EFSA working group on BPA assessment protocol

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BPA assessment Protocol

Workshop on BPA hazard assessment protocol
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European Food Safety Authority

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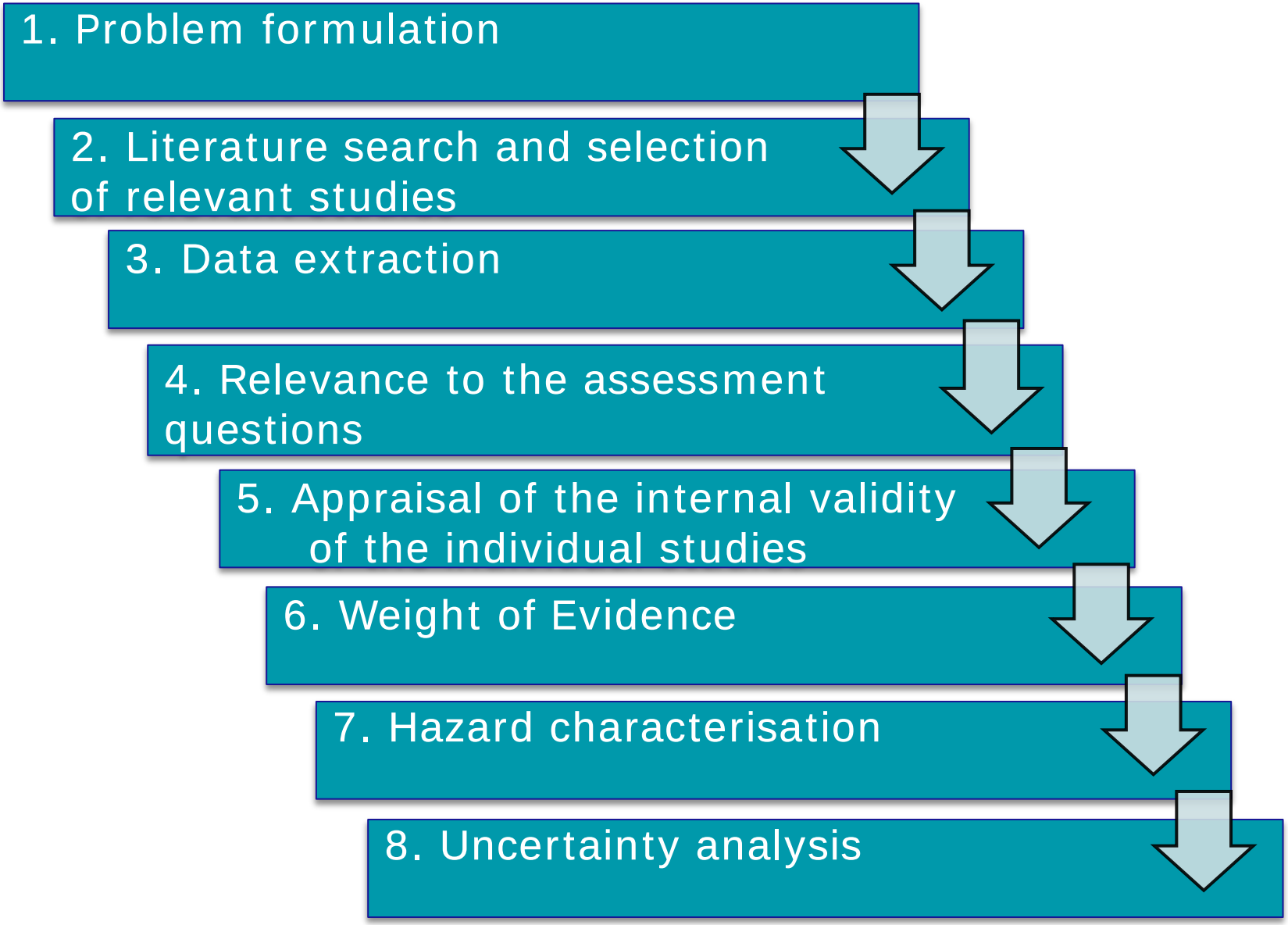
BPA Hazard Assessment Protocol



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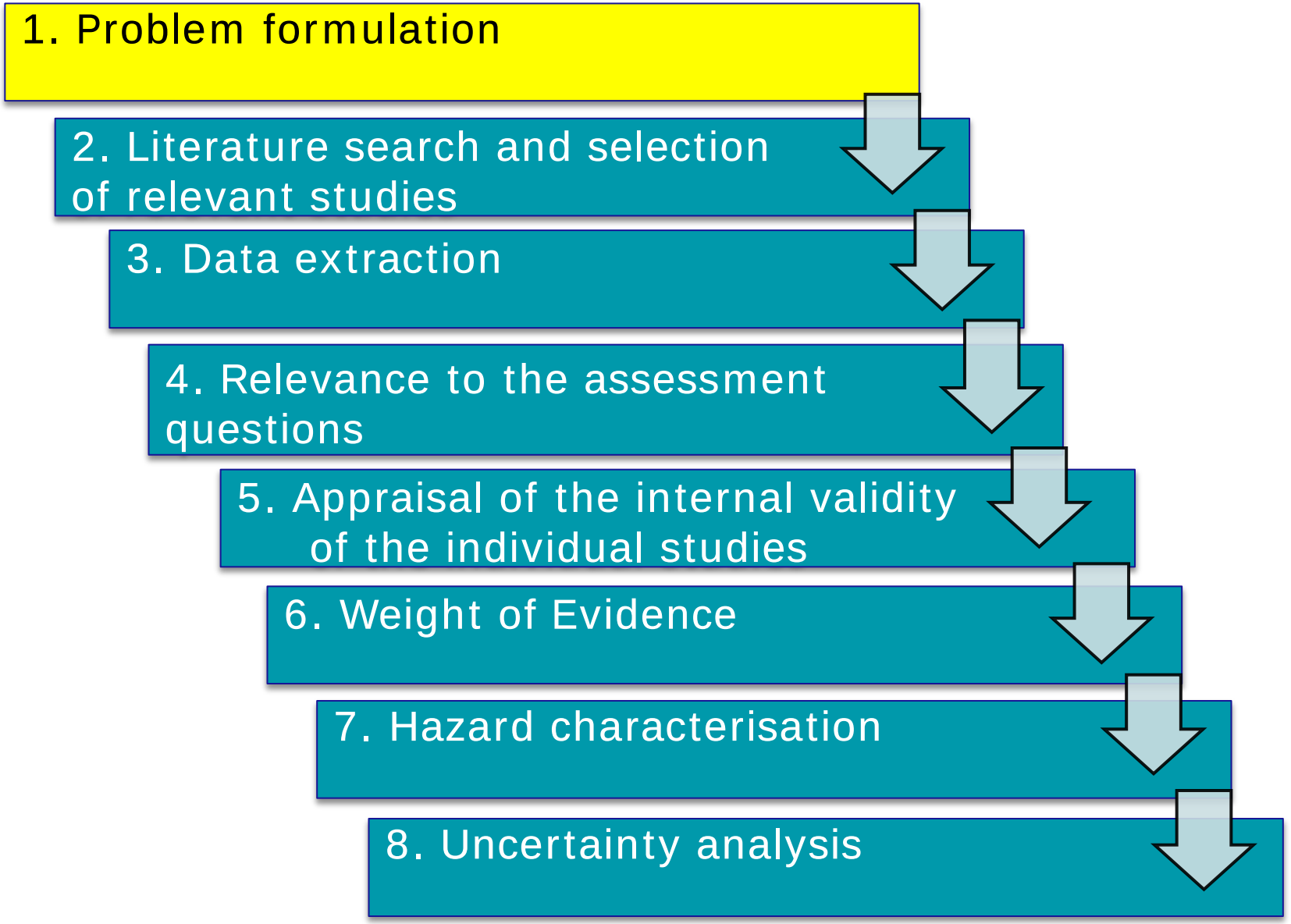


1a. Problem formulation

Objectives of the hazard assessment

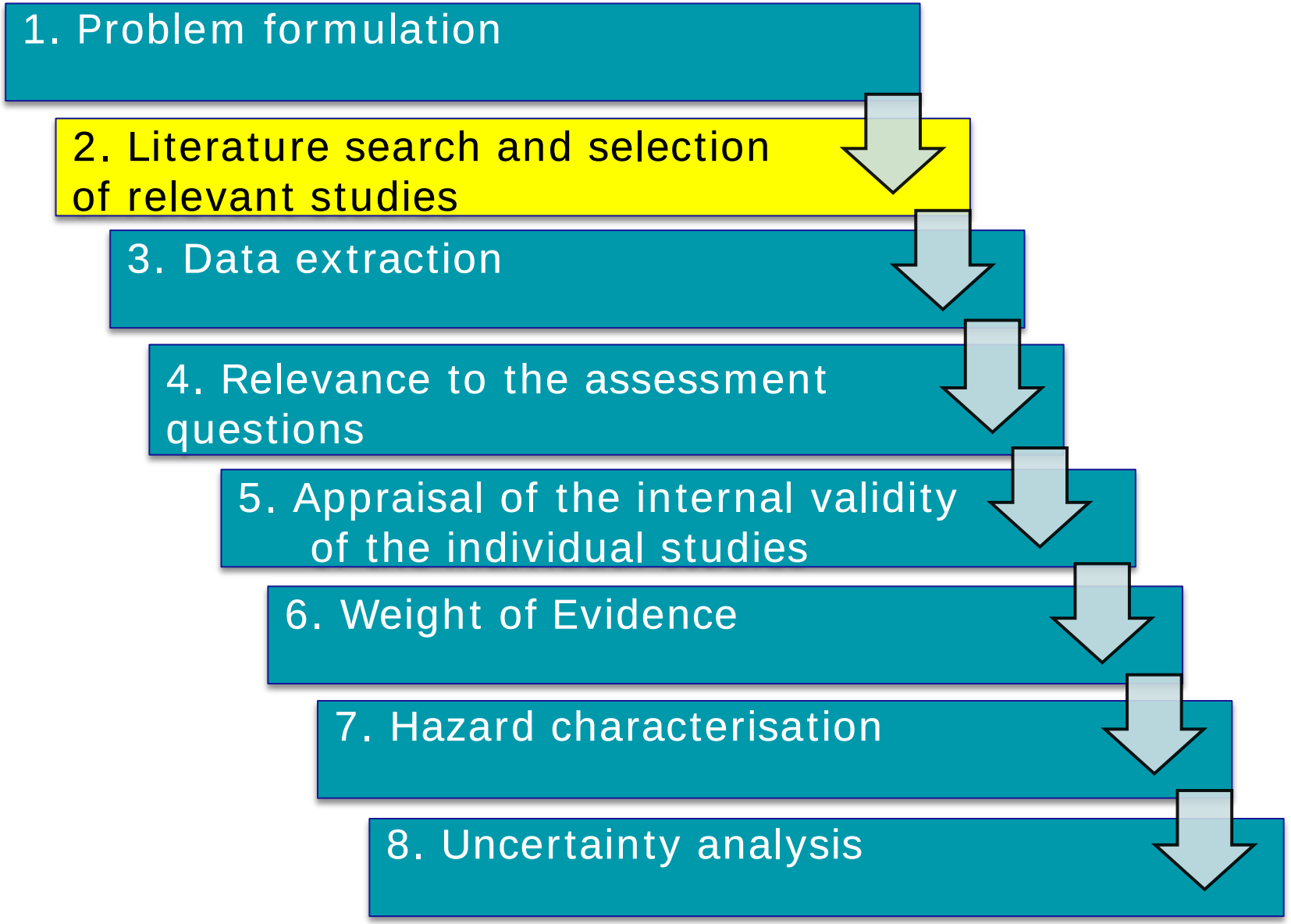
the evaluation will cover

i. the **adverse effects in humans** associated with the exposure to BPA via any route and

ii. the **adverse effects in animals** after oral or subcutaneous exposure to BPA at doses below the oral cut-off dose of 10 mg/kg bw per day (based on the benchmark dose lower confidence interval (BMDL10) in mice calculated by EFSA in 2015) and the subcutaneous cut-off dose of 0.5 mg/kg bw per day (based on the ratio of oral bioavailability and of subcutaneous systemic availability) and

iii. the **human and animal toxicokinetics** of BPA.

BPA Hazard Assessment Protocol



2a. Data collection and selection of studies

Literature Search Strategy

- 4 Databases
  - PubMed
  - Scopus
  - Web of Science CoreTM Collection
  - Toxline + DART (TOXNET platform)

Call for data

- To allow inclusion of studies originally not in English

2b. Data collection and selection of studies

Human studies: Inclusion/Exclusion criteria

Study design	In	• Cohort studies • Case-control studies (retrospective and nested) • Toxicokinetic studies (narrative approach)
	Out	• Cross-sectional studies • Animal studies • *In vitro* studies
Population	In	• All populations groups, all ages, males and females
	Out	• /
Exposure/ intervention	In	• All routes of exposure • All studies during pregnancy including those with single spot urine samples • Studies in which levels of BPA have been measured in human biological samples more than once
	Out	• Bio-Monitoring • Studies with single spot urine samples in non pregnant individuals
Language	In	• English
Time	In	• From 01/01/2013
Publication type	In	• Primary research studies (i.e. studies generating new data)
	Out	• Secondary research studies* • Expert opinions, editorials, and letters to the editor • PhD Theses • Extended abstracts, conference proceedings

* They will be collected separately and used to obtain additional references

2c. Data collection and selection of studies

Single measurement studies

- studies in which levels of BPA have been measured in human biological samples only once will not be included as exposure assessment is uncertain
- exception: studies in pregnant women which could be relevant for time windows of exposure.

2d. Data collection and selection of studies

Animal studies: Inclusion/Exclusion criteria

Study design	In	• All mammalian animals • Toxicokinetic studies (narrative approach)
Study design		
Population	In	/
	Out	• Non-mammalian animals
Exposure/ intervention	In	• Sub-cutaneous and oral • Studies in which levels of BPA have been measured in biological matrices • At least one dose below 10 mg/kg bw (oral) and 0.5 mg/kg bw (s.c.)
	Out	• Exposure routes other than oral and subcutaneous • Mixtures
Language	In	• English
Time	In	• From 01/01/2013
Publication type	In	• Primary research studies (i.e. studies generating new data)
	Out	• Secondary research studies* • Expert opinions, editorials, and letters to the editor • PhD Theses • Extended abstracts, conference proceedings

* They will be collected separately and used to obtain additional references

2e. Data collection and selection of studies

Mode of Action studies (MoA): Inclusion/Exclusion criteria

Study design	In	• *In vitro* studies • *In vivo* studies on animals and on human oriented mode of action
	Out	
Exposure/ intervention	In	• All routes of exposure • For *in vitro* studies: At least one dose level below 100 nM
	Out	• Mixtures
Language	In	• English
Time	In	• From 01/01/2013
Publication type	In	• Primary research studies (i.e. studies generating new data)
	Out	• Secondary research studies* • Expert opinions, editorials, and letters to the editor • PhD Theses • Extended abstracts, conference proceedings

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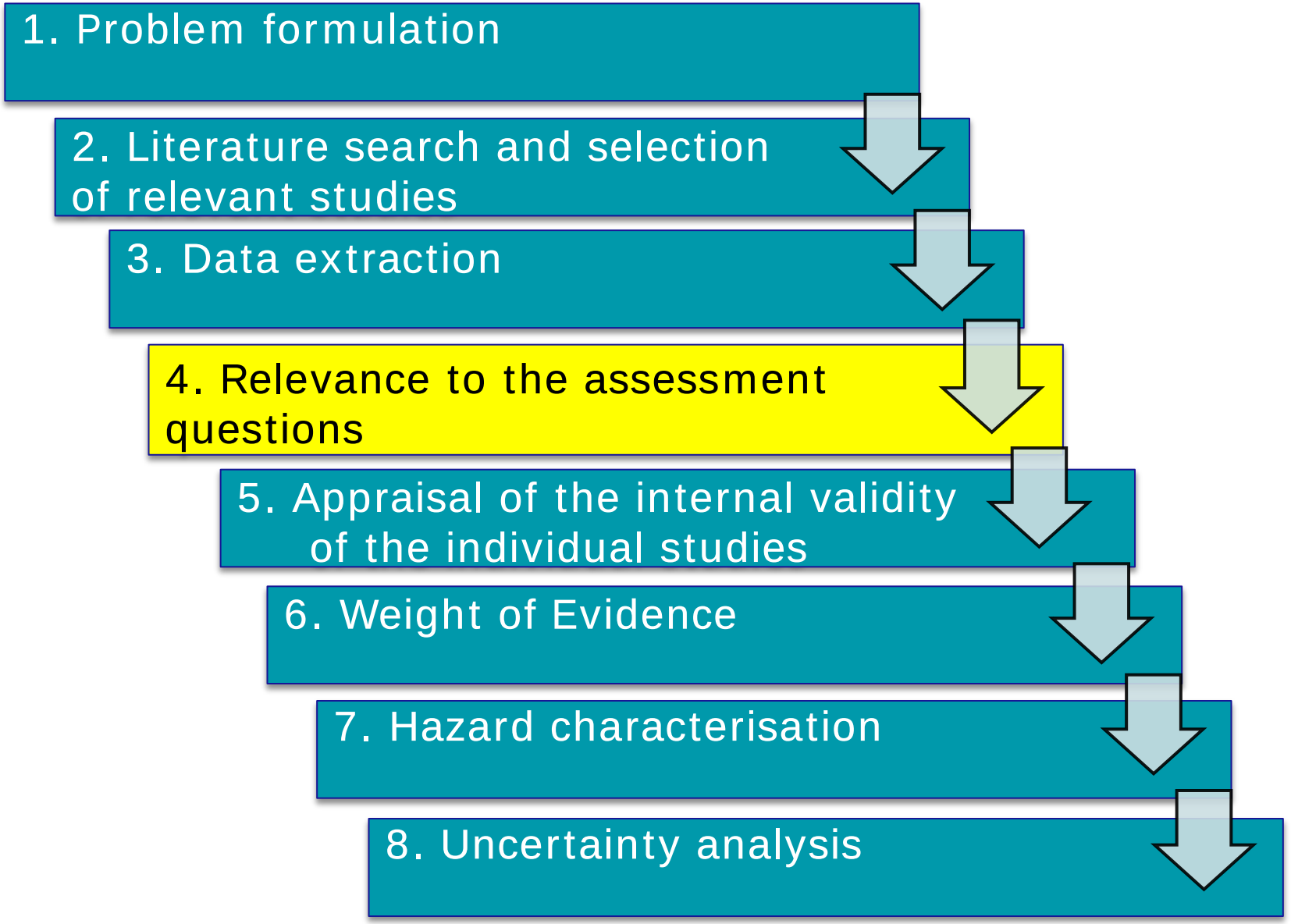

4. Relevance to the assessment questions

- Relevance to be evaluated at the level of the individual study in relation to the specific hazard sub-question asked (see Annex 2)
- Not to be confused with relevance to human health, considered after WoE
- There will be three possible judgements: yes (relevant), unclear and no (not relevant)

➢ Studies with no relevance will not be further considered in the assessment

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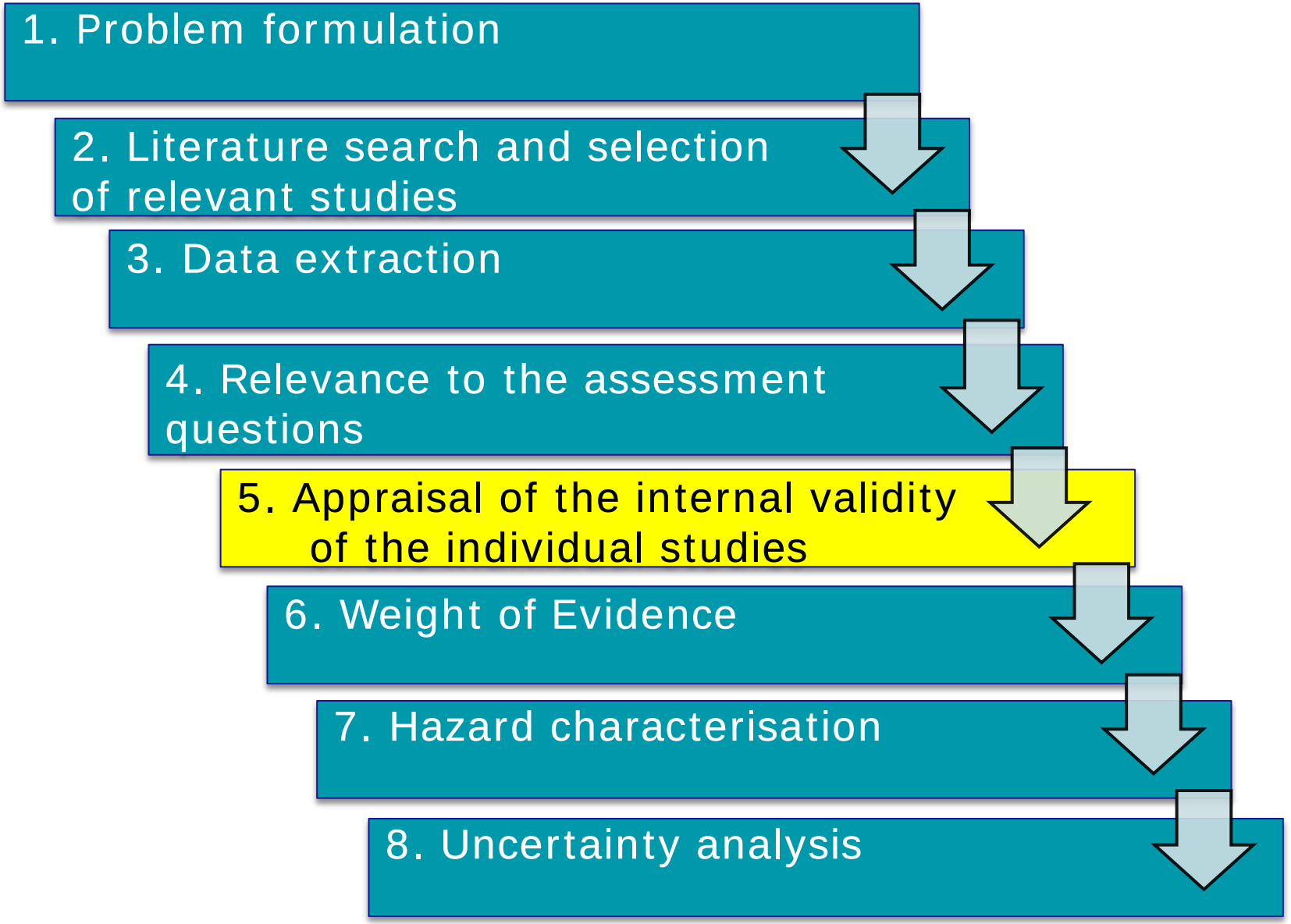

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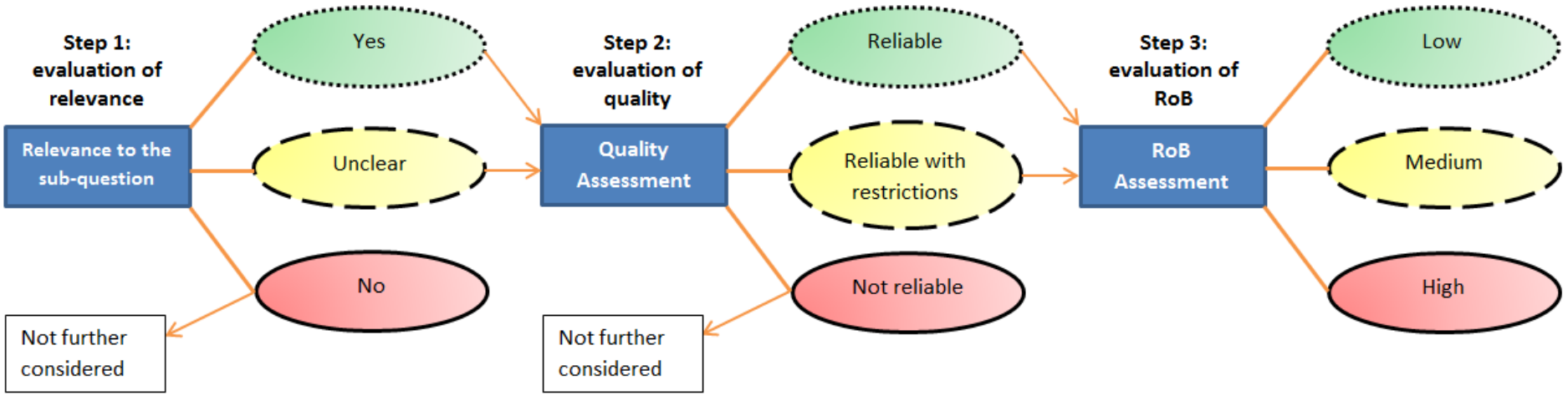
Process flow chart: individual study appraisal scheme



5. Appraisal of the internal validity of the individual studies

Conclusions on internal validity of human and animal studies

		Quality rating	
		Reliable without restrictions	Reliable with restrictions
Risk of Bias rating	Low RoB	Tier 1	Tier 2
	Medium RoB	Tier 2	Tier 3
	High RoB	Tier 3	Not further considered

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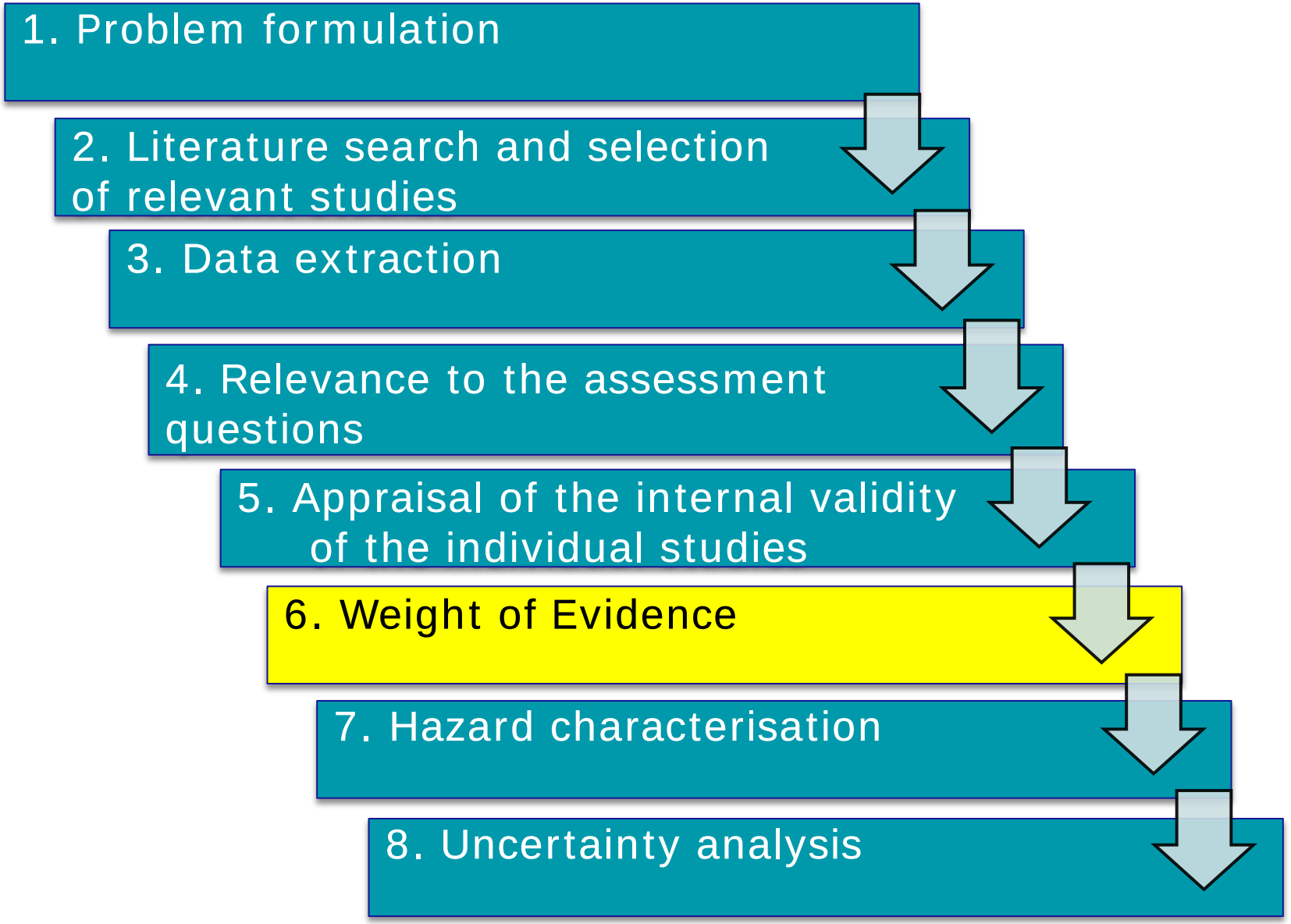
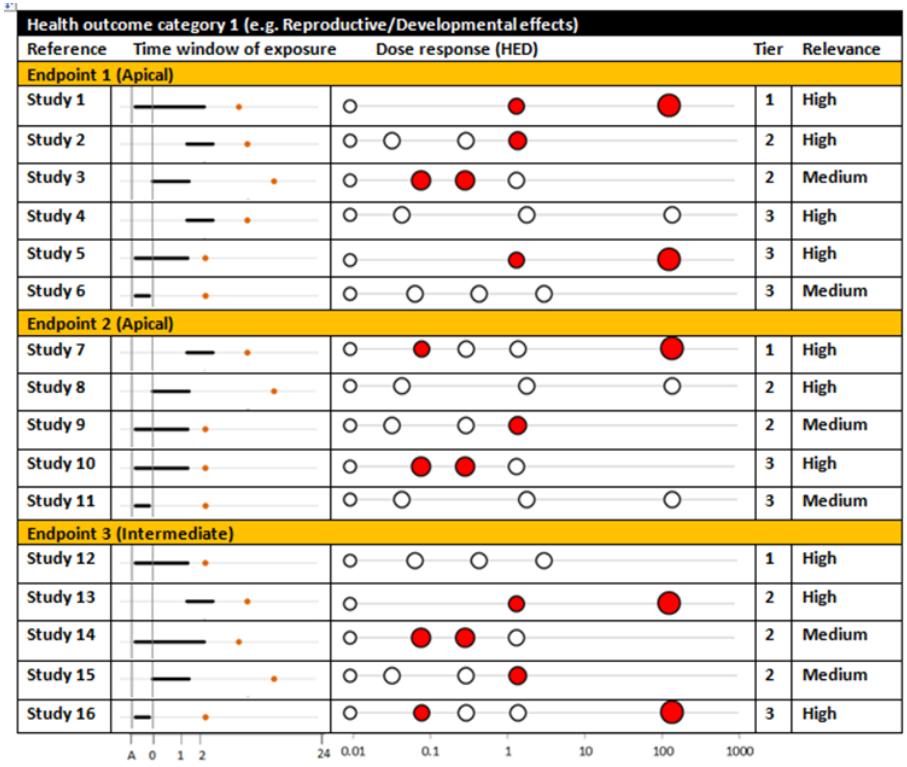


6a. Weight of evidence (WoE)

i. Assembling the evidence

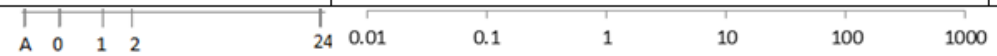
- studies will be sorted as follows:
  - a) health outcome category
  - b) related “apical” and “intermediate” endpoints will be grouped together
  - c) the order will take into account the tier of internal validity
- the graph will contain information on: **time window of exposure, duration of exposure, the doses tested, dose response, magnitude of effect, statistical significance**
- all the dose levels will be converted into Human Equivalent Doses
  - the HEDF will be updated to consider newly published evidence (see annex 1 for more info)

Figure 3: Graphical representation of the results



6a. Weight of evidence (WoE)

Health outcome category 1 (e.g. Reproductive/Developmental effects)				
Reference	Time window of exposure	Dose response (HED)	Tier	Relevance to the sub-question
Endpoint 1 (Apical)				
Study 1			1	High
Study 2			2	High
Study 3			2	Medium
Study 4			3	High
Study 5			3	High
Study 6			3	Medium
Endpoint 2 (Apical)				
Study 7			1	High
Study 8			2	High
Study 9			2	Medium
Study 10			3	High
Endpoint 3 (Intermediate)				
Study 11			1	High
Study 12			2	High
Study 13			3	Medium



Pre and postnatal time (months)
A= start of gestation

HED (µg/kg bw per day)

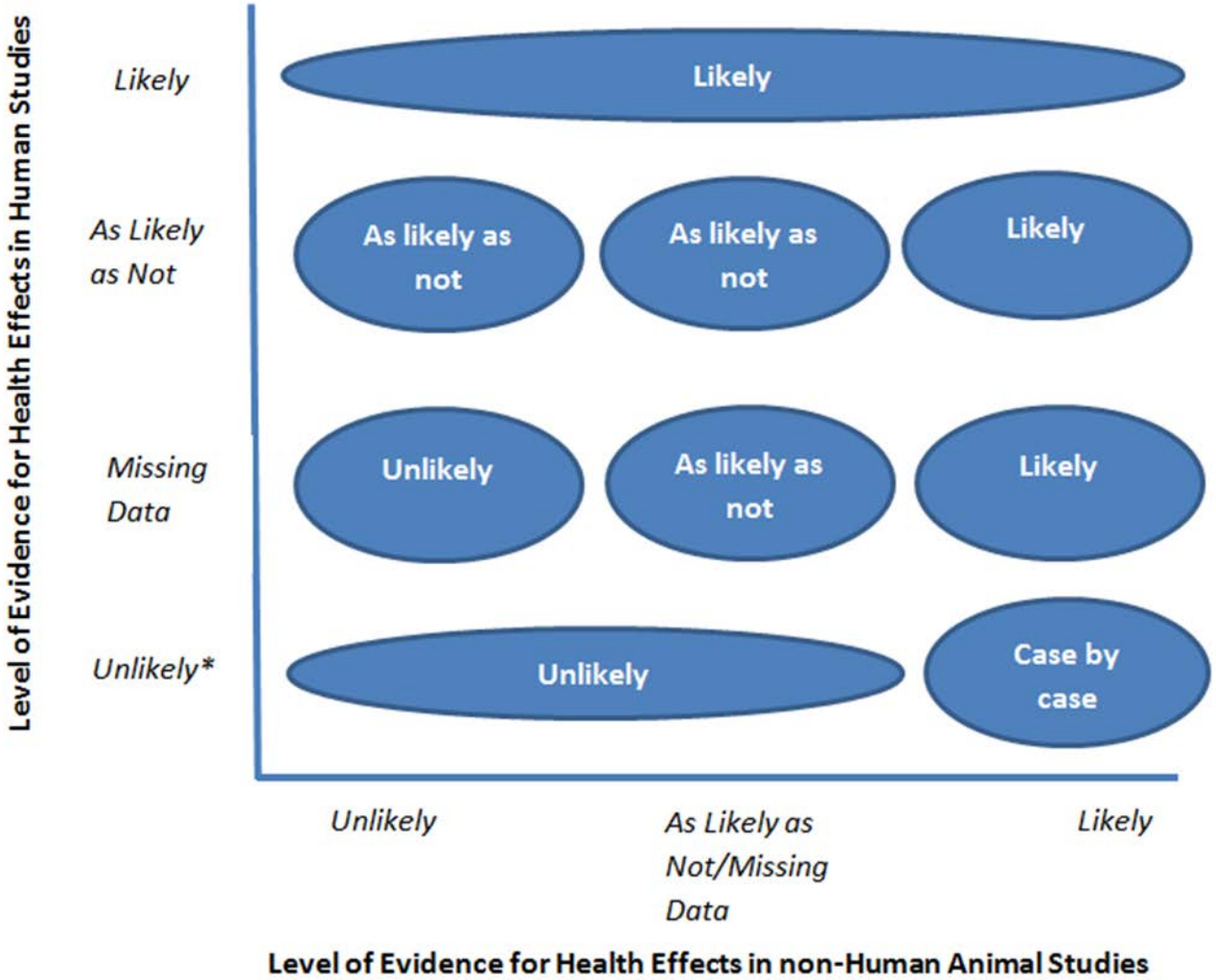
6b. Weight of evidence (WoE)

Criteria used to weigh the evidence

a. the overall internal validity of the studies that show/don't show an effect
b. the consistency of the results between different studies within the same species/population or across species/populations)
c. the dose-response relationships
d. the magnitude of effects
e. the biological plausibility of effects on interrelated endpoints or MoA
f. the relevance of the results to the question of interest

6c. Weight of evidence (WoE)

ii. Integrating the evidence integration of human and animal evidence (adapted from NTP OHAT)



Decision Procedure and Adversity

- Proposal from 2 independent reviewers per health outcome category
- Presentation and discussion in the WG
- Presentation and discussion in the Panel

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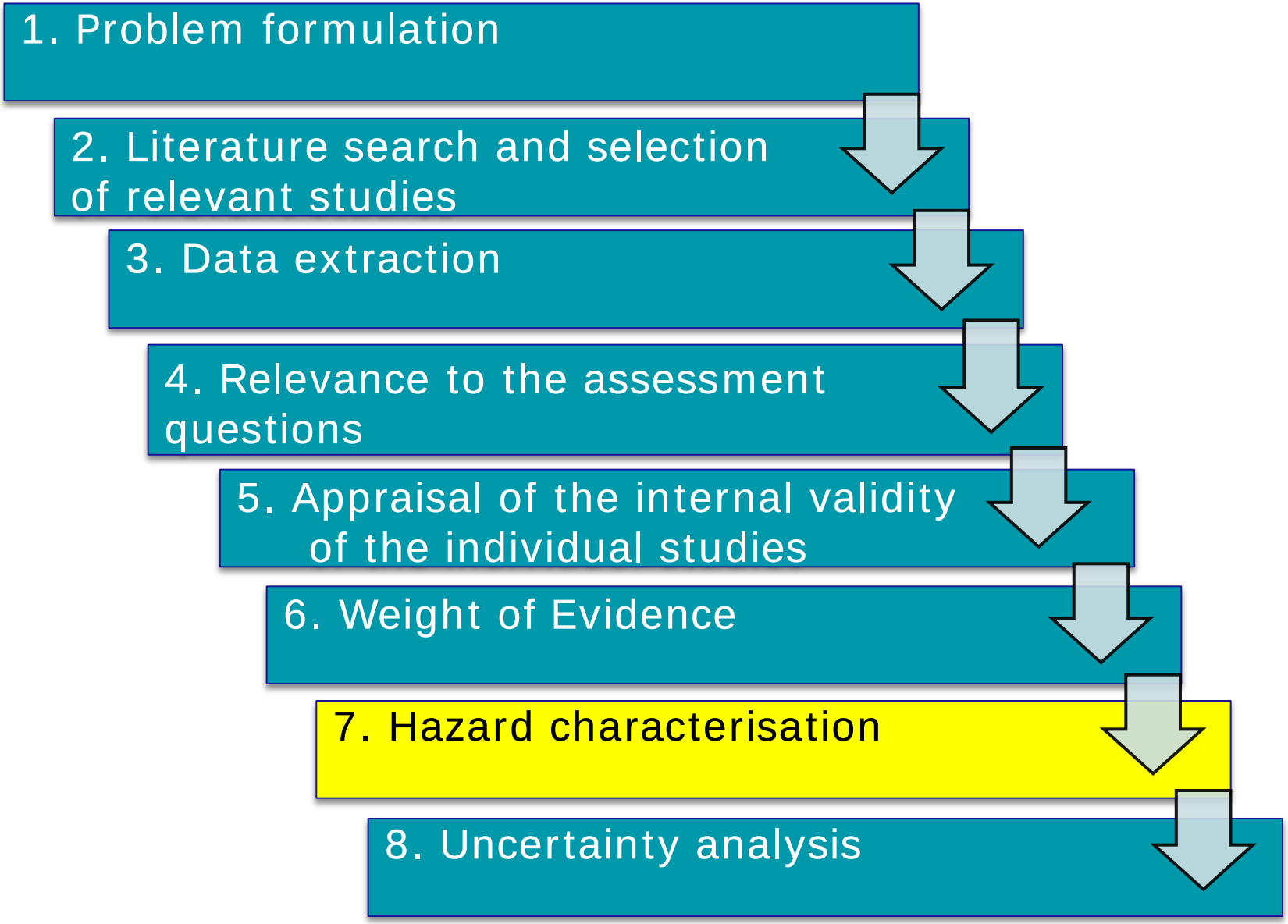

7a. Hazard characterisation

Setting the TDI

- only effects classified as “likely” after the integration of human and animal evidence will be used for hazard characterisation
- data on toxicokinetics will support the extrapolation of results from animal studies to humans and will support the selection of appropriate uncertainty factors
- MoA studies may support this process
- The “classical” approach with uncertainty factors will be used considering the HED procedure which accounts for interspecies differences in toxicokinetics

- **all effects classified as “likely” and “as likely as not” will be used in the analysis of uncertainty**

7b. Hazard characterisation using human data

How to deal with human data to derive a dose-effect relationship

- Because of methodological constraints, an estimate for human **exposure** is only possible by summing up urinary conjugated and unconjugated BPA (surrogate for the exposure).
- A **dose-response** relationship will be established if data allow it and by appropriate statistical methods a reference point will be derived for the TDI.
- No need for **inter-species** assessment factor
- **Intra-species** factor: depending on the population in which the outcome has been observed, an adjustment factor for the whole population could be needed.
- An **additional uncertainty factor** might be necessary to cover for uncertainty in the database

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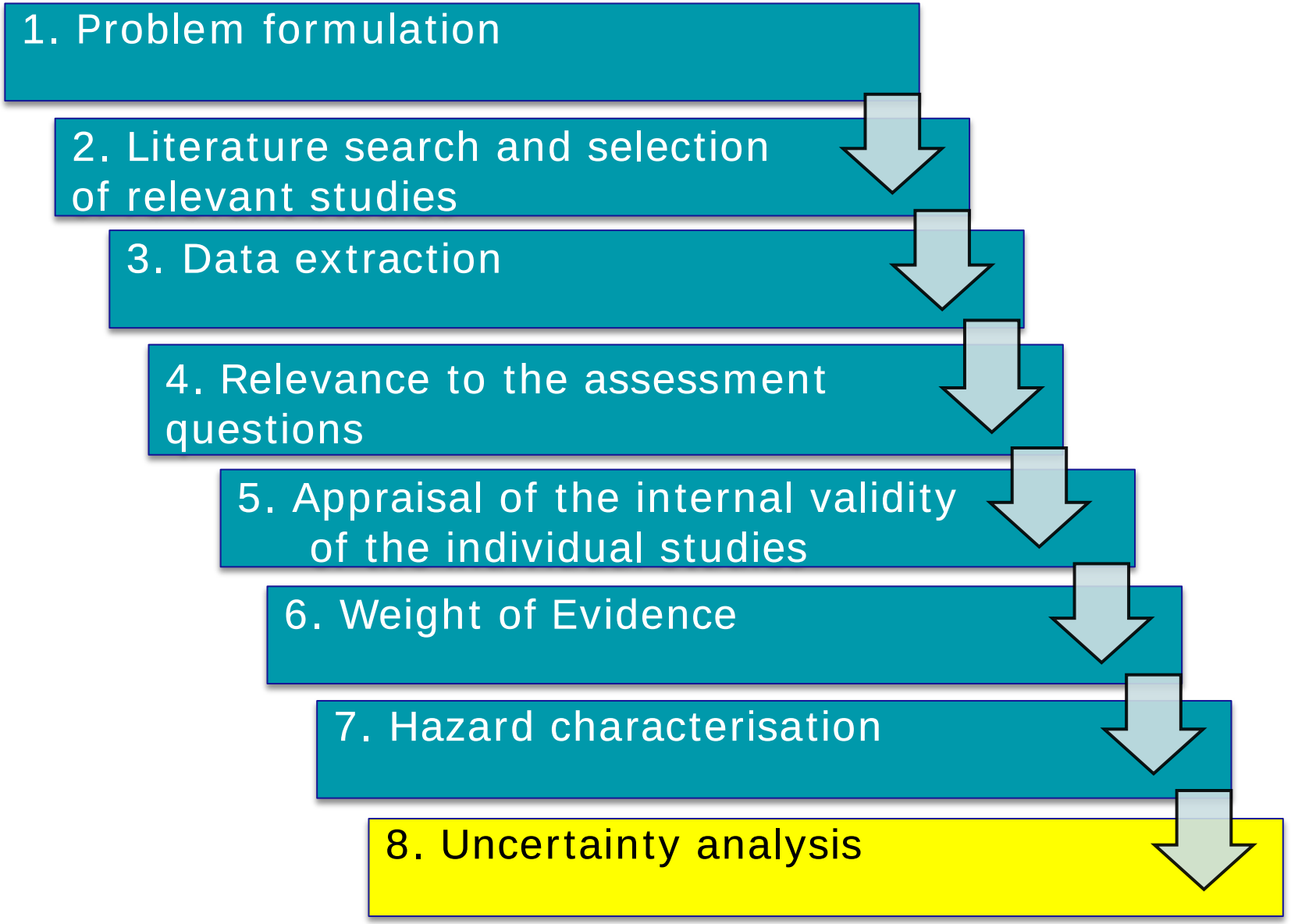
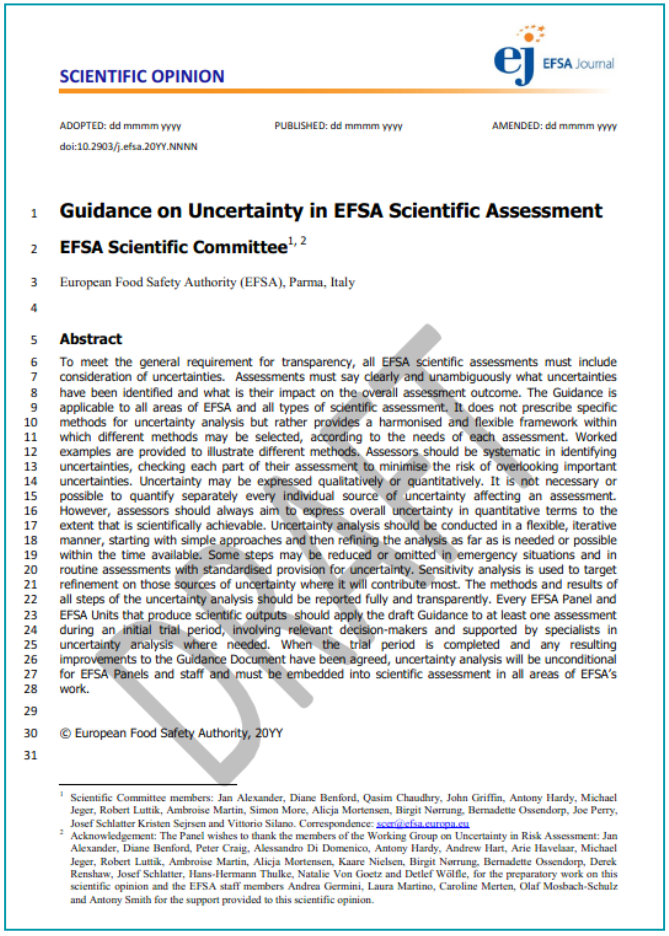


8. Uncertainty analysis

- guidelines described in the current draft guidance document will be followed
- partially addressed during the assessment of the individual studies
- special consideration for the situation where only effects are present which were classified “as likely as not”
- informal Expert Knowledge Elicitation to be used

Figure 5: Uncertainty guidance document (EFSA, 2017)



SCIENTIFIC OPINION

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Guidance on Uncertainty in EFSA Scientific Assessment

EFSA Scientific Committee[1, 2]

European Food Safety Authority (EFSA), Parma, Italy

Abstract

To meet the general requirement for transparency, all EFSA scientific assessments must include consideration of uncertainties. Assessments must say clearly and unambiguously what uncertainties have been identified and what is their impact on the overall assessment outcome. The Guidance is applicable to all areas of EFSA and all types of scientific assessment. It does not prescribe specific methods for uncertainty analysis but rather provides a harmonised and flexible framework within which different methods may be selected, according to the needs of each assessment. Worked examples are provided to illustrate different methods. Assessors should be systematic in identifying uncertainties, checking each part of their assessment to minimise the risk of overlooking important uncertainties. Uncertainty may be expressed qualitatively or quantitatively. It is not necessary or possible to quantify separately every individual source of uncertainty affecting an assessment. However, assessors should always aim to express overall uncertainty in quantitative terms to the extent that is scientifically achievable. Uncertainty analysis should be conducted in a flexible, iterative manner, starting with simple approaches and then refining the analysis as far as is needed or possible within the time available. Some steps may be reduced or omitted in emergency situations and in routine assessments with standardised provision for uncertainty. Sensitivity analysis is used to target refinement on those sources of uncertainty where it will contribute most. The methods and results of all steps of the uncertainty analysis should be reported fully and transparently. Every EFSA Panel and EFSA Units that produce scientific outputs should apply the draft Guidance to at least one assessment during an initial trial period, involving relevant decision-makers and supported by specialists in uncertainty analysis where needed. When the trial period is completed and any resulting improvements to the Guidance Document have been agreed, uncertainty analysis will be unconditional for EFSA Panels and staff and must be embedded into scientific assessment in all areas of EFSA’s work.

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[1] Scientific Committee members: Jan Alexander, Diane Benford, Qasim Chaudhry, John Griffin, Antony Hardy, Michael Jeger, Robert Luttik, Ambroise Martin, Simon More, Alicja Mortensen, Birgit Nørrung, Bernadette Ossendorp, Joe Perry, Josef Schlatter Kristen Sejrsen and Vittorio Silano. Correspondence: scer@efsa.europa.eu

[2] Acknowledgement: The Panel wishes to thank the members of the Working Group on Uncertainty in Risk Assessment: Jan Alexander, Diane Benford, Peter Craig, Alessandro Di Domenico, Antony Hardy, Andrew Hart, Arie Havelaar, Michael Jeger, Robert Luttik, Ambroise Martin, Alicja Mortensen, Kaare Nielsen, Birgit Nørrung, Bernadette Ossendorp, Derek Renshaw, Josef Schlatter, Hans-Hermann Thulke, Natalie Von Goetz and Detlef Wölfle, for the preparatory work on this scientific opinion and the EFSA staff members Andrea Germini, Laura Martino, Caroline Merten, Olaf Mosbach-Schulz and Antony Smith for the support provided to this scientific opinion.

Thank you for your attention

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Annex 1a: Determination of HEDF

- HEDF (= AUCAnimal/AUCHuman) values were calculated from experimentally determined serum/plasma AUCs of unconjugated BPA from adult and neonatal animals for a common gavage or injection dose of 100 µg/kg bw and from AUCs for human adults in the study of Thayer et al. (2015).

- The HED represent the multiples of BPA dose (D) in an animal species by a specified route that a human would require to obtain an equivalent AUC from oral administration (D × HEDF = HED).

- For comparison, the comparable dose adjustment factors (DAF) are shown which were derived by using the US EPA default of animal-to-human body weight ratios raised to the ¼ power.

Annex 1b: Determination of HEDF

Determination of HEDF for human adults

Species-Route	AUC-Adult (nmol × h × l^{-1})	HEDF-Adult (oral)	DAF Adult $bw^{1/4}$ Scaling
Mouse-oral	0.244	0.011 (= 0.244/23)	0.14 = $(0.025/70)^{1/4}$
Mouse IV injection	54	2.34 (= 54 /23)	
Rat-oral	2.6	0.7211 (= 2.6/23)	0.24 = $(0.25/70)^{1/4}$
Rat IV injection	95	4.1 (= 95 /23)	
Monkey-oral	1.5	0.065 (= 1.5/23)	0.55 = $(6.6/70)^{1/4}$
Monkey IV injection	180	7.53 (=180/23)	
Human-oral	23	n.a.	n.a.
Thayer et al. 2015	(reference value)		

Annex 1c: Determination of HEDF

Determination of HEDF for human infants

Species-Route	AUC-Neonate (nmol × h × l^{-1})	HEDF-Neonate (oral)
Mouse-oral	26	0.23 (= 26/80)
Mouse SC injection	26	0.23 (= 26/80)
Rat-oral	56	0.7 (= 56/80)
Rat SC injection	930	11.6 (= 930/80)
Monkey-oral	5.7	0.088 (= 5.7/80)
Monkey IV injection	190	2.375 (=190/80)
Human-oral	(reference value)	?
0-3 months	80	
older than 6 months	23	
AUC (=23) taken from Thayer et al. (2015) and adjusted by age specific factors derived after Mielke and Gundert-Remy (2009)		

Annex 2a: Hazard assessment sub-questions

#	HA step	Hazard assessment sub-questions	Approach
1	Hazard Identification	Is there new evidence with regards to any association between exposure to BPA at any pre- and/or postnatal life stage and general toxicity (e.g. liver and kidney), or reproductive and developmental, neurological, immune, cardiovascular, metabolic, mammary gland or carcinogenic outcomes in humans?	Systematic
2	Hazard Identification	Is there new evidence with regards to any association between exposure to BPA at any pre- and/or postnatal life stage and general toxicity (e.g. liver and kidney) or reproductive/developmental, neurological, immune, cardiovascular, metabolic mammary gland or carcinogenic outcomes at doses below the oral cut-off value of 10 mg BPA/kg bw per day or 0.5 mg/kg bw per day subcutaneous in mammalian animals?	Systematic
3	Hazard Identification	Is there new evidence with regards to BPA genotoxicity in vitro or in vivo?	Narrative
4	Hazard Identification	Is there new evidence with regards to an association between exposure to BPA at any pre- and/or postnatal life stage and any outcome not mentioned in Q1 in humans?	Systematic
5	Hazard Identification	Is there new evidence with regards to an association between exposure to BPA at any pre- and/or postnatal life stage and any outcome not mentioned in Q2 in mammalian animals?	Systematic

Annex 2b: Hazard assessment sub-questions'

6	Hazard Identification	What is the new evidence with regards to the MoA of BPA arising from in vitro studies at concentrations lower than 100 nM?	Narrative
7	Hazard Identification	Is there new evidence with regards to the MoA of BPA arising from other studies?	Narrative
8	Hazard characterisation	Is there new evidence with regards to BPA toxicokinetics in humans?	Narrative
9	Hazard characterisation	Is there new evidence with regards to BPA toxicokinetics in experimental mammalian animal species/strains?	Narrative
10	Hazard characterisation	Does the new evidence on the toxicokinetics of BPA in humans and experimental mammalian animals still support the same HED factors used in the 2015 EFSA opinion on BPA?	Informed by sub-questions 8 and 9
11	Hazard characterisation	What is the dose-response relationship for relevant outcomes in humans?	Informed by sub-questions 1 and 4
12	Hazard characterisation	What is the dose-response relationship for relevant outcomes in experimental animals according to the new evidence?	Informed by sub-questions 2, 3 and 5

Annex 3a: Appraisal of the internal validity

Internal validity of individual Human studies

Quality evaluation (adapted from NTP OHAT)

#	Question	Rating*
1	**KEY A** Can we be confident in the exposure characterisation (methods)?	
2	**KEY B** Can we be confident in the outcome Assessment (methods)?	
3	**KEY C** Was the time-window between exposure and outcome assessment appropriate?	
4	Do the statistical methods seem appropriate?	
Overall assessment of quality (Reliable (R), Reliable with restrictions (RR)		

*++, +, -, - -

RoB evaluation (adapted from NTP OHAT)

#	Question	Rating*
1	**KEY A** Did selection of study participants result in appropriate comparison groups?	
2	**KEY B** Did the study design or analysis account for important confounding and modifying variables?	
3	Were outcome data completely reported without attrition or exclusion from analysis?	
4	Was the exposure characterised consistently across study groups?	
5	Was the blinding applied and measurement consistent across study groups?	
6	Were all measured outcomes reported?	
Overall rating (Low, medium or High RoB)		

*++, +, -, - -

Annex 3b: Appraisal of the internal validity

Internal validity of individual Animal studies

Quality evaluation (adapted from SciRAP)

#	Quality element	Rating*
1	The test compound or mixture was unlikely to contain any impurities that may significantly have affected its toxicity	
2	**KEY A** A concurrent negative control group was included.	
3	**KEY B** Reliable and sensitive animal model	
4	Animals were individually identified.	
5	Housing conditions are appropriate	
6	The number of animals per sex in each cage was appropriate for the study type and animal model.	
7	Possible contaminants in the test system (e.g. phytoestrogens).	
8	An adequate number of doses was selected	
9	**KEY C** The timing and duration of administration were appropriate	
10	**KEY D** Reliable and sensitive test methods for the selected endpoints.	
11	**KEY E** Appropriate time points for measurements	
12	**KEY F** Appropriate statistics and number of animals per dose group	
Overall assessment of quality (Reliable (R), Reliable with restrictions (RR) and Not reliable (NR))		

RoB evaluation (adapted from NTP OHAT)

#	Question	Rating**
1	**KEY A** Was administered dose or exposure level adequately randomised?	
2	Was allocation to study group adequately concealed	
3	Were experimental conditions identical across study groups?	
4	**KEY B** Were outcome data completely reported without attrition or exclusion from analysis?	
5	Can we be confident in the exposure characterisation?	
6	**KEY C** Can we be confident in the outcome Assessment?	
7	Were all measured outcomes reported?	
Overall rating ((Low, medium or High RoB))		

*Fulfilled, partially fulfilled or not fulfilled

**++, +, -, - -