Meeting

EFSA 2023 re-evaluation of the risk to public health from bisphenol A (BPA) in foodstuffs

This is a paper for discussion.

This does not represent the views of the Committee and should not be cited.

Introduction

1. In December 2021, the European Food Safety Authority (EFSA) Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) published a draft opinion re-evaluating the health risks arising from the presence of Bisphenol A (BPA) in food. The Panel proposed a significant reduction in the current temporary Tolerable Daily Intake (TDI) of 4 µg/kg body weight (bw)/day to 0.04 ng/kg bw. This reduction would mean that both mean and high level consumers for all age groups would exceed the new TDI by 2-4 orders of magnitude.

2. Following public consultation EFSA published the final opinion on the re-evaluation of BPA in April 2023. The Panel established a new TDI of 0.2 ng BPA/kg bw per day in their finalised opinion. Although this new TDI is higher than the initially proposed level of 0.04 ng.kg bw, it still means that both mean and high level consumers for all age groups would exceed the new TDI by 2-3 orders of magnitude.

3. Both, the European Medical Agency (EMA) and the Bundesamt fuer Risikobewertung (BfR) provided comments to EFSA, highlighting diverging views. As the diverging views could not be resolved, according to the respective founding regulations, EFSA and the EMA/BfR are obliged to present a joint document to the European Commission (EC) clarifying the contentious scientific issue and identifying relevant uncertainties in the data. These documents are required to be publicly available.

4. The COT discussed the draft EFSA opinion at their extraordinary meeting in February 2023 and provided comments to EFSA; the papers prepared for the February COT meeting provide more detail on the EFSA approach and the key endpoints. The following paper briefly summarises the derivation of the new TDI, highlighting the changes to the draft opinion, where applicable and the diverging opinions, both by EMA and the BfR. Please note, the paper predominantly highlights the diverging views by the EMA and BfR; for EFSA's responses to these comments, please see the original documents for detail (the links are provided in Annex A).

5. Following the meeting the Secretariat are proposing that small groups of Members (with Secretariat support) tackle the key endpoints and issues (immunotoxicity, reproductive toxicity, modelling etc) with a detailed discussion at the full Committee in July or September.

Background

6. BPA is a monomer used in the manufacture of polycarbonates, epoxy resins and other polymeric materials, as well as in thermal printing in certain paper products. Polycarbonates are used in food contact materials such as reusable beverage bottles, infant feeding bottles, tableware and storage containers. Epoxy resins are used in the protective linings of food and beverage cans and vats (EFSA, 2021).

7. BPA is authorised for use as a monomer in plastic food contact materials in accordance with Commission Regulation (EU) No 10/2011/EU1 on plastic materials and articles intended to come into contact with foodstuffs and retained UK legislation. The specific migration limit for BPA is 0.05 mg/kg, reduced from 3 mg/kg following the EFSA 2015 evaluation of BPA.

2015 EFSA evaluation of BPA

8. In 2015, the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) established a temporary TDI (tTDI) of 4 µg/kg body weight (bw)/day (EFSA, 2015). The toxicity of BPA was evaluated using a weight of evidence approach. "Likely" adverse effects reported in animal studies were considered to be in the kidney and mammary glands. These underwent benchmark dose (BMDL10) response modelling. A BMDL10 of 8,960 µg/kg bw per day was calculated for changes in mean relative kidney weight in a two generation toxicity study in mice. No BMDL10 could be calculated for mammary gland effects. Using data on toxicokinetics, the BMDL10 was converted

to a Human Equivalent Dose (HED) of 609 µg/kg bw per day. The CEF Panel applied a total uncertainty factor (UF) of 150 (for inter- and intra-species differences and uncertainty in mammary gland, reproductive, neurobehavioural, immune and metabolic system effects) to establish a temporary TDI (t-TDI) of 4 µg/kg bw per day.

9. The CEF panel compared this t-TDI with exposure estimates and concluded that there was no health concern for any age group from dietary exposure and low health concern from aggregated exposure. The CEF Panel noted considerable uncertainty in the exposure estimates for non-dietary sources, whilst the uncertainty around dietary estimates was relatively low.

2023 Re-evaluation of BPA

10. In 2016, the CEP Panel received a new mandate from the EC to re-evaluate the risks to public health related to the presence of BPA in foodstuffs but also 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.

11. In particular, the re-evaluation should take into consideration the 2017 US National Toxicology Program (NTP)/Food and Drug Administration (FDA) study and all other new available information not previously evaluated by EFSA and which fulfil the criteria laid down in an established protocol.

12. The re-evaluation of BPA 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 TDI on the basis of the new information available.

13. The re-evaluation was performed by a systematic approach and in accordance with a pre-established protocol, which underwent public consultation. Studies from 1 January 2013 to 15 October 2018 were included in the evaluation, and EFSA launched a call for evidence to obtain human or animal data relevant to the risk assessment. Although some studies were published after the cut of date, the NTP CLARITY study and its associated Grantee studies were also included in the evaluation. For genotoxicity the literature search was extended until 21 July 2021 and the studies in the 2015 evaluation were re-assessed.

14. EFSA used a health outcome category (HOC) cluster approach to assess the data, which worked through the database by endpoint rather than by study

(so a particular study could occur in several endpoint clusters). For example, the HOC General Toxicity would have a cluster Liver toxicity and within that the endpoints alanine amino transferase (ALT) and aspartate amino transferase and (AST), and gamma-glutamyl transpeptidase (γ-GTP).

Evaluation of studies

Toxicokinetics

15. Two studies in mice, three studies in rats, three studies in ewes and two studies in humans were considered for toxicokinetic effects, with the studies in mice and rats not contributing to a better understanding. The human studies showed that BPA was absorbed to nearly 100% and pre-systemically metabolised to a great extend to glucuronide and sulfate conjugates. The areas under the curve (AUC) adjusted for dose were clearly different and the median AUC from both studies was 15.7 nM x h, which was 4-fold higher than the modelled AUC value calculated in the 2015 opinion. EFSA decided to use the median value of the AUCs for the calculation of the human equivalent dose factor (HEDF), as both modes of administration used in these studies was realistic for human exposure. In order to calculate the HEDF, the AUC data from the 2015 opinion were used for mice, rats, monkeys and dogs; for ewes the data reported in the current opinion was used, resulting in HEDFs of 0.0155 (mice), 0.1656 (rats), 0.095 (monkeys), 0.1395 (dogs), 0.1197 (ewes' gavage) and 0.4357 (ewes' diet).

Target organs & MoA

16. The mammary gland, prostate and uterus appeared to be the target organs for BPA induced toxicity. No human data were available; however assessment of the animal data provided the following conclusions. The effect on mammary gland weight was considered 'not likely', while effects on mammary gland and prostate histology as well as uterus weight showed effects that were not consistent across studies and hence considered 'as likely as not (ALAN)'. In addition, lesions in the mammary gland were inconsistent in the developmental exposure period with no increase in pre-neoplastic lesions ('not likely') but higher incidence in neoplastic lesions ('likely'), while in the developmental and developmental and adult exposure periods an increase in pre-neoplastic lesions ('ALAN') was reported but no increase in neoplastic lesions ('not likely'). These effects contributed to the overall conclusion of 'ALAN'. Mode of Action (MoA) studies in mammary gland addressing epigenetic effects, changes in gene expression and changes in hormone receptor levels suggested various MoAs of

BPA. Enhanced susceptibility to tumorigenesis in rodents has been reported in studies on prostate cancer, after co-treated with very high levels of oestradiol (E2) and testosterone, while developmental and chronic exposure to BPA without additional treatment with sex hormones did not demonstrate a direct tumorigenic effect. The non-neoplastic changes in gland cellular anomalies, squamous metaplasia and cystic endometrial hyperplasia in uterus histology were considered adverse by EFSA and therefore considered as ‘likely’ effects. While studies on uterine cells suggested various MoAs potentially involved in the induction of proliferative changes, the results in rodent studies did not demonstrate a tumorigenic activity of BPA.

Genotoxicity

17. Based on the data available from the 2015 EFSA opinion and any new information published since, EFSA concluded that BPA does not induce gene mutations in bacteria, while it does induce DNA strand breaks, clastogenic and aneugenic effects in mammalian cells *in vitro*. Oxidative stress related mechanism(s) have been suggested to possibly be responsible for the DNA damage and clastogenic activity of BPA *in vitro*. In contrast to the consistent *in vitro* findings, the available *in vivo* studies provide limited and inconsistent evidence for DNA and chromosomal damage. No evidence of aneugenicity in germ cells has been reported. Hence, EFSA concluded that there is no evidence supporting an *in vivo* genotoxic hazard through direct interaction with DNA and that it is ‘unlikely’ to ‘very unlikely’ that BPA presents a genotoxic hazard through a direct mechanism. Therefore, EFSA concluded that the available information allows for the establishment of a health based guidance value (HBGV).

18. The available literature indicated that several organs as well as haematological parameters were potential targets of BPA toxicity. No human studies were available (within this HOC), but clusters with relevant endpoints were identified in animal studies, i.e. body weight and effects on the liver, kidney, lung, thyroid, parathyroid, pituitary glands, adrenal glands, bone marrow and on haematological parameters. However, none of these effects were considered ‘very likely’ or ‘likely’ by EFSA. While there were effects noted at least on one exposure period, there were inconsistencies hence the effects were judged ‘ALAN’. The pivotal effect on the liver and kidney in the previous opinions were found at higher doses, hence the likelihood of effects assigned as ‘likely’ was not negated by the ‘ALAN’ effects at lower doses in studies assessed in the current evaluation. Mode of action (MoA) studies suggested oxidative stress as a potential pathogenic mechanism, but EFSA noted that other mechanisms may be operational as well.

Metabolic effects

19. Based on human data, none of the metabolic endpoints, i.e. obesity, cardiometabolic effects, thyroid effects, type 2 diabetes mellitus (T2DM), gestational diabetes mellitus, showed effects that were considered ‘likely’ or ‘very likely’. Data on positive association between BPA and obesity and T2DM was considered ‘ALAN’, whole a positive association between exposure and cardiometabolic effects, thyroid effects and gestational diabetes mellitus was considered ‘not likely’. Based on the animal data no metabolic effects were considered ‘very likely’. Uric acid was considered a ‘likely’ effect in the adult exposure period, as increased levels were observed in the liver of mice and in the serum of mice and rats. The other metabolic endpoints were considered ‘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. Several plausible MoAs were indicated for metabolic effects form animal and *in vitro* studies.

Neurotoxicity

20. Data from the recent literature review supported the fact that the central nervous system is a target of BPA toxicity. Human data thereby did not support an association between BPA exposure and impaired neurodevelopment, but animal data showed effects that were considered ‘likely’ for neurotoxicity by EFSA. ‘Likely’ effects were found for dendric spine density of pyramidal cells in hippocampus (CA1 and dentate gyrus areas) after developmental exposure, number of neurons in hippocampus (CA1 and CA3 areas) and dendritic spine density in pyramidal cells in medial part of the prefrontal cortex (PFC) after exposure during the growth phase/young age. In addition, effects were seen on nervous system functionality, i.e. a ‘likely’ effect on acetylcholinesterase (AChE) activity during the adult exposure period. ‘Likely’ effects were also noted on behaviour, for anxiety/emotionality during all exposure periods. The endpoint learning/memory also showed ‘likely’ effects of BPA from developmental and growth phase/young age and effects on sensory-motor coordination and salt preference in adults. Several MoAs have been proposed but the association between the identified effects of BPA with brain structure, function and development have not been sufficiently explored to draw conclusions.

Cardiotoxicity

21. No case-control or cohort studies were available for cardiotoxicity and therefore EFSA considered the evidence to be inadequate. Based on animal data,

the evidence of an effect of BPA on cardiotoxicity was considered as ‘not likely’ in the majority of endpoints and in a few endpoints as inadequate in one or more exposure period. The endpoints considered were absolute and relative heart weight, incidence of cardiac lesions, cardiac structural changes (measured by echocardiography), effects on cardiac function (measured by echocardiography), blood pressure and atherosclerotic lesions.

Reproductive toxicity

22. New evidence has been published since the last evaluation, indicating that reproduction is a target of BPA toxicity. Based on human data none of the endpoints were considered ‘very likely’ or ‘likely’ by EFSA. An association between maternal BPA exposure and impaired pre- and post-natal growth, shorter duration of gestation and preterm delivery, reduced male fertility and pubertal development when exposed during childhood was considered ‘not likely’. Effects seen on reduced female fertility and pre-eclampsia during adult and pubertal development when exposed during pregnancy was considered ‘ALAN’. Effects seen in animal data on both male and female reproduction were considered as ‘likely’, with ‘likely’ effects on ovary weight and histology and uterus histology after developmental exposure, on ovary histology after developmental and adult exposure, on implantation rate after growth/young age exposure and on ovary histology (follicle counts) after adult exposure. Effects on epididymis histology (exfoliated germ cells and inflammation) were seen on male reproduction after developmental and adult exposure, on testis histology (decreased seminiferous tubule diameter) after growth phase/young age exposure and on sperm (motility, viability, and acrosome reaction) after adult exposure and considered ‘likely’. Developmental effects were also noted, i.e. body weight, bone development, mammary gland histology, mammary gland weight (developmental exposure), mammary gland histology (developmental and adult exposure) and body weight and first oestrus (growth phase/young age exposure). However, the results were less consistent and therefore judged as ‘ALAN’. Supporting evidence for plausible MoAs were available, such as oestrogen and androgen receptor (AR) interactions and associated downstream and cross-stream effects including epigenetic changes.

Immunotoxicity

23. Data confirmed that the immune system is a target for BPA toxicity. Asthma/allergy, including data from the exposure periods pregnancy and childhood were identified as relevant endpoints in human studies. Based on these

studies a positive association between BPA exposure and asthma/allergy was considered ‘ALAN’, based on the exposure assessment in these studies and the overview of the observed effect patter on asthma and wheeze. In animal studies, five clusters of relevant endpoints were identified, i.e. innate, cellular and humoral immunity, inflammation and allergic lung inflammation, with allergic lung inflammation, cellular immunity and inflammation showing effects that were judged as ‘likely’. All other endpoints were noted to show effects, but the data was less consistent and hence EFSA considered those effects as ‘ALAN’. The effect noted on the production of specific IgE in response to an allergen was deemed adverse by EFSA as it is a crucial parameter in inducing allergic reactions in the respirator tract. Other effects supported the likelihood of this effect. Th17 cells and their cytokines play a pivotal role in cellular immune response and are involved in the development of inflammatory conditions, such as autoimmunity and lung inflammation. In vivo evidence of immunotoxicity was supported by MoA data. In vitro studies indicated the ability of BPA to induce immune deregulation, possibly leading to an increased susceptibility to develop inflammatory disease.

Derivation of the TDI

24. EFSA performed benchmark dose (BMD) analysis for dose-response modelling on all endpoints that were considered ‘very likely’ or likely’ in accordance with the EFSA guidance. A cut-off value of maximum 10 was applied for the ratio between the lowest dose tested (>0) and the BMD lower confidence interval (BMDL) for selection of the Reference Point (RP). Studies with a ratio ≥ 10 were considered inadequate for BMD analysis but were considered in the uncertainty analysis, as were studies that were considered ‘ALAN’. BMD analysis was performed with the administered dose, without conversion to HED, HED converted values were however used to compare the different modelling outcomes.

25. More than one BPA induced effect was identified by EFSA, with adverse effects seen in a similar dose range for other endpoints as for increase of Th17 cells. Reproductive and developmental effects, i.e. the ratio of primordial and total ovarian follicles, sperm motility, and metabolic effects, i.e. uric acid, had BMDLs up to 7-fold higher than the BMDL for Th17 cells. However, the increase in percentage of Th17 cells in the immune system was considered the most sensitive and hence the critical effect. In the report on diverging views between EFSA and the BfR, EFSA further clarified that the selection of the pivotal study (Luo et al., 2016) was based on a risk of bias scrutiny of scientific papers. The conclusions from the present opinion were not solely based on one study but

drawn from the WoE of the entire data set. The effect on Th17 cells was the most sensitive observed, even if the differences in doses with the other effects were relatively small. Furthermore, the effects described in the Luo et al. study were confirmed by more recently published studies (see Section 3.13; comment 30 Annex N of the opinion). Potential bias through background contamination was taken into account during the appraisal of the study's internal validity and the uncertainty of the dose at which the effect occurs was taken into account in the uncertainty analysis (UA).

26. After conversion of the doses from the Luo et al. (2016) study, the lowest BMDL40 was identified as a HED of 8.2 ng/kg bw per day and used as reference point (RP) for the derivation of a HBGV. EFSA did not apply an uncertainty factor (UF) for inter-species variability in toxicokinetics as this was already accounted for in the conversion to HED. The default UF of 2.5 and 10 were applied for inter-species toxicodynamic differences and intra-species variability in toxicokinetics and toxicodynamics, respectively.

27. EFSA undertook a structured uncertainty analysis using Expert Knowledge Elicitation (EKE) to identify and quantify (by expert judgement) the impact of the uncertainties on the hazard assessment. One major source of uncertainty was the large number of non-standard studies and endpoints, and the possibility that some endpoints had no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) lower than the RP and hence could be more sensitive. However, EFSA was unable to calculate BMDLs for these endpoints. The uncertainty analysis included any endpoints considered 'ALAN', 'likely' or 'very likely'. The overall uncertainty was expressed as the probability that the estimated lowest BMD for effects in animals which were relevant/adverse to humans were below any given dose. Sensitivity analysis showed that the probability for lower doses was predominantly driven by allergic lung inflammation, followed by cellular immunity, which included increased percentage of Th17 cells. In their response to the BfR EFSA noted that the main impact on the low TDI was the new evidence available and the resulting RP, with the UA confirming that a RP in this range was reasonable when taking into account all evidence and uncertainties.

28. Averaging across experts the probability that the lowest BMD for endpoints occurring in animals which were relevant to humans was below the RP of 8.2 ng/kg bw per day (HED) was 57 – 73%, the overall range of probabilities given by individual experts was even wider (44 – 98%), with the lowest probability being 27 – 43%. As there was sufficient uncertainty in the hazard assessment, EFSA considered it justifiable to include an additional UF of 2 when setting the TDI

to account for the uncertainties affecting the RP and the possibility that other endpoints are more sensitive. The additional UF needed to be large enough to cover its median estimate for the lowest estimated BMD, such that it is equally probable (50%) that the lowest estimated BMD is higher or lower.

29. The increase in Th17 cells is considered an intermediate endpoint and for it to be considered in risk assessment it needs to have a causal relationship with an adverse outcome. EFSA noted that while this endpoint does not have an established relevant quantitative adverse outcome pathway (AOP), the information reviewed indicated that an increment in Th17 cell percentage and their cytokine IL17 was linked to inflammation. Hence, it meets the definition of adversity set by EFSA and the WHO. EFSA did not consider it necessary to apply an additional UF to account for the use of an intermediate rather than apical endpoint as there was a lack of relevant quantitative data or specific guidance on risk assessments based on RPs which are considered intermediate endpoints.

30. Applying an overall UF of 50 to the RP, EFSA established a TDI of 0.2 ng/kg bw per day.

31. Comparing the newly derived TDI to the exposure estimates in the 2015 opinion, resulted in exceedances for mean and 95th percentile dietary exposures in all age groups by 2- to 3-fold. While the exposure assessment in the 2015 opinion may not accurately represent the current dietary exposure, even considering the uncertainties, as the TDI was exceeded, EFSA concluded that there was a health concern from dietary exposure to BPA for all age groups.

Diverging views

EMA

32. EMA did not agree with EFSA’s revised TDI due to the two agencies different scientific approaches to risk assessment and methodology for quantifying the risk, i.e. the adverse effect definition, the intermediate versus apical endpoint, the approach applied for consideration of studies and the risk assessment approach including the clinical relevance/extrapolation from animal studies for use in humans.

33. The agencies have diverging views on what can be considered sufficient scientific evidence to demonstrate that an intermediate endpoint in animals is causally associated with an adverse effect in humans. Furthermore, the agencies disagreed on the method for quantifying the risk and establishing an exposure

level considered safe in humans.

Adverse effect definition and intermediate versus apical endpoint

34. EMA did not dispute mice showed an increased **Th17 cell count** and reduced ovarian follicle counts, however EMA consider the pathogenesis, mode of action and clinical histopathology of organ damage based on biological significance and plausibility within the design and scope of studies performed. Hence, the implications of these observations for human exposure require evidence of causality. According to EMA there is insufficient evidence to support EFSAs claims that Th17 increases in mice lead to an increased risk of IgE-mediated immune disorders in humans. Furthermore, EMA does not consider an isolated observation of reduced ovarian follicle counts in a single study to signify impaired fertility in humans. Therefore, EMA and EFSA did not agree on what constitutes “a clear causal relationship” between intermediate and apical endpoints, and hence an adverse effect.

35. According to EMA there is no evidence from the studies included by EFSA that the observed increase in Th17 cells results in any adverse outcome (AO). Studies within the NTP CLARITY-BPA program, including studies on the toxicity of BPA provided no evidence for immunotoxicity at low doses. Hence, the current scientific understanding does not support a causal link between Th17 cells and IgE- mediated allergy, especially as no causal link has been demonstrated in any animal or human study. In EMA’s view some effects in animals are not seen in humans and this has implications for the translation of findings from and intermediate endpoint in animals to health effects in humans. In the absence of quantitative data to support animal to human extrapolation, any conclusion on relevance for humans is challenging, if not substantiated by data.

36. EFSA agreed that there was no direct causal link, however based on the weight of evidence assessment of a large number of studies and cluster endpoints for immunotoxicity evidence for a link between Th17 cells and several AOs exist as TH17 cells and their ILs are involved in diseases with an inflammatory pathogenesis. EFSA acknowledge that it might be appropriate to apply specific factors when extrapolating, however in this assessment extrapolation factors were not quantified due to the lack of relevant quantitative data and specific guidance. Hence the HEDF concept was applied.

37. Both agencies agreed that the studies applied for the reduction in **ovarian follicle counts** and sperm motility were not fertility studies compliant with OECD and ICH guidelines.

38. However, EMA does not consider there to be any scientific evidence that reduced follicle count or reduced sperm motility as single endpoint observed in the studies used by EFSA would result in reduced fertility in humans. The studies are predominantly mechanistic and not looking at toxicological endpoint causative for adverse effects on fertility. EMA also notes that there is evidence that rodents are able to compensate for reduced sperm motility and hence it is not possible to solemnly draw a conclusion on fertility in male rodents on this endpoint alone.

39. While both agencies may accept intermediate endpoints, for EMA to accept such intermediate endpoints to be sufficient as reference points, direct evidence demonstrating that the intermediate endpoint is in the causal pathway, and closely causally associated with the adverse effect, is required. This evidence needs to be within the design and scope of the studies.

Approaches to study inclusion

40. EMA utilises toxicokinetic data from good laboratory practice (GLP) studies to calculate safety margins from NOAELs and predictions of human exposure with preliminary pharmacokinetic data to assess harmful levels of medicines to humans. Robust non-GLP studies can be considered to support the evaluation and may contribute to the final assessment. For impurities accepts reference to scientific literature and allows calculations of permissible daily exposures based on that data.

41. EMA considers BPA a leachable impurity, should it appear in medicines, as the migration would be in trace amounts into liquid-containing packaging due to contact with essential packaging material.

42. In the post-authorisation phase (for safety signals), other non-clinical, clinical and observational studies from the scientific literature are also considered, if they are sufficiently robust.

Risk assessment approaches

43. Within EFSAs risk assessment framework, the TDI is established as a protective dose at which no health effect occurs.

44. For medicine approval, EMA performs a risk assessment based on quantification of risk, establishing doses at which exposures to substances (e.g. excipients and leachable impurities) would not have any adverse effects when administered to patients.

45. For studies to be considered to quantify risk they need to provide reliable evidence, i.e. apical endpoints to avoid uncertainty, a clear causality between exposure and adverse effect taking into account biological significance and plausibility, human relevance of the observed effect and data integrity.

BfR

46. Both, EFSA and the BfR acknowledged that the interpretation of available information and risk assessment are linked to the tools and methodologies applied, resulting in the divergence of opinion. The key points of divergence were the adverse effect definition, the inclusion/exclusion of scientific information, apical versus intermediate endpoint (reference point acceptability, adversity, relevance), reproductive toxicity endpoints, uncertainty analysis and choice of HEDF.

Adverse effect definition

47. The BfR agreed that there is evidence that BPA has an effect on Th17 cell counts and other effects on the immune system, however they did not consider the evidence convincing on the relationship between BPA mediated increase in Th17 cells and adverse outcomes in animals and humans. No adverse apical effects were reported in either the study from which the endpoint was derived (Luo et al., 2016), other long term studies (CLARITY report) or epidemiological studies. Furthermore, no endorsed adverse outcome pathway exists for this endpoint. Hence, the BfR concluded that the increase in Th17 cells in the spleen does not seem sufficiently justified and is therefore not suitable for the derivation of a HBGV. The selection of this endpoint by EFSA is not in agreement with the WHO/ICPS definition of adversity, according to the BfR and hence appears to lead away from considering the evidence for human health risks related to a certain exposure to a substance towards considering possible adversity which might manifest *in vivo* eventually.

48. The BfR considers EFSA to use conservative worst-case assumptions in every step of the risk assessment process, resulting in an over-conservative HBGV. Overall, the BfR does not agree with the hazard characterisation by EFSA and therefore does not support the TDI and subsequent risk characterisation.

Inclusion/exclusion of scientific evidence

49. In the opinion of the BfR, a hazard assessment and weight of evidence (WoE) approach solemnly based on studies from a specific publication period

could be biased by the time period the studies were performed. Hence, this is a methodological short coming, and the BfR would have considered additional studies beyond that time-frame at least for the identified critical endpoints.

50. In addition, the BfR identified some studies that were classified as Tier 3 for formal reasons, such as missing information on the purity of BPA, but were otherwise of good quality, while studies that use inappropriate housing materials and/or feed but claim to assess very low doses of BPA were classified as Tier 1. The latter studies have a high likelihood of background contamination and hence the BfR would disqualify such studies for a quantitative assessment.

Apical versus intermediate endpoints

51. The role of Th17 cells is context dependent and not yet fully understood in mice and humans, with a genetic link between increased IL-17A levels and disease in humans still missing (Li et al., 2018; Zwicky et al., 2020). The typical histological effect expected to result from increased Th17 cells percentage and activity, i.e. inflammation, was not detected in numerous animal studies (Tyl et al., 2008; Delclos et al., 2014; CLARITY project) at doses up to five times higher than the BMDL40 from the study selected by EFSA (Luo et al., 2016). EFSA considered BPA effects on inflammation to be not likely in exposure regimes ‘developmental’, ‘developmental and adult’, and ‘adult’ and only one study (Ogo et al., 2018) was considered relevant and likely for effects of BPA on neutrophils in epididymis during the exposure period ‘growth phase/young age’. In humans, except for plaque psoriasis and a few related diseases, e.g. psoriatic arthritis, many trials targeting the IL-17A pathway have fallen short of expectations. Hence, the BfR does not consider there to be strong evidence that the administered doses in the study selected by EFSA lead to adverse immune outcomes in healthy animals. The lack of reliable epidemiological studies, e.g. repeated 24-hour urine samples, does not allow for a definite conclusion on human effects.

52. While the BfR does not question the use of an intermediate endpoint as such, they did expressed concern for the use of the selected intermediate endpoint for setting a HBGV, when not accompanied by the observation of corresponding apical effects in the relevant *in vivo* data.

53. The BfR also questioned the dosing in the by EFSA selected study. There are several studies reporting delectable serum BPA levels in animals from control and/or vehicle groups, even when care was taken to minimise contamination via e.g., housing material. In the BfRs view, the selected study had likely background

contamination originating from polycarbonate cages and non-controlled standard chow, with the standard chow potentially exerting estrogenic activity which hampers the interpretation of the BPA effects. The BfR would not have classified this study as Tier 1 and would not have included it in the WoE.

54. The BfR noted that an assessment of course should follow the predetermined hazard assessment protocol. However, if that protocol leads to debatable conclusion, the this needs to be considered when discussing the final outcome of the assessment.

Reproductive toxicity endpoints

55. In addition to the issues on e.g, diet, cages/bedding, described by EFSA, the BfR had further reservation on the study by Hu et al. (2018) from which the ovarian follicle endpoint was derived. They did not consider the study to be Tier 2 due to shortcomings including the absence of reporting of follicle absolute numbers and lack of blinding during conduct of follicle counts. The latter was of particular importance to the BfR since classification of follicle stages is somewhat subjective and there is a clear risk of bias without blinding. Differently to EFSA the BfR did not consider the method cited by Hu et al. on follicle counting as evidence that they did perform their analysis blinded to treatment. Hence, based on these shortcomings and combined with the low effect size, the BfR considered the study by Hue et al. not reliable and hence would have allocated the study in Tier 3 and excluded it from the WoE. Both EFSA and the BfR however agreed that there were ovary effects based on the WoE.

56. The BfR acknowledge effects on sperm motility, but rated the study applied by EFSA for derivation of a HBGV as Tier 3 due to unknown background contamination. By assessing more recent studies and performing BMDL modelling on more recent studies, the BfR derived much higher HBGVs compared to the one identified by EFSA based on the Wang et al. (2016) study.

57. In contrast to EFSA, the BfR considered epididymal sperm count as a likely endpoint.

Uncertainty analysis

58. The BfR pointed out that a quantitative or semi-quantitative uncertainty assessment should rely on observed data, not expert judgement. For a data rich assessment, like BPA, the WHO/IPCS (2018) provide a suitable methodology for uncertainty characterisation. EFSA explained in their initial response to the public consultation (PC) why they did not apply the WHO/IPCS software tool.

59. The main contribution to the low TDI however stems from the choice or RP and therefore this point is more of a general methodological nature. The divergence between the BfR and EFSA concerns many aspects of the hazard characterisation, resulting in a TDI the BfR considers several order of magnitudes lower compared to what the BfR would expect.

60. The updated uncertainty analysis performed by EFSA does not properly address and account for the shortcoming in the hazard characterisation of BPA, potentially due to the methodology applied (EKE analysis).

Choice of HED factor

61. It is well known that in contrast to humans, BPA undergoes extensive enterohepatic recycling in rodents due to differences in the molecular mass threshold for biliary elimination. Hence, the blood concentration and elimination half-lives are increased in rodents (EFSA 2007; 2008; Collet et al., 2015).

62. In the study by Doerge et al. (2011) levels of free BPA were only observed above the detection limit within the first three measurement points, and inly in one or two of the twelve mice investigated per time point. This resulted in the AUC of free BPA and thus the HEDF to be very low compared to other studies. The ratio of overall to free BPA in serum also differed significantly from other studies, EHR was not covered. In other studies, the ratio of/the concentration/time profile of free BPA in serum mirrored the concentration/time course of total BPA in numerous studies, including studies with intervenors application. The BfR therefore consider the Doeger et al. study to be inadequate to derive and select a realistic HEDF.

63. According to the BfR, the studies by Sieli et al. (2011) and Taylor et al. (2011) should have been considered instead. Tayler et al. and others have clearly shown a clear linearity of the concentration of unconjugated BPA in serum after 24 hours (oral application, wide dose range (2 – 1000,000 µg/kg bw)). The linear dose-adjusted concentration/time profiles at 400 and 1000,000 µg/kg bw, respectively, match, with the exception of the last time point where analytical problems may have occurred. The BfR considers the results plausible with respect to the low solubility of BPA in water. BPA administered in fat or rodent chow will only slowly change into the aqueous environment of the stomach and intestine and therefore saturation of enzymes in intestinal cells seen in vitro would be unlikely in vivo, even at comparable doses. EFSA has argued that these studies would not be suitable as the doses applied (up to 13,000 – 100,000 µg/kg bw) might be above linear dose range. Already in 2015 EFSA considered that the AUCs

in those two studies were not increasingly proportional to the dose used and hence the observation pointed to a non-linear relationship. In addition, due to possible limitations of intestinal enzymes, the AUC of unconjugated BPA in the serum may be higher at higher doses, even if linearly dose adjusted.

64. In the view of the BfR the HEDF for mice should be corrected, with a realistic HEDF being 10 – 100 times higher. The BfR also did not think that this fact was sufficiently considered in the uncertainty assessment, partly due to the process of the uncertainty assessment used by EFSA (EKE) but also the date being used in the first step.

Question on which the views of the Committee are sought

Members are asked to consider the following questions:

i. Do Members have any comments on the EFSA BPA opinion or diverging views by the EMA and BfR?

ii. Do Members agree with the Secretariats proposal of smaller groups to tackle the key points of the EFSA opinion?

a. Are there any additional key points that the immunotoxicity, reproductive toxicity, BMD modelling and the uncertainty analysis that need to be considered?

b. Do Members consider any endpoints not relevant to BPA toxicity and hence would not need to be included in a more detailed consideration of the EFSA opinion?

c. Do Members require any additional external expertise?

iii. Does the Committee have any further comments?

Secretariat

May 2023

Abbreviations

AChE	Acetylcholinesterase

ALAN	As likely as not
ALT	Alanine amino transferase
AO	Adverse outcome
AOP	Adverse outcome pathway
AST	aspartate amino transferase and
AUC	Area under curve
BMD	Benchmark dose response modelling
BMDL	BMD lower confidence interval
BPA	Bisphenol A
bw	Body weight
EKE	Expert Knowledge Elicitation
HBGV	Health based guidance value
HED	Human equivalent dose
HEDF	Human equivalent dose factor
HOC	Health outcome cluster
GLP	Good laboratory practice

g-GTP	Gamma-glutamyl transpeptidase
PC	Public consultation
PFC	Prefrontal cortex
LOAEL	Lowest observed adverse effect level
MoA	Mode of action
NOAEL	No observed adverse effect level
RP	Reference point
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
(t-)TDI	(temporary) Tolerable daily intake
UA	Uncertainty analysis
UF	Uncertainty factor
WoE	Weight of evidence

Organisations

BfR	Bundesamt fuer Risikobewertung

CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EC	European Commission
EFSA	European Food Safety Authority
EMA	European Medical Agency
FDA	Food and Drug Administration
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IPCS	International Programme on Chemical Safety
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
WHO	World Health Organisation

Annex A to TOX/2023/25

EFSA's 2023 re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs.

Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs | EFSA (europa.eu)

Annex N – comments from public consultation.

downloadSupplement (wiley.com)

The report on divergent views between EFSA and EMA on EFSA's updated bisphenol A assessment can be accessed using this link:

ema-efsa-article-30.pdf (europa.eu)

The report on diverging views between EFSA and BfR on EFSA updated bisphenol A assessment can be accessed using this link:

Report on diverging views between EFSA and BfR on EFSA bisphenol A (BPA) opinion (europa.eu)

The summaries of the draft opinion provided to the COT at the extraordinary meeting on 10th February 2022 can be accessed using this link:

COT Meeting: 10th February 2022 | Committee on Toxicity (food.gov.uk)

Please note, EFSA has since amended the HBGV, so some of the information may be out of date.

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