An integrated approach to testing and assessment of chemical mixtures in the environment: The advent of Adverse Outcome Pathway footprinting

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Background

- Human environmental exposures are typically to mixtures of chemical, biological, and physical stressors
- Rarely are hazard and dose-response data available for chemical mixtures of interest (e.g., component proportions; relevant doses), and, 'traditional' assay data may also be lacking for individual mixture component chemicals
- This lack of available assessment relevant information may lead to under-estimation of risk to human health and the environment due to mixture exposures
- The time and resources needed to conduct traditional chemical by chemical analyses that inform phenotypic outcomes are not conducive for informing a broad landscape of current human health assessment concerns
- Integration of data from New Approach Methodologies (NAM) may provide opportunities to evaluate hazards associated with exposure to mixtures containing data-poor component chemicals

Bold new world with NAMs…

Conceptual Approach to Integrated Testing and Assessment (IATA) of Mixtures

(A) Problem formulation
- Screening/Prioritization?
- Hazard identification/grouping?
- Mixtures dose-response assessment?

(B) 'Fit-for-purpose' toolbox
- *Data mining* - exposure and hazard data
- *Cheminformatics* – (Q)SAR/read-across
- *High-throughput exposure modeling* – ExpoCast
- High-throughput TK – IVIVE/reverse dosimetry
- *Bioactivity* – ToxCast, Tox21, REACH
- *Adverse Outcome Pathway 'Footprinting'*

(C) WOE for mixture chemicals
- AOPs available for chemicals of interest?
- Anchor chemical(s) identified?
- D-R data available for mixture chemicals?

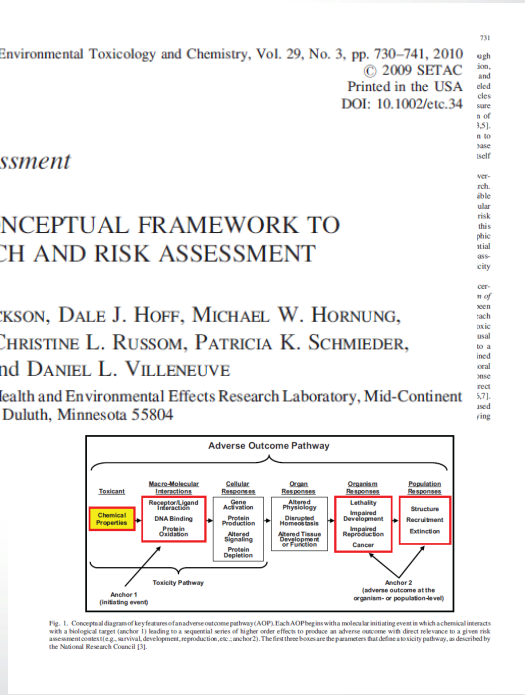
(D) Component-based Mixtures RA
- Apical and/or key event-based PODs
- D-R curves suitable for potency eval
- Multiple active AOPs/MOAs

Adverse Outcome Pathway (AOP)

- A way to organize potentially diverse streams of biological information to inform a given source to health outcome continuum
- Based on biological plausibility and/or statistical inference
- Data used in an AOP may span different levels of biological organization relevant to human health and/or ecological assessment (e.g., molecular, cellular, tissue/organ, up to whole organismal and/or population).

Key Principles of AOPs

- ❖ AOPs are chemical agnostic
- ❖ AOPs are commonly simplifications of complex biology
- ❖ AOPs are nodal/modular
- ❖ AOPs are evergreen; typically dynamic and evolving
- ❖ Multiple AOPs (i.e., AOP network) typically involved in phenotypic expression of bioactivity



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Hazard/Risk Assessment

ADVERSE OUTCOME PATHWAYS: A CONCEPTUAL FRAMEWORK TO SUPPORT ECOTOXICOLOGY RESEARCH AND RISK ASSESSMENT

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Fig. 1. Conceptual diagram of key features of an adverse outcome pathway (AOP). Each AOP begins with a molecular initiating event in which a chemical interacts with a biological target (anchor 1) leading to a sequential series of higher order effects to produce an adverse outcome with direct relevance to a given risk assessment context (e.g., survival, development, reproduction, etc.; anchor 2). The first three boxes are the parameters that define a toxicity pathway, as described by the National Research Council [3].

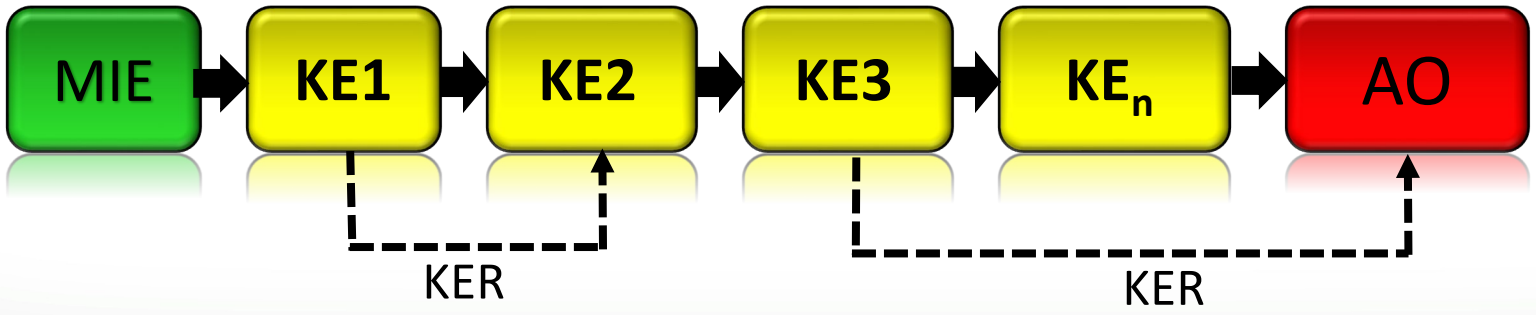
AOP Development

Key event (KE)

- *Functional qualitative unit of observation (i.e., what happened)*
- Observable Δ in biological state (measurable)
- Essential (but not necessarily sufficient to induce AO alone)

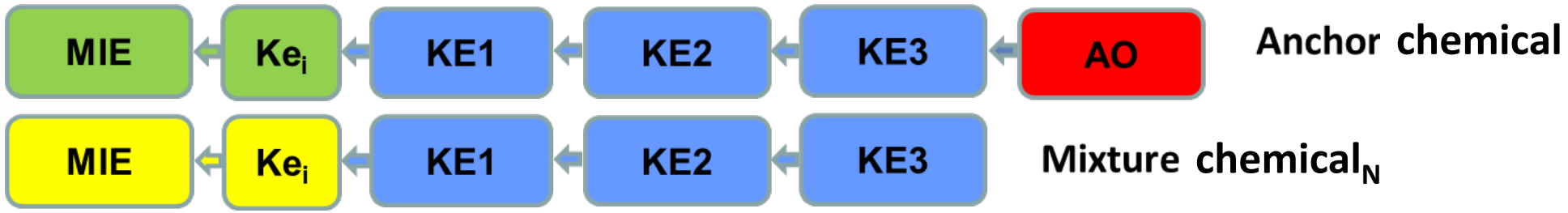
Key event Relationship (KER)

- *Functional unit of quantitative inference/extrapolation (i.e., relationship between direction and magnitude of Δ in a KE and other members of AOP)*
- State of $KE_{up/down}$ has some causal relationship to one or more other $KE_{up/down}$ and or $AO_{up/down}$
- Supported by biological plausibility and weight-of-evidence



AOP "Footprinting" Concept

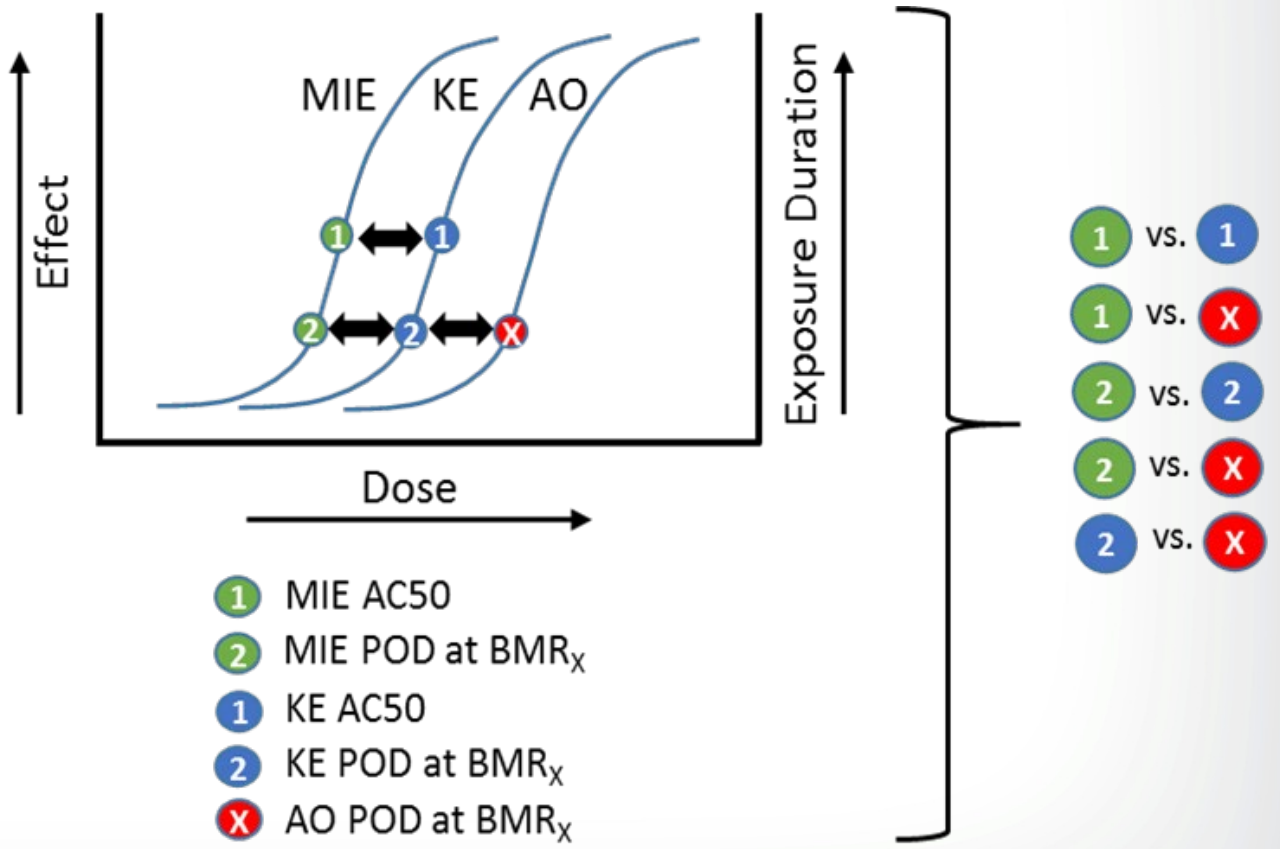
- **In contrast to AOP theory which posits a chemical agnostic description of the MIE to AO pathway, the footprinting approach first requires identification of well-characterized (hazard and dose-response) chemical(s) as the "anchor" or "index" for each operative AOP**
- AOP footprinting is the stepwise profiling and comparison of AOPs at the level of key events moving backward from the most downstream key event to the molecular initiating event



- The goal is to identify the key event(s) within each AOP suspected of contributing to a given adverse outcome at which similarity between mixture chemicals can confidently be determined. These key events are identified as the 'footprint' for a given AOP
- Mixture chemicals are then assigned to the appropriate 'footprint' category, and the key event dose-response relationship(s) (KER) for each chemical within a category are then used to evaluate mixture additivity

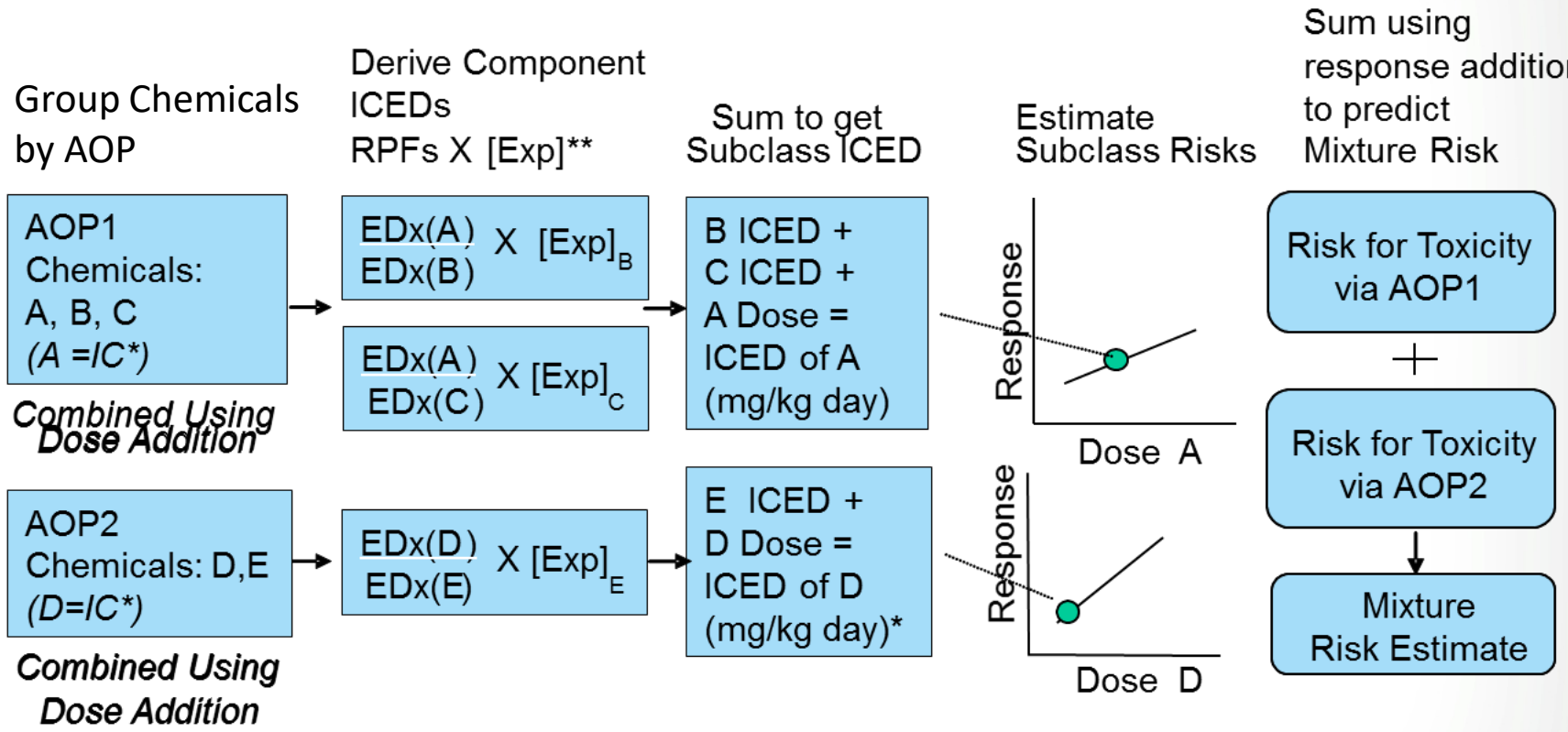
Footprint Identification and KER evaluation

- A key to identifying the 'footprint' is the WOE supporting the hazard and dose-response relationship to the AO (i.e., if the KE went away would incidence and/or severity of the AO change?)
- For most AOPs, there may be greater confidence in a KE 'footprint' if it is mechanistically proximal to the AO, however this will be dependent on KE data available
- Quantitatively, benchmark doses (BMD) at biologically-informed benchmark response levels (BMR) are ideal for comparisons
- If BMD modeling is not feasible, effect level calls (e.g., LOELs, LOTELs) based upon biological understanding and/or statistical significance could be used as comparator



Mixtures Assessment Approach: Integrated Addition

- Toxicity outcomes are rarely a single pathway phenomenon
- Non-pharmaceutical chemicals not designed based on fidelity of biology (i.e., environmental chems typically induce a messy network of perturbations and endpoints!)
- Integrated Addition method ideal for evaluating diversity of AOPs
- Entails integration of dose- and response-additive approaches

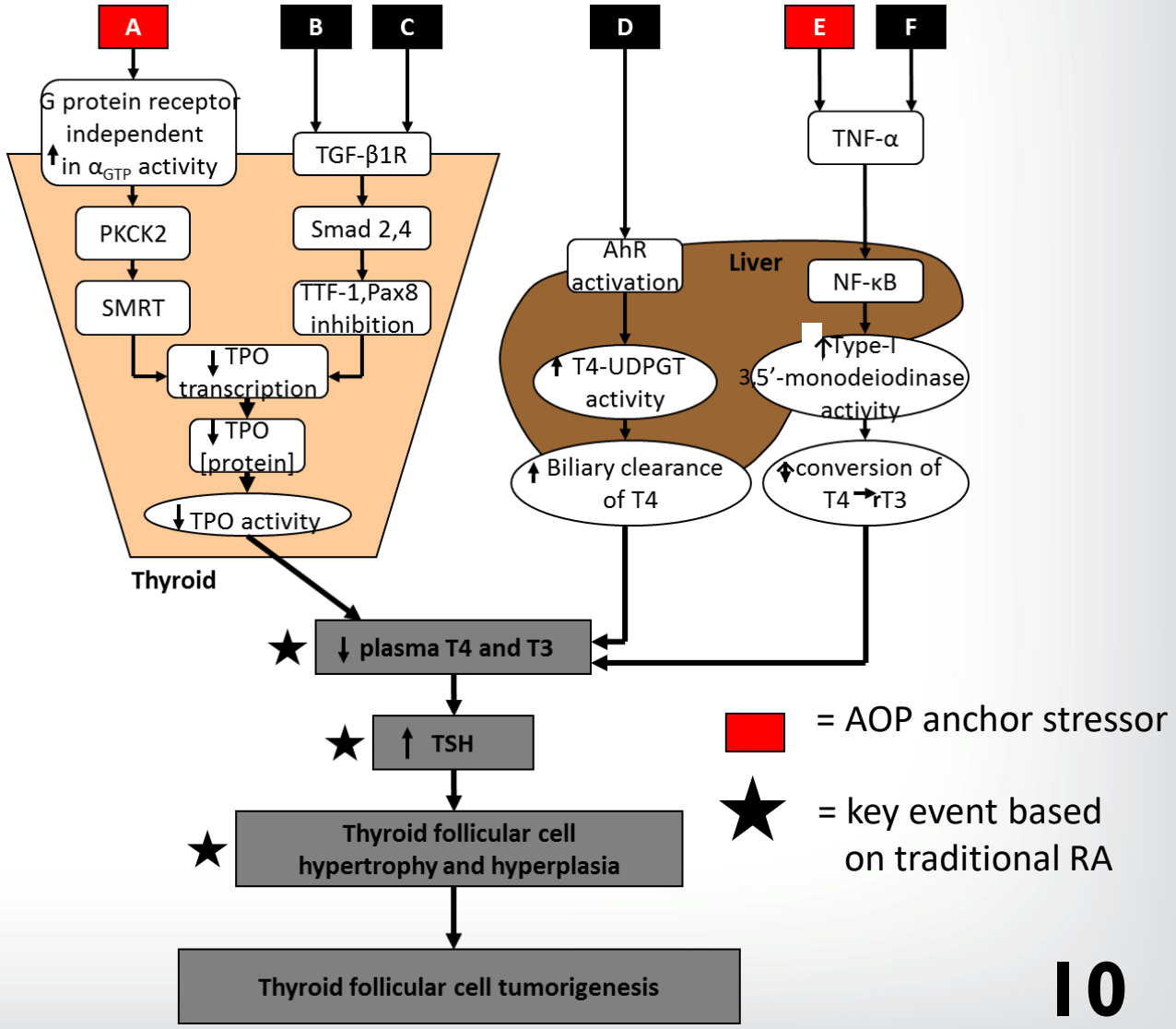


*IC = Index (AOP Anchor) Chemical

** [Exp]= Exposure Dose; Internal dose metric such as Total Absorbed Dose is desirable

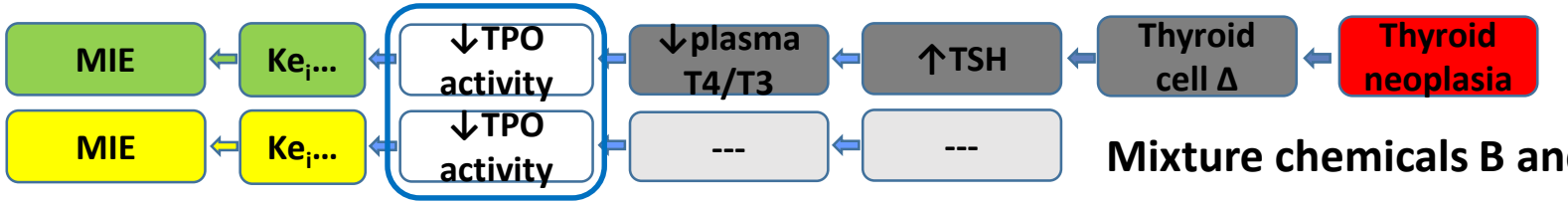
AOP Footprinting Conceptual Example

- Hypothetical mixture of six chemicals
- Two of six chemicals (e.g., A and E) have a replete AOP database including in vivo data indicating an exposure-response relationship resulting in thyroid follicular cell tumorigensis
- Two of the other four chemicals have alternative toxicity testing data streams supporting WOE for bioactivity up to T3/T4 perturbations in vitro
- The remaining two chemicals have alternative toxicity testing data supporting WOE for perturbations in hepatocellular processes involved in thyroid hormone economy/homeostasis



Thyroid AOP Footprint

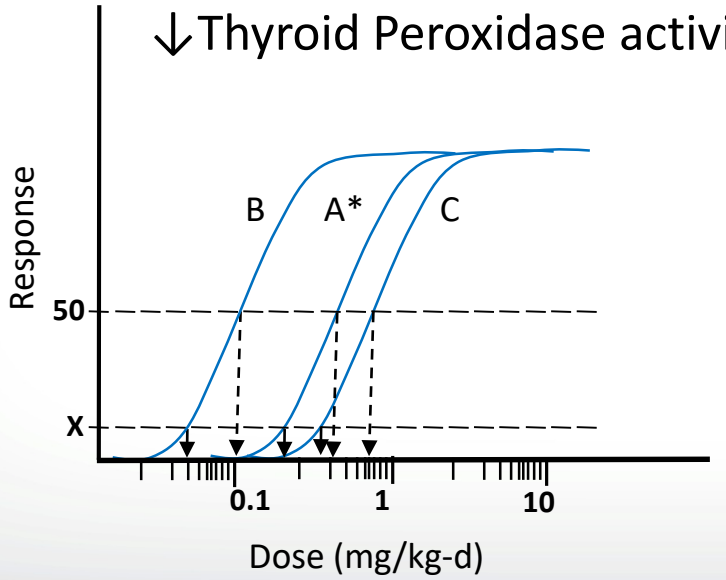
Example Thyroid AOP footprint evaluation



AOP Anchor chemical A

Mixture chemicals B and C

Dose-response modeling of AOP footprints

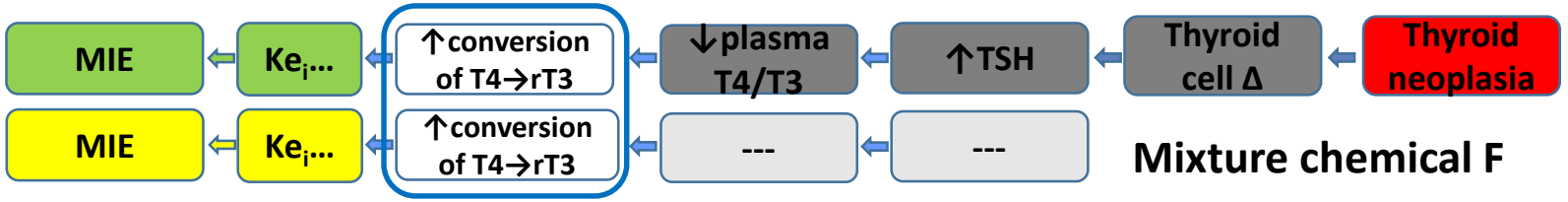


Chemical	BMD_X	BMD_{50}
A*	0.18	0.32
B	0.03	0.1
C	0.27	0.65

* = anchor chemical for AOP

Liver AOP Footprint

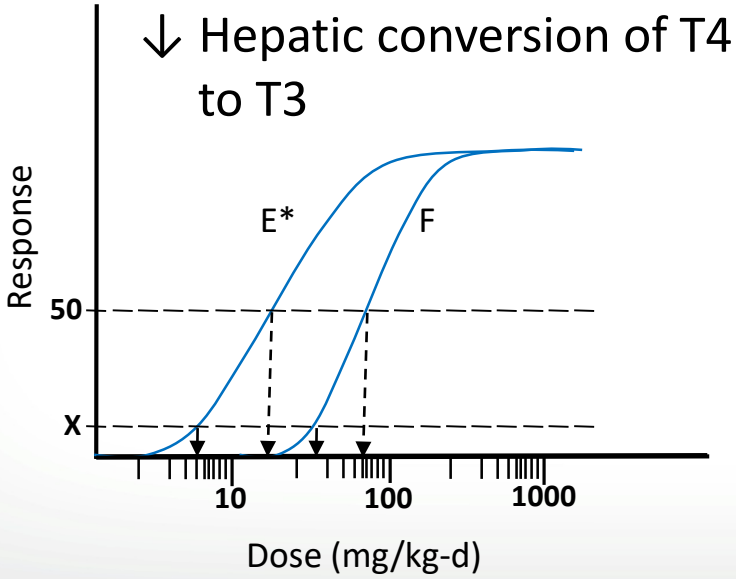
Example Liver AOP footprint evaluation



AOP Anchor chemical E

Mixture chemical F

Dose-response modeling of AOP footprints

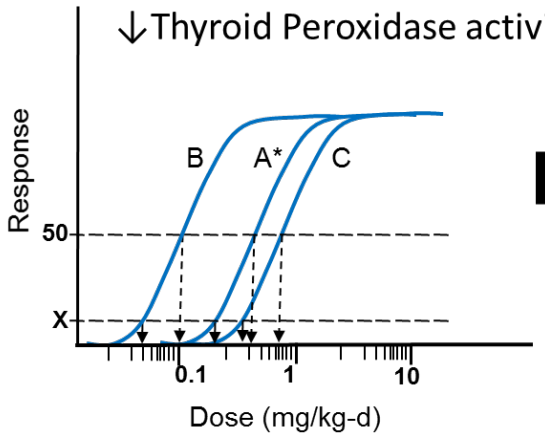


Chemical	BMD_X	BMD_{50}
E*	4	16
F	27	65

* = anchor chemical for AOP

Integrated Addition

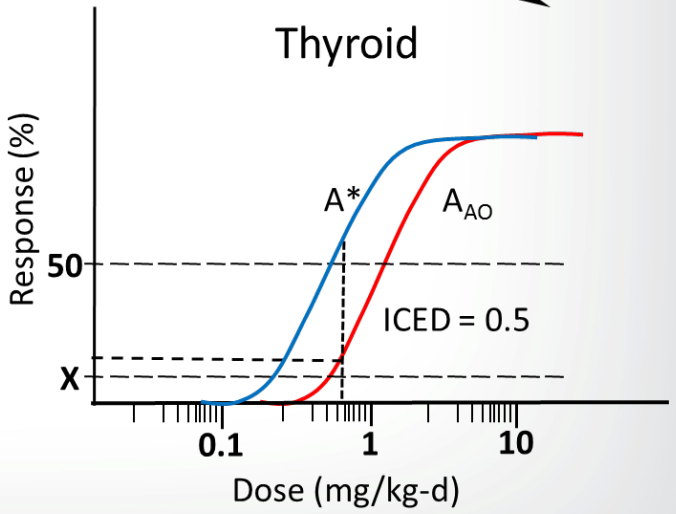
- Chemicals are evaluated based on the assumption of dose additivity within "common" footprint groupings and relative potency factors are derived (e.g., where RPF = BMD_X of the AOP anchor / BMD_X of AOP member chemical N)
- An index chemical (i.e., AOP anchor) equivalent dose (ICED) is calculated for each chemical and summed within footprint groupings
- ICED(s) are then used to estimate AO response due to mixture exposure based off of AOP anchor dose-response function



Chemical	BMD_{50}	RPF_{50}	Exp	ICED
A*	0.32	1	0.003	0.003
B	0.1	3	0.002	0.06
C	0.65	0.5	0.8	0.4
Total ICED				0.5

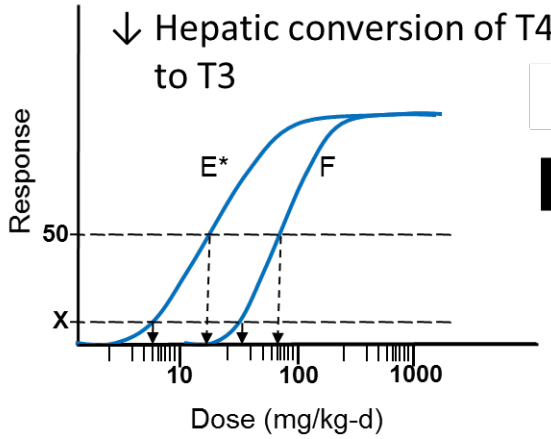
= dose-response of AOP anchor for thyroid follicular cell tumorigenesis

= dose-response of AOP anchor for ↓thyroid peroxidase (TPO) activity



Integrated Addition

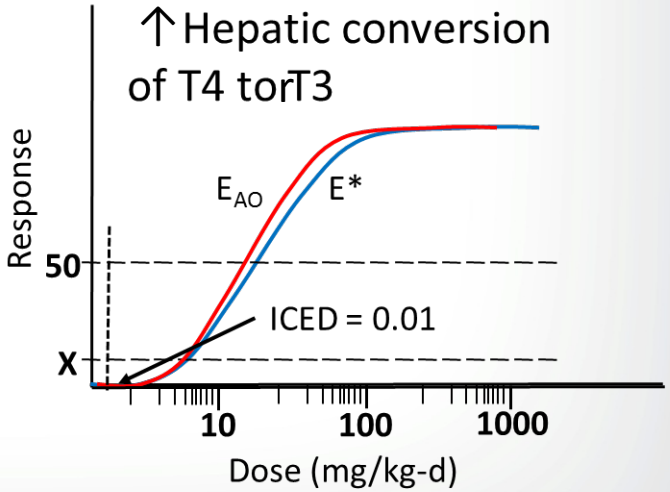
- Same RPF exercise for the liver compartment
- For mixture stressor D there is no AOP anchor
- Available information is non-apical (ends at T4-UDPGT activity in hepatocytes in vitro)
- Uncertain contribution to overall cancer mixture risk
- Until further AOP data becomes available (i.e., downstream key events), integrating stressor D into mixtures evaluation is difficult in a relative potency factor approach



Chemical	BMD_{50}	RPF_{50}	Exp	ICED
E*	16	1	0.01	0.01
F	65	0.25	0.0005	0.0001
			Total ICED	0.01

= dose-response of AOP anchor for thyroid follicular cell tumorigenesis

= dose-response of AOP anchor for ↑hepatic conversion of T4 to T3



Moving Forward

NAM/AOP footprinting

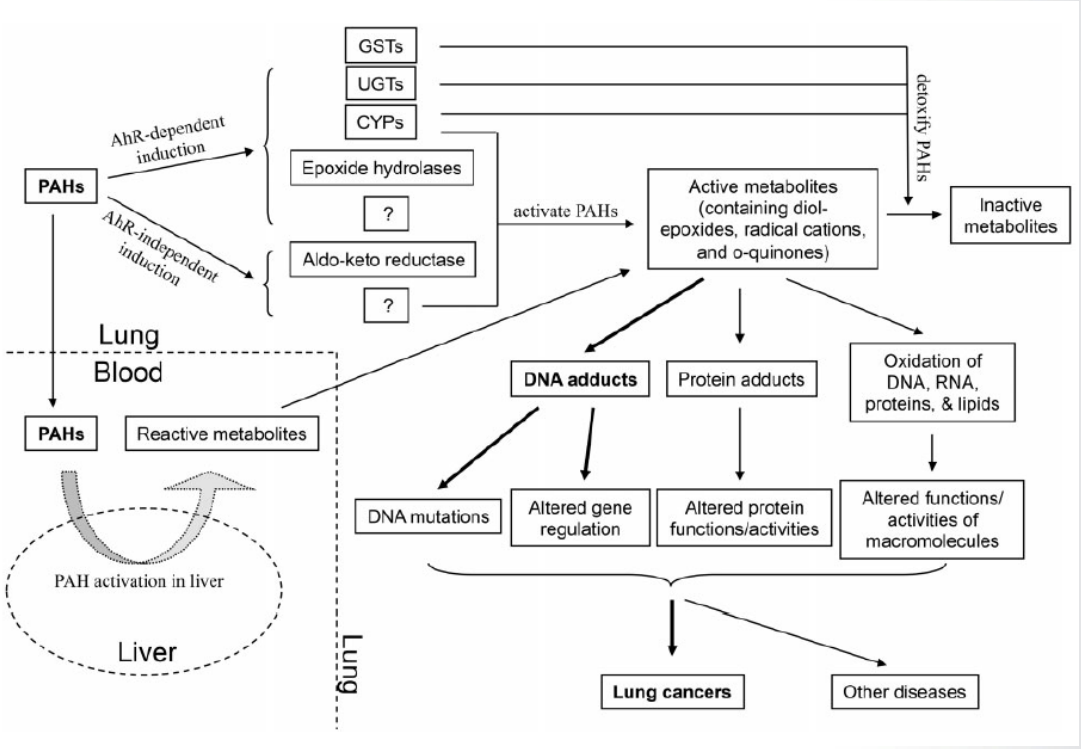
- Confidence in NAM data streams in general? SAR/Read-across ± HT/HC ADME/TK ± In vitro bioactivity, etc.
- Metabolic competence of cell-based (in vitro) information?
- Footprint events are only as good as the WOE demonstrating importance in AO (e.g., inhibitor studies, transgenic models; what is known about chemical class?)

Mixtures Assessment

- Whole mixture>>Sufficiently similar mixture>Component-based (phenotypic)>Component-based (NAM)
- Ab initio presumption of dose-additivity within AOP footprint grouping(s) (careful about deviations)
- Situations will arise where mixture chemicals may not have sufficient WOE for quantitative evaluation via use of NAM data, however, decisions on AOP footprint membership can still inform potential for additivity
- AOP anchor chemicals are key to estimations of mixture risk

Potential for AOP in Mixtures Assessment

- Decisions regarding hazard grouping (e.g., AOP/MOA) and component-based mixtures dose-response assessment could potentially be made at a level of biological detail where data can be rapidly/efficiently generated
- There is no known application of AOP in mixtures assessment. This conceptual approach significantly advances the utility of AOP information in a risk assessment context
- Potential for expanding the number of assessments for chemicals that have limited or no traditional toxicity data and sets the stage for incorporating additional data streams in the future
- *The key will be development of case studies across diverse chemical and biological space!*



Multiple mechanisms by which PAHs cause lung cancer. (Moorty et al. 2015, Tox Sci 145(1):5-15)

Acknowledgement

For all of the times I have trapped Glenn Rice into conversations and musings about the marriage between NAM, AOP, and Mixtures Risk Assessment over the past few years, my sincerest gratitude!!!

"The core of the committee's vision for the future involves the mapping of toxicity pathways in human tissues, and the identification of critical pathway perturbations responsible for toxic responses."

"Dose-response relationships for pathway perturbations can then be described quantitatively through biologically-based modeling of toxicity-pathway circuitry and human pharmacokinetics."

"When the vision is fully implemented, regulation will be based on avoidance of biologically significant perturbations of key human toxicity pathways, rather than on the current practice of assessing human health risks based on high-dose responses in animals and the use of questionable assumptions to extrapolate such findings to low-dose risks in people."

NRC (2007)