

SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of boric acid (E 284) and sodium tetraborate (borax) (E 285) as food additives¹

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS)^{2,3}

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ABSTRACT

The Panel on Food Additives and Nutrient Sources added to Food (ANS) provides a scientific opinion re-evaluating the safety of boric acid (E 284) and sodium tetraborate (borax) (E 285) as food additives in the EU. These additives are authorised in EU for use as preservatives of sturgeon eggs (caviar) up to a maximum concentration of 4 g boric acid/kg. Previous evaluations have been performed by the Scientific Committee for Food (SCF) in 1979, 1988 and 1992, Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1962, EFSA (2004, 2005a, b, 2012) and TemaNord (2002). The Panel concluded that boric acid and sodium tetraborate do not raise concern for genotoxicity. Feeding studies in rats, mice and dogs have demonstrated that the male reproductive system is adversely affected by boric acid and sodium tetraborate. The adverse effects found with boric acid are similar to those obtained from other borates indicating that the boron is the toxicologically active species. The data on toxicokinetics do not indicate differences between laboratory animals and humans. The Panel concluded that based on the NOAEL of 9.6 mg boron/kg bw/day, derived from a developmental toxicity study in rats, and application of an uncertainty factor of 60, a group ADI of 0.16 mg boron/kg bw/day can be established. Exposure to boron from its use as food additive at the highest is 0.04 mg boron/kg bw/day for children, 0.01 mg boron/kg bw/day for adolescents, 0.01 mg boron/kg bw/day for adults, and 0.01 mg boron/kg bw/day for the elderly. Exposure estimates to boron from its use as food additive at the highest 95th percentile for consumers only, for children, adolescents, adults and the elderly would be 0.56, 0.37, 0.13 and 0.15 mg/kg bw/day, respectively. The Panel concluded that it is unlikely that a regular exceedance of the ADI occurs.

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¹ On request from the European Commission, Question No EFSA-Q-2011-00468 and EFSA-Q-2011-00469, adopted on 3 October 2013.

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³ Acknowledgement: The Panel wishes to thank the members of the Working Group B on Food Additives and Nutrient Sources added to Food: Fernando Aguilar, Riccardo Crebelli, Birgit Dusemund, David Gott, Torben Hallas-Møller, Jürgen König, Oliver Lindtner, Daniel Marzin, Inge Meyland, Alicja Mortensen, Iona Pratt, Paul Tobback, Ine Waalkens-Berendsen, Ruud Woutersen for the preparatory work on this scientific opinion and EFSA staff: Joanne Gartlon for the support provided to this scientific opinion.

Suggested citation: EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added to Food, 2013). Scientific Opinion on the re-evaluation of boric acid (E 284) and sodium tetraborate (borax) (E 285) as food additives. EFSA Journal 2013;11(10):3407, 52 pp. doi:10.2903/j.efsa.2013.3407

Available online: www.efsa.europa.eu/efsajournal

KEY WORDS

boric acid, E 284 (CAS Registry Number 10043-35-3), sodium tetraborate, borax, E 285 (CAS Registry Number 1330-43-4), food preservatives

SUMMARY

Following a request from the European Commission, the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to re-evaluate the safety of boric acid (E 284) and sodium tetraborate (E 285) when used as food preservatives.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that has become available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

Boric acid and sodium tetraborate (borax) are authorised as food additives in the EU. They are authorised for use as preservatives of sturgeon eggs (caviar) up to a maximum concentration of 4 g boric acid/kg food by Commission Regulation (EU) 1129/2011. Previous evaluations relevant for assessing the safety for use in food have been performed by the Scientific Committee for Food (SCF) in 1979, endorsed in 1988 and published in 1992 (SCF, 1992)), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1962), EFSA (2004, 2005a, b, 2012) and TemaNord (2002). In addition, boron for use in food and drinking water has been evaluated by the SCF (1979, 1988, 1992, 1998), the Expert Group on Vitamins and Minerals (EVM, 2003) and the World Health Organization (WHO, 2011). The purity criteria for boric acid and sodium tetraborate for use as food additives have been specified in Commission Regulation (EU) 231/2012. Boric acid and sodium tetraborate are also included in Regulation (EU) No 609/2013 regarding substances that may be added for specific nutritional purposes in foods for particular nutritional purposes (PARNUTS).

The Panel noted that the specifications for sodium tetraborate do not contain an assay percentage value.

In 1990, the SCF evaluated boric acid and concluded that boric acid and its salts were toxicologically acceptable for use only as a preservative in genuine caviar. The evaluation noted that a long-term study in rats had demonstrated some accumulation of boric acid in some organs. The evaluation also reported that the compound is nephrotoxic (SCF, 1992).

Studies in animals have shown that boron is readily absorbed following oral exposure in rats (Ku et al., 1991; Usuda et al., 1998), rabbits (Draize and Kelley, 1959), sheep (Brown et al., 1989) and cattle (Owen, 1944; Weeth et al., 1981; EPA, 2004). Absorption of borates is approximately 95 % in rats following ingestion (IPCS, 1998; Vanderpool et al., 1994, as reported in EPA, 2004).

Boron appears rapidly in the blood and body tissues (liver, muscle, colon, testis, epididymides, seminal vesicles, prostate, adrenals) of several mammalian species following ingestion (IPCS, 1998). Most of the boron in blood is associated with the plasma fraction (Moseman, 1994). Boron is distributed throughout the tissues and organs of animals and humans, resulting in concentrations between 0.05 and 0.6 mg/kg fresh weight (Nielsen, 1986, 1989, as reported in EFSA, 2004), with lower levels found in fatty tissues (BfR, 2005). Small quantities of boric acid have also been detected in faeces, saliva, milk and perspiration (Kingma, 1958). The highest levels are found in bone, which possibly represents a second kinetic compartment, as elimination kinetics for bone differ from those of soft tissue and body fluids (EVM, 2003).

The excretion of boron compounds is similar in humans and animals and is predominantly (> 90 %) via urine, regardless of the route of administration (IPCS, 1998). Excretion of boron is relatively rapid and it has a half-life of elimination of 24 hours or less (Nielsen, 1986, 1989; Litovitz et al., 1988, as reported in EFSA, 2004; IPCS, 1998).

In a 90-day study with Sprague–Dawley rats, a pronounced reduction in testicular weights in males of the 87.5 mg boron/kg body weight (bw)/day group was observed (Weir and Fisher, 1972). The effects observed were similar in rats given either boric acid or sodium tetraborate via the diet. The Panel

considered 26.3 mg boron/kg bw/day (equivalent to 152 mg boric acid/kg bw/day) as the No Observed Adverse Effect Level (NOAEL) in this study. Female B6C3F1 mice exposed to boric acid in the diet for 90 days appeared to be less sensitive than males to the lethal effects of boric acid: 8/10 males and 6/10 females in the high-dose group died, and 1/10 males in the lower dose group. Histopathological changes included a dose-related increased incidence of extramedullary haematopoiesis in the spleen in males and females, and hyperkeratosis and acanthosis of the stomach in eight males and three females at the highest dose (IPCS, 1998; ECETOC, 1995).

Boric acid or sodium tetraborate fed to beagle dogs for 90 days at 0, 0.44, 4.38 or 43.75 mg boron/kg bw/day demonstrated a significant decrease in testes weight in the mid- and high-dose groups. Four out of 5 high-dose dogs showed complete testes atrophy and the remaining high-dose dog had partial testes atrophy (Weir and Fisher, 1972). Paynter (1963, reported in USDA, 2006) reported similar testes lesions in dogs exposed to 268 mg sodium tetraborate/kg bw/day for 90 days.

Reversibility of testicular lesions was evaluated in F344 rats dosed with boric acid for 9 weeks and assessed for recovery up to 32 weeks post treatment. Inhibited spermiation was exhibited at 5.6 µg boron/mg tissue, whereas inhibited spermiation progressed to atrophy at 11.9 µg boron/mg testes, with no boron accumulation in the testes to levels greater than those found in the blood during the 9-week period. After treatment, serum and testis boron levels in all dose groups fell to background levels. Inhibited spermiation was reversed by 16 weeks post treatment, but focal atrophy was detected that did not show recovery up to 32 weeks post-treatment (Ku et al. 1993).

Genotoxicity studies in bacteria and in mammalian cells *in vitro*, addressing several genetic end-points (gene mutation, chromosomal aberrations, micronuclei, sister chromatid exchanges), as well as an *in vivo* micronucleus test in mice, published after the previous EFSA evaluations (EFSA, 2004, 2005a, b), do not provide any indications of genotoxic potential of boric acid and sodium tetraborate. Thus, based on the available evidence, the Panel concluded that boric acid and sodium tetraborate do not raise concern for genotoxicity.

Microscopic changes in the tissues of mice, rats and dogs exposed to boric acid or sodium tetraborate for 2 years involved primarily the kidneys and testes. Glomerular and tubular damage has been noted in the kidneys. Glomerular damage consisted of changes in the permeability of the capillaries, and tubular damage consisted of cellular vacuolisation and shedding of cells into the tubular lumen. In male reproductive organs, the effects of boric acid and sodium tetraborate included inhibition of spermiation in stage IX and X tubules, followed by germ cell loss, changes in epididymal sperm morphology and caput sperm reserves, decreased serum testosterone levels and testicular atrophy. In males, the effects on reproductive organs have been reported in oral exposure studies at doses as low as 29 mg boron/kg bw/day in dogs exposed for 2 years to dietary boric acid or borax (Weir and Fisher, 1972).

Results from animal experiments demonstrate that boric acid adversely affects fertility and development. Feeding studies in different animal species (rats, mice and dogs) have consistently demonstrated that the male reproductive system is the principal target in experimental animals, although effects on the female reproductive system have also been reported. Testicular damage ranging from mildly inhibited spermiation to complete atrophy has been demonstrated following oral administration of boric acid. In a 2-year study in rats, effects on male fertility were observed at lower dose levels compared to dose levels where signs of general toxicity appeared. For this 2-year study, 17.5 mg boron/kg bw/day was considered a NOAEL for male and female fertility (EFSA, 2004).

In 1998, the IPCS concluded that the mechanism by which boric acid and borates induce testicular atrophy is unclear. However, as boron does not accumulate in the testes, it may be possible that decreased testosterone production is due to CNS-mediated mechanisms. It is further reported that it is unlikely that hormone changes cause testicular atrophy as spermatogenesis has been shown to be maintained even in the presence of significantly decreased intra-testicular testosterone levels (ECETOC, 1995).

Developmental toxicity of boric acid was investigated in the rat, the rabbit and the mouse. In the rat, developmental toxicity (decreased fetal weight at 13.7 mg boron/kg bw/day) occurred in the absence of marked maternal toxicity. For developmental toxicity in rats, a NOAEL of 9.6 mg boron/kg bw/day (equivalent to 55 mg boric acid/kg bw/day) has been derived by Price et al. (1996a).

The adverse effects of boric acid on development and fertility observed across species were very similar, both in nature and effective doses. Further, the adverse effects obtained with boric acid are comparable to those obtained from other borates, thus indicating that boron is the toxicologically active species. The available data on toxicokinetics do not indicate differences between laboratory animals and humans. It is not known whether there are significant differences in the toxicodynamics between humans and laboratory animal models and, in the absence of such knowledge, it must be assumed that the effects seen in animals could occur in humans. On the basis of toxicokinetic and toxicodynamic considerations, it is assumed that the animal data are relevant to humans. This is further underlined by the fact that (1) there are indications that boric acid is able to cross the human placenta and that (2) up to now, epidemiological studies in humans are insufficient to demonstrate the absence of an adverse effect of inorganic borates on fertility (ECHA, 2010).

In order to set a group acceptable daily intake (ADI), the Panel considered that this should be expressed as boron equivalents. The Panel identified the study of Price et al. (1996a) as the pivotal study and agreed with the NOAEL defined by the authors of this study. The NOAEL is 55 mg boric acid/kg bw/day (equivalent to 9.6 mg boron/kg bw/day). As the pivotal effect that serves as the basis for the ADI is developmental toxicity, pregnant women are the subgroup of interest in this regard (WHO, 2009). The glomerula filtration rate (GFR) being the critical physiological process for boron toxicokinetics, the default toxicokinetic UF for human variability was adjusted by the EFSA Panel on Nutrition, Dietetic Products and Allergies (NDA) and the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) on the clearance in pregnant women. This approach leads to a toxicokinetic UF factor of 1.8 instead of 3.2, whereas the intraspecies toxicodynamic UF of 3.2 remained unchanged. This ultimately resulted in an uncertainty factor (UF) of 60 instead of 100.

When applying an UF of 60 (EFSA, 2004, 2005a, b, 2012), the group ADI for boric acid and sodium tetraborate, expressed as boron equivalents, is 0.16 mg boron/kg bw/day.

The results of several human case studies described in the literature and collected from poisoning centres demonstrated that the average dose of boric acid required to produce clinical symptoms is presumed to be within the range 100 mg to 55.5 g, which is far above the ADI established by the Panel.

Exposure to boric acid and sodium tetraborate from its use as a food additive in caviar is based on the assumption that the consumption data from the EFSA Comprehensive Food Consumption Database reported for the food category “fish roe” cover the consumption of caviar. The Panel noted that these estimates are conservative, since it is unlikely that all foods falling into this food category actually are caviar. Although the EFSA Comprehensive Food Consumption Database provides information on food consumption from 32 different dietary surveys carried out in 22 different European countries, only those countries with statistically reliable data on the consumption of the food category “fish roe” were included in exposure estimate calculations at the mean and at the 95th percentile. Using the maximum permitted level (MPL) of 4 g/kg food of boric acid or sodium tetraborate expressed as boric acid, these exposure estimates result in mean exposures to boric acid and sodium tetraborate in the range 0.00–0.21 mg boric acid/kg bw/day for children, 0.00–0.06 mg boric acid/kg bw/day for adolescents, 0.00–0.08 mg boric acid/kg bw/day for adults and 0.00–0.05 mg boric acid/kg bw/day for the elderly. Exposure estimates at the 95th percentile could only be calculated for Denmark and Sweden (Denmark only for the elderly) and are in the range 2.81–3.17 mg boric acid/kg bw/day for children, 1.47–2.11 mg boric acid/kg bw/day for adolescents, 0.72–0.74 mg boric acid/kg bw/day for adults and 0.88 mg boric acid/kg bw/day for the elderly.

The estimates refer to the exposure to boric acid and sodium tetraborate expressed as boric acid. Based on its boron content of 17.5 %, the highest average exposure to boron across European Member States is 0.04 mg/kg bw/day for children, 0.01 mg/kg bw/day for adolescents, 0.01 mg/kg bw/day for adults and 0.01 mg/kg bw/day for the elderly and the very elderly. The exposure to boron from the use of boric acid and sodium tetraborate as food additives at the highest 95th percentile, for consumers only, would be 0.56, 0.37, 0.13 and 0.15 mg/kg bw/day for children, adolescents, adults and the elderly, respectively.

The Panel also noted that this exposure scenario is only valid for its present single authorisation for caviar, whereas a detailed exposure assessment would be needed if the authorisation would be extended to other products (e.g. fish roe other than sturgeon eggs).

The Panel identified a NOAEL of 55 mg boric acid/kg bw/day (equivalent to 9.6 mg boron/kg bw/day). When applying an UF of 60, the group ADI for boric acid and sodium tetraborate, expressed as boron equivalents, is 0.16 mg boron/kg bw/day.

For children and adolescents, at the highest 95th percentile, exposure estimates indicate exceedance of this ADI. However, exposure to boron from its use as a food additive in the form of boric acid and sodium tetraborate in caviar is unlikely to occur on a regular basis. Therefore, the Panel noted that even at high consumption and in consumers only, it is unlikely that a regular exceedance of the ADI occurs.

However, the Panel also noted that exposure to boron from its natural occurrence in the diet and from other sources (food supplements, food contact materials, feed for food-producing animals, cosmetics, oral hygiene products, etc.) may already lead to exposures exceeding the ADI.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008⁴ of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under Regulation (EU) No 257/2010⁵. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU⁶ of 2001. The report “Food additives in Europe 2000”⁷ submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010⁵ of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008⁴ of the European Parliament and of the Council on food additives

⁴ OJ L 354, 31.12.2008, p. 16.

⁵ OJ L 80, 26.03.2010, p. 19.

⁶ COM(2001) 542 final.

⁷ Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers. TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of boric acid (E 284) and sodium tetraborate (E 285) when used as food additives. In the present opinion, boric acid and sodium tetraborate concentrations are also expressed as boron equivalents.

Boric acid and sodium tetraborate are authorised as food additives in the EU. They are authorised for use as preservatives of sturgeon eggs by Commission Regulation (EU) 1129/2011.⁸ They are also found in many non-food products. Previous evaluations relevant for assessing the safety for use in food have been performed by the Scientific Committee for Food (SCF, 1992), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1962), EFSA (2004, 2005a, b, 2012) and TemaNord (2002).

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that has become available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

2. Technical data

2.1. Identity of the substances

2.1.1. Boric acid

Boric acid (E 284) is an inorganic compound with the molecular formula H_3BO_3 and molecular weight of 61.84 g/mol. Boric acid contains 17.5 % in weight of boron. The Chemical Abstracts Service (CAS) Registry Number for boric acid is 11113-50-1 and the European Inventory of Existing Commercial chemical Substances (EINECS) number is 234-343-4. According to the United States Adopted Names Council (USAN), boric acid has as CAS Registry Number 10043-35-3. The EINECS number is 233-139-2 (ChemIDplus, accessed 2011). The chemical name is boric acid. The structural formula of boric acid is given in Figure 1.

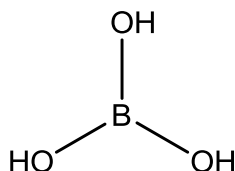


Figure 1: Structural formula of boric acid

Boric acid is a weak acid, with a pK_a of 9.15. At low concentrations in aqueous solutions below pH 7, boric acid exists predominantly as undissociated boric acid (B(OH)_3); above pH 10, the metaborate anion B(OH)_4^- becomes the main species in solution. Between pH 6 and pH 11 and at high concentrations ($> 0.025 \text{ mol/L}$), highly water-soluble polyborate ions such as $\text{B}_3\text{O}_3(\text{OH})_4^-$, $\text{B}_4\text{O}_5(\text{OH})_4^{2-}$ and $\text{B}_5\text{O}_6(\text{OH})_4^-$ are formed (IPCS, 1998; WHO, 2009).

Boric acid is colourless, odourless and exists in the form of transparent crystals or white granules or powder. It is slightly unctuous to the touch. It occurs in nature as the mineral sassolite. Boric acid is water soluble at 47 200 mg/L at 20 °C (ECHA, 2010). It is slightly soluble in ethanol (Haynes, 2010).

Synonyms are boracic acid, orthoboric acid and borofax.

⁸ Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) N°1333/2008 of the European Parliament and of the Council establishing a Union list of food additives. OJ L 295, 12.11.2011.

Boric acid has a melting point of approximately 171 °C (Commission Regulation (EU) 231/2012⁹) and a boiling point of 300 °C (International Chemical Safety Cards, 1994; IUCLID, 2000). It has a specific gravity of 1.51 and a relative density in water of 1.4 at 15 °C (International Chemical Safety Cards, 1994). Two values for the partition coefficient ($\log P_{\text{octanol/water}}$) have been found in the literature (0.757 at 25 °C and 0.175) (Strong, 2001; EVM, 2002; ECHA, 2010).

2.1.2. Sodium tetraborate

Sodium tetraborate (E 285) is an inorganic compound that exists in anhydrous and hydrated forms: the chemical formula of the anhydrous form is $\text{Na}_2\text{B}_4\text{O}_7$; its CAS Registry Number is 1330-43-4, its EINECS Registry number is 215-540-4 and its molecular weight is 201.27 g/mol. The chemical formula of the decahydrate form is $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$; its CAS Registry Number is 1303-96-4, its EINECS Registry number is identical to that of the anhydrous form and its molecular weight is 381.42 g/mol. The structural formula of sodium tetraborate anhydrous is given in Figure 2.

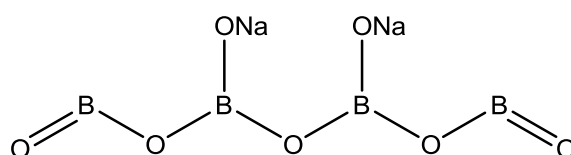


Figure 2: Structural formula of sodium tetraborate anhydrous

Sodium tetraborate decahydrate has a specific gravity of 1.73; it is soluble in cold water (47.1 g/L at 20 °C), very soluble in hot water and insoluble in acids and in ethanol. It is a colourless, monoclinic crystalline salt; it also occurs as a white powder. It readily effloresces, especially on heating. It loses all water of hydration when heated above 320 °C. The anhydrous form is slightly soluble in methanol; the decahydrated form is insoluble in ethanol (IPCS, 1998; Haynes, 2010).

The Panel noted that in literature the term ‘borax’ is used for a number of closely related minerals or chemical compounds that differ in their crystal water content, but usually borax refers to sodium tetraborate decahydrate. Commercially sold borax is usually partially dehydrated.

2.2. Specifications

Commission Regulation (EU) No 231/2012⁹ of 9 March 2012 laid down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008⁴ of the European Parliament and of the Council. There are no JECFA specifications for boric acid or sodium tetraborate.

⁹ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

Table 1: Specifications for boric acid (E 284) according to Commission Regulation (EU) No 231/2012⁹

	Specifications
Description	Colourless, odourless, transparent crystals or white granules or powder; slightly unctuous to the touch; occurs in nature as the mineral sassolite
Assay	Content not less than 99.5 %
Identification	
Melting point	At approximately 171 °C
Burns with a green flame	
pH of a 3.3 % aqueous solution	Between 3.8 and 4.8
Purity	
Peroxides	No colour develops with added KI solution
Arsenic	Not more than 1 mg/kg
Lead	Not more than 5 mg/kg
Mercury	Not more than 1 mg/kg

Table 2: Specifications for sodium tetraborate (E 285) according to Commission Regulation (EU) No 231/2012⁹

	Specifications
Description	Powder or glass-like plates becoming opaque on exposure to air; slowly soluble in water
Assay	–
Identification	
Melting range	Between 171 °C and 175 °C with decomposition
Purity	
Peroxides	No colour develops with added KI solution
Arsenic	Not more than 1 mg/kg
Lead	Not more than 5 mg/kg
Mercury	Not more than 1 mg/kg

The Panel noted that the specifications for sodium tetraborate do not contain an assay percentage value.

2.3. Manufacturing process

The manufacturing process of boric acid was described in EHC 204 (IPCS, 1998):

“Boric acid is produced mainly from sodium- or calcium-containing borate ores. The mined ore is crushed and ground before being reacted with sulfuric acid in the presence of a hot aqueous recycled liquor containing some boric acid. The resultant slurry contains insoluble gangue and either calcium or sodium sulfate by-product. After separation of unwanted insoluble gangue, recovery of the boric acid product is similar to that for borax (personal communication from Borax US to the IPCS, 1995).”

The manufacturing process of sodium tetraborate was described in EHC 204 (IPCS, 1998). *“Disodium tetraborate (borax) containing 5 or 10 molecules of water is produced mainly from sodium-containing borate ores. The mined ore is crushed and ground before dissolution in a hot recycled aqueous solution containing some borax. Insoluble gangue (clay particles) present in the hot slurry is separated off to produce a clear concentrated borax solution. Evaporative cooling of this solution to selected temperatures results in crystallisation of the desired products, which are then separated from the residual liquor and dried (personal communication from Borax US to the IPCS, 1995).”*

2.4. Methods of analysis in food

EHC 204 (IPCS, 1998) reports that the preferred method for analysis of boron in food is inductively coupled plasma atomic emission spectroscopy and that inductively coupled plasma methods are also the preferred method for the analysis of the low levels of boron found in biological and environmental samples. It is indicated that colorimetric methods must be used with caution (IPCS, 1998). Fast quantitation analysis by gas chromatography–mass spectrometry (GC-MS) has also been described (Lin-Min Zeng et al., 2010).

2.5. Reaction and fate in food

EHC 204 states that boric acid and sodium tetraborates are considered stable except for dehydration, which occurs at high temperatures (IPCS, 1998).

2.6. Case of need and proposed uses

Maximum permitted levels (MPLs) of boric acid or sodium tetraborate expressed as boric acid have been defined in Annex II of Regulation (EC) No 1333/2008¹⁰ on food additives for use in foodstuffs.

Currently, boric acid and sodium tetraborate are authorised food additives for preserving sturgeon eggs (caviar) in the EU with a MPL of 4 g boric acid or sodium tetraborate expressed as boric acid/kg.

The purity criteria for boric acid and sodium tetraborate for use as food additives have been specified in Commission Regulation (EU) 231/2012.⁹

2.7. Information on existing authorisations and evaluations

Boric acid and sodium tetraborate for use in food have been previously evaluated by the SCF in 1979, endorsed in 1988 and published in 1992 (SCF, 1992), EFSA (2004a, b, 2005, 2012) and JECFA (1962). In addition, boron in natural mineral waters has been evaluated by the SCF (1998), the EVM (2003) and the WHO (2011). The NDA Panel derived a tolerable upper intake level (UL) value of 10 mg boron/person/day for adults, and 3, 4, 5, 7 and 9 mg boron/day for children aged 1–3, 4–6, 7–10, 11–14 and 15–17 years of age, respectively derived by extrapolating from the UL for adults on a body surface area basis (EFSA, 2004). Taking into account the aforementioned UL values, the CONTAM Panel concluded that it is very unlikely that intake by the general population including children older than 14 years would exceed these levels even at the highest reported levels in bottled water. For children from 1 to 14 years of age, a maximum limit of 1.5 mg boron/L in bottled water would protect these children from exceeding the UL (EFSA, 2005).

Boric acid and sodium tetraborate may be used as mineral substances in the manufacture of food supplements according to Commission Regulation (EC) No 1170/2009 of 30 November 2009¹¹. No limits for the use of boric acid and sodium tetraborate in food supplements have been set in the EU legislation.

In 1990, the SCF evaluated boric acid and concluded that boric acid and its salts are toxicologically acceptable for use only as a preservative in genuine caviar. The opinion noted that a long-term study in rats had demonstrated some accumulation of boric acid in some organs. The evaluation also reported that the compound is nephrotoxic (SCF, 1992).

In the 1998 SCF opinion on boron in natural mineral waters, a NOAEL of 9.6 mg/kg bw was established based on decreased average fetal body weight in a rat developmental toxicity study (Price

¹⁰ Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 354, 31.12.2008, p. 16.

¹¹ Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. Official Journal L 314, 01.12.2009 pp.36-42.

et al., 1996a). The usual UF of 100 was applied, leading to the establishment of a tolerable daily intake (TDI) of 0.1 mg boron/kg bw/day for humans. The SCF opinion (SCF, 1998) concluded that assuming a “consumption of 2 litres of natural mineral water/person/day and the allocation of 10 % of the TDI to this source of exposure would lead to a guideline value of 0.3 mg/L”.

The WHO Guidelines for drinking water (WHO, 2011) report a TDI of 0.17 mg/kg body weight, based on a benchmark dose (5 % lower confidence limit) (BMDL₀₅) of 10.3 mg/kg bw/day for developmental toxicity, i.e. decreased fetal body weight in rats, and a UF of 60 (i.e. 10 for interspecies variation and 6 for intraspecies variation).

Using the aforementioned NOAEL of 9.6 mg/kg bw/day (SCF, 2000), EFSA (2004, 2005a, b) established a UL of 0.16 mg/kg bw/day using a UF of 60 calculated following the approach proposed by Dourson et al. (1998).

A scientific opinion on the safety evaluation of the active substances sodium borohydride and palladium acetate for use in active food contact materials by the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) has recently been published (EFSA, 2012). This opinion deals with the risk assessment of sodium borohydride (CAS No 16940-66-2) and palladium acetate (CAS No 3375-31-3) reduced to palladium (CAS No 7440-05-3) in plastic during the manufacturing process, when used in combination as an oxygen-absorbing system in food contact materials. The CEF Panel used the UL of 0.16 mg boron/kg bw/day, equivalent to 10 mg boron/person/day in adults, as established by the EFSA NDA Panel in 2004 (EFSA, 2004). Migration of boron into food was up to 0.09 mg/kg. Based on the default assumption for food contact materials that an adult may consume daily up to 1 kg of food in contact with food contact materials containing boron, the migration of 0.09 mg/kg food would correspond to an intake of 0.09 mg boron/adult/day, which is 111 times lower than the UL set by the NDA Panel. The CEF Panel concluded that there is no safety concern for the consumer from the use of these substances in oxygen-absorbing systems in food contact materials.

In 2002, boric acid and sodium tetraborate were evaluated by TemaNord. Boric acid and sodium tetraborate were not recommended for wide use as food additives, but due to their restricted use in sturgeons eggs (genuine caviar), exposure was considered to be limited and no further action was recommended (TemaNord, 2002).

In 2008, the 29th meeting of the Codex Committee on Fish and Fishery Products noted that the Committee on Food Additives had responded “*that the use of boric acid was not allowed since JECFA had not established an ADI for boric acid and that further scientific evidence was needed for its re-evaluation*” (Codex Alimentarius, 2008).

The use and sale of sodium tetraborate (boric acid) as a food ingredient is not permitted in Canada, the USA, Australia, New Zealand and Hong Kong (Canadian Food Inspection website, accessed 18 April 2011).

The IPCS defined an acceptable range of boron intake for adults as 1–13 mg/day (IPCS, 1998). The FNB (2001) developed ULs of 20 mg boron/day for adults, 17 mg boron/day for pregnant women aged 14–18 years and 20 mg boron/day for pregnant women aged 19–50 years; a UL for infants was judged indeterminable (EPA, 2004).

In 2005, the German Bundesinstitut für Risikobewertung (BfR) performed a health assessment on the addition of boric acid or sodium tetraborate to food supplements. However, due to a lack of data on total intake including from non-food sources, and regarding the toxicological characterisation they advised against using boron in the form of boric acid or sodium tetraborate in food supplements (BfR, 2005).

Boric acid and sodium tetraborate are also included in Regulation (EU) No 10/2011¹² on plastic materials and articles intended to come into contact with food. A specific migration limit (SML(T)) of 6 mg boron/kg food is applied for boric acid only in Annex II, and for the combined total of boric acid, anhydrous sodium tetraborate and sodium tetraborate in Annex III. The SML(T) value was derived from the group TDI of 0.1 mg boron/kg bw/day established by the SCF (1998) and the assumption that a 60-kg adult ingests 1 kg of food daily containing these substances (Directive 2002/72/EC).

Regulation (EU) No 609/2013,¹³ regarding substances that may be added for specific nutritional purposes in PARNUTS, states that boric acid and sodium tetraborate may be used in dietetic foods as sources of boron.

Various organisations have derived reference values for maximum intakes of boron without associated adverse effects. The IPCS derived a TDI 0.4 mg boron/kg bw/day (IPCS, 1998). ECETOC derived a TDI based on fertility and developmental effects of 19.2 mg boron/day for a 60-kg adult (ECETOC, 1995). The Expert Group on Vitamins and Minerals defined a safe UL for daily consumption over a lifetime of 0.16 mg boron/kg bw/day, equivalent to 9.6 mg boron/day for a 60-kg adult (EVM, 2003). The US Food and Nutrition board assigned a UL of 20 mg boron/day based on a 60-kg adult (≥ 19 years old) (FNB, 2001). The EPA defined a reference dose (RfD) (i.e. the dose in humans that is likely to be without an appreciable risk of deleterious non-cancer effects during a lifetime) of 0.2 mg boron kg bw/day (Allen et al., 1996; EPA, 2004).

Upper limits have been established for the use of boric acid, borates and tetraborates in various categories of cosmetic products in the EU, including oral hygiene products, which are not more than 5 % in talc, not more than 0.1 % in oral hygiene products, and not more than 3 % in other products (IPCS, 1998).

The following MRLs for boron have been defined: 0.2 mg boron/kg bw/day for acute (≤ 14 days) and intermediate (16–364 days) exposures; no MRL has been defined for chronic exposures (ATSDR, 2011).

In the EU, boric acid and sodium tetraborate are classified as class 1B reproductive toxicants (Regulation (EC) No 790/2009¹⁴).

2.8. Exposure assessment

2.8.1. Food consumption data used for exposure assessment

2.8.1.1. EFSA Comprehensive European Food Consumption Database

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was created, using existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data from the most recent national dietary survey in their country at the level of consumption by the individual consumer from the most recent national dietary survey in their country (see EFSA guidance on Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011a)).

Overall, the food consumption data gathered at EFSA were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

¹² Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.01.2011, p. 1.

¹³ Commission Regulation (EU) No 609/2013 of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control. OJ L 181, 29.3.2013, p. 35.

¹⁴ Commission Regulation (EC) No 790/2009 of 10 August 2009 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures. OJ L 235, 5.9.2009, p. 1.

The ANS Panel considered that chronic dietary exposure to boric acid and sodium tetraborate needs to be assessed. Therefore, as suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011a), dietary surveys with only one day per subject were not considered for the calculation of exposure to boric acid and tetraborate, as they are not adequate to assess repeated dietary exposure. Similarly, subjects who participated for only one day in the dietary studies where the protocol prescribed more reporting days per individual were excluded. Calculations were performed using individual body weights.

The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents, adults and the elderly.

For the present assessment, food consumption data were available from 28 different dietary surveys carried out in 17 different European countries. Due to the limited data reported on food consumption of fish roe, the population groups and countries as listed in Table 3 were considered for the exposure estimates of boric acid and tetraborate.

Table 3: Population groups considered for the exposure estimates of boric acid and sodium tetraborate

Population	Age range	Countries with chronic food consumption statistics
Children ¹⁵	From 36 months up to and including 9 years of age	Denmark, Finland, France, Germany, Sweden
Adolescents	From 10 up to and including 17 years of age	Belgium, Cyprus, Denmark, France, Germany, Italy, Sweden
Adults	From 18 up to and including 64 years of age	Belgium, Denmark, Finland, France, Germany, Ireland, Italy, Latvia, Spain, UK
The elderly ¹⁵	Older than 65 years	Belgium, Germany, Denmark, Finland, France

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b).

Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Annex II of Regulation (EC) No 1333/2008⁴, part D, to perform exposure estimates.

2.8.2. Exposure to boric acid and sodium tetraborate from its use as food additive

Exposure estimates have been performed using the MPL combined with national consumption data for the food category “fish roe” for the four population groups: children, adolescents, adults, and the elderly and the very elderly. Details of national consumption surveys are presented in Table 3.

The Panel noted that its estimates should be considered as being conservative as it is assumed that all foods reported within the category “fish roe” are caviar and that they contain the preservative boric acid and sodium tetraborate added at the MPL.

In this scenario, for all populations, data from the Comprehensive database were used by the Panel to calculate the mean and high-level exposures to boric acid and sodium tetraborate based on the EU food nomenclature (FCS) and using the MPL.

High-level exposure (95th percentile of consumers only) was calculated for those population groups and those countries only where the number of consumers was sufficient (Denmark and Sweden).

¹⁵ The terms “children” and “the elderly” correspond, respectively, to “other children” and the combined “elderly” and “very elderly” in the Guidance of EFSA on the ‘Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment’ (EFSA, 2011a).

Table 4 summarises the anticipated exposure of all four population groups to boric acid and sodium tetraborate.

As caviar is the only food where the use of boric acid and sodium tetraborate is authorised, this food is the only contributor to the exposure from its use as food additive. As no data on normal use levels were available to the Panel, exposure estimates were calculated by using the MPL only.

Table 4: Summary of anticipated exposure to borate and sodium tetraborate expressed as boric acid using the MPL in four population groups (mg/kg bw/day)

	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly and the very elderly (> 65 years)
Mean exposure	0.00–0.21	0.00–0.06	0.00–0.08	0.00–0.05
High level ¹⁶	2.81–3.17 ^(a)	1.47–2.11 ^(a)	0.72–0.74 ^(a)	0.88 ^(b)

(a): Data from Denmark and Sweden.

(b): Data from Denmark.

2.8.3. Exposure from other sources

2.8.3.1. Exposure from the diet

The major source of exposure to boron is resulting from its natural occurrence in foods. Two recent reports (Hunt et al., 1991; Anderson et al., 1994) provide an adequate indication of the amounts of boron found in various foods. The richest sources of boron are fruits, vegetables, pulses, legumes and nuts. Dairy products, fish, meats and most grains are poor sources of boron. Based on the United Kingdom National Food Survey (MAFF, 1991), the mean dietary intake of boron in the United Kingdom ranges from 0.8 to 1.9 mg/day. It should be noted that increased consumption of specific foods with high boron content will increase boron intake significantly; for example, one serving of wine or avocado provides 0.42 or 1.11 mg, respectively (Anderson et al., 1994). Moreover, for the population obtaining their drinking water from the 10 % of the public water systems that provide water containing > 0.4 mg boron/L, water used for drinking and cooking may be the major, or a significant, source of boron. Based on the preceding values, the mean daily intake of boron in the diet is judged to be near 1.2 mg/day. Mineral water consumption may also contribute to the overall exposure to boron (EFSA, 2005b).

As boric acid and borates are listed in Annex II of Council Regulation (EEC) No 2377/90¹⁷ with no maximum residue limit for all food-producing animal species, no estimates can be calculated for this source of exposure. However, the Panel noted that the use of boric acid and borates in animal feed will also contribute to the overall exposure.

Multivitamin–multimineral supplements for human use, listed as being for non-prescription use, contain up to 150 µg of boron from calcium borate, magnesium borate and sodium borate, all in magnesium oxide (Health Canada, 2007). Boric acid and sodium borate may be used as mineral substances in the manufacture of food supplements according to Regulation (EC) No. 1170/2009 of 30 November 2009.¹⁸

¹⁶ Typically 95th percentile of consumers only.

¹⁷ Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. OJ L 224, 18.8.1990, p. 1.

¹⁸ Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements OJ L 314, 1.12.2009, pp. 36–42.

Body-building supplements contain 1.5–10 mg boron per serving, with a median of 4 mg boron per serving. These supplements could result in daily exposures of 1.5–30 mg boron, as some are taken up to three times a day.

2.8.3.2. Exposure from sources other than foods

Food contact materials may also contribute to the exposure to boric acid and sodium tetraborate, as they are included in Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food. Based on the assumption that a 60 kg adult ingests 1 kg food daily containing these compounds and in respect to the specific migration limit of 6 mg boron/kg food, exposure estimates would be increased for adults by 0.1 mg boron/kg bw/day. For children with a body weight of 15 kg and the same assumption of ingestion of 1 kg food daily, this use would result in additional exposure of 0.4 mg boron/kg bw/day.

Boric acid, borax and other borates are used in a great array of consumer goods. Products in which boron compounds are used include soaps and detergents (as a bleaching agent in the form of perborate), preservatives, adhesives, porcelain, enamel, leathers, carpets, artificial gems, high-contrast photographic materials, wicks, electric condensers, fertilisers, insecticides and herbicides (Moore, 1997). Sodium borate and boric acid are also widely used in numerous cosmetic products, including make-up, skin and hair care preparations, deodorants, moisturising creams, breath fresheners and shaving creams, at concentrations up to 5 % (FDA, 1981; Beyer et al., 1983). Boric acid exposures from some personal care products, estimated by the EU Subcommittee on “Cosmetic Ingredients”, include 0.09–0.46 mg boric acid/kg bw/day for oral hygiene products, 0.03 mg boric acid/kg bw/day for eye products and 0.25 mg boric acid/kg bw/day for deodorants (SCC opinion XXIV/1820/95, as reported in IPCS, 1998).

The EU permits boric acid in certain consumer products (e.g. 3 % boric acid in eye products, up to 0.5 % in oral hygiene products) (SCC opinion XXIV/1820/95). Usage data provided by the industry (EU Technical Guidance Document, 1995, as reported in EVM, 2002) allow the estimation of total exposure to boric acid from consumer products as 22.8 mg/day per person, equivalent to 4 mg boron/day. If absorption across the dermis is assumed to range from 1 to 10 %, the absorbed boron dose from the source is 0.04–0.4 mg boron/day. The Task Group felt that 0.1 mg/day would serve as a reasonable average estimate of boron exposure from consumer products.

3. Biological and toxicological data

3.1. Absorption, distribution, metabolism and excretion

3.1.1. Absorption

The Panel considered that due to its ionisation properties (pK_a of 9.15) the unionised form of boric acid should be absorbed by a diffusion process in the stomach where the pH is acidic.

At the low concentrations and near-neutral pH found in most biological fluids, monomeric $B(OH)_3$ would be the predominant species present (with some $B(OH)_4^-$), regardless of whether the boron source is boric acid or one of the borates (IPCS, 1998).

At low concentrations, inorganic borates can be converted to boric acid at physiological pH in the aqueous layer overlying mucosal surfaces prior to absorption (EFSA, 2004). Therefore, borates and boric acid have similar absorption potentials (BfR, 2005).

Sodium tetraborate was administered orally to male rats ($n = 20$; 13 weeks old, bw 240–260 g) at 11 different doses ranging from 0 to 4 mg boron/kg bw (equivalent to 0–23 mg boric acid/kg bw) (Usuda et al., 1998). Recovery of boron in 24-hour urine accounted for 99.6 ± 9.7 % of the administered dose, demonstrating essentially total bioavailability of the orally administered boron dose in rats. The mean

plasma half-life was 4.64 ± 1.19 hours and the volume of distribution was 14.2 ± 3.0 l/kg bw (EPA, 2004).

The EPA review of boron reports that studies in animals have shown that boron is readily absorbed following oral exposure in rats (Ku et al., 1991; Usuda et al., 1998), rabbits (Draize and Kelley, 1959), sheep (Brown et al., 1989) and cattle (Owen, 1944; Weeth et al., 1981) (EPA, 2004). Absorption of borates is approximately 95 % in rats following ingestion (IPCS, 1998). Using mass spectrometry and the boron-10 isotope, Vanderpool et al. (1994, as reported in EPA, 2004) showed that fasted rats fed 20 µg of boron-10 isotope in the diet eliminated 95 % of the boron-10 isotope in the urine and 4 % in the faeces within 3 days after dosing, which suggests that > 90 % of orally administered boron is absorbed from the gastrointestinal tract.

3.1.2. Distribution

Boric acid measured as boron was evenly distributed throughout the body fluids, via passive diffusion (IPCS, 1998; EVM, 2003; EFSA, 2004). Boron appeared rapidly in the blood and body tissues of several mammalian species following ingestion (IPCS, 1998). Most of the boron in blood was associated with the plasma fraction (Moseman, 1994). Boron administered at various dose levels was distributed throughout the tissues and organs of animals and humans at concentrations between 0.05 and 0.6 mg/kg fresh tissue (Nielsen, 1986, 1989, as cited in EFSA, 2004) with lower levels found in fatty tissues (BfR, 2005), which is substantiated by its partition coefficient of -0.757 (IUCLID, 2000). Kingma (1958) reported that small quantities of boric acid have also been detected in faeces, saliva, milk and perspiration. The highest levels were found in bone, which possibly represents a second kinetic compartment, as elimination kinetics for bone differ from those of soft tissue and body fluids (EVM, 2003).

Adult male F344 rats (30 animals/group) were fed a control diet (<20 mg boron/kg) or a diet containing 9000 mg boric acid/kg (equivalent to 1 575 mg boron/kg diet and approximately 68 mg boron/kg bw/day) for up to 7 days (Ku et al., 1991, as reported in IPCS, 1998). Six animals per group were killed 1, 2, 3, 4 and 7 days after the start of exposure and boron levels were determined for all major tissues and organs. The boron concentrations were reported to be comparable in all tissues examined (plasma, liver, kidney, muscle, colon, brain, testis, epididymis, seminal vesicles, prostate, adrenals). Most tissues appeared to reach steady-state boron levels (12–30 mg boron/kg tissue) within 3–4 days following administration, levels being 3- to 20-fold higher than the levels in controls. Adipose tissue contained only 20 % of the boron found in other tissues (3.78 mg/kg tissue). Bone boron levels (47.4 mg/kg tissue) indicated greater accumulation in the bones than in other tissues and levels continued to increase throughout the exposure period. The higher affinity of boron for bone was reported to be indicative of a second kinetic component in which a small percentage of the boron absorbed may be sequestered. The elimination kinetics for bone also differ from those for soft tissue and body fluids (IPCS, 1998). The EPA evaluation on boron cited additional references in support of these findings, i.e. accumulation of boron in bones in rats (Forbes and Mitchell, 1957), lack of accumulation in the testes (Lee et al., 1978; Treinen and Chapin, 1991) and the absence of appreciable accumulation of boron in the epididymis (Treinen and Chapin, 1991; EPA, 2004).

Various rat tissue samples and blood samples from the 7-day study of Ku et al. (1991) and a 28-day study of Treinen and Chapin (1991) were measured for boron content (Moseman et al., 1994). After 28 days on the diet, all boron detected in the blood appeared to be associated with plasma. For both studies it was reported that levels of boron in the soft tissues were not substantially higher than those found in the blood and that fat tissues contained significantly less boron than levels found in soft tissues. Levels found in the testes were not higher than those found in other tissues. On a diet of 9 000 mg boric acid/kg (equivalent to 68 mg boron/kg body weight/day), the boron content in bone increased 20-fold after 1 day and was increased 50-fold after 1 week on the diet but decreased rapidly when the rats were taken off the diet. However, levels in the bone were still three times control levels 5 months after terminating the boron diet. Based on the studies of Treinen and Chapin (1991) and Ku et al., (1991), the ECETOC evaluation (1995) reported that, after 28-day or 9-week exposures to high

levels of boron in either the drinking water or the diet, the return to steady-state levels within a few days. The EPA evaluation (2004) of boron reported that, after 3 and 6 weeks' exposure to boric acid in drinking water at doses of 0, 2, 12.5, and 25 mg/rat/day (equivalent to 0, 0.35, 2.2, 4.4 mg boron/rat/day), solid tissues (kidney excluded) demonstrated boron contents which varied less than 25 % within any given dose time group (Naghii and Samman, 1996, as reported in EPA, 2004).

Young (60–70 days old) adult male F344 (CDF (F334/CrlBr) rats were administered a powdered diet containing 0, 3 000, 4 500, 6 000 or 9 000 mg boric acid/kg (corresponding to 300, 450, 600 or 900 mg boric acid/kg bw/day and 52.5, 78.8, 105 or 157.5 mg boron/kg bw/day) for 9 weeks. Control diet contained 20–40 mg/kg boric acid (equivalent to 3.5–7 mg boron/kg). Serum and bone boron concentrations were measured at weekly intervals and at 8, 16, 24 and 32 weeks after exposure terminated. The concentration of boron in the bone was increased in all treated groups to up to four times the levels found in serum. After exposure ceased, serum and urinary levels of boron were again within the range of control values. In comparison, boron levels in bone were still three times control values up to 32 weeks after exposure ceased (Chapin et al., 1997).

Magour et al. (1982, as reported in IPCS, 1998) determined the tissue levels of boron in Wistar rats administered 20 mg boron/kg bw/day as sodium tetraborate (equivalent to 177 mg sodium tetraborate/kg bw/day and 114 mg boric acid/kg bw/day) in drinking water. Exposure was initiated at 3 weeks of age and continued for 21 days. Blood boron levels rose continuously during the treatment period, reaching a maximum level of approximately 3.2 µg/g or 0.32 mg/100 ml blood. In another experiment, intraperitoneal (i.p.) administration of 42 mg boron/kg bw to 3-week-old or 3-month-old female Wistar rats resulted in generally higher tissue levels in the older rats and slightly higher tissue–blood ratios across a 4-hour post-administration period, but the differences were subtle and transient.

Elimination kinetics of boron from bone are different from those from soft tissue and body fluids, suggesting a second kinetic compartment in bone in which a small percentage of absorbed boron is stored (EFSA, 2004).

3.1.3. Metabolism

Inorganic borates, in low concentrations, are converted to boric acid at physiological pH in the aqueous layer overlying mucosal surfaces prior to absorption. Boric acid is not metabolised due to the energy barrier (523 kJ/mol) required to break the boron–oxygen bond in inorganic borates (Emsley, 1989; EVM, 2003; BfR, 2005). Therefore, the ionic species in the systemic circulation are expected to be the same across species (IPCS, 1998). This is supported by evidence in both human and animal studies, where more than 90 % of an administered dose of borate is excreted as boric acid (IPCS, 1998) and the observation that no organic boron compounds have been reported as metabolites (ECETOC, 1995).

Boric acid can form complexes with the hydroxyl, amino and/or thiol groups of different biomolecules. This complex formation depends on the concentration and is reversible (Moore, 1997).

3.1.4. Excretion

Boric acid is the only boron compound that has been identified in the urine and it tends to account for > 90 % of ingested boron (EPA, 2004). The primary route of elimination is by glomerular filtration (Dourson et al., 1998; Murray, 1998) and > 90 % of the administered dose is excreted via urine, regardless of the route of exposure or administration (EFSA, 2004; ECETOC, 1995). Borates, eliminated primarily in the form of boric acid in the urine, have reported half-lives of approximately 12 hours (USDA, 2006) or a range of 14–19 hours (FNB, 2001).

Using mass spectrometry and the boron-10 isotope, Vanderpool et al. (1994, as cited in EPA, 2004) showed that fasted rats (strain, sex and number not stated) fed 20 µg of boron-10 isotope in the diet eliminated 95 % of the boron-10 isotope in the urine and 4 % in the faeces within 3 days of dosing, producing a 77 % increase in the ratio of boron-10 isotope to boron-11 isotope in the urine. Moreover,

boron-10 in the liver peaked within 3 hours of dosing, with over 90 % recovery and a 56 % increase in the boron-10 to boron-11 isotope ratio, which returned to normal within 24 hours. This result suggests that > 90 % of orally administered boron is absorbed from the gastrointestinal tract within 3 hours and that absorption is complete within 24 hours (EPA, 2004).

Male F344 rats (number not stated) were exposed to boric acid at concentrations ranging from 3 000 to 9 000 mg/kg (equivalent to 525–1 575 mg boron/kg) in the diet for up to 9 weeks (Ku et al., 1993, as reported in IPCS, 1998). Urinary boron was elevated (450–600 µg boron/mg creatinine) 24 hours after the end of dosing. Three to four days post-treatment, urinary boron levels in all groups had returned to average control levels (8.97 ± 1.95 µg boron/mg creatinine for days 1, 7 and 14 post treatment). Based on this study, the ECETOC (1995) reported that, assuming first-order kinetics, a half-life of less than 12 hours can be calculated.

Elimination kinetics (especially route of elimination and terminal half-life) appears to be similar in humans and rats (IPCS, 1998). The 3- to 4-fold higher clearance in rats compared to humans arises from the higher glomerular filtration rate in rats (EFSA, 2004).

A pharmacokinetic study investigated the urinary elimination of boric acid in Sprague–Dawley female rats (non-pregnant and pregnant) (U.S. Borax, 2000; Vaziri et al., 2001, as reported in EPA, 2004). Prior to gavage administration of boric acid, three groups of 10 non-pregnant and 10 or 11 pregnant rats were given an initial 7-day diet supplemented with boron on gestation day 9. The aim of this initial diet was to achieve steady-state boron conditions for rats given a diet comparable to that ingested by humans in terms of boron content. This diet supplemented with boron was designed to deliver a dose of approximately 0.3 mg/kg bw/day boric acid (0.05 mg boron/kg bw/day). On the morning of day 8, the diet for all rats was changed to a low-boron casein diet containing 0.2 mg boron/kg diet for a total of 24 hours. This low-boron casein-based diet was used to minimise the dietary contribution of boron on the day of gavage and to minimise cross-contamination of the urine with boron in the diet. After 24 hours on the low-casein diet, groups of pregnant and non-pregnant rats were given a single oral dose of 0.3, 3.0 or 30 mg boric acid/kg bw (approximately equivalent to 0.052, 0.52 and 5.2 mg boron/kg bw, respectively) by gavage in deionised water. According to the authors, the low dose was an estimate of the high-end human dietary dose level, and the highest dose tested was approximately half of NOAEL from the rat developmental toxicity study of Price et al. (1996a).

Blood samples were taken 3 hours after the gavage dosing and 12 hours after the first sampling. Plasma concentrations of boron were considerably lower 15 hours after dosing compared to 3 hours after dosing (EPA, 2004). Urine was also collected during the 2 blood collections. The urinary concentration of boron at the high-dose was significantly higher in pregnant rats compared with non-pregnant rats, but not at the low- and mid-dose; the volume was not significantly different. The amount of boron (µg/12 hours) excreted in the urine increased proportionately with increasing dose and during the 12-hour collection period was higher (32–73 %) in pregnant rats compared to the non-pregnant rats at the high-dose level. The authors attributed this to the higher dose of boron administered to pregnant rats due to their larger body weight and to the higher fractional excretion of boron (boric acid clearance/creatinine clearance) in the pregnant rats which was statistically significant at the high-dose level. The percentage of administered dose of boric acid recovered in the urine was significantly higher in the low-dose group compared to the mid-dose and high-dose groups for both the non-pregnant and pregnant animals and higher in the pregnant compared to the non-pregnant rats across dose groups, which was statistically significant at the high-dose only. Although this study used a diet low in boron, it contributed to the overall dose of boric acid, and these amounts were not included in the nominal dose levels. When dietary contribution from the low boron diet was included in the dose, the actual dose levels were approximately 0.4, 3.1 and 30.1 mg boric acid/kg (equivalent to 0.07, 0.54 and 5.3 mg boron/kg). At the low dose, the dietary concentration of boron contributed another 27 % and 33 % to the overall dose given to non-pregnant and pregnant rats, respectively, whereas at the mid and high doses, the diet contributed 3 % and 0.3 %, respectively, to the total dose.

The authors suggested that the incremental increase at the low dose may explain the greater recovery of the administered dose in the low-dose group.

The authors reported that boron clearance appeared to be independent of the dose within the range of dose levels tested. The average absolute clearance value for pregnant rats was 1.01 ml/min with a standard deviation of 0.2 ml/min (coefficient of variation (CV) = 0.2). Boron clearance was slightly higher in pregnant rats compared to non-pregnant rats, but the difference was not statistically significant. The rate of creatinine clearance did not vary significantly with the different doses of boric acid in either non-pregnant or pregnant rats. Creatinine clearance, normalised against body weight, however, was significantly greater in non-pregnant rats compared to pregnant rats. Urea clearance was not significantly different between non-pregnant and pregnant rats. There were no consistent differences in the rate of urea clearance with the different doses of boric acid (U.S. Borax, 2000; Vaziri et al., 2001, as reported in EPA, 2004).

In a similar study by the same group (Vaziri et al., 2001, as reported in EPA, 2004), six pregnant and six non-pregnant rats (12 weeks old; body weight approximately 200–250 g) were administered a low level of boron in the diet for 7 days followed by a high single oral dose of 30 mg/kg bw boric acid (equivalent to 5.3 mg boron/kg bw), which resulted in plasma levels of 1.82 ± 0.32 and 1.78 ± 0.32 µg boron/mL among pregnant and non-pregnant rats in the first blood sample taken 3 hours after dosing. The plasma concentration curves indicated a one-compartment model and there was no evidence of saturation kinetics. The estimated half-life of boric acid in non-pregnant and pregnant rats were 2.9 and 3.2 hours, respectively, which are not statistically different.

3.1.5. Human studies

3.1.5.1. Absorption

Borates are readily, and almost completely (> 90 %), absorbed from the human gut by an undefined mechanism (EVM, 2003; EFSA, 2004; EPA, 2004). Estimates of absorption of borates in humans indicated that at least 92 % of a single oral dose was absorbed (IPCS, 1998; Murray and Schlekot, 2004).

Average levels of boron in the blood have been reported in various studies in human adults: 0.11 mg/L (range 0.04–0.36 mg/L), 0.03 mg/L, 0.097 mg/L, 0.031 ± 0.006 mg/L and 0.057 mg/L (Moseman, 1994). The ECETOC (1995) evaluation reported background levels of boron in human whole blood from a number of oral studies (control values) as ranging from 0.022–0.66 mg boron/L and 0.057–0.75 mg boron/L. This evaluation reported additional studies where levels of boron in different human tissues have been measured using different techniques (ECETOC, 1995).

Normal human blood boron concentrations reached a level of 0.3 mg/mL (Fail et al., 1998). Normal blood levels in children and infants range 0–1.25 mg/L (ATSDR, 2011; Moseman, 1994).

Normal values for human whole blood (based on current analytical measurements) ranged from approximately 0.02 to 0.06 mg boron/L. Culver and Hubbard (1996) reported that a number of studies in humans showed that entire ingested doses of borates tend to be absorbed systematically and excreted almost entirely in the urine, with a half-life of approximately 20 hours. Based on human studies they estimated that an intake of 100 mg boron/day would reach equilibrium of input and output within less than 2 weeks (Culver and Hubbard, 1996).

In a review of 782 cases of accidental boric acid exposure (age range 2 weeks to 98 years), serum levels ranged from 0 to 59.4 mg boron/L. These levels did not correlate with the estimated amount of boric acid ingested (Litovitz et al., 1988, as reported in IPCS, 1998).

3.1.5.2. Distribution

Boric acid measured as boron did not accumulate in the blood of regularly exposed workers (Culver et al., 1994, as reported in EFSA, 2004). Accumulation of boron in the skeletal bones of humans has been reported (Alexander et al., 1951; Forbes et al., 1954, as reported in EPA, 2004).

An average of 61 mg boron/kg (range 16–138 mg boron/kg) was found in 116 samples of human bone analysed from cadavers aged 5 months to 75 years. There was no correlation between age and boron content (Alexander, 1951). Another study reported that bone tends to have the highest levels of boron, soft tissues have the same levels approximately as blood and fat, muscle, heart and lung tissues have lower amounts (Moseman, 1994).

It has been shown that boron compounds can cross the human placenta (IPCS, 1998; EVM, 2003; EFSA, 2004) and some data indicated that it is also present in breast milk (BfR, 2005).

3.1.5.3. Metabolism

As in animals, boric acid does not seem to be metabolised in humans due to the high energy required to break the boron–oxygen bonds (Murray and Schlekat, 2004).

3.1.5.4. Excretion

The excretion of boron compounds is similar in humans and animals and is predominately by excretion of > 90 % of the administered dose, regardless of the route of administration (IPCS, 1998). Excretion of boron is relatively rapid and it has a half-life of elimination of 24 hours or less (Nielsen, 1986, 1988; Litovitz et al., 1988, as reported in IPCS, 1998; IPCS, 1998). In adults, average normal levels of boron in the urine have been reported in various studies as 0.92 mg/L (range 0.04–6.6 mg/L), 1.89 mg/L (range 0.47–7.8 mg/L) and 0.75 mg/L (0.16–2.9 mg/L). Furthermore, it was stated that following ingestion of a single dose of 2.5 g boric acid (equivalent to 0.44 g boron), boric acid was detected in the urine within 2 hours and up to 24 hours thereafter. (Moseman, 1994)

The kinetics of elimination of boron have been studied in human volunteers given boric acid via the i.v. or oral routes (Jansen et al., 1984a, as reported in EPA, 2004; Schou et al., 1984). Jansen et al., (1984a, as reported in EPA, 2004). Approximately 60–75 % of 750 mg boric acid (equivalent to 60–75 % of 131 mg boron) in a water solution was eliminated in urine within 24 hours; 93 % of the dose was excreted within 96 hours after administration. The half-life for elimination in these studies was approximately 21 hours. This value is supported by case reports of boric acid poisoning. The case reports of nearly 800 patients accidentally or intentionally poisoned with boric acid demonstrated an elimination half-life of 13.4 hours (range 4–27.8 hours) (Litovitz et al., 1988, as reported in IPCS, 1998). The authors reported that incomplete or inconsistent patient histories (especially with regard to total dose or dose-to-sample interval) most likely contributed to the variation in the half-life estimates.

The ECETOC evaluation reported background levels of boron in human urine from a number of oral studies (control values) as ranging from 0.38 to 1.9 µg boron/mL/day (ECETOC, 1995). However, higher concentrations for the general population ranging 0.07–0.66 mg/100 ml have also been reported (ATSDR, 2011). More than 50 % of an ingested amount of boron can be found in the urine within 24 hours, with the remaining amount detectable in the urine for up to 4 days (ATSDR, 2010).

A number of studies on the excretion of boron in the urine are reported by the EPA (2004). Six volunteers were administered approximately 131 mg boron as boric acid (equivalent to 748 mg boric acid) in both water (750 mg) and in a water-emulsifying ointment (740–1473 mg, approximately 130–258 mg boron). An average of 92–94 % of administered boron was excreted in the urine within 96 hours (Schou et al., 1984, as reported in EPA, 2004). In another study, two women ingested approximately 62 mg boron as boric acid (equivalent to 352 mg boric acid) (in addition to 80–140 mg of boron in food) and excreted more than 90 % of the ingested boron in the urine in the first week after dosing (Kent and McCance, 1941, as reported in EPA, 2004). Volunteers ($n = 10$) who drank spa waters containing approximately 100 mg daily dose of boron for 2 weeks had over 90 % absorption of

boron based on urinary excretion data (Job, 1973, as reported in EPA, 2004). Supplementation of the normal diet of male volunteers ($n = 8$) with 10 mg boron/day for 4 weeks resulted in 84 % recovery in the urine (Naghii and Samman, 1997).

Adult males ($n = 6$) consumed an aqueous solution of boric acid and, at 12 hours, the urinary elimination accounted for 52.7 ± 4.9 % (mean $\pm$ standard deviation (SD)) of the dose (range 46.4–58.9 %); at 24 hours, the cumulative urinary elimination accounted for 66.9 ± 6.4 % of the dose (mean $\pm$ SD), with a range of 57.1–75.0 % (Jansen et al., 1984b, as reported in EPA, 2004). These data demonstrated a marked similarity among this limited sample of adult men in the renal elimination of boric acid. Similar findings were reported by Astier et al. (1988, as reported in EPA, 2004). In another study, it was reported that 92–93 % of an administered oral dose (352 mg as boric acid) in humans was eliminated in urine during the first week following administration, with minor elimination pathways including saliva, sweat and faeces (Kent and McCance, 1941, as reported in EPA, 2004).

Jansen et al. (1984a, as reported in EPA, 2004) evaluated boron clearance daily in seven adult males exposed through dietary intake over 3 days and in the same subjects after 20-minute i.v. infusion of 28.52 mg boric acid per minute (equivalent to 5 mg boron), or a total dose per subject of 570–620 mg boric acid (equivalent to 100–109 mg boron). In the dietary intake phase, urine was collected at 12-hour intervals, and blood was sampled twice per day to determine basal levels of boron. There were no restrictions on diet during this period. For the infusion phase, subjects stayed in a metabolic ward for 12 hours after receiving the i.v. dose. Blood samples were drawn at 0, 0.42, 0.67, 2, 4, 6, 8, 10 and 12 hours, for a total of nine blood samples from each subject during the 12-hour period. After release from the metabolic ward, each subject had a blood sample drawn at 9 am and 4 pm daily for 5 days. Renal clearance was calculated as the total amount of boron excreted per minute in the urine, divided by the area under the plasma boron concentration–time curve ($\text{mg boron}_{\text{urine}}/\text{AUC-min}$), normalised to body surface area. For the dietary exposure phase, the urinary excretion of boric acid during any 12-hour period ranged from 1.52 to 18.1 mg, consistent with large variations in dietary intake of boron. Plasma concentrations during this 72-hour period ranged from < 0.10 to 0.46 mg boric acid/L (< 0.018 – 0.081 mg boron/L). In contrast, following boric acid infusion, plasma boron rose to peak concentrations 25 minutes after the start of the infusion at 10 – 20 mg/mL, approximately 100 times the basal concentration. Virtually all of the administered dose (99 %) was eliminated in the urine over 120 hours.

Healthy male individuals were given a specific diet containing boron for 3 days. The average intakes for each of the days were 4.56, 1.87, 4.75 mg/day. The urinary excretion during the 42-day collection period averaged 3.20 ± 0.41 mg/day. The authors observed that when the intake of boron was low, urinary boron loss was significantly lower than when boron intake was higher (Sutherland et al., 1998).

In several young men given oral doses of 100–150 g of boric acid (equivalent to 17.5–26.3 g boron), approximately 77–83 % of the administered boron compounds were found in the urine. In subsequent similar experiments, perspiration was found to contain about 1.5 % of a 1- to 3-g dose of boric acid. About 1 % of a 3-g dose could be accounted for in the faeces (Moseman, 1994).

In humans, approximately 100 % of boron ingested in the concentration range 0.4–3 mg/day was excreted in faeces and urine (Hunt et al., 1997, as reported in EFSA, 2004). At higher concentrations, supplementation with 10 mg of boron/day resulted in the recovery of 84 % of the dose in the urine (Samman et al., 1998, as reported in EFSA, 2004).

The renal clearance of boron normally consumed in the daily diet of non-pregnant and pregnant women was measured in 32 healthy women aged between 18 and 40 years, including 16 women in their second trimester (14–28 weeks) and 16 age-matched non-pregnant women (U.S. Borax, 2000; Pahl et al., 2001, as reported in EPA, 2004). The subjects were asked to collect all urine for the next 22 hours (24 hours from the baseline). A 24-hour blood sample was also collected. Urine from each individual was pooled over the initial 2-hour period and over the following 22-hour period. Urinary

clearance was measured by quantifying the amount of boron (mg) in the urine and blood. The urinary clearance of boron in humans was determined for all individuals and presented as ml blood cleared of boron per minute per kg body mass. The average clearance rate for boron in pregnant women was 1.02 ± 0.55 ml/min/kg bw (mean $\pm$ SD; range 0.252–2.028 ml/min/kg bw) and the average clearance rate for boron in non-pregnant women was 0.80 ± 0.31 ml/min/kg bw (mean $\pm$ SD; range 0.229–1.358 ml/min/kg bw). These results showed that pregnant women clear boron more effectively than non-pregnant women, which is consistent with the normal increase in renal blood flow and glomerular filtration rate during pregnancy. Individual body weights and clearance values from U.S. Borax (2000) were used to calculate boron clearance in units of ml/min. The average boron clearance for these subjects was 66.1 ml/min, with a standard deviation of 32.4 ml/min. The clearance values had high variability, with a CV of 0.49. It was noted that this high level of variability in the results of this study could be due to the estimation of boron intake based on the 24-hour dietary intake diary carried out by the test individuals. Boron intake estimates were based on the renal excretion of boron in 24 hours which was 1.3 mg boron/day, from which an average consumption was estimated at 0.02 mg boron/kg bw/day (EPA, 2004).

Studies have indicated that boron can be reabsorbed in kidney tubules in pregnant and non-pregnant women and in rats (Murray and Schlegel, 2004).

Boron compounds have been found in human breast milk (BfR, 2005). Concentrations in human breast milk of 4 μ g/L have been reported (Hunt et al., 2005, as reported in WHO, 2009). After administration of 1–13 g of boric acid, 10–285 mg/L was detected in human breast milk (Moseman, 1994).

In summary, in animals and humans, boric acid and sodium tetraborate are rapidly absorbed and excreted in the urine unchanged.

3.2. Toxicological data

3.2.1. Acute oral toxicity

Acute oral LD₅₀ values for boric acid and sodium tetraborate in mice and rats are in the range of approximately 400–900 mg boron/kg bw (EVM, 2003; EFSA, 2004) (equivalent to 2285–5143 mg boric acid/kg bw and equivalent to 3 540–7 965 mg sodium tetraborate/kg bw). Symptoms and signs include depression, ataxia, convulsions and weight loss. Damage to the kidneys and nerve cells has been observed on histopathological examination (EVM, 2002). Acute oral LD₅₀ values in the range of 200–350 mg boron/kg bw (equivalent to 1 143–2 000 mg boric acid/kg bw and equivalent to 1 770–3 098 mg sodium tetraborate/kg bw) were reported for guinea pigs, dogs, rabbits and cats (EFSA, 2004).

3.2.2. Short-term and subchronic toxicity

3.2.2.1. Mice

In a 13-week study conducted by the US National Toxicology Program (NTP, 1987; Dieter, 1994), B6C3F1 mice (10 animals per sex per group) were exposed to boric acid in the diet at concentrations of 0, 1 200, 2 500, 5 000, 10 000 or 20 000 mg/kg diet. These concentrations produced estimated consumptions of approximately 0, 194.3, 400, 806, 1 606 or 3 217 mg boric acid/kg bw/day (approximately 0, 34, 70, 141, 281 or 563 mg boron/kg bw/day) for males and 0, 269, 554, 1 108, 2 217 or 4 434 mg boric acid/kg bw/day (approximately 0, 47, 97, 194, 388 or 776 mg boron/kg bw/day) for females. Female mice were less sensitive than male mice to the lethal effects of boric acid; 8/10 males and 6/10 females in the high-dose group died, and 1 out of 10 males in the lower dose group. Clinical signs of toxicity were a thin, hunched appearance, dehydration, foot lesion and scaly tails. A dose-related decrease in body weight gain was observed. Histopathological changes included a dose-related incidence of minimal to mild extramedullary haematopoiesis of the spleen in males and females hyperkeratosis and acanthosis of the stomach in eight males and three females receiving the highest dose (ECETOC, 1995; IPCS, 1998).

3.2.2.2. Rats

In a 90-day study, albino Sprague–Dawley rats (10 animals per sex per group) received boric acid or sodium tetraborate in the diet as boron equivalents at concentrations of 0, 52.5, 175, 525, 1 750 or 5 250 mg boron/kg diet (equivalent to 0, 2.6, 8.8, 26.3, 87.5 or 262.5 mg boron/kg bw/day; 0, 14.9, 50.2, 150.3, 500 or 1 500 mg boric acid/kg bw/day; 0, 23.0, 77.9, 232.8, 774.4 or 2323 mg sodium tetraborate/kg bw/day) (Weir and Fisher, 1972). The effects observed with boric acid were similar to those observed with sodium tetraborate. All high-dose animals died within 3–6 weeks. Growth and food conversion were reduced at doses of 1 750 mg boron/kg diet and above. In animals receiving 87.5 mg boron/kg bw/day, body weights in males and females were reduced 44–55 % and 10–13 %, respectively. In this dose group, absolute organ weights, including the liver, spleen, kidneys, brain, adrenals and ovaries, were also significantly decreased. Relative organ-to-body weights of the adrenals and kidneys were significantly increased, but relative weights of the liver and ovaries were decreased. A pronounced reduction in testicular weights in males in the 87.5 mg boron/kg bw/day group was also observed. The Panel considered the NOAEL to be 26.3 mg boron/kg bw/day (equivalent to 150.3 mg boric acid/kg bw/day).

Thirty-, 60- and 90-day studies were performed in Sprague–Dawley rats administered drinking water containing sodium tetraborate at levels equivalent to 0.3, 1.0 or 6.0 mg boron/L (equivalent to 1.7, 5.7 or 34.3 mg boric acid/L) (Dixon et al., 1976). Average daily consumption of 35 ml of drinking water resulted in a highest estimated dose of 0.84 mg boron/kg bw/day, at which level no adverse effects were reported. In a similar study in which rats were also administered 0, 0.3, 1.0 and 6.0 mg/L boron as boric acid (equivalent to 0, 1.7, 5.7 and 34.3 mg boric acid/L) for 6 months, increased blood aldolase activity and minor testicular effects were reported at the highest dose (Krasovskii et al., 1976, as reported in ECETOC, 1995). The Panel noted that the dose levels associated with effects in the drinking water studies of Dixon et al. (1976) and Krasovskii et al. (1976, as reported in ECETOC, 1995) were not consistent, and testicular effects were noted at higher concentrations in dietary studies.

Sodium tetraborate was administered to male Sprague–Dawley rats (18 animals per group) in the diet at concentrations of 0, 500, 1 000 or 2 000 mg boron/kg diet (equivalent to approximate doses of 0, 30, 60 or 125–131 mg boron/kg bw/day; 0, 171, 343 or 714–748 mg boric acid/kg bw/day; 0, 266, 531 or 1 106–1 159 mg sodium tetraborate/kg bw/day) for 30 or 60 days (Lee et al., 1978; Dixon et al., 1979; IPCS, 1998). Body weights were not consistently affected by treatment; organ weights were not affected in the 30 mg/kg bw/day group. At 60 and 125–131 mg boron/kg bw/day, absolute liver weights were significantly lower after 60 days; epididymal weights were significantly lower (37.6 % and 34.8 %, respectively) after 60 days, but not after 30 days. Weights of prostate, spleen, kidney, heart and lung were not changed at any dose.

Long Evans rats (15 animals per group) were administered sodium tetraborate at levels of 0, 150 or 300 mg boron/L (equivalent to 0, 1 328 or 2 655 mg sodium tetraborate/L) in the drinking water for 70 days; basal diet contained approximately 54 µg boron/g feed (Seal and Weeth, 1980, as reported in EPA, 2004). Approximate boron intake for the treated rats was 23.7 and 44.7 mg boron/kg bw/day, respectively (EPA, 2004). Sodium tetraborate treatment at both doses produced significant decreases ($P < 0.05$) in body weight, testis, seminal vesicle, spleen and right femur weight and plasma triglyceride levels. Spermatogenesis was impaired and haematocrit was decreased slightly at the highest dose. From this study, a NOAEL was not identified but a LOAEL of 23.7 mg boron/kg bw/day was reported (EPA, 2004).

Reversibility of testicular lesions was evaluated in adult F344 rats (60–70 days old; body weight 200–220 g) dosed with 3 000, 4 500, 6 000 or 9 000 mg boric acid/kg in feed (equivalent to 26, 38, 52 or 68 mg boron/kg bw/day) for 9 weeks and assessed for recovery up to 32 weeks post treatment (Ku et al., 1993). Inhibited spermiation was exhibited at 3 000 and 4 500 mg boric acid/kg feed (5.6 µg boron/mg testes), whereas inhibited spermiation progressed to atrophy at 6 000/9 000 mg boric acid/kg feed (11.9 µg boron/mg testes), with no boron accumulation in the testes to levels greater than those found in blood during the 9-week period. After treatment, serum and testis boron levels in all dose

groups fell to background levels. Inhibited spermiation at 4 500 mg boric acid/kg feed was reversed by 16 weeks post treatment, but focal atrophy was detected and did not recover up to 32 weeks post treatment (Ku et al., 1993, as cited in IPCS, 1998; Moore, 1997; EFSA, 2004).

Young (60–70 days old) male F344 rats (CDF (F334/CrlBr) (six animals per group) were administered a powdered diet containing 0, 3 000, 4 500, 6 000 or 9 000 mg boric acid/kg (corresponding to 0, 300, 450, 600 or 900 mg boric acid/kg bw/day; equivalent to 0, 52.5, 78.8, 105 or 157.5 mg boron/kg bw/day) for 9 weeks (Chapin et al., 1997). Control diet contained 20–40 mg boric acid/kg (equivalent to 3.5–7 mg boron/kg). Serum calcium, phosphorus, potassium and chloride and blood and bone boron concentrations were measured at weekly intervals and at 8, 16, 24 and 32 weeks after exposure terminated. In a related study, male and female young adult F344 (CDF (F334/CrlBr) rats were administered 0, 200, 1 000, 3 000 or 9 000 mg boric acid/kg (corresponding to 0, 20, 100, 300 or 900 mg boric acid/kg bw/day; equivalent to 0, 3.5, 17.5, 52.5 or 157.5 boron/kg bw/day) in the diet for 12 weeks. Serum calcium, phosphorous, magnesium and bone boron and bone strength were measured at weekly intervals. In the first study, total serum calcium levels were unchanged by boric acid treatment. However, in the second study, slightly reduced total serum calcium levels were observed in males only. In both studies, compared with controls, phosphorus was reduced, potassium was unchanged and chloride was increased by 1 %; magnesium was reduced in the second study by 19 %. The concentration of boron in the bone was increased in all treated group up to four times the levels found in serum. After exposure ceased, serum and urinary levels of boron were again within the range of control values. In comparison, boron levels in bone were still three times control values up to 32 weeks after exposure ceased.

Male albino Sprague–Dawley rats (24 animals per group) were exposed to either 0, 100, 275 or 400 mg boric acid/kg bw/day (equivalent to 0, 17.5, 48.1 or 70 mg boron/kg bw/day). Kidneys were removed on day 10, 30 or 45, after sacrificing the rats. Kidney weights, kidney boron concentration and histopathological changes were determined. Significant accumulation of boron in kidney tissue was seen in the experimental groups, but there was a significant drop in boron concentration on the 45th day compared with the 30th day. Histopathological degenerative changes were observed particularly in the proximal tubular cells and were dose and time dependent. The authors concluded that “*subacute boric acid exposure caused dose-dependent histopathological changes in kidney tissue*” of all dose groups (Sabuncuoglu et al., 2006).

3.2.2.3. Dogs

Boric acid or sodium tetraborate was fed to Beagle dogs (five animals per sex per group) for 90 days at a dietary concentration of 0, 17.5, 175 or 1 750 mg boron equivalents/kg diet, equivalent to a weight- dose of 0, 0.44, 4.38 or 43.75 mg boron/kg bw/day (equivalent to 0, 2.5, 25 or 250 mg boric acid/kg bw/day and 0, 3.9, 38.8 or 387 mg sodium tetraborate/kg bw/day) (Weir and Fisher, 1972). At the two highest doses, testicular effects were observed in males. In males given boric acid, testis weight was significantly lower than controls in the mid-dose and high-dose groups (25 and 40 % reductions, respectively). In males given sodium tetraborate, testis weights in the low, mid-, and high-dose groups were 80, 85, and 50 % of controls, respectively. Four out of five high-dose dogs had complete testicular atrophy, and the remaining high-dose dog had “partial” atrophy. No other clinical or microscopic signs of toxicity were reported in any animals (IPCS, 1998). Health Canada (2007) has queried the significance of the results reported by Weir and Fisher (1972), citing the following arguments: “*irregular sacrifice schedules, dose level inconsistencies between studies, and variability in the actual intake of test compounds*”. The Panel noted that testicular effects were observed at all doses tested, but acknowledged that there were inconsistencies in the study design such as in the dose levels, the irregular autopsy schedules, and the variability in the actual intake of the test compounds.

3.2.3. Genotoxicity

The genotoxicity of boric acid and sodium borate was previously evaluated by the EFSA NDA Panel (EFSA, 2004). In this opinion EFSA noted that boric acid was not mutagenic in *Salmonella typhimurium* with or without exogenous metabolic activation (S-9 fraction) when tested at doses up to

20 000 µg/plate (Haworth et al., 1983; Benson et al., 1984; NTP, 1987; IPCS, 1998) and not mutagenic in the L5178Y mouse lymphoma TK^{+/−} assay with or without S-9 (NTP, 1987; Rudd, 1991).

Boric acid was also negative in *in vitro* assays for chromosomal aberrations or SCEs in Chinese hamster ovary cells with or without metabolic activation systems (NTP, 1987), and it did not induce unscheduled DNA synthesis (UDS) in primary cultures of male F344 rat hepatocytes (Bakke, 1991, as reported in EPA, 2004).

Similarly, crude or refined sodium tetraborate, tested at concentrations of 400–3 200 µg/mL, tested negative in assays for mutagenicity in V79 Chinese hamster cells up to 1.6 mg/mL (Landolph, 1985).

In vivo, boric acid did not prove to be genotoxic in a bone marrow micronucleus assay in Swiss–Webster mice (10 animals per sex per group) (O’Loughlin, 1991, as reported in EFSA, 2004, and EPA, 2004). In this study, boric acid was administered in deionised water orally for two consecutive days at 900, 1 800 or 3 500 mg/kg bw (equivalent to 158, 315, 613 mg boron/kg bw).

Based on these findings, the EFSA NDA Panel concluded that, overall, the available data indicated that boric acid and sodium tetraborate are not genotoxic (EFSA, 2004).

Since the NDA opinion was published, the following additional genotoxicity studies on boric acid and borates have been published.

In a series of studies by Türkez and coworkers (2007, 2008, 2010a, b, 2012a, b), human peripheral blood lymphocyte cultures were exposed to either boric acid or sodium tetraborate at concentrations of 2.5, 5 or 10 µmol/L for 72 hours and SCE and micronuclei (MN) assays performed. Neither compound at any concentration significantly affected SCE or MN rates (Türkez, 2008). In another study in which human peripheral blood cultures were exposed to boron compounds at concentrations ranging from 5 to 500 mg/L (equivalent to 28.6–2857 mg boric acid/L) and SCE, MN and chromosomal aberration tests performed, it was concluded that boron compounds are not genotoxic (Türkez et al., 2007); similar results were also reported for boric acid tested at 5, 10, 15 and 20 mg/L (equivalent to 0.88, 1.8, 2.6 and 3.5 mg boron/L) in human blood cultures (Türkez et al., 2012a). However, it was concluded, based on measurements of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase, total glutathione, malondialdehyde and total antioxidant capacity, that boron compounds cause oxidative stress (Türkez et al., 2007). However, in a similar study in which human blood cultures were treated with 5 and 10 mg boric acid/L (equivalent to 0.88 and 1.8 mg boron/L) and 5 and 10 mg sodium tetraborate/L (equivalent to 0.6 and 1.1 mg boron/L), no increases in SCEs, chromosomal aberrations or MN were observed, but it was reported that boric acid and sodium tetraborate increased total antioxidant capacity in erythrocytes (Geyikoglu and Türkez, 2008). Additional studies from the same author investigating the use of boric acid as an antimutagenic agent were identified (Türkez and Geyikoglu, 2010; Türkez et al., 2010; Türkez et al., 2012b). The Panel judged these studies not relevant for genotoxic evaluation.

In another study, human peripheral lymphocytes were treated with boric acid at concentrations of 400, 600, 800 or 1 000 µg/mL (equivalent to 70, 105, 140 or 175 µg boron/mL) for 24 and 48 hours (Arslan et al., 2008). Boric acid did not increase SCEs at any of the concentrations tested at both treatment times compared to concurrent untreated and vehicle controls. Due to the high cytotoxicity at 1000 µg/mL for 48 hours, SCEs were not evaluated for this concentration. Boric acid induced structural, but not numerical, chromosomal aberrations at all concentrations and incubation periods. However, the Panel noted unusual high incidences for human lymphocytes, of chromatid and chromosome aberrations in the untreated and solvent treated control cultures. On this basis the study was judged not adequate for the genotoxic evaluation of boric acid.

Induction of SCEs by sodium tetraborate in human lymphocytes *in vitro* has also been evaluated by Pongsavee (2009). In this study, lymphocyte cultures from 40 donors were treated with 0.1, 0.15, 0.2, 0.3 or 0.6 mg/mL without S-9 (equivalent to 0.06, 0.10, 0.13, 0.19 or 0.40 mg boric acid/mL and to

0.01, 0.017, 0.023, 0.03 or 0.07 mg boron/mL). The author concluded that sodium tetraborate at concentrations of 0.15, 0.2, 0.3 and 0.6 mg/mL significantly inhibited lymphocyte proliferation *in vitro* and induced SCEs. The Panel noted the unusual experimental design of the study, involving a large number of different blood donors, the abnormally low incidence of spontaneous SCEs (0.74/cell), and some puzzling results of statistical analysis (0.74 ± 0.07 vs. 2.20 ± 0.08 (not significant), 40 observations). Thus, the results of this study are considered of questionable reliability and of limited biological significance.

In a more recent study, male Sprague–Dawley rats (10 animals per group) were fed 100 mg boron/kg bw/day as boric acid (equivalent to 571 mg boric acid/kg bw/day) or sodium tetraborate (equivalent to 885 mg sodium tetraborate/kg bw/day) for 4 weeks in order to determine effects on lipid peroxidation, antioxidant activity and DNA damage (Ince et al., 2010). The control group consisted of 10 male Sprague–Dawley rats which were fed a normal diet containing 6.4 mg boron/kg. Both sodium tetraborate and boric acid caused decreases in malondialdehyde, DNA damage, the protein carbonyl content level in blood, and in reduced-glutathione (GSH) concentration in the liver, Cu–Zn superoxide dismutase, and catalase activity in the kidney. Both boron compounds increased -GSH concentration in blood and the vitamin C level in plasma. The authors concluded that boron supplementation at this level in the diet, decreases lipid peroxidation and enhances the antioxidant defence mechanism. For boric acid and sodium tetraborate, there were no differences in oxidant/antioxidant balance and the biochemical parameters except for serum vitamin A and liver GSH concentrations. The Panel noted that this study, albeit suggesting a possible protection mechanism against DNA damage, has limited relevance for the evaluation of boric acid and sodium borate genotoxicity.

In conclusion, the Panel noted that no reliable indication of genotoxicity is provided by studies on boric acid or sodium borate published after the previous EFSA evaluation (EFSA, 2004). Thus, based on the available evidence, the Panel concluded that boric acid and sodium borate do not raise concern for genotoxicity.

3.2.4. Chronic toxicity and carcinogenicity

3.2.4.1. Rodents

In a 2-year study, B6C3F1 mice (50 animals per sex per group) were fed a diet containing 0, 2 500 or 5 000 mg boric acid/kg diet (NTP, 1987, as reported in EPA, 2004). These concentrations led to doses of approximately 0, 275 or 550 mg boric acid/kg bw/day (equivalent to 0, 48 or 96 mg boron/kg bw/day). No clinical signs of toxicity were observed. Compared with controls, body weights were 10–17 % lower in males of the high-dose group after 32 weeks and in females of the high-dose group after 52 weeks. For males, survival at the end of the study was 41/50, 30/50, and 22/50 in control, low-, and high-dose groups respectively and this decrease was statistically significant. For females, survival was 33/50, 33/50, and 37/50 in control, low-, and high-dose groups respectively (NTP, 1987). The Panel noted the low mortality rate in the male control group. The testes showed testicular atrophy and interstitial cell hyperplasia at both dose levels. Also a dose-related increase in incidence of splenic lymphoid depletion in males was observed. An increased incidence of hepatocellular tumours in low-dose male mice was considered by NTP as non-associated to boric acid. Overall, the NTP concluded that this study produced no evidence of carcinogenicity of boric acid, although the low number of surviving males may have reduced the sensitivity of the study (NTP, 1987; Dieter, 1994; IPCS, 1998 as cited in EFSA, 2004).

In a 2-year study, rats (35 animals/sex/group) were administered sodium tetraborate or boric acid as boron equivalents at concentrations of 0, 117, 350 or 1 170 mg/kg in the diet (Weir and Fisher, 1972) (equivalent to 0, 5.9, 17.5 or 58.5 mg boron/kg bw/day; 33.7, 100 or 334 mg boric acid/kg bw/day; 52.2, 155 or 518 mg sodium tetraborate/kg bw/day); control groups contained 70 animals per sex. High-dose animals had coarse hair coats, scaly tails, hunched posture, swollen and desquamated pads of the paws, abnormally long toenails, shrunken scrotum, inflamed eyelids, and bloody eye discharge. These signs became frequent and more pronounced during the first year but did not change thereafter.

At the high dose, both boric acid and sodium tetraborate caused a lower food consumption during the first 13 weeks of the study and animals had suppressed growth throughout the 2 years. At this high dose, serum chemistry and urine values were normal, but the Packed Cell Volume and haemoglobin levels were significantly lower in males and females fed sodium tetraborate and in females fed boric acid compared to controls. Furthermore, the absolute and relative weights of the testes were significantly lower, and relative weight of the brain and thyroid were higher, than in controls. The seminiferous epithelium was atrophied, and the tubular size in the testes was decreased at 58.5 mg boron/kg bw/day. No treatment-related effects were observed at lower dose levels. An increased incidence of tumours was not observed (Weir and Fischer, 1972; IPCS, 1998). The NOAEL for this study was reported to be 17.5 mg boron/kg bw/day (EFSA, 2004).

The EVM (2003) concluded that there is no evidence of carcinogenicity from oral exposure to boron compounds in rats and mice (EVM, 2003). The Panel agreed with this conclusion.

3.2.4.2. Dogs

In a 2-year study, beagle dogs (4 animals/sex/group; bw not stated) were fed diets containing boric acid or sodium tetraborate at concentrations of 0, 58, 117 or 350 mg/kg diet as boron equivalents (Weir and Fisher, 1972) to provide doses of 0, 1.5, 2.9, or 8.8 mg boron/kg bw/day (EFSA, 2004) (equivalent to 8.29, 16.7 or 50 mg boric acid/kg bw/day). These doses of boric acid and sodium tetraborate had no remarkable effects on appearance, behaviour, appetite and elimination, body weight, food consumption, necropsy findings and absolute and relative organ weights. Consequently, additional groups received 1170 mg/kg diet of either boric acid or sodium tetraborate as boron equivalents (equivalent to 29 mg boron/kg bw/day or 166 mg boric acid/kg bw/day) until effects were noticed for both compounds after 38 weeks and the exposures terminated. In this study, no effects were observed on general appearance, body weight, food consumption, organ weights, haematology, or serum chemistry. Two dogs sacrificed at week 26 had testicular degeneration including atrophy of the seminiferous epithelium and spermatogenic arrest which appeared to be due to ingestion of sodium tetraborate; boric acid also caused testicular degeneration. Changes in testicular morphology occurred in males of both 1170 mg boron equivalents/kg diet groups at 38 weeks. After terminating exposure to 166 mg boric acid/kg bw/day, 2 dogs were placed on a control diet for 25 days and at the end of this period testes/body weight ratios were comparable to controls. In one of the dogs, microscopic examination revealed the seminiferous tubules to be lined by thick, moderately active spermatogenic epithelium. Based on the findings in this dog, the authors concluded that testicular degeneration appeared to be reversible and that spermatogenesis appeared to resume within a short period after boric acid withdrawal. Various organisations (ECETOC, 1995; IPCS, 1998; Health Canada, 1997; EFSA 2004) have questioned the validity of this study for risk assessment due to the small and variable number of dogs used (1–2 dogs at each of three time points) and the presence of testicular lesions in the controls (1 of 4 controls had slight to severe seminiferous tubular atrophy, another had moderate to severe atrophy, whereas a third had a detectable but insignificant reduction in spermatogenesis and 5 % atrophic seminiferous tubules). In conclusion, microscopic changes in the tissues of mice, rats, and dogs exposed to boric acid involve primarily the kidneys and testes. Glomerular and tubular damage have been noted in the kidneys. Glomerular damage consisted of changes in permeability of the capillaries, and tubular damage consisted of cellular vacuolisation and shedding of cells into the tubular lumen. In male reproductive organs, effects of boron have been noted in mice, rats, and dogs. Reproductive effects observed include inhibition of spermiation in stage IX and X tubules, followed by germ cell loss, changes in epididymal sperm morphology and caput sperm reserves, decreased serum testosterone levels, and testicular atrophy.

In summary, in males, effects on reproductive organs have been reported in oral exposure studies at doses of 29 mg boron/kg bw/day in dogs exposed for 2 years to dietary boric acid or sodium tetraborate (Weir and Fisher, 1972). For rats the NOAEL was reported to be 17.5 mg boron/kg bw/day (EFSA, 2004).

3.2.5. Reproductive and developmental toxicity

3.2.5.1. Reproductive toxicity

Testicular lesions have been observed in rats, mice, and dogs administered boric acid or sodium tetraborate in food or drinking-water (Truhaut et al., 1964; Weir and Fisher, 1972; Green et al., 1973; Lee et al., 1978; NTP, 1987; Ku et al., 1993, as reported in IPCS, 1998).

Mice

In a multigeneration continuous-breeding experiment (Fail et al., 1990, 1991), Swiss CD-1 mice (F0 generation) were fed boric acid in the diet at 0 (38 pairs), 1 000 (19 pairs), 4 500 (20 pairs) or 9 000 (20 pairs) mg/kg feed for 27 weeks (equivalent to doses of 0, 110, 598 and 1 269 mg boric acid/kg bw/day and 0, 19.2, 104.7 and 222.1 mg boron/kg bw/day for males, and 0, 182.3, 846.2, 1 660 mg boric acid/kg bw/day and 0, 31.9, 148.1 and 290.5 mg boron/kg bw/day for females). Treatment with boric acid significantly impaired fertility: no males or females in the high-dose groups were fertile. At the mid-dose, the number of litters per pair, number of live pups per litter, proportion of pups born alive and pup weight adjusted for litter size were all decreased compared with controls. The trend towards a lower fertility index at the mid-dose level started with the first litter and progressed in severity with subsequent matings. Animals from different treatment groups were cross-mated to determine the affected sex at this dose. When mid-dose males were mated with control females, mating and fertility indices were significantly depressed, with only one pair in that group producing a live litter; these indices were not affected when control males were mated with mid-dose females, confirming that the male was the affected sex. At F0 necropsy, sperm motility was significantly reduced in all exposed groups (by 12 % for low-dose, 32 % for mid-dose, and 47 % for high-dose). Low-dose and mid-dose animals from the F1 generation were exposed during gestation and lactation. The fertility of the low-dose F1 mice was not affected, but the litter-adjusted body weights of the F2 pups were significantly decreased (by 3.3 %) relative to controls. The low-dose was considered the LOAEL for an 8 % decreased sperm motility in the F0 males, 26 % increased uterine weight and 8 % increased kidney/adrenal weight in F1 females, and a 3.3 % reduction in litter-adjusted birth weight in the F2 pups. This study had no NOAEL, but the magnitude of the changes at the low dose is small and indicates that this dose level is close to the NOAEL (IPCS, 1998). The Panel noted that the LOAEL in this study is 19.2 mg boron/kg bw/day.

The IPCS (1998) noted that the unique effects in the adult F1 female mice (increased uterine weight, reduced cycle length) are consistent with an effect on female reproduction that was not seen in the F0 females, giving rise to concerns of the possibility of unique transgenerational or functional developmental toxicity in the absence of an available dose-response (IPCS, 1998).

Fail et al. (1989, as reported in IPCS, 1998) used both CD-1 mice and wild deer mice (*Peromyscus maniculatus*) to characterise boric acid effects on fertility and to test the reversibility of these effects. Adult CD-1 mice were exposed to boric acid in the feed at 0, 1 000, 4 500 or 9 000 mg/kg diet (equivalent to 150, 674 or 1 349 boric acid mg/kg bw/day; 0, 26.3, 118 or 236 mg boron/kg bw/day) for 27 weeks. Males in the mid- and high-dose groups had testicular atrophy and decreased spermatogenesis. Fertility was decreased in animals receiving the mid-dose and completely absent in the high-dose group. The same doses were used to test toxicity and reversibility in wild deer mice. The ability to produce offspring was significantly reduced by boric acid and none of the males in the high-dose group produced litters. Weight gains were similar for the treated and control wild deer mice. Although body and organ weights were similar between the mid-dose and control groups at necropsy, the testis and total accessory sex organ weights were significantly lower in the high-dose group compared to controls. These two species demonstrated different sensitivity to boric acid. The CD-1 mice had body weight loss as early as 5 weeks at the mid-dose level, whereas wild deer mice did not lose weight even at the high dose. Tissue pathology findings at the mid-dose were significant in CD-1 mice but not in wild deer mice. Wild deer mice were found to recover from boric acid exposure.

In follow-up studies, male Swiss CD-1 mice (17–20 animals per group) were exposed to dietary boric acid at 0 or 9 000 mg/kg diet (equivalent to 222 mg boron/kg bw and 1 269 mg boric acid/kg bw/day) for 9 weeks (Fail et al., 1998). Testicles were significantly smaller in the treated males compared to controls. In a similar study with male deer mice, after exposure to 0, 4 500 or 9 000 mg boric acid/kg diet (equivalent to 0, 111 or 222 mg boron/kg bw) for 8 weeks, dose-related testicular weight changes were observed. In both studies, males fed 4 500 mg/kg diet were infertile when mated to virgin, non-treated females. For the deer mice, infertility was completely reversible; reversibility was not tested in Swiss mice. At 9 000 mg boric acid/kg diet, severe degeneration was present after 9 weeks. Dose-response testicular degeneration was also observed for deer mice treated with 4 500 and 9 000 mg boric acid/kg diet for 8 weeks. Leydig cells in Swiss mice and deer mice appeared to be normal (PA Fail, Center for Life Sciences and Toxicology, Research Triangle Park, North Carolina, personal observation, 1996). The authors concluded that doses of 4500 mg boric acid/kg diet (equivalent to 111 mg boron/kg bw/day) caused infertility and disrupted spermatogenesis in males of both mouse species when exposure periods of 8 weeks or longer were tested.

Groups of Swiss CD-1 male mice (10 animals/group; 12–14 weeks old) were given boric acid by gavage at concentrations of 0, 120, 400 and 1200 mg/kg bw/day (equivalent to 0, 21, 70, 210 mg boron/kg bw/day) from study days 3–20 (Harris et al., 1992 as reported in ECETOC, 1995). Female animals (10 animals/group; 12–14 weeks old) were exposed to the same levels for days 0–20. Matings occurred during days 8–12 and necropsies occurred on day 20 for males and day 21 for females. At the highest dose tested, significant histopathological changes in the testis were noted including germ cell loss and tubular disruption. At the mid- and high-dose groups, reduced testis weight was also noted. For the females, 2 out of 10 animals at the top dose died but no effects on reproductive parameters were reported.

Rats

In addition to multigenerational reproductive toxicity studies, short-term studies reporting the results for relevant reproductive end-points are also reported.

Wistar rats (10 animals/sex/group) were given a single dose of boric acid by gavage at doses of 3000, 4000, 5000 mg/kg bw (equivalent to 525, 700, 875 mg boron/kg bw/day). Thirty days after the exposure, testicular lesions were discovered in the surviving animals of the mid- and high-dose groups, which included decreased testis volume, decreased number of sperm cells, persistence of Sertoli cells and hyperplasia of Leydig cells (Caujolle et al., 1962, as reported in ECETOC, 1995). The NOAEL was reported to be 525 mg boron/kg bw.

Caujolle et al. (1962) also exposed male and female rats to 200, 400 or 800 mg boric acid/kg bw/day (equivalent to 35, 70 or 140 mg boron/kg bw/day) by gavage for 30 days. Animals were observed for four generations for 1 year following exposure. Males exposed to the highest dose were sterile. Sterility and reduced fertility were observed in males given 400 mg boric acid/kg bw/day when mated with treated females, but sterility was not observed when exposed males were mated with control females. Histopathological changes to the testes were observed at 400 and 800 mg boric acid/kg bw/day. Sertoli cells were persistent but degenerate, whilst Leydig cells proliferated especially when tubular atrophy was increased. Slight histopathological changes were noted in the testes of males given the lowest dose. No lesions were found in female reproductive organs. The authors stated that animals of subsequent generations displayed normal fertility (no further details given) (Caujolle et al., 1962, as reported in ECETOC, 1995).

In a three-generation reproduction study in rats ($n = 8$ males and 16 females) rats were fed boric acid or sodium tetraborate in the diet at concentrations of 0, 117, 350 or 1 170 mg boron equivalents/kg diet (approximately equivalent to 0, 11.7, 35 or 117 mg boron/kg bw/day and 0, 67, 200, 669 mg boric acid/kg bw/day). Before the initiation of each breeding phase, the rats were fed these diets for 14 weeks. For both compounds, at the 2 lowest doses there were no adverse effects and the overall fertility indices were higher compared to controls. In addition, no gross abnormalities were observed

in organs examined from parents or weanlings from these lower concentration groups (Weir and Fisher, 1972). For both compounds, the highest dose caused sterility in males and decreased ovulation in females. At this high dose, lower body weight gains in both sexes, decreased food efficiency in females, and testicular degeneration in males were also observed (NPIC, website accessed 2011). The IPCS evaluation considered that the small group size, low control fertility rates (approx. 60 %), limited data reported, and inappropriate statistics limited the applicability of these data for risk assessment (Weir and Fischer, 1972 as reported in IPCS, 1998).

Male Sprague-Dawley rats (18 animals/group) were fed diets containing 0, 500, 1000 or 2000 mg sodium tetraborate/kg diet (approximately 0, 2.8, 5.7 or 11.3 mg boron/kg bw/day; 0, 16, 32.3, or 64.6 mg boric acid/kg bw/day) for 30 or 60 days (Lee et al., 1978, as reported in IPCS, 1998; Dixon et al., 1979). Significant decreases ($P < 0.05$) in the weight of liver, testes (including decrease in seminiferous tubular diameter and a reduction in spermatocytes and spermatogenic cells) and epididymis were observed at the 1 000 and 2 000 mg/kg dietary levels. Secondary to the loss of germ cells, the activities of enzymes found primarily in spermatogenic cells were significantly decreased, and enzyme activities associated with premeiotic spermatogenic cells were significantly increased (Lee et al., 1978; Dixon et al., 1979). Mean plasma follicle-stimulating hormone (FSH) levels were significantly elevated in a dose-dependent manner in all treatment groups in this study after 60-day exposures. FSH levels in animals exposed to the highest dose tested were still elevated 12 months after treatment termination, due to atrophied testes and no recovery of spermatogenesis. Plasma Luteinising Hormone (LH) levels were not significantly elevated, and mean plasma testosterone levels were within the normal range throughout the study (Lee et al., 1978; Dixon et al., 1979). At the end of the treatment period, male rats (five animals per group) were placed on normal diets and serial mating studies commenced. Each male was mated with a series of untreated virgin females in sequential 7-day periods for up to 12 weeks. Male fertility was significantly reduced at 1 000 and 2 000 mg/kg diet (Lee et al., 1978; Dixon et al., 1979). Although most effects were reversible 5 weeks after treatment, sterility in the 2 000 mg/kg diet group persisted for 60 days. At the lowest dose, no effects on reproduction were observed. For both treatment periods, at 1 000 mg/kg diet, infertility was observed in the first 3–5 weeks of breeding which then returned to approximate control levels. At 2 000 mg/kg diet for 30 days, dosed animals showed a significant reduction in fertility for 8 weeks with only partial recovery observed at 7–9 weeks. At 2 000 mg/kg diet for 60 days, dosed animals showed a significant reduction in fertility with no recovery up to 20 weeks. There were no dose-related decreases in litter size or fetal deaths, which was reported to indicate that boron caused germinal aplasia rather than a dominant lethal effect. In an EPA evaluation, a LOAEL of 50 mg boron/kg bw/day and a NOAEL of 25 mg boron/kg bw/day were defined for this study based on dose-related tubular germinal aplasia, which was reversible at low doses (EPA, 2004).

Dixon et al. (1976, as reported in EPA, 2004) studied the effects of sodium tetraborate on reproduction in male rats following acute and subchronic exposure. In the acute study, adult male Sprague–Dawley rats (10 animals per group) were given single oral doses of sodium tetraborate at 0, 45, 150 or 450 mg boron/kg bw (equivalent to 0, 257, 857 or 2 571 mg boric acid/kg bw). Fertility was assessed by serial mating trials in which each male was mated with a series of untreated virgin females in sequential 7-day periods (for up to 70 days). The females were sacrificed 9 days after the end of their breeding periods (when they could be 9–16 days' pregnant), and uteri and fetuses were examined. Male rats were sacrificed on days 1 and 7, and at subsequent 7-day intervals, for histopathological examination of the testes. No effect on male fertility was found at any dose in this study. Testicular lesions were not reported. For this study, an EPA evaluation of boron reported a NOAEL of 450 mg boron/kg bw for reproductive effects in male rats following single-dose oral exposure.

In the subchronic study, male Sprague–Dawley rats ($n = 10$ animals per group) were given 0, 0.3, 1.0 or 6.0 mg boron/L as sodium tetraborate (equivalent to 0, 1.7, 5.7 or 34 mg boric acid/L), in the drinking water for 30, 60 or 90 days (Dixon et al., 1976). The estimated highest exposure level provided 0.84 mg boron/kg bw/day (equivalent to 4.8 mg boric acid/kg bw/day). Based on this estimate, the lower two levels provided 0.042 and 0.14 mg boron/kg bw/day (equivalent to 0.24 and 0.8 mg boric acid/kg bw/day). For all doses and exposure durations, there were no noticeable

reproductive effects or changes in serum chemistry, plasma levels of FSH and LH, or weight of the body, testes, prostate or seminal vesicles. Fructose, zinc and acid phosphatase levels in the prostate were unchanged. After treatment, male rats were housed with a non-treated virgin female rat for 7 days which was then replaced by other virgin female rats for 7-day periods for up to 70 days. Each female rat was sacrificed and the uteri and fetuses examined. Breeding studies revealed no effects on male fertility. For this study, an EPA (2004) evaluation on boron reported the dose of 0.84 mg boron/kg bw/day, the highest dose tested, as the NOAEL (EPA, 2004). The Panel agreed with this conclusion.

The IPCS (1998) and EVM (2003) summarised that male reproductive effects from boron have been noted in mice, rats, and dogs and included inhibition of spermiation in stage IX and X tubules, followed by germ cell loss, changes in epididymal sperm morphology and caput sperm reserves, decreased serum testosterone levels, and testicular atrophy. Male reproductive effects have been reported in 2 year oral exposure studies using boric acid or sodium tetraborate at doses of 29 mg boron/kg bw/day in dogs (Weir and Fisher, 1972) and in rats the LOAEL for reproductive toxicity was 58.5 mg boron/kg bw/day (IPCS, 1998). The NOAEL for the three-generation rat study was reported as 17.5 mg boron/kg bw/day (Weir and Fisher, 1972). The Panel agreed with these conclusions.

Furthermore, the Panel agreed with ECETOC (1995) that dogs are not an appropriate model for reproductive studies and, therefore, did not take into account the data from the 2-year dog study (see section 3.2.4.2) for risk assessment given the low number of animals involved and the large variability of the dogs.

3.2.5.2. Developmental toxicity

Mice

The developmental toxicity of boric acid in Swiss albino CD-1 mice ($n = 26\text{--}28$) provided throughout gestation days 0–17 (0, 0.1, 0.2 or 0.4 % in feed) at estimated average doses of 0, 248, 452 or 1 003 mg boric acid/kg bw/day (equivalent to 0, 43, 79 or 175 mg boron/kg bw/day) was investigated (Field et al., 1989; Heindel et al., 1992; ECETOC, 1995). At sacrifice on gestation day 17, the groups contained 27, 27, 27 and 26 pregnant dams respectively (Moore, 1997). There was a dose-related decrease in average fetal body weight per litter, which was significant at 452 and 1 003 mg boric acid/kg bw/day ($P < 0.05$). Compared with controls, mice in the high-dose group showed an increase in resorptions per litter (19 % compared to 6 % in control) and fetal weight was reduced by 33 %. There was a significant increase in the number of malformed fetuses per litter at the high-dose group in comparison to controls ($P < 0.05$). In offspring of mice exposed to 452 or 1003 mg boric acid/kg bw/day, there was an increased incidence of skeletal malformations (particularly short rib XIII). There was a dose-dependent decrease in the incidence of rudimentary or full rib(s) at lumbar I (considered an anatomical variation). These changes occurred at doses for which there were also signs of maternal toxicity as maternal body weights of mice in the high-dose group were also 10–15 % below control values in the later days of treatment even though food and water consumption were not reduced. Relative kidney weight was increased significantly ($P < 0.05$) and minor renal lesions (renal tubular dilatation and/or regeneration) in randomly selected dams were also observed as follows: 0/10 in control, 2/10 in low-dose, 8/10 in mid-dose and 10/10 in high-dose groups. The authors surmised that since the renal lesions were minor and occurred at lower incidence in the low dose-group compared to the higher dose groups, that the low dose level of 248 mg/kg bw/day should be near the range of a NOAEL for maternal toxicity. There was no NOAEL for maternal toxicity (Field et al., 1989; Heindel et al., 1992).

Cherrington and Chernoff (2002, as reported in EPA, 2004) evaluated the developmental toxicity of boric acid in pregnant CD-1 mice in three different experiments. In the first experiment, mice were dosed daily from gestation days 6–10 by gavage with either 0, 500, or 750 mg boric acid/kg bw (equivalent to 0, 88, 131 mg boron/kg bw) (10 animals/group; control group 6 animals). The second experiment consisted of 160 timed pregnant animals weighed on gestation day 6 and assigned to 1 of

10 groups: controls treated on each of gestation days 6–8 (1 group); controls treated only on a single gestation day 6, 7, or 8 (3 groups); and groups of dams treated with a gavage dose of 400 mg/kg bw twice daily (total dose 800 mg boric acid/kg bw/day; equivalent to 140 mg boron/kg bw/day) on each of gestation days 6–8 (1 group) or only on a single gestation day 6, 7, 8, 9, 10 (4 groups). The third experiment consisted of either a single or 2 gavage doses of 750 mg/kg bw (equivalent to 131 mg boron/kg bw) each on gestation day 8. In the group with a single gavage dose on gestation day 8, 52 pups from 4 control litters and 33 pups from 3 boric acid-dosed litters were examined. For the group with 2 gavage doses of 750 mg/kg bw each on gestation day 8, 103 controls and 94 boric acid-treated fetuses were examined, weighed, and stained with alizarin red and alcian blue for skeletal evaluation on gestation day 17. From the first experiment, results indicated that 400 mg/kg bw daily doses resulted in decreased rib length, whilst daily doses of 750 mg/kg bw resulted in decreased rib length and femur length. Fetal body weight was not significantly decreased at either of these doses. In the second experiment, the results for the gestation days 9 and 10 daily exposures were not presented due to the lack of a concurrent control. Fetal body weight was reduced in all boric acid treatment groups (single days at gestation days 6, 7, 8, 9, or 10 and consecutive days from gestation days 6 to 8). Femur length was decreased on gestation day 7 and in fetuses exposed during gestation days 6–8. Cervical ribs were observed in fetuses exposed on gestation day 6, 7, or 8. From the third experiment, results indicated that the 2 doses of 750 mg/kg each on gestation day 8 significantly increased frequency of 11 separate malformations over background incidence. The most prevalent malformations were those associated with rib development. In contrast, the single dose on this day produced only increased incidences of unilateral thoracic vertebrae and cervical rib formation/ossification differences. These results demonstrate a separation of the effects of boric acid on fetal body weight and rib malformation with respect to the timing of the dose.

Rats

In Sprague-Dawley rats (26–28 animals/group), Heindel et al. (1992) investigated the developmental toxicity of boric acid provided in the diet throughout gestation days 0–20 at estimated average doses of 0, 78, 163 or 330 mg/kg bw/day (equivalent to 0, 13.6, 28.5 or 57.7 mg boron/kg bw/day). The dosing regimen allowed plasma boron levels to reach steady state before implantation. An additional group of rats received boric acid at 539 mg/kg bw/day on gestation days 6–15 only (i.e. post implantation). Maternal effects included a significant ($P < 0.05$) and dose-related increase in relative liver and kidney weights at 163 mg boric acid/kg bw/day and above. Only treatment with 539 mg boric acid/kg bw/day significantly increased prenatal mortality. Average fetal body weight per litter was significantly reduced ($P < 0.05$) in a dose-related manner in all treated groups compared with controls. The percentage of malformed fetuses per litter and the percentage of litters with at least one malformed fetus were significantly increased at 163 mg boric acid/kg bw/day and above. Malformations consisted primarily of anomalies of the eyes, the CNS, the cardiovascular system and the axial skeleton. The most common malformations were enlargement of lateral ventricles in the brain and agenesis or shortening of rib XIII. The percentage of fetuses with variations per litter was reduced relative to controls at 78 and 163 mg boric acid/kg bw/day (due to a reduction in the incidence of rudimentary or full ribs at lumbar 1 which is common for the species and strain) but was increased in rats exposed to 539 mg/kg bw/day. The variation with the highest incidence among fetuses was wavy ribs. The LOAEL of 78 mg/kg bw/day (lowest dose tested) for rats occurred in the absence of maternal toxicity; based on decreased fetal weight, a NOAEL was not found in this study.

Price et al. (1996a) followed up the Heindel et al. (1992) study in Sprague-Dawley (CD) rats to determine a NOAEL for fetal body weight reduction and to determine whether the offspring would recover from prenatal body weight reductions during postnatal development (IPCS, 1998). Skeletal malformations and variations were also studied to further characterise the low end of the [dose-response](#) curve and to determine whether skeletal defects incidences in the offspring changed during postnatal life. Boric acid was administered in the diet to female Sprague-Dawley rats (27–30 animals per group; representing 88–100 % pregnancy rate) from gestational days 0 to 20 at doses of 0, 19, 36, 55, 76 or 143 mg boric acid/kg bw/day (equivalent to 0, 3.3, 6.3, 9.6, 13.3 or 25.0 mg boron/kg bw/day). On gestational day 20, dams were terminated and uterine contents examined (Price et al., 1996a). The

only effect noted in the dams was an increase in relative kidney weight at the highest dose tested on gestation day 20 ($P < 0.05$), but this effect was no longer apparent at postnatal day 21. On gestational day 20, fetal body weights were statistically significantly reduced ($P < 0.05$) in both male and female offspring in the 76 (94 % of control) and 143 mg (88 % of control) boric acid/kg bw/dose groups only. These deficits in body weight were no longer evident at birth (approximately gestation day 22). Incidences of short rib XIII (a malformation) and wavy rib (a variation) were increased in the 76 and 143 mg boric acid/kg bw/day dose groups relative to control litters (Price et al., 1996a). In the high-dose group, the incidence of rudimentary extra rib on lumbar 1 (a variation) that was deemed biologically but not statistically significant. The incidence of extra rib on lumbar 1 (full and/or rudimentary) was 3.1, 1.3, 2.8, 0.9, 1.5, 0.2 % of fetuses for the controls through to the highest group respectively.

In a second stage of the same study, the same doses of boric acid (as above) were given in the diet to female Sprague–Dawley rats (25–29 animals per group), but exposure was terminated at birth and dams were allowed to deliver and rear their litters until postnatal day 21. On postnatal day 0, there were no effects of boric acid on offspring body weight, nor were any differences seen up to postnatal day 21. There was a significant increase in the incidence of skeletal malformations relative to controls in the 19 and 143 mg/kg bw/day groups. Variations in the incidence of short rib and wavy rib were not dose related and the percentage of pups per litter with short rib XIII was elevated only in the 144 mg boric acid/kg bw/day dose group. Also, on postnatal day 21 there was no incidence of extra rib on lumbar 1 in control or treated animals (Price et al., 1996a).

The Panel concluded that the NOAEL for this study is 55 mg boric acid/kg bw/day (equivalent to 9.6 mg boron/kg bw/day) based on a decrease in fetal body weight.

Rabbits

The developmental toxicity of boric acid was investigated in artificially inseminated New Zealand White rabbits. Boric acid in aqueous solution was administered by gavage to New Zealand White rabbits (18–23 pregnant /group) throughout gestation days 6–19 at doses of 0, 62.5, 125 or 250 mg boric acid/kg bw (equivalent to 0, 11, 22 or 44 mg boron/kg bw) (Heindel et al., 1994; Price et al., 1996b, EPA, 2004). Food consumption, body weight and clinical signs were monitored throughout the study. At the highest dose, maternal food consumption decreased during treatment, but increased at doses of ≥ 125 mg boric acid/kg bw/day after treatment. Maternal body weight (gestation days 9–30), weight gain during treatment, gravid uterine weight and number of ovarian corpora lutea decreased at 250 mg boric acid/kg bw/day, but maternal corrected gestational weight gain increased at ≥ 125 mg boric acid/kg bw/day (b et al., 1996). No treatment-related clinical signs of toxicity were observed during the study, except for vaginal bleeding noted in 2–11 does per day on gestation days 19–30 at the high dose; these does had no live fetuses on day 30. Vaginal bleeding was also observed in one female in the low-dose group and in one in the mid-dose group. Two maternal deaths occurred (one each at the low- and mid-dose), but were not treatment-related. At the high-dose, maternal relative kidney weight (but not absolute) was increased (without histopathological changes) whilst liver weight remained comparable in treated groups and controls. At gestation day 30, the animals were sacrificed and pregnancy status, number of resorptions, fetal body weight, viability, and external, visceral and skeletal malformations were examined. Developmental toxicity was not observed at doses less than 250 mg boric acid/kg bw/day. At 250 mg boric acid/kg bw/day, 90 % of implants were resorped (control 6 %), 73 % of litters were lost (i.e. no live fetuses in litter) (control 0 %), live litter size was reduced to 2.3 fetuses per litter (control 8.8) and average fetal body weight per litter was 92 % of control value. The incidence of late fetal deaths was low in all groups (≤ 2.8 % per litter). Consequently, there were only 14 live fetuses (six live litters) available for evaluation in the high-dose group, compared with 153–175 live fetuses (18–23 live litters) in the other groups (Price et al., 1996b).

Overall incidence of malformations at 250 mg boric acid/kg bw/day was 81 % per litter (control 26 %); visceral malformations were increased at the high-dose but skeletal malformations were not. Malformations of the cardiovascular system were the most frequently observed abnormality; at

250 mg boric acid/kg bw/day the incidence of fetuses per litter with major cardiovascular defects was 72 % (control 3 %) (Heindel et al., 1994). The most prevalent cardiovascular malformation (interventricular septal defect) was observed in 57 % of high-dose fetuses compared to 0.6 % among controls. The LOAEL was 250 mg boric acid/kg bw/day and the NOAEL was 125 mg boric acid/kg bw/day for developmental toxicity (prenatal mortality and malformation). The authors highlighted that the kinetics of the feeding studies in rodents would differ to those of the gavage study in the rabbit and consequently, despite using similar doses, exposures at target sites could differ considerably. Kinetic data were not available for the gavage study.

The adverse effects of boric acid on development and fertility observed across species were very similar, both in nature and effective doses. Further, the adverse effects obtained with boric acid are comparable to those obtained from other borates thus confirming that boron is the toxicologically active species. The available data on toxicokinetics do not indicate differences between laboratory animals and humans. It is not known whether there are significant differences in the toxicodynamics between humans and laboratory animal models and in the absence of such knowledge it must be assumed that the effects seen in animals could occur in humans. On the basis of toxicokinetic and toxicodynamic considerations it is assumed that the animal data are relevant to humans. This is further underlined by the fact that (1) there are indications that boric acid is able to cross human placenta and that (2) up to now, epidemiological studies in humans are insufficient to demonstrate the absence of an adverse effect of inorganic borates on fertility (ECHA, 2010). The Panel agreed with these conclusions.

3.2.6. Hypersensitivity, allergy and intolerance

In an OECD TG 406 study for skin sensitisation performed in the guinea pig according to GLP boric acid was found to be non-sensitising (IUCLID, 2000).

3.2.7. Human studies

3.2.7.1. Acute and repeated toxicity

Data from accidental poisonings indicate that the human is 2–3 g in infants, 5–6 g in children and 15–20 g in adults (for example, this would be equivalent to 2.6–3.5 g of boron in adults) (Dixon et al., 1976; Siegel and Wason, 1986; EVM 2003; EPA, 2004, ECETOC, 1995). Doses of 200–640 mg boric acid/kg bw have been reported to be lethal, but these values have not been fully verified (Fail et al., 1998). Another report stated that the lowest lethal dose after single accidental oral ingestion of boron acid by humans ranged from approximately 98 to 650 mg boron/kg bw (Stokinger, 1981; Teshima et al., 1992, as cited in EFSA, 2004). Death has been reported when boron was given intravenously at 0.5 mg boron/kg bw (EFSA, 2004).

3.2.7.2. Reproductive and developmental effects

Reproductive effects

There are numerous epidemiological studies on the effects of boric acid and boron exposure, primarily from occupational exposures with a degree of exposure through inhalation (European Borates Association, 2010); some of these studies are reported below.

Yazbeck et al. (2005) studied the correlation between boron blood levels and boron levels in drinking water in a group of 180 healthy individuals from Northern France. Men living in areas with more than 0.30 mg boron/L (equivalent to 1.71 mg boric acid/L) in drinking water had elevated but not significant boron blood levels compared with those living in municipalities with boron water levels of less than 0.30 mg/L (159.1 versus 123.0 ng/g; $P > 0.05$). Based on indicators such as birth rates, mortality rates and sex ratio, the authors concluded that the results of this study indicated that consumption of boron in drinking water at the levels found in the regions studied did not induce any deleterious effects.

A study was conducted to investigate the potential reproductive effects of sodium tetraborate exposure on men working in a sodium tetraborate mining and production factory located in California (Whorton et al., 1994a, b, as reported in IPCS, 1998). The study group comprised 542 men of which 72 % were eligible to participate. Data were collected via questionnaires and telephone follow-up interviews. In total, the study group fathered 529 births compared to an expected number of 466.6. The individuals were divided into groups based on the levels of exposure, but no adverse effects were associated with high exposures. There was a slightly higher and non-significant ratio of female to male births (i.e. 52.7 % female birth compared to the normal ratio value of 48.8 %). The authors overall concluded that there did not appear to be any hazardous effects of sodium tetraborate exposure on fertility.

In a 3-week study in post-menopausal women receiving a low-boron diet (0.33 mg/day; equivalent to 1.9 mg boric acid/day) or this diet supplemented to 3.33 mg boron/day (equivalent to 19.0 mg boric acid/day), the supplement had no effect on blood levels of minerals, or steroids (Beattie and Peace, 1993). In female volunteers supplemented with 3 mg boron (equivalent to 17 mg boric acid/day)/day for 1 year, no adverse effects were identified (Meacham et al., 1994).

Plasma oestradiol and testosterone levels were increased in adult male volunteers supplementing their diet with 10 mg boron/day for 4 weeks (Naghii and Samman, 1997; as reported in EPA, 2004). Mean total plasma testosterone concentration increased non-significantly after 4 weeks of supplementation. In comparison, the mean plasma 17β oestradiol concentration increased significantly, and the ratio of 17β oestradiol to testosterone increased significantly after supplementation. Plasma total cholesterol, triglyceride concentrations, or distribution among LDL and HDL fractions were not altered.

In a related study, 8 males ingested 10 mg boron as sodium tetraborate (equivalent to 57 mg boric acid or 88.5 mg sodium tetraborate) in capsule form, with their breakfast meal daily for 7 days (Naghii et al., 2011). Mean baseline plasma concentrations of boron were 0.036 ± 0.021 mg/L, within 24 hours of supplementation they were 0.066 ± 0.028 mg/L, after 7 days they were 1.32 ± 0.057 mg/L. After this supplementation period, the mean plasma free testosterone concentrations significantly increased whilst the mean plasma oestradiol decreased. Concentrations of inflammatory biomarkers (high sensitive CRP, TNF-alpha, interleukin 1-beta) also decreased substantially.

In a supplementation study, 4 men and 9 postmenopausal women, of whom 5 were given oestrogen therapy, and 1 woman prior to menopause, ate a diet with a boron content of 0.25 mg (equivalent to 1.4 mg boric acid) over 63 days (Nielsen et al., 1992, as reported in BfR, 2005). They were then given the same diet for 49 days which had been supplemented with 3 mg boron/day as sodium tetraborate (equivalent to 17 mg boric acid). Only in the case of the women who were undergoing hormone treatment, did the 17β -oestradiol serum contents of 99 ± 15 pg/mL prior to boron supplementation then increase to 157 ± 27 pg/mL ($p = 0.02$) after supplementation. It is well known that oestrogen administration leads to an increase in the copper plasma concentration. The copper concentration in plasma rose from 146 ± 9 µg/dl to 159 ± 11 µg/dl ($p = 0.04$). The authors suspected that elevated 17β -oestradiol values could be due to greater resorption of 17β -oestradiol or reduced degradation or excretion caused by boron. The study also revealed that the administration of oestrogens raises the serum concentrations of the immunoreactive ceruloplasmin and triglycerides and that the concentrations of these compounds in serum, as a consequence of boron supplementation, increased in all test persons. It was concluded that boron not only amplifies some oestrogen effects but also imitates them but they also acknowledged that the results need to be verified in larger patient groups.

A pregnant female (24 years old) who had ingested 250 mg boric acid (equivalent to 43.8 mg boron) during her 18th week of pregnancy had serum boron levels of 7.9 mg boron/L after ingestion which then reduced to 0.42 mg boron/L 28 hours after ingestion. An elimination half-life of 6 hours was reported. The female gave birth to a normal baby at gestation week 39 and up until 5 months of age, the baby showed no developmental delays (Harchelroad and Peskind as reported in Moore, 1997).

The EPA (2004) evaluation on boron reported that various case reports indicated that newborn infants (number not given) who ingested 4.5–14 g boric acid showed CNS symptoms. Doses of about 500 mg

boron/kg bw/day (equivalent to 2857 mg boric acid/kg bw/day) showed CNS involvement with headaches, tremors, restlessness and convulsions followed by weakness, coma, and death. Histological examination of 2/11 infants revealed degenerative changes in brain neurons, congestion, and oedema of brain and meninges with perivascular haemorrhage and intravascular thrombosis.

3.2.8. Mechanistic studies

Yoshizaki et al. (1999) reported that all parameters of epididymal sperm analysis were affected in males administered 500 mg boric acid/kg bw/day for 3 weeks and sperm number, motility, velocity and amplitude of lateral head displacement were also affected at 150 mg boric acid/kg bw/day. Morphological examinations revealed atrophy of seminiferous tubules and multinucleated giant cells in the testis in males administered 500 mg boric acid/kg bw/day. The NOAEL in this study was 50 mg boric acid/kg bw/day (equivalent to 8.8 mg boron/kg bw/day).

4. Discussion

The present opinion deals with the re-evaluation of the safety of boric acid (E 284) and sodium tetraborate (E 285) when used as food preservatives.

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations and reviews, additional literature that has become available since then and the data available following a public call for data. The Panel noted that not all original studies on which previous evaluations or reviews were based were available for re-evaluation by the Panel.

Boric acid and sodium tetraborate (borax) are authorised as food additives in the EU. They are authorised for use as preservatives of sturgeon eggs (caviar) up to a maximum concentration of 4 g boric acid/kg food by Commission Regulation (EU) 1129/2011. Previous evaluations relevant for assessing the safety for use in food have been performed by the Scientific Committee for Food in 1979, endorsed in 1988 and published in 1992 (SCF, 1992), Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1962), EFSA (2004ab, 2005, 2012) and TemaNord (2002). In addition, boron for use in food and drinking water has been evaluated by the SCF (1979, 1988, 1992, 1998), EVM (2003) and the WHO (2011). The purity criteria for boric acid and sodium tetraborate for use as food additives have been specified in the Commission Regulation (EU) 231/2012. Boric acid and sodium tetraborate are also included in Regulation (EU) No 609/2013 regarding substances that may be added for specific nutritional purposes in foods for particular nutritional purposes (PARNUTS). Boric acid and sodium borate may be used as mineral substances in the manufacture of food supplements according to Regulation (EC) No. 1170/2009 of 30 November 2009.

In 1990, the SCF evaluated boric acid and concluded that boric acid and its salts were toxicologically acceptable for use only as a preservative in genuine caviar (SCF, 1992). The evaluation noted that a long-term study in rats had demonstrated some accumulation of boric acid in some organs. The evaluation also reported that the compound is nephrotoxic (SCF, 1992).

In 2002, boric acid and sodium tetraborate were evaluated by TemaNord (TemaNord, 2002). Boric acid and sodium tetraborate were not recommended for wide use as food additives, but due to their restricted use in sturgeons eggs (genuine caviar), exposure was considered to be limited (it was calculated that at the maximum permitted use level of 4 g/kg, 10 g caviar would be required to reach the TDI for boron) and not to pose any adverse health effects. Therefore no further action was recommended (TemaNord, 2002).

In 2004, EFSA's former Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA Panel) defined a UL value for boron (in the form of boric acid and sodium tetraborate) of 10 mg/day for adults, based on decreased fetal body weights in rats following maternal exposure during pregnancy (EFSA, 2004).

Furthermore, the EFSA's former Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) delivered a statement on boric acid and sodium borate as nutrient sources of boron. The AFC Panel based its findings on the NDA opinion of the same year which had derived a UL of 10 mg/day for adults. As this UL applied to the intake of boron in the form of boric acid and borates and considering boric acid and sodium borate to be safe, the AFC Panel considered them suitable for use in foods for particular nutritional uses, food supplements and foods intended for the general population, provided that the UL was not exceeded (EFSA, 2005b).

Boric acid and sodium tetraborate are also included in Regulation (EU) No 10/2011¹⁹ on plastic materials and articles intended to come into contact with food. A specific migration limit (SML(T)) of 6 mg boron/kg food is applied respectively for boric acid only in Annex II, and for the combined total of boric acid, anhydrous sodium tetraborate and barium tetraborate in Annex III. The SML(T) value was derived from the group TDI of 0.1 boron mg/kg bw/day established by SCF and the assumption that 60 kg adult ingests 1 kg food daily containing these substances (Directive 2002/72/EC²⁰).

Studies in animals have shown that boron is readily absorbed following oral exposure in rats (Ku et al., 1991; Usuda et al., 1998), rabbits (Draize and Kelley, 1959), sheep (Brown et al., 1989) and cattle (Owen, 1944; Weeth et al., 1981; EPA, 2004). Absorption of borates is approximately 95 % in rats following ingestion (IPCS, 1998; Vanderpool et al., 1994, as reported in EPA, 2004). The Panel considered that due to its ionisation properties (pKa of 9.15) the unionised form of boric acid should be absorbed by a diffusion process in the stomach where the pH is acidic.

Boric acid is taken up from the gastrointestinal tract via passive diffusion (IPCS, 1998; EVM, 2003; EFSA, 2004). Boron appears rapidly in the blood and body tissues (liver, muscle, colon, testis, epididymides, seminal vesicles, prostate, adrenals) of several mammalian species following ingestion (IPCS, 1998). Most of the boron in blood is associated with the plasma fraction (Moseman, 1994). Boron is distributed throughout the tissues and organs of animals and humans at concentrations between 0.05 and 0.6 mg/kg fresh weight (Nielsen, 1986, 1989, as reported in EFSA, 2004), with lower levels found in fatty tissues (BfR, 2005). Kingma (1958) reported that small quantities of boric acid have also been detected in faeces, saliva, milk and perspiration. The highest levels are found in bone, which possibly represents a second kinetic compartment, as elimination kinetics for bone differ from those of soft tissue and body fluids (EVM, 2003).

The excretion of boron compounds is similar in humans and animals and is predominantly (> 90 % of the administered dose) via the urine, regardless of the route of administration (IPCS, 1998). Excretion of boron is relatively rapid and it has a half-life of elimination of 24 hours or less (Nielsen, 1986, 1988; Litovitz et al., 1988, as reported in EFSA, 2004; IPCS, 1998). In adults, average normal levels of boron in the urine have been reported in various studies as 0.92 mg/L (range 0.04–6.6 mg/L), 1.89 mg/L (range 0.47–7.8 mg/L) and 0.75 mg/L (0.16–2.9 mg/L) (Moseman, 1994). In another study reported by Moseman (1994), it was stated that, following ingestion of a single dose of 2.5 g boric acid (equivalent to 0.44 g boron), boric acid was detected in the urine within 2 hours and up to 24 hours thereafter.

In a 90-day study with Sprague–Dawley rats, a pronounced reduction in testicular weights in males of the 87.5 mg boron/kg bw/day group was observed (Weir and Fisher, 1972). The effects observed were similar in rats given either boric acid or sodium tetraborate via the diet. The Panel considered 26.3 mg boron/kg bw/day (equivalent to 152 mg boric acid/kg bw/day) as the NOAEL in this study. Female B6C3F1 mice exposed to boric acid in the diet for 90 days appeared to be less sensitive than males to the lethal effects of boric acid: 8/10 males in the high-dose group and 6/10 females in the high-dose group died, and 1 out of 10 males in the lower dose group. Histopathological changes included a dose-related increased incidence of extramedullary haematopoiesis in the spleen in males and females,

¹⁹ Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food OJ 15.1.2011, 21, p. 1

²⁰ Commission Directive 2002/72/EC of 6 August 2002 relating to plastic materials and articles intended to come into contact with foodstuffs OJ 15.08.2002, 220, p. 18

and hyperkeratosis and acanthosis of the stomach in eight males and three females at the highest dose (IPCS, 1998; ECETOC, 1995).

Boric acid or sodium tetraborate fed to beagle dogs for 90 days at dietary concentrations of 0, 0.44, 4.38 or 43.75 mg boron/kg bw/day demonstrated a significantly decrease in testes weight in the mid- and high-dose groups. Four out of five dogs in the high-dose group showed complete testes atrophy and the remaining dog had partial testes atrophy (Weir and Fisher, 1972). Paynter (1963 as reported in USDA, 2006) described similar testes lesions in dogs exposed to 268 mg sodium tetraborate/kg bw/day for 90 days.

Reversibility of testicular lesions was evaluated in F344 rats dosed at 3 000, 4 500, 6 000 or 9 000 mg boric acid/kg in the feed (equivalent to 26, 38, 52 or 68 mg boron/kg bw/day) for 9 weeks and assessed for recovery up to 32 weeks post treatment (Ku et al., 1993). Inhibited spermiation was exhibited at 3 000 and 4 500 mg boric acid/kg feed (5.6 µg boron/mg testes), whereas inhibited spermiation progressed to atrophy at 6 000 and 9 000 mg boric acid/kg feed (11.9 µg boron/mg testes), with no boron accumulation in the testes to levels greater than found in blood during the 9-week period. After treatment, serum and testis boron levels in all dose groups fell to background levels. Inhibited spermiation at 4 500 mg boric acid/kg feed was reversed by 16 weeks post treatment, but focal atrophy was detected that did not recover up to 32 weeks post treatment (as cited in IPCS, 1998; Moore, 1997; EFSA, 2004).

Genotoxicity studies in bacteria and in mammalian cells *in vitro*, addressing several genetic end-points (gene mutation, chromosomal aberrations, micronuclei, SCEs, UDS), as well as an *in vivo* micronucleus test in mice, did not provide any indication of a genotoxic potential for boric acid and sodium tetraborate. Positive results were reported in a single *in vitro* cytogenetic assay, where treatment with high doses of boric acid increased chromosomal aberration incidences. The Panel noted that in this study the clastogenic activity of boric acid was associated with marked cytotoxicity and cell death. Moreover, under the same treatment conditions, boric acid did not increase the incidence of SCEs, a hallmark of DNA damage. Overall, based on the weight of available evidence, the Panel reaffirmed the view of the NDA Panel (EFSA, 2004) that boric acid and sodium tetraborate do not raise concern for genotoxicity.

The IPCS (1998) and EVM (2003) summarised that male reproductive effects caused by boron have been noted in mice, rats, and dogs and included inhibition of spermiation in stage IX and X tubules, followed by germ cell loss, changes in epididymal sperm morphology and caput sperm reserves, decreased serum testosterone levels and testicular atrophy. Male reproductive effects have been reported in 2-year oral exposure studies using boric acid or sodium tetraborate at doses of 29 mg boron/kg bw/day in dogs (Weir and Fisher, 1972). In rats, the LOAEL for reproductive toxicity was 58.5 mg boron/kg bw/day (IPCS, 1998). The NOAEL for the three-generation rat study was reported as 17.5 mg boron/kg bw/day (Weir and Fisher, 1972). The Panel agreed with these conclusions.

Furthermore, the Panel agreed with ECETOC (1995) that dogs are not an appropriate model for reproductive studies and, therefore, did not take into account the data from the 2-year dog study (see section 3.2.4.2) for risk assessment given the low number of animals involved and the large intraspecies variability.

In 1998, the IPCS concluded that the mechanism by which boric acid and borates induce testicular atrophy is unclear. The authors of the IPCS (1998) evaluation considered that, as boron does not accumulate in the testes, it may be possible that decreased testosterone production is due to CNS-mediated mechanisms. It is further reported that it is unlikely that hormone changes cause testes atrophy, as spermatogenesis have been shown to be maintained even in the presence of significantly decreased intratesticular testosterone levels (ECETOC, 1995).

Developmental toxicity of boric acid was investigated in the rat, the rabbit and the mouse. In the rat, developmental toxicity (decreased fetal weight at 13.7 mg boron/kg bw/day) occurred in the absence

of marked maternal toxicity. For developmental toxicity, a NOAEL of 55 mg boric acid/kg bw/day (equivalent to 9.6 mg boron/kg bw/day) was reported (Price et al., 1996a).

The adverse effects of boric acid on development and fertility observed across species were very similar, both in nature and effective doses. Furthermore, the adverse effects obtained with boric acid are comparable to those obtained for other borates thus confirming that boron is the toxicologically active species. The available data on toxicokinetics do not indicate differences between laboratory animals and humans. It is not known whether there are significant differences in the toxicodynamics between humans and laboratory animals. In the absence of such a knowledge, it was assumed that the effects seen in animals would also occur in humans. On the basis of toxicokinetic and toxicodynamic considerations it is assumed that the animal data are relevant to humans. This is further underlined by the fact that (1) there are indications that boric acid is able to cross human placenta and that (2) up to now, epidemiological studies in humans are insufficient to demonstrate the absence of an adverse effect of inorganic borates on fertility (ECHA, 2010).

In order to establish a group ADI the Panel considered that it should be expressed as boron equivalents. The Panel considered the study of Price et al. (1996a) as the pivotal study and identified a NOAEL of 55 mg boric acid/kg bw/day. This NOAEL is equivalent to 9.6 mg boron/kg bw/day.

The use of an UF of 60 by the Panel was in agreement with that previously used by the NDA Panel (EFSA, 2004) and the CEF Panel (EFSA, 2012). The UF was calculated following the approach proposed by Dourson et al. et al., (1998). The default toxicokinetic (factor 4) and toxicodynamic (factor 2.5) uncertainty factors for extrapolation from animals to humans were considered appropriate for boron. Intraspecies variation (toxicokinetics) for boron relates primarily to variations in clearance. As the pivotal effect that serves as the basis for the ADI is developmental toxicity, pregnant women are the subgroup of interest in this regard (WHO, 2009). The glomerula filtration rate being the critical physiological process for boron toxicokinetics, the default toxicokinetic UF for human variability was adjusted by the NDA and CEF panel on the clearance in pregnant women. This approach leads to an intraspecies toxicokinetic UF of 1.8 instead of 3.2, whereas the intraspecies toxicodynamic UF of 3.2 remained unchanged. This ultimately resulted in an UF of 60 instead of 100.

When applying an uncertainty factor (UF) of 60, the group ADI for boric acid and sodium tetraborate, expressed as boron equivalents, is 0.16 mg boron/kg bw/day.

Exposure to boric acid and sodium tetraborate from its use as a food additive in caviar is based on the assumption that the consumption data from the EFSA Comprehensive Food Consumption Database reported for the food category “fish roe” cover the consumption of caviar. The Panel noted that these estimates are conservative since it is unlikely that all foods falling into this food category actually is caviar. Although the EFSA Comprehensive Food Consumption Database provides information on food consumption from 32 different dietary surveys carried out in 22 different European countries, only those countries with statistically reliable data on the consumption of the food category “fish roe” were included for exposure estimate calculations on average and at the 95th percentile. Using the MPL of 4 000 mg/kg caviar, these exposure estimates result in average exposure to boric acid and sodium tetraborate expressed as boric acid in the range 0.00–0.21 mg/kg bw/day for children, 0.00–0.06 mg/kg bw/day for adolescents, 0.00–0.08 mg/kg bw/day for adults and 0.00–0.05 mg/kg bw/day for the elderly and the very elderly. Exposure estimates at the 95th percentile could be calculated only for Denmark and Sweden (in Denmark only for the elderly) and are in the range 2.81–3.17 mg/kg bw/day for children, 1.47–2.11 mg/kg bw/day for adolescents, 0.72–0.74 mg/kg bw/day for adults and 0.88 mg/kg bw/day for the elderly and the very elderly.

The estimates listed in Table 4 and as indicated above refer to the exposure to boric acid and sodium tetraborate expressed as boric acid. Based on a boron content of 17.5 % in boric acid, the highest average exposure to boron from its use as a food additive across European Member States is 0.04 mg/kg bw/day for children, 0.01 mg/kg bw/day for adolescents, 0.01 mg/kg bw/day for adults and 0.01 mg/kg bw/day for the elderly and the very elderly. The exposure estimates to boron from its use

as food additive at the highest 95th percentile for consumers would be 0.56, 0.37, 0.13 and 0.15 mg/kg bw/day for children, adolescents, adults, and the elderly and the very elderly, respectively.

Based on these conservative assumptions, it is unlikely that the ADI of 0.16 mg boron/kg bw is exceeded by the use of boric acid and sodium tetraborate as food additive at its present authorisation. For children and adolescents at the highest 95th percentile, exposure estimates indicate exceedance of this ADI.

However, an exposure to boron from its use as food additive in the form of boric acid and sodium tetraborate used in caviar is unlikely to occur on a regular basis. In fact, to reach exposures at the ADI via consumption of caviar containing borates at the MPL, an adult with a body weight of 60 kg would have to consume 13.7 g caviar every day. Therefore, the Panel noted that even at high consumption and in consumers only, it is unlikely that a regular exceedance of the ADI occurs. The Panel also noted that this exposure scenario is only valid for its present single authorisation for caviar, whereas a detailed exposure assessment would be needed if the authorisation would be extended to other products (e.g. fish roe other than sturgeon eggs).

Moreover, the Panel noted, that exposure to boron from its natural occurrence in the diet and from other sources (food supplements, food contact materials, feed for food-producing animals, cosmetics, oral hygiene products, etc.) may already lead to exposures exceeding the ADI.

The Panel noted further that the specifications for sodium tetraborate do not contain an assay percentage value.

CONCLUSIONS

The Panel identified a NOAEL of 55 mg boric acid/kg bw/day (equivalent to 9.6 mg boron/kg bw/day). When applying an uncertainty factor of 60, the group ADI for boric acid and sodium tetraborate, expressed as boron equivalents, is 0.16 mg boron/kg bw/day.

The highest average exposure to boron from its use as a food additive across European Member States is 0.04 mg/kg bw/day for children, 0.01 mg/kg bw/day for adolescents, 0.01 mg/kg bw/day for adults and 0.01 mg/kg bw/day for the elderly and the very elderly. The exposure estimates to boron from its use as food additive at the highest 95th percentile for consumers would be 0.56, 0.37, 0.13 and 0.15 mg/kg bw/day for children, adolescents, adults and the elderly, respectively.

For children and adolescents at the highest 95th percentile, exposure estimates indicate exceedance of this ADI. However, exposure to boron from its use as a food additive in form of boric acid and sodium tetraborate used in caviar is unlikely to occur on a regular basis. Therefore, the Panel noted that even at high consumption and in consumers only, it is unlikely that a regular exceedance of the ADI occurs. The Panel also noted that this exposure scenario is only valid for its present single authorisation for caviar, whereas a detailed exposure assessment would be needed if the authorisation would be extended to other products (e.g. fish roe other than sturgeon eggs).

Based on these conservative assumptions, it is unlikely that the ADI of 0.16 mg boron/kg bw/day is exceeded by the use of boric acid and sodium tetraborate as food additive at its present authorisation.

However, the Panel also noted that exposure to boron from its natural occurrence in the diet and from other sources (food supplements, food contact materials, feed for food-producing animals, cosmetics, oral hygiene products, etc.) may already lead to exposures exceeding the ADI.

DOCUMENTATION PROVIDED TO EFSA

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GLOSSARY/ABBREVIATIONS

ADI	acceptable daily intake
AFC	Scientific Panel on Food additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
AUC	area under the curve
BMDL ₀₅	benchmark dose lower confidence limit 5 %
CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CNS	central nervous system
CONTAM	EFSA Panel on Contaminants in the Food Chain
CRP	C-reactive protein
CV	coefficient of variation
DNA	deoxyribonucleic acid
EC	European Commission
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemical
EFSA	European Food Safety Authority
EHC	Environmental Health Criteria
EINECS	European Inventory of Existing Commercial chemical Substances
EPA	Environmental Protection Agency
EU	European Union
EVM	Expert Group on Vitamins and Minerals
FCS	Food Classification System
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GC-MS	gas chromatography–mass spectrometry
GFR	glomerular filtration rate
GSH	reduced glutathione
HDL	high-density lipoprotein
IPCS	International Program on Chemical Safety
IUCLID	International Uniform Chemical Information Database
i.v.	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LD ₅₀	lethal dose 50
LDL	low-density lipoprotein
LH	lutinising hormone
LOAEL	lowest observed adverse effect level

MN	micronuclei
MPL	maximum permitted use level
NDA	EFSA Panel on Nutrition, Dietetic Products and Allergies
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
PARNUTS	Foods for Particular Nutritional Purposes
RfD	RfD (the human dose that is likely to be without an appreciable risk of deleterious non cancer effects during a lifetime)
SCE	sister chromatid exchange
SCF	Scientific Committee for Food
SD	standard deviation
SML	specific migration limit
TDI	tolerable daily intake
UDS	unscheduled DNA synthesis
UF	uncertainty factor
UL	tolerable upper intake level
USAN	United States Adopted Names Council
WHO	World Health Organization