Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies
on a request from the Commission related to the Tolerable Upper Intake
Level of Boron (Sodium Borate and Boric Acid)

(Request N° EFSA-Q-2003-018)

(adopted on 8 July 2004)

SUMMARY

Boron occurs in foods as borate and boric acid. Boron has not been established to be an essential nutrient for humans and no specific biochemical function for boron has been identified in higher animals or man. There is some evidence that, in humans, boron intake within the usual dietary range may influence the metabolism and utilisation of other nutrients, particularly calcium, and may have a beneficial effect on bone calcification and maintenance. Recommended intakes for boron have not been established.

Studies of dietary deprivation of boron in animals have reported adverse effects (e.g. on growth, serum steroid hormone concentrations and bone calcification) that can be corrected by increasing boron intake. The effects of low boron intakes are more marked when accompanied by low status for other nutrients (e.g. vitamin D, magnesium).

Data on boron intake in EU countries are limited. In the UK mean intake in adults from food is estimated at 1.5 mg/day, with the 97.5 percentile of 2.6 mg/day, while mean intake from water is estimated to be in the range of 0.2-0.6 mg/day. The main dietary sources of boron are plant foods, and foods rich in boron include fruits, leafy vegetables, mushrooms, nuts and legumes, as well as wine, cider and beer. Supplements may contain 1.5-10 mg boron/dose.

Boron as borate is readily absorbed (>90%) from the human gut and is evenly distributed throughout the tissues and organs, and can cross the placenta, of animals and humans. Absorbed boron is readily excreted in urine.

Ingestion of boron at dose levels of greater than 13 mg/kg body weight/day in short and long term studies in a number of animal species (e.g. mouse, rat, dog, pig) has been shown to result in a range of adverse effects, with developmental and reproductive effects being the most critical. Studies of boron in mice and rats and in vitro showed no evidence of carcinogenicity or genotoxicity.

There are many case reports of boron intoxication in humans. Ingestion of boric acid at daily dose levels of 0.14-0.43 g boric acid/kg body weight (equivalent to about 25-76 mg boron/kg body weight) over periods ranging from days to weeks, resulted in a variety of symptoms, the most common being gastrointestinal effects such as vomiting, diarrhoea and abdominal pain.

It was considered that the data on adverse effects of boron in humans were not adequate for establishing a tolerable upper intake level (UL). The UL was based on the adverse effect occurring at the lowest intake levels in animals, i.e. decreased foetal body weight in rats resulting from maternal boron intake during pregnancy. The no observed adverse effect level (NOAEL) for this effect (9.6 mg/kg body weight/day) was extrapolated to humans by...
dividing by an uncertainty factor of 60 (to allow for variability between rats and humans and between-person variability in humans) to give an UL of 0.16 mg/kg body weight/day, which is equivalent to an UL of 10 mg/person/day in adults. This UL also applies to pregnant and lactating women. UL values for children were derived by extrapolating from the UL for adults on a body surface area basis, giving values (mg/day) of 3, 4, 5, 7 and 9 for children aged 1-3, 4-6, 7-10, 11-14 and 15-17 years of age, respectively. These UL values apply only to the intake of boron as boric acid and borates.

Based on the limited data available, boron intakes from food and water in EU are below the UL. The consumption of some supplements containing boron may lead to intakes which exceed the UL.

KEY WORDS
Boron, sodium borate, boric acid, tolerable upper level.

BACKGROUND

In addition, and as announced in its White Paper on Food Safety, the Commission aims to put forward a proposal for harmonising legislation concerning the addition of vitamins and minerals to foods.

With a view to provide scientific support to the European Commission’s legislative work in this field, the Scientific Committee on Food (SCF) issued, from October 2000 to April 2003, a series of opinions on tolerable upper intake levels of individual vitamins and minerals and safety factors in relation to their use in fortified foods and food supplements (available on the Internet at: http://europa.eu.int/comm/food/fs/sc/scf/out80_en.html).

The SCF opinions covered 22 out of the 29 nutrients, which were considered to be within their mandate for this task. The SCF did not have sufficient time to adopt opinions for the following vitamins and minerals: vitamin C, chloride, fluoride, iron, phosphorus, potassium and sodium. In addition, during the decision making process for the adoption of Directive 2000/46/EC on food supplements the Parliament requested that boron, nickel, silicon, vanadium and tin should be allowed to be used in food supplements. Therefore, the European Food Safety Authority is asked to provide scientific opinions on the remaining 12 vitamins and minerals in accordance with the present terms of reference.

TERMS OF REFERENCE
With respect to the outstanding 12 vitamins and minerals, the European Food Safety Authority is asked 1) to review the upper levels of daily intakes that are unlikely to pose a risk

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of adverse health effects; 2) to provide the basis for the establishment of safety factors, where necessary, which would ensure the safety of fortified foods and food supplements containing the aforementioned nutrients.

ASSESSMENT

1. INTRODUCTION

Boron is a naturally occurring element that is found in the form of borates in the oceans, sedimentary rocks, coal, shale, and some soils. Boron has an atomic number of 5 and a relative atomic mass of 10.811; it exists in two allotropic forms, a brown amorphous powder and very hard brilliant crystals. In nature it is found only in compounds, for example with sodium and oxygen in borax (Na$_2$B$_4$O$_7$·10H$_2$O). In aqueous solution at near-neutral pH, monomeric boric acid [B(OH)$_3$] is the most common species present, regardless of whether the boron source is boric acid (H$_3$BO$_3$) or borate. Boron occurs in food as borate or boric acid. For comparative purposes doses of boron compounds are expressed as boron equivalents.

Boron is widely distributed in nature, with concentrations of about 10 mg/kg in the earth’s crust (range 5 mg/kg in basalts to 100 mg/kg in shales) and about 4.5 mg/litre in the ocean. The most important commercial borate products and minerals are borax pentahydrate, borax, sodium perborate, boric acid, colemanite, and ulexite.

Boron compounds can be found in the form of boric acid, borax and other borates in a wide range of consumer products, including boron-silicate glass, soaps, detergents, preservatives, adhesives, porcelain, cosmetics, enamel, leathers, carpets, artificial gemstones, high-contrast photographic material, wicks, electric condensers, fertilisers, insecticides, and herbicides (Moore et al., 1997).

Boron has long been known to be essential for the growth of vascular plants, with a number of functions which include sugar transport, cell wall synthesis and RNA metabolism. It is proposed that boron has an unique membrane function in plants (Nielsen, 1986; FNB, 2001; EGVM, 2002 and 2003). There is also some evidence to support the essentiality of boron in animals.

The WHO established an acceptable safe range of population mean intakes for boron of 1-13 mg/day (WHO, 1996; IPCS, 1998).

In 1996 the SCF adopted an opinion on trace elements including boron in natural mineral waters, in which a NOAEL of 9.6 mg/kg body weight was established. The usual safety factor of 100 was applied leading to the establishment of a tolerable daily intake (TDI) of 0.1 mg boron/kg body weight for humans. The SCF 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 Scientific Committee on Toxicity and Ecotoxicity set a TDI of 0.3 mg boron/kg body weight (CSTE, 1996).

The FNB established a tolerable upper intake level of 20 mg boron/day for adults (FNB, 2001). The EGVM upper safe level is 0.16 mg boron/kg body weight/day (EGVM, 2003).

2. NUTRITIONAL BACKGROUND

2.1 Physiological effects

Boron has not been established to be an essential nutrient for humans and no specific biochemical function for boron has been identified in higher animals or man (FNB, 2001). Recommended intakes for boron have not been established (SCF, 1993; FNB, 2001).

Studies of low boron diets (0.3-0.4 mg/day) in man have reported adverse effects on biological functions that can be corrected by increasing boron intake to about 3 mg/day (Nielsen et al., 1987a and b, and 1988a; Nielsen, 1990a.). Several studies have reported that intake of boron may influence the metabolism and utilisation of calcium, copper, magnesium, nitrogen, glucose, triglycerides, reactive oxygen and oestrogen and may have effects on the function of several body systems, including blood, brain and bone (Nielsen, 1997; Hunt, 1997; Penland, 1998; FNB, 2001). Perhaps the best-documented effect of boron is on calcium metabolism or utilization, and thus, bone calcification and maintenance (Nielsen, 1998; Nielsen et al., 1987b; Hunt et al., 1997; Pizzorno and Murray, 1999; Meacham et al., 1994).

Studies of dietary deprivation of boron in animals have reported adverse effects on biological functions, including depressed growth, reduced serum steroid hormone concentrations, changes in plasma and organ calcium and magnesium concentrations, plasma alkaline phosphatase and bone calcification, that can be corrected by increasing boron intake (Nielsen, 1989 and 1990b; Nielsen et al. 1988a, b and c, and 1990; Hunt and Nielsen, 1981; Hunt, 1988). The effects of low boron intakes are more marked when accompanied by low status for other nutrients (e.g. cholecalciferol, magnesium) (Benderdour et al., 1998; McCoy et al., 1994; Hunt, 1989; Hegsted et al., 1991; Nielsen, 1987a and b).

Although boron deprivation has been reported to cause developmental defects in fish (Rowe and Eckhert, 1999; Eckhert, 1998) and frogs (Fort et al., 1999 and 2002), such effects have not been found consistently in rodent models (Lanoue et al., 1998 and 1999).

2.2 Absorption, distribution, metabolism and elimination

There is no evidence from metabolic studies for homeostatic control of boron in humans (Samman et al., 1998; FNB, 2001), although some reports have suggested that regulation of body content occurs (Hunt and Stoecker, 1996; Sutherland et al., 1998).

Boron as borate is readily, and almost completely absorbed (>90%) from the human gut. The mechanism has not been defined. Essentially 100% of boron ingested in the range 0.4-3 mg/day is excreted in faeces and urine and there is no evidence of boron accumulation (Hunt et al., 1997). Supplementation with 10 mg of boron/day resulted in the recovery of 84% of the dose in the urine (Samman et al., 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. Boron as boric acid is evenly distributed throughout the body fluids, via passive diffusion. Boron administered at
various dose levels is distributed throughout the tissues and organs of animals and humans at concentrations between 0.05 and 0.6 mg/kg fresh weight and several times these concentrations in bones (Nielsen, 1989 and 1986). In rats dosed with 2, 12.5 and 25 mg boron/rat/day most tissues appeared to reach steady-state boron levels (12-30 mg boron/kg tissue) by 3-4 days; these levels were 3- to 20-fold above control values (IPCS, 1998; Naghii and Samman, 1993, 1996 and 1997b). Adipose tissue took up only 20% as much boron as other tissues (3.78 mg/kg tissue). Bone boron levels (47.4 mg/kg tissue) indicated greater uptake in bone than in other tissues (30-40 mg/kg tissue) after a 7 day feeding experiment in rats. In addition boron levels continued to increase throughout the 7 days.

It has been shown that boron compounds can cross the human placenta (IPCS, 1998).

Borate compounds are not metabolised by biological systems, because of the considerable energy required to break the boron-oxygen bond (Emsley, 1989).

The primary route of elimination is by glomerular filtration (Murray, 1998; Dourson et al. 1998) and >90% of the administered dose is excreted via urine, regardless of the route of exposure or administration. The 3- to 4-fold higher clearance in rats compared to humans arises from the higher glomerular filtration rate in rats. In humans, excretion is relatively rapid, with a half-life of elimination of 24 hours or less (Nielsen, 1986 and 1988; Litovitz et al., 1988). Boron does not accumulate in the blood of regularly exposed workers (Culver et al., 1994).

Elimination kinetics of boron from bone is different from soft tissue and body fluids (EGVM, 2002; IPCS, 1998; Chapin et al., 1997), suggesting a second kinetic compartment in bone in which a small percentage of absorbed boron is stored.

### 2.3 Food sources and other sources

Boron is present in aquatic and terrestrial plants but does not bioaccumulate through the food chain. The greatest exposure to boron for most populations comes from food. The daily intake of boron by humans can vary widely depending on the proportions of various food groups in the diet (Nielsen, 1988; Naghii and Samman, 1996; EGVM, 2002 and 2003). Foods rich in boron include fruits, leafy vegetables, mushrooms, nuts and legumes as well as wine, cider and beer. Meat, fish and dairy products are poor sources (Meacham and Hunt, 1998; Anderson et al., 1994; Hunt et al., 1991; MAFF, 1994).

Water, in particular mineral waters, can be an important source of boron. The average boron content of drinking water in Germany was estimated as 23.1 µg/L (Becker et al., 1997). In one study it was reported that bottled water can contain up to 4.35 mg boron/litre, with an average boron content of 0.75 mg/litre (Moore et al., 1997).

### 2.4 Typical intakes

Data on dietary intakes of boron are limited. Boron is not included in the nutrient databases for dietary surveys.

Mean (1.5 mg/day) and 97.5 percentile (2.6 mg/day) boron intakes in adults in the UK have been estimated from analysis of samples from the 1994 Total Diet Study using consumption data from the 1986/87 Dietary and Nutritional Survey of British Adults (MAFF, 1997). In the

report of the UK Expert Group on Vitamins and Minerals (EGVM, 2003), the exposure assessment revealed a mean intake for water (0.2-0.6 mg/day) for supplements (up to 2.0 mg/day), and for cosmetics and consumer products (up to 0.47 mg/day). Thus the estimated maximum daily intake of boron was 5.67 mg/day. Vegetarians were identified as a potential high intake group.

Rainey et al. (1999) have calculated the mean dietary intakes for a typical US population as 1.17 mg/day for men, 0.96 mg/day for women and 1.01 mg/day for pregnant women. Vegetarian adults had a mean intake of 1.47 mg/day for men and 1.29 mg/day for women. The IPCS (1998) reported an average intake of boron for humans as 0.44 µg/day from ambient air, 0.2-0.6 mg/day from drinking water, and 1.2 mg/day from the diet. Coffee and milk are low in boron, but make up 12% of the total boron intake by virtue of the volume consumed (Rainey et al., 1999).

Body building supplements have been reported to contain 1.5-10 mg boron/dose, resulting in possible daily intakes of 1.5-30 mg boron (EGVM, 2003).

3. HAZARD IDENTIFICATION

3.1 Animal toxicity data

3.1.1 Acute and short-term toxicity

The oral LD₅₀ values for boric acid and borax for mice and rats are in the range of 400-900 mg boron/kg body weight. Acute oral LD₅₀ values in the range of 200-350 mg boron/kg body weight for boric acid or borax exposure for guinea pigs, dogs, rabbits and cats (IPCS, 1998; Wang et al., 1984; Weir and Fisher, 1972; Smyth et al., 1969; Pfeiffer et al., 1945).

A short-term experiment (4 weeks) was performed to determine the specificity of the effect of boron on steroid hormones and to determine subsequent changes in plasma lipids in rats. Addition of boron (as boric acid) to the drinking water to provide 2 mg boron/rat/day, did not affect body or testicular weight. The addition of boric acid (2 mg boron/rat/day) to the drinking water resulted in significant elevations in the plasma 1,25-dihydroxyvitamin D concentration at week 2 and the plasma testosterone at week 4 relative to the control group. After 2 weeks, there was a significant decrease in plasma triacylglycerol and HDL-cholesterol concentrations in rats fed boric acid relative to their counterparts in the control group. However, at week 4 only HDL₃-cholesterol was significantly lower (Naghii and Samman, 1997a).

In a 13-week study conducted by the US National Toxicology Program (NTP, 1987) mice (10/sex/dose) were exposed to boric acid in the diet at concentrations sufficient to produce estimated intakes of approximately 0, 34, 70, 141, 281 or 563 mg boron/kg body weight/day for males and 0, 47, 97, 194, 388 or 563 mg boron/kg body weight/day for females. Deaths occurred at high doses (8/10 high dose males and 6/10 high dose females and 1/10 males of the 281 mg boron/kg body weight/day died). Clinical signs of toxicity were a thin, hunched appearance, dehydration, foot lesions, and scaly tails. A dose-related decrease in body weight gain was observed. Histological effects included a dose-related incidence of minimal to mild extramedullary haematopoiesis of the spleen in males and females, hyperkeratosis and acanthosis of the stomach at the highest dose level, and testicular lesions.

Non-accidental mortality at the end of the toxicity study was increased significantly in the males. The only significant lesions were seen in the testes of male mice (NTP, 1987; Dieter, 1994; IPCS, 1998).

Lee et al. (1978) fed borax in the diet to male Sprague-Dawley rats (18/dose) at dose levels equivalent to 0, 30, 60 or 125-131 mg boron/kg body weight/day for 30 or 60 days. Body weights were not consistently affected by treatment. Organ weights were not affected in the 30 mg/kg body weight/day, but at 60 and 125-131 mg/kg body weight/day, absolute liver and epididymis weights were significantly lower than the controls after 60 days but not after 30 days.

In a 90-day study, Sprague-Dawley rats (10/sex/dose) received 0, 2.6, 8.8, 26.3, 87.5 or 262.5 mg boron/kg body weight/day in the diet as boric acid or borax. All high dose animals died within 3-6 weeks. Body weights in males and females were reduced in animals receiving 87.5 mg boron/kg body weight/day. Absolute organ weights -including the liver, spleen, kidneys, brain, adrenals and ovaries- in this dose groups were also decreased. Relative weights of the adrenals and kidneys were significantly increased, but the relative weights of liver and ovaries were significantly decreased at the 87.5 mg boron/kg body weight/day. A pronounced reduction in testicular weights was also seen (Weir and Fisher, 1972).

In a 90-day drinking water study with male rats, the highest dose of 6 mg boron/L (as borax) (0.426 mg boron/kg body weight/day) caused no effects on fertility and reproduction or the weights of the testes or prostate (Dixon et al., 1979).

In a toxicity study beagle dogs were fed a diet containing boric acid or borax for 90 days or 2 years. In the 90-day study the dose levels were 0, 0.44, 4.58 or 43.75 mg boron/kg body weight/day. Testis weights were significantly lower than controls in the middle and upper dose groups. Testicular microscopic structure was not different from control and middle dose, however in the high dose group (43.75 mg boron/kg body weight/day) 4 out of 5 dogs had complete atrophy, and the remaining dog had one-third of tubules showing some abnormality. In the 2-year study the dogs (4/sex/group) received boric acid or borax in the diet at dose levels equivalent 0, 1.5, 2.9, or 8.8 and an additional group of dogs received 29 mg boron/kg body weight/day for 38 weeks. No effects were observed on general appearance, body weight, food consumption, organ weights, haematology, or serum chemistry. Changes in testicular morphology occurred in males in the highest dose groups (Weir and Fisher, 1972).

The effects of boron on bone growth were studied (Seffner et al., 1990) in growing pigs exposed to boron (4 or 8 mg/kg body weight per day) in two studies. They reported dose-related thinning of the cortex of the humerus and a reduction (significant at 8 mg/kg body weight/day) in bone-derived serum alkaline phosphatase, suggesting reduced osteoblast activity.

3.1.2 Long-term toxicity and carcinogenicity

A 2-year study was conducted in mice (50/sex/day) which received approximately 0, 275, or 550 mg boric acid/kg body weight/day (0, 48.1, or 96.3 boron/kg/day) in the diet. No clinical signs of toxicity were observed. Body weights were 10-17% lower in males of the high dose group after 32 weeks and in females after 52 weeks. Non-accidental mortality at the end of the study was 9/50, 20/50, and 23/50 in control, low-, and high-dose and this increase was
statistically significant in males. The lesions in male mice appeared in the testes which 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. Survival of the male mice was significantly reduced. An increased incidence of hepatocellular tumours in low dose male mice was considered by NTP as non-associated to boric acid. Overall, 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).

In a 2-year study, rats (35/sex/dose) were administered doses equivalent to 0, 5.9, 17.5, or 58.5 mg boron/kg body weight/day in the diet as borax or boric acid. 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. Serum chemistry and urine values were normal; the packed cell volume (PCV) and haemoglobin levels were significantly lower than in controls. 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 body weight/day. No treatment-related effects were observed at lower dose levels, the NOAEL for this study was 17.5 mg boron/kg body weight/day. An increased incidence of tumours was not observed (Weir and Fischer, 1972; IPCS, 1998).

Based on the lack of human data and on the results of the aforementioned animal studies, boron was classified by the US EPA as a Group D chemical (not classifiable as to human carcinogenicity) (US EPA, 1994).

### 3.1.3 Reproductive and developmental studies

In a multigeneration continuous-breeding experiment (Fail et al., 1990 and 1991), Swiss CD-1 mice (F0 generation) were fed boric acid in the diet at 0, 1000, 4500, or 9000 mg/kg feed for 27 weeks, which gave calculated doses of 0, 19.2, 104.7, and 222.1 mg boron/kg body weight/day for males and 0, 31.9, 148.1 and 290.5 mg boron/kg body weight/day for females. Treatment with boric acid significantly impaired fertility; all males and females in the high-dose groups were infertile. At the middle 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. The trend towards a lower fertility index at this dose level was more apparent with subsequent matings. Animals from different treatment groups were cross-mated. When mid-dose males were mated with controls 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 the F0 necropsy, sperm motility was significantly reduced in all exposed groups (by 12%, 32%, and 47%, from low- to high-dose groups, respectively). 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 a LOAEL for decreased sperm motility in the F0 males, 26% increased uterine weights and 8% kidney weight/adrenal weight in the F1 females and a 3.3% reduction in litter-adjusted birth weight in the F2 pups. This study provides no NOAEL, but the changes in the
low dose were minor and this indicates that this dose level is close to the NOAEL (IPCS, 1998).

Fail et al. (1989 and 1990) utilised CD-1 mice both to characterise the effects of boric acid on fertility and to test the reversibility of these effects. Adult CD-1 mice were exposed to boric acid in the feed for 27 weeks at 0, 1000, 4500, or 9000 mg/kg diet (doses were not given). The males at the high and mid-doses had testicular atrophy and decreased spermatogenesis. Fertility was diminished in animals receiving the mid-dose and completely absent in the high-dose group (IPCS, 1998).

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 in Sprague-Dawley rats at dose levels of 60 and 125-131 mg boron/kg body weight/day for 30 or 60 days (Lee et al., 1978). Mean plasma follicle stimulating hormone (FSH) levels were significantly elevated in a dose-dependent manner in all treatment groups in this study (60 to 125-131 mg boron/kg body weight/day) after 60-days of intake. FSH levels in animals receiving the highest dose tested (125-131 mg boron/kg body weight/day) were still elevated 12 months after treatment termination, owing to atrophied testes and no recovery of spermatogenesis. Plasma luteinizing hormone (LH) levels were not significantly elevated, and mean plasma testosterone levels were within the normal range throughout the study (Lee et al., 1978; IPCS, 1998).

The reversibility of testicular lesions was evaluated by Ku et al. (1993a) in an experiment in which F-344 rats were dosed at 3000, 4500, 6000, or 9000 mg boric acid/kg diet (26, 38, 52 and 69 mg boron/kg body weight/day) in the feed for 9 weeks and assessed for recovery up to 32 weeks post treatment. Inhibited spermiation was exhibited at 38-52 mg boron/kg body weight/day (5.6 µg boron/mg tissue), whereas inhibited spermiation progressed to atrophy at 52-68 mg boron/kg body weight/day (11.9 µg boron/mg tissue). Boron did not accumulate 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 38 mg/boron/kg body weight/day was reversed at 16 weeks post-treatment, but focal atrophy was detected that did not recover up to 32 weeks post-treatment (IPCS, 1998).

The development of the boron-induced testicular lesion was investigated by Treinen and Chapin (1991), who fed boric acid at a level of 0 or 60.9 mg boron/kg body weight/day (estimated by the authors) to male F-344 rats and sacrificed six treated and four control male rats at intervals from 4 to 28 days after the start of the boric acid intake. In half of the treated rats, there was inhibition of spermiogenesis in 10-30% of stage IX tubules at 7 days and inhibition in all stages IX and stage X tubules after 10 days of exposure. At 28 days there was significant loss of spermatocytes and spermatids from all tubules in exposed rats, and basal serum testosterone levels were significantly decreased from 4 days on (IPCS, 1998).

In a three-generation reproduction study performed in conjunction with the long-term toxicity study, it was found that 58.5 mg boron/kg body weight/day produced testicular atrophy and complete suppression of fertility in rats. Lower doses (17.5 or 5.9 mg boron/kg body weight/day) did not reduce fertility (Weir and Fisher, 1972). Due to the small group size the data of the study are of limited value for a risk assessment (IPCS, 1998).

A study by Ku et al. (1993a) found no detectable treatment-related changes in testicular structure in rats following consumption of 17.5 mg boron/kg body weight/day for up to 9
weeks. In a follow-up study to explore and identify the mechanism for the testicular toxicity of boric acid, Ku et al. (1993b) evaluated several end-points in cell culture systems following *in vitro* boric acid exposure. The data suggest an effect of boric acid on the DNA synthesis activity of mitotic and meiotic germ cells and, to a lesser extent, on energy metabolism in Sertoli cells. The effect on DNA synthesis occurred at boron concentrations that were similar to the serum levels recorded when testis atrophy was observed. These observations show that boric acid interferes with the production and or maturation of early germ cells, and offers an explanation for atrophy, but not for inhibited spermiation (IPCS, 1998).

Additional mechanistic studies by Ku and Chapin (1994) showed that testicular toxicity and CNS hormonal effects were not due to selective boron accumulation in testis or brain/hypothalamus. Changes in testis phosphorus, calcium, and zinc levels did not precede atrophy. *In vitro* studies showed no effect on steroidogenic functions of isolated Leydig cells. The authors showed that inhibited spermiation was not due to increased testicular cyclic adenosine monophosphate or reduced serum protease plasminogen activators. Effects of boric acid were also seen in Sertoli-germ cells co-cultures on Sertoli cell energy metabolism (lactate secreted by Sertoli cells is a preferred energy source for germ cells) and DNA/RNA synthesis (germ cells synthesise DNA/RNA, and boric acid impairs the synthesis of these nucleic acids in the liver). The most sensitive *in vitro* end-point was DNA synthesis in mitotic/meiotic germ cells; energy metabolism in germ cells was affected to a lesser extent, which was manifested *in vivo* as a decrease in early germ cell/Sertoli cell ratio prior to atrophy of the testes. The mechanisms of inhibited spermiation are still not defined (IPCS, 1998).

Heindel et al. (1992) also investigated the development toxicity and teratogenicity of boric acid in mice at 0, 43, 79, or 175 mg boron/kg body weight/day in the diet. There was a significant dose-related decrease in average foetal body weight per litter at 79 and 175 mg boron/kg body weight/day. Offspring of mice receiving 79 or 175 mg boron/kg body weight/day during gestation days 0-17, showed an increased incidence of skeletal (rib) malformations. These changes occurred at doses for which there were also signs of maternal toxicity (increased kidney weights and pathology); the LOAEL for developmental effects (decreased foetal body weight per litter) was 79 mg boron/kg body weight/day, and the NOAEL for developmental effects was 43 mg boron/kg body weight/day (IPCS, 1998).

Sprague-Dawley rats were fed diets giving intakes of 0, 13.6, 28.5 or 57.7 mg boron/kg body weight/day as boric acid from gestation days 0 to 20 (Heindel et al., 1992). An additional group of rats received boric acid at 94.2 mg boron/kg body weight/day on gestation days 6-15 only. Maternal effects included a significant and dose-related increase in relative liver and kidney weights at 28.5 mg/kg body weight/day and higher. Treatment with 94.2 mg boron/kg body weight/day significantly increased prenatal mortality. Average foetal body weight per litter was reduced significantly in a dose-related manner in all treated groups compared with controls. The percentage of malformed foetuses per litter and the percentage of litters with at least one malformed foetus were significantly increased at 28.5 mg boron/kg body weight/day. Malformations consisted primarily of abnormalities of the eyes, the CNS, the cardiovascular system, and the axial skeleton. The most common malformations were enlargements of lateral ventricles in the brain and agenesis or shortening of rib XIII. The percentage of foetuses with variations per litter was reduced relative to controls at 13.6 and 28.5 mg boron/kg body weight/day, but was significantly increased in rats receiving the 94.2 mg boron/kg body weight/day. The LOAEL of 13.6 mg boron/kg body weight/day for rats occurred in the absence of maternal toxicity; a NOAEL was not established (IPCS, 1998).
Price et al. (1996a) did a follow-up to the Heindel et al. (1992) study in Sprague-Dawley (CVD) rats in order to determine a NOAEL for foetal body weight reduction and to determine whether the offspring would recover from prenatally reduced body weight during postnatal development. Skeletal malformations and variations were also studied to further characterise the low end of the dose-response curve (phase 1) and to determine whether the incidence of skeletal defects in offspring changed during postnatal life (phase 2). Boric acid was administered in the diet to CD rats from gestation day 0 to 20. In phase 1, uterine contents were examined on gestation day 20. During phase 1, the intake of boric acid was 0, 3.3, 6.3, 9.6, 13.3 or 25 mg boron/kg body weight/day. For these treatment dose groups, foetal body weights were 99, 98, 97, and 88% of controls; the reduction was significant only at 13.3 and 25 mg boron/kg body weight/day on gestation day 20. During phase 1, the incidences of short rib XIII (a malformation) and wavy ribs (variation) were increased at 13.3 mg boron/kg body weight/day or more relative to the control litters. During phase 2, the intake of boric acid during gestation was 0, 3.3, 6.5, 9.8, 12.9 or 25.3 mg boron/kg body weight/day. At birth, boric acid intake stopped and dams were allowed to deliver and rear their litters until postnatal day 21. On postnatal day 0 of phase 2, there were no effects of boric acid on offspring body weight, nor were any differences seen through postnatal day 21. On postnatal day 21 of phase 2, the percentage of pups per litter with short rib XIII was elevated only in the 25.3 mg boron/kg body weight/day group, and there was no-treatment-related increase in wavy ribs or extra ribs on lumbar 1 observed in these pups on day 21. The NOAEL for phase 1 is 9.6 mg boron/kg body weight/day based on a decrease in foetal body weight and the LOAEL was 13.3 mg boron/kg body weight/day. The NOAEL for phase 2 was 12.9 mg boron/kg body weight/day, and the LOAEL was 25.3 mg boron/kg body weight/day (IPCS, 1998).

Price et al. (1996b) investigated the developmental toxicity and teratogenicity of boric acid in rabbits at doses of 0, 10.9, 21.9, or 43.7 mg boron/kg body weight/day given by gavage. Developmental effects in rabbits exposed to 43.7 mg boron/kg body weight/day included a high rate of prenatal mortality, increased number of pregnant females with no live foetuses, and fewer live foetuses per live litter on postnatal day 30. Malformed live foetuses per litter were increased significantly at 43.7 mg boron/kg body weight/day, primarily because of the incidence of foetuses with cardiovascular defects, the most prevalent of which was interventricular septal defect. Skeletal variations observed were extra rib on lumbar 1-position and misaligned sternebrae. The NOAEL for maternal and developmental effects was 21.9 mg boron/kg body weight/day and the LOAEL was 43.7 mg boron/kg body weight/day (IPCS, 1998).

In a 2-year study (Weir and Fisher, 1972) groups of 4 male and 4 female beagle dogs were fed diets containing boric acid or borax to provide doses of 0, 1.45, 2.93, or 8.75 mg boron/kg body weight/day. No evidence of toxicity was observed. An additional group of dogs (4 male and 4 female) was fed diets containing boric acid or borax doses of 0 or 29.3 mg boron/kg body weight/day for 38 weeks. The authors stated that boric acid caused testicular degeneration in dogs, including spermatogenic arrest and atrophy of the seminiferous epithelium. The background lesions in testis and its accessory organs were also high, so the results were equivocal (IPCS, 1998).

3.2 Genotoxicity

Boric acid was not mutagenic in Salmonella enterica var. Typhimurium with or without exogenous metabolic activation (S-9 fraction) (Haworth et al., 1983; Benson et al., 1984; NTP, 1987; IPCS, 1998). Boric acid was not mutagenic in the L5178Y mouse lymphoma tk
assay with or without S-9 (NTP, 1987; Rudd, 1991). Boric acid did not induce unscheduled DNA synthesis (UDS) in primary cultures of male F344 rat hepatocytes (Bakke, 1991).

Crude or refined borax were negative in assays for mutagenicity in V79 Chinese hamster cells, C3H10T \( \frac{1}{2} \) mouse embryo fibroblasts and diploid human foreskin fibroblasts (Landolph, 1985).

Similarly, boric acid was negative in \textit{in vitro} assays for chromosomal aberrations or sister chromatid exchanges (SCEs) in Chinese hamster ovary cells with or without metabolic activation systems (NTP, 1987).

\textit{In vivo}, boric acid was negative in a micronucleus assay on Swiss-Webster mice (O’Loughlin, 1991). In this study, boric acid was administered in deionized water orally for 2 consecutive days at 900, 1800 or 3500 mg/kg. No induction of chromosomal aberrations or mitotic spindle abnormalities in bone marrow erythrocytes was observed.

Overall, the available data indicate that boric acid and borax are not genotoxic.

### 3.3 Human toxicity data

#### 3.3.1 Acute and short-term toxicity

The lowest lethal dose after single accidental oral ingestion of boron acid by humans ranged from approximately 98-650 mg boron/kg body weight (Stokinger, 1981; Teshima \textit{et al.}, 1992), dermal exposure revealed a lower lethal dose of 1457 mg boron/kg body weight, whereas death has been reported when boron was given intravenously at 0.5 mg boron/kg body weight. Litovitz \textit{et al.} (1988) stated that the potential lethal dose is 3-6 g for infants and 15-20 g for adults expressed as boric acid.

There are data from several case reports of intoxication with boron as boric acid from inappropriate use of medical preparations (Goldbloom and Goldbloom, 1953; Linden \textit{et al.}, 1986; Litovitz \textit{et al.}, 1988; Stokinger, 1981; Kliegel, 1980; Stokinger and Spiegl, 1953; Naghii and Samman, 1997b; IPCS, 1998; Teshima \textit{et al.}, 1992; Stein \textit{et al.}, 1973; Gordon \textit{et al.}, 1973). Symptoms included gastrointestinal disturbances, generalised or alternating focal seizure disorders, irritability, granular degeneration of tubular cells, exfoliate dermatitis, epilepsy, cardio-circulatory collapse, CNS effects such as oedema and congestion of the brain, hair loss, lethargy, anorexia and mental confusion.

The toxic dose varied with the duration of intake, 4-10 weeks of intake revealed toxic effects at an average daily ingestion of 0.143-0.429 g boric acid/kg body weight, equivalent to 25-76 mg boron/kg body weight (O’Sullivan and Taylor, 1983).

Linden \textit{et al.} (1986) published a retrospective review of 364 cases of boric acid ingestion reported to the Rocky Mountain Poison and Drug Center in Denver, CO, USA, between 1983 and 1984. Vomiting, diarrhoea, and abdominal pain were the most common symptoms given by 276 cases. Of the 72 cases reported in 1984, 79% were asymptomatic, whereas 20% had mild gastrointestinal symptoms.

The average oral dose of boric acid required to produce clinical symptoms of intoxication with boron is still unclear (Goldbloom and Goldbloom, 1953; IPCS, 1998). In a 3-week study
in post-menopausal women receiving either a low boron diet (0.33 mg/day) or this diet supplemented to 3.33 mg/day, the supplement had no effect on blood levels of minerals, or steroids (Beattie and Peace, 1993).

3.3.2 Reproductive effects

An ecological study assessed boron exposure from drinking water and fertility among residents in two geographical regions in Turkey. Drinking water from Region I contained 2.05-29 mg boron/L and Region II had a range of 0.03-0.40 mg boron/L (IPCS, 1998; Sayli et al., 1998). No statistical analyses were performed. The results of this descriptive study suggest that fertility, as measured by the ability to produce a live birth, was not adversely affected for residents of high boron drinking water and soil area.

Fertility following inhalation exposure to boron was assessed in a descriptive study. Whorton et al. (1994) estimated the standardised birth rate (SBR) to assess fertility in 542/720 occupational workers in a borax mine in California, USA. No significant trend was observed for SBR in relation to exposure for quintiles of mean exposure levels ranging from <0.82 mg boron/m$^3$ to >5.05 mg boron/m$^3$.

4. DOSE-RESPONSE ASSESSMENT

The data on the toxicity of boron in humans are sparse and not suitable for dose-response assessment.

Limited long-term carcinogenicity studies in mice and rats showed no evidence of carcinogenicity. In addition, in vitro and in vivo studies at gene or chromosome level showed no evidence of genotoxicity.

There are several short-term and long-term toxicity studies in a number of animal species (e.g. mouse, rat, dog and pig). The animal experiments reveal developmental and reproductive effects as the most critical adverse effects. The reproductive effects were observed both in repeated dose toxicity studies and reproduction studies. The reproductive effects found at dose levels of 58.5 mg boron/kg body weight/day in a 2-year toxicity study in rats consisted of atrophy of seminiferous epithelium and decreased size of testicular tubules, which was not observed at 17.5 mg boron/kg body weight/day (Weir and Fisher, 1972). Developmental effects produced by boron included short ribs, variation in the number of ribs and decrease in foetal body weight. The LOAEL for decreased foetal body weight was 13.3 mg boron/kg body weight/day with a NOAEL of 9.6 mg/kg body weight/day (Price et al., 1996a).

CONCLUSIONS AND RECOMMENDATIONS

1. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL (UL)

1.1 Adults

The human database on boron is not adequate for establishing an UL and there are no human data on developmental and reproductive effects comparable to those observed in animals. In consequence, the UL is based on the most sensitive end-point detected in the animal studies,
i.e. the NOAEL for decreased foetal body weight in rats following maternal exposure during pregnancy. Normally, a default uncertainty factor of 100 would be applied to a NOAEL derived from an animal study, but this factor should be modified if relevant data are available. In the case of boron, critical analysis of the existing data on boron toxicokinetics allows use of the approach proposed by the International Programme on Chemical Safety (IPCS, 1994) in which the 10-fold interspecies and human variability factors are subdivided into toxicokinetic and toxicodynamic aspects. The default toxicokinetic uncertainty factor for extrapolation from animals to humans was appropriate for boron and was retained. The glomerular filtration rate (GFR) in pregnant women is $144 \pm 32$ mL/min (Dourson et al., 1998). Human variability in GFR during pregnancy was calculated as the ratio of the mean GFR divided by (the mean GFR minus two times the standard deviation) $(144/80)$, i.e. 1.8. The default toxicokinetic uncertainty factor for human variability was then adjusted from the default value of 3.2 to 1.8 based on variability in GFR, which is the critical physiological process involved in boron clearance. There are no data on species differences or human variation in boron toxicodynamics, and the default factors for these aspects were retained. The resulting combined overall uncertainty factor was 60. Dividing the NOAEL of 9.6 mg boron/kg body weight/day by the uncertainty factor of 60 gives a daily intake of 0.16 mg/kg body weight/day, which gives a UL of 10 mg boron/person/day for adults.

The UL only applies to the intake of boron in the form of boric acid and borates.

1.2 Pregnancy and lactation

As the UL is based on a NOAEL for adverse effects from reproductive studies and multigeneration studies, the UL of boron applies also to pregnant and lactating women.

1.3 Children and adolescents

There are no data on adverse effects of boron intakes on children and adolescents. However, the multigeneration studies in animals do not indicate that young animals are more susceptible than adults. Therefore, in the absence of adequate data the Panel chose to extrapolate the UL from adults to children on a surface area (body weight$^{0.75}$) basis. The reference weights derived by the SCF (SCF, 1993; SCF, 2000) are used as a basis for the calculations of surface area and UL.

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<tr>
<th>Age (years)</th>
<th>Tolerable Upper Intake Level (UL) for boron (mg/day)</th>
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2. RISK CHARACTERISATION

The limited data on boron intake in EU countries indicate that the intakes of boron from food and drinking water are below the UL.

The intake of some supplements containing boron may lead to intakes that exceed the UL.
3. **RECOMMENDATIONS**

Data on intake of boron from different sources in Europe is very limited and should be collected for different European countries.

**REFERENCES**


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PANEL MEMBERS


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