NTP
National Toxicology Program

Combined Exposures and Mixtures Program

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National Institute of Environmental Health Sciences

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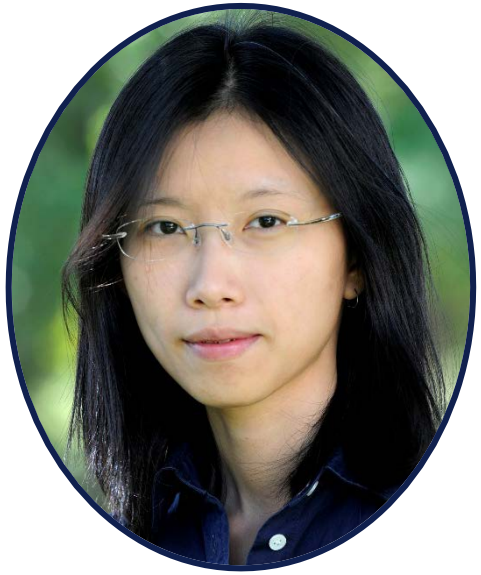

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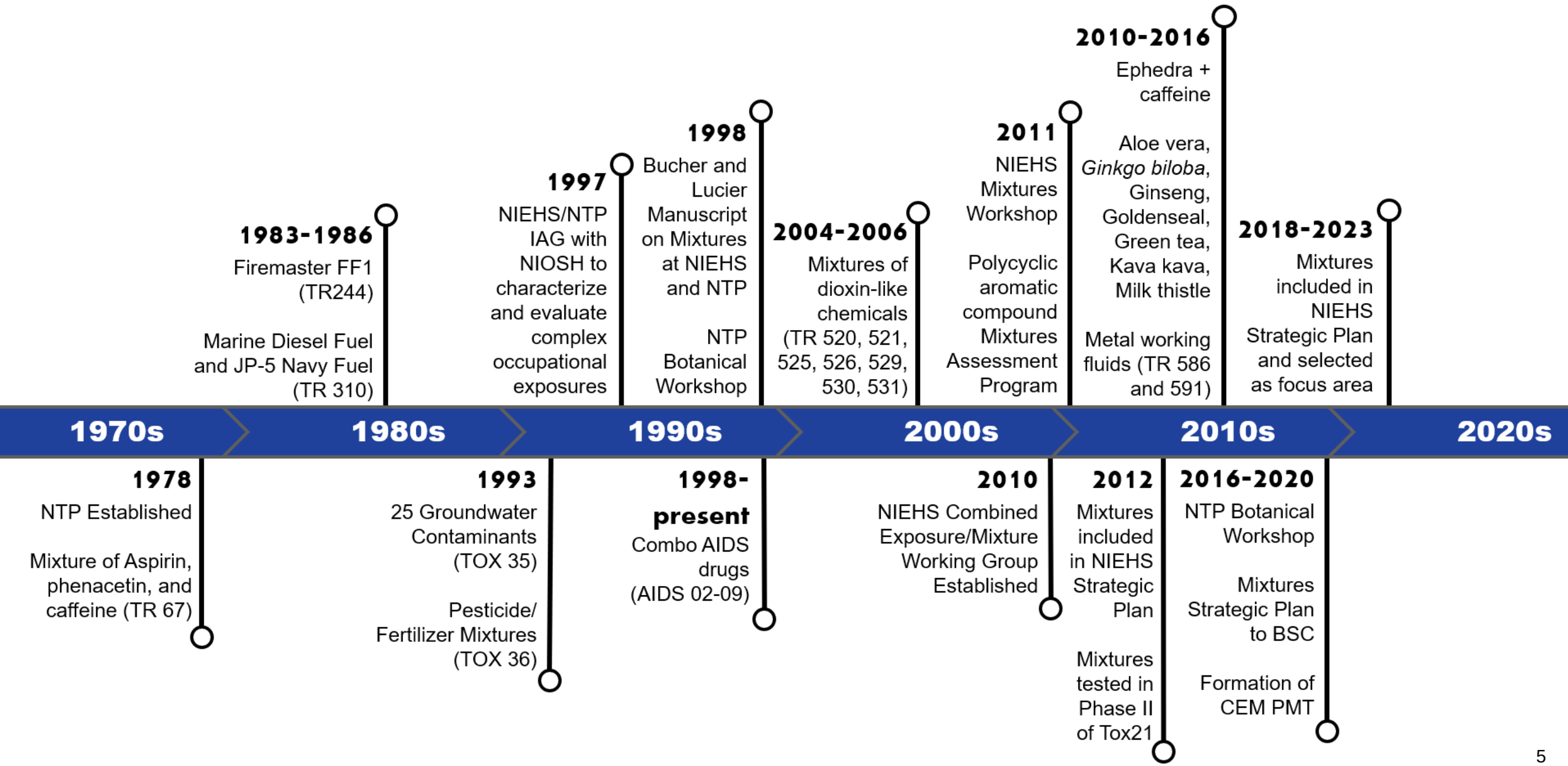
Problem statement

- Challenges persist in characterizing exposure to mixtures, evaluating their toxicity and hazard, and assessing associated risk.
- There is inconsistent use of available mixture methods and uncertainties in their application.
- Lack of harmonized terminology and methods comparisons complicate information synthesis and impede the use of mixtures data in decision-making.

Definitions

- Defined mixture – A mixture in which all components are identified and quantified.
- Complex mixture – A mixture of many constituents with some unidentified fraction (e.g., effluent sample, diesel exhaust).
- Exposome – Totality of exposures over a lifetime.
- Whole mixture approach – Considers the complete mixture. A whole mixture can be simple (containing few constituents) or complex.
- Component-based approach – Considers the components (aka constituents) in order to understand the mixture.

Historical Perspective



Mixture Risk Assessment Context

Objective 1

Problem formulation: **Identifying mixture of interest**

Data availability and quality

inadequate

No quantitative assessment

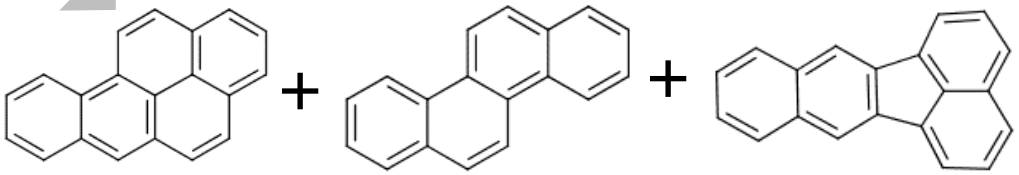
Objective 2

Whole mixture approach

Sufficient similarity

Objective 3

Component-based approach



Predictive models of mixture toxicity based on defined assumptions (dose addition, response addition)

comparison

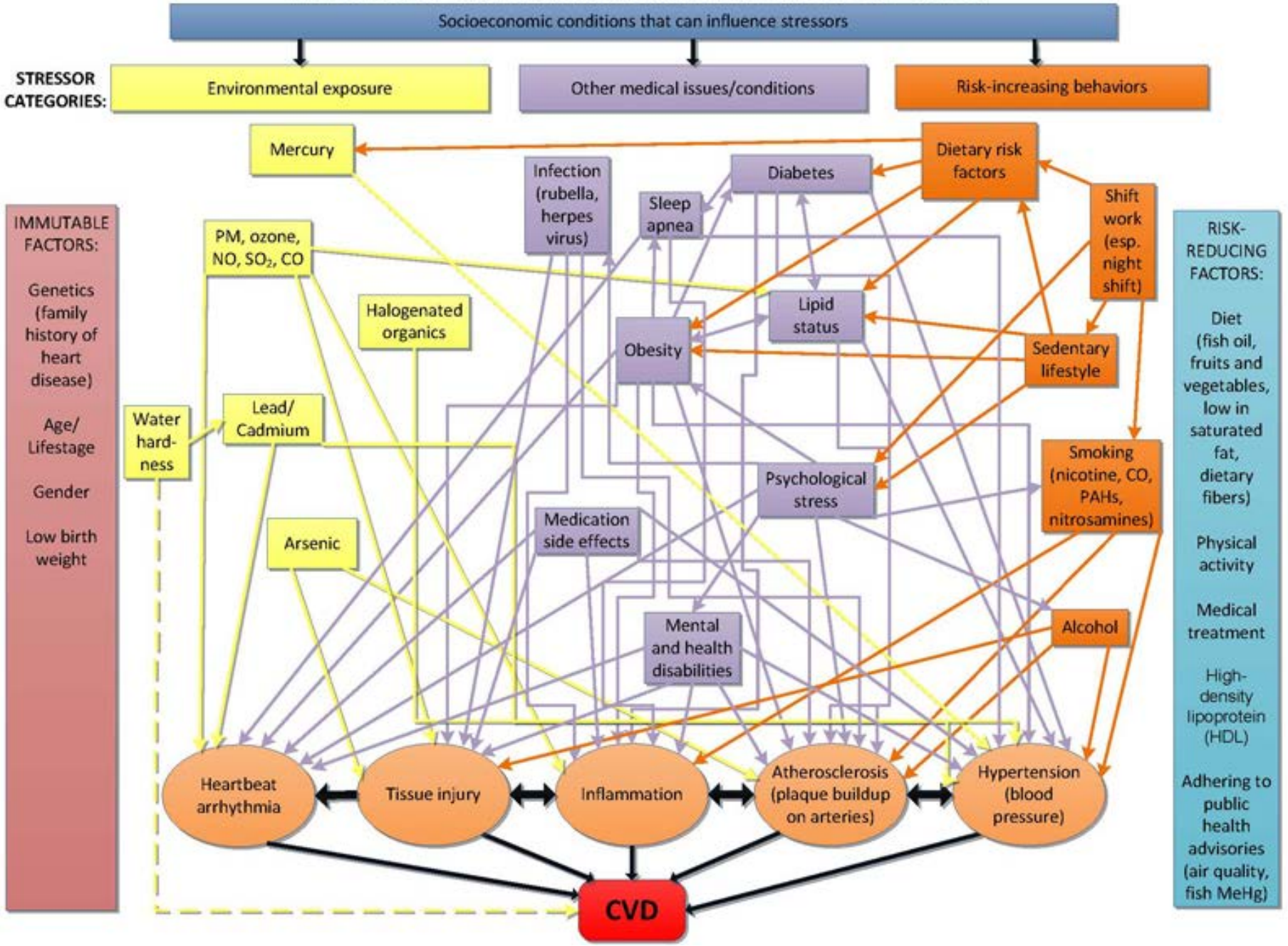
Objectives

1. Develop and apply a disease-centered systems biology approach for prioritizing mixtures for toxicological and hazard characterization to inform cumulative risk evaluation.
2. Develop and apply methods for complex mixture testing and data interpretation to inform risk assessment of whole mixtures.
3. Apply component-based approaches by experimentally evaluating defined mixtures and using predictive modeling approaches (e.g., dose addition, response addition) and compare the results with alternative whole mixture evaluation.

Objective 1: Disease-Centered Systems Biology Approach

- Currently, chemicals are grouped based on similar mechanism of action (e.g., dioxins, organophosphates, polycyclic aromatic compounds) or co-occurrence (Superfund site) due to:
  - Legislative mandates (e.g., Food Quality Protection Act)
  - Pragmatism
  - Scientific support for the use of dose addition with chemicals that have similar mechanisms of action
- These approaches for determining which chemicals to include in mixtures risk assessments are not necessarily the most protective or the most scientifically sound.

FACTORS INFLUENCING THE RISK OF CVD:



Developed by C. Menzie for EPA Cumulative Risk Assessment Workshop

Objective 1: Disease-Centered Systems Biology Approach

Hypothesis: Chemicals that target disparate signaling pathways contribute cumulatively to disease development, and their joint action can be estimated using mixture modeling approaches.

Approach: Develop mixtures projects focused on diseases that are priority areas of interest for DNTP

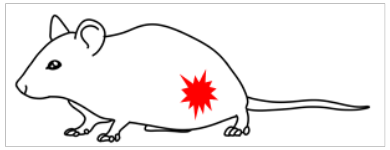
- Cancer
- Cardiovascular disease

Objective 1: Disease-Centered Systems Biology Approach

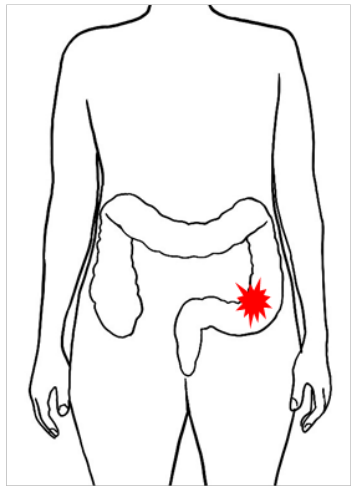
Disease-centered approach

1 Disease of interest: Colon cancer

2 Model and chemical selection; study design

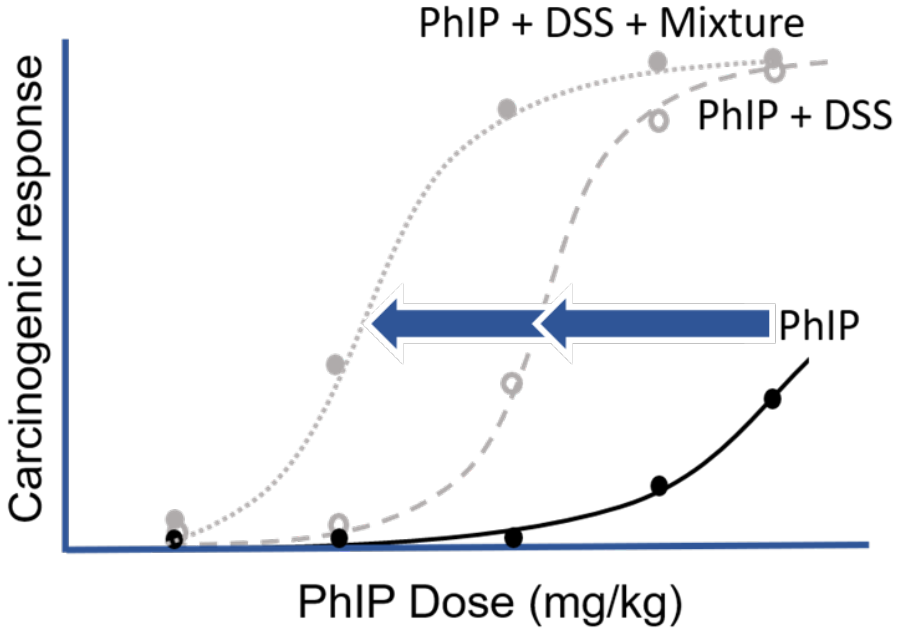


3 Data analysis: Treatment comparison



	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
Study 1	PhIP D-R					
Study 2	PhIP D-R	DSS				
Study 3	PhIP D-R	DSS	Atrazine + Cadmium + Bisphenol A			
Control		DSS				
Control			Atrazine + Cadmium + Bisphenol A			

Termination & data collection



Objective 2: Whole Mixtures

Develop and apply methods for complex mixture testing and data interpretation to inform risk assessment of whole mixtures

- Apply targeted and non-targeted chemical analyses, in vivo bioassays, and literature review methods for complex mixture testing and data interpretation to inform risk assessment.
- Develop methods for complex mixture evaluation including sufficient similarity, polypharmacokinetics, and bioassay-guided fractionation to identify toxic constituents.
- Provide DNTP research support for the Botanical Safety Consortium – a public-private partnership aimed at developing a toolbox of in vitro assays for identifying hazards associated with botanical ingredients.

Objective 2.1: Whole Mixture Testing and Analysis

Apply targeted and non-targeted chemical analyses, in vivo bioassays, and literature review methods

- Botanical testing program (e.g., *Garcinia cambogia*, black cohosh extract, *Echinacea purpurea*)
- Woodsmoke cancer hazard evaluation
- Personal care products health hazard evaluations (in coordination with Consumer Products and Therapeutics Program)

Objective 2.2: Methods – Sufficient Similarity

Chemical data

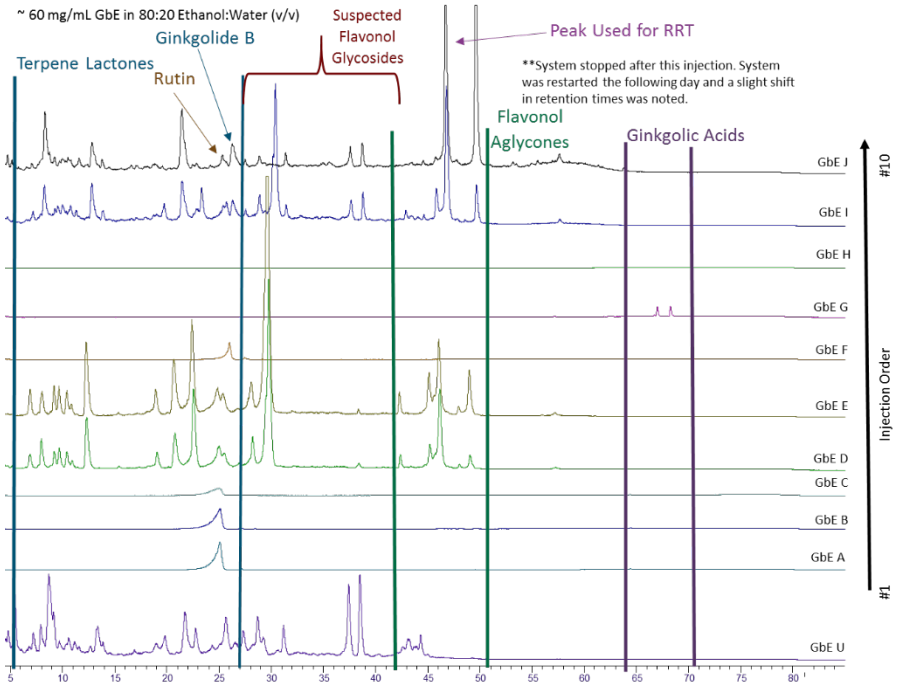
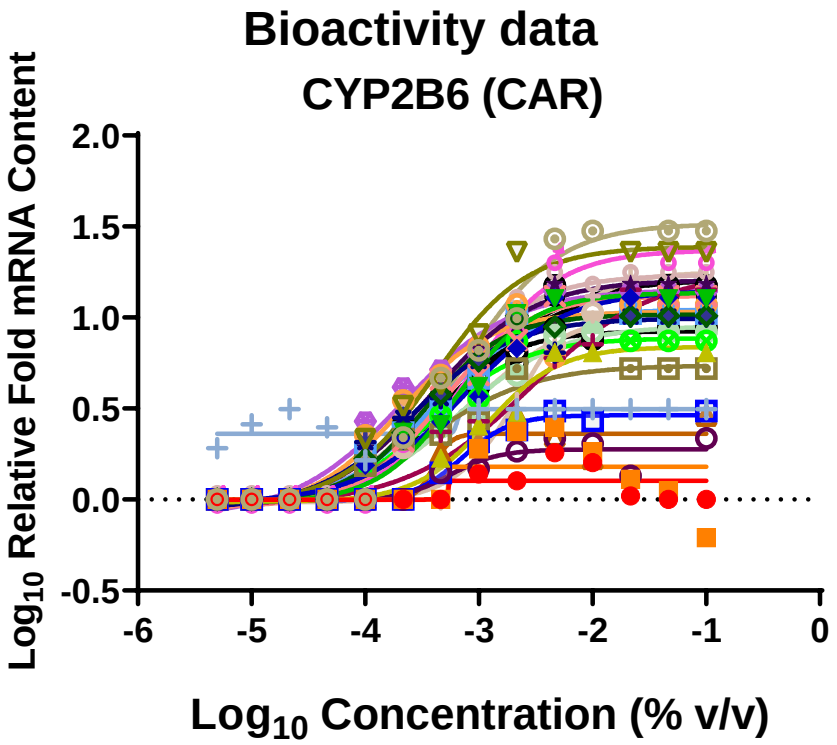
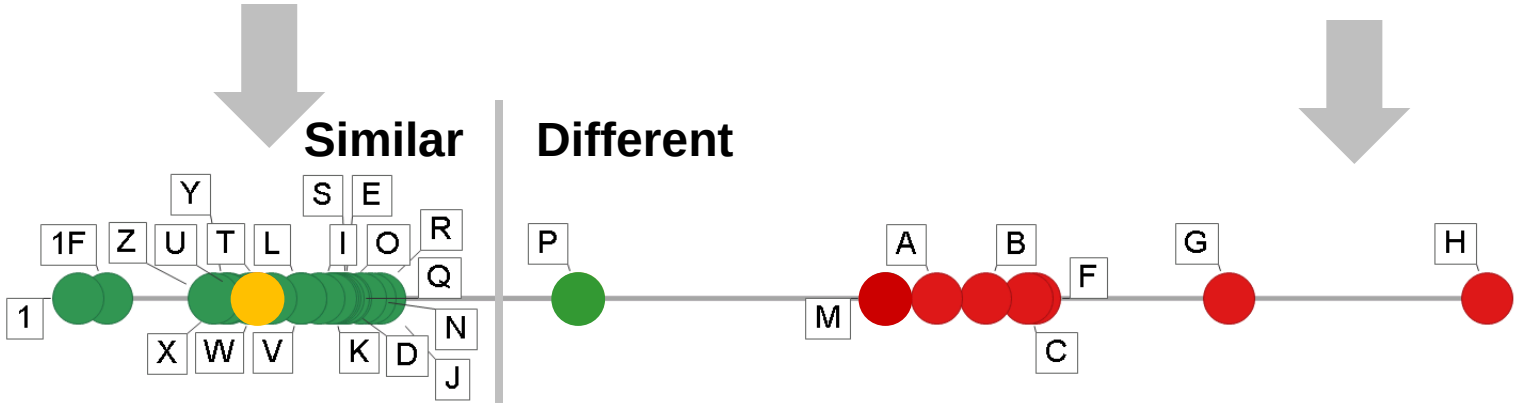


Figure 2. Non-Targeted Fingerprint Chromatograms of First Set of GbE Samples (Not Hydrolyzed), HPLC-ELSD

Bioactivity data





Catlin NR, et al. Food Chem Toxicol. 2018; 118:328-339

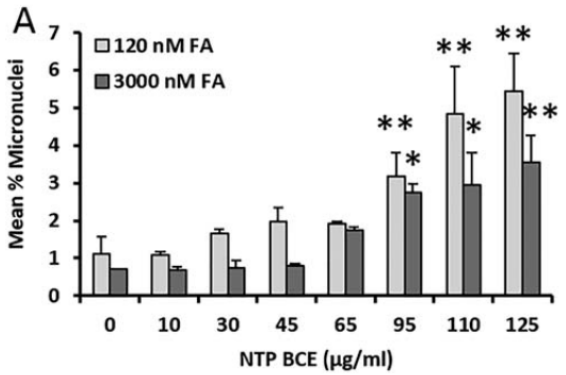
Objective 2.2: Methods – Bioassay-Guided Fractionation

Extraction

Bioassay

Active extract

Separation



Bioassay

Isolation/
Identification

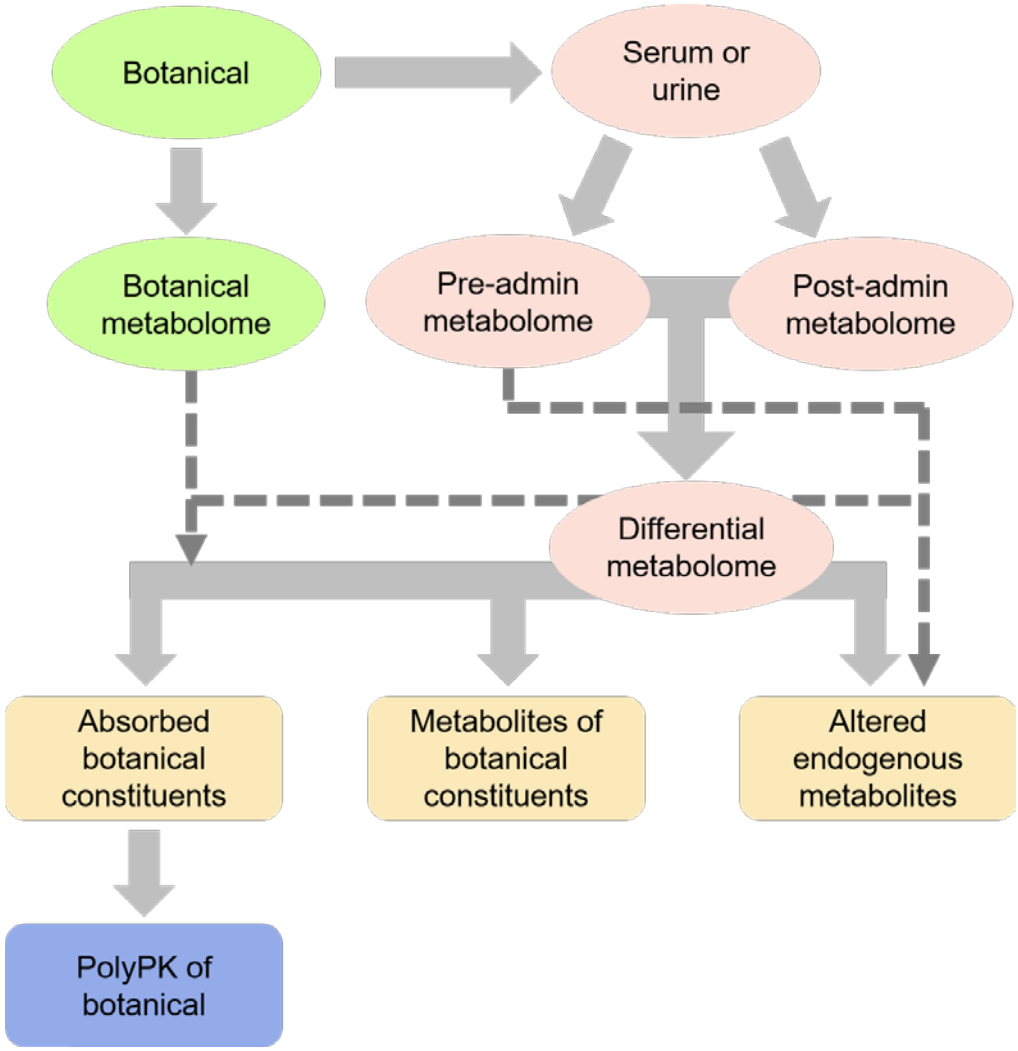
Chemical Structure

Active fraction

Roberts et al., 2019. Food and Chemical Toxicology. 124: 431-438.
Smith-Roe et al., 2018. Environmental and Molecular Mutagenesis 59:416-426.

Objective 2.2: Methods - Polypharmacokinetics

Standard practice	Recommendations
• Rarely assess ADME in animal studies	• Regularly assess ADME in animal studies
• Follow ‘marker’ constituents	• Follow toxicologically important constituents (identify active constituents) or employ polypharmacokinetics
• Drug-botanical interactions rarely evaluated with emphasis on clinical assessment	• Leverage *in silico* and *in vitro* approaches to identify potential drug-botanical interactions
• Animal to human dose comparisons rely on administered dose	• Animal to human dose comparisons based on systemic exposure (e.g., C_{max}, AUC, PBPK modeling)



Waidyanatha et al., 2018. Food and Chemical Toxicology. 121: 194-202.

Objective 2.3: Botanical Safety Consortium

Objectives

BOTANICAL SAFETY CONSORTIUM

- Engage with a broad group of global stakeholders to leverage the best scientific approaches
- Establish the appropriate levels of chemical characterization for complex botanical ingredients
- Identify pragmatic, fit-for-purpose *in vitro* & *in silico* assays to evaluate botanical safety
- Evaluate the application of these tools via comparison to the currently available safety information
- Integrate these tools and approaches into a framework that will facilitate robust evaluation of botanical ingredients

Objective 2.3: Botanical Safety Consortium

https://botanicalsafetyconsortium

The **BOTANICAL SAFETY CONSORTIUM** will provide a sound scientific basis for integrating existing botanical safety & toxicity information with the latest toxicological tools.

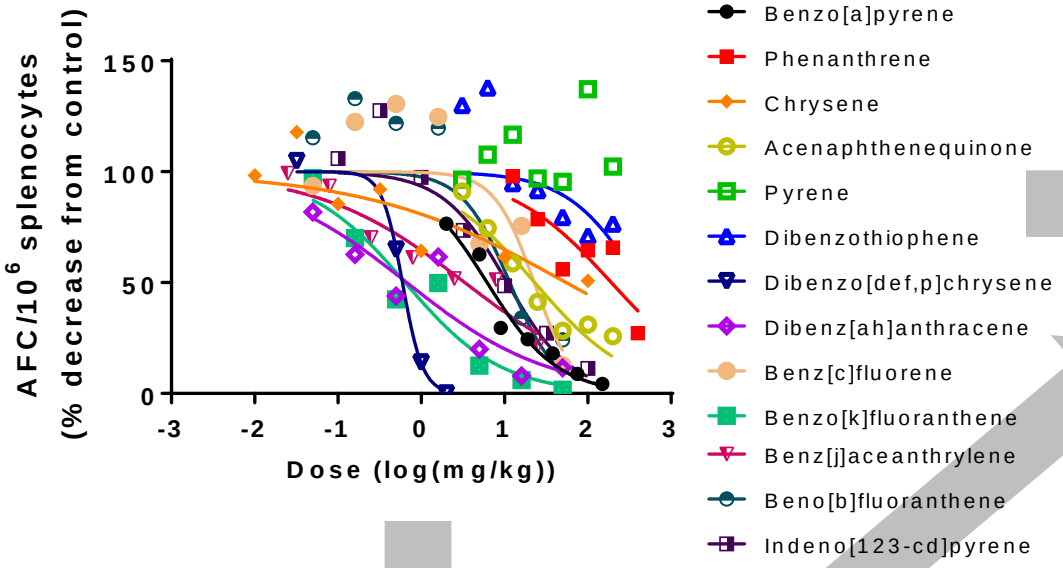
Objective 3: Strengthen Component-Based Approaches

- Component-based approaches incorporate dose-response data from individual chemicals to predict mixture effects.
- They represent the current default approach for mixtures risk assessment, despite notable limitations and challenges:
  - Only consider a small subset of individual chemicals for which dose-response data are available
  - Involve assumptions about chemical behavior, such as:
    - Joint action assumption (i.e., dose addition or response addition)
    - Lack of chemical interactions
- A whole mixture approach is favored by risk assessors and should be developed and compared to the current component-based approach

Polycyclic Aromatic Compound Mixtures Assessment Program

Component-Based Approach

Individual chemical dose-response data

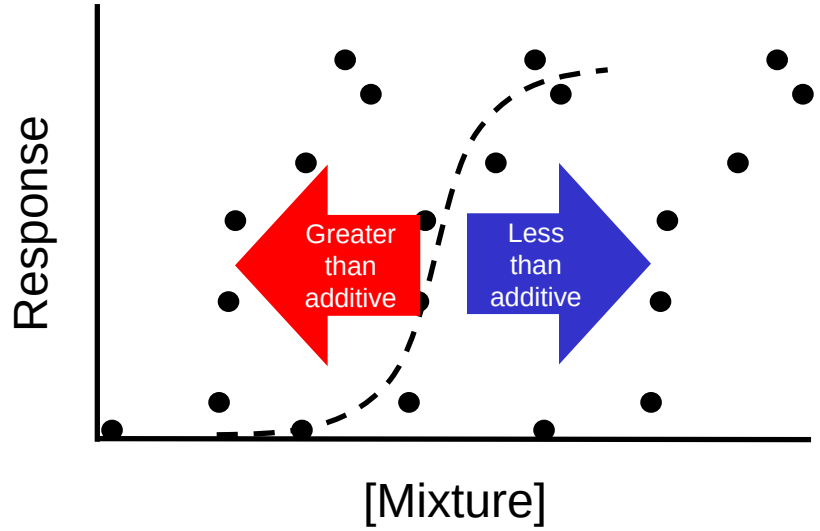


Design mixture studies

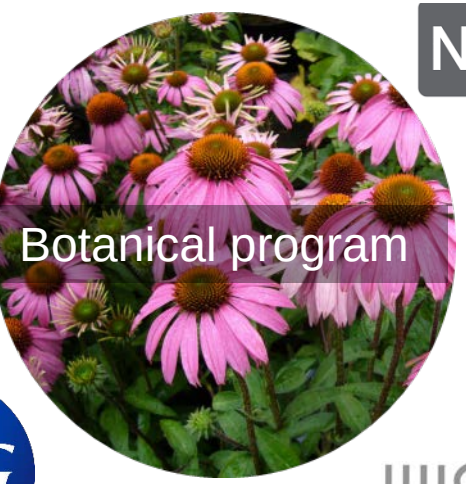
- Ray design
  - Select ratio(s) of chemicals (e.g., equipotent based on ED50)
  - Select doses of the mixture that are predicted to span 0 to 100% effect based on an assumption of dose addition

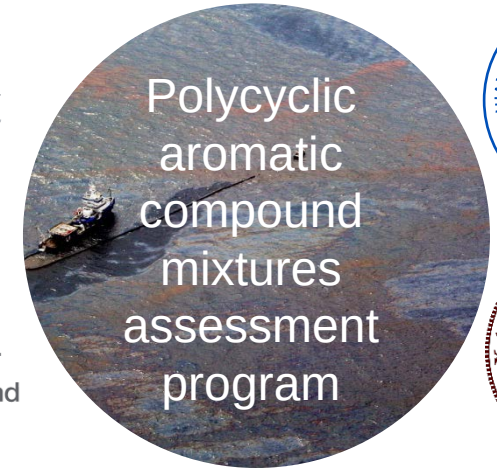
Predict mixture responses

- Dose addition
  - Relative Potency Factor
  - Other (e.g., Altenburger, Webster, Gennings, Hertzberg)
- Response addition



Stakeholder Engagement





Milestones

Short-term (0-1 year)	Medium-term (2-3 years)	Long-term (4-5 years)

Disease-based systems biology projects on cancer and cardiovascular disease

Project development	Hypothesis testing	Evaluation and communication

Botanical testing program

Data analysis	Reporting	Evaluation (state-of-the-science)

Complex mixture methods development

Complete existing case studies	Toolbox recommendations

Botanical Safety Consortium

Botanical library and assays	Testing	Framework

Component-based approach (Polycyclic Aromatic Compound Mixtures Assessment Program)

Component-based studies	Reporting and whole mixture	Evaluation (state-of-the-science)

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Workshop Steering Committee

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Thank you!