Report on diverging views between EFSA and BfR on EFSA updated bisphenol A assessment

Table of Contents

1. BACKGROUND

EFSA has conducted a re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs, which resulted in an EFSA opinion adopted by the CEP panel during its plenary meeting on 6-7 December 2022. A draft of this opinion underwent a public consultation (PC) from 15 December 2021 to 22 February 2022 (https://connect.efsa.europa.eu/RM/s/publicconsultation2/a0l1v00000E8BRD/pc0109). In this context BfR provided several comments highlighting divergences related to various aspects of EFSA's scientific assessment. EFSA answered in detail to all the comments received in Annex N of the opinion (EFSA CEP Panel, 2023).

The founding Regulation of EFSA stipulates that where there is a substantive divergence over scientific issues that cannot be resolved between EFSA and another body, the two bodies are obliged to cooperate with a view to either resolving the divergence or presenting a joint document to the European Commission clarifying the contentious scientific issues and identifying the relevant uncertainties in the data. This document shall be made public (Article 30 of Regulation (EC) No 178/2002).

In this context, a meeting was held between the two bodies on 11 January 2023 to discuss the diverging views on the scientific opinion and to identify opportunities for possible convergence.

This document focuses on the key points of scientific divergence and has been written jointly between the two bodies for the attention of the European Commission (EC).

2. POINTS OF SCIENTIFIC DIVERGENCE

Divergences related to the following items were identified and discussed in the meeting:

a) Adverse effect definition in the BPA assessment,
b) Inclusion and exclusion of scientific evidence in the BPA assessment,
c) Apical endpoints vs intermediate endpoints associated with BPA exposure: reference point (splenic Th17 cell percentage increase) acceptability, adversity and relevance,
d) Reproductive toxicity endpoints,
e) Uncertainty analysis (UA) as applied in the BPA assessment,
f) Choice of the human equivalent dose (HED) factor.

3. DISCUSSION

Specific points of discussion are presented below:

a) Adverse effect definition in the BPA assessment

EFSA selected as reference point (RP) to establish the health-based guidance value (HBGV) of BPA an intermediate endpoint, i.e. splenic Th17 cell percentage increase.

BfR acknowledged that there is evidence that BPA can have this and other effects on the immune system. However, BfR stated that no convincing evidence on the relationship between the BPA-mediated increase of the splenic Th17 cell percentage after stimulation of splenic cells *ex vivo* and adverse outcomes in animals and humans (e.g. inflammation)

exists. Neither the study from which the endpoint was derived (Luo et al., 2016), nor other long-term exposure studies (see e.g. Tyl et al., 2008, Delclos et al., 2014, studies conducted in the CLARITY project) or epidemiological studies report adverse apical effects like inflammation. No endorsed adverse outcome pathway exists for this endpoint. Thus, BfR considers that the novel intermediate endpoint "Th17 cell percentage increase in the spleen" does not seem sufficiently justified and established as predictor for an adverse health outcome in animals or humans and considers it not suitable for derivation of an HBGV.

Along these lines, BfR stated that the selection of this endpoint is not in agreement with the WHO/IPCS definition of adversity[1] (WHO/IPCS, 2009), used by EFSA.

BfR is of the opinion that the intermediate endpoint "splenic Th17 cell percentage increase" in animal models is currently not sufficiently justified as a predictor of adverse health outcome in animals and humans. Therefore, BfR considers the selection of this intermediate endpoint a paradigm shift: it leads 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. BfR considers this is not in line with general practices.

In addition, according to BfR, conservative worst-case assumptions are used in every step of the risk assessment process (e.g. the choice of the effect of concern, the weight of evidence (WoE) process as a whole, the choice of the toxicokinetic factor and the approach of quantifying remaining uncertainties), resulting in an over-conservative HBGV.

BfR does not agree with the hazard characterisation carried out by EFSA and consequently does not support the TDI and following risk characterisation.

EFSA highlighted that the definition of adversity used (WHO/IPCS, 2009) makes no mention, nor requirement for apicality of an effect to be considered adverse. In setting the HBGV, EFSA takes into account the effects which have a relationship with possible apical adverse effects and, therefore, potentially toxicologically relevant. However, such effects do not necessarily need to relate to an apical endpoint in a one-to-one causal association. EFSA includes the use of intermediate endpoints considered having a clear causal correlation with an adverse outcome (AO). EFSA deems that by weighting the overall body of evidence according to the applied protocol, it is possible to identify a link between an intermediate effect and an adverse outcome, even though the AO is not necessarily expressed within the design and scope of the studies considered and is not necessarily confirmed in a single (guideline) study.

The evidence reviewed in the opinion (Section 3.1.3), as well as the increasing scientific evidence on BPA effects (see responses to comment 30 in Annex N), was considered to clearly show that an increment in the Th17 cell percentage and their interleukins indicate enhancement of differential polarisation of the immune system consistent with proinflammation, and are involved in various immune-mediated disorders related to inflammatory pathogenesis both in animals and humans (e.g. psoriasis, diabetes, multiple sclerosis, neutrophilic asthma, etc.). Such evidence was also present in several animal

[1] "Changes in the morphology, physiology, growth, development, reproduction or lifespan of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences" (Annex 1 of WHO/IPCS, 2009).

studies considered in the current opinion which however were not included in WoE as they were judged as Tier 3. Moreover, there are also human studies that link Th17 cell-related effects to inflammation. In particular, there is clinical success of targeting the IL-17/IL-23 axis in chronic inflammation of body lining, while for internal organs this is less clear (Zwicky et al, 2020).
EFSA additionally noted that the lack of effect in the few available long-term studies does not necessarily mean that there is a lack of effect. In fact, it is well known that different species and strains have different sensitivity toward immune disorders. As reported in the opinion (Section 3.3 - Risk characterisation), 'for studying immunotoxicity, very often intermediate parameters are being used'. 'It is known that the developing immune system is especially vulnerable to chemical insults and that effects during developmental stages may have consequences later in life. This is well known for immune-mediated conditions such as allergy and autoimmunity (Dietert, 2014, Hessel et al., 2014)'.
Therefore, based on the current definitions from WHO/IPCS used by EFSA, intermediate endpoints, such as Th17 cell increase, can be considered adverse effects and, depending on the available information, may be used as a RP to establish HBGVs.
Finally, EFSA reiterated that the NTP CLARITY-BPA program did not specifically investigate Th17 cells in mice.

b) Inclusion and exclusion of scientific evidence in the BPA assessment.

BfR criticised the exclusion of multiple data from the WoE approach due to a restriction of the detailed study analysis to a narrow publication time window and considers that evidence from older studies was not sufficiently considered by EFSA at several points. Even if an initial literature search was carried out with a defined publication time-frame, BfR would have considered additional studies beyond (i.e. both before and after) that time-frame, at least for the identified critical endpoints. In the opinion of BfR, a hazard assessment based solely on studies from a specific publication period could be biased by the time period the studies were performed. BfR considers this as a methodological shortcoming e.g. for toxicokinetics, immunotoxicity and reproduction toxicity clusters.

In addition, BfR identified some studies that were classified as Tier 3 for formal reasons, such as missing information on BPA purity, but were of good quality overall. In contrast, studies that claim to assess very low-dose BPA effects but use inappropriate housing materials and/or feed were classified as Tier 1, even though background contamination is highly likely. In contrast to EFSA, BfR considers such unintended contamination relevant as it disqualifies the respective studies for a quantitative assessment.

EFSA explained in detail in the responses to comments 91, 27, 52-b and 53-a in Annex N of the opinion that the time-frame was the one set in the hazard assessment protocol, developed and published *a priori* to the BPA re-evaluation.

EFSA highlighted that performing a safety assessment using a systematic approach implies necessarily a predefined cut-off date for the literature to be considered and consequently the production of a data-gap in the evaluation. EFSA also pointed out that, despite this, additional literature published after 2018 was taken into account and referred to, where appropriate, in the response to the comments received during the PC (see Annex N of the opinion). Furthermore, regarding Immunotoxicity the single studies from 2015 opinion and 2016 Immunotoxicity statement were evaluated and considered of no impact on the overall conclusion. Additionally, specific literature published after 2018 was referred to in the opinion for improving the description of the clusters and endpoints grouping and mechanistic issues.

As regards the methodology used to assess the internal and external validity of the studies collected in the timeframe 01 January 2013 to 15 October 2018, a testing phase was carried out comparing the new methodology with the one used in 2015, and the comparability was considered sufficient and robust to not re-evaluate the literature already assessed. Determination of the TDI was conducted according to the methodologies described in EFSA guidance documents (EFSA Scientific Committee, 2017a,b; 2018a,b).

EFSA also pointed out that the exclusion of studies from WoE based on the lack of information on purity was done according to the hazard assessment protocol used for the BPA assessment (see Annex A to the opinion). This protocol underwent a PC before being published. Impurities are considered a key criterion in the evaluation because they may significantly affect the toxicity.

c) Apical endpoints vs intermediate endpoints associated with BPA exposure: reference point (splenic Th17 cell percentage increase) acceptability, adversity, and relevance.

According to **BfR** insufficient data exist to establish quantitative evidence or a causal link between the intermediate endpoint (increased Th17 cell percentage) and an adverse outcome, as well as for a transfer of the observed effects to other species including humans. The role of Th17 cells is context dependent and not yet fully understood in mice and humans. So far, a genetic link between increased IL-17A levels and disease in humans is missing (Li et al., 2018). Except for human plaque psoriasis and a few related diseases such as psoriatic arthritis, many trials targeting the IL-17A pathway in humans have fallen far short of expectations (Zwicky et al., 2020). In numerous animal studies on BPA, the typical histological adverse effect expected to result from increased Th17 cell percentage and activity – inflammation – was never detected even in doses up to 5 orders of magnitude higher than the BMDL40 from Luo et al (2016). This holds true both for rats and mice (see e.g. Tyl et al., 2008, Delclos et al., 2014, or the studies conducted in the CLARITY project). EFSA (2023) also considered BPA effects on inflammation as 'Not likely[2]', in the exposure regimes "developmental", "developmental & adult" and "adult". There was only one study (Ogo et al., 2018) in which EFSA considered relevant and 'Likely[3]' the effects of BPA on neutrophils in epididymis during the exposure period growth phase/young age.
Therefore, there is strong evidence that the administered doses in Luo et al. (2016) do not lead to adverse immune outcomes in healthy animals. Regarding animal models of disease, according to BfR, the study quality is still very low and results are inconsistent. The lack of reliable epidemiological studies (BfR agrees that e.g. repeated 24-hour urine samples would be required) prevents a definite judgement of human effects.

EFSA highlighted that even in the absence of a quantitative Adverse Outcome Pathway (AOP), evidence for a link between Th17 cells and adverse outcomes exists (Lynde et al. 2014; Martin et al., 2013) as reported in Section 3.1.3 of the opinion. Additionally, the probability of this association was quantified during the expert's knowledge elicitation

[2] Not Likely: There is very low confidence in the body of evidence for an association between exposure to the substance and health effect/s (e.g. there is evidence showing consistent no effects).

(EKE) performed in the context of the UA exercise (see Section 3.2.3 and Appendix D in the opinion).

EFSA further clarified that the selection of the pivotal study Luo et al. (2016) was based on a risk of bias scrutiny process of scientific papers. The conclusions from the present opinion were not based on one study, i.e. the Luo et al. (2016), but are drawn on the WoE of the entire data set, considering all studies available within the time frame (please refer also to the response to comment 236 in Annex N of the opinion). EFSA highlighted that besides the immunotoxicity studies, also studies in other health outcome categories HOCs), i.e. in reproductive toxicity and metabolism, reported effects at doses that are within 7 fold compared to the effects observed on Th17 cells (i.e., on primordial ovarian follicles, sperm motility and uric acid). The effect on Th17 was the most sensitive observed, even if the differences in doses with the other effects were relatively small. The effects identified in Luo et al. (2016) were also confirmed in more recently published studies, showing effects at the same dose range (Section 3.1.3 and response to comment 30 in Annex N). Also, the relevance in humans, the evidence related to allergy, the species difference, as well as human variability concerning immune response, have been addressed in the opinion.

Finally, EFSA does not dispute the not likely effects identified in the WoE related to the inflammation in some exposure categories. However, EFSA made clear that the study in which effects of BPA on IL-6 and neutrophils in epididymis were considered relevant and likely during the exposure period growth phase/young age, was a high-quality Tier 1 study.

BfR expressed concerns on the use of the selected intermediate endpoint for setting a TDI/HBGV based on the available data. In the BPA assessment, BfR does not question the use of an intermediate endpoint as such, but of an intermediate endpoint not accompanied by the observation of corresponding apical effects in the relevant *in vivo* data.

EFSA reiterated that no guidance on how to deal with intermediate endpoints has been developed yet; however, their use to establish a HBGV is not new to EFSA RAs (e.g., copper, cadmium and polyfluoroalkyl substances (EFSA Scientific Committee, 2023; EFSA CONTAM Panel, 2011; EFSA CONTAM Panel, 2020), when a clear causal relationship with an adverse outcome is identified, as already specified above. This is also the case for Th17 cells percentage (see point a).

BfR considered questionable the dosing in the study on which the HBGV was based (Luo et al. 2016) due to the likely background contamination originating from polycarbonate cages and non-controlled standard chow. There are several publications reporting detectable BPA levels in serum of animals from control and/or vehicle groups, even when care was taken to minimise contamination via e.g. housing materials (Bauer et al., 2012; Churchwell et al., 2014; Petzold et al., 2014; Heindel et al., 2015; Camacho et al., 2019). In BfR´s view, the use of standard chow potentially exerting estrogenic activity hampers the interpretation of the BPA effects. Consequently, BfR would not consider the classification of this study as Tier 1 and would not include it in the WoE.

EFSA pointed out that the background contamination due to polycarbonate cages reported in Luo et al. (2016) was taken into account during the appraisal of the study's internal validity (see Annex E of the opinion), and it was considered not to be a bias. This appraisal was also done according to the criteria set in the hazard assessment protocol. EFSA also highlighted that, background contamination (by e.g. BPA, phytoestrogens, others) is well known, and it may occur even under the most carefully controlled conditions, such as in the NTP CLARITY study. In response to the public consultation the

potential exposure of animals held in polycarbonate cages was estimated based on the study Howdeshell et al. (2003), and considered to be negligible; in addition, considering that controls and test animals were treated in the same way, it was considered as not relevant (see response to comment 45-a3 in Annex N). Additionally in the Luo et al. (2016) study, treatment effects showed a clear dose response. Even if it is not known whether background exposure to phytoestrogens affected the Th17 cells, again, controls were treated similarly to the test animals. The uncertainty of the dose at which the effect occurs was taken into consideration in the UA section.

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

EFSA reiterated that the methodology was designed for cross-checked conclusions, not to be followed blindly. As a matter of fact, the hazard assessment protocol underwent several revisions during the evaluation process to allow a flexible, fit for purpose but scientifically sound methodology. Each study was discussed in detail and judged by two experts, and in a second step, by the whole working group, until a common consensus was reached and subsequently discussed and adopted by the CEF Panel. Furthermore, the BPA assessment protocol was submitted for PC and published in 2017 (EFSA, 2017).

d) Reproductive toxicity endpoints

BfR expressed reservations regarding the study of Hu et al. (2018), from which the endpoint 'ratio of primordial and total ovarian follicles' was derived. In addition to the issues noted by EFSA (e.g. diet, cages, bedding not described), BfR considered further shortcomings as too serious for the study to be allocated to Tier 2, as already detailed in the PC. These shortcomings include the absence of reporting of follicle absolute numbers and the lack of blinding during conduct of the ovarian follicle counts. The latter issue was of particular importance to BfR since classification of follicle stages is somewhat subjective and there is a clear risk of bias doing this type of analysis without blinding. BfR is of the opinion that the reference to another paper (Hernandez-Ochoa et al., 2010) in the material and methods section of Hu et al. (2018) relates to the scoring of follicle stages according to morphological criteria. In the view of BfR, the fact that Hernandez-Ochoa et al. (2010) analysed the impact of AhR knockout on follicular development in mice 'without knowledge of genotype' does not mean that Hu et al. (2018) performed their analysis blinded as well. Based on the shortcomings mentioned above and combined with the low effect size, BfR considered the study by Hu et al. (2018) as not reliable. Due to this, BfR would have allocated this study to Tier 3, instead of Tier 2, and therefore would have excluded it from the WoE and not taken forward for BMD (Benchmark Dose) analysis.

The shortcomings identified in Hu et al. (2018) were all assessed by **EFSA** in the internal validity appraisal (Annex E) and, following the assessment, the experts did not consider it justifiable to downgrade the study tier. As noted in Annex E and reported in the response to comment 95-a4 of Annex N, EFSA, in contrast to BfR, concluded that, based on the follicle counting method cited by Hu et al. (2018) (i.e. Hernandez-Ochoa et al. 2010), Hu et al. (2018) did perform their analysis blinded to treatment. EFSA also remarked that the relevant endpoint was the ovarian follicle counts ratio rather than the absolute follicle number. EFSA and BfR agreed that there were ovary effects based on the WoE.

Regarding the endpoint sperm motility, **BfR** acknowledged the effects but assessed more recent studies and performed a BMD analysis with these studies. In doing so, studies in the same species and with similar exposure regimens were grouped and calculated together using the respective study as a covariate. As a result, BfR derived much higher HBGV values compared to the one identified by EFSA based on Wang et al. (2016). The latter study was rated Tier 3 by BfR due to unknown background contamination. In contrast to EFSA (2023), BfR considered the parameter "epididymal sperm count" as a likely endpoint.

EFSA stated that the different result on the endpoint 'sperm motility' was due to the use of different methodologies for assessing the studies. EFSA could not apply a covariate analysis when applying the BMD approach due to the fact that the studies available during the time span considered had a high variability in the design and were therefore not suitable to be used with such approach.

e) Uncertainty analysis as applied in the BPA assessment

BfR made a point that a quantitative or semi-quantitative uncertainty assessment should rely on the observed data, and not on expert judgement. Moreover, for a data-rich situation such as the one for BPA, a suitable methodology for uncertainty characterisation has been provided by WHO/IPCS (2018). BfR also considers that the main contribution to the low TDI stems from the choice of the RP and therefore this point is more of a general methodological nature. However, in the end, the divergence between BfR and EFSA concerns many aspects of the hazard characterisation, adding up to a TDI newly derived by EFSA (2023), which in the opinion of BfR is several orders of magnitude lower compared to what BfR would expect. According to BfR, the updated UA performed by EFSA - maybe due to the methodology applied (EKE analysis) - does not address and properly account for this shortcoming in the hazard characterisation of BPA.

EFSA pointed out that the main impact on the low TDI was due to the RP, which was based on new evidence considered with respect to the previous assessment (EFSA CEF Panel, 2015). The UA confirmed that a RP in this range was reasonable when taking account of all the evidence and uncertainties, and that an additional uncertainty factor of 2 was justified to achieve a reasonable (about 50%) probability of protecting against the most sensitive endpoint that is relevant and adverse for humans. EFSA pointed out that it had responded in detail to BfR's concerns about the subjectivity of expert judgement in its responses to the comments received in the PC (Annex N of the Opinion). In short, all scientific assessment necessarily involves expert judgement, and this would also be true of any other method of choosing a critical endpoint and determining a RP (including choice of the BMR and modelling options for BMD analysis). The UA conducted for this Opinion was based on careful and structured consideration of all the available evidence and associated uncertainties, elicited quantitative judgements by a formal elicitation procedure and combine those judgements by appropriate probability calculations to quantify the overall uncertainty about the RP. In all these respects it was superior to a conventional narrative assessment, both in terms of rigour and transparency, for the reasons explained in detail in Annex N of the Opinion.

EFSA also pointed out that in its responses to the PC (to comment 91-c and 220 in Annex N) it had responded in detail to BfR's suggestion to conduct UA with APROBA (plus), which is the software tool described in WHO/IPCS (2018) and explained why it was not applied.

f) Choice of the HED factor

BfR considers the study of Doerge et al. (2011) inadequate for deriving and selecting the human equivalent dose factor (HEDF) for mice due to several shortcomings and provides the following explanation.

It has been known for a very long time that in contrast to humans, BPA undergoes extensive enterohepatic recycling (EHR) in rodents due to differences in the molecular mass threshold for biliary elimination in rats and humans. Due to the EHR, the blood concentrations and elimination half-lives in rodents are increased. For more details, please compare e.g. EFSA (2007) and EFSA (2008). A more recent study comparing different species confirmed results from former studies (Collet et al., 2015). In Doerge et al. (2011), levels of free BPA above the detection limit were only observed within the first three measurement points and only in one or two of the twelve mice investigated per time point. Hence, the EHR was not covered by the data of this study. Accordingly, the area under the curve (AUC) of free BPA and thus, the HEDF, was very low compared to other studies (Sieli et al., 2011; Talyor et al., 2011). In addition, the ratio of overall BPA to free BPA in serum is very different from the other studies. Also in all other studies, including intravenous application (Doerge et al. 2012; Sieli et al., 2011; Talyor et al., 2011; Collet et al., 2015), the concentration/time profile of free BPA in serum mirrored the concentration/time course of total BPA. This is not the case in Doerge et al. (2011), because the study did not cover EHR. BfR concluded that Doerge et al. (2011) is not suitable for derivation of a realistic HEDF.

In BfR´s view, the studies from Sieli et al. (2011), Taylor et al. (2011) should have been considered instead. EFSA has argued that the named studies would not be suitable for HEDF derivation, because the doses applied (up to 13,000 – 100,000 µg/kg bw) might be above a linear dose range. Moreover, EFSA argued that due to possible limitation of intestinal enzymes (Hanioka et al., 2022), the AUC of unconjugated BPA in serum might be higher at higher doses, even if linearly dose adjusted. However, BfR stated that Taylor et al. (2011) and others (compare EFSA (2008); EFSA (2010)) have clearly shown a high linearity of the concentrations of unconjugated BPA in serum measured 24 h after oral administration over a wide dose range (2 – 100,000 µg/kg bw). Also, the linearly dose-adjusted concentration/time profiles after oral administration of 400 and 100,000 µg/kg bw, respectively, match perfectly – apart from the last time point, where analytical problems may have occurred (Taylor et al., 2011). This result seems plausible with respect to the low solubility of BPA in water. BPA administered in fat (e.g. corn oil) or rodent chow as in many studies considered in the EFSA opinion, will only slowly change into the aqueous environment of the stomach and intestine. Hence, saturation of the enzymes in the intestinal cells as seen *in vitro* (Hanioka et al., 2022) is unlikely *in vivo* even in comparably high doses.
Hence, BfR does not support EFSA´s argumentation not to use the studies suggested by the BfR. In the view of the BfR, the HEDF for mice should be corrected. A realistic HEDF is between 10 and 100 times higher, consequently leading to a TDI being 10 to 100 times higher.

BfR also does not agree that this fact is sufficiently taken into consideration in the uncertainty assessment. This is partly due to the process of uncertainty assessment used by EFSA (EKE). But it is also BfR's belief that in the first step, data should be used for the

hazard assessment. In doing so, BfR considers evident from the data that the HEDF is at least 10 times higher.

EFSA highlighted that the rationale for the choice of Doerge et al. (2011) and the non-suitability of the studies indicated by BfR for the selection of the HEDF are clearly explained in a specific section of the opinion (Section 3.1.1.4.1 Further clarifications on the selection of the HED factor) and addressed in the response to comment 45-a2 in Annex N.

EFSA further clarified that the possibility that the HEDF could be 10 times higher, was taken into account in a quantitative way in the revised UA (see Appendix D).

As reported in Annex N, already in the PC to the 2014 EFSA draft opinion, the derivation of the HEDF from the study of Doerge et al. (2011) was critically commented. EFSA had therefore revised the calculation of the AUC, which resulting in a higher value that was then used for the published opinion in 2015.

The analysis of the toxicokinetic data of Taylor et al. (2011) showed that for the study with 400 µg/kg bw the AUC (0-infinity) was 2.3-fold higher than the AUC (0 to 24h) indicating an analytical problem with the last data point which leaded to unreliable AUC-values and half-life estimates. For the study with 100,000 µg/kg bw, the use of the corn oil vehicle, in addition to the very high dose, made it not possible to separate both the kinetics of absorption and the kinetics of distribution from the elimination process (EFSA CEF Panel, 2015).

Already in 2015, it was observed that the AUCs in the studies of Taylor et al. (2011), and Sieli et al. (2011) were not increasing proportional to the doses used. This observation pointed at a non-linear relationship.

The results of a recently published study on *in vitro* metabolism of BPA by microsomes from mouse, rat and human may give an explanation on the mechanism behind this observation. The study reports on the *in vitro* enzyme kinetics in microsomes for the glucuronidation of BPA. The results clearly show that the concentration of BPA in the gastrointestinal tract after a dose of 100,000 µg/kg bw is several orders of magnitude higher than the Km for glucuronidation in intestinal microsomes in mice (Hanioka et al., 2020). Hence, at this dose a smaller fraction of BPA is undergoing pre-systemic metabolism in the gut wall than at lower doses. The authors reported also on Km-values for liver microsomes in mice for which the Km value is also much lower than the concentration of BPA reaching the liver, indicating that a smaller fraction of BPA is undergoing pre-systemic metabolism in the liver than at lower doses. Hence, the plasma concentrations of BPA at the high dose are not linearly related to the dose, implying that the resulting AUC is increasing more that the increase in dose, with the result that the AUC cannot be linearly adjusted to a dose of 100 µg/kg bw. For these reasons, and because the experimental studies in humans were performed with much lower doses (30 µg/kg and 100 µg/kg), the AUCs from the study of Taylor et al. (2011) cannot be used for the calculation of the HEDF.

The analysis of the toxicokinetic data of Sieli et al. (2011) indicated an extremely irregular concentration-time profile in which the kinetics of absorption and the distribution cannot be separated from the elimination process. This was explained by the vehicle corn oil and the administration by feed. Therefore, the AUCs were considered not appropriate for derivation of HEDF. The AUC after the dose of 20,000 µg/kg in the Sieli et al. (2011) study amounted to 900 nM x h, which when scaled linearly down to a dose of 100 µg/kg would be 4.5 nM x h.

When comparing the AUC derived from the study of Doerge et al. (2011) and the AUC derived from the study of Sieli et al. (2011), a high discrepancy could be observed which could not be further explained. The CEF Panel therefore decided in 2015 to use the AUC derived from the Doerge study (2011) for the calculation of the HEDF.

Also for the doses used in the Sieli et al. study (2011) the results of the study of Hanioka et al. (2020) are of interest. The Km value of the gastrointestinal microsomes is far below the concentration of BPA in the gastrointestinal tract after the doses of 20,000 µg/kg bw and of 13,000 µg/kg bw, respectively. Hence, the plasma concentrations of BPA at the high dose are not any more linearly related to the dose, implying that the resulting AUC is increasing more that the increase in dose, with the result that the AUC cannot be linearly adjusted to a dose of 100 µg/kg bw. For these reasons and because the experimental studies in humans were performed with much lower doses (30 µg/kg and 100 µg/kg), the AUCs from the study of Sieli et al. (2011) cannot be used for the calculation of the HEDF.

The study of Collet et al. (2015) is a study with intravenous administration of BPA. Because of the intravenous administration, there is no pre-systemic elimination in the enterocytes of the gastrointestinal tract and in the hepatocytes, both of which are major determinants of the systemic availability of BPA. Hence, although the study results, in particular AUC and clearance, are interesting, they cannot be used for calculating an HEDF for oral administration of the dose.

Given that no new experimental toxicokinetic data in mice following oral exposure have been published since 2013 which could shed light on the observed discrepancies, the CEP Panel decided to stick to the decision made by the CEF Panel in 2015. The CEP Panel based the calculation of the HEDF for the extrapolation from mice to humans on the AUC derived from the data of Doerge et al. (2011) and the median of the experimentally obtained AUCs in human volunteers (Teeguarden et al., 2015; Thayer et al., 2015), adjusted to a dose of 100 µg/kg bw.

3. CONCLUSIONS

Both BfR and EFSA acknowledged that the interpretation of the available evidence and the assessment of the risks are intrinsically linked to the tools and methodologies used, resulting in the divergences of opinion between the two institutions on several points, as described above. On that basis, it is not possible to achieve convergence for the differences of opinion between the two bodies regarding the hazard characterisation for BPA.

Both BfR and EFSA also acknowledged the importance of further constructive dialogue between EU Agencies, Member State national authorities as well as risk communication and management experts for future alignment of the methodologies applied, as foreseen by the One Substance One Assessment approach under the EC Chemical Strategy for Sustainability.

4. REFERENCES

Bauer SM, Roy A, Emo J, Chapman TJ, Georas SN, and Lawrence BP, 2012. The effects of maternal exposure to bisphenol A on allergic lung inflammation into adulthood. Toxicol Sci 130 (1), 82-93. DOI: 10.1093/toxsci/kfs227

Camacho L, Lewis SM, Vanlandingham MM, Olson GR, Davis KJ, Patton R, Twaddle NC, Doerge DR, Churchwell MI, Bryant MS, Mclellen FM, Woodling K, Felton RP, Maisha MP, Juliar BE, Gamboa da Costa G and Delclos KB, 2019. NTP CLARITY-BPA report (2018). A two-year toxicology study of bisphenol A (BPA) in Sprague-Dawley rats: CLARITY-BPA core study results. Food and Chemical Toxicology, 132. 110728

Churchwell MI, Camacho L, Vanlandingham MM, Twaddle NC, Sepehr E, Delclos KB, Fisher JW, and Doerge DR, 2014. Comparison of life-stage-dependent internal dosimetry for bisphenol A, ethinyl estradiol, a reference estrogen, and endogenous estradiol to test an estrogenic mode of action in Sprague Dawley rats. Toxicol Sci 139 (1), 4-20. DOI: 10.1093/toxsci/kfu021

Collet SH, Picard-Hagen N, Lacroix MZ, Puel S, Viguié C, Bousquet-Melou A, Toutain PL and Gayrard V, 2015. Allometric scaling for predicting human clearance of bisphenol A. Toxicology and Applied Pharmacology, 284(3), 323–329. doi:10.1016/j.taap.2015.02.024

Delclos K.B., Camacho L., Lewis S.M., Vanlandingham M.M., Latendresse J.R., Olson G.R., Davis K.J., Patton R.E., da Costa G.G., Woodling K.A., Bryant M.S., Chidambaram M., Trbojevich R., Juliar B.E., Felton R.P., and Thorn B.T. (2014): Toxicity Evaluation of Bisphenol A Administered by Gavage to Sprague Dawley Rats From Gestation Day 6 Through Postnatal Day 90. Toxicological Sciences 139 (1), 174-197. DOI: 10.1093/toxsci/kfu022

Dietert RR. Developmental Immunotoxicity, Perinatal Programming, and Noncommunicable Diseases: Focus on Human Studies. Adv Med. 2014; 2014:867805. doi: 10.1155/2014/867805

Doerge DR, Twaddle NC, Vanlandingham M and Fisher JW, 2011. Pharmacokinetics of bisphenol A in neonatal and adult CD-1 mice: Inter-species comparisons with Sprague-Dawley rats and rhesus monkeys. Toxicology Letters, 207(3), 298–305. doi:10.1016/j.toxlet.2011.09.020.

Doerge DR, Twaddle NC, Vanlandingham M, and Fisher JW (2012): Pharmacokinetics of bisphenol A in serum and adipose tissue following intravenous administration to adult female CD-1 mice. Toxicology Letters 211 (2), 114-119. DOI: 10.1016/j.toxlet.2012.03.008

EFSA (2007): Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) related to 2,2-BIS(4-HYDROXYPHENYL)PROPANE. EFSA Journal 5 (1), 428. DOI: 10.2903/j.efsa.2007.428

EFSA (2008): Toxicokinetics of Bisphenol A - Scientific Opinion of the Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food (AFC). EFSA Journal 6 (7), 759. DOI: 10.2903/j.efsa.2008.759

EFSA (2017): Gundert-Remy U, Bodin J, Bosetti C, FitzGerald RE, Hanberg A, Hass U, Hooijmans C, Rooney AA, Rousselle C, van Loveren H, Wölfle D, Barizzone F, Croera C, Putzu C and Castoldi AF, 2017. Bisphenol A (BPA) hazard assessment protocol. EFSA supporting publication 2017:EN-1354. 76, pp.doi:10.2903/sp.efsa.2017.EN-1354

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2023. Scientific opinion on the re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal 2023;21(4):6857, doi: 10.2903/j.efsa.2023.6857

EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2015. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal 2015;13(1):3978, 1040 pp. doi:10.2903/j.efsa.2015.3978.

EFSA Panel on Contaminants in the Food Chain. Scientific Opinion on the risk to human health related to the presence of perfluoroalkyl substances in food. EFSA Journal 2020;18(9):6223, 391pp.https://doi.org/10.2903/j.efsa.2020.6223

EFSA Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on tolerable weekly intake for cadmium. EFSA Journal 2011;9(2):1975. [19pp.] doi:10.2903/j.efsa.2011.1975

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen KH, More S, Mortensen A, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Aerts M, Bodin L, Davis A, Edler L, Gundert-Remy U, Sand S, Slob W, Bottex B, Cortiñas Abrahantes J, Court Marques D, Kass G and Schlatter J, 2017a. Update: use of the benchmark dose approach in risk assessment. EFSA Journal 2017;15(1):4658, 41 pp. doi:10.2903/j.efsa.2017.4658

EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter J, Silano V, Solecki R, Turck D, Benfenati E, Chaudhry Q, Craig P, Frampton G, Greiner M, Hart A, Hogstrand C, Lambre C, Luttik R, Makowski D, Siani A, Wahlstroem H, Aguilera J, Dorne J, Fernandez Dumont A, Hempen M, Valtuena Martínez S, Martino L, Smeraldi C, Terron A, Georgiadis N and Younes M, 2017b. Guidance on the use of the weight of evidence approach in scientific assessments. EFSA Journal 2017;15(8):4971, 69 pp. doi:10.2903/j.efsa.2017.4971

EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter J, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Martino L, Merten C, Mosbach-Schulz O and Hardy A, 2018a. Guidance on uncertainty analysis in scientific assessments. EFSA Journal 2018;16(1):e05123, 39 pp. doi:10.2903/j.efsa.2018.5123

EFSA Scientific Committee, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Younes M, Craig P, Hart A, Von Goetz N, Koutsoumanis K, Mortensen A, Ossendorp B, Germini A, Martino L, Merten C, Mosbach-Schulz O, Smith A and Hardy A, 2018b. The principles and methods behind EFSA's Guidance on Uncertainty Analysis in Scientific Assessment. EFSA Journal 2018;16(1):e05122, 235 pp. doi:10.2903/j.efsa.2018.5122

EFSA Scientific Committee, More SJ, Bampidis V, Benford D, Bragard C,Halldorsson TI, Hernandez-Jerez AF, Bennekou SH, Koutsoumanis K, Lambre C, Machera K, Mullins E,Nielsen SS, Schlatter JR, Schrenk D, Turck D, Younes M, Boon P, Ferns GAA, Lindtner O, Smolders E,Wilks M, Bastaki M, de Sesmaisons-Lecarre A, Ferreira L, Greco L, Kass GEN, Riolo F and Leblanc J-C, 2023. Scientific Opinion on the re-evaluation of the existing health-based guidance values for copperand exposure assessment from all sources. EFSA Journal 2023;21(1):7728, 117 pp.https://doi.org/10.2903/j.efsa.2023.7728ISSN:1831-4732

Hanioka N, Isobe T, Tanaka-Kagawa T, Jinno H, and Ohkawara S, 2022. *In vitro* glucuronidation of bisphenol A in liver and intestinal microsomes: interspecies differences in humans and laboratory animals. Drug Chem Toxicol 45 (4), 1565-1569. DOI: 10.1080/01480545.2020.1847133

Heindel JJ, Newbold RR, Bucher JR, Camacho L, Delclos KB, Lewis SM, Vanlandingham M, Churchwell MI, Twaddle NC, McLellen M, Chidambaram M, Bryant M, Woodling K, Gamboa da Costa G, Ferguson SA, Flaws J, Howard PC, Walker NJ, Zoeller RT, Fostel J, Favaro C, and Schug TT, 2015. NIEHS/FDA CLARITY-BPA research program update. Reprod Toxicol 58, 33-44. DOI: 10.1016/j.reprotox.2015.07.075

Hernandez-Ochoa I, Barnett-Ringgold KR, Dehlinger SL, Gupta RK, Leslie TC, Roby KF and Flaws JA, 2010. The ability of the aryl hydrocarbon receptor to regulate ovarian follicle growth and estradiol biosynthesis in mice depends on stage of sexual maturity. Biol Reprod. 2010;83(5):698-706.

Hessel EV, Tonk EC, Bos PM, van Loveren H, Piersma AH. Developmental immunotoxicity of chemicals in rodents and its possible regulatory impact. Crit Rev Toxicol. 2015 Jan;45(1):68-82. doi: 10.3109/10408444.2014.959163.

Howdeshell KL, Peterman PH, Judy BM, Taylor JA, Orazio CE, Ruhlen RL, vom Saal FS and Welshons WV, 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environmental Health Perspectives, 111(9),1180-1187. doi: 10.1289/ehp.5993

Hu Y, Yuan DZ, Wu Y, Yu LL, Xu LZ, Yue LM, Liu L, Xu WM, Qiao XY, Zeng RJ, Yang ZL, Yin WY, Ma YX and Nie Y, 2018. Bisphenol A initiates excessive premature activation of primordial follicles in mouse ovaries via the PTEN signaling pathway. Reproductive Sciences, 25(4), 609–620.

Li J, Casanova JL, and Puel A, 2018. Mucocutaneous IL-17 immunity in mice and humans: host defense vs. excessive inflammation. Mucosal Immunol 11 (3), 581-589. DOI: 10.1038/mi.2017.97

Luo SM, Li Y, Li YP, Zhu QX, Jiang JH, Wu CH and Shen T, 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.

Lynde CW, Poulin Y, Vender R, Bourcier M, Khalil S., 2014. Interleukin 17A: toward a new understanding of psoriasis pathogenesis. J Am Acad Dermatol. 71:141-50.

Martin DA, Towne JE, Kricorian G, Klekotka P, Gudjonsson JE, Krueger JG, Russell CB, 2013. The emerging role of IL-17 in the pathogenesis of psoriasis: preclinical and clinical findings. J Invest Dermatol. 133:17-26.

Ogo F.M., de Lion Siervo G.E.M., Staurengo-Ferrari L., de Oliveira Mendes L., Luchetta N.R., Vieira H.R., Fattori V., Verri Jr W.A., Scarano W.R., and Fernandes G.S.A. (2018): Bisphenol A Exposure Impairs Epididymal Development during the Peripubertal Period of Rats: Inflammatory Profile and Tissue Changes. Basic & Clinical Pharmacology & Toxicology 122 (2), 262-270. DOI: 10.1111/bcpt.12894

Petzold S, Averbeck M, Simon JC, Lehmann I, and Polte T, 2014. Lifetime-dependent effects of bisphenol a on asthma development in an experimental mouse model. PLoS ONE 9 (6). DOI: 10.1371/journal.pone.0100468

Sieli PT, Jašarević E, Warzak DA, Mao J, Ellersieck MR, Liao C, Kannan K, Collet SH, Toutain PL, Saal FSVS and Rosenfeld CS, 2011. Comparison of serum bisphenol a concentrations in mice exposed to bisphenol a through the diet versus oral bolus exposure. Environmental Health Perspectives, 119(9), 1260-1265. doi: 10.1289/ehp.1003385.

Taylor JA, vom Saal FS, Welshons WV, Drury B, Rottinghaus G, Hunt PA, Toutain PL, Laffont CM and VandeVoort CA, 2011. Similarity of bisphenol A pharmacokinetics in rhesus monkeys and mice: Relevance for human exposure. Environmental Health Perspectives, 119(4), 422-430. doi: 10.1289/ehp.1002514.Teeguarden JG, Twaddle NC, Churchwell MI, Yang X, Fisher JW, Seryak LM and Doerge DR, 2015. 24-hour human urine and serum profiles of bisphenol A following ingestion in soup: Individual pharmacokinetic data and emographics. Data in Brief, 4, 83–86. doi:10.1016/j.dib.2015.03.002.

Teeguarden JG, Twaddle NC, Churchwell MI, Yang X, Fisher JW, Seryak LM and Doerge DR, 2015b. 24-hour human urine and serum profiles of bisphenol A: Evidence against sublingual absorption following ingestion in soup. Toxicology and Applied Pharmacology, 288(2), 131–142. doi:10.1016/j.taap.2015.01.009

Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, Garantziotis S, Kissling GE, Easterling MR, Bucher JR and Birnbaum LS, 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environment International, 83, 107–115. doi:10.1016/j.envint.2015.06.008

Tyl R.W., Myers C.B., Marr M.C., Sloan C.S., Castillo N.P., Veselica M.M., Seely J.C., Dimond S.S., Van Miller J.P., Shiotsuka R.N., Beyer D., Hentges S.G., and Waechter J.J.M. (2008): Two-Generation Reproductive Toxicity Study of Dietary Bisphenol A in CD-1 (Swiss) Mice. Toxicological Sciences 104 (2), 362-384. DOI: 10.1093/toxsci/kfn084

Wang HF, Liu M, Li N, Luo T, Zheng LP and Zeng XH, 2016. Bisphenol A impairs mature sperm functions by a CatSper-relevant mechanism. Toxicological Sciences, 152(1), 145–154.

WHO/IPSC (World Health Organisation & International Programme on Chemical Safety), 2009. Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food. 689 pp. https://apps.who.int/iris/bitstream/handle/10665/44065/WHO_EHC_240_eng.pdf.

WHO/IPSC (World Health Organisation & International Programme on Chemical Safety), 2018. Guidance document on evaluating and expressing uncertainty in hazard characterization. 2nd ed. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/259858

Zwicky P, Unger S, and Becher B, 2020. Targeting interleukin-17 in chronic inflammatory disease: A clinical perspective. J Exp Med 217 (1). DOI: 10.1084/jem.20191123