COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

EFSA public consultation on the update of hexabromocyclododecanes (HBCDDs) in food

Background

1. The European Food Safety Authority (EFSA) launched a public consultation on the update of hexabromocyclododecanes (HBCDDs) in food.

2. Paragraphs 6- 17 provide a brief overview of previous assessments by EFSA in 2011 and COT in 2015, focusing on the rationale for and derivation of the reference point, approaches taken, and any updated information COT had available.

3. Paragraphs 18- 36 provide an overview of the 2020 EFSA opinion, focusing on toxicity data, rationale for, and derivation of, the reference point and approach taken and any additional information and deviations from the approach in the 2011 EFSA opinion.

4. The deadline for the public consultation is 25th November 2020. Could Members who wish to comment, please send their contributions to the Secretariat by **Wednesday 18th November 2020**.

5. Members are asked to please indicate which sections of the EFSA opinion their comments are referring too.

Previous assessments

EFSA, 2011

6. The full EFSA 2011 evaluation can be found here. The paragraphs below predominantly focus on the considerations behind the derivation of the reference point (RP).

7. EFSA noted at the time that all *in vivo* studies were carried out with technical HBCDDs mixtures containing more than one stereoisomer and that the isomer profile found in food differs substantially from the material tested.

8. The main target of HBCDDs toxicity in animals were the liver, thyroid hormone homeostasis, reproductive, nervous and immune system. The two available

epidemiological studies did not show any association between levels of HBCDD in blood and bone mineral density in elderly women or between HBCDD in human milk and neonatal blood levels of thyroid stimulating hormones (TSH).

9. EFSA considered that effects on the thyroid weight in female rats from a 28-day study, and neurodevelopmental effects on behaviour in male mice from a single exposure (Eriksson et al., 2006) were the most critical endpoints, and calculated a $BMDL_{10}$ of 1.6 mg/kg bw per day and a $BMDL_{10}$ of 0.93 mg/kg bw per day, respectively. Due to reservations about the numerical estimate of the $BMDL_{10}$ from the female rat study, the Panel decided to use the $BMDL_{10}$ of 0.93 mg/kg bw per day as the reference point. A benchmark response (BMR) of 10% was chosen to avoid extrapolation beyond the observable range.

10. While EFSA noted there were uncertainties with the study by Eriksson et al. (2006), such as the single administration on PND10 and a potential bias through lack of appropriate accounting of the litter effect, there were also arguments supporting the use of the study. The study provides the lowest dose resulting in developmental effects on behaviour and covered the relevant neurodevelopmental period due to exposure at PND10. Hence, EFSA concluded the study should be considered in the assessment.

11. The available toxicokinetic data indicated a slower elimination of HBCDDs in humans compared to rodents, with half-lives of 23 to 219 days (sum of α-, β-, γ-HBCDD) for humans and half-lives of 2 to 6 days (α-HBCDD) and 17 days (γ-HBCDD) for mice. Thus, EFSA considered the body burden more appropriate for a direct comparison of internal effect doses in humans and animals. Assuming an absorbed fraction of 0.85 in mice and applying the $BMDL_{10}$ of 0.93 mg/kg bw per day, EFSA calculated a corresponding body burden for mice of 0.79 mg/kg bw.

12. Assuming a worst-case scenario by applying the longest half-life identified in humans (219 days) and an absorption of 100%, in the absence of robust information, EFSA calculated an estimated chronic human dietary intake of 0.003 mg/kg bw per day (3 µg/kg bw per day) would result in an equivalent body burden.

13. Due to the limitations and uncertainties in the data base, EFSA did not consider it appropriate to establish a HBGV but instead applied the margin of exposure approach (MOE). Usually an MOE of 100 is considered sufficient to cover uncertainties and variabilities with respect to kinetic and dynamic inter- and intraspecies differences. Since EFSA's MOE approach was based on body burden comparison, the potential kinetic differences between animals and humans have been accounted for and by focussing on the body burden associated with a BMDL for neurobehavioral effects in mice during relevant periods of brain development and applying it to the entire human life span individual differences in susceptibility have been accounted for. Therefore, the calculated MOE should be sufficient to cover interspecies differences in dynamics (factor 2.5) and individual differences in kinetics (factor 3.2) and an MOE larger than 8 (2.5 x 3.2) should indicate no health concern.

14. All estimated MOEs were > 8 and hence EFSA concluded that the estimated exposures of HBCDD were not of concern to human health. Additional calculations based on biomarkers of exposure supported the conclusion.

COT, 2015

15. COT last evaluated HBCDD in 2015/2016 as part of their work reviewing government advice for infants and young children. COT noted at the time that none of the new data available since EFSAs last evaluation provided information on the mode of action of HBCDD or an improved basis for extrapolation of experimental animal data to humans. Therefore, the data did not offer an alternative approach for deriving the reference point to that taken by EFSA.

16. While COT agreed with EFSA in their 2015 statement that "interspecies differenced in toxicokinetics were accounted for by the body burden approach and that the use of data relating to a critical point of development reduced uncertainties in the risk assessment", COT also concluded that the "MOE should be rather higher than 8 to provide reasonable assurance of safety.

17. In the 2016 Addendum, the COT concluded that the estimated exposures of infants and young children from breast milk and food did not indicate a toxicological concern, however exposures from dust did.

Draft EFSA opinion, 2020

18. The draft EFSA opinion can be found here and at Annex A. The paragraphs below predominantly summarise EFSAs consideration of the new data available since the last evaluation in 2011 and the difference in approach for the derivation of a reference point (RP).

19. EFSA noted that most studies published since 2011 were still conducted without information on the stereoisomer composition of HBCDDs. A study by Gannon et al. (2019a) used HBCDD mixture enriched with α-HBCDD (81%) and studies by Maurice et al. (2015) and Bernhard et al. (2016) were performed with α-HBCDD alone; results of these studies confirmed the primarily liver and endocrine related effects reported in earlier 28-day studies.

20. The new data available from 28-day studies reported increased liver weight and hepatocellular hypertrophy at doses ≥ 20 mg/kg bw per day in rats and mice (Maranghi et al., 2013; Rasinger et al., 2014, 2018; Bernhard et al., 2016; Gannon et al., 2019a), the effects could be due to increased adipogenesis based on induced PPAR gamma expression. Liver lesions, such as increased vacuolation in hepatocytes, increased pyknotic nuclei, lymphocytic infiltration and hyperaemic vessels were reported at doses of 49.5 µg/kg bw per day and 199 µg/kg bw per day in mice (Rasinger et al., 2018). However, EFSA noted that there was no difference in

the incidence of liver lesions between the doses, which varied by three orders of magnitude, and hence did not consider the data any further.

21. In contrast to previous studies, effects on the thyroid, such as increased weight, follicular hypertrophy, hyperplasia and/or colloid depletion, were only reported at doses ≥ 20 mg/kg bw per day. Since changes in thyroid hormone levels were observed at the same dose, EFSA did not consider the changes in the thyroid gland and circulating thyroid hormone concentrations for the derivation of a RP.

22. Changes in testosterone (199 mg/kg bw per day) and oestradiol (not dose related) concentrations in mice and a reduction of growing ovarian follicles (≥ 20 mg/kg bw per day) in rats were observed.

23. Effects on lipid and sugar metabolism was reported at low doses in male mice exposed by oral gavage (Yanagisawa et al., 2014; Xie et al., 2019). However, effects in one study were only observed in combination with a high fat diet and while effects HBCDD-induced lipid accumulation was reported in human preadipocytes, the adipogenic effect appeared less potent than in mice and did not involve stimulation of PPARG-mRNA expression. EFSA noted that both studies only administrated one dose and that the relevance of the effects to humans were unclear and hence did not consider the studies further for the derivation of a RP.

24. No new developmental and reproductive studies were identified by EFSA.

25. Any studies published since 2011 on effects on the immune system confirmed previous observations; the effects were observed at doses around 20 mg/kg bw per day in rats.

26. Most of the new studies regarding effects on the nervous system focused on cellular and molecular effects on glutamatergic and dopaminergic neurons in the hippocampus and striatum, not neurobehavioral effects. Although there is extensive discussion regarding possible modes of action for neurobehavioral effects, EFSA concluded studies since 2011 were generally consistent with effects on the constitutive androstane receptor (CAR) and pregnane-X-receptor (PXR) in the liver of rodents. *In vitro* evidence suggests that thyroid hormone mediated developmental processes in the brain could be affected by HBCDDs. A study by Miller-Rhodes et al. (2014) reported significant changes in multiple neurobehavioral tests in the offspring of pregnant rats exposed to HBCDDs. The effects were observed at all doses, but no clear dose-response relationship could be identified. Effects on spatial learning and memory were observed in a study by Zhang et al. (2017a) exposing rats to HBCDDs (0.3, 3 and 30 mg/kg bw per day) from PND10 to PND70. This study is supportive of the study by Eriksson et al. (2006) on which the previous derivation of the RP is based, however, it was not further considered by the EFSA Panel due to limitations in the study; for example the number of animals were low, the data were poorly displayed and general information on animal health, locomotor activity and anxiety were not reported. A study by Pham-Lake et al. (2017) reported no explicit

behavioural abnormalities in 2-month old male mice dosed with 25 mg/kg bw per day by gavage for six weeks. EFSA noted that these findings do not necessarily contradict the findings by Eriksson et al. and Zhang et al. but that the time windows of exposure and endpoints evaluated differ. One study by Maurice et al. (2015) exposed female rats to α-HBCDD and reported significant effects on motor activity and anxiety in the offspring at low doses (22 ng/mg bw per day) but not in higher doses (66 ng/kg bw per day).

27. EFSA concluded that the slight induction of DNA strand breaks in some *in vitro* tests were most likely due to oxidative stress and that overall, the new *in vitro* genotoxicity data does not change the previous assessment that HBCDDs are not genotoxic *in vitro* or *in vivo*. EFSA previously concluded that the available data did not indicate HBCDDs to be carcinogenic in mice and no new carcinogenicity studies have been identified by EFSA since to revise this judgment.

28. While EFSA acknowledged the increasing number of epidemiological studies and research conducted in the field of adverse events related to HBCDDs exposure, the Panel concluded that the limitations related to exposure, study design, sample size, effects direction and lack of validity did not allow for any of the data to be used as basis for a risk assessment. Several endpoints, such as neurodevelopment and thyroid dysfunction in children and subfertility, type 2 diabetes, severe endometriosis, ovarian endometrioma and breast cancer metastasis in adults, were assessed in longitudinal and cross-sectional studies. Results from the one longitudinal study assessing internal exposure were not statistically significant or were not able to be replicated at a later follow up point in the same study. No concordant findings were identified in the single available cross-sectional study with the same endpoint/research question. One large longitudinal study in a European population reported statistically significant results for an association between HBCDDs and type 2 diabetes, however no assessment of internal exposure was included. Significant associations between lower α-HBCDD concentration and the risk of having a child born with congenital hypothyroidism and free androgen index and sex hormone binding globulin were reported in the available cross-sectional studies. Overall, EFSA considered the currently available data to be characterised by relatively small sample sizes, considerable heterogeneity (population, exposure, endpoints), varying methodological quality and effect inconsistency. Furthermore, EFSA considered it difficult to confirm the postulated association due to the lack of the same endpoint in either the paediatric or adult population and potential confounding due to possible underlying associations with other contaminants was hardly addressed analytically.

29. Overall, EFSA still considered the neurodevelopmental effects on behaviour, supported by mechanistic studies, the critical effect on which to base their risk characterisation.

30. EFSA did not consider the human data sufficient as a base for their risk assessment, neither did the Panel consider any of the new studies appropriate. In line with the 2011 evaluation, EFSA therefore performed BMD modelling on the data

(horizontal locomotion, rearing and total activity in mice) by Eriksson et al. (2006), applying the new EFSA Guidance on the use of the BMD approach (EFSA SC, 2017). However, EFSA noted that the BMDL for horizontal locomotion and total activity were below the lowest dose administered and decided to apply the NOAEL/LOAEL approach instead of BMD modelling and identified a LOAEL of 0.9 mg/kg bw for spontaneous behaviour.

31. In line with the 2011 assessment, EFSA considered the body burden, rather than daily exposure as the starting point of the assessment. In mice, the body burden was calculated assuming an oral absorption of 83%. As the body burden was not calculated at steady state but PND10 and the first day of administration and corresponding to the start of the critical point in brain development, EFSA decided not to apply the elimination rate in the calculation. Taking the LOAEL of 0.9 mg/kg bw per day and adjusting by the mouse oral bioavailability of 83%, the body burden in mice was estimated to be 0.747 mg/kg bw.

32. For human chronic dietary intake EFSA assumed 100% absorption and a half-life of 219 days, in the absence of any robust information and due to uncertainties in the methods, respectively, and calculated a value of 0.00235 mg/kg bw per day (2.35 µg/kg bw per day.

33. EFSA concluded that derivation of a HBGV was not appropriate due to the limitations in the database, including lack of information on stereoisomer composition and only sporadic effects in repeat dose reproductive studies, and applied an MOE approach instead.

34. In line with the 2011 evaluation, EFSA considered the potential toxicokinetic differences sufficiently covered by the application of a body burden comparison between animals and humans and therefore concluded that the calculated MOE would be sufficient to cover interspecies toxicodynamic effects (a factor of 2.5). Furthermore, the consideration of 100% absorption and application of worst-case half-life negates the necessity of an uncertainty factor to cover individual toxicokinetic differences. EFSA did recognise potential differences in individual susceptibility in the sub-population of infants and children and therefore the MOE should also cover individual differences in dynamics (a factor of 3.2). EFSA also applied an additional uncertainty factor of 3 to account for the extrapolation from a LOAEL to a NOAEL. No additional factor was deemed necessary for limitations in the database as EFSA noted that repeat does reproductive toxicity studies only showed sporadic effects, carcinogenicity studies were only done in mice and based on the mode of action and genotoxicity findings EFSA considered carcinogenicity unlikely to be a critical effect.

35. EFSA therefore concluded that an MOE higher than 24 (2.5 x 3.2 x 3) would indicate a low health concern.

36. In line with the 2011 evaluation, EFSA concluded that the current dietary exposure to HBCDDs across European countries does not indicate a concern for human health. An exception are breastfed infants with high milk consumption, for which the lowest MOEs could indicate a concern to health.

Questions for the Committee

a) Do Members have any comments on the content of the 2020 draft EFSA opinion on HBCDDs

b) Do Members have any comments on EFSA's overall conclusion

Secretariat
October 2020

This is a background paper for discussion. It does not reflect the views of the Committee and should not be cited

Annex A to TOX/2020/55

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Link to draft EFSA opinion

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