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

Endocrine Disruption and Human Health Effects

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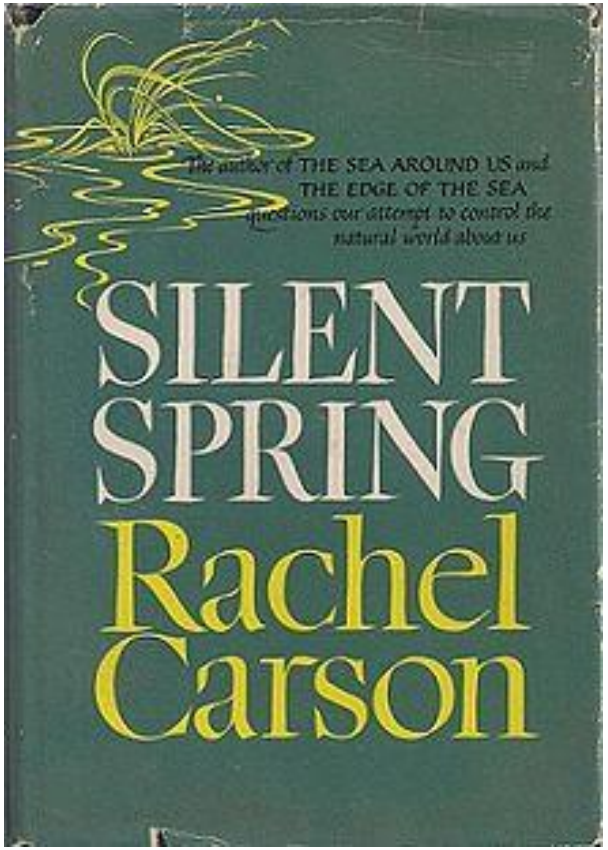
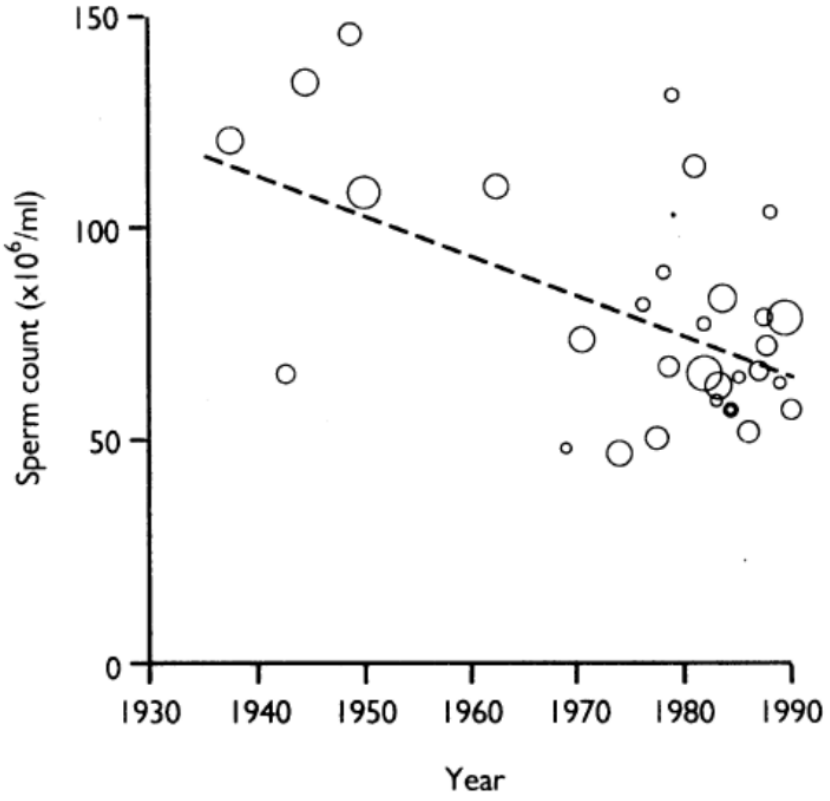
Silent Spring (1962)

- Environmental effects of pesticides, particularly DDT on birds.
- Prompted by USDA ant eradication program, with widespread areal spraying of agricultural areas with DDT and other pesticides in fuel oil.
- Suggested issues with reproduction.
- Carson introduced the term ‘biocides’, indicating that pesticides do not only kill pests but are detrimental to all life forms.
- Resulted in the creation of USEPA in 1970.



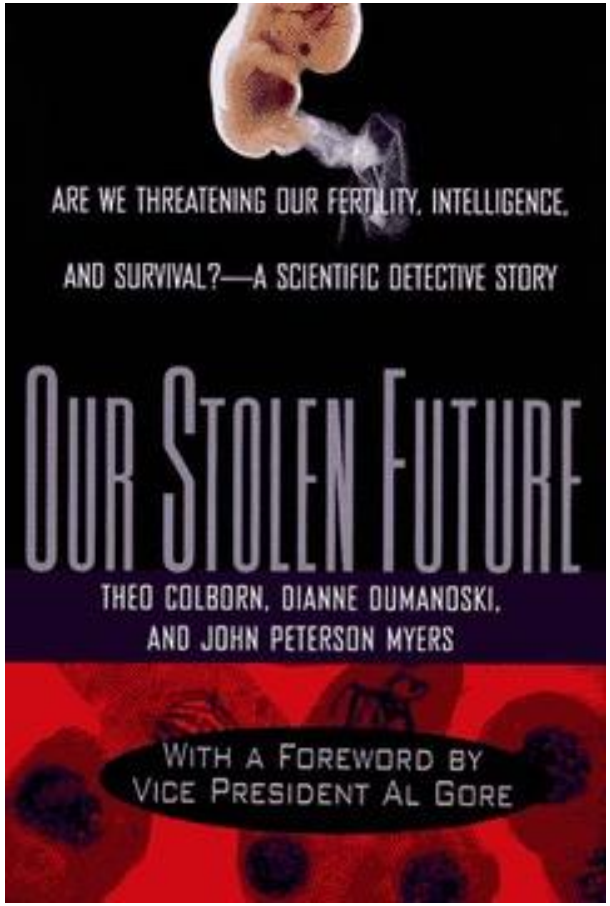
Carlsen and Skakkebaek (BMJ 1992)

- Human sperm count has declined during 20th century.
- Linear regression suggestive of zero sperm before 2100.
- Study quality questioned: different sampling and storage duration, different counting methods.
- Causation unclear – association with increase in chemical production.



Our Stolen Future (1996)

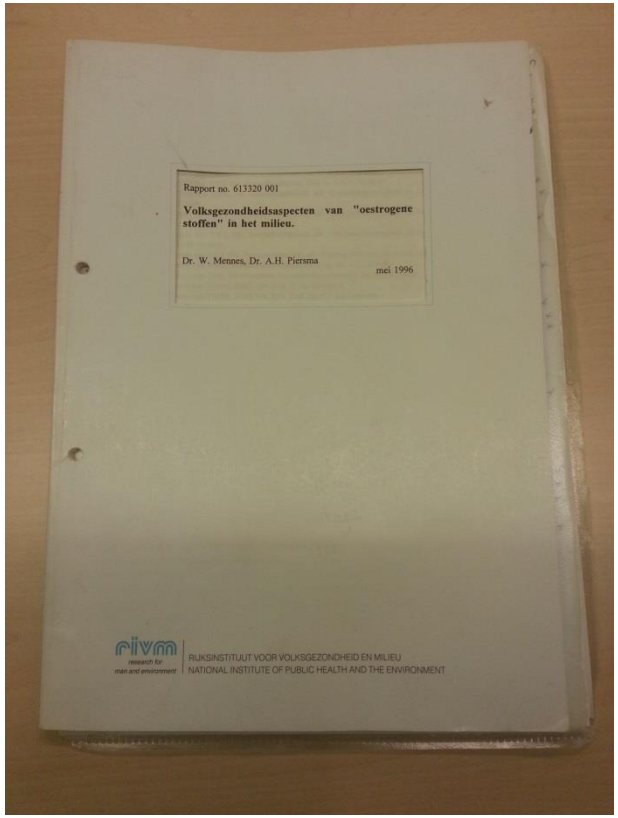
- Development of the Endocrine Disrupter Hypothesis.
- Contaminants might have been interfering with the hormonal control of development in wildlife and in people. Examples include Bald Eagles, Florida (1952); river otters, England (late '50s); mink, Michigan (mid-60's); Herring Gulls, Michigan (1970); alligators, Florida ('80s); seals, northern Europe (1988), dolphins, Mediterranean (early '90s), and sperm counts of men worldwide (1992).
- Includes DDT, DES, PCBs, nonylphenol, BPA
- Generated large research budgets, and spread beyond reproduction over all major public health issues, including e.g. obesity, diabetes, and neurodevelopment.



Mennes & Piersma (1996)

- Public health aspects of "estrogenic substances" in the environment

- Weighing the evidence for endocrine disruptor mediated human health effects (e.g. sperm quality, mammary and testicular cancer)
- No evidence for causal relationship
- Exogenous estrogen exposure is small as compared to endogenous and nutritional estrogen exposure
- Many confounding factors (life style, nutrition) may play a role
- More research needed on
  - the occurrence of health effect trends
  - causality of the association with exogenous endocrine exposures



Mennes & Piersma (1996)

- Compounds addressed in detail:

- Phytoestrogens
- Organochlorine insecticides
- PCBs
- PCDDs/PCDFs
- Alkylphenols
- Bisphenol-A
- Chlortriazines
- Vinclozolin
- Phthalates

Bron	oestrogeen equivalenten (µg/d)
Exogene oestrogenen	
morning after pil[1]	333500
anticonceptie pil[1]	16675
post-menopauze therapie[1]	3350
bioflavonoïden	102
oestrogene organochloor-milieucontaminanten	0,0000025
Endogeen oestradiol-17β[2]	
Vrouwen:	
vroeg in cyclus	81
rondom ovulatie	445 - 945
laat in cyclus	270
Mannen	45
anti-oestrogenen	
organochloorverbindingen	0,00008 - 0,00012
PAKs in voeding	0,0012 - 0,005
Indol-3-carbazol	0,00025 - 0,00128

Weybridge 1996, WHO/IPCS 2002 definition

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

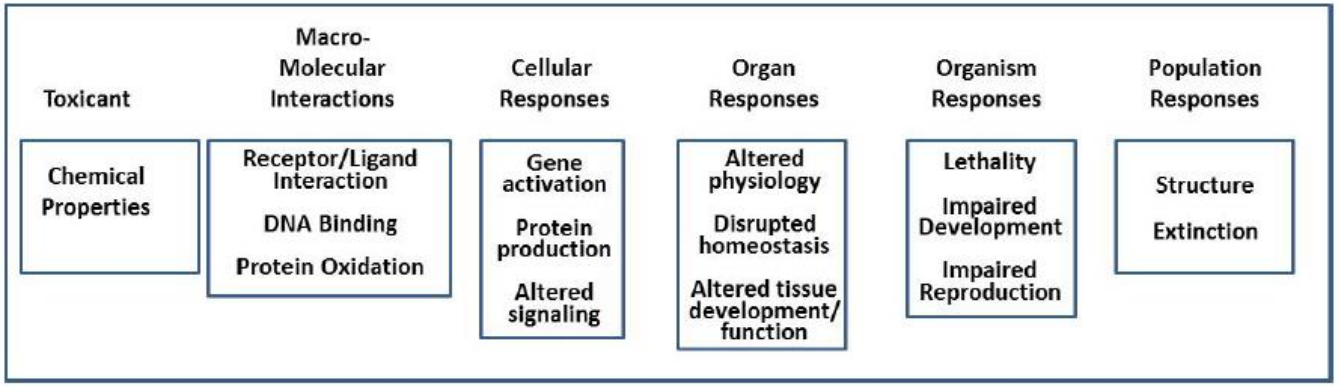
- Exogenous substance/mixture
- Alters function of the endocrine system
- Consequently causes
- Adverse health effects
- Intact organism (or progeny or (sub)-populations)

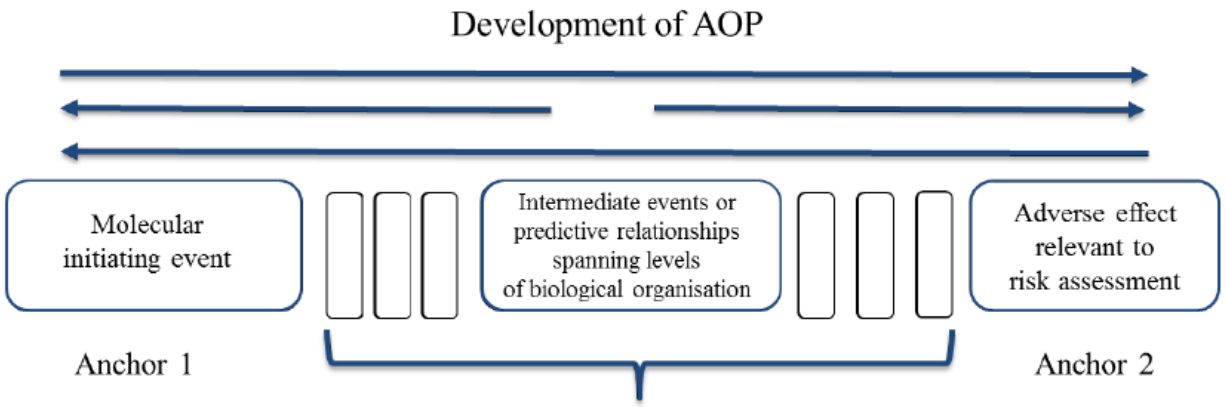
Issues with endocrine disruption

- Endocrine function versus adverse health effect
- Adaptive versus adverse
- Hazard versus risk
- Causality
- Low dose effects / Non-Monotonic Dose-Response
- Methods
- Novel end points

Endocrine function versus adverse health effect

Adverse Outcome Pathways

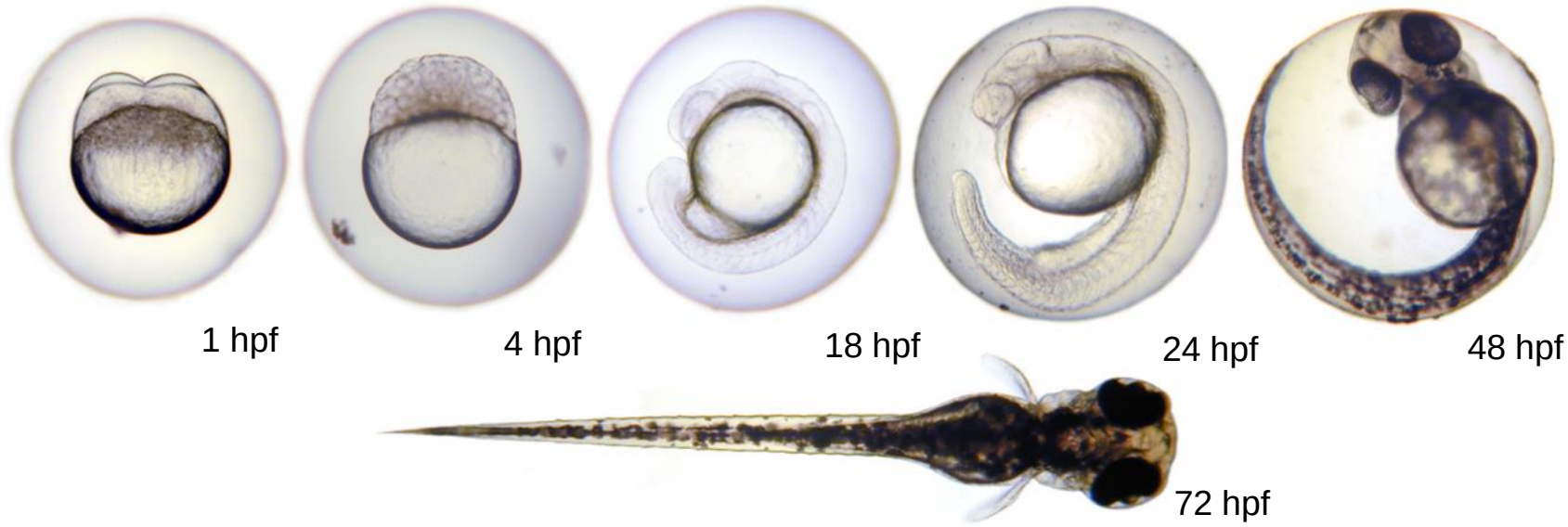




Some observations - 1

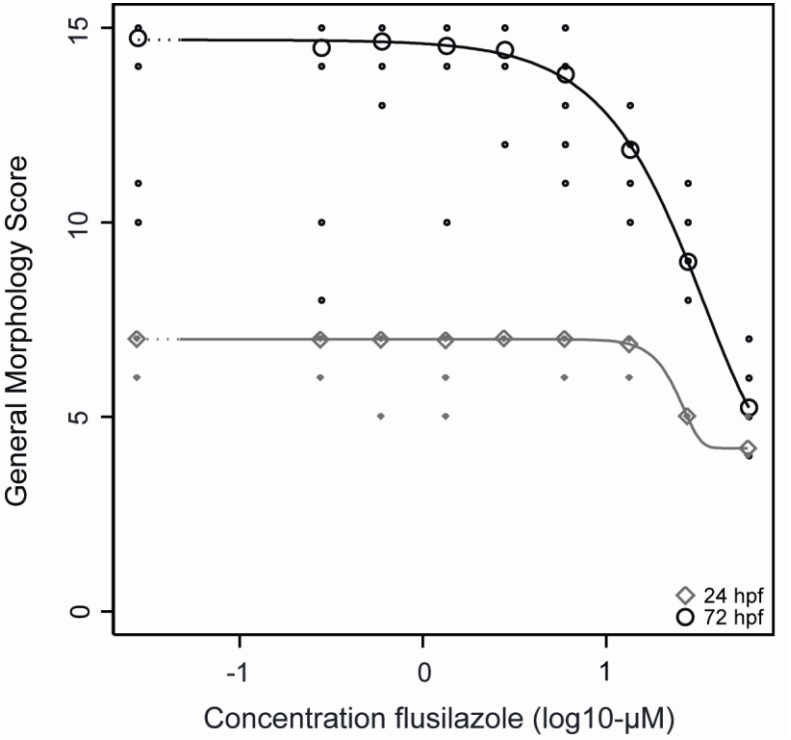
- Classical regulatory toxicology is largely based on effects that are generally considered adverse.
  - Death, body and organ weight, clinical signs etc.
- We are now able to detect a large variety of subtle changes in gene expression and biochemical parameters that may be adaptive or may indicate an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

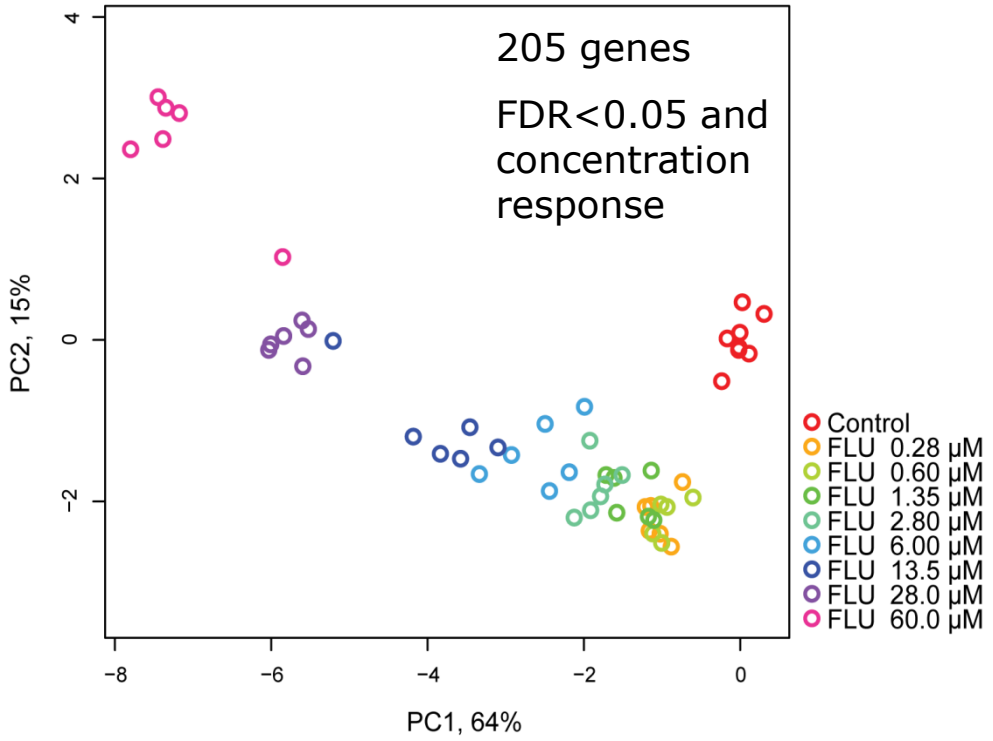
Zebrafish Embryotoxicity Test (ZET)



Hermsen et al., 2011

Principal Component Analysis - Flusilazole





Hermsen et al., 2011

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 in the hypothalamic – pituitary – gonadal axis

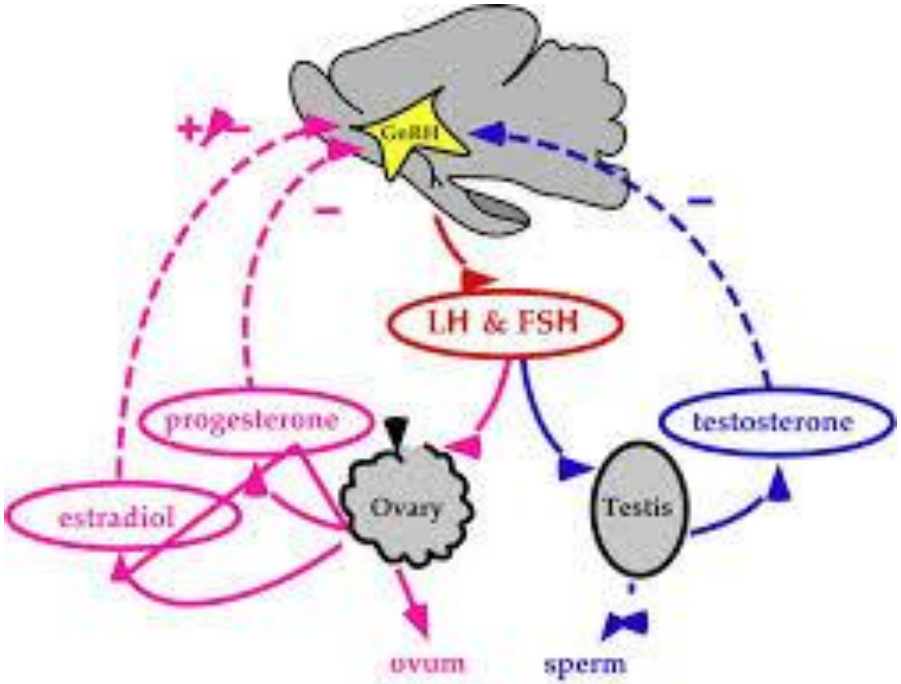


Figure 3

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REVIEW ARTICLE

Reproductive toxicants have a threshold of adversity

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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

- Current animal test protocols for hazard assessment are primarily based on monitoring adverse health effects.
- Only recently, endocrine parameters have been introduced into a so far limited number of animal test protocols.
- Still, a causal relationship between endocrine and adverse effects is difficult to prove, even if both effect types are observed simultaneously.

Some observations - 4

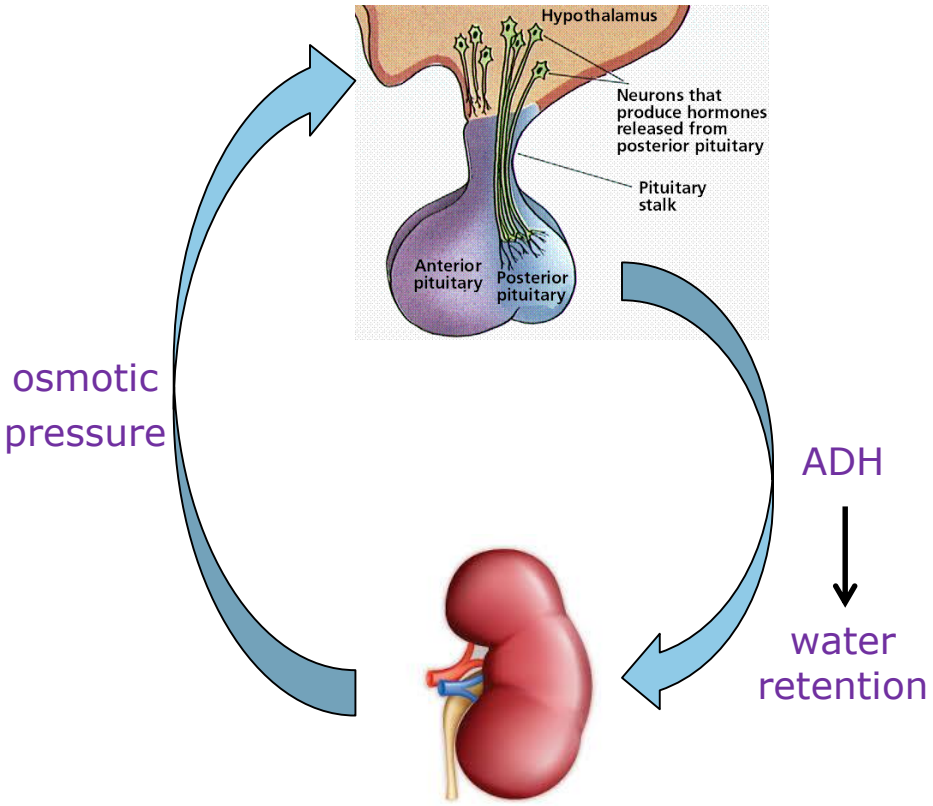
- We are now able to detect very low concentrations of xenobiotics in biological samples
- There is general concern about any exposure, secondary to the faulty assumption that hazard equals risk

- Cf. Paracelsus (1493-1541): *Alle Ding' sind Gift, und nichts ohn' Gift; allein die Dosis macht, daß ein Ding kein Gift ist.*

- Tr. *All things are poisons, and nothing is without poison; only the dose makes the thing not a poison.*

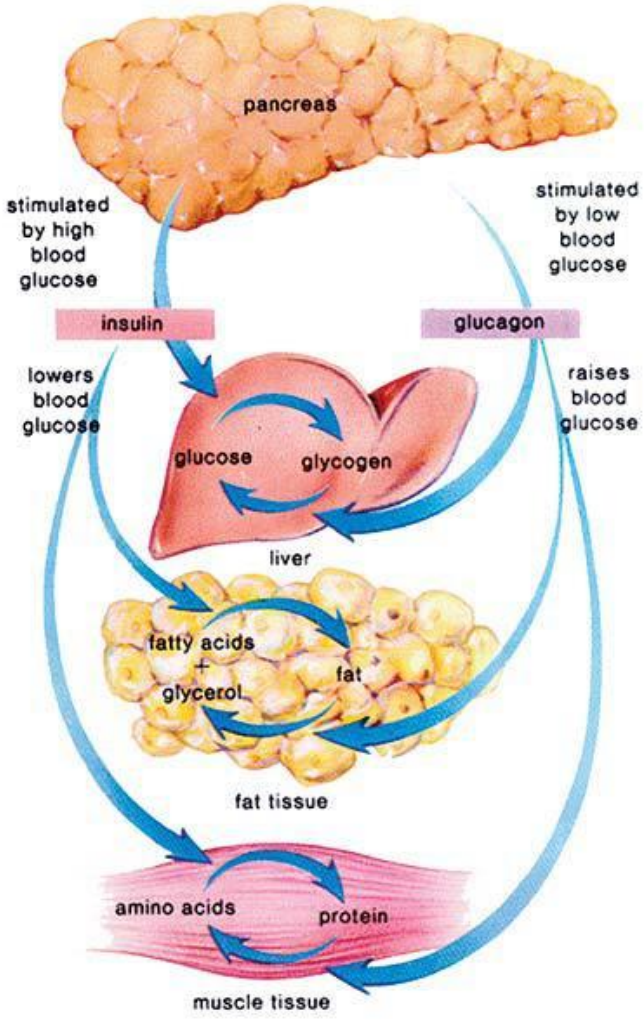
Compound X:

- Endocrine disruption via inhibition of ADH production.
- Causes electrolyte imbalance in blood and tissues, and edema in all body tissues.
- Electrolyte imbalance and brain edema cause death.
- LD50 in man around 100 g/kg bw/day.



Compound Y:

- Endocrine disruption via induction of insulin production and release.
- Decreased insulin production through reduced insulin promotor gene expression.
- Insulin resistance and diabetes mellitus type 2.
- Prevalence 9% worldwide in 2014.
- 7th leading cause of death in 2030 (WHO).



Hazard versus Risk

- Cf. Genotoxic carcinogen risk assessment:
  - Linear extrapolation of an effective high dose to zero for determining $1:10^6$ added risk
  - Alternative: Threshold model
  - Exposure estimate
- Cf. Classification and Labelling (GHS system):
  - Intrinsic toxic property of compound – hazard based
  - Limit dose 1000 mg/kg bw/day – potency consideration
- EU criteria for ED discussion:
  - Hazard based or risk-based?
  - Potency discussion

OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals

Note: Document prepared by the Secretariat of the Test Guidelines Programme based on the agreement reached at the 6th Meeting of the EDTA Task Force

Level 1 Sorting & prioritization based upon existing information	•Physical & chemical properties, e.g., MW, reactivity, volatility, biodegradability •Human & environmental exposure, e.g., production volume, release, use patterns •Hazard, e.g., available toxicological data
Level 2 *In vitro* assays providing mechanistic data	•ER, AR, TR receptor binding affinity •Transcriptional activation •Aromatase & Steroidogenesis *in vitro* •Aryl hydrocarbon receptor recognition/binding •High Through Put Prescreens •Thyroid function •Fish hepatocyte VTG assay •QSARs; Others (as appropriate)
Level 3 *In vivo* assays providing data about single endocrine Mechanisms and effects	•Uterotrophic Assay (estrogenic related) •Hershberger Assay (androgenic related) •Non-receptor mediated hormone function •Fish VTG assay (estrogenic related) •Others (e.g. thyroid)
Level 4 *In vivo* assays providing data about multiple endocrine mechanisms and effects	•Enhanced OECD 407 (endpoints based on endocrine mechanisms) •Male and female pubertal assays •Adult intact male assay •Fish gonadal histopathology assay •Frog metamorphosis assay
Level 5 *In vivo* assays providing data on effects from endocrine & other mechanisms	•1-generation assay (TG415 enhanced) •2-generation assay (TG416 enhanced) •Reproductive screening (TG421 enhanced) •Partial and full life cycle assays in fish, birds, amphibians & invertebrates (development & reproduction) •Combined 28 day/reproduction screening test (TG 422 enhanced)

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.

Novel end points

- Classical: EAT
  - Estrogen/Androgen/Thyroid
- Novel:
  - Metabolic syndrome
    - High blood pressure
    - Diabetes
    - Obesity
  - DOHAD: Developmental Origins of Health And Disease
    - Epigenetic changes
    - Metabolic programming
    - Behaviour and cognition
    - Many diseases of ageing implemented

Conclusions

- Endocrine disruption refers to adverse health effects mediated by a disturbance of hormone homeostasis.
- Endocrine disruption refers to situations raising serious concerns for public health.
- Endocrine disruption has been associated with a range of proposed adverse health effects.
- Causality is clear for potent compounds with relatively high exposures (DES, EE_2).
- In many cases of suggested endocrine disruption causality is uncertain.
- Current testing paradigms are focused on adverse effects, not on mechanism of action.
- Novel adverse health effects may need to be included in testing paradigms (metabolic syndrome, behavior, immune system).

Thank you