

ToxStrategies

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/>)

- Member of Tox21, RoB and Education workgroups

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

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

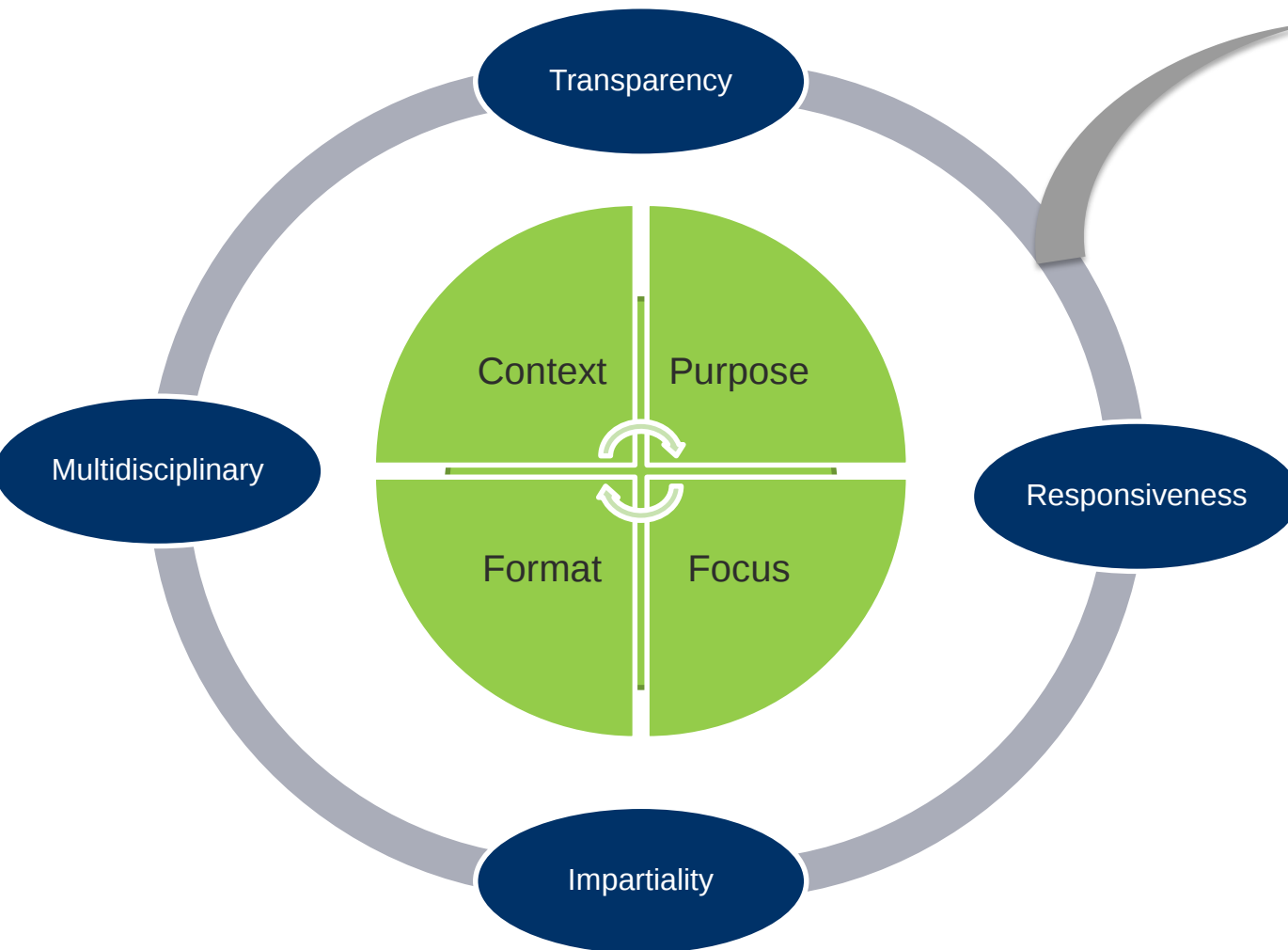
Body of Evidence Assessment

- 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

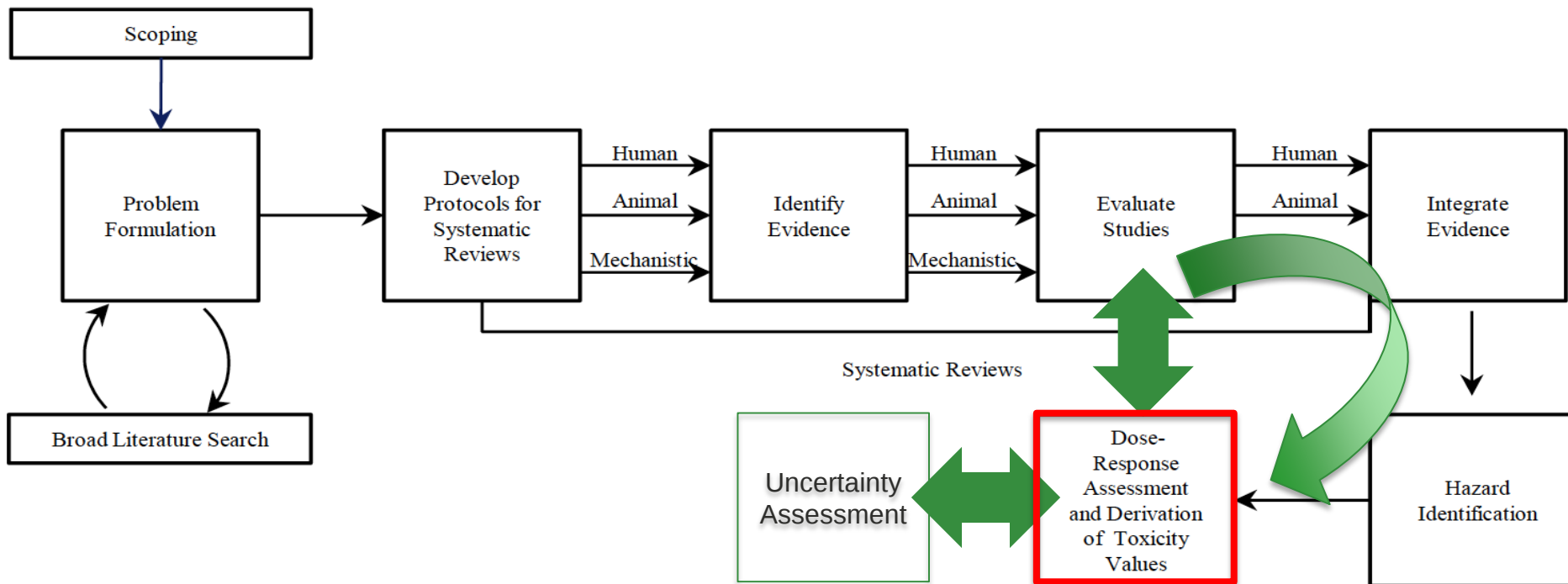
Problem Formulation Framework



1. PECO (and rationale)
2. Guidance for protocol

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

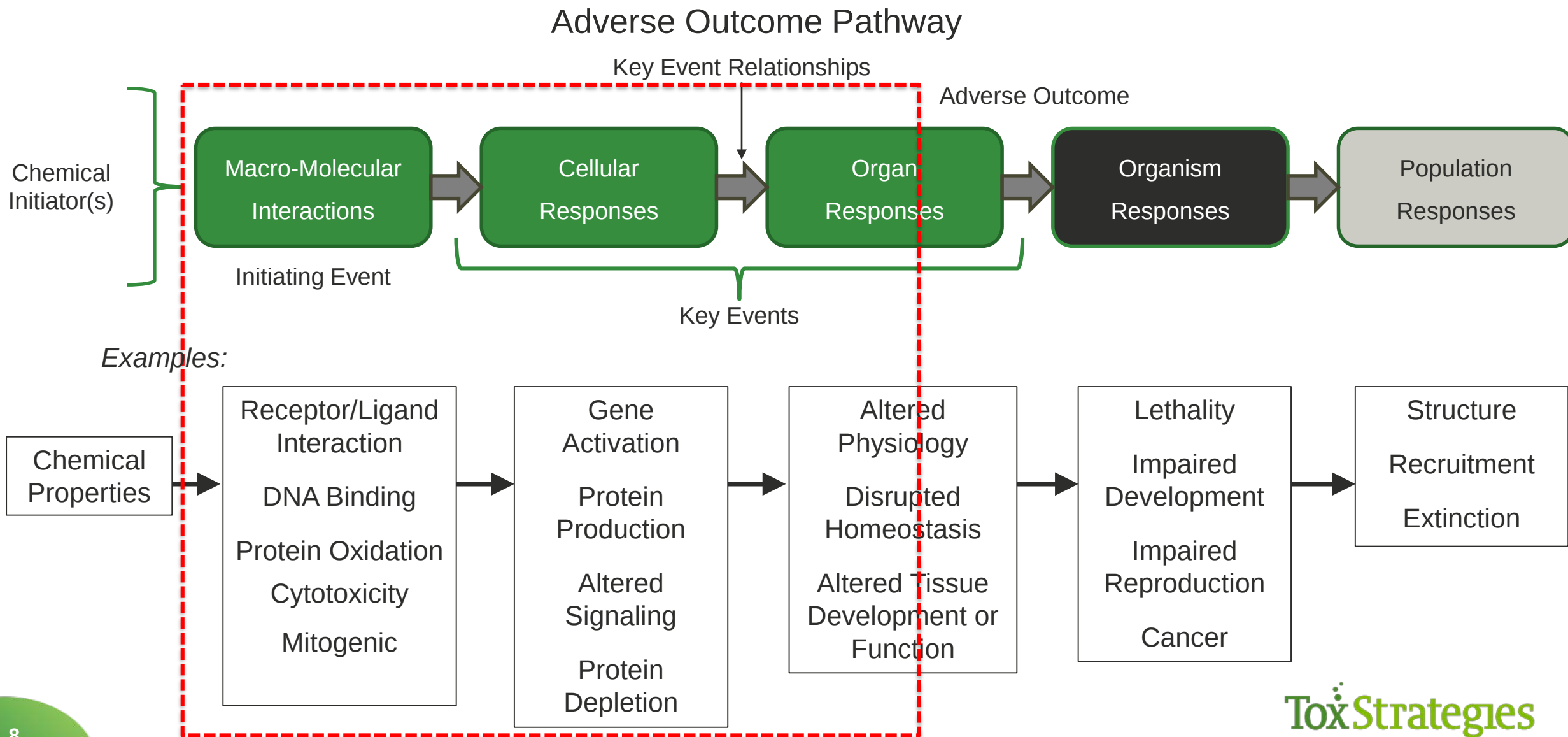
Figure S-1. Systematic Review in the context of the USEPA IRIS process. Adapted from NRC (2014)



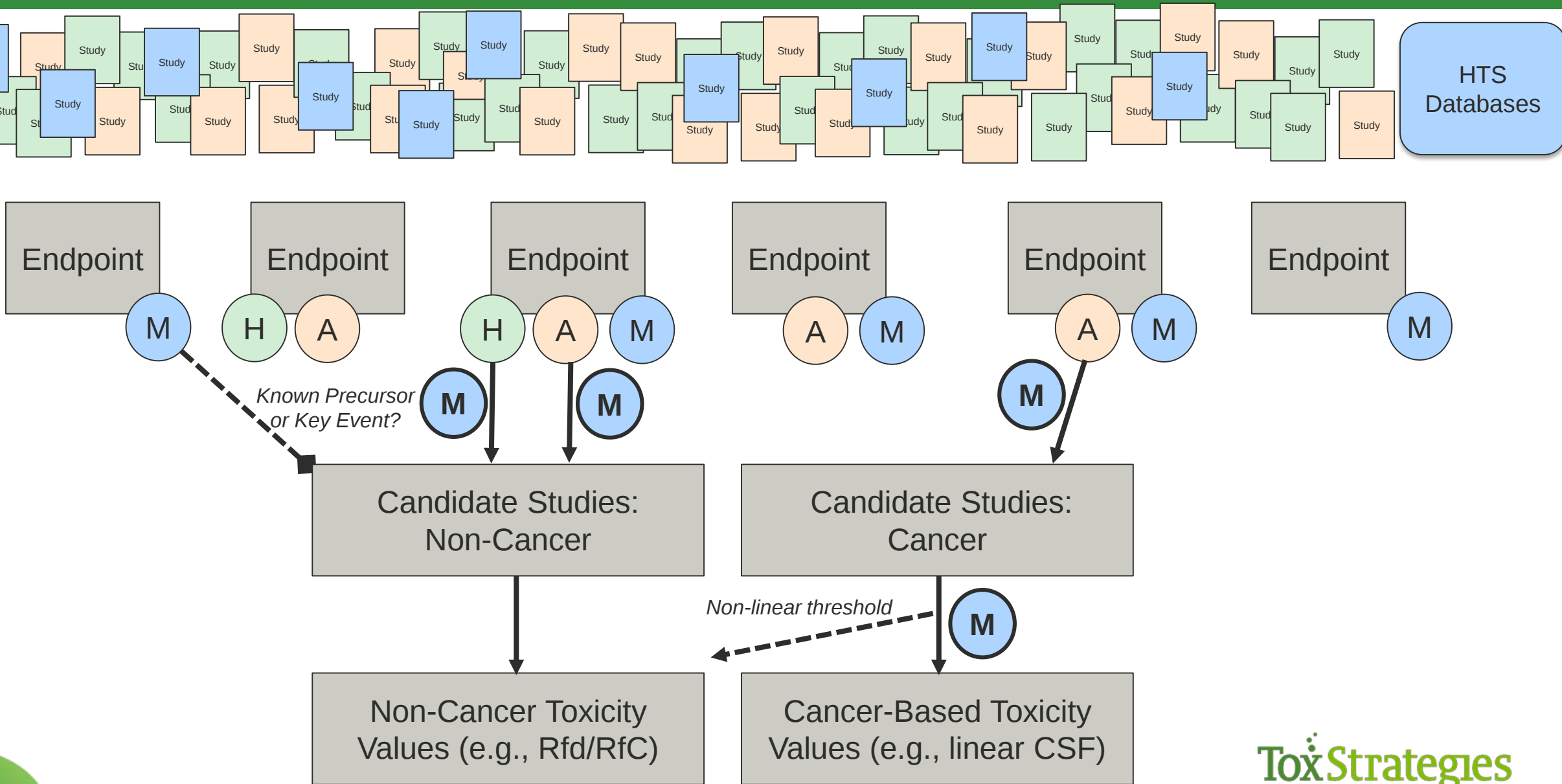
Problem formulation ultimately relates to the derivation of toxicity values

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

What is Mechanistic Data?

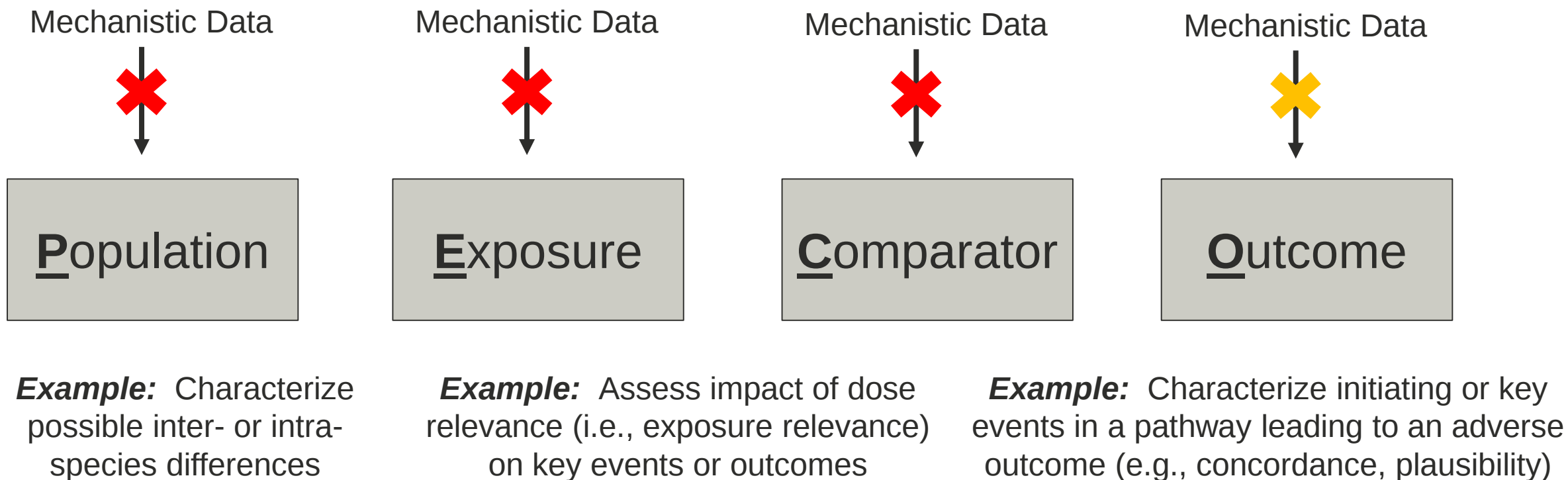


Use of Mechanistic Data in Risk Assessment

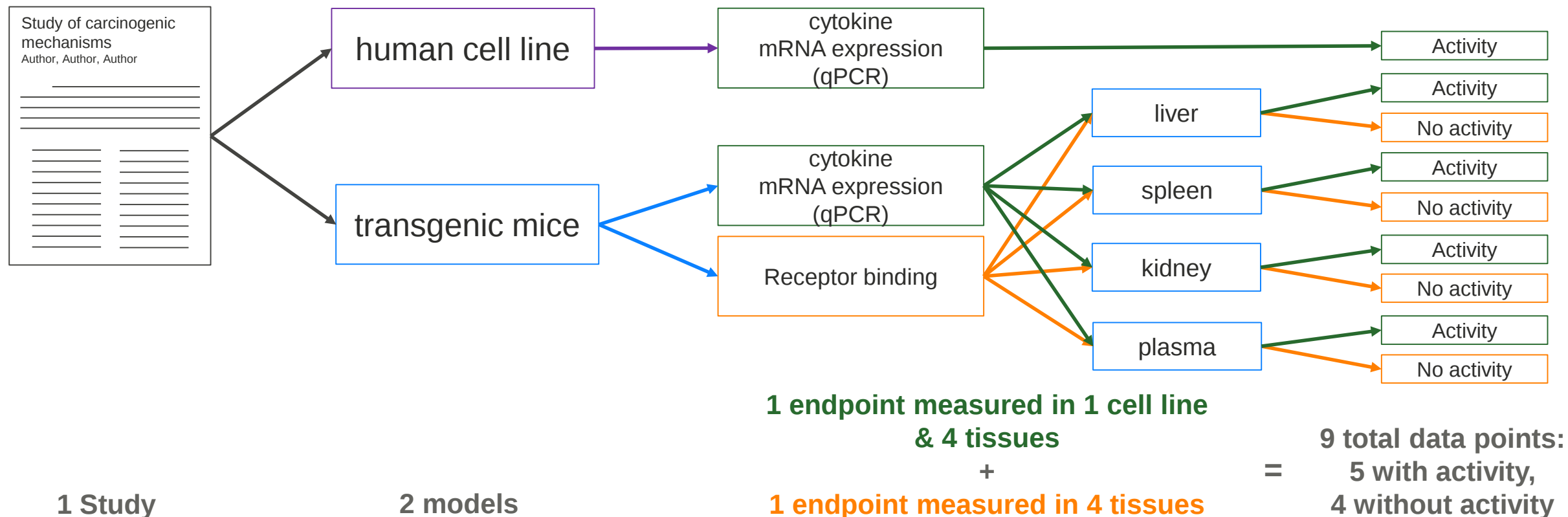


Mechanistic Data (Often) has Contextual Application to a Standard PECO

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



Mechanistic Data are Complex



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

Difficult to plan a priori

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

“Induces chronic inflammation”

Acute v. chronic?

In vivo v. vitro?



“Modulates receptor-mediated effects”

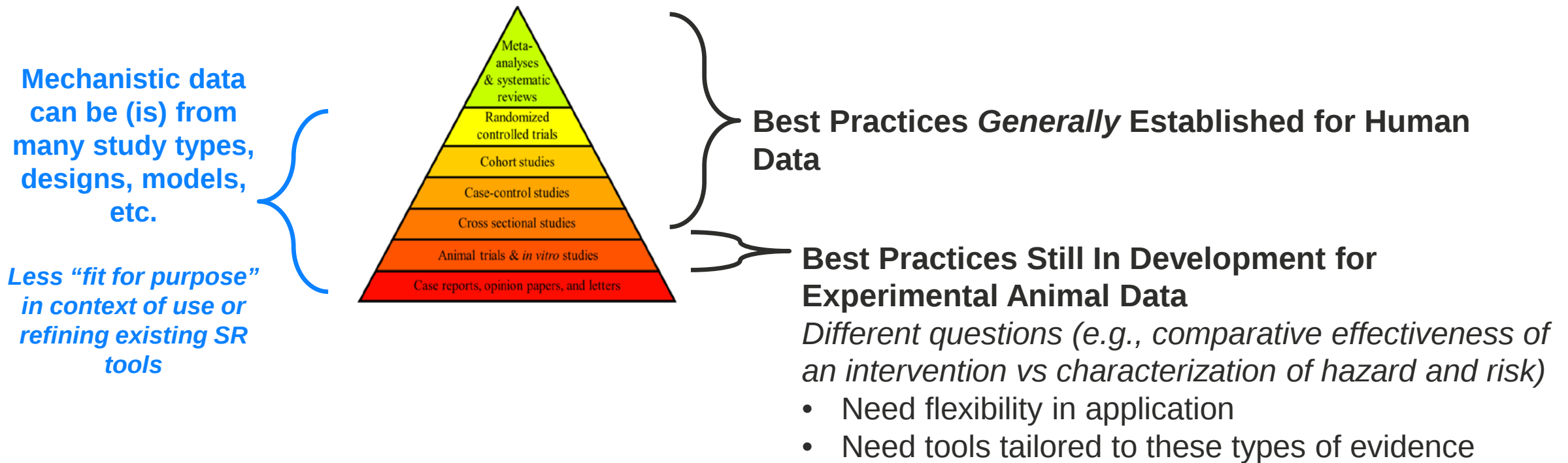
Any receptor?

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

Best Practices Evolving for Systematic Review



Difficult to guide operationalization in the protocol

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

ToxStrategies

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

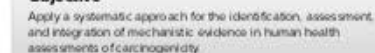


Figure 1: Workflow of the proposed framework.

The diagram illustrates the workflow of the proposed framework, which is divided into four main stages: Data Collection, Data Preprocessing, Model Training, and Model Deployment. Each stage is represented by a box, and the flow is indicated by arrows. A legend on the right side of the diagram lists the components of each stage.

- Data Collection:** Includes Data Source, Data Collection, and Data Storage.
- Data Preprocessing:** Includes Feature Selection, Feature Extraction, and Feature Transformation.
- Model Training:** Includes Model Selection, Model Training, and Model Evaluation.
- Model Deployment:** Includes Model Deployment, Model Monitoring, and Model Maintenance.

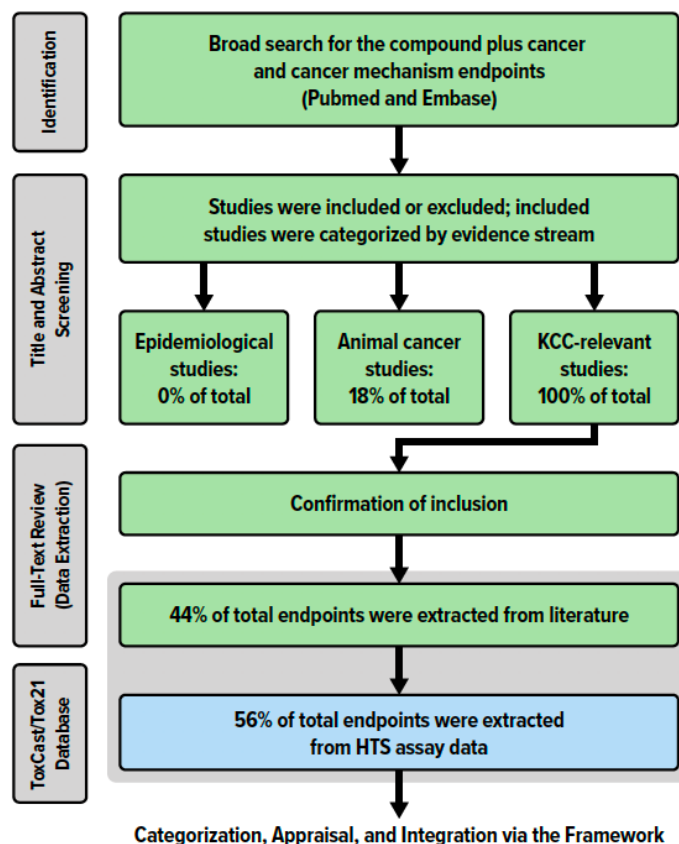
The flow starts with Data Collection, followed by Data Preprocessing, then Model Training, and finally Model Deployment. The legend on the right side of the diagram lists the components of each stage.

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

font: 10pt sans-serif; text-align: left; padding-left: 10px;">
 1. **Identify the main components of the system.**

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?

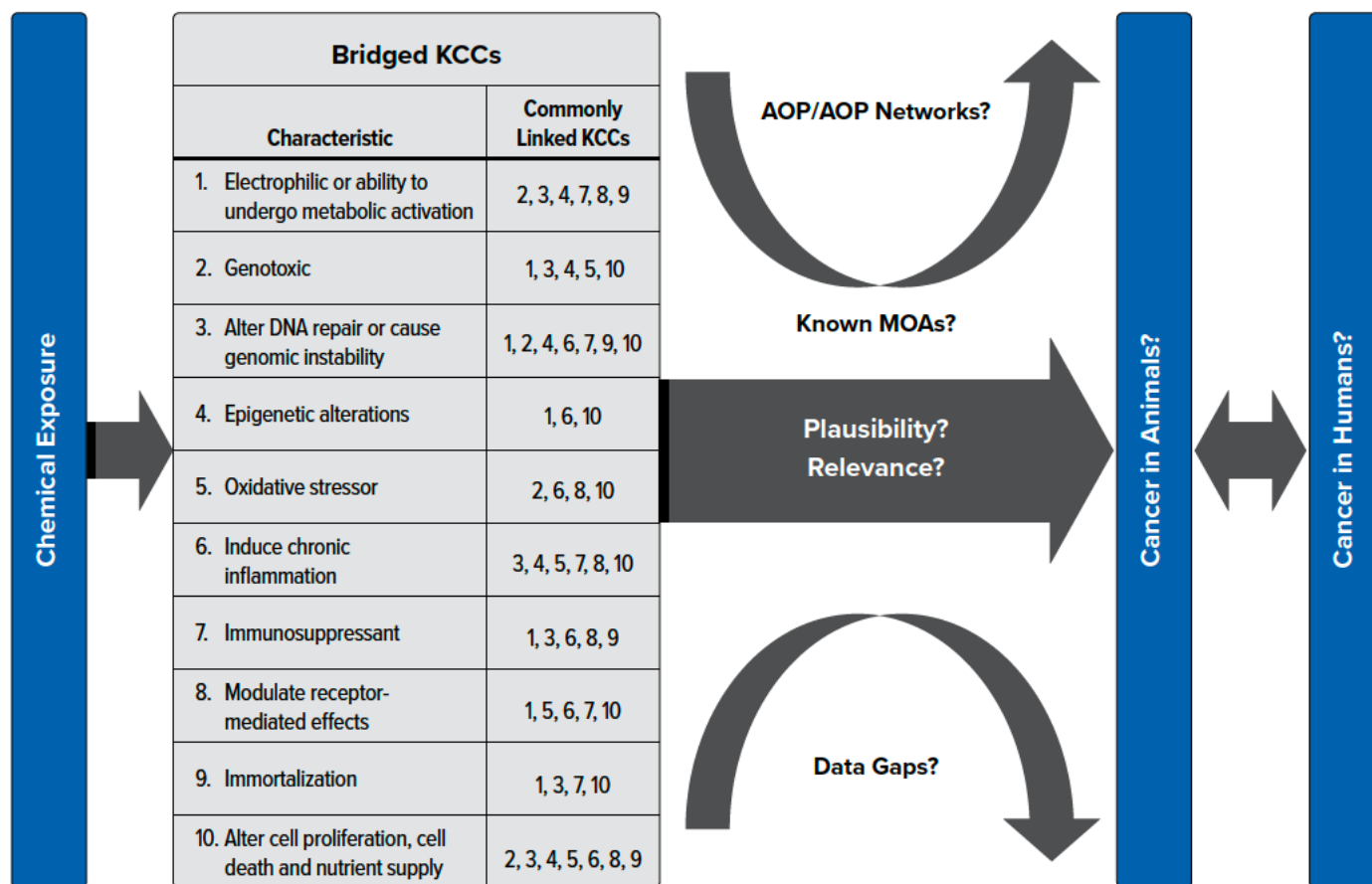
Which types of data are relevant for inclusion?

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?

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?

Example: Critical Appraisal of Mechanistic Data

Tox Strategies

Application of Mechanistic Data Quality Criteria in Assessment of the Relationship Between Congenital Heart Defects and TCE Exposure—A Case Study

Authors: Wikoff, D¹; Urban J²; Chappell G²; Haws, L²

¹ Fort Washington, Asheville, NC 28804. ² Fort Washington, Austin, TX 78743.

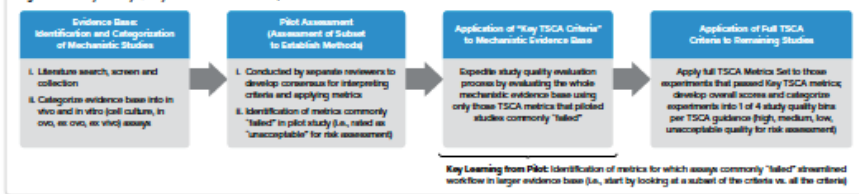
Introduction

- Tools for evaluating the quality of mechanistic studies** – especially *in vitro* experiments – within the systematic review paradigm are not well established. The current study develops a taxonomy of existing tools and requires development of new tools for appraising validity.
- One recently proposed tool is that from the US EPA-CRPT's 2008 Application of Systematic Reviews (CRPT-ASR) [10].
 - The evidence base for trichloroethylene (TCE) and congenital heart defects (CHD) in offspring presents an opportunity to explore study quality tools for mechanistic data. Previously a Risk of Bias (RoB) tool was developed for this purpose, and used to assess study quality [Willock et al., 2016¹¹]. Findings indicated the limited support for human and animal data suffered from high risk of bias, however, tools to evaluate the quality of mechanistic data were not available.
- Quality based on the Klimisch reliability scoring system, in which toxicology studies are assigned to one of four scores for reliability. Only 2 evaluation criteria are considered for each metric (‘‘0’’, ‘‘1’’, or ‘‘2’’) and the results in an automatic weighting of evaluation quality category.
- Study Quality Assessment (Fig 1)**
- All quality assessments were carried out by two PhD scientists (GC, AL) with experience conducting and/or assessing *in vivo* and *in vitro* studies. In cases of conflict, a third scientist (DW) was consulted to reach a consensus.
 - Study quality evaluation was conducted at the experimental level; many studies reported multiple experiments.
 - A pilot assessment was conducted in which the two analysts independently assessed a subset of 10 *in vitro* experiments. All decisions, disagreements, and refinements were documented. A similar pilot was

Objective

To evaluate the utility of the TSCA study quality tool for mechanistic data using the TCE-QHD mechanistic evidence base as a case study.

Figure 1. Summary of Study Quality Evaluation Process Developed for TCE-CHD Mechanistic Evidence Base



Methods

Development of TCE-CHD Evidence Base (Literature Search)

- * Any studies reviewing TCE exposure (in vivo or in vitro) relative to any mechanistic aspect of biomolecular and/or physiological pathways of cardiac development were included.

Selection of Screening Criteria:

- Primary Tool: TSCSA study quality tools
 - The TSCSA study quality tool is comprised of 7 domains, defined by 25 metrics (in vivo; per Table C-16 of TSCSA SR guidelines) or 24 metrics (in vitro; per Table C-14 of TSCSA SR guidelines).
 - Studies/experiments with any metric scoring a "4" are characterized as "unsatisfactory" for risk assessment and are excluded from further evaluation. For the remaining experiments, overall quality was determined by the weighted scoring calculations and categorizations based on Tables C-8 (in vitro) and C-9 (in vivo).
- Secondary Tools: two additional tools were selected for comparison purposes and applied to a subset of data.
 - SciRAP – a scoring method developed for quantifying reporting and methodological quality evaluations; the developers recommend using scores to rank study quality. Each evidence base is evaluated on a case-by-case basis, and no default score cutoffs are provided for characterizing evidence. There are 2 metrics, each with 3 levels of quality, none of which include a mechanism for excluding low quality studies from the evidence base.
 - ToxTool (Toxicological data Reliability Assessment Tool) – developed to evaluate toxicology study quality based on the Klimisch reliability scoring system, in which toxicology studies are assigned to 5 levels of reliability based on the quality of the data. The tool is applied to each study, and the "Y" or "N" for any of the "2-6 Risk Criteria" results in an automatic lowering of evaluation quality category.

Study Quality Assessment (Figure 1)

- All quality assessments were carried out by two PhD scientists (GC, JC) with experience conducting animal assessment in vivo and in vitro studies. In cases of conflict, a third scientist (DW) was consulted to facilitate a consensus solution.
- Study quality evaluation was conducted at the experimental level, many studies reported multiple experiments.
- A pilot assessment was conducted in which the two analysts independently reviewed a subset of 10 in vivo experiments. All decisions, interpretations, and refinements were documented. A similar pilot was conducted for the in vitro experiments.
- Based on findings of the pilot assessment, a tiered approach was used to assess in vivo studies quality:
 - Key Metrics Evaluation: The five Key Metrics were assessed; those studies that scored "4" on any of these metrics were removed from further study quality evaluation.
 - Complete Study Quality Evaluation: Those studies that scored 1-3 on all of the Key Metrics were then further evaluated using all of the quality metrics.
- A subset of the data were also subjected to quality evaluation using SciRAP and ToolTip methods for comparison purposes.

Results

Mechanistic Evidence Base: 22 studies were identified that tested TCE in an experimental model designed to examine a mechanistic aspect of cardiac development. These studies comprised a total of 68 unique experiments (Table 3).

Experiment type	Number of studies	Study title & Evidence Synthesis of Experimental Studies in Published TCR
In vitro	5	UNEP OPPY TCR in New Toxicity Study Methods (Table 2) (5)
In vitro (pre)	30	UNEP OPPY TCR in New Toxicity Study Methods (2) (30) UNEP TCF in Toxicology in Other Toxicity Studies (2) (30) ECOTEC TCF in Assessing Reliability of <i>In Vitro</i> Toxicity Studies (2) (30)
In vitro (pre)	28	UNEP OPPY TCR in New Toxicity Study Methods (2) (28) UNEP TCF in Toxicology in Other Toxicity Studies (2) (28) ECOTEC TCF in Assessing Reliability of <i>In Vitro</i> Toxicity Studies (2) (28)
In vitro (pre)	3	UNEP OPPY TCR in New Toxicity Study Methods (2) (3)
In vitro (pre)	10	UNEP OPPY TCR in New Toxicity Study Methods (2) (10)
Unpublished results	1	UNEP OPPY TCR in New Toxicity Study Methods (2) (1)
Total	68	-

Pilot Assessment:

- All 10 *in vitro* experiments scored a "4" (unsuitable for use in the assessment) in at least one Metric
- Five TSCA study metrics that the pilot experiments most commonly scored "4's" were identified as "Key Metrics" (Table 2). The list of commonly failed metrics was used to expedite subsequent evaluations of the mechanistic evidence base by focusing on these five (vs. all 25 for each study) as a means of improving efficiency and workflow.

Table 2. Metrics identified in criteria per risk evaluation using toxicology study quality metrics for in vitro experiments																																																																																																			
1. <i>Cell viability</i>	2. <i>Cell morphology</i>	3. <i>Cell proliferation</i>	4. <i>Cell death</i>	5. <i>Cell cycle</i>	6. <i>Cell differentiation</i>	7. <i>Cell migration</i>	8. <i>Cell adhesion</i>	9. <i>Cell signaling</i>	10. <i>Cell communication</i>	11. <i>Cell metabolism</i>	12. <i>Cell growth</i>	13. <i>Cell survival</i>	14. <i>Cell function</i>	15. <i>Cell response</i>	16. <i>Cell behavior</i>	17. <i>Cell phenotype</i>	18. <i>Cell genotype</i>	19. <i>Cell phenotype</i>	20. <i>Cell genotype</i>	21. <i>Cell phenotype</i>	22. <i>Cell genotype</i>	23. <i>Cell phenotype</i>	24. <i>Cell genotype</i>	25. <i>Cell phenotype</i>	26. <i>Cell genotype</i>	27. <i>Cell phenotype</i>	28. <i>Cell genotype</i>	29. <i>Cell phenotype</i>	30. <i>Cell genotype</i>	31. <i>Cell phenotype</i>	32. <i>Cell genotype</i>	33. <i>Cell phenotype</i>	34. <i>Cell genotype</i>	35. <i>Cell phenotype</i>	36. <i>Cell genotype</i>	37. <i>Cell phenotype</i>	38. <i>Cell genotype</i>	39. <i>Cell phenotype</i>	40. <i>Cell genotype</i>	41. <i>Cell phenotype</i>	42. <i>Cell genotype</i>	43. <i>Cell phenotype</i>	44. <i>Cell genotype</i>	45. <i>Cell phenotype</i>	46. <i>Cell genotype</i>	47. <i>Cell phenotype</i>	48. <i>Cell genotype</i>	49. <i>Cell phenotype</i>	50. <i>Cell genotype</i>	51. <i>Cell phenotype</i>	52. <i>Cell genotype</i>	53. <i>Cell phenotype</i>	54. <i>Cell genotype</i>	55. <i>Cell phenotype</i>	56. <i>Cell genotype</i>	57. <i>Cell phenotype</i>	58. <i>Cell genotype</i>	59. <i>Cell phenotype</i>	60. <i>Cell genotype</i>	61. <i>Cell phenotype</i>	62. <i>Cell genotype</i>	63. <i>Cell phenotype</i>	64. <i>Cell genotype</i>	65. <i>Cell phenotype</i>	66. <i>Cell genotype</i>	67. <i>Cell phenotype</i>	68. <i>Cell genotype</i>	69. <i>Cell phenotype</i>	70. <i>Cell genotype</i>	71. <i>Cell phenotype</i>	72. <i>Cell genotype</i>	73. <i>Cell phenotype</i>	74. <i>Cell genotype</i>	75. <i>Cell phenotype</i>	76. <i>Cell genotype</i>	77. <i>Cell phenotype</i>	78. <i>Cell genotype</i>	79. <i>Cell phenotype</i>	80. <i>Cell genotype</i>	81. <i>Cell phenotype</i>	82. <i>Cell genotype</i>	83. <i>Cell phenotype</i>	84. <i>Cell genotype</i>	85. <i>Cell phenotype</i>	86. <i>Cell genotype</i>	87. <i>Cell phenotype</i>	88. <i>Cell genotype</i>	89. <i>Cell phenotype</i>	90. <i>Cell genotype</i>	91. <i>Cell phenotype</i>	92. <i>Cell genotype</i>	93. <i>Cell phenotype</i>	94. <i>Cell genotype</i>	95. <i>Cell phenotype</i>	96. <i>Cell genotype</i>	97. <i>Cell phenotype</i>	98. <i>Cell genotype</i>	99. <i>Cell phenotype</i>	100. <i>Cell genotype</i>

SDG	Target	Measurable Indicators	SDG Definition of "Inequality" or the "Target"
8	8.10: Promote sustainable consumption and production patterns	8.10.1: The number of people consuming at least one sustainable consumption and production (SCP) product 8.10.2: The frequency of consumption and production activities, comparing to the base and sustainable consumption and production of people	8.10: Promote sustainable consumption and production patterns 8.10.1: The number of people consuming at least one sustainable consumption and production (SCP) product 8.10.2: The frequency of consumption and production activities, comparing to the base and sustainable consumption and production of people
10	10.4: Improve financial inclusion and access to financial services	10.4.1: The percentage of the adult population having access to financial services 10.4.2: The percentage of the adult population having access to financial services, comparing to the base and sustainable financial inclusion and access to financial services	10.4: Improve financial inclusion and access to financial services 10.4.1: The percentage of the adult population having access to financial services 10.4.2: The percentage of the adult population having access to financial services, comparing to the base and sustainable financial inclusion and access to financial services
16	16.6: Enhance public access to information and transparency	16.6.1: The percentage of the adult population having access to information and transparency 16.6.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency	16.6: Enhance public access to information and transparency 16.6.1: The percentage of the adult population having access to information and transparency 16.6.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency
17	17.1: Enhance public access to information and transparency	17.1.1: The percentage of the adult population having access to information and transparency 17.1.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency	17.1: Enhance public access to information and transparency 17.1.1: The percentage of the adult population having access to information and transparency 17.1.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency
22	22.1: Enhance public access to information and transparency	22.1.1: The percentage of the adult population having access to information and transparency 22.1.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency	22.1: Enhance public access to information and transparency 22.1.1: The percentage of the adult population having access to information and transparency 22.1.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency
26	26.1: Enhance public access to information and transparency	26.1.1: The percentage of the adult population having access to information and transparency 26.1.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency	26.1: Enhance public access to information and transparency 26.1.1: The percentage of the adult population having access to information and transparency 26.1.2: The percentage of the adult population having access to information and transparency, comparing to the base and sustainable public access to information and transparency

Assessment of Study Quality Across the Evidence Base

- 22% of the evidence base was of acceptable quality for risk assessment (Figure 2). In all cases except one, the assessment in a given study was similarly categorized for either human or environmental risk.
- 44 of the 68 experiments included at least one unacceptable score in a Key Metric. Of those remaining after Key Metric assessment, all experiments were "acceptable" for risk assessment following assessment with all TSCA metrics.
- Of the 24 experiments that were "acceptable" for risk assessment as "high" quality and 10 scored as "medium" quality. No studies scored as "low" quality.
- Very few of the experiments involving in vivo or cell culture in vitro models were judged to be sufficiently quality for risk assessment. By contrast, a larger proportion of the *in vivo* and *in vitro* experimental models were scored to be of sufficient quality to be retained for further consideration in risk assessment and exposure.
- The most commonly "labeled" metrics were those associated with reporting on the preparation and storage of the test substance (Metric 3), source element of data analysis (Metrics 22 and 23) and end use of the test substance (Metric 14, most relevant to cell culture experiments) (Figure 2). More than 1/2 of the experiments scored "4" on multiple metrics.

Comparison of Quality Using Secondary Tools:

- Across the three looks, the ToxicTox outcomes for the selected sites usually more consistently reflect the TSCA results than SciRep, although the general dichotomy in study quality between the two approaches is more apparent as the number of studies in each approach increases (Table 2)
- Two of the three looks (TSCA and ToxicTox) allowed for direct comparisons of overall study designations.
- Comparison of the overall quality designations were similar with respect general categorization, though two of the three would have been designated in the lowest of all categories under TSCA relative to the second lowest in ToxicTox.
- Comparison of findings to the output from SciRep are less direct, requiring the analysts to interpret the output relative to the overall scores. Visual inspection generally shows convergence (e.g., more red in Study 10 vs. Study 2 across graphics).

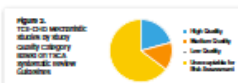


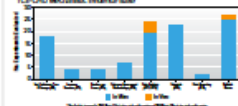
Figure 2. TCA-CO₂ evidence base: study quality evaluation of macrobenthic studies using TCA systematic review guidelines



Figure 8. TCA-CHO metabolic studies by model type and study quality category based on meta-analytic review Calzavara 2015



Figure 8. Mean study quality metric scores ("unexplained" across non-Asian, non-Hispanic populations)



Overall Findings and Lessons

- The TSCA study quality metrics provide a relatively thorough method for assessing study quality in mechanistic studies.
- The metrics and metric scoring provide a transparent means of assessing quality as well as a tool to facilitate decisions regarding relevance or risk to human health.
- The heterogeneity of mechanistic study designs provide significant challenges in context of a "one size fits all" quality evaluation approach. Particular challenge was met when assessing external validity study metrics for *in vivo* studies of mechanistic parameters.
- Evaluation of the quality of mechanistic *in vitro* studies has many challenges related to implementation and workflow:
 - Resource-intensive process with 25 metrics to consider.
 - Difficult to achieve consistent evaluation output between reviewers and raters.
 - Pilot exercises are critical to determine consensus/agreement on metric criteria refinement, interpretation, and application.
- The breadth and depth of evidence analyst expertise plays a critical role in making quality determinations across metrics.
- A critical role to assess study quality without a significant consideration of the PQCO.
- The content of the research question is critical for establishing chemical-specific parameters and defining/interpreting the study quality metrics and criteria used to score the evidence base.
- The TSCA study quality metrics, as well as SciRAP and ToxToolbox, generally provide a measure of internal validity through their significant focus on reporting elements. These tools do not provide explicit guidance on how to relate to external or conduct validity in a manner that is consistent with established considerations of such as (e.g., mammalian model systems relevant to human risk assessment) nor mammalian models.

Conclusions

- The TSCA study quality metrics provide a relatively thorough method for assessing study quality in mechanistic studies as well as providing a transparent process for determining the relative weight of each component of risk assessment. Topic-specific refinements and development of operating procedures improved the utility of this tool.
- The network and utility of assessing study quality of mechanistic data should be enhanced during problem formulation and protocol development. The specific use of mechanistic data in a risk assessment (e.g., hazard identification, MoA evaluation) should be identified a priori as this is critical to impact of the data on the assessment for assessing study quality as well as to ensuring that resources are used to provide meaningful output to inform risk assessment.
- The TSCA study quality metrics were developed in the case of TSC-C140. It is unlikely that evaluation of the mechanistic data would have changed the earlier risk of low study qualifications related to development of toxicity tests. TSCs are not intended to be a quantitative risk assessment for TSCs.

TABLE 3. Composition of *in vitro* stage quality evaluation media

Study	Experimental Model & Design	Primary Study Population/Outcomes	Outcomes	Limitations
2	In vivo model of depressive and anxiety disorders based on 12 OGD surgical shock and surgery lesions	• Stress: 12 OGD • Interpopulation: High-Quality Study • Consensus: Results from 12 OGD patients have the best treatment	• Stress: 9 (90%) High-Quality Study • Interpopulation: Moderate-to-High-Quality Study • Consensus: Results about outcomes for 12 OGD patients	• Reporting Quality Score: 90 out of 100 • Methodological Quality Score: 90, and of 90 • Consensus: 90% Confirmed 
3	In vitro: Protein activity in brain-derived neurotrophic factor and BDNF	• Stress: Not evaluated multiple studies • Interpopulation: Comprehensive for the study • Consensus: Results from patients have the best treatment for 12 OGD	• Stress: 2 (20%) High-Quality Study • Interpopulation: Not evaluated 0 • Consensus: Results may be the best result in the reported literature	• Reporting Quality Score: 90 out of 100 • Methodological Quality Score: 90, and of 90 • Consensus: 90% Confirmed 
4	In vitro: Brain neurotrophic factor and BDNF	• Stress: Not evaluated multiple studies • Interpopulation: Comprehensive for the study • Consensus: Results from patients have the best treatment for 12 OGD	• Stress: 2 (20%) High-Quality Study • Interpopulation: Not evaluated 0 • Consensus: Results may be the best result in the reported literature	• Reporting Quality Score: 90 out of 100 • Methodological Quality Score: 90, and of 90 • Consensus: 90% Confirmed 

1. www.epiupdate.com is a website dedicated to the dissemination of information relating to epidemiological research, with a focus on HIV and AIDS.
2. www.cdc.gov is the website of the United States Centers for Disease Control and Prevention, which is the main source for information on infectious diseases.
3. www.who.int is the website of the World Health Organization, which is the main source for information on infectious diseases.
4. www.unicef.org is the website of the United Nations Children's Fund, which is the main source for information on infectious diseases.
5. www.medicines.org.uk is the website of the United Kingdom Medicines Division, which is the main source for information on infectious diseases.
6. www.fda.gov is the website of the United States Food and Drug Administration, which is the main source for information on infectious diseases.
7. www.hhs.gov is the website of the United States Department of Health and Human Services, which is the main source for information on infectious diseases.
8. www.dhs.gov is the website of the United States Department of Homeland Security, which is the main source for information on infectious diseases.
9. www.doe.gov is the website of the United States Department of Energy, which is the main source for information on infectious diseases.
10. www.dia.gov is the website of the United States Department of Information, which is the main source for information on infectious diseases.

Challenges in Identification, Selection, and Appraisal

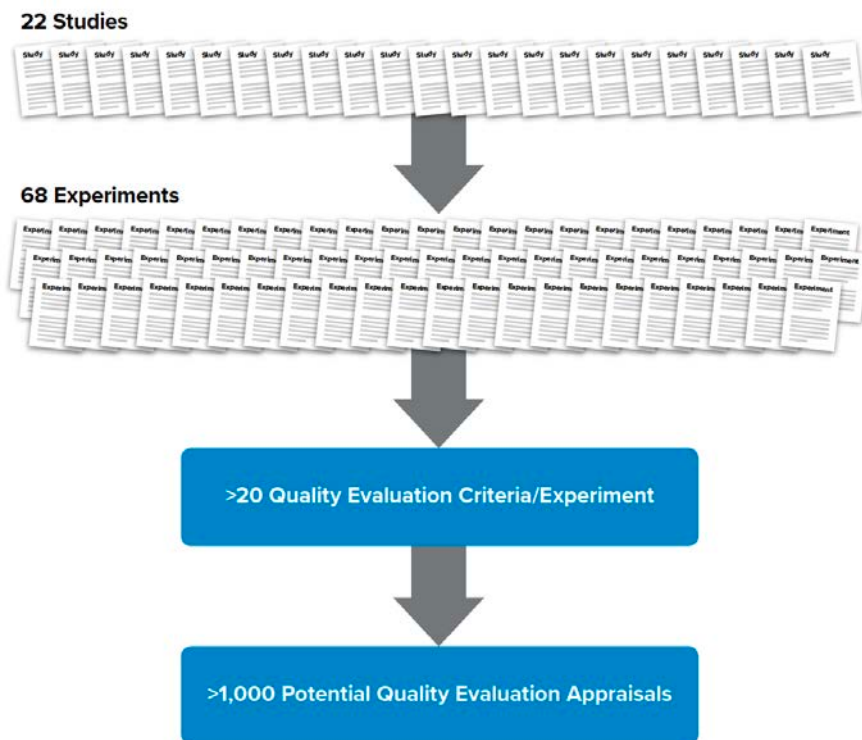


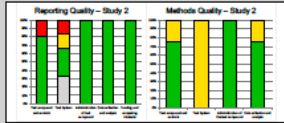
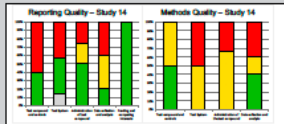
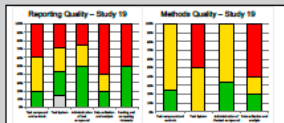
Table 1. TCE-CHD Mechanistic Studies: Breakdown of Experiment Types

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?

Challenges in Critical Appraisal

Study number	Experiment Model & Endpoint	Study Quality Evaluation Outcomes		
		TSCA	ToxRTTool	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?

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

ToxStrategies

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 developing 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 subfactors 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 IPC3 or U.S. EPA. In this paper, 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.

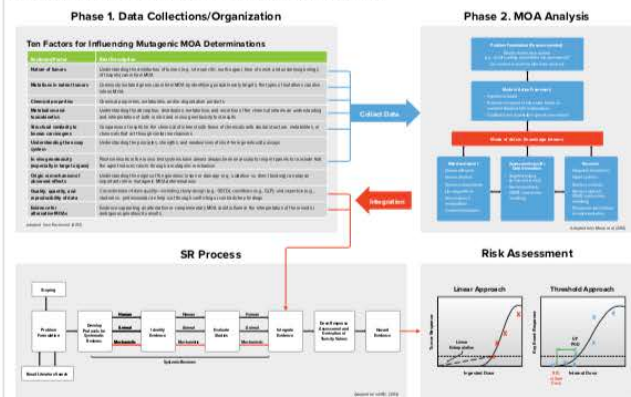
Phase 2

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).

Figure 1. An Approach to Integrating Mechanistic Information in Human Health Risk Assessment

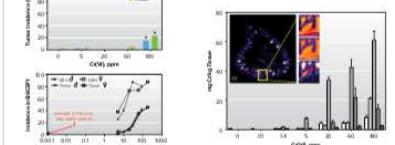


Phase 1: Data Collection & Organization

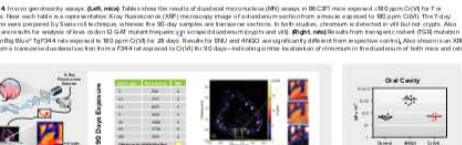
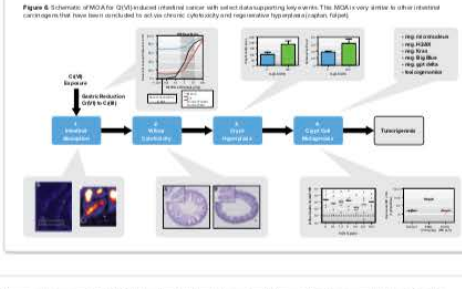
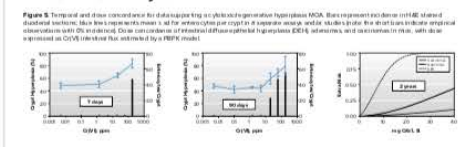
Table 1. Use of 10 Factors from Eastmond (2012) to Inform MOA Analysis for Hexavalent Chromium (Cr(VI)).	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues.
Factor 1: Nature of Tumors Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues. Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.
Factor 2: Mechanistic Information Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues. Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.
Factor 3: Genotoxicity Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues. Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.
Factor 4: Molecular Information Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues. Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.
Factor 5: Pharmacokinetics Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues. Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.
Factor 6: Molecular Information Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.	Table 2. Summary of In Vivo Genotoxicity Results in Target Tissues. Cr(VI) is a known human carcinogen, classified as a Group 1 carcinogen by IARC. It is associated with lung, nasal, and kidney tumors in humans and rodents. In rodents, it is also associated with liver, spleen, and bone marrow tumors.

Table 3. Comparison of In Vivo Data and Critical Effects Between Cr(VI) and Cr(III).		
Parameter	Cr(VI)	Cr(III)
Exposure route	Oral (gavage) and intraperitoneal (i.p.)	Oral (gavage) and intraperitoneal (i.p.)
Exposure dose	0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000, 5000, 10000, 20000, 50000, 100000, 200000, 500000, 1000000, 2000000, 5000000, 10000000, 20000000, 50000000, 100000000, 200000000, 500000000, 1000000000, 2000000000, 5000000000, 10000000000, 20000000000, 50000000000, 100000000000, 200000000000, 500000000000, 1000000000000, 2000000000000, 5000000000000, 10000000000000, 20000000000000, 50000000000000, 100000000000000, 200000000000000, 500000000000000, 1000000000000000, 2000000000000000, 5000000000000000, 10000000000000000, 20000000000000000, 50000000000000000, 100000000000000000, 200000000000000000, 500000000000000000, 1000000000000000000, 2000000000000000000, 5000000000000000000, 10000000000000000000, 20000000000000000000, 50000000000000000000, 100000000000000000000, 200000000000000000000, 500000000000000000000, 1000000000000000000000, 2000000000000000000000, 5000000000000000000000, 10000000000000000000000, 20000000000000000000000, 50000000000000000000000, 100000000000000000000000, 200000000000000000000000, 500000000000000000000000, 1000000000000000000000000, 2000000000000000000000000, 5000000000000000000000000, 10000000000000000000000000, 20000000000000000000000000, 50000000000000000000000000, 100000000000000000000000000, 200000000000000000000000000, 500000000000000000000000000, 1000000000000000000000000000, 2000000000000000000000000000, 5000000000000000000000000000, 10000000000000000000000000000, 20000000000000000000000000000, 50000000000000000000000000000, 100000000000000000000000000000, 200000000000000000000000000000, 500000000000000000000000000000, 1000000000000000000000000000000, 2000000000000000000000000000000, 5000000000000000000000000000000, 10000000000000000000000000000000, 20000000000000000000000000000000, 50000000000000000000000000000000, 100000000000000000000000000000000, 200000000000000000000000000000000, 500000000000000000000000000000000, 1000000000000000000000000000000000, 2000000000000000000000000000000000, 5000000000000000000000000000000000, 10000000000000000000000000000000000, 20000000000000000000000000000000000, 50000000000000000000000000000000000, 100000000000000000000000000000000000, 200000000000000000000000000000000000, 500000000000000000000000000000000000, 1000000000000000000000000000000000000, 2000000000000000000000000000000000000, 5000000000000000000000000000000000000, 10000000000000000000000000000000000000, 20000000000000000000000000000000000000, 50000000000000000000000000000000000000, 100000000000000000000000000000000000000, 200000000000000000000000000000000000000, 500000000000000000000000000000000000000, 1000000000000000000000000000000000000000, 2000000000000000000000000000000000000000, 5000000000000000000000000000000000000000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 200, 500, 1000, 200, 500, 1000, 200, 500, 1000, 2000, 5000, 100, 200, 500, 1000, 2000, 5000, 100, 200, 50000000000000000000	

Figure 2. Tumor incidence in lung cancer. The graph shows the incidence of lung cancer in humans and rodents exposed to Cr(VI). The incidence is significantly higher in humans than in rodents, and increases with dose. The graph also shows the incidence of lung cancer in humans and rodents exposed to Cr(III). The incidence is significantly lower in humans than in rodents, and increases with dose.



Phase 2: MOA Analysis



Risk Assessment

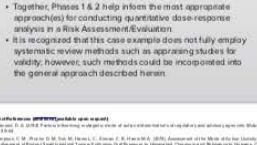
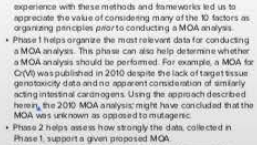
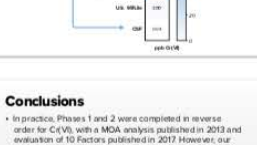
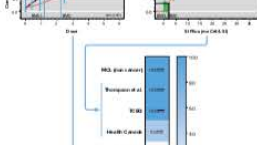
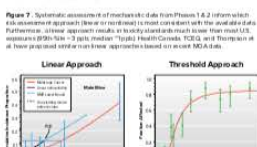


Figure 7. Schematic of MOA for Cr(VI)-induced cancer with selected supporting keywords. The diagram shows a flow from Cr(VI) exposure to DNA damage, which leads to mutations and cancer. The diagram also shows the role of Cr(VI) in DNA damage and the resulting mutations.

Figure 7. Schematic of MOA for Cr(VI)-induced cancer with selected supporting keywords. The diagram shows a flow from Cr(VI) exposure to DNA damage, which leads to mutations and cancer. The diagram also shows the role of Cr(VI) in DNA damage and the resulting mutations.

Figure 7. Schematic of MOA for Cr(VI)-induced cancer with selected supporting keywords. The diagram shows a flow from Cr(VI) exposure to DNA damage, which leads to mutations and cancer. The diagram also shows the role of Cr(VI) in DNA damage and the resulting mutations.

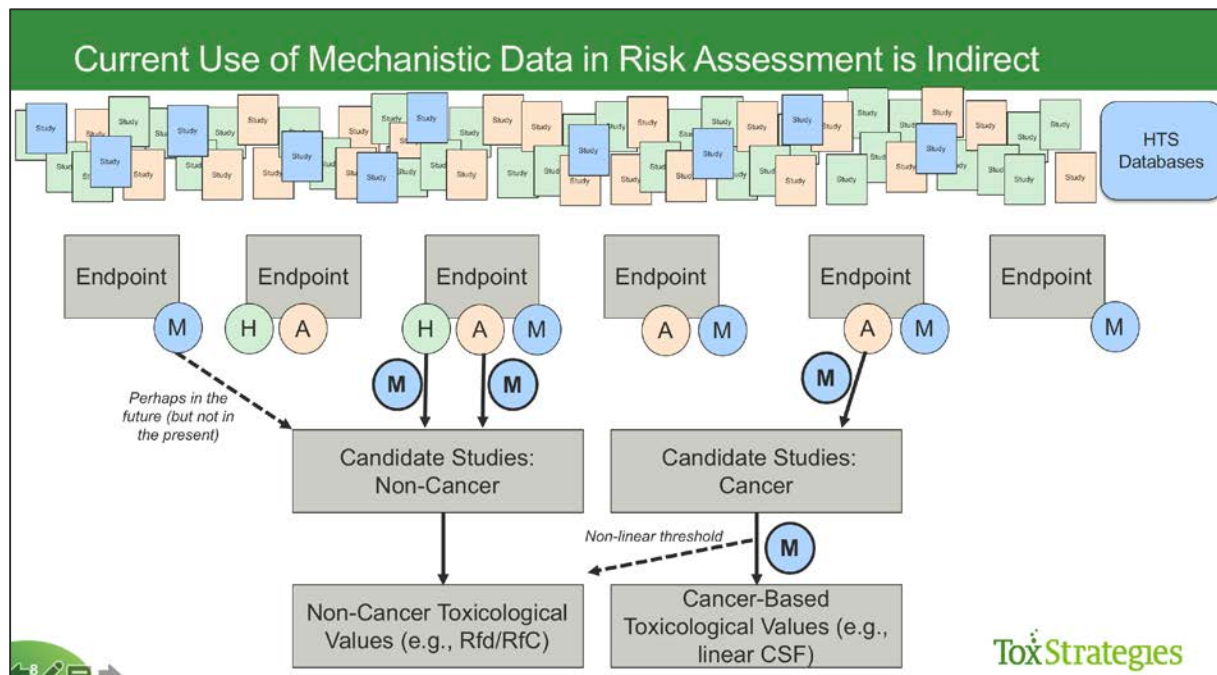
Potential impact on problem formulation:

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

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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?*

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

Thank you!

Grace Chappell, Chad Thompson, Jon Urban, Susan Borghoff, Julia Rager, Seneca Fitch, Mark Harris, Laurie Haws