

# Breakout Report back

Group 3: Mixtures Toxicology



# (1) Benefits to using mechanistic data

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- We are defining “mechanistic” here as mode of action data
  - For example, with the Key Characteristics, chemicals can have different mutagenic mechanisms, but we are talking about genotoxicity as an umbrella term
- Mechanistic data can be considered for the current US EPA mixtures guidance to decide which model to use (dose addition or response addition)
- When there is no other data, mechanistic data is useful for grouping chemicals
- Can be used to develop testable hypotheses and identify possible interactions
- Can inform biomarker development
- Helpful in developing PBPK models
- Helpful in designing epidemiology studies



# (1) Challenges to using mechanistic data

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- Requires better defining the tipping point from adaptive to adverse
- Cannot use a KC generally, need specifics, in order to use for grouping chemicals (can't just say receptor-based activity, need to specify which receptor)
- Need to be able to extrapolate from *in vitro* to *in vivo*
- Need to establish that the biological endpoint is occurring at the exposure levels relevant to humans (and in combinations that are relevant)
  - Continuous versus episodic exposure, timing, duration
- Incorporating individual variability



## (2) Progression of cancer development

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### Tipping point to malignancy? Evidence?

- Is the focus here on chemical regulation or early detection/treatment of cancer?
- We need more mixtures data to answer this question
  - Is the tipping point different for a KC versus combinations of KCs
  - Start simple – looking at combinations of chemicals that have different mechanisms of mutagenicity to observe the joint action
- Hanahan and Weinberg hallmarks are specific to solid tumors, so tipping might be different for hematopoietic cancers



### Necessary or sufficient? Individually or in combo?

- We don't know which of the KCs are more critical in cancer development
- Component-based studies can help us to determine this
  - Series of binary studies combining chemicals with known mechanism of action related to target pathways



## (3) Tools and technologies

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- Programs to link NAM data to cancer data should be cancer type-specific
- Support for tiered approaches that screen in vitro and test in vivo
  - Look for patterns for grouping and chemicals that stand out with very different signatures – use to prioritize for inclusion
- Two tracks
  - Hypothesis based: develop programs to understand the joint action of pathways
  - ‘Below threshold testing’: test whole mixtures, assume safety at levels where you get no significant gene expression changes (aka impatient approach); caveat - may only be applicable for some cancer types
- Need to provide biological context on results to decision-makers (cannot just provide upstream data)



## (3) Technologies and platforms

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### *In vitro*

- Important to look for and distinguish agonism and antagonism, stimulation and suppression, in receptor-based assays



## (3) Technologies and platforms

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### -omics data (*in vitro* or *in vivo*)

- key issue is translating the signatures into modes, mechanisms and pathways
  - Need to provide context on omics data to decision-makers
  - We have been using these approaches for points of departure, not connecting to pathways
  - Currently developing case studies to get from points of departure to identifying key pathways (e.g., PFAS mixtures)
  - Mixtures is one of the first places it can be really useful – looking at not the chemical identity but biological signature (useful for sufficient similarity – identifying response patterns)
    - Link patterns to known bad actors
    - Compare mixtures responses to those with known patterns
    - Useful for grouping chemicals
  - Caveat: transcriptional changes do not always precede biological changes leading to cancer



## (4) Building scientific confidence

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- Need to build bridges between known adverse outcomes (apical) and early stage biomarkers (non-apical, key events)
  - Focus on tumors that we know about (including the surrounding milieu)
- Provide scientific basis for interpretation of assay measurements
  - We are measuring a tiny fraction of the biological response – need to explain why that measurement is relevant to the disease of interest
  - Need to make the chain of connection between the measurement and disease
  - Not all endpoints are directly related to human disease (nipple retention), but they can still provide information on an adverse pathway
- Learn from the case studies that are being developed with other endpoints (e.g., EuroMix)



## (4) Risk communication

- Need to think about risk communication to regulators and the public (two very different groups)
  - Need to involve risk communication scientists – we are not the appropriate group to tackle this
    - Honesty is key (about certainty/uncertainty)
- We can still do risk assessment (without building all the bridges between data and disease), but we will have to communicate the uncertainty – there is less confidence in the risk assessment
- For NAMs, can give a probability that a chemical is a carcinogen, but not a probability that you will get cancer
  - Need to consider all the other factors (e.g., disease state, genetic background, other exposures)
  - Difficult for us to communicate to the public before the risk assessor community has confidence in NAMs
- We are dealing with population effects with cancer
- Communicate windows of increased susceptibility



## (5) What should we be studying?

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- Both (carcinogen and non-carcinogen) approaches are important – should not be either/or
- Focus on the biology of the particular tumor you are investigating – we do not yet have enough information to pursue a generalized approach
- Carcinogen needs to be defined, however, the definition depends on the context of the experiment
  - In a DMBA/TPA study, both are carcinogens
- CFSAN perspective - lesson from Delaney act - labeling things with overly rigid definitions is not science



## (5) What should we be studying?

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### Non-carcinogens (pros and cons)

- Difficult to find chemicals that target the hallmarks and are non-carcinogens
- Does not matter whether or not chemicals are classified as carcinogens or non-carcinogens
- Body of literature on co-carcinogens indicate that below thresholds with different target organs and different mechanisms, you typically get response addition



## (6) How should we be studying joint action?

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### Disease-centered approach considerations

- Developing projects specific to tissue and cancer type was considered to be very important! (from there, we could secondarily employ a pathway-based approach)
- Recommended cancer type(s) for study:
  - Most prevalent
  - Most deadly
  - Most well-understood
  - Good animal model
  - Good database
  - Some discussion of cancer clusters to identify risk factors
- Recommendation for one disease-centered, hypothesis-based approach and one more agnostic (combining chemicals with different mechanisms)



## (6) How should we be studying joint action?

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### Pathway-driven approach considerations

- Priority pathway combinations:
  - Could be non-specific in terms of cancer
    - Caution for the pathway approach is that the functional genetic mutations are different in different cancers so there is caution in looking at one pathway in different cancers