Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

COT statement on tetrabromobisphenol A - Review of toxicological data

Introduction

1. In 2003, the COT considered data on some brominated flame retardants (BFRs) in fish from the Skerne-Tees river system. In the course of the discussions, Members noted a need to consider other BFRs, such as tetrabromobisphenol A (TBBPA). As the Food Standards Agency is planning to conduct a survey of TBBPA in fish and shellfish during 2004, the Committee was invited to consider the toxicological data in advance of receiving the results of the survey.

2. Decabromodiphenyl ether (decaBDE) and tetrabromobisphenol-A (TBBPA) have been found commonly in marine fish and shellfish but studies have shown that decaBDE and TBBPA are present in the eggs of wild birds [1,2] a finding that was unexpected. There appear to be no data on BFRs in hen eggs; they are being included in the survey to assess whether the unknown source of the BFRs in the wild bird eggs is also affecting free range hen eggs.

3. TBBPA has not been evaluated by the Scientific Committee on Food (SCF) or the Joint FAO/WHO Expert Committee on Food Additives (JECFA). However it is currently under evaluation under the Existing Substances Regulations. A draft risk assessment is available[3]. Many of the studies referred to are unpublished and not freely available.

4. The COT evaluation of the toxicology of TBBPA was based primarily on the EU risk assessment. The data described in this statement are summarised from the EU risk assessment unless otherwise indicated.

Background

5. BFRs are structurally diverse chemicals used in plastics, textiles and other materials to enhance their flame-retardant properties. TBBPA may be present as an additive or may be reacted into the polymer matrix. Reactive flame retardants are covalently bonded to the plastic itself whilst additives are mixed into the material. This means that reactive materials are less likely to leach or volatilise whereas additives are more easily released. Where TBBPA is used as an additive flame retardant it is generally used with antimony trioxide

for maximum performance. Antimony trioxide is generally not used in conjunction with TBBPA in reactive flame retardant applications.

6. TBBPA is produced by the bromination of bisphenol A (BPA) in the presence of either a hydrocarbon solvent (with or without water), 50% hydrobromic acid or aqueous alkyl monoethers. TBBPA is not produced in the EU however 13,800 tonnes were used in the EU during 1999 (620 tonnes in the UK in 2001).

7. The primary use of TBBPA (approximately 90% of use) is as a reactive intermediate in the manufacture of epoxy and polycarbonate resins. When used as a reactive intermediate it becomes covalently bound in the polymer and is effectively lost. The only potential for exposure is from unreacted TBBPA which may exist where excess has been added during the production process. It is used as a reactive flame retardant in polycarbonate and unsaturated polyester resins. It may also be used as an additive flame retardant in the manufacture of acrylonitrile-butadiene styrene resins, high impact polystyrene and phenolic resins. As an additive flame retardant TBBPA does not react chemically with the other components of the polymer and therefore may leach out of the polymer matrix. Additive use accounts for approximately 10% of the TBBPA used.

Toxicological profile as described in the EU risk assessment

Toxicokinetics

8. Toxicokinetic data are available for the rat but not for other experimental animals. TBBPA is well absorbed from the gastrointestinal tract. Systemic distribution of TBBPA is low with most distributed directly to the liver and eliminated in the faeces via the bile. There is some evidence to suggest that the compound undergoes enterohepatic recirculation. TBBPA undergoes metabolism via glucuronidation and/or sulphation. The half-life in blood is about 20 hours and 95% of administered dose was reported to be eliminated in the faeces within 72 hours, with only a small percentage eliminated in the urine. Repeat dose studies have shown no potential for bioaccumulation in fat. A toxicokinetic study in pregnant animals indicated no significant transfer of TBBPA or its metabolites to the fetus.

9. TBBPA has been detected in samples of human serum or plasma. In Sweden the concentrations ranged from <0.5 to 3.8 ìg/kg lipid, and one study estimated the half-life of TBBPA in serum to be 2.2 days. In Norway, the range was <0.4 ng/kg lipid to 1.8 ìg/kg lipid. A further study in Norway analysed the level of TBBPA in pooled serum samples at six timepoints during 1977 to 1999. TBBPA was not detected in samples from 1977-1981 but was found to increase from 0.44 ìg/kg lipid in 1986 to 0.65 ìg/kg lipid in 1999. The range of TBBPA in pooled samples from 8 groups of individuals collected in 1998 was 0.34 – 0.71 ìg/kg lipid. In Japanese volunteers, the maximum blood concentration detected was 12 ìg/kg lipid. There is no information on blood concentrations of TBBPA in the UK population.

10. Limited data demonstrate that TBBPA has also be detected in human breast milk. Concentrations of 0.29 – 0.94 ìg/kg lipid were detected in two of four samples collected from West Berlin during 1998/99; 11 ìg/kg lipid was detected in a single sample from the Faroe Islands, but no information was available on the possible reason for this high value. Analysis of pooled samples of breast milk collected from Norway during 2001 found TBBPA was present at 0.010 – 0.1 ìg/kg lipid.

Acute and sub-acute toxicity

11. TBBPA is of low acute toxicity via oral administration. The LD_{50} in the rat is >50,000 mg/kg body weight (bw) and in the mouse is >10,000 mg/kg bw. There is no information available on the acute toxicity of TBBPA in humans.

12. In a recent study conducted to OECD guidelines, by MPI research [4], rats were administered 0, 100, 300 or 1000 mg/kg/day TBBPA in corn oil by gavage for 13 weeks, followed by a 6-week recovery period. There were no treatment-related deaths, no effects on body weight gain, and no abnormal ophthalmic observations. A functional observational battery (FOB) and assessment of motor activity at week 12 revealed no evidence of neurobehavioural effects. Platelet counts were significantly reduced (by 17%) in the high dose males at 13 weeks, but not at the end of the recovery period. There were no other haematological changes. The values were comparable to control at the end of the recovery period, other markers of liver toxicity were normal and there were no abnormal histopathological findings in the liver.

13. There was a reduction in T4 levels in all treated males and females on day 33 and in males at day 90, with no clear dose-response relationship. There were no reported differences in TSH and T3 at any timepoint, and the T4 levels were comparable to control following the recovery period.

14. Overall, the EU risk assessment concluded there were no clear toxicologically significant effects up to the highest nominal dose of 1000 mg/kg bw/day. However, it was noted that due to the relative insolubility of TBBPA, it was only partially dissolved at the highest level. In view of this the EU risk assessment concluded that only 50% of the TBBPA would be available for absorption and suggested that the nominal dose of 1000 mg/kg bw/day was equivalent to an actual dose of 500 mg/kg bw/day.

15. Two dietary studies in rats reported no treatment-related effects at TBBPA doses up to 100 mg/kg bw/day for 90 days and up to 75 mg/kg bw/day for 28 days. Two short term (3 – 28 days) studies designed to determine the effect of TBBPA on the liver indicated no clear evidence of hepatotoxicity up to doses of 2250 mg/kg. Some significant changes were apparent in one study, including reduced liver glutathione content, following 7 days of TBBPA administration at 750 and 1125 mg/kg bw/day, and indicators of porphyrogenic action at 50 and 250 mg/kg bw/day [5]. The relationship to dose and treatment time was not consistent, and there were no accompanying histological changes.

A further study investigated the effect of daily gavage dosing, up to 28 days, of TBBPA (10-250 mg/kg bw/day) on the kidneys in rats. No dose related or toxicologically significant effects were reported.

16. Repeated dermal exposure to 50% of the maximum non-irritating dose of TBBPA did not induce bromacne (analogous to chloracne) in the skin.

17. There is no information on the effect of repeated exposure to TBBPA in humans.

Mutagenicity and carcinogenicity

18. TBBPA was not mutagenic in reverse mutation assays with *Salmonella typhimurium* or *Saccharomyces cerevisiae* in the presence or absence of metabolic activation and up to cytotoxic concentrations. A chromosomal aberration test in human peripheral lymphocytes in the presence and absence of S9 also gave negative results. TBBPA has also been tested for its ability to induce intragenic recombinations in mammalian cells. The study did not show an increase in the number of revertant colonies. TBBPA has not been tested for mutagenicity *in vivo.* Overall, in view of the negative data and absence of structural alerts for genotoxic potential, there are no concerns for genotoxicity. There are no carcinogenicity data for TBBPA.

Reproductive toxicity

19. A comprehensive developmental study by Hass *et al*. [6] was recently conducted according to the proposed OECD guideline TG426 [7]. TBBPA was administered to rats by gavage at 0, 50 and 250 mg/kg bw/day from gestational day 7 to postnatal day 17. Observations included endpoints associated with endocrine effects (anogenital distance at birth, time of nipple development and sexual maturation) and a range of neurobehavioural tests at timepoints up to the age of 7 months. The authors concluded the study demonstrated that TBBPA caused developmental neurotoxicity at 250 mg/kg bw/day, expressed as decrease of habituation capability and impairment of learning and memory in both sexes. However the draft EU risk assessment questioned the relevance of the observations and considered that the study showed no significant treatment-related toxicity.

20. Data from a recent 2-generation reproductive toxicity study in rats [8], conducted to OECD guidelines, indicate that TBBPA has no toxicologically significant effects on fertility or neurodevelopment at doses of up to 1000 mg/kg. Observations on neurodevelopment were conducted in the F_2 generation and included neurobehavioural studies of motor activity, learning and memory, and neuropathological evaluation and morphometric measurements. Significant reductions in the levels of T4 were observed in F_0 males at 100 and 1000 mg/kg and in F_0 females at 1000 mg/kg. Levels of T4 were reduced in F_1 males and females at 100 and 1000 mg/kg. Levels of T3 were reduced in F_0 males from 1000 mg/kg. There was no effect on TSH. The authors suggested that these findings indicated that TBBPA can induce UDP-glucuronosyltransferase, the enzyme involved in metabolism of T4.

21. In a study of developmental neurotoxicity, TBBPA was administered as a single oral dose of 0.75 or 11.5 mg/kg bw to 10-day old male mice. No differences in performance were observed in behavioural tests conducted on the mice at the age of 2 and 4 months [3]. This study was conducted by Eriksson and colleagues, who reported that some polybrominated diphenyl ethers produced significant effects when tested using this protocol, as discussed in the COT statement on BFRs in fish from the Skerne-Tees river system [9].

22. There are no human data available on the reproductive toxicity of TBBPA.

Other studies

In vivo

23. Following oral exposure of pregnant rats to TBBPA (5 mg/kg/bw) on days 10-16 of gestation there were no effects on maternal bodyweight, total litter size, or number of resorptions [10]. There was no effect on total and free T4 plasma levels in dams or offspring and no effect on T3 in the dams. T3 was not detected in the plasma of offspring. An increase in TSH levels was reported in the dams and offspring compared to controls, but this was only significant in the offspring.

In vitro

24. TBBPA has shown evidence of weak oestrogenic activity in some, but not all, *in vitro* assays using cell lines and recombinant yeast models [11-15].

COT evaluation

The reported effects of TBBPA on thyroid hormones differed between studies. As TBBPA was reported to inhibit the binding of ^{125}I-T3 ($1x10^{-10}$ M) to the thyroid hormone receptor in the range $1x10^{-6}$ to$1x10^{-4}$ M [15], TBBPA would be expected to have thyroid hormone agonist effects and to inhibit thyroid stimulating hormone (TSH). However, this did not appear to be the case. Given the lack of consistency in the data, it was considered difficult to ascertain whether TBBPA had significant thyroid effects. A reduction in T4 levels in one study had been reported but levels had returned to baseline following a recovery period, which indicated that any effect was reversible. The relevance of data suggesting that TBBPA may displace T4 from transthyretin (TTR), a major T4 binding protein in the rat, was unclear in view of the limited understanding of the role of TTR. It was possible that induction of UDP-glucuronosyltransferase could account for the findings, but that needed to be demonstrated.

25. No long-term carcinogenicity study was available. However, the results of mutagenicity studies were negative and the subchronic studies provided no indication of a mechanism to suggest that TBBPA could lead to carcinogenesis of relevance to humans following life-time exposure.

26. TBBPA had been shown to have weak estrogenic activity in some in vitro assays. The lack of effects in a recent 2-generation reproductive toxicity study in rats suggests that TBBPA is not an endocrine disruptor *in vivo* at doses relevant to human exposure.

27. Discussions focussed on results of neurotoxicity studies in rats [4,6,8]. Hass *et al*. [6] reported a significant reduction in habituation at a dose of 250 mg/kg bw/day. There was no consistent pattern of effects across the dose groups, which raised doubts about the biological plausibility of the effect. In contrast, reduced habituation was not observed in the MPI study [8], at 100 and 1000 mg/kg bw/day. A decrease in parietal cortical thickness was observed at 1000 mg/kg bw/day at day 11. Additional data, not described in the EU risk assessment, showed that this effect was not present at day 60 [16], and hence the MPI study was not considered to provide evidence of neurotoxicity. Detailed consideration was therefore given to the comparison of these studies.

28. The two studies used slightly different end points at slightly different ages (postnatal days 21, 27 and 84 in the Hass study and postnatal days 17, 21 and 60 in the MPI study). Both studies showed the expected increase in spontaneous activity after weaning, and both showed significant habituation activity in the controls over the test period. Absolute activity values are not comparable between the two studies due to methodological differences, but the percentage changes over time should be comparable.

29. The MPI study showed less habituation in the controls than the Hass *et al.* study, which would have reduced its ability to detect a toxicant-induced fall in habituation, particularly at postnatal day 21. However the MPI study did have sufficient power to detect as marked a fall in habituation as was seen in the Hass *et al.* study at post-natal day 21 - yet failed to do so at 100 and 1000 mg/kg. The rather more modest decline in habituation, seen in the Hass *et al.* study at post-natal day 84 was not reproduced in the MPI study at post-natal day 60. Overall, the lack of effect in the MPI study, and the lack of internal consistency in the results of Hass *et al.* indicates that the findings of a reduced habituation at 250mg/kg by Hass are likely to have been due to chance.

30. The EU draft risk assessment report concluded that its risk characterisation for oral exposure should be based on a repeat dose study in which rats were administered TBBPA in corn oil. No significant toxicological effects were reported in this study at the highest dose of 1000 mg/kg bodyweight/day. Based on the limited solubility of TBBPA in the dosing vehicle (corn oil) the EU report assumed that only 50% of the TBBPA was bioavailable. However the Committee considered that assumptions on absorption should not be based on solubility and therefore the highest dose of 1000 mg/kg bw/day could be used as the basis for deriving a Tolerable Daily Intake (TDI).

31. A total uncertainty factor of 1000 would be required. This comprises the default uncertainty factor of 100 to allow for inter- and intra-species variation and an additional factor of 10 for the absence of chronic toxicity studies.

Conclusions

32. The available data on TBBPA do not raise specific toxicological concerns. There is a lack of long term or carcinogenicity studies, but this is not considered essential to the evaluation in view of the absence of relevant effects in the available studies.

33. No clear adverse effects were observed at doses up to 1000 mg/kg bw/day, the highest dose tested in a 90-day study and in a two-generation reproductive toxicity study. This dose level may be used as the basis for deriving a Tolerable Daily Intake (TDI).

34. An uncertainty factor of 100 is applied to allow for inter- and intra-species variation. An additional factor of 10 is required because of the absence of chronic toxicity studies. The Committee therefore recommended a TDI of 1mg/kg bw/day.

COT statement 2004/02
July 2004

References

1. Herzke D., Berger U., Nygård T. and Vetter W. (2003). Organochlorines, organobromines and their metabolites in eggs of Norwegian birds of prey. Dioxin 2003, *Organohalogen Compounds*, **60-65**.

2. de Boer J., Allchin C., Zegers B., Boon J. P., Brandsma S. H., Morris S., Kruijt A. W., van der Veen I., van Hesselingen J. M. and Haftka J. J. H. (2002). HBCD and TBBP-A in sewage sludge, sediments and biota, including interlaboratory study. RIVO Report No. C033/02. September 2002.

3. 2,2',6,6'-Tetrabromo-4,4'-isopyrilidene diphenol (tetrabromobisphenol A). Draft EU Risk Assessment, November 2003.

4. MPI Research (2002a). A 90-day oral toxicity study of tetrabromobisphenol-A in rats with a recovery group (Unpublished report).

5. Szymanska JA, Piotrowski JK and Frydych B (2000). Hepatotoxicity of tetrabromobisphenol A: effects of repeated dosage in rats. *Toxicology*, **142**: 87-95.

6. Hass H, Wamberg C, Ladefoged O, Dalgaard M, Rye Lam H, Vinggard A (2003). Developmental neurotoxicity of tetrabromobisphenol A in rats. Unpublished.

7. Organisation for Economic Co-operation and Development (OECD) (2003). Draft test guideline no. 426 – developmental neurotoxicity testing.

8. MPI Research (2002b). An oral two generation reproductive, fertility and developmental neurobehavioural study of tetrabromobisphenol-A in rats (Unpublished report)

9. Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (2003). Statement on brominated flame retardants in fish from the Skerne-Tees rivers system.

10. Meerts IATM, Assink Y, Cenjin PH, Weijers BM, van den Berg HHJ (1999). Distribution of the flame retardant tetrabromobisphenol A in pregnant and fetal rats and effect on thyroid hormone homeostasis. *Organohalogen Compounds*, **40**: 375-378.

11. Samuelson M, Olsen C, Holme JA, Meussen-Elholm E, Bergmann A, Hongslo JK (2001). Estrogen-like properties of brominated analogs of bisphenol A in the MCF-7 human breast cancer cell line. *Cell Biology & Toxicology*, **17**: 139-151.

12. Körner W, Hanf V, Schuller W, Bartsch H, Kreienberg R, Hagenmaier H (1996). Validation and application of a rapid in vitro assay for assessing

the estrogenic activity of halogenated phenolic compounds. *Organohalogen Compounds*, **27**: 297-302.

13. Miller D, Wheals BB, Beresford N, Sumpter JP (2001). Estrogenic activity of phenolic additives determined by an in vitro yeast bioassay. *Environmental Health Perspectives*, **2**: 133-138.

14. Olsen CM, Meussen-Elholm ETM, Holme JA, Hongslo JK (2003). Effects of the environmental oestrogens bisphenol A, tetrachlorobisphenol A, tetrabromobisphenol A, 4-hydroxybiphenyl and 4,4'-dihydroxybiphenyl on oestrogen receptor binding, cell proliferation and regulation of oestrogen sensitive proteins in the human breast cancer cell line MCF-7. *Pharmacology & Toxicology*. **92**: 180-188.

15. Kitamura S, Jinno N, Ohta S, Kuroki H, Fujimoto N (2002). Thyroid hormonal activity of tetrabromobisphenol A and tetrachlorobisphenol A. *Biochemical & Biophysical Research Communications*, **293**: 554-559.

16. MPI Research (2003). Amendment to the final report. An oral two generation reproductive, fertility and developmental neurobehavioural study of tetrabromobisphenol A in rats (Unpublished report).