NTP
National Toxicology Program

Extending a Risk-of-Bias Approach to Address *In Vitro* Studies

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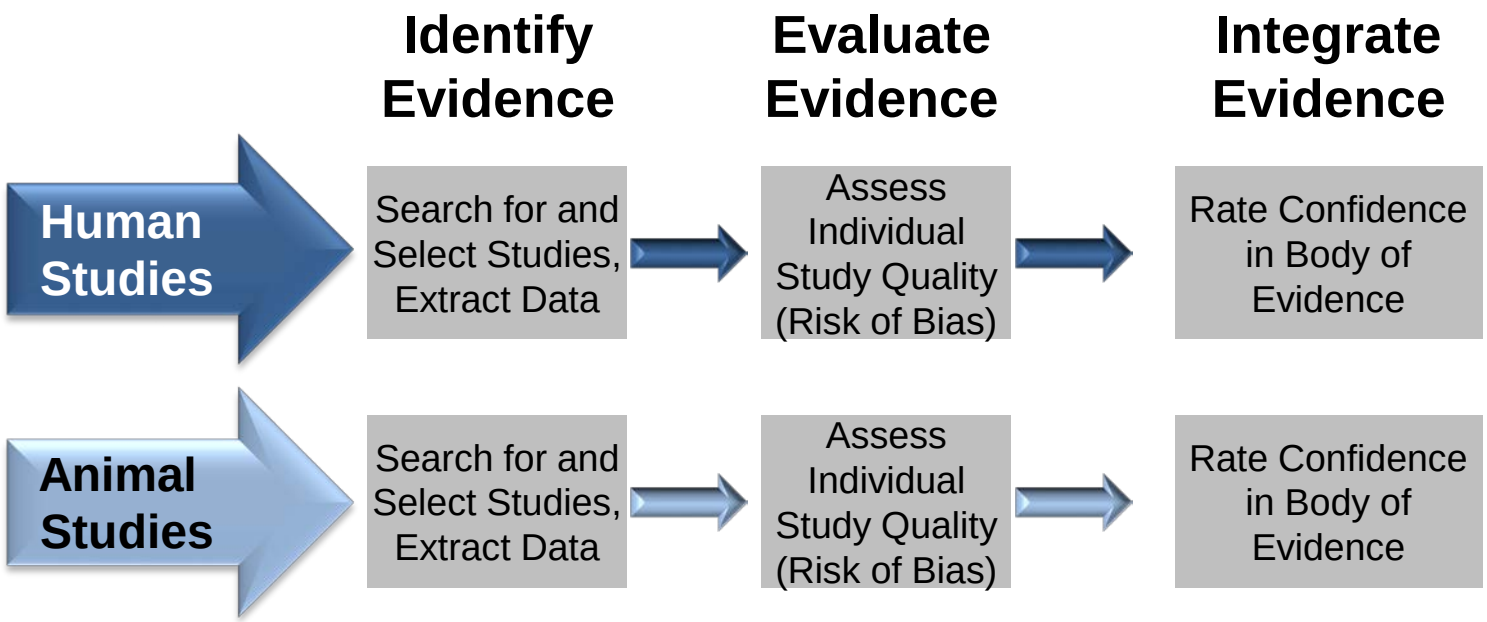
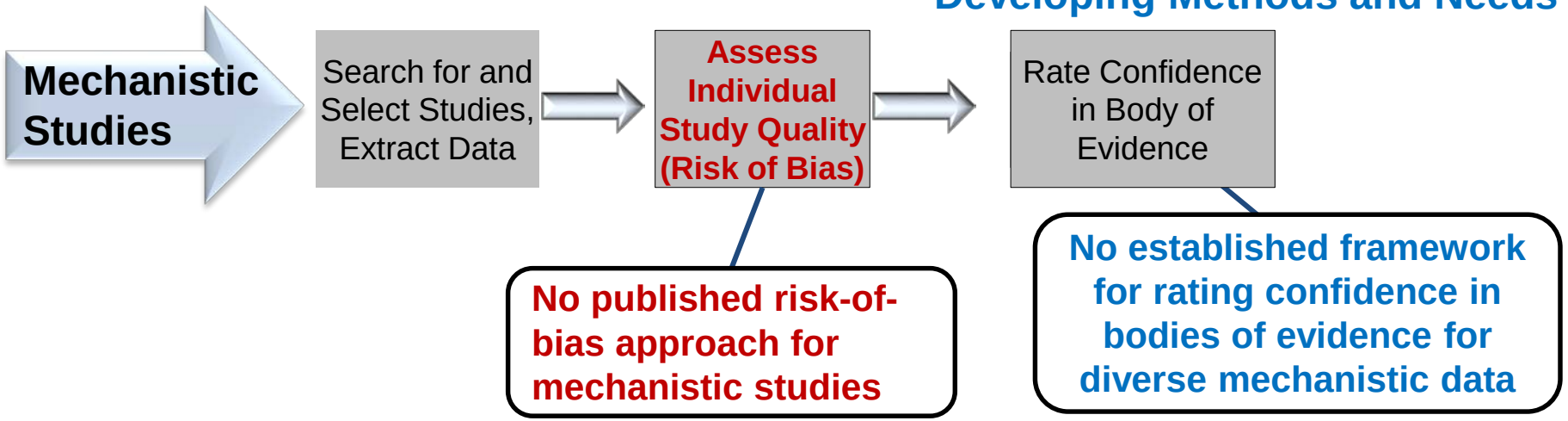
Lack of Methods for Mechanistic Studies



Established Methods

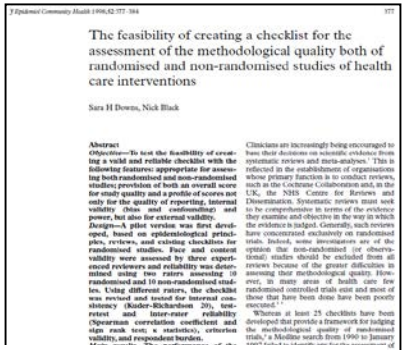
Developing Methods and Needs



Assessing Risk of Bias

- **Published approaches and risk of bias tools**
  - Established tools for randomized controlled trials
  - Multiple tools for observational human studies
  - Emerging tools for animal studies
- **What about Mechanistic studies?**

OHAT project to extend risk of bias approach to in vitro exposure studies


The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions

Sara H Downes, Nick Black

Abstract

Objective—To test the feasibility of creating a valid and reliable checklist with the following features: appropriate for assessing both randomised and non-randomised studies; provision of both an overall score for study quality and a profile of scores not only for the quality of reporting, internal validity (bias and confounding) and power, but also for external validity.

Design—A pilot version was first developed, based on epidemiological principles, reviews, and existing checklists for randomised studies. Face and content validity were assessed by three experienced reviewers and reliability was determined using two raters assessing 10 randomised and 10 non-randomised studies. Using different raters, the checklist was revised and tested for internal consistency (Kuder-Richardson 20), test-retest and inter-rater reliability (Spearman correlation coefficient and sign rank test; κ statistics), criterion validity, and respondent burden.

Clinicians are increasingly being encouraged to base their decisions on scientific evidence from systematic reviews and meta-analyses.[1] This is reflected in the establishment of organisations whose primary function is to conduct reviews, such as the Cochrane Collaboration and, in the UK, the NHS Centre for Reviews and Dissemination. Systematic reviews must seek to be comprehensive in terms of the evidence they examine and objective in the way in which the evidence is judged. Generally, such reviews have concentrated exclusively on randomised trials. Indeed, some investigators are of the opinion that non-randomised (or observational) studies should be excluded from all reviews because of the greater difficulties in assessing their methodological quality. However, in many areas of health care few randomised controlled trials exist and most of those that have been done have been poorly executed.[2]

Whereas at least 25 checklists have been developed that provide a framework for judging the methodological quality of randomised trials,[3] a Medline search from 1990 to January


Collaborative Approach to Meta Analysis and

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Animal Data from Experimental Studies


SYRCLE's risk of bias tool for animal studies


THE COCHRANE COLLABORATION®


Methods Guide
for Comparative Effectiveness Reviews

Assessing the
in Systematic


AHRQ

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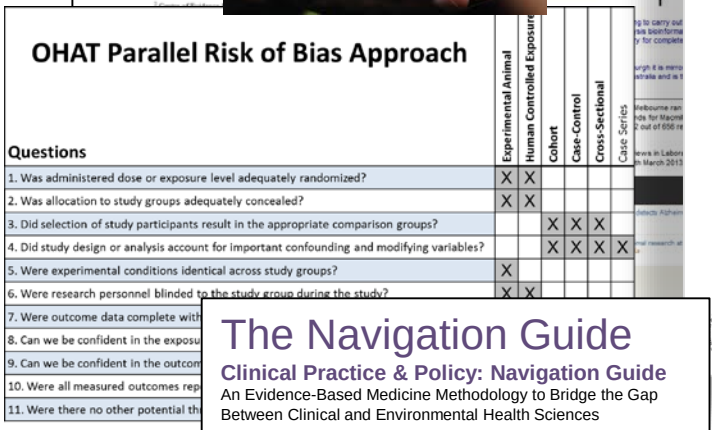
OHAT Parallel Risk of Bias Approach

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	X				
7. Were outcome data complete with						
8. Can we be confident in the exposu						
9. Can we be confident in the outcom						
10. Were all measured outcomes rep						
11. Were there no other potential th						

March 2012.
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EHC047-EF.
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ahrq.gov/

The Navigation Guide

Clinical Practice & Policy: Navigation Guide

An Evidence-Based Medicine Methodology to Bridge the Gap Between Clinical and Environmental Health Sciences


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s Assessment Tool: for Non-
nterventions (ACROBAT-NRSI)

Julian PT Higgins and Barney C Reeves

n, 2014 Cochrane Colloquium

In vitro Studies Are Subset of Mechanistic Data

- Mechanistic data – 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* exposure studies

- This project focused on studies with ***in vitro exposure*** regimens

A "Parallel" Approach Across Evidence Streams

- Predefined set of questions to address


Human studies


Animal toxicology studies

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

Study Design Determines Which Questions Apply

Approach to *In Vitro Exposure Studies* Based on Experimental Animal

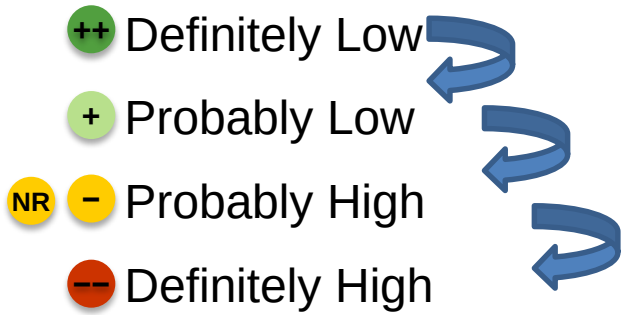
Same set of questions from experimental animal applied to studies with *in vitro* exposure regimens

Risk-of-Bias Questions	*In Vitro* Exposure	Experimental Animal	Human Controlled Exposure	Cohort	Case-Control	Cross-Sectional	Case Series
1. Was administered dose or exposure level adequately randomized?	X	X	X				
2. Was allocation to study groups adequately concealed?	X	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	X					
6. Were research personnel blinded to the study group during the study?	X	X	X				
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	X	
8. Can we be confident in the exposure characterization?	X	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	X
10. Were all measured outcomes reported?	X	X	X	X	X	X	X
11. Were there no other potential threats to internal validity	X	X	X	X	X	X	X

Criteria Define How to Reach Rating Decisions

- **Risk-of-bias questions** cover key topics consistent with other published approaches for evaluating human and animal studies

- **Specific criteria** provide guidance for answering each risk-of-bias question
  - There are separate criteria for each study design
  - Criteria contain detailed guidance that defines the evidence from a study report to determine each risk-of-bias rating



- At minimum the guidance must distinguish between the 4 ratings

Extending Risk-of-Bias Approach to *In Vitro* Studies

- Starting point
  - Questions and criteria from experimental animal risk of bias tool used as model

- Criteria adapted to address *in vitro* exposure regimens
  - Multiple rounds of review and discussion with NTP expert group addressed issues such as:

    1) **Applicability of questions**

    2) **Developing criteria and editing language for the criteria**

    3) **Where specific issues should be covered**

    4) **Were there other internal validity issues to be added/or were not addressed?**

***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

Extending Methods to *In Vitro* Studies

First Example Consideration in Developing Criteria

- **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

- Applies to potential differences between cells across different groups
- If homogeneous cell suspension
  - No variation or difference between groups
  - Therefore, no need for randomization

Note: lack of variation in homogeneous cell suspension also applies to question on need for allocation concealment

Extending Methods to *In Vitro* Studies

2nd Example Consideration in Developing Criteria

- **Were experimental conditions identical across study groups?**
  - Housing **or cell culture** conditions and husbandry practices should be identical across control and experimental groups
  - Include use of the same vehicle in control and experimental animals **or cells**

In vitro study applicability

- Applies to potential differences between cells across different groups
- Identical conditions include:
  - Same media for controls and experimental culture wells
  - Same solvent (i.e., used to dissolve treatment chemicals) for control cells
  - Culture plates must be uniformly incubated and handled
    - Same medium and schedule for changes, washes
    - Same time spent out of incubator
    - Same incubator and plate conditions
      (e.g., incubator plate location effects, plate edge-effects, etc.)

Extending Methods to *In Vitro* Studies

"In vitro" – specific criteria across the questions

1) **randomization** – no variation = no impact if homogeneous cell suspension
2) **allocation concealment** – no variation = no impact if homogeneous suspension
3) **participant selection** – NA
4) **confounding** – NA
5) **experimental conditions** – same media, solvent, incubator, plate conditions
6) **blinding during study** – robotic systems eliminate need; otherwise may apply
7) **incomplete data** – includes evidence of well or plate loss without explanation
8) **exposure characterization** – purity, stability, solubility, volatility of substance
9) **outcome assessment** – acceptable or well established methods and blinding unless automated/no handling between experiment and measurement
10) **reporting** – covers whether all measured outcomes were reported
11) **other** – project specific considerations (e.g., appropriate statistical methods)

Summary

- The OHAT risk-of-bias tool uses a parallel approach to assess individual study quality/internal validity on an outcome basis
  - Single set of questions
  - Study-design specific criteria for human and experimental animal studies
  - Method posted on OHAT Website (http://ntp.niehs.nih.gov/go/38673)

- Project extended the risk of bias approach to *in vitro* studies
  - Criteria adapted to address *in vitro* exposure regimens through multiple rounds of review and discussion with NTP expert group
  - The tool presents one potential approach for assessing internal validity

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  - David Allen
  - Scott Auerbach
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  - Stephen Ferguson
  - Michael Paris
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  - Judy Strickland
  - Ray Tice
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Thank You