Meeting

EFSA consultation on update of the risk assessment of polybrominated diphenyl ethers (PBDEs) in food

This is a paper for discussion.

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

Introduction

1. EFSA was asked by the European Commission to update its 2011 risk assessment of polybrominated diphenyl ethers (PBDEs) in food, focusing on ten congeners: BDE-28, -47, -49, -99, -100, -138, -153, -154, -183 and -209. EFSA's 2011 risk assessment focused on eight congeners and was only able to draw conclusions on four of them due to limitations in the available toxicology data. The congeners considered in this new opinion are the same eight as considered previously, plus BDE-49 and -138.

2. EFSA released its draft updated risk assessment for public consultation on 8 June 2023 and the deadline for comments is 20 July 2023. A link to the consultation can be found at Annex A. If Members have any additional comments following the meeting, please return them to the Secretariat by Tuesday 18th July, specifying the relevant section where possible.

3. Members are invited to provide comments on the draft opinion, to be submitted to EFSA, and also to advise on whether they agree with EFSA's approach to assessing the risks of PBDEs.

Background

4. The PBDEs previously had widespread use as flame retardants in construction materials, furniture, and electric and electronic equipment. They were applied as technical mixtures known as PentaBDE, OctaBDE and DecaBDE at

concentrations typically ranging between 5 and 30% by weight. These technical mixtures were named according to their predominant bromine substitution pattern but could contain other congeners too. They are not chemically bound to the plastics or textiles and can leach out. The production and use of PentaBDE and OctaBDE was phased out in Europe in 2004, and restrictions on DecaBDE followed in subsequent years. Most other regions of the world subsequently adopted similar restrictions, and production and use of PBDEs has been phased out world-wide. However, many products which contain PBDEs are still in use and the PBDEs can leach out of these products either during their use or when they are disposed of.

5. PBDEs are widespread in the environment as they are persistent, and they bioaccumulate in the food chain. PBDEs are listed in Annex A of the Stockholm Convention, with specific exemptions for use or production.

6. The 2011 EFSA opinion focused on the eight isomers BDE-28, -47, -99, -100, -153, -154, -183 and -209 as they considered these most relevant to dietary exposure based on the compositions of the technical PBDE mixtures and data on occurrence in the environment and in food. Subsequently a European Commission Recommendation recommended also including BDE-49 and -138 in the monitoring of brominated flame retardants in food.

7. While the 2011 EFSA opinion considered eight isomers it could only assess the risks of four of them due to a lack of toxicology data. At that time neurodevelopmental effects in mice were identified as the critical endpoint for the four congeners BDE-47, -99, -153 and -209 and BMDL10s based on either locomotion or total activity, depending on the isomer, determined from studies in mice administered single oral doses on postnatal day 10. For BDE-47, -99 and -153, as these have much longer half-lives of elimination in humans than mice, the BMDL10s were converted to the levels of chronic dietary intake in humans expected to lead to the same body burdens. These calculations were based on assuming 100% oral absorption in humans and using the upper ends of the ranges of estimated half-lives of elimination in humans. For BDE-209, as the difference between the half-life of elimination in laboratory animals and humans was smaller the BMDL10 in mice was used directly. EFSA calculated margins of exposure (MOEs) and considered MOEs greater than 2.5 to not be of concern. This was based on allowing an MOE of 2.5 for interspecies variation in toxicodynamics. The reduction from 100 was due to the interspecies toxicokinetic differences being accounted for in the use of the body burden approach, intraspecies differences being accounted for by using the upper end of the ranges of human

half-lives and the conservative assumption of 100% oral absorption by humans, and a margin for intraspecies differences in toxicodynamics not being considered required due to the endpoint being based on a sensitive life-stage. EFSA concluded that there were no concerns at estimated dietary intakes for BDE-47, -153 and -209, but there was a potential concern for BDE-99.

8. The COT previously evaluated the risks from PBDEs in the diets of infants and young children (COT, 2015, 2017). While the COT had some reservations with the studies, it considered that the BMDL10s calculated by EFSA provided a reasonable basis for risk assessment in the context of a limited database. However, the COT considered that it would be appropriate to perform the same calculation for BDE-209 as for BDE-47, -99 and -153, in order to allow for extrapolation from a single dose to chronic exposure. Therefore, the COT additionally converted the BMDL10 for BDE-209 in mice to the level of chronic dietary intake in humans expected to lead to the same body burden, and used that to calculate MOEs. The COT agreed with the EFSA 2011 opinion that interspecies differences in toxicokinetics were accounted for by the body burden approach, and that the use of a relatively high elimination half-life for humans, and of data relating to a critical period of development, reduced uncertainties in the risk assessment. However, the COT considered that MOEs should be rather higher than 2.5 to provide assurance of safety. The COT identified possible concerns for infants from exposure of some of the PBDEs from dust, breast milk or food, and possible concerns for toddlers from exposure to some of the PBDEs from dust and soil, though it was noted that the use of the PBDEs had been largely phased out and options for risk management were limited.

Draft EFSA update of the risk assessment of PBDEs in food

Toxicokinetics

9. Studies in rats and/or mice indicate oral bioavailabilities of 75–90% for BDE-47, about 50% for BDE-99, about 73% for BDE-100, about 77% for BDE-154 and 10–26% for BDE-209. Studies that addressed the distribution of BDE-47, -85, -99, -100, -153, -154 and -209 in mice or rats are described in the draft opinion. Adipose tissue tended to be the tissue with the highest concentrations; however, for BDE-47 and -209 there is evidence of selective distribution to the liver. Following repeated exposure of PBDEs to rats and/or mice accumulation was seen mainly in adipose tissue and the liver. Terminal half-lives ranged from 2.5 to 75.9

days.

10. Maternal transfer of PBDEs has been shown by studies in rats. Studies showed that BDE-47 and -209 and/or their debrominated metabolites (octa- and nona-BDE) are transferred to the offspring both in utero and via lactation, and similar results were also shown for BDE-28, -66, -85, -99, -100, -138, -153, -154 and -183.

11. The principle metabolic pathways of PBDEs are oxidation and debromination. Phase II metabolism has also been shown: glucuronide- and sulphate-conjugates of 2,4-dibromophenol (2,4-DBP) have been detected in rats exposed to BDE-47, and unconjugated 2,4,5-tribromophenol (2,4,5-TBP), as well as glucuronide, sulphate and glutathionyl conjugates of 2,4,5-TBP, have been detected in rats exposed to BDE-99.

12. Regarding human data, information on absorption is only available for BDE-209, for which a PBK model predicted an oral absorption of 0.286. PBDEs are present in cord blood and breast milk, showing maternal transfer in humans. The transfer of OH-PBDE metabolites (5-OH-BDE-47 and 6-OH-BDE-47) across the placental membrane has also been reported.

13. Studies with human liver microsomes and primary hepatocytes have shown that the main metabolic pathway in humans is CYP-mediated hydroxylation. A comparison of the results of studies using rat microsomes and human microsomes showed that the metabolism of BDE-47 and -99 is catalysed by different CYPs in rats and humans and produces different hydroxylated metabolites. In humans the primary CYP responsible for the production of hydroxylated metabolites of BDE-47, -99 and -100 is CYP2B6, whereas in rats the metabolism of BDE-47 is mediated by CYP1A1, CYP2B2 and CYP3A1 and the metabolism of BDE-99 is mediated by CYP3A1. Phase II metabolism of the OH-derivatives has been shown in humans, with glucuronide and sulphate conjugates formed.

14. Half-lives of elimination had been estimated for a number of the PBDEs using different methodologies. These were 1100 days for BDE-28, 510-664 days for BDE-47, 280-1040 days for BDE-99, 573-670 days for BDE-100, 2380-2700 days for BDE-153, 480-1214 days for BDE-154 and 94-100 days for BDE-183. In addition, the half-lives were estimated for several BDEs based on biomonitoring studies. These were 7-15 days for BDE-209, 18-39 days for nonaBDEs and 37-91 days for octaBDEs. Alternative half-lives were also estimated for BDE-209 using oral human PBK models. These were 15 days from a model without enterohepatic

circulation and 18 days from a model including enterohepatic circulation.

Toxicity in laboratory animals

15. In EFSA's previous opinion in 2011 it was concluded that the main targets of toxicity in subchronic and chronic toxicity studies in rats and mice were the liver, thyroid hormones, and the nervous, reproductive and immune systems. The new EFSA draft opinion has considered the adverse effects of PBDEs endpoint by endpoint, considering data already reviewed in the 2011 opinion together with studies published since the 2011 assessment.

Effects on the liver

16. Repeat dose exposures of BDE-47, -209, -15 and DE-71 to rodents resulted in effects including increased liver weight, centrilobular hepatocyte hypertrophy, lymphocytic infiltration in periportal areas, lipid accumulation/steatosis, changes in serum clinical chemistry and microsomal enzyme induction. Similar effects plus inflammation and necrosis were observed in offspring exposed *in utero* and/or postnatally until weaning. Increases in oxidative stress markers were noted in the liver of rat offspring exposed *in utero* and during lactation to BDE-99.

17. For BDE-47 the LOAEL in adult mice was 0.45 mg/kg bw/day, based on histopathological changes. The LOAEL in adult rats was 0.03 mg/kg bw/day, based on lipid accumulation/steatosis. The NOAEL for mice offspring exposed *in utero* and via lactation was 0.002 mg/kg bw/day, based on hepatic steatosis and injury. The NOAEL for rat offspring exposure *in utero*, via lactation and directly on postnatal days (PND) 12-21 was 0.1 mg/kg bw/day, based on increased absolute and relative liver weight and hepatocellular hypertrophy.

18. For BDE-209 the NOAEL for adult rats was 5 mg/kg bw/day, based on increased liver weight and liver toxicity. The LOAEL for rat offspring exposed in utero and by lactation was 0.7 mg/kg bw/day (maternal dose).

19. For BDE-15 the LOAEL in adult mice was 1.2 mg/kg bw/day, based on hepatocellular hypertrophy and histopathological changes.

20. Fourteen-week studies were conducted on the technical product DE-71 (which consists of 43% BDE-47, 43% BDE-99, 8% BDE-100, around 2% each of BDE-153, -154, and -85, and 1% each of BDE-28 and -183) in adult rats and mice. The NOAEL was 0.007 mg/kg bw/day in adult rats and 3.6 mg/kg bw/day in adult

mice, based on increased relative and absolute liver weight and hepatocellular hypertrophy. In adult mice exposed for 2 years, the LOAEL was 2.1 mg/kg bw/day, based on histopathological findings such as fatty changes and focal necrosis. In a study of offspring rats exposed *in utero*, during lactation and for 2 years postweaning, the LOAEL was 2.1 mg/kg bw per day based on hepatocellular hypertrophy.

21. EFSA also noted that the technical product PentaBDE caused fatty degeneration in the liver and had a porphyrogenic effect, and the technical product OctaBDE had effects on haem biosynthesis and the levels of porphyrins.

Effects on thyroid hormones

22. Repeat dose exposures of adult rats to BDE-47, BDE-209 and the technical product DE-71 resulted in increased thyroid weight, changes in thyroid hormone homeostasis and changes in follicle structure. In addition, reductions in T3 and/or T4 levels, with increased serum TSH in some cases, were also observed in rat offspring exposed via the dams during gestation and lactation, and for BDE-209 increases in thyroid weight and changes in follicle structure were also observed.

23. The lowest LOAEL for BDE-47 in adult rats was 0.026 mg/kg bw/day, based on decreased T3. The lowest LOAEL in offspring was 0.1 mg/kg bw/day, based on decreased T3 and T4 levels.

24. For BDE-99, reduced T4 levels were observed in pregnant rats at 0.06 mg/kg bw/day and in their offspring on PND22 at 0.3 mg/kg bw/day.

25. For BDE-209, the lowest LOAEL in rats was 0.7 mg/kg bw/day, based on increased thyroid weight in adults and follicular cell hypertrophy in their offspring.

26. For the technical product DE-71, the lowest LOAELs in rats were 3.6 mg/kg bw/day in adults, based on decreased T4 levels, and 0.1 mg/kg bw/day in offspring exposed *in utero*, during lactation and directly for 13 weeks postweaning, based on decreased T3 and T4 levels.

Effects on lipid and sugar metabolism

27. Studies available at the time of the 2011 EFSA evaluation indicated that exposure to BDE-47 or -99 via injection affected lipid and glucose metabolism. A small number of studies on PBDEs in rats and mice using oral dosing have subsequently been published using BDE-47 and -209 and the technical product

DE-71.

28. Exposure to BDE-47 resulted in decreased body weight with a LOAEL of 0.2 mg/kg bw/day in mice, and changes in liver and serum lipids, serum glucose and serum insulin levels. However, the results were contradictory between different studies for almost all of these parameters. Decrease serum insulin triglycerides were observed in mouse pups exposed in utero, with a LOAEL of 1 mg/kg bw/day (maternal dose).

29. For BDE-209, increased fasting glucose was observed in mice fed a high fat diet. The text in the EFSA draft is contradictory in two places and it is unclear if EFSA considered the LOAEL to be 0.005 or 0.01 mg/kg bw/day (the two tested dose levels in the study).

30. For DE-71, increased glucose tolerance and reduced insulin sensitivity were observed in mice exposed *in utero*, with a LOAEL of 0.1 mg/kg bw/day (maternal dose).

Other effects observed in repeat dose studies

31. Some studies have become available reporting other adverse effects for BDE-209 and the technical product DE-71 in rats or mice.

32. For BDE-209 these include effects on the kidney (LOAEL = 22.8 mg/kg bw/day as the maternal dose in rat offspring exposed *utero* and via lactation), heart (LOAEL = 5 mg/kg bw/day in rats), adrenal gland (LOAEL = 600 mg/kg bw/day in rats) and haematological effects (LOAEL = 1000 mg/kg bw/day in rats).

33. For the technical product DE-71, one study reported that exposure of rats *in utero* and via lactation resulted in effects on cardiovascular reactivity and osmoregulatory responses. In F1 rats exposed for 2 years hydronephrosis in the kidney was observed in males at 10.7 mg/kg bw/day and females at 36 mg/kg bw/day, and effects on the thymus, forestomach, parotid salivary gland, adrenal cortex and preputial gland were also observed in males at 36 mg/kg bw per day. In addition, a significant increase in the incidence of chronic active inflammation of the prostate was observed in males at 10.7 and 36 mg/kg bw/day. In mice exposed for two years forestomach hyperplasia was observed at 21 mg/kg bw/day in males and 71 mg/kg bw/day in females, and adrenal cortex hypertrophy was observed in both sexes at 71 mg/kg bw/day.

Reproductive and developmental toxicity

34. A range of effects of PBDEs on reproduction and development have been reported.

35. Exposures of male rats and mice to BDE-47, -99 and -209 have resulted in decreased serum testosterone and oestradiol, though in two studies with BDE-47 increases in serum testosterone were observed. In female mice exposed to BDE-47 increased serum testosterone level and increased serum testosterone:oestradiol ratio were observed.

36. Exposures of male rats and mice to BDE-47 also resulted in effects on testes including changes in the organisation of the seminiferous tubules, germ cell loss, increased numbers of multinucleated giant cells and decreased numbers of spermatids and daily sperm production, as well as effects on sperm motility. The lowest LOAEL for apical effects was 0.03 mg/kg bw/day in rats based on effects on testes. Exposure of pregnant rats during gestation and lactation resulted in effects in the male offspring including decreased testes weight, sperm count and motility, with a lowest LOAEL of 0.2 mg/kg bw/day. Following the exposure of pregnant rats on gestation day (GD) 6 there were also effects on female offspring including decreased ovarian weight and alteration of folliculogenesis, with a LOAEL of 0.14 mg/kg bw/day.

37. Repeat dose exposures of male rats to BDE-99 resulted in changes in testicular weights, damage to sperm, degeneration of seminiferous tubule epithelium and decreased spermatozoa. Administration of BDE-99 to pregnant rats on GD6 resulted in female reproductive tract changes in the F1 generation, with a LOAEL was 0.06 mg/kg bw. Administration to pregnant mice during gestation resulted in changes in the male reproductive organs, including decreased anogenital index, increased incidence of cryptorchidism, effects on Leydig cells and spermatogenic cells and on sex hormone levels, with a LOAEL of 0.2 mg/kg bw/day. It was also reported to result in incomplete or delayed ossification and internal variations, and impaired spermatogenesis in male offspring.

38. Repeated exposures of male rats or mice to BDE-209 resulted in male reproductive toxicity, including decreased testis and epididymis weights, degeneration of seminiferous tubules, decreased germ cell proliferation, decreased sperm count and viability, and increases in sperm malformations. Exposures during gestation and/or lactation resulted in similar effects in male

offspring, resulting in decreased mating and fertility index and decreased litter size. The lowest LOAEL was 5 mg/kg bw/day. BDE-209 was shown to affect testicular histopathology, steroidogenesis and germ cell dynamics. BDE-209 was embryotoxic in mice when Mice were exposed to ≥750 mg/kg bw/day during gestation, resulting in post-implantation loss, resorptions and decreases in live fetuses/litter. A significant reduction in anogenital distance was reported at 1,500 mg/kg bw per day in mice exposed during gestation.

39. Exposures of rats or mice *in utero* to BDE-99 or -209 affected steroidogenesis (decrease in the activities of 3β- and 17β-HSD in testis) and induced significant reductions in serum and intratesticular levels of testosterone at weaning and adulthood. Offspring of rats exposed to BDE-99 on GD10-18 showed decreases in circulating oestradiol and testosterone at weaning and adulthood; the LOAEL was 1 mg/kg bw/day. The LOAEL for BDE-209 was 500 mg/kg bw per day, based on significant reductions in serum testosterone and in the activities of 3β- and 17β-hydroxysteroid dehydrogenase.

40. For BDE-3, decreased in germ cells and sperm count were reported in male mice, with a lowest LOAEL of 1.5 mg/kg bw/day. Exposure of male rats *in utero* affected testis development, and reduced anogenital distance was observed at doses ≥100 mg/kg bw/day. Exposure of male rats *in utero* or postnatally resulted in decreased circulating testosterone levels, with a LOAEL of 50 mg/kg bw/day.

41. Repeated exposures of rats and mice to the technical product DE-71 resulted in decreased testis and epididymis weights, decreased sperm count and motility and increased testis germinal atrophy in males, with a lowest LOAEL of 71 mg/kg bw/day. A statistically significant increase in LH, and non-statistically significant increases in testosterone, androstenedione and oestrone, were observed in male rats exposed for 3 days to 60 mg/kg bw per day. Male rats exposed *in utero* and during lactation to DE-71 until weaning at 30.6 mg/kg bw/day (maternal dose) showed decreased anogenital distance and delayed preputial separation. Postnatal exposures of rats affected the reproductive organs in males at doses ≥30 mg/kg bw/day and caused a delay in vaginal opening in females at 60 mg/kg bw/day.

Immunotoxicity

42. For BDE-47, morphological changes were observed in the spleen and thymus in juvenile mice at 0.45 mg/kg bw/day.

43. Exposure of mice to BDE-209 for up to 10 months resulted in reduced leucocytes (monocytes), decreases in cytokine production and lower CD8-T and CD4-T cells proliferation, as well as weaker antigen-specific responses to Listeria infection. Exposure for 2 years resulted in morphological changes in the spleen. Exposure of rats to BDE-209 resulted in morphological changes in the gastrointestinal tract, impaired barrier function, and inflammation characterised by the upregulation of inflammatory mediators including interleukins. Postnatal exposure of mice (postnatal days 56-76) to 400 mg/kg bw per day resulted in decreased splenic and thymus weight. The lowest LOAEL for BDE-209 was 4 mg/kg bw/day, based on reduction of leukocytes, decreases of cytokine production and lower CD8-T cell proliferation in mice.

44. The technical product DE-71 was reported to alter splenic lymphocyte populations (but not cellular or humoral immune responses) in rats exposed *in* utero and during lactation and postweaning. In mice and rats exposed to DE-71 for 14 weeks thymus atrophy was observed. The lowest LOAEL for DE-71 was 0.018 mg/kg bw/day, based on decreased splenic $CD4^{+}CD8^{+}$ cells in a 28-day study in mice.

45. The technical mixture PentaBDE was reported to have effects on the immune system of adult mice and the development of the immune system of their offspring, with a NOAEL of 50 mg/kg bw/day. DecaBDE had more limited effects on the immune system in a single study.

Neurotoxicity

46. All of the PBDE congeners and technical mixtures that have been tested have shown neurobehavioural effects in rats and mice such as alterations in locomotion and spontaneous activity, anxiety and learning and memory abilities.

47. For BDE-47 the lowest LOAEL was 0.03 mg/kg bw/day (as the maternal dose), based on impaired learning in mice offspring which had been exposed *in utero* and during lactation when faced for the first time with the Barnes maze (at eight weeks of age).

48. For BDE-99 the lowest LOAEL was 0.06 mg/kg bw, based on increased locomotor activity in 71 day-old rats exposed *in utero* via a single oral dose to dams on gestation day 6. This is lower than the LOAEL used in EFSA’s 2011 assessment, which was 0.8 mg/kg bw, based on reduction of total activity in mice offspring tested at 2 and 4 months of age which were exposed *in utero* via a single oral dose on gestation day 10.

49. A single study was available for BDE-153, for which the LOAEL was 0.9 mg/kg bw and the NOAEL was 0.45 mg/kg bw, based on total activity in mice.

50. For BDE-183, -203 and -206, the only studies available tested single dose levels an thus did not provide information on dose-response.

51. For BDE-209, the lowest LOAEL was 1 mg/kg bw/day, based on impaired spatial learning and memory in adult rats that had been dosed in the early postnatal period. However, contrasting with this finding was a developmental neurotoxicity study on technical DecaBDE material containing 97.51% BDE-209, which was conducted in accordance with OECD test guideline 426. This study reported no effects in neurobehavioural observations which included startle response, learning and memory tests, nor on motor activity nor in neuropathological or morphometric measurements, at maternal doses up to 1000 mg/kg per day.

52. EFSA cited a systematic review of the association between PBDEs and measures related to learning, memory and attention in laboratory animals, which concluded that that there was a “moderate” level of evidence that exposure to BDE-47, -99 and -209 affects learning, measured as latency in the Morris water maze trial, while for other effects and for BDE-153, -203 and -206, and the technical product DE-71, the evidence was considered “low” or “very low.”

Genotoxicity

53. Negative results were reported for several PBDEs in the Ames test, and BDE-47 and -209 were also tested in *in vivo* gene mutation assays with negative results.

54. The technical product DecaBDE was negative in an *in vitro* gene mutation assay and an *in vitro* chromosome aberration assay.

55. BDE-47 produced positive results in *in vitro* micronucleus tests in SH-SY5Y cells and in V79 cells (these cell lines were noted by EFSA to express CYP enzymes). In contrast, negative results were reported for BDE-47, -99, -100, -153, -154 and -209 in HepG2 cells. Two metabolites of BDE-47, 6-OH-BDE-47 and 6-MeOH-BDE-47, were also positive in *in vitro* micronucleus tests. However, negative results were reported in *in vivo* micronucleus tests in bone marrow or peripheral blood for BDE-47, -209 and the technical product DE-71. EFSA noted that there was no evidence of toxicity to the bone marrow in these studies; however, evidence elsewhere on the toxicokinetics and systemic toxicity of these

substances indicate that the bone marrow would have been exposed.

56. *In vitro* tests indicated the ability of some PBDEs to cause single and double strand DNA breaks (BDE-47, -99, -100, -153, -154 and -209), and hydroxylated or methoxylated metabolites of BDE-47 and -49 were also positive in *in vitro* Comet tests. Some evidence indicates that PBDEs can damage DNA indirectly via reactive oxygen species. BDE-47 and -209 induced DNA strand breaks alongside increases in 8-OHdG or markers of oxidative stress. The Comet assay carried out in the presence of formamidopyrimidine-DNA glycosylase (FPG) suggested that BDE-47 and -209 increased purine oxidation.

57. Overall, while there was some evidence for genotoxicity *in vitro*, there was no evidence for genotoxicity *in vivo*, and EFSA concluded that the ten PBDEs under consideration are not genotoxic *in vivo*.

Carcinogenicity

58. No carcinogenicity studies were available on individual PBDEs. Studies were available in rats and mice on the technical product DecaBDE, which contained 94–97% BDE-209. An increase in liver adenoma in Fischer 344/N rats, and an increase in liver adenoma and carcinoma in male B6C3F1 mice, were observed. EFSA noted that the dose levels tested were very high.

59. Studies were also available in rats and mice on the technical product DE-71 (which comprised BDE-99: 42%, BDE-47: 36%, BDE-100: 10%, BDE-154: 4%, BDE-153: 3%, BDE-85: 2%). In male and female Han Wistar rats there were increased incidences of hepatocholangioma, hepatocellular adenoma or hepatocellular carcinoma (combined). In the males there were also increased incidences of thyroid gland follicular cell adenoma and pituitary gland (pars distalis) adenoma. In the females there were also increased incidences of cholangicarcinoma of the liver. In the females there was also evidence of uterine stromal polyps or stromal sarcoma, which were possibly related to treatment. The LOAEL for carcinogenicity was 2.1 mg/kg bw/day.

60. DE-71 increased the incidence of hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma in male B6C3F1/N mice, and of hepatocellular adenoma and hepatocellular carcinoma in the females. The NOAEL was 2.1 mg/kg bw/day.

Observations in humans

Thyroid disease

61. EFSA noted a growing body of epidemiological research in this area. However, there was currently weak evidence for any associations from the available studies, which were characterised by a small number of prospective studies, generally short follow-up periods, relatively small sample sizes, considerable heterogeneity in the assessed populations, exposures, and outcomes, and varying methodological quality. There were also inconsistencies in the direction of effect. For example, some studies reported increases in TSH, while others reported increases in free T3, free T4, total T3 or total T4.

62. Associations were reported for BDE-47, -99, -100, and -153, mostly in individual studies. For BDE-47 and -99, there were associations with reduced TSH and total T3 in two studies. For BDE-47 there were associations across all of the thyroid function biomarkers, and there were partially also for BDE-99, but there were discordant effect directions for both these congeners. EFSA concluded that the currently available evidence could not be used for hazard characterisation.

Neurotoxicity

63. Epidemiological studies on neurodevelopmental outcomes tended to evaluate different endpoints using different methods. EFSA focused on three domains that had the highest level of comparability between studies: cognitive function, autism and ADHD.

Cognitive function

64. Eight cohort studies assessed the association between levels of PBDEs and cognitive function, including intelligence, using various of the Wechsler scales. The WPPSI-III, WISC-III and WISC-IV were used, along with their subscales.

65. BDE-28 was analysed in 6 cohort studies and one showed a positive signal for the full-scale IQ at 8 years (per 10-fold increase; -7.9 points; 95% CI: -13.6, -2.3).

66. BDE-47 was assessed in all 8 studies and showed a positive signal for an effect on full-scale IQ in 2 different studies, although at different time points, 2 years and 5 years. It also showed a positive signal at 2 years follow-up for the verbal IQ subscale. In addition, a 10-fold increase in BDE-47 exposure was associated with significantly lower full scale IQ scores in boys, after adjusting for confounding factors (-4.4 points; 95% CI: -7.9, -0.9), and childhood BDE-47 levels

were associated with visual spatial abilities (virtual Morris water maze time and visual space memory retention) at 8 years.

67. BDE-99 was assessed in all 8 studies and two statistically significant associations were reported for the IQ components at two different timepoints (2 years and 6–8 years); however, the associations were in opposite directions.

68. BDE-100 was assessed in all 8 studies and gave four positive signals arising from two studies at three different timepoints. BDE-100 levels were statistically significantly associated with full-scale, performance and verbal IQ at 2 years, performance IQ at 3 years, and with spatial perception and reasoning at 6-8 years.

69. BDE-153 was assessed in 7 studies and two statistically significant associations were identified in two different studies at different timepoints: full-scale IQ at 2–8 years in one study and verbal memory at 13–15 years in the other.

70. There were no statistically significant associations observed for BDE-154 and -183 in the studies that assessed them.

71. For the sum of PBDEs, five statistically significant associations were reported across three different studies. These were for full-scale IQ (2 studies), verbal IQ (1 study), performance IQ (1 study) and processing speed (1 study).

72. Summarising, EFSA noted that BDE-28, -47, -99, -100, -153 or the sum were associated with full-scale IQ in three studies.

Autism

73. Five studies investigated autism either as a binary endpoint or through the social responsiveness scale. These included 1 case-control study, 1 cohort study, 1 nested case-control study and 1 cross-sectional study. Due to the small number of studies, small sample sizes, and lack of replication of the few postulated associations, EFSA found the data to provide a limited basis for risk characterisation.

ADHD, hyperactivity and attention

74. Fifteen papers evaluated the associations of PBDE levels with ADHD or ADHD subscales.

75. Statistically significant associations between prenatal maternal PBDE concentrations and ADHD scales were reported in one cohort study at 5 years (sum of PBDEs associated with the child behaviour checklist (CBCL) and the continuous performance test (CPT) and at 7 years (sum of PBDEs associated with DSM-IV). However, the other studies reported no associations between prenatal or human milk PBDEs levels and ADHD scores, clinical ADHD diagnosis or dichotomous ADHD symptoms.

76. Childhood sum PBDE levels were associated with the DSM-IV ADHD scores in one cohort study at 7 years of age, but the other studies reported null associations.

77. Twelve papers assessed inattention or hyperactivity subscales through a range of tools, and four of these reported associations with prenatal exposure to PBDEs. An association between sum of PBDEs and increased CBCL-attention problems and DSM-IV-inattention problems was reported at 5 and 7 years in one cohort study. Another cohort study reported an association between BDE-47 and increased BASC-hyperactivity problems. Another cohort study reported an association between BDE-47 and increased CBCL-attention problems in boys. And another cohort study reported an association between BDE-47 and -153 and increased CBCL-attention problems. Five other studies that studied associations between prenatal maternal levels of PBDEs and attention or hyperactivity scores reported null associations.

78. For childhood exposures, three studies reported statistically significant associations: one for the sum of PBDEs and teacher reported DSM-IV-inattention, BASC hyperactivity, and BASC-attention problem scores; one for BDE-28 and -153 with BASC-hyperactivity scores; and one for BDE-47 levels and dichotomous DSM-IV attention deficit symptoms. However, four other studies did not identify associations between childhood PBDE levels and hyperactivity or attention problems.

79. EFSA concluded that due to the inconsistencies in the results the epidemiological data on ADHD could not be used for hazard characterisation at present.

Lipid and sugar metabolism

80. EFSA found that the evidence on associations between diabetes-related endpoints and PBDEs had grown since the 2011 evaluation, with longitudinal studies now available, though the number of studies per specific endpoint was

limited. In general the available data were characterised by relatively small sample sizes, considerable heterogeneity in the assessed populations, exposures, and outcomes, varying methodological quality, and inconsistency in the effects reported. Therefore, EFSA concluded that the currently available epidemiological data on diabetes could not be used for risk characterisation.

81. For obesity, EFSA also noted a growing body of epidemiological research. However, there was a relatively small number of studies overall, and a small number of prospective studies that assessed diverse populations. Of these, there were a small number of statistically significant findings related to prenatal PBDEs levels and obesity attributes (BMI, waist circumference, % body fat) and these were in the direction of an inverse association which was not consistent with the data on type 2 diabetes or cardiometabolic risk factors. Therefore, the currently available epidemiological data on obesity could not be used for hazard characterisation.

Cardiovascular effects

82. There was a relatively small evidence base on PBDE levels and endpoints or risk factors related to cardiovascular disease, with both the number of studies and sample sizes being small and the endpoints under study being diverse. The only single statistically significant associations were related to cholesterol and triglycerides, were from small studies, and had not been replicated. Therefore, EFSA concluded that the currently available epidemiological data on cardiovascular effects and risk factors could not be used for hazard characterisation.

Effects on male reproduction

83. EFSA found that there is a growing body of epidemiological research on associations between PBDE exposure and endpoints related to the male reproductive system. However, there were still only a small number of prospective studies, and there were variable follow-up periods, relatively small sample sizes, a lot of heterogeneity in the assessed populations, exposures and outcomes, varying methodological quality, and inconsistency in the effects. There was a relative lack of data on clinical endpoints, while conclusions could also not be drawn on associations with semen quality and sex hormones, which were assessed in a larger number of studies but with a high risk of bias and without replication of the results. Therefore, EFSA concluded that the currently available epidemiological data on effects on male reproduction could not be used for

hazard characterisation.

Effects on female reproduction

84. EFSA also found a growing body of epidemiological research on associations between PBDEs exposure and endpoints related to the female reproductive system. Two domains with more data were focused on: pubertal development and included endpoints related to fertility and/or assisted reproduction techniques.

85. Pubertal development in girls was assessed in one cohort study and two cross sectional studies. All three reported some statistically significant results, but in opposite directions. The two cross sectional studies reported associations with premature pubertal development, while the longitudinal study reported an association with delayed pubertal development.

86. Two cohort studies and 2 cross-sectional studies were on women undergoing assisted reproduction techniques. Two statistically significant associations were reported for failed implantation, but with opposite effect direction, and single statistically significant inverse associations were reported for pregnancy and live birth. Five studies (3 cohort studies, 2 cross-sectional studies assessed endpoints related to fertility in couples not attending a fertility clinic. The two cross sectional studies reported the statistically significant adverse associations with time to pregnancy, pregnancy loss, and prematurity.

87. EFSA concluded that due to the small number of prospective studies, short follow up periods, relatively small sample sizes, multiple comparisons and inconsistency in the effects, the currently available epidemiological data on effects on female reproduction could not be used for hazard characterisation.

Birth outcomes

Outcomes measured before or at birth

88. The evidence on associations between PBDE levels during pregnancy and birth outcomes has grown since the 2011 EFSA evaluation. More studies are now available, in particular on birth weight (21 studies), but also birth length, head circumference and gestational age (≥8 studies each). For other specific endpoints, the number of studies was small.

89. For birth weight, cohort studies on PBDEs measured during or before pregnancy showed inconsistent results, with 5 studies showing reductions in birth weight and 6 studies showing no association. Of the 5 studies reporting associations, BDE-47 was associated with birth weight reduction in 3 of them and BDE-99 and -100 in 2 of the studies. The cross-sectional studies mostly reported null associations, with one exception. Two overlapping nested case-control studies also reported associations with reductions in birth weight and increased risk of fetal growth restriction. EFSA considered the evidence inconsistent.

90. For birth length, most cohort studies did not show associations, with the exception of one which reported an association between with maternal preconceptional BDE-28 exposure and reduced birth length. Two case-control studies also reported associations with reduced birth length, one for BDE-28 and -100 and one for BDE-207, -208, -209 and the sum of 19 PBDEs.

91. Three studies reported associations between PBDE exposure and reduced head circumference, two cohort studies and one cross-sectional study. PBDE exposure was negatively associated with gestational age in one cohort study and one case-control study.

92. EFSA concluded that, for these endpoints, the number of studies were small and the findings were heterogeneous, and thus the evidence was insufficient. There were also too few studied to draw conclusions on weight-for-length, Apgar score, placental size, sex ratio or neural tube defects.

Outcomes measured postnatally

93. EFSA noted that for the outcomes measured in the early years after birth (anogenital distance, 2nd to 4th digit ratio and infant growth), the number of studies for each outcome was small, the population size in each study was small (n 300), and results were generally not consistent between studies. Associations with reduced anogenital distance in boys were reported in two cohort studies, though the PBDE congeners for which statistically significant associations were found were not the same.

Effects on the immune system

94. Six studies had been published investigating associations with clinical endpoints such as atopy and/or asthma in childhood. There were no consistently-observed statistically significant associations. EFSA noted that the available data

were characterised by only a small number of prospective studies, short follow up periods, relatively small sample sizes, considerable heterogeneity in the assessed populations, exposures and outcomes, varying methodological quality, and inconsistency in effects. Therefore, they concluded that the currently available epidemiological data on effects on the immune system could not be used for hazard characterisation.

Carcinogenicity

95. The body of epidemiological evidence on cancer consisted of one cohort study, one nested case control and eight cross-sectional studies where the PBDE exposure was assessed at the same time or in some cases later than the outcome ascertainment. The populations studied were diverse. The cancers studied included thyroid, prostate and breast cancers, leukaemia and non-Hodgkin lymphoma.

96. For thyroid cancer, 5 studies were identified, of which 2 were longitudinal cohort studies. A statistically significant association between BDE-28 and thyroid cancer was reported in one nested case-control study and also in one cross-sectional study in China. There was no consistent association observed in the remaining studies.

97. For breast cancer, one nested case control and 5 cross-sectional studies were identified. Statistically significant results were seen only in one cross-sectional study.

98. Overall, EFSA considered that the epidemiological evidence on cancer was characterised by only a very small number of prospective studies, short follow up periods, relatively small sample sizes, substantial heterogeneity in the assessed populations, exposures, and outcomes, varying methodological quality, and inconsistency in the effects. Therefore, they concluded that the currently available evidence coming from human studies on cancer could not be used for hazard characterisation.

Other endpoints

99. EFSA also considered the epidemiological evidence related to other endpoints, which were addressed in small number of studies. These included mortality (2 studies), lung function (3 studies), hyperuricemia (1 study), celiac disease (1 study), microbiome (3 studies), inflammatory and oxidative stress

related biomarkers (8 studies), and epigenetics related biomarkers (15 studies). No clear conclusions appear to have been drawn based on the limited evidence available.

Mode of action

100. EFSA focused its consideration of modes of action the endpoints of most relevance for the hazard characterisation, and therefore activation of biotransformation enzymes regulated via AHR, CAR and PXR, neurotoxicity, thyroid hormone signalling, reproductive and other endocrine related effects, and secondary genotoxicity via oxidative stress.

AhR mediated effects

101. There is some evidence that PBDEs and their metabolites can act as ligands of the AhR and have both agonistic and antagonistic effects, generally at micromolar concentrations. EFSA found it difficult to assess if experimental results were influenced by any contamination with dioxins. BDE-47, -49, -100, -153 and -154, including some of their metabolites, appear to be the congeners most likely to affect AhR activity. EFSA noted that the technical product DE-71 did not induce AhR dependent liver tumours in rats. EFSA observed that it appears that several PBDEs are able to inhibit TCDD-induced AHR activity.

Activation of CAR and PXR

102. There is some evidence that PBDEs can activate CAR/PXR-dependent gene expression, at least at relatively high exposure levels (micromolar in cell culture). PXR has higher sensitivity than CAR to PBDEs. EFSA considered that CAR/PXR-dependent biotransformation enzyme induction could accelerate the metabolism of steroid and thyroid hormones, which would provide a possible explanation for decreased concentrations of circulating oestradiol, testosterone and T4 as reported in several studies.

Neurotoxicity

103. There is a body of evidence that PBDEs interfere with mitochondrial calcium homeostasis, leading to oxidative stress and apoptosis. This is seen both *in vitro* at low µM concentrations in relevant cellular systems, and *in vivo* at dose levels relevant for neurobehavioural effects. There are also observations of changes in neurotransmitters, or in the expression of related genes, and in cell

migration and differentiation. The effects appear to be common across the different PBDE congeners. There is some evidence to indicate that OH-metabolites are more active than the parent congeners, but the data available do not allow a clear identification of the relative potencies of different PBDEs.

104. There is also evidence to indicate the involvement of thyroid hormones in the neurotoxicity of BDE-209, but there is a lack of data on whether thyroid hormones are similarly involved for other PBDEs.

Effects on the thyroid hormone system

105. Further studies have been published since EFSA's 2011 evaluation to support the conclusions made that many PBDEs have the potential to interfere with thyroid hormone signalling at different levels. There is also further evidence that PBDEs are more potent following their metabolism through hydroxylation, methoxylation and sulfation changing in thyroid hormone function.

106. Several OH-PBDEs, MeO-PBDEs and PBDE sulfates have been shown to bind strongly to thyroid hormone transport proteins, including TTR. Binding affinities tend to increase with bromination substitutions up to 4 bromine atoms. Evidence from *in silico* models indicates that binding of these metabolites to TTR is mostly by hydrophobic interactions but that positively charged Lys15 is important for the coordination.

107. There is substantial evidence that PBDEs alter levels of thyroid hormones and thyroid hormone-dependent functions by inducing changes in their metabolism. Increased expression of CAR and PXR play a role but there is also evidence to suggest the involvement of some other too. These include inhibition of sulfotransferase (SULT), which occurs at nanomolar concentrations (and OH-PBDEs appear to be particularly potent inhibitors of SULT), and reduced expression of enzymes involved in the biosynthesis of thyroid hormones. There are variable reports on PBDEs or their metabolites affecting the conversion of T4 to T3, but some suggesting increased expression of deiodinases while others show inhibited activity or no effects. The gene for OATP2, which transports T4 into the liver, is a CAR target, which suggests that transport of T4 into the liver might also be accelerated by PBDEs.

108. The potential of PBDEs and their metabolites to modify function of THRs has been assessed, but not clear conclusions could be drawn. While it appears that some PBDEs can bind to THRs, the effects on THR activities are less clear.

109. Overall, EFSA concluded that there is good evidence that PBDEs and their OH-metabolites affect the thyroid hormone system by competing with T4 for thyroid binding proteins and by altering expression and activities of enzymes that metabolise thyroid hormones. Such effects could explain or contribute to the effects of PBDEs on thyroid hormone homeostasis and possibly neurodevelopment.

Reproductive effects

110. There is evidence for the involvement of endocrine effects, oxidative stress, mitochondrial dysfunction, apoptosis, oxidative damage to DNA, changes in gene expression and epigenetic mechanisms in the generation of adverse effects on reproduction. PBDEs can modulate the endocrine system by interfering with several hormonal signalling pathways simultaneously: binding to androgen receptor, disruption of oestrogenic and testosterone synthesis signalling pathways, and modulation of the thyroid hormone receptor. It was shown that thyroid dysfunction, with alteration in thyroid homeostasis, can induce impairment of testicular steroidogenesis and cause suppression of spermatogenesis, which may result in reduced fertility and infertility.

Genotoxicity

111. Although EFSA concluded that the PBDEs are not genotoxic *in vivo*, they considered the mode of action that may be responsible for DNA damage observed *in vitro*. Studies on BDE-47, -99 and -209 and their OH-metabolites *in vitro* showed that DNA strand breaks or micronuclei they induced were primarily mediated by oxidative stress. Increases in levels of reactive oxygen species were demonstrated, as well as changes in superoxide dismutase (SOD) level and increases in MDA content. The enhancement of single strand breaks by Fpg in the Comet assay was in line with oxidatively damaged DNA.

Identification of critical effects and dose response assessment

Identification of critical effects

112. The evidence from the available human data did not provide sufficient basis for risk assessment. Therefore, EFSA considered the data from studies in

laboratory animals to use in the hazard characterisation.

113. Targets for PBDE toxicity were the liver, and the thyroid hormone, reproductive, nervous and immune systems, and on lipid and sugar metabolism. Studies on the technical products DE-71, PentaBDE, OctaBDE and DecaBDE were not considered suitable for identifying points of departure as they are complex mixtures, often with limited information on the congener profile, and also with little or no information on impurities such as dioxin-like compounds. In addition, they are not representative of the balance of PBDE congeners that humans are exposed to via food.

114. Carcinogenicity was not considered a critical effect. Carcinogenicity studies had not been conducted on individual PBDE congeners but only on the technical products DecaBDE and DE-71. The increases in liver adenoma in Fischer 344/N rats and in liver adenoma and carcinoma in male B6C3F1 mice exposed to DecaBDE were observed at very high doses >1000 mg/kg bw/day and were likely due to CAR activation, which is a well-documented key event for rodent liver tumour development but not relevant to humans. The carcinogenic effects observed for DE-71 in Wistar Han rats (liver, thyroid and uterine tumours) and in B6C3F1/N mice (liver tumours) may be due to oxidative damage and changes in hormone homeostasis, and the liver tumours were likely due to CAR activation. The thyroid tumours observed may have resulted from chronic stimulation of the thyroid gland by TSH. EFSA noted that PBDEs are not genotoxic *in vivo*.

115. EFSA focused on the effects on neurodevelopment, reproduction/development and thyroid hormones. Changes in sex and thyroid hormones were considered to be key events in the adverse outcome pathways leading to neurodevelopmental and reproductive effects but are not apical outcomes. Therefore, changes in serum sex and thyroid hormone concentrations were not considered appropriate for the establishment of the points of departure.

116. EFSA identified the critical studies regarding effects on neurodevelopment as follows:

- For BDE-47 three studies were considered and one was selected due to its controlled method of dosing, dams were dosed daily during gestation and lactation, the higher relevance to humans of the endpoints investigated, all relating to learning and memory abilities, and the robustness of the effects observed in the learning and memory performance reported in this study. The results of the selected study were also in concordance with those from other studies related to the assessment of the effects of BDE-47 on learning

and memory.
- For BDE-99 three studies were considered and one was selected as exposed rat dams were exposed during gestation and lactation and tested a higher number of animals than one of the other studies.
- For BDE-153 EFSA identified the same critical study as in the 2011 opinion since no new data were available.
- For BDE-209 four studies were considered and one was selected as neonatal rats were dosed daily from PND 5-10, a period of exposure that encompass a critical window of the brain development, and tested up to PND 70 and PND 75, and the higher relevance to humans of the endpoints investigated, all relating to learning and memory abilities.

117. EFSA identified the critical studies regarding effects on reproduction and development as follows:

- For BDE-47 the lowest doses at which effects were observed are from studies in which adult males were exposed repeatedly. In three studies the LOAEL was the same, based on changes in cell organisation of the seminiferous epithelium in two studies and based based on decreased relative seminiferous epithelial thickness and increased apoptotic germ cells in the third. The same types of effect had also been reported at higher doses in animals exposed *in utero* and during lactation.
- For BDE-99 two studies were identified with relevant effects on reproduction and/or development following single exposures of rats on gestation day 6, based on decreased number of spermatids and sperm production in male offspring in one study and based on an increased fraction of dams with resorptions in the other. In the second study histopathological changes were also observed in ovaries in the F1 generation, and resorptions were also reported in female F1 mated with untreated males. There were also effects on prenatal development shown in three studies. These were resorptions, incomplete ossification, liver and heart hypertrophy, and malformations.
- For BDE-153 no relevant studies of effects on reproduction or development were identified.
- For BDE-209 two studies from the same research group were considered which both showed reproductive effects following repeated exposure. These effects were reduced sperm cell number and motility, and increased sperm cell malformations, in both studies.

Dose-response analysis

118. EFSA performed benchmark dose modelling in accordance with its latest guidance (2022). Taking into account the guidance, which recommends defining the BMR as a percent change in the response relative to the control group, EFSA selected a BMR of 10% for the neurodevelopmental effects, a BMR of 10% for daily sperm counts, a BMR of 5% for sperm motility and epithelial thickness of seminiferous epithelium, a BMR of 20% for apoptotic gonadal cells and a BMR of 10% for changes in ossification.

- For BDE-47, the lowest BMDL10 was 0.023 mg/kg bw/day for reproductive effects (impaired spermatogenesis following repeated exposure), while a BMDL10 of 0.15 mg/kg bw/day was calculated for neurodevelopmental effects (impaired spatial learning and memory following repeated exposure).
- For BDE-99, the lowest BMDL10 was 0.05 mg/kg bw for developmental effects (increased fetal resorption following a single dose on gestation day 6), while a BMDL10 of 0.43 mg/kg bw/day was calculated for neurodevelopmental effects (reduction in level of anxiety following repeated exposure).
- For BDE-153, the BMDL10 was 0.1 mg/kg bw from the single neurodevelopmental study available (based on impaired learning and memory following a single exposure on postnatal day 10).
- For BDE-209, the lowest BMDL5 was 0.91 mg/kg bw/day for reproductive effects (decreased sperm motility after repeated exposure), while a BMDL10 of 1.59 mg/kg bw/day was calculated for neurodevelopmental effects (impaired learning and memory after repeated exposure).

Estimation of body burdens at the BMDLs

119. Since PBDEs accumulate with repeated exposure body burdens, rather than the daily intakes, were considered as the appropriate dose metric for the risk assessment.

120. In the 2011 opinion, EFSA used a one-compartment approach to estimate the body burdens at the BMDLs. In this new draft opinion, a tissue-level approach was considered more accurate and was used instead. In the tissue-level approach, strain/species specific tissue concentrations following an exposure close to that encountered in the critical study are used to estimate the concentrations in the various tissues and organs. These are then summed, taking into account the different weights of all the organs and tissues to determine the total body burden.

- For BDE-47, the BMDL10 calculated for reproductive effects corresponded to a body burden of 0.123 mg/kg bw. The BMDL10 for neurodevelopmental effects corresponded to a body burden of 0.806 mg/kg bw.
- For BDE-99, the BMDL10 for developmental effects corresponded to a body burden of 0.0155 mg/kg bw. The BMDL10 for neurodevelopmental effects corresponded to a body burden of 1.443 mg/kg bw.
- For BDE-153, BMDL10 from the single neurodevelopmental study available corresponded to a body burden of 0.0125 mg/kg bw.
- For BDE-209, the BMDL5 for reproductive effects corresponded to a body burden of 0.0181 mg/kg bw. The BMDL10 for neurodevelopmental effects corresponded to a body burden of 0.0316 mg/kg bw.

Estimation of chronic human dietary intake levels corresponding to the body burdens at the BMDLs

121. The levels of chronic dietary intake in humans that would lead to the same body burdens as the BMDLs were calculated using the following equation:

Chronic human dietary intake = (body burden at the BMDL × Kel) / Fabs.

where,

Kel = elimination rate constant [ln(2)/(T1/2 in days)] (1/days).

Fabs = fraction of the chemical absorbed into the body.

122. Due to a lack of robust data, EFSA assumed the human oral absorption (Fabs) of BDE-47, -99 and -153 to be 100%. For BDE-209, the Panel used a value of s30% predicted by a human PBK model.

123. Instead of using the worst-case estimated human half-lives for BDE-47, -99 and -153, as in the 2011 EFSA evaluation, in this evaluation median half-lives of 510, 280 and 2700 days, respectively, from a more recent publication were used, as the methodology used was considered more robust. For BDE-209, whilst in the 2011 evaluation EFSA had concluded that the elimination half-lives of animals and humans did not differ by orders of magnitude, and therefore used the BMDL10 calculated in laboratory animals directly in risk characterisation, in this draft opinion EFSA have used the same approach as for BDE-47, -99 and -153, and have use an estimated human half-life of 15 days from two studies to estimate the chronic human dietary intake corresponding to the body burden at the BMDLs.

124. For BDE-47, for reproductive effects the resulting point of departure (POD, reference point) was concluded to be 168 ng/kg bw/day. For neurodevelopmental effects, the POD was concluded to be 1096 ng/kg bw/day.

125. For BDE-99, for developmental effects the resulting POD was concluded to be 38.4 ng/kg bw/day. For neurodevelopmental effects, the POD was concluded to be 3573 ng/kg bw/day.

126. For BDE-153, the resulting POD for neurobehavioural effects was concluded to be 3.2 ng/kg bw/day.

127. For BDE-209, for reproductive effects the resulting POD was concluded to be about 3,000,000 ng/kg bw/day. For neurodevelopmental effects, the POD was concluded to be about 5,000,000 ng/kg bw/day.

Approach to risk characterisation

128. EFSA considered the possibility of a combined risk from the PBDE congeners (dose addition). The four PBDEs for which there were suitable experimental data to derive a POD all affect neurodevelopment, and for three of these data are available showing effects on reproduction. In addition, EFSA noted the evidence that changes in the thyroid hormone system could be a mode of action in the effects of PBDEs on both neurodevelopment and reproduction. EFSA therefore concluded that BDE-47, -99, -153 and -209 should be included in a common assessment group.

129. For the other six PBDEs included in the assessment (BDE-28, -49, -100, -138, -154 and -183) no suitable toxicology data on which to derive PODs for risk assessment had been identified. However, *in vitro* mechanistic studies had been conducted in neural cells, comparing the results with BDE-47, and in some instances also BDE-99 and/or BDE-209, and these indicated that they may share common modes of action with BDE-47, -99, -153 and -209. Therefore, it was concluded that all ten PBDEs should be included in a common assessment group and dose-addition assumed.

130. EFSA considered the approach to take to the cumulative risk assessment. The hazard index approach was not considered appropriate as the evidence was only sufficient to establish HBGVs for three of the PBDEs. The establishment of a group HBGV was possible, with BDE-47 as the index chemical; however, relative potency factors could only be determined for two of the remaining PBDEs. It was concluded that calculating a total margin of exposure

(MOET) would be most appropriate approach.

131. Given the uncertainties present, and as a form of sensitivity analysis, EFSA took four different approaches (designated tiers) to the calculation of MOETs:

- Tier 1 – the lowest PODs for each BDE-47, -99, -153 and -209 were used. For the congeners with no identified PODs, the lowest POD for BDE-47 was applied, since it is the PBDE congener with the most toxicological data.
- Tier 2 – only the four congeners with PODs were considered, and their lowest PODs used.
- Tier 3 – only the four congeners with PODs for neurodevelopment were considered, using their PODs for neurodevelopment.
- Tier 3 – due to the uncertainties with the data and POD for BDE-153 it was excluded and only the three congeners with robust PODs for neurodevelopmental effects were considered, using their PODs for neurodevelopmental effects.

132. In interpreting the MOETs EFSA concluded that MOETs lower than 25 would raise a concern. This would allow for interspecies differences in toxicodynamics (factor of 2.5), intraspecies differences in toxicokinetics (factor of 3.2) and intraspecies differences in toxicodynamics (factor of 3.2). Toxicokinetic differences between species (usually addressed by a factor of 4) are addressed by the body burden approach used.

133. The approach differs from the previous assessment, which considered less than 2.5 to be of concern. In part, this is due to the use of median half-lives of elimination in humans in this evaluation rather than upper bound estimates in the previous evaluation.

Occurrence and exposure assessment

Occurrence in food

134. In total, 84,249 analytical results from 10,879 samples generated by either GC-MS or GC-ECD fulfilled the quality criteria applied and were used in the exposure assessment. Some of the occurrence data were provided by the UK when it was an EU Member State.

135. Left-censored data accounted for 51% of the results. LOQs and LODs were provided for 21% of the left-censored data. LODs or LOQs were provided for

4% and 75%, respectively, of the right-censored data.

136. The majority of the quantified results provided were for the “fish, seafood, amphibians, reptiles and invertebrates” food group. In this group, the highest concentrations were reported for BDE-47 and the lowest for BDE-138. Within this group fish offal had the highest concentrations. The second most level of quantifiable residue levels was for the group “meat and meat products.” In this group, the highest concentrations were reported for BDE-209 and the lowest for BDE-49 and -138.

137. Few data were available on PBDE concentrations in infant formula, and the percentage of left-censored data ranged 31-100%. For BDE-49 and -138 less than 6 results were reported, which were all left censored. Lower bound concentrations ranged between 0 and 0.006 µg/kg wet weight (for BDE-209). Upper bound concentrations ranged between 0.001 and 0.028 µg/kg wet weight (for BDE-209).

138. Data on the occurrence of PBDEs in European breastmilk were taken from pooled samples that were collected as part of WHO/UNEP field studies between 2014 and 2019. Median levels ranged between 0.007 µg/kg lipid (for BDE-49) and 0.326 µg/kg lipid (for BDE-47).

139. Data on the occurrence of metabolites of PBDEs in food were limited and only addressed MeO-BDEs in marine food. As there was only limited information on origin, levels and fate of MeO-BDEs in food, EFSA concluded that they could not address the significance of MeO-BDEs for risk assessment.

140. EFSA evaluated the effects of cooking and processing on PBDEs and found that most of the changes in concentration observed are due to changes in lipid content and moisture loss. An exception is that BDE-209 has been shown to undergo debromination during cooking to produce congeners with fewer bromine atoms; this was noted to be in line with degradation pathways observed in the environment. Only a small number of studies have addressed PBDE metabolites, and these indicated that the effects on MeO-PBDEs are similar way to the parent compounds. *In vitro* digestion models indicated that cooking may decrease the bioaccessibility of PBDEs, OH-PBDEs and MeO-PBDE, but the validity of the models has not been verified.

Dietary exposure assessment

141. EFSA’s dietary exposure assessments do not appear to have included UK consumption data, since the UK is no longer an EU Member State, though EFSA’s comprehensive database includes some UK consumption data from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the National Diet and Nutrition Survey (NDNS) that were provided to EFSA when the UK was an EU Member State.

142. EFSA estimated the mean and 95th percentile chronic dietary exposures to PBDEs separately for each consumption survey using data recorded at the individual level in the Comprehensive Database.

143. The highest mean exposure to PBDEs at the lower bound (LB) across all congeners was estimated for “toddlers” and “other children,” with exposures tending to decrease across increasing age groups. However, “Infants” were the least exposed age group for the LB scenario for all PBDEs except BDE-209.

144. Similarly, the mean exposures at the upper bound (UB) also tended to decrease with age, with “toddlers” having the highest exposure to all congeners. The least exposed age group to BDE-49 and -138 was “infants”, whilst for BDE-47 and -209 it was “adults”, and to BDE-28, -99, -100, -153, -154 and -183 it was the “elderly”.

145. Estimated exposures for “pregnant women” and “lactating women” were within the range of exposure estimates for other adults.

146. The highest exposure estimates were for BDE-209, followed by BDE-47.

147. The food groups “meat and meat products” and “fish, seafood, amphibians, reptiles and invertebrates” contributed the most to dietary exposures to BDE-28, -47, -99, -100, -153, -183 and -209. For BDE-49, -138 and -154, the main contributor was “animal and vegetable fats and oils and primary derivatives thereof.”

148. For formula-fed infants EFSA used a consumption scenario to estimate exposures. Based on previous EFSA guidance on default values, and considering the long half-lives of the PBDEs, EFSA used consumption values of 170 and 210 mL/kg bw/day for mean and P95 consumption, respectively. These were based on daily consumption levels of 850 mL for mean consumption and 1050 mL for high consumption for infants aged around 2 months with a body weight of 5 kg. Occurrence data for dry infant formula were adjusted to the liquid form by applying a dilution factor of 8. Exposures were not estimated for two congeners, BDE-49 and -138, due to the limited occurrence data for these two congeners.

The highest exposures were estimated to be to BDE-209.

149. For breast-fed infants EFSA used a different scenario to estimate exposures. In this case they based the assessment on infants aged three months, with and average daily consumption of 800 mL and a high consumption of 1,200 mL and a body weight of 6.1 kg. The milk was assumed to have a mean fat content of 3.5%. The highest exposures were estimated for BDE-209, followed by BDE-153, -47 and -99.

150. Dust is a significant non-dietary source of exposure, particularly for young children due to a higher intake as a result of greater hand-to-mouth contact and due to their lower body weights. However, two European studies which measured both dust and dietary exposure reported that diet provided over 90% of body burdens.

Risk characterisation

151. EFSA calculated the MOETs and applied a tiered approach to the risk characterisation, as described above. In order to address the fact that survey participants highly exposed to one congener will not necessarily be highly exposed to all other congeners, and to avoid underestimating the MOETs at P95, the individual MOEs were calculated for each congener for each survey participant, and then the MOETs at the mean and P95 exposure estimates were derived for each dietary survey and age group. This process was repeated for all the tiers.

152. The results of the MOET calculations can be found in Tables 45 and 46 of the draft EFSA opinion, on pages 302-303.

153. For tier 1, the MOETs for the mean exposure estimates were all great than 25 for the LB estimates, but they were all less than 25 (and thus of potential concern) for the UB exposures for all age groups. The MOETs for the P95 exposure estimates were greater than 25 for the LB estimates except for “toddlers” and “other children” at the median and maximum exposure estimates and “adolescents” at the maximum exposure estimate. For the UB estimated the MOETs were less than 25 for all age groups.

154. For tier 2, the MOETs for the mean exposure estimates were all greater than 25 for the LB estimates, but they were all less than 25 for the UB exposures for all age groups. The MOETs for the P95 exposure estimates were greater than 25 for the LB estimates except for “toddlers” and “other children” at the median and

maximum exposure estimates and “adolescents” at the maximum exposure estimates. For the UB estimated the MOETs were less than 25 for all age groups.

155. Thus, the results of tier 2 were similar to tier 1. This indicates that exposures to the PBDEs for which there are no toxicological data (BDE-28, -49, -100, 138, -154 and -183) have little effect on the risk assessment provided that the assumption that they are not more toxicologically potent than BDE-47 is reasonable.

156. For tier 3, the MOETs for the mean exposure estimates were all greater than 25 for the LB estimates, but they were all less than 25 for the UB exposures for all age groups. The MOETs for the P95 exposure estimates were greater than 25 for the LB estimates except for “toddlers” at the maximum exposure estimates. They were all below 25 for the UB estimated for all age groups.

157. For tier 4, the MOETs at the mean and P95 exposures were all far greater than 25 for all age groups.

158. EFSA considered that the exposure estimates at tier 1, 2 and 3 are of concern at the LB P95 estimates in some surveys of “toddlers,”, “other children” and “adolescents.” EFSA acknowledged that there are large uncertainties due to the lack of toxicological data for most congeners, and that the tier 4 exposures did not indicate a concern. However, tier 4 is not representative of dietary exposure to all the PBDEs under consideration. In comparing the results of tiers 1-3 with tier 4, EFSA noted the importance of the contribution of the POD for BDE-153 to the MOET. This was the lowest POD but also the one with the highest uncertainty.

159. For (exclusively) formula-fed infants, the tier 1, 2 and 3 estimates of MOET were all greater than 25 when considering the LB estimates and all less than 25 when considering the UB estimates. For tier 4 all estimated MOETs were greater than 25. EFSA noted the uncertainty in the exposure estimates, and therefore risks, due to the large differences between the LB and UB estimates.

160. For breastfed infants, the tier 1, 2 and 3 estimates of MOET were all less than 25. For tier 4, they were all above 25. EFSA noted that the low MOETs were driven by BDE-153 and its low POD. They considered the impact on body burdens, considering that it takes 3-4 half-lives to reach steady-state levels in the body, which would be 3 years or more for BDE-99, 4 or more years for BDE-47 and 22 years or more for BDE-153 in humans. They calculated the body burden of BDE-153 that would result from 6 months of breastfeeding and noted that this was 50

times lower than the body burden at the POD for BDE-153, indicating this was not a concern for the median breast milk concentrations used in the exposure assessment.

161. As an alternative approach, EFSA used data on concentrations of the PBDEs that had been measured in adipose tissue in adults and the concentrations measured in breastmilk fat to estimate body burdens and compare to the POD body burdens. The MOETs calculated at the different tiers were generally consistent with the results based on the estimates of dietary exposure. One notable aspect was that body burdens of BDE-209 were much greater than anticipated based on estimated dietary exposures. It was considered that the most likely explanation is significant exposure from additional non-dietary sources such as dust.

Uncertainty analysis

162. Using semi-formal structured methods of expert knowledge elicitation, it was concluded with more than 90% certainty that the 10 PBDEs considered are either not genotoxic *in vivo*, or that if they were this would be via an indirect mechanism with a threshold.

163. The key uncertainties were identified as being the use of left-censored occurrence data, the lack of occurrence data for some relevant food categories, uncertainty regarding the appropriateness of the POD for BDE-153, the lack of neurodevelopmental toxicity data for 6 of the 10 PBDEs considered and the lack of reproductive/developmental data for 7 of the PBDEs.

164. These uncertainties were taken into account in a quantitative uncertainty analysis using expert knowledge elicitation. For half of the dietary surveys considered, EFSA concluded with more than 70% certainty that the mean combined potency-adjusted exposure estimates for all ten PBDEs for “toddlers” raise a health concern for reproductive/developmental toxicity effects. At the P95 of the combined potency-adjusted exposure estimates there was more certainty of a health concern. For example, there was concluded to be more than 90% certainty of a health concern for reproductive/developmental effects at the P95 for “toddlers.”

165. The probability of a health concern for neurodevelopmental effects was considered lower than for reproductive/developmental effects. For half of the dietary surveys considered there was concluded to be more than 50% certainty at

the mean combined potency-adjusted exposure estimates for toddlers of concern for reproductive/developmental effects.

166. Overall, the draft EFSA opinion concludes that current dietary exposures to PBDEs in the European population raise a health concern.

Recommendations

167. Finally, EFSA made a number of recommendations in order to reduce uncertainties and refine the risk assessment, which can be summarised as follows:

- Surveillance of PBDEs should continue, using more sensitive analytical methods, including the analysis of more congeners than the 10 considered here.
- More occurrence data are required for infant formula, using more sensitive analytical methods, in order to enable a more robust exposure assessment for formula-fed infants.
- Further information is required on toxicokinetics in humans, e.g. on oral absorption, half-lives of elimination and metabolism. More data are required on the relation of maternal body burden to transfer to the fetus and during lactation. The data should be used to develop a toxicokinetic model for the PBDEs, which includes placental transfer and excretion into breastmilk.
- Further toxicological data are required to the PBDEs with limited data available at present, particularly BDE-153, in particular on reproduction/development and on neurobehavioural effects following perinatal exposure. Such studies should be conducted with individual PBDEs of high purity.
- Data are required to develop adverse outcome pathways (AOPs), particularly related to effects on neuro-behaviour, reproduction/development and effects on thyroid function.
- Further work is required to align effects observed in in animal studies with adverse effects in humans. Further research should focus on combined exposure to multiple PBDEs and other chemicals.

Questions on which the views of the Committee are sought

Members are asked to consider the following questions:

i. Do Members have comments on the draft EFSA opinion that they wish to be submitted to EFSA? Please can Members specify the sections of the draft opinion they are referring to or the specific lines of text where possible.

ii. Do Members agree with the approach taken to the risk assessment of PBDEs in the draft EFSA opinion?

iii. Considering the long half-lives of elimination (e.g. 2700 days for BDE-53, 510 days for BDE-47 and 280 days for BDE-99), do Members agree with the conclusion that the dietary exposures assessed raise a health concern for toddlers, with >70% certainty at mean exposure and >95% certainty at P95 exposure?

iv. Does the Committee have any further comments?

Secretariat

July 2023

References

COT (2015). Statement on the potential risks from polybrominated diphenyl ethers (PBDEs) in the infant diet. COT Statement 2015/01. March 2015.

PBDEstatementfinal.pdf (food.gov.uk)

COT (2017). Addendum to the 2015 COT statement on potential risks from polybrominated diphenyl ethers (PBDEs) in the infant diet: potential risks from polybrominated diphenyl ethers (PBDEs) in the diets of infants and young children. COT Statement 2017/3. November 2017.

[ARCHIVED CONTENT] Addendum to the 2015 COT statement on potential risks from polybrominated diphenyl ethers (PBDEs) in the infant diet | Food Standards Agency (nationalarchives.gov.uk)

Annex A to TOX/2023/34

EFSA's draft opinion for public consultation: Update of the risk assessment of polybrominated diphenyl ethers (PBDEs) in food.

Public Consultation Detail (1) (europa.eu)

Secretariat

July 2023