



TERA

TOXICOLOGY EXCELLENCE FOR RISK ASSESSMENT

Mode of Action and Dose-Response Evaluation of the Effect of Partially Hydrogenated Oils on LDL-Cholesterol

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Background

The FDA published a *Federal Register* notice tentatively determining that PHOs are no longer GRAS when used in food [Docket No. FDA-2013-N-1317]. Their rationale states that:

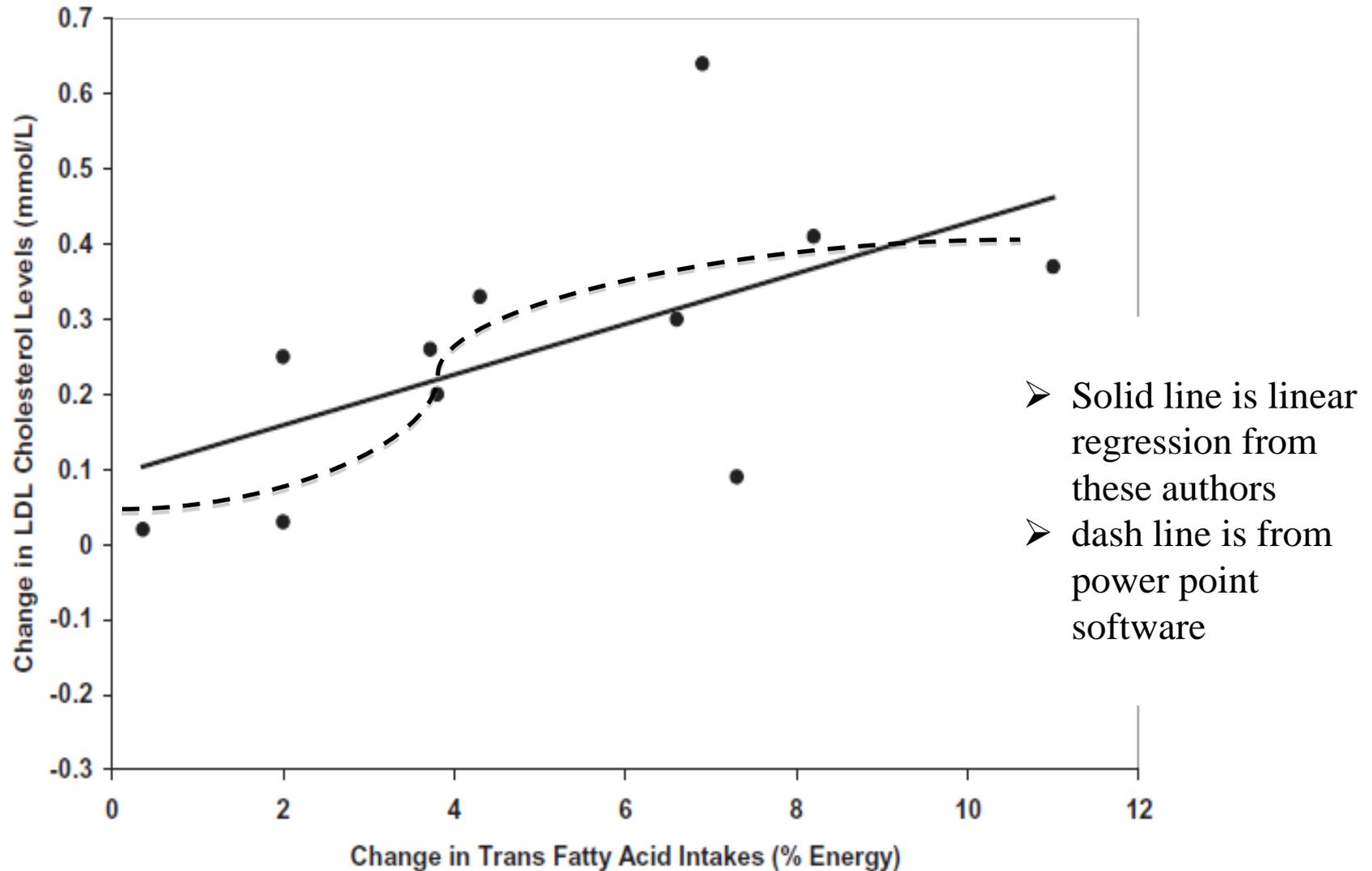
- Trans-fatty acids (TFA) affect lipid metabolism and pro-inflammatory effects, causing dose-dependent increases in coronary heart disease (CHD) events in humans.
- There is no threshold intake for industrial-produced TFA (iTFA) that would not increase an individual's risk of CHD.
- Therefore, the determination is based on evidence that the consumption of PHOs (the primary source of TFAs) could be harmful under any condition of use in food.

Disclosure

- TERA is a 501(c)3 nonprofit organization. Our mission 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.
- This work was funded by the ILSI North America PHO Task Force.
 - Mike Dourson's travel to the colloquium was paid by SOT
- ILSI North America is a public, nonprofit foundation that provides a forum to advance understanding of scientific issues related to the nutritional quality and safety of the food supply by sponsoring research programs, educational seminars and workshops, and publications. ILSI North America receives support primarily from its industry membership.
- Members of the PHO Task Force have seen interim versions of this work and provided scientific comments, but the conclusions are those of the TERA team

Trumbo and Shimakawa 2011

(9 studies, including 2 with 2 data points each)



Outline

- Mode of action evaluation – importance and approach
- Focus is on LDL based on the FDA notice, recognizing that CHD is *much* more complex.
- Dose-response analysis
 - Comparison of approach with published regressions
 - Meta regression – comparison with regression and meta-analysis

Key Questions

- What is the mode of action (MOA) and how does it help us understand interpolation or even low dose extrapolation?
- What does an analysis of variability in the human data tell us?
- What is the shape of the curve relating TFA exposure and LDL-cholesterol levels? Is there a threshold for adverse effect?



Challenges

- Bringing a risk assessment perspective to a nutritional question
 - Many more variables than epidemiology studies of industrial chemicals
 - Studies generally designed to answer yes/no questions – not with risk assessment or dose-response in mind
- With what is the TFA replaced?
 - Most replacements (SAT, 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
- Not all TFAs are equivalent – e.g., stearic acid, vaccenic acid

MOA EVALUATION

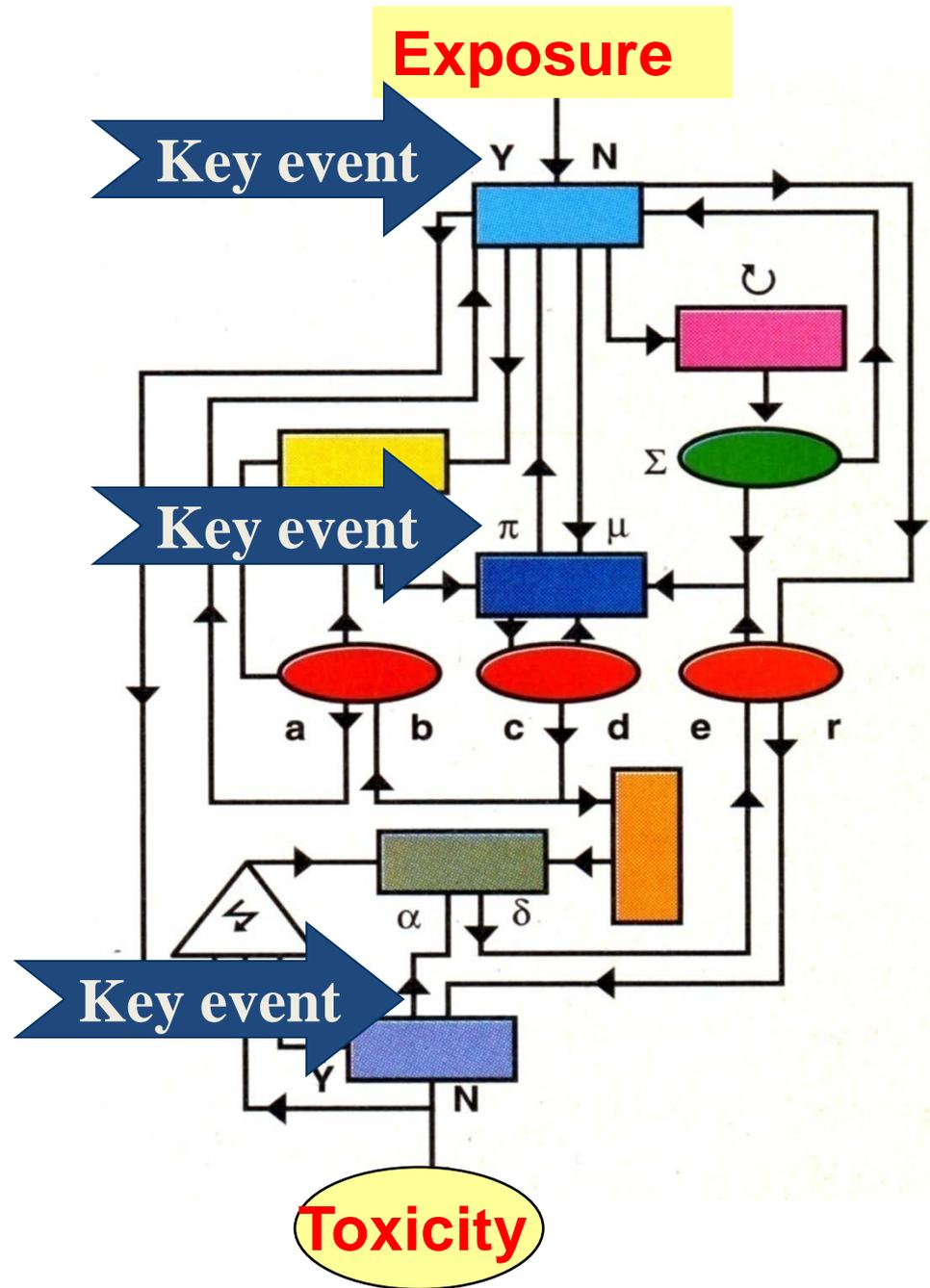
Mode of action

identification of **key events**, that is obligatory steps

... is not ...

Mechanism of action

more detailed understanding at biochemical & molecular level



Critical Aspects of MOA Analysis

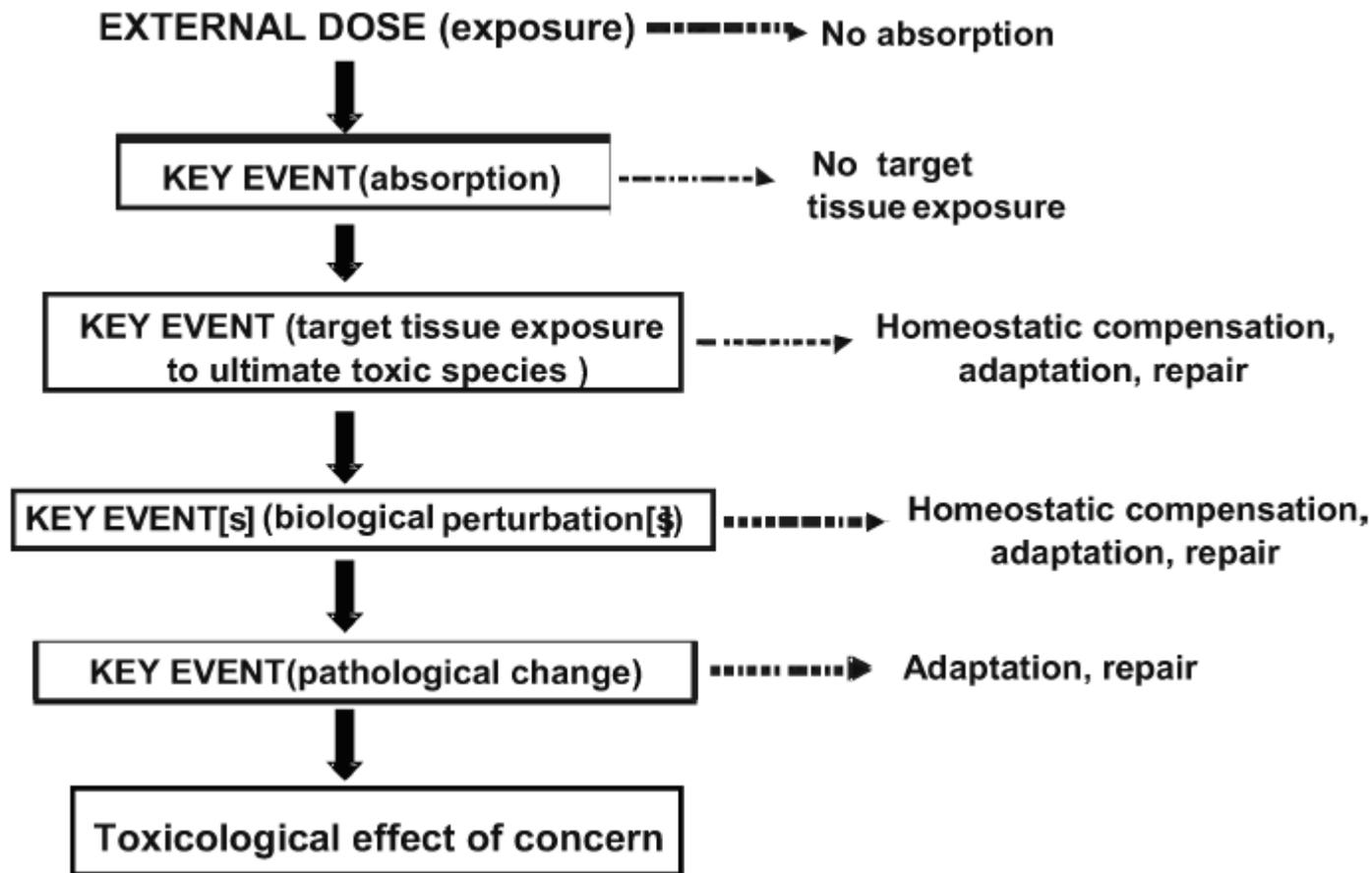
- Identify key events by which TFAs increase LDL levels
- Evaluation of causality (modified Hill criteria)
- Evaluation of human relevance
- Shape of the dose-response curve in the region of interest, including determination of thresholds for adverse effect, if appropriate

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 with that for critical effect
 - Temporal relationships for key events & critical effect
- Biological Plausibility & Coherence
- Strength, Consistency & Specificity
- Other Modes of Action
- Identify Uncertainties
- Conclusion

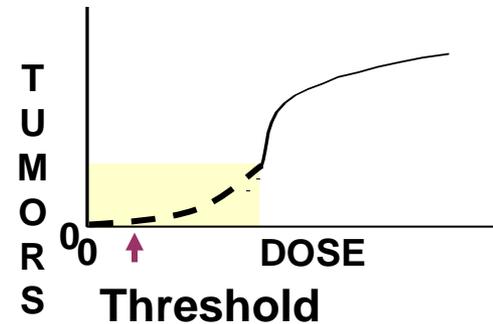
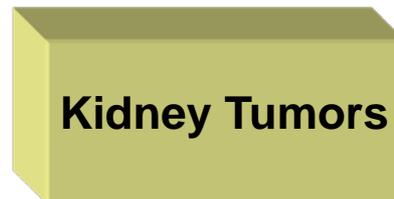
Key Events Dose-Response Framework – KEDRF





Shape of Dose-Response for Key Events...

...Determines that for Apical Effect



Key Event



Nonlinearity / Threshold



Dose – Response and Temporality

<i>Dose</i> (ng/kg bw/day)	<i>Temporal</i>		
	<i>Key event 1</i>	<i>Key event 2</i>	<i>Key event 3</i>
0.2 (2 ppm)	+ 4 weeks	+ 52 weeks	
1 (10 ppm)	++ 4 weeks	++ 52 weeks	+ 107 weeks
4 (40 ppm)	+++ 4 weeks	+++ 13 weeks	++ 52 weeks

Dose-Response

MOA Approach for TFA

- Elevated LDL levels result from either:
 - Increased LDL production
 - Increased release of VLDL
 - Decreased triglyceride uptake and metabolism
 - Increased recycling of LDL cholesterol (CETP)
 - Decreased LDL clearance
 - Decreased LDLR transcription (SREBP)
 - Increased degradation of the LDL receptor
 - Decreased membrane “fluidity”, which interferes with receptor activity

Proposed Key Events for Increased LDL by PHOs

Primary events (Not sequential)

Contributing Events

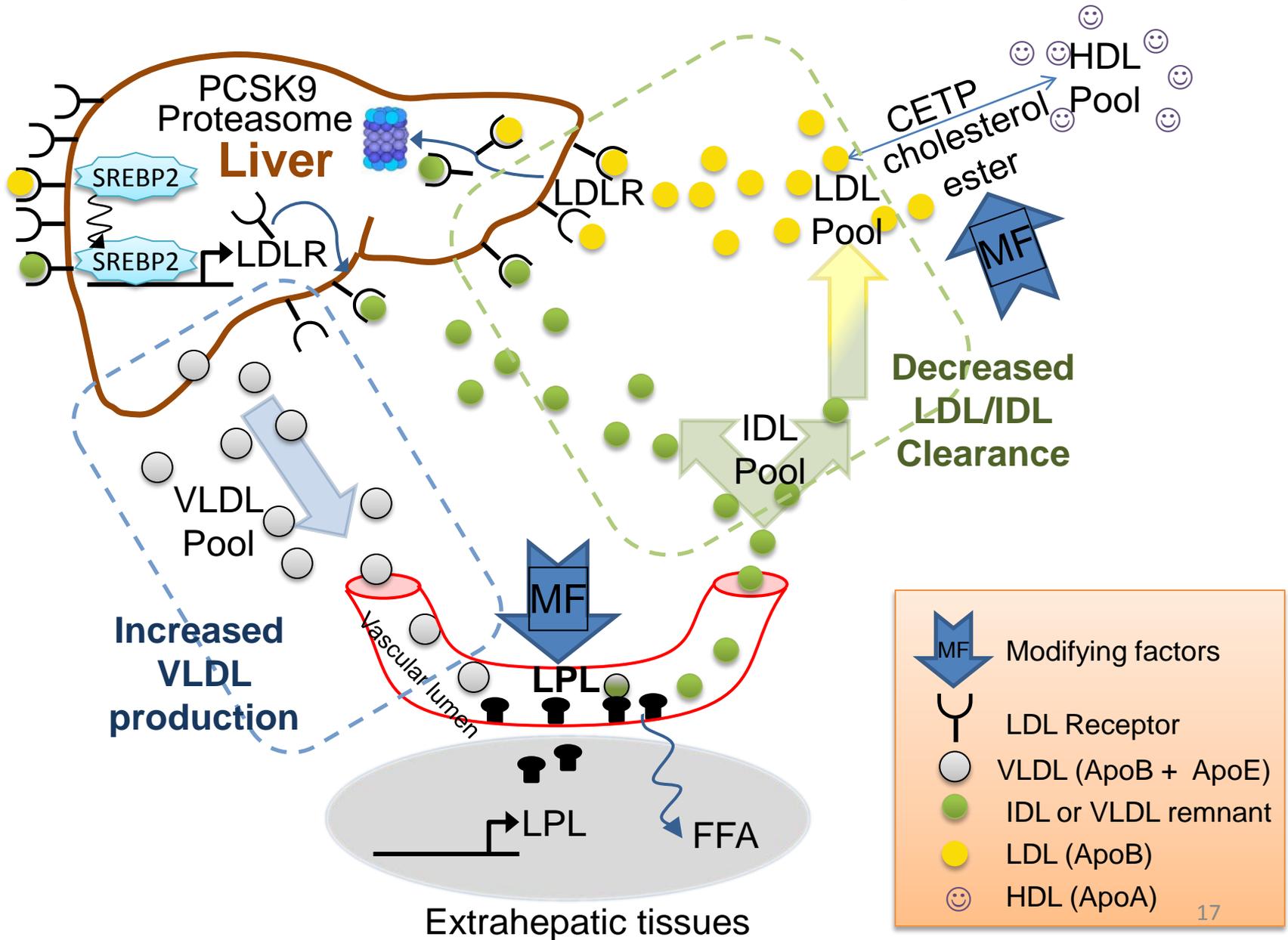
Increased VLDL Levels

- Increased lipitation of ApoB (e.g. by MTF; ApoCIII)
- Decrease proteasomal degradation of ApoB protein
- Cholesterol depletion (via SREBP transcription factors)
- Increased activity of CETB

Decreased LDL receptor activity

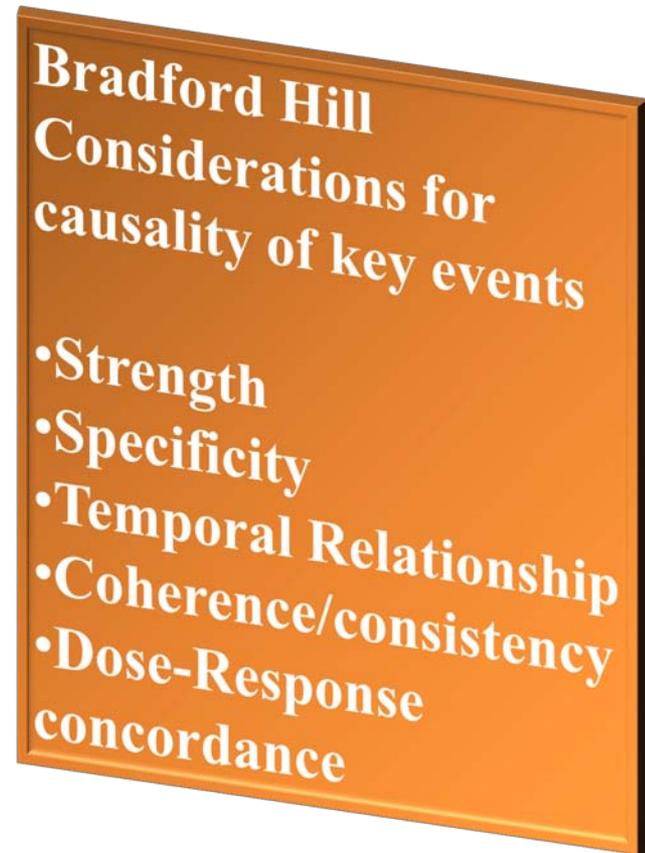
- Altered cholesterol esterfrication (ACAT)
- Decreased LDLR transcription (SREBP2)
- Decrease receptor number (↓ activity)
- Increased PCSK9 activity (degradation of LDLR)
- Altered membrane composition

MOA for Elevated LDLs by TFA



Mode of Action/Key Events Framework

- Hypothesis based
- Sequence of observable and quantifiable critical key events
- **Cause/effect relationship between key event and the apical effect is based on Bradford Hill considerations**
- Qualitative and quantitative species concordance





Data Consistent with TFA MOA

- TFAs increase LDL production
 - TFA increases the secretion rate of ApoB
 - CETP activity is significantly higher in the plasma of subjects fed trans fatty acid-rich diets.
 - LDL levels are significantly lower in animals devoid of CETP activity.
 - TFAs increase VLDL and/or ApoB secretion in vitro.
- TFAs decrease LDL receptor activity
 - Reduced LDLR expression (SERBP2)
 - Degradation of the LDL receptor (PCSK9)
 - Reduced activity due to altered membrane fluidity
 - Reduced receptor binding of IDL/LDL

Data Inconsistent with TFA MOA

- Evidence inconsistent with increased LDL production
 - High TFA diets do not always raise plasma VLDL levels and/or ApoB
 - CETP activity is not always higher in subjects fed diets high in *trans* fats.
- Evidence inconsistent with LDL receptor activity playing a key role:
 - VLDL/LDL production contributes more to increased LDL levels than LDL clearance
 - LDL levels increase in LDLR-knock-out mice fed high trans diets. LDLR KO mice do not have a change in receptor expression
 - LDL clearance is significantly affected when LDLR activity is reduced >50%
 - Regarding reduced membrane fluidity - no conflicting evidence identified, but supporting evidence is *in vitro* and has limitations –both treatment model (some done with synthetic membranes) and nonphysiologic concentrations.
 - TFA exposure does not always decrease LDLR binding of ¹²⁵I-LDL



Dose-temporality of key events

- Dose and temporality of key events are difficult to establish.
 - Dose:
 - Few relevant dose-response data available for key events, but clear inconsistencies not found
 - *In vivo* studies in animals and *in vitro* studies also did not investigate dose-response for TFAs.
 - Dose-response data for effects of TFA on LDL-C levels also show large variability
 - Temporality:
 - Limited data – only single time points investigated
 - However, no violations of temporality were located.
 - Events underlying the key events appear to have a rapid response to TFA exposure with some occurring within hours of treatment

Relationship of Biology to Shape of the Dose-Response Relationship

- The shape of the dose-response curve in the empirical region does little to inform the shape outside the range of the data.
- Non-threshold for adverse effect:
 - No safe exposure level; risk for adverse effect increases with a single molecule.
 - Risk for adverse effect exists at all doses.
 - Presumed to apply for chemicals that interact with DNA due to stochastic assumption
- Threshold for adverse effect:
 - The number of molecules must equal or exceed the threshold before an adverse response occurs.
 - Presumed to apply for chemicals that interact with cellular processes organs/tissues that have redundancy



Shape of the Dose-Response Relationship (Cont.)

- Evidence presented as supporting non-threshold:
 - LDL levels in clinical studies can be fit by linear regression. However,
 - Authors do not appear to have tested alternative dose-response formulations.
 - Variability among the studies was not considered in the regression.
 - Individual threshold may exist but individual variability means there is no *population* threshold. However,
 - Statement is logically incorrect, since populations are composed of individuals
 - Physiological limits exist: 1-inch high adult humans anywhere?

Shape of the Dose-Response Relationship (Cont.)

- Large variability between postprandial and fasting states in normal individuals imply wide range of normal.
- Every event in the transport, utilization and clearance of TFAs appears highly regulated at gene, protein or receptor levels.
- Plasma LDL levels are readily described by non-linear, rate-limited pharmacokinetics.
- Every aspect of TFA transport, utilization & clearance appears subject to feedback regulation implying homeostasis.
- **Thus, adverse effects would occur only when homeostasis is perturbed, i.e., when a threshold is reached or exceeded.**

META-REGRESSION

Key Goals and Questions

- Expand database used for regressions, particularly in the low-dose region
- Use meta-regression to improve the accuracy of the evaluation
 - placing greater weight on the studies with less uncertainty
- Evaluate the shape of the dose-response curve, including consideration of nonlinear models
- Use the data to inform our understanding of human variability

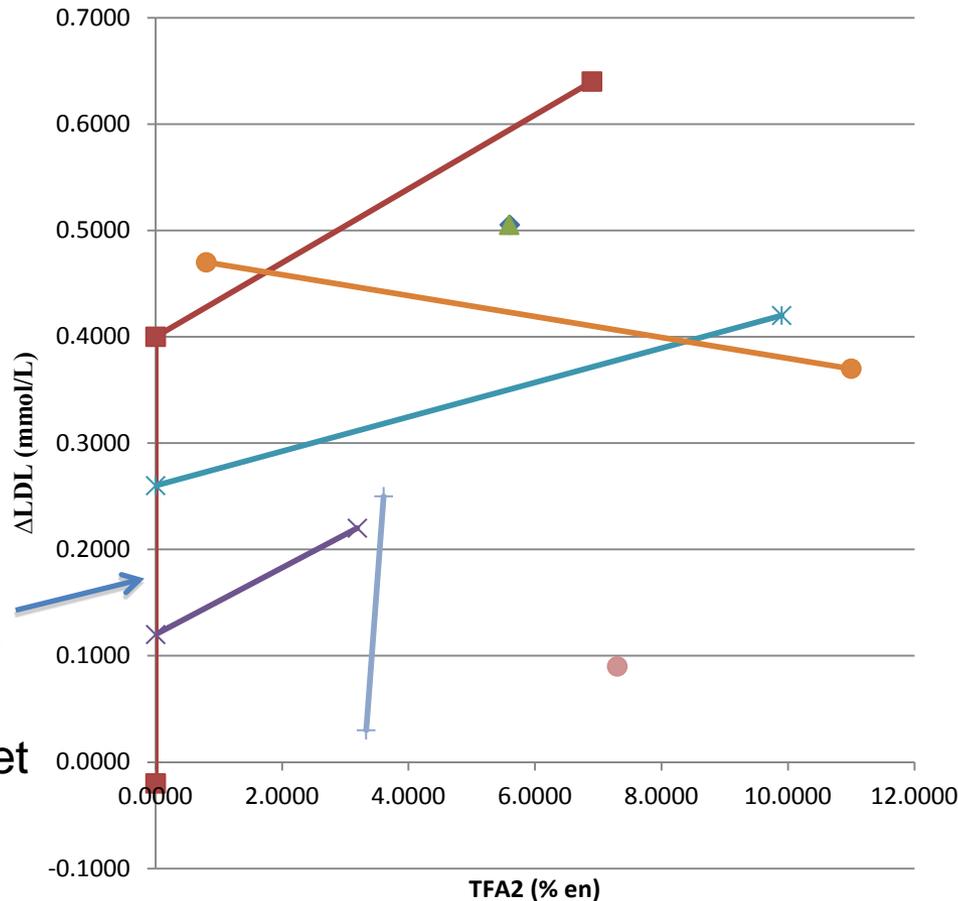


About Modeling and Shape of Dose-Response Curve

- Models might provide suggestions regarding the shape of dose response curve, but need mode of action to draw conclusions.
- Models generally are continuous and have difficulty fitting thresholds, or other biological discontinuities.
- Theoretically, zero TFA change results in zero increase in LDL, but
 - This assumes that all other "LDL influencers" are held constant.
 - Moreover, this does not suggest that a threshold for an adverse effect does not exist or that a single molecule of TFA can result in increased LDL. For example...

Change in LDL with TFA

TFA2 vs. Δ LDL

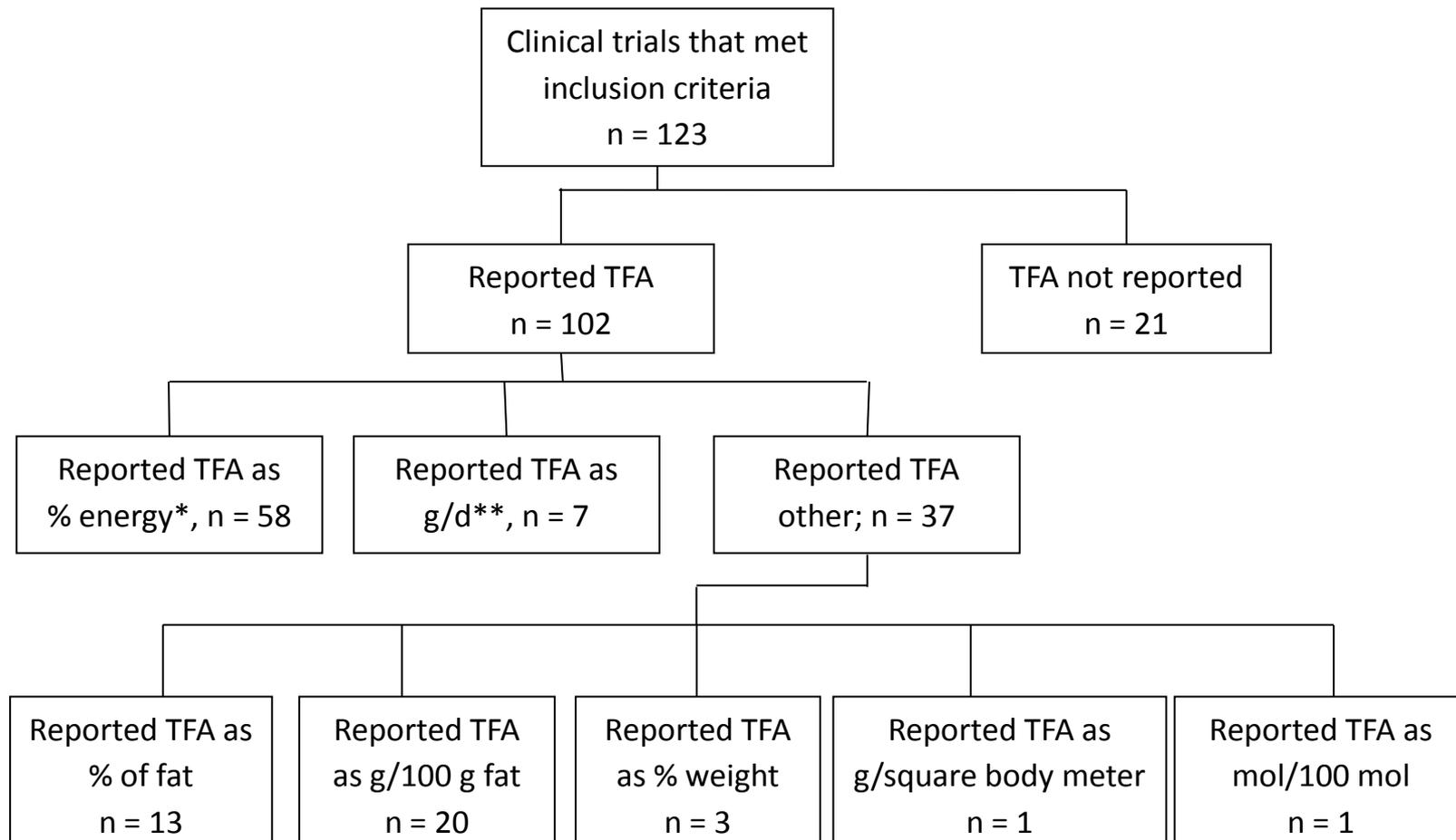


Studies: no TFA in
1st Compared Diet

- French et al 2002
- Sundram et al 1997
- Sundram et al 2003
- Sundram et al 2007
- Teng et al 2010
- Mensink & Katan 1990
- Noakes & Clifton 1998
- Lovejoy et al 2002

4 Studies: no
TFA in 2nd
Compared Diet

Clinical Trials Reporting TFA – BioFortis Evidence Map



Information provided by ILSI and BIOFORTIS



Inclusion Criteria

- Randomized controlled trials
- Measure TFA content as % en
- Are conducted over a period of time ≥ 3 weeks (21 days)
- Include, at a minimum, LDL-C as an outcome measurement
- Provided a measure of variability (SD or SE) for LDL-C

- After preliminary analyses, focused on non-ruminant, controlled diets

Comparison of Inclusion Criteria

- Ascherio – No formalized criteria provided; randomized trials that directly compared the effects of TFAs with those of isocaloric amounts of cis fatty acids
- Brouwer – Original research; reported effects of iTFA, CLA or other rTFA on LDL-C and HDL-C; randomized controlled trial with parallel, crossover, or Latin square design; **≥13 days**
- Trumbo & Shimakawa - Randomized clinical studies and prospective observational cohort studies; measured LDL-C; crossover design; **≥3 wks**; diet described and statistical analysis provided; fiber included in diet assessment

Comparison with Previous Regression Work

- Previous search criteria and inclusion/exclusion were more limited.
 - A challenge – broader inclusion criteria makes it harder to make consistent comparisons – separating impacts of other fatty acids from those of TFAs



Meta-Regression Analysis

- Method of combining studies for estimating the relationship between exposure and response
- Reduces skewing effects of random error and small sample size within individual studies
- Can provide greater accuracy

Meta-Analysis Vs. Meta-Regression

- Both combine studies with weighting by power
- Meta-analysis (e.g., Mensink, Chowdhury), addresses questions such as:
 - Does the exposure have a biological effect?
 - How large is that effect?
- Meta-analysis cannot address the dose-response questions:
 - At what dose does an effect begin to occur?
 - How big is the change in effect for a given change in dose?
- Univariate and multivariate models: can include additional study-level explanatory variables to define the dose-response curve



Our Meta-Regression Analysis

- Input data: 44 data points from 22 publications
 - Each data point reflects difference between lowest nonruminant TFA group and a “test group”
- Linear and non-linear curves (power model, Hill model)
- Possible further evaluations
 - PHOs and non-PHOs
 - Healthy vs. nonhealthy
- **Results shown here are preliminary; additional modeling work is in progress.**

Results

- Limited high-quality low-dose data
- Large variability in the input data, particularly at low TFA intake, indicating TFA level is not the primary determinant of LDL-C levels at low TFA intake.
- Hill model could explain 70% of the variability in the data.
 - Consistent with LDL receptor binding playing a key role in the MOA
- No statistical difference between Hill coefficient of 1 (Michaelis-Menten model, steep initial slope) and coefficient of 3.6 (flat initial slope).
 - Mathematical modeling cannot inform our understanding of the shape of the dose-response curve and whether a threshold exists
 - Hill coefficient of 1 is consistent with several aspects of the MOA having M-M kinetics.
 - Hill coefficient of 3.6 is consistent with threshold-like behavior for the relationship between change in TFA and change in LDL
 - **Threshold and linear models were not significantly different**

Figure 2: LDL Change vs TFA Change; Expected Study Means; for Linear Model, Best Fitting Model, and "Bounding Acceptable" Hill Model

Low Dose Region

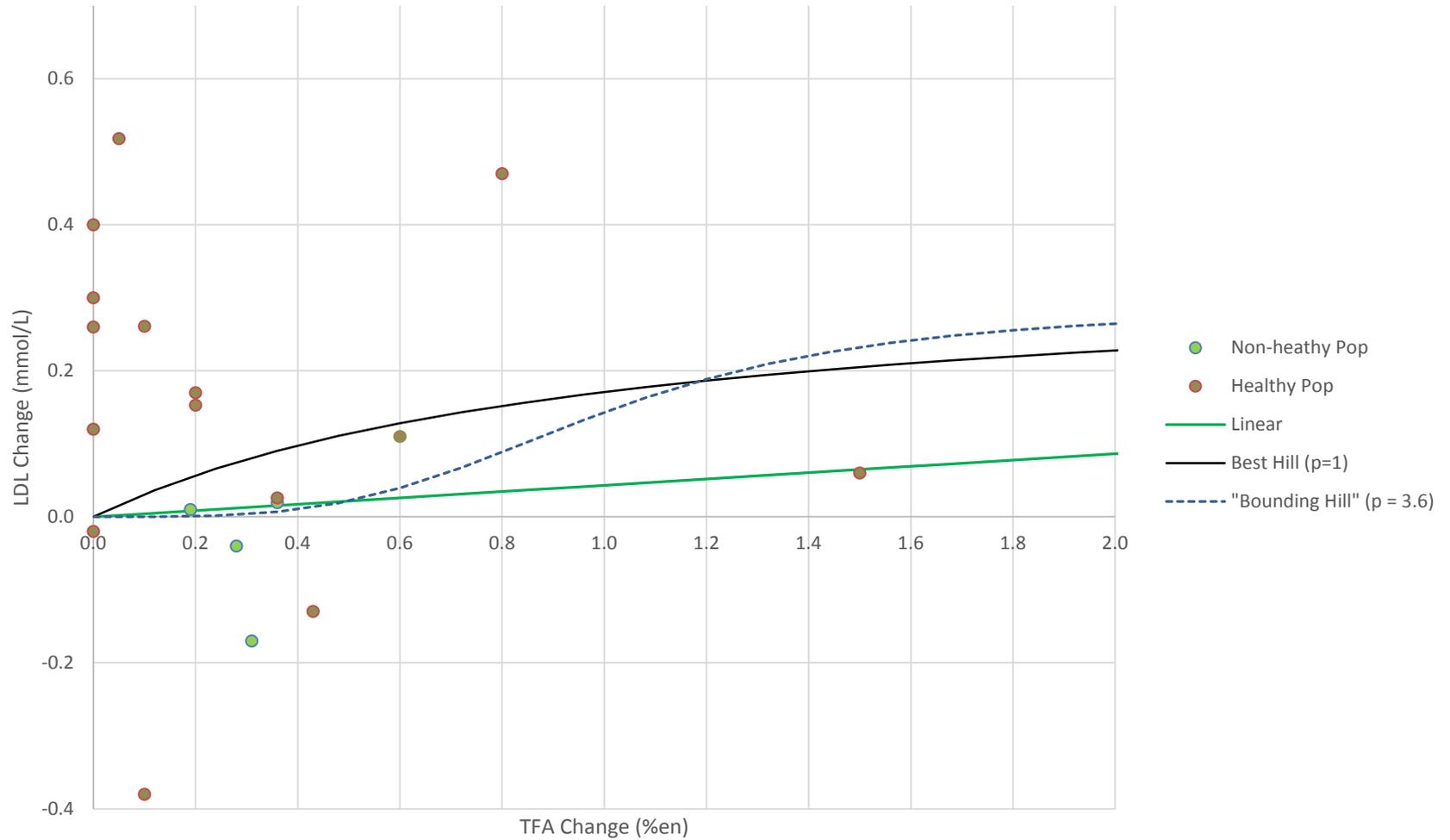
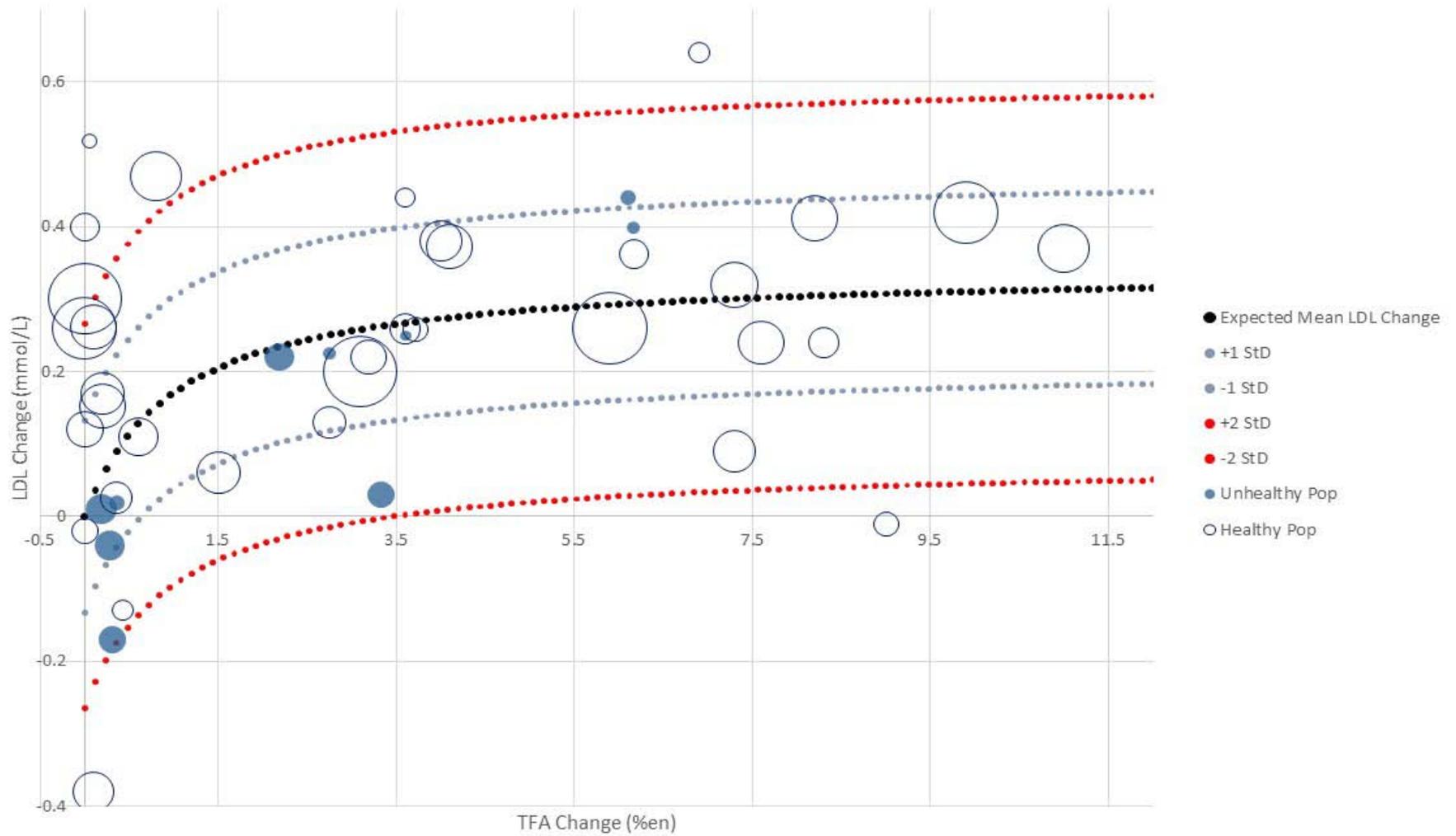


Figure 3: LDL Change vs TFA Change; Expected Study Mean and StD Intervals; for Best Fitting Model
Full Dose Region





Summary

- Data are insufficient to rigorously apply the modified Hill criteria for evaluating MOA, but a nonlinear dose-response is expected
 - Such data otherwise suggest a threshold in the dose for adverse effects,
 - Based on the high degree of regulation of pathways within a homeostatic range and large individual variability.
- Meta-regression weighs studies by degree of uncertainty; this improves our understanding of dose-response.
- Applying risk assessment techniques to nutritional issues can aid in understanding and offer new perspectives.

Acknowledgements

TERA team

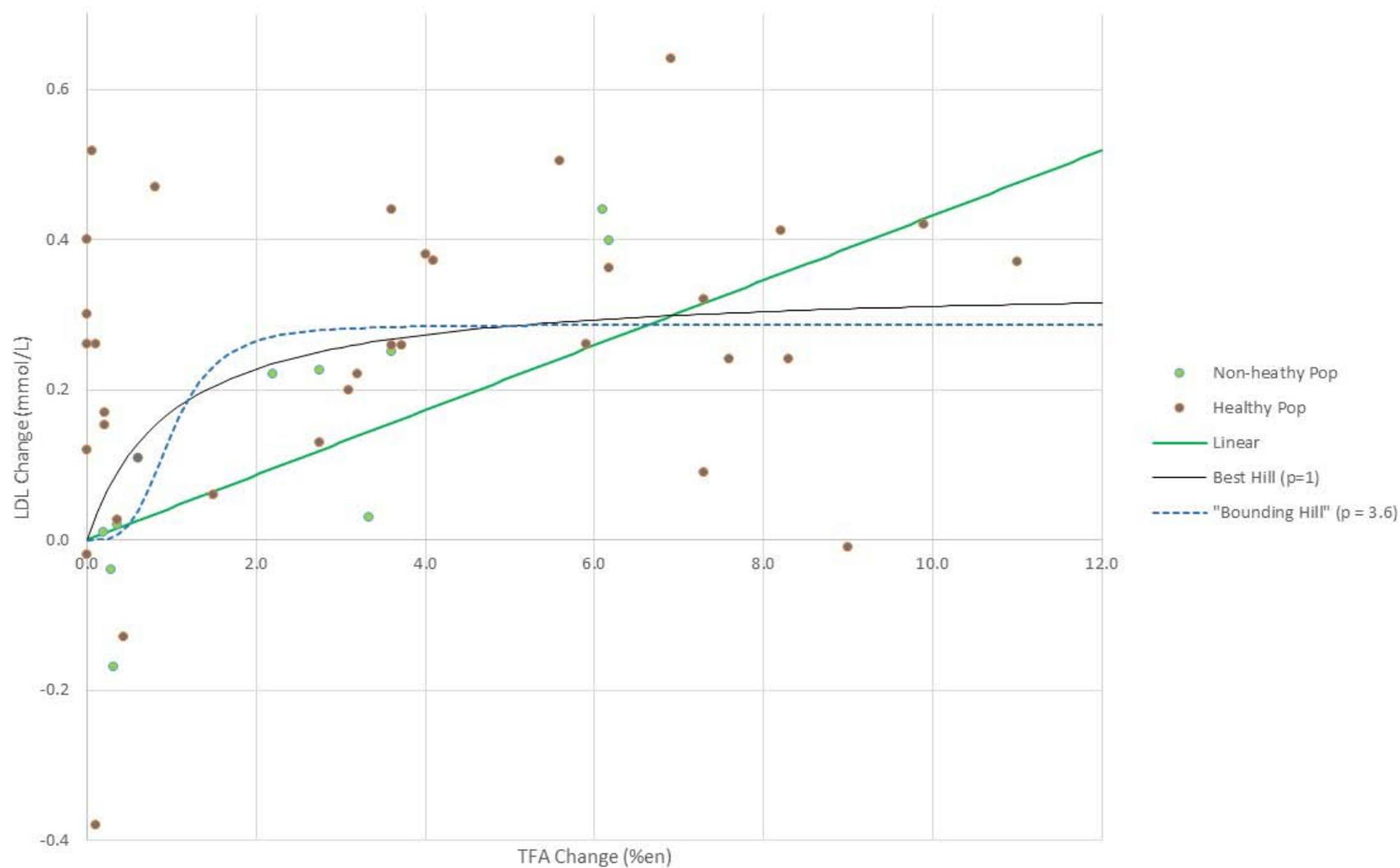
- Melissa Vincent
- Bruce Allen, BCA Associates
- John Reichard
- Ann Parker

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- ILSI North America PHO Task Force Cochairs – Kristin Rubin, Steve Hermansky, Richard Lane
- Alison Kretser, Mansi Krishnan – ILSI North America

This work was funded by the ILSI North America PHO Task force, but the conclusions are those of TERA.

- Extra slides

Figure 1: LDL Change vs TFA Change; Expected Study Means; for Linear Model, Best Fitting Model, and "Bounding Acceptable" Hill Model
Full Dose Region



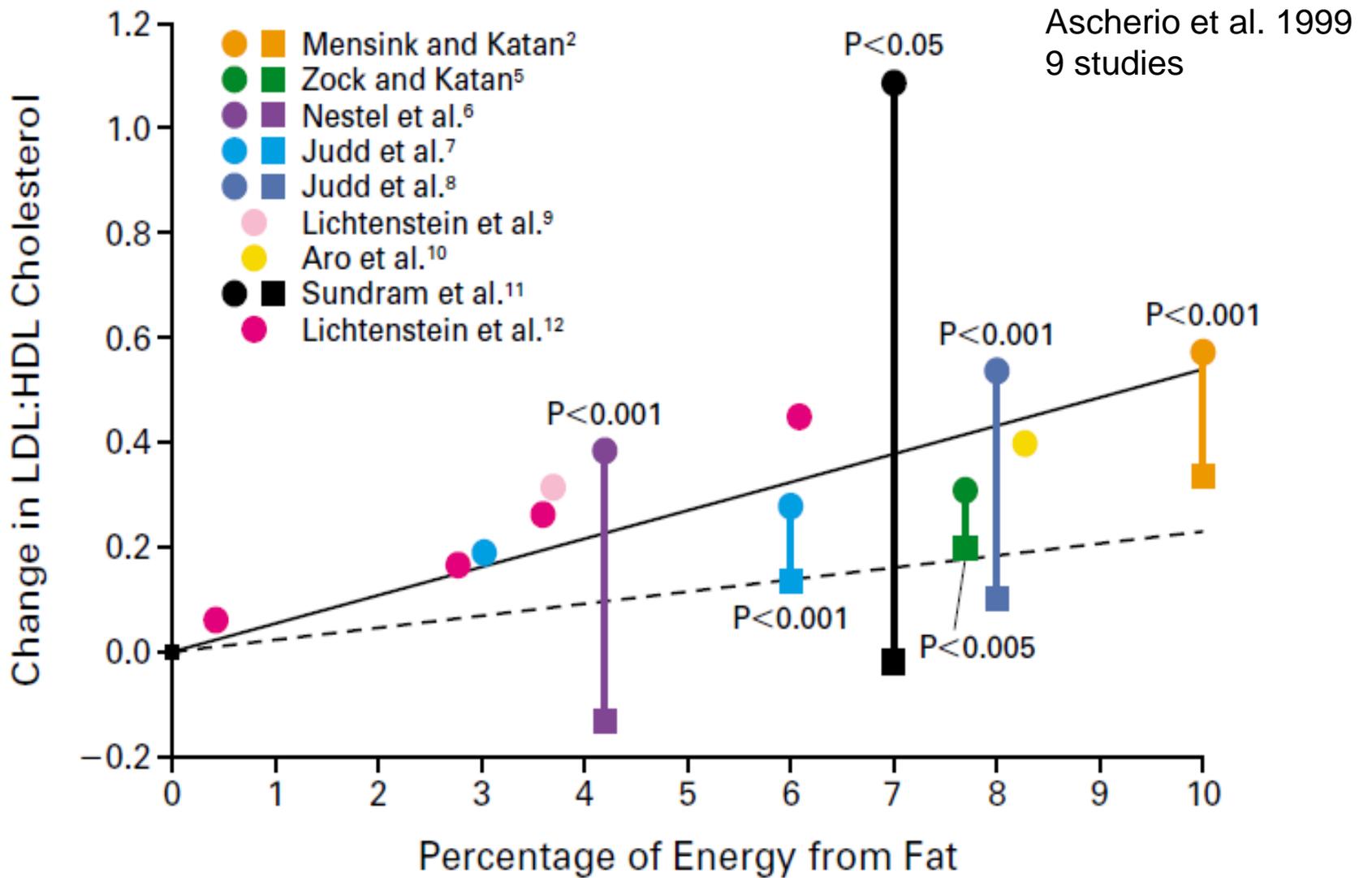


Figure 1. Results of Randomized Studies of the Effects of a Diet High in Trans Fatty Acids (Circles) or Saturated Fatty Acids (Squares) on the Ratio of LDL Cholesterol to HDL Cholesterol.

A diet with isocaloric amounts of cis fatty acids was used as the comparison group. The solid line indicates the best-fit regression for trans fatty acids. The dashed line indicates the best-fit regression for saturated fatty acids.



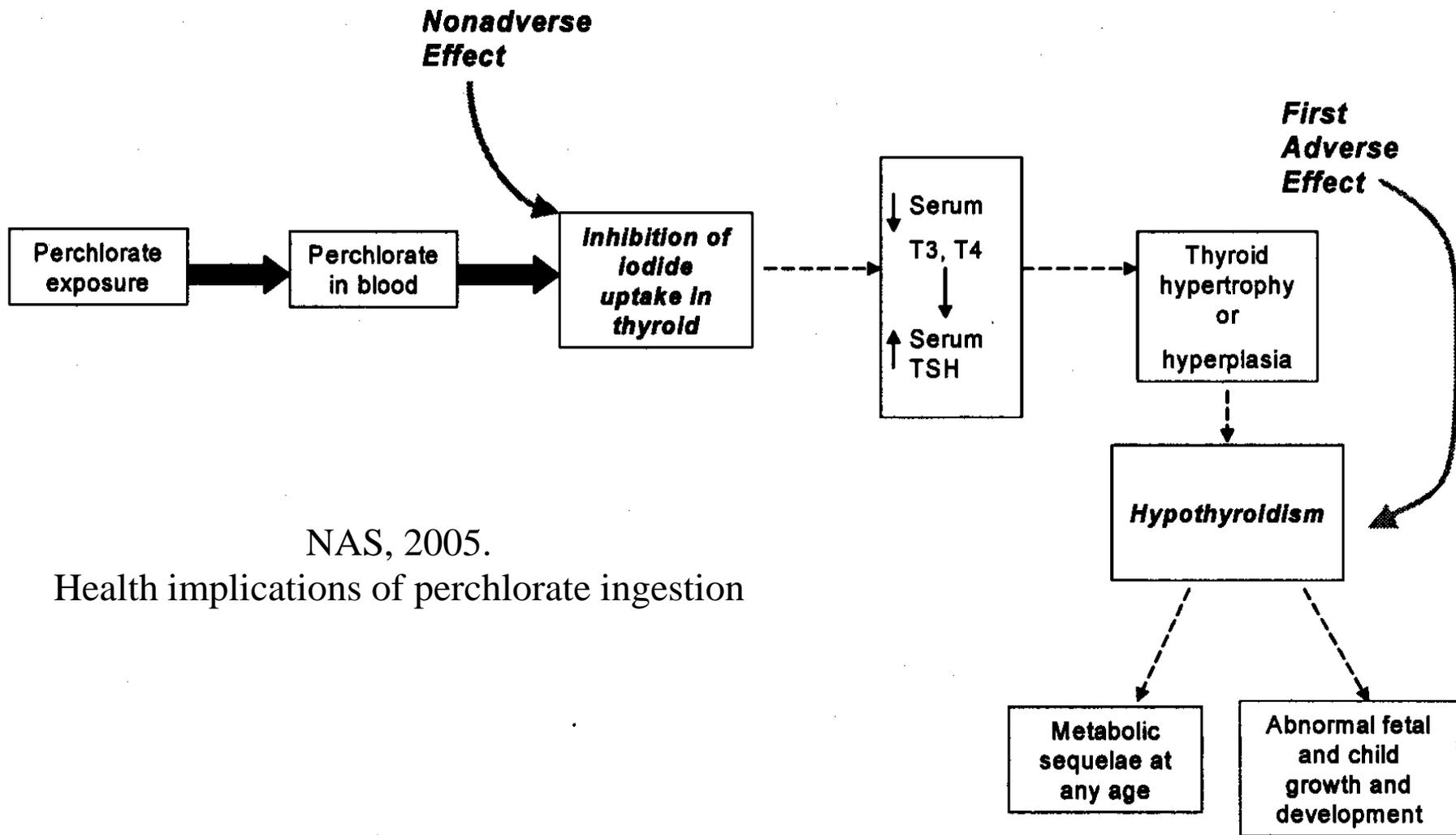
Dose-Response Assessment

- Dose-response determines a quantitative relationship between exposure and the likelihood of effects
 - Both in the range of observation,
 - And inference/extrapolation
- Inference/extrapolation requires consideration of
 - Mode of action
 - Animal-human differences
 - Human variability



Traditional: Critical Effect

- Risk assessment is... preventive medicine. Thus, toxicologists, epidemiologists, *and clinicians* are needed in judgment of critical effect
 - conduct hazard identifications collaboratively
 - Focus on effects of medical significance
- Critical effect is... the first adverse effect, or its known precursorr, that occurs as dose rate increases (EPA, 2013).



NAS, 2005.

Health implications of perchlorate ingestion

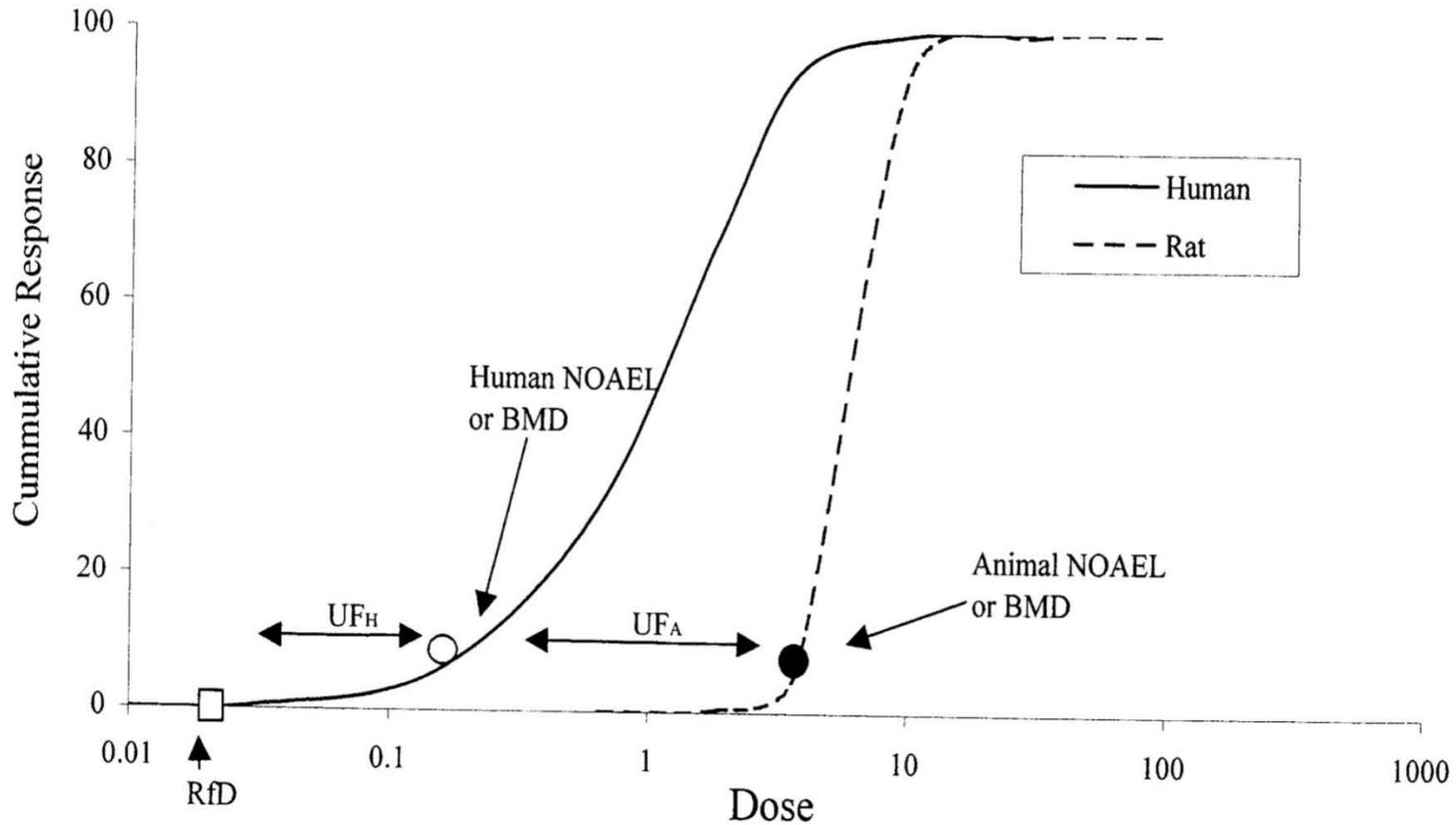
FIGURE 5-2 Committee's suggested mode-of-action model for perchlorate toxicity in humans indicating first adverse effect in the continuum.

Traditional: Uncertainty Factors

- Uncertainty factors for within human variability, experimental animal to human extrapolation, LOAEL to NOAEL, subchronic to chronic, and lack of certain data.
- Misconceptions:
 - Studies with small “n” are not useful.
 - The variability of the human population is large; an uncertainty factor of 10-fold with human data is often not enough.

Factor of 10 Enough?

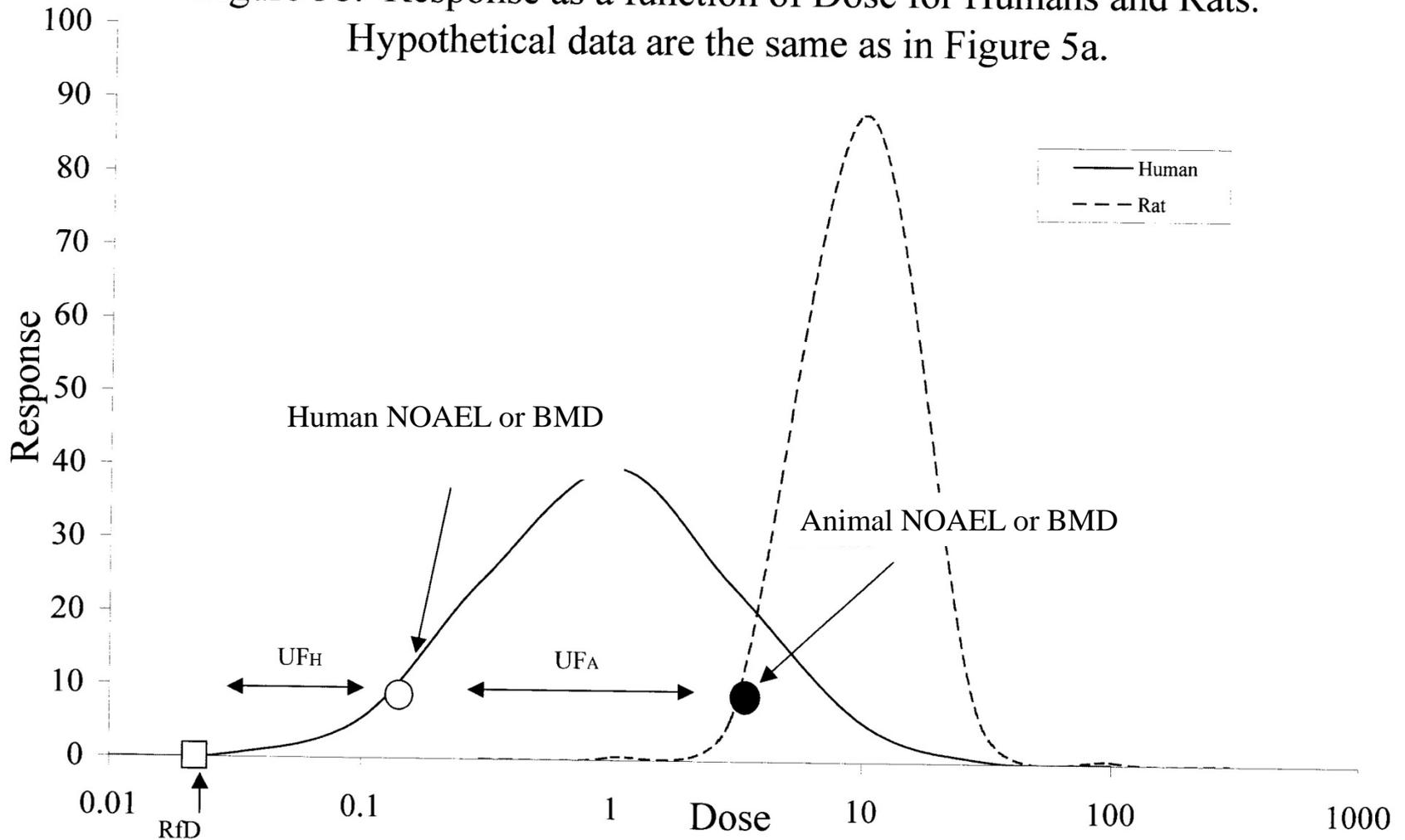
Figure 5a. Cumulative Response as a function of Dose for Humans and Rats. Data are hypothetical, but approximate real situations.



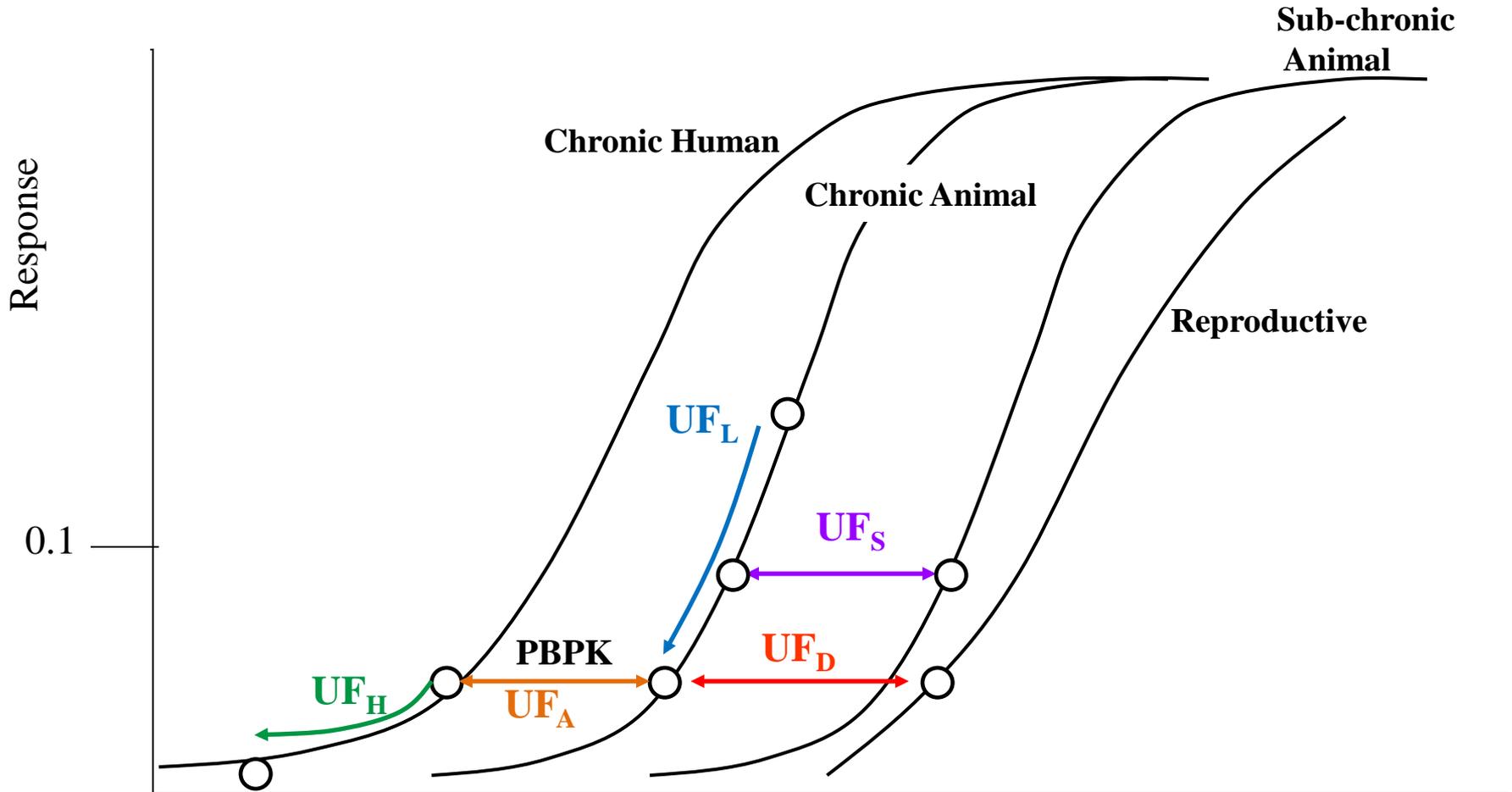
Dourson, M.L., G. Charnley and R. Scheuplein, 2002

Factor of 10 Enough?

Figure 5b. Response as a function of Dose for Humans and Rats. Hypothetical data are the same as in Figure 5a.

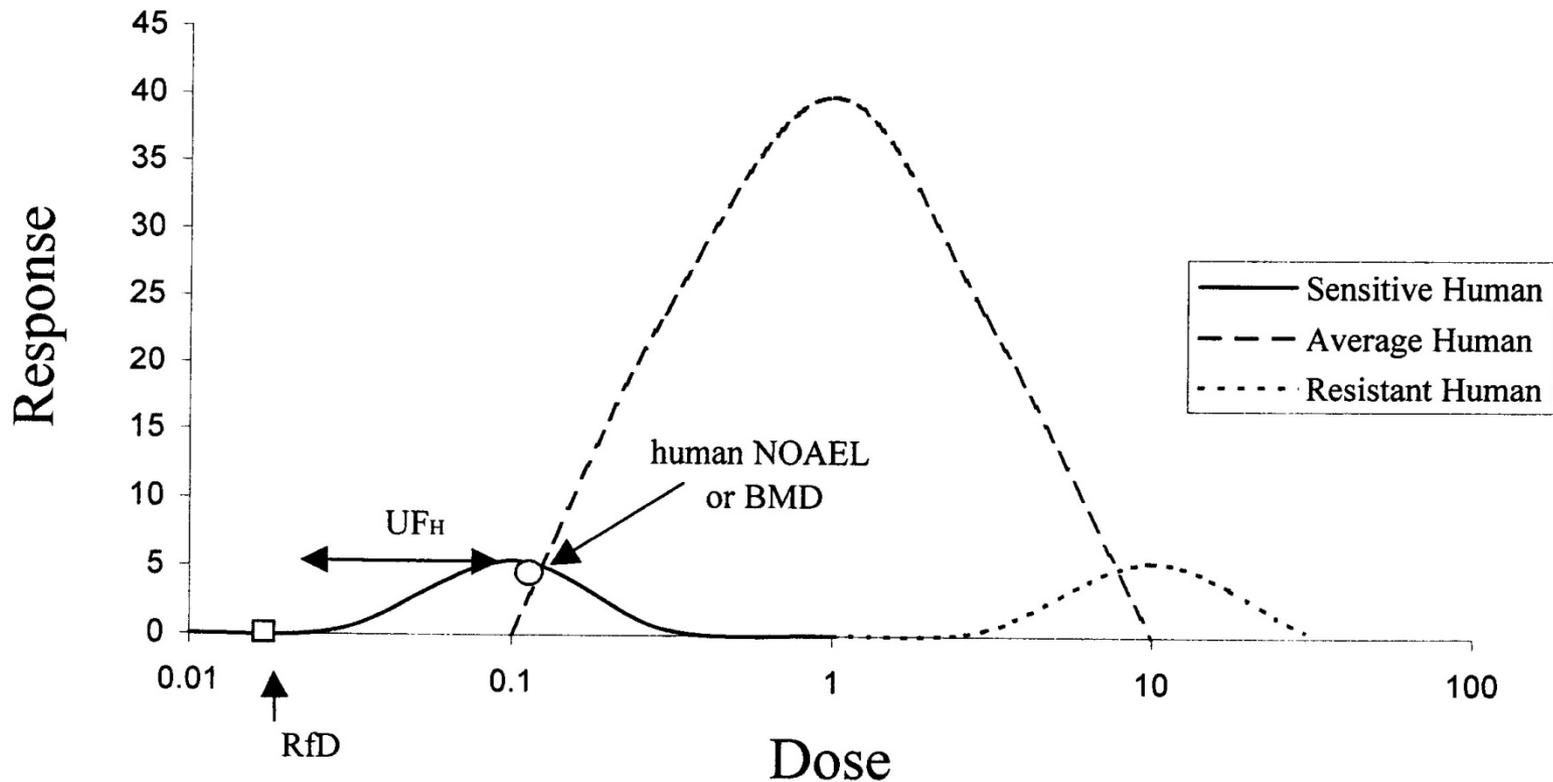


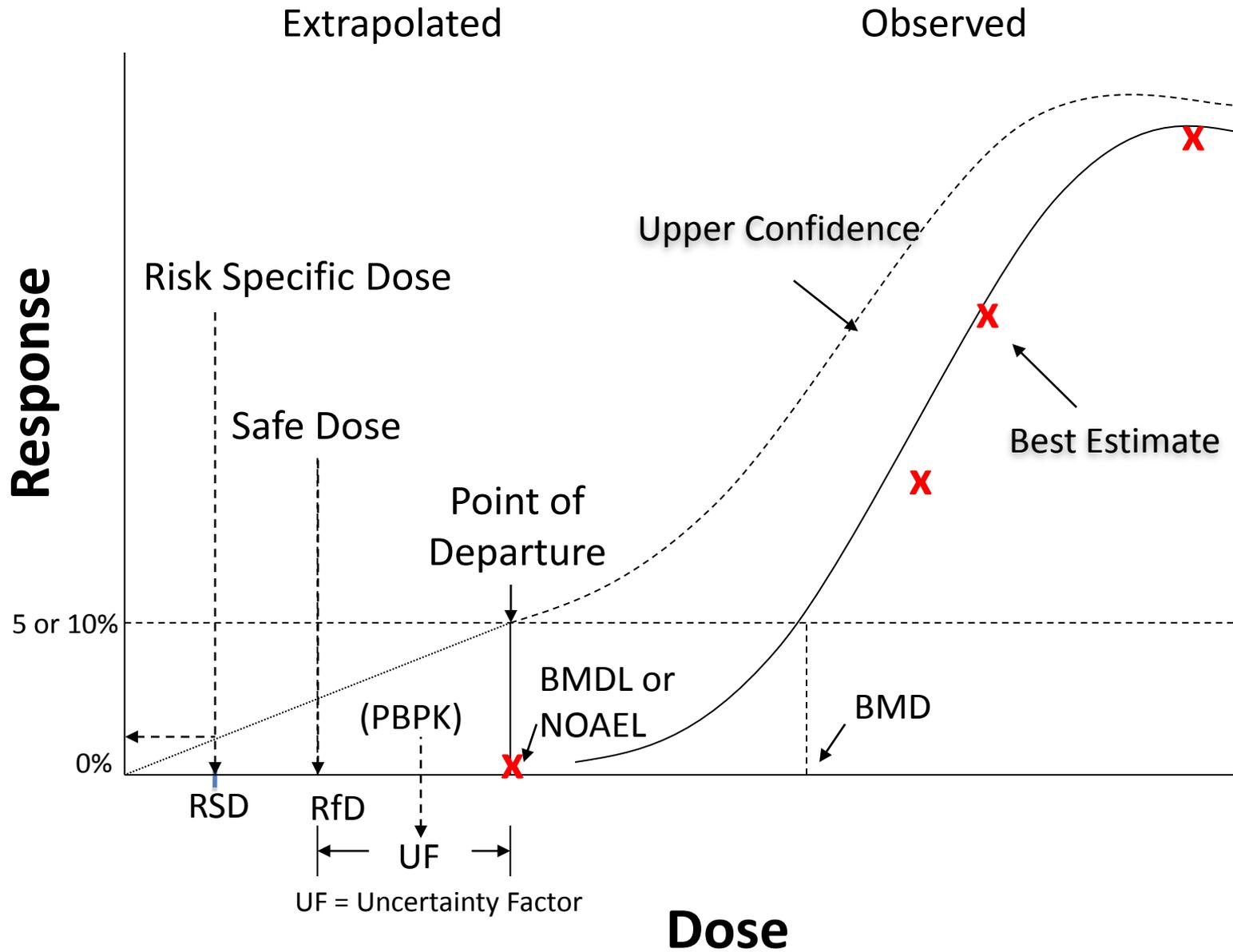
Areas of Uncertainty to Consider in Noncancer Dose Response Assessment



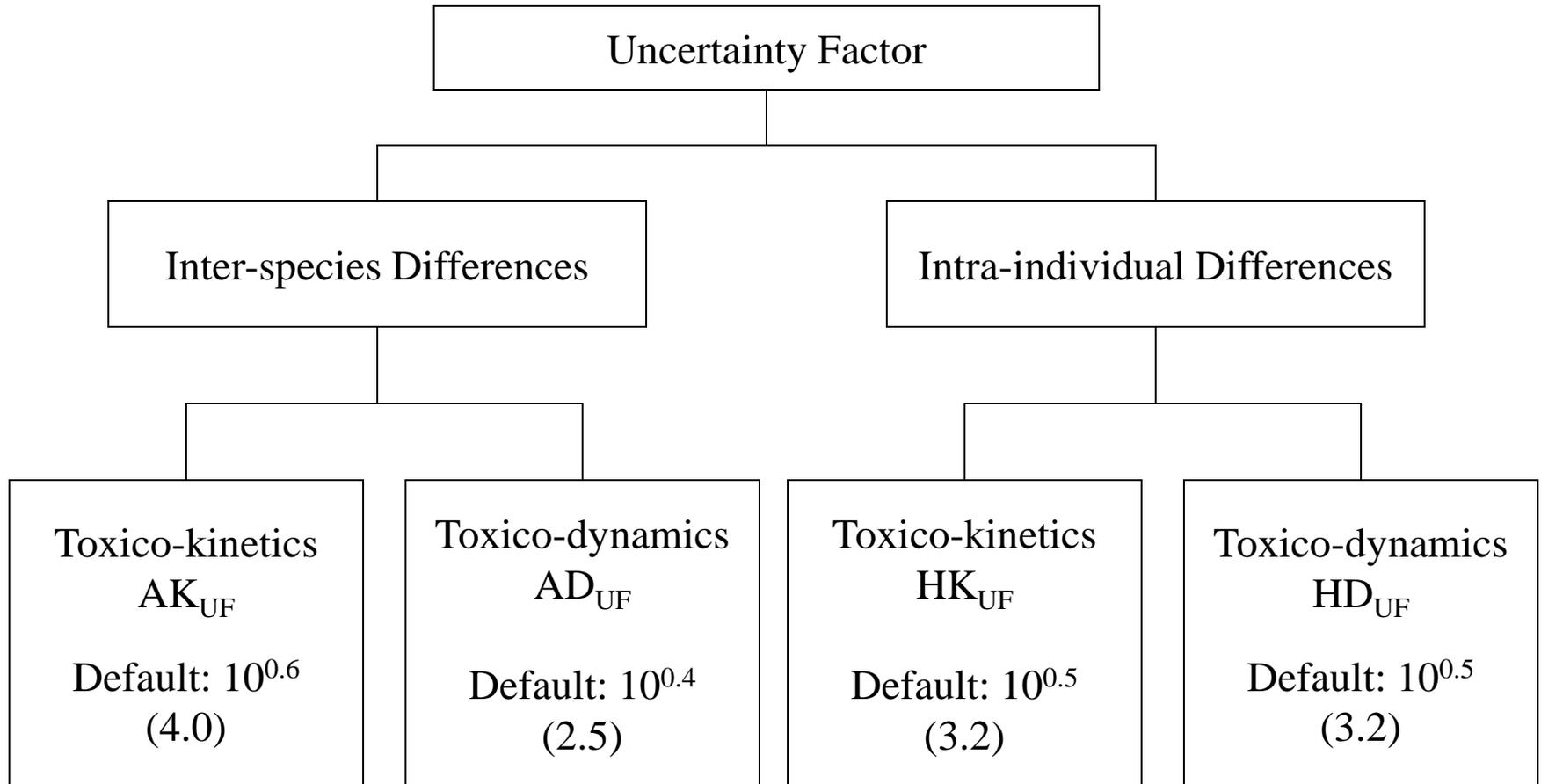
Factor of 10 Enough?

Figure 6a. Response as a function of dose for humans of different sensitivities. Hypothetical data for humans are the same as in Figure 5b.





Contemporary: Chemical Specific Adjustment Factor (CSAF)



Contemporary: BMD

- Clear advantages and disadvantages exist in the use of a benchmark dose (BMD)
 - Uses responses near the range of observation.
 - Includes a measure of variability in the response.
 - Determines a consistent measure of response.
 - Applies to fewer, more robust, toxicity data sets.
 - Accounts for more dose response of critical effect

Physiologically-Based Pharmacokinetics

- It is now routine to ask folks whether or not a PBPK model is available for the chemical of interest.
- Numerous PBPK papers; some have been given top awards (RASS of SOT papers of the year):
 - Sweeney, L. et al. (2001). Proposed occupational exposure limits for select glycol ethers using PBPK models and Monte Carlo simulation. *Toxicol. Sci.* 62(1):124-139.
 - Kirman, C.R., et al. (2004). Addressing nonlinearity in the exposure-response relationship for a genotoxic carcinogen: cancer potency estimates for ethylene oxide. *Risk Anal.* 24:1165-1183.

Categorical Regression

RfD Definition

"without appreciable risk"
"is likely to be"
"deleterious effect"

Regression model

$r < 10^{-2}$
 $P(*) > 0.95$
severity = moderate or frank

New RfD Definition

$P (r < 10^{-2} \text{ at dose} < \text{RfD}) > 0.95$
where $r = P (\text{severity} > 1)$

Hertzberg R.C. and M.L. Dourson, 1993

Aldicarb Clinical Studies

Frequency of Clinical Signs or Blood Cholinesterase

Study	Dose (mg/kg- day)	Group Size	Clinical Signs	Blood Cholinesterase Inhibition^a
Haines, 1971	0.025	4	1 Apprehension	4 Whole blood
	0.05	4	1 Runny nose ^c	4 Whole blood
	0.10	4	4 Weakness and sweating, Nausea in 2 individuals	4 Whole blood
Wyld et al., 1992 ^b	0	22	0	0 Plasma & 0 RBC
	0.010	8	2 Headaches ^c	0 Plasma & 0 RBC
	0.025	12	1 Sweating	12 Plasma & 11 RBC
	0.050	12	1 Sweating	1 Plasma & 1 RBC
	0.06	1	1 Sweating	1 Plasma & 1 RBC
	0.075	3	1 Lightheadedness	3 Plasma & 3 RBC

Effect Categories of Aldicarb Exposure in Humans

Frequency of Categories of Effect Associated with Aldicarb Exposure in Humans						
Study	Dose (mg/kg/day)	Group Size	Frequency of Responders within Categories of:			
			NO Effects	Non- adverse Effects	Adverse Effects	Frank Effects
Wyld	0.0	22	22 (22)	0 (0)	0 (0)	0 (0)
Wyld	0.010	8	8 (0)	0 (0)	0 (0)	0 (0)
Wyld	0.025	12	0 (0)	8 (11)	4 (1)	0 (0)
Wyld	0.025	4	0 (0)	0 (3)	4 (1)	0 (0)
Wyld	0.50	12	0 (0)	1 (11)	11 (1)	0 (0)
Wyld	0.50	4	0 (0)	0 (4)	4 (0)	0 (0)
Wyld	0.075	4	0 (0)	0 (2)	4 (2)	0 (0)
Haines	0.10	4	0 (0)	0 (0)	2 (2)	2 (2)

Categorical Regression for Aldicarb

