Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

Claude Lambre'

Chair of the EFSA Scientific Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel)

24 January 2022

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Welcome & Introduction

Welcome

- Participants: Registered stakeholders
- Chair: Claude Lambré (CEP chair)
- Presenters and Q&A panellists:
  - Members of EFSA Working Group on BPA re-evaluation
  - EFSA staff members

Draft scientific opinion on the re-evaluation of the risks to public health related to the presence of BPA in foodstuffs

- **24 Nov. 2021**

  The EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) endorsed for public consultation the draft scientific opinion.

- **15 Dec. 2021 to 22 Feb. 2022**

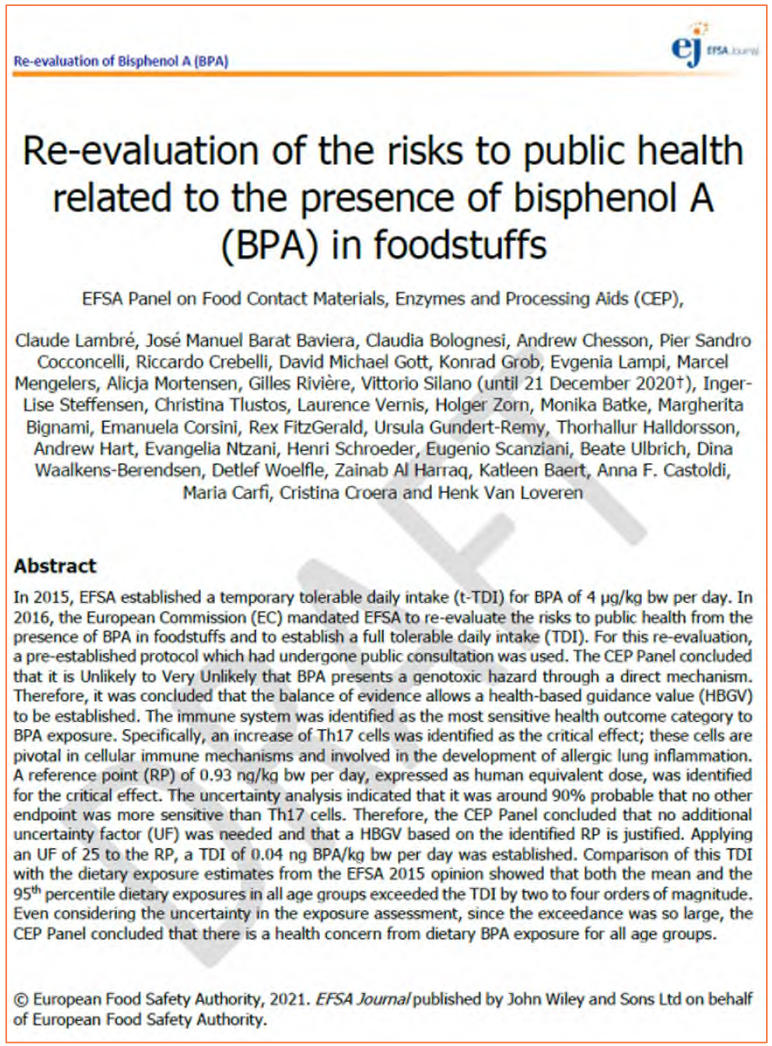
  Public consultation open.

  Interested parties can submit comments using the dedicated EFSA webpage.

  https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l1v00000E8BRD/pc0109

- **24 Jan. 2022**

  Meeting with stakeholders and other interested parties to gather feedback.



Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),

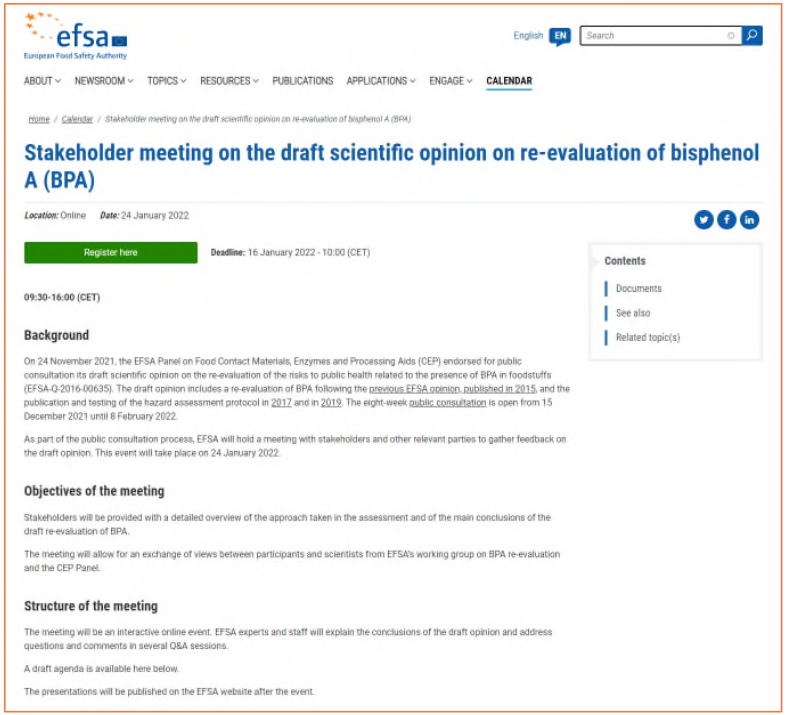
Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Laurence Vernis, Holger Zorn, Monika Batke, Margherita Bignami, Emanuela Corsini, Rex FitzGerald, Ursula Gundert-Remy, Thorhallur Halldorsson, Andrew Hart, Evangelia Ntzani, Henri Schroeder, Eugenio Scanziani, Beate Ulbrich, Dina Waalkens-Berendsen, Detlef Woelfle, Zainab Al Harraq, Katleen Baert, Anna F. Castoldi, Maria Carfì, Cristina Croera and Henk Van Loveren

Abstract

In 2015, EFSA established a temporary tolerable daily intake (t-TDI) for BPA of 4 μg/kg bw per day. In 2016, the European Commission (EC) mandated EFSA to re-evaluate the risks to public health from the presence of BPA in foodstuffs and to establish a full tolerable daily intake (TDI). For this re-evaluation, a pre-established protocol which had undergone public consultation was used. The CEP Panel concluded that it is Unlikely to Very Unlikely that BPA presents a genotoxic hazard through a direct mechanism. Therefore, it was concluded that the balance of evidence allows a health-based guidance value (HBGV) to be established. The immune system was identified as the most sensitive health outcome category to BPA exposure. Specifically, an increase of Th17 cells was identified as the critical effect; these cells are pivotal in cellular immune mechanisms and involved in the development of allergic lung inflammation. A reference point (RP) of 0.93 ng/kg bw per day, expressed as human equivalent dose, was identified for the critical effect. The uncertainty analysis indicated that it was around 90% probable that no other endpoint was more sensitive than Th17 cells. Therefore, the CEP Panel concluded that no additional uncertainty factor (UF) was needed and that a HBGV based on the identified RP is justified. Applying an UF of 25 to the RP, a TDI of 0.04 ng BPA/kg bw per day was established. Comparison of this TDI with the dietary exposure estimates from the EFSA 2015 opinion showed that both the mean and the 95th percentile dietary exposures in all age groups exceeded the TDI by two to four orders of magnitude. Even considering the uncertainty in the exposure assessment, since the exceedance was so large, the CEP Panel concluded that there is a health concern from dietary BPA exposure for all age groups.

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

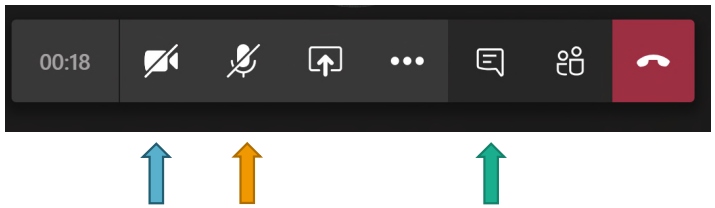
- Interactive online event to provide:
  - A detailed overview of the approach taken in the assessment
  - The main conclusions of the draft re-evaluation of BPA
- Participation open to all interested stakeholders that registered until 16 Jan. 2022 at 10h00 CET.
- EFSA experts and staff to explain the conclusions of the draft opinion and address questions and comments in two Q&A sessions.
- Possibility to exchange views between participants and scientists from EFSA's working group on BPA re-evaluation and the CEP Panel.
- The presentations given will be published on the EFSA website and the link provided after the event.



Agenda

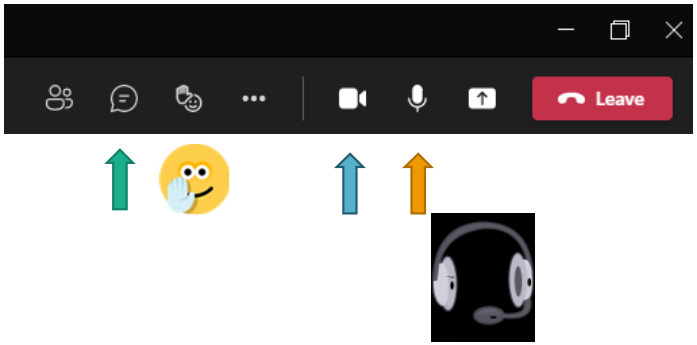
Time	No	Topic	Presenters
9:30	1	**Welcome and introduction (10')**	Claude Lambré (Chair CEP Panel)
9:40	2	**Background of the request (10')**	Valeriu Curtui (EFSA)
		Re-evaluation of Bisphenol A – Overview of the EFSA CEP Panel Risk Assessment	
9:50	3	Methodology (30')	Cristina Croera (EFSA)
10:20	4	Hazard identification - Part I (30') Toxicokinetics, general toxicity, metabolic effects and cardiotoxicity	Ursula Gundert-Remy (Member WG BPA re-evaluation)
10:50	5	Hazard identification - Part II (30') Genotoxicity, carcinogenicity and mammary gland proliferative effects, neurotoxicity and developmental neurotoxicity, and reproductive and developmental toxicity	Rex FitzGerald (Member WG BPA re-evaluation)
11:20	*Coffee break (20')*		
11:40	6	**Q&A session 1 (20')**	*Chair: Claude Lambré*
12:00	7	Hazard identification – Part III (25') Immunotoxicity	Henk van Loveren (Chair WG BPA re-evaluation)
12:25	8	Hazard and risk characterisation (25')	Katleen Baert (EFSA)
12:50	*Lunch break (100')*		
14:30	9	**Q&A session 2 (75')**	*Chair: Claude Lambré*
15:45	10	**Concluding remarks and closing of the meeting (15')**	Claude Lambré (Chair CEP Panel)
16:00	*End of the meeting*		

Good practice of conduct for participants during presentations & breaks



- This meeting is run remotely using the TEAMS Platform.
- This meeting will be recorded for internal purposes only and it will not be published on any communication platform.
- During the presentations, please switch off your camera and mute your microphone to avoid background noise.
- Use the chat function to write your questions and comments.
- During coffee and lunch breaks, please **remain connected**.

Good practice of conduct for participants during Q&A session



- Questions related to the **BPA hazard identification, characterisation and risk characterisation** will be addressed by the members of the EFSA Working Group on BPA re-evaluation and by EFSA staff during the Q&A sessions:
  - 11:40-12:00
  - 14:30-15:45
- When requested to talk, please turn on your camera and microphone and ensure no background noise by making use of headset.
- Write in the chat or use  if you would like to address a specific comment during the Q&A discussions.
- Please note that **questions related to matters not falling** within the scope of this meeting or within the remit of EFSA (e.g. risk management) will not be addressed.
- Relevant questions and/or comments that may not be addressed during this meeting due to time constraints can be submitted through the **public consultation platform** on EFSA website.

Next presentation → Background of the request

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

Background of the request

Valeriu Curtui

Head of Food Ingredients and Packaging Unit, EFSA

24 January 2022

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Overview of previous EFSA evaluations on BPA

2015

EFSA scientific opinion on BPA risk assessment

- The CEF Panel dealt with the assessment of the risk to public health associated with BPA exposure.
- Relative kidney weight in mice was considered as the most critical effect and used for the derivation of a **temporary Tolerable Daily Intake (t-TDI)** of **4 µg/kg bw per day.**
- The TDI was set as temporary due to **uncertainties** in the database and highlighted the need to fill **data gap (e.g., NTP Clarity chronic study).**
- By comparing this t-TDI with the exposure estimates, the CEF Panel concluded that there was no health concern for any age group from dietary exposure or from aggregated exposure. However, while the uncertainty around dietary estimates was relatively low, the CEF Panel noted considerable uncertainty in the exposure estimates for non-dietary sources (EFSA CEF Panel, 2015).

Overview of previous EFSA evaluations on BPA

2016

EFSA statement on BPA immunotoxicity based on two new studies

- Followed a request from the Dutch authorities for the **re-evaluation of the t-TDI** set in 2015.
- Evaluation of two additional studies on the **effects of exposure to BPA on the immune system** including investigation of potential allergic conditions.
- The CEF Panel confirmed its position that the studies available at that time suggested effects on the immune system, but that the studies were **not sufficiently robust** to take them forward for risk characterisation.

Overview of previous EFSA evaluations on BPA

2015

Scientific opinion on BPA risk assessment

Temporary Tolerable Daily Intake (t-TDI): 4 µg/kg bw per day

2016

Statement on BPA immunotoxicity based on two new studies

2016

New two step-mandate on BPA hazard re-evaluation by EC to EFSA on the basis of new evidence available and on the NTP CLARITY study report published in 2018

New two step-mandate on BPA hazard re-evaluation by EC to EFSA (2016)

Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002, the European Commission asks EFSA to:

- **establish a protocol** detailing the **criteria** for new study inclusion and for toxicological **evidence appraisal** for the re-evaluation of BPA, to ensure an efficient and transparent re-assessment of BPA.

Methodology

- **re-evaluate the risks** to public health related to the presence of BPA in foodstuffs. In particular, the re-evaluation should take into consideration new data available from the results of the **US National Toxicology Program (NTP)/Food and Drug Administration (FDA) study** due in 2017 as well as all other new available information not previously evaluated by EFSA and which fulfil the criteria laid down in an established protocol. This re-evaluation should seek to clarify the **remaining uncertainties** concerning the toxicological endpoints of BPA, especially those concerning the mammary gland, reproductive, metabolic, neurobehavioural and immune systems and to establish a **full tolerable daily intake (TDI)** on the basis of the new information available.

Hazard identification, characterisation & Risk characterisation

Mandate allocation

Mandate from the **European Commission** (EC, EFSA-Q-2016-00635) to set up a BPA hazard assessment protocol and to re-evaluate the safety for consumers of BPA.

EFSA has allocated this task to the EFSA **CEP Panel** (EFSA Scientific Panel on Food Contact Materials, Enzymes and Processing Aids)

Working Group composition

Members of **EFSA's Working Group** on BPA re-evaluation:

- Henk Van Loveren (Chair)
- Monika Batke (External)
- Margherita Bignami (CONTAM Panel)
- Claudia Bolognesi (CEP Panel)
- Emanuela Corsini (External)
- Riccardo Crebelli (CEP Panel)
- Rex FitzGerald (External)
- David Gott (CEP Panel)
- Ursula Gundert-Remy (FAF Panel)
- Thorhallur Halldorsson (EFSA SC)
- Andy Hart (External)
- Evangelia Ntzani (CONTAM Panel)
- Eugenio Scanziani (External)
- Inger-Lise Steffensen (CEP Panel)
- Henri Schroeder (External)
- Ine Waalkens-Berendsen (FAF Panel)
- Detlef Wölfle (FAF Panel)

Outputs developed

1st step: BPA hazard assessment protocol

2017: Publication of the protocol

Details the *a priori* approach used for an efficient, transparent and methodologically rigorous re-assessment of the BPA safety.

TECHNICAL REPORT

APPROVED: 30 November 2017

doi:10.2903/sp.efsa.2017.EN-1354

Bisphenol A (BPA) hazard assessment protocol

European Food Safety Authority (EFSA),
Ursula Gundert-Remy, Johanna Bodin, Cristina Bosetti, Rex FitzGerald, Annika Hanberg, Ulla Hass, Carlijn Hooijmans, Andrew A. Rooney, Christophe Rousselle, Henk van Loveren, Detlef Wölfle, Fulvio Barizzone, Cristina Croera, Claudio Putzu and Anna F. Castoldi

2nd step: Re-evaluation of BPA safety

2021: Draft opinion endorsed for Public consultation

Takes into consideration the new data from the US NTP/FDA study as well as all other new available information. Seeks to clarify the remaining uncertainties. No updates on exposure assessment.

Re-evaluation of Bisphenol A (BPA)

Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),

Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Vittorio Silano (until 21 December 2020†), Inger-Lise Steffensen, Christina Tlustos, Laurence Vernis, Holger Zorn, Monika Batke, Margherita Bignami, Emanuela Corsini, Rex FitzGerald, Ursula Gundert-Remy, Thorhallur Halldorsson, Andrew Hart, Evangelia Ntzani, Henri Schroeder, Eugenio Scanziani, Beate Ulbrich, Dina Waalkens-Berendsen, Detlef Woelfle, Zainab Al Harraq, Katleen Baert, Anna F. Castoldi, Maria Carfì, Cristina Croera and Henk Van Loveren

Next presentation → BPA Hazard Assessment Protocol

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

BPA hazard assessment protocol

Cristina Croera

BPA WG coordinator, EFSA

24 January 2022

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New two step-mandate on BPA hazard re-evaluation by EC to EFSA (2016)

- 1st step: BPA hazard assessment protocol


TECHNICAL REPORT

APPROVED: 30 November 2017

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The '2017 methodology'

- 2nd step: Re-evaluation of BPA safety
- Protocol testing phase →


TECHNICAL REPORT

APPROVED: 24 October 2019

doi:10.2903/sp.efsa.2019.EN-1732

Testing the study appraisal methodology from the 2017 Bisphenol A (BPA) hazard assessment protocol

European Food Safety Authority (EFSA)

Cristina Croera, Monika Batke, Emanuela Corsini, Rex E. FitzGerald, David Gott, Evangelia Ntzani, Ursula Gundert-Remy, Thorhallur Halldorsson, Henri Schroeder, Eugenio Scanziani, Inger-Lise Steffensen, Beate Ulbrich, Ine Waalkens-Berendsen, Detlef Wölfle, Fulvio Barizzone, Federica Barrucci, Ellen Van Haver, Anna F. Castoldi and Henk Van Loveren

Final step: hazard and risk characterisation

Hazard assessment protocol: systematic approach

1. Problem formulation
2. Literature search & selection studies
3. Appraisal of the internal validity
4. Appraisal of the external validity
5. Data extraction
6. Weighing the body of evidence
7. Selection of the effects for HC
8. Uncertainty analysis

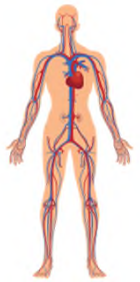
WG tasks

1. Problem formulation

- **Aim** of this hazard assessment:

To assess whether the new scientific evidence (published after 31/12/2012, and not previously appraised by the EFSA), still supports the previous t-TDI for BPA of 4 µg/kg bw per day.

- Decision should be based on the evaluation of:
  - (i) **adverse effects in humans** associated with the exposure to BPA via any route;
  - (ii) **adverse effects in animals** after exposure to BPA via any route;
  - (iii) human and animal **toxicokinetics** of BPA



BPA Hazard Assessment protocol

1. Problem formulation
2. Literature search and selection of relevant studies
3. Appraisal of the internal validity
4. Appraisal of the external validity (fo animal studies only)
5. Data extraction
6. Weighing the body of evidence
7. Selection of the effects for the hazard characterisation
8. Uncertainty analysis

2. Literature search and selection of relevant studies

- **SYSTEMATIC REVIEW**

- All human and animal toxicity data potentially providing a point of departure for setting a TDI

- **NARRATIVE REVIEW:**

- Human cross-sectional studies
- Human and animal *in vitro* and mechanistic studies (MoA studies)
- Toxicokinetic studies

2. Literature search and selection of relevant studies

- **Literature search:**

➢ from 2013 till 15 October 2018→ for all the health outcome categories (HOCs), *apart for Genotoxicity* (*till July 2021 + studies evaluated in the 2015 opinion*)

- Two steps **selection process** (using Distiller software):

1. Screening of Title + Abstract: screen for relevance to the general scope of the assessment

2. Screening of Full text:
   - **General inclusion criteria**, e.g. availability of full text in English, primary study
   - **Specific inclusion criteria** for human, animal and in vitro studies, related e.g. to study design and routes of exposure and cut-off doses

BPA Hazard Assessment protocol

1. Problem formulation
2. Literature search and selection of relevant studies
3. Appraisal of the internal validity
4. Appraisal of the external validity (for animal studies only)
5. Data extraction
6. Weighing the body of evidence
7. Selection of the effects for the hazard characterisation
8. Uncertainty analysis

3. Appraisal of the internal validity

Internal validity: relates as to whether a human or animal study answers correctly the research question free from bias.

- Two aspects considered for **risk of bias (RoB)**:
  a. Introduction of a systematic difference between the control and the exposed group only (e.g., non-randomised allocation of animals to study groups)
  b. Introduction of a bias potentially affecting to the same extent the control and the exposed study groups (e.g., the reliability of the method used to test the outcome)
- Appraisal conducted **per study** (group of endpoints), except if there was any endpoint triggering different scoring, even in one question
- Appraisal performed **using Distiller software**

Tool for human data (case-control and cohort study design), adapted from NTP/OHAT, 2015

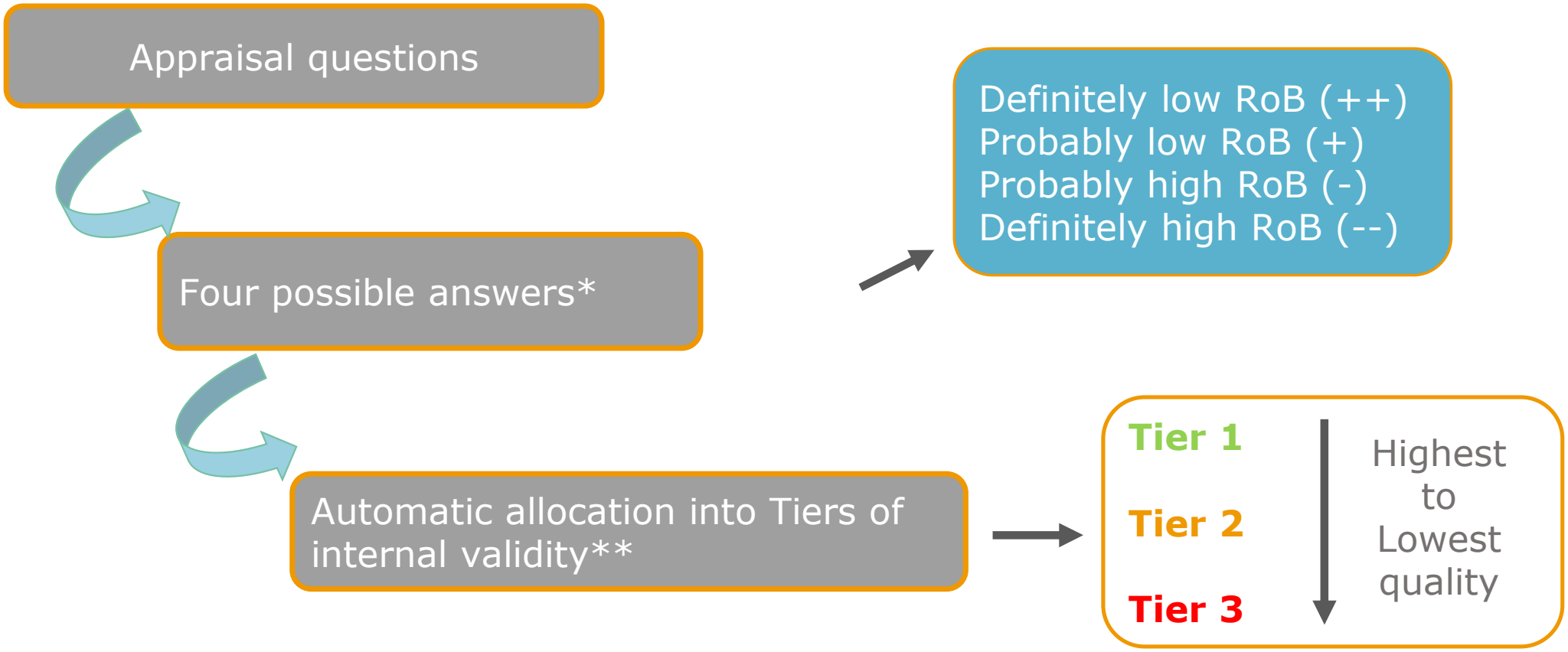
#	Key Q	Question	Domain	Rating (++, +, -, --)
1	A	Did selection of study participants result in appropriate comparison groups?	Selection	
2		Were outcome data completely reported without attrition or exclusion of experimental units from analysis?	Attrition	
3	B	Can we be confident in the exposure characterisation?	Detection	
4	C	Can we be confident in the outcome assessment?	Detection	
5	D	Did the study design or analysis account for important confounding and modifying variables?	Confounding	
6		Were all measured outcomes reported?	Selective reporting	
7	E	Do the statistical methods seem appropriate?	Other sources of bias	

3. Appraisal of the internal validity

Tool for animal data, adapted from OHAT, 2015

#	Key Q	Question	Domain	Rating (++, +, -, --)
1		Was the administered dose or exposure level adequately randomised?	Selection	
2		Was the allocation to study group adequately concealed	Selection	
3		Were the experimental conditions identical across study groups?	Performance	
4		Were outcome data completely reported without attrition or exclusion from analysis?	Attrition	
5		Can we be confident in the exposure characterisation?	Detection	
	A	Sub-question: Did the test compound contain any impurities?		
6		Can we be confident in the outcome assessment?	Detection	
	B	Sub-question: Were the outcome assessors adequately blinded to the study group?		
7		Were all measured outcomes reported?	Selective reporting	
8		Were the statistical methods and the number of animals per dose group appropriate?	Other sources of bias	
	C	Sub-question: Was the number of animals per dose group appropriate?		

3. Appraisal of the internal validity



*According to the Guidelines on the RoB rating for each internal validity question (adapted from NTP-OHAT, 2015) and to a Catalogue of decisions developed by experts and EFSA staff for making the decisions of the experts as much objective as possible

**According to pre-defined rules for Tier allocation set up in the Distiller software

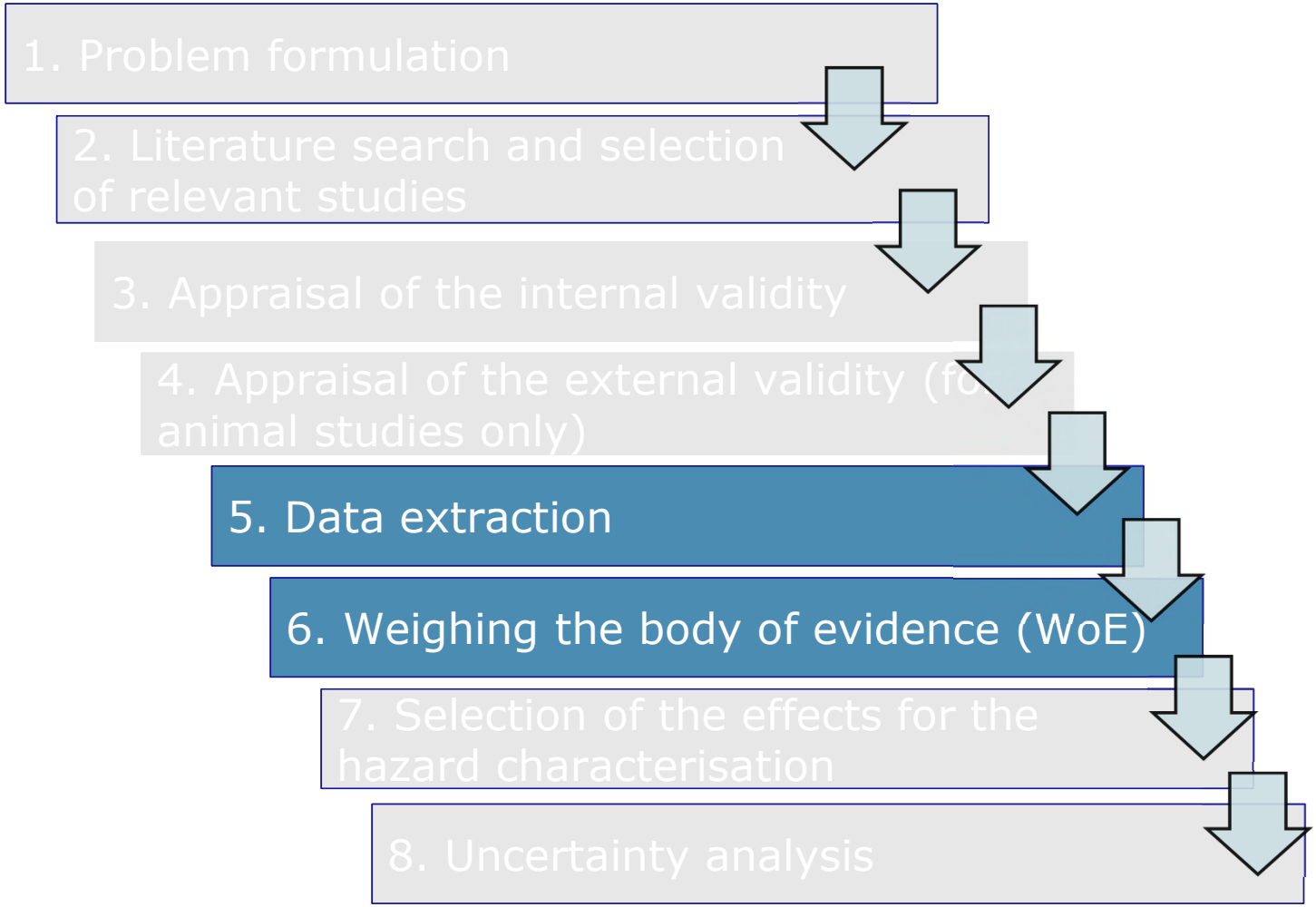
4. Appraisal of the external validity (for animal studies only)

It refers to the **relevance** of measuring a given **endpoint** (i.e., an apical or intermediate measured outcome) in an **animal model** with respect to human health.

- **Two questions to answer:**
  - **Q1:** Is the **animal model** relevant for human health outcomes?
  - **Q2:** Is/are the **endpoint/s** relevant for human health outcomes?

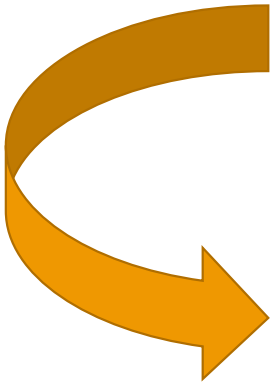
- **Three possible ratings** according to the SciRAP tool guidance criteria (www.scirap.org; Beronius et al., 2014):
  - **Directly relevant** (only for Q2), **indirectly relevant** or **not relevant**

BPA Hazard Assessment protocol



5. Data extraction

- Following the appraisal for internal and external validity:
  - The **data extraction** of the **studies results** for **relevant endpoints** was performed.
  - These endpoints were then **grouped into structural and/or functional clusters, for each health outcome category** (HOC).



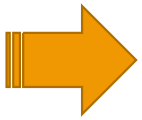
- General toxicity
- Immunotoxicity
- Metabolic effects
- Neurotoxicity and developmental neurotoxicity
- Reproductive and developmental toxicity
- Cardiotoxicity
- Carcinogenicity and mammary gland proliferative effects
- Genotoxicity

5. Data extraction

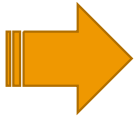
- Following the appraisal for internal and external validity:
  - The **data extraction** of the **studies results** for **relevant endpoints** was performed.
  - These endpoints were then **grouped into structural and/or functional clusters, for each health outcome category** (HOC).

E.g., for the human studies:

HOC:
Immunotoxicity



Relevant endpoints:
Acute bronchiolitis
Acute otitis media
Allergic diseases
Asthma
Atopic dermatitis
Atopy
Bacterial colonisation
Bronchitis
Chest infections
Croup
Eczema
Forced respiratory volume in 1s (FEV_1)
Fraction of exhaled nitric oxide (FENO)
IgE
PC20
Pneumonia
Rashes, eczema or hives
Rhinitis (allergic)
Wheeze



Cluster:
Asthma/allergy

5. Data extraction: human and animal studies

Data extraction form for human cohort and case-control studies

RefID number
Author
Year of the study
Study type
Study subjects and sex
Exposure time
Age (in case of exposure during childhood)
Matrix analysed for BPA determination
BPA exposure level
Health outcome category
Cluster
Endpoints
Direction of the effect
Description of the results

Data extraction form for experimental animal studies

Study identification	RefID number
	Author
	Year of the study
Animal model	Species/(sub)strain/sex
Exposure	Period of exposure (pre-mating, mating, gestation, lactation, adult)
	Duration of exposure (e.g., GD0-GD20)
	Route of administration (diet, drinking water, gavage, s.c., i.p., i.v., dermal, inhalation, intratesticular)
	Dose regimen (dose level or concentration of BPA per group; documentation of details for dose conversion when conducted)
	Time of measurement
Results	Results per dose or concentration and at a specific time point (statistically significant increase, statistically significant decrease, no change)
Cluster (endpoint)	The cluster/s allocation of the relevant endpoint/s addressed in the study is/are reported

6.Weighing the body of evidence (WoE)

❑ **1st step:**

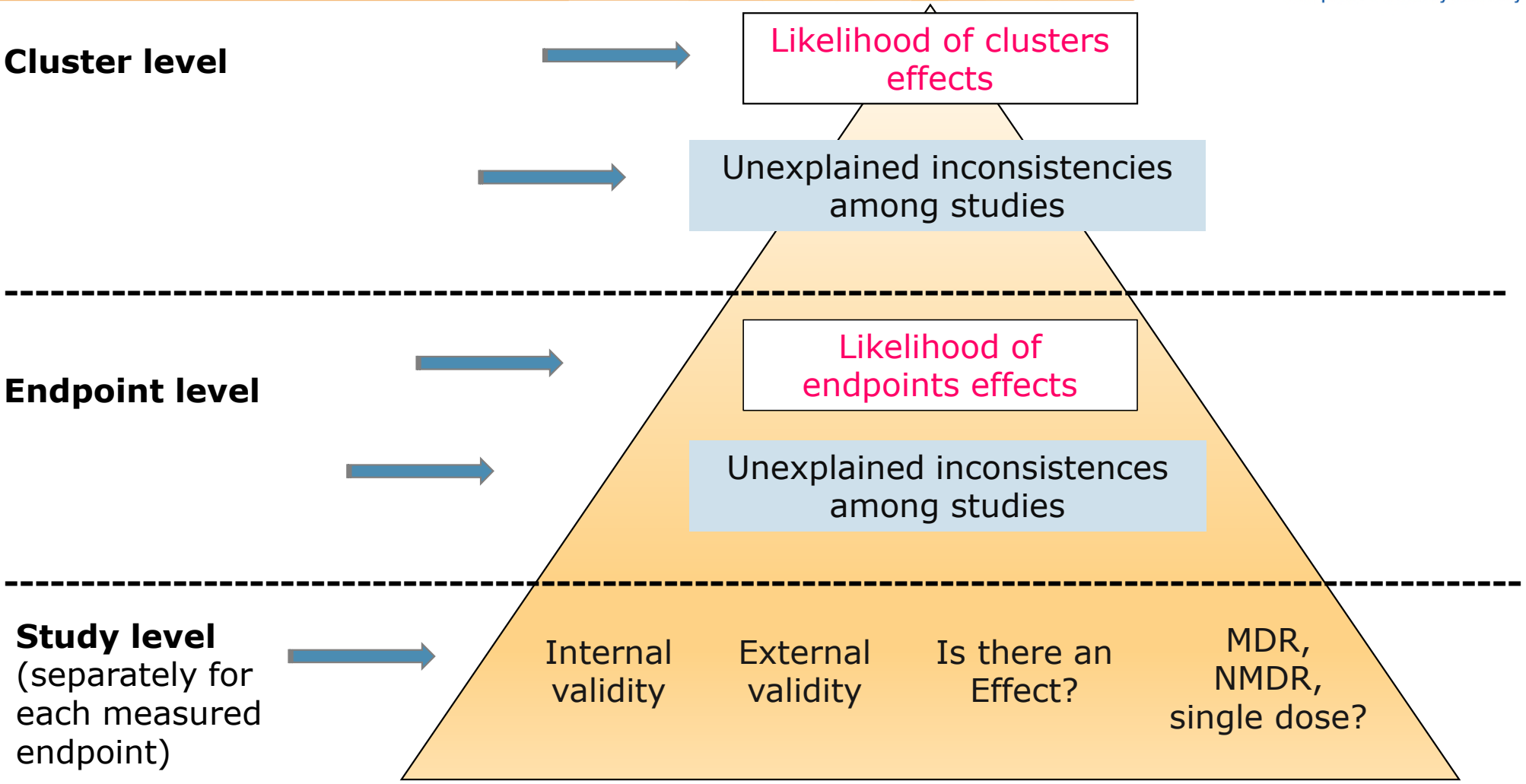
Assessment of the **level of confidence** in the overall **body of evidence** of the data extracted

Likelihood level of confidence for an association between exposure to BPA and a health effect

- Separately for **animal and human** studies
- Separately for **different exposure categories**:
  - *Pregnancy (human) or Developmental exposure* (pre-natal and/or post-natal until weaning) *(animals)*
  - *Developmental and adult exposure* (pre-natal and post-natal in pups until adulthood) *(animals)*
  - *Childhood (human) or Growth phase/young age exposure (animals)*
  - *Adult exposure* (after puberty) *(human and animal)*
  - *Indirect (germline) exposure (animals)*

- For each **HOC**
- At the **endpoint** level
- At the **cluster l**evel

6.Weighing the body of evidence (WoE)*



* Modified version of NTP-OHAT approach (2015) and of the VKM risk assessment of energy drinks and caffeine (VKM, 2019)

6.Weighing the body of evidence (WoE)

Scale of likelihoods	Definition
Very Likely (VL)	There is **very high confidence** in the body of evidence for an association between exposure to the substance and health effect/s
Likely (L)	There is **high confidence** in the body of evidence for an association between exposure to the substance and health effect/s
As likely as not (ALAN)	There is **low confidence** in the body of evidence for an association between exposure to the substance and health effect/s
Not likely	There is **very low confidence** in the body of evidence for an association between exposure to the substance and health effect/s
Inadequate evidence	There is **insufficient evidence** available to assess if the exposure to the substance is associated with and health effect/s or data are missing

6. Weighing the body of evidence (WoE)

❑ **2nd step**:

- **Integration of human and animal likelihoods for a cluster health effect,** for the selection of effects for Hazard characterization.

Human evidence						
	Very likely	VL	VL	VL	VL	VL
	Likely	L	L	L	L	VL
	ALAN	ALAN	ALAN	ALAN	L	VL
	Not likely	Not likely	Not likely	ALAN	L	VL
	Inadequate evidence	Non classifiable	Not likely	ALAN	L	VL
		Inadequate evidence	**Not likely**	**ALAN**	**Likely**	**Very likely**
		Animal evidence				

6. Weighing the body of evidence (WoE): Integration of Likelihoods

- Example for the HOC Metabolic effects:

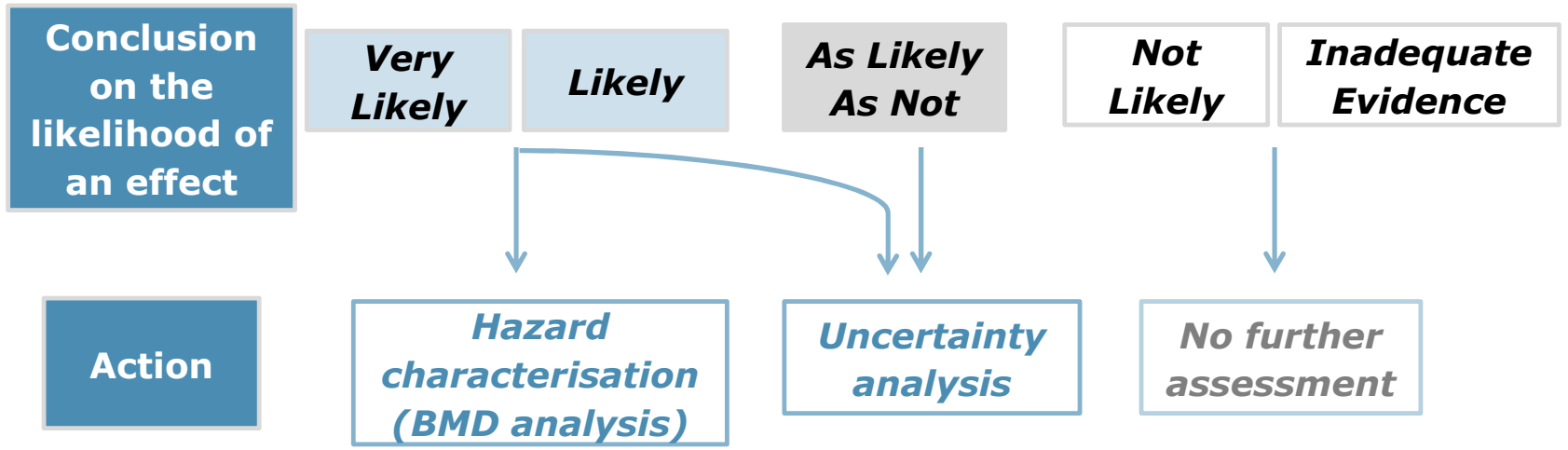
Human studies				**Animal studies**			**Application of the matrix and outcome of the integration Human-Animal**
HoC	**Endocrine, nutritional and metabolic effects**			**Metabolic effects**			**Metabolic effects of BPA**
Cluster 1	Obesity	Likelihood		Obesity	Likelihood		**Final likelihood of the 2 stream**
Exposure category	Exposure during pregnancy	Not Likely		Developmental (pre-natal and/or post-natal until weaning)	ALAN		
				Developmental and adult (pre-natal and/or postnatal in pups until adulthood)	ALAN		
	Exposure during childhood	Not Likely		Growth phase/young age	ALAN		
	Exposure during adulthood	ALAN		Adult exposure (after puberty)	Not Likely		
				Indirect (germline) exposure	Not Likely		
Overall likelihood		**ALAN**			**ALAN**		**ALAN**

BPA Hazard Assessment protocol

1. Problem formulation
2. Literature search and selection of relevant studies
3. Appraisal of the internal validity
4. Appraisal of the external validity (fo animal studies only)
5. Data extraction
6. Weighing the body of evidence
7. Selection of the effects for the hazard characterisation
8. Uncertainty analysis

7. Selection of the effects for the hazard characterisation

8. Uncertainty analysis



- Studies investigating **Very likely** or **Likely** adverse effects relevant to humans, with at least 1 ctrl+ two BPA dose levels, were considered for **benchmark dose (BMD) analysis.**
- **Very likely, Likely or ALAN** clusters were included in the **uncertainty analysis (UA).**

8. Uncertainty analysis

- The uncertainty analysis was conducted **in accordance with EFSA's guidance on uncertainty analysis**, using a combination of methods appropriate to each step of the assessment (a 'case-specific' uncertainty analysis, EFSA Scientific Committee, 2018a).

- **Aim:**

  - To assess whether other effects of BPA may potentially occur after exposure to lower doses than the endpoint on which the reference point (RP) is based and, if so, inform a decision on what size of additional uncertainty factor would be suitable to take those effects into account.

Two levels of uncertainties:

- Uncertainties in hazard and risk characterisation with potential establishement of an UF (semi-quantitative approach).
  - An **expert knowledge elicitation** (EKE) was conducted for each of the VL, L and ALAN clusters, providing a **distribution quantifying uncertainty about the estimated lowest BMD** for effects in that cluster that occur in animals and are relevant and adverse for humans.

- Uncertainties in the protocol (e.g., literature search, publication bias, internal validity, BMD analysis, HED factors, etc.), identifying *high, medium, low* potential contribution to the overall uncertainty (qualitative approach).

Next presentation → BPA Hazard Identification Part I

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

Hazard Identification - Part I

Ursula Gundert-Remy

EFSA Working Group on BPA re-evaluation

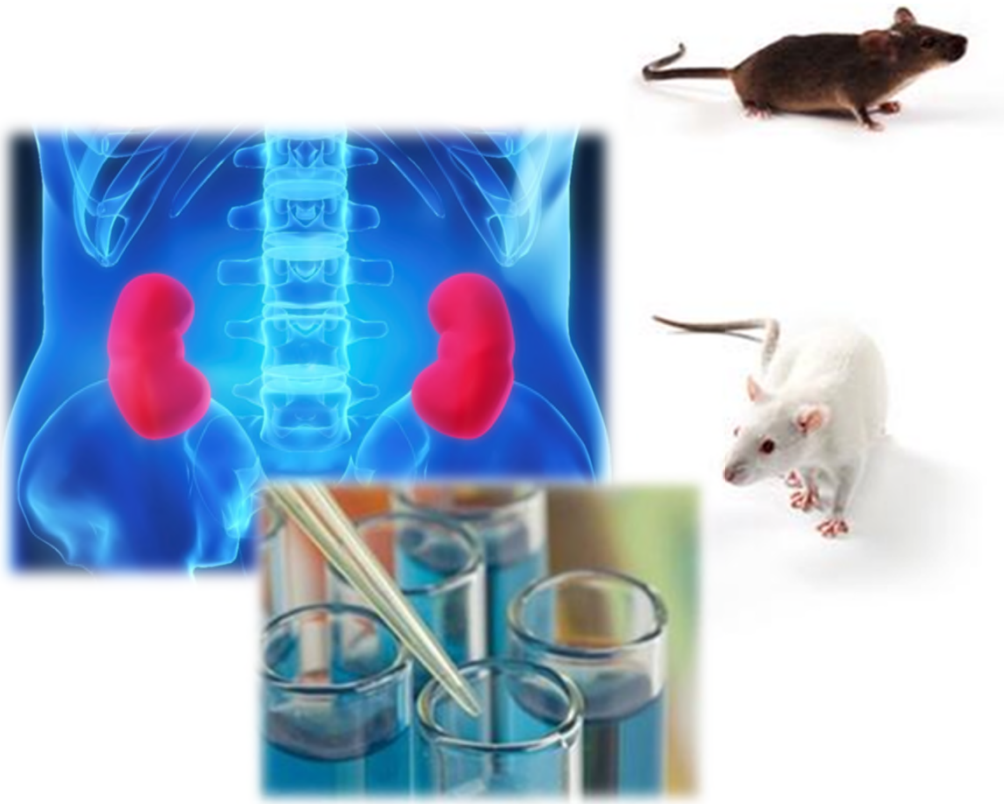
24 January 2022

Trusted science for safe food

efsa
European Food Safety Authority

Hazard Identification

- **Toxicokinetics**
- **General toxicity**
- **Metabolic effects**
- **Cardiotoxicity**



Hazard Identification: Toxicokinetics

- The new studies in **mice** and **rats** did not add relevant information to existing knowledge on the kinetics of BPA with respect to hazard assessment.

- New studies in **ewes** showed that the relative systemic availability was higher when BPA was administered via pellets compared with nasogastric tubing. This finding may be explained by contact of pellets with buccal mucosa and buccal absorption of BPA.

- As already reported in the 2015 EFSA opinion, **human** data showed that BPA is absorbed to nearly 100% and is pre-systemically metabolised to a great extent to glucuronide and sulfate conjugates.

Hazard Identification: Toxicokinetics

- New studies in **humans** not available for the EFSA opinion 2015 showed that the BPA concentration in the systemic circulation is low.
- The dose-corrected AUCs were clearly different in the two studies available (Thayer et al., 2015 and Teeguarden et al., 2015).
- The most probable explanation would be that, in the study with dose-corrected higher Cmax and higher AUC (administration of BPA via a cookie), the contact time with the buccal mucosa could be longer compared with the other study (administration via soup).
- Because of the longer contact time more BPA could have been absorbed through the mucosa without undergoing pre-systemic elimination.

Hazard Identification: Toxicokinetics

- The CEP Panel decided to use the median value of the AUCs from two human studies for the calculation of the **Human Equivalent Dose Factor (HEDF)**.
- AUC data were used from the 2015 EFSA opinion (EFSA CEF Panel, 2015) for mice, rats, monkeys and dogs. For ewes and humans, the experimental data reported in the current opinion were used.

Species (oral route)	AUC (nM × h)	HEDF (AUC animal/ AUC human)
Human (Thayer et al., 2015 and Teeguarden et al., 2015b) (median)	15.7	
Mouse (n=1)(*) (Doerge et al., 2011)	0.244	0.0155
Rat (mean) (Doerge et al., 2010)	2.6	0.1656
Ewe – gavage (mean) (Guignard et al., 2016)	1.88	0.1197
Ewe – diet (mean) (Guignard et al., 2016)	6.84	0.4357
Rhesus monkey (mean) (Doerge et al., 2010)	1.5	0.095
Dog (mean) (Gayrard et al., 2013)	2.19	0.1395

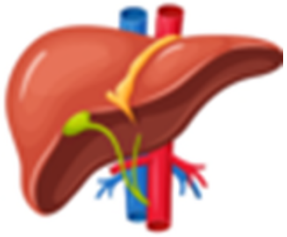
(*) Every time point concentration measurement was done from pooled blood of n= 12 mice and one AUC was derived.

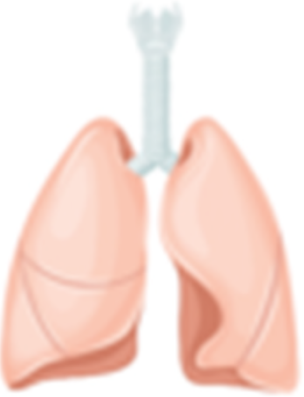
Hazard Identification: General toxicity

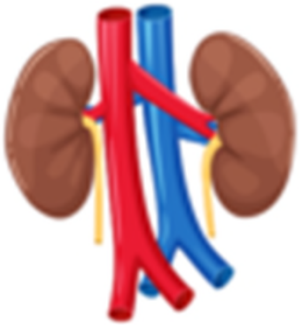
- Several organs are potential targets of toxicity for BPA and haematological parameters can be affected by this compound.

- No **human studies** were available, while 10 clusters of relevant endpoints were identified in **animal studies**:

  - Body weight (evaluated under the health outcome category (HOC) Metabolic effects)
  - Effects on organs (liver, kidney, lung, thyroid, parathyroid, pituitary gland, adrenal gland, bone marrow)
  - Effects on haematological parameters







Hazard Identification: General toxicity

- An increase of absolute **kidney weight**, along with changes in **clinical biochemistry**, was reported when exposed during adulthood, while no changes in kidney weight were observed for the other exposure periods, and therefore judged as **As Likely As Not** (**ALAN)**.

- **Histological findings in kidney** (renal tubule cysts, nephropathy and hyperplasia of the transitional epithelium) following BPA exposure during developmental life stages until weaning or adulthood were inconsistently observed in the evaluated studies, and therefore judged as **ALAN.**

- **Liver weight** changes: inconsistent outcomes were reported and considered as **Not Likely** (developmental exposure until weaning) or **ALAN** (developmental until adulthood and adult exposure).

- During the developmental until weaning or adulthood exposure periods, **histological findings in liver** (angiectasis, cystic degeneration, hepatodiaphragmatic nodules, mononuclear cell infiltration, fatty change) were not consistently observed and showed no clear dose–responses. Therefore, the CEP Panel considered the histological effects as **ALAN**.

Hazard Identification: General toxicity

- Several changes in **clinical biochemistry parameters** potentially **associated with liver toxicity** (liver enzyme activities, bilirubin levels) were observed. However, the results from studies with developmental until weaning or adulthood exposure are not consistent regarding these findings and were judged as **ALAN**.

- Considering the **other clusters in this HOC** there were only few effects reported in the WoE assessment such as (effects at very high doses of BPA at or above 25000 µg/kg bw per day (i.e. increase of relative lung weight and hypercellularity in bone marrow), inconsistent effects (i.e. hyperplastic changes in thyroid, parathyroid and pituitary gland and hypertrophic changes in adrenal gland), and effects without a clear dose-response (e.g. in haematology) that were all judged as **ALAN** or **Not Likely**.

Hazard Identification: General toxicity – integrated likelihood

- Overall, none of the evaluated clusters' effects was considered Very Likely or Likely.
- In each of the evaluated clusters there were less consistent results among the available studies and, therefore, these effects were judged as **As Likely As Not (ALAN)** in all the clusters.

D: Developmental (pre-/post-natal until weaning) exposure
D&A: Developmental until adulthood exposure
G: Growth phase / young age exposure
A: Adult exposure
I: Indirect (germline) exposure

Human stream		Animal stream		Integrated likelihood
Cluster	Overall likelihood	Cluster	Overall likelihood	
		Body weight	ALAN (D, D&A, G)	ALAN
		Kidney effects	ALAN (D, D&A, A)	ALAN
		Liver effects	ALAN (D, D&A, A)	ALAN
		Lung effects	ALAN (D)	ALAN
		Thyroid gland effects	ALAN (D, D&A)	ALAN
		Parathyroid effects	ALAN (D, D&A)	ALAN
		Pituitary gland effects	ALAN (D, D&A)	ALAN
		Adrenal gland effects	ALAN (D)	ALAN
		Bone marrow effects	ALAN (D, D&A)	ALAN
		Haematological parameters	ALAN (D, D&A)	ALAN

Modes of action (MoAs) for General toxicity

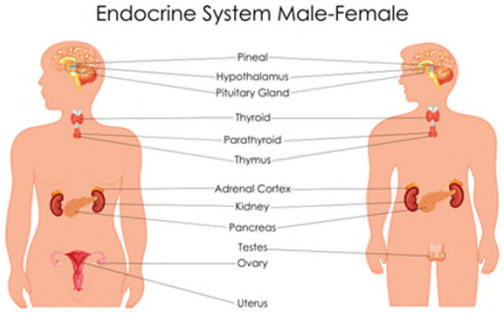
- **Oxidative stress**
  - as a potential pathogenetic mechanism for **kidney** damage.
  - leads to impaired mitochondrial function in liver cells and **liver** toxicity.

- **Epigenetic changes via DNA methylation** may have an impact on enzyme expression and hepatic metabolism.

Hazard Identification: Metabolic effects

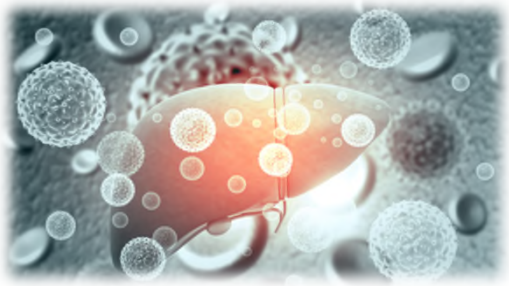
- In the **human studies**, five clusters of relevant endpoints were identified:

  - Obesity
  - Cardiometabolic effects
  - Thyroid effects
  - Type 2 diabetes mellitus (T2DM)
  - Gestational diabetes mellitus



- In the **animal studies**, eight clusters of relevant endpoints were identified:

  - Obesity
  - Fat deposition in the liver
  - Glucose regulation
  - Blood lipids
  - Uric acid
  - Type 1 diabetes mellitus (T1DM)
  - Other metabolic hormones
  - Thyroid hormones



Hazard Identification: Metabolic effects

- Based on the **human data**, none of the metabolic clusters showed effects that were considered Likely or Very Likely.
  A positive association between BPA exposure and obesity and T2DM was judged as **ALAN**, while a positive association between BPA exposure and cardiometabolic effects, thyroid effects and gestational diabetes mellitus was judged as **Not Likely**.

- Based on the **animal data**, no metabolic clusters were considered Very Likely. The cluster and endpoint uric acid was considered **Likely** (in the adult exposure period), as increased levels were observed in liver or in serum of mice or rats after BPA exposure. → This endpoint was taken forward for BMD analysis.
  The other metabolic clusters were considered either **ALAN** (obesity, fat deposition in the liver, glucose regulation, blood lipids and T1DM) or **Not Likely** (other metabolic hormones and thyroid hormones), in one or more exposure periods.

Animal to human extrapolation and adversity of increased uric acid

- **Increased uric acid** associated with BPA exposure was not only observed in animals but also observed in humans (Hu et al., 2019).
- Uric acid concentration is related to **elevated blood pressure** in animals (Mazzali et al., 2001) and humans (Borghi et al., 2015; Johnson et al., 2018)
- A causal relationship between increased uric acid and elevated blood pressure is plausible because lowering the uric acid concentration in plasma led to a decrease in the elevated blood pressure (Johnson et al., 2019).
- An increase in systolic blood pressure is related to **vascular mortality** (Prospective Studies Collaboration, 2002).

Hazard Identification: Metabolic effects – integrated likelihood

Human stream		Animal stream		Integrated likelihood
Cluster	**Overall likelihood**	**Cluster**	**Overall likelihood**	
Obesity	ALAN (A)	**Obesity**	ALAN (D, D&A, G)	ALAN
Thyroid effects	Not Likely (P)	**Thyroid hormones**	Not Likely (D, D&A, A)	Not Likely
Cardiometabolic effects	Not Likely (P)			Not Likely
T2DM	ALAN (A)			ALAN
Gestational Diabetes Mellitus	Not Likely (A)			Not Likely

P: Exposure during pregnancy;
C: Exposure during childhood;
A: Adult exposure

D: Developmental (pre-/post-natal until weaning) exposure
D&A: Developmental until adulthood exposure
G: Growth phase / young age exposure
A: Adult exposure
I: Indirect (germline) exposure

Hazard Identification: Metabolic effects – integrated likelihood

Human stream		Animal stream		Integrated likelihood
Cluster	Overall likelihood	Cluster	Overall likelihood	
		Uric Acid	**Likely** (A)	**Likely**
		T1DM	ALAN (G, A)	ALAN
		Fat deposition in the liver	ALAN (D, G, A)	ALAN
		Glucose regulation	ALAN (D, A, I)	ALAN
		Blood lipids	ALAN (A)	ALAN
		Other metabolic hormones	Not Likely (D, D&A, G, A)	Not Likely

P: Exposure during pregnancy;
C: Exposure during childhood;
A: Adult exposure

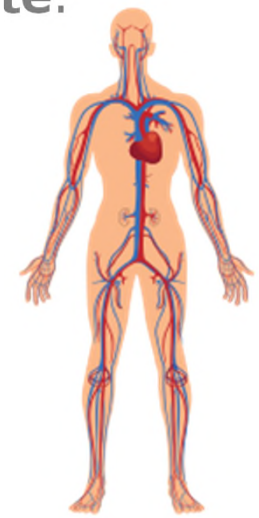
D: Developmental (pre-/post-natal until weaning) exposure
D&A: Developmental until adulthood exposure
G: Growth phase / young age exposure
A: Adult exposure
I: Indirect (germline) exposure

Modes of action for Metabolic effects

- MoA data in animals showed that BPA could increase the formation of hepatic uric acid by increasing the activity of the enzyme xanthinoxidase.
- Evidence for plausible MoAs were available for the effects of BPA on obesity, fat deposition in the liver and glucose regulation, taken from animal and in vitro studies.
- MoA data on T1DM were very limited and the results depended on the animal model used.

Hazard Identification: Cardiotoxicity

- No **human studies** were available. Therefore, the evidence for a positive association between BPA exposure and cardiotoxicity in human was considered **Inadequate**.



- In the **animal studies**, six clusters of relevant endpoints were identified:
  - Absolute and relative heart weight
  - Incidence of cardiac lesions
  - Cardiac structural changes
  - Effects on cardiac function
  - Blood pressure
  - Atherosclerotic lesions

- Based on the **animal studies**, the evidence of BPA effects was judged as **Not Likely** in the majority of the cardiotoxicity clusters, and in few clusters as **Inadequate**, in one or more exposure periods. Given the functional relationship between the endpoints, the outcome of the WoE was considered biologically plausible.

Next presentation → Hazard identification Part II

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

Hazard Identification - Part II

Rex FitzGerald

EFSA Working Group on BPA re-evaluation

24 January 2022

Trusted science for safe food

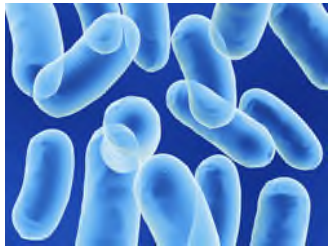
European Food Safety Authority

Hazard Identification

- **Genotoxicity**
- **Carcinogenicity and mammary gland proliferative effects**
- **Neurotoxicity and developmental neurotoxicity**
- **Reproductive and developmental toxicity**

Hazard Identification: Genotoxicity

- The analysis of the available literature data indicate that:
  - BPA does not induce gene mutations in bacteria,
  - BPA induces DNA strand breaks, clastogenic and aneugenic effects in mammalian cells *in vitro,*
  - oxidative stress-related mechanism(s) are plausibly involved in this DNA damaging and clastogenic activity.

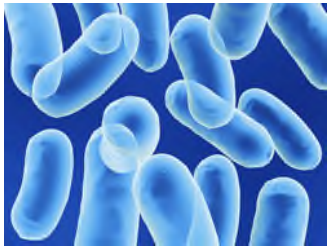


- In contrast with consistent positive *in vitro* findings, the ***in vivo* findings** in several studies with high/limited reliability were inconsistent → the evidence does not support an *in vivo* genotoxic hazard posed by BPA through direct interaction with DNA.

Uncertainty Analysis: Genotoxicity

- The CEP Panel concluded that it is Unlikely to Very Unlikely (5 – 30% probability) that BPA presents a genotoxic hazard, the causes of which include a direct mechanism. Accordingly, it was concluded that it is Likely to Very Likely (70 - 95% probability) that BPA either presents a genotoxic hazard only through indirect mechanism(s) or is not genotoxic.

- Considering the WoE for probabilities closer to either 70% or 95% that BPA does not present a genotoxic hazard by a direct mechanism, the CEP Panel concluded that probabilities close to 95% are more strongly supported by the evidence than probabilities close to 70% and, therefore, the balance of evidence allows a HBGV to be established.



Hazard Identification: Carcinogenicity and mammary gland proliferative effects

- The analysis of the available literature data indicates that, in the HOC "Carcinogenicity and mammary gland proliferative effects", the following organs are targets of BPA-induced toxicity:

- **Mammary gland**
- **Prostate**
- **Uterus**

Hazard Identification: Carcinogenicity and mammary gland proliferative effects

- Human data → No human studies were available

- Animal data → five clusters with relevant endpoints:
  - **mammary gland weight**
  - **mammary gland histology**
  - **prostate histology**
  - **uterus weight**
  - **uterus histology**

- For <u>histology</u>, four subclusters were defined:
  - non-neoplastic changes
  - pre-neoplastic lesions
  - neoplastic lesions
  - proliferation and apoptosis

Hazard Identification: Carcinogenicity and mammary gland proliferative effects

- **Mammary gland weight:** No effects in female rats treated with BPA up to 2 years of age were observed, therefore the cluster was judged as **Not Likely**.

- **Mammary gland histology:**
  - Several histological changes, e.g., longitudinal growth, terminal end buds (TEBs), branching, gland density and mammary gland scores, were reported in female and/or male rats and mice after BPA exposure during the developmental until weaning or adulthood period. Due to the diversity of these outcomes, assessed at different timepoints and doses, the CEP Panel considered the induction of these **non-neoplastic effects** as **ALAN**.
  - Regarding **pre-neoplastic lesions,** the CEP Panel noted an increase of atypical foci (unusual epithelial growth patterns) at the lowest dose in female rats following treatment during developmental until adult exposure (**ALAN**) but no effect after developmental exposure until weaning (**Not Likely**) in female rats in the chronic study.
  - Regarding **neoplastic lesions,** adenoma and/or adenocarcinoma incidence was increased at the lowest dose in female rats following treatment during developmental until weaning period (**Likely**) but no statistically significant effect was found in female rats after long-term exposure from in utero until adulthood (**Not Likely**).
  - These effects contributed to the **overall judgement ALAN** of the cluster mammary gland histology.

Hazard Identification: Carcinogenicity and mammary gland proliferative effects

❑ Prostate histology:

- **Non-neoplastic changes** related to inflammation and hyperplasia following developmental exposure until weaning were graded as **ALAN** due to inconsistencies in the outcome among studies. In the developmental and adult exposure period these effects were considered as **Not Likely** and in the adult exposure period the non-neoplastic changes were **Inadequate** due to the limitations in the study database.

- **Pre-neoplastic lesions**, i.e., atypical hyperplasia and dysplasia, were considered as **ALAN** after developmental exposure and **Unlikely** after developmental until adult exposure in rat studies.

- **Proliferation and apoptosis** were considered as **ALAN** following developmental exposure in two rat studies.

- No evidence of induction of **neoplastic lesions** were seen in any of the exposure periods

- These effects contributed to the **overall judgement ALAN** of the cluster Prostate histology.

Hazard Identification: Carcinogenicity and mammary gland proliferative effects

❑ Uterus histology:

- Some **non-neoplastic changes,** i.e., gland cell anomalies, endometrial cystic hyperplasia and squamous metaplasia after developmental exposure to BPA in rats, were considered as **Likely**.
  - ➢ *These endpoints were taken forward for BMD analysis*
- Other **non-neoplastic changes** i.e., uterine dilatation, endometrial hyperplasia and squamous metaplasia after developmental and adult exposure, and gland nests and gland nest density after adult exposure, were considered **ALAN** .
- **Apoptosis** was considered **ALAN** after developmental and adult exposure period and **Not Likely** after developmental exposure.
- No evidence of induction of **neoplastic lesions** were seen in any of the exposure periods, since a negative dose trend (no adverse effect) was observed at two years with a significant decrease at the highest dose (developmental exposure), while a positive trend without statistical significance at the higher doses was observed at one year (developmental and adult exposure). Therefore, neoplastic lesions in the uterus were considered as **Not Likely**.
- These effects contributed to the **overall judgement ALAN** of the cluster Uterus histology.

Hazard Identification: Carcinogenicity and mammary gland proliferative effects - Integrated likelihood

Human stream		Animal stream		Integrated likelihood
Cluster	Overall likelihood	Cluster	Overall likelihood	
		Effects on Mammary gland weight	Not likely (D)	Not likely
		Effects on Mammary gland histology	ALAN (D, D&A)	ALAN
		Effects on Prostate histology	ALAN (D)	ALAN
		Effects on Uterus weight	ALAN (D)	ALAN
		Effects on Uterus histology	**Likely** (D)	**Likely**

D: Developmental (pre- / post-natal until weaning) exposure
D&A: Developmental until adulthood exposure
G: Growth phase / young age exposure
A: Adult exposure

Modes of Action (MoAs) for Carcinogenicity and mammary gland proliferative effects

Mammary gland proliferative effects:

- Epigenetic effects, changes in gene expression and changes in hormone receptor levels suggested various MoAs of BPA possibly involved in the induction of proliferative/morphological changes. Some *in vivo* studies indicated that stromal-epithelial interactions may play a crucial role in the BPA-induced developmental changes in the mammary gland. *In vitro* studies provided some support for the hypothesis that BPA contributes to a higher susceptibility to mammary gland carcinogenesis

Prostate cancer:

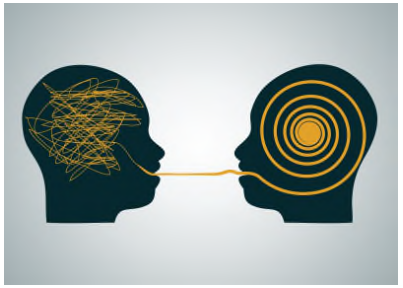
- BPA can enhance the susceptibility to tumorigenesis in rodents co-treated with very high levels of E2 and testosterone, while developmental and chronic exposure to BPA without additional sex hormones did not demonstrate a direct tumorigenic effect.

Uterine cancer:

- *In vitro* MoA studies on uterine cells indicated that BPA increases the proliferation rate. Other *in vitro* studies suggested that BPA modulates various mechanisms underlying the onset, growth and invasion of uterine tumours. However, the results of rodent studies did not demonstrate a tumorigenic activity of BPA.

Hazard Identification: Neurotoxicity and developmental neurotoxicity

- The analysis of the available literature data indicates that the central nervous system is a target of toxicity for BPA.
- The **human data** considered endpoints from the cluster **Neurodevelopment**
- In the **animal studies**, three **clusters** of endpoints were defined:
  - **Neuromorphology**
  - **Nervous system functionality**
  - **Behaviour**



Hazard Identification: Neurotoxicity and developmental neurotoxicity

- In the **neuromorphology** cluster, the endpoint dendritic spine density of pyramidal cells in hippocampus (CA1 and dentate gyrus areas) was judged as **Likely** after developmental exposure, while the endpoints number of neurons in hippocampus (CA1 and CA3 areas) and dendritic spine density in pyramidal cells in the medial part of the PFC were judged as **Likely** after exposure during the growth phase/young age.

- In the **nervous system functionality** cluster, the endpoint AChE activity was judged as **Likely** during the adult exposure period.

- In the **behaviour** cluster, the endpoint anxiety/emotionality was judged as **Likely** during all exposure periods (developmental, growth phase/young age, adult and exposure through the male germline). Furthermore, the endpoint learning/memory was judged as **Likely** during the developmental and growth phase/young age exposure, while the endpoints sensory-motor coordination and salt preference were judged as **Likely** during adult exposure.

  - *All these endpoints were taken forward for BMD analysis*

WoE for Neurotoxicity and developmental neurotoxicity- Integrated likelihood

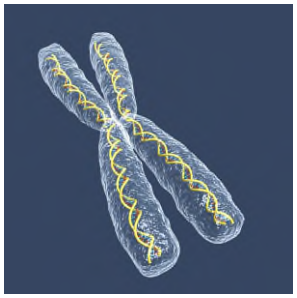
Human stream		Animal stream		Integrated likelihood
Cluster	Overall likelihood	Cluster	Overall likelihood	
Neurodevelopment (behaviour after developmental exposure)	Not likely (P)	**Behaviour**	**Likely** (D, G, A, I)	**Likely**
		Neuromorphology	**Likely** (D, G)	**Likely**
		Nervous system functionality	**Likely** (A)	**Likely**

P: Exposure during pregnancy
C: Exposure during childhood

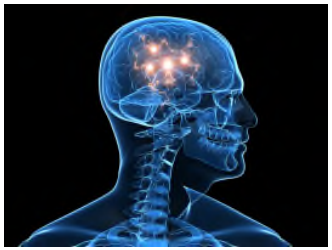
D: Developmental (pre- / post-natal until weaning) exposure
G: Growth phase / young age exposure
A: Adult exposure
I: Indirect (germline) exposure

MoAs studies for Neurotoxicity and developmental neurotoxicity

- Possible mechanisms of action that link the identified effects of BPA on various endpoints related to brain structure, function and development have not been sufficiently systematically explored in the literature to draw conclusions.



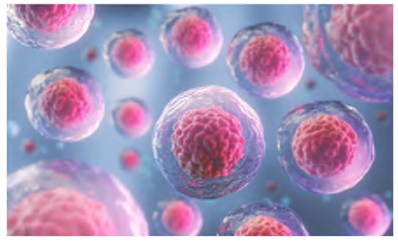
- There is evidence for an involvement of steroid-hormone-dependent pathways (oestrogen, androgen, corticosterone); oxidative stress, mitochondrial function and calcium regulation; gene expression changes through DNA methylation and other signaling pathways (canonical and non-canonical Wnt pathways, kinases).



Hazard Identification for Reproductive and developmental toxicity

- The analysis of the available literature data indicate that the reproductive system is a target of toxicity for BPA.

- Five relevant clusters of endpoints were defined in the **human studies**:
  - Fetal and post-natal growth
  - Prematurity
  - Pre-eclampsia
  - Male fertility and female fertility



- In the **animal studies**, three clusters of relevant endpoints were defined:
  - Developmental toxicity
  - Female reproductive toxicity
  - Male reproductive toxicity

Hazard Identification: Reproductive and developmental toxicity

- Based on the animal data, both **female and male reproductive toxicity** clusters showed effects that were judged as **Likely**:

  - In the **female reproductive toxicity** cluster: the endpoints ovary weight and histology and uterus histology were judged as **Likely** after developmental exposure, ovary histology after developmental and adult exposure, implantation rate after growth phase/young age exposure, and ovary histology (follicle counts) after adult exposure.

  - In the **male reproductive toxicity** cluster: the endpoints epididymis (exfoliated germ cells and inflammation) were judged as **Likely** after developmental exposure (pre-natal and/or post-natal until adult), testis histology (decreased seminiferous tubule diameter) after growth phase/young age exposure, and sperm (motility, viability and acrosome reaction) after adult exposure.

    - *These endpoints were taken forward for BMD analysis.*

Hazard Identification: Reproductive and developmental toxicity

- In the **developmental toxicity cluster**, effects were noted but were less consistent, and therefore **judged as ALAN**:

  - i.e., the endpoints bone development, mammary gland histology, body weight (in the developmental exposure), mammary gland weight and mammary gland histology (in the developmental and adult exposure) as well as body weight and age at first oestrus (in the growth phase/young age exposure).

Animal to human extrapolation and adversity of reprotoxicity endpoints

Focus on endpoints showing low BMDL values:

- Effects on ovary (altered follicle maturation, decreased number of implantations and fertilization rate) were reported only in mice studies of limited quality. In rats, the CLARITY-BPA study reported only increased number of ovary follicle cysts at the two highest dose levels.
- There was relatively consistent evidence for sperm effects at low dose (rodent adult exposure).
- All these effects are considered adverse and relevant for humans

WoE for Reproductive and developmental toxicity- Integrated likelihood

Human stream		Animal stream		Integrated likelihood
Cluster	Overall likelihood	Cluster	Overall likelihood	
		Developmental toxicity	ALAN (D, D&A,G)	ALAN
Fetal and Post-natal Growth	Not Likely (P)			Not Likely
Pubertal/Endocrine	ALAN (P)			ALAN
Female fertility	ALAN (A)	**Female reproductive toxicity**	**Likely** (D,D&A,G,A)	**Likely**
Male fertility	Not Likely (A)	**Male reproductive toxicity**	**Likely** (D&A,G,A)	**Likely**
Prematurity	Not Likely (P)			Not Likely
Pre-eclampsia	ALAN			ALAN

P: Exposure during pregnancy
C: Exposure during childhood
A: Adult exposure

D: Developmental (pre- / post-natal until weaning) exposure
D&A: Developmental until adulthood exposure
G: Growth phase / young age exposure
A: Adult exposure

MoAs studies for Reproductive and developmental toxicity

- Supporting evidence was available for a variety of plausible MoAs of BPA on reproductive toxicity.
- They include ER and AR interactions and associated downstream and cross-stream effects, including epigenetic changes.
- The role of BPA-induced generation of oxidative stress in reproductive effects has been less explored.

Coffee break

Q&A session

Following presentation → BPA Hazard Identification Part III: Immunotoxicity

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

Hazard Identification - Part III: Immunotoxicity

Henk Van Loveren

Chair EFSA Working Group on BPA re-evaluation

24 January 2022

Trusted science for safe food

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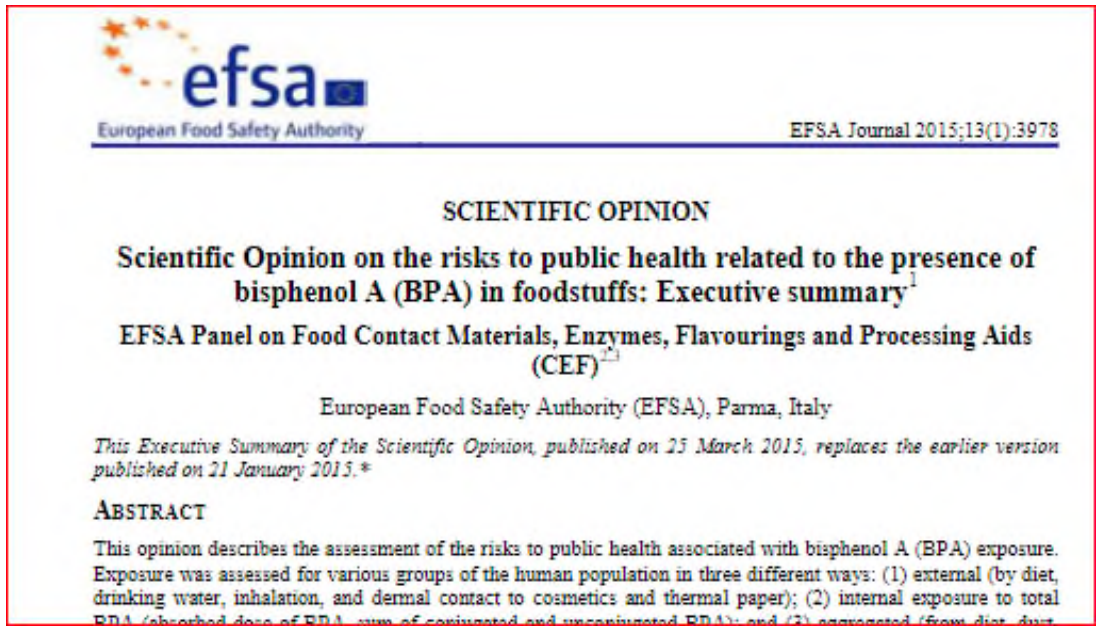
European Food Safety Authority

2015 EFSA Opinion on BPA: Immunotoxicity



European Food Safety Authority

EFSA Journal 2015;13(1):3978

SCIENTIFIC OPINION

Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

*This Executive Summary of the Scientific Opinion, published on 25 March 2015, replaces the earlier version published on 21 January 2015.**

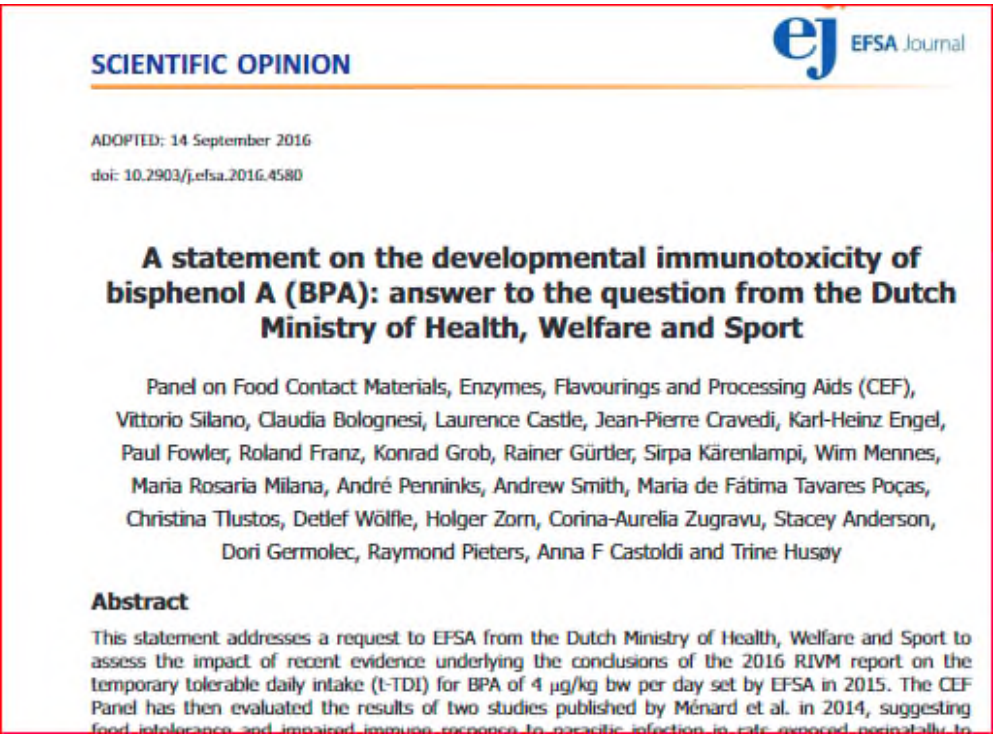
ABSTRACT

This opinion describes the assessment of the risks to public health associated with bisphenol A (BPA) exposure. Exposure was assessed for various groups of the human population in three different ways: (1) external (by diet, drinking water, inhalation, and dermal contact to cosmetics and thermal paper); (2) internal exposure to total

- **Human studies:** Indication that BPA exposure may be linked to immunological outcomes, although a causal link could not be established.
- **Studies in animals:** Lent support to the possibility of effects on the immune system. An effect of concern was, in particular, increased Immunoglobulin E (IgE) and allergic lung inflammation. However, like the human studies, the animal studies suffered from shortcomings.

↳*The CEF Panel did not take these effects forward for the risk characterisation.*

2016 EFSA statement on immunotoxicity



SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 14 September 2016

doi: 10.2903/j.efsa.2016.4580

A statement on the developmental immunotoxicity of bisphenol A (BPA): answer to the question from the Dutch Ministry of Health, Welfare and Sport

Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), Vittorio Silano, Claudia Bolognesi, Laurence Castle, Jean-Pierre Cravedi, Karl-Heinz Engel, Paul Fowler, Roland Franz, Konrad Grob, Rainer Gürtler, Sirpa Kärenlampi, Wim Mennes, Maria Rosaria Milana, André Penninks, Andrew Smith, Maria de Fátima Tavares Poças, Christina Tlustos, Detlef Wölfle, Holger Zorn, Corina-Aurelia Zugravu, Stacey Anderson, Dori Germolec, Raymond Pieters, Anna F Castoldi and Trine Husøy

Abstract

This statement addresses a request to EFSA from the Dutch Ministry of Health, Welfare and Sport to assess the impact of recent evidence underlying the conclusions of the 2016 RIVM report on the temporary tolerable daily intake (t-TDI) for BPA of 4 μg/kg bw per day set by EFSA in 2015. The CEF Panel has then evaluated the results of two studies published by Ménard et al. in 2014, suggesting

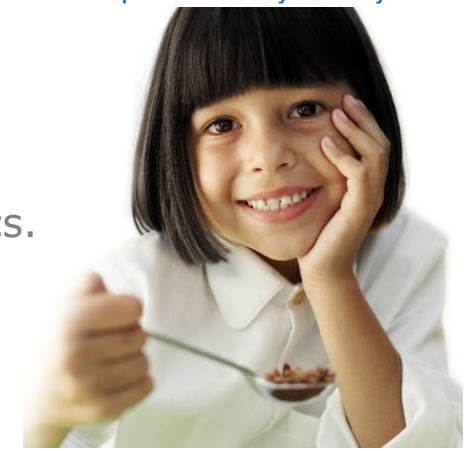
Two additional studies on the effects of BPA exposure to the immune system, in which potential allergic conditions were investigated.

↳ *The CEF Panel confirmed its position that the studies available at that time suggested effects on the immune system, but that the studies were not sufficiently robust to take them forward for risk characterization.*

Immunotoxicity: New studies & clusters

Defined clusters (n. of new unique studies)	Exposure periods assessed
Human studies	
• **Asthma/allergy (9)**	- Pregnancy - Childhood
Animal studies	
• **Allergic lung inflammation (3)**	- Developmental (pre- / post-natal until weaning)
• **Cellular immunity (16)**	- Developmental and adult (pre- / post-natal in pups until adulthood)
• **Inflammation (12)**	- Growth phase/young age
• **Humoral immunity (2)**	- Adult
• **Innate immunity (10)**	

Human studies: Asthma / allergy

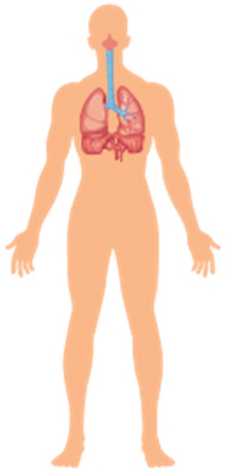


- Exposure during pregnancy: **As Likely As Not (ALAN)**
  - Seven studies, all Tier 3 (i.e., lowest level of internal validity).
  - 2.836 participants with populations of comparable size but varied characteristics.
  - Four studies including a European-descent population.
- Exposure during childhood: **ALAN**
  - Four studies, all Tier 3 (i.e., lowest level of internal validity).
  - 1.546 participants with populations of comparable size but varied characteristics.
  - Only one study including a European-descent population.
- Overall conclusion
  - Small number of studies.
  - Suboptimal exposure assessment in all studies (single spot urine sample).
  - Considerable heterogeneity in the assessed populations, exposure levels and endpoints.

⇨ Evidence for a positive association between BPA exposure and allergy was considered as **ALAN**.

Animal studies: Allergic Lung Inflammation

- Developmental (pre- / post-natal until weaning) exposure: **Likely**
  - Two studies (both Tier 2).
  - Ovalbumin-specific IgE increase in serum was judged **Very Likely**.
  - Mast cell PGD2, lung CysLT and lung IL-17 were also judged Likely.
- Adult exposure: **Likely**
  - One study (Tier 1).
  - Increase in eosinophils in the bronchoalveolar lavage fluid was considered **Likely**.
  - Increase in cytokines IL-4 & IL-33 was considered Very Likely.


Respiratory System

- Overall conclusion
  - Production of specific IgE in response to an allergen and increased eosinophils in BAL were deemed as adverse as they are crucial parameter in allergic reactions in the respiratory tract. Other effects in that cluster supported the likelihood of this effect.

⇨ The CEP assigned a likelihood level of **Likely** to allergic lung inflammation effects of BPA.

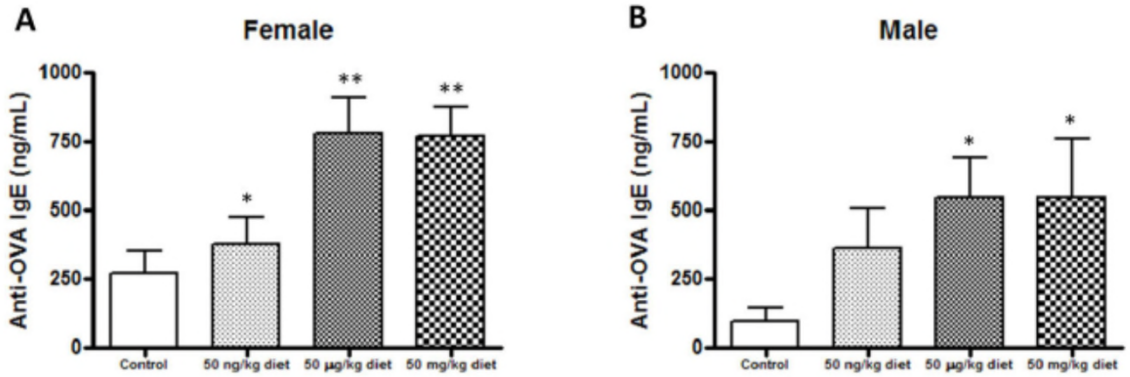


Figure 1.
Anti-OVA IgE in sera of female (A) and male (B) offspring with perinatal exposure to BPA and subsequent adult OVA challenge. Note that the y-axes in A and B are the same to facilitate comparison between female and male offspring. Bars represent mean ± SEM. $*P < 0.05$ and $**P < 0.0001$ compared to respective control (open bar). Females: n = 6 (control), n = 11 (50 ng), n = 13 (50 µg), n = 14 (50 mg). Males: n = 8 (control), n = 9 (50 ng), n = 10 (50 µg), n = 4 (50 mg).

O'Brien et al. (2014a). Perinatal bisphenol A exposure beginning before gestation enhances allergen sensitization, but not pulmonary inflammation, in adult mice. Journal of Developmental Origins of Health and Disease, 5(2), 121–131. doi:10.1017/S204017441400004X [RefID 5462].

Animal studies: Cellular immunity

- Developmental (pre- / post-natal until weaning) exposure: **Likely**
  - Eleven studies (seven Tier 1, two Tier 2 and two Tier 3).
  - Increase in Th17 cells and associated cytokines (IL-17, IL-21, IL-23) was judged **Likely**.
- Developmental and adult (pre- / post-natal in pups until adulthood) exposure: **Not Likely**
  - Four studies (three Tier 1, one Tier 3), with no consistent effects observed.
- Adult exposure: **Not Likely**
  - Four studies (two Tier 1, one Tier 2 and one Tier 3), with no consistent effects observed.
- Overall conclusion
  - Likely effect of BPA during the developmental exposure period for the endpoint Th17 cells.

⇨ The CEP assigned a likelihood level of **Likely** to cellular immunity effects of BPA.

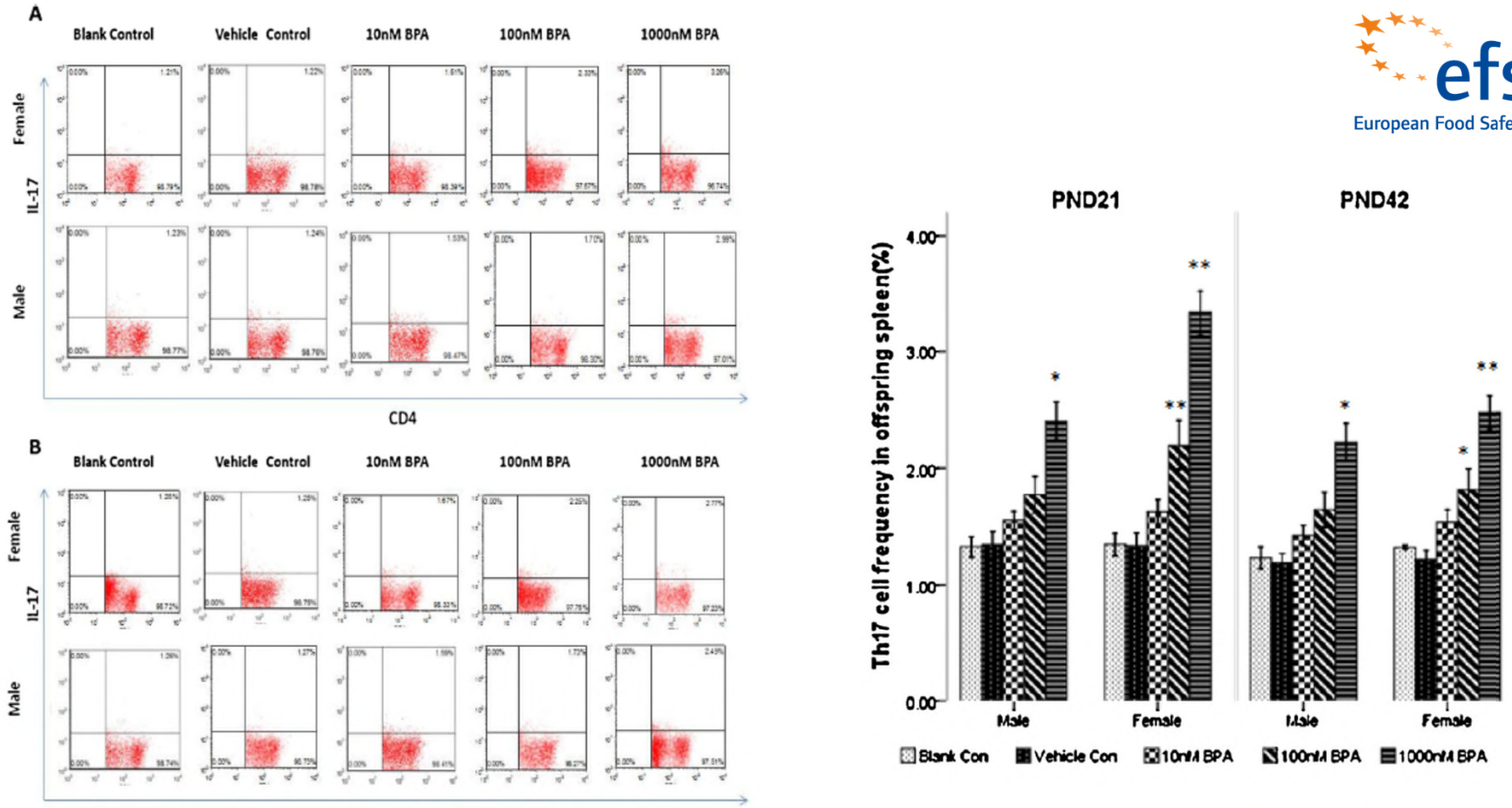
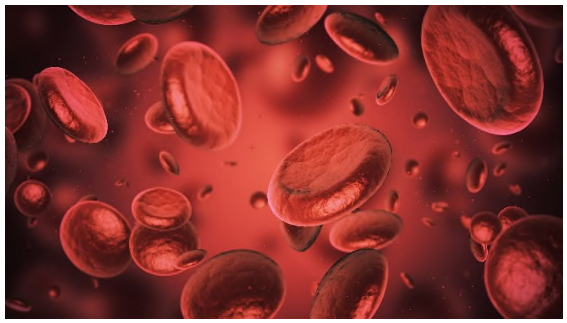


Fig. 3. Effects of maternal exposure to BPA during gestation and lactation on Th17 cell frequency in the spleen in offspring mice. At PND21 and PND42, offsprings were sacrificed, spleens were collected, and Th17 cell frequency in spleen was analyzed in males and females by FCM. (A) and (B) are representative flow cytometry plots for CD4 and IL-17 staining of T17 cell frequency in spleen on PND21 and PND42, respectively. (C) Quantification of the frequency of Th17 cells in males and females on PND21 and PND42. Data are expressed as mean ± SEM (n = 10 except 11 for 1000 nM BPA group). *$p < 0.05$ compared with blank control group for the same gender, **$p < 0.01$ compared with blank control group for the same gender.

Luo et al. (2016). Gestational and lactational exposure to low-dose bisphenol A increases Th17 cells in mice offspring. Environmental Toxicology and Pharmacology 47, 149–158. doi:10.1016/j.etap.2016.09.017 [RefID 4679]

Animal studies: Inflammation

- Developmental (pre- / post-natal until weaning) exposure: **Not Likely**
  - Five studies (three Tier 1, one Tier 2 and one Tier 3), with insufficient evidence to support an effect.
- Developmental and adult (pre- / post-natal in pups until adulthood) exposure: **Not Likely**
  - Two studies (both Tier 1), with generally no effects observed.
- Growth phase/young age exposure: **Likely**
  - One study (Tier 1).
  - Increase of neutrophils in epididymis was judged **Likely.**
  - Increase of IL-6 in epidydimis also judged Likely.



- Adult exposure: **Not Likely**
  - Four studies (one Tier 1, three Tier 2), considered to have insufficient evidence.
- Overall conclusion
  - Likely effect of BPA in the exposure period growth phase/young age for the endpoint neutrophils in epididymis.

⇨ The CEP assigned a likelihood level of **Likely** to the cluster inflammation.

Animal studies: Humoral immunity

- Developmental (pre- / post-natal until weaning) exposure: **ALAN**
  - One study (Tier 1)
  - A decrease of IgA was observed, but with only one dose and with non-independent endpoints.
- Developmental and adult (pre- / post-natal in pups until adulthood) exposure: **Not Likely**
  - One study (Tier 1), with no consistent, transient or with no clear dose-response effects observed.
- Overall conclusion

⇨ The CEP assigned a likelihood level of **ALAN** to humoral immunity effects of BPA.

Animal studies: Innate immunity

- Developmental (pre- / post-natal until weaning) exposure: **ALAN**
  - Seven studies (four Tier 1, two Tier 2 and one Tier 3).
  - No endpoints with a consistent effect following BPA exposure, except from a decrease in lysozyme production with only one dose of BPA.
- Developmental and adult (pre- / post-natal in pups until adulthood) exposure: **Not Likely**
  - Three studies (all Tier 1), with no consistent effects shown.
- Growth phase/young age exposure: **Not Likely**
  - One study (Tier 2), with no effects observed.
- Adult exposure: **Not Likely**
  - One study (Tier 2), with either no effects observed or no clear dose-response effects observed.
- Overall conclusion

⇨ The CEP assigned a likelihood level of **ALAN** to innate immunity effects of BPA.

Weight of Evidence assessment of BPA effects on immunotoxicity – Integrated likelihood

Human stream		Animal stream		Integrated likelihood
Cluster	Overall likelihood	Cluster	Overall likelihood	
Asthma/ allergy	ALAN (P, C)	**Allergic lung inflammation**	**Likely** (D, A)	**Likely**
		Cellular immunity	**Likely** (D)	**Likely**
		Inflammation	**Likely** (G)	**Likely**
		Humoral immunity	ALAN (D)	ALAN
		Innate immunity	ALAN (D)	ALAN

P: Exposure during pregnancy
C: Exposure during childhood

D: Developmental (pre- / post-natal until weaning) exposure
G: Growth phase / young age exposure
A: Adult exposure

Allergic lung inflammation

- Allergic lung inflammation may be brought about by serum IgE levels, specific for certain respiratory allergens.
- When IgE, bound on the membrane of mast cells through specific IgE receptors, is cross-linked by the allergen, the mast cells release various products such as histamine, serotonin as well as inflammatory mediators.
- This in turn leads to attraction of inflammatory cells, such as eosinophils, causing damage in the respiratory tract.
- Inflammation may ultimately lead to reduced capacity of the respiratory function of the lungs.
- Production of IgE is regulated by an array of cytokines, released from T lymphocytes as well as from other cell types, such as the mast cells or the inflammatory cells themselves. In addition, the recruitment of inflammatory cells is regulated by pro-inflammatory mediators, produced by different cell types.

Cellular immunity

- T cells have a central role in both cell-mediated and humoral immune responses.
- They recognise antigens presented by antigen-presenting cells (APCs) via the surface-expressed T-cell receptor.
- Multiple phenotypes of T cells have been identified with different functions. CD4+ T cells are divided into Th1, Th2, Th9, Th17 and T regulatory (Treg) groups, each with a specific profile of cytokine production.
- The functions of these T cells include promotion of inflammation by cytokine production (Th1 and Th17 cells); helping B cells (Th2 cells); regulation of immunosuppressive responses (Treg).
- T helper 17 cells play a role in host defence against extracellular pathogens by mediating the recruitment of inflammatory cells to infected tissues.
- Th17 cells play a critical role in the induction of tissue inflammation, tissue destruction, and participate in various immune diseases including asthma.

Modes of Action studies for immunotoxicity

- Increased number of antigen-presenting (dendritic) cells was observed after intratracheal exposure to BPA. Effects on non-specific cells, such as antigen presenting cells and epithelial cells, through presentation of antigens to T lymphocytes or release of mediators influence the regulatory homeostasis of the immune system.

- This may lead to suppression of T regulatory cells and subsequent stimulation of Th17 cells, ultimately leading to enhanced production of IgE and inflammatory mediators.

- After being cross-linked at the surface of mast cells, IgE may lead to the release of additional inflammatory mediators that may lead to inflammatory reactions that include eosinophils.

- It is currently not clear how BPA interacts with the various cells comprising the immune system or cells such as epithelial cells or fibroblasts, of which the mediators influence the immune system, but a role for GR, ER2 and ERRA and subsequent activation of transcription factors are plausible.

Other inflammation

- In addition to effects in the respiratory tract, **an inflammatory effect was also seen in the epididymis after exposure to BPA, which may be brought about by partly similar mechanisms.**

Conclusion on immunotoxicity hazard of BPA

- The CEP Panel considers that a hazard exists for adverse effects of BPA on the immune system, that may result, depending on the dose, in inflammatory reactions such as in the respiratory tract.
- *In vivo* evidence was supported by MoA studies. *In vitro* studies indicated the ability of BPA to induce immune deregulation, possibly leading to an increased susceptibility to develop inflammatory diseases.
- Effects were noted both after exposure during developmental stages as well as at adulthood, hence the hazard was considered to exist throughout the different life stages.
- Using a WoE approach, the CEP Panel assigned a likelihood level of **Likely** to the following clusters and endpoints:
  - **Cellular immunity**: Increased number of Th17 cells was considered Likely. Although Th17 cells are T cells, and therefore were put in the cluster cellular immunity, they play a role in allergic responses, and therefore the effect on Th17 cells is consistent with the effect on specific IgE.
  - **Allergic lung inflammation**: In particular, BPA-induced effects on serum Ovalbumin-specific IgE was considered Very Likely. It is deemed as adverse as it is a crucial parameter in inducing allergic reactions in the respiratory tract. Furthermore, effects on eosinophils in the bronchoalveolar lavage fluid were considered Likely. Other effects in that cluster supported the likelihood of this effect.
  - **Inflammation:** BPA effects on neutrophils in epididymis were considered Likely**.**

⇨ These endpoints were brought forward for Benchmark Dose analysis.

Benchmark Dose Analysis: Immunotoxicity

BPA exposure led to a dose-related increment of Th17 cells in mice. This effect was consistent with associated cytokines (IL-17, IL-21 and IL-23), as well as with effects of BPA in the cluster of allergic lung inflammation. Aberrant regulation of Th17 cells plays a significant role through their cytokines in the pathogenesis of asthma. The CEP Panel notes that increment of Th17 cells is an intermediate endpoint, and some reserve capacity will exist, but is considered adverse.

A default Benchmark response (BMR) of 5% is suggested to be used in EFSA Guidelines. However, deviating from this default is possible, based on toxicological or statistical considerations.

In the mouse study, the coefficient of variance in the control groups was around 20%.

While considering that in the human population, for individuals a 20% increase may not necessarily imply an adverse condition for that person, given the pivotal role of Th17 cells in lung allergy, the CEP Panel considered that if the population at large showed a 20% increment in Th17 cells, individuals that are in the higher segment of the normal range, will be put out of the normal range, and as a consequence numbers of lung allergy cases would be expected to go up.

Based on these considerations, the CEP Panel considered 20% BMR would be in line with the variability noted in the animal study and the wider normality range in humans and considered it as adverse.

Conclusions

- The newly evaluated information on immunotoxicity was found to be in line with earlier observations and indicates now more firmly that there is a hazard with respect to adverse outcomes of BPA exposure on the immune system.
- Based on the human, animal and *in vitro* data available, BPA appeared to promote interwoven pathways involved in immune deregulation that play a role in immune-related disorders.
- Endpoints related to cellular immunity, allergic lung inflammation and inflammation were brought forward for benchmark dose analysis.

Next presentation → Hazard and risk characterisation

Stakeholder meeting on the draft scientific opinion on re-evaluation of bisphenol A (BPA)

Hazard and risk characterisation

Katleen Baert

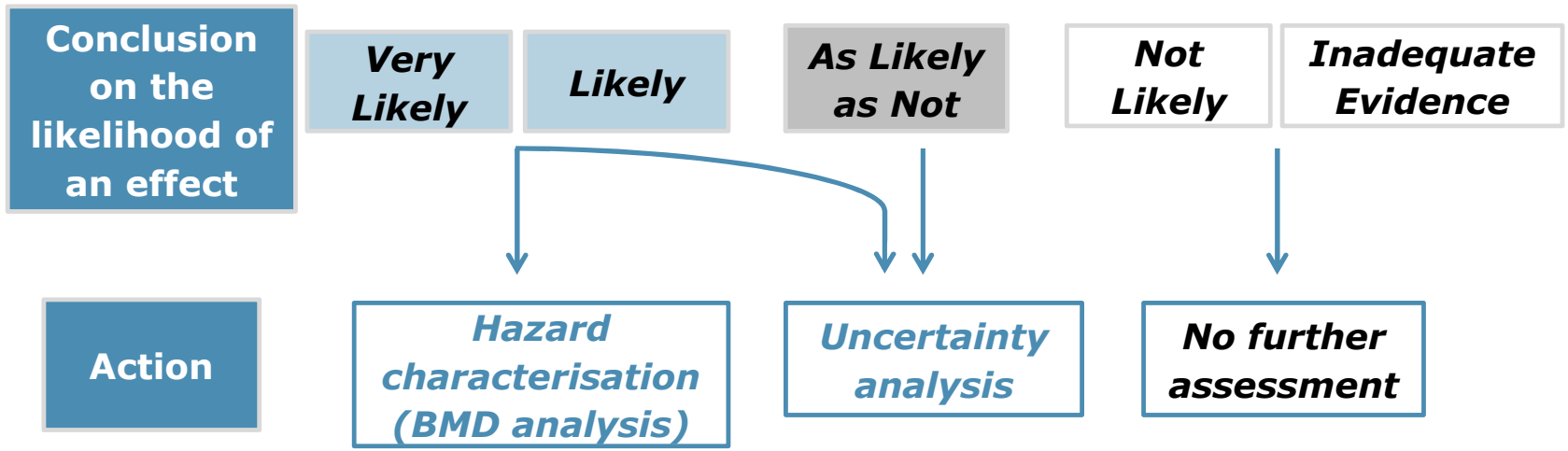
Food Contact Materials Team Leader, EFSA

24 January 2022

Trusted science for safe food

European Food Safety Authority

Selection of the effects for the hazard characterisation and the uncertainty analysis (UA)



- Studies investigating **Very likely** or **Likely effects**, with at least 1 ctrl+ two BPA dose levels, were considered for **benchmark dose (BMD) analysis.**

- **All ALAN, Likely** and **Very likely** clusters were included in the **uncertainty analysis (UA).**

Endpoints brought forward for BMD analysis

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Immuno-toxicity

- Effect on Th17 cells
- Effect on neutrophils in epididymis
- Effect on eosinophils in the bronchoalveolar lavage fluid
- Effect on serum OVA-specific IgE

Metabolic effects

- Hepatic and serum uric acid

Neurotoxicity and developmental neurotoxicity

- Anxiety/emotionality
- Learning and memory
- Male sexual behaviour
- Salt preference
- Dendritic spine density
- Number of neurons in hippocampus
- AChE activity

Reproductive and developmental toxicity

- Ovary weight
- Uterus histology*
- Ovary histology
- Decreased implantation incidence
- Epididymis histology
- Testis histology
- Effects on sperm

In total **24 studies** were brought forward for BMD analysis; several studies providing multiple datasets.

* Endpoint also considered under carcinogenicity and mammary gland proliferative effects.

BMD analysis

Selection of the Benchmark response (BMR):
details provided in Annex I

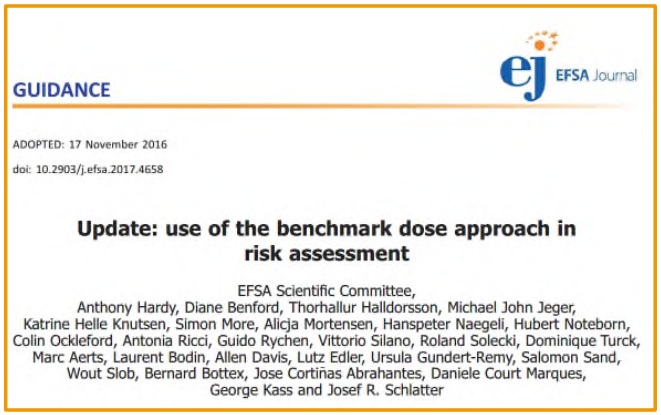
BMD analysis: R-package PROAST (EFSA tool)

Apply cut-of value of 10 for **ratio of lowest non-zero dose and BMDL:***

Is lowest dose/BMDL >10? → yes → BMDL not used for selection of reference point (RP); study considered in uncertainty analysis

no

BMDL values converted to Human Equivalent Dose (**HED**) and considered for **selection of RP**


GUIDANCE

EFSA Journal

ADOPTED: 17 November 2016

doi: 10.2903/j.efsa.2017.4658

Update: use of the benchmark dose approach in risk assessment

EFSA Scientific Committee,
Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael John Jeger, Katrine Helle Knutsen, Simon More, Alicja Mortensen, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Vittorio Silano, Roland Solecki, Dominique Turck, Marc Aerts, Laurent Bodin, Allen Davis, Lutz Edler, Ursula Gundert-Remy, Salomon Sand, Wout Slob, Bernard Bottex, Jose Cortiñas Abrahantes, Daniele Court Marques, George Kass and Josef R. Schlatter

* This cut-off value is used in the BMDS software to discard datasets for BMD analysis (US EPA, 2020). In the absence of EFSA guidance, the CEP Panel decided to apply this cut-off value to bring a study forward for selection of the RP

Endpoints brought forward for selection reference point (RP)

Immuno-toxicity	• Effect on Th17 cells • Effect on neutrophils in epididymis
Metabolic effects	• Hepatic uric acid
Neurotoxicity and developmental neurotoxicity	• Anxiety/emotionality • Learning and memory • Dendritic spine density
Reproductive and developmental toxicity	• Ovary weight • Ovary histology • Epididymis histology • Effects on sperm

In total, **19 BMDL values** for **10 endpoints** were brought forward for selection of the RP.

Lowest BMDL values after conversion to HED

Reference	Endpoint	Species	BMR	Group	BMDL* (ng/kg bw per day)	BMDU* (ng/kg bw per day)
Luo et al. (2016) [RefID 4679]	Th17 cells	Mouse	20%	F PND21	0.93	11.5
		Mouse	20%	F PND42	2.64	27.7
		Mouse	20%	M PND21	4.65	52.5
		Mouse	20%	M PND42	5.43	52.4
Hu et al. (2018) [RefID 11119]	Ratio of primordial and total follicles	Mouse	5%		14.9	5410
Ma et al. (2018) [RefID 12637]	Hepatic uric acid	Mouse	20%		24.6	6185
Wang HF et al. (2016) [RefID 7618]	Motility (effects on sperm)	Mouse	20%		53	1159

* BMDL and BMDU values converted to human equivalent dose (HED)

BMDL: lower confidence limit of the benchmark dose; BMDU: upper confidence limit of the benchmark dose; BMR: benchmark response; F: female; M: male; PND: post-natal day.
For the calculation of the BMDL/BMDU values expressed as HED, a HEDF of 0.0155 was used for studies in mice and a HEDF of 0.1656 for studies in rats.

BMD Analysis: Effect on Th17 cells

Reference: Luo et al., 2016 [RefID 4679]

Endpoint: Th17 cell frequency in the spleen of offspring mice (%).

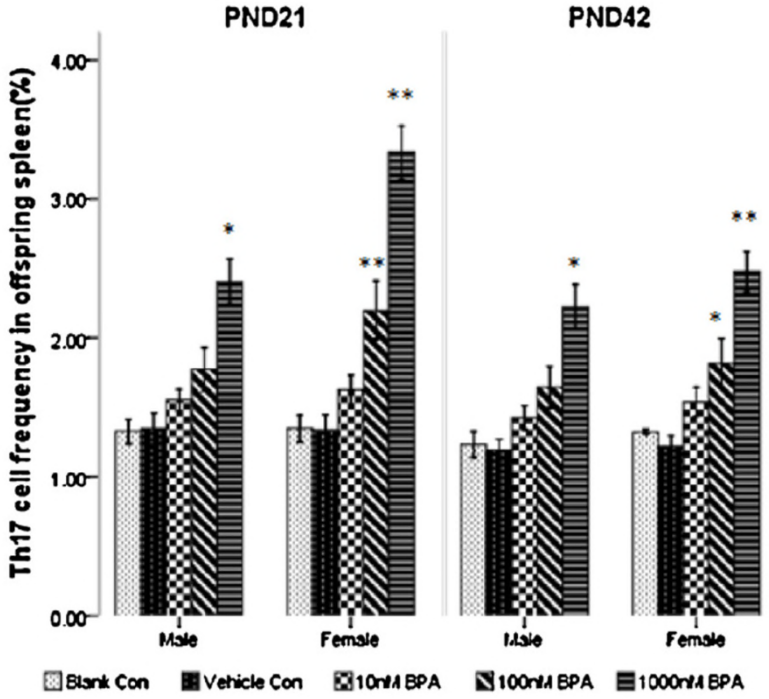
Outcome: A dose-related increment of Th17 cells.

This effect was consistent with effects on:

- cellular immunity based on Th17 cells and associated cytokines (IL-17, IL-21 and IL-23),
- effects of BPA in the cluster of allergic lung inflammation.

Benchmark response (BMR): 20%

BMDL	60 ng/kg bw per day
BMDL (HED)	0.93 ng/kg bw per day



Luo et al. (2016). Gestational and lactational exposure to low-dose bisphenol A increases Th17 cells in mice offspring. Environmental Toxicology and Pharmacology 47, 149–158. doi:10.1016/j.etap.2016.09.017 [RefID 4679]

BMD Analysis: Ovalbumin-specific IgE

Reference: O'Brien et al., 2014a [RefID 5462]

Endpoint: Effect of BPA on ovalbumin-specific IgE in male and female offspring mice

Due to a litter effect in the dataset and the lack of information to take the litter effect into account, no BMD analysis was performed on this endpoint.

Study was used in the uncertainty analysis

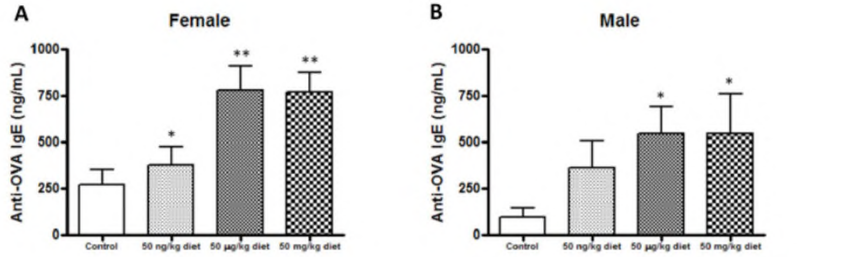


Figure 1.
Anti-OVA IgE in sera of female (A) and male (B) offspring with perinatal exposure to BPA and subsequent adult OVA challenge. Note that the y-axes in A and B are the same to facilitate comparison between female and male offspring. Bars represent mean ± SEM. $*P < 0.05$ and $**P < 0.0001$ compared to respective control (open bar). Females: n = 6 (control), n = 11 (50 ng), n = 13 (50 µg), n = 14 (50 mg). Males: n = 8 (control), n = 9 (50 ng), n = 10 (50 µg), n = 4 (50 mg).

O'Brien et al. (2014a). Perinatal bisphenol A exposure beginning before gestation enhances allergen sensitization, but not pulmonary inflammation, in adult mice. Journal of Developmental Origins of Health and Disease, 5(2), 121–131. doi:10.1017/S204017441400004X [RefID 5462].

BMD Analysis: Ratio of primordial and total follicles

Reference: Hu et al. (2018) [RefID 11119]

Endpoint: Ratio of primordial and total follicles in adult mice

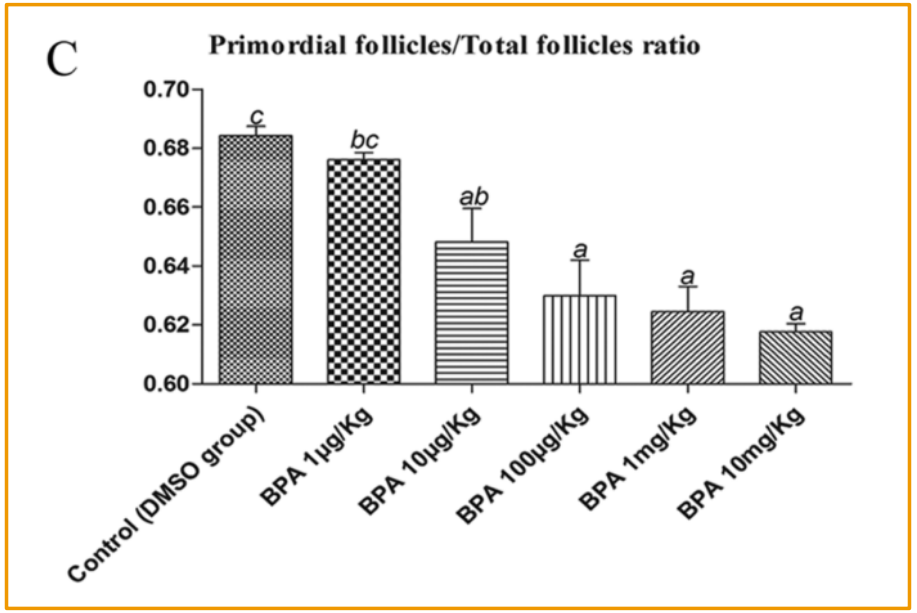
Outcome:

Dose-dependent decrease of the ratio of primordial and total follicles.

BMR:

The default value for continuous data of a 5% change in mean response compared with the controls

BMDL	960 ng/kg bw per day
BMDL (HED)	14.9 ng/kg bw per day



Hu et al. (2018). Bisphenol A initiates excessive premature activation of primordial follicles in mouse ovaries via the PTEN signaling pathway. Reproductive Sciences, 25(4), 609–620. doi: 10.1177/1933719117734700. [RefID: 11119]

BMD Analysis: Hepatic uric acid

Reference: Ma et al. (2018) [RefID 12637]

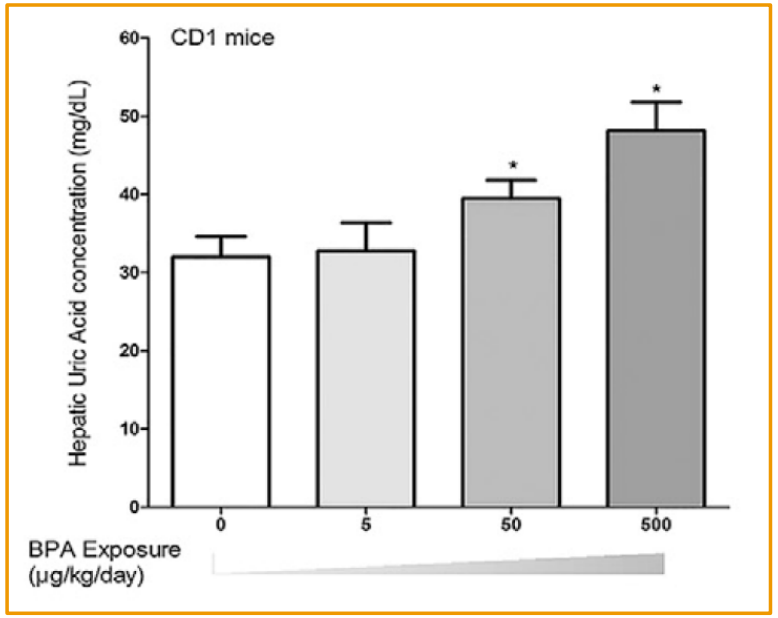
Endpoint: Hepatic uric acid concentrations in mice

Outcome: Dose-dependent increase of uric acid concentration in liver due to an increased activity of xanthine oxidase in the liver.

BMR:

For the control group the coefficient of variation was 21%. A BMR of 20% change in mean response compared with the controls was considered to be appropriate.

BMDL	1590 ng/kg bw per day
BMDL (HED)	24.6 ng/kg bw per day



Ma et al. (2018). Bisphenol A promotes hyperuricemia via activating xanthine oxidase. FASEB Journal, 32(2), 1007–1016. doi: 10.1096/fj.201700755R. [RefID: 12637]

BMD Analysis: Effects on sperm

Paper: Wang HF et al. (2016) [RefID 7618]

Endpoint: Sperm motility during adult exposure.

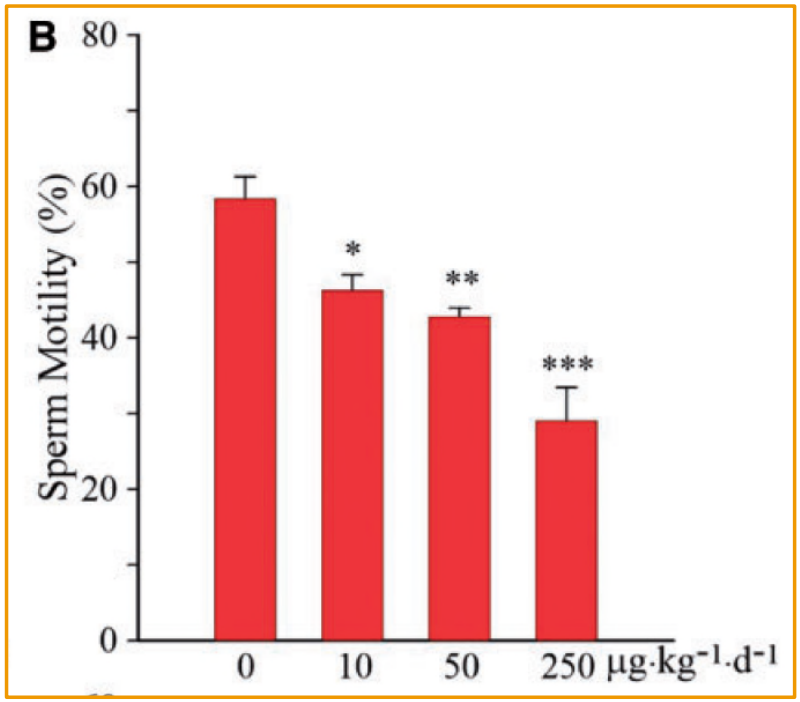
Outcome:

BPA exposure led to a dose-dependent decrease of sperm motility in mice.

BMR:

A BMR of 20% was selected, justified by the nature of the endpoint and the variability observed in the control group. The BMR of 20% corresponds approximately to the coefficients of variation of the control groups.

BMDL	3410 ng/kg bw per day
BMDL (HED)	53 ng/kg bw per day



Wang et al. (2016). Bisphenol A impairs mature sperm functions by a CatSper-relevant mechanism. Toxicological Sciences, 152(1), 145–154. doi: 10.1093/toxsci/kfw070. [RefID: 7618]

Lowest BMDL values after conversion to HED

Reference	Endpoint	Species	BMR	Group	BMDL* (ng/kg bw per day)	BMDU* (ng/kg bw per day)
Luo et al. (2016) [RefID 4679]	Th17 cells	Mouse	20%	F PND21	0.93	11.5
		Mouse	20%	F PND42	2.64	27.7
		Mouse	20%	M PND21	4.65	52.5
		Mouse	20%	M PND42	5.43	52.4
Hu et al. (2018) [RefID 11119]	Ratio of primordial and total follicles	Mouse	5%		14.9	5410
Ma et al. (2018) [RefID 12637]	Hepatic uric acid	Mouse	20%		24.6	6185
Wang HF et al. (2016) [RefID 7618]	Motility (effects on sperm)	Mouse	20%		53	1159

* BMDL and BMDU values converted to human equivalent dose (HED)

BMDL: lower confidence limit of the benchmark dose; BMDU: upper confidence limit of the benchmark dose; BMR: benchmark response; F: female; M: male; PND: post-natal day.
For the calculation of the BMDL/BMDU values expressed as HED, a HEDF of 0.0155 was used for studies in mice and a HEDF of 0.1656 for studies in rats.

Lowest BMDL values after conversion to HED

Reference	Endpoint	Species	BMR	Group	BMDL* (ng/kg bw per day)	BMDU* (ng/kg bw per day)
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Wang HF et al. (2016) [RefID 7618]	Motility (effects on sperm)	Mouse	20%		53	1159

Reference Point i.e., lowest BMDL value from all endpoints

* BMDL and BMDU values converted to human equivalent dose (HED)

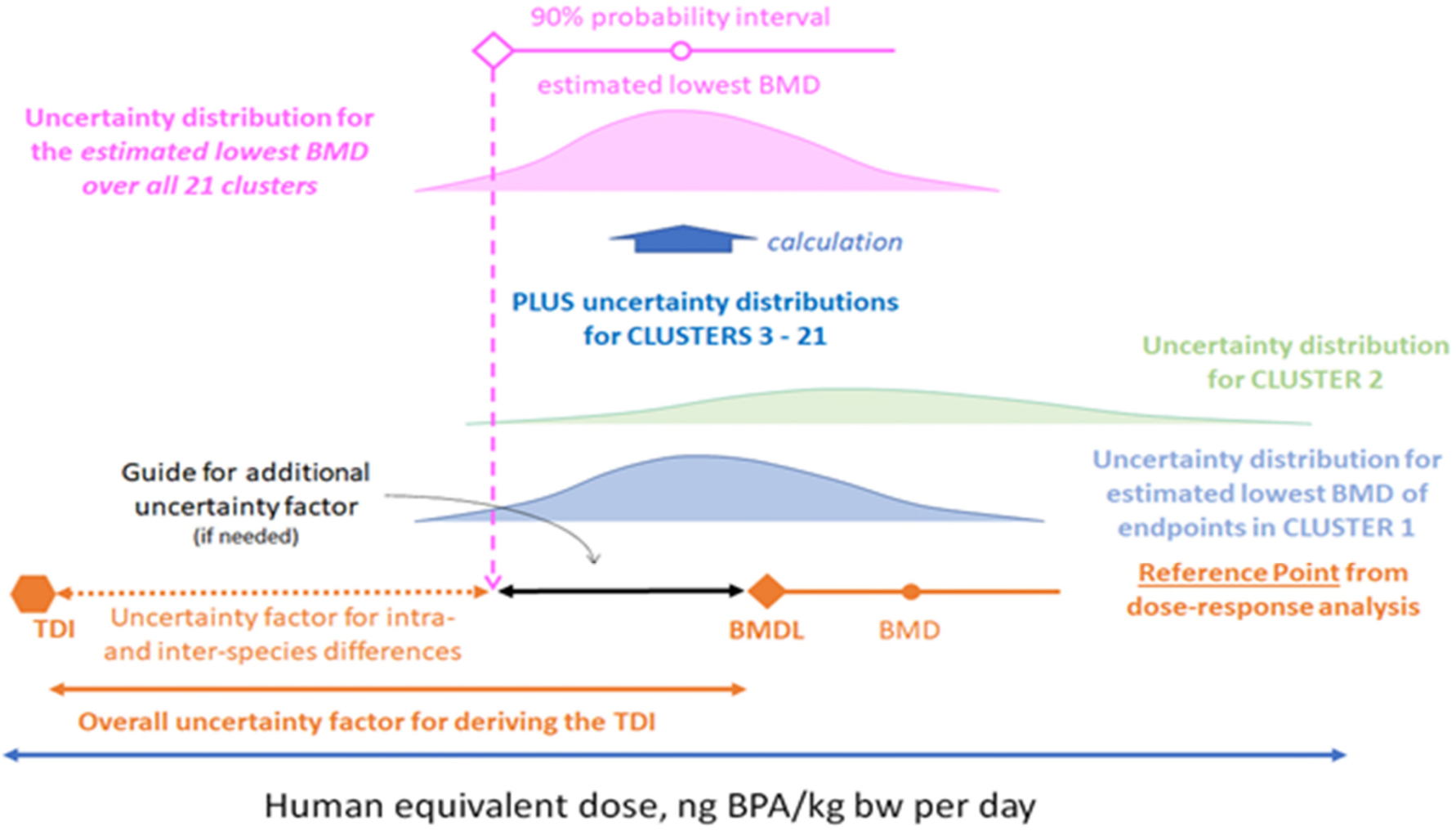
BMDL: lower confidence limit of the benchmark dose; BMDU: upper confidence limit of the benchmark dose; BMR: benchmark response; F: female; M: male; PND: post-natal day.
For the calculation of the BMDL/BMDU values expressed as HED, a HEDF of 0.0155 was used for studies in mice and a HEDF of 0.1656 for studies in rats.

Uncertainty Analysis (UA)

- **Aim**: determine what additional uncertainty factor (if any) is needed to take account of the possibility that effects other than the critical effect *might be more sensitive*.

- **In total, 21 clusters of endpoints** rated ALAN, Likely or Very Likely in the WoE assessment were considered in the UA.

Graphical representation of approach UA



Uncertainty analysis (UA)

Two questions for each cluster of effects:

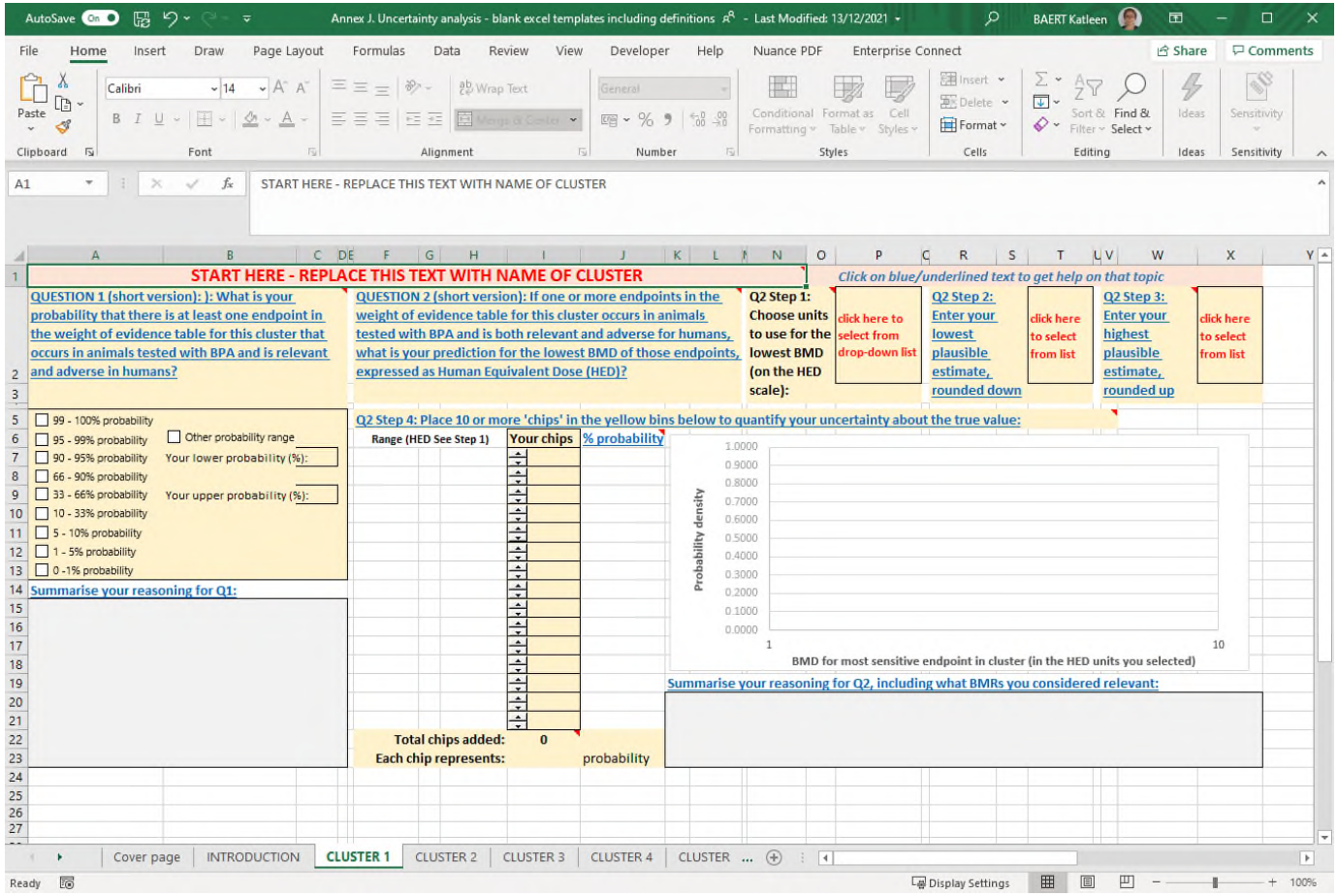
- **Q1**: What is your probability that there is at least one endpoint in the weight of evidence (WoE) table for this cluster that occurs in animals tested with BPA and is relevant and adverse in humans?

- **Q2**: If one or more endpoints in the WoE table for this cluster occurs in animals tested with BPA and is both relevant and adverse for humans, what is your prediction for the lowest BMD of those endpoints, expressed as HED?

UA: Elicitation of probability judgements from individual experts

Steps:

- For each cluster: 2 or 3 experts were asked to answer both questions independently
- Discussion among experts regarding their judgements
- Experts had possibility to revise their judgements.
- Expert's judgements were combined to produce a distribution quantifying uncertainty about the estimated lowest BMD across all clusters.

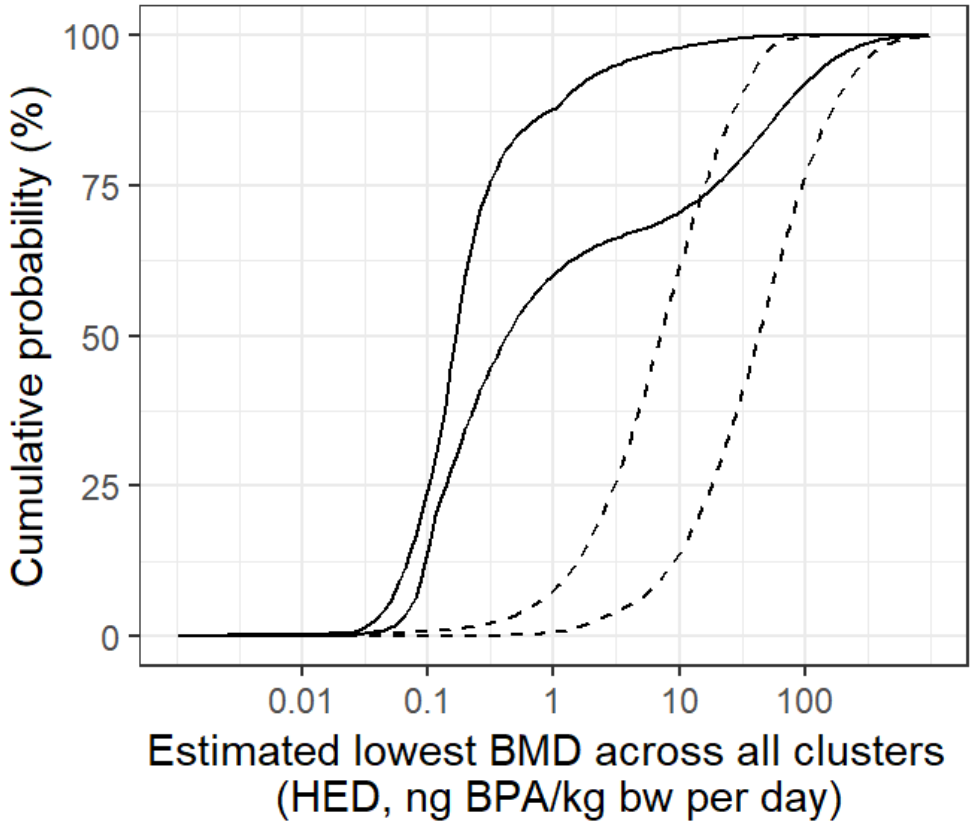


UA: Sensitivity analysis

What is the effect on the lowest estimated BMD when a cluster is omitted?

The estimates for the lowest BMD increased markedly when allergic lung inflammation is excluded

The assessment of allergic lung inflammation cluster was reviewed by the whole working group



solid curves: combining all the assessed clusters
dashed curves: excluding allergic lung inflammation

UA: Review allergic lung inflammation

***Q1**: What is your probability that there is at least one endpoint in the weight of evidence (WoE) table for this cluster that occurs in animals tested with BPA and is relevant and adverse in humans?*

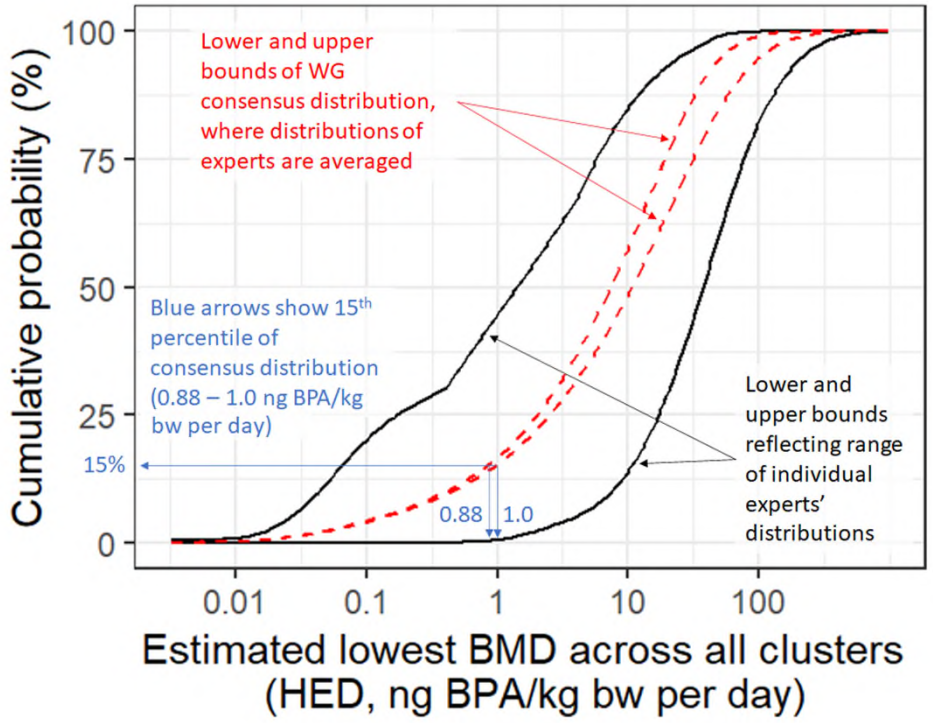
➡ Experts agreed on a consensus probability of 66% that at least one of the endpoints in this cluster that occurs in animals, is both relevant and adverse for humans

***Q2**: If one or more endpoints in the WoE table for this cluster occurs in animals tested with BPA and is both relevant and adverse for humans, what is your prediction for the lowest BMD of those endpoints, expressed as HED?*

➡ Elicitation of judgements from 14 experts separately

Uncertainty Analysis (UA)

- Lower and upper bounds quantifying uncertainty about the lowest BMD across all clusters (black curves)
- Distributions of experts were averaged for each cluster (red dashed curves)
- Experts agreed on the red dashed curves as their consensus distribution

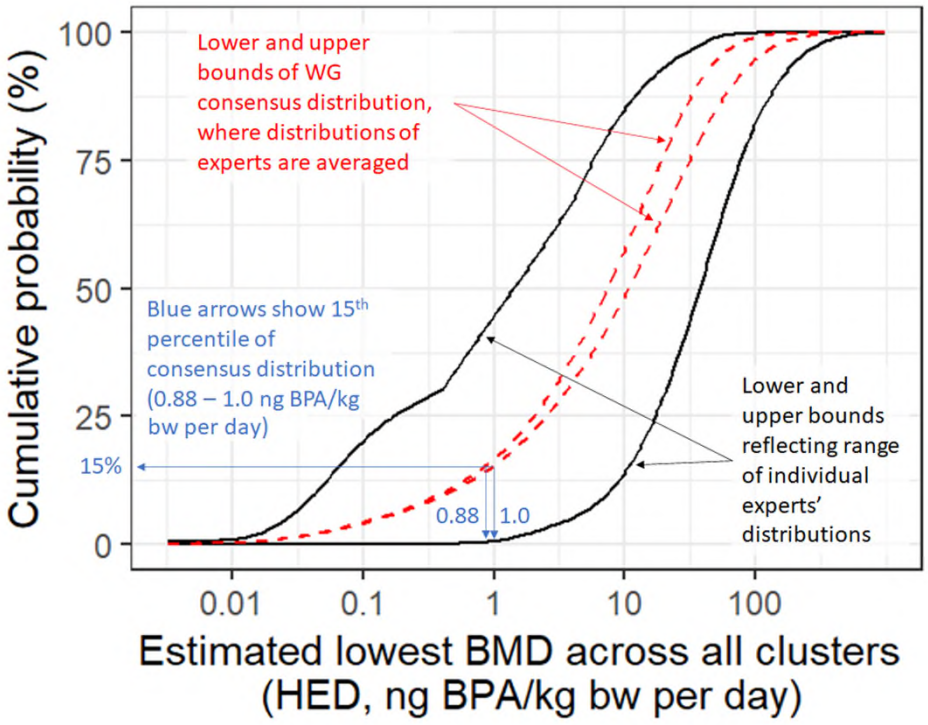


Cumulative distributions quantifying uncertainty about the estimated lowest BMD across all 21 clusters considered in the uncertainty analysis.

Uncertainty Analysis (UA)

- **The Reference Point (RP; 0.93 ng BPA/kg bw per day) is close to the 15th percentile** of both the lower and upper bounds of the consensus cumulative probability function.
- There was 85% probability that the estimated lowest BMD for all assessed clusters is above the RP.
- Large range of endpoints tested

⇨ **No additional uncertainty factor (UF)**



Cumulative distributions quantifying uncertainty about the estimated lowest BMD across all 21 clusters considered in the uncertainty analysis.

Health-based guidance value (HBGV)

Reference point (RP) for the critical effect: 0.93 ng/kg bw per day, expressed as human equivalent dose

Uncertainty factor of 25

- inter-species toxicodynamic difference (2.5)
- intra-human variability in toxicokinetics and toxicodynamics (10)

Uncertainty analysis: **no additional uncertainty factor**

Tolerable daily intake (TDI) of 0.04 ng BPA/kg bw per day

Risk characterisation

TDI: 0.04 ng BPA/kg bw per day

Dietary exposure estimates EFSA 2015 Opinion:

Average: 0.03-0.375 µg/kg bw per day across age groups

High (P95): 0.08-0.857 µg/kg bw per day across age groups

Both the average and high **dietary exposures** in all age groups **exceeded the TDI** by two to four orders of magnitude

- If TDI based on second most sensitive effect → TDI would have been exceeded by dietary exposure by two to three orders of magnitude.

Risk characterisation

TDI: 0.04 ng BPA/kg bw per day	⟺	Dietary exposure estimates EFSA 2015 Opinion

Uncertainties associated with the risk characterisation:

- The exposure assessment presented in the 2015 opinion may not fully represent the current dietary exposure

*Even considering the uncertainty in the exposure assessment, since the exceedance was so large, the CEP Panel concluded that there is a **health concern** from dietary BPA exposure for all age groups.*

Conclusions (1)

- After conversion to HED, the CEP Panel selected the lowest BMDL value of 0.93 ng/kg bw per day for the effect of BPA on Th17 cells in mice to be used as reference point (RP).
- The CEP Panel concluded that no additional UF was needed and that a HBGV based on the identified RP is justified.
- The CEP Panel applied the UF of 25 for inter-species toxicodynamic difference and intra-human variability in toxicokinetics and toxicodynamics and **established a TDI of 0.04 ng/kg bw per day**.

Conclusions (2)

- The comparison of the dietary exposure estimates from the 2015 EFSA opinion with the new TDI showed that both the average and high dietary exposures in all age groups (including all infants and toddler groups) exceeded the TDI by two to four orders of magnitude.

- Even considering the uncertainty in the exposure assessment, since the exceedance was so large, the CEP Panel concluded that there is a health concern from dietary BPA exposure for all age groups of the general population.

Lunch break

Q&A session

Following presentation → Concluding remarks and closure of the meeting

Concluding remarks and closing of the meeting

Claude Lambré

Chair of the EFSA Scientific Panel on Food Contact Materials, Enzymes and Processing Aids (CEP Panel)

Next steps

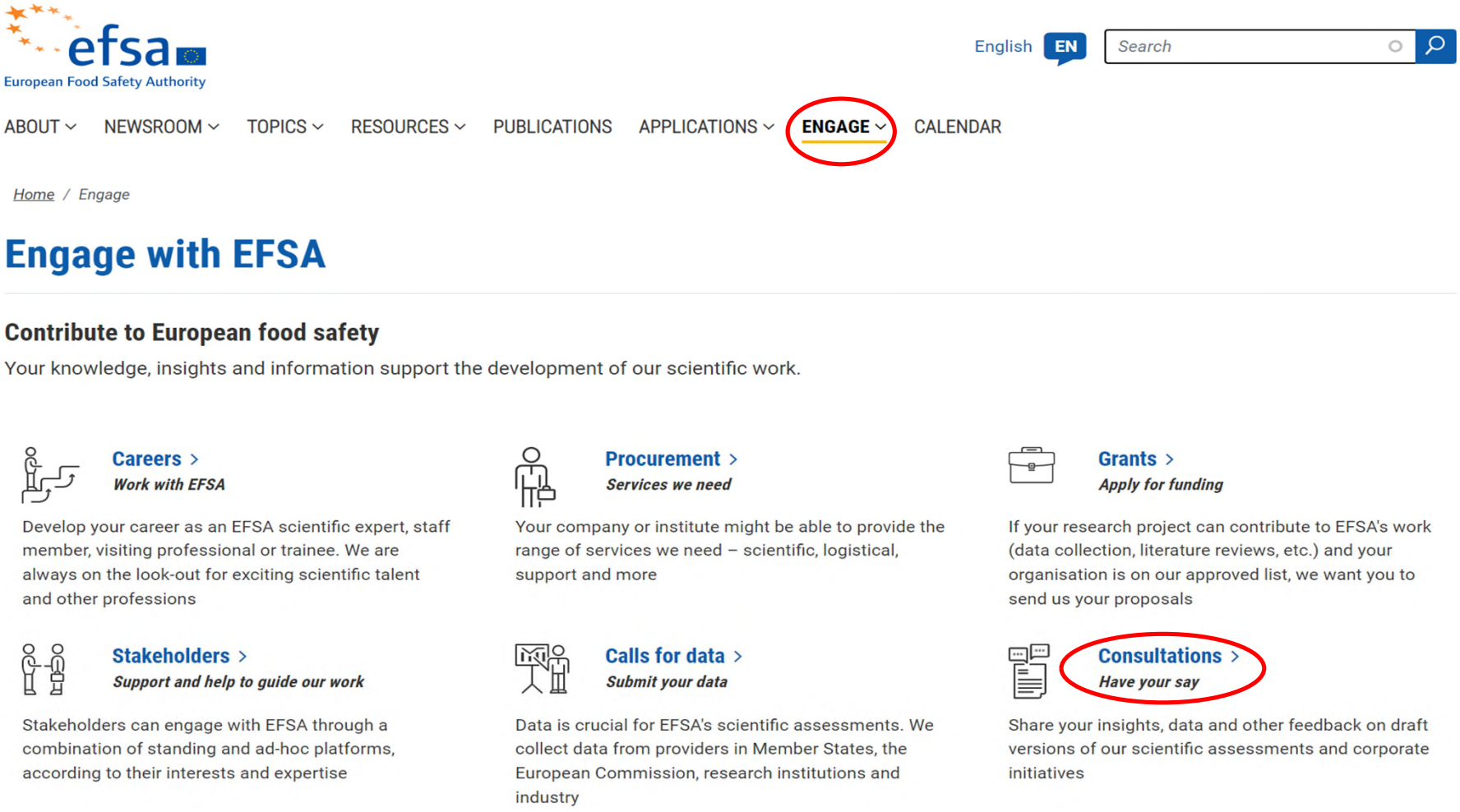
The draft opinion is available at the EFSA website for **PUBLIC CONSULTATION until 22 February 2022**

Remaining questions and/or comments that could not be addressed during this meeting can be submitted through the public consultation platform.

How to access the draft opinion and submit comments?

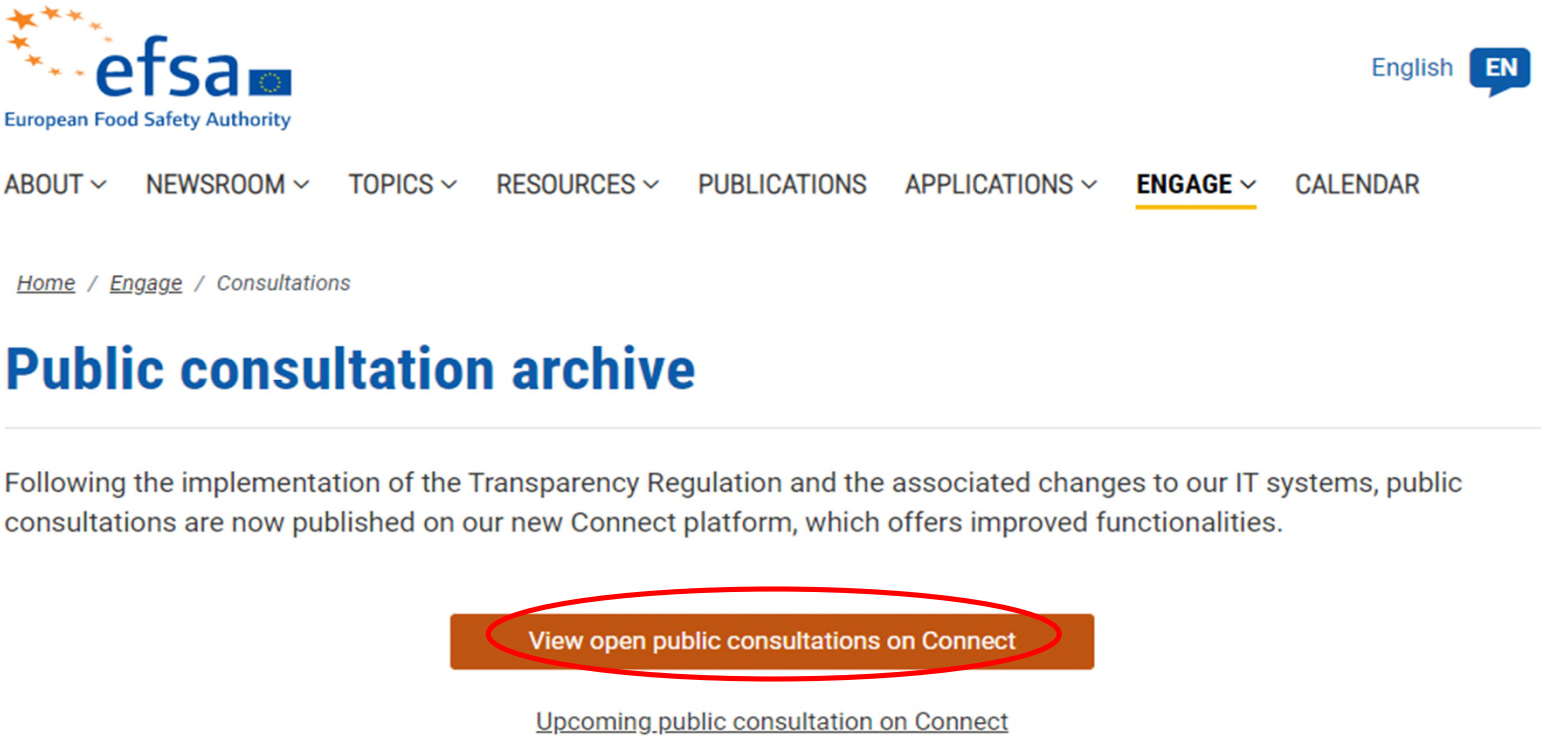
Next steps

EFSA website – https://www.efsa.europa.eu/en



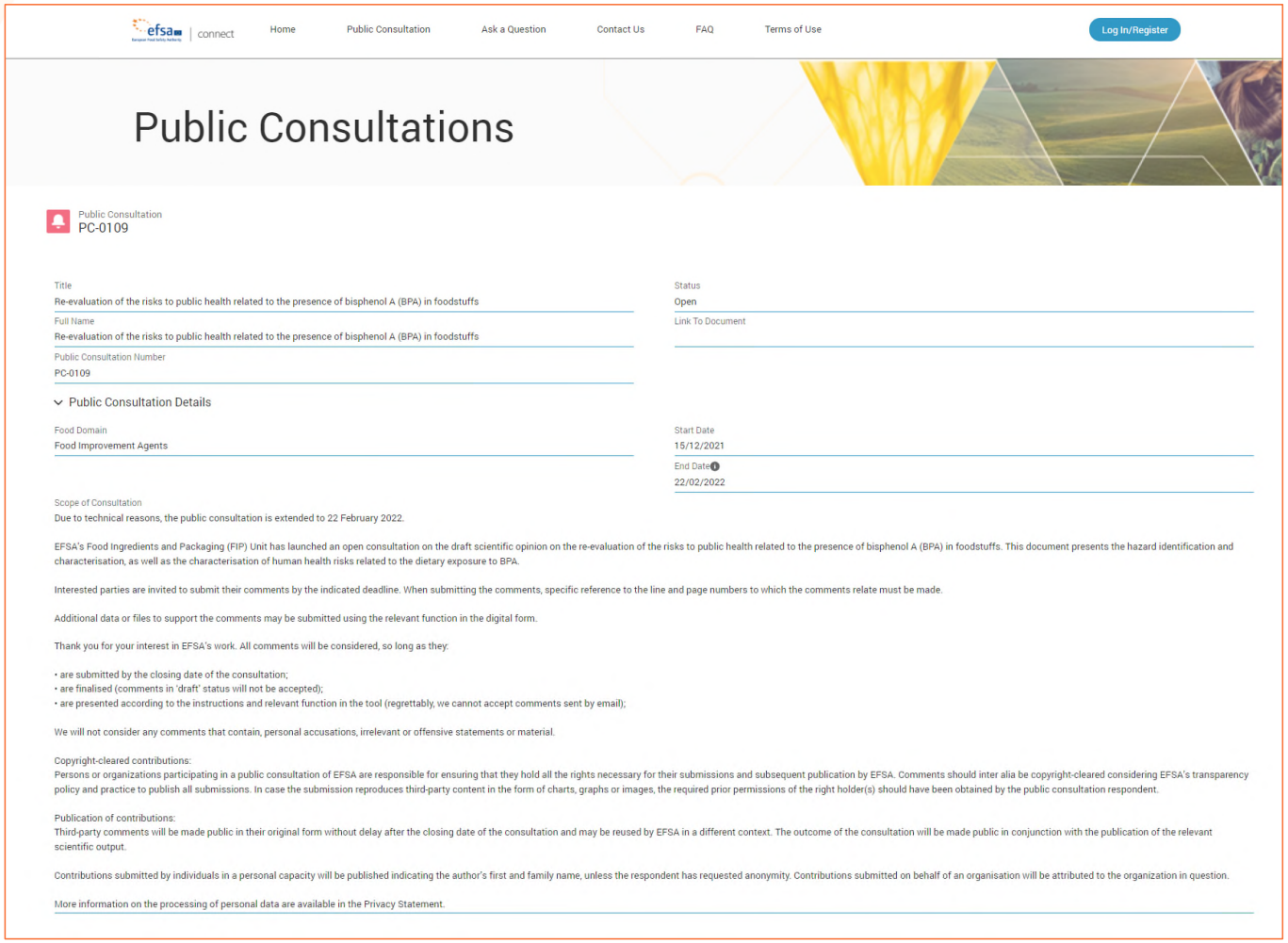
Next steps

EFSA website – https://www.efsa.europa.eu/en



A list will be displayed where you can find **all open public consultations**.
You can click on **PC-0109 (BPA draft opinion)** and leave a comment using the specific form provided.

Next steps



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Public Consultations

Public Consultation
PC-0109

Title
Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs

Full Name
Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs

Public Consultation Number
PC-0109

Status
Open

Link To Document

Public Consultation Details

Food Domain
Food Improvement Agents

Start Date
15/12/2021

End Date
22/02/2022

Scope of Consultation
Due to technical reasons, the public consultation is extended to 22 February 2022.

EFSA's Food Ingredients and Packaging (FIP) Unit has launched an open consultation on the draft scientific opinion on the re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. This document presents the hazard identification and characterisation, as well as the characterisation of human health risks related to the dietary exposure to BPA.

Interested parties are invited to submit their comments by the indicated deadline. When submitting the comments, specific reference to the line and page numbers to which the comments relate must be made.

Additional data or files to support the comments may be submitted using the relevant function in the digital form.

Thank you for your interest in EFSA's work. All comments will be considered, so long as they:

- are submitted by the closing date of the consultation;
- are finalised (comments in 'draft' status will not be accepted);
- are presented according to the instructions and relevant function in the tool (regrettably, we cannot accept comments sent by email);

We will not consider any comments that contain, personal accusations, irrelevant or offensive statements or material.

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Publication of contributions:
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Interested parties are invited to submit their comments by the **22 February 2022.**

Comments should be inserted according to the **instructions on the website** and using the relevant function in the tool (EFSA cannot accept comments sent by email).

Comments should be **finalised** (comments in 'draft' status will not be accepted).

Next steps

The comments submitted through the public consultation:

- ✓ Will be considered by the **EFSA Working Group on BPA** re-evaluation and the **CEP Panel**
- ✓ Based on their scientific merit, they may feed into the final version of the Opinion before its **adoption** (2022)

In parallel, an **EFSA Annex** will be developed:

- ➢ All original comments will be listed as submitted
- ➢ Reply to the comments will be provided including an explanation of the actions taken and the rationale

Thank you for attending our event

The presentations of today's event will be available on the EFSA website and the link to access them will be sent shortly after the meeting.

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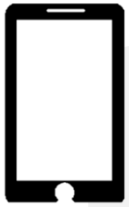

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