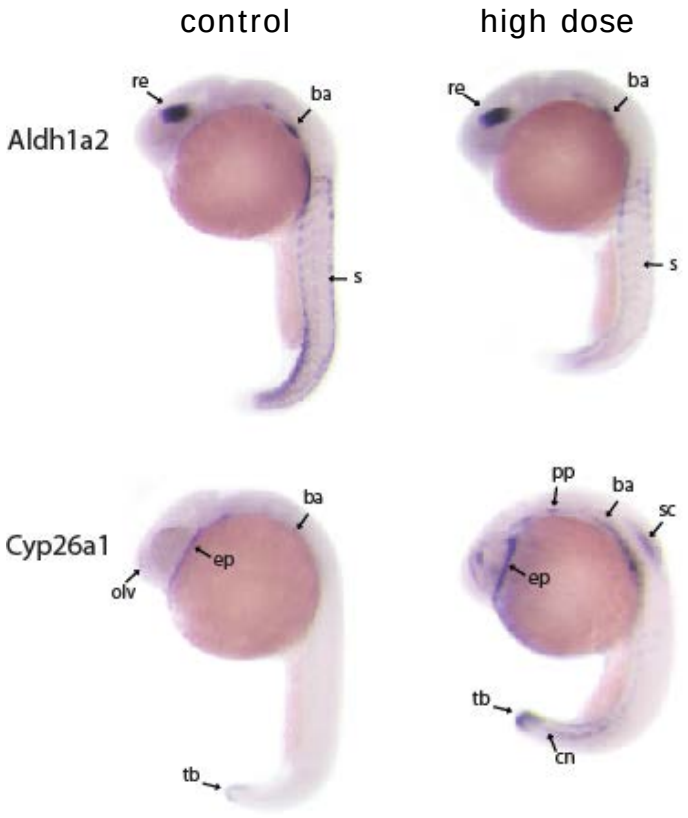
Principal Component Analysis - Flusilazole

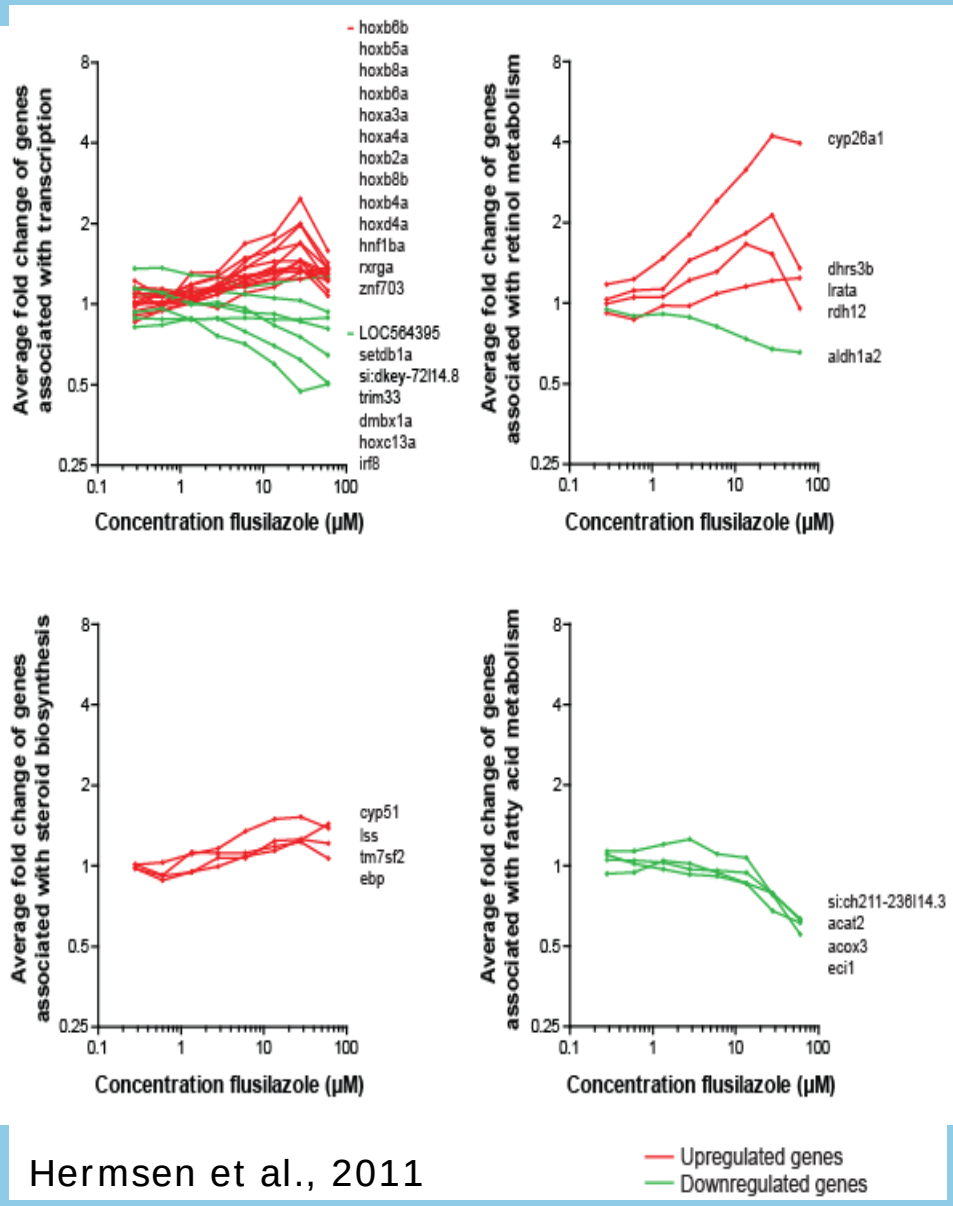




Hermsen et al., 2011

Selected gene set regulation - Flusilazole





Hermsen et al., 2011

Some observations - 2

- The organism has homeostatic mechanisms that enable compensation of effects of xenobiotic exposures precluding an adverse health effect to occur.
- Xenobiotic effects within the homeostatic range are not adverse per se.
- The threshold of adversity is crossed when homeostasis is overwhelmed, and adverse effects are then possible.

Homeostasis through feedback loop mechanisms

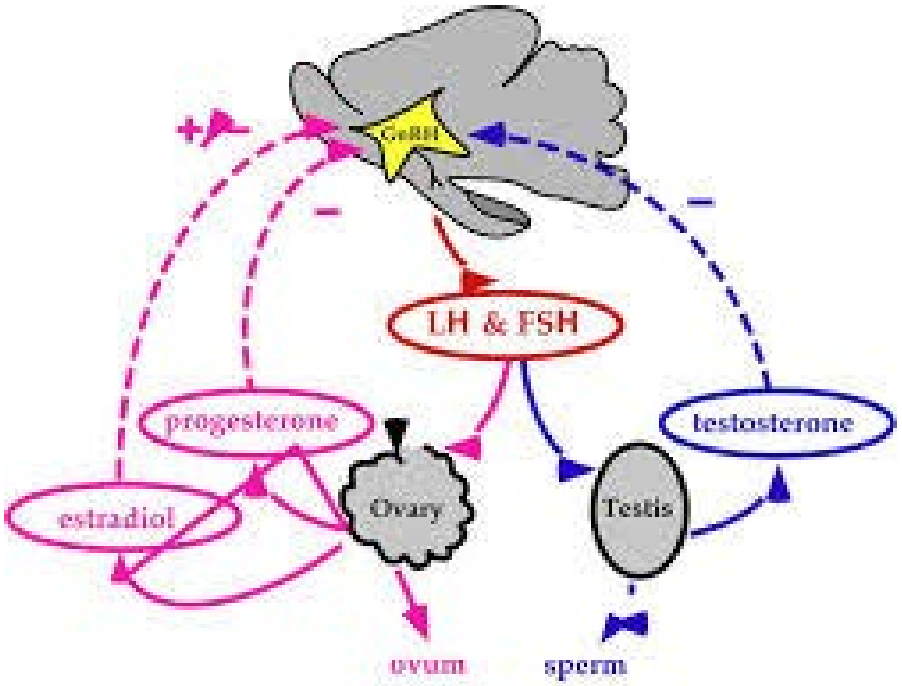


Figure 3

Critical Reviews in Toxicology, 2011; 41(6): 545–554

ISSN 1040-8444 print/ISSN 1547-6898 online
DOI: 10.3109/10408444.2011.554794

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REVIEW ARTICLE

Reproductive toxicants have a threshold of adversity

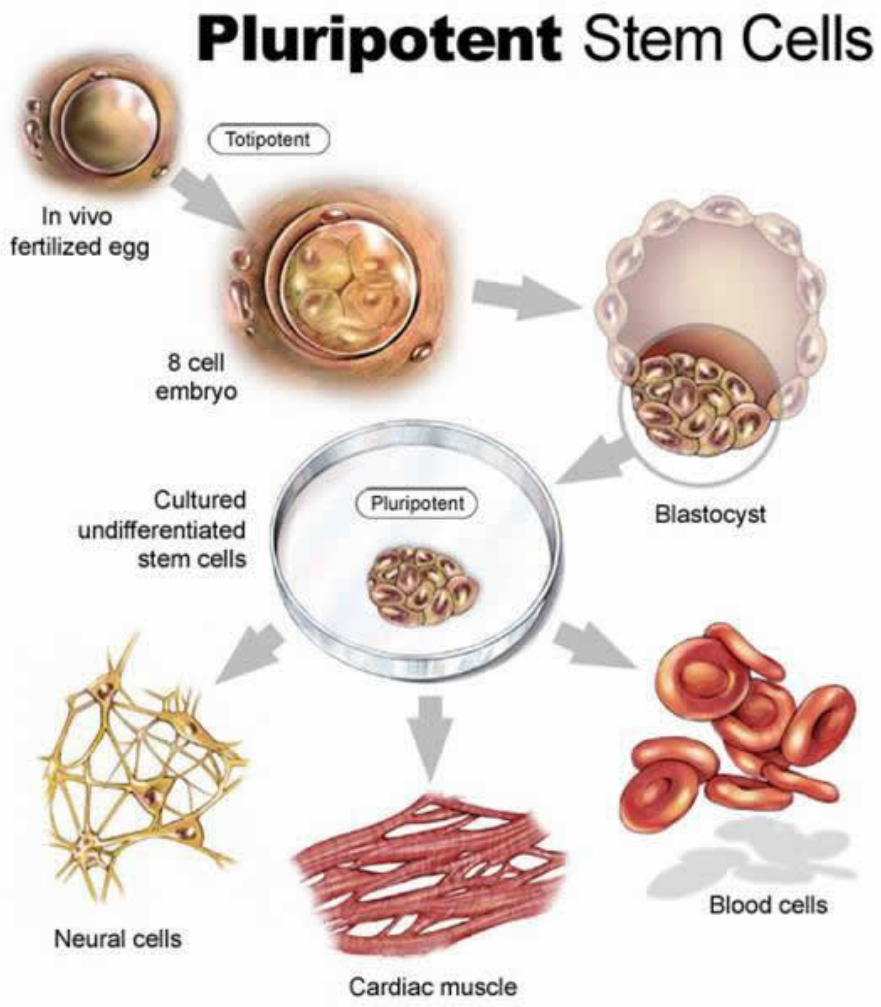
Aldert H. Piersma,[1,4] Lya G. Hernandez,[1] Jan van Benthem,[1] J. J. Andre Muller,[2] F.X. Rolaf van Leeuwen,[2] Theo G. Vermeire,[3] and Marcel T. M. van Raaij[2]

Taken together, evidence from a variety of human teratogens supports the notion that the impact of exogenous chemical exposures is highly dose dependent and demonstrates that low-dose exposure can often be nonadverse, suggesting that up to a threshold of adversity, the body can effectively neutralize hazards through homeostatic mechanisms. A wealth of experience with thousands of chemicals evaluated in animal studies for reproductive hazard and risk identification corroborates this position. It is therefore justified to use the threshold dose approach in the risk assessment of reproductive toxicants.

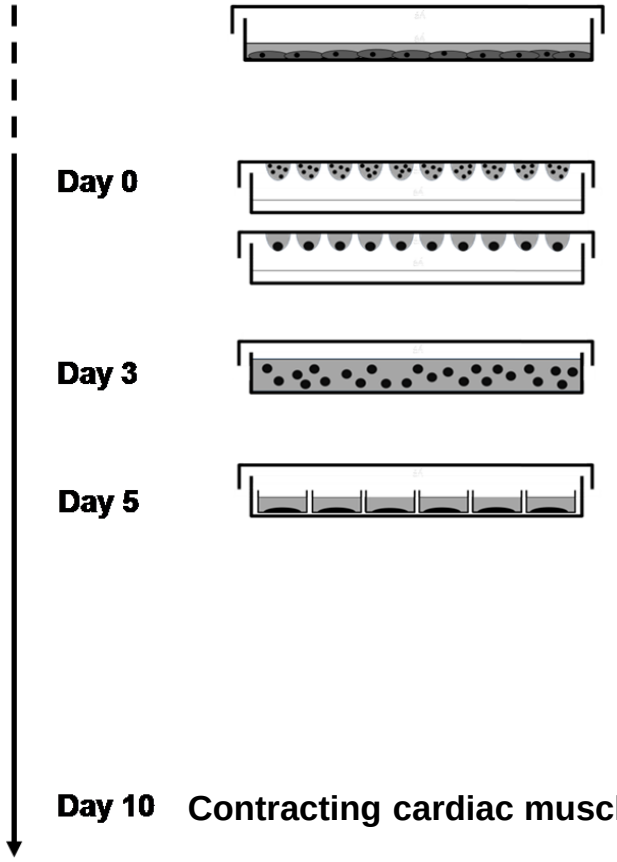
Some observations - 3

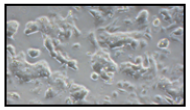
- We are now able to detect very low concentrations of xenobiotics in biological samples
- There is general concern about any exposure, secondary to the faulty assumption that hazard equals risk
- Paracelsus is forgotten (‘All compounds are poisons, it is the dose that makes the compound not a poison.’)

Pluripotent embryonic stem cells

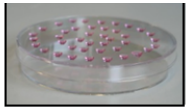


Embryonic Stem cell Test (EST)

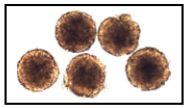




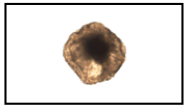
Embryonic stem cell culture



Hanging drop culture



Embryoid Bodies (day 3)



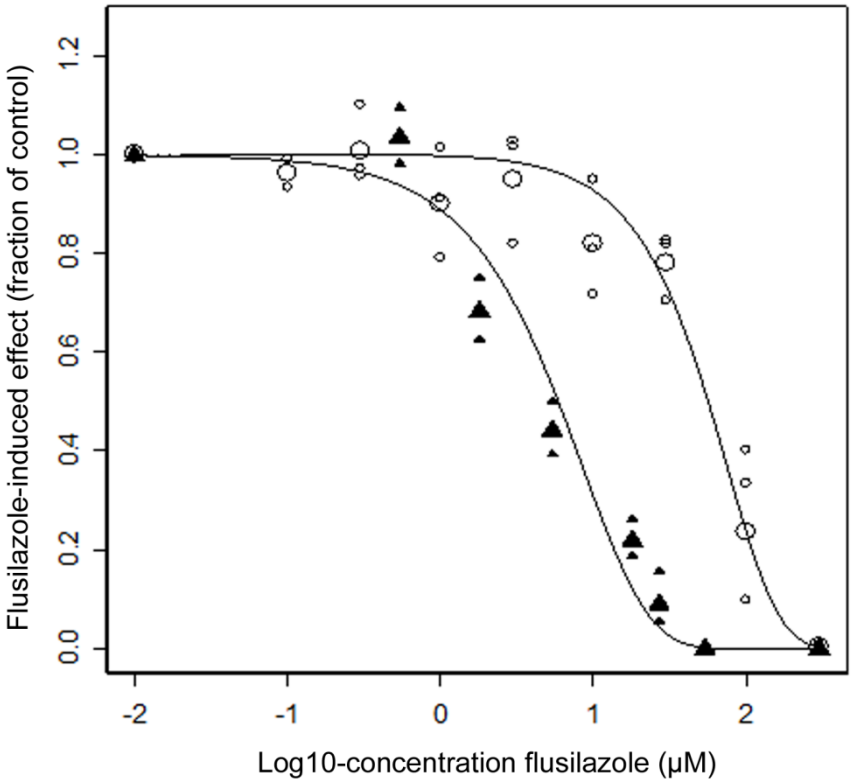
Embryoid Body (day 5)

Embryoid Body (day 10)

Van Dartel et al., 2009

Define threshold of adversity in vitro ?

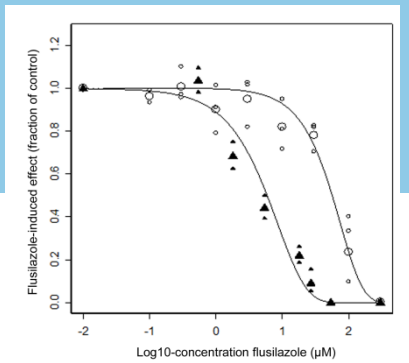
- Flusilazole dose-response
- EST classical dose-response on contracting muscle foci readout
- Differentiation more sensitive than proliferation
- Interpreted as indicative of a specific developmental toxicant

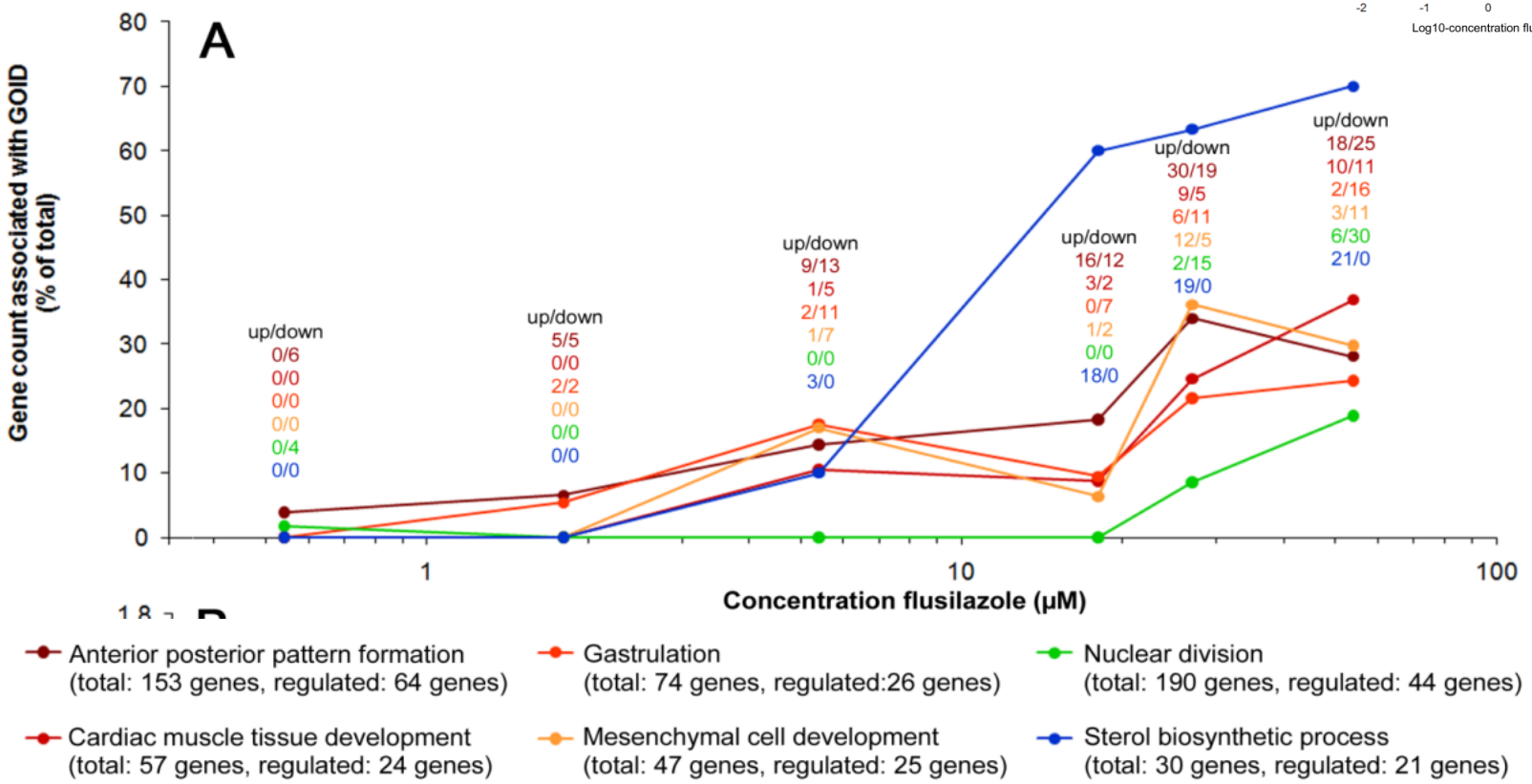


Concentration-response curves of ESC differentiation (▲) (n=2) and cell viability (○) (n=3) after flusilazole exposure.

Van Dartel et al., 2011

Flusilazole-induced GO term enrichment





Van Dartel et al., 2011

Some observations - 4

- Reported low-dose effects are often not reproducible.
- Study design and statistics are major issues of concern in many low dose studies.
- Findings from in vitro models lacking the homeostatic feedback mechanisms cannot be simply extrapolated to adversity and risk
- There is no time for reflection in view of the enormous amount of studies appearing continuously.

Developmental toxicity of butylbenzylphthalate

Exposure
GD6-15 (Δ)
GD6-20 (o)
Assessment
GD21

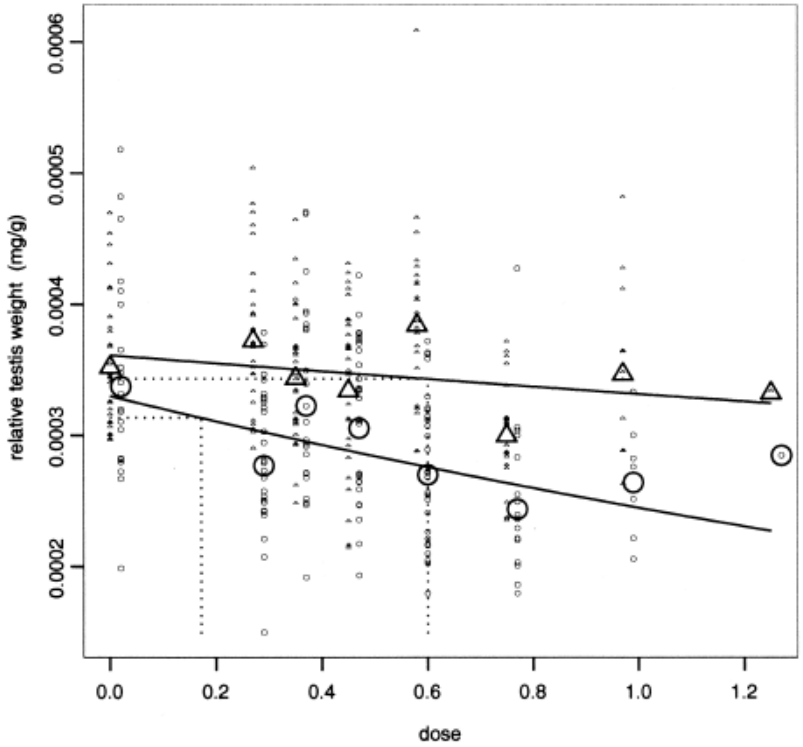
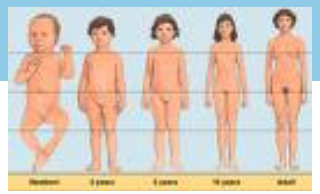


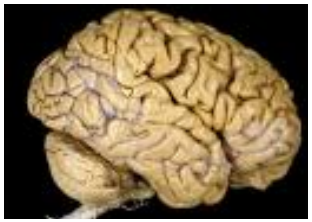
Fig. 12. Dose-response data and fitted model for relative fetal testis weight. For further explanation see legend of Fig. 1. Horizontal dashed line: 5% effect level.

Piersma et al., 2000

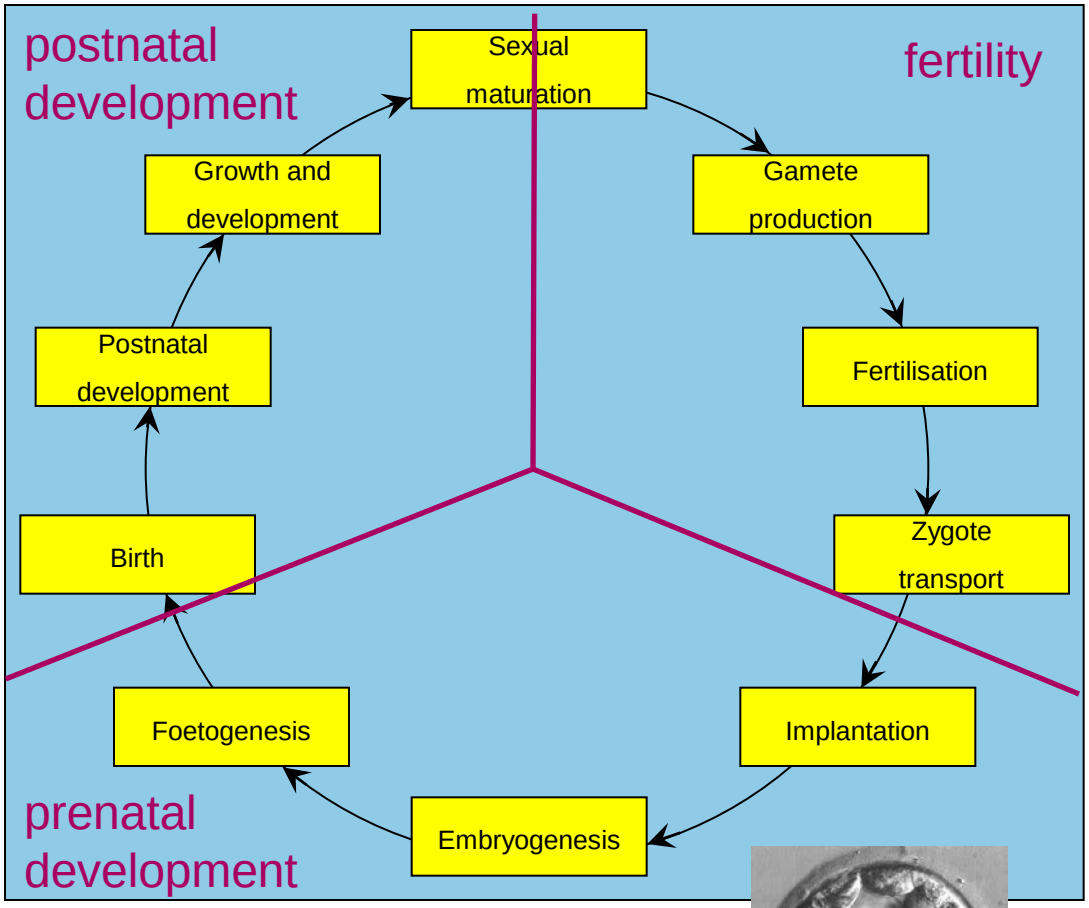
Needed

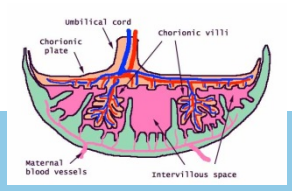
- Good quality studies
- Innovation of current hazard assessment tools
- Realistic interpretation of findings
  - Considering relevance of model (*pars pro toto*)
  - Considering adaptation vs. adversity
  - Considering compound potency
  - Considering human exposure levels
- Provide confidence instead of suspicion
- You cannot prove a negative, need to accept residual uncertainty





The Reproductive Cycle





Thank You

Dorien van Dartel

Sanne Hermsen

Esther de Jong

Ahmed Osman

Joshua Robinson

Sjors Schulpen

Peter Theunissen

Ilse Tonk

Aart Verhoef