

Strategies for Studying Combined Exposures and Mixtures

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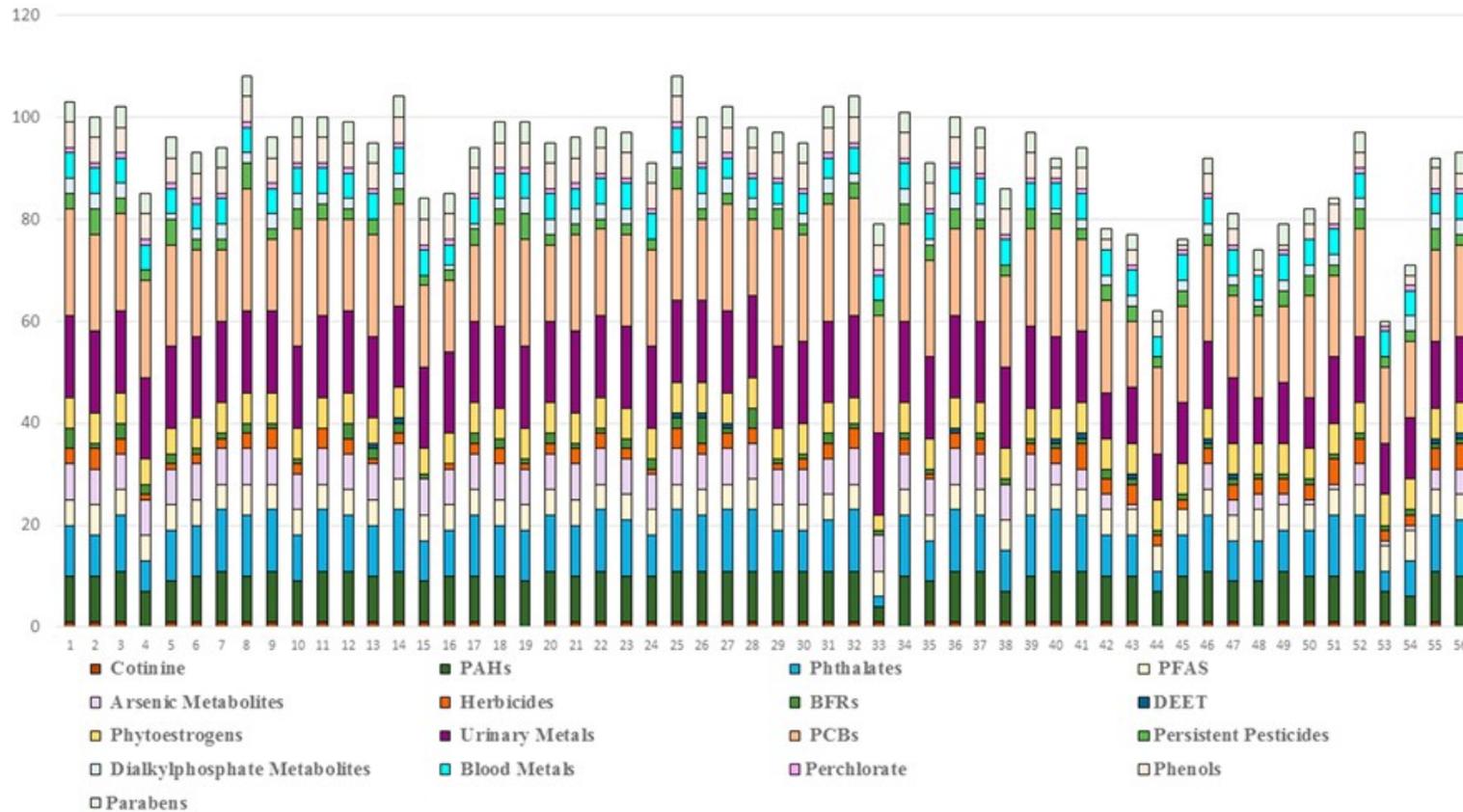


Outline

- Background
 - ← Clarifying questions
- Mixtures research areas
 - Component-based approaches
 - ← Clarifying questions
 - Whole mixture approaches
 - ← Clarifying questions
 - Systems biology approaches
 - ← Clarifying questions and discussion



Internal exposure



From: Rosofsky et al. 2017. Exposure to multiple chemicals in a cohort of reproductive-aged Danish women. *Environmental Research* 154:73-85

Background



-
- 135 biomarkers measured in blood, serum, or urine
 - 91 biomarkers detected on average (range of 60-108)
 - Some biomarkers detected in 100% of women (phytoestrogens, PCBs, hexachlorobenzene, perchlorate, PFAS, metals, and PAHs)



Personal passive sampling devices

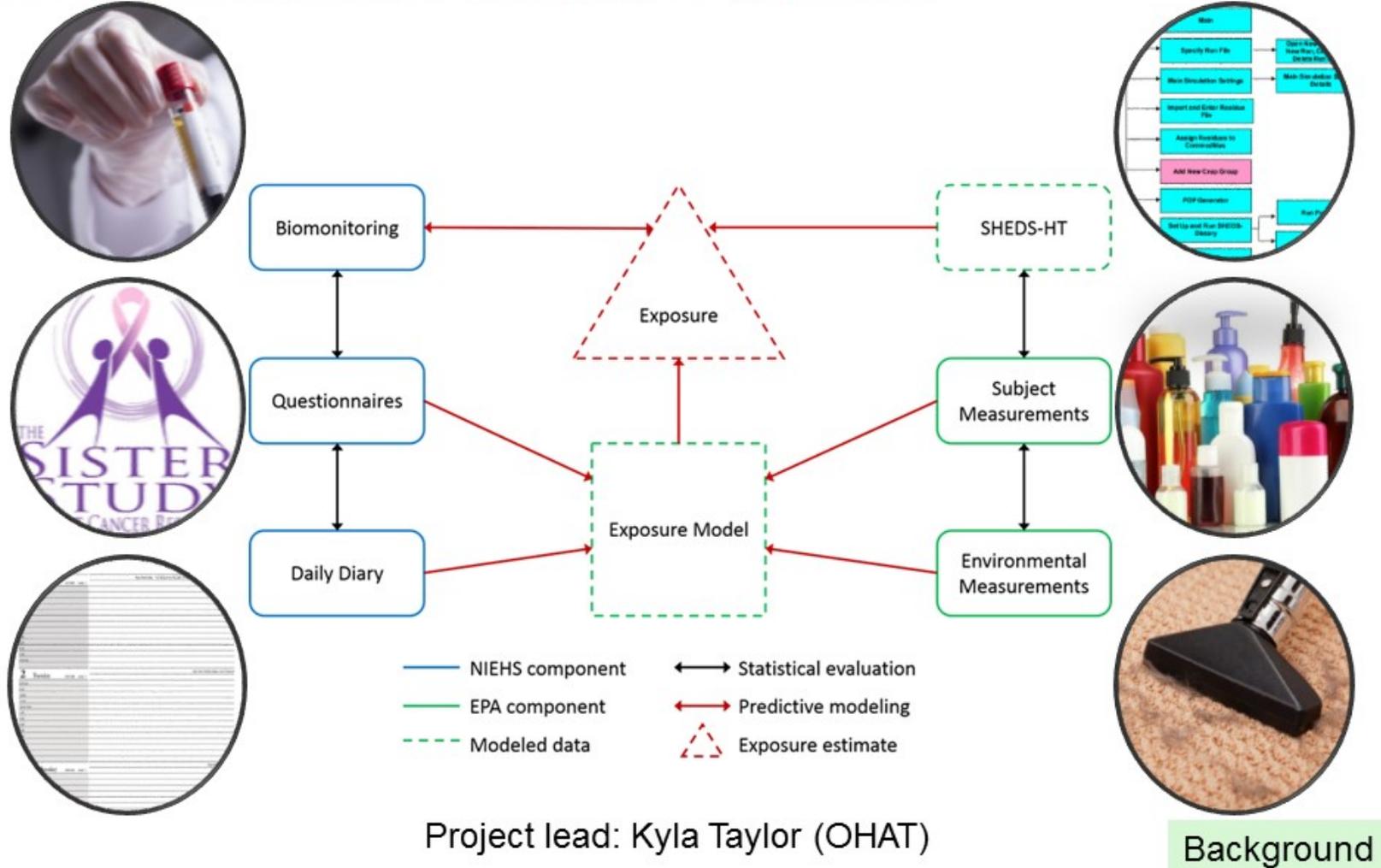


- Kim Anderson (OSU) – NIEHS Superfund grantee
- Methods for measuring ~ 1400 chemicals in extracts from passive sampling devices



NIEHS/EPA Exposure Study

Personal care and consumer products





Combined Exposures/Mixtures

- Defined (aka simple) mixture – All *components* are identified and quantified (e.g., pharmaceutical combination)
- Complex mixture – Many constituents with some unidentified fraction (e.g., source emission like diesel exhaust)
- Whole mixture – Often used interchangeably with complex mixture, but is defined here as consideration of an entire mixture without regard to component data (regardless of complexity)
- Exposome – Totality of exposures over a lifetime

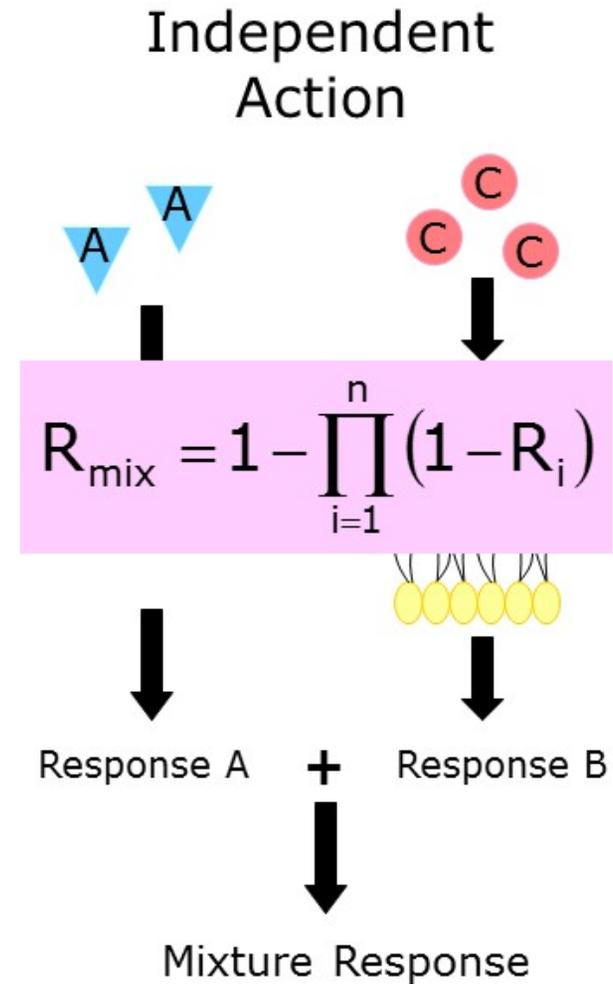
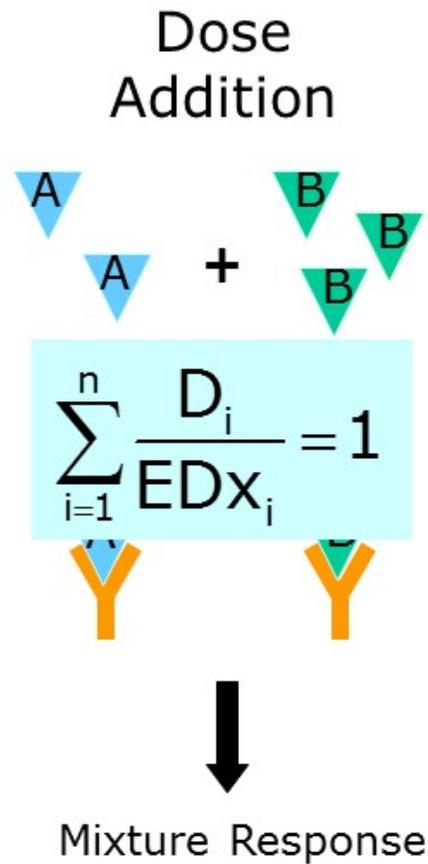


Defined mixture

- Aggregate – includes consideration of different routes
- Cumulative – addition of multiple components
 - Dose addition – Adding chemicals at the dose level; typically applied to chemicals with similar mechanisms of action
 - Independent action or response addition – Adding chemicals at the response level; typically applied to chemicals with different mechanisms of action



Concepts of additivity



Background



Implications of model selection

- Independent action – As long as all chemicals in the mixture are below their individual No Observed Adverse Effect Level (NOAEL), the mixture is not expected to have an adverse effect
- Dose addition – Chemicals below their NOAEL can contribute to a total dose that elicits an adverse effect.
- Therefore, *if* chemicals are **dose additive**, a cumulative risk assessment should be performed to protect human health

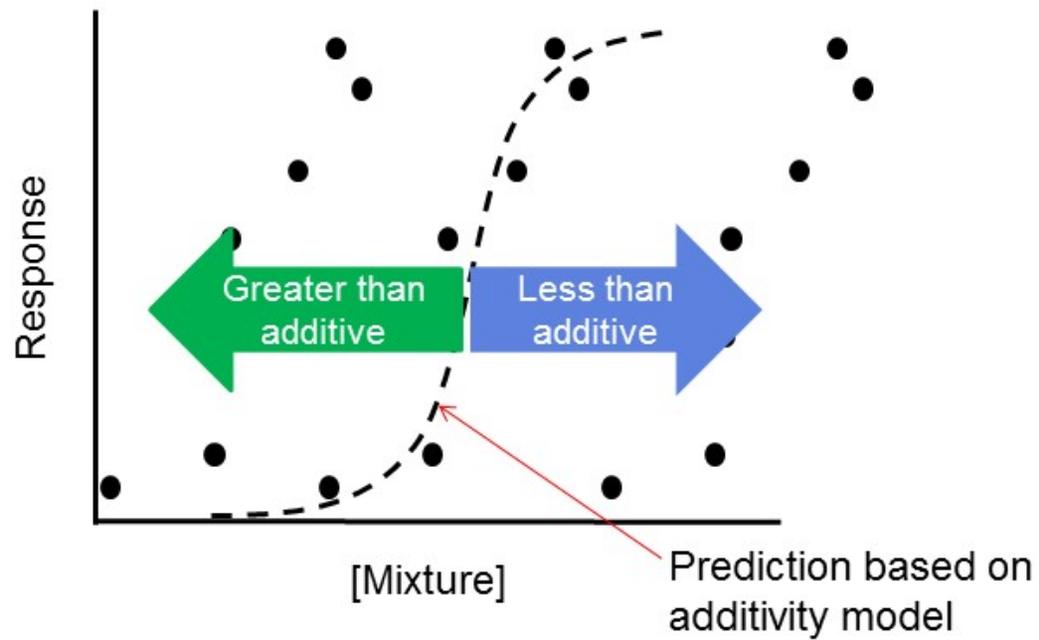


Defined mixture

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 - Independent action or response addition – Adding chemicals at the response level; typically applied to chemicals with different mechanisms of action
- Interaction – deviation from additivity
 - Greater than additivity (formerly synergy) – the combination produces an effect at a lower dose than predicted from the appropriate additivity model
 - Less than additivity (formerly antagonism) – the combination produces an effect at a higher dose than predicted from the appropriate additivity model



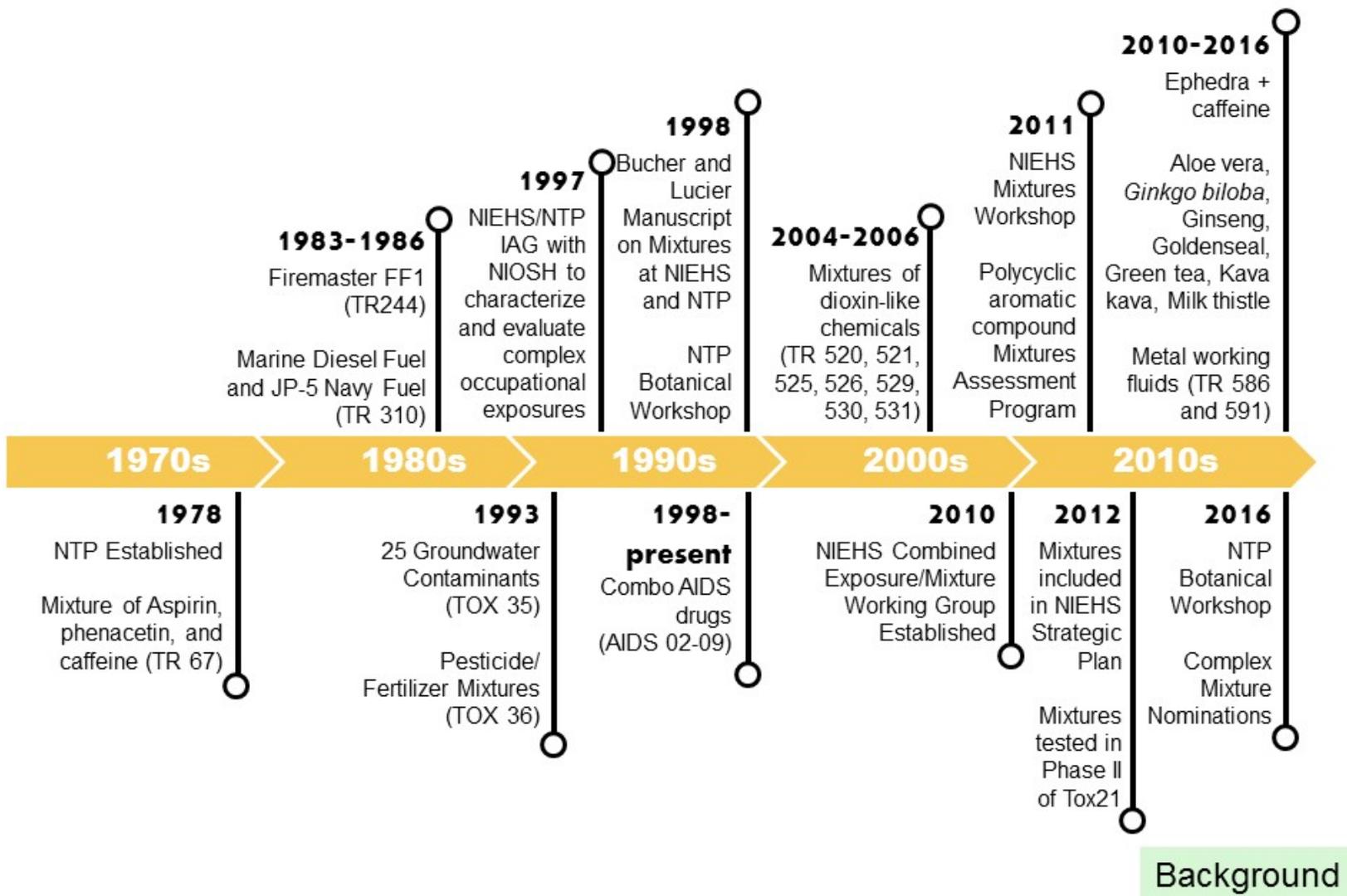
Characterizing interaction



Background

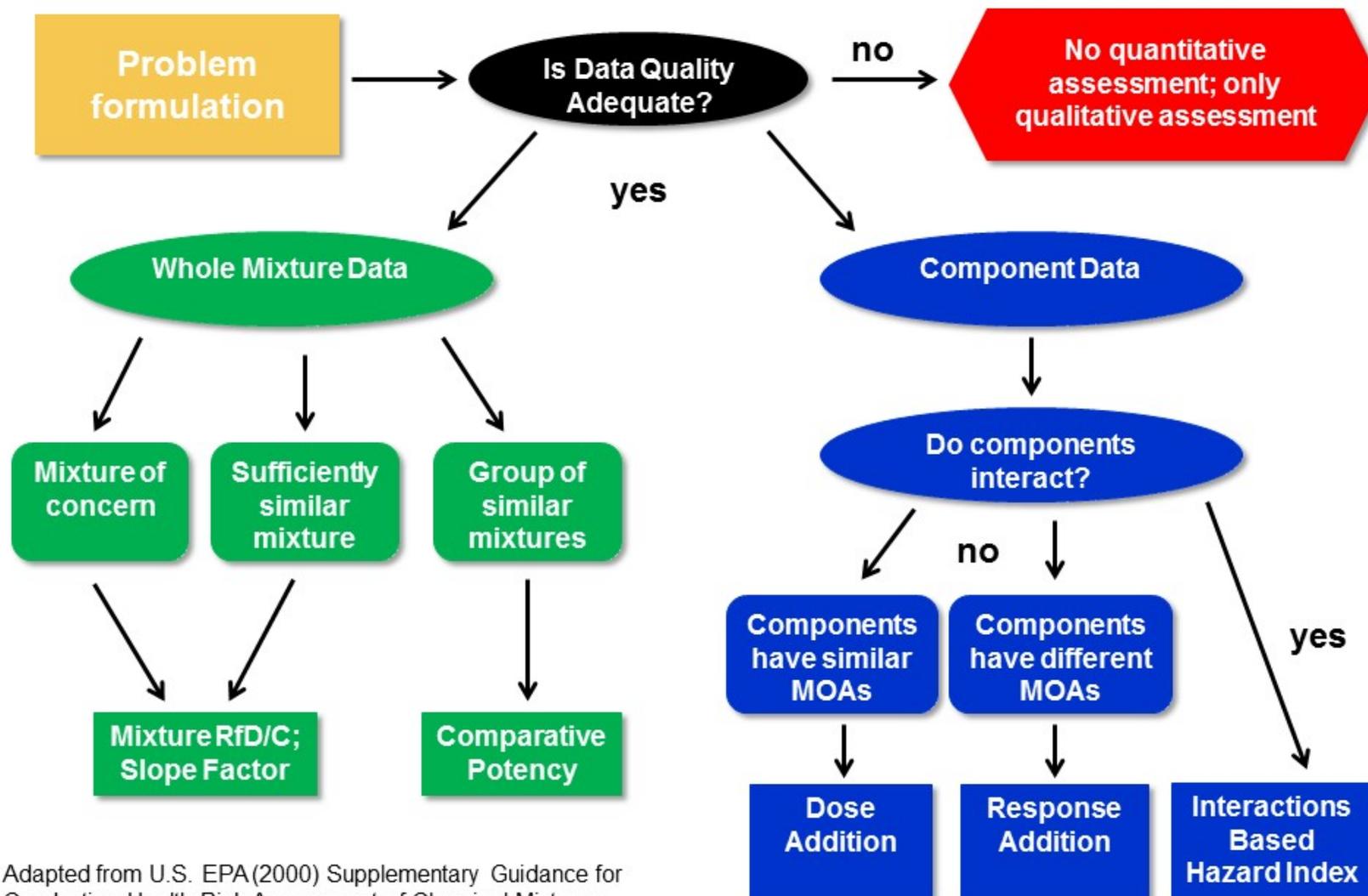


History of mixtures research at NTP





Understanding health effects of mixtures



Adapted from U.S. EPA (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures



Problem formulation: Choose your own adventure

1. Are you starting from an exposure (e.g., occupational, specific class of chemicals) or a disease or a population?
 - If exposure, use monitoring or modeling data to define the exposure profile
 - If disease, determine which stressors impact disease development
 - If population, characterize exposures within the population
2. Regardless of starting place, at some point, decisions regarding which exposures to include in the health evaluation are required



Deciding which stressors to include

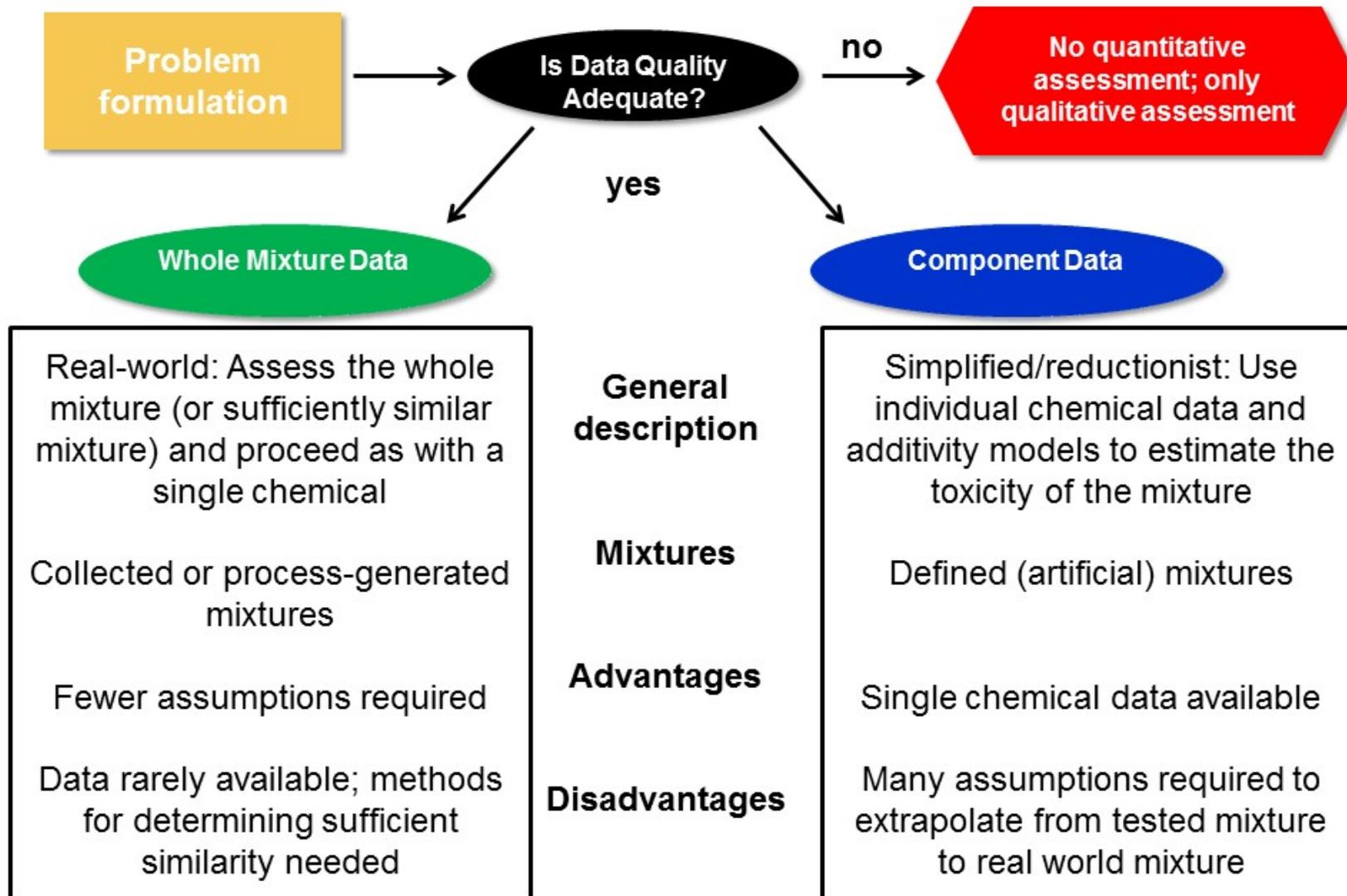
Considering biological similarity

Most similar	Chemicals share a...	Examples
	Common active metabolite	Benzyl butyl phthalate and dibutyl phthalate share the active metabolite monobutyl phthalate
	Molecular initiating event	Parathion and chlorpyrifos both inhibit acetylcholinesterase and elicit the same downstream key events
	Adverse outcome pathway	Perchlorates decreases synthesis of thyroid hormone, while dioxin increases elimination of thyroid hormone
	Target tissue	Ephedrine and caffeine are both cardiotoxic
	Disease	DES and tobacco smoke cause cancer in different tissues
Least similar		

Background

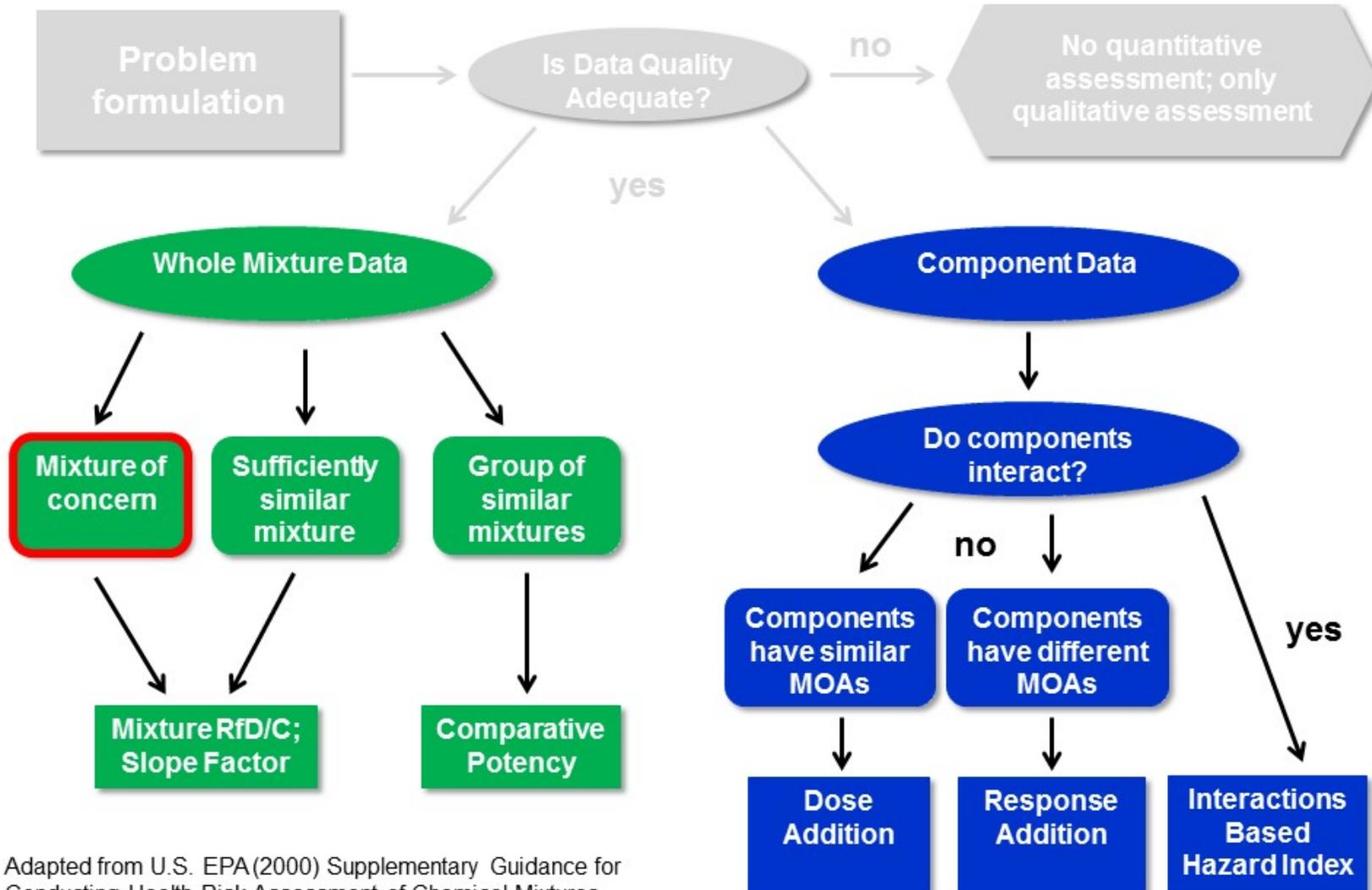


Understanding health effects of mixtures





NTP Mixtures Research



Adapted from U.S. EPA(2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures



Mixture of concern

Paradox

- Strong preference for data on the “mixture of concern”
 - Decreases assumptions involved in estimating health effects and risk from exposure to the mixture
- Defining and testing the mixture of concern is not actually possible in the case of complex mixtures

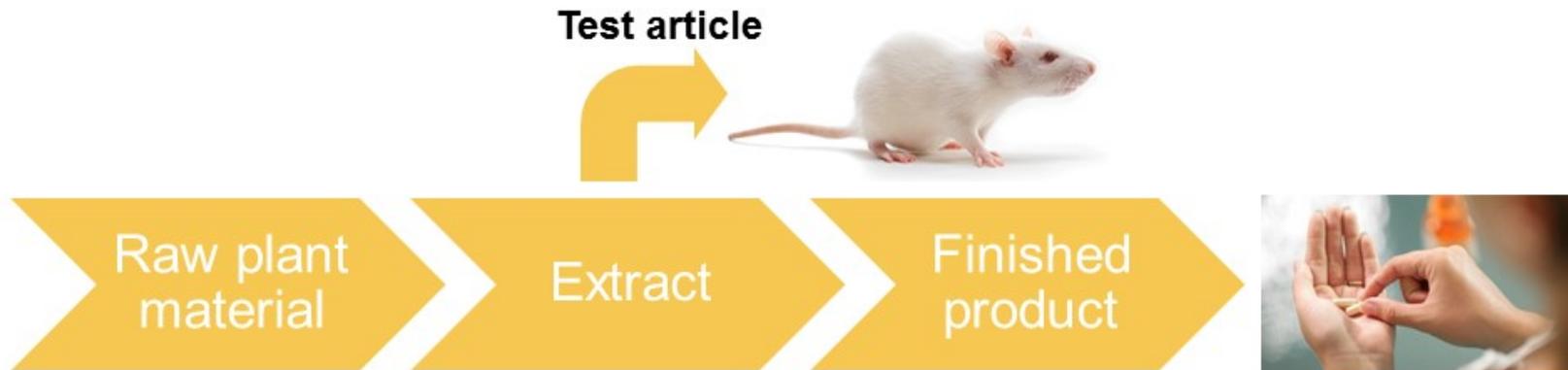


Background



Mixture of concern

Botanical dietary supplement example



Sources of variation

- Plant part (aerial, root, whole plant, leaf, seed)
- Climate
- Soil conditions
- Season
- Plant maturity
- Contaminants (mold, pesticides, metals)
- Co-harvested materials (other plants, soil)
- Adulteration
- Extraction process
- Solvents
- Adulteration
- Contamination
- Storage/shipping conditions
- Manufacturing process
- Excipients
- Combination with other botanicals
- Adulteration
- Contamination
- Storage/shipping conditions

Exposure

Background



Mixture of concern

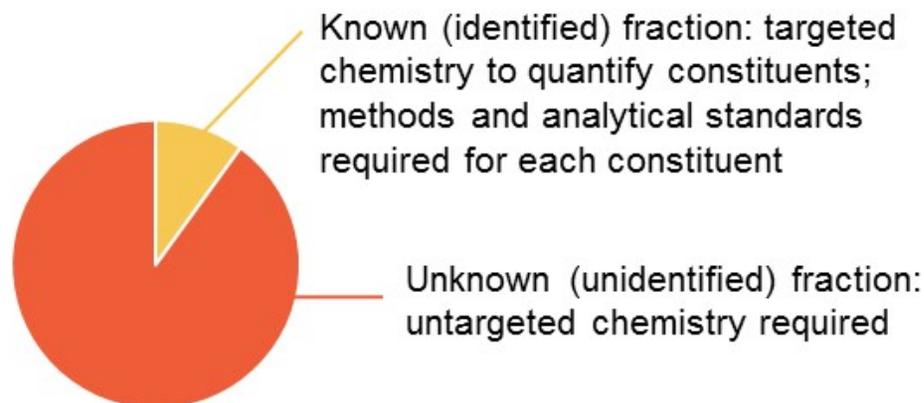
- Botanical dietary supplements
 - Aloe vera, bitter orange, ephedra and ephedra with caffeine, *Echinacea purpurea* extract, *Ginkgo biloba* extract, ginseng, goldenseal root powder, green tea extract, kava kava, milk thistle, senna
- Commercial mixtures
 - Flame retardants (e.g., Firemaster FF-1), metal working fluids (e.g., TRIM[®] VX)
- Fuels
 - Marine diesel fuel, Jet fuel (JP-5)
- Simulated environmental mixtures
 - Groundwater contaminants (25 chemicals)
 - Pesticide/fertilizer mixture



Whole mixture testing

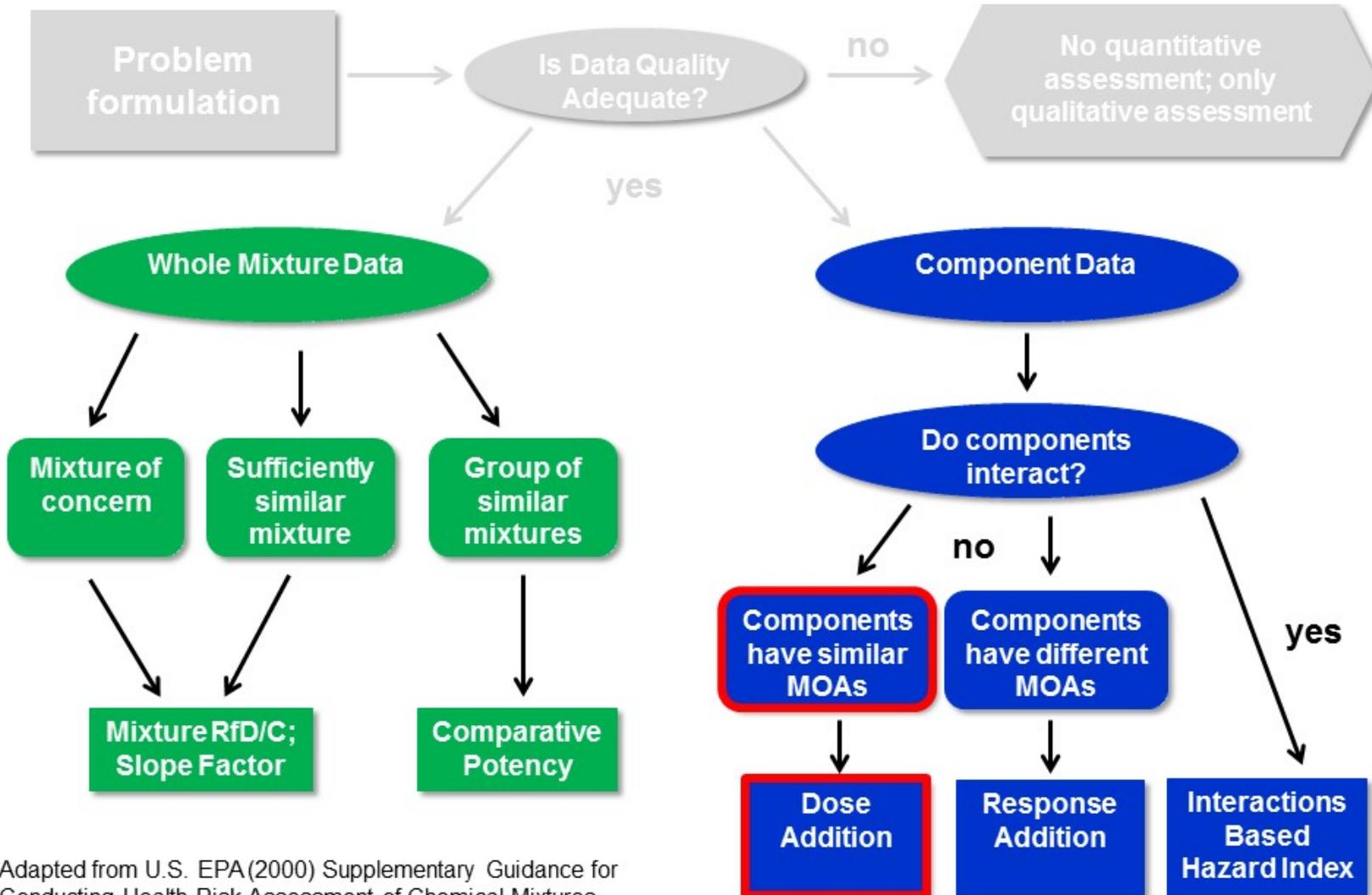
Key issues

- Chemical analysis
 - Targeted
 - Untargeted
- Test article selection
- Extrapolating findings to related whole mixtures
- Understanding which constituent(s) are driving observed effects
- Deciding which metabolite(s) to measure in TK/ADME studies





NTP Mixtures Research



Adapted from U.S. EPA(2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures

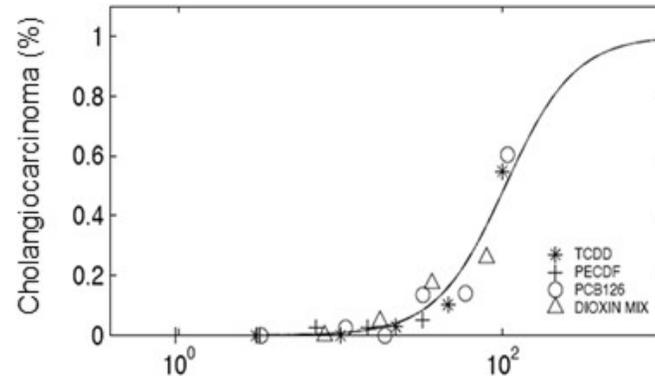


Dioxin mixture studies

Hypothesis: The carcinogenic effects of a mixture of dioxin-like chemicals can be predicted using individual dose-response data in a dose additive model.

Reference chemical $RPF = \frac{ED50_{TCDD}}{ED50_{TCDD}} = 1$ <p>TCDD</p>	$RPF = \frac{ED50_{TCDD}}{ED50_{PCB-126}} = 0.1$ <p>3,3',4,4',5-Pentachlorobiphenyl (PCB-126)</p>	$RPF = \frac{ED50_{TCDD}}{ED50_{PeCDF}} = 0.1$ <p>2,3,4,7,8-Pentachlorodibenzofuran (PeCDF)</p>
33 ng x 1 = 33 ng	+ 333 ng x 0.1 = 33 ng	+ 66 ng x 0.1 = 6.6 ng

Total TCDD (73 ng)



Walker *et al.*, 2005. Environ Health Persp 113:43-48

Background



- Dose additivity is an appropriate model for this group of chemicals – supporting the current risk assessment framework
- Component-based mixture predictions could be applied to chronic endpoints
- Notable interactions were not observed



Lessons from past NTP mixtures work

- Assessing whole mixtures without a strategy for comparing across related mixtures invites criticism of test article selection by industry and prevents partner agencies from effectively utilizing resulting data
- Hypothesis driven mixtures research (e.g., dioxin project) yields more interpretable and useful data than exploratory research (e.g., 25 chemical mixture low dose project)
 - A: The carcinogenic effects of a mixture of dioxin-like chemicals can be predicted using individual dose-response data in a dose additive model
 - B: If we build a mixture of 25 chemicals based on common exposure ratios and test at human-relevant doses will we see unexpected/impressive toxicity?



Evolution in mixtures toxicology

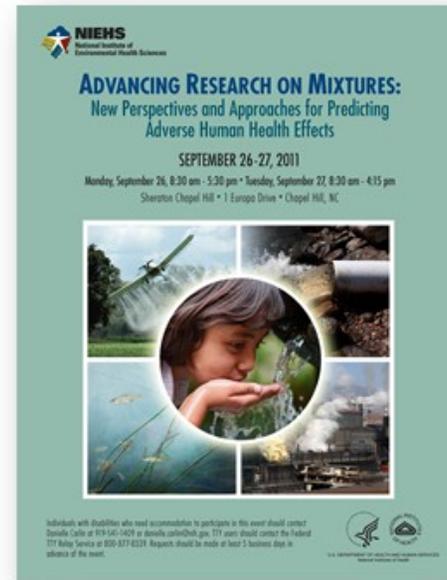
- 20th Century
 - Dose addition versus independent action – which model is more appropriate?
 - Looking for the unexpected – which chemicals might have (much) greater effects in combination than alone?
- 21st Century
 - Dose addition often viewed as a default approach – focus on defining the boundaries of its application
 - Prioritizing which chemicals to evaluate for cumulative effects viewed as more important than possible interactions
 - Developing whole mixture approaches
 - Adapting high throughput tools for use with mixtures



NIEHS Mixtures Workshop

Sept 26-27, 2011

- Goal: Identify and focus on key issues that present challenges in mixtures research
 - Use to inform the development of an intramural and extramural mixtures research strategy
- Multidisciplinary participation
 - Mixtures experts from statistics, biology/toxicology, epidemiology, exposure science, and risk assessment
- Format
 - Background presentation from invited speakers
 - Breakout sessions
- Comprehensive workshop report
 - <http://www.niehs.nih.gov/about/visiting/events/past/mtg/2011/mixtures/index.cfm>



Background



Key issues

- Improved exposure assessment (monitoring, modeling, and unbiased approaches)
 - Develop exposure technologies
 - Evaluate novel methods (e.g., EWAS, exposome)
- Tools and methods for prioritization of chemicals/mixtures
 - More use of exposure data (e.g., NHANES database)
 - High-throughput screening methods to assess interactions and mixtures
- Cross-disciplinary effort is required
 - Relative potency factors generated in toxicology studies to epidemiological assessments
 - Epidemiological findings for identification of important combinations for toxicological studies
- Bridging *in vitro* and *in vivo* approaches
 - Link *in vitro* responses to biologically-meaningful endpoints, which should be validated *in vivo*



Glenn Rice (USEPA)



Ray Yang (CSU)

Background



Key issues (continued)

- Development and validation of statistical methods
 - Predictive mixture toxicity models (e.g., component-based and sufficient similarity)
 - Assessment of multiple chemical associations in epidemiology
- Systems-based approaches for studying mixtures
 - Predict interactions of chemicals that target a common pathway or system without testing all potential chemical combinations
- Development/refinement of both “bottom-up” (component-based) and “top-down” (whole mixtures) approaches for predicting toxicity of mixtures
- Data collection and management (e.g., federated databases)
 - Raw data on both single chemicals and mixtures
 - Standardization and integration across datasets
 - Significant planning to establish the scope and implementation strategy



Chirag Patel (Stanford)



Linda Birnbaum and Nigel Walker

Background



NIEHS strategic plan – Goal 4

How combined environmental exposures affect disease pathogenesis

- a) Assess joint action of multiple environmental insults (e.g., chemicals, nonchemical stressors, and nutritional components), on toxicity and disease, and identify interactions resulting from combined exposures
- b) Study role of the human microbiome and its influence on environmental health, and explore role of microbiome in responses to environmental exposures
- c) Study interactions of infectious agents with environmental exposures
- d) Understand how nonchemical stressors, including socioeconomic, behavioral factors, etc., interact with other environmental exposures to impact human health outcomes, and identify preventive measures



Background



NIEHS CEM Working Group

Coordinating across NIEHS

- Meets quarterly to discuss mixtures projects throughout NIEHS
- Updates on mixtures-related activities (e.g., workshops, seminars)
- Development of a logic model to guide prioritization of NIEHS mixtures efforts



Danielle Carlin (DERT)

Background

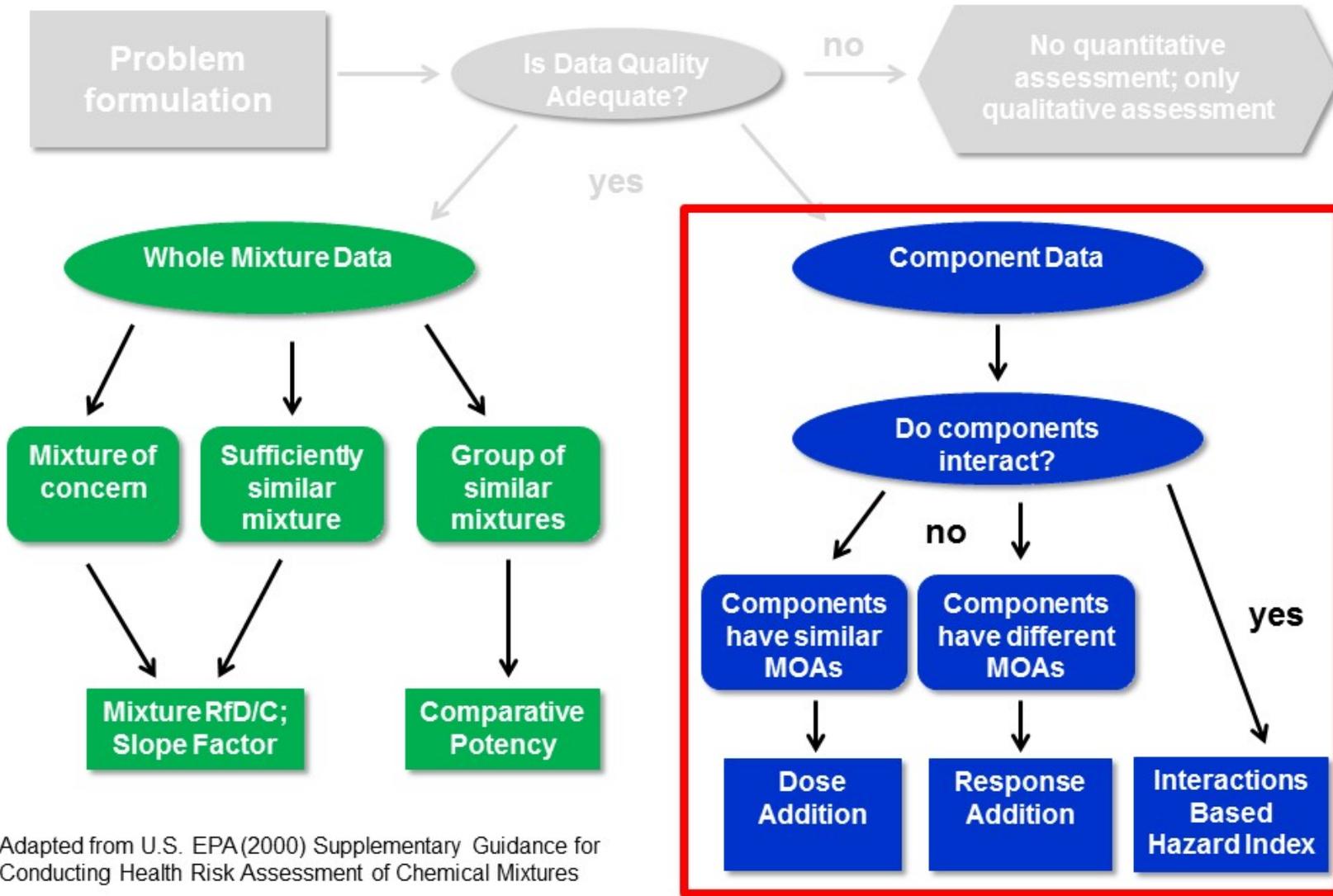


Outline

- **Background**  Clarifying questions
- Mixtures research areas
 - Component-based approaches
 - Whole mixture approaches
 - Systems biology approaches



Understanding health effects of mixtures



Adapted from U.S. EPA(2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures



Component-based approaches

Considerations

- Require dose-response data on all individual components and the defined mixture of interest
 - Uncertainty in individual component data will feed into uncertainty in cumulative assessment
- Relatively few examples of application, particularly with higher order mixtures (>10 components)
- Assumptions required
 - Chemicals adhere to a specific model of additivity (dose addition, independent action)
 - Chemicals do not interact
- Requires appropriate statistical methods for determining deviation from additivity



Application of component-based methods

Risk assessment

- Quantitative cumulative risk assessments of pesticides with a common mechanism of action
 - Organophosphates, N-methyl carbamates, triazines, choroacetanilides, pyrethrins/pyrethroids
- Guidance documents providing potency information on chemicals with similar mechanisms of action
 - Dioxin-like chemicals
 - Polycyclic aromatic hydrocarbons
- Screening level analysis of Superfund site chemicals (regardless of mechanisms) using a hazard index approach loosely based on dose addition concept

Component-based approaches



Polycyclic aromatic hydrocarbons (PAHs)

Defining the problem



- Multiple diverse sources of exposure to complex, dynamic mixtures
 - Very limited whole mixture cancer data available (e.g., coal tar), with significantly more data available for individual parent PAHs
 - Methods to compare across complex mixtures are still under development
- Multiple routes of exposure possible
- Many known toxicities associated with some chemicals in the class (cancer, neurotoxicity, immunotoxicity, developmental and reproductive toxicity)

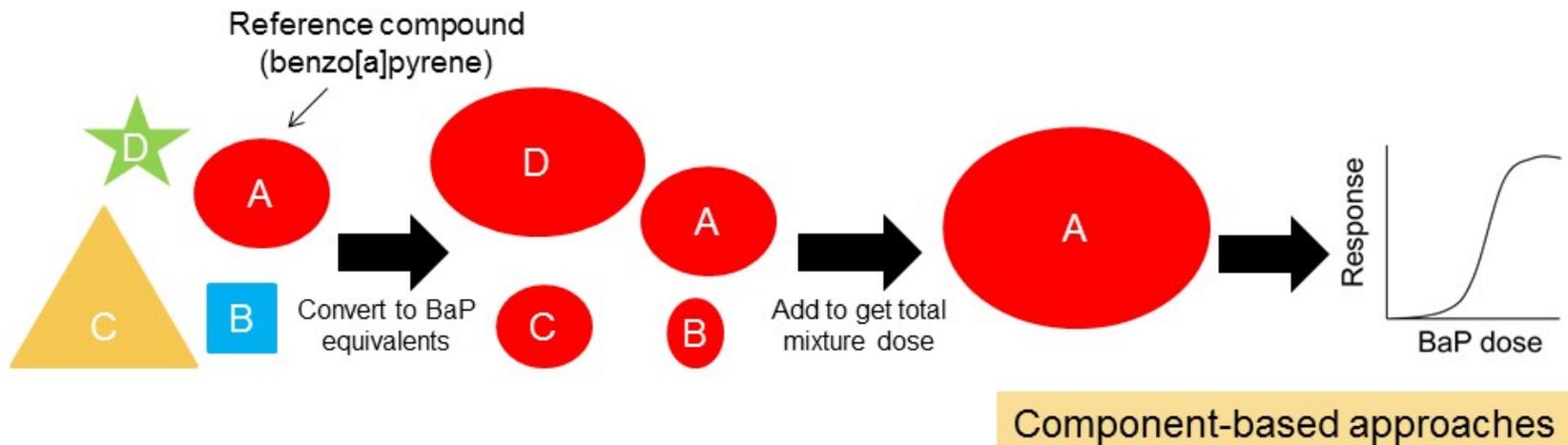
Component-based approaches



Cancer risk from PAH exposure

EPA IRIS

- 1993 Provisional Guidance for Quantitative Risk Assessment of PAHs
 - Provided relative potency information for 7 PAHs
 - Only addressed parent PAHs and cancer risk
 - Used extensively for risk assessment by EPA and other groups (e.g., seafood safety following Gulf Oil Spill)





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 - Used extensively for risk assessment by EPA and other groups (e.g., seafood safety following Gulf Oil Spill)
- 2010 *Draft* Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures
 - Update to 1993 guidance – providing relative potency information for 27 PAHs
 - 2001 Workshop to inform development of document
 - Reviewed by a Scientific Advisory Board in 2011

Component-based approaches



Uncertainties and limitations

2001 Workshop on Approaches to PAH Health Assessment

- The RPF approach only considers a small subset of PAHs (i.e., unsubstituted PAHs only, no heterocyclic compounds or nitro- or alkyl- substituted PAHs)
- There are no human toxicity data for any individual PAH
- The assumption of additivity may not be valid, and there may be interactions among PAHs or between PAHs and other components of a mixture (e.g., metals)
- PAHs may generally have a common mode of action (i.e., mutagenicity), but multiple modes of action for carcinogenesis are possible
- The approach is limited to the oral exposure route (i.e., a recommendation was made not to apply the factors to dermal and inhalation exposures)



Scientific Advisory Board Review

- The SAB recognized “the pragmatic need for the RPF approach” based on currently available data; however, the SAB recommends:
 - Strengthening the rationale for the RPF approach
 - Strengthening the rationale for the assumption that there are no interactions among PAHs at environmentally relevant doses
 - Generating RPF data only for PAHs with available cancer bioassay data (cancer endpoint data is not a substitute)
 - Developing a whole mixture approach
 - Working with the NTP to develop whole mixture datasets on relevant complex PAH mixtures

Component-based approaches



Nominations and scoping

- Motivation
 - 1984-2005 Multiple nominations for PAHs
 - 2010 Deepwater Horizon Oil Spill and SAB review of EPA RPF approach
- Considerations
 - Compared to many other chemical classes, there is a great deal of data available for PAHs, particularly benzo[a]pyrene
 - Responding directly to the SAB proposal for NTP to test multiple (10-12) complex mixtures in 2-year cancer studies would take too long and be prohibitively expensive
- Stakeholder input
 - Discussions with EPA colleagues
 - 2012 SOT Workshop: Sufficient similarity of whole representative mixtures or a relative potency factor approach: PAHs as a Case Study

Component-based approaches



NTP PAC* Mixtures Assessment Program

Addressing uncertainties

- “The RPF approach only considers a small subset of PAHs (i.e., unsubstituted PAHs only, no heterocyclic compounds or nitro- or alkyl- substituted PAHs)” – 2001 EPA Workshop
 - Evaluate a broader range of compounds, *hence Polycyclic Aromatic Compounds (PACs) instead of PAHs
 - Develop high throughput approaches that can be used to rapidly assess a large number of PACs
- “The assumption of additivity may not be valid, and there may be interactions among PAHs or between PAHs and other components of a mixture (e.g., metals)” – 2001 EPA Workshop
 - Challenge the assumption that PAHs do not interact
- “PAHs may generally have a common mode of action (i.e., mutagenicity), but multiple modes of action for carcinogenesis are possible” – 2001 EPA Workshop
 - Explore other “icities” and adverse outcome pathways

Component-based approaches



PAC Mixtures Assessment Program

Addressing uncertainties (continued)

- “Strengthening the rationale for the RPF approach” – EPA SAB
 - Apply the RPF approach to PACs
 - Investigate the limitations of the RPF approach and how broadly it can be applied to the class
- “Develop a whole mixture approach” – EPA SAB
 - Generate whole mixture chemistry and biological data to contribute to the development of a whole mixture approach
 - Compare results from whole mixture evaluation to component-based evaluation of PAC mixtures
- “Bridging *in vitro* and *in vivo* responses” and developing “high-throughput screening methods to assess interactions and mixtures” – 2011 NIEHS Workshop
 - Develop and evaluate *in vitro* approaches for PAC mixtures

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NTP Polycyclic Aromatic Compounds Research

Polycyclic Aromatic Compounds (PACs) are widespread environmental contaminants. There are many different types of

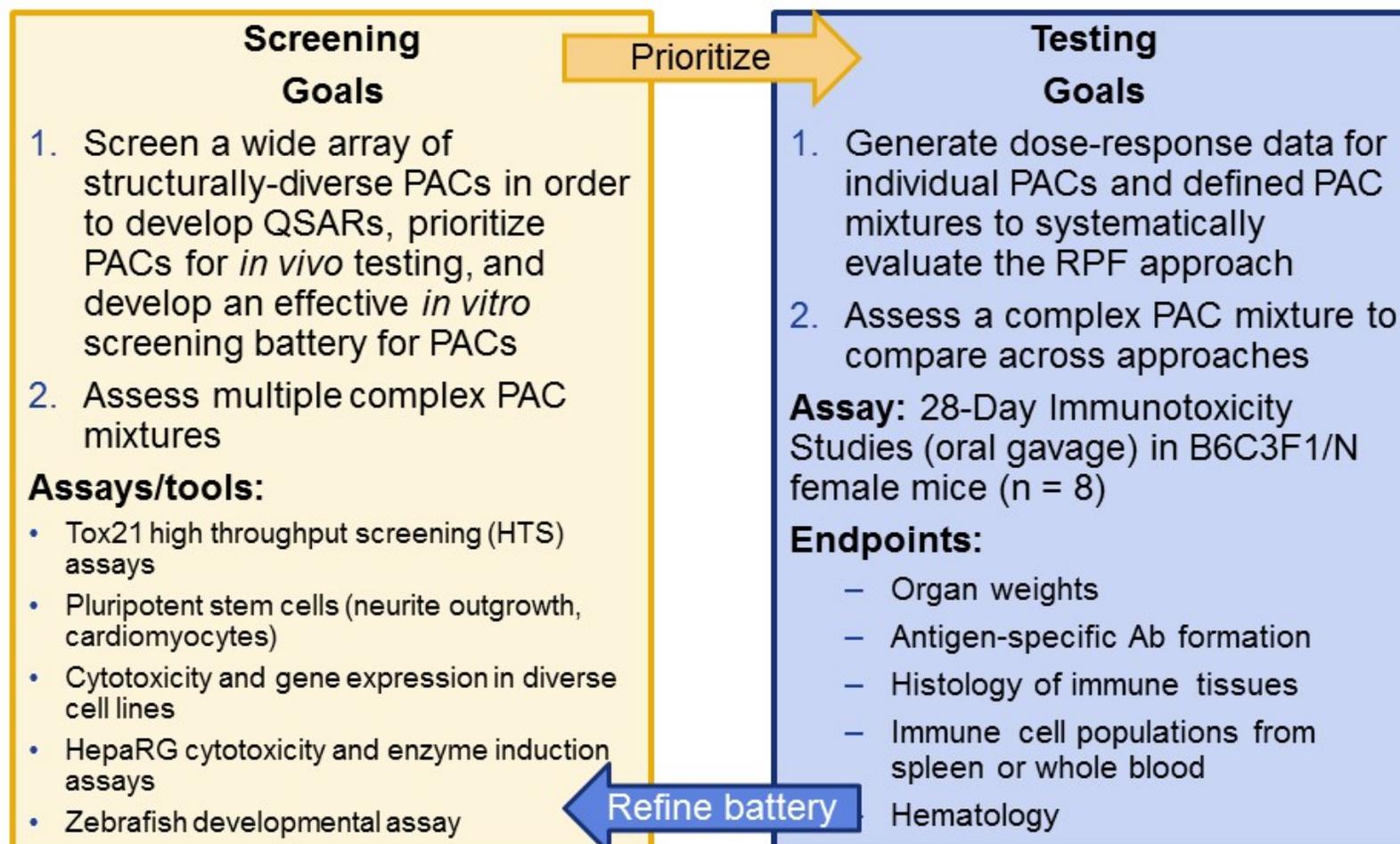


Component-based approaches



PAC Mixtures Assessment Program

Summary of approach





Comparison to cancer data

PAC	RPF*	HTS	Cyto	HepaRG	Neur	Cardio
Dibenz[a,c]anthracene	4	+++	++	+	-	-
Benzo[a]pyrene	1	+++	+++	+	-	-
Dibenz[a,h]anthracene	0.9	++	+++	-	-	NA
Benzo[b]fluoranthene	0.8	++	+++	+	-	+
Benz[a]anthracene	0.2	+++	-	-	-	+
Chrysene	0.1	++	+	-	-	NA
Benzo[k]fluoranthene	0.08	++	-	+	-	+
Anthracene	0	+	-	-	-	-
Pyrene	0	-	-	-	-	+
Phenanthrene	0	-	-	-	-	+

*2010 EPA draft "Development of a Relative Potency Factor (RPF) Approach for Polycyclic Aromatic Hydrocarbon (PAH) Mixtures"

Component-based approaches



Assessing a broad range of PACs

- Cytotoxicity in 13 cell lines
 - Assay selected based on preliminary data from *in vitro*/alternative animal assays demonstrating good correlation with *in vivo* cancer potency data
 - Cell lines included with the goal of covering maximum biological space (based on tissue of origin and gene expression)
- Assessing > 80 individual PACs and ~10 complex PAC mixtures



Nisha Sipes (BSB)



Scott Auerbach (BSB)



Erik Tokar (NTPL)

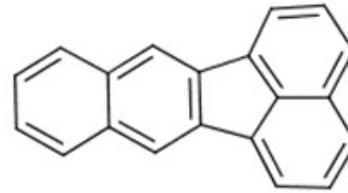
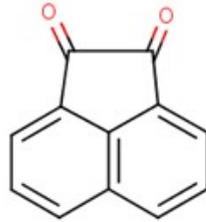
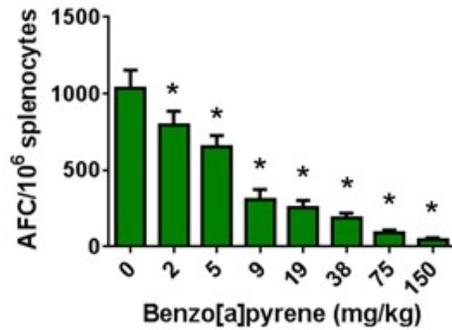


Stephen Ferguson (BSB)

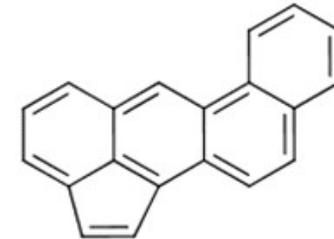
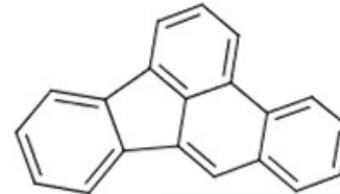
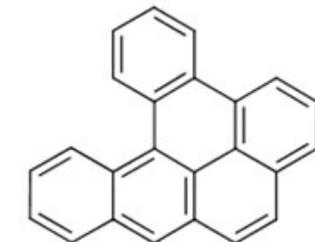
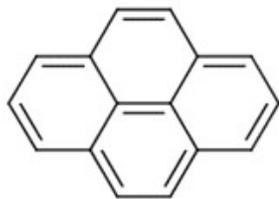
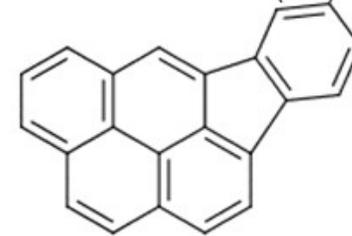
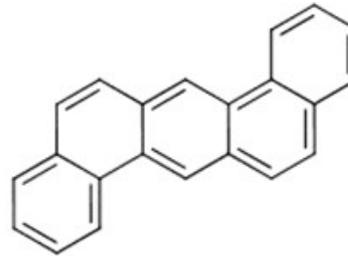
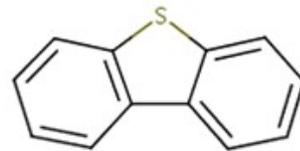
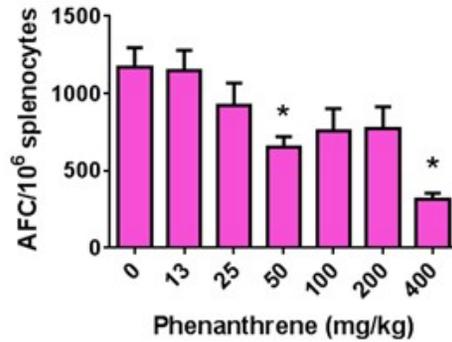
Component-based approaches



In vivo RPF assessment



Dori Germolec (TB)



Component-based approaches



Predicted versus observed

1. Generate dose-response data for individual PACs (in progress)
2. Input data into available component-based models (RPF, other dose addition models, independent action model)
3. Assess the toxicity of mixtures made up of individual chemicals (i.e., generate “observed” mixture data)
4. Compare observed mixture toxicity to predicted mixture response
 - Observed data matches RPF predictions → supports assumptions
 - Observed data deviates from predictions → potential interaction among constituents

Component-based approaches



Hypothesis-driven research

1. PAC mixtures will be dose additive and the Relative Potency Factor approach will provide a good approximation of mixture toxicity
 - Individual PACs will not interact when present in mixtures
2. Relative potency factors generated from immunotoxicity studies will be similar to those from carcinogenicity studies
3. Accounting for the toxicity of a subset of known components will adequately approximate the toxicity of a complex mixture
 - Other components (i.e., the unidentified fraction) will not meaningfully contribute to mixture toxicity

Component-based approaches



Relevance to other complex classes

Ongoing NTP class studies

- Phthalates
- Bisphenols
- Flame retardants
- Perfluorinated compounds
- Water disinfection byproducts

Component-based approaches



Health effects generally assessed on a chemical-by-chemical basis...

Better understanding of the application and limitations of component-based approaches will help to move from single chemical to cumulative assessments, bringing us closer to real-world exposures

Relative potency data from toxicology studies can be used in epidemiology studies to assess associations between a total class value (e.g., total PAC) and the health endpoint of interest

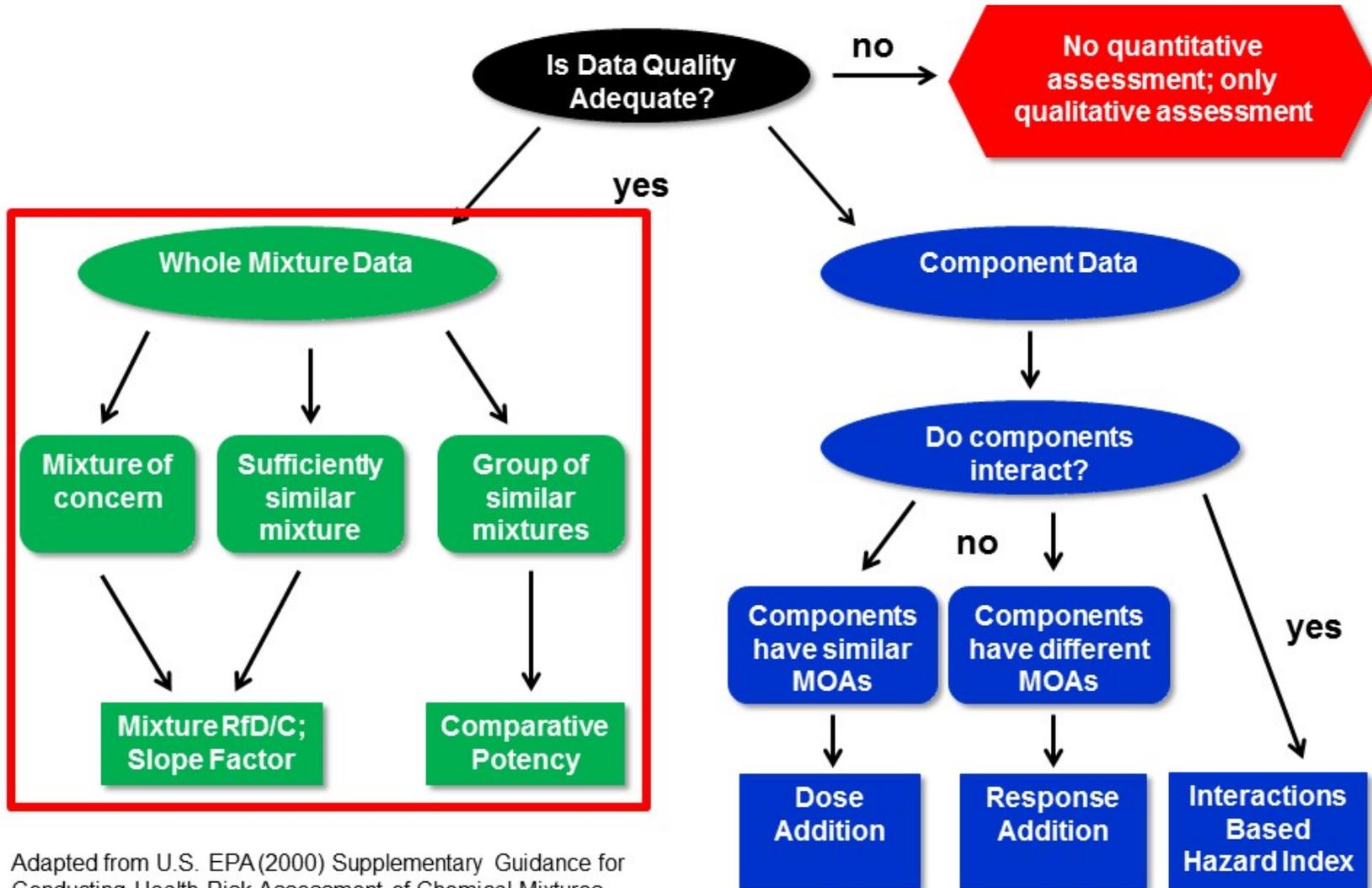


Outline

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- **Mixtures research areas**
 - **Component-based approaches**
 Clarifying questions
 - Whole mixture approaches
 - Systems biology approaches



Assessing risk from mixtures



Adapted from U.S. EPA (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures



Whole mixture approaches

Sufficient similarity

- There is no single “mixture of interest” for complex mixtures
- What are the options for evaluating the health effects of whole, complex mixtures?
 1. Assume the tested mixture is representative of the mixtures of interest (i.e., all complex mixtures tested at NTP)
 2. Develop an approach for determining whether or not a tested mixture is *sufficiently similar* to mixtures of interest



Sufficient similarity

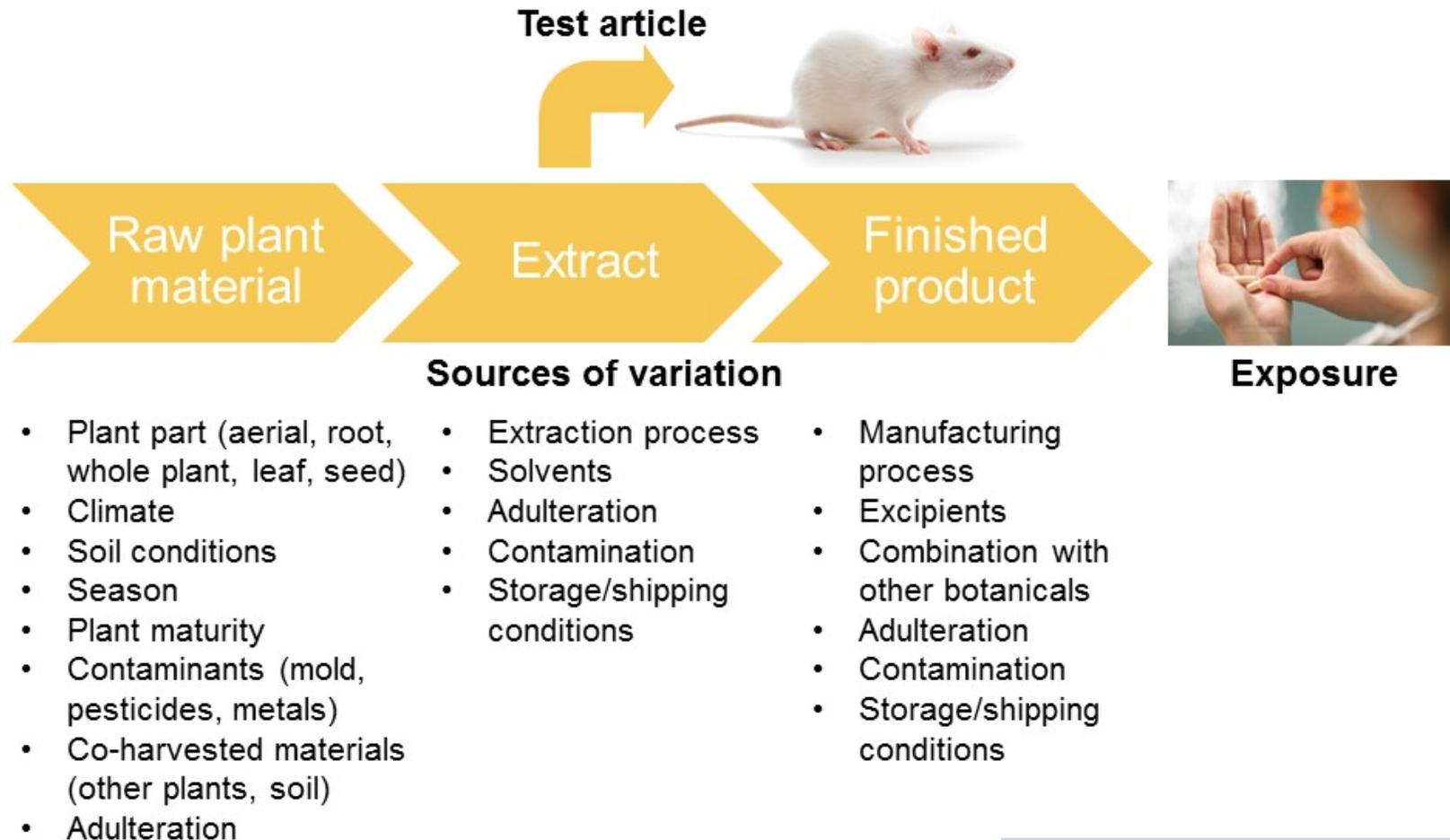
Definition

- Refers to a “mixture that is very close in composition to the mixture of concern, such that differences in their components and their proportions are small”
- “The toxicologic consequences of exposure to the two mixtures (i.e., the mixture of concern and the mixture on which data are available) will be identical or at least indistinguishable from one another”



Mixture of concern

Botanical dietary supplement example



Whole mixture approaches



Feedback from botanical technical reports

“The unique *Ginkgo biloba* leaf extract discussed in TR-578 is not representative of other *Ginkgo biloba* leaf extracts marketed in the United States, and is almost certainly not sold in the United States. It is incorrect to represent it as similar to other *Ginkgo biloba* leaf extracts based on the dissimilarity of its chemical composition to that of other commercially available *Ginkgo biloba* leaf extracts.” American Herbal Products Association (AHPA) public comments on TR-578 (slides), February 8, 2012

“The title of NTP TR 585 should be changed to accurately reflect that the green tea extract used in these studies is a unique ingredient that may or may not be similar to other green tea leaf extracts marketed in the United States...All statements in NTP TR 585 that claim or infer that the tested green tea extract is similar to other green tea extracts should be removed.” AHPA written comments on TR 585, May 8, 2014

“...we are concerned that NTP researchers may be erroneously basing its oral consumption toxicity analysis on an Aloe Vera product sample that is not reflective of the products currently marketed in the US and exported in large quantities.”
Congressional Inquiry, June 18, 2010

“The Committee urges NTP to be highly precise when describing the results of its studies on particular extracts of an herbal species to avoid any possible confusion about the relevance of such studies to other extracts of the species.” The United States Senate Appropriations Committee in report accompanying the fiscal year 2014 Labor, Health and Human Services and Education Appropriations spending bill

Whole mixture approaches



NTP selected an inappropriate test article that is not representative of anything else in the marketplace.



Exploring sufficient similarity

Ginkgo biloba extract

- Exposure: Popular botanical dietary supplement with an estimated 1.6 million Americans taking it in 2012*
- Toxicity: Major toxicity targets of liver, nose, and thyroid gland generally consistent across sex, species, and exposure period
 - Conclusions from 2-year studies: *Some* evidence of carcinogenicity in male and female rats based on thyroid tumors and *clear* evidence of carcinogenicity in male and female mice based on liver tumors
- Doubt: The test article had high levels of some constituents (15% terpene lactones compared with 6% recommended in standardized extract)



Whole mixture approaches



Exploring sufficient similarity

Ginkgo biloba extract

- How should we go about determining sufficient similarity?
- Does chemical similarity reflect biological similarity?
- Is there a clear point of divergence from similarity?
- How should scientific judgment be applied?
- Can unsupervised approaches be used to determine similarity?
- Can we develop similarity criteria?
- Are there methods that can be applied across different mixtures or is a case by case approach needed?

Whole mixture approaches



How similar is similar enough?

Case studies

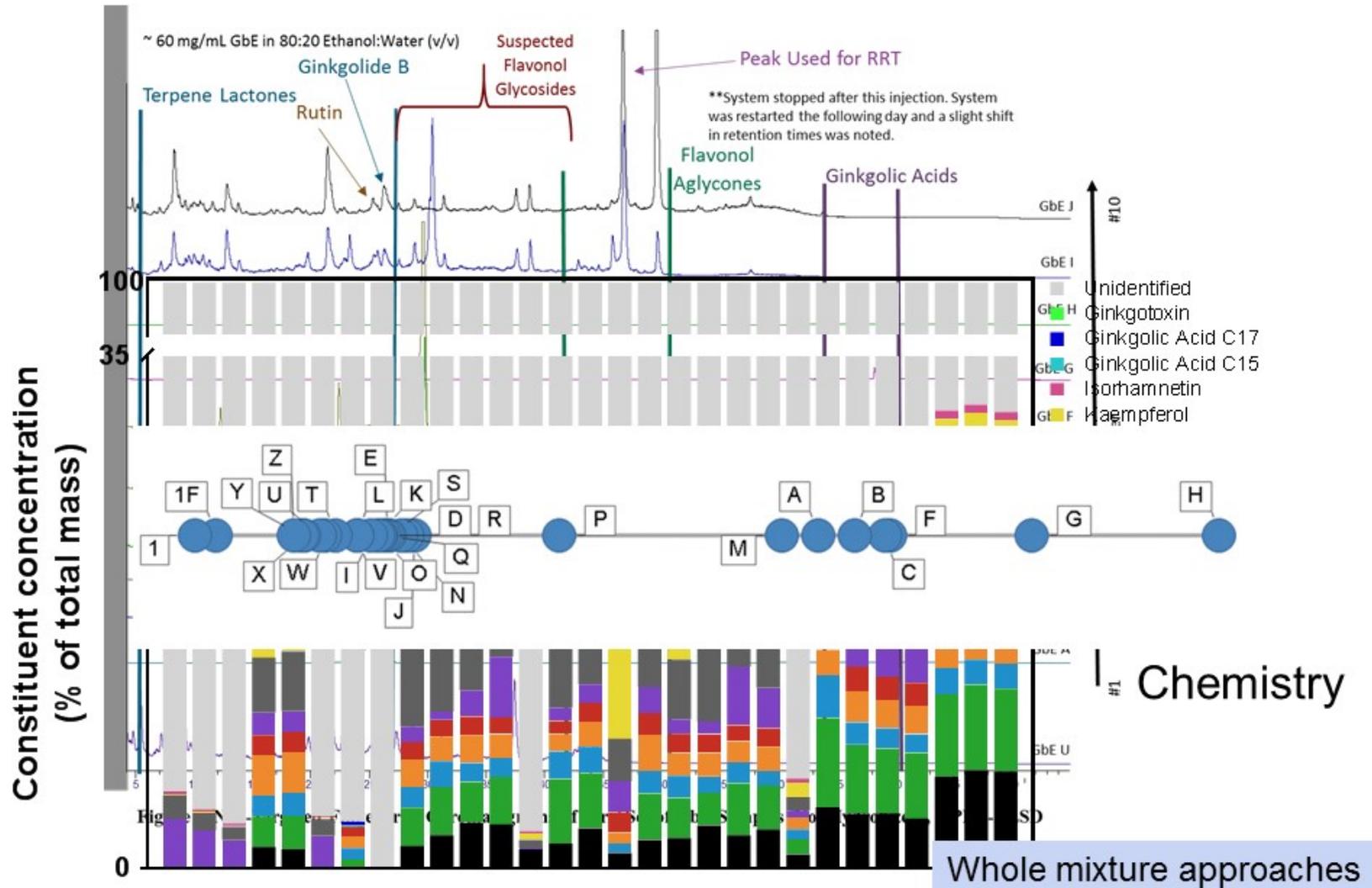
- *Ginkgo biloba* extract
 - Chemistry: Relatively large identified fraction; known marker constituents
 - Biology (NTP): Noted *in vivo* effects – hepatotoxicity, pathways identified
- Black cohosh extract
 - Chemistry: Large unidentified fraction; low confidence that marker constituents are associated with toxicity
 - Biology (NTP): Genotoxicity
- *Echinacea purpurea* extract
 - Chemistry: Large unidentified fraction
 - Biology (NTP): Weak activity – Enhanced immune response



Whole mixture approaches



Chemical similarity





Suramya Waidyanatha (POB)



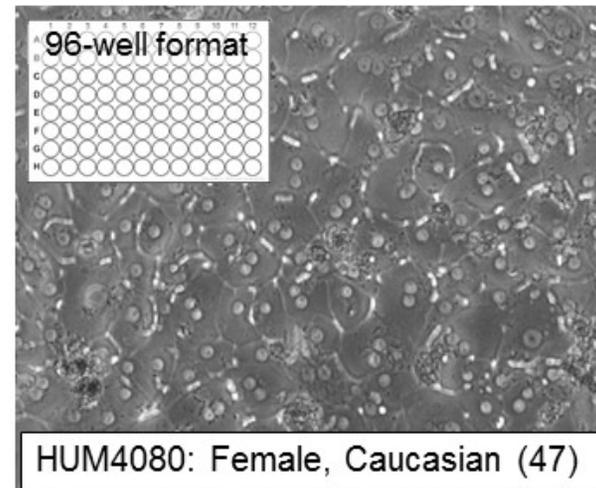
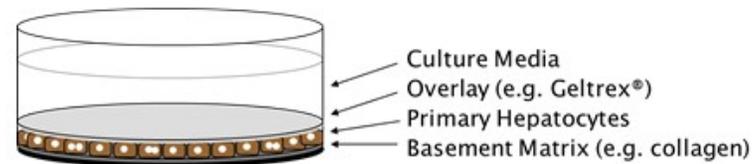
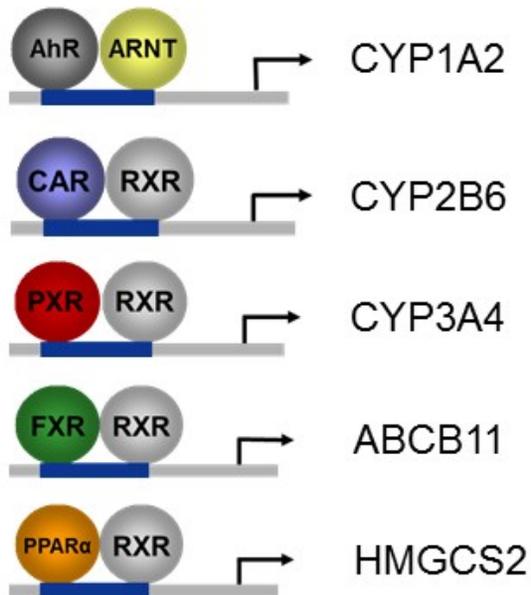
Brad Collins (POB)



Primary human hepatocytes

NTP Laboratories

- *In vitro* liver model used to predict drug metabolism and drug-drug interactions
- Endpoints of interest:



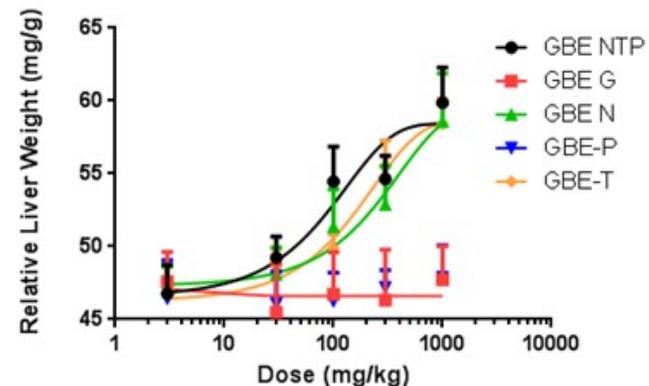
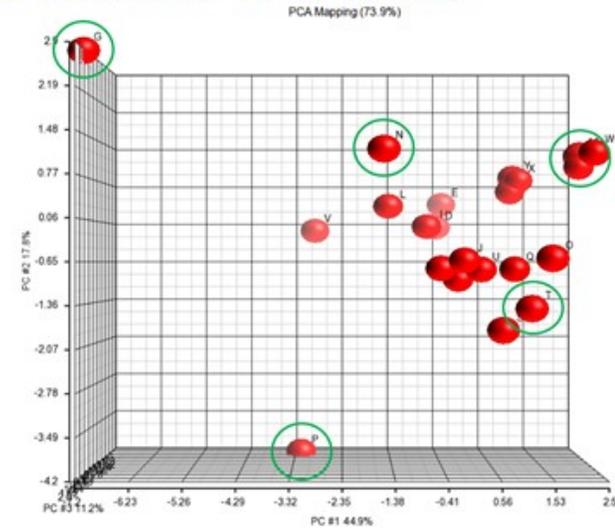
Whole mixture approaches



5-Day Rat Studies

Evaluation of a subset of *Ginkgo biloba* extracts

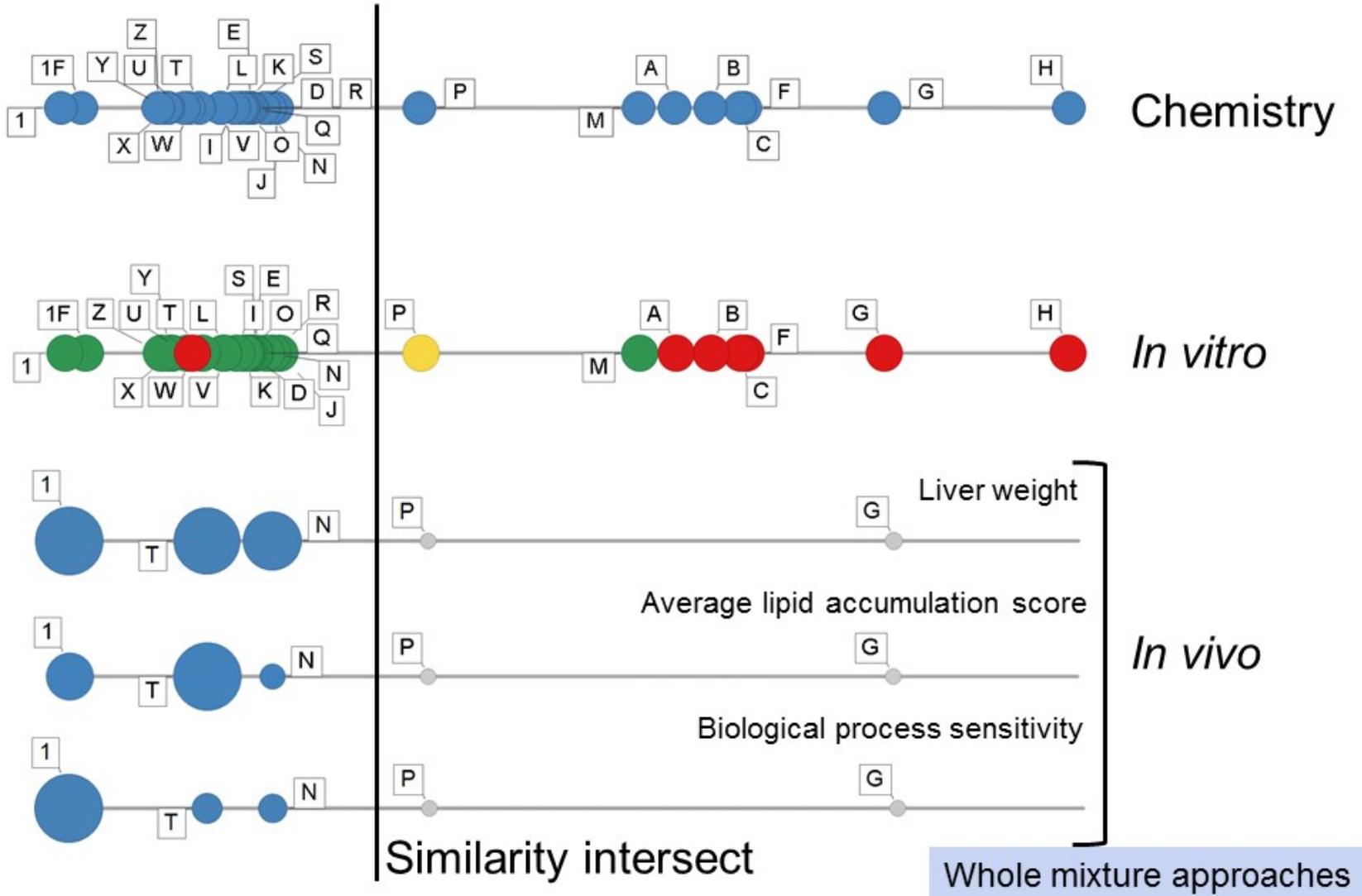
- Study design
 - 5 chemically-diverse lots
 - NTP (1), G, N, P, T
 - 5-Day oral gavage
 - Doses: 0, 3, 30, 100, 300, 1000 mg/kg/day
 - F344 rats
 - Endpoints
 - Organ weights
 - Clinical chemistry and hematology
 - Gene expression in liver



Whole mixture approaches



Determining sufficient similarity





Conclusions

- The NTP test article was most similar to NIST standards and EGb761[®] samples (gold standard in the marketplace)
- Based on chemistry alone, NTP selected an appropriate test article
 - A combination of chemistry and targeted *in vitro* analysis is recommended for future comparisons
- There were *Ginkgo biloba* extract bulk samples that did not resemble standardized extract, and others that were obviously adulterated with flavonol aglycones or unknown material
- In at least one case, it appears that a non-*Ginkgo biloba* extract constituent(s) is responsible for some biological activity (e.g., sample containing Gotu kola)
- Terpene lactones appear to be driving the hepatotoxicity observed in the *in vivo* studies

Whole mixture approaches



Relevance to other complex mixtures

NTP projects

- PAC-MAP
- Crumb rubber
- Glyphosate formulations
- Flame retardant formulations
- Metal working fluids
- All other botanical dietary supplements
- Personal care products
- Water disinfection byproducts

Whole mixture approaches



Developing whole mixtures approaches that can be broadly applied will have a huge impact on our ability to estimate health effects from exposure to complex mixtures

Whole mixture approaches provide a bridge between toxicology and epidemiology

Methods are also relevant to read-across efforts for single chemicals

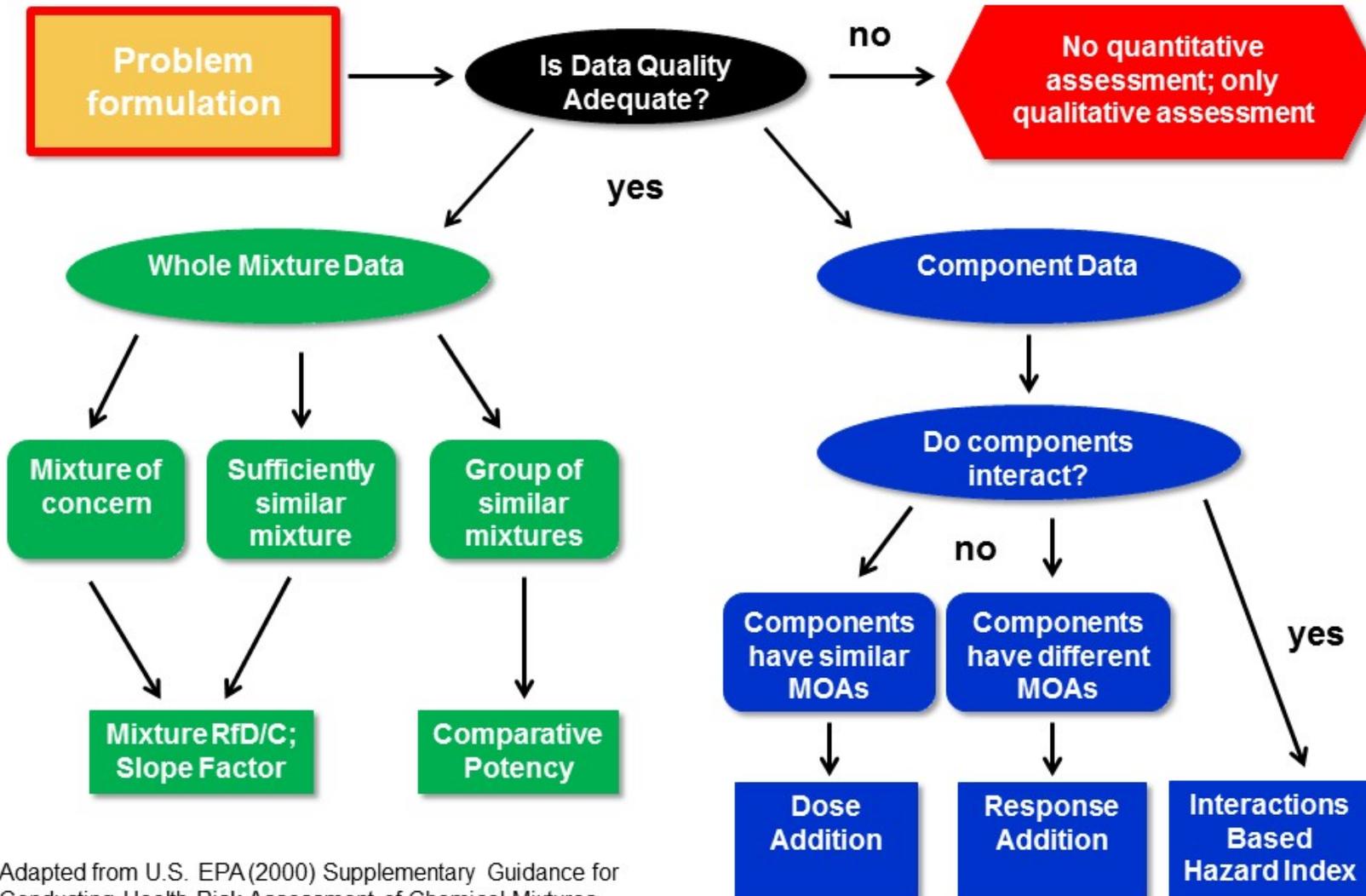


Outline

- Background
 - **Mixtures research areas**
 - Component-based approaches
 - **Whole mixture approaches**
 - Systems biology approaches
- ← Clarifying questions



Assessing risk from mixtures



Adapted from U.S. EPA (2000) Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures



Deciding which stressors to include

Considering biological similarity

Most similar	Chemicals share a...	Examples
	Common active metabolite	Benzyl butyl phthalate and dibutyl phthalate share the active metabolite monobutyl phthalate
	Molecular initiating event	Parathion and chlorpyrifos both inhibit acetylcholinesterase and elicit the same downstream key events
	Adverse outcome pathway	Perchlorates decreases synthesis of thyroid hormone, while dioxin increases elimination of thyroid hormone
	Target tissue	Ephedrine and caffeine are both cardiotoxic
	Disease	DES and tobacco smoke cause cancer in different tissues
Least similar		

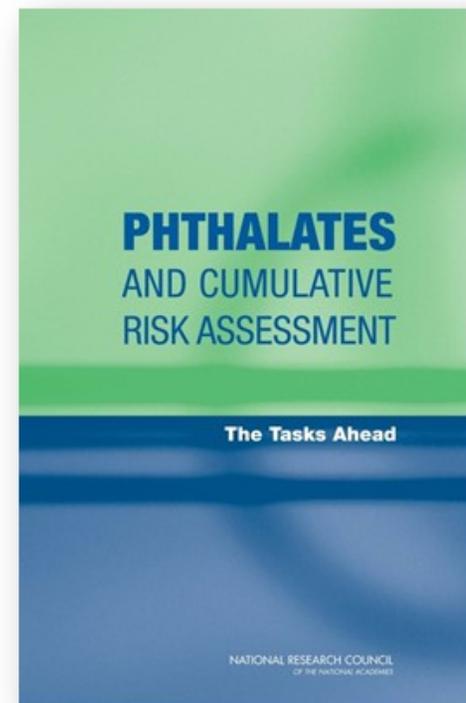
Systems biology approaches



Shifting paradigm

Background

- Standard practice: only perform cumulative risk assessments on chemicals that share molecular initiating events (e.g., organophosphates)
- In 2008, EPA asked the National Research Council to review the data on phthalates and determine whether or not a cumulative risk assessment should be performed
- NRC reviewed the data on phthalate mixtures and recommended including not only phthalates but other chemicals that disrupt androgen signaling



Systems biology approaches



Data supporting NRC conclusions

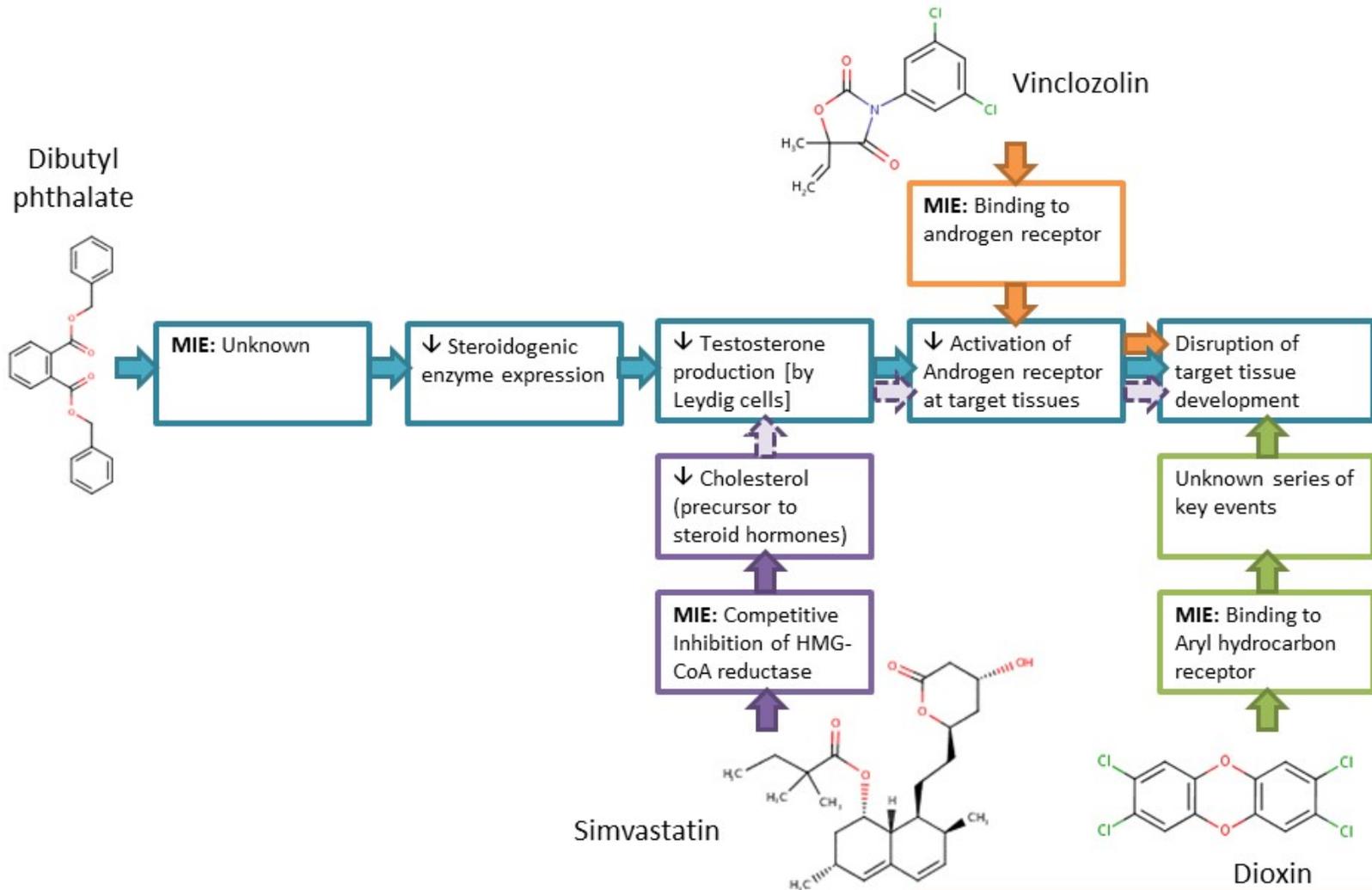
Phthalates and other antiandrogens

- Combinations of phthalates
 - Binary combinations: DBP+BBP, DBP+DEHP
 - 5 phthalate mixture: DBP+BBP+DEHP+DiBP+DPeP
 - 9 phthalate mixture:
DBP+BBP+DEHP+DiBP+DPeP+DHP+DHeP+DiHeP+DCHP
- Combinations of phthalates and other antiandrogens
 - Binary: DBP+linuron, DBP+procymidone
 - 7 chemical mixture: DBP+BBP+DEHP+linuron+prochlorz+procymidone+vinclozolin
 - 10 chemical mixture:
DBP+BBP+DEHP+DiBP+DiHeP+linuron+prochlorz+procymidone+vinclozolin

Conclusion from body of work: Dose addition generally provides a better fit than independent action, supporting inclusion of phthalates and other antiandrogenic chemicals in a cumulative risk assessment



Network of AOPs



MIE = Molecular Initiating Event

Systems biology approaches



Systems-based approaches

Relevant projects

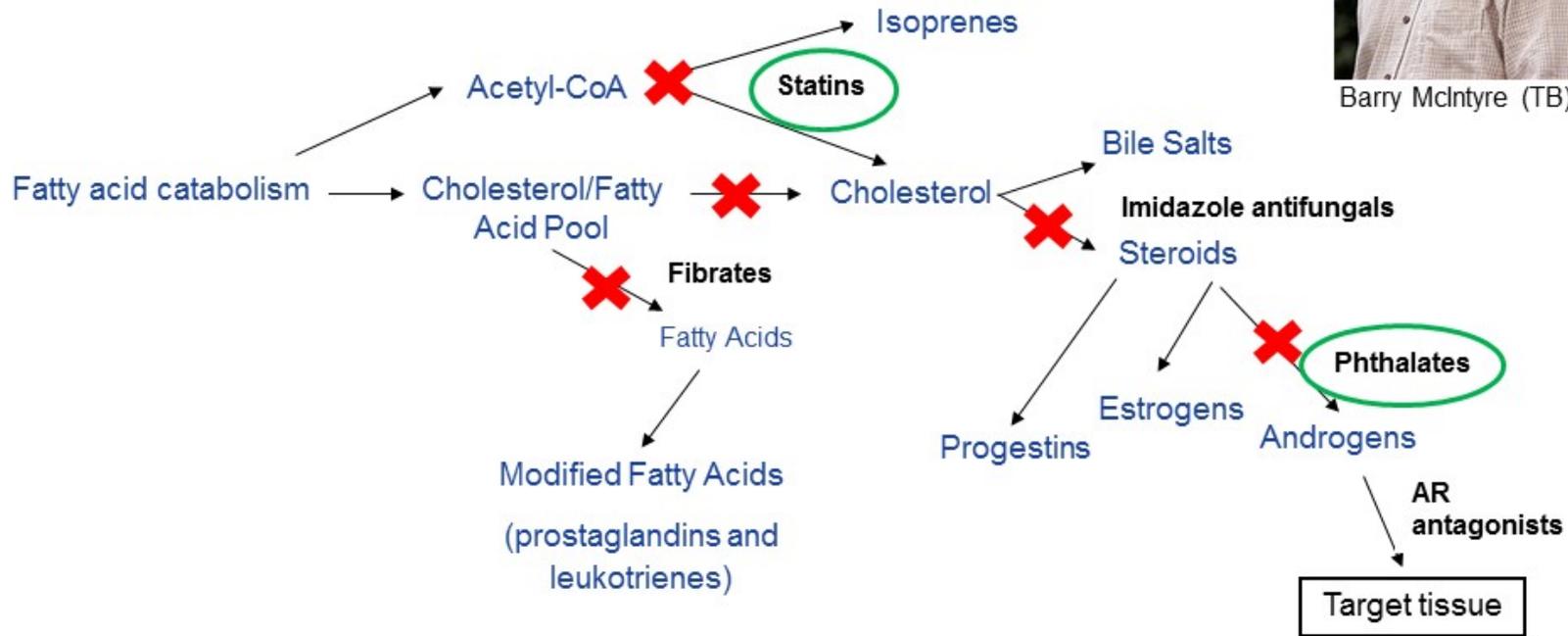
- Evaluating mixtures of chemicals that disrupt lipid signaling and steroid hormone production
- Biological Mechanisms/Pathways of the Combined Effects of Chemical and Non-chemical Stressors Associated with Atherosclerosis
- CNVERGE: Cancer Network and enVironmental Exposure Research aGEnda



Targeting lipid signaling



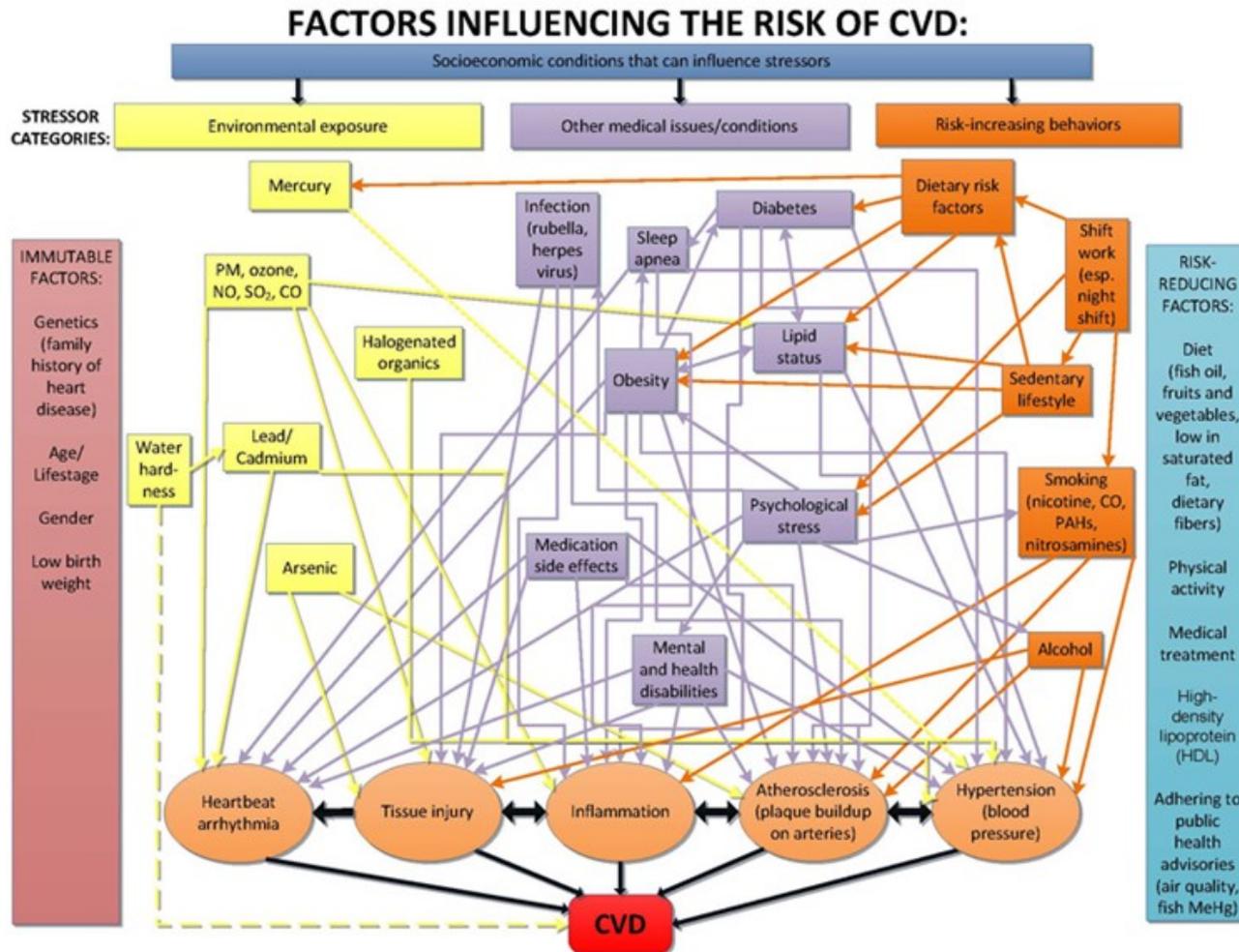
Barry McIntyre (TB)



Systems biology approaches



Disease-centered mixtures



Developed by C. Menzie for EPA Cumulative Risk Assessment Workshop

Systems biology approaches



Mixtures and Atherosclerosis Workshop

Spring 2018

Biological Mechanisms/Pathways of the Combined Effects of Chemical and Non-chemical Stressors Associated with Atherosclerosis



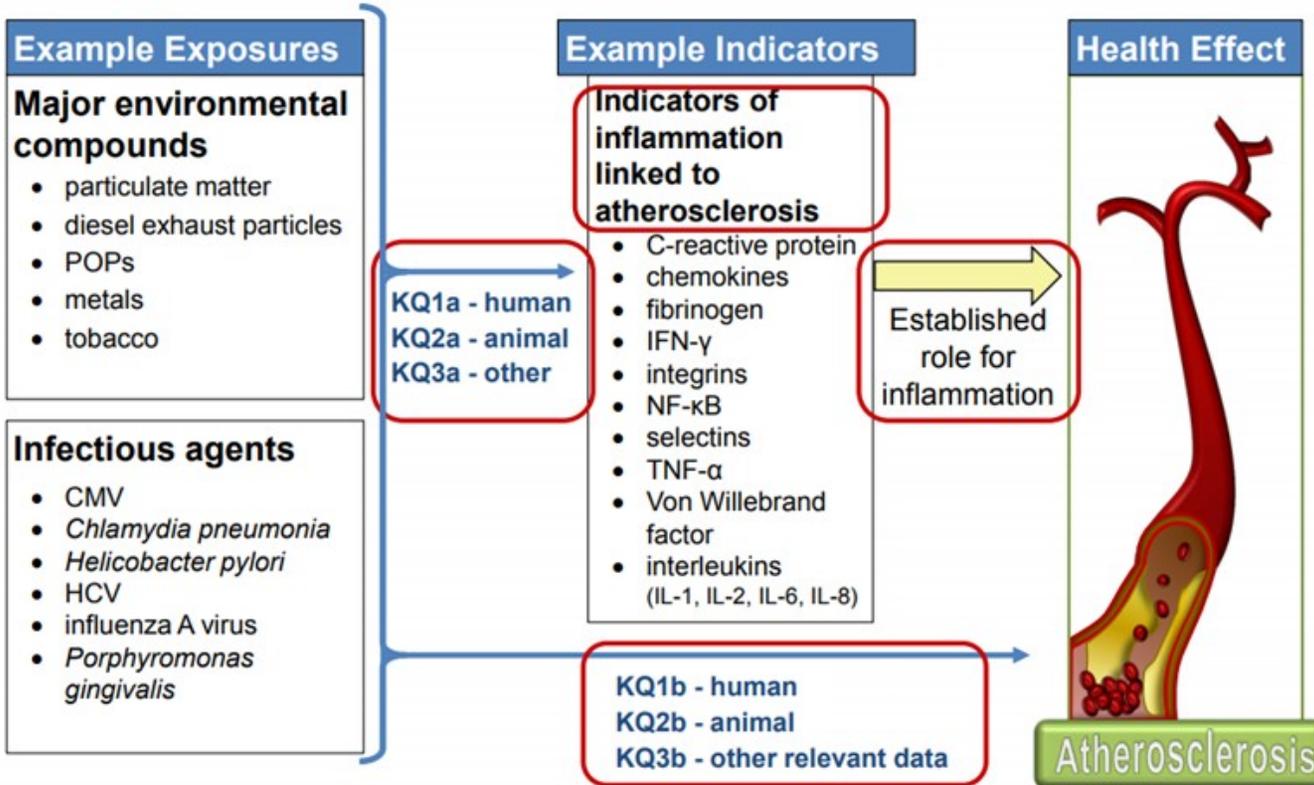
Organizers: Danielle Carlin (DERT) and Michelle Olive (NHLBI)

Systems biology approaches



Inflammation and atherosclerosis

Analytical Framework



Andy Rooney (OHAT)

Examine support for temporal sequence (i.e., exposure \rightarrow inflammation \rightarrow atherosclerosis)

Systems biology approaches



Cancer Pathways and Mixtures

CNVERGE background

- Halifax Project: “Assessing the Carcinogenic Potential of Low Dose Exposures to Chemical Mixtures in the Environment” Aug 8-9, 2013
 - Goal: Bring together cancer researchers and environmental scientists to identify mixtures that target the hallmarks of cancer
- NIEHS Workshop, Aug 25, 2015: “Halifax Project: Low Dose Theory Symposium”

Nicole Kleinstreuer (NICEATM)



Mark Miller (OD)

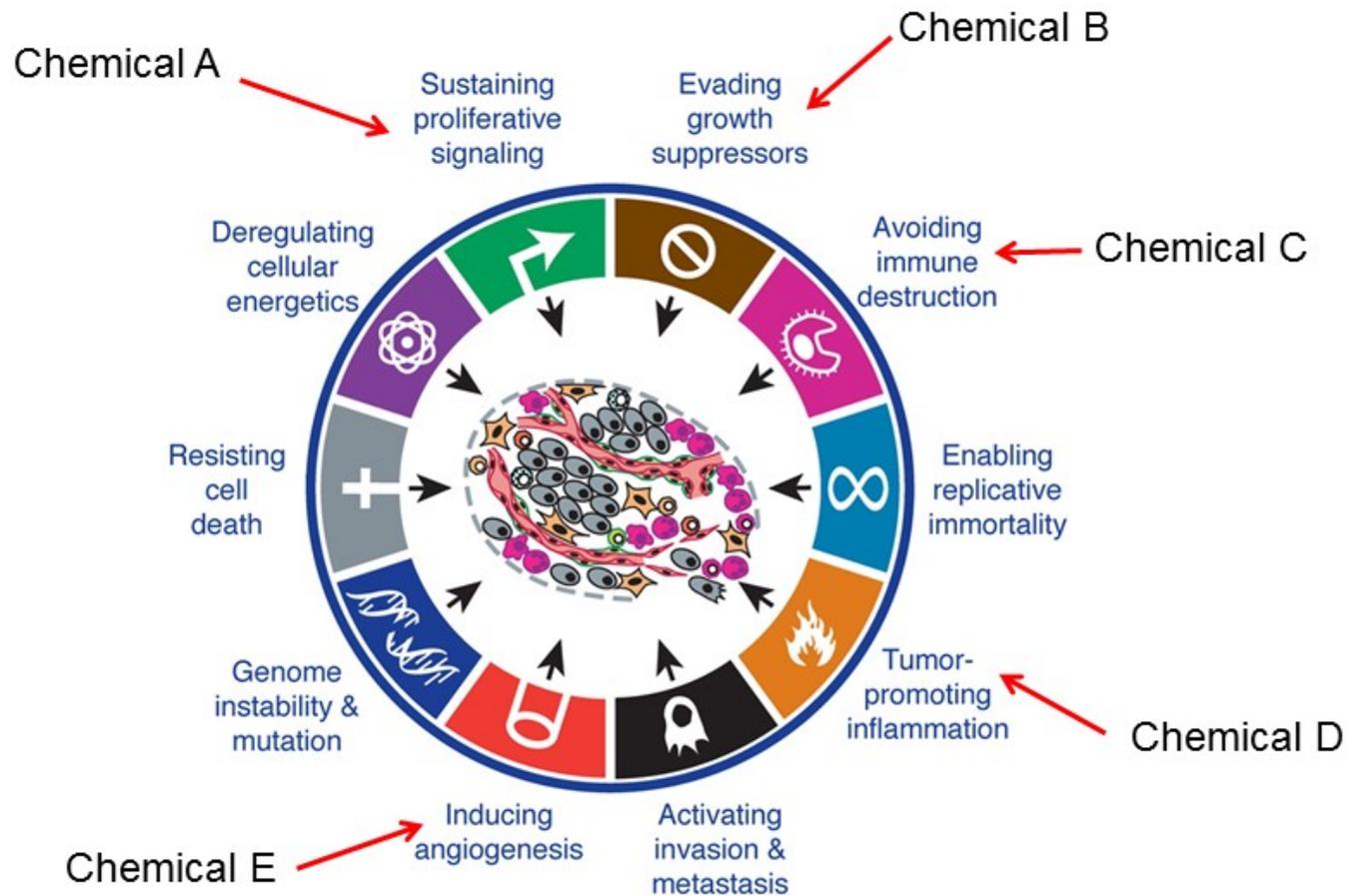


Systems biology approaches



Hypothesis

Chemicals present at low levels that would not be expected to elicit cancer, can contribute to the development of cancer by acting on different pathways



Hanahan and Weinberg, 2011. Cell 144: 646-674

Systems biology approaches



Environmental Working Group Nomination to NTP:
Experimentally evaluate the hypothesis proposed by
the Halifax Project



Path forward

- Focus on a specific cancer type (e.g., breast cancer)
 - Considerations for identification of animal model: human relevance, short timeframe to cancer development, incorporation of genetic instability hallmark
- Identify key molecular targets associated with each hallmark and develop *in vitro* assays for each target
- Build a list of chemicals that interact with each of the identified targets
 - Considerations: specificity, environmental relevance



Identifying targets and candidate chemicals

Hallmark	Molecular target	Candidate chemicals
Angiogenesis	↑ VEGF	Nicotine
Evading immune destruction	Complement system	PAHs
Sustaining proliferative signaling	AhR or ER activation	Bisphenol A, phytoestrogens
Evading growth suppression	p53 or Rb inhibition	Arsenite
Invasion and metastasis	↑ EMT pathway	NNK, hexachlorobenzene
Enabling replicative immortality	↑ hTERT	Nickel, acetaminophen
Resistance to cell death	↓ pro-apoptotic signaling	Sulfonamides
Inflammation	↑ cytokine signaling	Infectious agents, asbestos, silica
Reprogramming energy metabolism	Mitochondrial electron transport chain disruption	Organophosphates, pyrethroids
Genome instability	Oncogene mutation	Genotoxic agents or genetic predisposition

Systems biology approaches



Conclusions

- NTP is tackling big mixtures questions using the latest toxicology tools
- Efforts are concentrated in areas that will provide data to inform
 1. Application and refinement of component-based risk assessments
 2. Development and application of whole mixtures approaches
 3. Prioritization of chemicals for inclusion in cumulative risk assessment based on knowledge of biological systems



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Scott Masten
David Umbach (DIR)
Suramya Waidyanatha
Nigel Walker

All of NTP has been involved in mixtures work!





Outline

- Background
- **Mixtures research areas**
 - Component-based approaches
 - Whole mixture approaches
 - **Systems biology approaches**

 Clarifying questions



Questions for BSC

- Please comment on whether NTP is addressing the highest priority questions in mixtures toxicology to inform risk assessment.
- How would you rank the importance and tractability of the three areas discussed?
- What do you anticipate will likely be NTP's most challenging obstacles in achieving meaningful contributions to understanding mixtures toxicity?
- Are there additional areas in mixtures toxicology on which NTP should focus effort?
- Please comment on whether the selected test articles (noted below) are appropriate for exploring the stated challenge:
 - Uncertainties in the relative potency factor approach – PACs
 - Sufficient similarity of whole mixtures – botanicals
 - Systems-based prioritization of chemicals for cumulative risk assessment – chemicals that target the hallmarks of cancer