NTP
National Toxicology Program

Developing Rational Hypotheses for Testing Mixtures of Chemicals That Target Pathways of Carcinogenesis

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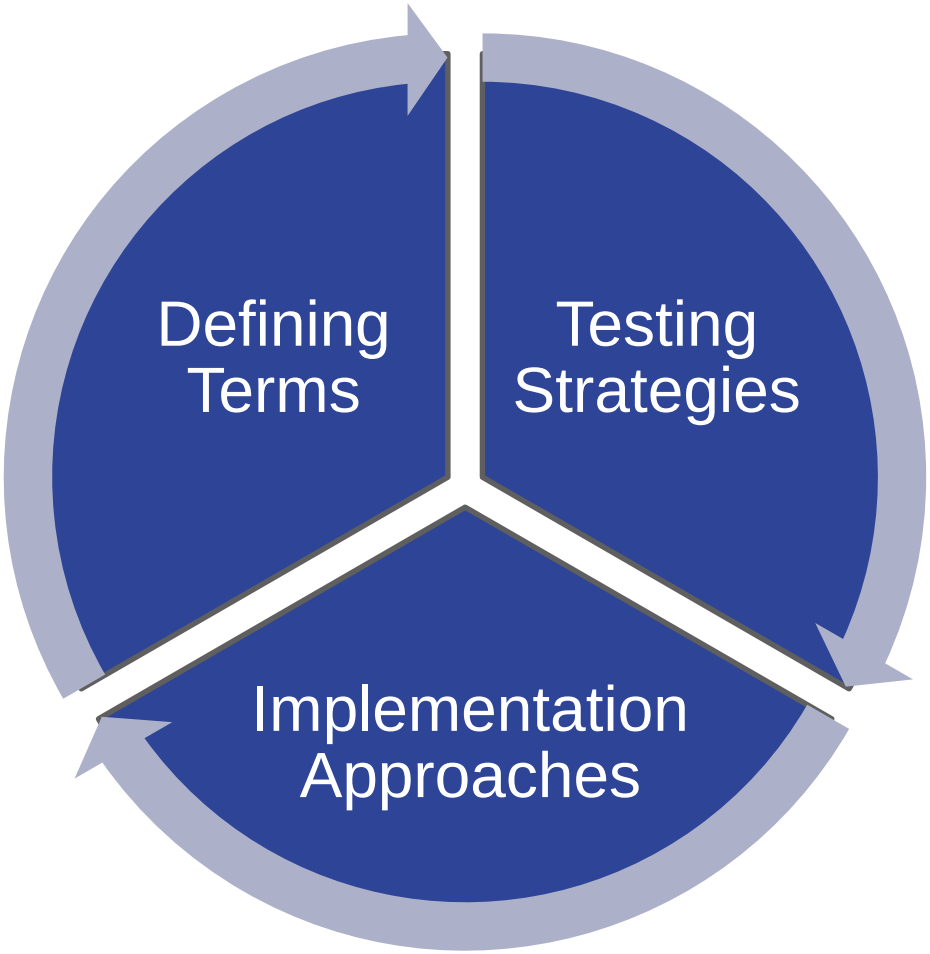
Breakout session questions

Mixtures

- Should we be addressing the joint action of co-carcinogens below their individual cancer thresholds, or focusing on chemicals that are not carcinogens but target the Hallmarks/Key Characteristics and could contribute to cancer development jointly?
- Can mixtures hypotheses be generalizable across cancer types? When should they be specific to tumor types/incidence based on ADME principles and knowledge of key events for that cancer type?

Iterative decision-making



Defining terms

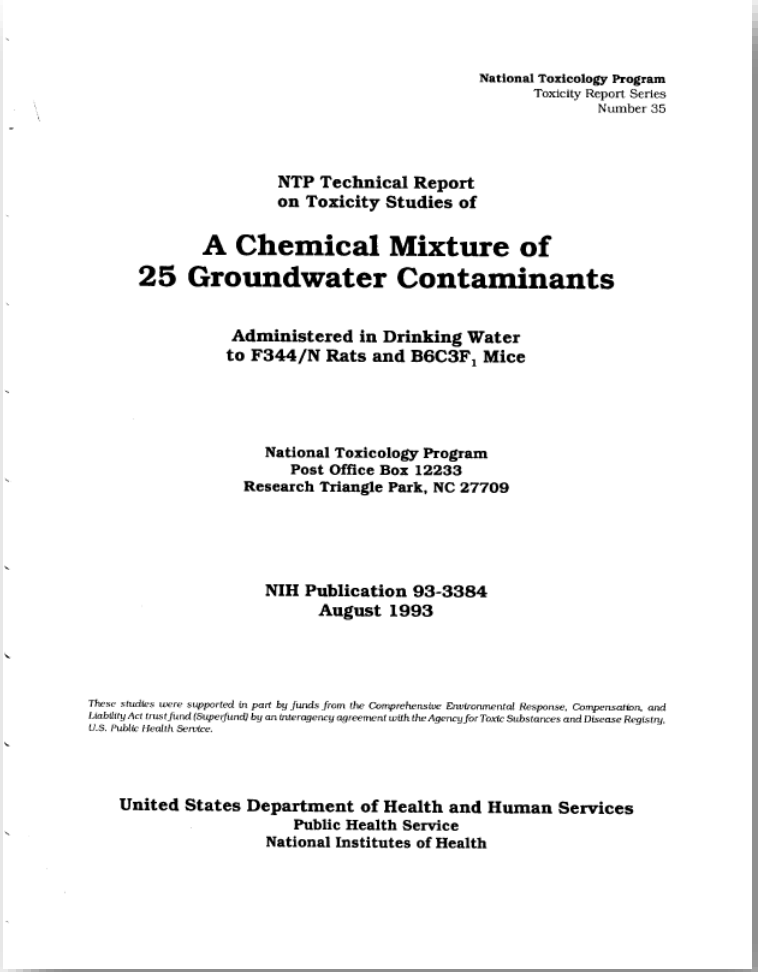
- Low dose
  - Environmentally-relevant levels
  - Below NOAEL
  - Less concerned about low dose, more concerned about understanding the joint action of multiple chemicals that converge on pathways leading to cancer
- Mixtures
  - Focus on chemicals that have not been identified as carcinogens
  - Only include non-genotoxic chemicals
  - Identify chemicals based on pathways
- Cancer
  - *De novo* cancer
  - Priming the conditions for cancer (e.g., decreased time to cancer with genetic predisposition, lower dose of carcinogen required)
  - Specific cancer type(s) or generalizable to all cancers

Requirements

- Tractable
  - Can be executed in a reasonable timeframe with a reasonable investment
- Interpretable
  - Upon completion of testing, knowledge is gained, regardless of outcome
- Impactful
  - Will either support current risk assessment paradigm as protective of human health or provide data to advance cancer risk assessment practice

Lessons learned

- **Goal:** Explore the potential for interactions among chemicals in complex mixtures
- **Approach:** Combine 25 chemicals at ‘environmentally-relevant’ levels, dose animals for 26 weeks, observe toxicity
- **Issue:** No hypothesis articulated for expected mixture responses
- **Outcome:** Uninterpretable data



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Number 35

NTP Technical Report
on Toxicity Studies of

A Chemical Mixture of 25 Groundwater Contaminants

Administered in Drinking Water
to F344/N Rats and B6C3F$_1$ Mice

National Toxicology Program
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Research Triangle Park, NC 27709

NIH Publication 93-3384
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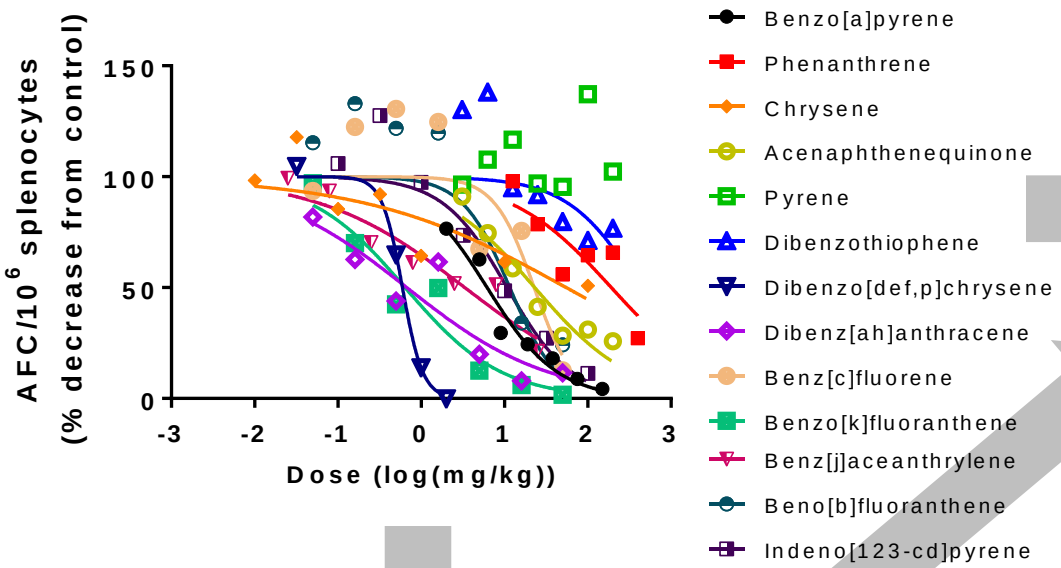
Hypothesis-based mixtures research

Individual chemical dose-response data

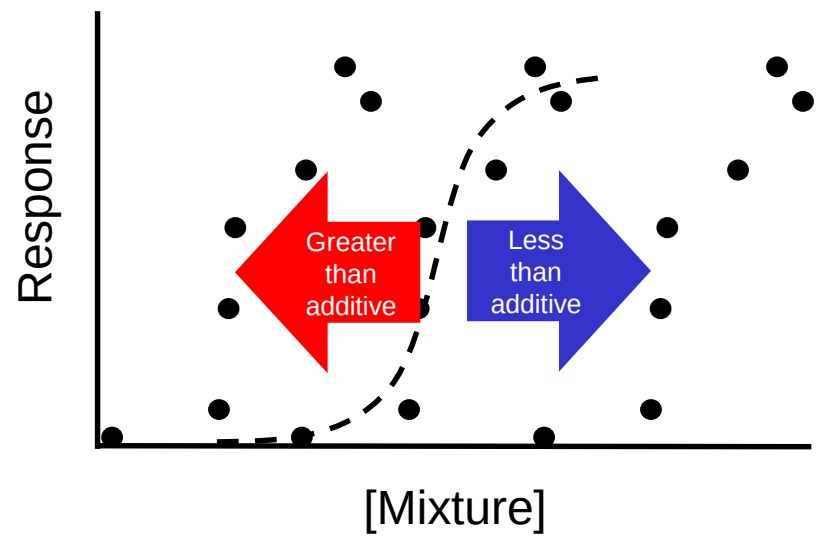


Design mixture studies

- 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



Breakout session questions

Mixtures

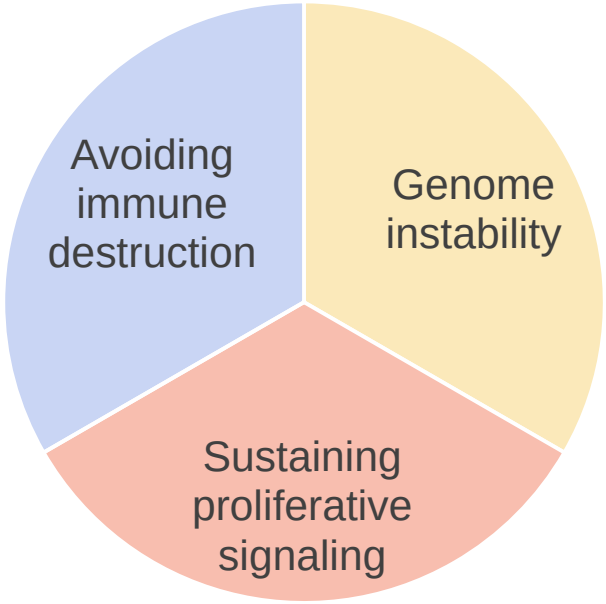
- Should we be addressing the joint action of co-carcinogens below their individual cancer thresholds, or focusing on chemicals that are not carcinogens but target the Hallmarks/Key Characteristics and could contribute to cancer development jointly?
  - What should we be studying (carcinogens or non-carcinogens)?
- Can mixtures hypotheses be generalizable across cancer types? When should they be specific to tumor types/incidence based on ADME principles and knowledge of key events for that cancer type?
  - How should we be studying them?

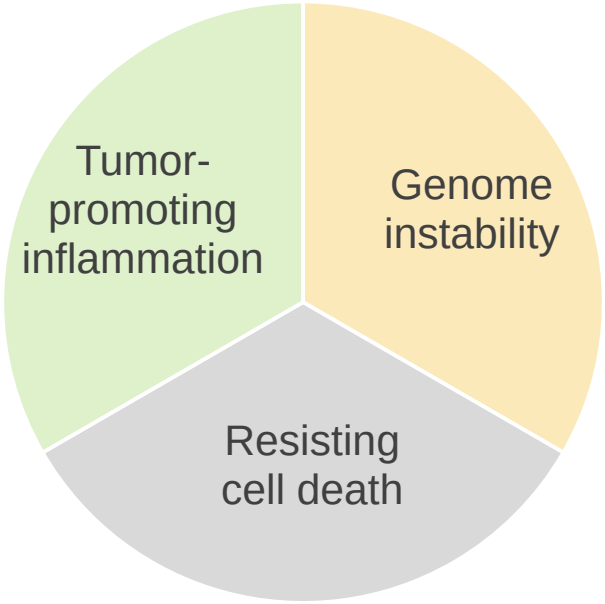
Implications for cancer risk assessment

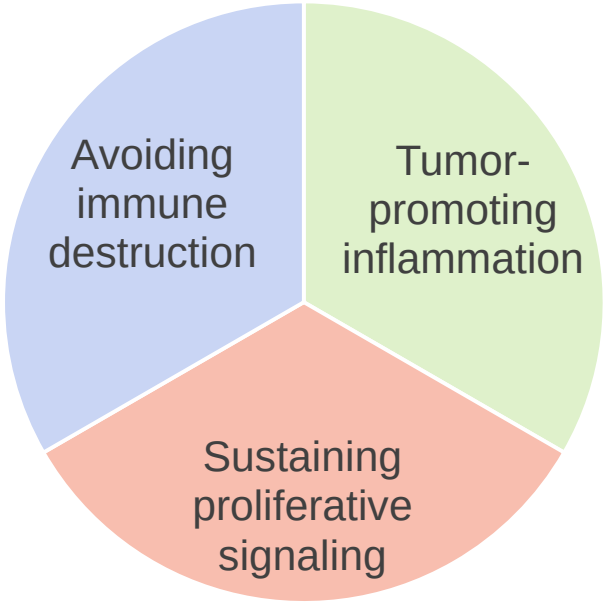
- Should we be addressing the joint action of co-carcinogens below their individual cancer thresholds?
  - Outcome 1: data supports the current paradigm of considering carcinogenic chemicals with divergent mechanisms of action as independent-acting
  - Outcome 2: data indicating that carcinogens with different mechanisms of action display dose-additive effects would imply that dose additivity (not response additivity) should be used as a default assumption for risk assessment of carcinogenic chemicals that co-occur (e.g., at a Superfund site)

Building on co-carcinogen research

- **Hypothesis:** Certain combinations of carcinogens will produce dose additive or greater-than-dose additive (i.e., synergistic) interactions when present jointly by targeting cooperative pathways
- **Research aim:** Identifying which causal pathway combinations are sufficient to elicit cancer







Credit to Michele La Merrill for introducing the topic of causal pies to this discussion

Learning from the literature

- Cursory review of the co-carcinogen research
  - Kinds of chemicals that have been included
  - Study types
  - Endpoints
- Can co-carcinogenic chemicals included in existing studies be mapped to pathways?

DATABASE ON BINARY COMBINATION EFFECTS OF CHEMICAL CARCINOGENS[a]

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are exposed to a multitude of chemi

to individual chemicals. As a result of synergistic interactions, the cancer risk of exposure to combinations or mixtures of even low levels of individual carcinogens may be much higher than expected. Identification of consistent patterns of synergistic interactions between specific types of chemicals is, thus, of the utmost importance for informed hazard assessment and for health protection.

Co-carcinogen research

Insult	'Dominant' Key Characteristics	Tissue	Model	Reference
UV radiation	Is genotoxic Is immunosuppressive	Skin	*In vitro* and *in vivo*	Accardi 2014 (review)
Human papilloma virus	Alters DNA repair Alters cell death			
DMBA	Is electrophilic (metabolized) Is genotoxic	Ovary	Rat	Chuffa 2013
EtOH	Induces oxidative stress			
Azoxymethane	Is genotoxic	Intestine	A/J Min/+ mouse model	Hansen 2019
Persistent organic pollutants (PCBs, HCH, PBDEs, PFAA)	Is immunosuppressive Modulates receptor-mediated effects			
MNU, DEN, BBN, DHPN, DMH	Is electrophilic Is genotoxic	Multi-organ	Rat	Ishii 2017
Diphenylarsinic acid	Modulates receptor-mediated effects Induces oxidative stress			
NMU	Is electrophilic Is genotoxic	Mammary	Rat	Randi 2006
Hexachlorobenzene	Modulates receptor-mediated effects			

Co-carcinogen research plan

- Review of co-carcinogen research
- Map substances from co-carcinogen literature to Hallmark/Key Characteristic pathways
  - Expert judgment
- Highlight examples of mixtures studies that include multiple cancer pathways (e.g., genetic instability + immunosuppression)
- Identify promising combinations for study (indications of potential for synergy of pathways)
- Design combination studies to advance the science:
  - Moving beyond binary combinations (including 3 or more pathways)
  - Quantitative evaluation of joint effects based on pathway combinations

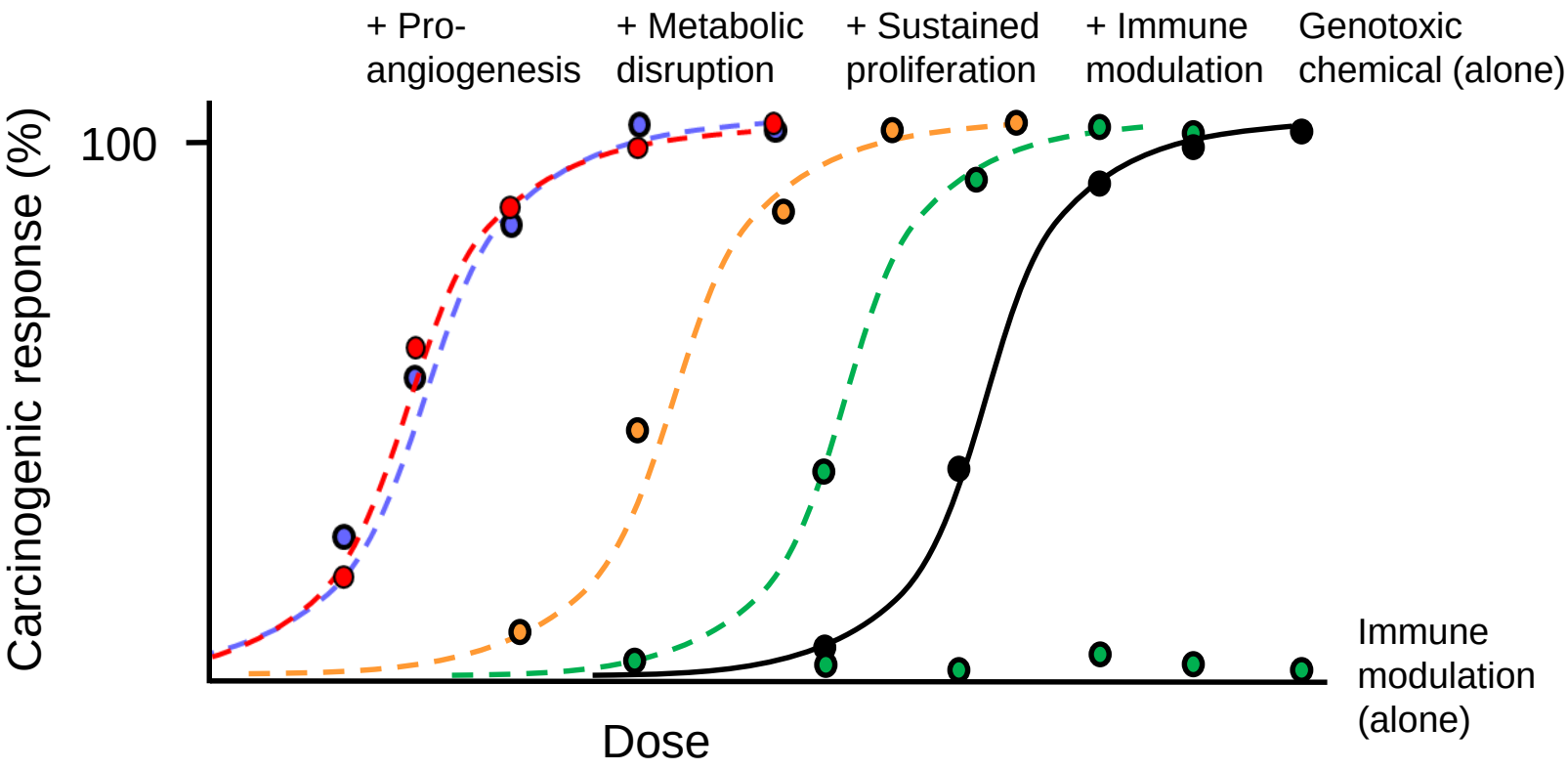
Implications for cancer risk assessment

- Or focusing on chemicals that are not carcinogens but target the Hallmarks/Key Characteristics and could contribute to cancer development jointly?
  - Outcome 1: data supports the current paradigm of not including non-carcinogenic chemicals in cumulative evaluations of carcinogens
  - Outcome 2: data indicating that non-carcinogenic chemicals contribute to carcinogenicity in some *predictable* way would imply that they should also be accounted for in a cumulative risk assessment of carcinogens

Non-carcinogen research plan

- Hypothesis: Non-carcinogenic chemicals that target Hallmark/Key Characteristic pathways can contribute to the development of cancer by creating optimal conditions (aka the perfect storm)
- Moving forward
  - Prioritize pathways for inclusion
    - Preference for “upstream” and “critical” pathways
    - Deprioritize pathways activated at later stages of cancer development
  - Screen environmental (non-carcinogenic) chemicals for pathway activation in battery of *in vitro* assays mapped to pathways
  - Evaluate single chemicals and mixtures to explore pathway interactions in complex systems (e.g., 3D tissue and animal models)

Non-carcinogen research plan



Breakout session questions

Developing a research program

- Can mixtures hypotheses be generalizable across cancer types? When should they be specific to tumor types/incidence based on ADME principles and knowledge of key events for that cancer type?

Disease-centered approach

Start with a cancer type

- Select a cancer of interest (e.g., breast cancer)
  - Ideal candidate: we know something about etiology, widespread occurrence (important public health concern)
- Identify a model that reflects the cancer type
- Identify pathways that are early stage events in the cancer type
  - For example, receptor-based pathway
- Select chemicals that target those pathways and are implicated for association with the cancer of interest (e.g., known presence in tissue)

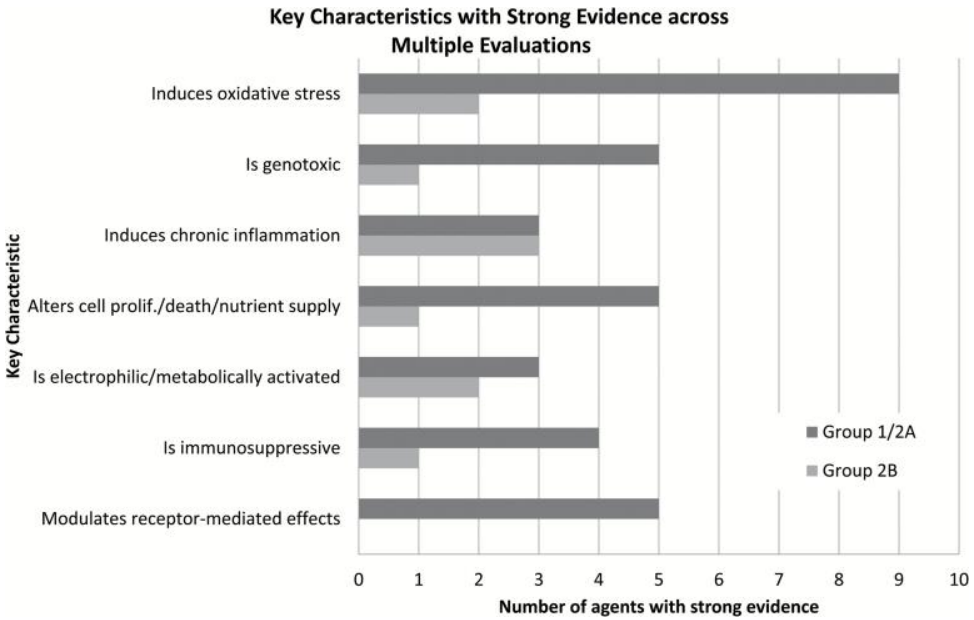
Start with model selection

- Start with the model (e.g., *ras*H2 mice)
- Identify a tissue where cancer is likely to develop in that model (e.g., lung)
- Select pathways
  - Pathway 1: Sustained proliferation/evasion of cell death (human *HRAS* transgene)
  - Pathway 2: TBD
  - Pathway 3: TBD
- Select chemicals that hit those pathways and that will reach the tissue of interest

Pathway approach

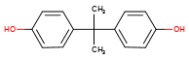
Start with pathway selection

1. Frequency distribution of combinations of Key Characteristics exhibited by carcinogens
2. Select the most common combination set
3. Select chemicals that target those pathways
4. Select a model (and cancer type) appropriate for those pathways



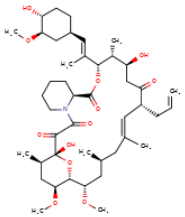
Guyton et al. 2018. Carcinogenesis. doi:10.1093/carcin/bgy031

Hypotheses via AOP



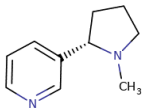
Increased proliferative signaling

ERα agonism → ↑PCNA/Ki-67 ↓p53/p21 transcription → Increased proliferation → Hyperplasia



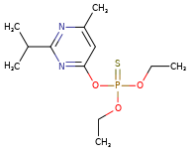
Decreased immune surveillance

Inhibition of Calcineurin → NFAT inhibition → ↓ IL-2, INF-γ expression → ↓T-cell activation/ proliferation → Immune suppression



Angiogenesis

nAChR Agonism → MAPK, PI3K/Akt, and NFκB → Endothelial cell proliferation → Capillary network formation → Pathological Angiogenesis

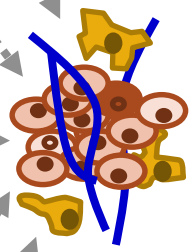


Oxidative stress

Metabolic activation → AChE Inhibition → Production of ROS → Lipid peroxidation → Oxidative damage

Decreased DNA repair capacity

unknown → ↓ expression of DNA repair genes → Increase in DNA damage



Molecular Initiating Event → **Key Event** → **Key Event** → **Key Event** → **Adverse Outcome** → **Adverse Outcome**

Challenges

- Cancer pathways (as described in the Hallmarks) are not fully understood and are definitely messy
  - Complex etiology
  - Differ depending on the cancer type
  - Are not discrete, but interdependent
- Environmental chemicals are messy
  - Often with more than one mechanism of action
  - Need to consider ADME
- Low-dose mixtures studies are notoriously difficult to interpret
- Timing is a key issue that adds complexity
  - The order of exposure matters

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Thank you!
Questions?