NTP
National Toxicology Program

Consideration of Internal and External Validity in Mechanistic Studies

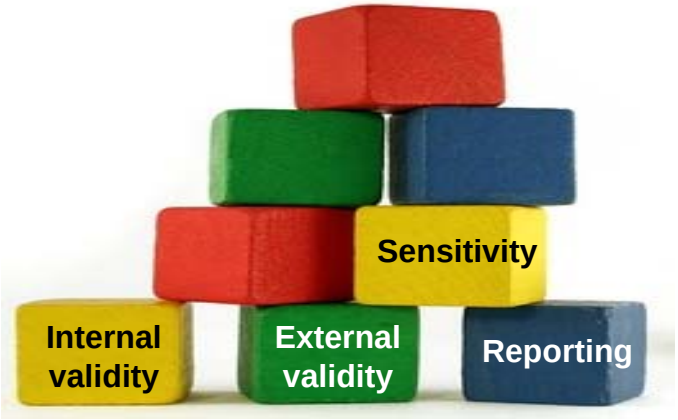
Andrew Rooney, PhD

Strategies and Tools for Conducting Systematic Reviews of Mechanistic Data to Support Chemical Assessments

December 10, 2018

Defining the Terms

- Internal Validity
  - Whether study design and conduct may bias results
  - Also called risk of bias
- External Validity
  - Extent study address the review question
  - Also called applicability, relevance
- Reporting Quality
  - Adequacy of reporting for evaluating study design, conduct, results
- Sensitivity
  - Whether study design and conduct impact ability to detect an effect



Diverse Study Types, Model Systems, Designs

- Where does it come from?
  - Wide variety of study types not intended to identify a disease phenotype
  - Studies directed at mechanisms (cellular, biochemical and molecular)
  - Includes in vitro and in vivo studies
- Assessing quality or risk of bias
  - Mechanistic studies with in vivo exposure could be addressed by tools for human and animal studies
  - What about studies with in vitro exposure regimes?

Shifting Focus to Mechanistic Studies

- Tools to Evaluate Human Studies
  - Established tools for randomized controlled trials
  - Active area of research for observational human studies (ROBINS-E., etc)
- Tools to Evaluate Animal Studies
  - Multiple tools (SYRCLE, Navigation Guide, OHAT)
- Tools and Emerging Approaches for Mechanistic Studies
  - Recent activity
    - Some tools (NTP/OHAT “use-case”, SciRAP)
    - What’s currently being done?
    - Need for a systematic review…

OHAT Risk of Bias "Use-case" in PFOA Evaluation

OHAT "Parallel" Approach Across Evidence Streams

- Features of OHAT risk-of-bias tool for assessing Internal Validity
  - Study design determines which questions are applicable
  - Evaluation is endpoint specific
- Predefined set of questions address
  - Human studies
  - Animal toxicology studies

Human Data

Experimental Animal Data

- Study design determines which questions apply

1.Randomization of exposure (experimental animal studies)

Risk-of-Bias Questions	Experimental Animal	Human Controlled Exposure	Cohort	Case-Control	Cross-Sectional	Case Series
1. Was administered dose or exposure level adequately randomized?	X	X				
2. Was allocation to study groups adequately concealed?	X	X				
3. Did selection of study participants result in the appropriate comparison groups?			X	X	X	
4. Did study design or analysis account for important confounding and modifying variables?			X	X	X	X
5. Were experimental conditions identical across study groups?	X					
6. Were research personnel blinded to the study group during the study?		X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X
9. Can we be confident in the outcome assessment (including blinding of assessors)?	X	X	X	X	X	X
10. Were all measured outcomes reported?	X	X	X	X	X	X
11. Were there no other potential threats to internal validity	X	X	X	X	X	X

4. Confounding (observational studies)

OHAT "Use-case" in PFOA/PFOS Evaluation

A "Parallel" Approach Across Evidence Streams

- Predefined set of questions address
  - Human studies
  - Animal toxicology studies
- Features of OHAT risk-of-bias tool
  - Study design determines which questions are applicable
  - Evaluation is endpoint specific

Use-case Explored Extending the Risk of Bias Approach from Experimental Animal Studies to Studies with an In Vitro Exposure Regime

Human Data

Experimental Animal Data

In Vitro Exposure Studies

Use-Case Methods Development Process

Extending the OHAT Risk-of-Bias Approach to In Vitro Studies

- Criteria adapted to address studies with in vitro exposure regimens
  - Multiple rounds of review and discussion with an NTP expert group addressed issues such as:
    - Applicability of questions
    - Where specific issues should be covered
    - Other issues not in the animal tool
    - Language for criteria
  - Applied to studies with an in vitro exposure regime

***In Vitro* Review Group**

- Scott Auerbach
- Warren Casey
- Michael Devito
- Stephen Ferguson
- Rick Paules
- Ray Tice
- Kristine Witt

Contractors

- David Allen
- Michael Paris
- Judy Strickland

Use-Case Adaptation Example

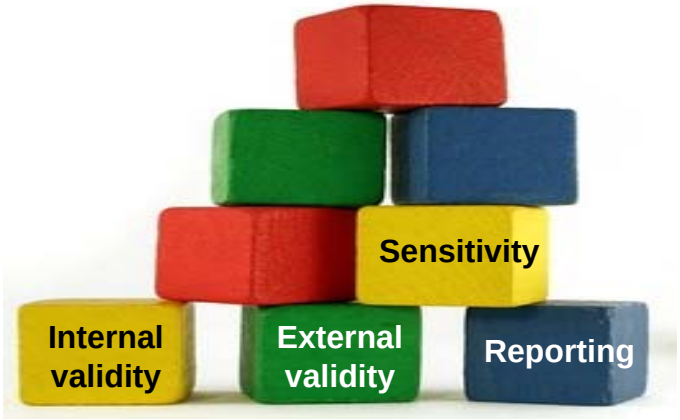
1) Was administered dose or exposure level adequately randomized?

- Helps to assure that treatment is not given selectively based on potential differences in human subjects, animals, **cells, or tissues**
- Requires each human subject, animal, **or cell** had an equal chance of being assigned to any study group including controls
- In vitro study applicability
  - Potential differences between cells that comprise different groups will depend on study design
  - If homogeneous cell suspension, then no variation or difference between groups … therefore, no need for randomization

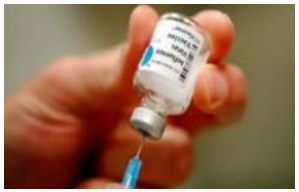
  - Used in NTP Monograph: Immunotoxicity Associated with Exposure to PFOA/PFOS (http://ntp.niehs.nih.gov/go/749926)

… Remember Other Study Quality Factors

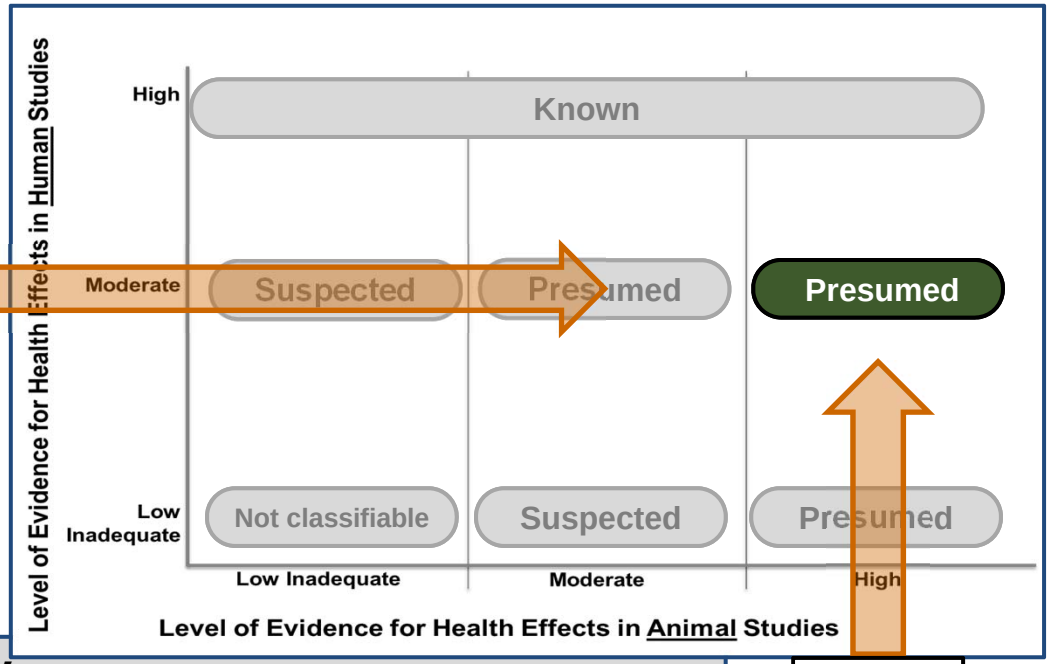
- Internal Validity
  - Whether study design and conduct may bias results
  - Also called risk of bias
- **External Validity**
  - Extent study address the review question
  - Also called applicability, relevance
- Sensitivity
  - Whether study design and conduct impact ability to detect an effect



Consider How Mechanistic Data Are Used in the Evaluation



Human Evidence



Animal

Mechanistic Data

Mechanistic Data Informed Conclusions in Evidence Integration Step in PFOA/PFOS Immunotoxicity Use-case

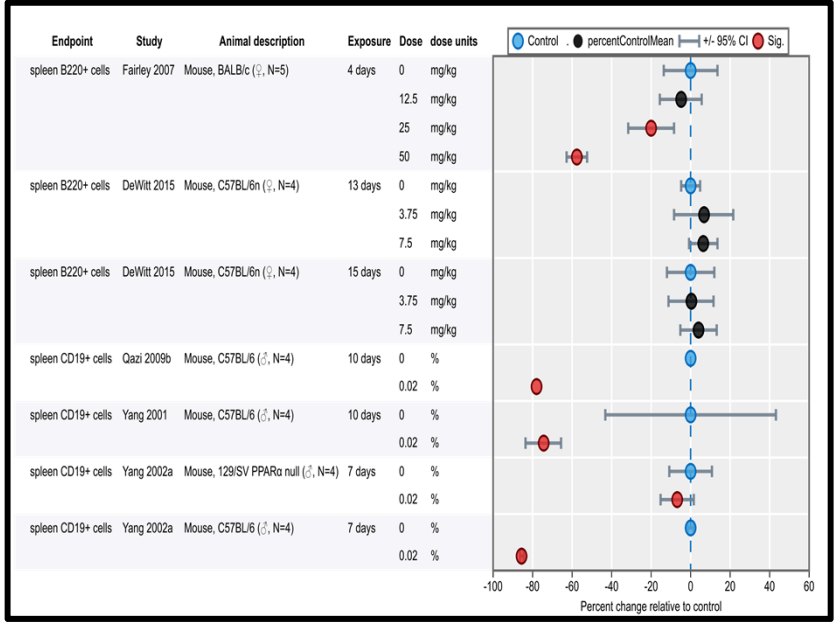
Informing Biological Plausibility

- Are there data showing PFOA-associated disruption of early events in the process leading to the antibody response?
- Were changes at same or lower concentrations as the observed effect?
- **Examples:** Key cell populations, cell signaling, activation

Mechanistic Data from Use-case

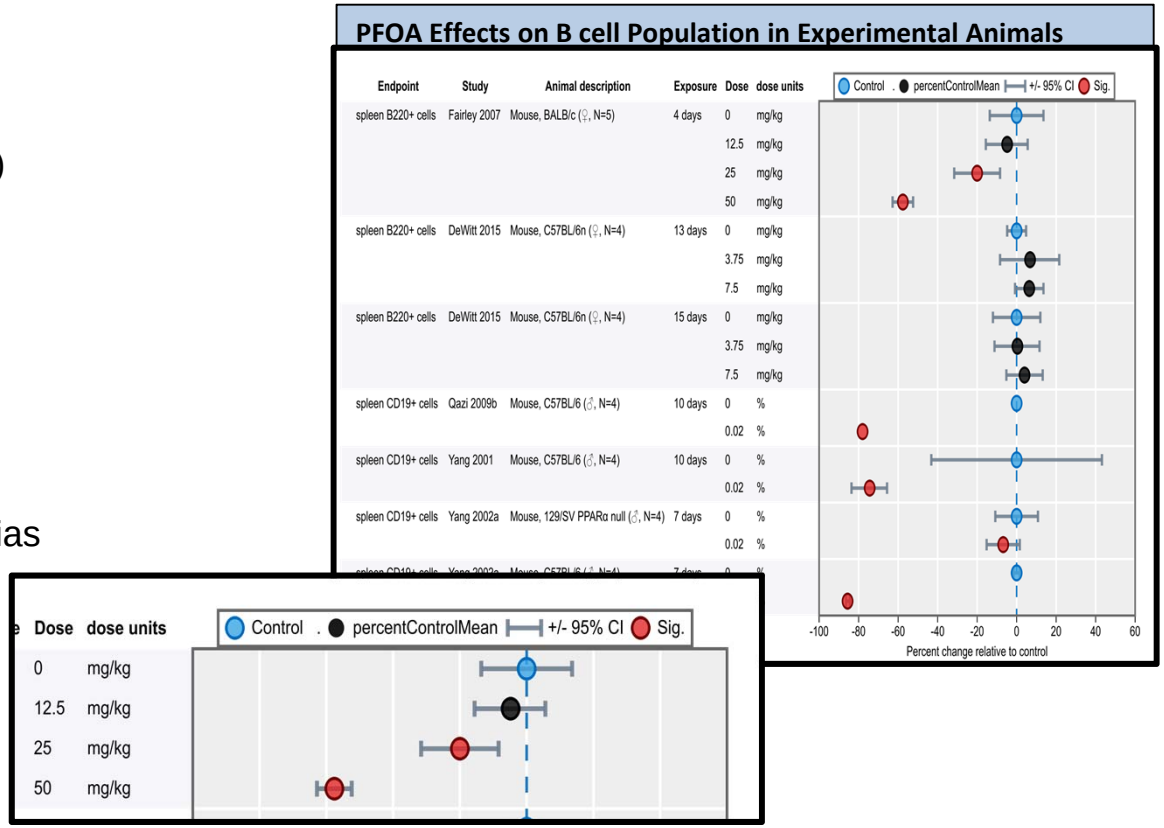
- **Mechanistic Data**
  - B cell and T cell numbers
  - Cytokines (IL-4, IL-6, IL-5)
  - Antigen presenting cells
- **Evaluate Evidence**
  - Magnitude
  - Dose-response
  - Consistency
  - **Internal Validty/ Risk of Bias**
  - Publication Bias
  - External Validity/ Applicability
    - Endpoint for Humans
    - Dose

PFOA Effects on B cell Population in Experimental Animals



Mechanistic Data from Use-case

- **Mechanistic Data**
  - B cell and T cell numbers
  - Cytokines (IL-4, IL-6, IL-5)
  - Antigen presenting cells
- **Evaluate Evidence**
  - Magnitude
  - Dose-response
  - Consistency
  - Internal Validity/ Risk of Bias
  - Publication Bias
  - **External Validity/ Applicability**
    - **Endpoint for Humans**
    - **Dose**

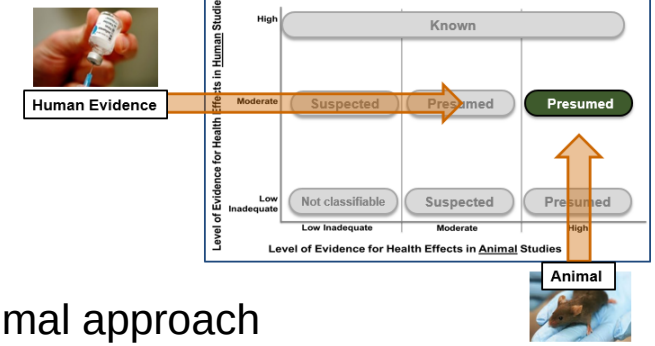


*the effective dose for mechanistic studies is **higher** than dose associated with effects in animal studies*

Lessons from Use-Case

Consideration of Mechanistic Data

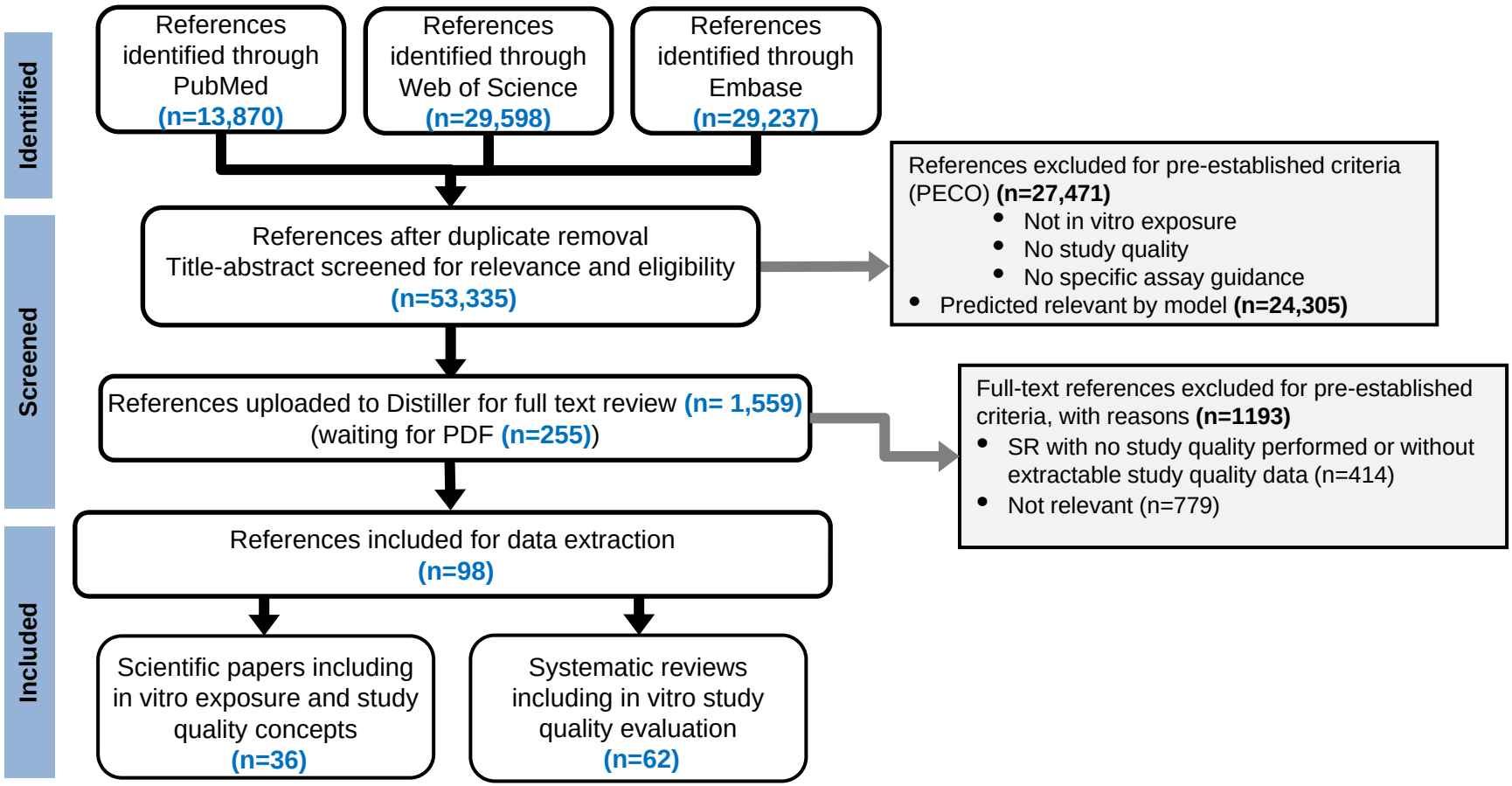
- Problem Formulation
  - Outlined use of mechanistic data
  - Followed human and animal evidence (iterative)
- Internal Validity
  - Assessed with risk of bias method extended from animal approach
  - Focused on endpoints with relevance to human and animal data
- External Validity
  - Critical to have plan for evaluating key mechanistic data
  - Dose and applicability were drivers in use of mechanistic data
- Use-case Represents An Approach
  - Active area of research
  - Systematic review of current practices…



Someone Needs to Do a Systematic Review…

- Systematic Review of Study Quality/Critical Appraisal Approaches Used to Assess In Vitro Studies
- NTP and Evidence-Based Toxicology Collaboration (EBTC) Effort
- Two Types of Published Studies
  - 1) Systematic reviews that considered and critically assessed in vitro studies
  - 2) Research papers, guidance, methods that provide guidance on how to critically assess in studies with an in vitro exposure regime

Initial Systematic Review Screening



Initial Results

Full Text Screening

- **Included**
  - Research Papers/Methods
    - 36 publications
  - Systematic Reviews
    - 62 SRs addressed in vitro exposure /study quality
- **Excluded**
  - Reviews
    - 200 general “reviews”
    - 177 stated “systematic reviews” without apparent study quality evaluation
- In Process …. still pulling PDFs (255)

Systematic Review Topics

- Dentistry
- Medical/Clinical
- Nutrition
- Toxicology/ Environmental Health
- General mechanisms

Research Paper Topics

- High-throughput screening
- Medical/Clinical
- Toxicology/ Environmental Health
- General mechanisms

Initial Results

Full Text Screening

- **Included**
  - Research Papers/Methods
    - 36 publications
  - Systematic Reviews
    - 62 SRs addressed in vitro expos
- **Excluded**
  - Reviews
    - 200 general “reviews”
    - 177 stated “systematic reviews” without apparent study quality evaluation
- In Process …. still pulling PDFs (255)

Developing Interactive Database

Study Quality Characterization

'Domains'	'Questions'	Short citat..	
Detection Bias	Were experimental conditions similar across all groups?	Bonniaud, e..	1
		Stead, et al ..	2
	Can we be confident in the outcome assessment?	Bouhifd, et ..	1
		Harbell, et ..	1
		Vesterinen,..	1
	Was there sufficient power to detect an effect?	Lovell, et al ..	1
		Mayhew, et..	1
		Valcu, et al ..	1
		Vesterinen,..	1
	Were there sufficient replicates to determine variability?	Bouhifd, et ..	1
		Caraus, et a..	1
		Lovell, et al ..	1
		Malo, et al (..	1
		McConnell, ..	1
		Valcu, et al ..	1
Exposure bias	Can we be confident in the exposure assessment?	Hsie, et al (..	1

Thank you

Questions?