Non-monotonic dose responses in studies of endocrine disrupting chemicals:
bisphenol A as a case study

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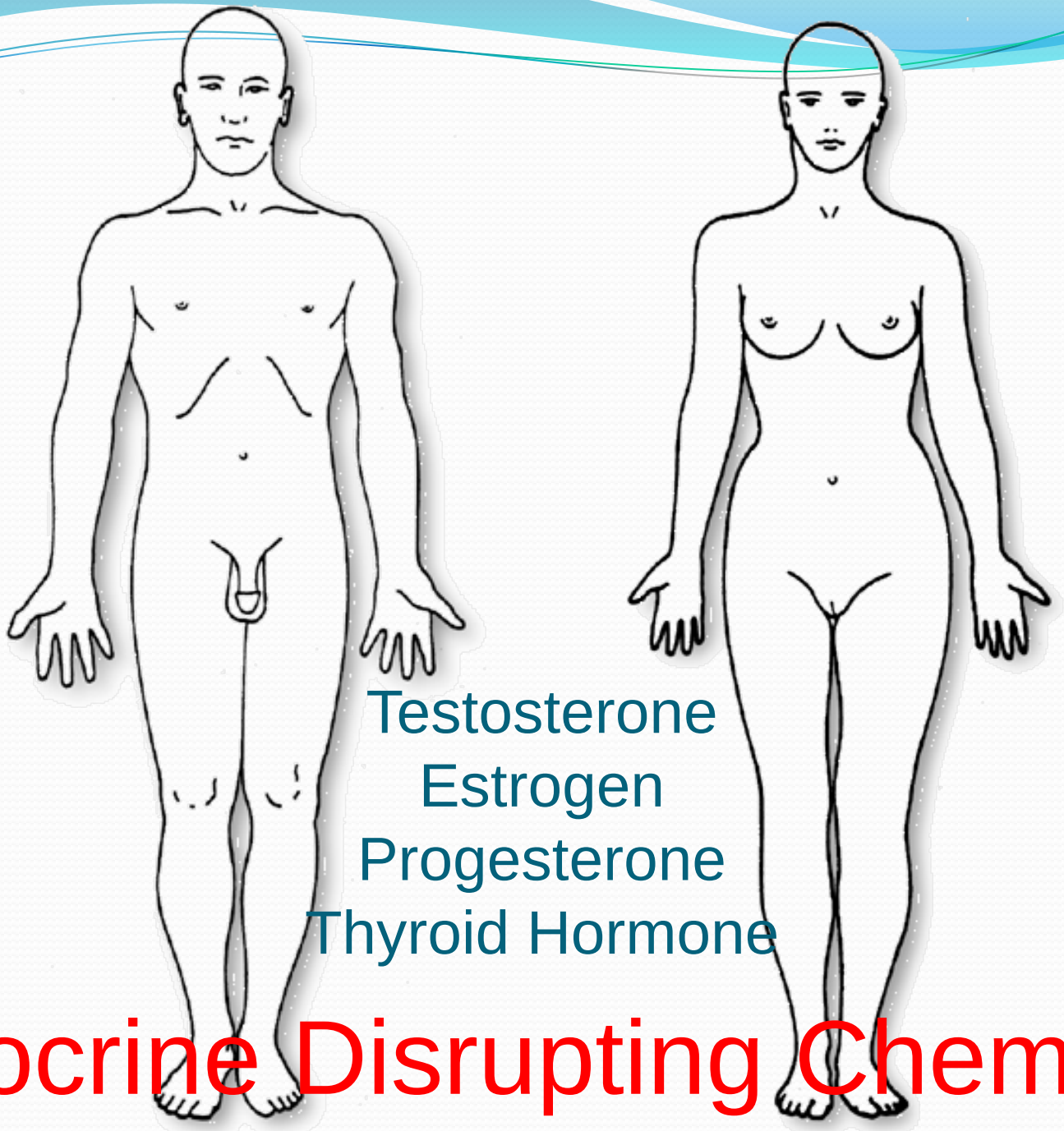
We live in a chemical stew



Testosterone
Estrogen
Progesterone
Thyroid Hormone

Endocrine Disrupting Chemicals

Wingspread (1991)

- A group of 21 scientists from many fields came together to weigh evidence reported on the effects of endocrine disruptors on sexual differentiation, reproductive function, neurobehavioral development and autoimmune diseases.

- "We are certain of the following: A large number of man-made chemicals that have been released into the environment, as well as a few natural ones, have the potential to disrupt the endocrine system of animals, including humans."

EDCs: The EPA Definition

- "An endocrine disruptor is an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior."

R E V I E W

Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses

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For decades, studies of endocrine-disrupting chemicals (EDCs) have challenged traditional concepts in toxicology, in particular the dogma of "the dose makes the poison," because EDCs can have effects at low doses that are not predicted by effects at higher doses. Here, we review two major concepts in EDC studies: low dose and nonmonotonicity. Low-dose effects were defined by the National Toxicology Program as those that occur in the range of human exposures or effects observed at doses below those used for traditional toxicological studies. We review the mechanistic data for low-dose effects and use a weight-of-evidence approach to analyze five examples from the EDC literature. Additionally, we explore nonmonotonic dose-response curves, defined as a nonlinear relationship between dose and effect where the slope of the curve changes sign somewhere within the range of doses examined. We provide a detailed discussion of the mechanisms responsible for generating these phenomena, plus hundreds of examples from the cell culture, animal, and epidemiology literature. We illustrate that nonmonotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Whether low doses of EDCs influence certain human disorders is no longer conjecture, because epidemiological studies show that environmental exposures to EDCs are associated with human diseases and disabilities. We conclude that when nonmonotonic dose-response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus, fundamental changes in chemical testing and safety determination are needed to protect human health. (Endocrine Reviews 33: 378–455, 2012)

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Abbreviations: AI, Androstenedione; AhR, aryl hydrocarbon receptor; BPA, bisphenol A; CDC, Centers for Disease Control and Prevention; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DES, diethylstilbestrol; EDC, endocrine-disrupting chemical; EPA, Environmental Protection Agency; ER, estrogen receptor; FDA, Food and Drug Administration; GLP, good laboratory practices; LOAEL, lowest observed adverse effect level; mER, membrane-associated ER; NHANES, National Health and Nutrition Examination Survey; NIS, sodium iodide symporter; NMDRC, nonmonotonic dose-response curve; NOEL, no observed effect level; NOAEL, no observed adverse effect level; NTP, National Toxicology Program; PIN, prostatic intraepithelial neoplasia; POP, persistent organic pollutants; ppb, parts per billion; SERM, selective ER modulator; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; WoE, weight of evidence.

Environmental Chemicals: Evaluating Low-Dose Effects

doi:10.1289/ehp.1205179


Linda S. Birnbaum

Regulatory Toxicology and Pharmacology

Commentary

Low dose effects and nonmonotonic dose responses of endocrine disrupting chemicals: Has the case been made?

Lorenz R. Rhomberg *, Julie E. Goodman

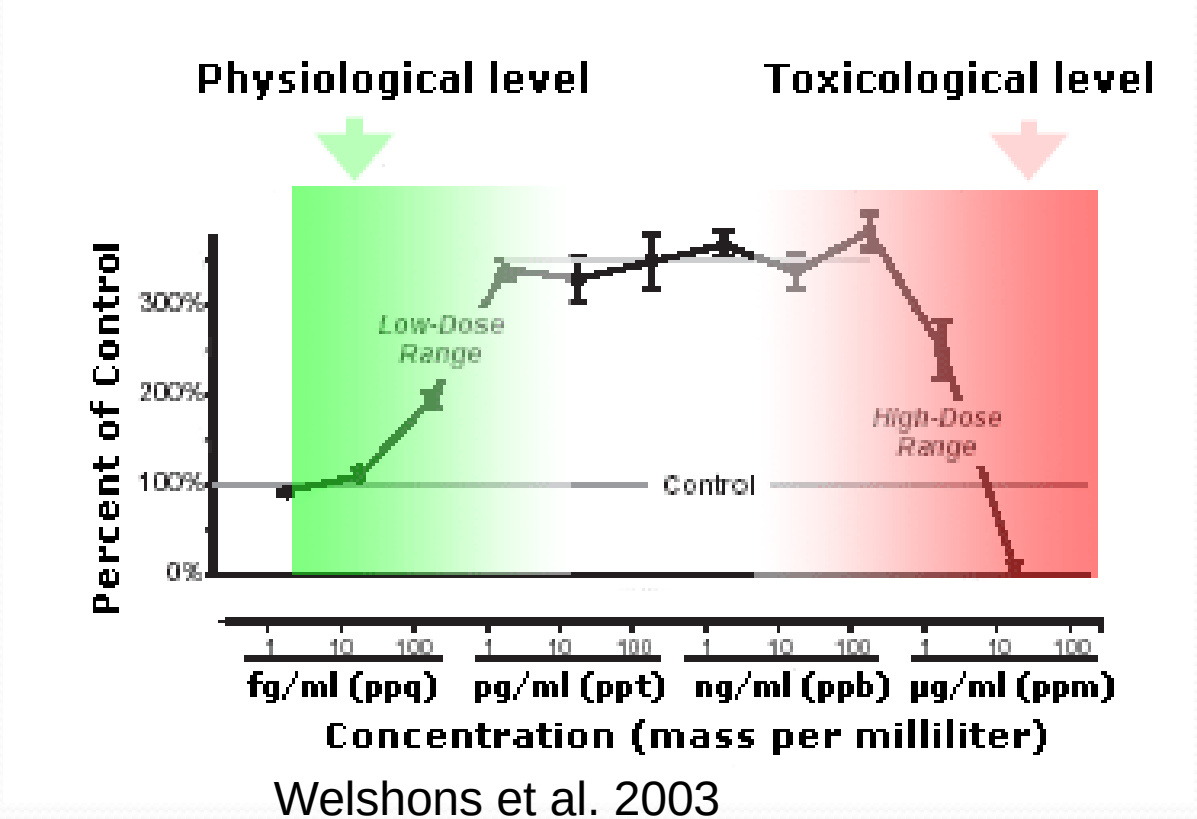
ARTICLE INFO

ABSTRACT

A brief review of our findings

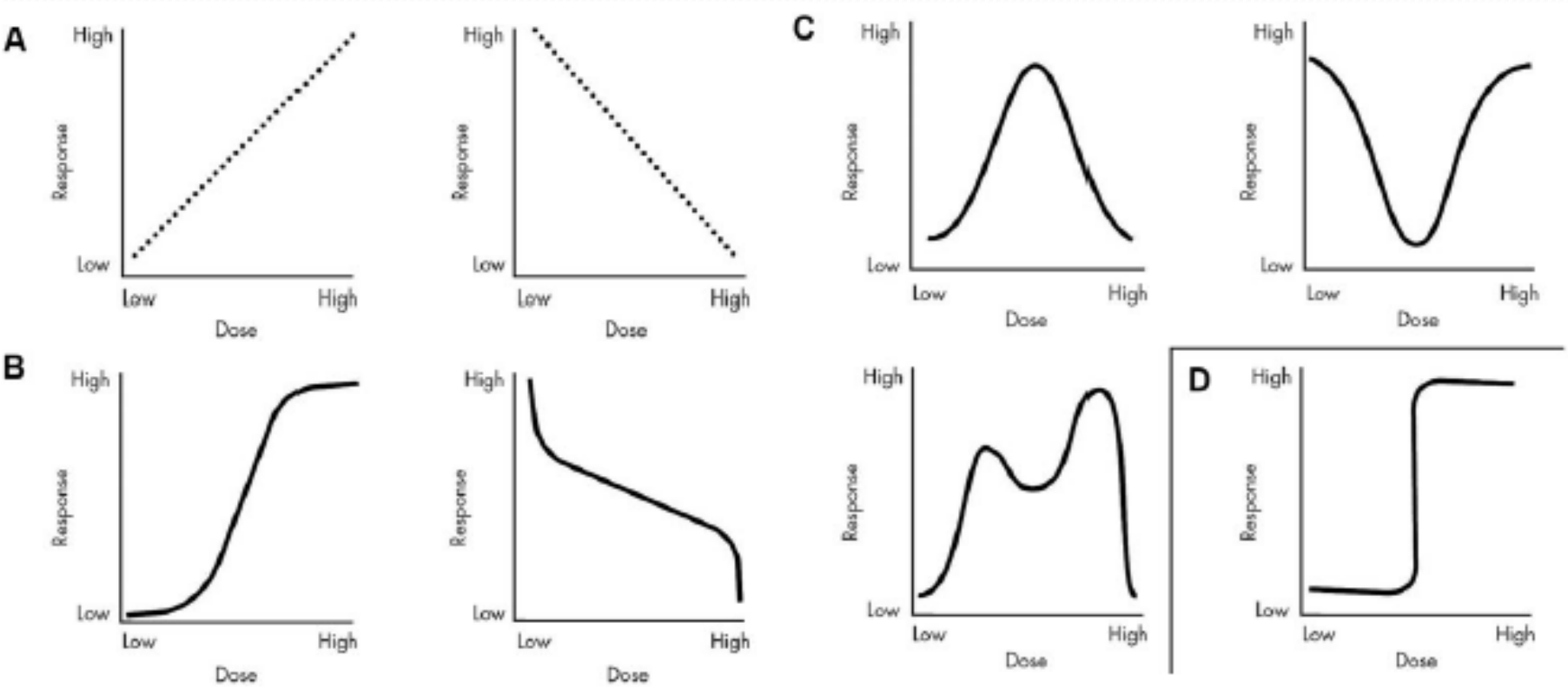
- The principles of endocrinology should be considered for study and regulation of EDCs
- Low dose effects have been observed for a number of EDCs
- Non-monotonic dose responses are common for both hormones and EDCs and have been observed in *in vitro*, *in vivo* (animal) and epidemiology studies.

Endocrine Principle: Hormones have non-linear, and often non-monotonic responses



Welshons et al. 2003

Non-monotonicity: a mathematical definition describing a curve shape



Mechanisms to produce NMDRCs

- Cytotoxicity
- Cell- and tissue-specific receptors and cofactors
- Receptor selectivity
- Receptor down-regulation & desensitization
- Receptor competition
- Endocrine negative feedback loops

Non-monotonic dose response curves are observed for hormones & EDCs

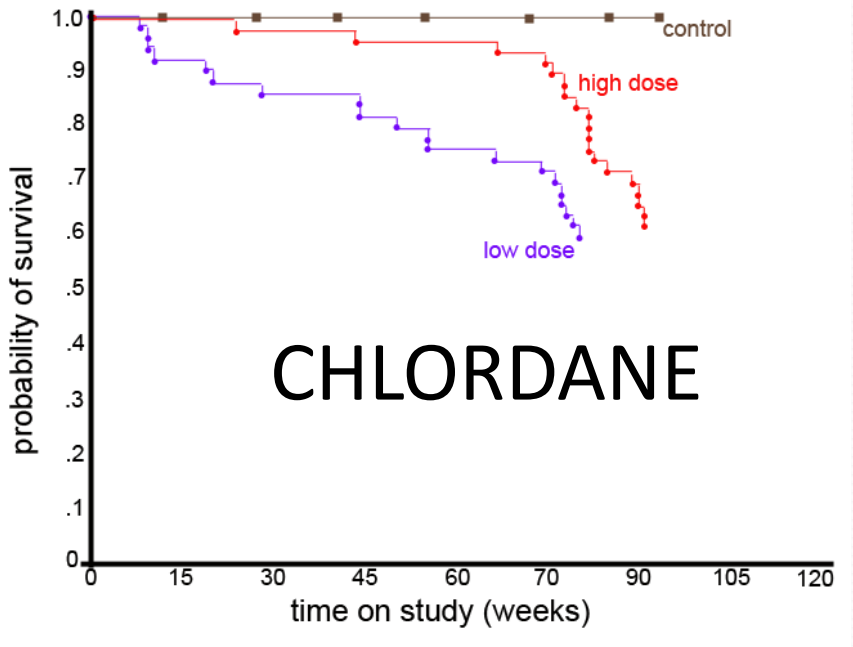
- More than 20 hormones
- More than 70 EDCs
- In cultured cells, laboratory animals and human populations
- For a variety of endpoints

Challenges to our conclusions

- Why aren't there any NMDRCs in guideline studies?
- Are NMDRCs relevant if they are only observed in some endpoints or tissues?
- Do NMDRCs occur in the range of human exposures?
- Can we distinguish NMDRCs from statistical flukes, particularly in epidemiology studies?
- Is there any evidence that endpoints that demonstrate NMDRCs are adverse?

- **Are NMDRCs 'common'? What is their frequency?**

Why aren't there NMDRCs in guideline studies?



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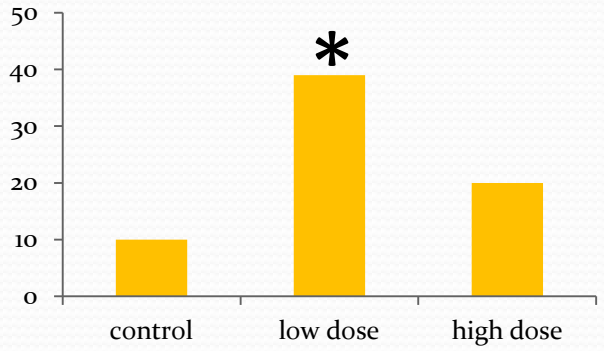
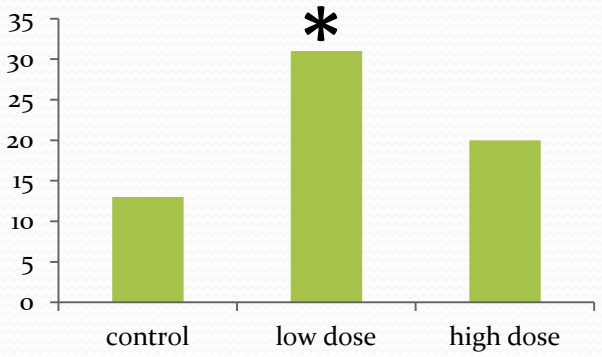
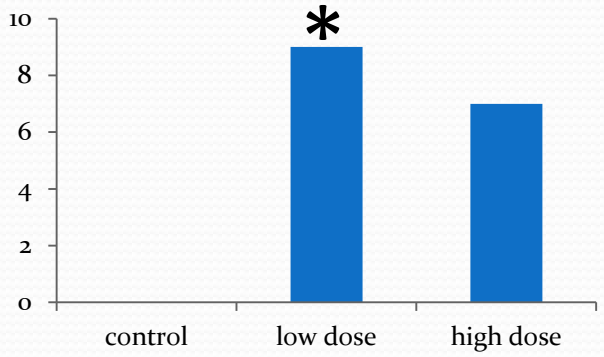
Hepatocellular carcinoma



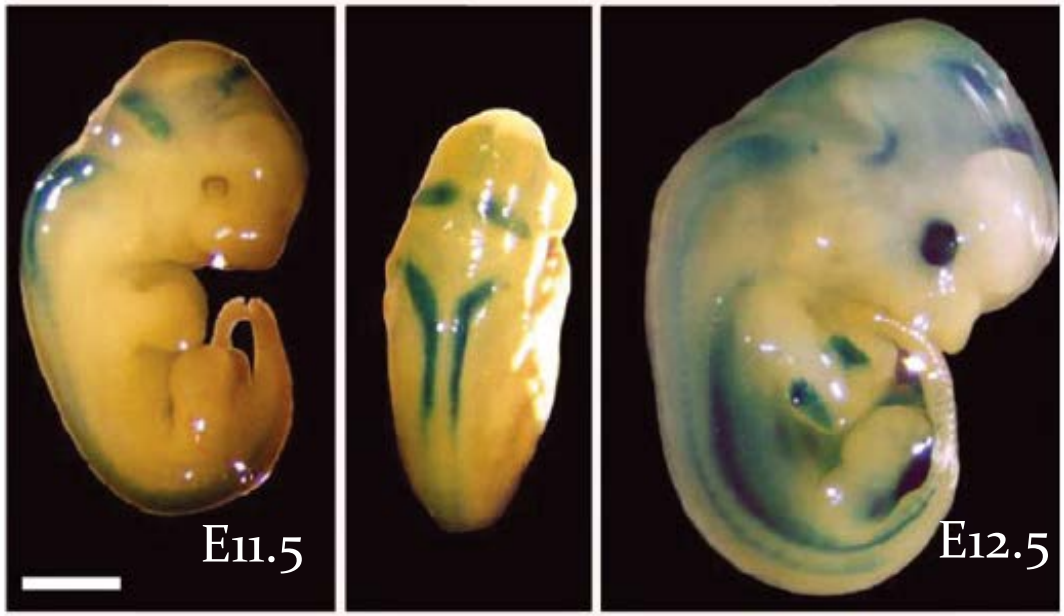
Pituitary chromophobe adenoma



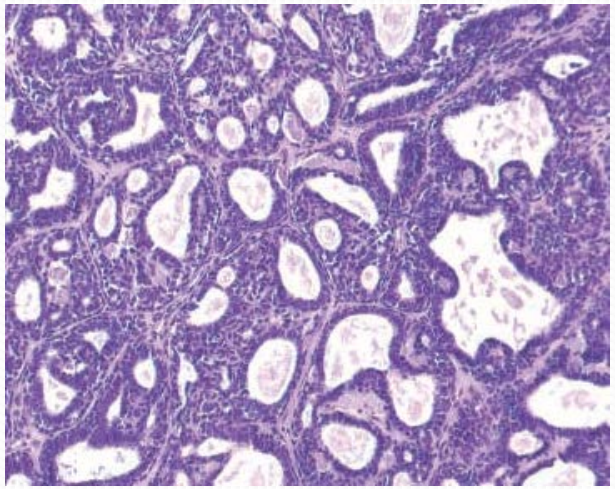
Thyroid C-cell carcinoma



If NMDRCs are only observed in certain tissues or for certain endpoints, are they relevant?

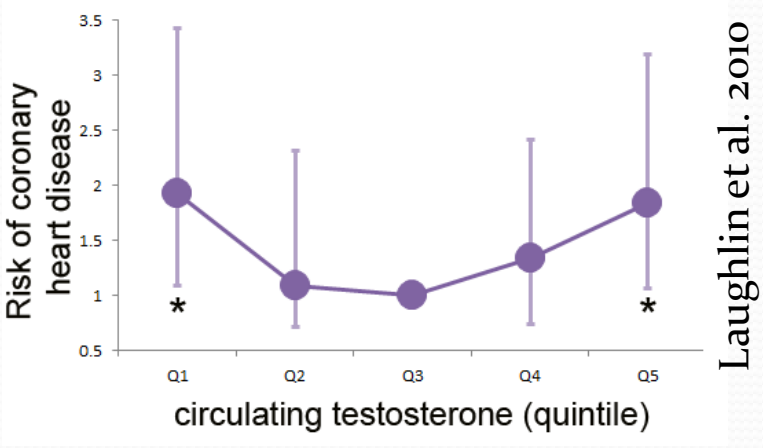


Expression of thyroid hormone receptor
Nucera et al. 2009

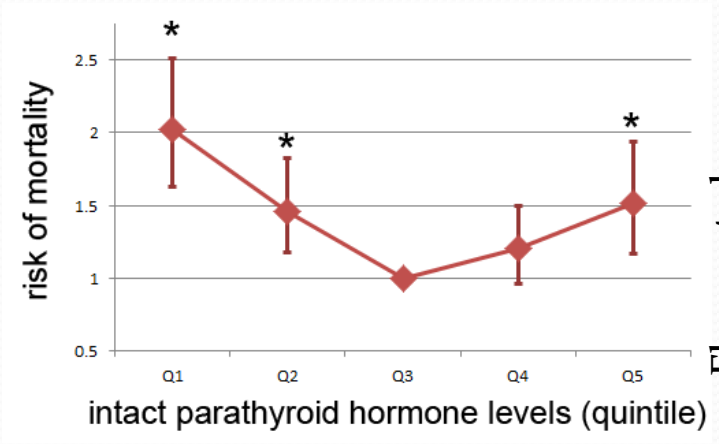


Maffini et al. 2004

Do NMDRCs occur in the range of human exposures?

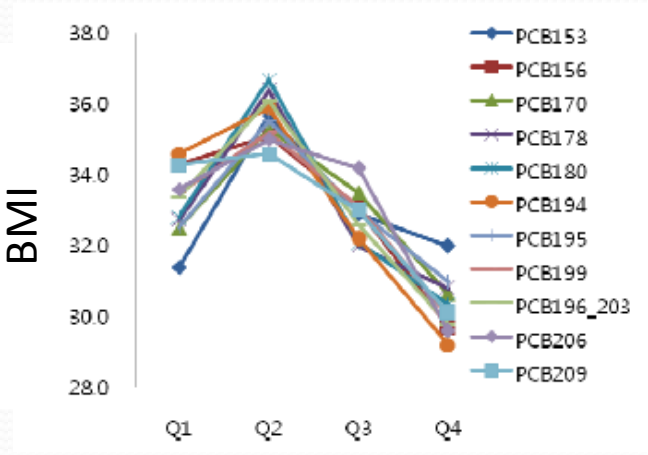


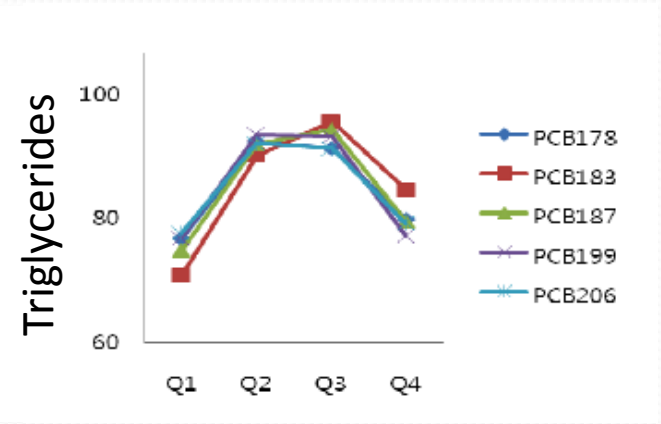
Laughlin et al. 2010



Floege et al. 2011

Lee et al. 2011





Is there any evidence that endpoints that demonstrate NMDRCs are adverse?

Defining ‘adverse’

- US EPA: “A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge.”
- WHO: “A change in morphology, physiology, growth, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences.”
- FDA & EFSA: no definition for ‘adverse’

Are there any NMDRCs for adverse effects?

- Fertility
- Neurobehaviors
- Aromatase activity
- Cholesterol levels
- Timing of puberty
- Expression of hormone receptors
- Organ weight
- Hormone levels
- Metamorphosis parameters
- DNA damage
- Dopamine transport
- Body weight
- Liver enzymes
- Tissue morphology (mammary gland)
- Gene expression
- Mitochondrial metabolism
- Ion transporter activity
- Infarct size after injury
- Protein levels
- Responses to allergens
- Fecundity
- Retinal neurogenesis
- Estrous cyclicity
- Anogenital distance
- Antioxidant enzyme activities

Are there any NMDRCs for adverse effects? Answers from epidemiology:

- Diabetes incidence
- BMI (obesity)
- Triglyceride levels, HDL cholesterol levels
- Telomere length in lymphocytes
- Atherosclerosis plaques
- Arthritis
- Endometriosis
- Fasting glucose levels
- Mental development scores (infants)
- Cytokines in umbilical cord blood
- Age at time of natural menopause
- Thyroid disease
- Hypertension incidence

What is the frequency of NMDRCs?

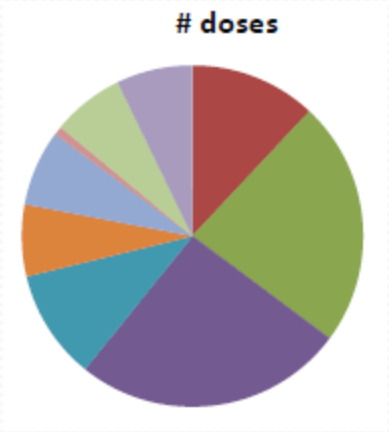
"Nonmonotonic dose-response relationships in Toxicological studies." JM Davis & DJ Svendsgaard 1994

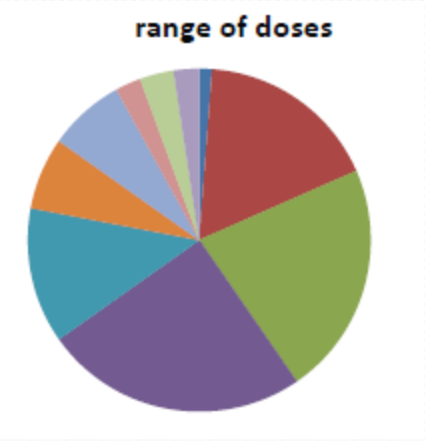
"The results of this study suggest that the incidence of U-shaped dose-response relationships is roughly on the order of 12% to 24%."

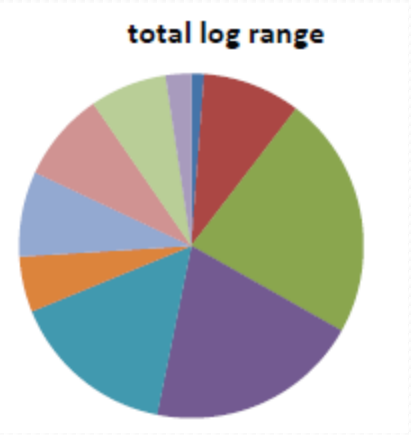
"There very likely is a bias against publishing, or even submitting for publication, 'anomalous' data of the type in question. If so, the attempt to determine the incidence of U-shaped relationships based on the published toxicology literature may… underestimate the true rate of occurrence of such data."

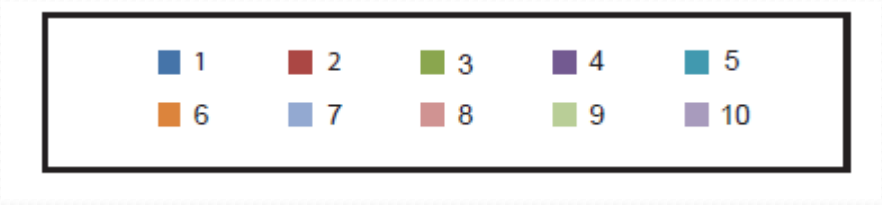
A pilot study: using BPA *in vitro* studies as a proof-of-principle

- 109 studies with 388 experiments were identified
- 93 studies with 250 dose response experiments were utilized in the analysis









Assessing non-monotonicity

1. Visual inspection of the curve, especially when no statistics had been performed for specific doses.
2. Statistical analysis in the manuscript indicates that lower doses produce significant effects compared to untreated controls, but higher doses do not.
3. Statistical analysis indicates that lower doses produce an increase in response compared to untreated controls and high doses produce a decrease in response compared to untreated controls, or vice versa.
4. Visual inspection suggests a U- or inverted U-shaped curve is present and statistical analysis indicates that higher doses are significantly different from the response at lower doses, regardless of whether the response at the higher doses is significantly different from what occurs in untreated controls.

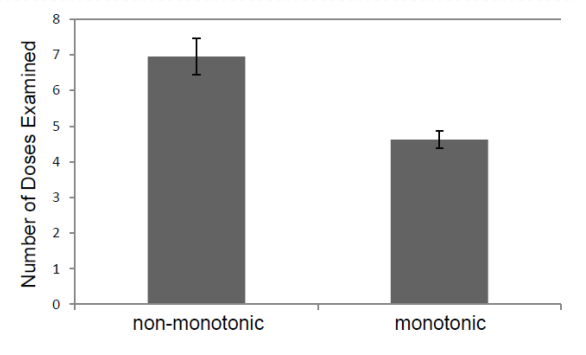
What is the frequency of NMDRCs?

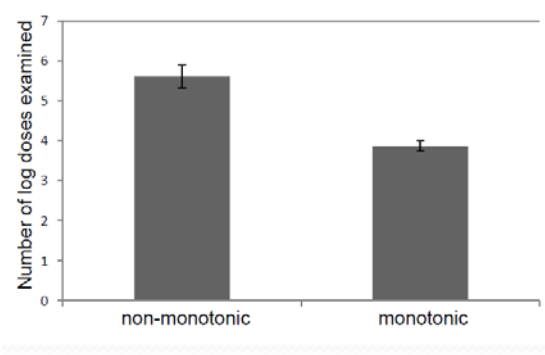
- Observed in 59/250 experiments (23.6%)
- Observed in 32/93 studies (34.4%)

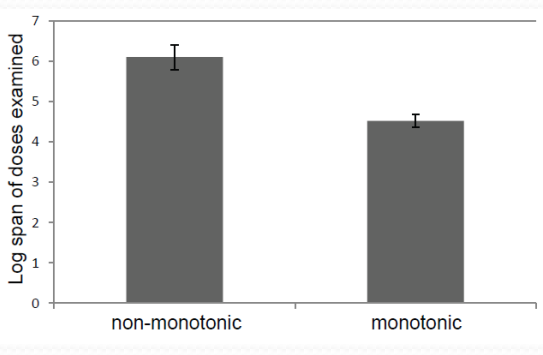
- Even when studies that did not include statistics were removed, NMDRCs were observed in 53/229 experiments (23.1%)

- **Thus, NMDRCs are 'common' in the BPA *in vitro* literature**

Study design influences the ability to detect NMDRCs

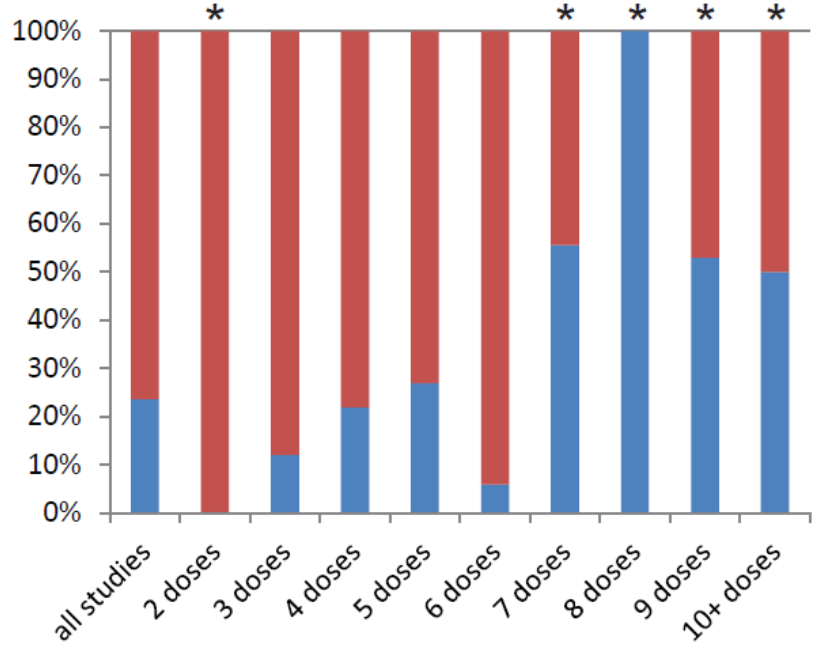


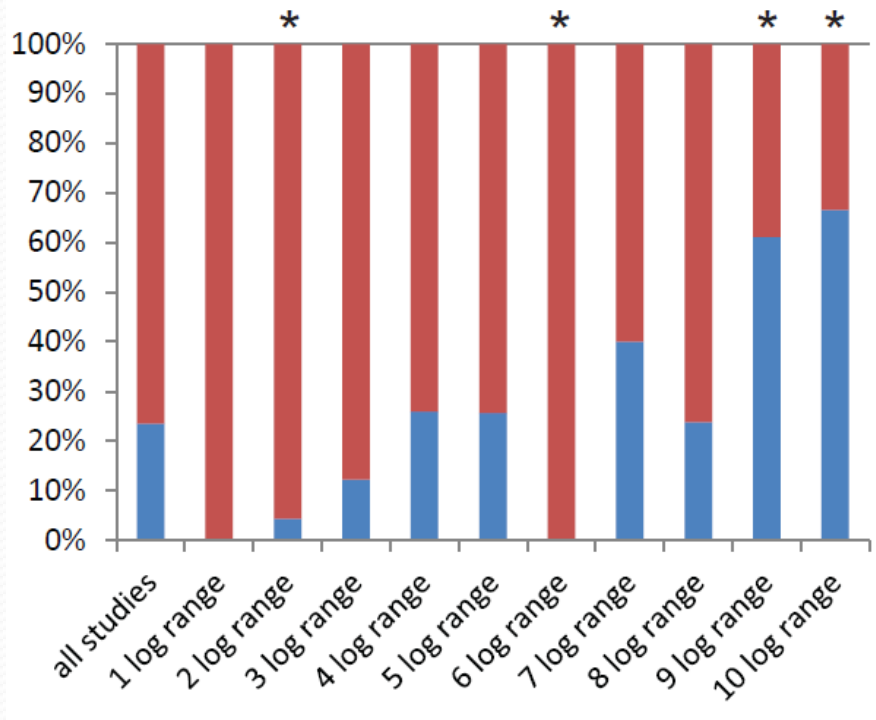




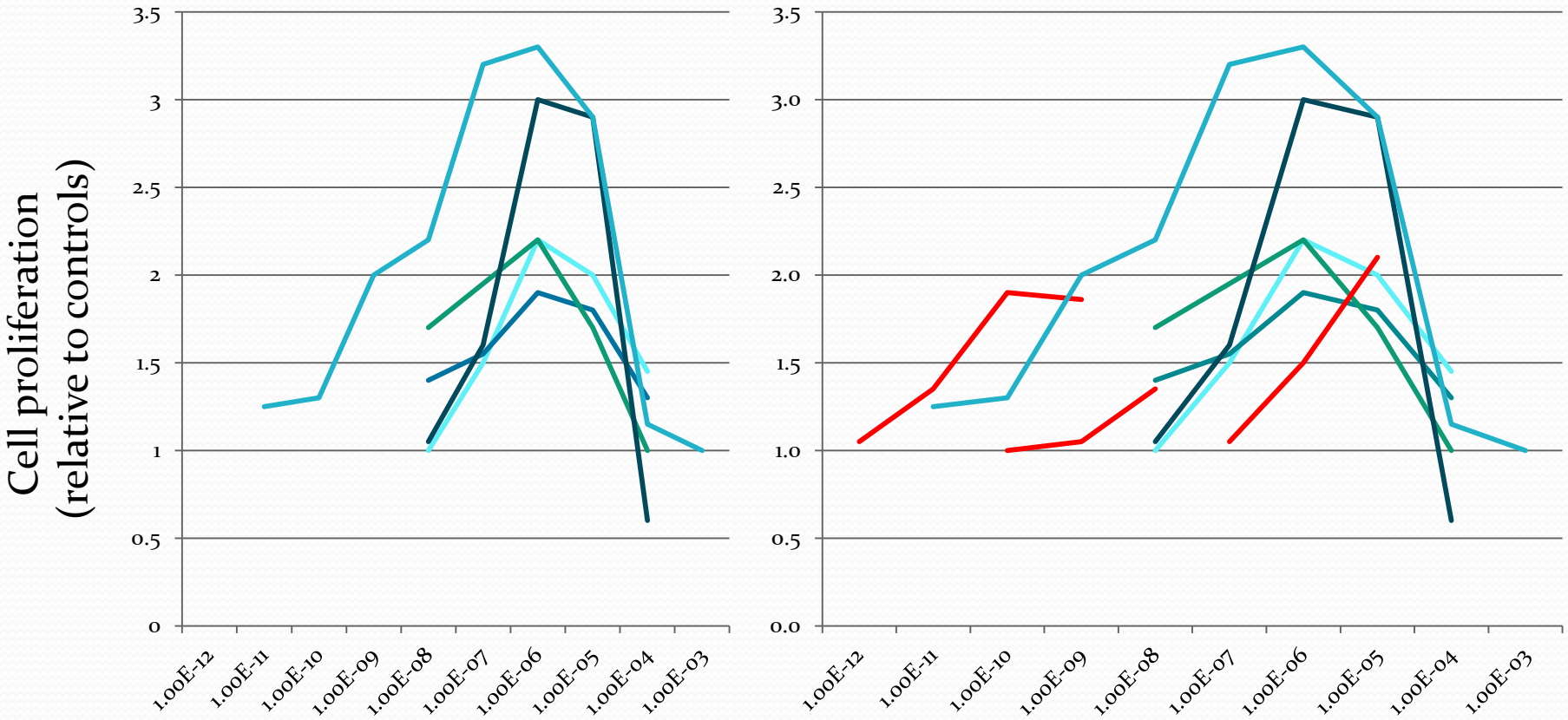
- Studies that report NMDRCs examine more doses, over a wider range of concentrations, than studies that do not.

Studies examining more doses, over a wider range of concentrations, have a higher probability of detecting NMDRCs

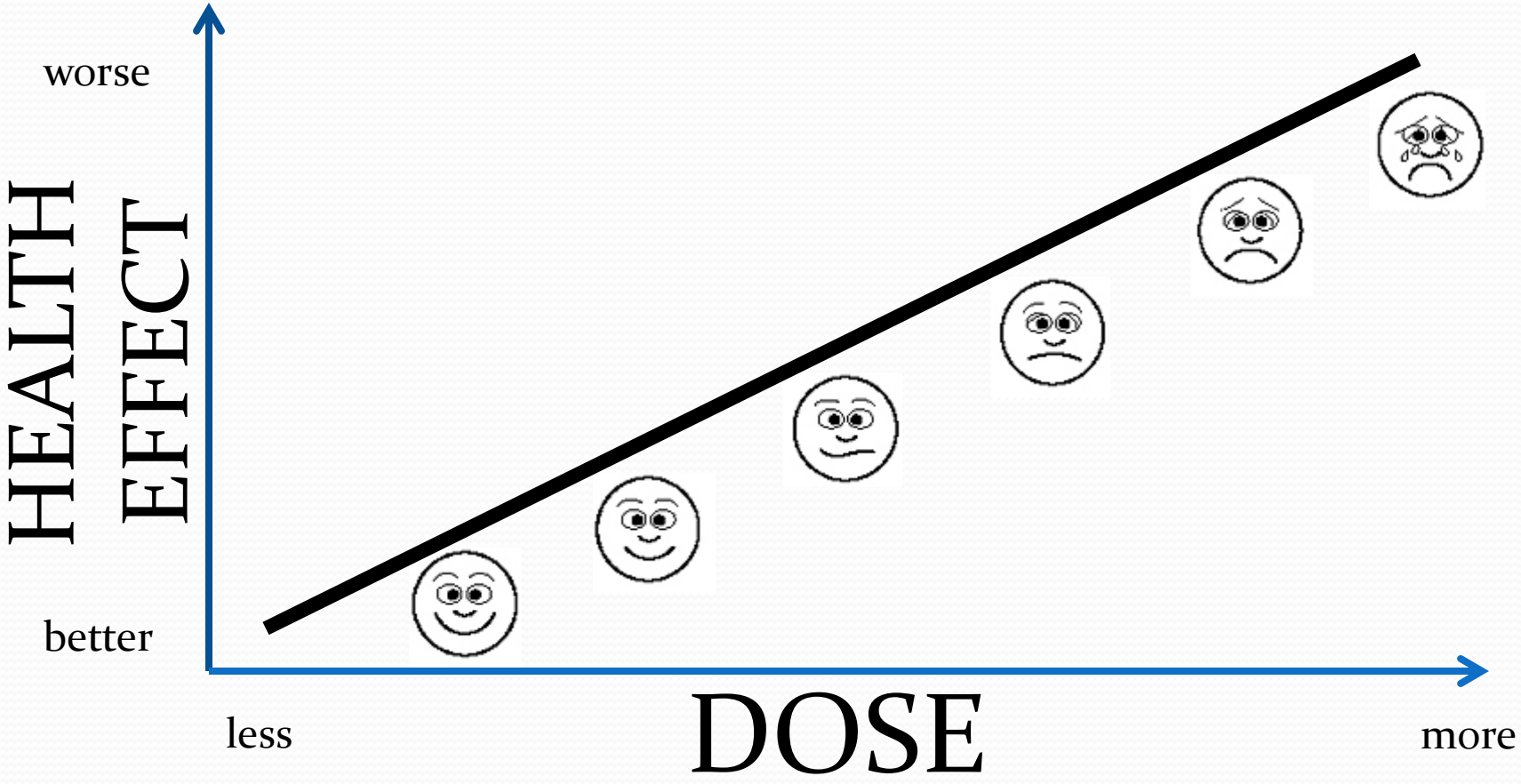




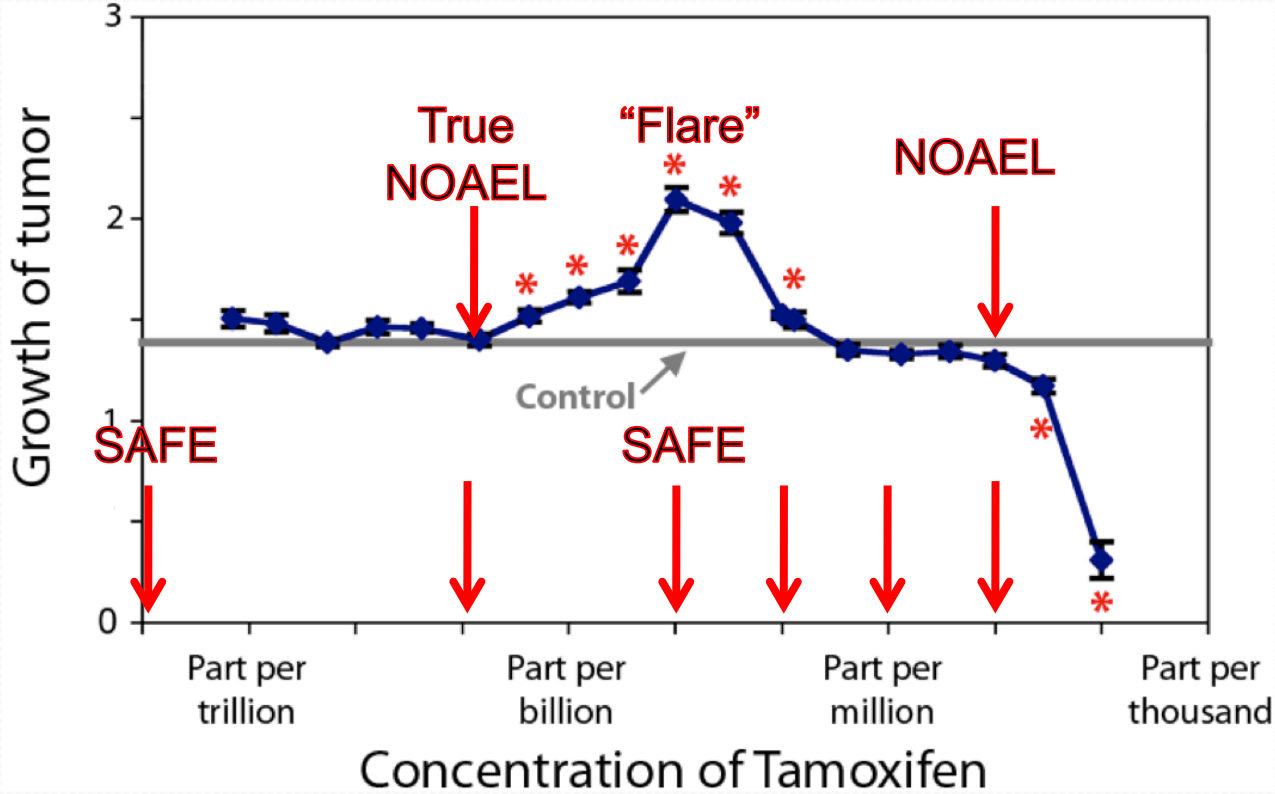
Are NMDRCs reproducible?



NMDRs question whether high doses can be used to predict the effects of low doses



A practical example, with implications for risk assessment: tamoxifen flare



Summary

- We identified hundreds of examples of NMDRCs for hormones and EDCs.
- NMDRCs are observed in cultured cells, in animals, and in human populations.
- We have addressed specific challenges to our findings with scientific evidence.
- In this pilot study of the BPA *in vitro* literature, more than 20% of experiments and 30% of studies report NMDRCs.
- Study design strongly influences whether NMDRCs are detected.
- NMDRCs challenge the idea that high doses can predict the effects of chemicals at lower doses.