United States Environmental Protection Agency

Chemical Mixtures: What Dose-Response Analysts Really Want

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Advancing Research on Mixtures: New Perspectives and Approaches for Predicting Adverse Human Health Effects
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Talk Outline

- Risk Assessments: Complex Mixture Toxicity Data
  - Example: Disinfection by-products and EPA's 4-lab study
  - Study Power at low doses
  - Which environmental mixtures to study?
  - Considering sufficient similarity?
- Risk Assessments: Mixture Component Data
  - Which Individual Components to test?
  - How to test more components?
  - Mode of action
  - Additivity

Whole Mixture vs. Component Approaches

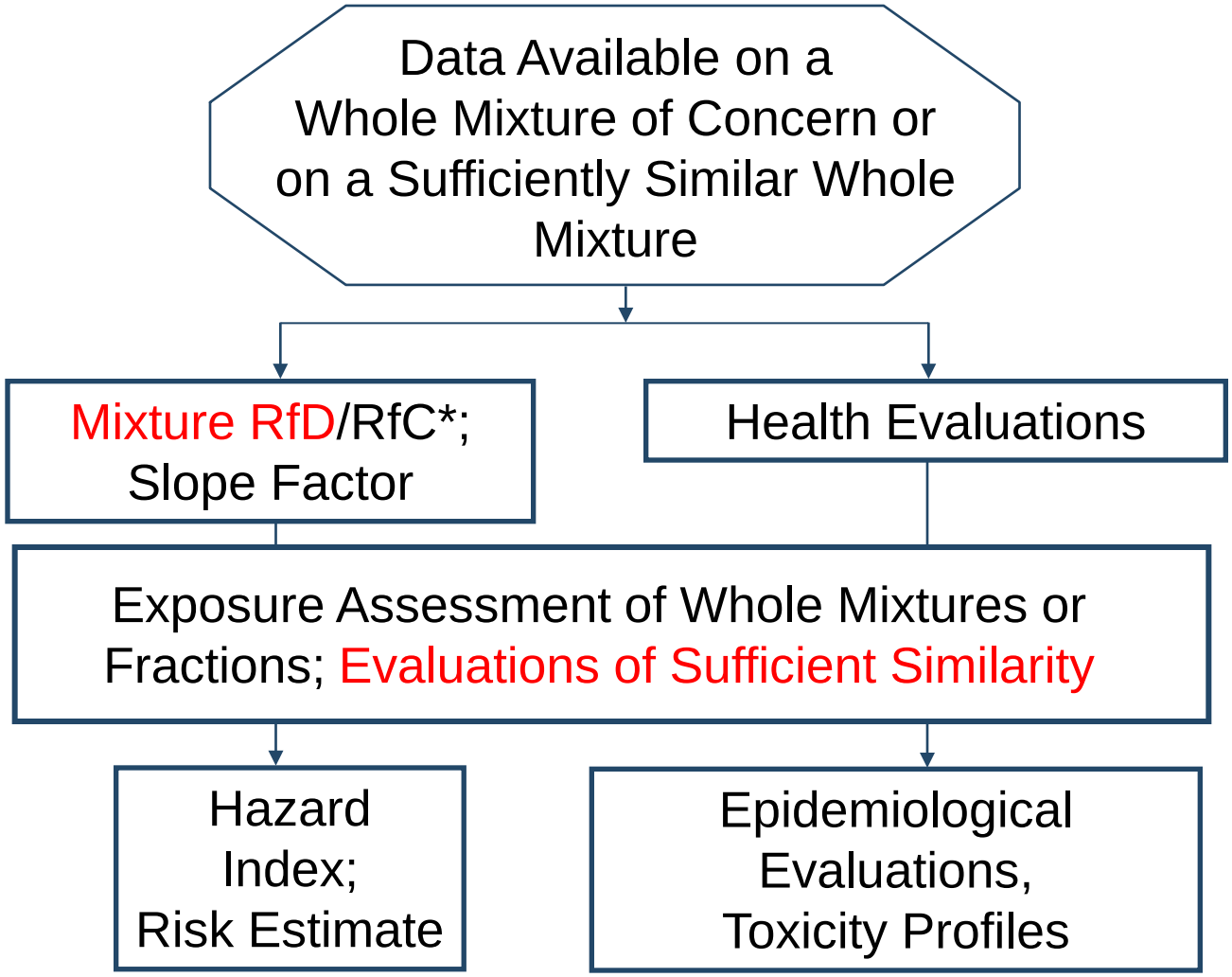
- Whole mixture based assessments (preferred)
  - Need good toxicity and exposure data on the whole mixtures
    - Some approaches forced to rely on available toxicity or exposure information
  - Need to evaluate whether tested mixture is sufficiently similar to mixture of concern (in environment)
- Component-based methods (practical)
  - Simple models describe complex biological processes
  - Need good toxicity and exposure data on individual components,
    - Some approaches forced to rely on available component toxicity or exposure information
  - Assumptions regarding combinability of component data

Complex Mixtures

- Hundreds of components
- Variable chemical composition over time, place or different conditions under which mixture produced

- Testing complex whole mixtures
  1. Test in low response region; near current human exposure levels
  2. Relative proportions of component chemicals similar to those measured in environmental samples
  3. Include unidentified components (Teuschler et al., 2002)

Flow Chart for Evaluating Whole Mixtures (Focus Today)

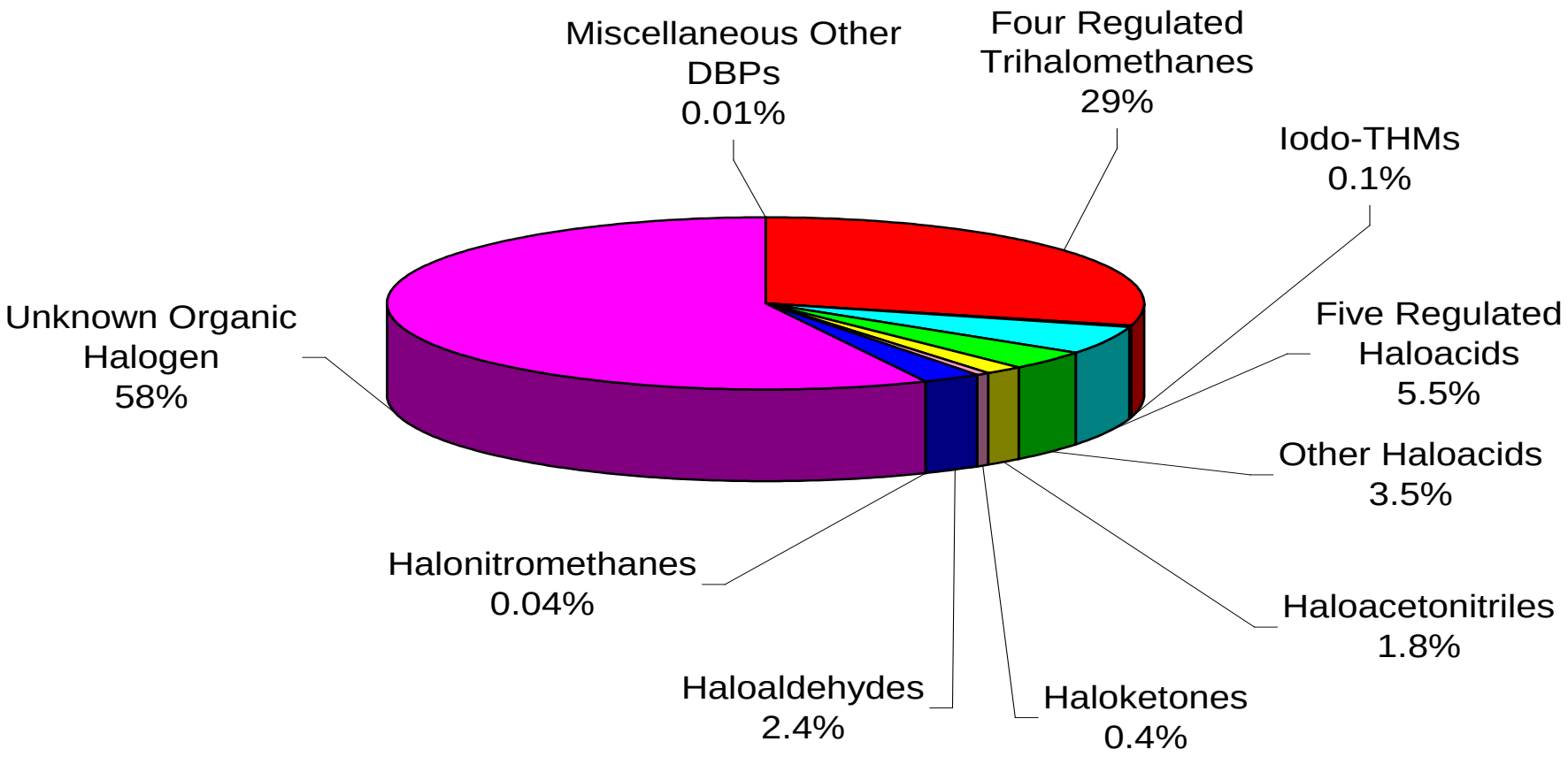


*Oral Reference Dose/Inhalation Reference Concentration

Chemical Disinfection of Drinking Water: Motivating Whole Mixture Example

- *"Most significant public health advance of the millennium."*
  Life Magazine, 1997
  - Dramatic reductions in waterborne disease morbidity and mortality
- Disinfectants react with organics and inorganics in source waters producing disinfection by-products (DBPs)
- Number, types and concentrations of DBPs in finished water depend on:
  - Disinfectant (e.g., chlorine, chloramine, ozone)
  - Source water characteristics, including seasonal composition changes
  - Treatment characteristics
  - Distribution system characteristics
- Most of US population exposed

Halogenated DBPs Produced Through Chlorination



> 600 chemicals identified as DBPs, approximately half of the mass of total organic halide resulting from chlorination remains unidentified

Source: Richardson et al., 2008

Possible DBP Human Health Risks

- Some epi studies report countervailing risks including spontaneous abortion & term low birth weight associated with DBP exposures

- Epidemiologic study results
  - Reproductive/developmental effects inconsistently observed
  - Effect magnitudes generally low
  - Are differences in epi study outcomes due to differences in DBP levels or composition, differences in source waters, disinfection methods/practice, exposure levels, populations examined, chance, etc.?

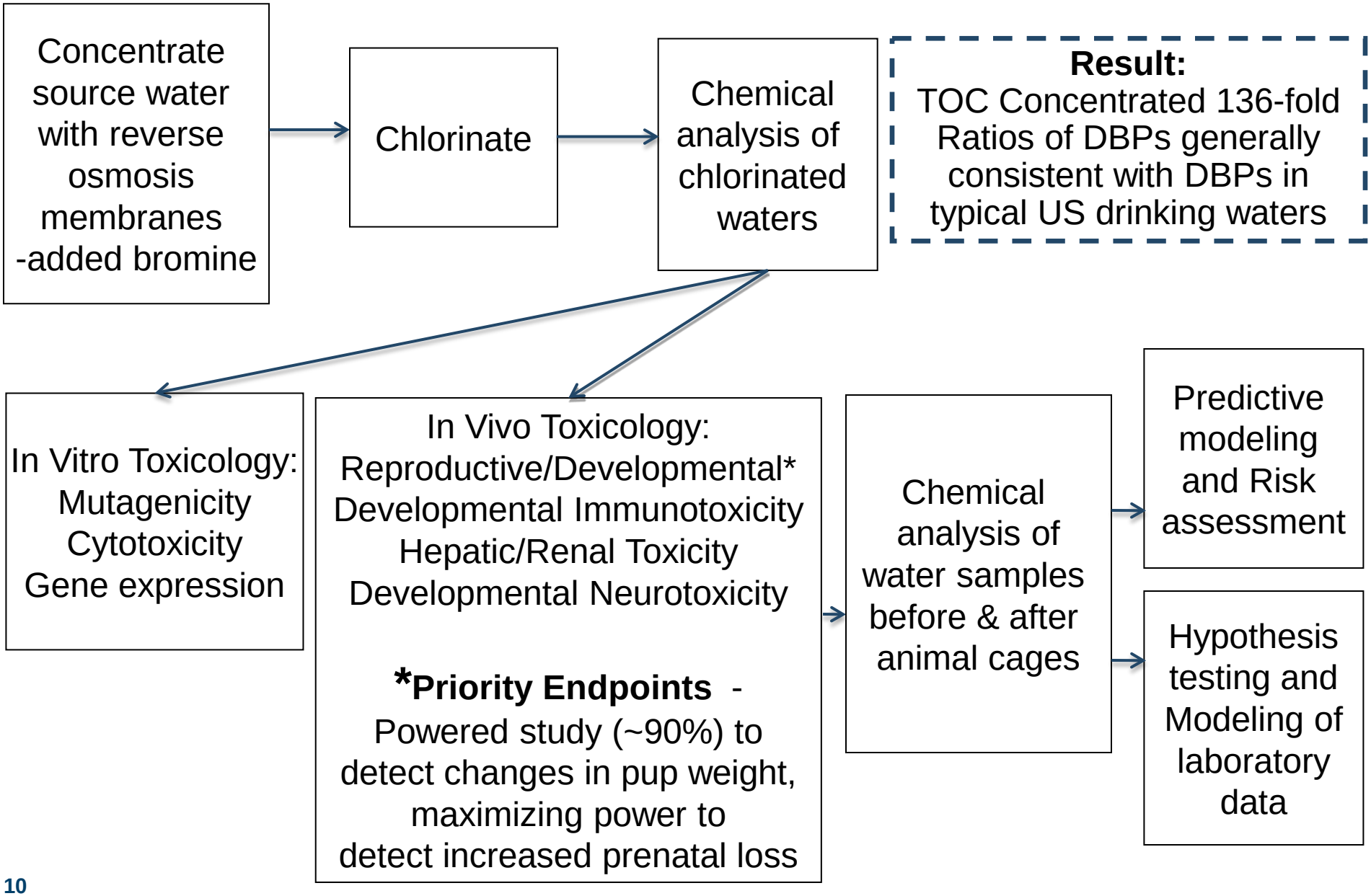
- Effects observed in epidemiology studies unexpected based on dose-response models of individual DBP data in animal bioassay
  - Magnitude of observed effects in animal studies relatively small
  - Using conventional approaches (e.g., response addition) effect magnitudes in positive epidemiology studies much larger

- Limited in vivo study of whole DBP mixtures in test species

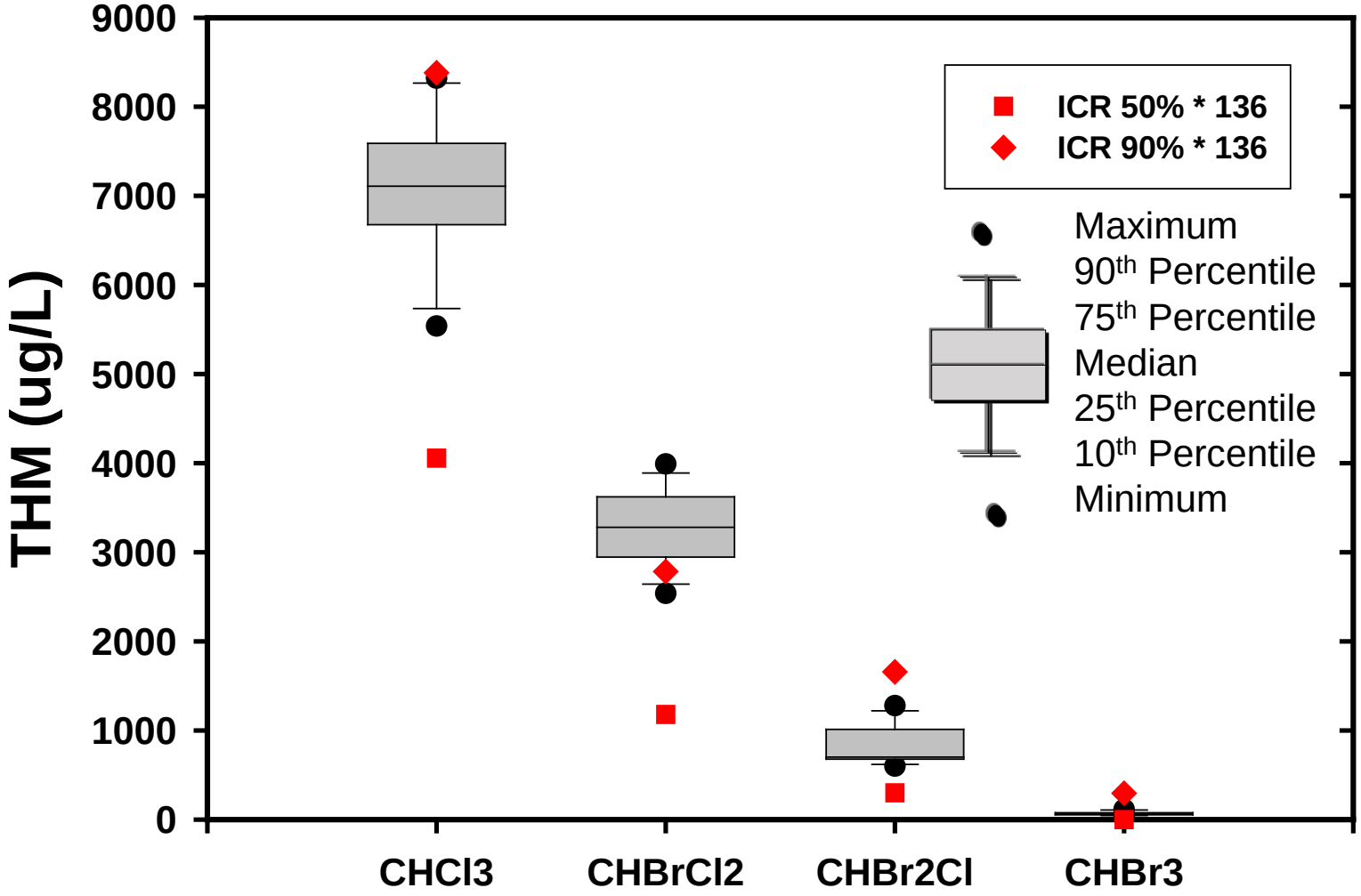
EPA's 4-Lab Study Experiments

- In vivo multigenerational study with whole mixture DBP concentrates administered via drinking water to rats
- Designed to address toxicology of:
  - "Real-world" DBP mixtures using concentrates
  - Defined DBP mixtures
- Challenge: develop methods to concentrate potable and palatable (to rats) disinfected water, maintaining DBPs in proportions consistent with environmental mixtures
- Expertise: drinking water treatment, analytical chemistry, toxicology, risk assessment

4-Lab DBP Treatment



Trihalomethane Concentrations in EPA's 4-Lab Study

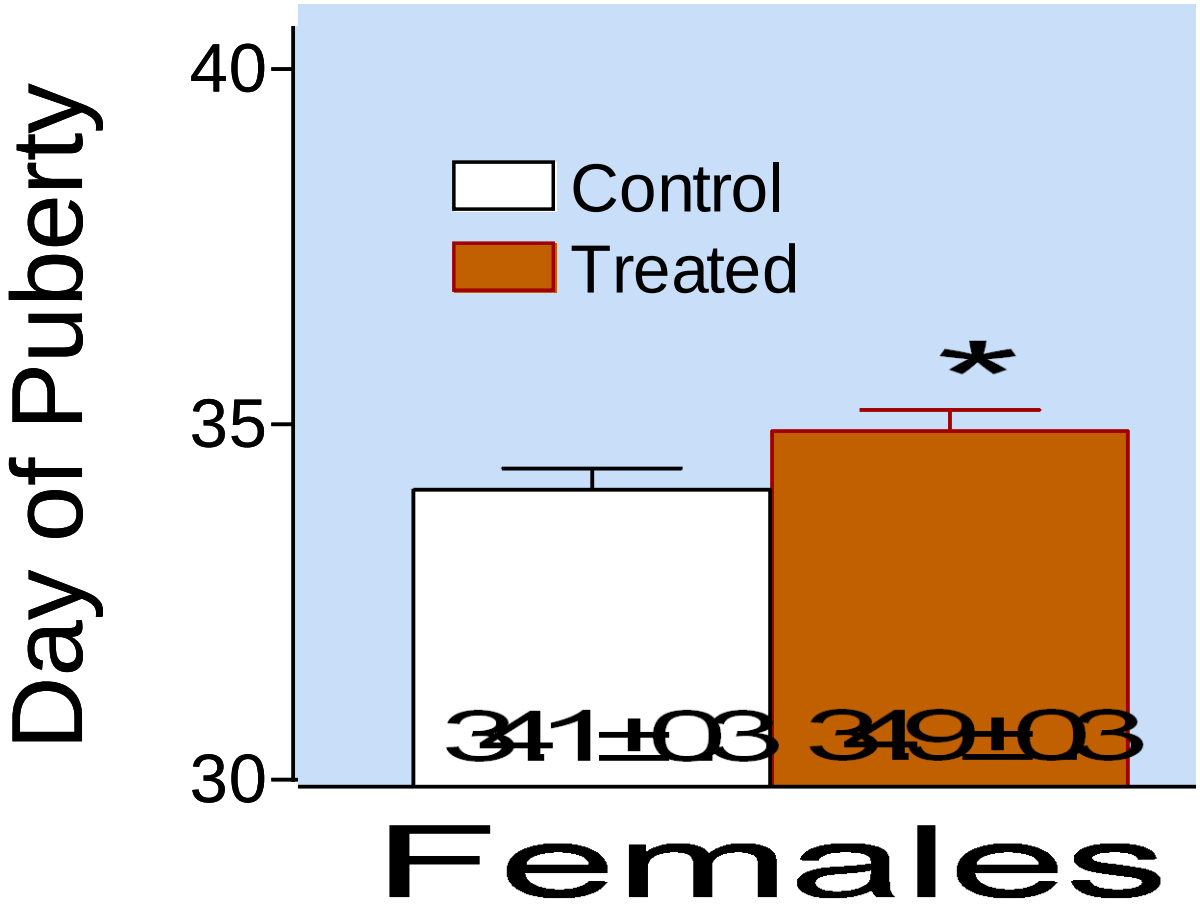


EPA intended DBP levels to be at high end of statistical distribution

ICR- Information Collection Rule- DBP Survey in US drinking waters

Source: Pressman et al., 2010

Female Rats Show Subtle Delays in Puberty Onset After Treatment with 4-Lab DBP Mixture- 1 of Few Positive Findings



* Statistically Significant $p < 0.05$

- Generally negative study
- Subtle effect: Puberty onset based on Day of Vaginal Opening (VO) <1 day delay
- Biological Significance ?
- Delayed VO also observed in defined mixtures study of regulated DBPs (trihalomethanes and haloacetic acids)

Source: Narotsky et al., 2008

What Could a Dose-Response Analyst do with 4-Lab Data? Mixture Reference Dose (RfDm)

$$RfD_m = \frac{NOAEL, LOAEL\ or\ BMDL}{UF_m}$$

Where:

RfD - "an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime."

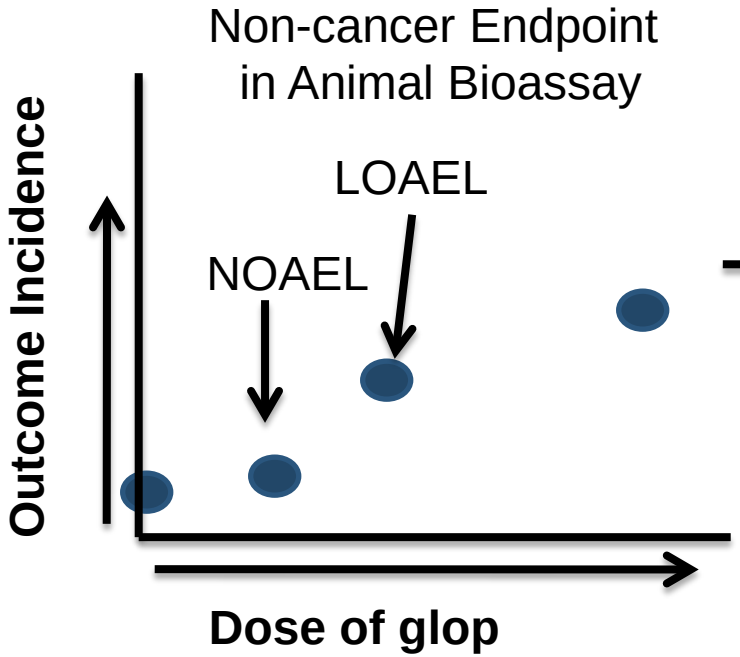
NOAEL/LOAEL = No/Lowest-Observed-Adverse-Effect Level

BMDL = Lower 95% confidence limit on an X% Effective Dose (e.g., ED_{10})

UF_m = Uncertainty Factors for the mixture (e.g., interspecies, intraspecies, exposure duration, NOAEL to LOAEL, data base deficiencies)

NOAEL, LOAEL or BMDL from experimental toxicity data on complex mixture dose response; serve as points of departure (POD)

Complex Mixture Test Results: What Dose-Response Analysts Do



Apply Uncertainty Factors (UFs) → Reference Dose

Pathologlogy of response:
Whether an outcome is adverse or a precursor event? What is pathway and how does it progress? What levels of biological organization are affected? Evidence supporting causal relationship between precursor event and adverse effect?

Uncertainty Factors intended to account for:

1. Variation in susceptibility in human population
2. Uncertainty animal to human extrapolation
3. Uncertainty extrapolating from subchronic to chronic data
4. Uncertainty extrapolating from a LOAEL rather than a NOAEL
5. Uncertainty associated with extrapolation when the database is incomplete.

Testing Complex Mixtures

- Resource intensive
  - Preparation of the complex mixture
  - Chemical characterization of prepared complex mixture
  - Toxicity studies on complex mixtures
- Limited numbers of complex mixtures can be tested
  - 4-Lab studied 1 water, 1 type of chemical disinfectant
- Develop library of test results
  - Organized source water and disinfectant combinations
  - Matrix for comparison to other drinking waters (Teuschler & Simmons, 2003)

Develop Library of Toxicity Information on Finished Water Matrix Scenarios

	Source Water Characteristics				
Disinfectant	Low Bromide, Low TOC	High Bromide, Low TOC	Low Bromide, High TOC	High Bromide, High TOC	Other Characteristics of Concern
Chlorine					
Ozone/ Post Chlorine					
Chloramine					
Other Disinfectant of Concern					

For Each Mixture:
- Chemical analysis
  - Known DBPs
  - TOX
  - %Brominated
  - %Chlorinated
  - Chromatograms
- Toxicity data
  - In vivo
  - In vitro
  - Associated Epi

Adapted from Teuschler & Simmons, 2003

So Many Different Environmental Mixtures, So Little Time, Money, Scientists…Which Mixtures to Test?

- Exposure information
  - Identify composition of mixture/s to which most people are exposed, then test those mixtures
- Epidemiology & toxicity information
  - Epi and Traditional Toxicology studies identify characteristics of mixtures or components of increased health concern
  - Can high through-put toxicology data (e.g., transcriptomic data) adequately compare different formulations of mixtures (either complex mixtures or defined mixtures)?
    - Need to evaluate whether (under what conditions) high through-put tests are relevant to whole animal toxicology
    - Consider trade-offs of characterizing differences among mixtures at different levels of biological organization
    - Group similar mixtures, subject “different” mixture groups to further testing

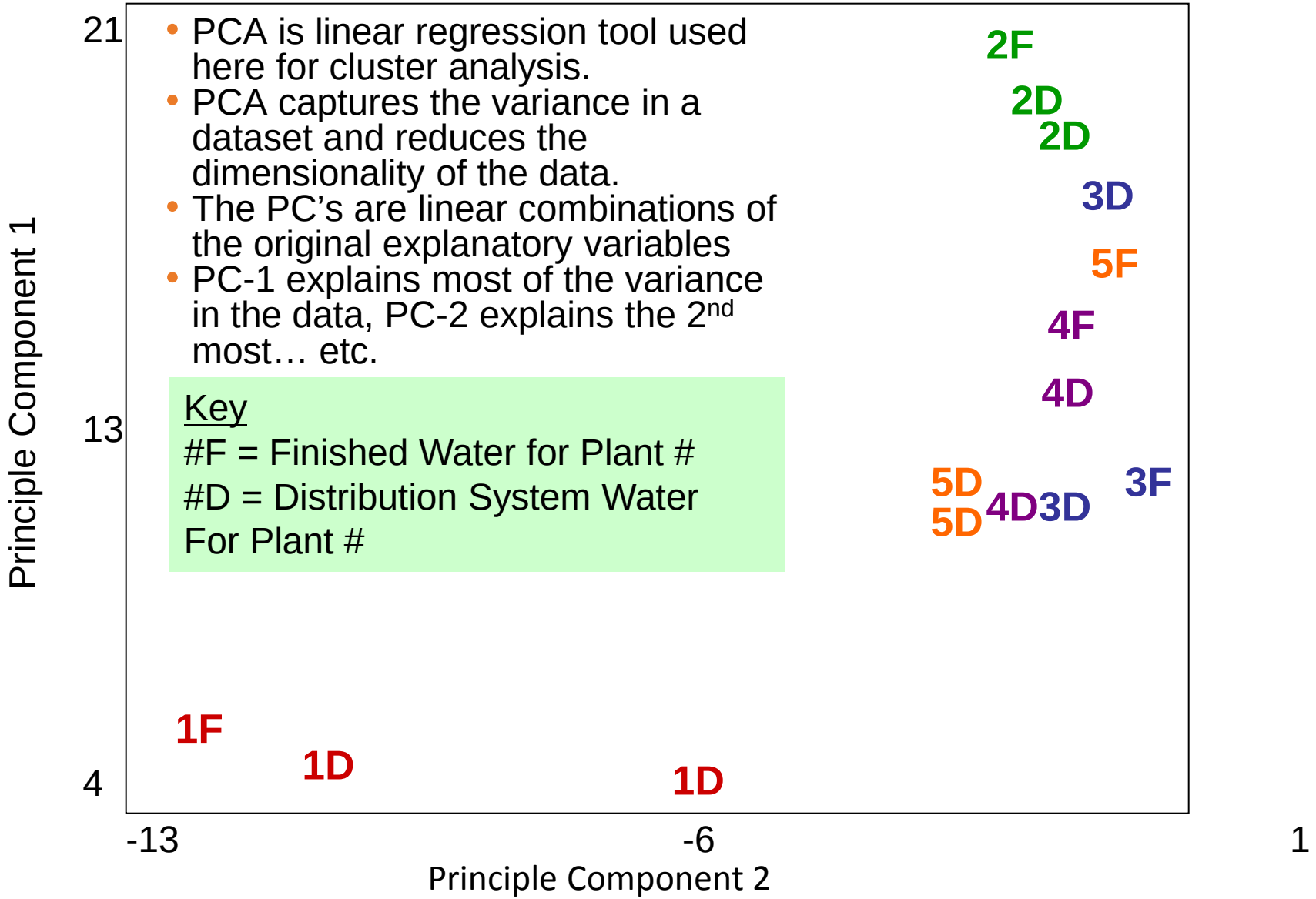
Development of Criteria for Evaluating Sufficient Similarity

- If toxicity data are not available for a mixture of concern, base the risk assessment on a sufficiently similar mixture (US EPA, 2000).
- Toxicological, chemical and statistical criteria are needed to clearly determine when two or more complex mixtures are sufficiently similar for risk assessment purposes.
- Example EPA research project on Drinking Water Disinfection By-Products (DBPs)
  - Used an example DBP data set for 5 drinking water treatment plants to explore development of sufficient similarity criteria for DBP mixtures
  - Chemical measures (e.g., Similar Bromide/Chlorine ratios, TOC levels in source water, concentrations of individual DBPs)
  - Toxicity outcome: mutagenicity
    - Research results published in 6 companion papers in the Journal of Toxicology and Environmental Health (Bull et al., 2009a,b; Feder et al., 2009a,b; Rice et al., 2009; Schenck et al., 2009)

Principal Components Analysis (PCA): Differences Between Mixtures from Ground Water Plant 1 and 4 Surface Water Plants 2-5

- PCA is linear regression tool used here for cluster analysis.
- PCA captures the variance in a dataset and reduces the dimensionality of the data.
- The PC's are linear combinations of the original explanatory variables
- PC-1 explains most of the variance in the data, PC-2 explains the 2nd most… etc.

Key
#F = Finished Water for Plant #
#D = Distribution System Water For Plant #



Interrelationships Among Source Water Characteristics, Disinfectants and Health Data

Source Water Characteristic	Water Treatment	Example Effects On DBP Types	Some Health Effects Of Concern
High Levels of Total Organic Carbon	Chlorine Added	Mostly Chlorinated DBPs, High Levels of Total Organic Halides (TOX)	Systemic Effects, Epidemiologic Data on Bladder Cancer
	Ammonia Added	Increased Proportions of Brominated DBPs, Less MX**, *New DBPs (Nitrosamines)	Toxicity of Brominated DBPs, Less Mutagenic, New Carcinogens
High Bromide Levels	Chlorine Added	Some Chlorinated, but Mostly Brominated DBPs	Increased Toxicity, Epidemiologic Data on Reproductive and Developmental Effects
	Ozone Added	Decreased Total TOX, Mostly Brominated DBPs, *New DBP (Bromate)	Toxicity of Brominated DBPs, New Carcinogen
Lower to Higher pH Levels (acidity)		MX Ring Form Shifts from Closed to Open	More to Less Mutagenicity, Respectively

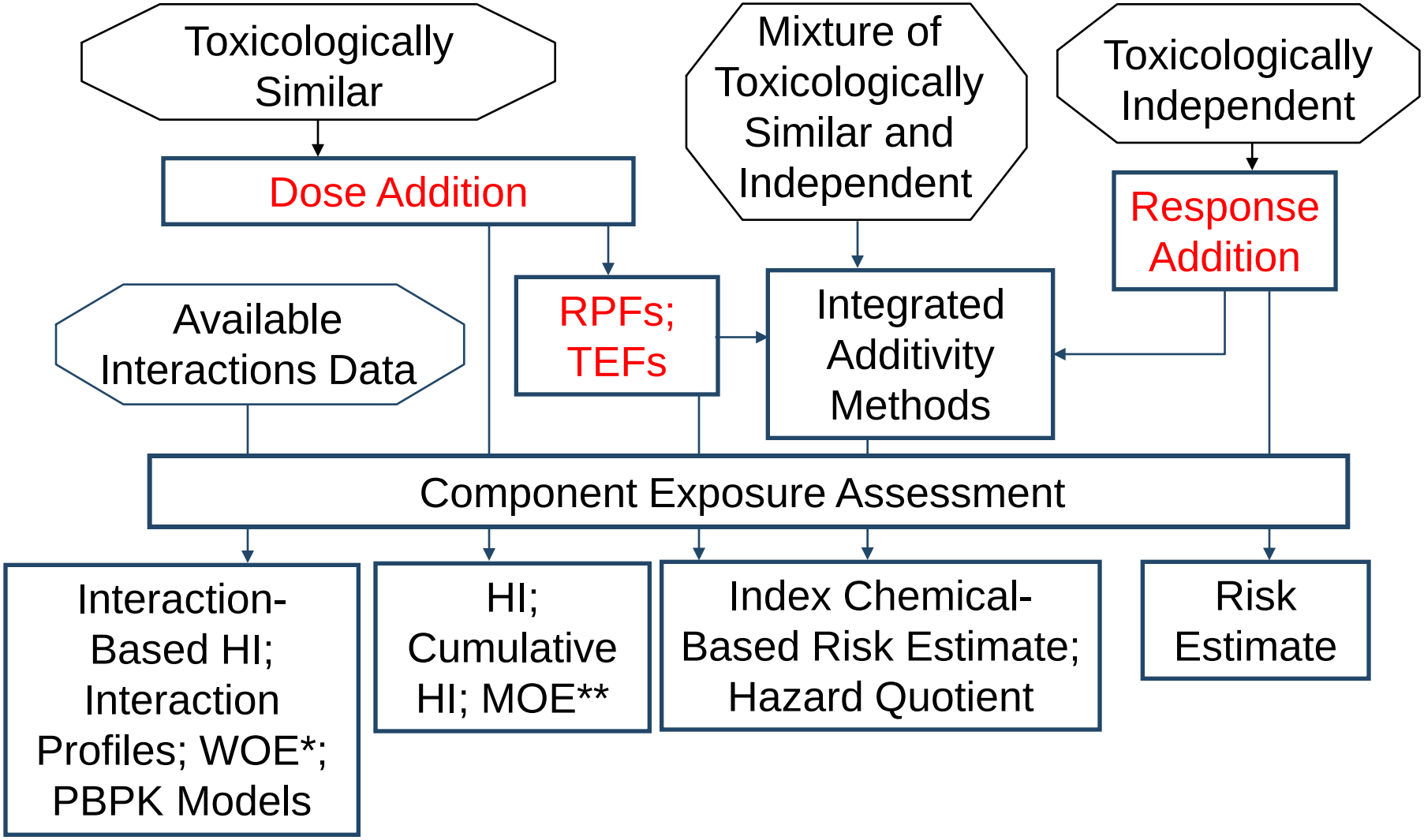
*New DBPs refers to DBPs that are not expected for the same source water treated with chlorine.
**MX = 3-chloro-4-(dichloromethyl) -5- hydroxy-2(5*H*) -furanone

Source: Rice et al. 2009

Sufficient Similarity

- Currently, unevenly undertaken/considered
- Testing different mixture formulations encountered in the environment is a *critical research gap* in environmental mixtures risk assessment
  - Test defined mixtures; develop toxicity signature profile(s) for defined chemical mixture classes
- Biostatistical methods available (more methods development useful!!)
  - Principal components analysis
  - Nonparametric statistical methods based on “bootstrap” resampling
- Could high through-put toxicology technologies improve basis for distinguishing among similar environmental mixtures (more toxicology information about a wider variety of mixtures)?
  - Informational gap is resource intensive to fill with animal data
    - How informative are high through-put data to distinguish among different types of effects or different effect severities?
    - How relevant are different high through-put results to whole animals or people?
    - Trade-offs implicit when evaluating at different levels of biological organization

Flow Chart for Evaluating Mixture Components



*Weight of Evidence for Toxicological Interactions
**Margin of Exposure

Key Concept: Additive Joint Toxic Action of Mixture Components

- Simple Similar Action (Dose Addition—e.g., Hazard Index [HI], Cumulative HI, Relative Potency Factors [RPFs], Toxicity Equivalence Factors [TEFs])
  - Addition of component doses, scaling factors for relative toxicity:
    - TEFs for Dioxins
    - RPFs for other chemical classes, e.g., organophosphates
  - Assumes common toxic mechanism/mode of action or similar toxicity of components
- Simple Dissimilar Action (Response Addition—e.g., Cancer Risk Sums)
  - Addition of component risks
  - Assumes toxicological and statistical independence

Source: U.S. EPA, 2000

Relative Potency Factor (RPF) Analysis

Concentration Chemical A1 (Index Chemical)

X RPF_{A1} =

(Note: RPF_{A1} = 1)

Index Chemical Concentration A1

Concentration Chemical A2

X RPF_{A2} =

Index Chemical Equivalent Concentration of A2

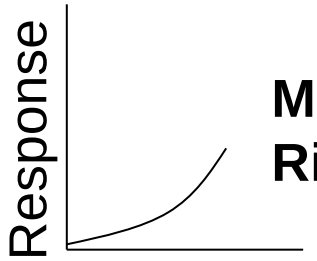
Concentration Chemical A3

X RPF_{A} =

Index Chemical Equivalent Concentration of A3

Sum index chemical equivalent concentration to estimate total mixtures exposure in units of the index chemical

Index Chemical Dose-Response



Mixture Risk

Equivalent Concentration

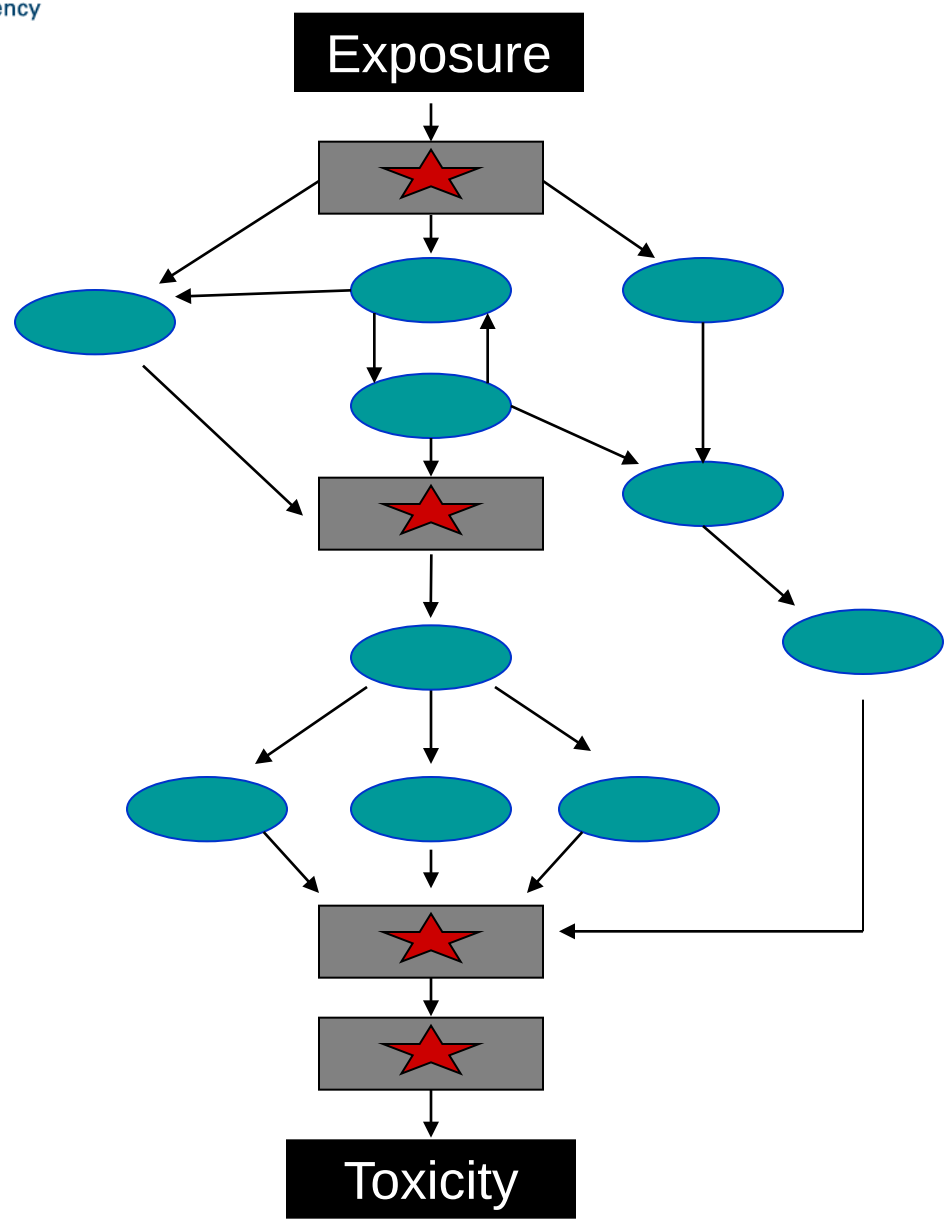
TEFs and RPFs: Key Questions

- How much do we know about component toxicities relative to index chemicals at environmentally relevant doses for key components of important mixtures?
  - Is the effect observed in the comparative tox assay relevant to humans? Are the exposure routes relevant?
  - Are there key components or component groups not addressed?
    - Unidentified components
- What do we know about mode of action?
  - Dose additivity (at environmental doses)?
  - Response additivity (at environmental doses)?
  - Mixed modes of action?
- How can high through-put data be used to evaluate assumptions of dose additivity?

MOA Data

- Mixture component models relatively simple
  - MOA data key to developing biologically-based dose-response models
  - MOA data can help determine if there are other groups of chemicals that can be evaluated using RPF approaches
  - From a risk assessment perspective, what is utility of additional MOA data for evaluating risks posed by components of well-studied mixtures?
    - Should certain chemicals be added to TEF approach?
      - Adding polychlorinated naphthalenes or polybrominated biphenyls to TEF approach for dioxin-like compounds
    - Change confidence in key assumptions; better evaluate biological basis of component methods
    - Unlikely to affect quantitative estimate of risk
  - Is empirical evidence of dose-addition sufficient?

Schematic of Toxic Events



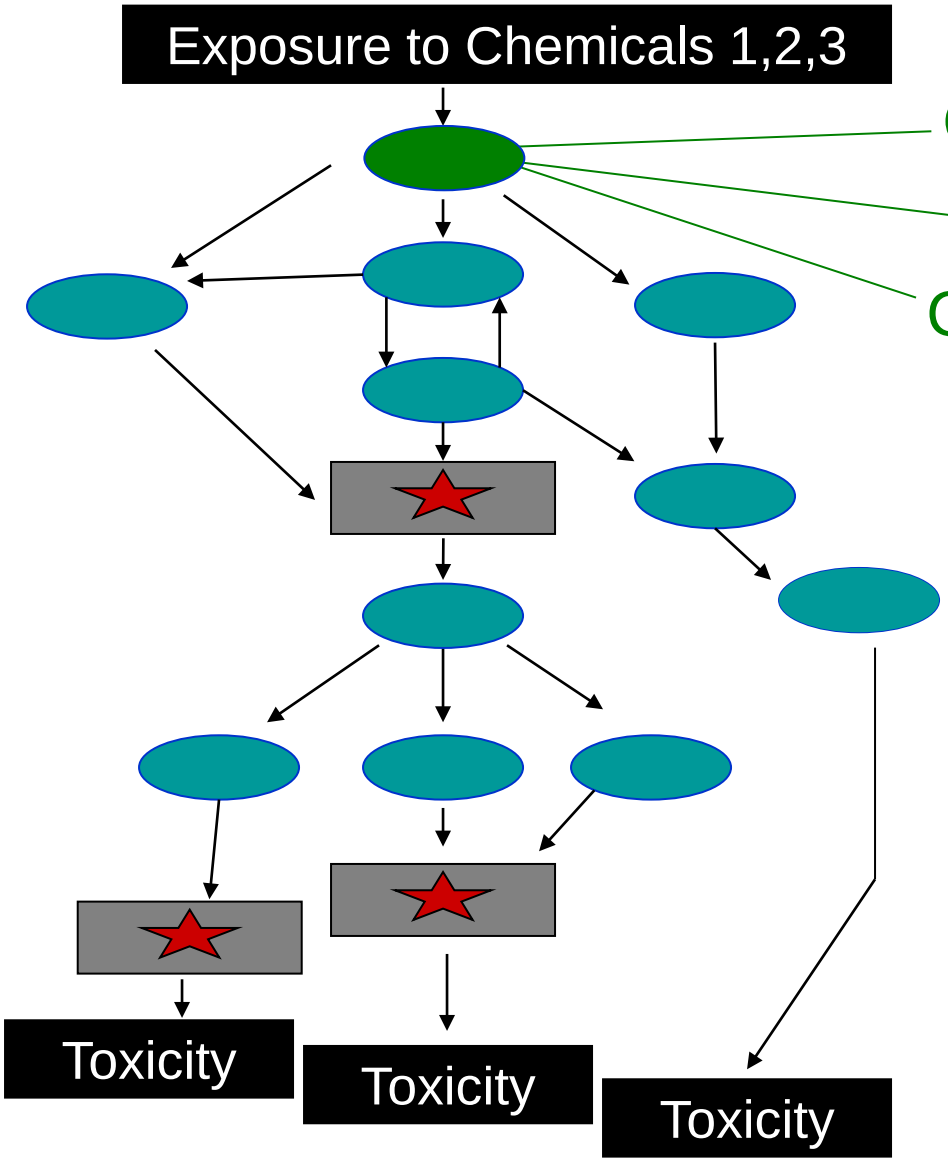
Event – Mechanism of Action, detailed understanding at biochemical and molecular level

Key Event – Mode of Action Identification of key and required steps

Toxicity Outcome – Observable adverse effect

Graphic used with permission of Jason Lambert & Linda Teuschler

Dose Addition via Same Mechanism of Action



Chemical 1 Toxic Action

Chemical 2 Toxic Action

Chemical 3 Toxic Action

Various adverse effects via a shared mechanism of action

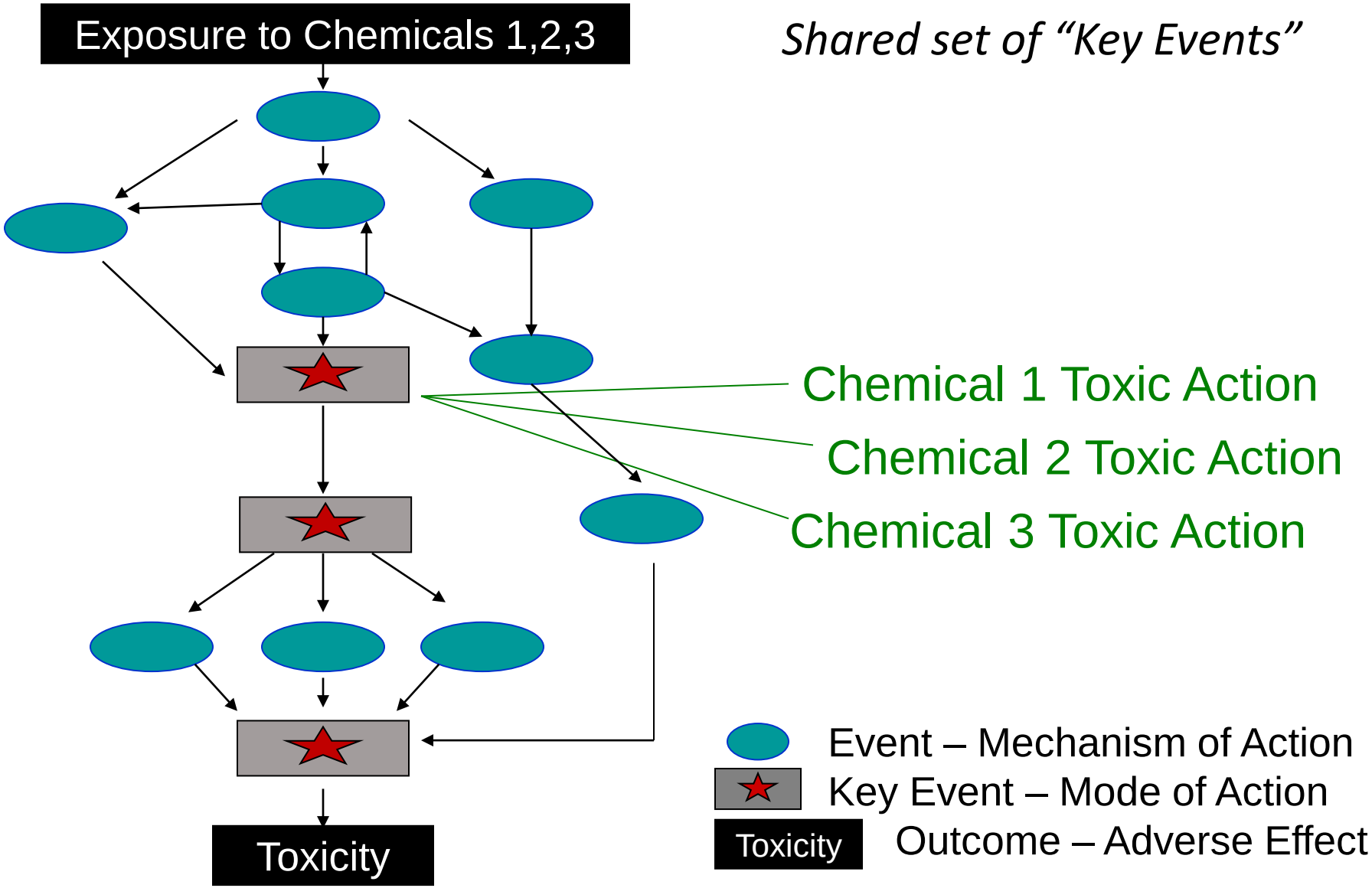
Event – Mechanism of Act

Key Event – Mode of Action

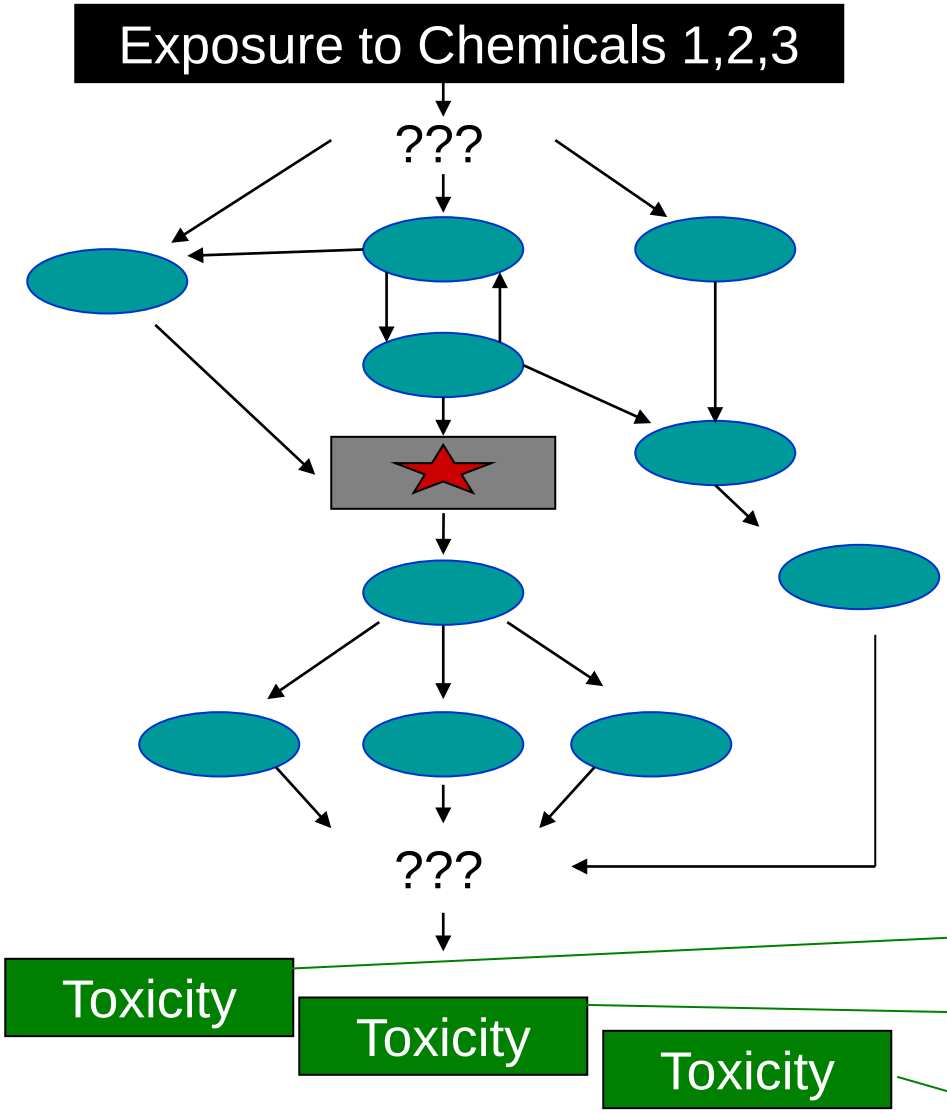
Toxicity Outcome -Adverse Effect

Dose Addition via Common Mode of Action

Shared set of "Key Events"



Dose Addition via Toxicological Similarity



Common target organ, tissue or system

Event – Mechanism of Action

Key Event – Mode of Action

Toxicity Outcome – Adverse Effect

Chemical 1 Toxic Action

Chemical 2 Toxic Action

Chemical 3 Toxic Action

What do Dose-Response Analysts Really Want?

- Chemically analyze complex mixtures being tested in epidemiology and toxicology studies
- Evaluate low-response region, include analysis of statistical power
  - Test environmentally-relevant component proportions
- Discuss/analyze biological significance of subtle effects observed at low doses/exposures
- Additional exposure analyses and testing of key components to fill data gaps in component approaches (e.g., dioxins, PAHs, phthalates)
- Collaborative interpretation/applications of high through-put technologies
  - Answer sufficient similarity questions
  - Answer MOA questions, if needed
  - Answer additivity questions
  - Prioritize mixtures for further testing

United States Environmental Protection Agency

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