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Alternatives Assessment 123 Webinar:

Mechanistic Data in a Systematic Review Framework: Developing Confidence in Bodies of Evidence

JANUARY 21, 2015

FACILITATED BY: JOEL TICKNER, SCD

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** If you would like to ask a question or comment during this webinar please type your question in the Q&A box located in the control panel.*

Goals

- Continuing education and dialog

- To advance the practice of alternatives assessment for informed substitution across federal, state, and local agencies through networking, sharing of experiences, development of common approaches, tools, datasets and frameworks, and creation of a community of practice.

Purpose of this call

- Alternatives assessment processes often suffer from significant gaps in toxicological data
- High throughput in-vitro screens provide a means to fill data gaps and serve as primary data
- The objective of the Tox 21 partnership, a multi-agency collaborative effort, is to shift the assessment of chemical hazards from traditional experimental animal toxicology studies to one based on target-specific, mechanism-based, biological observations largely obtained using *in vitro* assays, with the ultimate aim of improving risk assessment for humans and the environment and the design of safer chemicals
- A key goal is to develop better predictive tools

International Symposium on Alternatives Assessment

Advancing Science & Practice

NATCHER CONFERENCE CENTER | BETHESDA, MD | MARCH 5–6, 2015

Organized by The Lowell Center for Sustainable Production at University of Massachusetts Lowell

HOME ABOUT PROGRAM SPEAKERS REGISTRATION VENUE

The ***International Symposium on Alternatives Assessment: Advancing Science and Practice*** provides a collegial forum for governmental agency staff, university researchers, industry sustainability professionals, environmental health consultants and advocates to:

- Understand and discuss gaps in knowledge and methods confronting the use of alternatives assessment.
- Identify elements of a research agenda for alternatives assessment and a process for moving it forward.
- Advance and support the growing community of practice for alternatives assessment.

To view program and to register visit: www.saferalternatives.org

Speaker

Dr. Andrew Rooney

Office of Health Assessment and Translation,
National Institute of Environmental
Health Sciences

Discussion Questions

- What are the strengths and weaknesses of high throughput screening data for both data gap filling and as primary data to support alternatives assessment?
- How useful/usable is Tox 21 data for both comparing chemical alternatives and designing safer molecules at the present time.
- What are the challenges and opportunities to more effective integration of these data streams in chemical alternatives assessment?

Webinar Discussion Instructions

- Due to the number of participants on the Webinar, all lines will be muted.
- If you wish to ask a question, please type your question in the Q&A box located in the drop down control panel at the top of the screen.
- All questions will be answered at the end of the presentations.

NTP
National Toxicology Program

Mechanistic Data in a Systematic Review Framework: Developing Confidence in Bodies of Evidence

Andrew Rooney, PhD
Office of Health Assessment and Translation
National Institute of Environmental Health Sciences

January 21, 2015

Presentation Outline

- Background
  - Office of Health Assessment and Translation
  - Systematic review, mechanistic data, and environmental health questions
- Mechanistic Data in the OHAT Framework
  - Planning
  - Identifying the evidence
  - Evaluating the evidence
  - Integrating the evidence
- Challenges and Ongoing Methods Development
- Questions
  - SR on OHAT Website (http://ntp.niehs.nih.gov/go/38673)

Office of Health Assessment and Translation

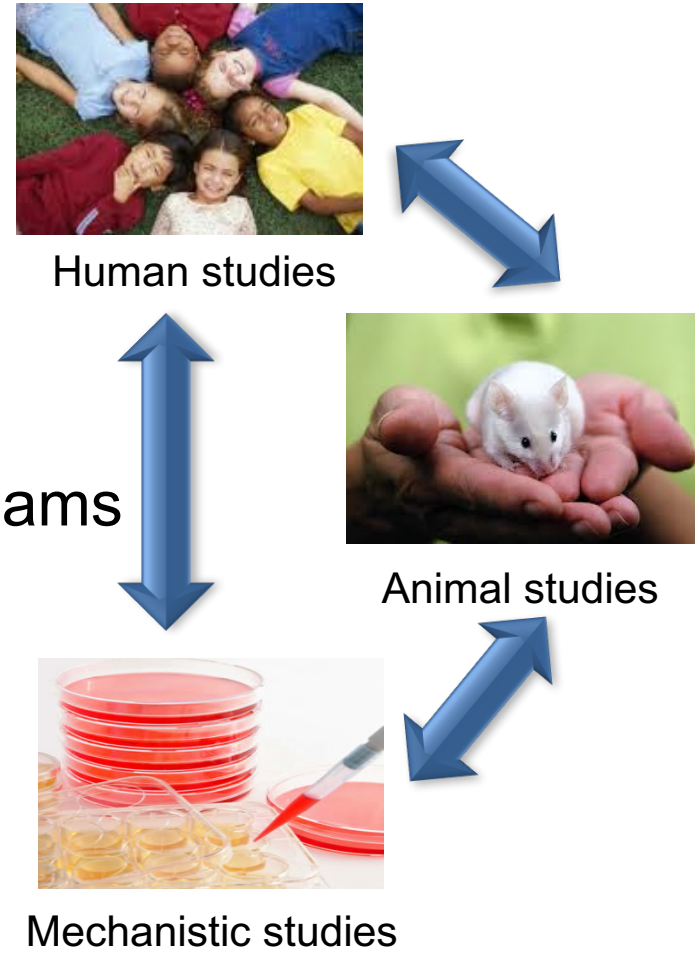
- **Conduct literature-based evaluations** to assess the evidence that environmental substances cause adverse health effects
  - Hazard or State-of-science evaluations
  - Provide opinions on whether substances may be of concern given current human exposure

- **Methods development**
  - Systematic review
  - Increasing integration of mechanistic data
  - Approaches to assess confidence in mechanistic data

OHAT Evaluations

Requirements for Environmental Health

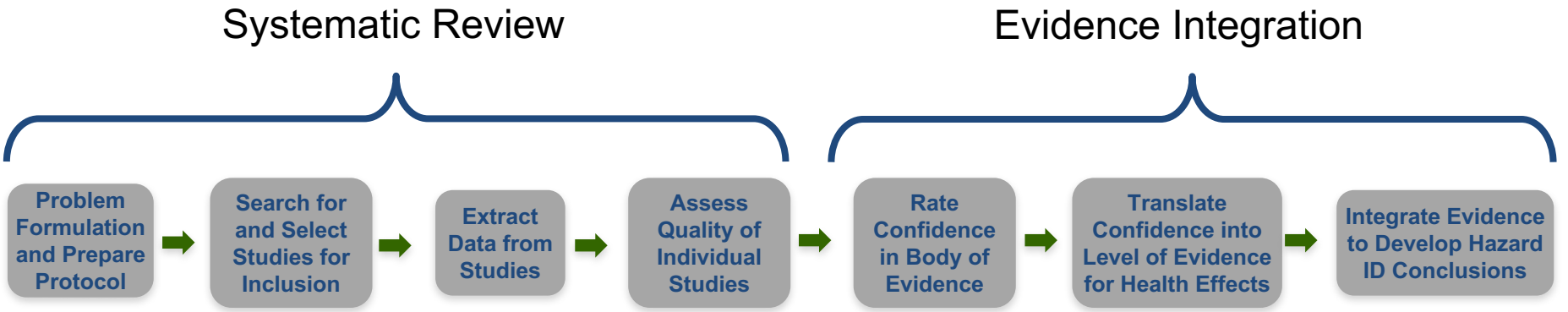
- Address breadth of relevant data
  - Wide range of human study designs
  - Animal studies
  - Mechanistic studies (*in vitro* and other relevant data)
- Procedure to integrate evidence streams
- Dual role for mechanistic data
  - 1) Integrate with human/animal evidence
  - 2) Potential to support decisions in absence of human or animal data



Alternatives often have small, primarily mechanistic data sets

Extends Existing Systematic Review Methods

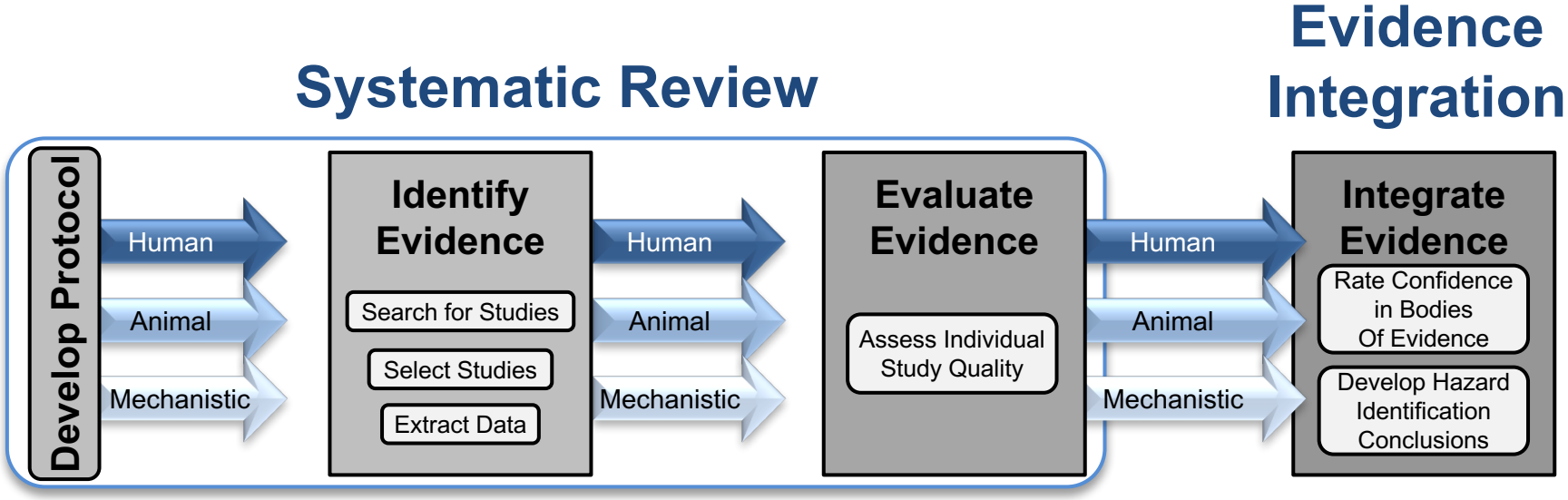
- **Evidence Integration**
  - The process for reaching conclusions on the NTP's confidence across a body of studies within an evidence stream (i.e., human and animal data separately) and then integrating those conclusions across the evidence streams with consideration of other relevant data such as supporting evidence from mechanistic studies
  - Lack of consensus on term "Weight of Evidence"? (Weed et al., 2005)



Systematic Review and Evidence Integration

Increased transparency and objectivity

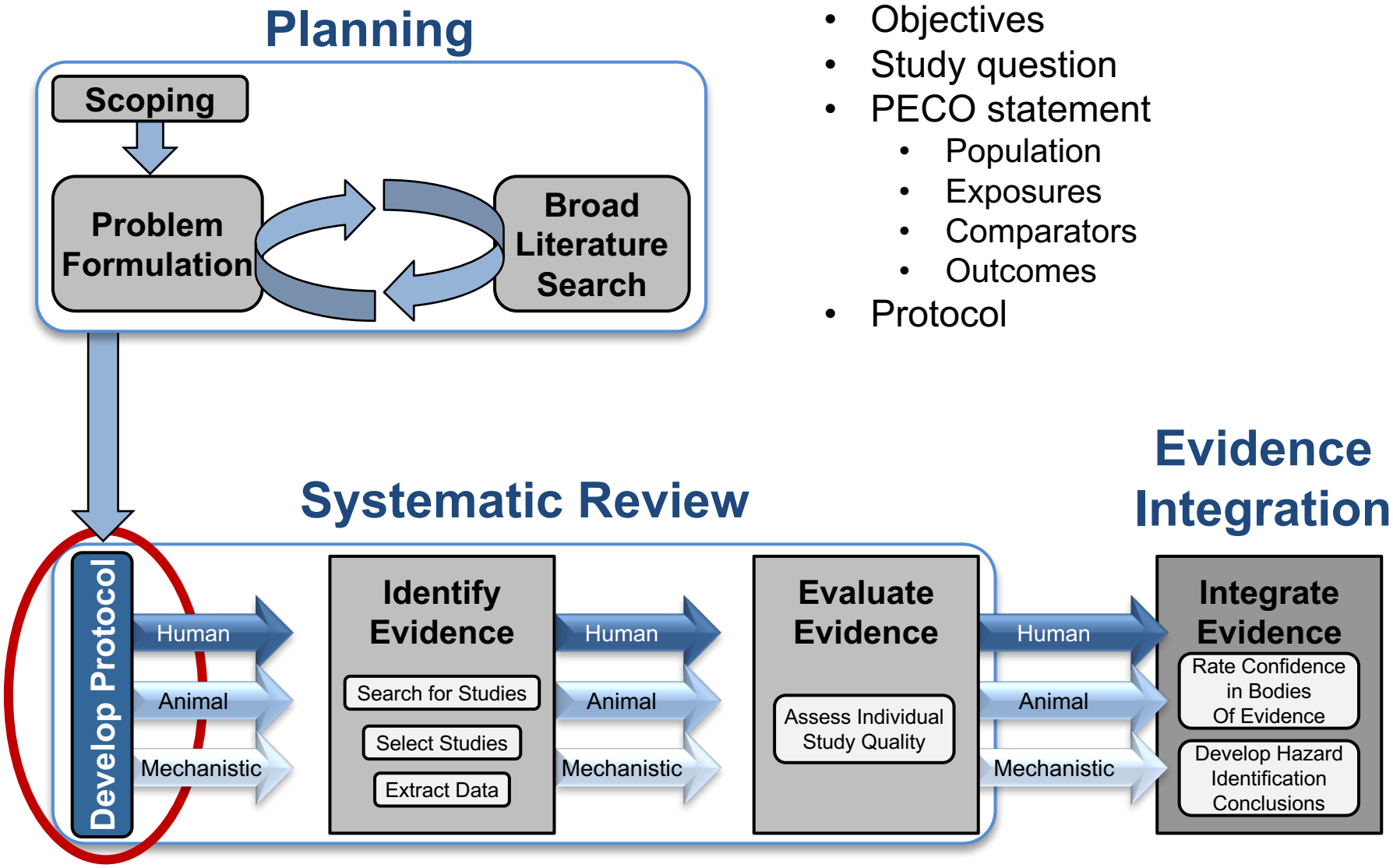
- Applied to all three evidence streams (human, animal, mechanistic)
- Framework for documenting the basis of scientific judgments
  - Individual study quality
  - Confidence in bodies of evidence
  - Hazard ID conclusions
- Procedures to integrate evidence streams



Planning the Evaluation

Planning step develops

- Objectives
- Study question
- PECO statement
  - Population
  - Exposures
  - Comparators
  - Outcomes
- Protocol



Example: Evaluation of PFOA/PFOS immunotoxicity

- PECOS for Human and non-human animal evidence
  - **Population:** Humans or animals without restriction on sex or life stage
  - **Exposure:** PFOA (CAS# 335-67-1) or PFOS (CAS# 1763-23-1) or their salts
  - **Comparator:** Humans or animals exposed to lower levels or vehicle
  - **Outcomes:**
    - *Primary outcomes:* Immune-related diseases and measures of immune function
    - *Secondary outcomes:* Immunostimulation and observational immune endpoints

- What about Mechanistic evidence?
  - **Outcomes:**
    - *Primary outcomes:* Measures of immune function after ***in vitro* exposure**
    - *Secondary outcomes:* Observational immune endpoints after ***in vitro* exposure**

Consider Supplementing PECO Statement

Mechanistic data should address relevant outcomes

- How broadly should one collect mechanistic evidence?
  - For narrow, well-defined outcomes
    - PECO for mechanistic data developed in protocol
    - May require technical experts to ID mechanisms and list of search terms
  - For multiple outcomes or general health effects reviews
    - 1) Identify relevant mechanistic data if clearly known
    - 2) Plan to supplement with outcome-relevant mechanistic data
      - After health effects are identified, additional search may be warranted
      - All changes are documented

Relevant outcomes may not be clear until after human and animal data are collected

Identifying the Evidence

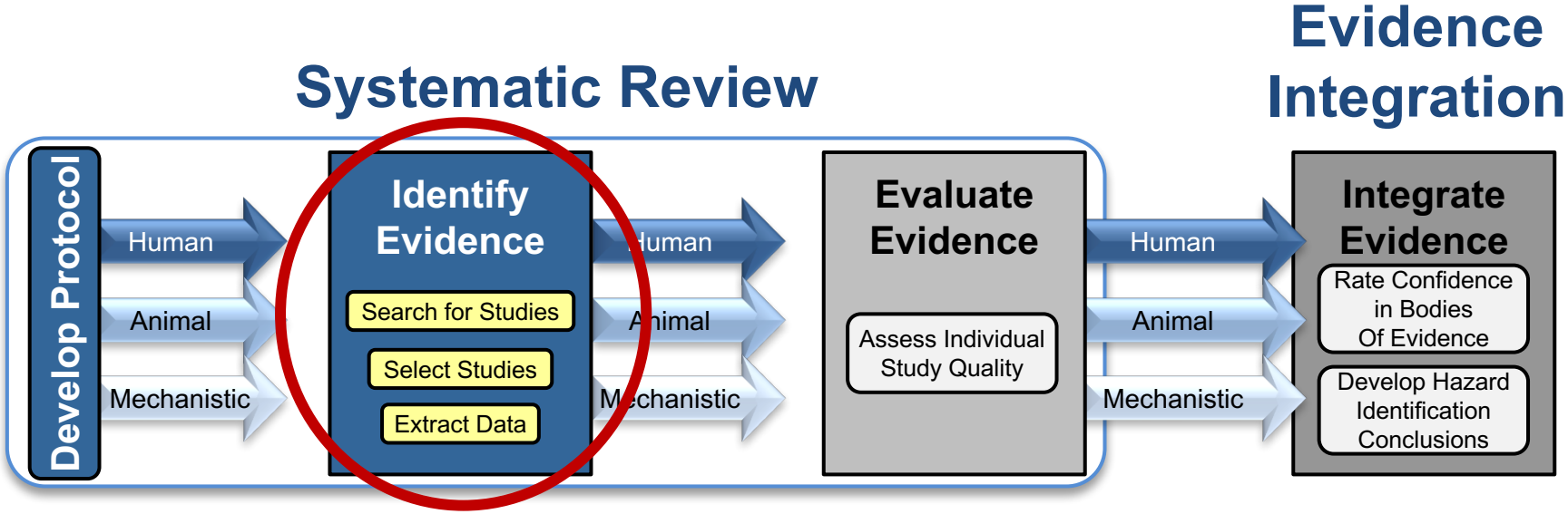
- Search for Studies
- Select Studies
- Extract Data

Challenge for Mechanistic Data

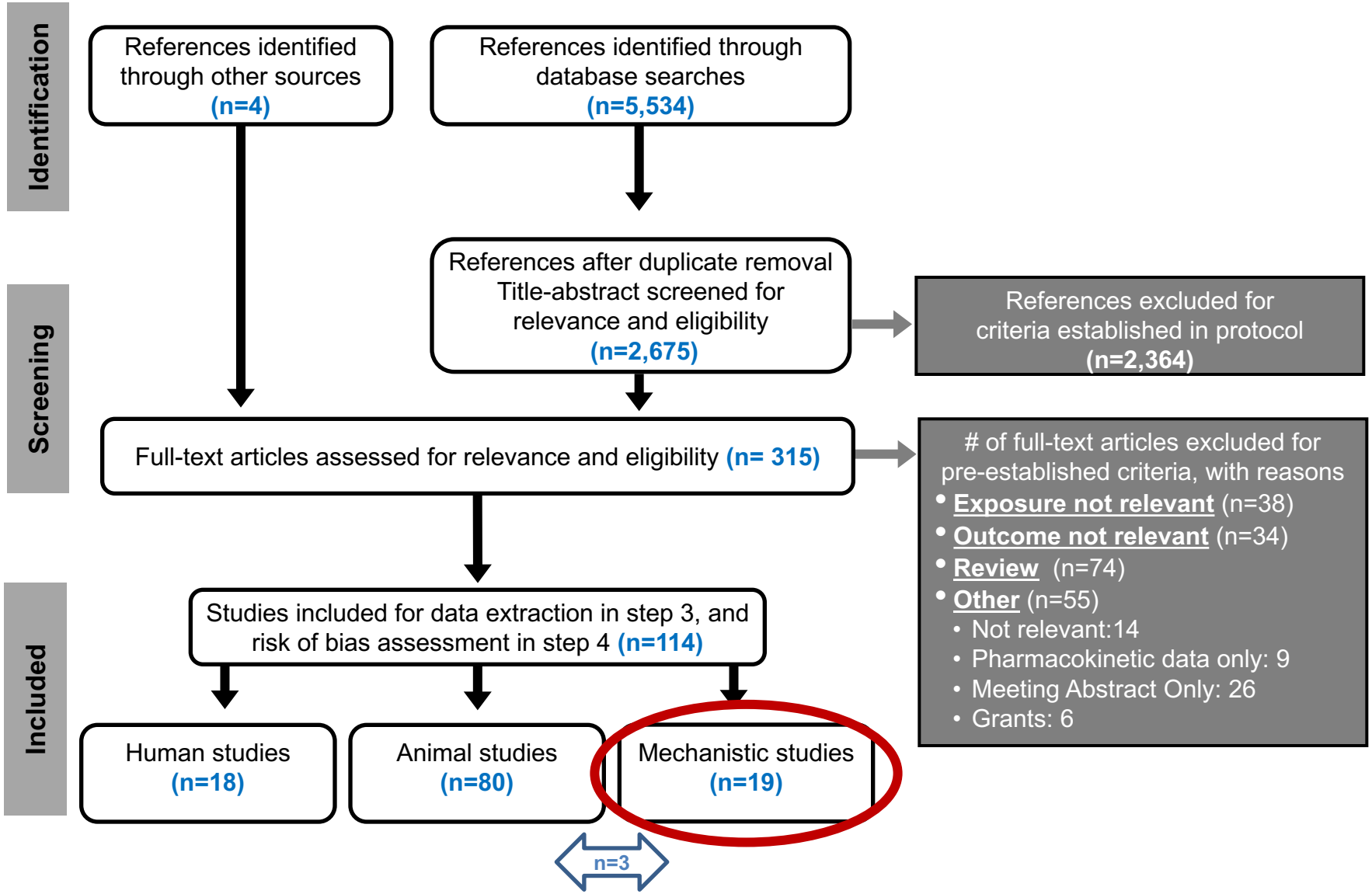
How narrowly do you define "relevant" studies?

Or

Which mechanistic data are relevant?

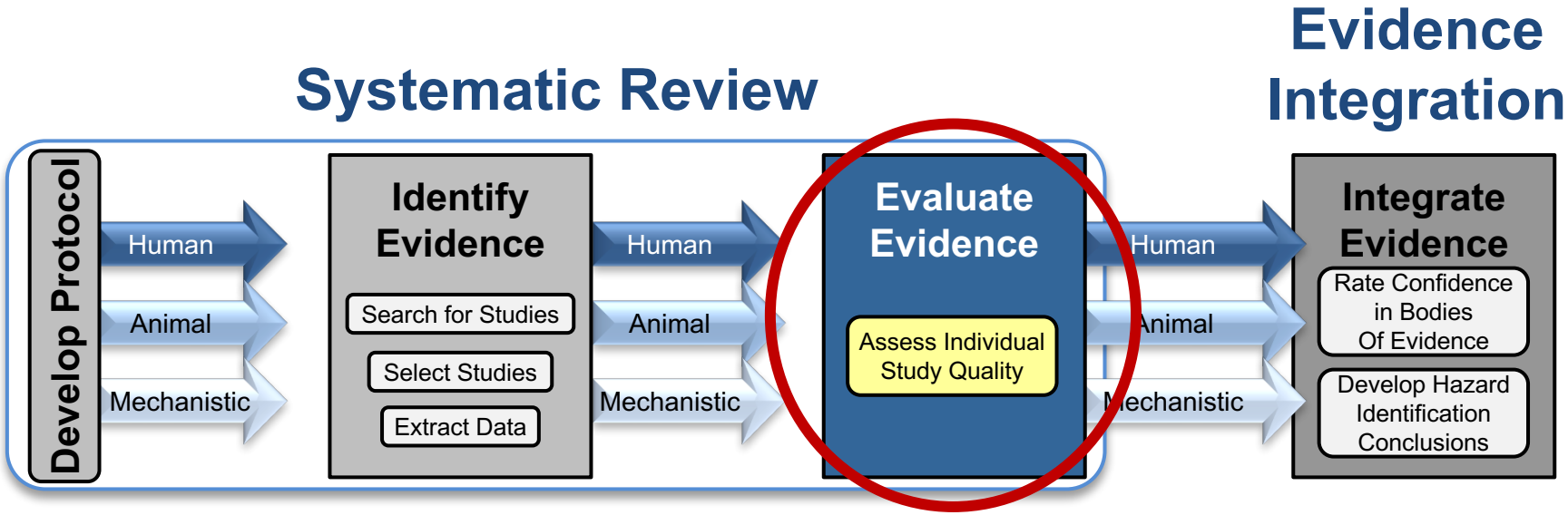


Selecting Studies: PFOA/PFOS Immunotoxicity



Assessing Individual Study Quality

- Multiple aspects of "quality" and "utility" are important
  - **Risk of bias or internal validity**
    How credible are findings based on study design and conduct?
  - **Reporting quality**
    How well was the study reported?
  - **Directness and applicability**
    How well does the study address the topic under review?



Published Approaches and Challenges

- Established risk of bias tools for randomized controlled trials
- Emerging methods on how to assess risk of bias for:
  - Observational human studies
  - Animal studies
  - Mechanistic studies?
    - OHAT draft approach

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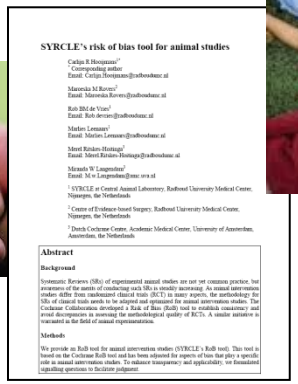
AHRQ EBPCs



SYRCLE's risk of bias tool for animal studies

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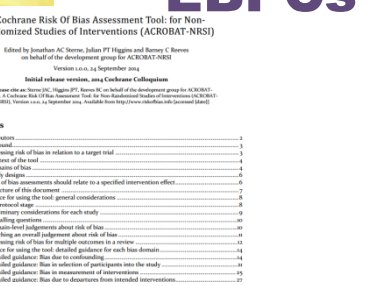
Abstract

Background

Systematic Reviews (SRs) of experimental animal studies are not yet common practice, but awareness of the merits of conducting such SRs is steadily increasing. As animal intervention studies differ from randomized clinical trials (RCT) in many aspects, the methodology for SRs of clinical trials needs to be adapted and optimized for animal intervention studies. The Cochrane Collaboration developed a Risk of Bias (RoB) tool to establish consistency and avoid discrepancies in assessing the methodological quality of RCTs. A similar initiative is warranted in the field of animal experimentation.

Methods

We provide an RoB tool for animal intervention studies (SYRCLE's RoB tool). This tool is based on the Cochrane RoB tool and has been adjusted for aspects of bias that play a specific role in animal intervention studies. To enhance transparency and applicability, we formulated signalling questions to facilitate judgment.



A Cochrane Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI)

Edited by Jonathan AC Sterne, Julian PT Higgins and Barney C Reeves on behalf of the development group for ACROBAT-NRSI

Version 1.0.0, 24 September 2014

Initial release version, 2014 Cochrane Colloquium

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Contents

A "Parallel" Approach Across Evidence Streams

- Predefined set of questions adapted from AHRQ to address


Human studies


Animal toxicology studies


In vitro/mechanistic studies

- Study design determines which questions are applicable
  - Answers equate to risk of bias rating for each question
  - Answers on 4-point scale from Clarity Group
  - Evaluation is endpoint specific

++	Definitely Low risk of bias
+	Probably Low risk of bias
–	Probably High risk of bias
––	Definitely High risk of bias

OHAT Risk of Bias Questions

Single set of Questions

1. Was administered dose or exposure level adequately randomized?
2. Was allocation to study groups adequately concealed?
3. Did selection of study participants result in appropriate comparison groups?
4. Did study design or analysis account for important confounding and modifying variables?
5. Were experimental conditions identical across study groups?
6. Were research personnel and human subjects blinded to study group during the study?
7. Were outcome data complete without attrition or exclusion from analysis?
8. Can we be confident in the exposure characterization?
9. Can we be confident in the outcome assessment?
10. Were all measured outcomes reported?
11: Were there other potential threats to internal validity?

Questions for Experimental Studies

1. Was administered dose or exposure level adequately randomized?
2. Was allocation to study groups adequately concealed?
3. Did selection of study participants result in appropriate comparison groups?
4. Did study design or analysis account for important confounding and modifying variables?
5. Were experimental conditions identical across study groups?
6. Were research personnel and human subjects blinded to study group during the study?
7. Were outcome data complete without attrition or exclusion from analysis?
8. Can we be confident in the exposure characterization?
9. Can we be confident in the outcome assessment?
10. Were all measured outcomes reported?
11. Other potential threats to internal validity?

Developing Each Risk of Bias Rating

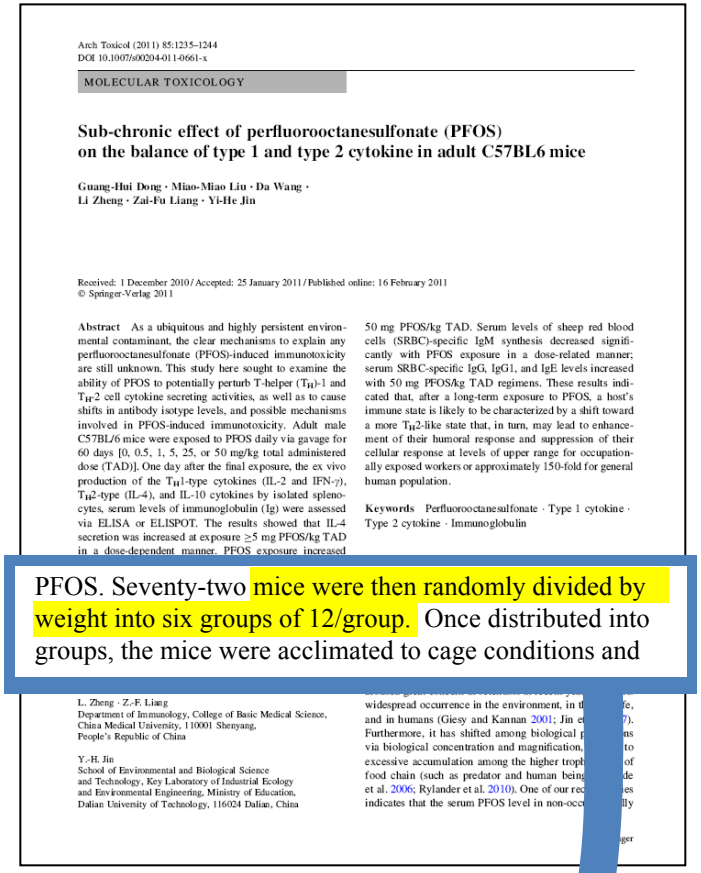
Evidence from Study Report or Author Contact

Specific Guidance

Guidance defines all 4 ratings for each question

1. Randomization

- ++ **Definitely Low risk of bias:** There is direct evidence that animals were allocated to any study group including controls using a method with a random component. Restricted randomization (e.g., blocked) will be considered low bias …
- \+ **Probably Low risk of bias:** There is indirect evidence that animals were allocated to any study group including controls using a method with a random component (i.e., authors state that allocation was random …
- – **Probably High risk of bias:** ….
- – – **Definitely High risk of bias:** …



Arch Toxicol (2011) 85:1235–1244
DOI 10.1007/s00204-011-0661-x

MOLECULAR TOXICOLOGY

Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice

Guang-Hui Dong · Miao-Miao Liu · Da Wang · Li Zheng · Zai-Fu Liang · Yi-He Jin

Received: 1 December 2010 / Accepted: 25 January 2011 / Published online: 16 February 2011
© Springer-Verlag 2011

Abstract As a ubiquitous and highly persistent environmental contaminant, the clear mechanisms to explain any perfluorooctanesulfonate (PFOS)-induced immunotoxicity are still unknown. This study here sought to examine the ability of PFOS to potentially perturb T-helper (T_H)-1 and T_H-2 cell cytokine secreting activities, as well as to cause shifts in antibody isotype levels, and possible mechanisms involved in PFOS-induced immunotoxicity. Adult male C57BL/6 mice were exposed to PFOS daily via gavage for 60 days [0, 0.5, 1, 5, 25, or 50 mg/kg total administered dose (TAD)]. One day after the final exposure, the ex vivo production of the T_H1-type cytokines (IL-2 and IFN-γ), T_H2-type (IL-4), and IL-10 cytokines by isolated splenocytes, serum levels of immunoglobulin (Ig) were assessed via ELISA or ELISPOT. The results showed that IL-4 secretion was increased at exposure ≥5 mg PFOS/kg TAD in a dose-dependent manner. PFOS exposure increased 50 mg PFOS/kg TAD. Serum levels of sheep red blood cells (SRBC)-specific IgM synthesis decreased significantly with PFOS exposure in a dose-related manner; serum SRBC-specific IgG, IgG1, and IgE levels increased with 50 mg PFOS/kg TAD regimens. These results indicated that, after a long-term exposure to PFOS, a host's immune state is likely to be characterized by a shift toward a more T_H2-like state that, in turn, may lead to enhancement of their humoral response and suppression of their cellular response at levels of upper range for occupationally exposed workers or approximately 150-fold for general human population.

Keywords Perfluorooctanesulfonate · Type 1 cytokine · Type 2 cytokine · Immunoglobulin

PFOS. Seventy-two mice were then randomly divided by weight into six groups of 12/group. Once distributed into groups, the mice were acclimated to cage conditions and

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widespread occurrence in the environment, in t… fe, and in humans (Giesy and Kannan 2001; Jin e… 7). Furthermore, it has shifted among biological p… ns via biological concentration and magnification, … to excessive accumulation among the higher troph… of food chain (such as predator and human being… de et al. 2006; Rylander et al. 2010). One of our rec… es indicates that the serum PFOS level in non-occ… lly

Was administered dose or exposure adequately randomized? | ++

Support for final rating: *"mice were randomly divided by weight"*

Extending Methods to Mechanistic Studies

- **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* /mechanistic applicability**
    - Potential differences between cells that comprise different groups will depend on study design
      - Finite cell strains with document number of population doublings
      - Primary cells from multiple donors
      - Homogeneous cell suspension

Risk of Bias for Experimental Studies

In vitro-specific Guidance

1. Was administered dose or exposure level adequately randomized?

Experimental Animal Studies

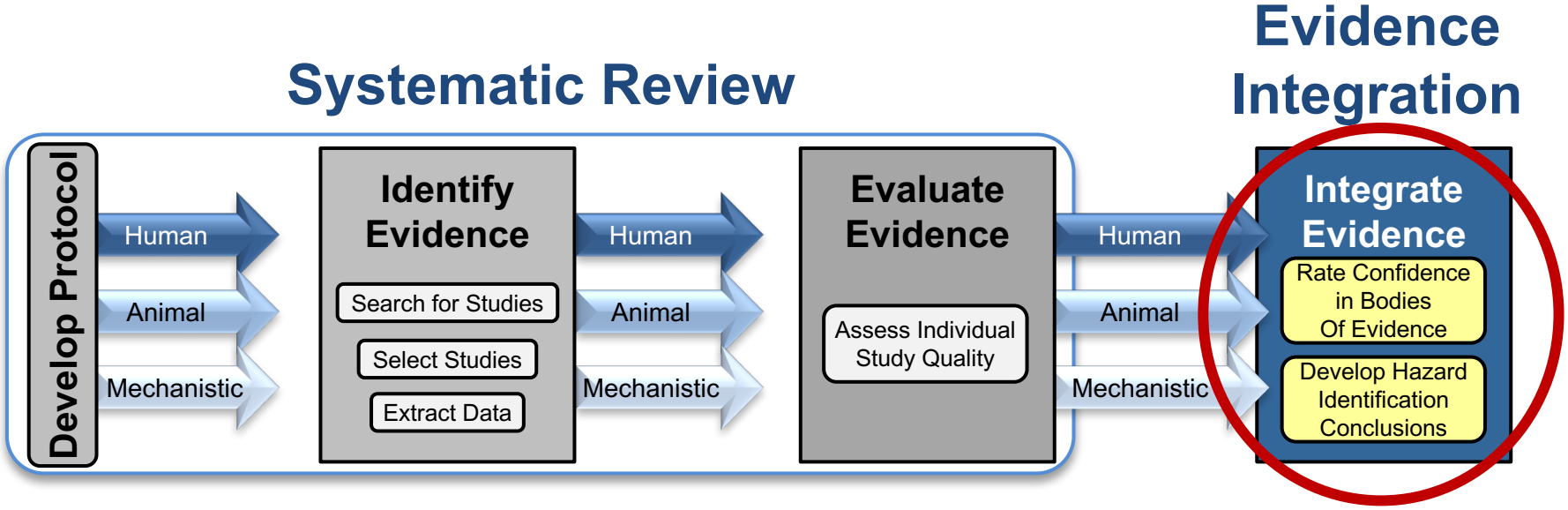
- Definitely low risk of bias
  - Direct evidence that animals were allocated to any study group including controls using a method with a random component.
  - Restricted randomization (e.g., blocked randomization) is considered acceptable
  - Requires concurrent controls

In vitro studies

- Definitely low risk of bias
  - Direct evidence that cells were allocated to any study group including controls using a method with a random component.
  - **OR** all cells in culture come from a homogenous cell suspension recently collected from cell culture vessels following appropriate techniques
  - Requires concurrent control

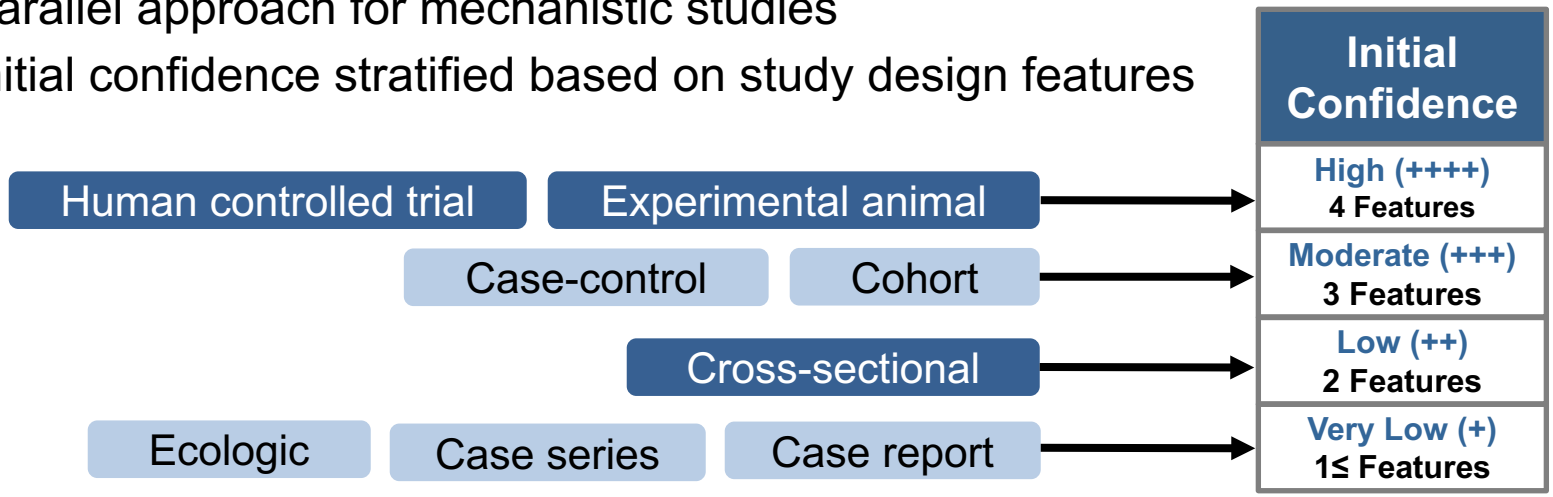
Evidence Integration

- **Rating Confidence in Bodies of Evidence**
- **Integrating Evidence Streams for Hazard ID**



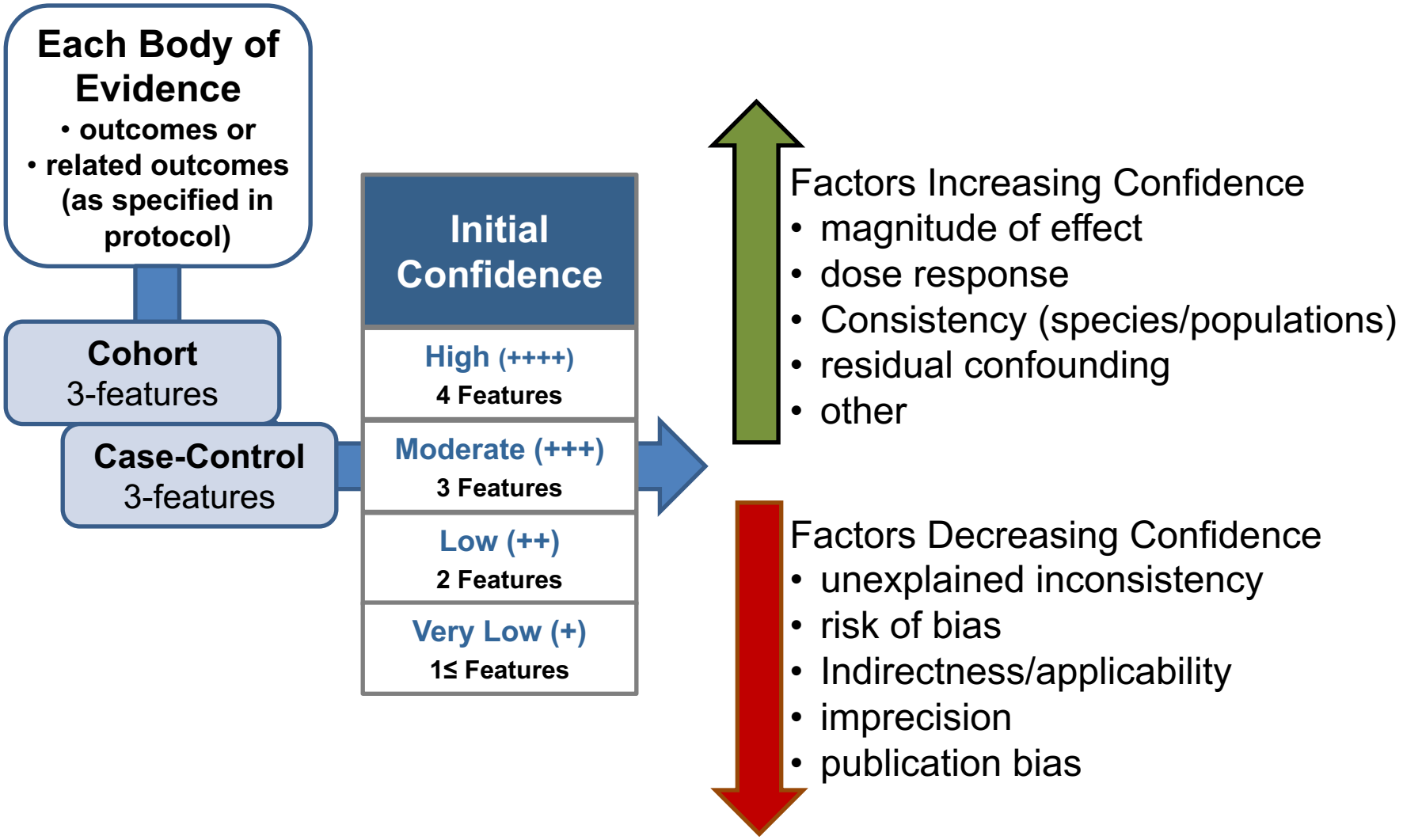
Rating Confidence in Bodies of Evidence

- **Developing confidence ratings**
  - How confident are you that findings from a group of studies reflect the true relationship between exposure to a substance and effect?
- **GRADE Working Group**
  - Widely accepted method for rating confidence in a body of evidence on healthcare interventions
- **OHAT Framework**
  - Guidance for human and animal studies
  - Parallel approach for mechanistic studies
  - Initial confidence stratified based on study design features



Rating Confidence in the Body of Evidence

Example from Human Observational Studies



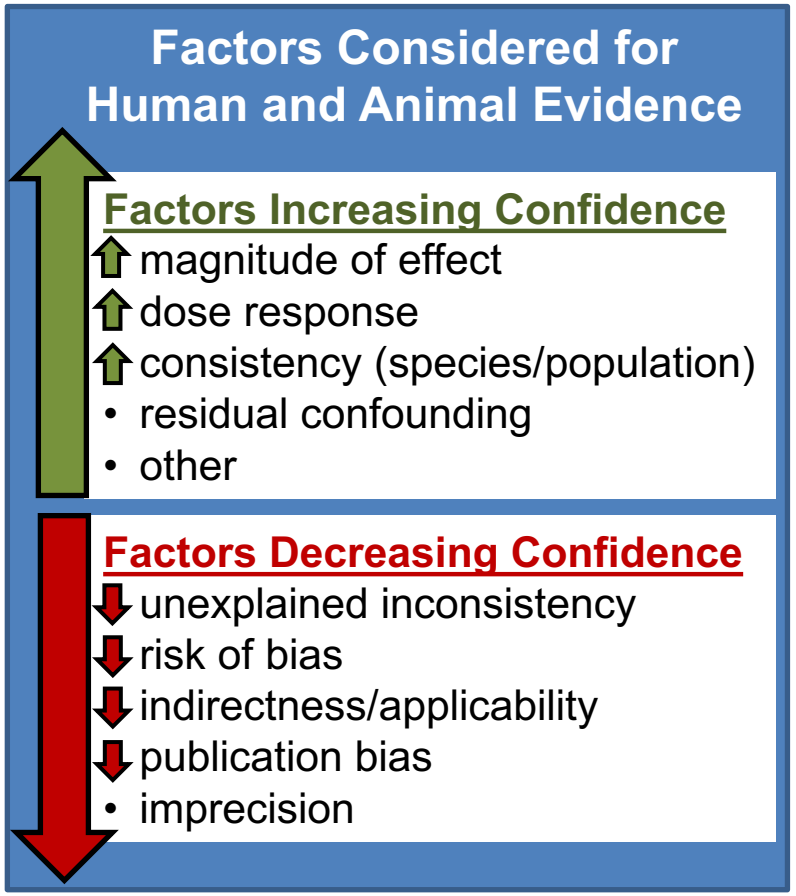
Rating Confidence in the Body of Evidence

Extending Methods to Mechanistic data

- **Rating confidence in bodies of evidence**
  - Factors increasing/decreasing confidence

- **Parallel factors for mechanistic data**
  - ↑ magnitude of effect ≈ potency
  - ↑ dose-response
  - ↑↓ consistency
  - ↓ risk of bias
  - ↓ directness/applicability ≈ relevance
    - pathway for human health
    - concentration for human exposure
  - ↓ publication bias

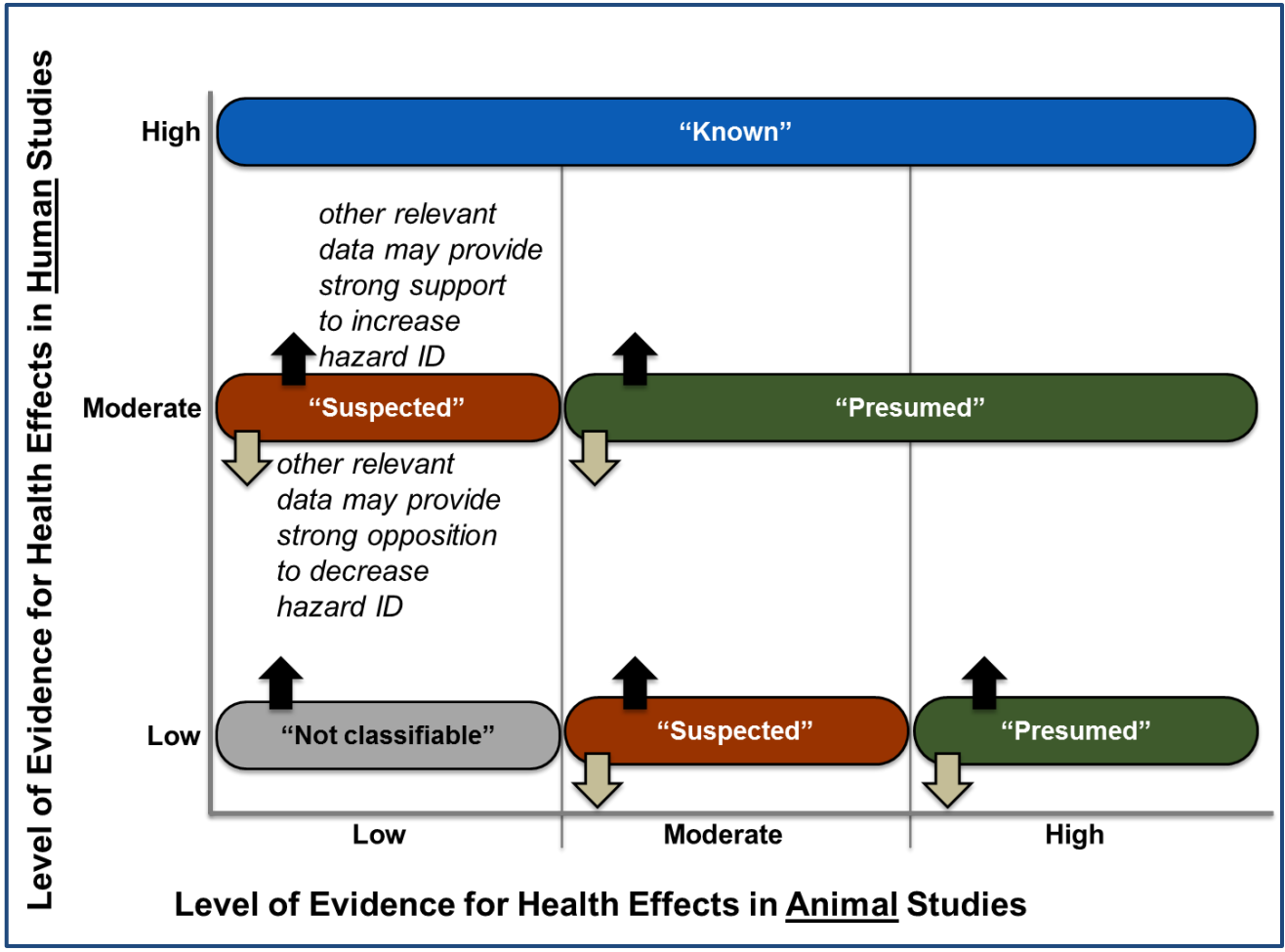
- **Other developing approaches**
  - Similarity profiles
  - Exploring utility of pathway approach (AOP)



Evidence Integration: Develop Hazard ID

Two part process

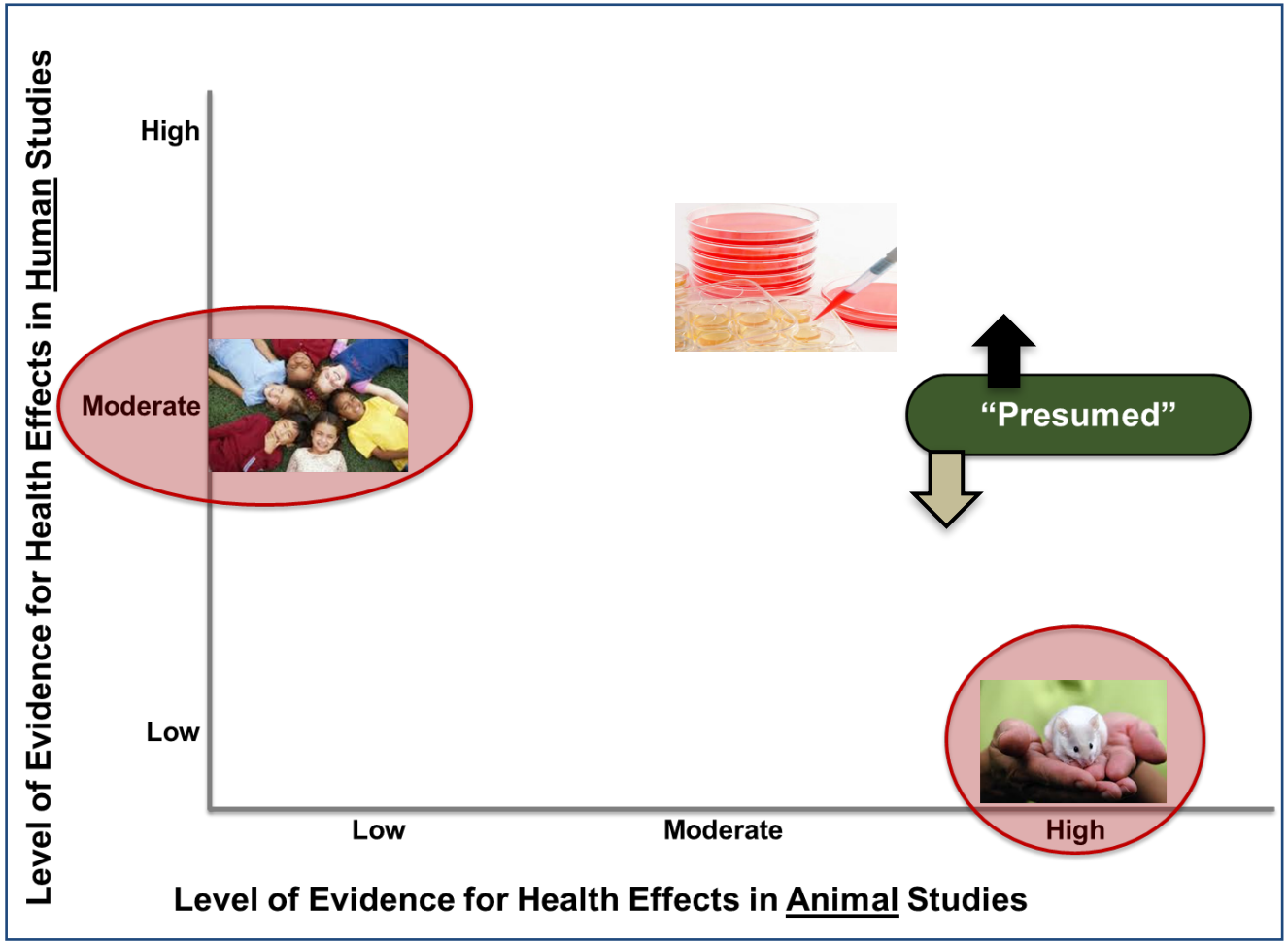
- Consider human and animal evidence together
- Consider impact of mechanistic data
  - *in vitro* data, or
  - upstream indicators



Evidence Integration: Develop Hazard ID

Two part process

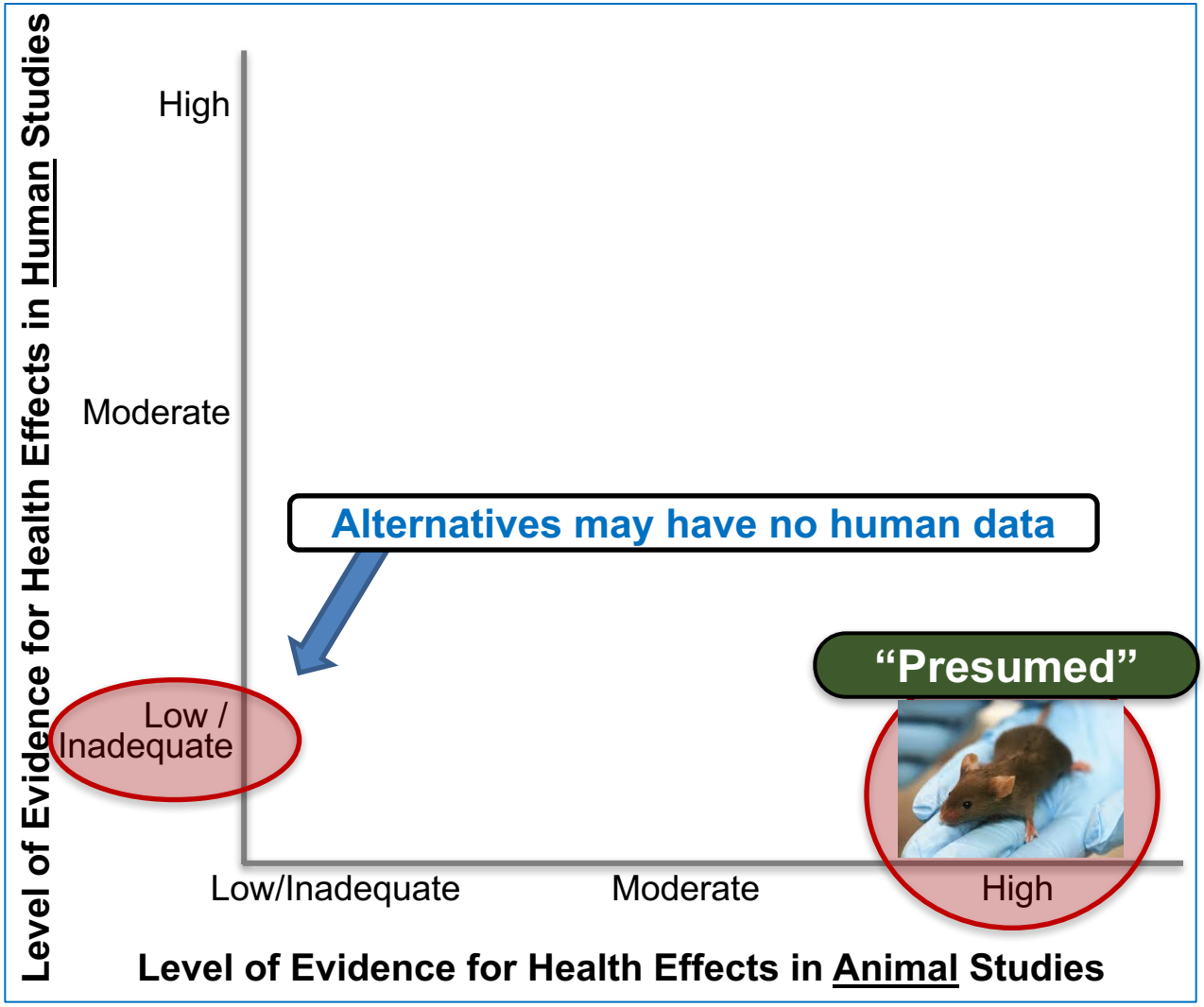
- Consider human and animal evidence together
- Consider impact of mechanistic data
  - *in vitro* data, or
  - upstream indicators
    - strong support?
    - strong opposition?



Evidence Integration: Develop Hazard ID

Level of Evidence Reflects Confidence in Data

- Low / Inadequate Level of Evidence
  - Low confidence in body of evidence for an association between exposure and health outcome
  - Or no data available



Evidence Integration: Develop Hazard ID

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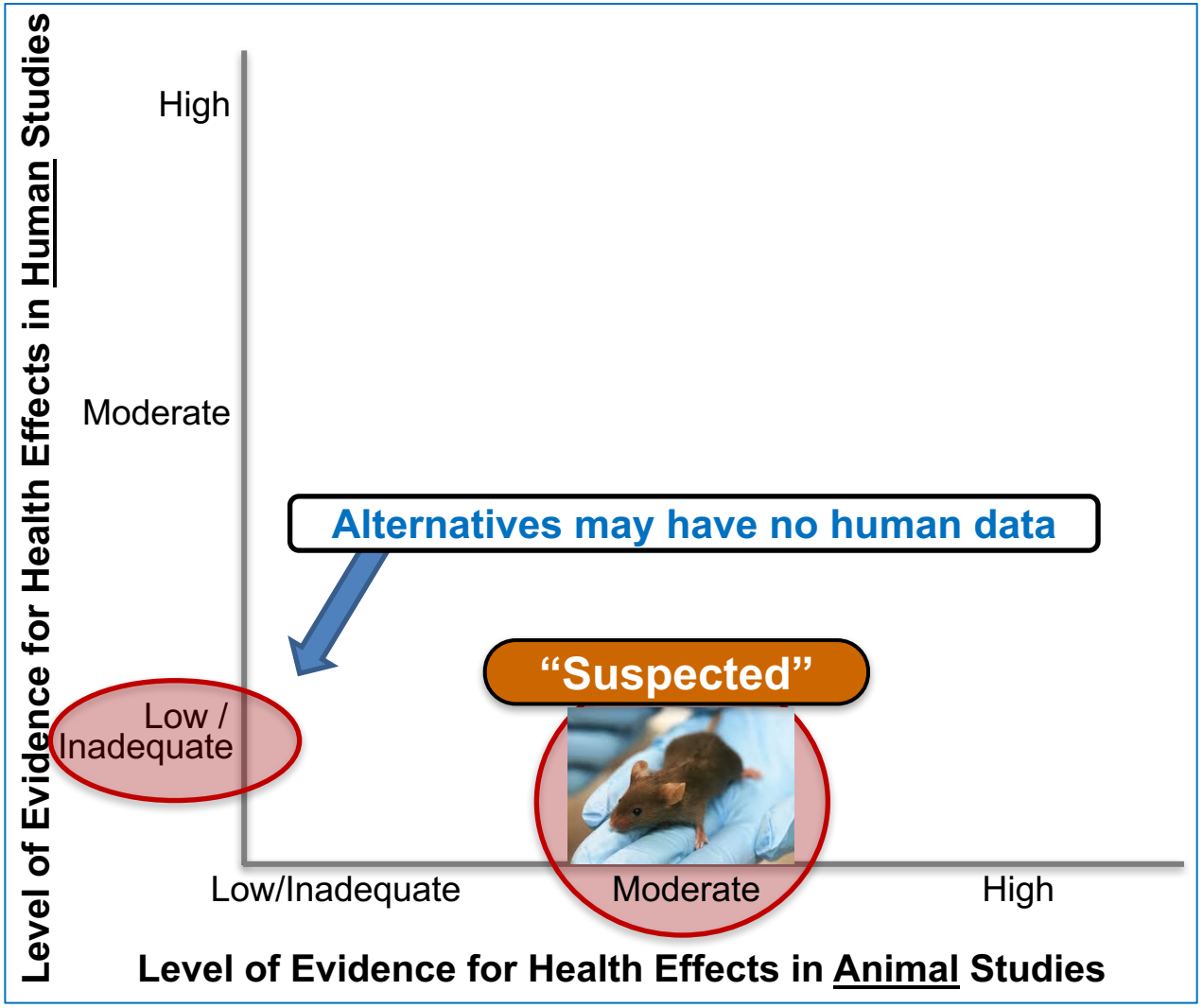
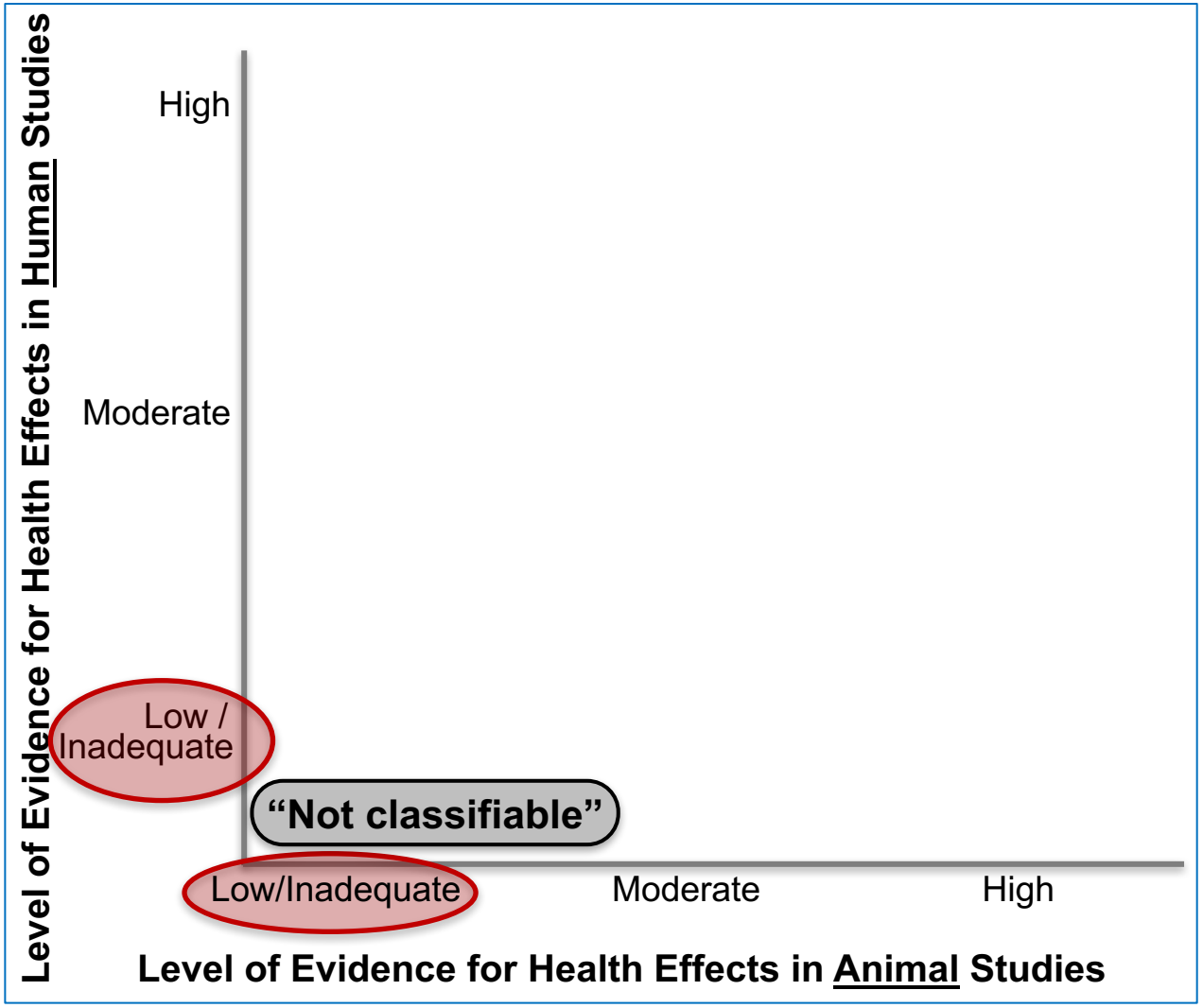


Evidence Integration: Develop Hazard ID

Level of Evidence Reflects Confidence in Data

- Low / Inadequate Level of Evidence
  - Low confidence in body of evidence for an association between exposure and health outcome
  - Or no data available

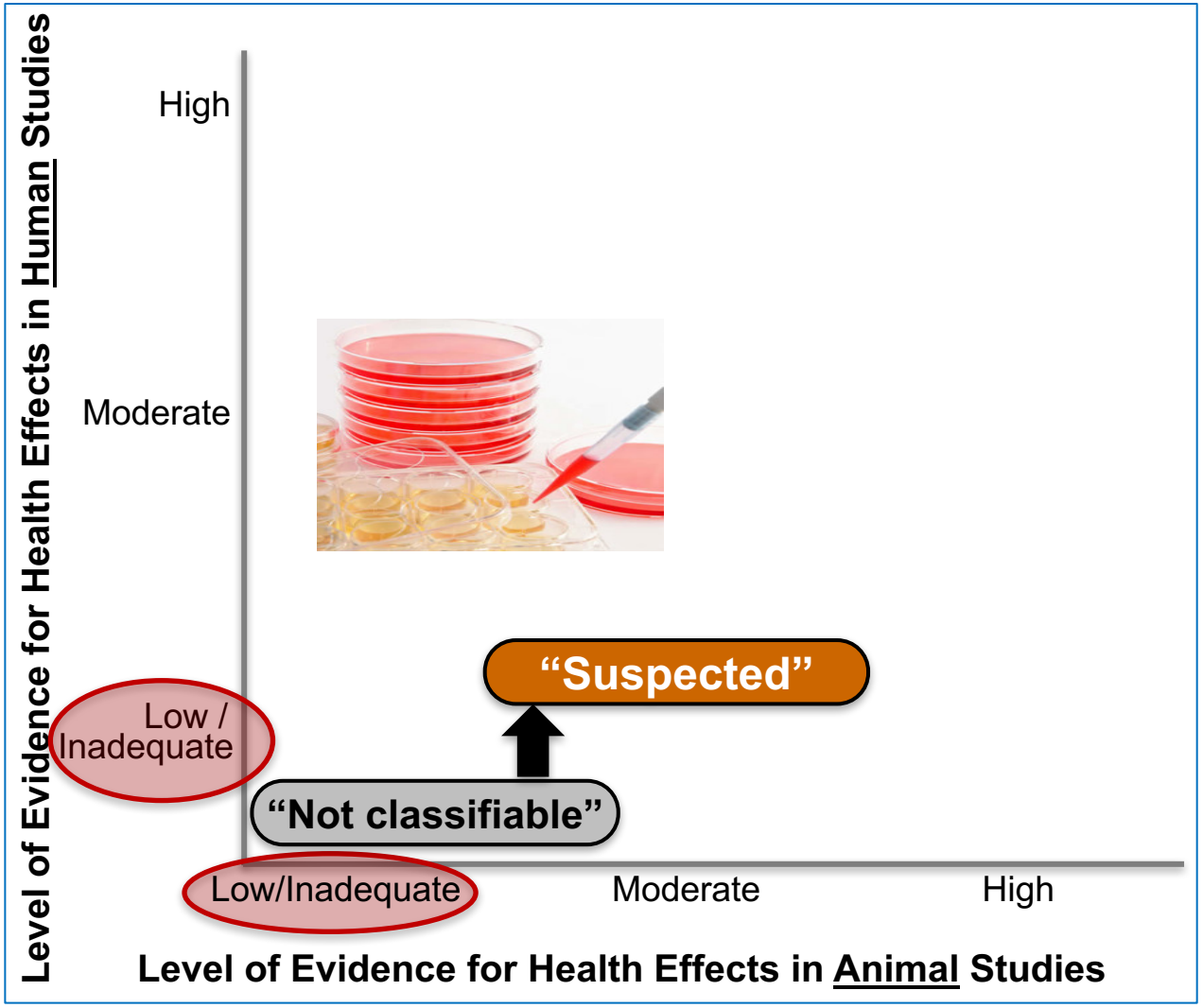
Alternatives may have no human data and no animal data



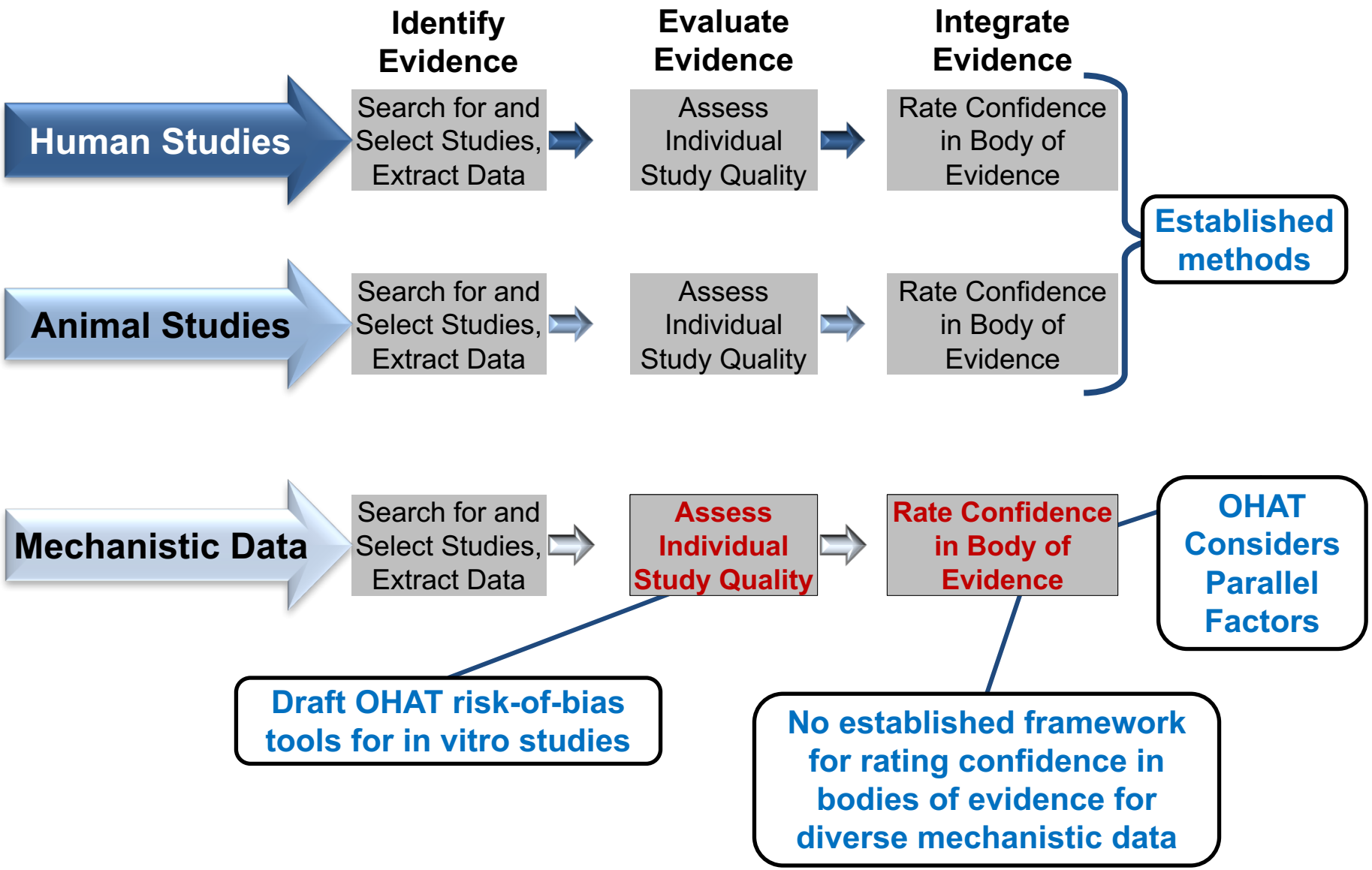
Evidence Integration: Develop Hazard ID

Challenging decisions in absence of human or animal data

- Mechanistic data
  - *in vitro* data, or
  - upstream indicators
    - It is envisioned that strong evidence for a relevant biological process from mechanistic data could result in a conclusion of "suspected" in the absence of human or animal data



Challenges for Mechanistic Data



Systematic Review and Mechanistic Data

- Systematic review procedures are being used to address questions in toxicology and environmental health
- The OHAT Framework uses a parallel approach for all three evidence streams (human, animal, mechanistic studies)
- Alternatives are likely to support lower confidence but still potential to support decision making
- Focus for methods development and refinement
  - ✓ Risk of bias tool for *in vitro*/mechanistic studies
  - ✓ Developing confidence ratings in mechanistic studies for integrating with human/animal effects
  - – Developing confidence ratings in mechanistic studies for use as stand-alone evidence

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  - **Jamie Dewitt,** ECU Department Pharmacology and Toxicology
  - **Tony Fletcher,** London School of Hygiene and Tropical Med.
  - **Dori Germolec,** NIEHS/NTP
  - **Christopher Lau,** US EPA, ORD/NHEERL
  - **Roberta Scherer,** Johns Hopkins, Bloomberg School Pub.H.

- **Office of Science Information Management**
  - Stephanie Holmgren

- **National Toxicology Program**
  - John Bucher, Associate Director
  - Mary Wolfe, Director OLPR
  - Lori White (OLPR)

- **NTP BSC Working Group**

- **Navigation Guide Work Group**

- **Interagency Comments**
  - ATSDR
  - EPA
  - DoD
  - FDA
  - NIOSH

- **Public Comments**

Thank You

Discussion Questions

- What are the strengths and weaknesses of high throughput screening data for both data gap filling and as primary data to support alternatives assessment?
- How useful/usable is Tox 21 data for both comparing chemical alternatives and designing safer molecules at the present time.
- What are the challenges and opportunities to more effective integration of these data streams in chemical alternatives assessment?

Next Webinars

Lowell Center for Sustainable Production
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Alternatives Assessment 124: The Use of QSARs, Read Across, and Analogue Approaches to Inform Decision Making in Alternatives Assessments
February 17, 2015, 12pm est

Presenters: Dr. Jay Tunkel and Ms. Cathy Rudisill, SRC, Inc.

Alternatives Assessments under REACH: Lessons Learned
Feb. 26, 2015, 11am est

Presenters:
Thierry Nicot and Denis Mottet, European Chemicals Agency
Tatiana Santos, European Environment Bureau
Julius Waller EPPA Inc.