Distinguishing between Mode and Mechanism of Action

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Conflict of Interest Statement

The research described in part was funded by different sponsors to the nonprofit organization Toxicology Excellence for Risk Assessment (TERA). Funding is ~2/3rd government and ~1/3rd industry. See http://www.tera.org/about/FundingSources.html.

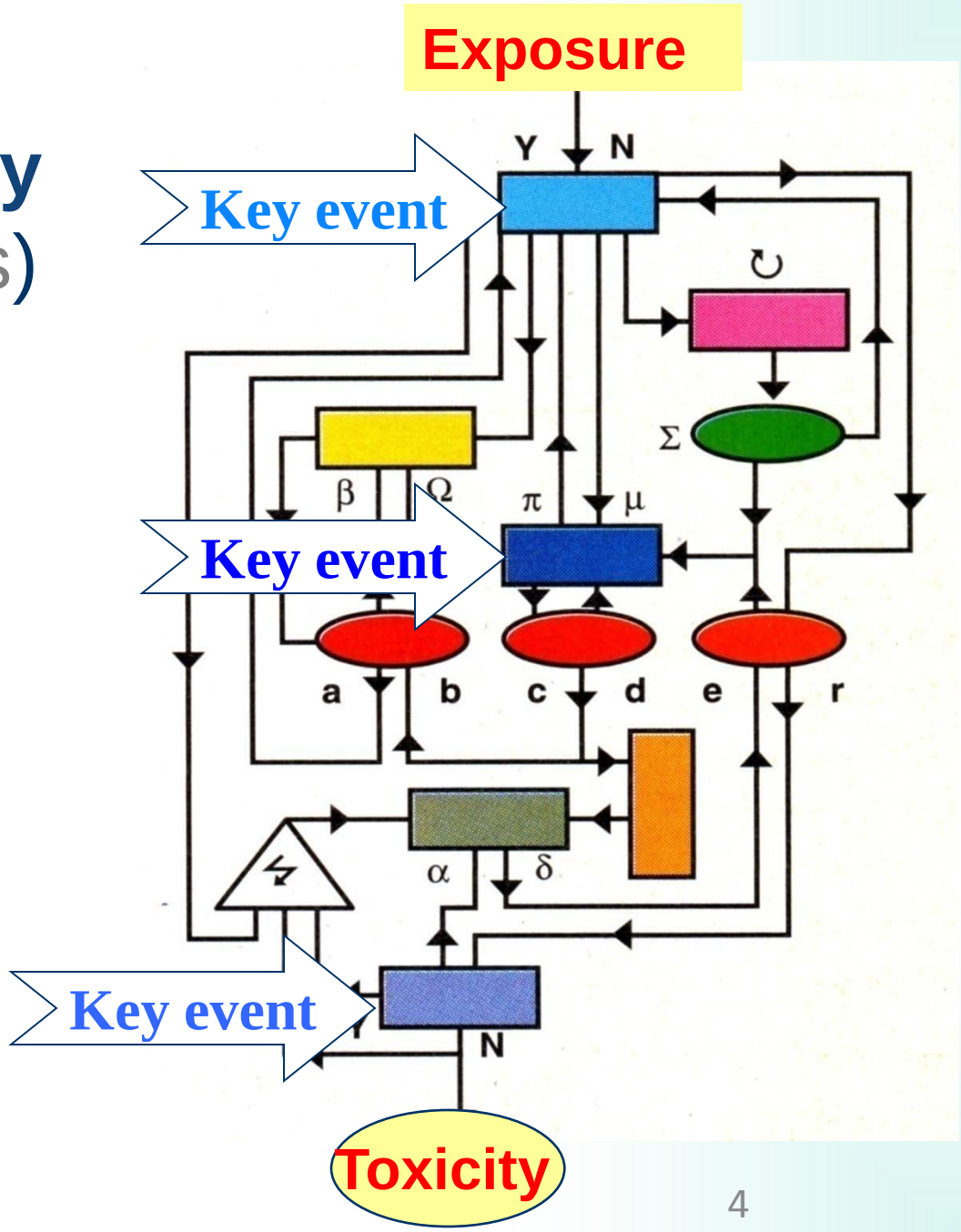
The mission of the TERA Center is *to support the protection of public health* by developing, reviewing and communicating risk assessment values and analyses; improving risk methods through research; and, educating risk assessors, managers, and the public on risk assessment issues.

Mode of action
(identification of **key & obligatory** steps)

… is not …

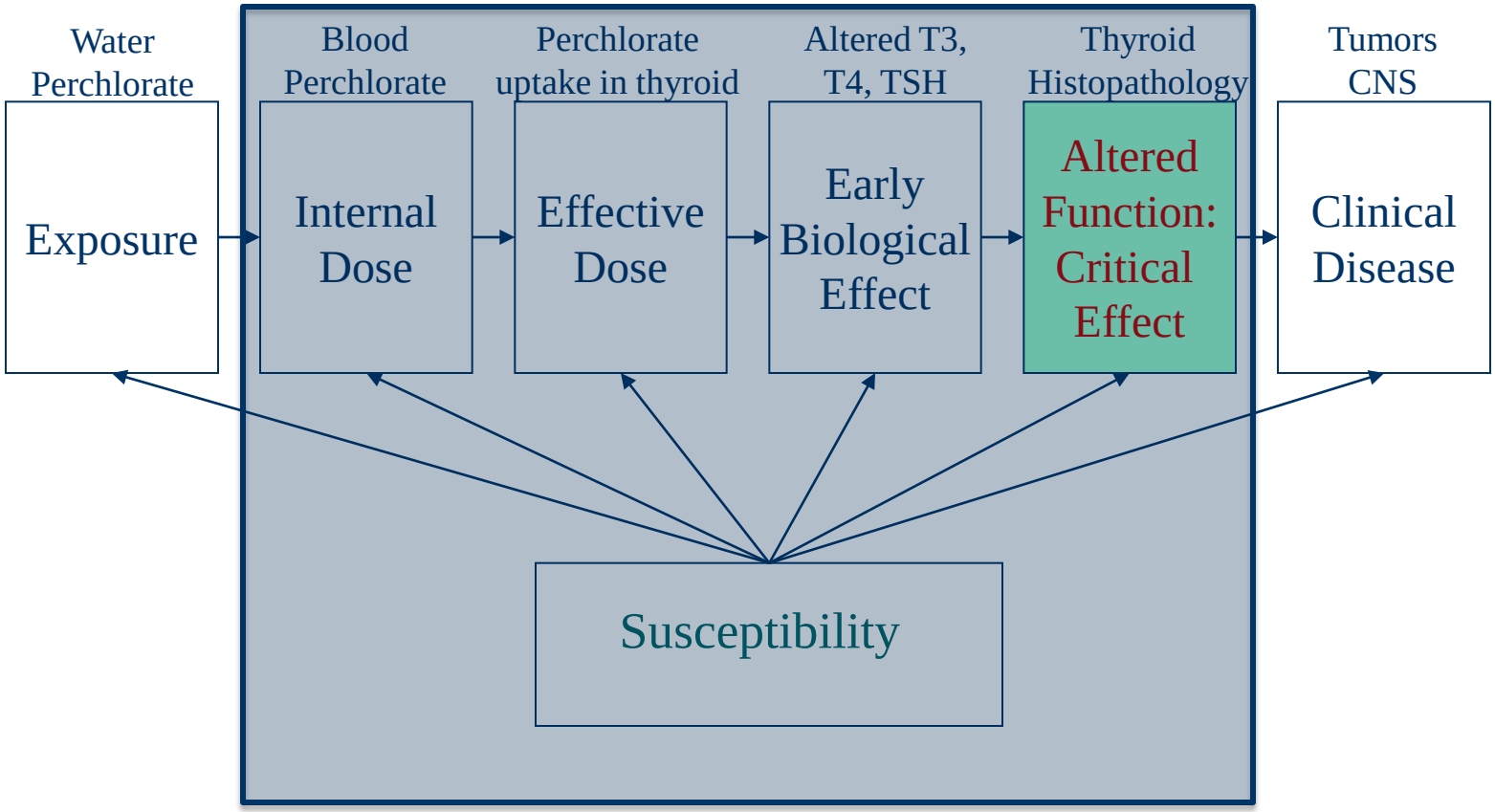
Mechanism of action
(more detailed understanding at biochemical & molecular level)

In part based on EPA (2005)



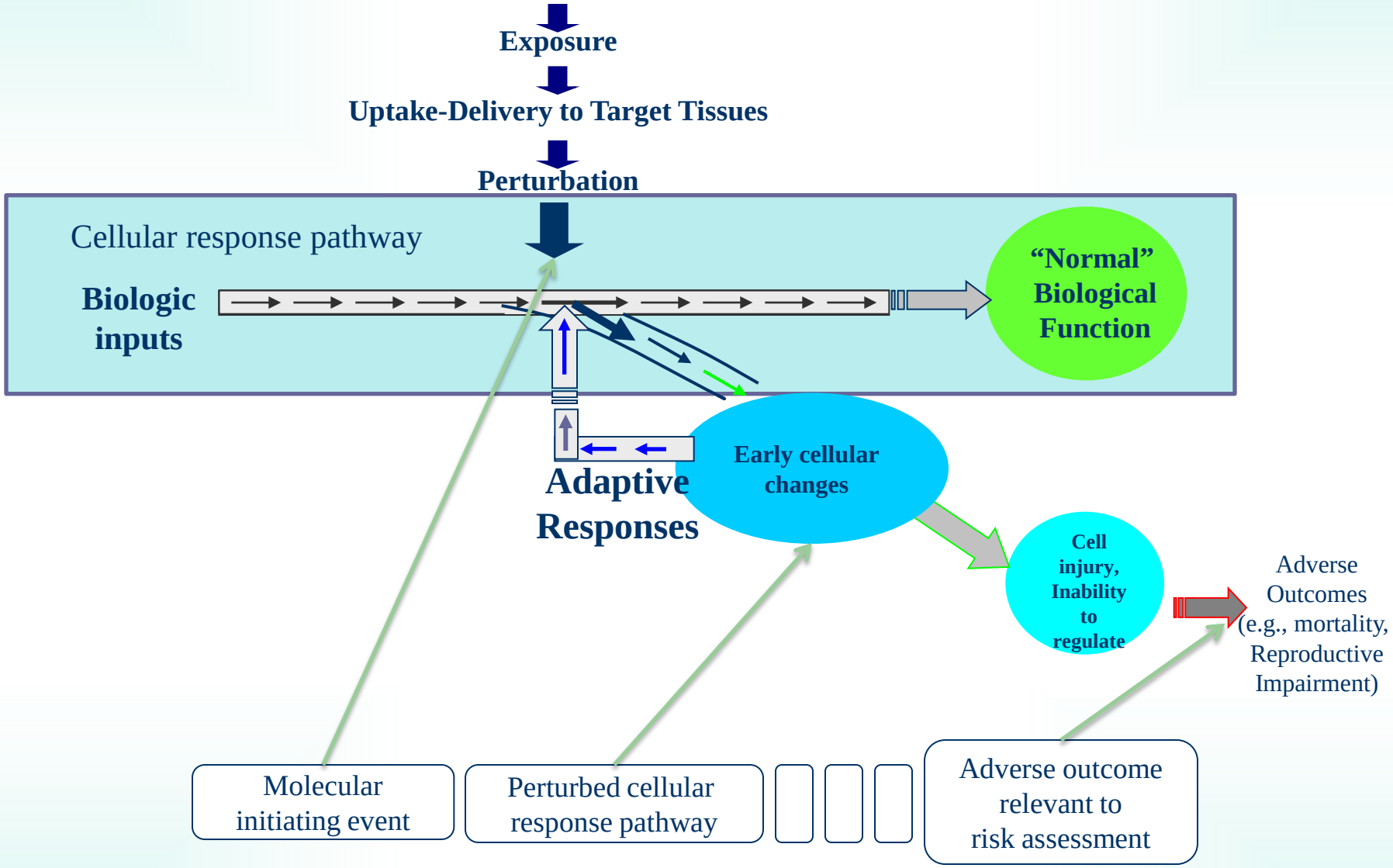
The Black Box of MOA Revealed

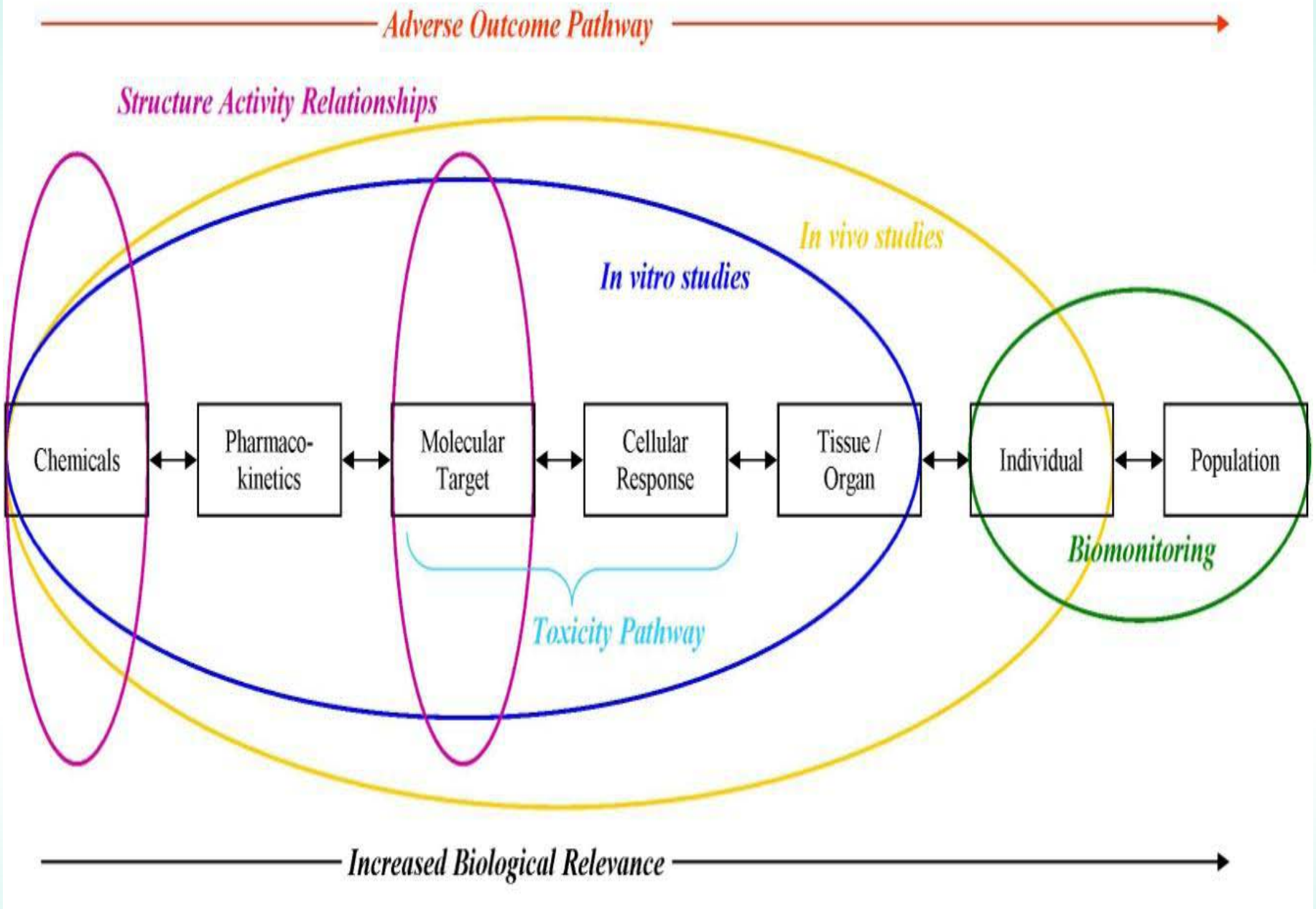
Exposure **Effect**



Adapted from Schulte (1989); Farland et al. 2000

Tox 21: Outcome Pathways of NAS





EPA 2011; Ankley et al. 2010.

MOA versus AOP

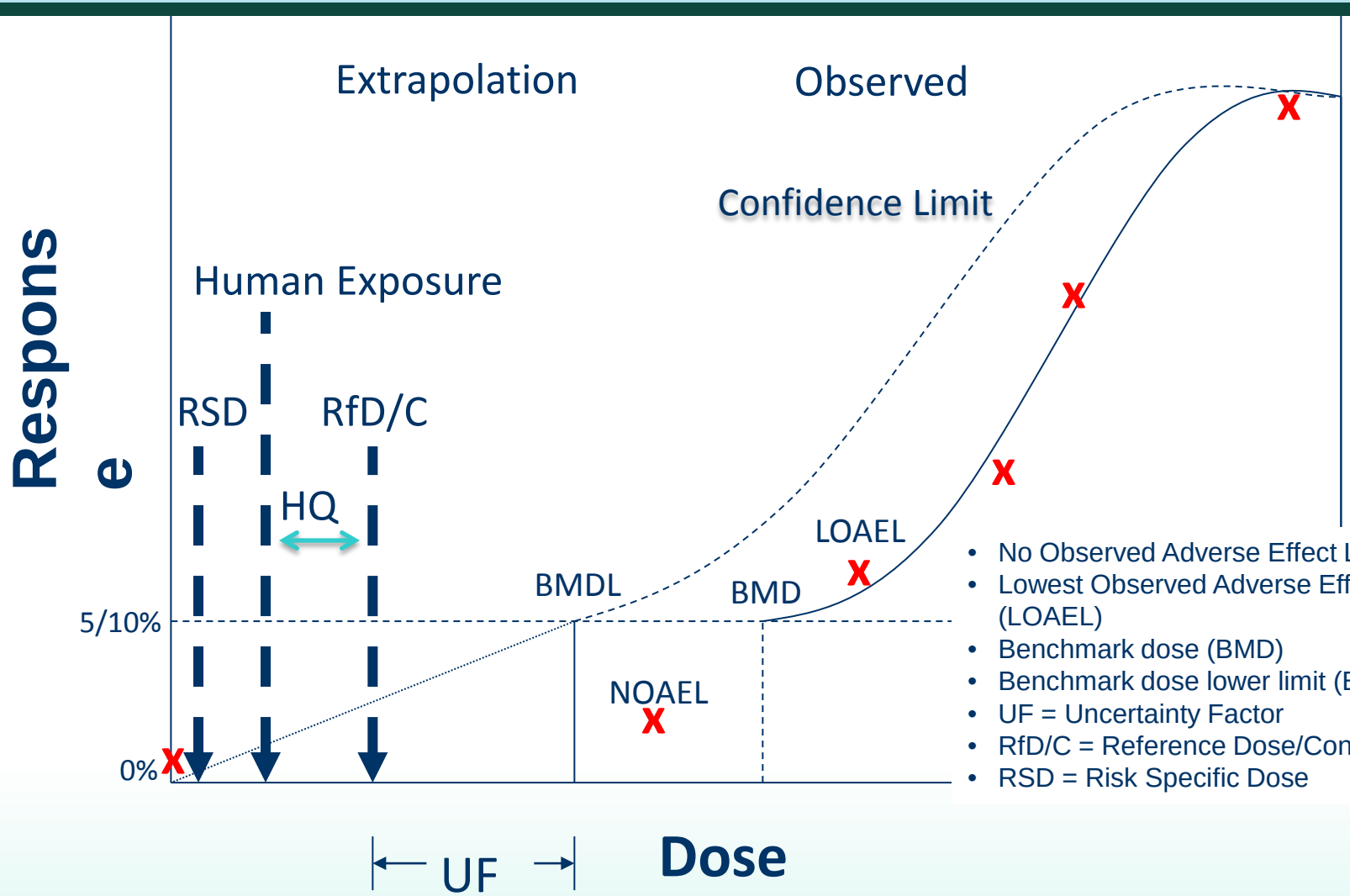
- From a risk assessor's perspective…
  - Adverse Outcome Pathway (AOP) reflects the inherent structure of the body for dealing with internal and external impingements. AOP is chemical-agnostic.
  - Mode of action reflects the key & obligatory steps through which a chemical interacts with the organism... And the organism's response. MOA is chemical-specific

Thresholds, Dose Response and MOA

- The question of thresholds is a biological one and cannot be resolved by mathematical model fitting
  - It is essentially impossible to mathematically determine whether a threshold exists. Any data consistent with no dose-related change in response are also consistent with a slight, nonzero dose-related change.

- The underlying presumption about shape of dose-response curve is different for cancer and noncancer
  - Default for cancer risk assessment –linear extrapolation (based on DNA-reactive MOA)
  - Default for noncancer – nonlinear or threshold extrapolation (based on non-DNA reactive MOA)
  - This results in a different burden of proof depending on the endpoint.

Typical Dose Response Approaches

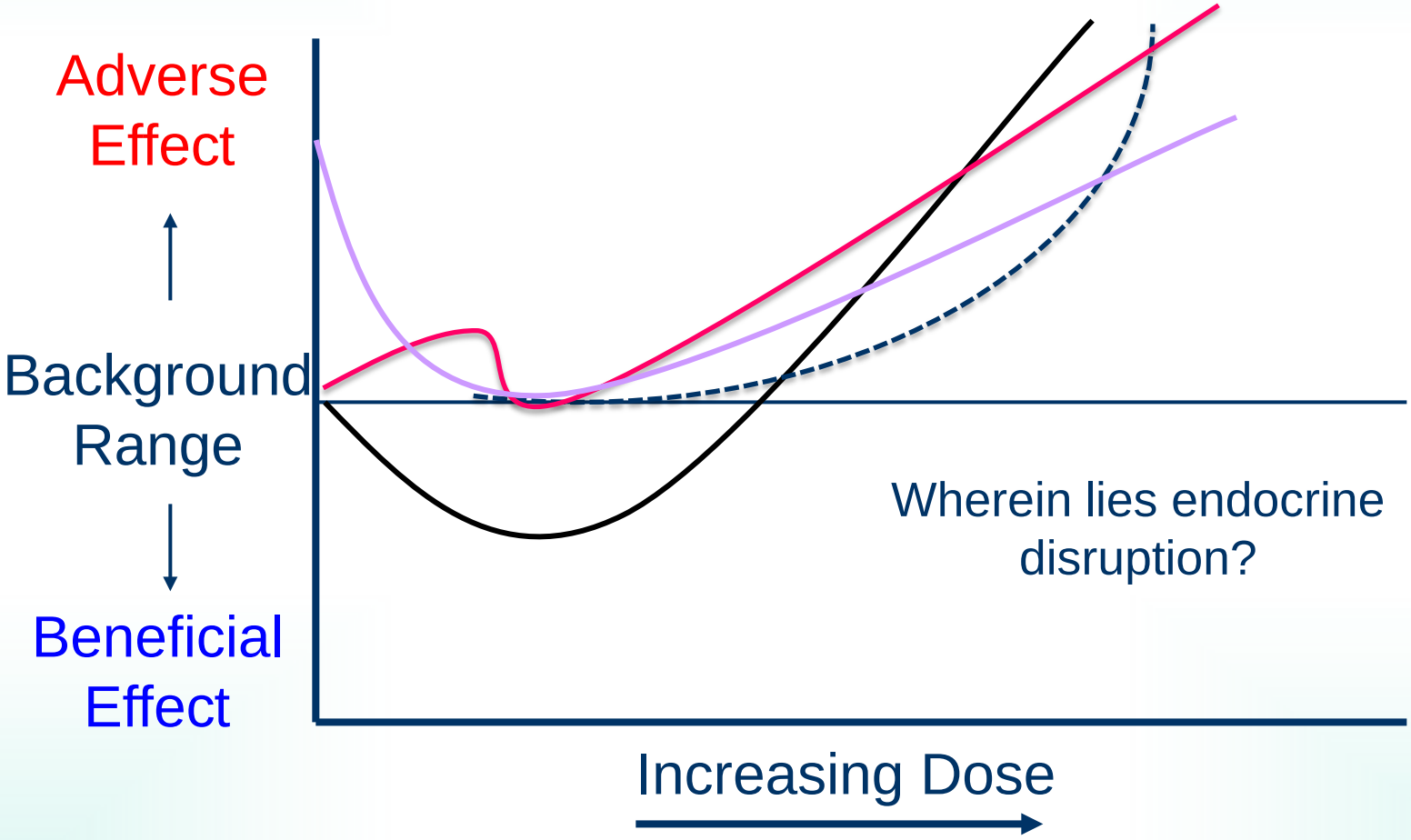


- No Observed Adverse Effect Level (NOAEL)
- Lowest Observed Adverse Effect Level (LOAEL)
- Benchmark dose (BMD)
- Benchmark dose lower limit (BMDL)
- UF = Uncertainty Factor
- RfD/C = Reference Dose/Concentration
- RSD = Risk Specific Dose

But Then Again… A Variety of Possibilities

Essentially (–), Hormesis (–), Toxicity (---)

Nonmontonic (–)



ILSI-IPCS-EPA
Mode of Action Framework

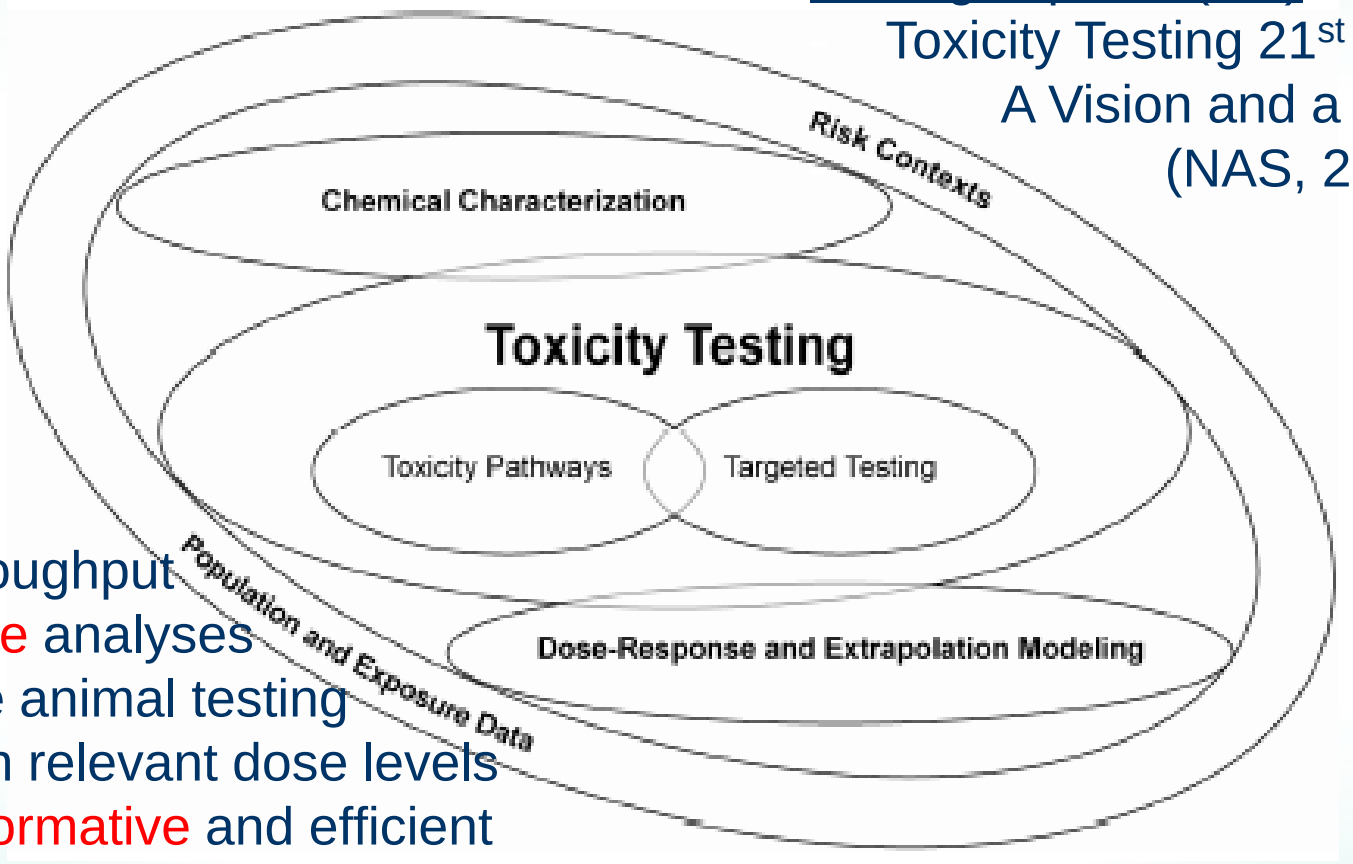
- Postulated Mode of Action
  - Identify sequence of key events on path to critical effect
- Experimental Support
  - Concordance of dose-response for key events for critical effect
  - Temporal relationships for key events & critical effect
- Biological Plausibility & Coherence
- Strength, Consistency & Specificity
- Other Modes of Action
- Identify Uncertainties
- Conclusion

Various publications over 15 years

The Future: Toxicology 21--- Systems Biology-based Toxicology Testing?

Driving impetus (US)

Toxicity Testing 21st Century: A Vision and a Strategy (NAS, 2007)



The Vision

- Cheaper
- High throughput
- Predictive analyses
- Minimize animal testing
- Focus on relevant dose levels
- More informative and efficient
- Characterize human variability
- Improve scientific basis of risk assessment
- Human cells – minimal interspecies extrapolation

Some Risk Assessment Uses of Systems Biology

- Hazard characterization:
  - Hypothesis generation for AOPs/MOAs (maturing)
  - Hypothesis testing of AOPs/MOAs (developing)
  - Endpoint identification (developing)
- Dose-response assessment:
  - Characterize dose-response on biomarker data (developing)
  - Decreased need for low dose extrapolation (developing)
  - Reduced extrapolation across species (immature)
- Exposure assessment
  - Use biomarkers of effect to combine exposures (immature)
  - High-throughput exposure assessments (EPA's ExpoCast program); RAIDAR and USETOX models – immature

Biomarker Applications

- Biomarkers of exposure
  - Quantify/verify exposure
  - Medical monitoring (intervention)
  - Cross-species extrapolation (kinetics)
- Biomarkers of effect
  - Medical monitoring (recovery and long-term effects)
  - Cross-species extrapolation of toxicodynamics
  - Evaluate mode of action hypotheses/help characterize AOP
  - Immediate precursors for dose-response
  - Low-dose response characterization
  - Mechanistic modeling
- Biomarkers of Susceptibility
  - Identify susceptible subpopulations
  - Characterize human variability

MOA for Acrylamide

- One MOA for tumors is direct DNA damage due to glycidamide.
- Other tumor MOAs are growth stimulation, oxidative stress and genotoxicity other than mutagenicity due to acrylamide.
- Mutagenicity and genotoxicity are only seen at doses higher than those that caused tumors. However, unmeasured mutagenicity might be occurring at low doses.
- Tumors are generally benign, occur late in life, and are more often in hormonally-active organs. Such tumor appearance is more consistent with manners of tumor formation that are different from direct mutation.

Biomarkers of Effect

Bowyer et al., 2008. Subchronic acrylamide exposure in Fischer 344 rats.

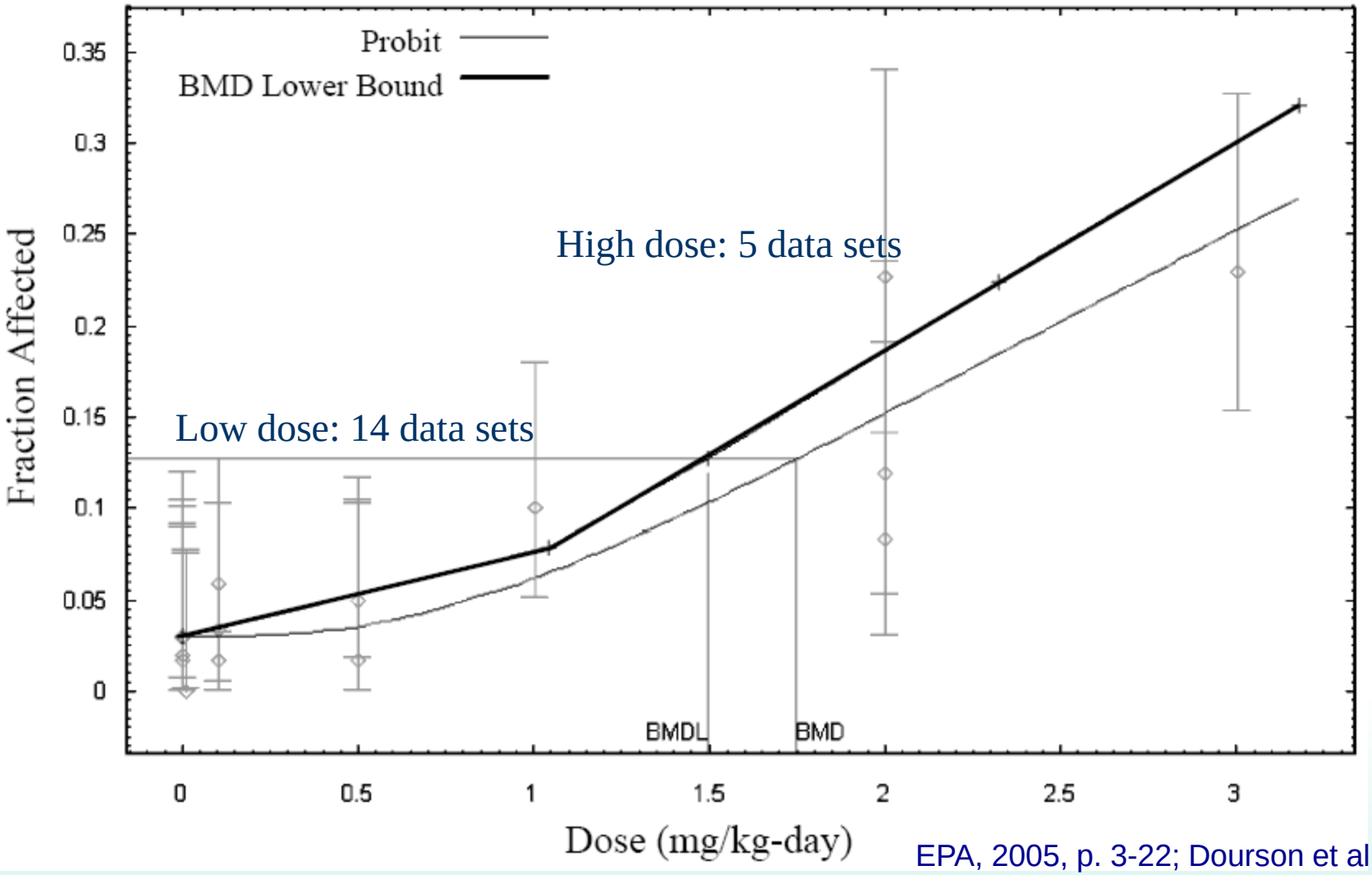
Region	Gene Expressed	Expression Levels		
		% Relative to Control[a]	Relative to GADPH	P value[d]
Thyroid	Glyceraldehydephosphate dehydrogenase (GAPDH)	83±12%	NA	NA
Thyroid	Thyroglobulin	102±18%	54.6-fold	0.97
Thyroid	Thyroid peroxidase	102±18%	0.278-fold	0.77
Thyroid	Sodium iodide symporter	142±22%	0.0218-fold	0.12
Thyroid	Type I 5′-deiodinase	142±38%	0.295-fold	0.48
Thyroid	Type II 5′-deiodinase	189±33%	0.0181-fold	0.034
Thyroid	Type III 5′-deiodinase	113±18%	0.00139-fold[b]	0.53
Thyroid	Mki67	109±14%	0.0619-fold	0.71
Pituitary	Thyroid stimulating hormone β	108%	12.31	0.30
Pituitary	Thyroid hormone receptor α	103%	8.53	0.57
Pituitary	Thyroid hormone receptor β	109%	8.74	0.73

Statistically significant

The authors think this argues against a hormone MOA, but does it?

Dual Mode of Action (MOA)

Probit model fitted to pooled-all thyroid tumor data shows different slopes between low & high doses.



EPA, 2005, p. 3-22; Dourson et al., 2008

Summary for Acrylamide MOA

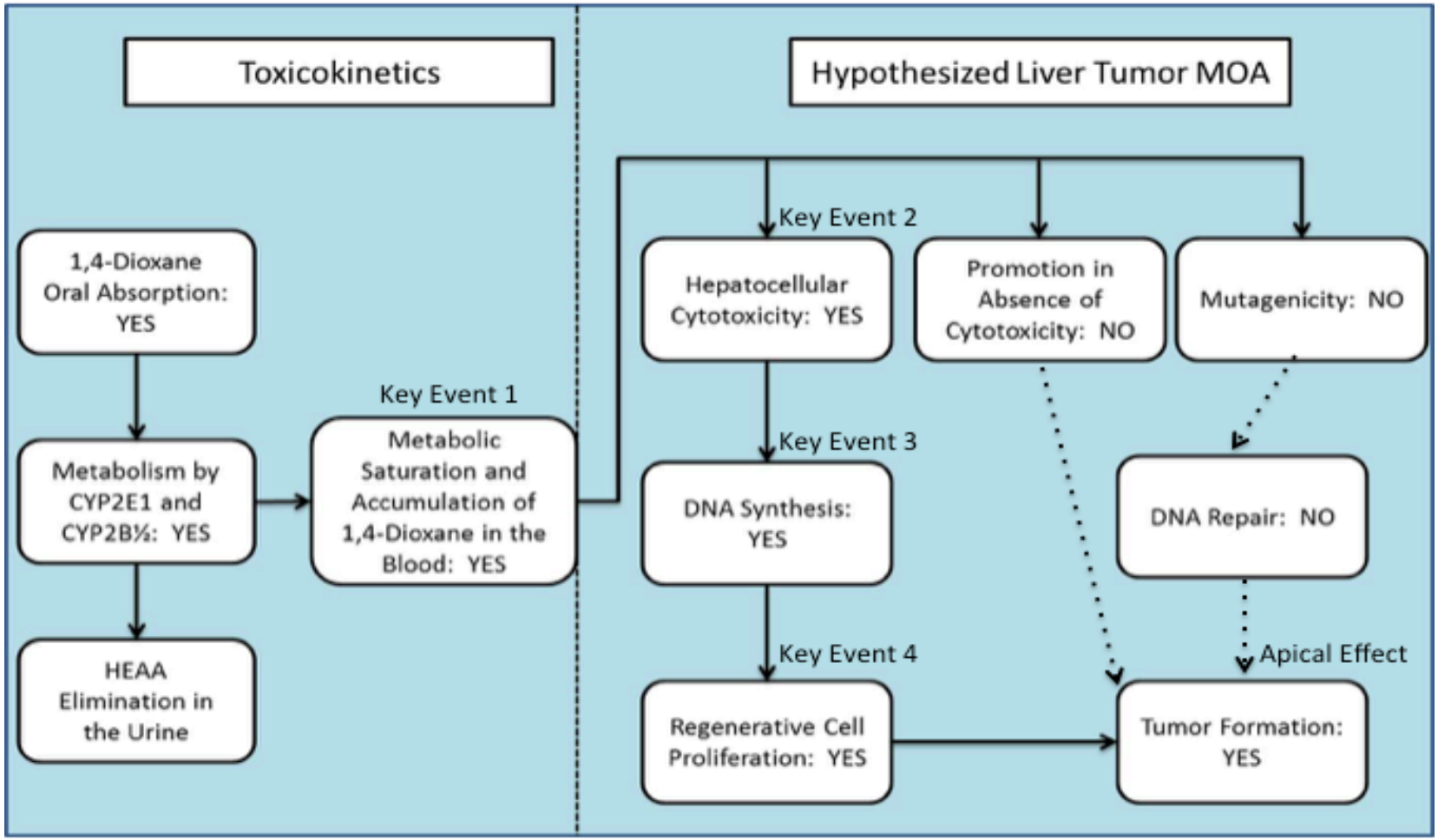
- **The weight of scientific evidence supports both a mutagenic and non-mutagenic manners of tumor formation are likely to contribute to thyroid tumors.**

- **A multiple MOA dose response assessment based on EPA (2005, page 3-22) and EPA (1998) is suggested for the management of exposures associated with this chemical.**

MOA for 1,4-Dioxane Liver Tumors

- Data from two mouse cancer bioassays, one 13-week mouse study, and seven rat cancer bioassays, show toxicity in the rodent liver.
- Observed liver toxicity is related to metabolic saturation.
- 1,4-dioxane is negative for mutagenicity and DNA repair, but does show DNA synthesis.
- Appearance of liver tumors occurs in species/strain with a high background incidence.

Figure 1. Mode of Action (MOA) for 1,4-Dioxane Induced Liver Tumors



EPA, 2013 and adapted by Dourson et al., 2014

EPA (2013) Figure 3-2. Plasma 1,4-dioxane levels in rats following i.v. doses of 3-5,600 mg/kg

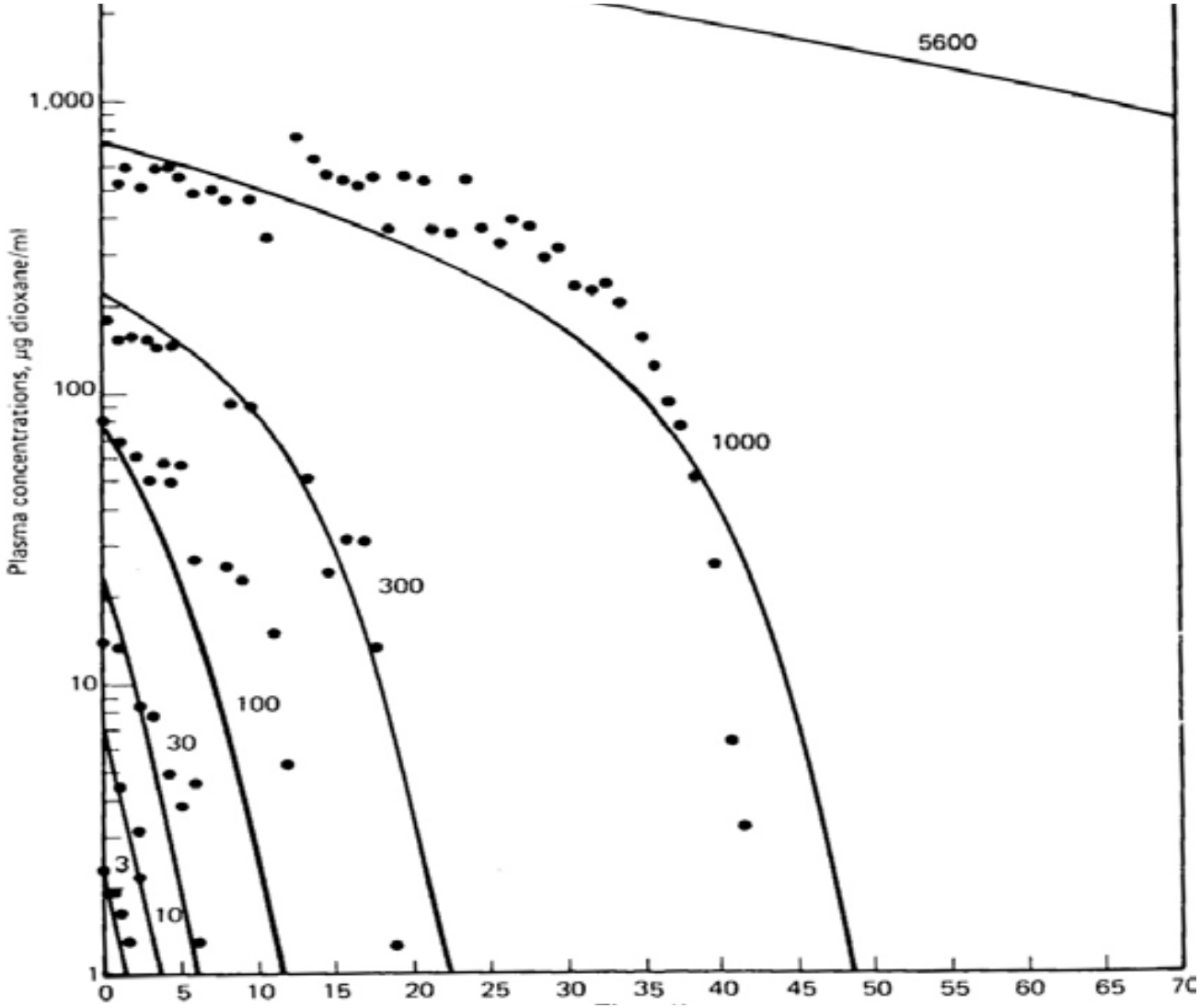
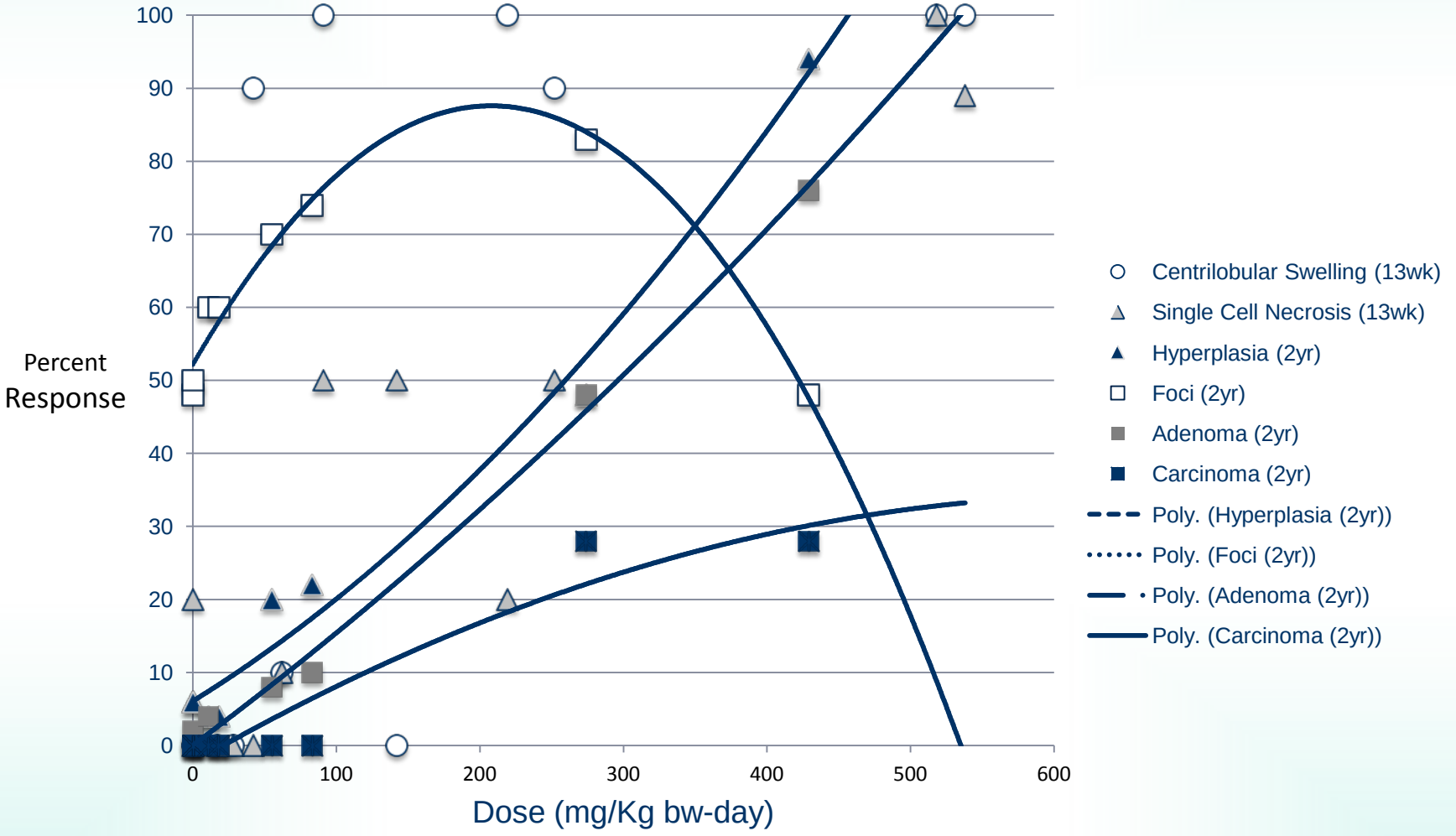


Figure 3. Pooled Incidence of 6 effects in F344 Male and Female Rats Given 1,4-Dioxane for either 13 Weeks or 2 Years. 13 Week doses have been adjusted to chronic equivalents (JBCR, 1990).



Alliance for Risk Assessment (*ARA*, 2015)

Summary for 1,4-Dioxane MOA

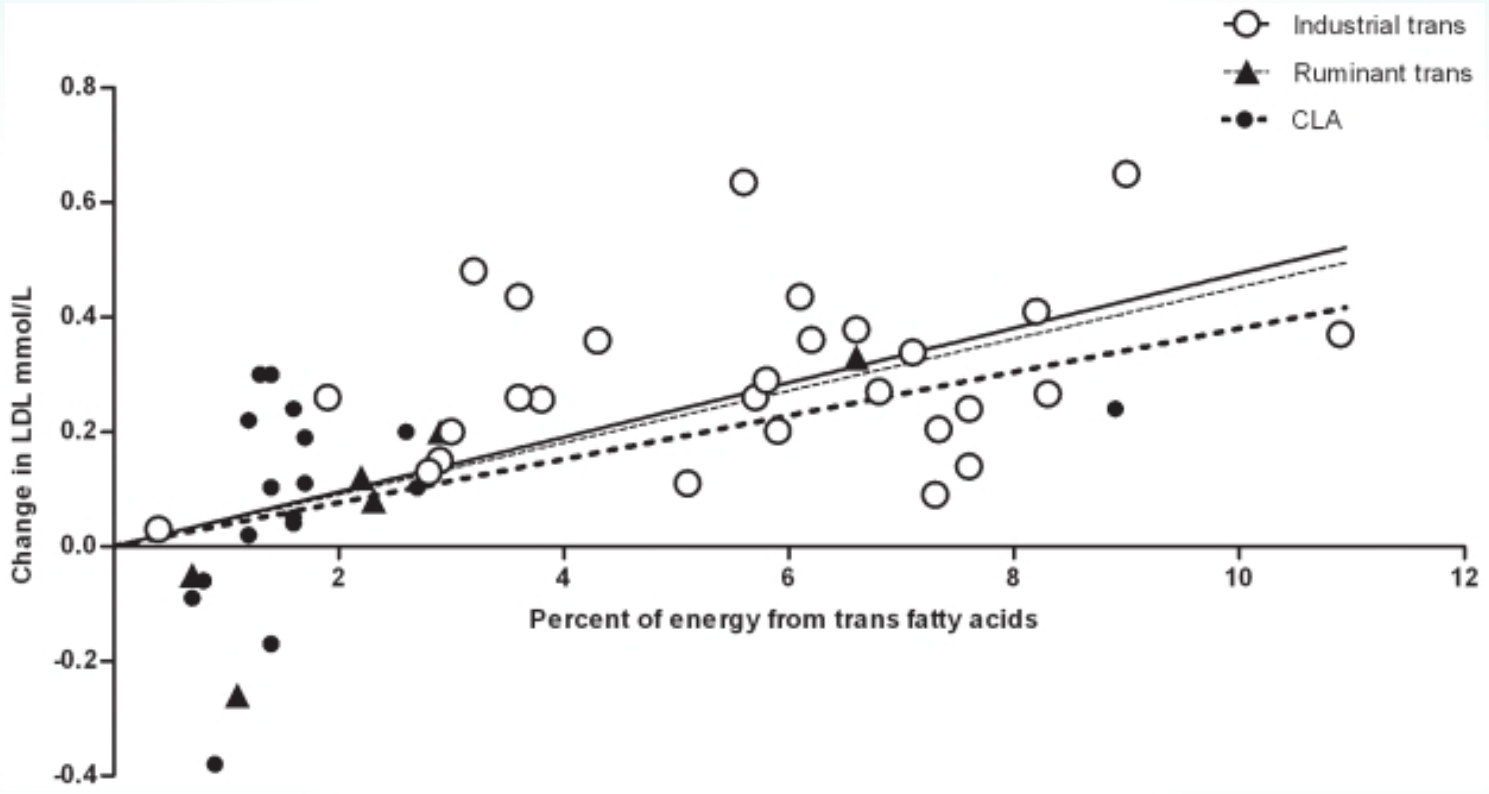
- Mutations induced by 1,4-dioxane, if any, are not a key event in tumor formation.
- The re-read of the mouse liver slides of the NTP (1978) clearly shows noncancer liver toxicity proceeding tumor formation.
- Metabolism saturates at oral doses above 100 mg/kg-day.
- 1,4-Dioxane is the toxic moiety.
- 1,4-Dioxane causes a regenerative hyperplasia in rat and mouse liver ahead of liver tumors in both dose rate and time.

MOA for Trans Fatty Acids

- Bringing a risk assessment perspective to a macronutrient
  - More variables than epidemiology of industrial chemicals
  - Studies generally not designed to do risk assessment
  - With what is the TFA replaced? Most replacements (SAT, *cis*-MUFA, PUFA) are not neutral – some are beneficial.
  - If rest of diet held constant, total energy is not constant.
  - Substantial variability across studies in fatty acid distribution

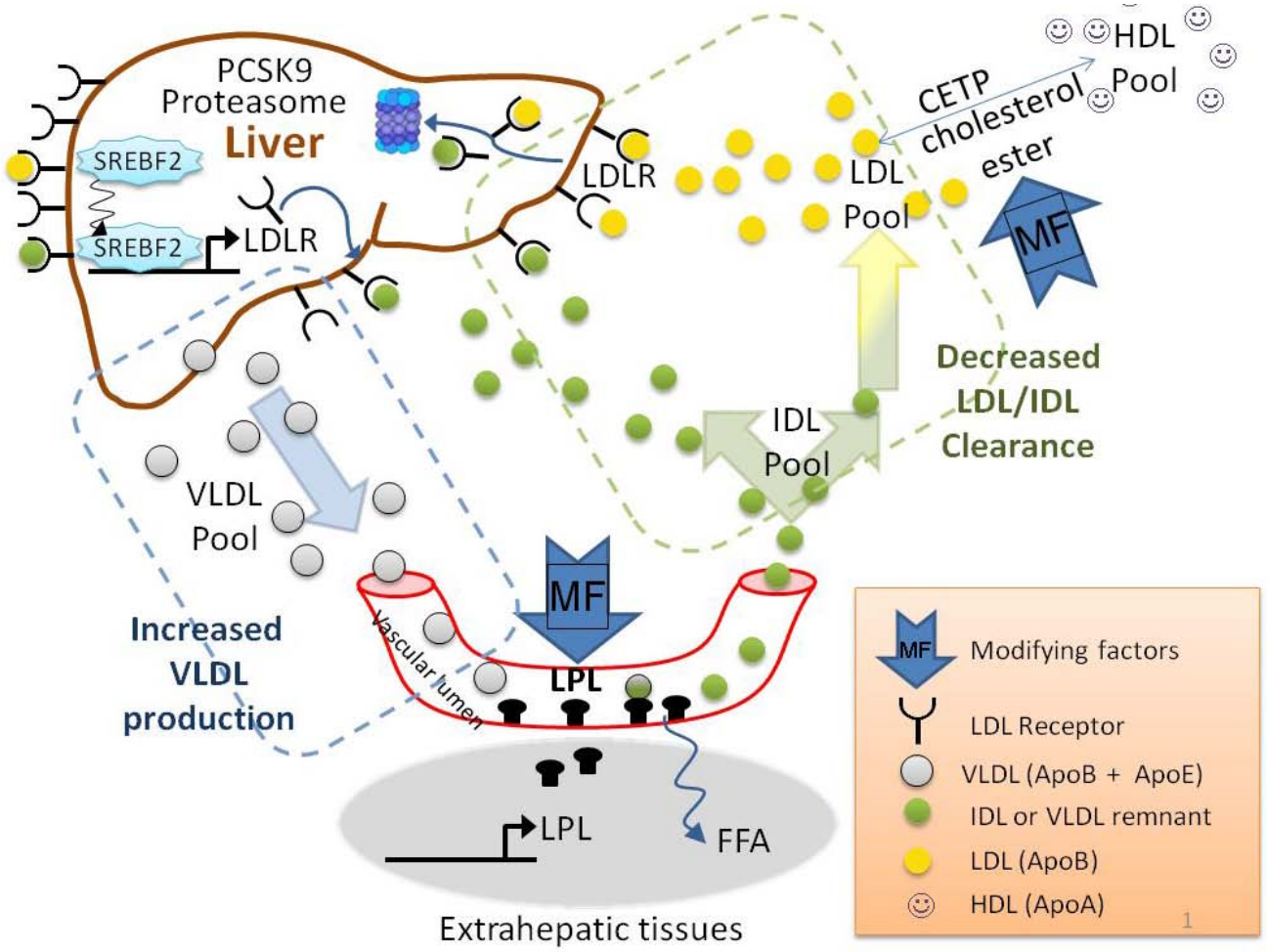
- Focus is on LDL based on the FDA notice, recognizing that coronary heart disease is *much* more complex.

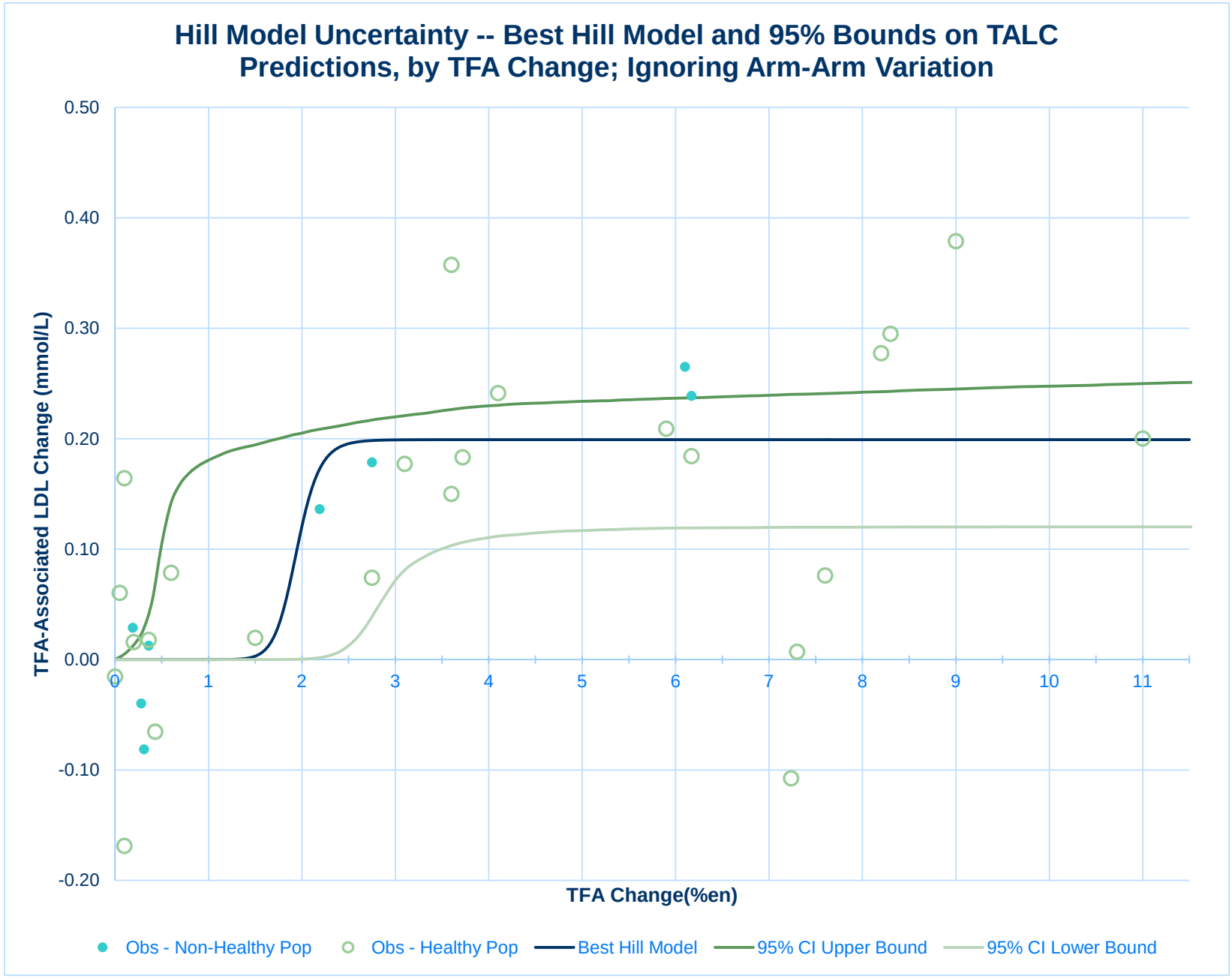
- What is the shape of the curve relating TFA exposure and LDL-cholesterol levels? Is there a threshold for adverse effect?



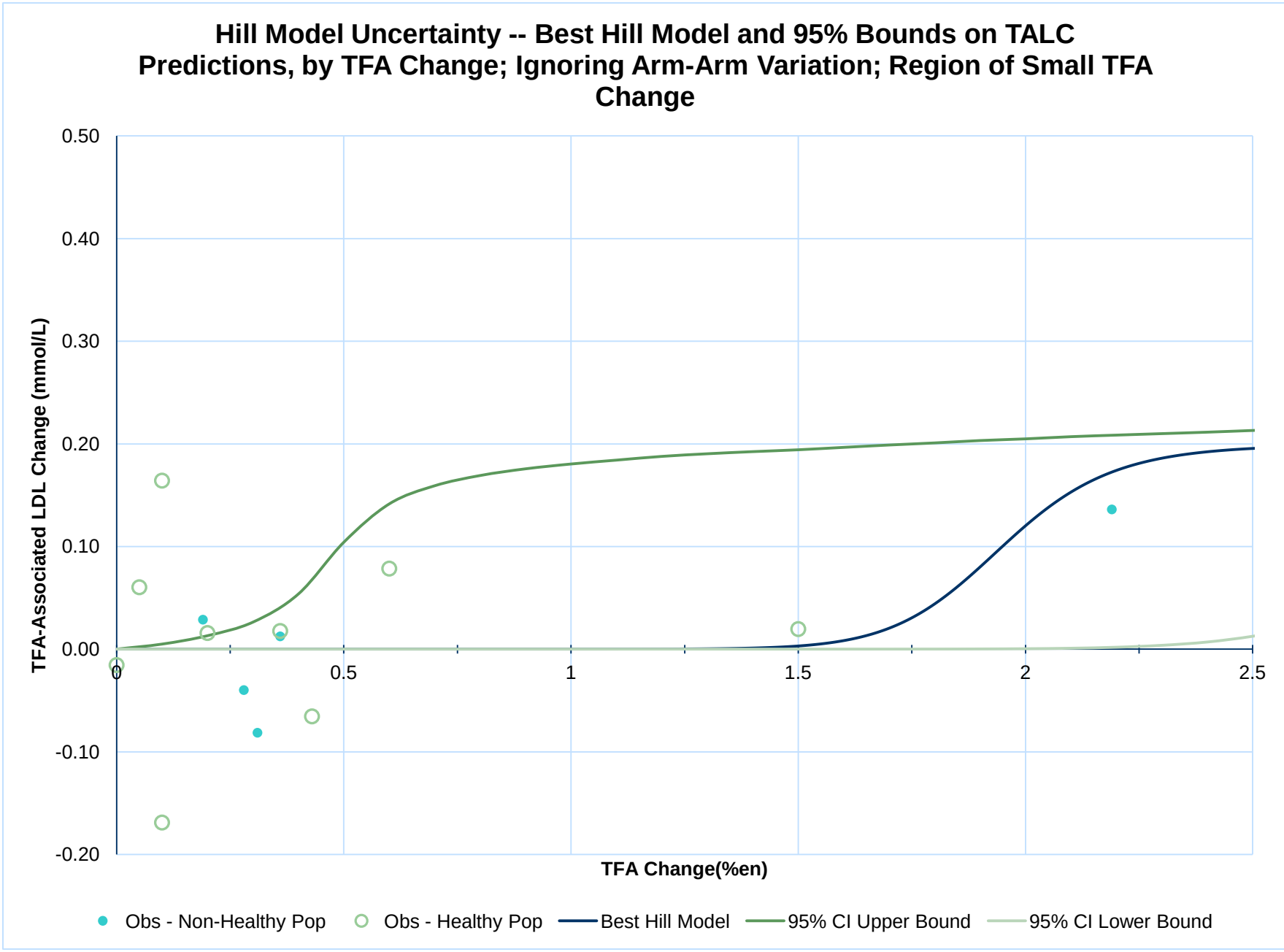
Brouwer et al. (2010): Change in TFA and LDL vs. cis-MUFAs diet; 39 studies

Biology of LDL Control

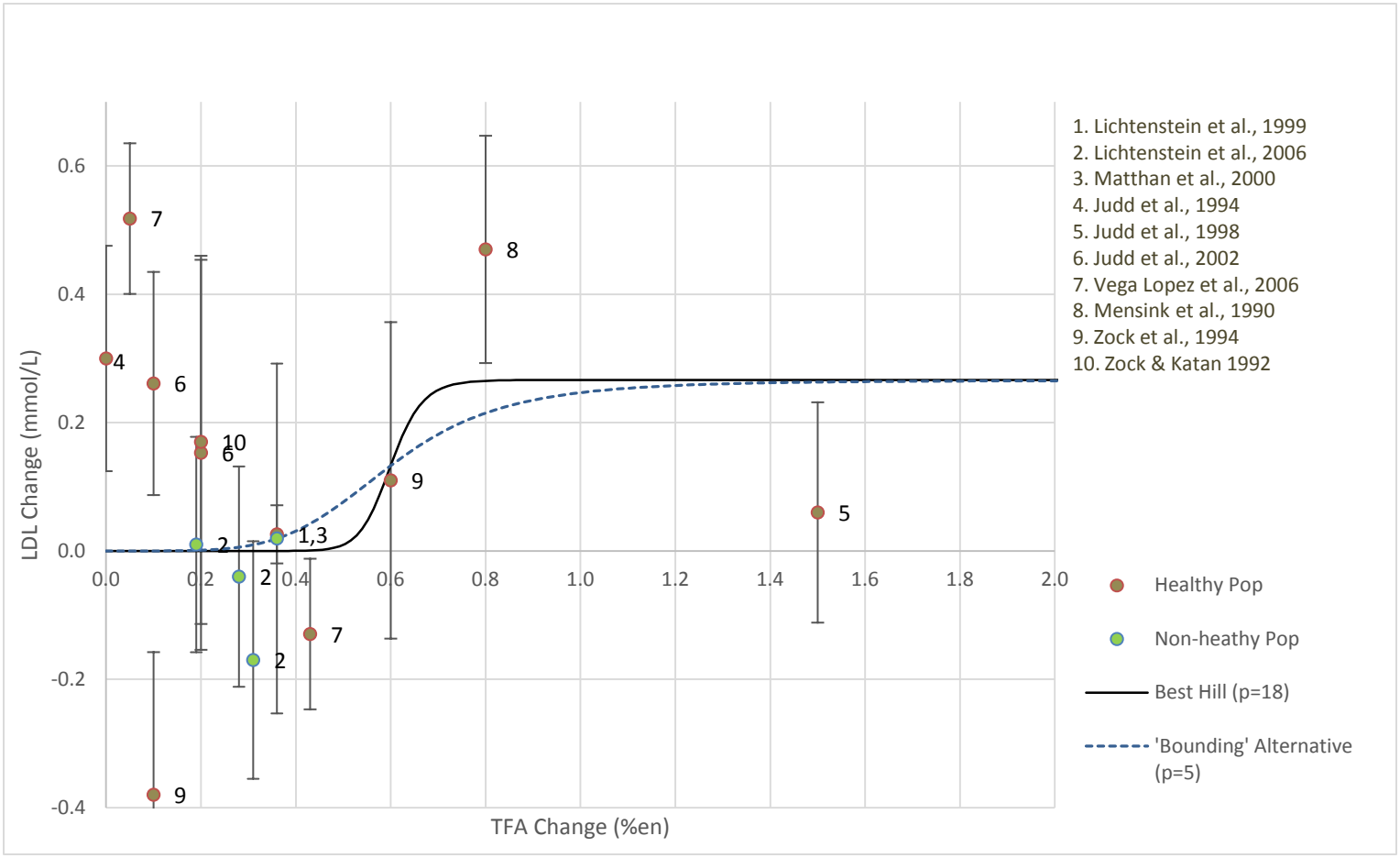




Haber et al., 2015


Hill Model Uncertainty -- Best Hill Model and 95% Bounds on TALC Predictions, by TFA Change; Ignoring Arm-Arm Variation; Region of Small TFA Change
TFA-Associated LDL Change (mmol/L)
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
0
0.5
1
1.5
2
2.5
TFA Change(%en)
Obs - Non-Healthy Pop
Obs - Healthy Pop
Best Hill Model
95% CI Upper Bound
95% CI Lower Bound

Low-Dose Studies with Error-bars



Haber et al., 2015

Summary for TFAs

- **Elevated LDL levels result from either:**
  - **Increased LDL production**
  - **Decreased LDL clearance**
- **A substantial database supports this MOA, although the key events are likely to be interdependent, rather than sequential.**
- **Both key events are *functions of non-linear biological processes* including rate-limited pharmacokinetic clearance, receptor mediated transcription and both positive and negative feedback loops**

Summary

- **Mode of action (identification of key & obligatory steps) *is not …*Mechanism of action (more detailed understanding at biochemical & molecular level), *is also not …*Adverse Outcome Pathway.**
- **Mode of Action is sufficient for risk assessment purposes.**
- **Defining sufficiency for Mode of Action is a judgment call by experts and incorporates data from multiple sources.**
- **Peer review is a necessary part of the overall determination of a chemical's Mode of Action.**