Intake of free sugars, chronic metabolic diseases and dental caries - Appraisal: risk of bias

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efsa
European Food Safety Authority

SUB-QUESTIONS 5 AND 6 –LITERATURE SEARCHES

Databases

Database	Platform	Types of studies
Cochrane Library. Cochrane Central Register of Controlled Trials (CENTRAL)	Wiley	Intervention studies
Cochrane Library. Cochrane Database of Systematic Reviews (CDSR)	Wiley	Systematic reviews
Cochrane Library. Database of Abstracts of Reviews of Effects	Wiley	Systematic reviews
Embase	Elsevier	Systematic reviews, intervention studies, observational studies
PubMed	NLM	Systematic reviews, intervention studies, observational studies
Scopus	Elsevier	Systematic reviews, intervention studies, observational studies

SUB-QUESTIONS 5 AND 6 –LITERATURE SEARCHES

❑ Date limits

Sub-Q	Endpoints	Date limit	Systematic review
5	Adipose tissue	Intervention and observational studies: December 2011	Te Morenga et al., 2012
5	Blood pressure	Interventions: August 2013	Te Morenga et al., 2014
		Observational studies: no date limit	-
5	Blood lipids	Interventions: August 2013	Te Morenga et al., 2014
		Observational studies: no date limit	-
5	All other endpoints	Intervention and observational studies: no date limit	-
6	Dental caries	Intervention and observational studies: November 2011	Moynihan and Kelly, 2014

SUB-QUESTIONS 5 AND 6 – DATA EXTRACTION

Data to be extracted from each study included

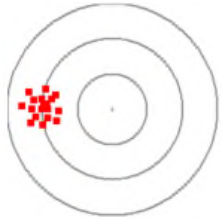
- Characteristics of the studies (e.g. study design)
- Key-elements (e.g. population, intervention/exposure, comparator, outcomes/endpoints, setting and duration)
- Results
- Aspects related to the internal validity of the studies (e.g. confounders, randomisation)
- Funding source

How

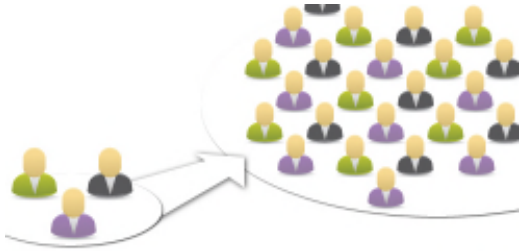
- In the original units of measurement
- Using pre-defined forms
- By one EFSA staff/WG expert
- Data quality checks

SUB-QUESTIONS 5 AND 6 - APPRAISAL OF INDIVIDUAL STUDIES

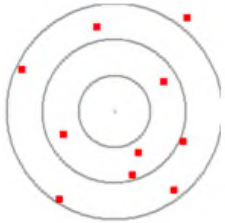
- **Internal validity** (bias): the degree to which bias or a systematic error, or deviation from the truth, in results or inferences is minimised in the study of interest. Bias can vary in:
  - **magnitude** (small or large impact on effect estimate)
  - **direction** (under- or overestimation of the true effect)



- **External validity**



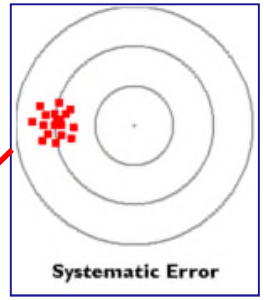
- **Precision** (random error)



(FORMAL) APPRAISAL OF RISK OF BIAS

1. Question (problem) formulation and definition of eligibility criteria for study selection
2. Search for eligible studies
3. Selection of studies for inclusion/exclusion
4. Data extraction from included studies
5. Appraisal of individual studies
6. Evidence synthesis (integration)
7. Interpretation of results in light of all identified uncertainties (focus on BODY of evidence)

formal appraisal



- For each study 'unit'
- By outcome

Using **Critical Appraisal Tools** (**CATs**)

SUB-QUESTIONS 5 AND 6 - APPRAISAL OF INDIVIDUAL STUDIES

Internal validity or risk of bias (RoB)

- To be appraised using a customised version of the **OHAT/NTP tool**

Reasons for the choice:

- developed to facilitate consideration of RoB across evidence streams and study types
- covers human intervention and observational studies (any design)
- clear guide for evaluators with examples
- more experience within EFSA
- consistency across EFSA assessments

* https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

THE OHAT/NTP TOOL FOR RISK OF BIAS ASSESSMENT

- developed to provide a parallel approach to evaluating RoB across study designs in RA of chemicals
- 6 domains, plus 'other'
- questions address aspects relevant to specific study designs

Bias Domains and Questions	Experimental Animal[1]	Human Controlled Trials[2]	Cohort	Case-control[3]	Cross-sectional	Case Series
Selection Bias						
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 appropriate comparison groups?			X	X	X	
Confounding Bias						
4. Did the study design or analysis account for important confounding and modifying variables?			X	X	X	X
Performance Bias						
~~5. Were experimental conditions identical across study groups?~~	X					
6. Were the research personnel and human subjects blinded to the study group during the study?	X	X				
Attrition/Exclusion Bias						
7. Were outcome data complete without attrition or exclusion from analysis?	X	X	X	X	X	
Detection Bias						
8. Can we be confident in the exposure characterization?	X	X	X	X	X	X
9. Can we be confident in the outcome assessment?	X	X	X	X	X	X
Selective Reporting Bias						
10. Were all measured outcomes reported?	X	X	X	X	X	X
Other Sources of Bias						
11. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	X	X	X	X	X	X

[1]Experimental animal studies are controlled exposure studies. Non-human animal observational studies could be evaluated using the design features of observational human studies such as cross-sectional study design.
[2]Human Controlled Trials (HCTs): studies in humans with a controlled exposure, including Randomized Controlled Trials (RCTs) and non-randomized experimental studies
[3]Cross-sectional studies include population surveys with individual data (e.g., NHANES) and population surveys with aggregate data (i.e., ecological studies).

* https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

RoB RATING INSTRUCTIONS*

Question	Rating	RoB rating instructions
Bias domain: selection bias		
1. Was administered dose or exposure level adequately randomized?	++	There is direct evidence that subjects were allocated to any study group including controls using a method with a random component. Acceptable methods of randomization include: referring to a random number table, using a computer random number generator, coin tossing, shuffling cards or envelopes, throwing dice, or drawing of lots (Higgins and Green 2011). Restricted randomization (e.g., blocked randomization) to ensure particular allocation ratios will be considered low risk of bias. Similarly, stratified randomization and minimization approaches that attempt to minimize imbalance between groups on important prognostic factors (e.g., body weight) will be considered acceptable
	+	There is indirect evidence that subjects were allocated to study groups using a method with a random component (i.e., authors state that allocation was random, without description of the method used), OR it is deemed that allocation without a clearly random component during the study would not appreciably bias results. For example, approaches such as biased coin or urn randomization, replacement randomization, mixed randomization, and maximal randomization may require consultation with a statistician to determine risk-of-bias rating (Higgins and Green 2011)
	-/NR	There is indirect evidence that subjects were allocated to study groups using a method with a non-random component, OR there is insufficient information provided about how subjects were allocated to study groups (record "NR" as basis for answer). Note: Non-random allocation methods may be systematic, but have the potential to allow participants or researchers to anticipate the allocation to study groups. Such "quasi-random" methods include alternation, assignment based on date of birth, case record number, or date of presentation to study (Higgins and Green 2011).
	-	There is direct evidence that subjects were allocated to study groups using a non-random method including judgment of the clinician, preference of the participant, the results of a laboratory test or a series of tests, or availability of the intervention (Higgins and Green 2011)

* https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

CUSTOMISATION

Criteria that may require customisation:

Confounding →

Blinding →

Exposure assessment →

Outcome assessment →

Table 1. Adapted from OHAT RoB tool (source: OHAT Handbook - January 9, 2015)[8]

Bias Domains and Questions	Int.	Obs.
Selection Bias		
1. Was administered dose or exposure level adequately randomized?	X	
2. Was allocation to study groups adequately concealed?	X	
3. Did selection of study participants result in appropriate comparison groups?		X
Confounding Bias		
4. Did the study design or analysis account for important confounding and modifying variables?		X
Performance Bias		
5. Were the research personnel and human subjects blinded to the study group during the study?	X	
Attrition/Exclusion Bias		
6. Were outcome data complete without attrition or exclusion from analysis?	X	X
Detection Bias		
7. Can we be confident in the exposure characterization?	X	X
8. Can we be confident in the outcome assessment?	X	X
Selective Reporting Bias		
9. Were all measured outcomes reported?	X	X
Other Sources of Bias		
10. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	X	X

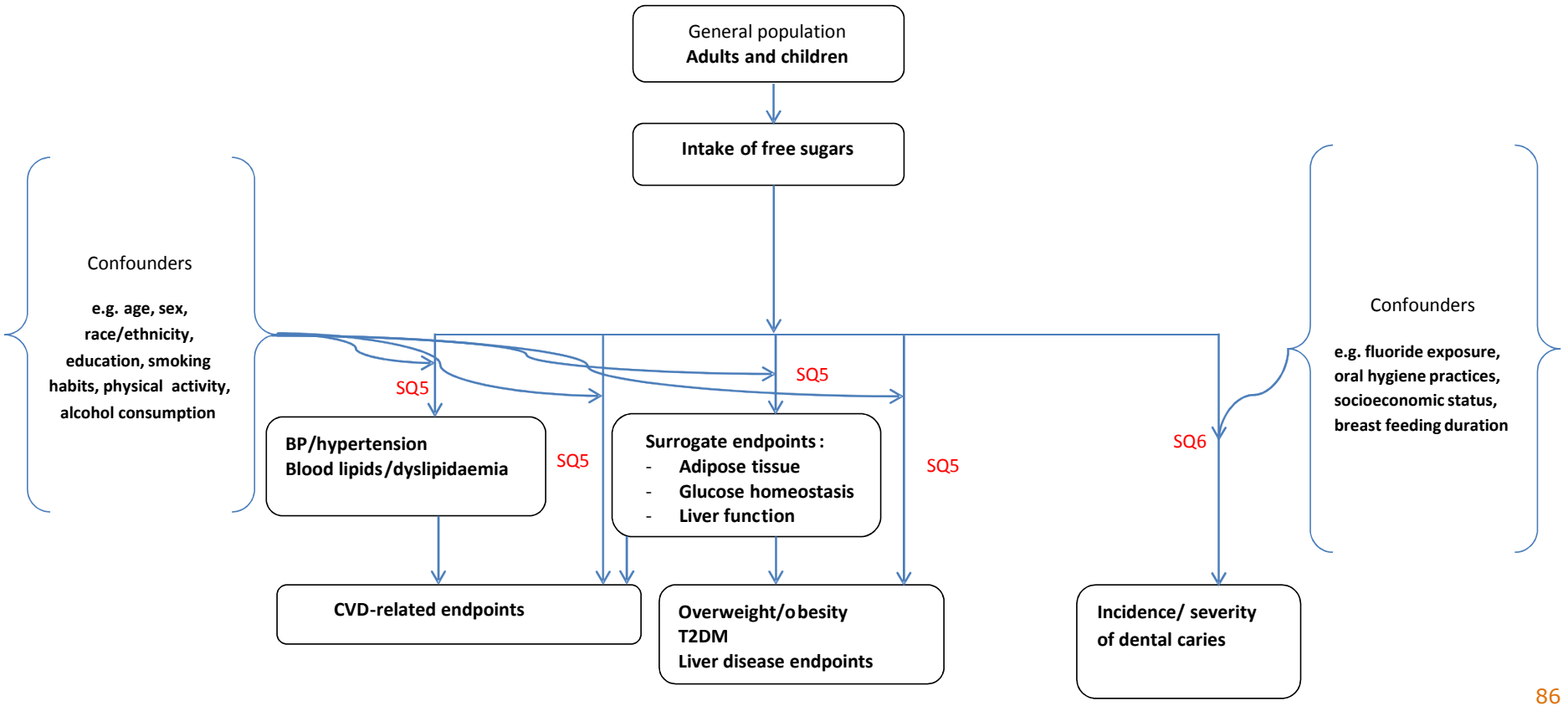
SUB-QUESTIONS 5 AND 6 - APPRAISAL OF INDIVIDUAL STUDIES

Question 4: potential confounders

- Some identified *a priori* based on available literature
- Additional confounders may be identified by the reviewers
- Apply to observational studies only
- Adjustment for mediators in the causal pathway between the intake of free sugars and disease-related endpoints: potential source of over-adjustment bias

- If NOT addressed by randomisation in intervention studies: to be considered under "other risk of bias"

SUB-QUESTIONS 5 AND 6: CONCEPTUAL FRAMEWORK



SUB-QUESTIONS 5 AND 6 - APPRAISAL OF INDIVIDUAL STUDIES

Question 7: confidence in the exposure characterisation

- refers to the confidence on the methods used to characterise the exposure as defined by the authors

- **NOT** to the extent to which the exposure investigated on each study reflects the intake of free sugars from all dietary sources

Factors affecting misclassification of subjects/accuracy of intake estimates:

- Method used
- Accuracy of the method used
- Systematic changes in habitual intakes

SUB-QUESTIONS 5 AND 6 - APPRAISAL OF INDIVIDUAL STUDIES

Question 8: confidence in the outcome assessment

- Confidence in the outcome requires valid, reliable, and sensitive methods to assess the outcome applied consistently across groups

- Outcome misclassification or measurement error may be unrelated to the exposure (non-differential) or related to the exposure (differential)

- Factors affecting misclassification of subjects in relation to the outcome assessment:
  - Objectivity of the outcome assessment
  - Consistency of the measurement
  - Blinding of outcome assessors (for knowledge of exposure)

* https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

ANSWER FORMAT FOR THE RISK OF BIAS QUESTIONS

++ ***Definitely Low** risk of bias*:
There is direct evidence of low risk-of-bias practices
(May include specific examples of relevant low risk-of-bias practices)

\+ ***Probably Low** risk of bias*:
There is indirect evidence of low risk-of-bias practices OR it is deemed that deviations from low risk-of-bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias.

\- NR ***Probably High** risk of bias*:
There is indirect evidence of high risk-of-bias practices OR there is insufficient information (e.g., not reported or "NR") provided about relevant risk-of-bias practices

-- ***Definitely High** risk of bias*:
There is direct evidence of high risk-of-bias practices
(May include specific examples of relevant high risk-of-bias practices)

- It encourages judging the direction and magnitude of bias, when possible
- Ideally: looking at empirical evidence for bias
- If no clear rationale for judging the direction of bias, no guessing…

SUB-QUESTIONS 5 AND 6 - APPRAISAL OF INDIVIDUAL STUDIES

Appraisal (and customisation) to be done:

- At outcome level
- By two mutually independent experts

In case of discrepancies:

- to be discussed at the WG
- selection of the most conservative judgement (highest RoB) if no agreement is reached

SUMMARISING RoB FOR EACH STUDY (BY OUTCOME)

- **Tabular summary** for each study, including the key elements and a summary of the results of the critical appraisal
- Two options for **combining the scores** for each study (to be decided):
  - Use of an algorithm
  - Consider RoB separately in the WoE and uncertainty analysis

EXAMPLES OF VISUAL SUM…SSMENT

Table 9. Example of a Visual Summary of Risk of Bias Ratings for Animal Studies

Across studies

Risk of Bias Question	Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Study 7	Study 8	Study 9	Study 10
Randomization	+	–	++	++	–	++	+	+	++	–
Allocation concealment	–	–	–	–	–	–	+	–	–	–
Confounding (design/analysis)	++	+	++	++	++	+	++	++	++	++
Unintended exposure	+	+	+	+	+	+	+	+	+	+
Identical experimental conditions	++	++	+	+	++	++	++	++	++	+
Adhere to protocol	+	+	+	+	–	+	+	+	+	+
Blinding of researchers during study	–	–	–	–	–	–	+	–	–	–
Missing outcome data	–	+	++	++	--	–	+	–	–	+
Assessment of confounding variables	+	+	++	++	++	–	+	+	++	++
Exposure characterization	++	–	+	+	–	–	+	+	–	–
Outcome assessment	+	+	+	+	+	+	++	+	+	–
Blinding of outcome assessors	+	+	+	+	++	+	+	+	+	+
Outcome reporting	+	+	+	++	--	+	+	+	+	–

Key:

- *Definitely low risk of bias* (++)
- *Probably low risk of bias* (+)
- *Probably high risk of bias* (–)
- *Definitely high risk of bias* (--)

Studies are evaluated on all applicable risk of bias questions based on study design. The rating or a… on an outcome basis prior to determining the tier from 4 options: definitely low risk of bias (++), p… bias (-), or definitely high risk of bias (--).

Individual study

Safety of isoflavones from food supplements in menopausal women

Breitman et al., 2003 – Ref ID: 5800

Animal model	**Species**	Rats
	Strain (source)	Sprague-Dawley from Charles River Canada (Montreal, Quebec)
	Number of animals	Total 50 (sham, n=10; OVX , n=40)
	Age (weight):	90-day (not reported)
	Diet:	AIN 93G containing 0.2% Ca (Dyets, Inc., Bethlehem, PA) devoid of isoflavones
	Dosing method:	Dietary administration
	Funding source	Dairy Farmer's of Canada for graduate student funding and the J.P. Bickell Foundation for project funding.
	Authors' conflicts of interest	Not reported
Dosing	**Intervention**	SHAM: isoflavone-free diet OVX control: isoflavone-free diet High-Ca: OVX, 2.5% Calcium IF: OVX, isoflavone extract, 1.6 g/diet IF + High-Ca: OVX, isoflavone extract, 1.6 g/diet + 2.5% Ca
	Start of intervention since OVX	1 week
	Duration	8-week
Statistical analysis	**Statistical analysis**	Kruskal–Wallis one-way ANOVA on ranks followed by Tukey's post hoc test was used to detect differences among groups. Differences were considered statistically significant if $P < 0.05$.
Results	**Uterus Relative weight (g/kg bw) Mean ± SD**	SHAM 1.65 ± 0.27* OVX 0.21 ± 0.05 High-Ca 0.28 ±0.04 IF 0.19 ± 0.04 IF HighCa 0.23 ± 0.09 (#): different from control ($P < 0.05$)

Risk of Bias Appraisal — **Tier: 1**

Bias domain	Question	Score	Judgement
Confounding	Key question A: Did the study design or analysis account for important confounding and modifying variables assessed consistently across groups using valid and reliable measures?	++	Body weight was measured each week. Age of the rats was similar among all groups.
	Did researchers adjust or control for other exposures that are anticipated to bias results?	+	No information about the cages used. An AIN diet was used for all groups.
	Were experimental conditions identical across study groups?	++	
Attrition/ exclusion	Were outcome data incomplete due to attrition or exclusion from analysis?	++	No loss of animals
Information/ detection	Were the outcome assessors blinded to study group or exposure level?	+	No information about blinding.
	Were confounding variables assessed consistently across groups using valid and reliable measures?	++	
	Key question B: Can we be confident in the outcome assessment?	++	
Selective reporting	Were all measured outcomes reported?	++	
Repeated measurements	Key question C: Are the repeated measurements (if any) on the experimental units treated appropriately?	++	

EFSA isoflavones case-study for PROMETHEUS (2015)

OHAT/NTP Handbook

* https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

SUMMARISING RoB FOR EACH STUDY (BY OUTCOME)

OHAT/NTP tool applies an algorithm to classify each study (by outcome) into '**tiers of RoB**':

- Tier 1
- Tier 2
- Tier 3

The algorithm is based on the identification of '**key criteria**'

Limitations of the approach:
- Loss of info
- No account for 'impact' of bias

Table 6. Example of Approach for Determining Tiers of Study Quality for Individual Observational Studies

- **Tier 1**: A study must be rated as "definitely low" or "probably low" risk of bias for key elements AND have most other applicable items answered "definitely low" or "probably low" risk of bias.

  Example of key risk of bias elements for observational human studies:
  - Can we be confident in the exposure characterization?
  - Can we be confident in the outcome assessment?
  - Does the study design or analysis account for important confounding variables?

- **Tier 2**: Study meets neither the criteria for 1st or 3rd tiers.
- **Tier 3**: A study must be rated as "definitely high" or "probably high" risk of bias for key elements AND have most other applicable items answered "definitely high" or "probably high" risk of bias.

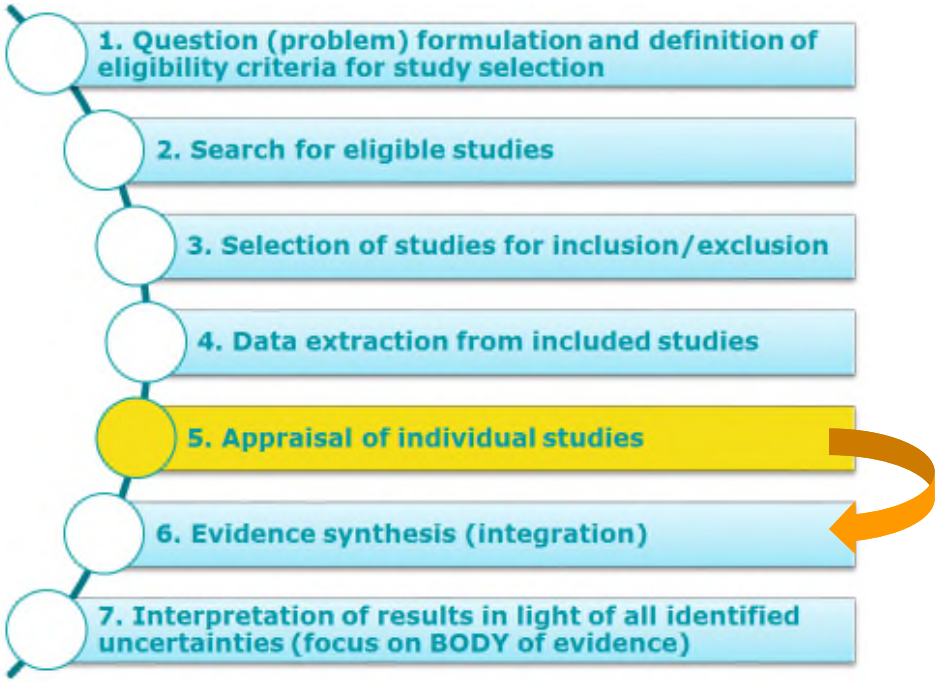
		Risk of Bias Domains and Ratings										
		Key Criteria			Other RoB Criterion							
Category	Guidance	Can we be confident in the exposure characterization?	Can we be confident in the outcome assessment?	Did the study design or analysis account for important confounding and modifying variables?	Other RoB criteria	Other RoB criteria	Other RoB criteria	Other RoB criteria	Other RoB criteria	Other RoB criteria	Other RoB criteria	Other RoB criteria
1st tier	– "definitely low" or "probably low" risk of bias for key items AND – "definitely low" or "probably low" risk of bias for most other applicable criteria	+	++	+	-	+	+	+	+	+	+	-
2nd tier	example 1	-	+	++	++	–	+	-	+	+	+	+
	study does not meet criteria for "low" or "high" example 2	+	++	+	+	-	-	-	--	+	-	+
	example 3	--	-	--	++	-	+	+	+	+	+	+
3rd tier	– "definitely high" or "probably high" risk of bias for key items AND – "definitely high" or "probably high" risk of bias for most other applicable criteria	--	-	-	--	+	-	-	+	--	+	--

Risk of bias response options for individual items

++	Definitely low risk of bias	--	Definitely high risk of bias
+	Probably low risk of bias	-	Probably high risk of bias

Studies are evaluated on all applicable risk of bias questions based on study design. The rating or answer to each risk of bias question is selected on an outcome basis prior to determining the tier from 4 options: definitely low risk of bias (++), probably low risk of bias (+), probably high risk of bias (-), or definitely high risk of bias (--).

ACCOUNTING FOR RESULTS OF APPRAISAL IN THE ANALYSIS



Possible ways (OHAT/NTP based on Higgins and Green 2011):

- restrict primary analysis to studies with lower RoB and perform a sensitivity analysis to show how conclusions might be affected if studies at high RoB were included
- present multiple (stratified) analysis
- present all studies and provide a narrative discussion of RoB, ideally through a structured approach

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Other ways possible

APPRAISAL RISK OF BIAS