United States Environmental Protection Agency

Introduction to EPA's Human Health Risk Assessment Practices for Chemical Mixtures

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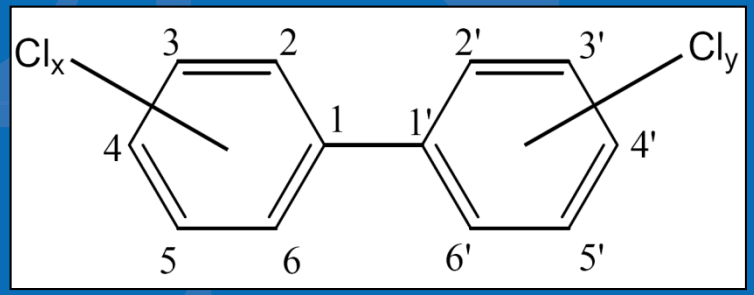

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- The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.
- I have no conflicts of interest to disclose.

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Public Science Meeting on PCB Mixture Assessment Methods

- **Introduction to EPA's human health risk assessment practices for chemical mixtures**
  - *Glenn Rice, U.S. EPA*

- **Mixtures modeling: methods considered for the assessment of PCBs**
  - *Laura Carlson and Jeff Gift, U.S. EPA*

- **Methods for estimating relative potency values**
  - *Grace Patlewicz, U.S. EPA*

- **Overview of the Mixture Similarity Tool (MiST)**
  - *Graham Glen and Joanne Trgovcich, ICF*

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Talk Outline

1. Introduction
2. Component Methods
3. Whole Mixture Methods
4. Sufficient Similarity Methods

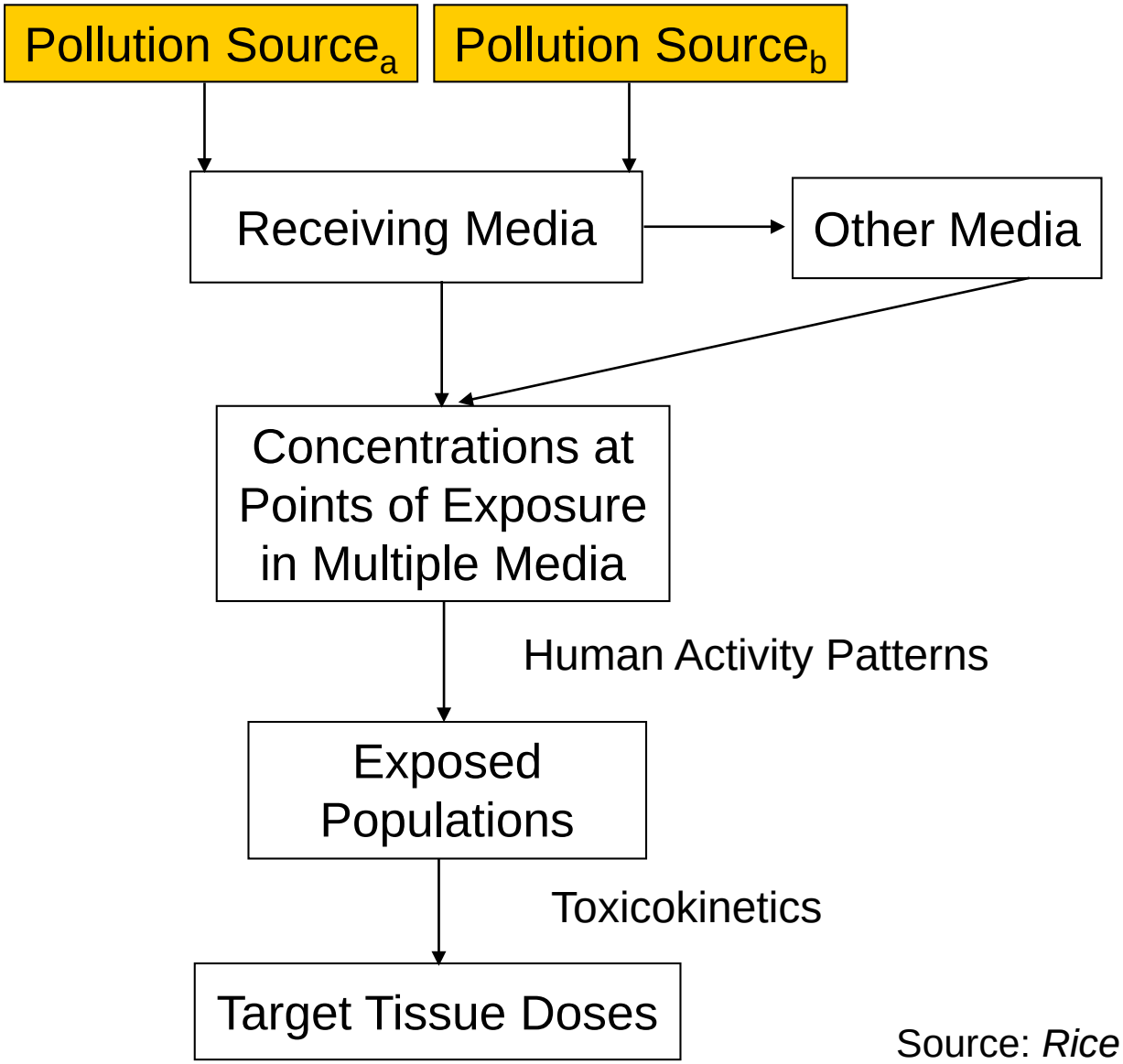
Chemical Mixtures

Any combination of two or more chemical substances regardless of source or of spatial or temporal proximity

- Combinations range from a few to 100's of chemicals
- Some components of complex mixtures may not be identified chemically

US EPA (1986) Chemical Mixtures Risk Assessment Guidelines
US EPA (2000) Supplementary Chemical Mixtures Guidance

Mixture Exposure Assessment Conceptual Model



Source: *Rice et al. (2008)*

Mixtures Fate and Transport

- Environmental mixtures can change over time

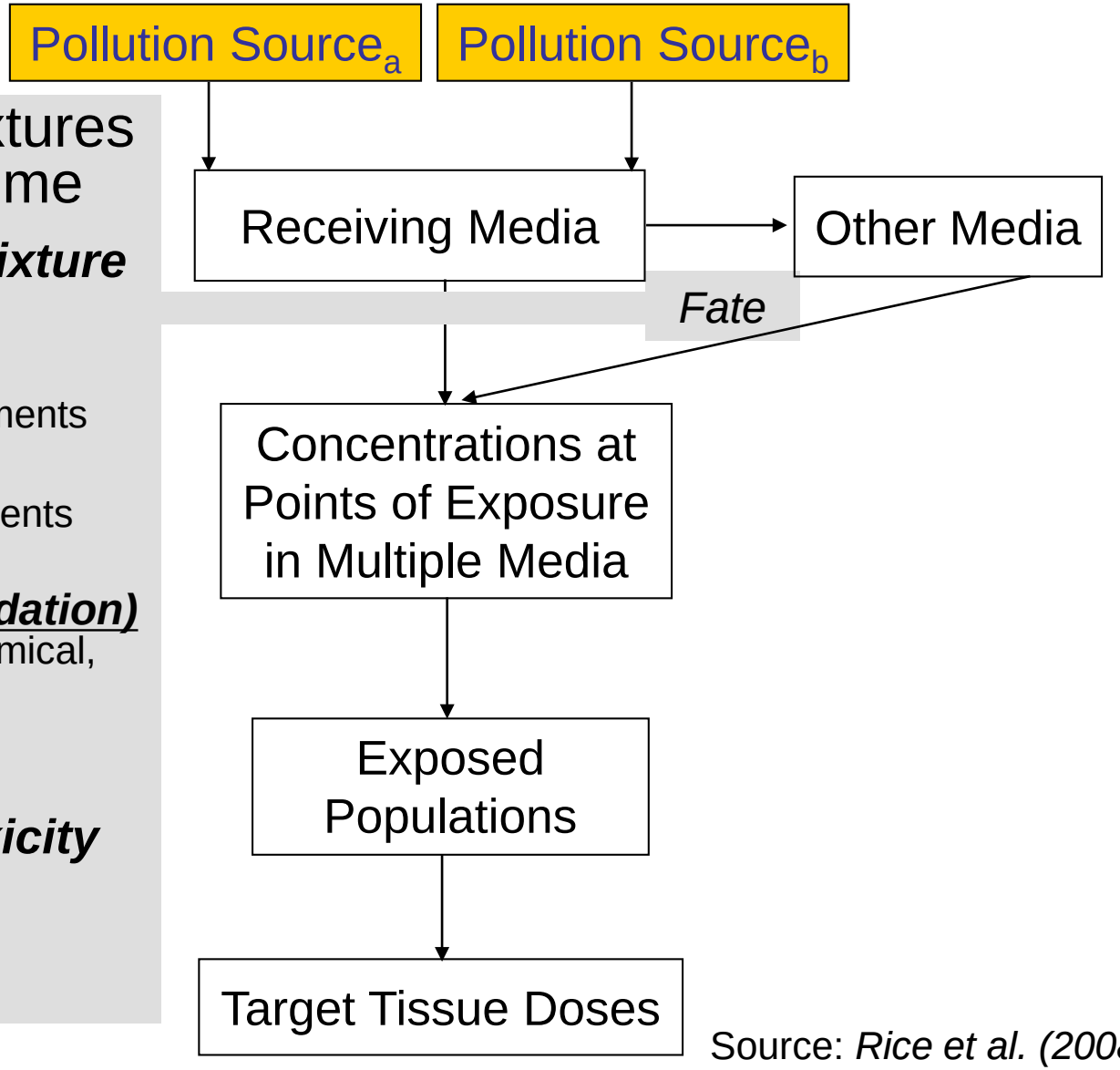
Differential fate of mixture components

Transport
through individual compartments

Partitioning
transfer between compartments (abiotic and biotic)

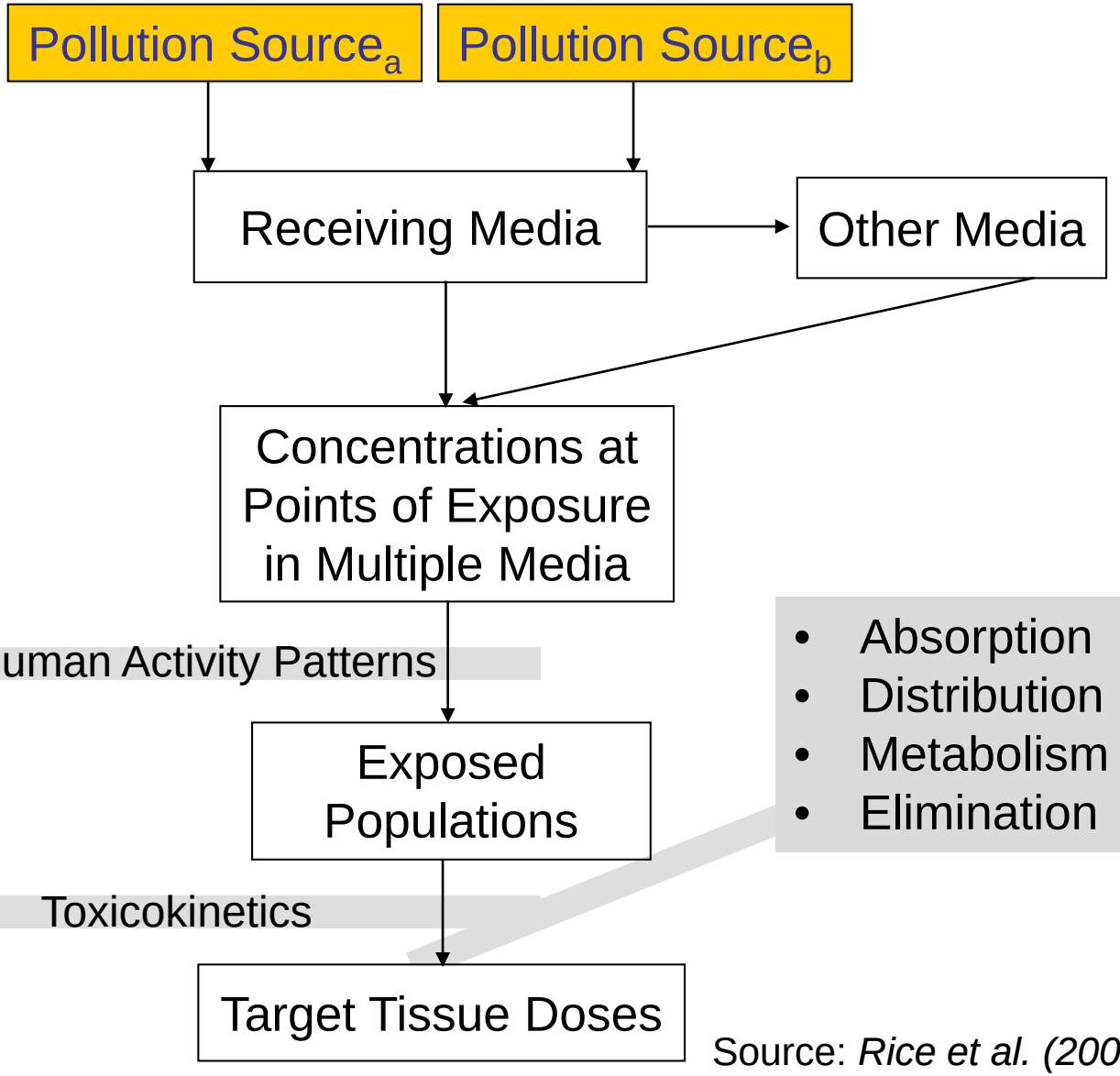
Transformation (degradation)
mediated by biological, chemical, physical agents

Changes can affect composition and toxicity of mixture



Source: *Rice et al. (2008)*

Mixture Exposures



Humans exposed concurrently and sequentially to many chemicals

- various routes of exposure
- over varying periods of time

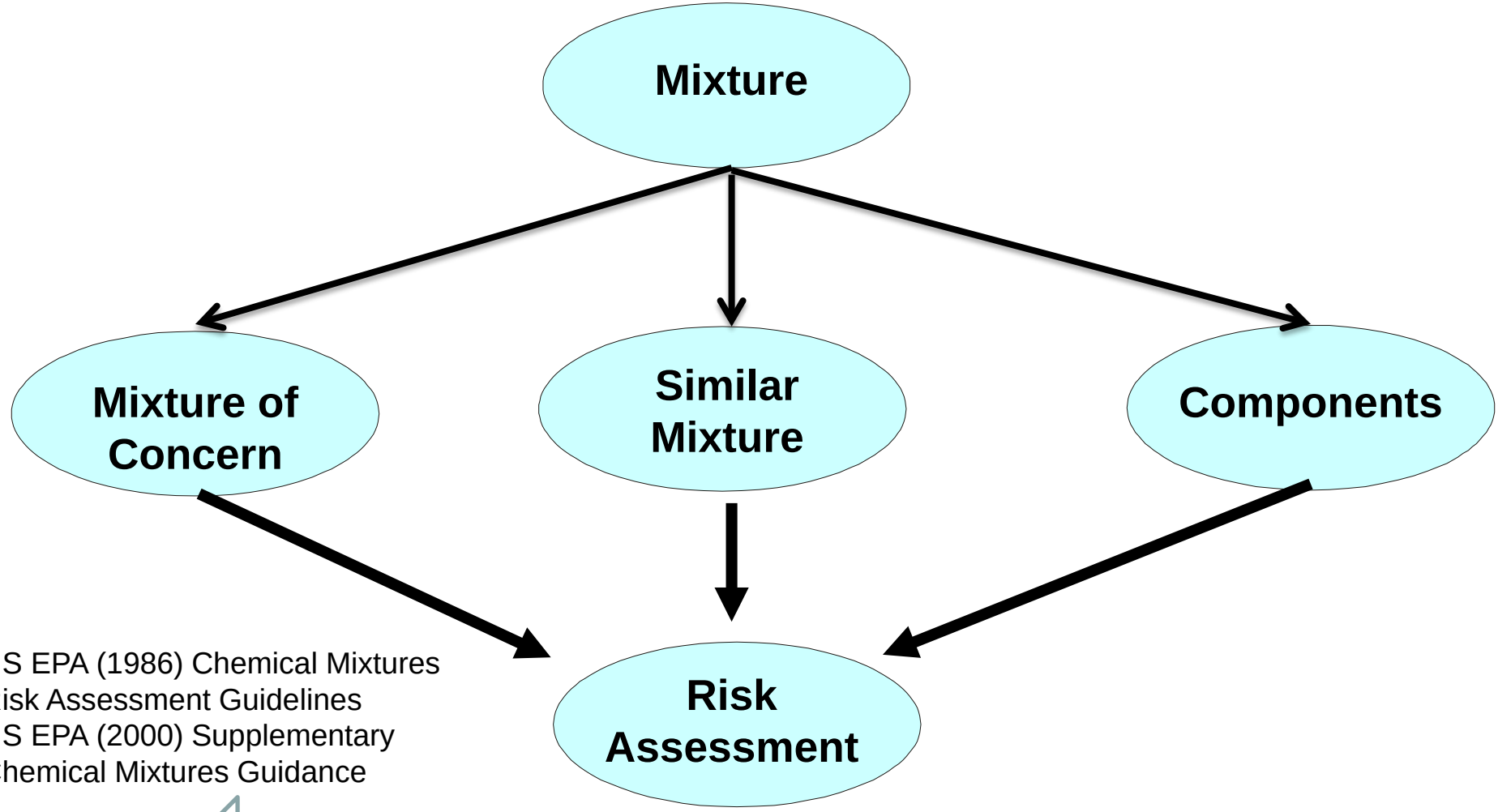
Primary exposure routes:

- ingestion
- dermal absorption
- inhalation

Source: *Rice et al. (2008)*

Chemical Mixtures Health Risk Assessment: Approaches

Choice of approach is data-driven



US EPA (1986) Chemical Mixtures Risk Assessment Guidelines
US EPA (2000) Supplementary Chemical Mixtures Guidance

Increasing Confidence

Component Methods

Generally, based on 1 of 3 assumptions regarding joint toxic action

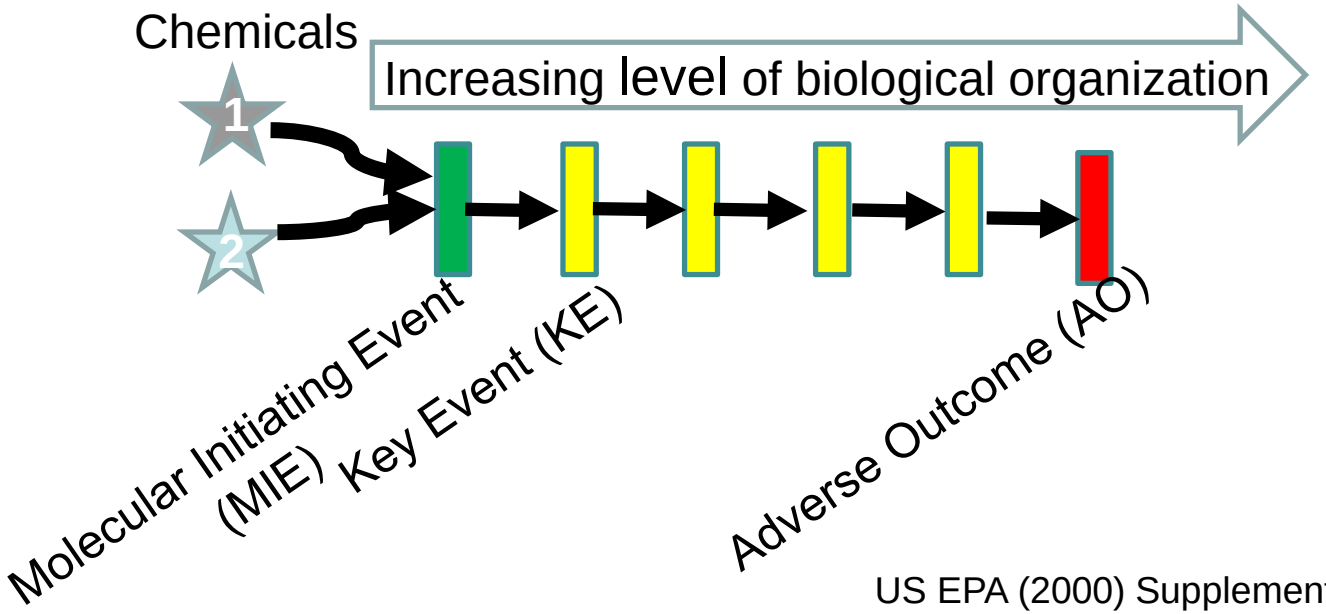
1. Simple Similar Action
2. Simple Dissimilar Action
3. Toxicological Interaction

US EPA (2000) Supplementary Chemical Mixtures Guidance

Additive Joint Toxic Action: Simple Similar Action

Dose addition: hazard index (HI), toxicity equivalence factors (TEFs), relative potency factors (RPFs)

- Addition of component doses, scaled for relative toxicity
- Assumes components affect same pathway of toxicity (i.e., common MOA)

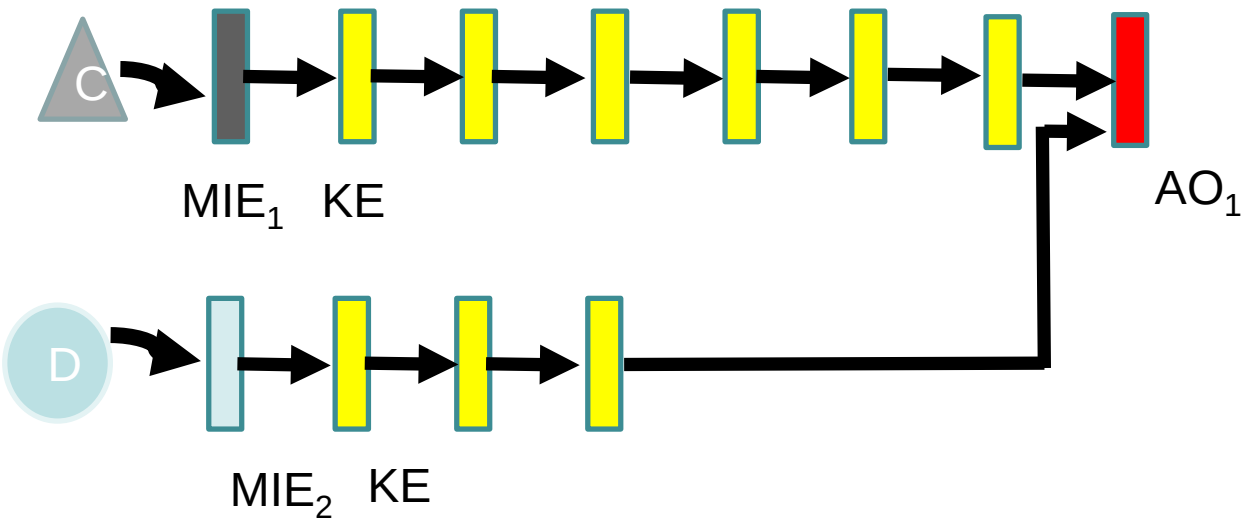


US EPA (2000) Supplementary Chemical Mixtures Guidance

Mixture of two chemicals, Chemical 1 and Chemical 2, act as toxicodynamic clones, affect same adverse outcome through same mode of action; doses add at the MIE in this hypothetical toxicity pathway

Simple Dissimilar Action

- Response addition—cancer risk sums
  - Addition of component risks
  - Assumes toxicological and statistical independence
- Effects addition—cumulative effects
  - Addition of biological responses across components
  - Assumes toxicological similarity across components



Mixture of 2 toxicologically independent chemicals affect same adverse outcome through different and independent pathways

US EPA (2000) Supplementary Chemical Mixtures Guidance

Toxicological Interactions

- Toxicological interactions are defined here as any toxic response that is greater than or less than those observed under the specified type of additivity, including new responses (not observed when chemicals dosed individually)
- Interaction effects
  - Types of Interactions
    - Chemical-Chemical
    - Toxicokinetic
    - Toxicodynamic
  - Many applicable terms (e.g., inhibition, masking, etc.)
  - Most common terms refer to descriptors that are:
    - greater than additive (i.e., synergism)
    - less than additive (i.e., antagonism)
- Interaction-Based Hazard Index Method

US EPA (2000) Supplementary Chemical Mixtures Guidance
Hertzberg, R.C.; MacDonell, M.M. Synergy and other ineffective mixture risk definitions. *Sci. Total. Environ.* **2002**, *288*, 31–42.

Dose Addition: Relative Potency Factors (RPFs) Generalized Index Chemical Method

Formula for estimating the Index Chemical Equivalent Dose (ICED). The product of the RPF and the dose of the individual chemical is summed to express the mixture dose for *n* chemicals in terms of the index chemical:

$$ICED = \sum_{i=1}^{n} \left[RPF_i \; X \; D_i \right]$$

where,

ICED = mixture dose expressed as dose of the index chemical

D_i = dose of the *i* th mixture component (*i* = 1,…,*n*), and

RPF_i = relative potency factor is a toxicity proportionality constant relative to the index chemical for the *i* th mixture component (*i* = 1,…,n).

US EPA (2000) Supplementary Chemical Mixtures Guidance

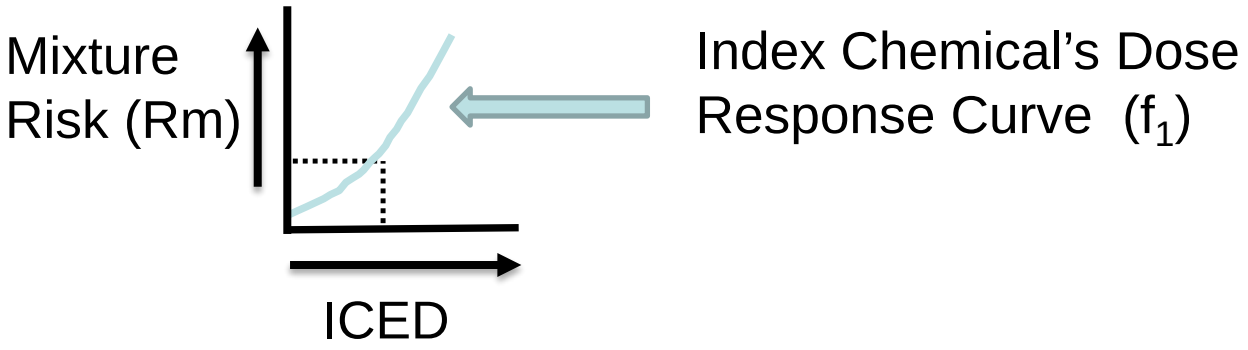
Dose Addition: Relative Potency Factors (RPFs) Generalized Index Chemical Method

RPF Method for 2 Chemical Mixture

$$Rm = f_1(D_1 + RPF_2D_2) = f_1(ICED)$$

Where:

RPF_i	Scales the dose (D) of chemical 2 for its relative potency to index chemical (Chemical 1),
Rm	predicted mixture risk
ICED	Index Chemical Equivalent Dose
f	Index Chemical Dose-Response Function



US EPA (2000) Supplementary Chemical Mixtures Guidance

Methods to Calculate RPFs

For mixture components, chemical i and index chemical, the Relative Potency Factor (RPF_i) may be estimated as:

1) the ratio of equally toxic doses of the 2 chemicals, e.g.,

$$RPF_i = \frac{ED_X\left(Index\ Chemical\right)}{ED_X\left(Chemical_i\right)}$$

ED_x = The "Effective Dose" at which an x% response is observed.

2) the ratio of potency factors of the 2 chemicals, e.g.,

$$RPF_i = \frac{Dose\ Coefficient\left(Chemical_i\right)}{Dose\ Coefficient\left(Index\ Chemical\right)}$$

US EPA (2000) Supplementary Chemical Mixtures Guidance

Comparison of TEFs and RPFs

Toxicity Equivalence Factor	Relative Potency Factor
Specific Type of RPF	**Generalized Case**
All health endpoints	May be limited
All routes	May be limited
All timeframes of exposure	May be limited
Encompasses all doses	May be limited to specific dose range
Implies more abundant data	May be based on lower quality/ fewer data are available
Implies greater certainty about mechanism of action	Assumes similar mode of action May be more accurate because application can be constrained given available data
One TEF set for all scenarios	Can generate different RPF sets for various scenarios

Source: US EPA (2000) Supplementary Chemical Mixtures Guidance

Whole Mixtures

- "Whole mixtures" typically represent the combination of chemicals in the exposure being assessed.
  - Operationally defined in some situations
- The composition of the mixture including the component chemicals and their proportions might be fully known, partially known, or unknown.

Rice, G.E., I. Eide, P. I. Feder, C. Gennings. 2018. Chapter 15: Assessing Human Health Risks Using Information on Whole Mixtures. In Chemical Mixtures and Combined Chemical and Nonchemical Stressors Exposure, Toxicity, Analysis, and Risk. Eds. C. V. Rider and J.E.Simmons. Springer International. Cham Switzerland

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Whole Mixture Methods

- Risk assessors generally have more confidence in assessments based on whole mixture methods than those based on component methods
- Generally, fewer data on whole mixtures
- Applicability concerns
  - composition of tested mixture may differ from environmental mixture of concern
  - complications associated with measuring mixture, and preparing mixture for toxicological testing environmental mixtures (e.g., collecting, concentrating and storing)

Procedure to Derive Whole Mixture Health Risk Values

1. Data collection and evaluation of mixture that was tested
   - Epidemiology/human data
   - In vivo toxicology data
   - In vitro toxicology data
2. Evaluate stability of the mixture that was tested
   - Variability in components and their relative proportions
     - Across sources
     - Over time within a medium
     - Across media (e.g., uptake and retention of toxic compounds in food web)
3. Is the mixture on which health effects data are available sufficiently similar to mixture of concern (i.e., mixture encountered in the environment)?

Source: US EPA (2000) Supplementary Chemical Mixtures Guidance

Procedure to Derive Whole Mixture Health Risk Values (Part 2)

4. Conduct dose-response assessment
   - Use single chemical procedures (e.g., RfD, slope factors)

5. Characterize uncertainties
   - Relevance of observed health effects in the study of tested mixture to those anticipated through environmental exposures

Source: US EPA (2000) Supplementary Chemical Mixtures Guidance

Whole Mixture Reference Dose (RfDm)

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

Where:

RfD	=	Reference dose
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, database deficiencies)

NOAEL, LOAEL or BMDL typically from experimental toxicity data on complex mixture dose-response

Reference Dose "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."

Source: US EPA (2000) Supplementary Chemical Mixtures Guidance

Mixture Case Study: Technical and Weathered Toxaphene

- Manufacture of insecticide, piscicide, and acaricide begins mid-1940s
- Complex mixture of hundreds of chlorinated terpenes (ATSDR, 2014)
- U.S. EPA cancelled registration for most uses in 1982 and all registered uses in 1990 (ATSDR, 2014; US EPA, 2018)
- Most European Nations ban toxaphene during 1980s (Barbini, 2007)

- Following environmental release, toxaphene congeners undergo differential transformation and degradation via abiotic and biotic (e.g., soil microbes) processes, resulting in different mixtures of persistent toxaphene congeners, commonly termed “weathered toxaphene”

ATSDR (Agency for Toxic Substances and Disease Registry). (2014). Toxicological profile for toxaphene [ATSDR Tox Profile]. Atlanta, GA: U.S. Department of Health and Human Services. http://www.atsdr.cdc.gov/ToxProfiles/tp94.pdf
Barbini, (2007). Determination of toxaphene residues in fish foodstuff by GC-MS. Bull Environ Contam Toxicol 79: 226-230. http://dx.doi.org/10.1007/s00128-007-9179-6
US EPA (2018). Provisional Peer-Reviewed Toxicity Values for Technical Toxaphene (CASRN 8001-35-2) Weathered Toxaphene, and Toxaphene Congeners. EPA/690/R-18/002

Derivation of a Provisional Chronic p-RfD for Technical Toxaphene

Effect: thyroid cytoplasmic vacuolation, male rats, 25−29 wks dietary exposure

Point of Departure: **$BMDL_{10}$ (HED) = 0.0092 mg/kg-day**

$UF_A = 3\ (10^{0.5})$	accounts for uncertainty in characterizing toxicokinetic or toxicodynamic differences between rats and humans
$UF_D = 3\ (10^{0.5})$	accounts for uncertainty: potentially more sensitive immune effects following chronic exposure
$UF_H = 10$	accounts for intraspecies (human-to-human) variability in susceptibility

$$RfD = \frac{BMDL_{10}}{UF_A \times UF_D \times UF_H}$$

9×10^{-5} mg/kg-day *= 0.0092 mg/kg-day ÷ 100*

Chu (1988) Reproduction study of toxaphene in the rat. J Environ Sci Health B 23: 101-126.
US EPA (2018) Provisional Peer-Reviewed Toxicity Values for Technical Toxaphene (CASRN 8001-35-2) Weathered Toxaphene, and Toxaphene Congeners. EPA/690/R-18/002

Derivation of Screening* Provisional Chronic p-RfD for Weathered Toxaphene

Effect: thyroid cytoplasmic vacuolation, male rats, 25−29 wks dietary exposure

Point of Departure: **$BMDL_{10}$ (HED) = 0.0092 mg/kg-day**

$UF_A = 3 (10^{0.5})$	accounts for uncertainty in characterizing toxicokinetic or toxicodynamic differences between rats and humans
$UF_D = 10$	accounts for uncertainty associated with the limited available data comparing the toxicity of technical toxaphene and weathered toxaphene
$UF_H = 10$	accounts for intraspecies (human-to-human) variability in susceptibility

*EPA judged that the toxicity data for weathered toxaphene or individual toxaphene congeners are inadequate to derive noncancer provisional toxicity values. However, available information was judged adequate to be of limited use to risk assessors, and in such cases, EPA develops a “screening value.”

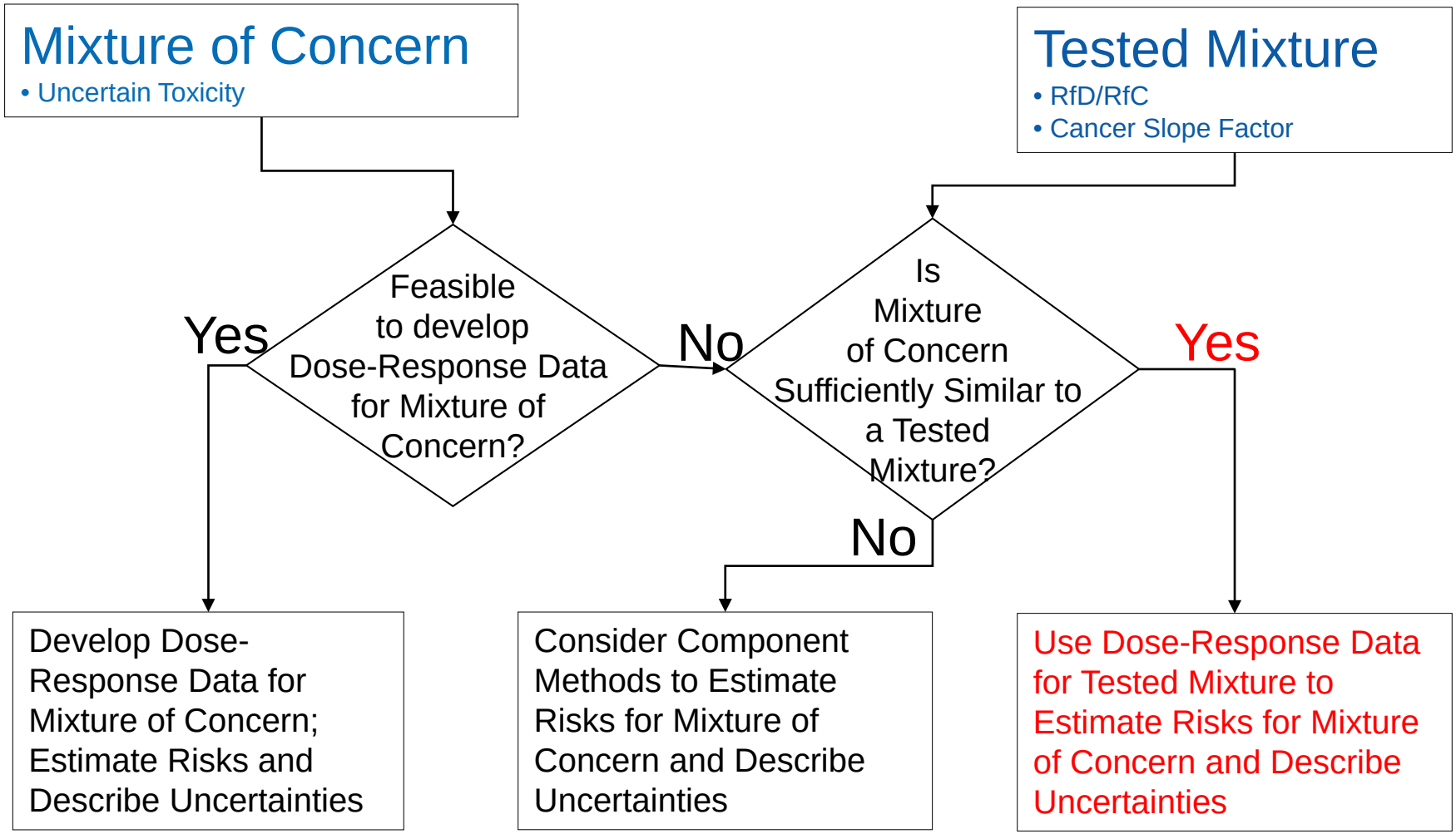
Sufficient Similarity

EPA 2000

- If toxicity data are not available for a mixture of concern, the risk assessment can be based on surrogate toxicity information obtained from testing a *sufficiently similar* mixture
- Describes general principles, but no methods
  - A mixture is sufficiently similar to another when its components are not very different and are in about the same proportions.
  - Few differences in environmental fate, uptake, bioavailability and pharmacokinetics.
  - Expected toxicological consequences of exposure to the two mixtures are nearly identical.

Source: US EPA (2000) Supplementary Chemical Mixtures Guidance

Sufficient Similarity



Mixtures considered sufficiently similar when expected health consequence of exposure to 2 mixtures nearly identical

Source: Rice, G. E., I. Eide, P. I. Feder, C. Gennings. 2018 . Assessing Human Health Risks Using Information on Whole Mixtures. Chapter 15 in Chemical Mixtures and Combined Chemical and Nonchemical Stressors: Exposure, Toxicity, Analysis, and Risk Editors C.V. Rider and J.E. Simmons. Springer International Publishing AG

Sufficient Similarity Approaches

Sufficient similarity methods not included in EPA (2000) Guidance

Some Published Sufficient Similarity Approaches

- Feder et al. 2009: Uses principal component analysis
- Feder et al. 2009: Uses statistical bootstrap method
- Marshall et al. 2013: Uses equivalence testing methods
- Caitlin et al. 2018: Additional NTP tox. Testing of botanical supplement. Uses strength-of-evidence approach, empirical equivalence testing, and visual interval evaluation

Marshall et al. 2013 methods provide a reasonable and defensible approach for evaluating similarity among PCB Mixtures (discussed further in presentation 2)

Marshall et al. 2013 “An empirical approach to sufficient similarity: combining exposure data and mixtures toxicology data” Risk Analysis 33(9) 1582-1596.

Feder et al., 2009 “Evaluating sufficient similarity for drinking-water disinfection by-product (DBP) mixtures with bootstrap hypothesis test procedures”. J Toxicol Environ Health A.;72(7):494-504.

Feder et al 2009. “Evaluating sufficient similarity for disinfection by-product (DBP) mixtures: multivariate statistical procedures.” J Toxicol Environ Health A. 2009;72(7):468-81.

Caitlin 2018. How similar is similar enough? A sufficient similarity case study with Ginkgo biloba extract. Food Chem Toxicol. 118:328-339.

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Summary

- Basing risk assessments on whole mixtures dose-response data considered more reliable than basing assessments on component dose-response data
- Component data approaches most often used in assessments of environmental mixtures; RPF method has been used to evaluate hazard and cancer risks for multiple mixtures
- When available (infrequent), reference doses and cancer slopes can be derived from whole mixture dose-response data and used in risk assessments
- A goal of sufficient similarity approaches is to evaluate the confidence in using dose-response data for a tested mixture in assessments of other mixtures that have either not been tested or been subjected to limited testing

Future Directions

- Additional approaches for evaluating similarity are needed
- Outputs from new approach methodologies (NAMs) could be used to generate hazard assessment data and dose-response data to address similarity among mixtures
    - o toxicogenomics
    - o proteomics
    - o metabolomics
    - o chemoinformatics
    - o bioinformatics
    - o cell-based bioactivity screening assays
- Informativeness of the NAM data for addressing similarity among mixtures will depend on relationship between the NAM endpoint/s and human disease (i.e., confidence in understanding the relationship).

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  - Ingvar Eide, *Retired, Statoil ASA*

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