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Problem Formulation: Lessons and Tools from Practical Applications Involving Systematic Review of Mechanistic Data

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Disclosure

Travel to meeting supported by the NAS

Practitioner of systematic review (SR) and evidence-based methods applied in the fields of toxicology and risk assessment (including chemicals, food ingredients/ contaminants, etc.)

Vice-Chair, Science Advisory Committee, Evidence Based Toxicology Collaboration (http://www.ebtox.org/)

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Associate Editor (Systematic Review), Toxicological Sciences; active peer reviewer of SR for other journals

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Objectives

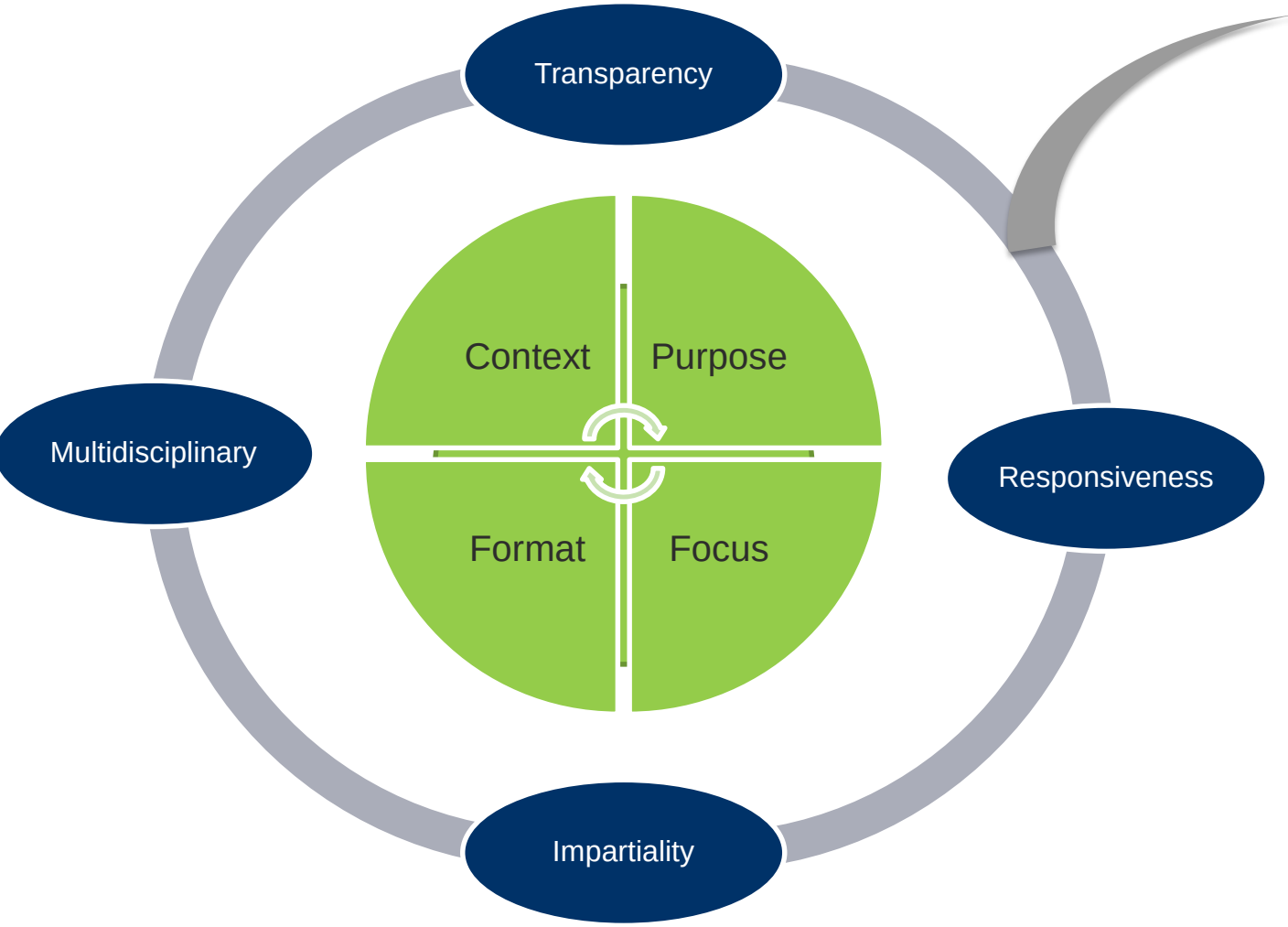
1. Highlight the complexities of problem formulation as it relates to use of mechanistic data in risk assessment facilitated by systematic review
2. Provide an appreciation of the challenges encountered during the practice of systematically reviewing mechanistic data (and potential solutions that can be addressed during problem formulation)

Common Components of Systematic Review

Problem Formulation	• Scoping, scientific needs/objectives, feasibility • Develop PECO question/statement and context
Protocol Development	• Determine methods for selecting, appraising, and evaluating evidence • Document methods (*a priori*)
Identify Evidence Base	• Implement search strategy (syntax, databases, etc.) • Screen and select studies via inclusion/exclusion criteria
Individual Study Assessment	• Extract data • Critical appraisal for risk of bias (internal validity) and possibly other elements of study quality/relevance
Body of Evidence Assessment	• Qualitative synthesis and integration - includes assessment of confidence (consistency, magnitude, dose-response, etc.) • Quantitative synthesis and integration (e.g., meta-analyses, meta-regression)
Reporting	• Comprehensive documentation of approach, findings, and conclusions in a public forum

Wikoff DS, Miller GW. 2018. Systematic reviews in toxicology. Toxicol Sci 163(2):335–337.

Problem Formulation Framework



1. PECO (and rationale)

2. Guidance for protocol

A.A. Rooney, P. Whaley; E. Aiassa; N. Randall; B. Beverly; D. Wikoff. A Problem Formulation Framework to Support Optimal Use of Scientific Resources in Conducting Chemical Risk Assessments. In Preparation

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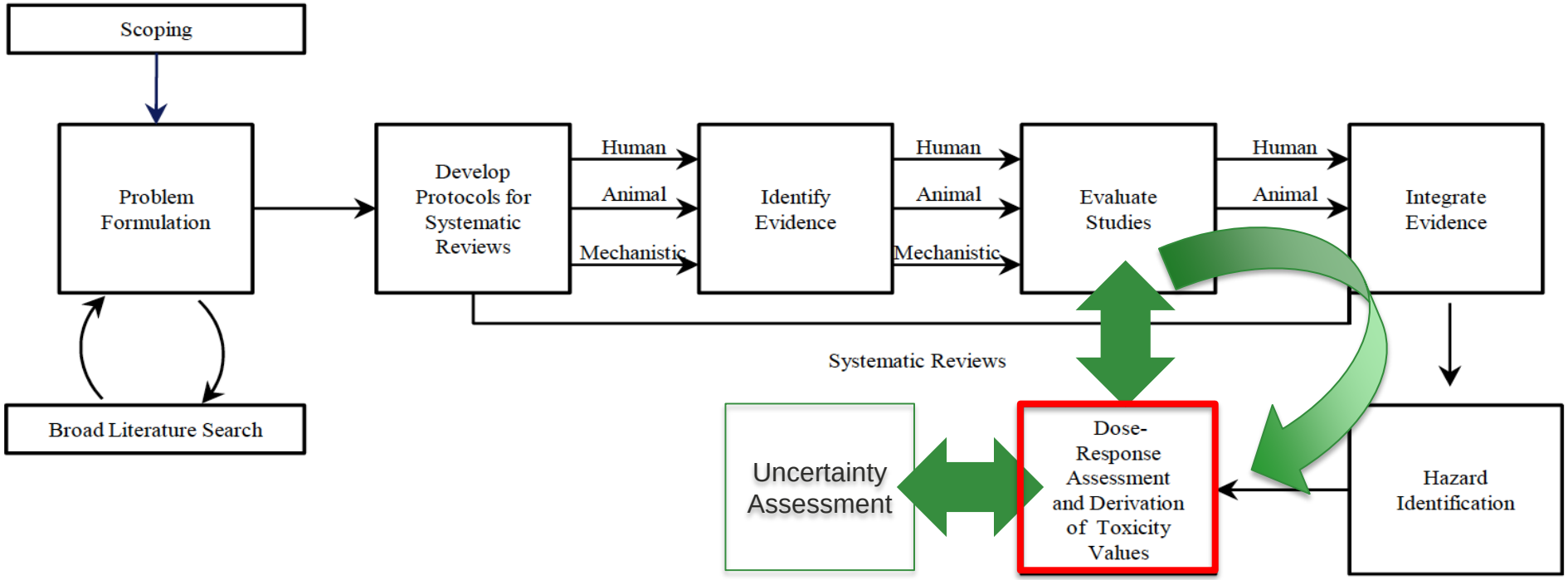
Problem Formulation in Risk Assessment (*Facilitated by Systematic Review*)

Figure S-1. Systematic Review in the context of the USEPA IRIS process. Adapted from NRC (2014)



Problem formulation ultimately relates to the derivation of toxicity values

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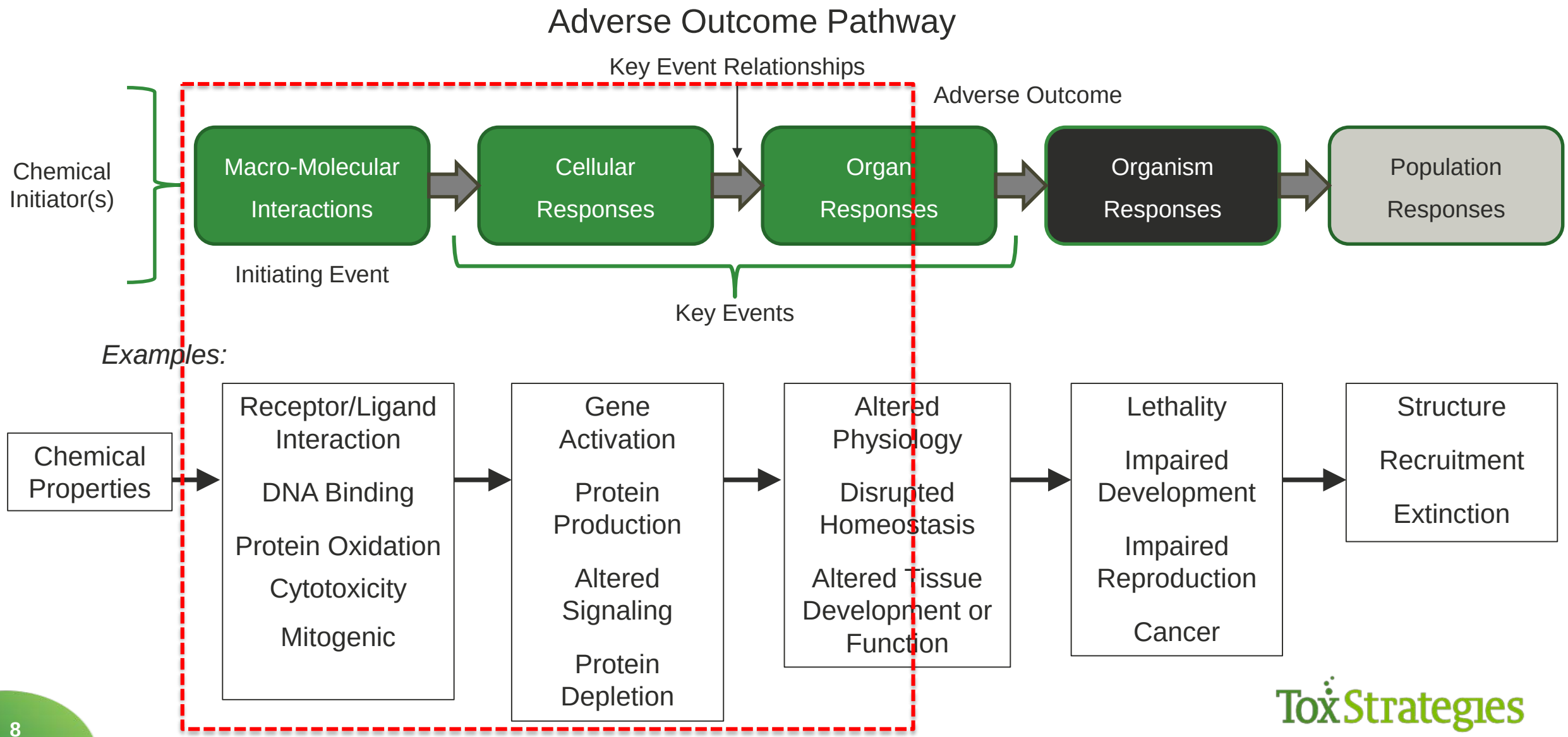
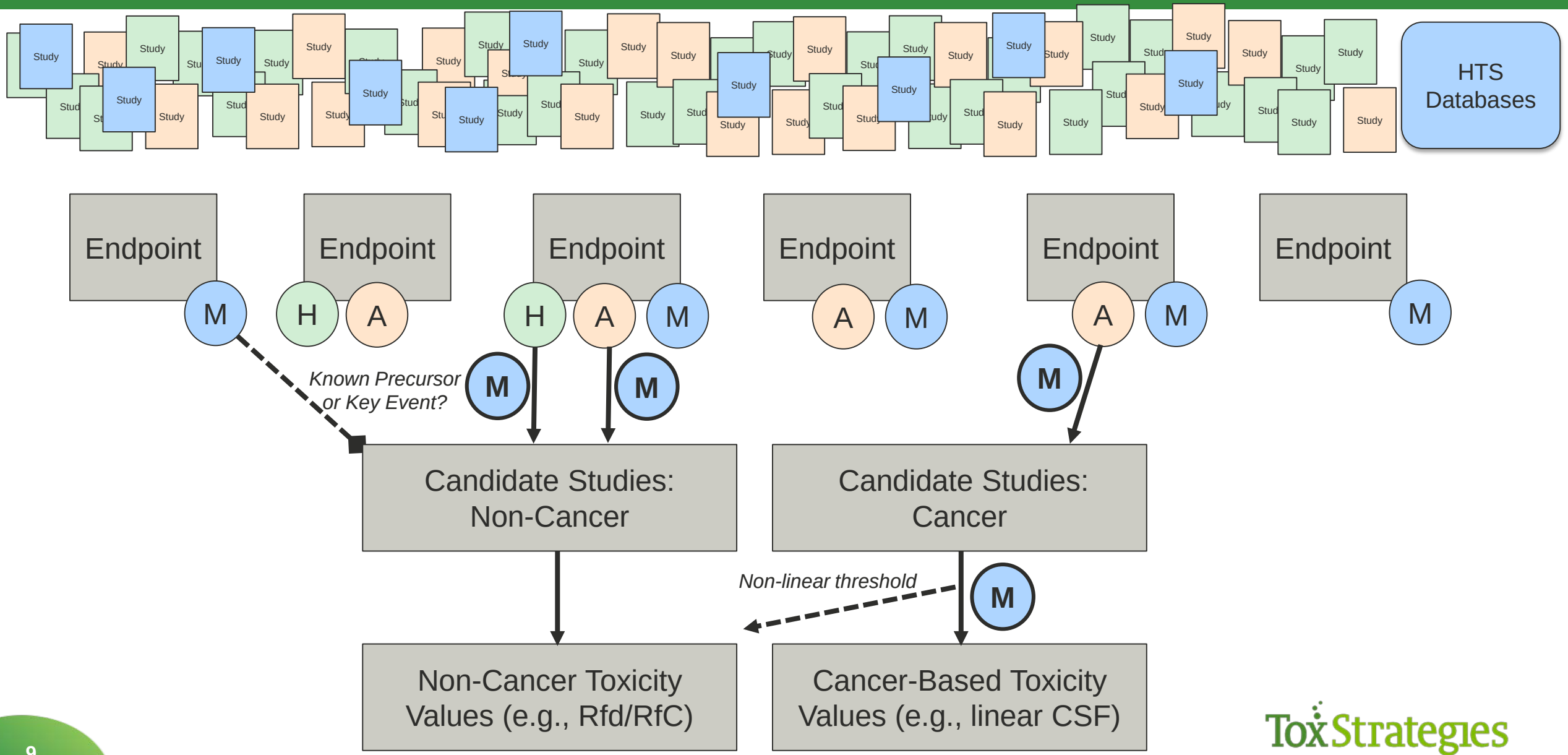
Why is problem formulation (often) challenging for mechanistic data?

What is Mechanistic Data?

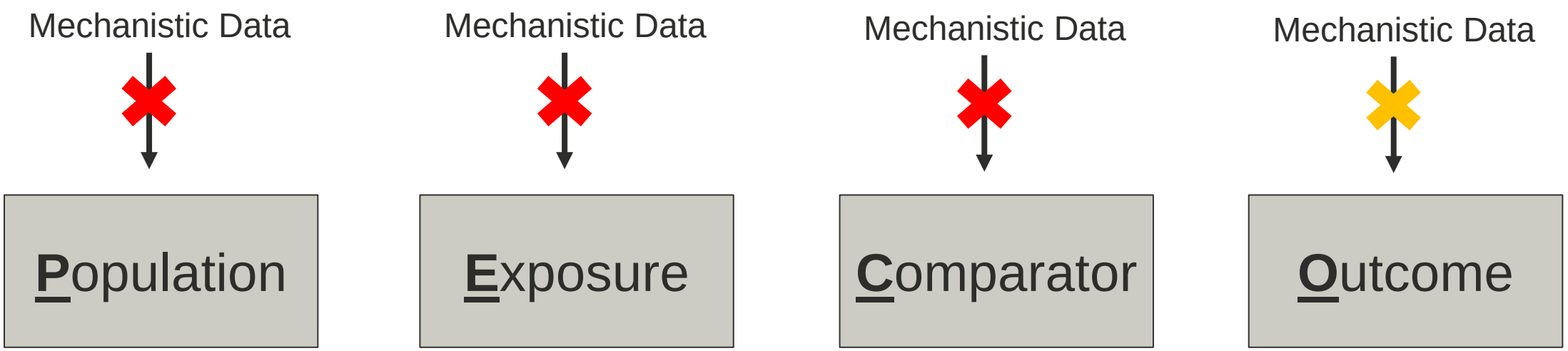


Use of Mechanistic Data in Risk Assessment



Mechanistic Data (Often) has Contextual Application to a Standard PECO

If it is assumed that the standard PECO involves the potential for an adverse effect (outcome) in humans…



Example: Characterize possible inter- or intra-species differences

Example: Assess impact of dose relevance (i.e., exposure relevance) on key events or outcomes

Example: Characterize initiating or key events in a pathway leading to an adverse outcome (e.g., concordance, plausibility)

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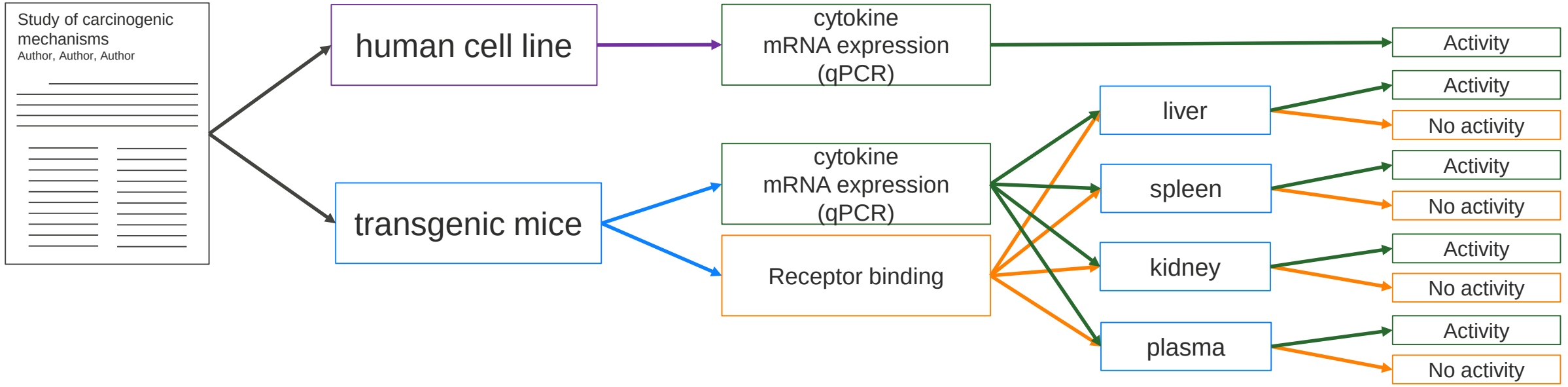
Mechanistic Data are Complex



1 endpoint measured in 1 cell line & 4 tissues + 1 endpoint measured in 4 tissues

=

9 total data points: 5 with activity, 4 without activity

1 Study **2 models**

Requires expertise, training, resources, established workflow, flexible tools, etc.

Difficult to plan a priori

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Evidence Identification Can be Challenging (*which impacts problem formulation*)

"Induces chronic inflammation"
Acute v. chronic?
In vivo v. vitro?

"Modulates receptor-mediated effects"
Any receptor?
Directionality?

Agent 1-1			Agent 1-2			Agent 1-3			Agent 1-4		
curated	built-in v1.2	built-in v1.21	curated	built-in v1.2	built-in v1.21	curated	built-in v1.2	built-in v1.21	curated	built-in v1.2	built-in v1.21
264	117	117	348	155	155	149	31	31	21	15	15
444	241	365	391	341	433	223	156	193	25	14	28
41	36	231	47	46	299	16	23	139	2	3	22
17	408	408	58	255	255	20	198	198	1	4	4
178	122	6169	74	51	262	191	160	804	3	2	18
329	10	40	113	12	33	135	10	26	7	0	0
247	208	597	94	83	205	144	80	268	5	1	6
99	12	69	179	36	161	72	16	64	1	0	0
85	2	2	173	25	25	104	5	5	2	0	0
239	174	428	217	196	263	172	138	261	2	3	8
1359	1178	6390	973	1079	1097	837	667	1443	42	35	45

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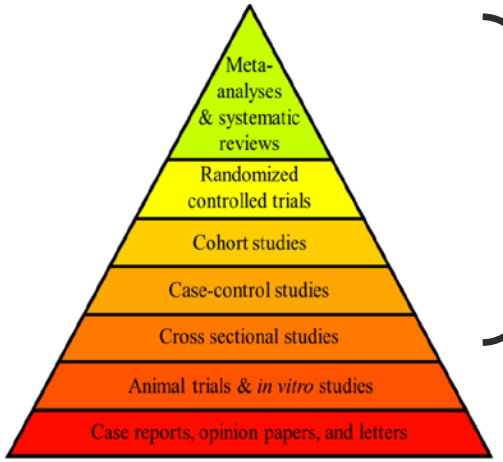
Best Practices Evolving for Systematic Review

Mechanistic data can be (is) from many study types, designs, models, etc.

Less "fit for purpose" in context of use or refining existing SR tools



Best Practices *Generally* Established for Human Data

Best Practices Still In Development for Experimental Animal Data

Different questions (e.g., comparative effectiveness of an intervention vs characterization of hazard and risk)

- Need flexibility in application
- Need tools tailored to these types of evidence

Difficult to guide operationalization in the protocol

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Challenges During Practice (*and Solutions During Problem Formulation*)

Example: Selection, Appraisal, and Integration for Specific Purpose

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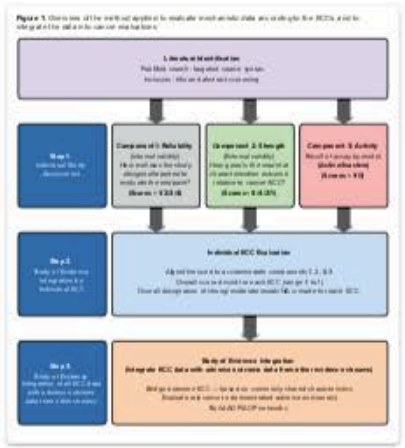
Systematic Evaluation and Integration of Data Relevant to Mechanisms of Carcinogenesis: Practical Experience in the Identification, Assessment, and Integration of Mechanistic Evidence in Human Health Assessments

G.A. Chappell, S.J. Borghoff, S.H. Fitch, C.L. Doepker, D. Wikoff



Background

- Mechanistic data provide valuable information regarding characterization of carcinogenicity
- The evaluation and integration of mechanistic data includes complexities:
  - heterogeneity of data types, collection, and reporting
  - understanding the human relevance of endpoints measured in animal and/or cell-based models, or even in silico approaches
  - potentially large evidence base with many endpoints to integrate
- Wikoff et al., (2018) recently published a framework which builds on the key characteristics of carcinogen (KCC) approach by **quantitatively integrating mechanistic** data in a reproducible manner using elements important to risk assessment (e.g., quality, relevance, weight-of-evidence). The output includes an overall conclusion regarding KCC activity as it relates to carcinogenic responses, as well as identification of data gaps and prioritized research needs.

Objective

Apply a systematic approach for the identification, assessment, and integration of mechanistic evidence in human health assessments of carcinogenicity

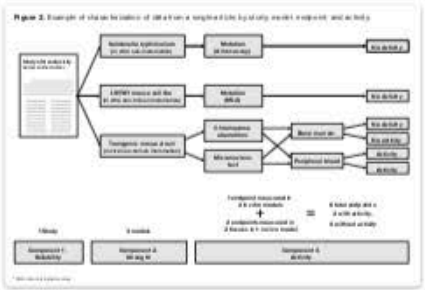
Methodology

Literature Identification

- The PubMed database was searched using syntax that included the agent name(s) in addition to terms relating to the ten KCCs (**Table 1**).
- Titles and abstracts from all articles returned via the searches were screened using DistillerSR (Evidence Partners, Ottawa, Canada), and were either included or excluded based on the following criteria (**Table 2**).
- High-throughput screening (HTS) data available through the ToxCast/Tox21 databases were extracted from the U.S. Environmental Protection Agency (U.S. EPA) Summary Files database (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data; invitrodb_v2; which was released October 2015 and remains the most up-to-date publicly available version; accessed October 2017 for this study).

Individual Study Assessment (Step 1 of Framework)

- The full text of all included articles were reviewed, and mechanistic data were evaluated and scored for reliability, strength, and activity (**Figure 1**) per Wikoff et al., (2018).
  - Reliability (i.e. study quality)
    - Reliability was scored on a scale of 1 to 4 (1=reliable without restriction; 2=reliable with restriction; 3=not reliable; 4=not assignable).
  - Strength
    - This ranking represents the confidence in the predictive capability of the model to predict activity of the KCC in humans.
    - The strength of the model was scored highest for human in vivo studies (either epidemiological or controlled exposure; score=6), followed by in vivo mammalian animal models (score=4), in vitro assays in either human or non-human mammalian cells (score=2), and non-mammalian systems (either in vitro or in vivo) received the lowest score (score=1).
  - Activity
    - For each endpoint, activity scores of 0 or 1 were assigned to indicate a response relative to control or background respectively (**Figure 2**).
- ***Across all KCCs, collectively >150 endpoints were evaluated and scored.***



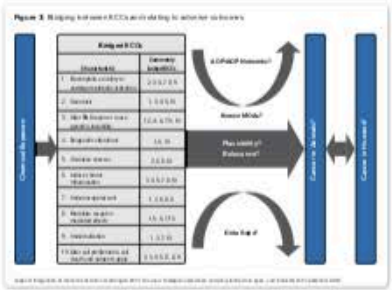
Body of Evidence Integration: Individual KCCs (Step 2 of Framework)

- These elements were then quantitively integrated to provide a weighted score for each KCC, which facilitates the assessment of the body of evidence in the context of observed tumorigenic responses in animals and humans.

- The integrated scores for each KCC are then translated into categorical designations for overall activity of each KCC as related to the agent. This designation is a relative measure that is used to inform the basis of an overall characterization score of activity for the KCC (i.e., not carcinogenic potential) along with expert judgement (which involves consideration of variables such as model, dose, cytotoxicity, etc.) using the criteria in **Table 3**.

Body of Evidence Integration — KCC Data and Other Evidence Streams (Step 3 of Framework)

- Bridging between KCCs (**Figure 3**)
  - The KCCs are recognized to occur as a set of (commonly linked) events, and are, thus, considered in relation to each other (Smith et al., 2016).
- Anchoring to adverse outcome
  - KCC data are also then considered relative to any carcinogenic outcomes observed in experimental animals and humans, which allows for the mechanistic KCC to be evaluated in the context of the biological plausibility of a link with observed or potential adverse outcomes (or key events) related to carcinogenicity.



Results

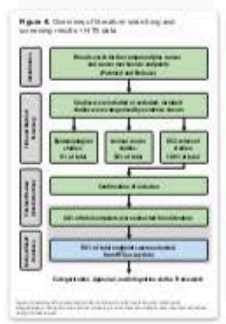
Categorization of all articles returned via literature searching was conducted based on review of the titles and abstracts. KCC-relevant data were extracted and scored from the literature sources, and data available in the ToxCast/Tox21 screening program was also extracted and incorporated in the review. All extracted data were then scored for reliability, strength, and activity. (see **Figure 4**)

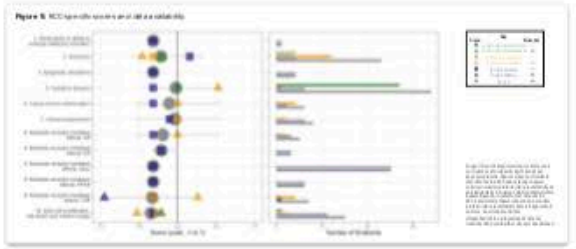
Integration of evidence per KCC

- Overall, nearly all of the integrated scores for each KCC were indicative of a lack of activity, particularly when accounting for study reliability and strength of the model tested.
  - When model types were evaluated individually, the integrated data for some specific model types demonstrated activity (e.g., non-human mammalian in vivo data for KCC #5; see **Figure 5**).
  - Substantial variation was observed in the number of endpoints per KCC.
  - Strength of evidence for activity ranged from "no activity" to "weak evidence of activity" across the KCCs.

Integration of evidence for KCCs with other data streams

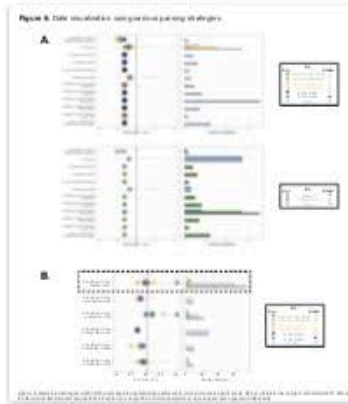
- When the KCC-related mechanistic data were integrated with other available data streams related to cancer outcomes, the agent is unlikely to pose a carcinogenic risk to humans (see **Table 4**).





Overall Findings

- The methodology, process, and results of this assessment represent a case study of practical experience with the implementation of a systematic review of mechanistic evidence into human health assessments.
- In addition to presenting how the KCC data can be systematically integrated with other evidence streams, this case study demonstrates that systematic evaluation of mechanistic data may include substantial complexity, and that consistent evaluation of such data requires a clearly defined process executed by a skilled multidisciplinary team.
- Evaluation of mechanistic data related to the KCC, together with consideration of evidence from experimental animal cancer studies, can be valuable in cases of limited epidemiological data.
- The expertise of the screener impacts which studies will be included in the evidence base and how such will be appraised.
- The Wikoff et al., framework provides a feasible and flexible solution for assessing and integrating mechanistic data in systematic assessments.
  - Rapid incorporation of all data provides a quick overview of activity per KCC.
  - Data visualizations are helpful in facilitating the evidence to decision process.
    - Data source (e.g., literature vs. HTS data) may be especially important for data-poor agents (**Figure 6A**)
    - Using KCC sub-categories can provide additional information (**Figure 6B**)



Conclusion

The Wikoff et al., framework provides a feasible and flexible solution for assessing and integrating mechanistic data in systematic assessments of potential carcinogenicity

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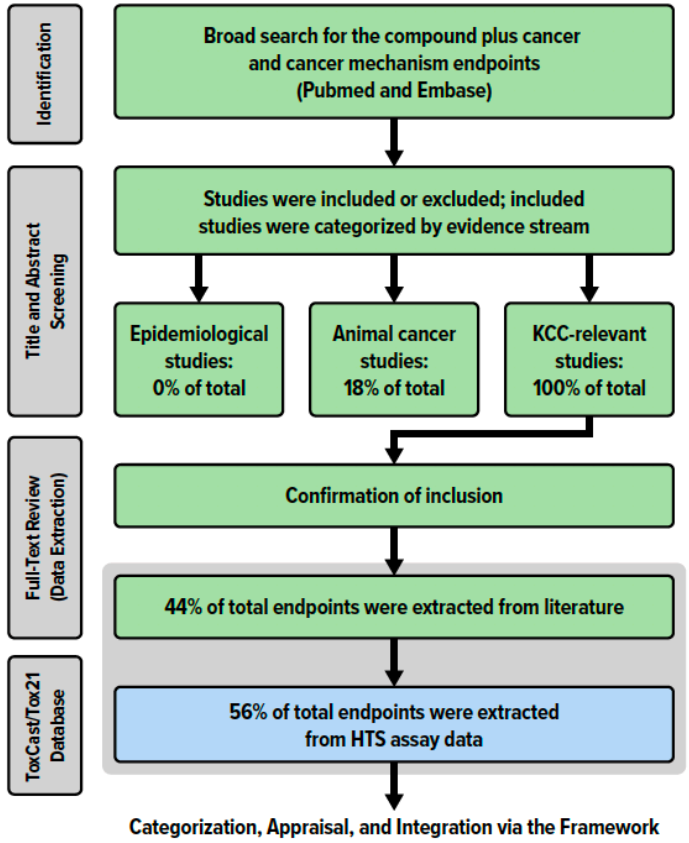
Challenges in Identification, Selection, and Extraction of Mechanistic Data

Figure 4. Overview of literature searching and screening results + HTS data.



Legend: Summary of the number of articles returned via initial searching and subsequent categorizations during title and abstract screening, and number of endpoints based on data extraction during full text review.

Potential impact on problem formulation:

Are there "mapped" mechanisms or assays to the endpoint of interest?

Which types of data are relevant for inclusion?

What level of scoping, piloting, and refinement may be needed?

Do we have appropriate expertise?

Do we have appropriate tools to facilitate an accurate review?

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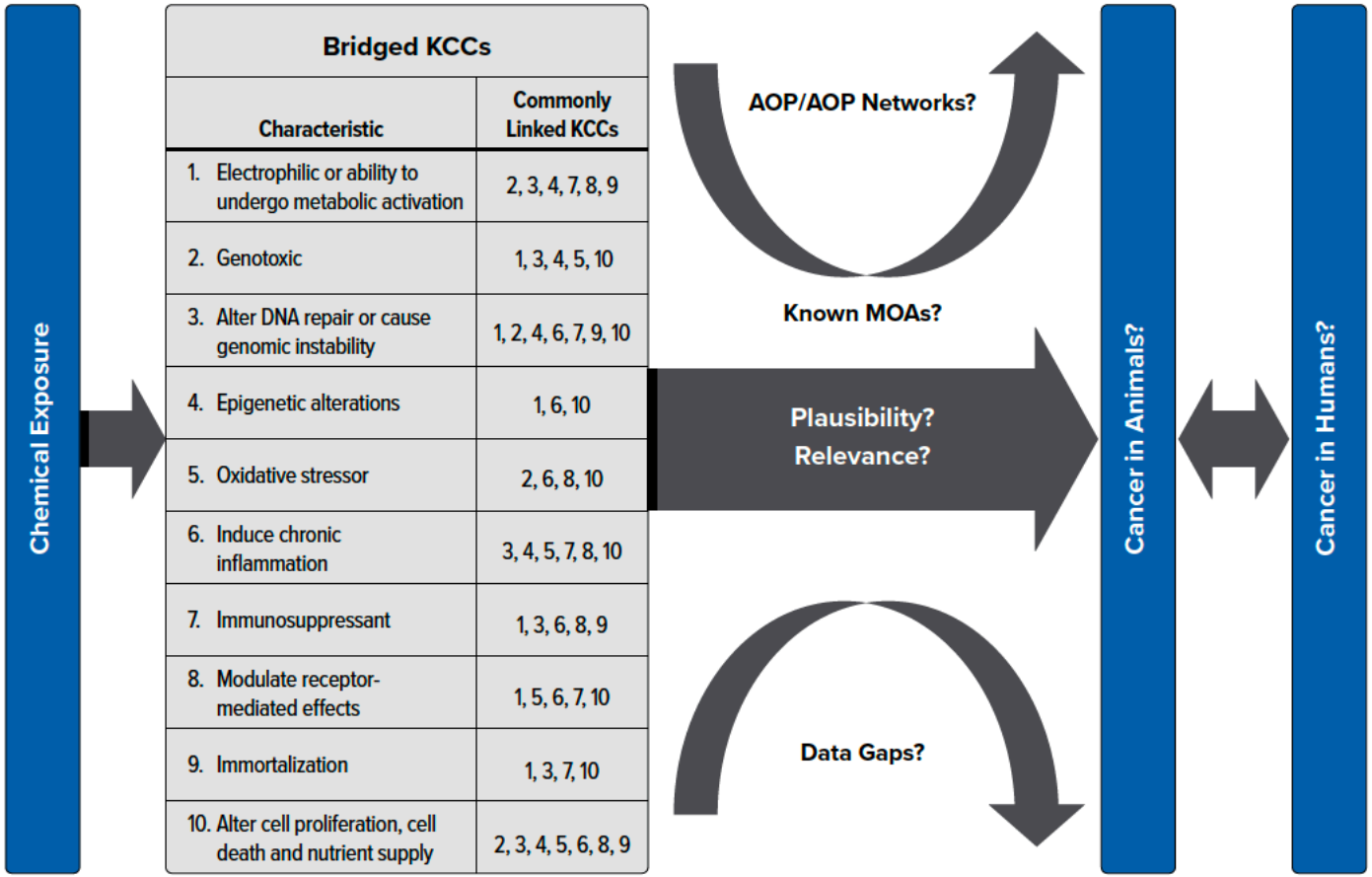
Challenges with Integration



Potential impact on problem formulation:

Are integration approaches available?

Will the a-priori decisions provided for collection and evaluation of all necessary information?

What kind of experts will we need?

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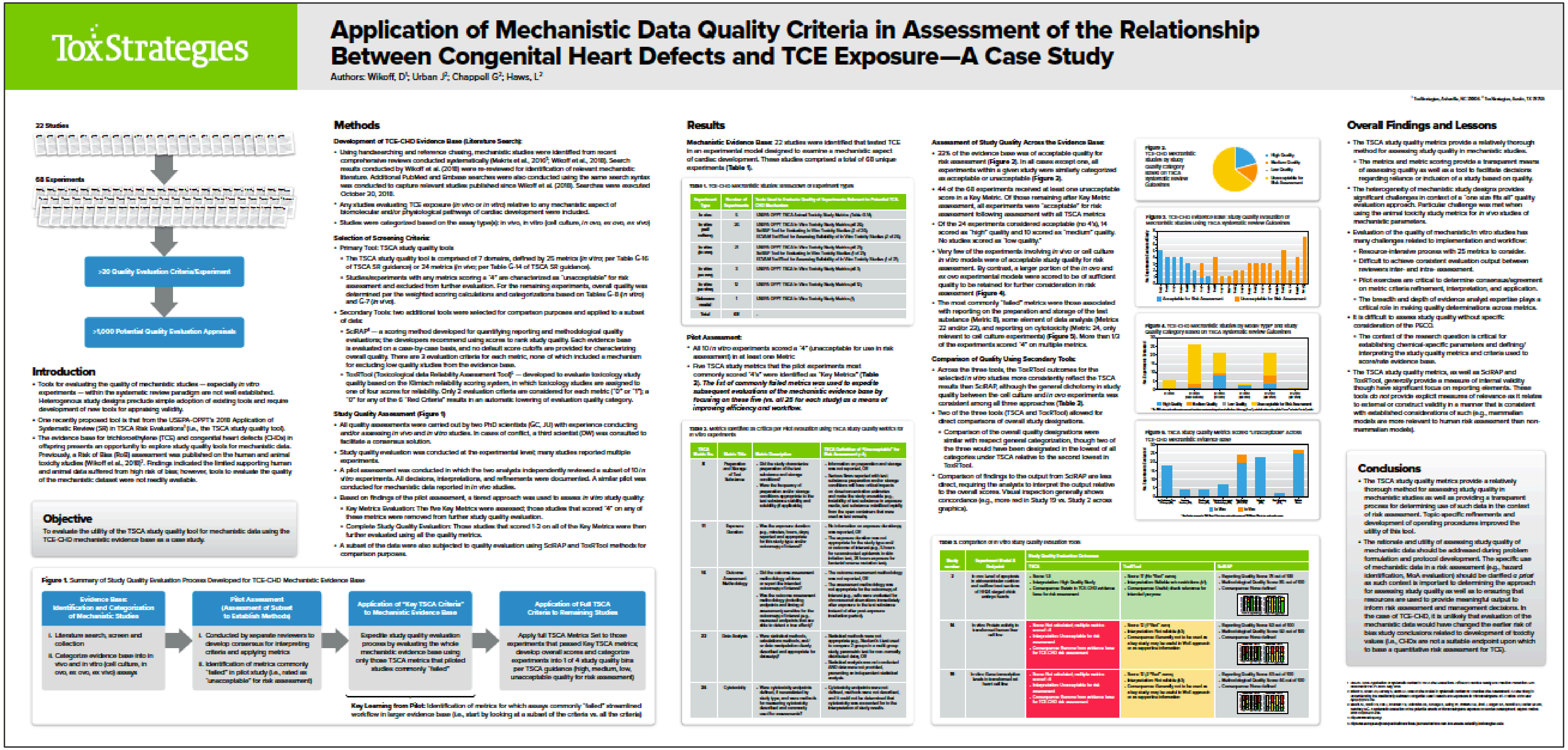
Example: Critical Appraisal of Mechanistic Data



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Challenges in Identification, Selection, and Appraisal

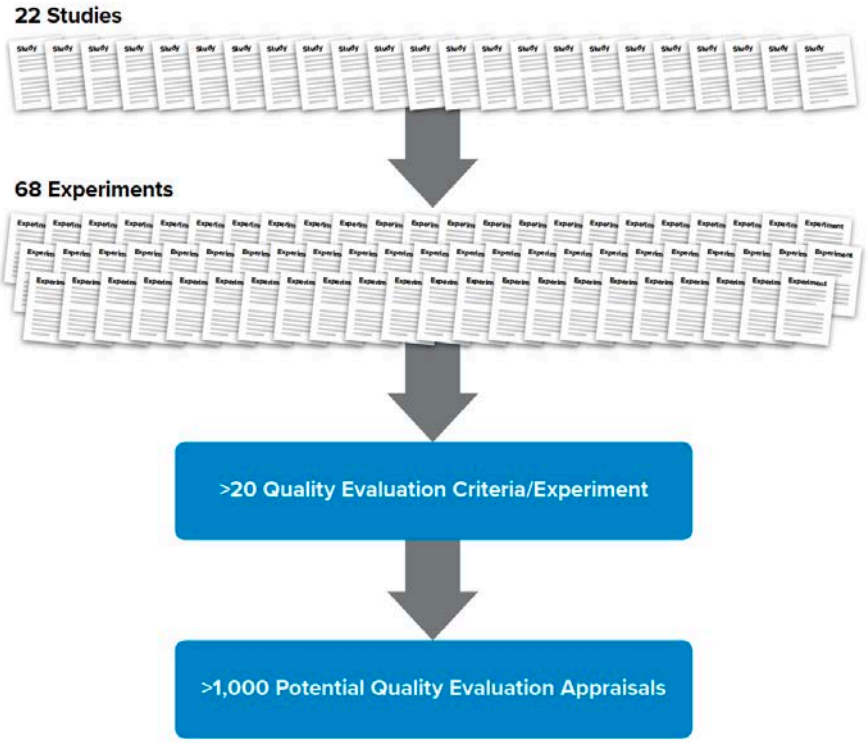

Table 1. TCE-CHD Mechanistic Studies: Breakdown of Experiment Types

Experiment Type	Number of Experiments	Tools Used to Evaluate Quality of Experiments Relevant to Potential TCE-CHD Mechanism
In vivo	5	USEPA OPPT TSCA Animal Toxicity Study Metrics (Table G-14)
In vitro (cell culture)	26	USEPA OPPT TSCA In Vitro Toxicity Study Metrics (all 26); SciRAP Tool for Evaluating In Vitro Toxicity Studies (2 of 26); ECVAM ToxRTool for Assessing Reliability of In Vitro Toxicity Studies (2 of 26)
In vitro (in ovo)	21	USEPA OPPT TSCA In Vitro Toxicity Study Metrics (all 21); SciRAP Tool for Evaluating In Vitro Toxicity Studies (1 of 21); ECVAM ToxRTool for Assessing Reliability of In Vitro Toxicity Studies (1 of 21)
In vitro (ex ovo)	3	USEPA OPPT TSCA In Vitro Toxicity Study Metrics (all 3)
In vitro (ex vivo)	12	USEPA OPPT TSCA In Vitro Toxicity Study Metrics (all 12)
Unknown model	1	USEPA OPPT TSCA In Vitro Toxicity Study Metrics (1)
Total	68	-

Potential impact on problem formulation:

How do we identify which data are relevant to the endpoint? Or to risk assessment? Do we have the time or resources to conduct a given level of appraisal? Do we have the expertise?

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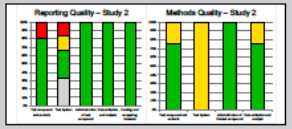
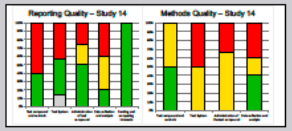
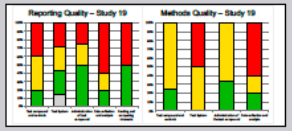
Challenges in Critical Appraisal

Study number	Experiment Model & Endpoint	Study Quality Evaluation Outcomes		
		TSCA	ToxRTool	SciRAP
2	In ovo: Level of apoptosis in atrioventricular cushion and outflow tract sections of HH24 staged chick embryo hearts	• Score: 1.3 • Interpretation: High Quality Study • Consequence: Retain in TCE-CHD evidence base for risk assessment	• Score: 17 (No "Red" zeros) • Interpretation: Reliable w/o restrictions (k1) • Consequence: Useful; check relevance for intended purpose	• Reporting Quality Score: 78 out of 100 • Methodological Quality Score: 85 out of 100 • Consequence: None defined 
14	In vitro: Protein activity in transformed human liver cell line	• Score: Not calculated; multiple metrics scored =4 • Interpretation: Unacceptable for risk assessment • Consequence: Remove from evidence base for TCE-CHD risk assessment	• Score: 12 (1 "Red" zero) • Interpretation: Not reliable (k3) • Consequence: Generally not to be used as a key study; may be useful in WoE approach or as supportive information	• Reporting Quality Score: 50 out of 100 • Methodological Quality Score: 50 out of 100 • Consequence: None defined 
19	In vitro: Gene transcription levels in transformed rat heart cell line	• Score: Not calculated; multiple metrics scored =4 • Interpretation: Unacceptable for risk assessment • Consequence: Remove from evidence base for TCE-CHD risk assessment	• Score: 12 (2 "Red" zeros) • Interpretation: Not reliable (k3) • Consequence: Generally not to be used as a key study; may be useful in WoE approach or as supportive information	• Reporting Quality Score: 43 out of 100 • Methodological Quality Score: 46 out of 100 • Consequence: None defined 

Potential impacts on problem formulation:

What will we do with the appraisal output? How will we consider "quality" of mechanistic in the risk assessment? How will appraisal compliment relevance?

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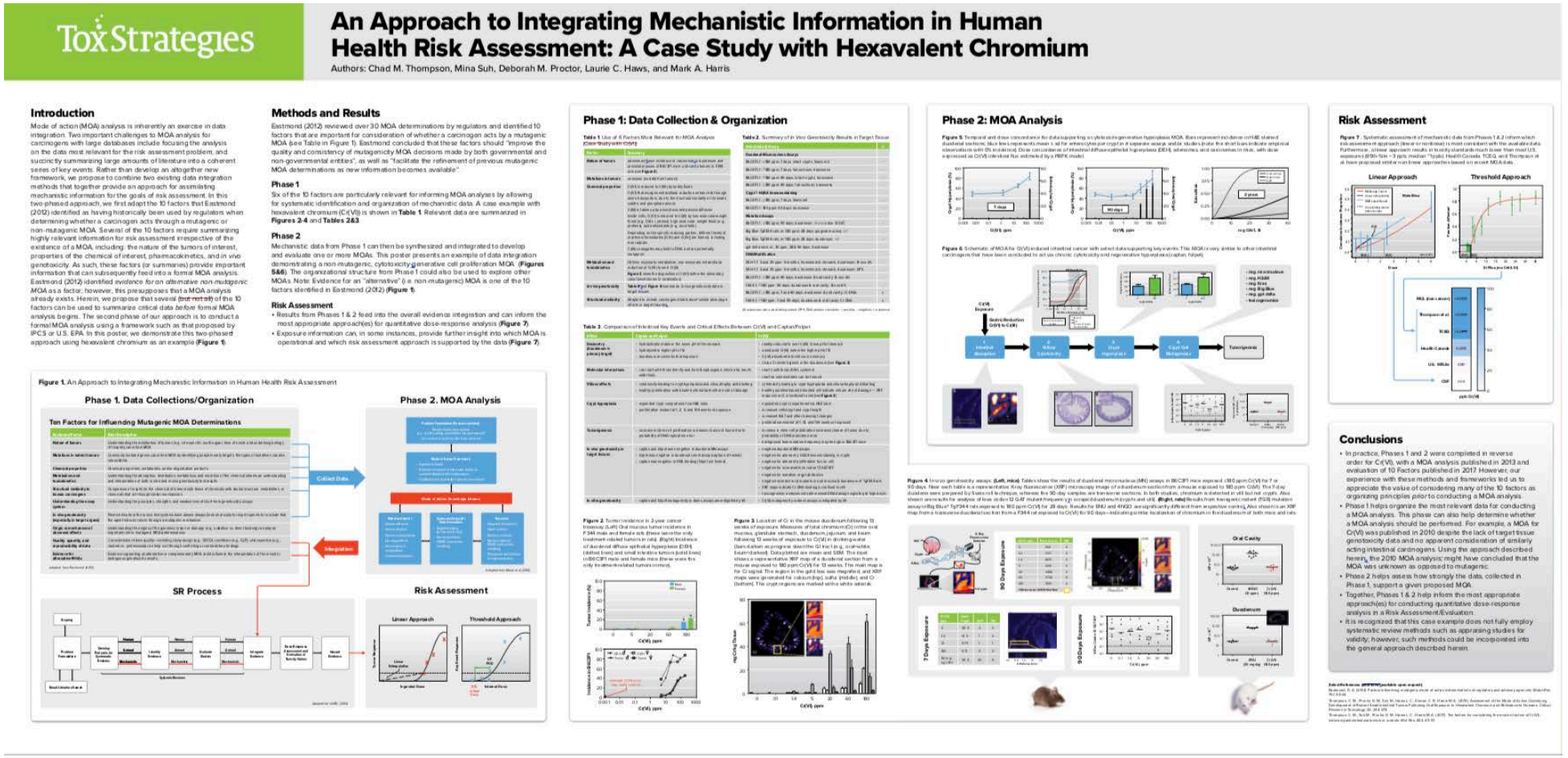
Example: Structured Evaluation of Mechanistic Data in MoA to Support Risk Assessment



An Approach to Integrating Mechanistic Information in Human Health Risk Assessment: A Case Study with Hexavalent Chromium

Authors: Chad M. Thompson, Mina Suh, Deborah M. Proctor, Laurie C. Haws, and Mark A. Harris

Introduction

Mode of action (MOA) analysis is inherently an exercise in data integration. Two important challenges to MOA analysis for carcinogens with large databases include focusing the analysis on the data most relevant for the risk assessment problem, and succinctly summarizing large amounts of literature into a coherent series of key events. Rather than develop an altogether new framework, we propose to combine two existing data integration methods that together provide an approach for assimilating mechanistic information for the goals of risk assessment. In this two-phased approach, we first adapt the 10 factors that Eastmond (2012) identified as having historically been used by regulators when determining whether a carcinogen acts through a mutagenic or non-mutagenic MOA. Several of the 10 factors require summarizing highly relevant information for risk assessment irrespective of the existence of a MOA, including: the nature of the tumors of interest, properties of the chemical of interest, pharmacokinetics, and in vivo genotoxicity. As such, these factors (or summaries) provide important information that can subsequently feed into a formal MOA analysis. Eastmond (2012) identified evidence for an alternative non-mutagenic MOA as a factor; however, this presupposes that a MOA analysis already exists. Herein, we propose that several (but not all) of the 10 factors can be used to summarize critical data *before* formal MOA analysis begins. The second phase of our approach is to conduct a formal MOA analysis using a framework such as that proposed by IPCS or U.S. EPA. In this poster, we demonstrate this two-phased approach using hexavalent chromium as an example (**Figure 1**).

Methods and Results

Eastmond (2012) reviewed over 30 MOA determinations by regulators and identified 10 factors that are important for consideration of whether a carcinogen acts by a mutagenic MOA (see Table in Figure 1). Eastmond concluded that these factors should "improve the quality and consistency of mutagenicity MOA decisions made by both governmental and non-governmental entities", as well as "facilitate the refinement of previous mutagenic MOA determinations as new information becomes available."

Phase 1

Six of the 10 factors are particularly relevant for informing MOA analyses by allowing for systematic identification and organization of mechanistic data. A case example with hexavalent chromium (Cr(VI)) is shown in **Table 1**. Relevant data are summarized in **Figures 2-4** and **Tables 2&3**.

Phase 2

Mechanistic data from Phase 1 can then be synthesized and integrated to develop and evaluate one or more MOAs. This poster presents an example of data integration demonstrating a non-mutagenic, cytotoxicity-generative cell proliferation MOA (**Figures 5&6**). The organizational structure from Phase 1 could also be used to explore other MOAs. Note: Evidence for an "alternative" (i.e. non-mutagenic) MOA is one of the 10 factors identified in Eastmond (2012) (**Figure 1**).

Risk Assessment

- Results from Phases 1 & 2 feed into the overall evidence integration and can inform the most appropriate approach(es) for quantitative dose-response analysis (**Figure 7**).
- Exposure information can, in some instances, provide further insight into which MOA is operational and which risk assessment approach is supported by the data (**Figure 7**).

Phase 1: Data Collection & Organization

Phase 2: MOA Analysis

Risk Assessment

Conclusions

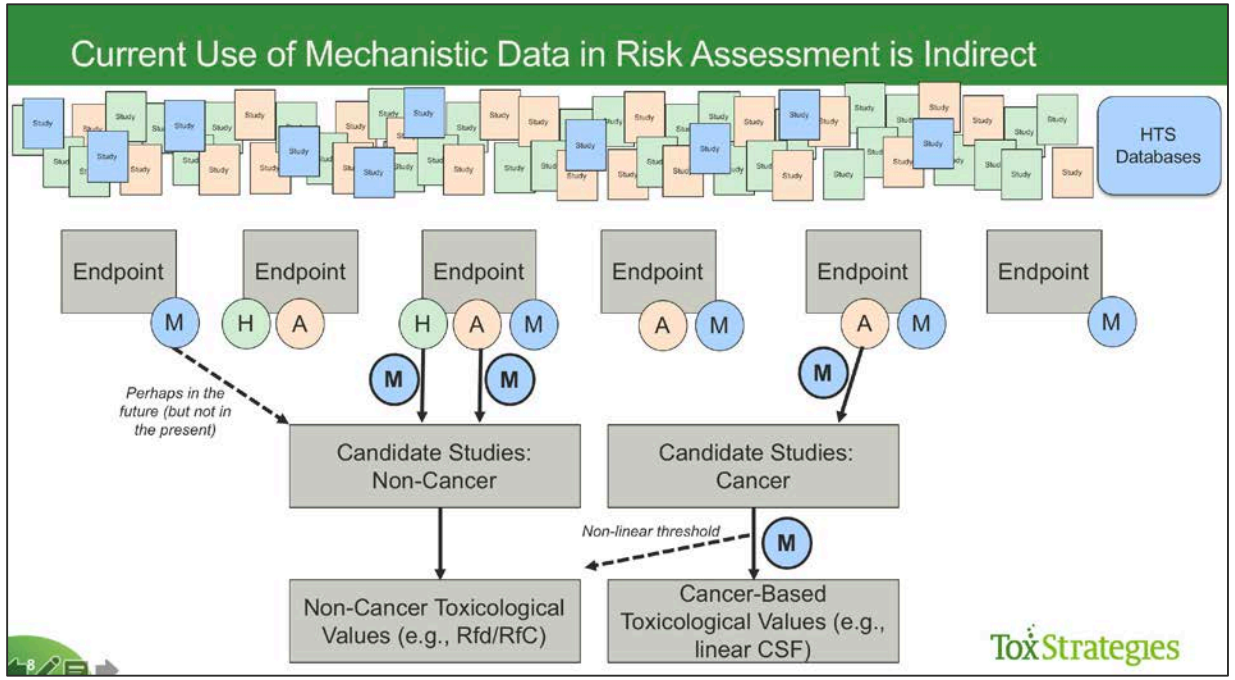
- In practice, Phases 1 and 2 were completed in reverse order for Cr(VI), with a MOA analysis published in 2013 and evaluation of 10 Factors published in 2017. However, our experience with these methods and frameworks led us to appreciate the value of considering many of the 10 factors as organizing principles prior to conducting a MOA analysis.
- Phase 1 helps organize the most relevant data for conducting a MOA analysis. This phase can also help determine whether a MOA analysis should be performed. For example, a MOA for Cr(VI) was published in 2010 despite the lack of target tissue genotoxicity data and no apparent consideration of similarly acting intestinal carcinogens. Using the approach described herein, the 2010 MOA analysis might have concluded that the MOA was unknown as opposed to mutagenic.
- Phase 2 helps assess how strongly the data, collected in Phase 1, support a given proposed MOA.
- Together, Phases 1 & 2 help inform the most appropriate approach(es) for conducting quantitative dose-response analysis in a Risk Assessment/Evaluation.
- It is recognized that this case example does not fully employ systematic review methods such as appraising studies for validity; however, such methods could be incorporated into the general approach described herein.

Potential impact on problem formulation:

What existing frameworks exist to help structure the evaluation? How will mechanistic data be used in risk assessment?

Conclusions and Recommendations For Problem Formulation

Intent for Mechanistic Data Must be Understood and Very Well-Defined



- **Identify potential hazard?**
- **Characterize potential hazard?**
- **Characterize MoA to inform dose-response methods or selection of key events, etc.?**

How will it be determined which mechanistic data are relevant? Important? Necessary? Appraised? Integrated?

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Conclusions From Practice

- **Complexity is often difficult to fully accommodate *a priori***
  - Considerations of systematic mapping or iterative determination for systematic review of mechanistic data may be important
- **Use (and volume) of mechanistic data are likely to determine most suitable approaches for selection and critical appraisal**
  - Considerations for aspects not covered by internal validity (e.g., relevance)
- **Rely on existing guidance/frameworks where possible**
- **Appreciate the role of staff training, piloting, and subject matter expertise**

Overall Recommendations

Recognize the iterative process associated with development of best practiceS

"We are looking for rigor, not rigidity." – Dr. Gary Miller

Wikoff DS, Miller GW. 2018. Systematic reviews in toxicology. Toxicol Sci 163(2):335–337.

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Thank you!

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