Technical Report

No 63

Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings

February 1995

ISSN-0773-8072-63

Technical Report No. 63

Reproductive and General Toxicology of some Inorganic Borates and Risks Assessment for Human Beings

February 1995

ISSN-0773-8072-63

Brussels, February 1995

ECETOC Technical Report No. 63

TOXICOLOGY OF INORGANIC BORATES

CONTENTS

SUMMARY AND CONCLUSIONS

A review of the toxicology of some inorganic borates is provided together with a risk assessment for man. The toxicology data base is largest for boric acid (H_3BO_3) and borax ($Na_2B_4O_7.10H_2O$). Limited data are available for sodium perborate (mono and tetrahydrate) and boric oxide and even less is available (acute data only) for borax pentahydrate and anhydrous borax.

The toxicological end-points of concern identified for both boric acid and borax from animal studies were fertility and developmental toxicity. Generally, these effects were observed in more than one species. Data were available on fertility from dog and rat studies on both borax and boric acid. In addition, mouse data were available on boric acid.

For both boric acid and borax, the lowest NOAEL for fertility in male and females rats was determined to be 17 mg boron/kg bw. Effects seen at the LOAEL included testicular toxicity, reduced spermiation in males and decreased ovulation in females. At higher dose levels testicular atrophy was observed. A lower NOAEL was available from a dog study (8.8 mg boron/kg bw) but the data were considered unsuitable for risk assessment purposes. For female mice, the NOAEL for fertility was around 27 mg boron/kg bw. Effects included reduced ovulation and decreased pup weights in the second generation offspring.

For developmental toxicity, only data on boric acid were available from rat, mouse and rabbit studies. The rat was confirmed as the most sensitive species with a NOAEL of 9.6 mg boron/kg bw, based on reduced foetal body weight and skeletal effects observed at the LOAEL.

Taking NOAELs for fertility and developmental toxicity, an uncertainty factor (UF) was applied to derive a tolerable daily intake for a 60 kg human. Justification for an uncertainty factor of 30 is presented, taking into account the nature of the hazard, adequacy of the data base and detailed knowledge of how borates are absorbed, distributed and excreted without liver metabolism. Limited human data were also available for consideration. A tolerable daily intake of borates for a 60 kg person was calculated to be 34 mg boron/day and 19.2 mg boron/day which could be ingested without the risk of fertility (testicular) or developmental effects respectively.

Based on the tolerable daily intake of 19.2 mg boron and taking into consideration the maximum boron intake from diet is 7 mg/d from food, mineral waters and other beverages including wine, up to 12 mg boron could be obtained from other sources including drinking water without exceeding the total daily intake. With a drinking water standard at the current EC Guide Level of 1 mg boron/l,

the total boron intake from food and water is well below the calculated total daily intake. Therefore, the current drinking water Guide Level of 1 mg boron/l is considered to be sufficiently conservative and there is no need for it to be reduced even further to 0.3 mg boron/l as recently recommended by WHO (1993).

The overall conclusion is that, at high doses, boric acid and borax cause adverse effects on fertility and developmental toxicity in animals models. Preliminary investigations have been carried out to try to identify the mechanism concerning the testicular effects observed, but very little relevant work has yet been done to establish the cause of developmental toxicity effects. The precise mechanism of action is unclear but it is known that borates are not metabolised, neither do they accumulate in the body except for low deposits in bone. At borate concentrations found in the environment either as a food constituent or when present in fresh waters and in some drinking waters, the risk assessment has demonstrated that exposure is not likely to cause any undue health risk to human beings.

SECTION 1. INTRODUCTION

The element boron (B) is widely distributed in nature in low concentrations. Because of its high affinity for oxygen, boron always occurs in nature bound to oxygen in the form of inorganic borates. Apart from their occurrence in a few commercially exploitable deposits (mainly as sodium or calcium borate minerals), the borates are present everywhere at low concentrations in rocks (< 100 mg B/kg), soils (<10-20 mg B/kg), fresh waters (< 1 mg B/l) and sea water (5 mg B/l).

Throughout this review the term "borate" is used as an abbreviation for the boron-oxygen substance under consideration. The term "boron" is used to express data as the equivalent boron (B) content of a borate, and is not intended to mean elemental boron. The term is also used to compare the effect of an equivalent B content of one borate with another when discussing doses applied in animal studies (details for conversion are given in section 2.4). Except for sodium perborate the toxicological effects are likely to result from the ultimate chemical species in aqueous solution, namely undissociated boric acid (see section 2.1).

Borates are in extensive commercial use. The nature of the product or end use will determine the extent of exposure to consumers and the environment. In some of the larger applications such as glass wool (insulation), enamels, ceramics and borosilicate glass, the borate becomes fixed into a water-insoluble matrix with little or no environmental impact. Applications where slow leaching into the environment will occur include adhesives, flame retardants and timber preservatives. The borates enter the aqueous environment most readily when used or discharged directly in the form of water-soluble inorganic borates. These include perborate-containing detergents, boronated fertilisers, additives to corrosion inhibitors in anti-freeze formulations, biocides for cutting fluids, insecticides and as buffers/preservatives for cosmetic and pharmaceutical preparations.

This last mentioned application has been used for more than 100 years, until superseded by other products considered medically more effective. During this period there have been occasionally problems associated with the misuse of borates, resulting in poisoning in adults, children and babies. Currently boric acid is permitted in a range of cosmetic products in the European Union (EU) at the following levels; talcum powder (5%), oral hygiene products (0.5%) and other products (3%).

Borates are taken up naturally in all life forms and are found especially in fruit, vegetables, nuts and wine. Although borates are essential plant micronutrients, their essentiality for animals is not proven, but it does appear that borates may be nutritionally important for animals and man. During

the past 5 years, largely through US Department of Agriculture dietary studies on animals and human beings, there has been renewed interest in the nutritional importance of boron. For example, it is suggested (Nielsen, 1992) that inadequate dietary boron (≤ 0.2 mg B/d) may be one factor that contributes to susceptibility to bone loss or osteoporosis. Further work is still required to establish whether there is an essential requirement for boron in man.

The concentration of borates in fresh waters is under scrutiny because of the widespread use of perborates in laundry detergents, since borate is not removed during the sewage treatment process. The EC is currently revising its guideline values of drinking water, which for boron is at present set at 1 mg B/l. The WHO (1993) has recently published a new guideline value for boron in drinking water of 0.3 mg B/l, following a risk assessment based on a 1972 study investigating the reproductive effects of borates in dogs (Weir and Fisher, 1972). In the light of more recent data, this guideline will be subject to further review and WHO have indicated that boron will be given top priority for re-evaluation at the first opportunity.

The objective of this Task Force is to present a comprehensive review of the toxicology of inorganic borates and to focus mainly on the reproductive toxicity of borates including recently published data. Because similar effects have not been observed in man, extrapolation of animal data will be required and a risk assessment will be presented based on estimated human exposures to borates from a variety of sources. Finally, a suitable safe level of borate in drinking water will be recommended. A companion ECETOC document will consider the ecotoxicological properties of the borates to provide similar guideline values for environmental parameters.

SECTION 2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, PRODUCTION AND USES

2.1 IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES

Borax, the principal naturally occurring commercial source of the element boron, with the formula $Na_2B_4O_7.10H_2O$, can be considered as the basis of inorganic borates. Borates are odourless white crystalline granules or powders. Identity and physical/chemical properties of the seven major inorganic borates discussed in this report are summarised in Table 1.

Of particular interest in a toxicology review is an understanding of what species of chemical interacts with biological tissue and the consequences of any such interaction. Because boric acid is stable and a very weak acid (pKa 9.15), the undissociated acid (H_3BO_3) is the predominant species in aqueous solution at physiological pH. This applies also to boric oxide and sodium borates. As a consequence, the toxicology of all these substances is likely to be similar on an equivalent boric acid basis (as boron). Furthermore, it is known that boric acid can form complexes with carbohydrates and proteins (Kliegel, 1980). For the series of boron-containing compounds identified in Table 1 (except sodium perborate), it is assumed that the final species in question will either be the undissociated boric acid or a complex of boric acid with a carbohydrate or protein.

Different considerations of toxicology apply to sodium perborate, a peroxygen compound, that readily generates hydrogen peroxide in addition to sodium borate in biological systems. Thus, sodium perborate will provide a local environment with a pH of around 10, which may be partially responsible for some of the acute inflammatory and tissue reactions described in this review.

2.2 PRODUCTION

Limited published data are available on production statistics of the inorganic borates, as follows:

- the estimated total world production of boron minerals and compounds in 1991 was almost 3 million tonnes (Lyday, 1992);
- the world's annual capacity (expressed in tonnes per annum B_2O_3) to make the major boron chemicals in 1989 (CEH, 1993) was

  USA, 750,000 of sodium borates and 195,000 of boric acid,

  Western Europe, 30,000 of sodium borate, 56,000 of boric acid.

Table 1 Identity, Physical and Chemical Properties

Physical/Chemical Properties	Boric Acid[a]	Borax[a]	Borax Pentahydrate[a]	Anhydrous Borax[a]	Boric Oxide[a]	Sodium Perborate Tetrahydrate[b]	Sodium Perborate Monohydrate[c]
Chemical Formula	H_3BO_3	$Na_2B_4O_7.10H_2O$	$Na_2B_4O_7.5H_2O$	$Na_2B_4O_7$	B_2O_3	$NaBO_3.4H_2O$	$NaBO_3.H_2O$
Chemical Name	Orthoboric acid	Disodium tetraborate decahydrate	Disodium tetraborate pentahydrate	Disodium tetraborate	Diboron trioxide	Sodium perborate tetrahydrate	Sodium perborate monohydrate
Synonyms	Boracic acid	Borax decahydrate, Borax 10 Mol	Borax 5 Mol, Sodium tetraborate pentahydrate	Sodium tetraborate, Borax Glass	Boron trioxide, Anhydrous boric acid	PBS4, PBST	PBS1, PBSM
CAS Reg. No.	10043-35-3	1303-96-4	12179-04-3	1330-43-4	1303-86-2	10486-00-7	10332-33-9
EINECS No.	233-139-2	215-540-4	215-540-4	215-540-4	215-125-8	234-390-0	234-390-0
Physical Form	White crystalline granules or powder	White crystalline granules or powder	White crystalline granules or powder	White vitreous granules	White vitreous granules	White crystalline powder	White crystalline powder
Molecular Weight	61.83	381.37	291.35	201.27	69.62	153.9	99.8
Specific Gravity (20°C)	1.51	1.73	1.81	2.37	1.83		
Bulk Density (kg m^{-3})	880	935	1,000-1,150	1,075-1,380	975-1,090	700-900	500-650
Melting Point Closed Space (°C) Anhydrous Form (°C)	 171 450 (crystal)	 > 62 742 (crystal)	 < 200 742 (crystal)	 742 (crystal)	 450 (crystal)	 Decomp.	 Decomp.
Boron Content (%)	17.48	11.34	14.85	21.49	31.06	7.03	10.8
Available Oxygen (%wt)	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	10.0	15
Water Solubility (%w/w)	4.72 (20°C) 27.53 (100°C)	4.71 (20°C) 65.63 (100°C)	3.6 (20°C) - 50.15 (100°C)	2.48 (20°C) - 34.5 (100°C)	Rapidly hydrates to boric acid	23 g/l (20°C) - 37 g/l (30°C)	15g/l (20°C) - 24g/l (30°C)
pH of Aqueous Solution (20°C)	6.1 at 0.1% 3.7 at 4.7%	9.24 (constant)	9.24 (constant)	9.24 (constant)	Rapidly hydrates to boric acid	10 at 1.5%	10 at 1.5%
pK_a (pK_1) (20°C)	9.15[d]						
Pow (25°C)	0.175[e]	Not applicable (dissociates)	Not applicable (dissociates)	Not applicable (dissociates)	Not applicable (Rapidly hydrates to boric acid)	Not applicable (decomposition)	Not applicable (decomposition)

References

a Borax Consolidated (1992)
b Interox Chemicals (1982a)
c Interox Chemicals (1982b)
d Dawber and Matusin (1982)
e Barrès (1967)

The European consumption of sodium perborate tetrahydrate in 1993 was 620,000 tonnes (CEFIC, 1994).

2.3 USES

Borates are in widespread use in industrial, agricultural and consumer products. The specific end uses of each substance are outlined below.

2.3.1 Boric Acid

Boric acid is an important source of soda-free boric oxide (B_2O_3) in vitreous systems such as glass, enamels and ceramic glazes. It is a flame retardant with effective anti-smoulder characteristics. The high-purity form is used in the production of capacitors, and for the control and emergency shutdown of nuclear reactors (absorption of neutrons by ^{10}B isotope). Boric acid is also used as a preservative in some cosmetic and pharmaceutical preparations. In admixture with borax it is a useful buffer in eye lotions.

2.3.2 Borax

Borax is generally the sodium borate selected for applications in aqueous solutions, e.g., anti-corrosion additive in vehicle cooling systems or in cutting fluids. Borax is also used for cosmetic/pharmaceutical applications.

2.3.3 Borax Pentahydrate

Borax pentahydrate is the major borate raw material for the glass, ceramics and enamel industries. It is used as the feedstock for the production of sodium perborate bleach.

2.3.4 Anhydrous Borax

Anhydrous borax is borax from which the water of crystallisation has been removed by fusion. The resultant molten glass is cooled and crushed to form a granular product, but the glassy nature of the particles can still be discerned. The finished product has a higher bulk density than either borax or borax pentahydrate and is preferred to these materials in the glass, ceramic and enamel industries if furnace capacity and/or storage capacity is at a premium.

2.3.5 Boric Oxide

Boric oxide is prepared by the dehydration of boric acid. As in the case of anhydrous borax, the water is removed by fusion and the glassy product is crushed to form a coarse granular or a powder product. The glassy nature of the material can be discerned in the coarse form.

Boric oxide is used in the manufacture of ferroboron and other master alloys. It is used for heat treatment in the metallurgical industries.

Boric oxide is hygroscopic, absorbing moisture from the atmosphere to form boric acid.

2.3.6 Sodium Perborate Tetrahydrate

Sodium perborate tetrahydrate is used primarily as a bleaching agent in detergent powders and in bleaching powders. It is used to a smaller extent as a mild disinfectant and deodorant in cosmetic and pharmaceutical preparations (Martindale, 1977).

2.3.7 Sodium Perborate Monohydrate

Sodium perborate monohydrate is used primarily as a bleaching agent in detergent powders.

2.4 CONVERSION FACTORS TO BORON EQUIVALENT

Studies reported in the literature have been conducted with different boron compounds. The boron content of each compound is determined by its chemical composition. Conversion factors have to be applied in order to compare the real doses of boron. Therefore the equivalent of boron (B) to the doses used in the studies are given throughout this report. The calculations were based on following data:

1 part boric acid, H_3BO_3, contains 0.175 parts of boron, B.
1 part borax, $Na_2B_4O_7.10H_2O$, contains 0.113 parts of boron, B.
1 part sodium perborate tetrahydrate, $NaBO_3.4H_2O$, contains 0.070 parts of boron, B.
1 part sodium perborate monohydrate, $NaBO_3.H_2O$, contains 0.108 parts of boron, B.

2.5 ANALYTICAL METHODS

A variety of techniques is available for the analysis of borates, as boron, including absorption and emission spectrophometry and both atomic and mass spectrometry. Before carrying out the appropriate analytical procedure on biological materials, it is generally necessary to prepare the sample by extracting the borate from unwanted components, that may interfere with the colorimetric or other instrumental methods of analysis. A detailed review of these methods is available in the companion ECETOC report on the ecotoxicity of borates (ECETOC, 1995).

The British Standards Institution (1979) has published a Standard in 29 parts (BSI, 1979) for the analysis of the commercial inorganic borates and perborates, which is also recognised by the International Organisation for Standardisation.

SECTION 3. TOXICOKINETICS IN ANIMALS AND MAN

This section reviews the relevant data on absorption, distribution, metabolism and excretion of borate. Although absorption describes the processes involved in the transfer of a substance from the site of administration to the systemic blood circulation, it is possible, particularly for substances administered by the oral route, to use urinary levels of the substance as an indicator of absorption. This may be helpful in the absence of specific blood/plasma values.

A representative set of blood and urine concentrations in animals are summarised in Tables 2 and 3. Normal blood boron concentrations in man are generally within a fairly narrow range and these concentrations are usually lower than those seen in untreated animals. Urinary boron output reflected boron intakes. In view of the large amount of available data, not all the studies referred to in the Tables will be discussed in the text. Salient features of the toxicology will be discussed by reference to appropriate studies.

3.1 ABSORPTION

3.1.1 Animal Studies

3.1.1.1 Oral

Ingested borate is readily absorbed by various species. For example, eighteen 45kg sheep were fed supplemental boron as sodium borate to provide total doses, equivalent to 1.7 or 4.4 mg B/kg bw/d for 11 days during which time faecal boron and urinary boron estimations were made. Total daily boron intakes including diet were 30mg for the control group and 75 or 200mg for the supplement group. Urinary boron excretion was proportional to exposure and accounted for 72 and 85% respectively of the total boron excreted (faecal and urine) over the observation period in the supplement groups (Brown *et al*, 1989).

Owen (1944) measured absorption in two cows receiving 18-23 g/d of borax in their feed, equivalent to 5.6-7.3 mg B/kg bw/d, for 42 days. Boron was excreted in urine, faeces and milk with no adverse findings reported. Weeth *et al* (1981) conducted similar studies for 10 days and confirmed these findings on heifers receiving water containing borax at 15, 30, 60 or 120mg B/l, equivalent to 2.8, 4.6, 7.7 and 13.8 mg B/kg bw/d. In this study boron plasma levels were also measured, and these were shown to increase with exposure in a curvilinear fashion, as were urinary boron levels.

Table 2 Concentration as Boron in Blood

Species	Route	Dose	Blood*	References
Human being	Inhalation	untreated	0.04 - 0.40 µg B/g	Imbus *et al*, 1963
	Inhalation (Preceeding 10 d) Pre-shift Post-shift	untreated untreated 0.38 mg B/kg bw/d (5d)	0.02 µg B/g 0.072 µg B/g 0.239 µg B/g	Culver *et al*, 1994a
	Oral	control 1.41 mg B/kg bw/d (14 d)	1.4 µg B/ml 2.9 µg B/ml	Job, 1973
	Dermal	control 0.9 mg B/kg bw (single dose)	0.06 µg B/ml 0.19 µg B/ml	Stüttgen *et al*, 1982
	i.v.	control 1.50 mg B/kg bw (single dose)	0.04 µg B/ml (plasma) 0.4 µg B/ml (steady state, plasma) 3.5 µg B/ml (peak, plasma)	Jansen *et al*, 1984b
	Diet	untreated	0.0 - 0.35 µg B/ml (serum)	Linden *et al*, 1986
	Diet	untreated untreated	0.097 µg B/g 0.022 µg B/g	Clarke *et al*, 1987a Clarke *et al*, 1987b
	Diet	untreated	0.022-0.66 µg B/g	Barr *et al*, 1993
	Diet	untreated	0.031 µg B/g	Woittiez and Iyengar, 1988
	Diet	untreated	0.14 - 0.74 µg B/ml	Ward, 1987
	Diet	untreated	0.057 µg B/ml	Abou-Shakra *et al*, 1989
Dog	Oral	control 8.8 mg B/kg bw/d (2 y)	2.1 µg B/ml 4.8 µg B/ml	Weir and Fisher, 1972
Rat	Oral	control 94 mg B/kg bw/d (7 d)	1.94 µg B/g (plasma) 16 µg B/g (plasma)	Ku *et al*, 1991
	Oral	control 61 mg B/kg bw/d (28 d)	0.16 µg B/g (plasma) 11.7 µg B/g (plasma)	Treinen and Chapin, 1991
	Oral	control 68 mg B/kg bw/d (7 d)	< 4 µg B/ml 17.3 µg B/ml	Ku *et al*, 1993a

* Whole blood unless stated otherwise

Table 3 Concentration as Boron in Urine

Species	Route	Dose	Urine	References
Human being	Inhalation	untreated	0.7 µg B/ml (0.04 - 6.6)	Imbus *et al*, 1963
	Inhalation (Preceeding 10 d) Pre-shift Post-shift	untreated untreated 0.38 mg B/m^3 (5 d)	1.34 µg B/mg creatinine 2.11 µg B/mg creatinine 9.39 µg B/mg creatinine	Culver *et al*, 1994a
	Oral	control 1.41 mg B/kg bw/d (14 d)	1.13 µg B/ml/d 62.8 µg B/ml/d	Job, 1973
	Oral	control 3 mg B/d	0.7 mg B/d 1.1 - 2.8 mg B/d	Meachan *et al*, 1994
	i.v.	control 1.7 mg B/kg bw (single dose)	1.24 µg B/ml/d 1.53 µg B/ml/d	Jansen *et al*, 1984b
	Diet	untreated	0.38 - 0.7 µg B/ml/d	Ward, 1987
	Diet	untreated	0.75 µg B/ml (0.15 -2.98)	Abou-Shakra *et al*, 1989
	Diet	untreated	1.9 µg B/ml (0.47 - 7.8)	Minoia *et al*, 1990
Dog	Oral	control 1.5 mg B/kg bw 2.9 mg B/kg bw 8.8 mg B/kg bw (2 y)	8.6 µg B/ml 62.6 µg B/ml 58.6 µg B/ml 158.6 µg B/ml	Weir and Fisher, 1972
Rat	Oral	control 68 mg B/kg bw (7 d)	9.0 µg B/mg creatinine 500.0 µg B/mg creatinine	Ku *et al*, 1993a
Mouse	*i.v.*	control 25 mg B/kg bw (single dose)	53 µg B/mouse 302 µg B/mouse	Farr and Konikowski, 1963

3.1.1.2 Dermal

Dermal absorption across intact skin of animals appears to be very low. Dermal absorption across non-intact skin varies according to the vehicle used, with absorption being high with an aqueous vehicle but lower with oil-based vehicles.

Doses of 1-2 ml of preparations containing 3% boric acid were applied either as an aqueous jelly or as an oleaginous ointment to intact skin of anaesthetized rats, covering areas of 3-28 cm^2. Low or no excretion of boron in urine was detected by this route but absorption was demonstrated when a similar experiment was conducted using damaged skin. Urinary boron levels were increased 4 to 8-fold when boric acid was applied as an ointment and 34-fold when applied as an aqueous jelly. A parallel study measuring the rate of dermal absorption suggested slow boron absorption when presented as an ointment, but rapid absorption when presented as an aqueous jelly (Nielsen, 1970). Overall, the urinary excretion of boric acid was 1% of the given dose when applied as an ointment and 23% when applied as an aqueous jelly (Neilsen, 1970).

Draize and Kelley (1959) studied dermal absorption in rabbits of 5% boric acid presented as an aqueous solution or in talc and as a pure substance. Preparations were applied to 10-15% of the body surface with an occlusive dressing for 1.5 hours daily for 4 days. Insignificant amounts of boric acid were absorbed across intact and slightly abraded skin of the rabbit as measured by urinary boron excretion; more seriously damaged skin did permit absorption of boric acid in this study.

3.1.2 Human Studies

3.1.2.1 Oral

Absorption of ingested borate appears to be rapid and virtually complete. In a study in which human volunteers were given a single dose of 500 mg boric acid, equivalent to 1.5 mg B/kg bw for a 60kg person, more than 90% of the ingested boron was excreted *via* the urine over a 96-hour period (Schou *et al*, 1984). This corroborates the early work of Job (1973) who reported a similar urinary excretion of boron in volunteers drinking curative SPA waters with a high boron content (providing a total of 1.7mg B/kg bw/d) for two weeks. Jansen *et al* (1984a) measured the absorption of 3% boric acid by 6 male volunteers, after taking it either in an aqueous solution or as waterless water-emulsifying ointment spread onto biscuits. Urinary boron was measured over 96 hours following administration. Intake of boric acid when delivered as an ointment varied amongst the volunteers from 740 mg to 1,470 mg and took between 13 and 80 minutes for it to be

consumed. The boric acid dose from the aqueous solution was 750 mg. In both instances more than 92% of the borate was excreted *via* the urine within 96 hours.

Sodium perborate monohydrate, buffered by sodium hydrogen tartrate, is marketed in dentistry as a bacteriostatic mouthwash to be used twice daily against gingivitis. A 1.7 g dose, consisting of a mixture of 1.2 g of perborate and 0.5 g anhydrous sodium hydrogen tartrate, is dissolved in 30 ml warm water, circulated in the mouth for about 3 minutes and is then spat out. Tests on both patients and healthy human volunteers have established that boron is not taken up through the mucous membrane, but probably by ingestion of residual amounts after treatment. In the study the highest measured blood boron concentration (0.32 μg/ml), from a pretreatment level of 0.14 μg/ml (Edwall *et al*, 1979).

3.1.2.2 Dermal

Dermal absorption across intact skin is minimal in human infants (Friis-Hansen *et al*, 1982) and adults (Beyer *et al*, 1983). Absorption across abraded skin (Stüttgen *et al*, 1982) varies with the carrier vehicle used. Thirty one males with either normal or diseased skin conditions, such as psoriasis, eczema or urticaria, were given a single application of an emulsifying ointment containing 3% boric acid, providing doses ranging from 3-127 mg B to normal subjects and 37-89 mg B to the patients with diseased skin conditions. No increases in urinary boron was observed in any of the treated adults. When 3% boric acid was applied as an aqueous jelly to 6 male volunteers, all with severe skin conditions, urinary boron levels increased within 24 hours and then decreased, suggesting evidence for dermal absorption through non-intact skin when boric acid was presented in the aqueous form. Blood boron levels were also measured in these 6 subjects and increases were observed within a few hours of dosing. On the other hand, a small group of infants with nappy dermatitis was treated with boric acid ointment and no increase in urinary or blood boron levels was observed, in agreement with the response in adults to the same ointment.

Friis-Hansen *et al* (1982) measured blood boron levels in 22 newborn infants treated with an oily ointment containing the equivalent of 3% boric acid. Ointment (3 g) was applied to the napkin region over 4-5 days providing a total dose of 15.7 mg B. Mean plasma boron concentrations fell over 5 days from a pre-treatment value of 0.49 down to 0.29 μg B/ml, compared with 0.62 falling to 0.21 μg B/ml in 10 untreated neonates. Such decrease of boron levels is expected in neonates due to their initial reduced dietary intake, the boron levels in milk being around 0.1-0.2 μg/ml, and therefore this study indicates no absorption of boron when applied in an emulsifying ointment.

3.1.2.3 Inhalation

Borate appears to be absorbed by the respiratory tract as described in a study of process workers at U.S. Borax Inc., although the relative contribution to the absorption by the oral and inhalation routes cannot be readily ascertained (Culver *et al*, 1994a).

3.2 DISTRIBUTION

3.2.1 Animal Studies

At physiological pH, it is believed that undissociated boric acid distributes evenly throughout the body in plasma and tissues. Treinen and Chapin (1991) established that steady state tissue levels were attained at 3-4 days in a 28 day study on the rat receiving an estimated dose of 61 mg B/kg bw/d. The most complete study of boron distribution has been done in rats given 9,000 ppm boric acid in the diet (equivalent to approximately 94 mg B/kg bw/d) for up to 7 days (Ku *et al*, 1991). They reported that boron distributes at a comparable concentration in all tissues examined including plasma, liver, kidney, muscle, colon, brain, testis, epididymis, seminal vesicles, prostate and adrenals. Typical levels in tissues one day after start of exposure were 3 to 20-fold above controls, commensurate with this high exposure, with steady state conditions being reached within 3-4 days. Even after 28 days or 9 weeks exposure to high levels of boron in the diet or drinking water, the return to steady state conditions for urinary boron and serum boron occurred within a few days (Treinen and Chapin 1991, Ku *et al* 1993a). Boron concentrations in control mice have been measured. The highest concentrations of boron in control mice were 26 μg/g dry weight in bone (Massie *et al*, 1990) and 64 μg/g in fresh kidney medulla (Laurent-Pettersson *et al*, 1992).

The only significant accumulation of boron was in bone where amounts doubled compared with initial concentrations (Ku *et al*, 1991). Accumulation of boron in bone has also been observed by Forbes and Mitchell (1957). Corroborating evidence of distribution in total body water is from studies in mice, in which the plasma and red blood cell concentrations of boron were equivalent after an *i.p.* injection of borax (Locksley and Sweet, 1954). Pfeiffer *et al* (1945) reported accumulation in the brain, liver and fat of dogs given the relatively high single dose of 2,000 mg/kg bw of boric acid, equivalent to 350 mg B/kg bw.

3.2.2 Human Studies

In human beings, the deposition of boron has been observed in a range of tissues including bone (Alexander *et al*, 1951; Forbes *et al*, 1954), scalp, hair, finger and toe nails (Abou-Shakra *et al*,

1989; Krause *et al*, 1989; Ciba and Chrusciel, 1992), teeth (Ward *et al*, 1987) and lung (Sabbioni *et al*, 1990) which are summarised in Table 4.

Abou-Shakra *et al* (1989) measured boron content in human tissues by a modified mass spectrometry procedure. The data suggested that levels in whole blood and serum were homeostatically regulated but that higher concentrations were present in nails and hair, indicating slow accumulation over longer periods. Alexander *et al* (1951) measured boron levels in bone by thermal neutron capture from 116 samples taken from 33 human beings, with an age range of 5 months to 75 years. Values ranged from 16-138ppm by weight as bone ash. Forbes *et al* (1954) examined cadaver tissue of a 46 year old male for distribution of boron, cobalt and mercury in a range of organs and tissues. The only organ in which boron levels were higher than others was the skeleton, with 0.9ppm of fresh tissue, compared with 0.14 ppm in blood. Ward (1987) also examined boron levels in a range of human tissues (as dry weight) from 14 control subjects and 18 sufferers from rheumatoid arthritis using a prompt-gamma neutron activation technique. High boron values were found in bone, hair and teeth. Interestingly, boron levels in the rheumatoid arthritis sufferers were slightly lower when compared with controls. Boron values ranging from 0.9-30.5 μg/g (as dry weight) were found in blood, bone hair and nails, although considerable variation (0.8-38.3) was seen. Shuler *et al* (1990) measured the boron content in dried human tissues and found the greatest amounts occurring in spleen and liver. Levels in bone and nail were not presented.

3.3 METABOLISM AND EXCRETION

Boric acid and borates do not appear to be metabolised via the liver in man or animals, since no organic boron compounds have been reported as metabolites. Borates are excreted primarily in the urine regardless of the route of administration.

3.3.1 Animal Studies

Elimination times for animals have not been explicitly stated in the literature, but can be either calculated or estimated from published data. Farr and Konikowski (1963) measured urinary boron concentration in mice after *i.v.* injection of sodium pentaborate and reported a plasma clearance rate of 0.2 ml/min. Using their data and assuming first-order kinetics for elimination, the half-life for elimination in the mouse was in the order of 1 hour. The rat pharmacokinetic data from Ku *et al* (1993a) (see section 3.2.1) can also be used to calculate a half life < 12 hours, again assuming first-order kinetics.

Table 4 Tissue Distribution as Boron in Humans (μg B/g)

Lung	Hair	Bone	Nail	Kidney	Liver	Brain	References
	0.8-15.6 (dry)	0.9-30.5 (dry)	4.6-38.3 (dry)	0.7-4.9 (dry)			Ward, 1987
	4.3 (fresh)		16.6 (fresh)				Abou-Shakra *et al*, 1989
	1.03-1.56 (dry)						Krause *et al*, 1989
0.41 (fresh)							Sabbioni *et al*, 1990
				1.27 (dry)	2.25 (dry)	0.87 (dry)	Shuler *et al*, 1990
						20 (dry)*	Andrasi *et al*, 1993
	0-5 (dry)						Ciba and Chrusciel, 1992
0.07 (fresh)		0.9 (fresh)		0.25 (fresh)			Forbes *et al*, 1954
		16-138 (ash)					Alexander *et al*, 1951

* mean age of patients = 70 years

3.3.2 Human Studies

The renal blood clearance of sodium pentaborate was observed in 8 patients undergoing boron neutron capture therapy for intracranial malignant tumours and was found to average 39.1 ml/min per 1.73 m^2 surface area (Farr and Konikowski, 1963).

Elimination of boron from the blood is largely by excretion of greater than 90% of the administered dose *via* the urine, regardless of route of administration. Excretion is relatively rapid, occurring over a period of a few days, with a half-life of elimination of 24 hours or less. The kinetics of elimination of boron have been evaluated in human volunteers given boric acid *via* the *i.v.* and oral routes (Jansen *et al*, 1984b; Schou *et al*, 1984, respectively). Doses of 600 and 750 mg boric acid were given by the *i.v.* and oral routes, respectively. The half-life for elimination was approximately 21 hours in both these studies. This value is corroborated by case reports of almost 800 patients, accidentally or intentionally poisoned with boric acid and a very comparable elimination half-life was found ranging from 4 to 27.8 hours, with a mean of 13.4 hours (Litovitz *et al* , 1988). Astier *et al* (1988) reported a value of 28.7 hours in a single case of accidental boric acid ingestion in which a massive amount of 45g was administered in 2 doses over 20 hours to a patient suffering from arterial hypertension. Four hours after the first dose, plasma boron levels were 64 μg/ml which decreased by 50% within 24 hours and subsequently fell to less than 10 μg/ml within 5 days. Not all the dose could be accounted for by urinary excretion and it is possible that other excretory routes (eg sweat) could have been involved, in addition to loss of borate through vomiting.

3.4 BORON LEVELS IN HUMAN BEINGS

3.4.1 Exposed Workers

An occupational biological monitoring study was carried out in 1992 on 17 employees at the U.S. Borax Inc. mine in California (Culver *et al*, 1994a). During a typical work week, employees exposed to the highest level of sodium borate dust (mean airborne concentration 2.66 mg B/m^3, equivalent to an intake of about 0.38 mg B/kg bw/d) showed blood and urine boron levels commensurate with their exposure. During periods of non-exposure, *i.e.* week-ends or vacation, these returned rapidly to the levels found in non-exposed individuals.

3.4.2 Human Beings in High Boron Areas

Areas of Northern Chile are naturally high in boron and lithium minerals. Blood samples taken from 40 residents in these areas were analysed for boron and compared with boron levels in local

drinking waters. As expected, blood boron levels rose in proportion with boron exposure *via* the drinking water (Barr *et al*, 1993). Mean blood boron levels ranged from 0.022 to 0.66 μg/g in various locations in Chile. In the same areas the water boron levels ranged from 0.311 to 15.2 μg/ml (see Figure 1 and Figure 2). Due possibly to the low number of blood and water samples in the two mid-value regions, a straight line relationship is not necessarily appropriate, even though one was fitted. Instead, re-evaluation of the data to include more fully the mid-value regions, was accomplished by fitting an exponential curve to these data. By taking the mean values for each district, weighting could be given to each set of data points. The new curve shown in Figure 3 of boron in drinking water against boron in blood (Unilever, ESL, 1993) should be compared with the straight relationship proposed by Barr *et al* (1993). The curvilinear response, suggests a natural homeostatic mechanism may be operating.

Figure 1 Study Sites in Northern Chile

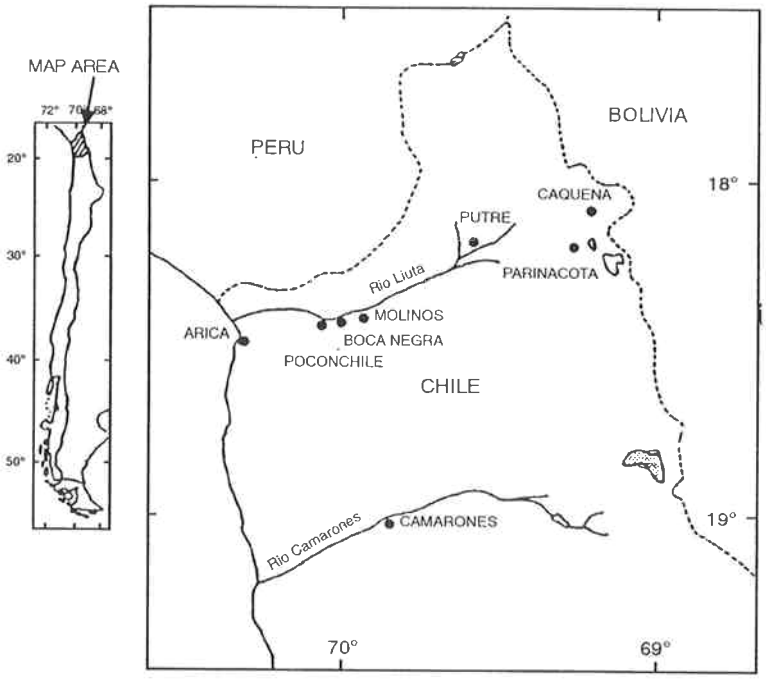


Figure 2 Relationships between concentrations in water and blood of boron. Barr *et al* (1993)

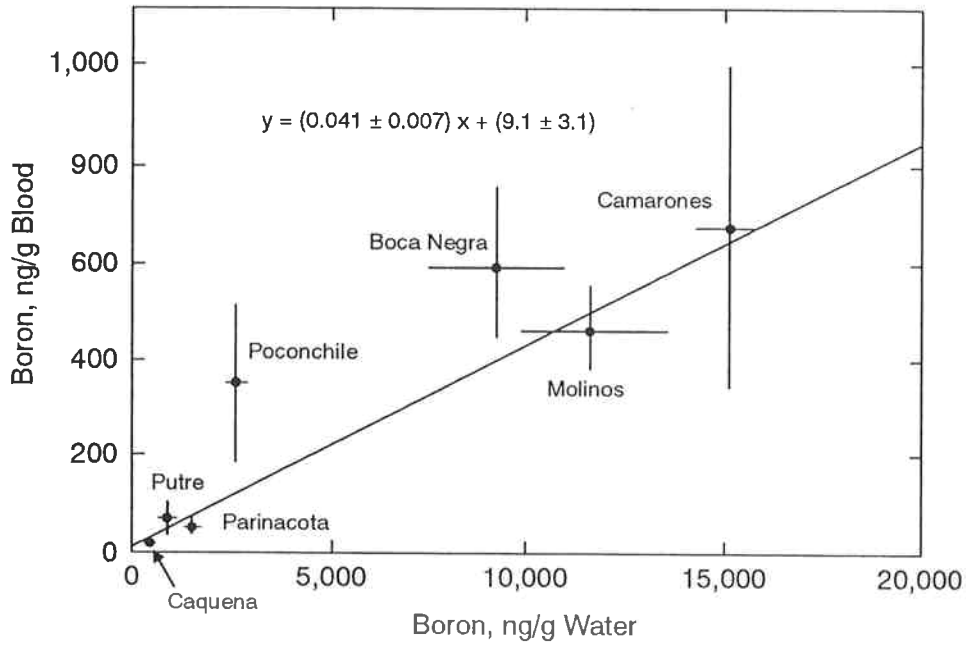
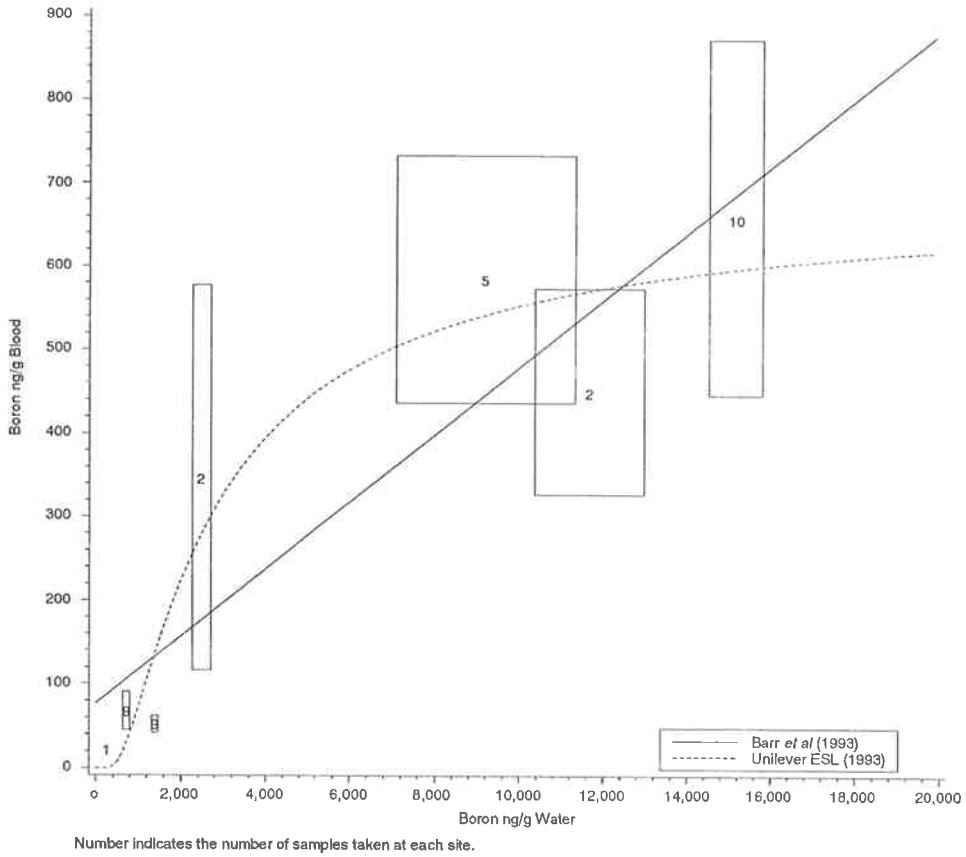


Figure 3 Relationship between concentrations in water and blood of boron



Number indicates the number of samples taken at each site.

SECTION 4. TOXICOLOGY

4.1 INTRODUCTION

Most of the information on the toxicology of the inorganic borates in both animals and man is derived from studies on boric acid and borax. Less is published about sodium perborate. The most comprehensive of the large number of reviews on all aspects was published as a book (Kliegel, 1980).

Many of the earlier data reported in the literature on the toxicity of boric acid and, to a lesser extent, of borax to human beings arose from the effects of medicinal treatments, long since obsolete, in hospitals since boric acid was introduced as an antiseptic (Lister, 1875). Other data have arisen through accidental misuse in hospitals, for example when 3% aqueous boric acid antiseptic solution was mistaken for distilled water and was used to prepare a milk feed. This information is scattered throughout the medical literature and has been tabulated (Kliegel, 1980). It is necessary to put the above mentioned historical medicinal use and misuse into proper context, because no cases of industrial intoxication have been reported (Casarett and Doull, 1980, 1986). The reason for this is that the toxic effects described in the literature are observed only if the borate is absorbed internally through ingestion or absorption through large areas of non-intact skin, e.g., rash or eczema, conditions not relevant to normal end-uses in industry.

An independent expert panel, appointed in the USA by the Cosmetic, Toiletry and Fragrance Association (CTFA), published a comprehensive safety assessment on borax and boric acid in cosmetic and pharmaceutical end-uses (Beyer *et al*, 1983). The panel concluded that borax and boric acid, in concentration of ≤ 5% are safe cosmetic ingredients when used as recommended. However, cosmetic formulations containing free borax or boric acid should not be used on infant or injured skin.

The pharmaceutical and medical literature also contains numerous reviews (Pfeiffer and Jenney, 1950; Caujolle, 1951; Goldbloom and Goldbloom, 1953; Valdes-Dapena and Arey, 1962; Levinskas, 1964; Jaspersen and Schlumpf, 1969; Rasi, 1973). More recent reviews on the toxicology of the borates are also available (Minoia *et al*, 1987; US-EPA, 1991; US-ATSDR, 1992; US-EPA, 1992; CEFIC, 1993; UNEP, 1993; and Culver *et al*, 1994b), together with an unevaluated database (RTECS, 1993).

At the present time, the most important topic for consideration is the reproductive effect of borates observed in laboratory animals, which is discussed in section 5.

4.2 ANIMAL STUDIES

Unless stated by the authors, ppm B in diet was converted into mg B/kg bw by the method of Lehman (1959). As mentioned in section 2.4, the doses of boron compounds used in the studies are given in this report as boron equivalent. As with many toxicology studies, the precise chemical form which interacts with biological tissue to cause an adverse reaction cannot always be unequivocally identified. The details provided are of the given dose of the starting material presented undiluted or in the stated vehicle.

4.2.1 Acute Toxicity

4.2.1.1 Oral

Acute oral toxicity is summarised in Table 5A.

The earliest systematic investigation of the acute toxicity of boric acid was carried out in order to assess its safety as a constituent of antiseptic ointments (Pfeiffer *et al*, 1945). The acute oral toxicity LD_{50} of boric acid in mice was 3,450 mg/kg bw and that in rats was 2,660 mg/kg bw. Oral doses of 200-2,000 mg/kg bw boric acid usually induced emesis in dogs, so that a subcutaneous injection of 30 mg of morphine had to be given before the boric acid. Under these conditions the acute oral toxicity LD_{50} of boric acid in dogs was around 2,000 mg/kg bw.

In another study, the acute oral toxicity (LD_{50}) of boric acid in rats was 3,000-4,000 mg/kg bw (equivalent to 524-700 mg B/kg bw), and that of borax was 4,500-6,000 mg/kg bw (equivalent to 510-680 mg B/kg bw). Symptoms of toxicity included signs of CNS depression, ataxia, convulsion and death and were similar for borax and boric acid (Weir and Fisher, 1972).

The acute oral toxicity (LD_{50}) of disodium tetraborate pentahydrate, $Na_2B_4O_7.5H_2O$, in rats was 2,403-4,207 mg/kg bw (equivalent to 357-624 mg B/kg bw). Symptoms of toxicity were similar to those described above for borax and boric acid (Reagan and Becci, 1985a).

The acute oral toxicity (LD_{50}) of sodium perborate tetrahydrate in rats is 2,243 mg B/kg bw (159 mg B/kg bw) (Dufour *et al*, 1971) and 2,100 mg/kg bw (149 mg B/kg bw) in another study (Chater, 1978). The acute oral toxicity (LD_{50}) in a single dose of 4% aqueous solution of sodium

Table 5A Acute Toxicity (oral)

Compound	Species	LD_{50} mg/kg bw	LD_{50} mg B/kg bw*	References
H_3BO_3	mouse	3,450	603	Pfeiffer *et al*, 1945
	rat	2,660	465	Pfeiffer *et al*, 1945
	rat	3,000-4,000	612	Weir and Fisher, 1972
	dog	2,000	350	Pfeiffer *et al*, 1945
$Na_2B_4O_7.10H_2O$	rat	4,500-6,000	595	Weir and Fisher, 1972
$Na_2B_4O_7.5H_2O$	rat	2,403-4,207	490	Reagan and Becci, 1985a
$NaBO_3.4H_2O$	mouse	3,425	243	Momma *et al*, 1986
	rat	2,243	159	Dufour *et al*, 1971
	rat	2,100	149	Chater, 1978
$NaBO_3.H_2O$	rat	> 650	>70	Mulinos *et al*, 1952
	rat	1,600-2,100	200	Moreno *et al*, 1987a
	rat	1,120	121	Glaza, 1988

* Mid point of range

perborate (tetrahydrate) by gavage on 10 male and 10 female mice gave an LD_{50} of 3,600 mg/kg bw (256 mg B/kg bw) for males and 3,250 mg/kg bw (231 mg B/kg bw) bw for females. Symptoms of toxicity included depression of spontaneous movements and irritation of the gastro-intestinal tract with diarrhoea (Momma *et al*, 1986). A relatively low oral dose of sodium perborate tetrahydrate of 50 mg/kg bw in dogs produced a strong vomiting reflex, ascribed to the generation of hydrogen peroxide in the stomach (Dufour *et al*, 1971).

The acute oral toxicity (LD_{50}) of sodium perborate monohydrate in rats is in the range of 1,600-2,100 mg/kg bw (171-225 mg B/kg bw) (Moreno *et al*, 1987a) and 1,120 mg/kg bw (120 mg B/kg bw) (Glaza, 1988). Symptoms of toxicity included depression, bloated abdomen and irritation of the gastro-intestinal tract with diarrhoea. Earlier work (Mulinos *et al*, 1952) had shown no deaths in rats given a single peroral dose through a stomach tube of sodium perborate monohydrate (2% aqueous solution) up to 650 mg/kgbw (equivalent to 70 mg B/kgbw). Examination of various body organs in these rats revealed no gross abnormalities. No data could be obtained on the acute oral effects of sodium perborate monohydrate in rabbits, because decomposition of the substance caused a bloated condition of the stomach.

4.2.1.2 Dermal LD_{50}

The data are summarised in Table 5B.

The acute dermal toxicity (LD_{50}) of boric acid in male and female rabbits is greater than 2,000 mg/kgbw. The test material, moistened with saline solution, was applied for 24 hours to clipped, abraded skin and the animals were observed for 14 days. No deaths occurred during the observation period (Weiner *et al*, 1982).

Table 5B Acute Toxicity (dermal)

Compound	Species	LD_{50} mg/kg bw	LD_{50} mg B/kg bw	Remarks and References
H_3BO_3	rabbit	>2,000	>350	24 h exposure, clipped abraded skin, (Weiner *et al*, 1982).
$Na_2B_4O_7.5H_2O$	rabbit	>2,000	>297	Reagan and Becci, 1985b.
$NaBO_3.H_2O$	rabbit	>2,000	>214	Severe irritation signs, all animals, (Moreno *et al*, 1987b).

The acute dermal toxicity (LD_{50}) of disodium tetraborate pentahydrate, $Na_2B_4O_7.5H_2O$, in male and female rabbits is greater than 2,000 mg/kg bw. No deaths occurred during the observation period (Reagan and Becci, 1985b).

The acute dermal toxicity (LD_{50}) of sodium perborate monohydrate in rabbits is greater than 2,000 mg/kgbw. Nine of the ten animals survived, with signs of severe irritation in all animals. One rabbit died with signs of systemic toxicity, including diarrhoea and congestion of internal organs (Moreno *et al*, 1987b).

4.2.1.3 Inhalation LC_{50}

The data are summarised in Table 5C.

The four hour acute inhalation exposure of rats to boric acid dust was studied (Bio/dynamics Inc., 1982). The test group consisted of 5 male and 5 female Sprague-Dawley rats. During the exposures to a nominal dust concentration of 16 mg/l, the animals were group housed without food or water. The mean airborne test material concentration measured was 0.16 mg/l (160 mg/m^3), and the mass median diameter of the dust particles was 8.52 μm. No rats died during the exposure period or the subsequent 14-day observation period. The exposure caused slight irritation of the

mucous membranes, but body weights and necropsy findings were not indicative of a response to exposure.

Table 5C Acute Toxicity (inhalation)

Compound	Species	LC_{50} mg/m³	LC_{50} mg B/m³	Remarks and References
H_3BO_3	rat	>160 (no deaths)	>28	4h exposure,aerosol,slight irritation of mucous membrane (Bio/dynamics, 1982).
$NaBO_3.4H_2O$	rat	>74 mg/m³ (highest dose level, no deaths)		No effect at 11 mg/m³ in air, irritation at 39 mg/m³ (Silaev, 1984).

The minimum irritating concentrations for rats of sodium perborate tetrahydrate in air was 39.2 mg/m³, with no effect up to 11.3 mg/m³; the LC_{50} was greater than 74 mg/m³ (Silaev, 1984).

4.2.1.4 Other Routes

The data are summarised in Table 5D.

The acute intravenous LD_{50} of a 5% aqueous solution of boric acid injected into mice was 1,780 mg/kg bw, and that injected into rats was 1,330 mg/kg bw (Pfeiffer *et al*, 1945).

Table 5D Acute Toxicity (other routes)

Compound	Species	LD_{50} mg/kg bw	LD_{50} mg B/kg bw	Remarks and References
H_3BO_3	mouse	1,780	311	*i.v.*, 5% aq solution (Pfeiffer *et al*, 1945).
	rat	1,330	232	*i.v.*, 5% aq solution (Pfeiffer *et al*, 1945).
$NaBO_3.H_2O$	rabbit	78	8.3	*i.v.*, 2% aq solution (Mulinos *et al*, 1952).
H_3BO_3	mouse	2,070	362	*s.c.* 5% aq solution (Pfeiffer *et al*, 1945).
	guinea pig	1,200	210	*s.c.* 5% aq solution (Pfeiffer *et al*, 1945).

Death by respiratory arrest was caused to an anaesthetized dog by intravenous injection of a dose of sodium perborate tetrahydrate corresponding to 500 mg/kg bw (Dufour *et al*, 1971).

The acute intravenous LD_{50} of a 2% aqueous solution of sodium perborate monohydrate injected into rabbits within 5 minutes was 78 mg/kg bw (Mulinos *et al*, 1952).

The acute subcutaneous LD_{50} of a 5% aqueous solution of boric acid injected into mice was 2,070 mg/kg bw, and that injected into guinea pigs was 1,200 mg/kg bw (Pfeiffer *et al*, 1945).

4.2.1.5 Irritation

Skin

The data are summarised in Table 6A.

The procedure for testing and evaluating the primary skin irritation of borax, $Na_2B_4O_7.10H_2O$, (10 ml, 5% in water (w/v)) or boric acid (5ml, 10% in water (w/v)) on rabbit skin (Roudabush *et al*, 1965) was identical to that described in the U.S. Regulations (21 CFR 191.11), except that the rabbits were not immobilised, and the 1-inch square cellulose pads were used instead of surgical gauze. The substance was applied to 6 rabbits with intact and 6 with abraded skin. In these tests, both borax and boric acid were mild skin irritants after 24 and 72 hours. A similar test procedure in the guinea pig included application of the aqueous solutions of the materials on cellulose pads held in contact with the skin under an appropriate sleeve. Six guinea pigs with clipped, depilated, intact abdomens and 6 with clipped, depilated, abraded backs were used with each material. Borax was a mild skin irritant and boric acid a moderate skin irritant in the guinea pig. The abraded rabbit skin was more sensitive than intact skin for both borax and boric acid, whereas the abraded guinea pig skin was less sensitive than intact skin for boric acid with no difference in the case of borax.

The primary dermal irritation of disodium tetraborate pentahydrate, $Na_2B_4O_7.5H_2O$, was determined by applying 0.5 g moistened with saline on two clipped sites on the backs of 6 rabbits. The contact time was 4 hours under an occlusive dressing, with observation for up to 76 hours (Reagan and Becci, 1985c). The substance was regarded to be non-irritant.

The detailed toxicological properties of boric oxide (B_2O_3) in rats, rabbits and dogs have been published (Wilding *et al*, 1959). Topical application of boric oxide dust (1g/25cm^2) to the clipped wetted backs of four rabbits produced erythema that persisted for 2 or 3 days.

Sodium perborate tetrahydrate was tested for skin irritation on six male rabbits. An area on the right flank of each animal was clipped and about 500 mg perborate, moistened to a paste with water, was applied to the test site (25x25 mm) under an occlusive dressing for 4 hours. The Draize

Table 6A Acute Toxicity (skin irritation)

Compound	Species	Dose and route of administration	Duration of exposure	Results and References
H_3BO_3	rabbit	5ml, 10% in water (w/v), intact and abraded skin	Not stated. (21 CFR 191.11)	Mild irritant after 24 and 72 h in primary irritation test (Roudabush *et al*, 1965).
	guinea pig	5ml, 10% in water (w/v), intact and abraded skin	Not stated.	Moderate irritant after 24 and 72 h in primary irritation test (Roudabush *et al*, 1965).
$Na_2B_4O_7.10H_2O$	rabbit	10ml, 5% in water (w/v), intact and abraded skin	Not stated. (21 CFR 191.11)	Mild irritant after 24 and 72 h in primary irritation test (Roudabush *et al*, 1965).
	guinea pig	10ml, 5% in water (w/v), intact and abraded skin	Not stated.	Mild irritant after 24 and 72 h in primary irritation test (Roudabush *et al*, 1965).
$Na_2B_4O_7.5H_2O$	rabbit	0.5 g, clipped back	4h	Non-irritant (Reagan and Becci, 1985c).
$NaBO_3.4H_2O$	rabbit	500 mg, paste with 0.2 ml water, on intact skin	4 h	Classified as non-irritant (Southwood, 1986a).
	guinea pig	in vegetable oil ointment	4 h	Non-irritant (Silaev, 1984).
	rat	solid applied to skin	12 daily applications	Moderately to severely irritant, but 1% solution non-irritating after 12d (Chater, 1978).
$NaBO_3.H_2O$	rabbit	0.5 g on skin	4h	Slightly irritating (Moreno *et al*, 1987c).
	rabbit	500 mg, paste with 0.2 ml water, on intact skin	4 h	Non-irritant (Southwood, 1986b)
B_2O_3	rabbit	1 g/25cm^2 dust applied to clipped wetted back	Not stated.	Produced erythema, that persisted for 2-3 d (Wilding *et al*, 1959)

scale was used to assess the degree of erythema and oedema at the site 1 hour and 1, 2 and 3 days after the removal of the dressing. No erythema or oedema were observed, and sodium perborate tetrahydrate was classified as non-irritant to rabbit skin (Southwood, 1986a). Using the same method, sodium perborate monohydrate gave a similar result and was also non-irritant to rabbit skin (Southwood, 1986b).

Sodium perborate tetrahydrate was non-irritating to the skin of guinea pigs, when applied as a paste in oil, for a 4 hour exposure (Silaev, 1984).

A study investigating the irritant effects of repeated exposures of sodium perborate tetrahydrate to rat skin found that the solid material was moderately to severely irritating over a course of 12 consecutive daily applications. However, even 12 consecutive applications of a 1% solution was found to be practically non-irritating (Chater, 1978).

In the OECD test for irritation/corrosion on the skin of rabbits, sodium perborate monohydrate was found to be slightly irritating (Moreno *et al*, 1987c).

Eye

The data are summarised in Table 6B.

Six young rabbits had 100 mg of boric acid applied to one eye of each rabbit. The eyes were rinsed after 24 hours and irritation scores in individual animals ranged from 0 to 18. No evidence of corrosion was noted. Changes noted in the coloration and texture of the eye itself were blistered appearance to the conjunctiva. The material was classified as Toxicity Category III (40 CFR 156) by ocular administration, defined as "Corneal involvement or irritation clearing in d 7 or less" (Doyle, 1989a).

Six young rabbits had 77 mg of borax, $Na_2B_4O_7.10H_2O$, applied to one eye of each rabbit. The eyes were rinsed after 24 hours and irritation scores in individual animals ranged from 0 to 39. No evidence of corrosion was noted. Changes noted in the coloration and texture of the eye itself were blanched appearance in conjunctiva, blistered appearance in conjunctiva and thickened appearance in conjunctiva. The material was classified as Toxicity Category II (40 CFR 156) by ocular administration, defined as "Corneal involvement or irritation clearing in 8-21 days" (Doyle, 1989b).

Six young rabbits had 100 mg of disodium tetraborate pentahydrate, $Na_2B_4O_7.5H_2O$, applied to one eye of each rabbit. The eyes were not rinsed, and the ocular reaction was recorded after 1, 24, and 72 hours and up to 10 days after treatment. Scores of up to 34 were recorded. Changes noted were the blistered areas throughout the conjunctiva with the most severe area where the substance had settled. The material was rated as causing severe irritation by ocular administration (US-EPA, 40 CFR 160, Pesticide Assessment Guidelines, Subdivision F Hazard Evaluation: Human and Domestic Animals, November 1982), defined as causing significant injury to the eye that persists for 21 days or more (Reagan and Becci, 1985d).

Table 6B Acute Toxicity (eye irritation in the rabbit)

Compound	Dose and route of administration	Remarks and References
H_3BO_3	100 mg in one eye	Irritation, clearing 7 d or less (Doyle, 1989a).
$Na_2B_4O_7.10H_2O$	77 mg in one eye	Corneal irritation, clearing 8-21 d (Doyle, 1989b).
$Na_2B_4O_7.5H_2O$	100 mg in one eye	Severe irritation >21 d (Reagan and Becci, 1985d).
$NaBO_3.4H_2O$	100 mg in one eye	Observed up to 21 d. Severe irritant to the rabbit eye (Southwood, 1986a).
	100 mg in one eye	Serious damage to unwashed left eye, but slight damage to eyes when rinsed after 4 or 30 seconds (Momma *et al*, 1986).
	50 mg in one eye	Acute conjunctivitis, corneal irritation at 48 hours, disappeared on d 10 (Silaev, 1984).
	6 mg (0.01 ml) in one eye.	Observed up to 21 d. Moderate irritant. Low volume eye test (Barber, 1987).
	1% solution in one eye	Non-irritant (Chater, 1978).
$NaBO_3.H_2O$	100 mg in one eye	Observed up to 21 d. Severe irritant to the rabbit eye (Southwood, 1986b).
	0.1 mg in one eye	Observed up to 21 d. Corrosive to unwashed eye (Moreno *et al*, 1987d).
B_2O_3	50 mg in one eye	Immediate conjunctivitis, caused by exothermic hydrolysis to boric acid (Wilding *et al*, 1959).

About 50 mg of boric oxide placed in the left eye of four rabbits produced immediate conjunctivitis, probably as a result of exothermic hydration of boric oxide to boric acid (Wilding *et al*, 1959).

Sodium perborate tetrahydrate was tested for eye irritation on two female rabbits and the Draize scale was used for assessment of irritation. The test substance (approximately 100 mg) was applied to the conjunctival sac of the left eye of each rabbit, the other eye was untreated. The eyes were examined after 1 to 2 hours, and up to 3 or 21 days after application for each of the animals, respectively. Effects on the eye included corneal opacity, severe conjunctival redness and discharge and necrosis of the nictitating membrane. Overall, sodium perborate tetrahydrate was considered as a severe irritant to the rabbit eye (Southwood, 1986a). Under the same test conditions, sodium perborate monohydrate was considered as a severe irritant to the rabbit eye (Southwood, 1986b).

When 50 mg of sodium perborate tetrahydrate was introduced into the rabbit eye, acute conjunctivitis developed. After 48 hours there was corneal irritation, which resolved itself by day 10 (Silaev, 1984).

The irritation of a small volume of sodium perborate tetrahydrate was assessed in three rabbits. The methods used were similar to those described above (Southwood, 1986a) except that the amount of test material used was less, in this case a volume of 0.01 ml was used (approximating to 6 mg). Although irritant effects such as conjunctival redness were seen, such effects had resolved within 7 days of the application of the test material. Sodium perborate tetrahydrate was considered as a moderate irritant to the rabbit eye under these conditions (Barber, 1987).

Instilling a 1% solution of sodium perborate tetrahydrate to the rabbit eye elicited no signs of irritation, and this strength of solution was considered to be non-irritant (Chater, 1978).

A test in rabbits investigated the effect of an eye washing procedure on the irritant effect of sodium perborate tetrahydrate. After instilling 100 mg of the test material into the left eye, groups of 3 rabbits received either no further treatment or the eye was washed 4 or 30 seconds later. The expected severe effects were seen when no eyewashing procedure was used, but when eyes were washed within either 4 or 30 seconds, there was no irritant effect whatever (Momma *et al*, 1986).

Sodium perborate monohydrate was found to be corrosive to the unwashed rabbit eye, even 14 days after exposure (Moreno *et al*, 1987d).

Skin Sensitisation

A skin sensitization test on sodium perborate monohydrate was carried out on 5 male and 5 female Hartley albino guinea pigs. Another similar group served as controls. Twenty four hours prior to each induction, an area of 5 x 5 cm on the left flank of each animal was clipped free of hair, 0.5 ml of the solid was applied to the depilated area and covered with an occlusive dressing. The challenge (5% dilution in distilled water) was applied to the depilated right flank 14 days after the induction. The test established that sodium perborate monohydrate did not cause skin sensitization (Moreno *et al*, 1987e).

4.2.2 Subacute/Subchronic

The data are summarised in Table 7.

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing 0, 1,200, 2,500, 5,000, 10,000 and 20,000 ppm boric acid for 90 days. Based on the feed consumption of controls in week 4 of the experiment, these doses are approximately 0, 34, 71, 142, 284 and 568 mg B/kg bw/d for males and 47, 98, 196, 392 and 784 mg B/kg bw/d for females. Doses of 1,200 and 2,500 ppm

Table 7 Subacute/Subchronic Toxicity (oral)

Compound	Species	Route	Dose of administration	Duration	Results and References
H_3BO_3	mouse 10 M and 10 F per group $B6C3F_1$	Diet	0, 34, 71, 142, 284, 568 (males) and 47, 98, 196, 392 and 784 mg B/kg bw/d (females)	90 d	Doses at lowest two levels well tolerated. At 568 mg B/kg/d 8 of 10 males died. At > 142 mg B/kg/d testicular atrophy in males (NTP, 1987).
$Na_2B_4O_7.10H_2O$ or H_3BO_3	rat 10 M and 10 F per group Sprague-Dawley	Diet	0, 2.6, 8.8, 26, 88 and 260 mg B/kg bw/d	90 d	100% mortality at highest dose. Atrophy of testes of males at 88 mg B/kg/d. At or below this dose, physical appearance as for controls (Weir and Fisher, 1972).
$Na_2B_4O_7.10H_2O$	male rat (number not specified) Sprague-Dawley	Drinking Water	0.3, 1.0 or 6.0 mg B/l Highest dose around 0.84 mg B/kg bw/d	30, 60 and 90 d	No adverse effects noted (Dixon *et al*, 1976).
	male rat 15 per group Long-Evans	Drinking Water	0, 150, 300 mg B/l	70 d	Adverse effects at both doses. Reduction in body weight, decrease in testes weight, etc. (Seal and Weeth, 1980).
	male rat 18 per group Sprague-Dawley	Diet	50, 100, 200 mg B/kg bw/d	30 and 60 d	No adverse reproductive effect at lowest dose. Loss of germinal cell elements observable at 2 higher doses (Lee *et al*, 1978).
$NaBO_3.4H_2O$	male rat 20 per group Wistar	Gavage	200 mg/kg bw/d, or 14.2 mg B/kg bw/d	6 d	No toxic effect (Dufour *et al*, 1971).
	rat 5 M and 5 F per group Wistar	Gavage	Limit test, 1,000 mg/kg bw/d, or 71 mg B/kg bw/d	28 d	Reduced bodyweight gain and reduced food consumption. Slight local irritating effect on gastric mucosa. Minor effects on blood parameters (Degussa, 1989).

appeared to be well tolerated. Eight out of 10 males that received 20,000 ppm and 6/10 females that received 10,000 ppm boric acid died before the end of the study. Animals at the high doses were thin and dehydrated, had a hunched posture, foot lesions and scaly tails. There was a significant reduction (10-20%) in final mean body weight in both males and females fed at the three highest doses. The main adverse symptoms observed at dose levels greater than 5,000 ppm boric acid were testicular atrophy in males and extramedullary haematopoiesis of the spleen in males and females (NTP, 1987).

Sprague-Dawley rats (10/sex/dose) were administered borax or boric acid in the diet for 90 days at 0, 52.5, 175, 525, 1,750 or 5,250 ppm as boron equivalents. These doses corresponded to about 0, 2.6, 8.8, 26, 88 or 260 mg B/kg bw/d, respectively. Both borax and boric acid produced 100% mortality in the highest-dose group. Rats fed 1,750 and 5,250 ppm boron had rapid respiration, inflamed eyes, swollen paws and desquamated skin on the paws and tail. Complete atrophy of the testes occurred in all males fed diets at 1,750 ppm boron. The physical appearance of rats receiving levels at or below 525 ppm were generally comparable with controls (Weir and Fisher, 1972).

Studies of 30, 60 and 90 days were carried out on rats exposed to drinking water containing borax at 0.3, 1.0 or 6.0 mg B/l. At an average daily consumption of 35 ml of drinking water, the highest estimated dose would represent 0.84 mg B/kg bw/d, and no adverse effects were observed at this level of exposure (Dixon *et al*, 1976).

In a study by Seal and Weeth (1980), male Long-Evans rats (15/dose) were offered borax in drinking water for 70 days at levels of 0, 150 and 300 mg B/l, which is assumed (US-EPA, 1992) to correspond to a total boron intake of 23.7 and 47.4 mg/kg bw/d, based on a bodyweight of 350 g and water intake of 49 ml/d. Both doses of borax produced significant decreases in body weight, and also decreases in weights of the testes, seminal vesicles, spleen, and right femur as well as in the levels of plasma glycerides. In addition, spermatogenesis was impaired in animals receiving the highest dose.

In both 30 and 60 day feeding studies male rats receiving 500 ppm boron as borax, approximately 50 mg B/kg/d, failed to show any adverse effects. At dose levels of 100 and 200 mg B/kg/d, testicular and plasma boron concentrations rose in a dose-dependent fashion, with loss of germinal cell elements (Lee *et al*, 1978).

Oral administration of a dose of 200 mg/kg bw of sodium perborate tetrahydrate (14 mg B/kg bw) in rats for 6 consecutive days produced no toxic effect. At 1,000 mg/kg bw (70 mg B/kg bw), slight reversible haematological changes were found (Dufour *et al*, 1971).

In a 28-day study, conducted under GLP and in accordance with the OECD-Guideline (Degussa, 1989), sodium perborate tetrahydrate was orally administered to rats at a dose of 1,000 mg B/kg bw (limit test). Salivation was observed in almost all treated rats; in males this was a reddish colour. In males food intake and body weight gain were decreased. Slight effects were seen in red blood cell parameters (decreased values) and number of platelets (increased) in both sexes and lymphocytes (decreased) only in males. Additionally the organ weights of brain, heart, kidneys and

testes were slightly reduced in males. Microscopic examination of tissues revealed a mild test substance related reduction of the splenic parenchyma in males only. In the stomach of males and females slight acanthosis and hyperkeratosis in the forestomach and hyperplasia of the fundic mucosa were recorded. There was no reported effect on the testes. In this study no specific toxic effects occurred. Most of the observed findings were considered to be of secondary nature, due to the local effects on the gastric mucosa. Red blood cells, platelets and lymphocytes (spleen) may be the target organ of toxicity for sodium perborate in rats. The reduced organ weights of the testes should be considered in the context with other reduced organ weights and can be explained with the reduction in food intake and body weight gain.

Sodium perborate tetrahydrate was tested for percutaneous irritation by applying a dose of 200 mg/kg bw/d for 20 days on the abraded skin of male and female New Zealand rabbits, covering approximately 10% of the body surface. A separate control group was dosed with water (Procter and Gamble, 1966a). The skin from animals in either group (test or control) was near normal. There was no microscopic finding indicative of a toxic response to the test substance. There were no statistically significant differences between the two groups in growth, organ/body weight ratios and blood values. One animal from each group died during the study, but cause of death was not determined. Indications of parasites were found in individual animals of each group.

In a further study, sodium perborate tetrahydrate was tested for percutaneous irritation by applying a dose of 50 mg/kg bw/d five times weekly over 90 days on the intact skin of New Zealand rabbits (Procter and Gamble, 1966b). A separate control group was dosed with water and no skin irritation was observed in either the test or control group. The blood values and organ/body weight ratios of the test group were within the normal range as obtained from the control animals. Variations in the growth rates were not significantly different from those of the control group. One animal from each group died during the study, but the cause of death was not determined. The tissues of the remaining animals showed no histopathologic evidence of toxicity in either group.

4.2.3 Chronic Toxicity and Carcinogenicity

Chronic Toxicity

Data are summarised in Tables 8A and B. For ease and clarification, some studies of 6 months duration are included in the general category of chronic toxicity tests.

Rats of 300 g weight were administered drinking water containing 0, 0.3, 1.0 and 6.0 mg/l of boron (as boric acid) for 6 months. The authors (Krasovskii *et al*, 1976) stated that these doses of boron

Table 8A Chronic Toxicity (oral)

Compound	Species	Route	Dose of administration	Duration	Results and References
H_3BO_3	male rat n=? white random-bred	Drinking water	0, 0.3, 1.0 and 6.0 mg B/l. Highest dose estimated as 0.3 mg B/kg bw/d	6 months	Some increased blood aldolase and and minor testicular effects at highest dose (see text for uncertainty about actual dose) (Krasovskii *et al*, 1976).
$Na_2B_4O_7.10H_2O$ or H_3BO_3	Sprague-Dawley rat 35M and 35F per group	Diet	0, 117, 350 and 1170 ppm B. Estimated as 0, 5.9, 17.5 or 58.5 mg B/kg bw/d	2 years	At two lowest doses, no change in organs after 2 years. At highest dose, suppressed growth and atrophy of testes of all males (Weir and Fisher,1972).
$Na_2B_4O_7.10H_2O$ or H_3BO_3	Beagle dog 4M and 4F per group	Diet	0, 58, 117 and 350 ppm B. About 0, 1.5, 2.9 or 8.8 mg B/kg bw/d.	2 years	No adverse effects noted (Weir and Fisher, 1972).
H_3BO_3	B6C3F$_1$ mouse 50M and 50F per group	Diet	0, 2,500 and 5,000 ppm around 0, 78 and 200 mg B/kg bw/d	2 years	Decreased body weight gain (10-17%) Testicular atrophy at 200 mg/kg (NTP, 1987).

Table 8B Chronic Toxicity (inhalation)

Compound	Species	Route	Dose of administration	Duration	Results and References
B_2O_3	4 to 70 albino rats per group (M and F)	Inhalation of aerosol	77 mg/m^3, or 24 mg B/m^3	24 wk; 6 h/d; 5 d/wk	No toxic signs observed (Wilding *et al*, 1959).
	3 dogs sex and strain unspec.	Inhalation of aerosol	57 mg/m^3, or 18 mg B/m^3	23 wk; 6 h/d; 5 d/wk	No toxic signs observed (Wilding *et al*, 1959).

corresponded to 0.015, 0.05 and 0.3 mg/kg bw/d and that at the highest dose of 0.3 mg/kg bw there was increased blood aldolase activity and some minor testicular effects. However, there is an uncertainty about actual dose levels in this paper which is discussed in more detail in Appendix A. Because if it is assumed that rats drink approximately 30 ml/d of water, then drinking water containing their highest concentration of 6.0 mg B/l would deliver a dose of 0.62 mg B/kg bw/d to each rat (not 0.3 mg B/kg bw as stated). Not only was there an uncertainty about actual dose levels, but other workers (Dixon *et al*, 1976) were unable to find any adverse effects at 6 mg B/l in drinking water (as borax) administered for 90 days.

Groups of 35 male and 35 female Sprague-Dawley rats received diets containing borax or boric acid at 0, 117, 350 and 1,170 ppm boron equivalent for 2 years; these doses were approximately 0, 5.9, 17.5 or 58.5 mg/kg bw/d of boron[1]. Lowered food consumption, retarded body weight gain, coarse hair coats, hunched position, swollen pads and inflamed bleeding eyes, as well as changes in haematologic parameters, were observed in animals receiving the highest dose of boric acid or borax. At 1,170 ppm boron animals exhibited decreased food consumption during the first 90 days and suppressed growth throughout the study. In addition shrunken scrotum and atrophic testes were observed in high dose males. The weights of the testes and testes-to-bodyweight ratio were significantly decreased, whereas the brain- and thyroid-to-bodyweight ratios were significantly higher than those of controls. No treatment-related effects were observed in rats treated with 117 or 350 ppm boron as borax or boric acid. Therefore, in rats the LOAEL in this study was 58.5 mg B/kg bw/d and the NOAEL was 17.5 mg/kg bw/d of boron (Weir and Fisher, 1972).

Two year dietary feeding studies were carried out by Weir and Fisher (1972) in groups of 4 young male and 4 young female beagle dogs containing 0, 58, 117 or 350 ppm boron as borax or boric acid (about 0, 1.5, 2.9 or 8.8 mg B/kg bw/d). No effects were found on bodyweight, food consumption, organ weights, organ-to-bodyweight ratios or clinical parameters. Therefore a NOAEL in dogs of 8.8 mg B/kg bw/d was defined. In the absence of symptoms at the above 3 doses, additional groups of dogs were given 1,170 ppm boron as borax or boric acid for up to 38 wk (about 29 mg B/kg bw/d). Both compounds were reported to cause testicular degeneration in males at this dose. The subsequent availability in 1992 of the full details of these dog studies (Weir and Fisher, 1961-1967) cast some doubt on their validity because of testicular effects on control animals (see section 5).

In a study on the inhalation toxicity of boric oxide, a group of 70 rats was exposed for 24 weeks (6h/d, 5 d/wk) to aerosols of boric oxide of average concentration of 77 mg/m^3, and another group

[1] Conversion of ppm diet to mg/kg bodyweight in this section according to Lehman, 1959.

of 20 rats to as high as 470 mg/m^3 for 23 weeks. A group of 3 dogs was also exposed to 57 mg/m^3 for 23 weeks. No toxic effects were observed in these studies, nor were there any deaths due to the inhalation of aerosol (Wilding *et al*, 1959).

Carcinogenicity

As part of a large study on spermicide constituents, Boyland *et al* (1966) injected intravaginally twenty BALB/C mice twice a week with 0.1 ml of 2% boric acid tragacanth suspension. The animals were treated in total with 100 injections and were observed for 20 months. An untreated control, a vehicle control and a positive control (0.3% DMBA = Dimethylbenzanthracene) were used. Marked chronic inflammation of the genital tract was present in nearly all treated animals. After 18 months one mouse of the boric acid group developed a squamous tumour of low grade malignancy of the vagina. In the positive control group 15 of 20 (75%) of the mice developed malignant tumours of the vagina and/or perineal skin within 8 months. No treatment-related incidence of tumours at other sites was seen in the mice treated with boric acid.

In a bioassay fifty B6C3F$_1$ mice per group and sex were administered 0, 2,500 and 5,000 ppm boric acid in the diet for 103 weeks (NTP, 1987). The average amount of boric acid consumed daily was approximately 400-500 mg/kg bw or 1,100-1,200 mg/kg bw respectively. This corresponds to 70-87 mg B/kg bw or 192-210 mg B/kg bw. The survival rate was significantly lower in the high dose males (22/50) and in low dose males (30/50) compared with the control group (41/50). Apart from a reduced body weight gain (10-17% compared with the controls) during the first year, no other treatment related clinical signs were observed in the high dose group. The histopathological findings showed no significant dose related increase in neoplasms. Testicular atrophy was observed in the males of the high dose group and in some mice there was interstitial cell hyperplasia. Under the conditions of this study, there was no evidence of carcinogenicity of boric acid in male and female mice.

4.2.4 Genotoxicity

Gene Mutation in Bacterial System

The data are summarised in Table 9A.

Boric acid did not induce gene mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 with and without activation by both rat and hamster liver microsomal preparation up to test concentrations of 20,000 µg/plate (Haworth *et al*, 1983, NTP, 1987).

Table 9A Gene Mutations in Bacterial Systems

Compound	Strain or Cell line	Metabolic Activation	Concentration (µg/plate)	Results	Reference
H_3BO_3	TA98, TA100, TA1535, TA1537	± S9	10-20,000	negative	Haworth *et al*, 1983
	TA98 , TA100	± S9	1-100	negative	Benson *et al*, 1984
	TA98, TA100, TA1535, TA1537	± S9	33-1,820	negative	NTP, 1987
	TA98, TA100, TA1535, TA1537, TA1538	± S9	10-2,500	negative (no toxicity to bacteria)	Stewart, 1991
	E. coli	± S9		(positive ?)	Demerec *et al*, 1951
	E. coli	± S9	1,000	negative	Iyer and Szybalski, 1958
$NaBO_3.4H_2O$	TA98, TA100, TA102	± S9 + catalase	10-2,000	with S9 or catalase:negative without S9: weakly positive in TA100 and TA102 (toxic to bacteria above 20 µg/plate).	Seiler, 1989

Negative results were also obtained in the *Salmonella typhimurium* strains TA98 and TA100, when tested at the non-toxic concentrations of 100 µg/plate boric acid or 100 µg/plate borax with and without rat S9 mix (Benson *et al*, 1984).

This result was confirmed by Stewart (1991) in a standard plate incorporation test using different *Salmonella typhimurium* strains. At dose levels between 10 and 2,500 µg/plate no dose related increases in the number of revertants and no toxicity were seen either in the presence or absence of metabolic activation containing 4 or 10% S9 mix.

Sodium perborate induced a weak mutagenic effect in the *Salmonella typhimurium* strains TA100 and TA102 but not with strain TA98. The survival rate in TA100 was reduced at concentrations above 20 µg/plate. At 100 µg/plate the number of revertants was increased by a factor of 1.5, whereas the corresponding survival rate was decreased to less than 15%. Both effects, mutation activity as well as toxicity disappear completely upon incubation with a rat liver homogenate or addition of 10 µg/plate catalase due to the decomposition of peroxygen constituents (Seiler, 1989).

In a back mutation test with sd-4 *Escherichia coli* (streptomycin dependent) Demerec *et al* (1951) reported mutagenic activity of boric acid, but the results were inconsistent and showed great variability. In the NTP report (1987) these results were assessed as negative since Iyer and Szybalski (1958) using *Escherichia coli* strain sd 4-73 failed to confirm these findings.

Gene Mutation in Mammalian Cells

The data are summarised in Table 9B.

Boric acid (H_3BO_3) was examined for mutagenic activity at the *thymidine kinase locus* (TK-locus) in the L5178Y mouse lymphoma cell in the presence and absence of an Aroclor induced rat-liver metabolic activation system. Only a slight reduction in cell growth was observed even at the maximum concentration of 5 mg/ml. Boric acid was not mutagenic in this assay (Rudd, 1991).

In an earlier NTP-study (1987) boric acid did not show mutagenic activity in the mouse lymphoma test under comparable conditions (1-5 mg/ml boric acid, ± S9).

In C3H/10T1/2 cells and in human diploid foreskin fibroblasts, refined borax at concentrations of 0.4 to 3.2 mg/ml was tested for mutation to ouabain resistance. An increase in Oua(r) mutant frequency was not observed in any experiment, but the test substance showed a dose-dependent cytotoxicity to both cell lines (up to 91%) (Landolph, 1985).

Table 9B Gene Mutations in Cultured Mammalian Cells

Compound	Cell line	Metabolic Activation	Concentration (µg/ml)	Results	Reference
H_3BO_3	L5178Y mouse lymphoma cells	± S9	1,000-5,000	Negative[a]	NTP, 1987
		± S9	up to 5,000	Negative[a] (cytotoxicity at 5 mg/ml)	Rudd, 1991
$Na_2B_4O_7.10H_2O$	L5178Y mouse lymphoma cells	no	400-3,200	Negative[b] (dose dependent cytotoxicity)	Landolph, 1985
	human diploid foreskin fibroblasts V79 Chinese hamster lung cells	no	400-3,200	Negative[c] (dose dependent cytotoxicity)	Landolph, 1985

a Increase of mutations at the thymidine kinase locus (TK locus)
b Increase of the frequency of the ouabain resistance (Oua(r))
c Increase of the frequency of 8-azaguanine resistance (Azg(r))

In an assay for mutation to 8-azaguanine resistance (Azg(r)) in V 79 Chinese hamster cells, refined borax caused a weak, but statistically insignificant increase in mutation frequency at a concentration of 0.8 mg/ml. At the other test concentrations (0.4, 1.6 and 3.2 mg/ml) increases in mutation frequency were not seen. The highest cytotoxicity produced was 99% at 3.2 mg/ml of refined borax (Landolph, 1985).

Primary DNA-Damage

The data are summarised in Table 9C

Primary DNA-Damage in Bacteria

The influence of sodium perborate on repair of damaged DNA was tested in *Escherichia coli p 3478* with repair deficient (pol A_1-) and repair proficient (pol A+) strains. At sodium perborate concentrations of 0.0015 and 0.0033 μ moles (equivalent to 0.0002 and 0.0004 μg/ml as $NaBO_3.4H_2O$) the pol A_1- strain was preferentially inhibited, suggesting DNA-induced damage by sodium perborate. In presence of catalase (50 μg/ml) the inhibition of both test strains was not complete (Rosenkranz, 1973), suggesting that peroxy species were broken down in the presence of catalase.

Unscheduled DNA-Synthesis (UDS) in Cultured Mammalian Cells

In the *in vitro* hepatocyte DNA Repair Assay, the ability of boric acid to induce unscheduled DNA synthesis (UDS) was tested in primary cultures of F-344 rat hepatocytes at concentrations between 5 and 5,000 μg/ml in two independent experiments. The test did not show any induction of unscheduled DNA synthesis (Bakke, 1991).

Sister Chromatid Exchange (SCE) in Cultured Mammalian Cells

In Chinese hamster ovary cells (CHO) boric acid was tested for induction of sister chromatid exchanges. In the absence of S9 mix the test concentration range was between 200 and 500 μg/ml and in presence of S9 mix between 250 and 2,000 μg/ml. No increase in SCE was observed (NTP, 1987).

Clastogenicity

The data are summarised in Table 9D

Table 9C Primary DNA - Damage and Indicator Tests

Compound	Test system and cell line	Metabolic Activation	Concentration (µg/ml)	Results	Reference
$NaBO_3.4H_2O$	DNA-repair test (*E. coli* p 3478, repair deficient)	± Catalase	0.12 and 0.27	Positive, inhibition of DNA-polymerase not complete with catalase	Rosenkranz, 1973
	DNA-repair test (*E. coli* p 3478, repair proficient)	± Catalase	0.12 and 0.27	Positive, inhibition of DNA-polymerase not complete with catalase	Rosenkranz, 1973
H_3BO_3	UDS test rat (hepatocytes)	-	5 - 5,000	Negative	Bakke, 1991
	SCE test (CHO cells) Chinese hamster ovary	± S9	250; 7,000 (± S9) 200; 500 (± S9)	Negative Negative	NTP, 1987

Table 9D Chrosomal Aberrations in Cultured Mammalian Cells and Micronuclei Induction in Mammals

Compound	Test system	Metabolic Activation	Concentration (µg/ml)	Results	Reference
H_3BO_3	Chromosomal aberration test (CHO cells) Chinese hamster ovary	+ S9 - S9	500 - 2,000 1,000 - 2,500	Negative Negative	NTP, 1987
$NaBO_3.4H_2O$	Chromosomal aberration test (CHO-K1 cells)	± S9	10 - 100	With S9 - negative Without S9 - positive	Seiler, 1989
H_3BO_3	Micronucleus test in mouse (*in vivo* tests)	-	900, 1,800 and 3,500 mg/kg (oral, for 2 days)	Negative	O'Loughlin, 1991

Chromosomal Aberrations in Cultured Mammalian Cells

In a chromosomal aberration test in CHO-cells, boric acid was tested in the presence of S9 mix at concentrations of 500, 1,000, 1,500 and 2,000 μg/ml and without S9 mix in concentrations of 1,000, 1,600, 2,000 and 2,500 μg/ml. No chromosomal damage was induced (NTP, 1987).

Sodium perborate was tested for chromosomal aberrations in CHO-K1 cells in concentrations between 10 and 100 μg/ml. An increase in aberrant metaphases was found in the absence of a metabolic activation system, whereas after pretreatment with rat liver S9-mix, no effect was observed (Seiler, 1989).

Micronucleus Induction in vivo

Boric acid was tested in the *in vivo* bone marrow micronucleus assay in Swiss-Webster mice. Ten mice per sex and group were orally dosed with boric acid in sterile deionised water at dose levels of 900, 1,800 or 3,500 mg/kg bw/d for 2 consecutive days. Clinical signs included rough fur and a hunched posture; no deaths occurred. Five mice per sex per group were killed at 24 and 48 hours after the final dose, respectively; all were evaluated for cytotoxicity and micronucleus formation in bone marrow erythrocytes. The percentage of Polychromatic Erythrocytes (PCE's) was not altered significantly and no increase in the incidence of micronuclei was observed (O'Loughlin, 1991).

Cell Transformation Test

In a cell transformation test with C3H/10T1/2 cells at concentrations of 0.8, 1.6 and 3.2 mg/ml, refined borax did not induce significant numbers of morphologically transformed foci. Also anhydrous borax was negative in a modified transformation assay (Landolph, 1985).

4.3 HUMAN DATA

4.3.1 Acute Effects (Non-Occupational Exposure)

There is no agreement about the lethal oral dose of boric acid or borax for human beings. Several of the older pharmaceutical reviews (Goldbloom and Goldbloom, 1953; Valdes-Dapena and Arey, 1962; Jaspersen and Schlumpf, 1969) make the unsubstantiated statement that the average acute lethal dose in man is 15 to 20 g. This appears to originate from the comment, again without supporting evidence, that "one must admit that 18 to 20 g is fatal to man" (Caujolle, 1951). The lowest lethal dose in a newborn baby is estimated to be about 5 g (Wong *et al*, 1964). Another

source (Gosselin *et al*, 1976) stated that the mean lethal dose of borax or boric acid in adults is not known for certain, but it is believed to be greater than 30 g; the main symptoms of acute borate poisoning include vomiting, diarrhoea, convulsions, depression of the CNS and, at a later stage, erythematous skin eruptions followed by extensive exfoliation.

Data on the acute effects of past medicinal treatments, with borax or boric acid in hospitals during the past 100 years or so, together with accidental misuse in hospitals has been analysed (Kliegel, 1980). Between 1846 and 1975 there were 121 deaths, of which 44 arose from oral ingestion, about 40 from occlusive treatment of non-intact skin and the remainder mainly from the washing out of body cavities, e.g., the bladder, with aqueous boric acid solution. By the nature of these treatments, it was often difficult to assess the actual dose administered.

A recent review of 784 cases of boric acid ingestions from two poison control centres in the USA for the period 1981 to 1985 (Litovitz *et al*, 1988) contrasted markedly from the earlier publications cited above. There were no fatalities. No patient developed severe toxicity symptoms, and 88% of the cases were asymptomatic. The authors concluded that acute boric acid ingestion produced minimal or no toxicity and that aggressive treatment is generally not necessary. No fatalities or severe manifestations of toxicity were observed, despite ingestions of up to 88.8 g.

Some adverse effects of sodium perborate in man have been summarised from 11 publications during the period 1934 to 1975 (Kliegel, 1980). About 30 cases involved the accidental single ingestion of around 5 g of sodium perborate bleach (95%), which often led to vomiting, diarrhoea and abdominal pain.

4.3.2 Acute Effects (Occupational Exposure)

Industrial intoxication has not been reported from exposure to the inorganic borates (Casarett and Doull, 1980, 1986).

In a study of workers at a sodium borate mine and processing plant, the acute effects of exposure were assessed in 79 exposed workers and 27 unexposed workers (Wegman *et al*, 1991; and Hu *et al*, 1992). Exposed workers were all those with a known pattern of exposure to borate dust, and the non-exposed workers were non-office workers without regular exposure. Data were collected on pre-existing conditions such as cold, allergy and smoking at a pre-study exposure interview. Each subject was investigated on 4 consecutive days. Exposures were monitored continuously with a personal aerosol monitor, and at the shift end by weighing deposits on air filters. As eye, nose and throat irritation may result from exposure to dusts of a "non-respirable" particle size, the aerosol

monitor used was validated as capable of use as a total dust sampler (Woskie *et al*, 1993). The clinical symptoms of respiratory and eye irritation were assessed hourly using a questionnaire, and peak expiratory flow was measured at this time. These methods allowed exposure and clinical data to be resolved into 15 minute periods, as well as to provide the 6 hour daily average. Analysis of the data was by logistic regression.

Exposed workers reported more frequent irritations than unexposed workers for a number of symptoms (nose, eye, throat irritation and breathlessness), but not for cough. These findings persisted when account was taken of smoking, age and presence of the common cold. The average 6 hour time weighted average exposure to sodium borate dust in the exposed group was 5.7 mg/m^3 (range 0.01 to 115 mg/m^3). The majority of exposures were between 1 and 10 mg/m^3 as sodium borate. Analysis indicated that short term peak exposures to dust were primarily responsible for the excess of symptoms reported. There was a clear dose-response demonstrated by an increasing incidence of clinical effects with increasing measured exposure levels, which was more marked using the 15 minute period compared to the 6 hour period. Individual nasal or respiratory symptoms were reported to a far greater extent than eye irritation. Symptoms were graded for severity, and most reported irritant signs were mild. Although exposure to borate dust could cause irritation between 1 and 10 mg/m^3, the effects were mild. There was no difference in the irritation recorded following exposure to borate dusts of different degrees of hydration. Thus anhydrous sodium borate was no more potent than the other borates in the workplace.

A dust exposure study on sodium perborate tetrahydrate was done on male and female human volunteers to establish a minimum irritation limit, which was set at 21 mg/m^3 (Silaev, 1984).

4.3.3 Chronic Effects (Non-Occupational Exposure)

In a thorough clinical trial conducted over 90 years ago on 12 healthy young male volunteers (Wiley, 1904), quantities of up to a maximum of 5 g/d boric acid (870 mg B/d) or equivalent of borax were administered through the diet. The length of the dietary trial ranged from 4 days to a maximum of about two weeks, with one exception. The results showed that at the dose rate of 4 or 5 g/d of boric acid or borax equivalent, there was a loss in appetite and feeling of fullness and uneasiness of the stomach, in some cases resulting in nausea. There was also often a dull and persistent headache. Both of these symptoms resulted in an inability to perform work of any kind. Although 0.5 g of boric acid/day was tolerated for a few days, similar adverse effects were obtained if this period was extended to 50 days. These adverse effects disappeared after the withdrawal of the borate from the diet.

Where sodium perborate was used repeatedly as a mouthwash against gingivitis or other infections of the bucosal cavity, symptoms included "brown hairy tongue", oedema of the lips, blistering and hypertrophy of the papillae of the tongue. Symptoms generally ceased when treatment with perborate was discontinued (Kliegel, 1980, pages 757-780).

4.3.4 Chronic Effects (Occupational Exposure)

Wegman *et al* (1991) assessed whether changes in respiratory health status could be detected over a period of seven years in workers at a sodium borate mine and processing plant. Data from an earlier study in 1981 were compared with results on the same workers in 1988. Forced vital capacity, forced expiratory volume and peak expiratory flow were measured, and other data collected by questionnaire. A total of 303 workers had acceptable data from both surveys. Taking a number of variables into account, including smoking and the expected natural decline in lung function over the study period, it was concluded that exposure to borate dust over the seven year period had not resulted in long-term health effects.

Irritant dermatitis caused by repeated contact with a number of cleaning agents containing alkaline substances and oxidising agents, including sodium perborate, was reported during a study of 541 members of a hospital cleaning department, of whom there was a high prevalence rate of occupational skin diseases of 15.3% (Hansen, 1983).

SECTION 5. REPRODUCTIVE TOXICOLOGY

5.1 INTRODUCTION

In several animal studies, there are reports of borax or boric acid interfering with reproductive processes. The most serious end-point which was repeatedly observed was testicular toxicity. This section reviews the studies in a chronological order which have addressed fertility and developmental toxicity endpoints, together with other studies in which observations were made of reproductive effects. Only those studies which are considered relevant for risk assessment are discussed in this chapter. For completeness, several other studies considered unsuitable for risk assessment are summarised in Appendix A.

As the precise definitions of key terms in toxicology have tended to vary with time, the terms used in this report are taken from Annex IV of the European Commission Directive 93/21/EEC adapting for the 18th time the Dangerous Substances Directive (67/548/EEC). According to Section 3.2.3.3. of Annex IV, reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. Thus, reproductive toxicity may be classified under two main headings: Effects on Fertility (male and female) and Developmental Toxicity.

To enable reasonable comparison between the data reported in this section, conversion factors have been used as described in section 4.2 to convert concentration of boron in the diet (e.g. ppm) to the actual dose to which the test species was exposed (in mg B/kg bw/d).

5.2 FERTILITY EFFECTS IN ANIMALS

5.2.1 Acute Exposure

Data on the effects on fertility after acute exposure to borates are summarised in Table 10A, with further details provided in the Appendix A. In addition, two more recent studies are highlighted here.

Dixon *et al* (1976) exposed groups of 10 adult male Sprague-Dawley rats to single oral doses of borax in order to determine reproductive effects during serial mating studies. According to the authors, the doses corresponded to 45, 150 and 450 mg B/kg bw. After treatment, each male rat was housed singly with a virgin female for a period of 7 days. After 7 days, the females were

Table 10A Fertility Effects after Acute Exposure

Compound	Species animals/group	Dose and Route mg B/kg bw	Duration of observation	NOAEL mg B/kg bw	LOAEL mg B/kg bw	Effects at LOAEL	Reversibility at Dose mg B/kg bw	References
H_3BO_3	rat (Wistar) 10 M + 10 F	525, 700 and 875 (gavage)	30 d	525	700	Decreased testis volume Decreased sperm cell number.	Not reported	Caujolle *et al*, 1962*
	rat 50 M	175, 350, 525, 700 and 875 (gavage)	130 d	350	525	Partial testicular tubular atrophy from d 30 onward.	At 525 and 700 but not at 875 after 130 d	Bouissou and Castagnol, 1965*
$Na_2B_4O_7.10H_2O$	rat (Sprague-Dawley) 10 M	45, 150 and 450 (gavage)	70 d	450	-	None.	-	Dixon *et al*, 1976
H_3BO_3	rat (Sprague-Dawley) 8 M	44, 88, 175 and 350 (gavage)	57 d	88	175	Disturbed tubular spermiation Changes in sperm parameters.	Reversible up to 350 after 57 d	Linder *et al*, 1990

* Discussed in Appendix A

replaced with new virgin females. The studies were usually terminated 70 days after exposure. The authors concluded that no significant effects on male fertility were apparent at any of the exposures tested.

The effect of acute exposure to boric acid on the male reproductive tract of the rat was investigated more recently by Linder *et al* (1990). In a time-response study, groups of Sprague-Dawley adult male rats (6 rats per group) were exposed by gavage to boric acid at doses of 0 (control) and 2,000 mg boric acid/kg bw (the dose was administered in two volumes on the same day). These doses corresponded to 0 and 350 mg B/kg bw, respectively. Rats were killed at 2, 14, 28 and 57 days post-exposure. In the 350 mg B/kg bw group sporadic but statistically significant differences ($p < 0.05$) in reproductive organ weights were noted when compared to controls. The authors stated that these differences were slight and of questionable biological significance. Histopathological changes in the testis and epididymis appeared from day 14 after exposure. These changes consisted of disturbed sperm maturation in the tubules and testicular debris in the epididymides. Recovery occurred from day 28 onwards but was not yet complete at termination of the recovery period (day 57 post exposure) since 2 out of 6 animals still had some retention of spermatids in the tubules. Abnormalities in sperm heads and tails and in sperm head counts were also observed from day 14 and changes in spermatozoa motility were noted. By day 57, all sperm parameters had returned to control levels confirming that the observed effects on sperm parameters were reversible at the dose level tested.

In a related dose-response study reported in the same publication, groups of Sprague-Dawley adult male rats (8 rats per group) were exposed by gavage to boric acid at doses of 0, 250, 500, 1,000 and 2,000 mg boric acid/kg bw (the dose was administered in two volumes on the same day). These doses correspond approximately to 0, 44, 88, 175 and 350 mg B/kg bw. All animals in the dose-response study were killed 14 days post exposure since this was identified as the interval at which maximum testicular damage was observed in the corresponding time-response study. No effect on reproductive organ weights was observed at any exposure level when compared to controls. Histopathological changes in the testes as well as changes in sperm parameters consistent with the time-response study were observed in animals exposed to 175 and 350 mg B/kg bw. No significant effect on serum hormones was found at any exposure level (see section 6 for details).

Summarising the two studies, the authors concluded that acute exposure to boric acid can adversely affect the testis (spermiation) and sperm quality in the adult male rat at dosages of 175 mg B/kg bw and above. These effects are reversible at the dose level tested in the time-response study (350 mg B/kg bw) since by day 57 post exposure, only minimal testicular changes

remained. Based on the non-detection of the above effects, the authors concluded that the no observed effect level for acute exposure of the rat to boric acid was 88 mg B/kg bw.

5.2.2 Repeated Exposure

Data on repeated exposure are summarised in Table 10B.

In an extensive series of experiments covering several regimes, Weir and Fisher (1972) investigated the chronic and reproductive effects in rats exposed to borax or boric acid in the diet.

In the rat chronic feeding study Weir and Fisher (1972), groups of Sprague-Dawley rats (35 male and 35 female per group) were exposed to borax or boric acid in the diet at levels of 117, 350 and 1,170 ppm boron for two years, corresponding approximately to 5.9, 17.5 and 58.5 mg B/kg bw. At 6 and 12 months, five rats of both sexes from each group were killed for intermediate investigation. At 2 years, all survivors were killed and necropsied. Atrophic testes were found in all males exposed to the high doses (58.5 mg B/kg bw) of both borax and boric acid at 6, 12 and 24 months. Microscopic examination of the tissue revealed atrophied seminiferous epithelium and decreased tubular size in the testes. The testes' weights and the testes/body weight ratios were significantly lower than those of control animals. Other changes at this dose which were reported by the study authors but which were not related to reproduction are discussed in Chapter 5. No gross or histopathologic alterations were reported by the authors in the animals exposed to either 5.9 or 17.5 mg B/kg bw throughout the 2 year exposure period. Based on the results from this chronic feeding study, the authors concluded that exposure of rats at levels up to 17.5 mg B/kg bw in the diet over a 2 year period was without adverse effect on fertility.

In the 3-generation reproductive study by Weir and Fisher (1972), Caesarean-derived rats were randomised into control and test groups (8 males and 16 females per group). The animals were exposed to borax or boric acid at 117, 350 and 1,170 ppm boron in the diet, corresponding to 5.9, 17.5 and 58.5 mg B/kg bw respectively. Prior to initiation of the first breeding phase, the rats were fed their respective diets for 14 weeks. Each generation was mated twice to produce two sets of offspring. Rats exposed to the high dose of either borax or boric acid (corresponding to a level of 58.5 mg B/kg bw) were sterile. Microscopic examination of the atrophied testes of all males in this group showed no viable sperm. The authors also reported evidence of decreased ovulation in the majority of ovaries examined from the females exposed to 58.5 mg B/kg bw and no litters were obtained from these high dose females when mated with control male animals. There were no adverse effects on reproduction reported at exposures of 5.9 and 17.5 mg B/kg bw. The authors reported no adverse effects on fertility, lactation, litter size, progeny weight or appearance in rats

Table 10B Fertility Effects after Repeated Exposure

Compound	Species animals/group	Dose and Route mg B/kg bw/d	Duration of Exposure	NOAEL mg B/kg bw/d	LOAEL mg B/kg bw/d	Effects at LOAEL	References
H_3BO_3	rat (Wistar) M + F	35, 70 and 140 (gavage)	30 d	-	35	Slight histopathological changes in testis.	Caujolle *et al*, 1962*
H_3BO_3	rat 50 M	140 (gavage)	30 d	-	140	Testicular atrophy and reduced tubular diameter. Partial atrophy remaining at 79 d after end of exposure.	Bouissou and Castagnol, 1965*
H_3BO_3 or $Na_2B_4O_7.10H_2O$	dog (beagle) 4 M + 4 F	1.5, 2.9 and 8.8 (diet)	2 years	8.8	-	None.	Weir and Fisher, 1972*
H_3BO_3 or $Na_2B_4O_7.1OH_2O$	dog (beagle) 4 M + 4 F	29.3 (diet)	38 wk	-	29.3	Testicular atrophy and spermatogenic arrest (more severe in borax group). Some evidence suggesting reversibility.	Weir and Fisher, 1972*
H_3BO_3 or $Na_2B_4O_7.1OH_2O$	rat (Sprague-Dawley) 35 M + 35 F	5.9, 17.5 and 58.5 (diet)	2 years	17.5	58.5	Testicular degeneration (atrophied seminiferous epithelium and decreased tubular size).	Weir and Fisher, 1972
H_3BO_3 or $Na_2B_4O_7.10H_2O$	rat (Sprague-Dawley) 8 M + 16 F	5.9, 17.5 and 58.5 (diet)	3 generation reproduction	17.5	58.5	Sterility. No viable sperm in atrophied testes. Decreased ovulation in females and no litters after mating with controls.	Weir and Fisher, 1972
$Na_2B_4O_7.10H_2O$	rat	0.3, 1.0, 6.0 mg B/l (drinking water)	30, 60 and 90d	0.84 (= 6 mg B/l)	-	No biologically significant change. No effects on male fertility in breeding studies.	Dixon *et al*, 1976*
H_3BO_3	rat	0.3, 1.0, 6.0 mg B/l (drinking water) = 0.015, 0.05 and 0.3 mg B/kg	6 months	0.015 (= 0.3 mg B/l)	0.05 (= 1.0 mg B/l)	Changes in sperm parameters.	Krasovskii *et al*, 1976*
$NaB_4O_7.10H_2O$	rat (Sprague-Dawley) 18 M	50, 100 and 200 (diet)	30 and 60d	-	50	Plasma FSH increased at 30 and 60 d (changes were dose and time related). Decreased tubular size at 60 d but no effect on male fertility.	Lee *et al*, 1978

* Discussed in Appendix A

Table 10B Fertility Effects after Repeated Exposure (cont.)

Compound	Species animals/group	Dose and Route mg B/kg bw/d	Duration of Exposure	NOAEL mg B/kg bw/d	LOAEL mg B/kg bw/d	Effects at LOAEL	References
H_3BO_3	mouse (B6C3F1) 10 M + 10 F	34, 71, 142, 284 and 568 (males) (diet)	13 wk	71	142	Significant reduction in weight gain. Degeneration and/or atrophy of seminiferous tubules.	NTP, 1987*
	mouse (B6C3F1) 50 M + 50 F	78 and 201 (diet)	2 years	78	201	Testicular atrophy and hyperplasia of interstitial cells.	NTP, 1987*
	rat (F344) 30 M	61 (diet)	4 wk	-	61	Inhibited spermiation from day 7. Lower serum basal testosterone level from d 4. Significant loss of spermatids at d 28.	Treinen and Chapin, 1991
	mouse (Swiss CD-1)	27, 111 and 220 for males (diet)	3 Gen. Repro. (Continuous Breeding Protocol)	-	27	Reduced sperm motility in parental mice (no effect on reproductive performance). Shorter oestrus cycles in F1. Slightly lower adjusted pup bw in F2 pups.	Fail *et al*, 1991
	mouse (Swiss CD - 1) 10 M + 10 F	21, 70 and 210 (gavage)	d 3 - d 20 (males) d 0 - d20 (females)	21 70	70 210	Reduced testis weight (males). Two of six litters totally resorbed (females).	Harris *et al*,1992*
	rat (F344) 6 M	26, 38, 52 and 68 (diet)	9 wk + 32 wk recovery	-	26	Midly inhibited spermiation by wk 5 Recovered by wk 16 of the recovery period. (NOAEL of 17 mg B/kg reported by authors from earlier 9 wk study).	Ku *et al*, 1993a

* Discussed in Appendix A

exposed to either 5.9 or 17.5 mg B/kg bw. Also, no gross abnormalities were observed in the organs examined from either parents or weanlings from these dose groups. Based on these study data, the authors concluded that exposure of rats at levels up to 17.5 mg B/kg bw in the diet in a 3-generation reproduction study was without adverse effect.

The results of additional studies conducted by the same authors on the chronic effects in dogs after dietary exposure to borates are summarised in Appendix A.

Lee *et al* (1978) exposed groups of Sprague-Dawley male rats (18 animals/group; 200 to 250g bw) to borax in the diet at levels of 500, 1,000 and 2,000 ppm boron for periods of 30 or 60 days. According to the authors, these doses corresponded approximately to 50, 100 and 200 mg B/kg bw. Rats exposed to either 100 or 200 mg B/kg bw showed significantly decreased epididymal weight after 30 and 60 days, absence of germinal elements and a reduction in spermatocytes, spermatids and spermatozoa. These effects were more pronounced in the high dose group (200 mg B/kg bw). At the end of 60 days, testes' weights were significantly decreased in the 100 and 200 mg B/kg bw groups. Mean seminiferous tubular diameter was significantly decreased at 60 days in a dose-related manner in all exposure groups. Also, selected testicular enzymes were affected in a dose and time related manner (increased activity for enzymes associated with pre-meiotic spermatogenic cells and decreased activity for enzymes associated with post-meiotic spermatogenic cells). Plasma FSH was increased in a dose and time related manner at all exposures but no significant effect was found on plasma testosterone or LH levels. At the end of the 30 and 60 days exposure periods, 5 males per group were serially mated with control females. Male rats exposed to 200 mg B/kg bw were sterile for 8-12 weeks and this was only partially reversed in wk 9 and 10 in the animals exposed for 30 days. Males exposed to 100 mg B/kg bw showed transient sterility which was completely reversible after a 3-4 week recovery period following exposure to the high boron diet. Fertility was not affected in the 50 mg B/kg bw group. The authors concluded that exposure of male rats to 50 mg B/kg bw for 30 and 60 days in the diet is without significant effects on fertility.

Treinen and Chapin (1991) studied the development of testicular lesions in mature male F344 rats (approximately 30 animals/group; 17 wk old) exposed to 9,000 ppm boric acid in the diet for 4 weeks, corresponding approximately to 61 mg B/kg bw based on animal weight and food consumption. Animals were killed after 4, 7, 10, 14, 21 and 28 days of exposure. Inhibited spermiation began from day 7 of exposure and increased in severity and number of animals affected afterwards. At day 10, all exposed animals were affected. By day 28, there was advanced epithelial disorganisation and a significant loss of spermatocytes and spermatids from tubules at all stages of spermatogenesis. Basal testosterone level in serum was decreased from day 4 and remained lower than controls throughout the observation period. However, stimulated testosterone

levels were no different between controls and exposed animals (see Section 6 for further details). In their discussion, the authors noted that the earliest effect produced by boric acid in this study was inhibition of spermiation (release of spermatozoa) which appeared by day 7.

Using a Continuous Breeding Protocol, Fail *et al* (1991) exposed male and female Swiss (CD-1) mice to boric acid for 27 weeks at levels of 0, 1,000, 4,500, or 9,000 ppm in the diet. The authors reported estimated average doses for males based on feed consumption and body weight to be 0, 27, 111 and 220 mg B/kg bw, respectively, for week 1. Fertility of the parent mice was totally eliminated at 220 mg B/kg bw and no litters, dead or alive, were produced by cohabited pairs receiving this top dose. During 14 weeks of cohabitation, fertility of the parental mice was partially reduced in the mid-dose group. For the first litter in this study, the initial fertility index (percentage of cohabited pairs having at least one litter) was not significantly different between the doses of 111 mg B/kg bw and 27 mg B/kg bw and the controls. As the duration of the study, and thus the dosing period, increased, the fertility index declined such that only one of the 20 females from the mid-dose group had a fourth litter. All other parameters of reproductive performance which were measured were significantly decreased ($p < 0.05$) at 111 mg B/kg bw compared to controls. These included the average numbers of litters per pair, the average number of live pups per litter, the average live pup weight and the adjusted live pup weight. This trend began with the first mating and progressed in severity through subsequent matings. The progressive fertility index (percentage of females in a group having four litters) was also decreased at 111 mg B/kg bw. Crossover mating trials of control animals and animals exposed to 111 mg B/kg bw confirmed the male as the predominantly affected sex. Indeed, reproductive success of exposed males with control females was similar to that when both males and females were exposed. Results at necropsy showed that 27 mg B/kg bw represented a NOAEL for body weight and for those reproductive organ weights measured (testis and epididymis). Histopathologic examination of tissues from parental animals revealed significant testicular effects in males exposed to either 111 or 220 mg B/kg bw. Analysis of motility, concentration and morphology of sperm demonstrated a clear dose-response relationship of exposure to boric acid. Sperm concentration was significantly depressed at 111 and 220 mg B/kg bw. Sperm motility was significantly lower than controls in all treatment groups although at the lowest dose of 27 mg B/kg bw, no significant changes were noted in the male testes. Reproductive effects on females were minimal and vaginal cytology was normal throughout the ovulatory cycle. When F1 animals were exposed to 27 mg B/kg bw, the males showed a slight but not significant decrease in sperm concentration compared to controls but no effects on motility or morphology were noted. The F1 females exposed to 27 mg B/kg bw had significantly shorter oestrus cycles than those of controls. Only the F1 animals of the control and low dose groups were mated after having been exposed to boric acid *in utero*, during lactation and postnatal growth. When these F1 animals were mated, fertility was normal. F2 offspring showed a slightly lower

adjusted mean bw which was statistically significant compared to controls at $p < 0.5$ (-3% compared to controls). According to the authors, the NOAEL for the parental generation in this study approached 27 mg B/kg bw, although at this dose, only the motility of epididymal sperm in the male animals was affected without any adverse effects on fertility. The F1 males (exposed to 27 mg B/kg bw) had lower though not statistically significant sperm concentrations than controls but again, fertility was unaffected. Due to the slightly lower adjusted mean bodyweights of pups in the F2 generation, a true NOAEL for the F2 generation was not established.

Ku *et al* (1993a) investigated testicular toxicity of boric acid in adult male F344 rats (6 animals/group; 200-220g bw) exposed for 9 weeks at levels of 3,000 ppm, 4,500 ppm, 6,000 ppm and 9,000 ppm in the diet. According to the authors, these doses corresponded approximately to 26, 38, 52 and 68 mg B/kg bw. Rats in the control and 4,500, 6,000 and 9,000 ppm boric acid dose groups were placed on control feed and followed for a 32 week recovery period. Animals exposed to 52 and 68 mg B/kg bw showed severe inhibition of spermiation by week 2 followed by testis and epididymis weight loss and finally, progression to atrophy in week 9 and 6, respectively. Rats exposed to 38 mg B/kg bw showed severe inhibition of spermiation by week 2 with some germ cell exfoliation observed only at week 9 and epididymis weight loss. The animals exposed to 26 mg B/kg bw showed only mildly inhibited spermiation by week 5 and this continued variably until the end of the exposure period. The authors stated that the NOAEL for testicular effects was approximately 17 mg B/kg bw (2,000 ppm boric acid) from an earlier unpublished 9 week pilot study. At 38 mg B/kg bw, partial recovery of inhibition of spermiation was seen at 8 weeks and full recovery was reported after 16 weeks. In the 52 and 68 mg B/kg bw groups, no evidence of recovery from atrophy up to 32 weeks after exposure was observed (for further study details, see Section 6).

5.2.3 Summary of Fertility Effects

Studies investigating acute oral exposure to borates in the rat as test species identified the male animal as the most sensitive sex. In a number of studies conducted over the last 30 years, it was confirmed that exposure of the male rat to boric acid or borax resulted in histopathological changes in the testes and/or in standard sperm parameters at 175 mg B/kg bw and above (see Table 10). At higher exposure levels (>525 mg B/kg bw), more serious effects were noted (i.e., atrophy of the testicular tubules). The effects appeared about 14 days post exposure and were reversible up to exposure levels of 700 mg B/kg bw after an observation period of about 130 days. In one study (Dixon *et al*, 1976; see below), reproductive performance in males was not affected up to single exposure levels of 450 mg B/kg bw.

Repeated exposure to borates either by gavage or in the diet produced histopathological lesions in the testis of the male rat at exposures of about 26 mg B/kg bw and above. At these doses, only very mild subtle effects were observed histologically i.e. mild inhibition of spermiation (Ku *et al*, 1993a). In some studies (Lee *et al*, 1978; Ku *et al*, 1993a), reversibility of the observed effects is clearly observed in the first weeks following exposure. In a 3 generation study sterility was clearly observed in the male rat at 58.5 mg B/kg bw with a NOAEL of 17.5 mg B/kg bw (Weir and Fisher, 1972). Similarly, the male mouse showed minor effects such as subtle changes in sperm motility at 27 mg B/kg bw/d; fertility was normal (Fail *et al*, 1991).

A possible effect of exposure to borates on female reproductive performance was suggested as early as 1962 by Caujolle *et al*. This was confirmed by Weir and Fisher (1972) who noted decreased ovulation and infertility in female rats exposed to 58.5 mg B/kg bw. In the mouse however, slight though statistically significant decreases in female reproductive parameters such as adjusted live pup weight were only noted at exposure levels of 111 mg B/kg bw in the parental generation. In the F1 generation, exposure of female mice to 27 mg B/kg bw resulted in shorter oestrus cycles than those of controls, but reproductive performance was unaffected.

5.3 DEVELOPMENTAL TOXICITY IN ANIMALS

This section reviews chronologically the available data from developmental toxicity studies (see Table 11).

5.3.1 Rabbit

In a developmental toxicity study conducted by NTP (1991) with artificially-inseminated New Zealand White rabbits (30 female animals/group) does were exposed to 0, 62.5, 125, or 250 mg boric acid/kg bw by gavage on days 6 to 19 of gestation which are the days of major organogenesis in this species. These doses correspond to levels of 0, 10.9, 21.8 and 43.5 mg B/kg bw, respectively. On gestational day 30, approximately 1-2 days before expected parturition, surviving female animals were killed. Dead and live foetuses were weighed and live foetuses were killed and examined for external, visceral and skeletal malformations and/or variations. The following observations were recorded.

Maternal Toxicity

Pregnant does exhibited no overt symptoms attributable to boric acid toxicity except that a decreased food intake (30% reduction vs. controls during exposure period) and vaginal

Table 11 Developmental Effects

Compound	Species animals/group	Dose and Route mg B/kg bw/d	Duration of Exposure	NOAEL mg B/kg bw/d	LOAEL mg B/kg bw/d	Effects at LOAEL	References
H_3BO_3	rabbit (New Zealand white rabbit) 30 F	10.9, 21.8 and 43.5 (gavage)	gd[1] 6 - 19 (killed on gd 30)	21.8	43.5	Prenatal mortality and decreased foetal bw. Increased incidence of cardiovascular effects. (Maternal toxicity).	NTP, 1991
H_3BO_3	mouse (Swiss CD-1) 30 F	43, 79 and 175 (diet)	gd 0 - 17 (killed on gd 17)	43	79	11% mean foetal bw reduction. Minor skeletal variations. (Maternal toxicity from 43 mg B/kg bw/d).	Heindel *et al*, 1992
H_3BO_3	rat (Sprague-Dawley) 30 F	13.7, 28 and 58 (diet)	gd 0 - 20 (killed on gd 20)	-	13.7	6 - 7 % mean foetal bw reduction. Minor skeletal variations. (No maternal toxicity).	Heindel *et al*, 1992
H_3BO_3	mouse (Swiss CD-1) 10 F	21, 70 and 210 (gavage)	gd 8 - 14 (killed on pnd[2] 4)	70	210	Complete resorption of implants.	Harris *et al*, 1992[3]
H_3BO_3	rat (Spraque-Dawley) 30 F (phase I)	3.3, 6.3, 9.6, 13.3 and 25 (diet)	gd 0 - 20 (killed on gd 20)	9.6	13.3	6% mean foetal bw reduction. Increased incidence of short rib XIII. Minor skeletal variations. (No maternal toxicity).	Price *et al*, 1994
H_3BO_3	rat (Sprague-Dawley) 30 F (phase II)	3.2, 6.5, 9.7, 12.9 and 25.3 (diet)	gd 0 - 20 (killed on pnd 21)	12.9	25.3	Increased incidence of short rib XIII. (No maternal toxicity).	Price *et al*, 1994

1 gd = gestational day
2 pnd = postnatal day
3 Discussed in Appendix A

bleeding was noted at 43.5 mg B/kg bw between gestational days 19 - 30. All high-dose animals with vaginal bleeding had no live foetuses at sacrifice. The authors considered 43.5 mg B/kg bw as the LOAEL for pregnant does and 21.8 mg B/kg bw as the NOAEL for maternal toxicity.

Developmental Toxicity

Rabbits were very susceptible to prenatal mortality at the highest dose in this study (43.5 mg B/kg bw) since 90% of implants/litter were resorbed in this group compared to 6% for controls. At 21.8 mg B/kg bw/d, no difference in percentage resorptions per litter were seen, compared to controls.

Average foetal bodyweight per litter was 92% of the controls at the high dose (43.5 mg B/kg bw) but even at this exposure, it did not reach statistical significance possibly due to the low number of pups surviving. An increased incidence of malformed live foetuses/litter was observed at 43.5 mg B/kg bw, primarily due to cardiovascular defects (72% for major defects of heart and/or great vessel in the high-dose group vs. 3% in controls).

There were no variations between groups concerning the incidence of skeletal malformations. The only skeletal variations of interest was a dose related reduction in the incidence of extra ribs on Lumbar I which the authors did not consider to be toxicologically important. Overall, the rabbit skeletal system was less sensitive to the developmental toxicity of boric acid. Since no definitive developmental effects were observed in animals exposed to either 10.9 or 21.8 mg B/kg bw, the authors concluded that 21.8 mg B/kg bw was the NOAEL for developmental toxicity.

5.3.2 Mouse

A study of developmental toxicity of boric acid in mice was carried out by Heindel *et al* (1992). Time-mated Swiss albino CD-1 mice (approximately 30 female animals/group) were exposed to boric acid at 0, 0.1, 0.2 or 0.4% in the diet for the entire period of gestation (days 0 to 17). Average daily doses reported were 0, 248, 452, or 1,003 mg boric acid/kg bw which corresponded to 43, 79 and 175 mg B/kg bw, respectively. On killing, maternal and foetal bodyweights were taken and foetuses were subsequently examined for external, visceral and skeletal malformations and/or variations. The following observations were recorded.

Maternal Toxicity

Maternal toxicity associated with exposure included effects on bodyweight gain and on organ weights. At the high dose (175 mg B/kg bw), boric acid caused a reduction in maternal weight gain during treatment but weight gain corrected for uterine weight was not affected. At this dose increased relative kidney weight and increased relative food intake were also noted. Mild renal lesions (tubular dilation with or without regeneration) were observed at all dose levels in the study. Based on their assessment of histologically apparent kidney lesions, the authors considered that a maternal NOAEL was not reached in this study.

Developmental Toxicity

Exposure to boric acid during gestation was not associated with pre-implantation loss in any of the dose groups, but increased post-implantation loss was noted at 175 mg B/kg bw.

At 175mg B/kg bw, foetal bodyweight was reduced by 33% (average of both sexes) compared to controls. At 79 mg B/kg bw, a less severe foetal weight reduction was observed (11%). Boric acid also had significant effects on foetal morphology. The incidence of malformed foetuses/litter was significantly increased at 175 mg B/kg bw but not at lower doses. The increase in malformations was primarily due to skeletal defects and included an increased incidence of short rib XIII. Looking at skeletal variations, the authors observed that the percentage of foetuses with variations/litter was significantly decreased in the low and mid-dose groups when compared with controls. Specifically, the presence of full or rudimentary rib(s) at Lumbar I was observed less frequently at 43, 79 and 175 mg B/kg bw than in controls. Since these anatomical variations for this species and strain are common, the authors concluded that 43 mg B/kg bw was the NOAEL for developmental toxicity in this study.

5.3.3 Rat

Heindel *et al* (1992) investigated the toxicity of boric acid to rats. Time-mated Sprague-Dawley rats (approximately 30 females/group) were exposed to boric acid at 0, 0.1, 0.2 or 0.4% in the diet for the entire period of gestation (days 0 to 20). Average daily doses reported for rats were 0, 78, 163 and 330 mg boric acid/kg bw which corresponded to 0, 13.7, 28 and 58 mg B/kg bw, respectively. To limit pre-implantation loss and hence allow expression of other types of foetal effects, an additional group of rats (14 females) was exposed to 0.8% boric acid, equivalent to 94 mg B/kg bw, on days 6 - 15 of gestation only. When the rats were killed, maternal and foetal bodyweights were

taken and foetuses were subsequently examined for external, visceral and skeletal malformations and/or variations. The following observations were recorded.

Maternal Toxicity

All females survived until scheduled termination and pregnancy rates were 90-100% for all groups. Maternal toxicity was observed at 28, 58 and 94 mg B/kg bw as shown by increased relative liver and kidney weights at 28 mg B/kg bw and above, and by decreased weight gain during treatment and gestation at 58 mg B/kg bw and above. According to the authors, the NOAEL for maternal toxicity was 13.7 mg B/kg bw.

Developmental Toxicity

Increased resorptions and foetal deaths were noted at 94 mg B/kg bw (36% resorptions/litter compared to 4% resorptions in the controls).

Decreased mean foetal bodyweights/litter were observed at all exposures. At the lowest dose level (13.7 mg B/kg bw), the mean foetal bodyweight reduction was 6-7% compared to the control weight (average for both sexes). The percentage of foetuses malformed/litter was increased at 28 mg B/kg bw and above. Specifically, the incidence of litters containing a foetus with a skeletal malformation was significantly increased at 28mg B/kg bw and above. The incidence of litters with one or more offspring with a visceral or external malformation was increased at 58 mg B/kg bw and at 94 mg B/kg bw, respectively. Anatomical variations were noted to occur with a lower incidence than controls at 13.7 and 28 mg B/kg bw, due primarily to a reduction in incidence for rudimentary or full rib(s) at Lumbar I. The authors noted that this variation is common for this animal species and strain. At 94 mg B/kg bw the incidence of anatomical variations was significantly increased above control levels. Based on the foetal bodyweight results, 13.7 mg B/kg bw was considered by the authors to be a LOAEL for the rat in this study.

A very recent study addressing the developmental toxicity of boric acid in the rat was reported by Price *et al* (1994). The objective was to determine a NOAEL for pre-natal effects and to evaluate post-natal recovery after exposure to boric acid during foetal development. In a first phase (Phase I), time-mated Sprague-Dawley rats (approximately 30 females/group) were exposed to boric acid at 0, 0.025, 0.050, 0.075, 0.1 or 0.2% in the diet for the entire period of gestation (days 0 - 20). Average daily doses reported were 19, 36, 55, 76 and 143 mg boric acid/kg bw bodyweight which were calculated to be 3.3, 6.3, 9.6, 13.3 and 25 mg B/kg bw, respectively. Phase I animals were

killed on day 20. For postnatal evaluation (Phase II), additional dams (approximately 30/group) were exposed to the same levels of boric acid in the diet also for the entire period of gestation. The calculated daily doses for Phase II animals were 3.2, 6.5, 9.7, 12.9 and 25.3 mg B/kg bw. These animals were allowed to deliver and rear their litters until they were killed on postnatal day 21. The average intake from the diet was less than 0.4 mg B/kg bw for control animals. In both phases, offspring were evaluted for post-implantation mortality, bodyweight and morphology (external, visceral and skeletal). The following observations were recorded.

Maternal Toxicity

All females survived until scheduled termination and no characteristic clinical signs were associated with boric acid exposure. Some minor effects of exposure on maternal weight and weight gain appeared to be secondary to reduced gravid uterine weight. Relative kidney weight was increased at the highest dose in Phase I but the effect was no longer evident by postnatal day 21 in the Phase II group. The authors concluded that there was little evidence of maternal toxicity at any of the doses tested (Phase I or II).

Developmental Toxicity

Developmental effects were found in foetuses from animals exposed to 13.3 mg B/kg bw and above (Phase I) which are mainly associated with foetotoxic activity. Specifically, a reduction in the mean foetal bodyweight per litter (6% compared to controls) was observed in Phase I foetuses at 13.3 mg B/kg bw. At this dose, skeletal changes which included an increased incidence of short rib XIII (considered a malformation by authors of this study) and an increased incidence of wavy rib (considered a variation) were also observed. At the high dose for Phase I animals (25 mg B/kg bw), the bodyweight reductions and skeletal changes were more pronounced. The reduction in incidence in extra rib on Lumbar I (a variation) which was noted in the previous rat study was not statistically significant here due to the low incidence in control animals (3.2% in controls in this study compared to 14% in the study from Heindel *et al*, 1992). The animals from the Phase II group which were killed on postnatal day 21 showed no reduction in pup bodyweight in any group compared to controls, which indicates full recovery in the offspring already by postnatal day 0 from treatment-related bodyweight effects. The rib variations observed in the foetuses (wavy rib) from Phase I were not observed in any dose group in Phase II. At the highest dose in Phase II (25.3 mg B/kg bw), an increased incidence of short rib XIII was observed. The authors concluded that the NOAELs for the prenatal and postnatal study phases (Phase I and Phase II) were 9.6 and 12.9 mg B/kg bw/d, respectively.

5.3.4 Summary of Developmental Effects

In the studies addressing developmental toxicity, the dose ranges selected for the animal studies overlapped (ranging from about 3 to 175 mg B/kg bw) permitting some comparison of the results obtained across species (mouse, rat and rabbit). Considering the LOAEL's for developmental effects (see Table 11), the rat is the most sensitive species for developmental toxicity showing a slight (about 6%) but statistically significant reduction in foetal bodyweight at 13.3 mg B/kg bw. At this dose, some skeletal changes were also noted. The LOAEL for the rabbit was defined by increased prenatal mortality and increased incidence of cardio-vascular malformations at 43.5mg B/kg bw. Finally, the developmental LOAEL in mice was defined by an 11% reduction in foetal bodyweight observed at 79 mg B/kg bw compared to controls. Some minor variations were also noted at this dose level. Thus the sensitivity of the species tested to boric acid exposure can be summarised as rat > rabbit > mouse for developmental toxicity. The developmental toxicity in rats was observed at a dose which did not induce any significant maternal toxicity. In contrast, developmental toxicity was observed in rabbits and in mice only at doses which produced maternal toxicity.

An overall NOAEL for all 3 species can be established unequivocally at 9.6 mg B/kg bw.

5.4 HUMAN REPRODUCTIVE DATA

Two early Russian studies reported reduced sexual function in men exposed occupationally to borate (Tarasenko *et al*, 1972) or through drinking water (Krasovskii *et al*, 1976). As it was not unequivocally established that exposure was solely to borates, these studies cannot be considered relevant to the risk assessment. A later critical review of reproductive effects of boric acid (Barlow and Sullivan, 1982) concluded that there was a lack of adequate evidence about the reproductive effects of this chemical in human beings based on the above studies. Nevertheless, concern about the reproductive effects in laboratory animals from ingestion of high levels of inorganic borates led to a study for potential adverse reproductive effects in male employees (Whorton *et al*, 1994) at the U.S. Borax mine and production facility in California.

The method employed was a questionnaire to examine any anti-fertility influence of occupational exposures by studying the reproductive performance in terms of live births to the wives of workers, subsequent to specific occupational exposures to sodium borate dust. The results were calculated as the statistic "standardized birth ratio" or SBR.

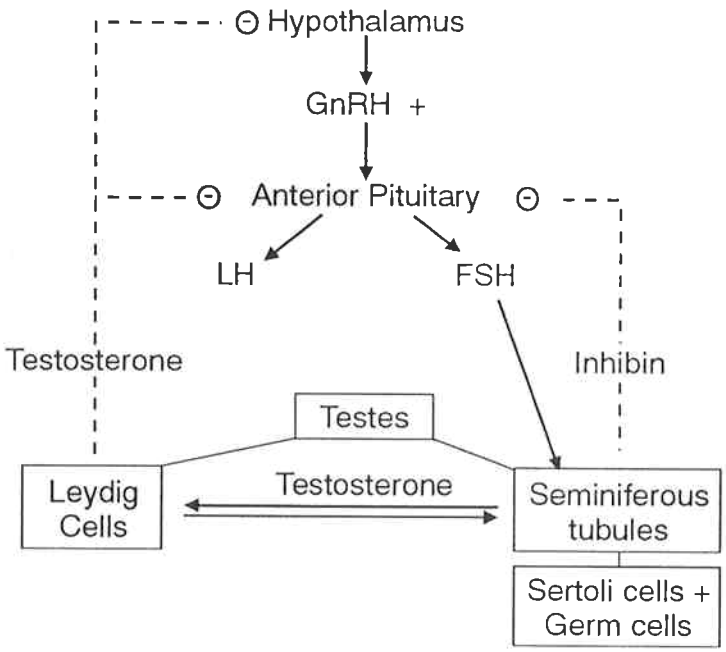
Of the 753 eligible male employees with more than 6 months service, 542 (72%) completed the questionnaire. The average length of employment of study participants was 16 years. Personal sampling data of dust exposures for the most exposed jobs were available since the early 1980's. Sodium borate dust exposure categories for 8-hours were arbitrarily assigned three categories described as "high" (> 8 mg/m^3), "medium" (3 - 8 mg/m^3) and "low" (< 3 mg/m^3). The range of exposure in one year was 2 to 35.7 mg/m^3 (sodium borates) which, for the high exposure group, is equivalent to a mean of 0.34 mg B/kg bw/d or 24 mg B/d for a 70kg worker.

The number of live children born to wives of the workers after exposure or job assignment was obtained from the questionnaires and compared to the number of births that would be expected if the study population had, adjusting for maternal age, parity, race and calendar time period, the same reproductive experience as a comparable non-exposed population (data from national fertility tables published for successive birth cohorts of women in the USA). The results showed that there were 529 observed births fathered by the participants when only 466.6 were expected from a statistically significant excess in the standardized birth ratio (SBR) of 113, significant to $p < 0.01$. The authors concluded that exposures to inorganic borates up to an estimated maximum of 24 mg B/d did not lead to a reduction in birth rate.

SECTION 6. MECHANISM OF ACTION OF TESTICULAR EFFECTS

6.1 GENERALISED SCHEME

The male reproductive system is regulated by a complex interaction between the central nervous system (CNS), hypothalamus, pituitary and testis, as illustrated below;



The hypothalamus synthesises and releases a peptide gonadotropin releasing hormone (GnRH), which is discharged by regular pulses into the blood stream. The timing of the pulses is critical to the release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) from the anterior pituitary into the general circulation. Local control of Leydig, Sertoli and germ cells appears to be modulated by numerous growth factors arising from within the testes (Heindel and Treinen 1989).

The seminiferous tubules comprise two major cell types, Sertoli cells and germ cells. As maturation of the germ cell occurs in close association with the Sertoli cells, it has been proposed that they play a pivotal role in cell to cell communication which is needed to control the movement of germ cells through the seminiferous tubules. This role includes involvement in spermiation (release of mature spermatozoa).

6.2 EXPERIMENTAL INVESTIGATIONS

A number of studies have been undertaken which help to provide some insight into the mechanism of action by which boron-induced testicular dysfunction occurs. The key findings are summarised in Table 12, which indicate that borates cause decreased testosterone production, reduced spermiation (due to accumulation of spermatid at stages VIII and IX) and germ cell loss. Treinen and Chapin (1991) have provided much of the evidence to support this hypothesis. Boric acid was administered to rats *via* the diet at 9,000 ppm (61 mg B/kg bw) for up to 4 weeks. The first pathological testicular effect (inhibition of spermiation) was seen on day 7. This progressed towards exfoliation of viable germ cells and eventually germ cell loss by day 28. No accumulation of boron in the testes was observed over the treatment period. Leydig cells were not affected by the boric acid treatment, even though basal serum testosterone concentration was decreased, suggesting the pituitary control of the Leydig cells and their function remained intact. This was in marked contrast to the effects on Sertoli cells both with respect to function (inhibition of spermiation progressing with increasing dose to testicular atrophy) and to the Sertoli cell structure (including epithelial disorganisation, peripheral spermatid nuclei and the presence of abnormal residual bodies).

Table 12 Mechanism of Action: Testicular Toxicity

Study type	Enzyme measurements	Hormone assays	Testicular histology	Reference
Acute rat, gavage	ND	FSH, LH not affected	↑ spermatids stages VIII/IX	Linder *et al*, 1990
30-60d rat, oral	↑ in premeiotic activity ↓ in spermiogenesis	↑ FSH and LH Testosterone unaffected	↓ spermatocytes, spermatids spermatozoa, germinal aplasia	Lee *et al*, 1978
28d rat, oral	ND	↓ testosterone	↓ spermiation germ cell loss	Treinen and Chapin, 1991
9 wk rat, oral	ND	↑ FSH and LH testosterone unaffected	↑ spermatid IX ↓ spermatozoa	Ku *et al*, 1993a
9 wk mouse, oral	ND	↓ testosterone	ND	Grizzle *et al*, 1989
16d gerbil, sc	↑ enzymes	ND	↓ spermiation germ cell loss	Sharma, 1978

ND = Not done

Two abstracts provide additional supporting evidence that the Sertoli cell is the main target cell in the testes. Grizzle *et al* (1989) administered 9,000 ppm boric acid in the diet to mice for 9 weeks (equivalent to 68 mg B/kg). A decrease in testosterone concentration was observed in the treated animals. Furthermore, challenge with hCG (human Chorionic Gonadotropin) stimulated testosterone

production in the control animals (23.5 ng/ml) but only slight increases were observed in the treated animals (5.3 ng/ml). Anderson *et al* (1992) reported endocrine responses in sera after 2, 9 or 14 days following administration of boric acid in the diet at 9,000 ppm (68 mg B/kg bw). Hormone testicular effects were assessed at basal levels and following GnRH and hCG challenge. No changes in hormone concentrations were observed after exposure to boric acid for 2 or 9 days but after 14 days the basal FSH concentration was increased and testosterone concentrations decreased in treated animals compared with the controls. The concentration of LH was unchanged. Stimulation by GnRH and hCG resulted in relatively higher FSH concentrations in treated rats but testosterone concentrations were unchanged. These data suggest that boric acid affects the testes, probably the Sertoli cells, resulting in indirect effects on either the pituitary or the Leydig cells.

In a further study Ku *et al* (1993a) investigated testicular toxicity in rats given boric acid in the diet for at levels up to 9,000 ppm for 9 weeks, followed by a 32 week recovery period. Mild inhibition of spermiation was demonstrated at 3,000 ppm with 25-50% of tubules with retained spermatid at stage IX, and severe widespread inhibition of spermiation at 4,500 ppm. Testicular atrophy occurred at 6,000 ppm. Although no accumulation of boron was observed in the testes, concentrations of testicular boron increased with dose in line with boron serum concentrations. As serum boron levels decreased following cessation of treatment, so did levels in the testes. Sperm head counts in the testes and epididymis were monitored with no adverse effect observed at 3,000 ppm. Increases in testes sperm head counts and decreased epididymal sperm counts were observed at 4,500 ppm (38mg B/kg). Partial recovery from these effects was observed after 8 weeks and total recovery after 16 weeks at 4,500 ppm. The recovery from the mild effects observed at 3,000 ppm was not monitored. The authors cited a NOAEL of 2,000 ppm (17 mg B/kg) for inhibition of spermiation based on a preliminary study. This recovery profile is consistent with data from Linder *et al* (1990) which showed significant recovery from inhibition of spermiation within 7 weeks following acute boric acid exposure to rats.

6.3 MECHANISTIC INVESTIGATION *IN VITRO* AND *IN VIVO*

In other recent publications (Ku *et al*, 1993b, Ku and Chapin, 1994) the possible mechanism of action of boron-induced testicular toxicity was investigated still further using *in vivo* and *in vitro* procedures but without any definitive result. Since inhibition of spermiation and possible germ cell atrophy had been demonstrated it was speculated that decreased testosterone production may be responsible for this.

The *in vivo* findings (Ku and Chapin, 1994) were based on investigations following exposure of rats to boric acid in the diet at levels up to 9,000 ppm for up to 28 days. The flavin content in the

testes, liver and brain was measured, as it has been demonstrated that exposure of rats to boric acid may induce riboflavinurea (Roe *et al,* 1972) which could result in riboflavin deficiency. Riboflavin (Vitamin B2) is the precursor to flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), both of which are essential co-enzyme derivatives. No significant or consistent changes in flavin content (FAD/FMN) with time were observed.

The effect of boric acid exposure was investigated in *in vitro* studies using co-cultures of primary Sertoli/germ cells to measure the intra-cellular cyclic AMP levels and DNA/RNA synthesis and, using single cultures of primary Leydig cells, testosterone production was measured (Ku *et al,* 1993b). No accumulation of cAMP in the co-cultures in the presence of boric acid was observed at concentrations up to 10mM boric acid (108 mg B/l). Accumulation was observed at 10 mM (which is 5 to 10 times the highest boron concentration from *in vivo* studies). A significant decrease of DNA synthesis was noted at exposures of 1-3 mM boric acid. The authors postulated that these changes might explain the germ cell atrophy seen *in vivo.* No changes were seen in testosterone production in isolated Leydig cells, suggesting a CNS-mediated effect of boric acid rather than a direct effect.

6.4 CONCLUSIONS

The mechanism of action by which borate is a testicular toxicant is not clear. Since it is established that boron does not accumulate in the testes even though it is present in testicular tissue during periods of exposure, it is possible that the continued decreased testosterone production arises *via* a CNS mediated mechanism. It is not likely that hormone changes can explain the testicular atrophy observed at high dose levels since it has been shown that spermatogenesis can be maintained in the presence of significantly decreased intra-testicular testosterone levels.

Inhibition of spermiation has been investigated and involvement of Sertoli cells is suggested, as effects on these cells can lead to testicular atrophy. The changes in serum hormone levels may reflect an indirect effect on the CNS mediated by paracrine and/or autocrine influences. Overall, the precise mechanism of boron activity on the testes remains to be elucidated.

SECTION 7. DIETARY EXPOSURE IN MAN

7.1 INTRODUCTION

To provide an objective basis for a risk assessment on the possible reproductive effects of exposure of man to the inorganic borates, it is essential to be aware of and to consider the role of daily boron exposure in food, beverages and drinking water.

Boron is a naturally occurring element present widely in the environment in the form of borates and functions as an essential micronutrient for the healthy growth of all plant life. More specific details about the boron content of plants and mineral water are given elsewhere (ECETOC, 1995). It should be noted here that boronated fertilisers are added - where necessary - to correct boron deficiency symptoms in plants and to optimise crop yields (Bergmann, 1988). Consequently, the natural daily dietary intake of borate through food and beverages forms an important part of the daily exposure for animals and human beings.

There are difficulties in quantifying the actual intake of dietary borate, because of the number of variables involved, and such estimates are necessarily only approximate. One obvious variable is the natural boron content of the soil on which the plants have been grown, and whether boronated fertilisers have been used. The daily intake of boron by man may also vary widely, depending on the proportions of various food groups in the diet. For example, fresh vegetables, fruit and wine have a relatively high boron content. There may also be a considerable elevation in the daily boron intake from drinking water and mineral water, whereas in the former case there are a few areas of the world with a naturally high environmental background level of borate or in the latter case geologically conditioned mineral waters which often contain significantly more borate. Finally, the improvement in analytical techniques during the past 40 years or so, combined with improved techniques for less adventitious contamination of samples, may explain why current estimates of daily intake are generally lower than in the older literature.

7.1.1 Nutritional Essentiality of Borates

In recent years, dietary studies on animals and man have been undertaken to establish the nutritional importance of boron since there is no firm evidence that it is an essential dietary component. Nielsen (1992) suggested that inadequate dietary boron (<0.2 mg B/d) may be one factor that contributes to susceptibility to bone loss or osteoporosis. Nielsen *et al* (1987) conducted a study on 12 postmenopausal women in which they received a conventional diet providing

0.2 mg B/d for 119 days followed by a boron supplement of 3 mg B/d, as sodium borate, for 24 days. Dietary boron had a marked effect on major mineral metabolism, which could be modified by the magnesium status of the individuals. The effect of the additional boron supplement was to reduce the urinary excretion of calcium and magnesium. Beattie and Peace (1993) investigated the influence of a low boron diet for 3 weeks on bone, major mineral, and sex steroid metabolism in 6 postmenopausal women. During a further 3 week observation period these volunteers were given a boron supplement of 3 mg B/d, as sodium tetraborate. Changes in boron intake from the basal dietary level of 0.33 mg B/d to the higher level of 3.33 mg B/d had no effect on minerals or steroids. Nevertheless, the low boron diet appeared to induce hyperabsorption of calcium since positive calcium balances were found in combination with elevated urinary calcium excretion.

Whilst the longer of these studies suggests a beneficial effect of boron may be apparent, neither study demonstrates essentiality. Thus, the role of boron in human nutrition remains to be defined.

7.2 FOOD AND WATER

7.2.1 **Food and Beverages** (including Mineral Water)

In the 1960s a detailed estimate of the daily dietary intake of borate in France for an adult in normal health, consuming about 2,800 calories per day, was reported to be about 7 mg boron (without alcoholic beverages), with an additional 3 to 4 mg boron provided by half a litre of wine (Ploquin, 1967). Any dietary variation in the weight of fresh fruit, vegetables and especially wine - all with a high boron content - clearly had a decisive influence on the actual result. In each case the food item was analysed raw if consumed raw, or after usual cooking.

Samples of U.K. diet for the year 1966/67 on foods including cereals, meat, fish, fats, fruits and preservatives, root vegetables and milk (in unspecified proportions), provided a mean of 2.8 ± 1.5 mg B/d, excluding beverages, except for milk (Hamilton and Minski, 1972, 1973).

This lower average daily dietary intake of boron was also found from a comprehensive study carried out on the mineral element composition of over 200 Finnish foods (Varo and Koivistoinen, 1980). The average supply for foods was estimated as 1.7 mg B/d (including dairy products, but excluding beverages). It was estimated that vegetable foods were an important source of food boron (60% of input).

The United States Food and Drug Administration prepared a mixed diet composite (USDIET-1), which included foods and beverages (prepared, cooked and ready-to-eat), to represent the intake of

25-30 year old males in the USA (Iyengar *et al*, 1987). Analytical results (Iyengar *et al*, 1990) of the above USDIET-1 (Southeast USA) and USDIET-2 (North Central USA) gave an average daily intake of 1.5 mg boron.

Based on the published United Kingdom national food survey, (UK Ministry of Agriculture, 1991) and applying the boron contents found in Finnish foods (Varo and Koivistoinen, 1980), daily dietary boron values of 0.8-1.9 mg were obtained as an estimate for the UK. It is worth noting that United Kingdom wine consumption (Gregory *et al*, 1990) could add a further 1.1-2.4 mg B/d, because of the high boron content of wine. Using the same method of calculating dietary boron levels, a recent UK demi-Vegetarian diet had an estimated intake of 2.8-5.5 mg B/d (Draper *et al*, 1993).

Details of an EU-average diet and an EU-average vegetarian diet have been published (EUROMONITOR, 1992). Taking these diets and applying the food and beverage boron contents from a large Finnish study (Varo and Koivistoinen, 1980), a range of boron intakes was estimated as 1.6 to 4.5 mg B/d for average and 2.4 to 7.0 mg B/d for vegetarian diets.

Table 13 Estimated Average Dietary Intake of Borate (mg B/d)

Country	Daily Intake	Reference
France	10[a]	Ploquin, 1967
Finland	1.7[b]	Varo and Koivistoinen, 1980
UK	2.8[b]	Hamilton and Minski, 1972
	0.8-1.9[b]	MAFF, 1991
	1.1-2.4[c]	Gregory *et al*, 1990
	2.8-5.5[d]	Draper *et al*, 1993
EU	1.6-4.5[e]	Euromonitor[g], 1992
	2.4-7.0[f]	Euromonitor[g], 1992
USA	1.5	Iyengar *et al*, 1990
Canada	1.3	Iyengar *et al*, 1988

[a] includes 0.5 litres of wine
[b] excludes beverages
[c] from wine consumption
[d] demi-vegetarian diet
[e] EU average diet
[f] EU vegetarian diet
[g] Boron values in food according to Varo and Koivistoinen, 1980

Mineral water

Analysis of the borate content of 25 commercially available but unspecified mineral waters (Graffmann *et al*, 1974) showed a range from <0.02 to 3.23 mg B/l, with an average of around 0.5 mg B/l.

Allen *et al* (1989) analysed 37 brands of bottled mineral water, 9 of U.S. origin and the rest of European origin for chemical composition. There was a wide scatter in the boron content of the mineral waters, with 8 brands containing >1 mg B/l, the highest being 4.3 mg B/l. The average boron content for the 37 was 0.13 mg B/l.

The daily intake of mineral water is generally less than that of drinking water, and estimated to be around 700 ml in Germany (Dieter, 1993). For regular drinkers of mineral water, there may be a daily intake of perhaps 0.3 mg B on average, or up to about 3 mg B in extreme cases.

7.2.2 Drinking Water

The borate content in fresh water used for the abstraction of drinking water in most parts of the world is generally less than 1 mg B/l and often less than 0.5 mg B/l. Drinking water is usually a blend of fresh and ground waters. Less data are available on ground waters used for this purpose, but in some parts of Southern Europe and particularly in areas of volcanic activity in Italy and Greece, the borate content is often greater than 1 mg B/l (ECETOC, 1995). Thus the overall contribution of dietary boron from drinking water assuming an average consumption of 2 l/d is generally less than 1 mg B.

There is a high natural background level of boron in the surface waters of some parts of the world, where the drinking water intake of borate may be substantially greater than 1 mg B/l. One recent published example is the arid region of northern Chile, which is rich in minerals and natural salts (see section 3.4.2). Water boron content at differing locations along the Loa river basin ranged from 3.99 mg B/l at Lequena to the exceptionally high 26 mg B/l at Qulilagua (Luis Cáceres *et al*, 1992). A further study of the same area (Barr *et al*, 1993) was carried out, in which the range of boron in the water supplies varied from 0.31 mg B/l to the highest value of 15 mg B/l. The authors estimated that a daily intake of 1,800 g of drinking water, the highest computed intake (from drinking water only) would be 27 mg B/d. The authors showed that a high boron content of drinking water tended to lead to a raised blood boron level in the sample of 40 human beings living in this area.

7.3 SUMMARY

Various studies indicate that the daily dietary intake of boron in man ranges from 1.3-4.5 mg B/d of which food and beverages are the major contributors. By comparison, the contribution of boron intake from drinking water is relatively low (less than 1 mg B/d).

SECTION 8. RISK ASSESSMENT OF BORATES

8.1 INTRODUCTION

Risk assessment plays a central role in the decision-making process for many chemicals. While risk assessment is not the sole input to risk management it certainly is an important one. The process of risk assessment includes a consideration of hazard identification, dose/response assessment, exposure assessment and risk characterisation.

The evaluation hereafter will focus mainly on the reproductive hazard of borates as this was identified as the critical toxicological endpoint. The dose-response analysis will permit the determination of a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL) which will then be used to calculate a Tolerable Daily Intake (TDI) of boron in the form of borates for a 60 kg person. The quantitative process to derive a tolerable daily intake includes the use of uncertainty factors or safety factors in order to weight various confidence parameters such as adequacy of data base, nature and severity of the effects, intra- and inter-species variations.

A risk characterisation will be presented for both the general population and specific worker populations such as borax miners, borate production workers and others using borates occupationally. Because of the nature of the critical effect and the fact that exposure of young children to testicular toxicants may present a risk, a need for a specific TDI for this population sub-group was reviewed by the Task Force. The exposure of this sub-group to boron *via* diet and water was considered to be lower (<4 mg B/d) than that for adults and that consequently they were not at risk.

8.2 HAZARD IDENTIFICATION

A large number of various animal toxicity studies are available for different inorganic borates covering acute, chronic, genotoxic, carcinogenic and reproductive endpoints. In addition to the older studies, various more recent studies are available which generally meet current standards. Most studies of borates have been done with boric acid and borax. Data on the toxicology of perborate and boric oxide are limited.

Borates were found to be neither genotoxic nor carcinogenic. However acute and repeated exposure studies consistently showed the testis as a target organ. This was confirmed in fertility

studies. Developmental effects were found which are mainly associated with foetotoxic activity. Both, reproductive and developmental aspects are taken as a basis for the following risk assessment, since they represent the most sensitive endpoints of toxicity for these compounds.

8.3 DOSE/RESPONSE ASSESSMENT

8.3.1 Fertility

In the present risk assessment four main studies are identified as being pivotal for determination of a NOAEL. These include a three-generation study in rats (Weir and Fisher, 1972), a two-generation study in mice (Fail *et al*, 1991), a 60-day study in rats (Lee *et al*, 1978) and a 9-week study with recovery (32 wk) in rats (Ku *et al*, 1993a). All these studies concern oral administration of boric acid and/or borax *via* the diet. For comparative purposes, doses are expressed as boron equivalents.

As discussed in Appendix A, the 2-year study in dogs was not used for risk assessment due to a number of inadequacies.

Reproductive Performance

In male and female rats a clear NOAEL of 17.5 mg B/kg bw was determined whereas at 58.5 mg B/kg bw sterility appeared together with atrophy of testes and decreased ovulation (Weir and Fisher, 1972). In a male rat study (Lee *et al*, 1978) a NOAEL was found at 50 mg B/kg bw. At 100 mg B/kg reproductive performance recovered partially after 4 weeks cessation of dosing. In the male and female mouse a NOAEL was found at 27 mg B/kg bw (Fail *et al*, 1991) and at a dose of 111 mg B/kg bw for 60 days decreased fertility was observed, but only from the second litter onwards. Sterility was observed at 220 mg B/kg bw.

From these studies, the lowest NOAEL in rodents for reproductive performance was 17.5 mg B/kg bw.

In man no effect on reproductive performance was found up to a level of 0.34 mg B/kg bw as shown in an epidemiology study (Whorton *et al*, 1994).

Testicular changes

Although a slight effect on sperm motility was seen in mice at 27 mg B/kg bw (Fail *et al*, 1991) all the other parameters measured were normal at this dose level. In rats a slight increase in a plasma reproduction-related hormone (follicle-stimulating hormone) was reported at 50 mg B/kg bw (Lee *et al*, 1978). These effects were minimal and a no-effect-level was not determined. However the authors stated that these levels approached the no-effect level.

Ku *et al* (1993a) found mild and reversible inhibition of spermiation in rats at 26 mg B/kg bw. The authors reported a NOAEL for testicular effects of 17 mg B/kg bw in a preliminary study.

Overall a NOAEL of 17 mg B/kg bw and a LOAEL of 26 mg B/kg bw could be established for subtle testicular changes in rodents.

There are no reliable results in human beings for adverse testicular effects.

8.3.2 Developmental Aspects

There are four recent and well conducted developmental toxicity studies performed in rats (Heindel *et al*, 1992; Price *et al*, 1994), mice (Heindel *et al*, 1992) and rabbits (NTP, 1991). In these studies the rat was found to be the most sensitive species with a NOAEL of 9.6 mg B/kg bw. At a LOAEL of 13.3 mg B/kg bw a small decrease in mean foetal bodyweight (6%) a decrease in skeletal variations and an increased incidence of short rib XIII was found. The latter effect is reported by the authors as a malformation, but they recognise it may be considered alternatively as a variation (Price *et al*, 1994). Certainly, there is no evidence from this study that presence of short rib XIII influenced the normal development of affected pups. For the risk assessment the NOAEL of 9.6 mg B/kg bw is used. There are no available data for human beings concerning developmental effects.

8.4 DERIVATION OF A TOLERABLE DAILY INTAKE (TDI)

8.4.1 Basic Considerations

The criteria for setting quantitative estimates of a TDI are based not only on the determination of a NOAEL but also on the extrapolation of this level to man based on uncertainty factors.

Uncertainty factors of 10 are generally applied as default values to allow for extrapolation from animal data to human beings and for interspecies differencies if insufficient data are available (Rubery *et al*, 1990; Lewis *et al*, 1990; Meistrich, 1992; Renwick, 1993). Uncertainty factors related to inter and intraspecies extrapolation including severity of the measured parameter have to be taken into account.

Uncertainty factors may be modified, taking the following into account:

Interspecies considerations

- Toxicokinetics are comparable in animals and man,
- Comparable mechanisms of action are identified in animals and man,
- Adequacy of the extrapolation of animal data to human beings,
- Data base is adequate.

Intraspecies considerations

- No sensitive subpopulation is expected based on metabolism, age or sex,
- Reproducibility of response within each animal species,
- Adequate human data available,
- A no-effect level has been defined,
- The effects are well defined,
- Recovery from effects.

8.4.2 Tolerable Daily Intake for Borates

The NOAEL and/or the LOAEL determined for boron at the specific endpoints of reproduction and development are adequate for determining a TDI, as a threshold for such effects exists.

Interspecies factor

The toxicokinetics of borates in man and animals is similar irrespective of the route of administration. Borates are not metabolised and so there is no potential source of variation among

species, with respect to metabolic competence. The same critical effects are found in all animal species examined.

Since borate distributes throughout body water, differences in distribution between animals and man are expected to be insignificant. In all animal species tested, the main target organ was the same. Although no data concerning the target organ and its sensitivity in human beings are available, the reproductive performance of mine workers exposed to sodium borate dusts was not impaired at the relatively low doses to which they were exposed.

Consequently an UF of 3, representing half-log base 10 is considered to adequately reflect the uncertainty in interspecies variation for borates toxicity. This UF is appropriate for both reproductive and developmental toxicity endpoints.

Intraspecies factor

Because borates are not metabolised the variability within the human population can be expected to be low. For boron a NOAEL has been determined for both developmental and fertility end points.

The subpopulation of woman of child-bearing age need to be considered with respect to the possible developmental effects. Also the possibility of testicular effects in the male need to be considered. These adverse effects are well defined in animals and some recovery has been demonstrated at the higer dose levels.

Based on the fact that the borates are not metabolised, the effects are similar in all test species and the no-effect levels are clearly established, the default UF 10 is not justified and can be reduced. However, taking into account the nature of the toxic endpoints, and that the effects may be severe at higher dose levels, it would be prudent to apply a UF 10 for the combination of intraspecies comparison and severity of effects.

8.4.3 Calculation of Tolerable Daily Intake of Boron for Reproductive Effects.

The TDI of boron of a 60 kg person for various reproductive effects is calculated on the basis of the relevant experimentally determined NOAEL in animals and the application of appropriate uncertainty factors as follows:

$$TDI = \frac{NOAEL \times 60kg\ bw}{(UF\ interspecies) \times (UF\ intraspecies)}$$

Fertility:

$$TDI = \frac{17.5mgB/kg\ bw \times 60kg\ bw}{3 \times 10} \qquad TDI = 35mgB/day$$

Testicular effects:

$$TDI = \frac{17mgB/kg\ bw \times 60kg\ bw}{3 \times 10} \qquad TDI = 34mgB/day$$

Developmental aspects:

$$TDI = \frac{9.6\ mgB/kg\ bw \times 60\ kg\ bw}{3 \times 10} \qquad TDI = 19.2mgB/day$$

8.5 EXPOSURE ASSESSMENT

8.5.1 General Population

The most significant route of exposure of borates for the general population is *via* diet and drinking water. Absorption by the dermal route does not normally occur across the intact skin and inhalation exposure is unlikely. Therefore these routes are considered negligible. In Europe the dietary intake of borates could be up to 5 mg B/d and occasionally up to 7 mg B/d for specific subgroups (vegetarians). The contribution from drinking water is generally less than 1 mg B/d (see Section 7).

It should to be noted that although additional exposure to borates species in other products may occur, exposure levels will be minimal and are not taken in account in this assessment.

8.5.2 Workforce

Workers may be exposed to borates during mining, processing and handling activities.

Occupational exposure of miners to borate dusts was demonstrated by their elevated boron blood levels. Culver *et al* (1994a) calculated the daily borate intake of miners exposed to borate

containing dust and found levels ranging from 5 to 24 mg B/d as a total daily intake including diet. This study is thought to represent the maximum likely exposure to borates. At current permissible exposure levels Time Weighted Average (TWA) for borates recommended by ACGIH are 1 mg/m^3 for borax pentahydrate and anhydrous borax and 5 mg/m^3 for borax decahydrate. With exposure at such a level, boron intake is at the maximum 6 mg B/d (assuming inhalation of 10 m^3 of air containing 5 mg/m^3 borax decahydrate and 100% absorption).

8.6 RISK ASSESSMENT

8.6.1 General Population

Overall Consumption

Considering the lowest TDI of 19.2 mg B/d as adequate for protection of normal population from developmental and testicular effects, the current exposure levels are thought to be sufficiently safe and no special measures should be recommended.

Drinking Water Guideline

Based on the same TDI of 19.2 mg B/d a safe level of boron in drinking water may also be calculated: considering the assumed adult human consumption of 2 l drinking water/d and the available dietary exposure to boron, a level of approximately 1 mg B/l in the drinking water (current EU Guide Level, Council Directive relating to the Quality of Water intended for Human Consumption, 80/778/EEC) would not pose any health risk to the public. The calculated boron intake from drinking water and food would be respectively 2 and 7 mg B/d at the maximum, leading to a total daily intake of 9 mg B/d. Based on these data, there is clearly no need to reduce the current EU Guide Level of 1 mg B/l in drinking water since this level is already conservative and provides additional safety margins when compared to the lowest TDI of 19.2 mg B/d as calculated above.

In the Drinking Water Health Advisory (US-EPA, 1992), it is recommend that the relative source contribution (RSC) from drinking water is based on annual exposure data or, if data are not available, a value of 20% should be assumed as the drinking water RSC. WHO will, wherever possible, use data based on mean levels in food, air and drinking water or intakes estimated on the basis of consideration of physical and chemical properties in order to derive a water guideline value. Where such information is not available, an arbitrary default value of 10% is used. In deriving the Drinking Water Guideline for boron, WHO used the 10% default value as the RSC.

The derivation of a Drinking Water Guideline value in this document has not used a default value as adequate data are now available on boron levels in foods, beverages, mineral and drinking water (see Section 7).

Since there are no other significant sources of exposure to boron, the current EU Guide Level for boron in Drinking Water remains sufficiently conservative.

8.6.2 Workforce

In the case of occupational exposure to a predominantly male workforce only fertility/testicular effects are relevant and the appropriate TDI should be 34 mg B/d. The available data on miners most heavily exposed to borates indicate that this TDI is not exceeded. For female workers the TDI of 19.2 mg B/d, based on developmental effects, is not exceeded provided current recommended exposure levels are achieved.

8.6.3 Conclusion

Having considered the exposure of human beings to borates in normal and occupational conditions it is apparent that, even considering a worst case scenario, there is still a margin of safety between the calculated TDI and the actual intake of boron. It is concluded that no risk of adverse reproductive effect, either fertility or developmental, is expected to occur at the current boron exposure levels.

APPENDIX A. FERTILITY EFFECTS IN ANIMALS

Studies considered unsuitable for use in Risk Assessment

Acute Exposure (see 5.2.1.)

In an early study by Caujolle *et al* (1962), Wistar rats were exposed to boric acid in a single dose by gavage. The animals were divided into three groups (10 males and 10 females per group; approx. 200g bodyweight; about 10 wk old) and exposed to levels of 3,000, 4,000 or 5,000 mg boric acid/kg bw, corresponding to 525, 700 and 875 mg B/kg bw respectively. Thirty days after exposure, testicular lesions were found in survivors from the middle and high dose groups which included decreased testis volume, decreased number of sperm cells, persistence of Sertoli cells and hyperplasia of Leydig cells. The authors concluded that the maximal tolerated dose (NOAEL) for acute exposure was 525 mg B/kg bw.

Bouissou and Castagnol (1965), exposed groups of adult male rats (50 animals/group; at least 7 wk old) to a single dose of 1,000, 2,000, 3,000, 4,000 or 5,000 mg boric acid/kg bw by gavage, corresponding to 175, 350, 525, 700 and 875 mg B/kg bw respectively. After this single exposure, killings were carried out every 10 or 15 days for 130 days. Results of this acute study showed that testicular tubular atrophy began to appear at the two highest doses from day 20 onwards and were also partially present in the 525 mg B/kg bw group from day 30. Testicular recovery was complete in the 525 and 700 mg B/kg bw dose groups but not in the 875 mg B/kg bw group after an observation period of 130 days.

Repeated Exposure (see 5.2.2.)

Caujolle *et al* (1962) exposed groups of male and female rats for 30 days to 200, 400 and 800 mg boric acid/kg bw by gavage, corresponding to 35, 70 and 140 mg B/kg bw, respectively. Full details of the study protocol were not provided but the authors reported that the animals were observed for four generations for one year following exposure. Results from the parental matings showed that all males exposed to 140 mg B/kg bw were sterile. Sterility appeared in males from the 70 mg B/kg bw dose group when these were mated with females from the same exposure group or from females exposed to 140 mg B/kg bw but not when they were mated with control females. The authors reported without quantification that reduced fertility was found when males exposed to 70 mg B/kg were mated with females exposed to 35 mg B/kg bw. Although the data reporting is inadequate, a qualitative appraisal of the mating results suggest an additional effect of exposure on

female reproductive performance. Histopathological testicular changes were frequently or constantly found in animals exposed to 70 and 140 mg B/kg bw. Sertoli cells were persistent but degenerate whereas Leydig cells proliferated especially when tubular atrophy increased. No spermatozoids were found in the epidydimal canals. Only occasional, slight histopathological testicular changes were found in male animals exposed to 35 mg B/kg bw. No lesions were found in female reproductivel organs. Although no further details were presented, the authors stated that animals of the next generations showed normal fertility.

Bouissou and Castagnol (1965) exposed adult male rats (50 animals; at least 7 wk old) to 800 mg boric acid/kg bw for 30 days by gavage, corresponding to 140 mg B/kg bw. Animals were killed every 6 days during exposure and then every 10 days during the recovery period (up to 109 d). Results showed that animals exposed to 140 mg B/kg bw for 30 days had testicular atrophy and reduced tubular diameter. These effects were observed in some animals at the initial kill, and increased in severity and in the number of affected animals thereafter until all test animals were affected. The number of affected animals only began to decrease from day 45 onwards (i.e. 75 days after study start) of the recovery period. One animal of the five remaining still showed partial atrophy 79 days after the end of the exposure period.

In the same publication, the authors briefly described a further study using immature male rats (2 and 3 weeks old) which were exposed to 800 mg boric acid/kg bw (corresponding to 140 mg B/kg bw) by gavage for 30 days. Adequate details of the study protocol and the observed results were not presented. Summarising their reported studies, the authors concluded that the effect of boric acid on testicular tissue was found to be minimal in the pre-puberty phase, maximal at puberty and moderate at the post-puberty phase.

In the chronic beagle dog study by Weir and Fisher (1972), test groups of animals (4 male and 4 female dogs per group) were exposed to borax or boric acid in the diet at levels of 58, 117 and 350 ppm boron for 2 years, corresponding approximately to 1.5, 2.9 and 8.8 mg B/kg bw. One male and one female dog from each group (including the control group) were killled and necropsied after 1 year. With two exceptions, all of the remaining animals were killed at the end of the 2 year period (week 104) and gross necropsies were performed. The remaining 2 dogs were killed at 117 weeks in order to evaluate the recovery from any potential effects. No remarkable changes were noted in the animals from any group throughout the 2 year feeding study with either boric acid or borax. No effects on bodyweight or food consumption were found and, at necropsy, no adverse findings were reported.

In a second dog study by the same authors, two additional test groups of animals (again 4 males and 4 females per group) were exposed to borax or boric acid at 1,170 ppm boron in the diet (corresponding to 29.3 mg B/kg bw) for a shorter exposure period of 38 weeks. In this test regime, two male and two female dogs from each group were killed at 26 weeks and most of the remaining animals were killed at 38 weeks. However, one male and one female dog exposed to the test substance were placed on control food during a 25 day period following exposure to evaluate reversibility of effects. Dogs exposed to 29.3 mg B/kg bw showed testicular changes similar to those observed in the rat study (see Section 5.2.2). Severe testicular atrophy and spermatogenic arrest were observed in two dogs killed at 26 weeks in the group exposed to borax. Microscopic examination of the dogs exposed to boric acid showed spermatogenic arrest and atrophy of the seminiferous tubules at 38 weeks. No other adverse effects were observed in the exposed animals for the test period of 38 weeks. Evidence from only one animal suggested that the testicular degeneration may be of a reversible nature.

Based on the results from both dog studies, the authors concluded that 8.8 mg B/kg bw was a NOAEL for chronic exposure in the dog. However, the numbers of dogs which were used by the authors in both of these studies were too low to provide adequate data for statistical analysis on reproductive endpoints. For example, only 4 male animals were used as the same controls for both tested compounds (boric acid and borax) in the 2 year study and of these, one dog was killed after 12 months, 2 were killed at 24 months and the remaining animal was killed at 27 months. Similarly, in the test groups for the three dietary feeding levels, less than 4 dogs were evaluated at any one time due to killing at different time intervals. A closer examination of the raw data revealed that testicular effects were also observed amongst some control animals (in the 38 week study). Some doubts may thus be raised on the validity of these results for the establishment of an animal NOAEL for this reproductive endpoint. Finally, there are difficulties associated with the use of dogs for reproductive studies such as their seasonal breeding performance, inbreeding factors and insufficient historical background data which altogether suggest that the dog is not an appropriate model (ICH, 1992). Because of these arguments, the use of the data from these dog studies for a quantitative risk assessment of reproductive toxicity should be cautioned.

In a subchronic study by the US National Toxicology Program (NTP, 1987) which formed part of a carcinogenicity programme (see section 4 for further details), B6C3F_1 mice (10 males and 10 females per group) were exposed to boric acid in the diet at levels of 0, 1,200, 2,500, 5,000, 10,000 and 20,000 ppm for 13 weeks. These levels corresponded to 0, 34, 71, 142, 284 and 568 mg B/kg bw for males and 0, 47, 98, 196, 392 and 784 mg B/kg bw for females based on reported feed consumption of controls at week 4 of the study. Over 60% mortality was observed at 20,000 ppm and a significant reduction in bodyweight gain in both males and females was reported

at the three highest exposures (5,000, 10,000 and 20,000 ppm). At doses > 5,000 ppm (142 mg B/kg bw for the male), degeneration or atrophy of the seminiferous tubules was observed.

In the follow-up carcinogenicity study (NTP, 1987), B6C3F$_1$ mice (50 males and 50 females per group) were exposed to boric acid in the diet at levels of 0, 2,500 and 5,000 ppm for 2 years. According to the authors, these levels corresponded approximately to 0, 78 and 201 mg B/kg bw respectively. At 201 mg B/kg bw, an increased incidence of testicular atrophy and interstitial cell hyperplasia was reported in males.

Harris *et al* (1992) investigated the reproductive toxicity of boric acid to Swiss CD-1 mice as part of an assessment of a short-term screening protocol. Groups of male animals (10 animals/group; 12-14 wk old) were exposed to boric acid by gavage at concentrations of 0, 120, 400 and 1,200 mg boric acid/kg bw from study day 3 to necropsy on day 20. Groups of female animals (10 animals/group; 12-14 wk old) were exposed to the same levels of boric acid from study day 0 to day 20. These doses correspond to 0, 21, 70 and 210 mg B/kg bw. Mating occurred during days 8 to 12 and necropsies took place on day 20 (males) and day 21 (females). At 210 mg B/kg bw, significant histopathological changes in testis were noted including germ cell loss and tubular disruption. Reduced testis weight was observed at 210 mg B/kg bw and also at 70 mg B/kg bw. Apart from the testis weight changes, the animals exposed to 70 mg B/kg bw were generally indistinguishable from controls. In the females, death occurred in 3 animals out of 10 at 210 mg B/kg bw but no significant effects on female fertility parameters were reported.

DRINKING WATER STUDIES

Dixon *et al* (1976) exposed groups of rats to borax in drinking water for 90 days at concentrations of 0.3, 1.0 or 6.0 mg B/l (numbers of animals used were not reported). Randomly selected animals were studied following 30, 60 and 90 days of exposure. The authors assumed that the animals drank approximately an average of 35ml water per day, and calculated the maximum boron daily exposure in this study to be 0.84 mg B/kg bw. The authors reported that this subchronic study failed to reveal any biologically significant change in clinical serum chemistry, bodyweight, organ weights (testis, prostate, seminal vesicles) or in hormone plasma levels (follicle-stimulating hormone, FSH and luteinizing hormone, LH). Further, breeding studies failed to reveal any effects on male fertility following exposure. The authors concluded that exposure of the male rat to boron at the levels in the study was without significant reproductive toxicity when administered subchronically in drinking water.

Krasovskii *et al* (1976) exposed groups of white male rats (number of animals not reported; + 300g bodyweight) to boric acid in drinking water for 6 months at concentrations of 0.3, 1 and 6 mg B/l. The authors stated that these doses corresponded to 0.015, 0.05 and 0.3 mg B/kg bw, respectively. At 0.3 mg B/kg bw, the gonad weight/bw ratio was decreased and exposure also caused a decline in the mobility of spermatozoids, a reduction of their number and in their acid and osmotic resistance. Rats exposed to 0.05 mg B/kg bw showed a reduction in the number of spermatozoids and a reduction in their mobility time and acid resistance. However at this dose, other parameters including weight factors, nature of spermatozoid motility and osmotic resistance were not affected. The authors concluded that 0.015 mg B/kg bw (equivalent to 0.3 mg B/l) was a no adverse effect level in this study.

Repeated exposure of male rats to boron in drinking water was only performed in two reported studies and these data appear controversial. The relevance of the subtle effects observed in the drinking water study by Krasovskii *et al* (1976) either statistically or biologically is questionable especially since, in a related study, Dixon *et al* (1976) did not observe any effects on male reproductive organ weights or on fertility at comparable or higher exposures. As both of these studies are inadequately reported, a clear no effect level cannot be defined.

DEVELOPMENTAL EFFECTS IN ANIMALS

Harris *et al* (1992) assessed the developmental toxicity of boric acid in Swiss CD-1 mice in a screening protocol. Dams (approximately 10 pregnant females/group; 12-14 week old) were exposed to boric acid by gavage at levels of 0, 120, 400 and 1,200 mg boric acid/kg bw during organogenesis (days 8-14). These doses corresponded to 0, 21, 70 and 210 mg B/kg bw, respectively. The pregnant animals were allowed to deliver and pups were examined on postnatal day 0, 1 and 4. Necropsy occurred for pups and dams on postnatal day 4. Complete resorption of implants occurred in all females exposed to 210 mg B/kg bw. At all other exposures, no effects occurred on implantation, number of live pups or pup weights.

BIBLIOGRAPHY

Abou-Shakra FR, Havercroft JM and Ward NI, 1989. Lithium and Boron in Biological Tissues and Fluids. Trace Elements in Medicine 6 (4), 142-146.

Alexander GV, Nusbaum R.E. and MacDonald N.S, 1951. The Boron and Lithium Content of Human Bones. J. Biol. Chem. 192, 489-496.

Allen HE, Halley-Henderson MA and Hass CN, 1989. Chemical composition of bottled mineral water. Arch. Environ. Health 44(2), 102-116.

Anderson SA, Sauls HR Pearce SW and Fail PA, 1992. Endocrine responses after boric acid exposure for 2, 9 or 14 days in cannulated male rats. Biology of reproduction 46, Suppl. 1, 124.

Andrasi E, Suhajda M, Saray I, Bezur L, Ernyei L and Reffy A, 1993. Concentration of elements in human brain: gliobastoma multiforme. The Science of the Total Environment 139, 399-402.

Astier A, Baud F and Fournier A, 1988. Toxicocinetique du bore apres une intoxication accidentelle massive par l'acide borique. Toxicokinetics of Boron after a massive Accidental Ingestion of Boric Acid. J. Pharm. Clin. 7, (2), 57-62.

Bakke JP, 1991. Evaluation of the potential of boric acid to induce unscheduled DNA synthesis in the *in vitro* hepatocyte DNA repair assay using the male F-344 Rat, SRI International, Study No. 2389-V500-91, 23 August 1991, Report to U.S. Borax Inc.

Barber JE, 1987. A bleaching agent: eye irritation study (low volume). Unpublished Report No CTL/L/1532, 11 August 1987. ICI, CTL.

Barlow SM and Sullivan FM, 1982. Reproductive Hazards of Industrial Chemicals. Academic Press, London (ISBN 0-12-078960-4), 126-135.

Barr RD, Clarke WB, Clarke RM, Venturelli J, Norman GR and Downing RG, 1993. Regulation of lithium and boron levels in normal human blood; environmental and genetic considerations. J. Lab. Clin. Med. 121(4), 614-619.

Barrès M, 1967. Contribution à l'étude de l'isopoly-condensation des borates alcalins par électrométrie et partages. Rev. Chim. Miner. 4, 803-838; Chem. Abstr. 1968, 69, 30628.

Beattie JH and Peace HS, 1993. The influence of a low boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in postmenopausal women. Br. J. Nutrition 69, 871-884.

Benson WH, Birge WJ and Dorough HW, 1984. Absence of mutagenic activity of sodium borate (borax) and boric acid in the *Salmonella* preincubation test. Environ. Toxicol. and Chemistry 3, 209-214.

Bergmann W, 1988. Ernährungsstörungen bei Kulturpflanzen (Nutritional Disorders in Crops). 2nd Edition, VEB Gustav Fischer Verlag Jena (ISBN 3-334-00248-9), Germany.

Beyer KH, Bergfeld WF, Berndt WO, Boutwell RK, Carlton WW, Hoffmann DK and Schroeter AL, 1983. Final report on the safety assessment of sodium borate and boric acid. J. of Am. College of Toxicol. 2(7), 87-125.

Bio/dynamics Inc., 1982. An acute inhalation toxicity of boric acid in the rat. Report, 29 June 1982, Project No. 82-7563, prepared for U.S. Borax Bio/dynamics Inc., P.O. Box 43, Mettlers Road, East Millstone, New Jersey, 08873, USA.

Borax Consolidated, 1992. Booklet on Borax products and their applications. Borax Consolidated Ltd, Guilford, Surrey, UK.

Bouissou H and Castagnol R, 1965. Action de l'acide borique sur le testicule du rat. Archives des Maladies Professionelles de Médecine du Travail et de Sécurité Sociale T28 6, 293-306.

Boyland E, Roe FJC and Mitchley BCV, 1966. Test of certain constituents of spermicides for carcinogenicity in genital tract of female mice. Brit. J. of Cancer 20, 184-189.

British Standards Institution, 1979. BS5688: Part 0: Boric acid, boric oxide, disodium tetraborates, sodium perborates and crude sodium borates for industrial use. BSI, 2 Park St, London W1A 2BS.

Brown TF, McCormick ME, Morris DR and Zeringue LK, 1989. Effects of Dietary Boron on Mineral Balance in Sheep. Nutr. Res. 9, 503-512.

Casarett and Doull, 1980. Toxicology, 2nd edition, MacMillan Publishing Co. Inc., New York, 440.

Caujolle F, 1951. Le bore en thérapeutique. Produits Pharmaceutiques 6, 117-124.

Caujolle F, Familiades C and Gout R, 1962. Limite de tolérance du rat à l'acide borique. Comptes rendus des scéances des l'Académie des Sciences 254, 3449-3551.

CEFIC, 1993. Review paper on boron ecotoxicity/toxicity, Peroxygen Sector Group, Conseil Européen des Fédérations de l'Industrie Chimique, Avenue E. Van Nieuwenhuyse 4, bte 2, B - 1160 BRUSSELS, Belgium.

CEFIC, 1994. Statistics of the Peroxigen Sector Group, Conseil Européen des Fédérations de l'Industrie Chimique, Avenue E. Van Nieuwenhuyse 4, bte 2, B - 1160 BRUSSELS, Belgium.

CEH, 1993. Boron minerals and chemicals. November 1993, in Chemical Economics Handbook, SRI International 717.1002 C.

Chater BV, 1978. Sodium perborate. Acute oral toxicity with histology and skin and eye irritation. Unpublished Report No: CTL/T/1150, 28 July 1978. ICI, CTL.

Ciba J and Chrusciel A, 1992. Spectrophotometric determination of boron in human hair with Azomethine H. Fresenius J. Anal. Chem. 342, 147-149.

Clarke WB, Webber CE and Koekebakker M. 1987a. Lithium and boron in human blood. J. Lab. Clin. Med. 109, 155-158.

Clarke WB, Koekebakker M, Barr RD, Downing RG and Fleming RF, 1987b. Analysis of ultratrace lithium and boron by neutron activation and mass-spectrometric measurement of ^{3}He and ^{4}He, Appl. Radiat. Isot. 38, 735-743.

Culver BD, Shen PT, Taylor TH, Lee-Feldstein A, Anton-Culver H and Strong PL, 1994a. The relationship of blood- and urine-boron to boron exposure in borax-workers and the usefulness of urine-boron as an exposure marker. Environ. Health Perspect. 102 (suppl. 7), 133-137.

Culver BD, Smith RG, Brotherton RJ, Strong PL, Gray TM, 1994b. Boron. In: Patty's Industrial Hygiene and Toxicology, John Wiley & Sons Inc., New York, N.Y. 4th Edition, Volume 2F, Chapter 42, 4411-4448.

Dawber JG and Matusin DH, 1982. Potentiometric and polarimetric studies of the reaction of boric acid and tetrahydroxyborate ion with polyhydroxy compounds. J. Chem. Soc. Faraday Trans. 1, 78, 2521-2528.

Degussa, 1989. Sodium perborate tetrahydrate. 4-Week oral toxicity study after repeated administration in rats, Study No.: 867666. Degussa AG, Hanau, Germany.

Demerec M, Bertani G and Flint J, 1951. A survey of chemicals for mutagenic action on *E. Coli*. Am. Naturalist 85, 119-136.

Dieter Dr H, 1993. Private communication. Bundesgesundheitsamt, Berlin, Germany.

Dixon RL, Lee IP and Sherins RJ, 1976. Methods to assess reproductive effects of environmental chemicals: Studies of cadmium and boron administered orally. Environ. Health Perspect. 13, 59-67.

Doyle RL, 1989a. Primary eye irritation of boric acid. Ref. 88-3444-21 of 7 February 1989, (Unpublished Report No. TX-089-006 to U.S. Borax) Hill Top Biolabs, Inc. Cincinnati, Ohio 45242, USA.

Doyle RL, 1989b. Primary eye irritation of Borax 10 Mol. Ref. 88-3443-21 of 30 January 1989, (Unpublished Report No. TX-089-007 to U.S. Borax) Hill Top Biolabs, Inc. Cincinnati, Ohio 45242, USA.

Draize JH and Kelley EA, 1959. The Urinary Excretion of Boric Acid Preparations following Oral Administration and Topical Applications to Intact and Damaged Skin of Rabbits, Toxicol. Appl. Pharmacol. 1, 267-276.

Draper A, Lewis J, Malhotra N and Wheeler E, 1993. The energy and nutrient intakes of different types of vegetarian: a case for supplements? British Journal of Nutrition 69, 3-19, Table 7.

Dufour JJ, Rogg C and Cerioli A, 1971. Rapport final d'une etude des effets toxiques et vomitifs du perborate de sodium. Institut Battelle, Centre de recherche, 7 route de Drize, 1227 Carouge/Geneva, Switzerland.

ECETOC, 1995. Ecotoxicology of some inorganic borates. Technical Report in preparation.

Edwall L, Karlén B and Rosén A, 1979. Absorption of boron after mouthwash treatment with Bocosept. Europ. J. Clin. Pharmacol. 15, 417-420.

EPA, 1991. Health and environmental effects document for boron and boron compounds, U. S. Environmental Protection Agency, Environ. Report EPA/600/8-91/015; Order No. PB91-233635, 269pp. Criteria Assess Off. Cincinnati, OH, USA.

EPA, 1992. Boron. Drinking water health advisory, Office of Water, U.S. Environmental Protection Agency, April 1992.

EUROMONITOR, 1992. European Marketing Data and Statistics 1992 (ISBN 0-86338-403 X).

Fail PA, George JD, Seely JC, Grizzle TB and Heindel JJ, 1991. Reproductive toxicity of boric acid in Swiss (CD-1) mice: assessment using the continuous breeding protocol. Fundam. Appl. Toxicol. 17, 225-239.

Farr LE and Konikowski T, 1963. The Renal Clearance of Sodium Pentaborate in Mice and Men. Clin. Chem. 9, 717-726.

Forbes RM, Cooper AR and Mitchell HH, 1954. On the Occurrence of Beryllium, Boron, Cobalt and Mercury in Human Tissues. J. Biol. Chem. 209, 857-865.

Forbes RM and Mitchell HHJ, 1957. Accumulation of Dietary Boron and Strontium in Young Adult Albino Rats. Arch. Ind. Health 16, 489-492.

Friis-Hansen B, Aggerbeck B and Jansen JA, 1982. Unaffected blood boron levels in newborn infants treated with a boric acid ointment. Food Chem. Toxicol. 20, 451-454.

Glaza SM, 1988. Acute oral toxicity study of sodium perborate monohydrate (grade A) in rats. Report HLA 71002522 to Solvay-Interox. Hazleton Laboratories America Inc. 3301 Kinsman Boulevard, Madison, Wisconsin 53704.

Goldbloom RB and Goldbloom A, 1953. Boric acid poisoning. Report of four cases and a review of 109 cases from the world literature. J. Pediat. 43, 631-643.

Gosselin RE, Hodge HC, Smith RP and Gleason MN, 1976. Clinical Toxicology of Commercial Products. The Williams & Wilkins Co., Baltimore, 4th Edition, Section III, 63-66.

Graffmann G, Kuzel P, Nösler H and Nonnenmacher G, 1974. Spurenbestimmung von Bor in Oberflächengewässern und Trinkwässern. Chemiker Zeitung 98, 499-504.

Gregory J, Foster K, Tyler H and Wiseman M, 1990. Table on consumption of wine and fortified wine. The Dietary and Nutritional Survey of British Adults, 1990, H.M Stationery Office, London.

Grizzle TB, Sauls HR, Dennis SW and Fail PA, 1989. Response of testis to HCG in Swiss mice (CD-1) following chronic exposure to boric acid (BORA). Biol. Reprod. (Suppl. 1)40, 153.

Hamilton EI and Minski MJ, 1972/1973. Abundance of the chemical elements in man's diet and possible relations with environmental factors. Sci. Total Environ. 1, 375-394.

Hansen KS, 1983. Occupational dermatoses in hospital cleaning women. Contact Dermatitis 9, 343-351.

Harris MW, Chapin RE, Lockhart AC and Jokinen MP, 1992. Assessment of a short-term reproductive and developmental toxicity screen. Fundam. Appl. Toxicol. 19, 186-196.

Haworth S, Lawlor T and Mortelmans K, 1983. Salmonella mutagenicity test results for 250 chemicals. Environ. Mutagen. Suppl. 1, 3-142.

Heindel JJ and Treinen KA, 1989. Physiology of the male reproductive system: endocrine, paraovine and autocrine regulation. Toxicologic Pathology 17, 2, 577-590.

Heindel JJ, Price CJ, Field EA, Marr MC, Myers CB, Morrissey RE and Schwetz BA, 1992. Developmental toxicity of boric acid in mice and rats. Fundam. Appl. Toxicol. 18, 266-277.

Hu X, Wegman DH, Eisen EA, Woskie SR and Smith RG, 1992. Dose related acute irritant symptom responses to occupational exposure to sodium borate dusts. Br. J. Ind. Med. 49, 706-713.

ICH, 1992. On detection of toxicity to reproduction for medicinal products. Step 2 of the ICH procedure. Medicines Control Agency, London.

Imbus HR, Cholak J, Miller LH and Sterling T, 1963. Boron, Cadmium, Chromium and Nickel in blood and urine. Arch. of Environ. Health 6, 286-295.

Interox Chemicals, 1982a. Product data sheet on SODIUM PERBORATE TETRAHYDRATE, FP 2.1.10-U.K.-2,6c-1282. Interox Chemicals Ltd, UK, P.O. Box 7, Warrington, Cheshire, England WA4 6HB.

Interox Chemicals Ltd, 1982b. Product data sheet on SODIUM PERBORATE MONOHYDRATE, FP 2.1.9-U.K.-2,6c-1282. Interox Chemicals Ltd, UK, P.O. Box 7, Warrington, Cheshire, England WA4 6HB.

Iyengar GV, Tanner JT, Wolf WR and Zeisler R, 1987. Preparation of a mixed human diet material for the determination of nutrient elements, selected toxic elements and organic nutrients: a preliminary report. Sci. Total Environ. 61, 235-252.

Iyengar GV, Clarke WB, Downing RG and Tanner JT, 1988. Lithium in biological and dietary materials. Trace Elem. Anal. Chem. Med. Biol. Proc. Int. Workshop, 5th, 267-269.

Iyengar GV, Clarke WB and Downing RG, 1990. Determination of boron and lithium in diverse biological matrices using neutron activation-mass spectrometry (NA-MS). Fresenius J. Anal. Chem. 338, 562-566.

Iyer VN and Szybalski W, 1958. Two simple methods for the detection of chemical mutagens. Appl. Microbiol. 6, 23-29.

Jansen JA, Schou JS and Aggerbeck B, 1984a. Gastro-intestinal absorption and *in vitro* release of boric acid from water-emulsifying ointments. Fd. Chem. Toxic. 22, 49-53.

Jansen JA, Andersen J and Schou JS, 1984b. Boric Acid Single Dose Pharmacokinetics after Intravenous Administration to Man. Arch. Toxicol. 55, 64-67.

Jaspersen HP and Schlumpf M, 1969. Borsäure-ja oder nein? Schw. Apth. Ztg. 107, 496-503.

Job C, 1973. Resorption und Ausscheidung von peroral zugeführtem Bor (in English, Absorption and Excretion of Orally Ingested Boron). Zeitschrift für angewandte Bäder - und Klimaheilkunde 20, 137-142.

Kliegel W, 1980. Bor in Biologie, Medizin, und Pharmazie. Springer-Verlag Berlin Heidelberg New York (ISBN 3-540-93411-1).

Krasovskii GN, Varshavskaya SP, and Borisov AI, 1976. Toxic and gonadotropic effects of cadmium and boron relative to standards for these substances in drinking water. Environ. Health Perspect. 13, 69-75.

Krause C, Chutsch M, Henke M, Huber M, Kliem C, Schulz C and Schwarz E., 1989. Umwelt-Survey Band I. Studienbeschreibung und Humanbiologisches Monitoring. WaBoLu-Hefte 5/1989, 304 pp; Chem. Abstr. 1991, 114, 87707e.

Ku WW, Chapin RE, Moseman RF, Brink RE, Pierce KD and Adams KY, 1991. Tissue disposition of boron in rats. Toxicol. Appl. Pharmacol. 111, 145-151.

Ku WW, Chapin RE, Wine RN and Gladen BC, 1993a. Testicular toxicity of boric acid (BA); Relationship of dose to lesion development and recovery in the F344 rat. Reprod. Toxicol. 7, 305-319

Ku WW, Shih LM and Chapin RE, 1993b. The effects of boric acid (BA) on testicular cells in culture. Reprod. Toxicol. 7, 321-331.

Ku WW and Chapin RE, 1994. On the mechanism of testicular toxicity of boric acid in rats; *In vivo* and *in vitro* studies. Environm. Health Persp. 102 supp 7, 99-105.

Landolph JR, 1985. Cytotoxicity and negligible genotoxicity of borax ores to cultured mammalian cells. Am. J. Ind. Med. 7, 31-43.

Laurent-Pettersson M, Delpech B and Hellier M, 1992. The mapping of natural boron in histological sections of mouse tissues by the use of neutron-capture radiography. Histochemical Journal 24, 939-950.

Lee IP, Sherins RJ and Dixon RL, 1978. Evidence for induction of germinal aplasia in male rats by environmental exposure to boron. Toxicol. Appl. Pharmacol. 45, 577-590

Lehman AJ, 1959. Appraisal of the safety of chemicals in foods, drugs and cosmetics. Association of Food and Drug Officials of the United States.

Levinskas GJ, 1964. Toxicology of boron compounds. In : Boron, Metallo-boron Compounds and Boranes. Ed. Adams RW, Wiley, 693-737.

Lewis SC, Lynch JR and Nikiforov AI, 1990. A New Approach to Deriving Community Exposure Guidelines from "No-Observed-Adverse-Effect Levels". Regulatory Toxicology and Pharmacology 11, 314-330.

Linden CH, Hall AH, Kulig KW and Rumack BH, 1986. Acute ingestions of boric acid. Clin. Toxicol. 24, 269-279.

Linder RE, Strader LF and Rehnberg GL, 1990. Effect of acute exposure to boric acid on the male reproductive system of the rat. J. Toxicol. Environ. Health 31, 133-146

Lister J, 1875. Recent improvements in the details of antiseptic surgery. The Lancet 603-605.

Litovitz TL, Klein-Schwartz W, Oderda GM and Schmitz BF, 1988. Clinical Manifestations of Toxicity in a Series of 784 Boric Acid Ingestions, Am. J. Emerg. Med. 6 (3), 209-213.

Locksley HB and Sweet WH, 1954. Tissue Distribution of Boron Compounds in relation to Neutron-capture Therapy of Cancer, Proc. Soc. Exp. Biol. Med. 86, 56-63.

Luis Cáceres V, Erika Gruttner D and René Contreras N, 1992. Water recycling in arid regions: Chilean case. Ambio 21(2), 138-144.

Lyday PA, 1992. Boron, annual report 1991, U.S. department of the interior, U.S. bureau of mines, September 1992, Table 1.

MAFF, 1991. UK Ministry of Agriculture, Fisheries and Food. Household food consumption and Expenditure 1991. Annual Report of the National Food Survey Committee, H.M. Stationery Office, Table B1.

Martindale, 1977. The Extra Pharmacopoeia. 27th Edition, The Pharmaceutical Press, London, 1202.

Massie HR, Aiello VR, Shumway AE and Armstrong T, 1990. Calcium, iron, copper, boron, collagen and density changes in bone with aging in C57BL/65 mice. Exp. Gerontol. 25, 469-481.

Meachan SL, Taper LJ and Volpe SL, 1994. The effects of boron supplementation on bone mineral density, dietary, blood and urinary calcium, phosphorus, magnesium and boron in female athletes. Environ. Health Persp. 102 (Suppl. 7) 79-82.

Meistrich ML, 1992. A Method for Quantitative Assessment of Reproductive Risks to the Human Male. Fund. Appl. Toxicol. 18, 479-490.

Minoia C, Gregotti C, Di Nucci A, Candura SM, Tonini M and Manzo L, 1987. Toxicology and health impact of environmental exposure to boron. G. Ital. Med. Lav. 9, 119-124.

Minoia C, Sabbioni E, Apostoli P, Pietra R, Pozzoli L, Gallorini M, Nicolaou G, Alessio L and Capodaglio E, 1990. Trace element reference values in tissues from inhabitants of the European Community I. A study of 46 elements in urine, blood and serum of Italian subjects. The Science of the Total Environment 95, 89-105.

Momma J, Takada K, Suzuki Y and Tobe M, 1986. Acute oral toxicity and ocular irritation of chemicals in bleaching agents. J. Food Hygienic Society of Japan 27, 553-560. (German translation available).

Moreno OM, Cerven DR, Cerven BW and Altenbach EJ, 1987a. Sodium perborate monohydrate - LD_{50} in rats. Report MB 87-8676 A to Solvay/Interox. MB Research Laboratories, Inc. Spinnerstown, PA 18968, USA.

Moreno OM, Cerven DR, Cerven BW and Altenbach EJ, 1987b. Sodium perborate monohydrate - acute dermal toxicity in albino rabbits. Report MB 87-8676 B to Solvay/Interox. MB Research Laboratories, Inc. Spinnerstown, PA 18968, USA.

Moreno OM, Cerven DR, Cerven BW and Altenbach EJ, 1987c. Sodium perborate monohydrate - OECD test for dermal irritation/corrosion in albino rabbits. Report MB 87-8676 C to Solvay/Interox. MB Research Laboratories, Inc. Spinnerstown, PA 18968, USA.

Moreno OM, Cerven DR, Cerven BW and Altenbach EJ, 1987d. Sodium perborate monohydrate - Acute eye irritation/corrosion in rabbits. Report MB 87-8676 C to Solvay/Interox. MB Research Laboratories, Inc. Spinnerstown, PA 18968, USA.

Moreno OM, Fritz LK, Cerven BW and Altenbach EJ, 1987e. Sodium perborate monohydrate. Skin sensitization study in guinea pigs. Project-No.: MB 87-8676 F to Solvay/Interox. MB Research Laboratories Inc. Spinnerstown, PA 18968, USA.

Mullinos MG, Higgins GK and Christakis GJ, 1952. On the toxicity of sodium perborate. J. Soc. Cosmet. Chem. 3, 297-302.

Nielsen GH, 1970. Percutaneous Absorption of Boric Acid from Boron-containing Preparations in Rats. Acta Pharmacol. et Toxicol. 28, 413-424.

Nielsen FH, Hunt CD, Mullen LM and Hunt JR, 1987. Effect of dietary boron on mineral, oestrogen and testosterone metabolism in postmenopausal women. FASEB J 1, 394-397.

Nielsen FH, 1992. Facts and fallacies about boron. Nutrition Today, May/June, 6-12.

NTP, 1987. Toxicology and carcinogenesis studies of boric acid in $B6C3F_1$ mice. National toxicology program technical report series No. 324, U.S. Department of health and human services; Chem. Abstr. 1988, 108, 107932f.

NTP, 1991. Price CJ, Marr MC, Myers CB, Heindel JJ and Schwetz BA. Final Report on the Developmental Toxicity of Boric Acid in New Zealand White Rabbits. National Toxicology Program, National Institute of Environmental Health Sciences, November 1991 (NTIS Accession No. PB92-129550); Laboratory Supplement, December 1991 (NTIS Accession No. PB92-129568).

O'Loughlin KG, 1991. Bone marrow erythrocyte micronucleus assay of boric acid in Swiss-Webster mice. Study No. 2389-C400-91, 19 August 1991, (Report to U.S. Borax Inc.) SRI International, USA.

Owen EC, 1944. The excretion of borate by the Dairy Cow. J. Dairy Res. 13, 243-248.

Pfeiffer CC, Hallman LF and Gersh I, 1945. Boric acid ointment. A study of possible intoxication in the treatment of burns. J. Amer. Med. Assoc. 128(4), 266-274.

Pfeiffer CC and Jenney EH, 1950. The pharmacology of boric acid and boron compounds. Bull. Natl. Form. Comm. 18, 57-80.

Ploquin J, 1967. Le bore dans l'alimentation. Bul. de la Société Scientifique d'Hygiène Alimentaire 55, (1-3), 70-113.

Price CJ, Marr MC and Myers CB, 1994. Determination of the NOAEL for developmental toxicity in Sprague-Dawley rats exposed to boric acid in feed on gestational days 0 to 20, and evaluation of postnatal recovery through postnatal day 21. Report 65C-5657-200. Research Triangle Park NC, USA.

Procter and Gamble, 1966a. Report BTS No 454. Professional and Regulatory Services, Procter and Gamble, Strombeek Bever, Belgium.

Procter and Gamble, 1966b. Report V1927-108. Professional and Regulatory Services, Procter and Gamble, Strombeek Bever, Belgium.

Rasi U, 1973. Contribution to the problem of the acute intoxication through boric acid and borates (in German). Ph.D. Thesis, University of Zürich, Switzerland.

Reagan EL and Becci PJ, 1985a. Acute oral LD_{50} study of 20 Mule Team, lot no. USB-12-84 sodium tetraborate pentahydrate in Sprague-Dawley rats. Report TX-85-5 of 1 February 1985 to U.S. Borax. Food & Drug Research Laboratories, Inc. Waverly, NY 14892-0107, USA.

Reagan EL and Becci PJ, 1985b. Acute dermal toxicity study of 20 Mule Team, lot no. USB-12-84 sodium tetraborate pentahydrate in New Zealand white rabbits. Report TX-85-6 of 20 February 1985 to U.S. Borax. Food & Drug Research Laboratories, Inc. Waverly, NY 14892-0107, USA.

Reagan EL and Becci PJ, 1985c. Primary dermal irritation study of 20 Mule Team, lot no. USB-12-84 sodium tetraborate pentahydrate in New Zealand white rabbits. Report TX-85-9 of 23 January 1985 to U.S. Borax. Food & Drug Research Laboratories, Inc. Waverly, NY 14892-0107, USA.

Reagan EL and Becci PJ, 1985d. Primary eye irritation study of 20 Mule Team, lot no. USB-12-84 sodium tetraborate pentahydrate in New Zealand white rabbits. Report TX-85-10 of 8 February 1985 to U.S. Borax. Food & Drug Research Laboratories, Inc. Waverly, NY 14892-0107, USA.

Renwick AG, 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. Food Additives and Contaminants 10(3), 275-305.

Roe DA, McCormick DB and Lin RT, 1972. Effects on riboflavin on boric acid toxicity. J. Pharm. Sci. 61, 1081-1085.

Rosenkranz HS, 1973. Sodium hypochlorite and sodium perborate; preferential inhibitors of DNA polymerase deficient bacteria. Mutation Research 21, 171-174.

Roudabush RL, Terhaar CJ, Fassett DW and Dziuba SP, 1965. Comparative acute effects of some chemicals on the skin of rabbits and guinea pigs. Toxicol. Appl. Pharmacol. 7, 559-565.

RTECS, 1993. Boric acid (CAS 10043-35-3) and Sodium borate decahydrate (CAS 1303-96-4) in : Registry of toxic effects of chemical substances. National Institute for Occupational Safety and Health, January 1993 .

Rubery ED, Barlow SM and Steadman JH, 1990. Criteria for setting quantitative estimates of acceptable intakes of chemicals in food in the UK. Food Additives and Contaminants 7(3), 287-302.

Rudd CJ, 1991 . Mouse lymphoma cell mutagenesis assay ($tk^{+/-}/tk^{-/-}$) of Boric Acid. Study No. 2389-G300-91, 23 August 1991. Report to U.S. Borax Inc. SRI International, USA.

Sabbioni E, Nicolaou GR, Pietra R, Beccaloni E, Coni E, Alimonti A and Caroli S, 1990. Inductively coupled atomic emission spectrometry and neutron activation analysis for the determination of element reference values in human lung tissue. Biological Trace Element Research 26-27, 757-768.

Schou JS, Jansen JA and Aggerbeck B, 1984. Human Pharmacokinetics and Safety of Boric Acid. Arch. Toxicol. Suppl. 7, 232-235.

Seal BS and Weeth HJ, 1980. Effect of boron in drinking water on the laboratory rat. Bull. Environm. Contam. Toxicol. 25, 782-789.

Seiler JP, 1989. The mutagenic activity of sodium perborate. Mutation Research 224, 219-227.

Shuler TR, Pootrakul P, Yarnsukon P and Nielsen FH, 1990. Effect of thalassemia/haemoglobin E disease on macro, trace and ultrartrace element concentrations in human tissue. J. Trace Elem. Exp. Med. 3, 31-43.

Silaev AA, 1984. Experimental determination of the maximum permissible concentration of sodium perborate in workplace air. Gig. Tr. Prof. Zabol. 6, 44 (in Russian, French translation).

Southwood J, 1986a. Sodium perborate tetrahydrate: Skin irritation and eye irritation studies, Report No CTL/T/2427. 10 January 1986. ICI, CTL.

Southwood J, 1986b. Sodium perborate monohydrate: Skin irritation and eye irritation studies, Report No CTL/T/2423. ICI, CTL.

Stewart KR, 1991. Salmonella/microsome plate incorporation assay of boric acid. Study No. 2389-A200-91, 12 August 1991. Report to U.S. Borax Inc. SRI International, USA.

Stüttgen G, Siebel Th and Aggerbeck B, 1982. Absorption of Boric Acid through Human Skin Depending on the Type of Vehicle. Arch. Dermatol. Res. 272, 21-29.

Tarasenko N Yu, Kasparov AA and Strongina OM, 1972. Effect of boric acid on the generative function in males. Gigiena Truda i Professionalnye Zabolevaniya 11, 13-16 (English translation).

Treinen KA and Chapin RE, 1991. Development of Testicular Lesions in F344 Rats after Treatment with Boric Acid. Toxicol. and Appl. Pharmacol. 107, 325-335.

UNEP, 1993. Boron and its inorganic compounds. United Nations Environment Programme, International Register of Potentially Toxic Chemicals, 1-109. Palais des Nations, CH-1211 Geneva 10, Switzerland.

Unilever, ESL, 1993. A statistical evaluation of the relationship of boron in drinking water against boron in blood. Unilever Research, UK.

US-ATSDR, 1992. Toxicological profile for boron, U.S. Department of Health & Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Final Version, July 1992, 1-86.

US-EPA, 1991. Health and environmental effects document for boron and boron compounds, U. S. Environmental Protection Agency, Environ. Report EPA/600/8-91/015; Order No. PB91-233635, 269pp. Criteria Assess Off. Cincinnati, OH, USA.

US-EPA, 1992. Boron. Drinking water health advisory, Office of Water, U.S. Environmental Protection Agency, April 1992.

Valdes-Dapena MA and Arey JB, 1962. Boric acid poisoning. J. Pediat. 61, 531-546.

Varo P and Koivistoinen P, 1980. XII. General discussion and nutritional evaluation. In: Mineral Element Composition of Finnish Foods 165. Ed. Koivistoinen P, Acta Agriculturae Scandinavica, Supplementum 22, Stockholm.

Ward NL, 1987. The determination of boron in biological materials by neutron irradiation and prompt gamma-ray spectrometry. J. Radioanalytical and Nuclear Chemistry. 110(2), 633-639.

Weeth HJ, Speth CF and Hanks DR, 1981. Boron Content of Plasma and Urine as Indicators of Boron Intake in Cattle. Am. J. Vet. Res. 42, 474-477.

Wegman DH, Eisen EA and Smith RG, 1991. Acute and chronic respiratory effects of sodium borate particulate exposures. Unpublished report to U.S. Borax Inc. 3 January 1991.

Weiner AS, Conine DL and Doyle RL, 1982. Acute dermal toxicity screen in rabbits; Primary skin irritation study in rabbits of boric acid. Ref. 82-0280-21 of 15 March 1982, Report No. TX-82-10 to U.S. Borax, Hill Top Research, Inc. Cincinnati, Ohio 45242, USA.

Weir RJ and Fisher RS, 1961-1967. Full toxicologic study reports on borax and boric acid. Volumes 1-8, prepared for U.S. Borax Hazleton Laboratories, Inc. Virginia, USA.

Weir RJ and Fisher RS, 1972. Toxicologic studies on borax and boric acid. Toxicol. Appl. Pharmacol. 23, 351-364.

Whorton MD, Haas JL, Trent L and Wong O, 1994. Reproductive effects of sodium borates on male employees; birth rate assessment. Occup. & Environ. Med. 51, 761-767.

Wilding JL, Smith WJ, Yevich P, Sicks ME, Ryan SG and Punte CL, 1959. The toxicity of boron oxide. Am. Ind. Hyg. Assoc. 20, 284,-289.

Wiley HW, 1904. Influence of food preservatives and artificial colors on digestion and health, I-Boric Acid and Borax. U.S. Department of Agriculture, Bureau of Chemistry - Bulletin 84, Part I, I-477. Washington, USA.

Woittiez JRW and Iyengar GV, 1988. Trace elements in human clinical specimens; evaluation of literature data to identify reference values. Trace element. Anal. Chem. Med. Proc. Int. Workshop, 229-235.

Wong LC, Heimbach MD, Truscott DR and Duncan BD, 1964. Boric acid poisoning: Report of 11 cases. Canadian Medical Association Journal 90, 1018-1023.

Woskie SR, Shen P, Finkel M, Eisen EA, Smith TJ, Smith R and Wegman DH, 1993. Calibration of a continuous-reading aerosol monitor (Miniram) to measure borate dust exposures. Appl. Occup. Environ. Hyg. 8(1), 38-45.

WHO, 1993. Guidelines for drinking water quality. 2nd edition. Vol 1. Recommendations. World Health Organisation, Geneva.

MEMBERS OF THE TASK FORCE

M. RICHOLD	UNILEVER RESEARCH UK - Bedford
C. LALLY	PROCTER & GAMBLE B - Brussels
V. GARNY	SOLVAY B - Brussels
W. STERZEL	HENKEL D - Düsseldorf
G. FULLER	BORAX CONSOLIDATED UK - Guildford
M. COLLINS	ICI UK - Runcorn
W. MAYR	DEGUSSA D - Hanau
W. HAEBLER (Secretary)	ECETOC B - Brussels

MEMBERS OF THE SCIENTIFIC COMMITTEE (Peer Review Committee)

W.F. Tordoir (Chairman), Head, Occupational Health and Toxicology Division	SHELL NL - Den Haag
H. Verschuuren, (Vice-Chairman), Head, Toxicology Department	DOW EUROPE CH - Horgen
O.C. Bøckman, Scientific Advisor	NORSK HYDRO N - Porsgrunn
N.G. Carmichael, Toxicology Director Worldwide	RHÔNE-POULENC F - Lyon
H. De Henau, European Technical Centre Professional and Regulatory Services	PROCTER AND GAMBLE B - Brussels
A. De Morsier, Head, Ecotoxicology	CIBA-GEIGY CH - Basel
P.A. Gilbert, Head, Environmental Division	UNILEVER UK - Port Sunlight
I.J. Graham-Bryce, Head, Environmental Affairs	SHELL NL - Den Haag
B. Hildebrand, Director, Experimental Toxicology	BASF D - Ludwigshafen
J.R. Jackson, Director, Medicine and Health Science	MONSANTO EUROPE B - Brussels
K. Künstler[1], Head, Biological Research	HENKEL D - Düsseldorf
H. Lagast[1], Chief Medical Officer	SOLVAY B - Brussels
E. Löser, Head, Institute of Industrial Toxicology	BAYER D - Wuppertal
R. Millischer, Chief Toxicologist	ELF ATOCHEM F - Paris
I.F.H. Purchase, Director, Central Toxicology Laboratory	ZENECA UK - Alderley Park

[1] Stewards responsible for primary peer review

LIST OF ECETOC PUBLICATIONS (continued inside back cover)

MONOGRAPHS

No.	Title
No. 1	Good Laboratory Practice. Oct 79
No. 2	Contribution to Strategy for Identification and Control of Occupational Carcinogens. Sep 80
No. 3	Risk Assessment of Occupational Chemical Carcinogens. Jan 82
No. 4	Hepatocarcinogenesis in Laboratory Rodents : Relevance for Man. Oct 82
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology). Dec 83
No. 6	Acute Toxicity Tests, $LD_{50}(LC_{50})$ Determinations and Alternatives. May 85
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies. Dec 85
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment. Feb 86
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals. Jun 87
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man. Aug 87
No. 11	Eye Irritation Testing. Jun 88
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity). Nov 89
No. 13	DNA and Protein Adducts: Evaluation of their Use in exposure Monitoring and Risk Assessment. Oct 89
No. 14	Skin Sensitisation Testing. Mar 90
No. 15	Skin Irritation. Jul 90
No. 16	Mutation Research, Special Issue: Early Indicators of Non-Genotoxic Carcinogenesis. Jun 91
No. 17	Hepatic Peroxisome Proliferation. May 92
No. 18	Evaluation of the Neurotoxic Potential of Chemicals. Sep 92
No. 19	Respiratory Allergy. Aug 93
No. 20	Percutaneous Absorption. Aug 93
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation. Sep 94
No. 22	Evaluation of Chemicals for Oculotoxicity. Nov 94

JACC REPORTS

No.	Title
No. 1	Joint Assessment of Commodity Chemicals, Melamine. Feb 83
No. 2	Joint Assessment of Commodity Chemicals, 1,4-Dioxane. Feb 83
No. 3	Joint Assessment of Commodity Chemicals, Methyl Ethyl Ketone. Feb 83
No. 4	Joint Assessment of Commodity Chemicals, Methylene Chloride. Jan 84
No. 5	Joint Assessment of Commodity Chemicals, Vinylidene Chloride. Aug 85
No. 6	Joint Assessment of Commodity Chemicals, Xylenes. Jun 86
No. 7	Joint Assessment of Commodity Chemicals, Ethylbenzene. Aug 86
No. 8	Joint Assessment of Commodity Chemicals, Methyl Isobutyl Ketone. May 87
No. 9	Joint Assessment of Commodity Chemicals, Chlorodifluoromethane. Oct 89
No. 10	Joint Assessment of Commodity Chemicals, Isophorone. Sep 89
No. 11	Joint Assessment of Commodity Chemicals, (HFA-132b) 1,2-Dichloro-1,1-Difluoroethane. May 90
No. 12	Joint Assessment of Commodity Chemicals, (HFA-124) 1-Chloro-1,2,2,2-Tetrafluoroethane. May 90
No. 13	Joint Assessment of Commodity Chemicals, (HFA-123) 1,1-Dichloro-2,2,2-Trifluoroethane. May 90
No. 14	Joint Assessment of Commodity Chemicals, (HFA-133a) 1-Chloro-2,2,2-Trifluoromethane. Aug 90
No. 15	Joint Assessment of Commodity Chemicals, (HFA-141B) 1-Fluoro 1,1-Dichloroethane. Aug 90
No. 16	Joint Assessment of Commodity Chemicals, (HCFC-21) Dichlorofluoromethane. Aug 90
No. 17	Joint Assessment of Commodity Chemicals, (HFA-142b) 1-Chloro-1,1,Difluoroethane. Feb 91
No. 18	Joint Assessment of Commodity Chemicals, Vinylacetate. Feb 91
No. 19	Joint Assessment of Commodity Chemicals, Dicyclopentadiene. Jul 91
No. 20	Joint Assessment of Commodity Chemicals, Tris-/Bis-/Mono-(2-ethylhexyl)phosphate. May 92
No. 21	Joint Assessment of Commodity Chemicals, Tris-(2-butoxyethyl)-phosphate. Mar 92
No. 22	Joint Assessment of Commodity Chemicals, Hydrogen Peroxide. Jan 93
No. 23	Joint Assessment of Commodity Chemicals, Polycarboxylate Polymers as Used in Detergents. Nov 93
No. 24	Joint Assessment of Commodity Chemicals, (HFC-125) Pentafluoroethane. May 94
No. 25	Joint Assessment of Commodity Chemicals, (HCFC-124) 1-Chloro-1,2,2,2-Tetrafluoroethane. Jul 94
No. 26	Joint Assessment of Commodity Chemicals, Linear Polydimethylsiloxanes (viscosity 10-100,000 centisokes). Sep 94
No. 27	Joint Assessment of Commodity Chemicals, *n*-Butyl Acrylate. CAS No. 141-32-2. Aug 94
No. 28	Joint Assessment of Commodity Chemicals, Ethyl Acrylate. CAS No. 140-88-5. Sep 94
No. 29	Joint Assessment of Commodity Chemicals, 1,1-Dichloro-1-Fluoroethane (HCFC-141b) CAS No 1717-00-6. Feb 95
No. 30	Joint Assessment of Commodity Chemicals, Methyl Methacrylate CAS No 80-62-6. In press
No. 31	Joint Assessment of Commodity Chemicals, 1,1,1,2-Tetrafluoroethane (HFC-134a) CAS No. 811-97-2. Feb 95

TECHNICAL REPORTS

No. Title

No. 1 Assessment of Data on the Effects of Formaldehyde on Humans. May 81
No. 2 The Mutagenic and Carcinogenic Potential of Formaldehyde. May 81
No. 3 Assessment of Test Methods for Photodegradation of Chemicals in the Environment. Aug 81
No. 4 The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man. Jul 82
No. 5 Toxicity of Ethylene Oxide and its Relevance to Man. Sep 82
No. 6 Formaldehyde Toxicology: an Up-Dating of the ECETOC Technical reports 1 and 2. Sep 82
No. 7 Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere. Sep 82
No. 8 Biodegradation Testing: An Assessment of the Present Status. Nov 83
No. 9 Assessment of Reverse-Phase Chromatographic Methods for Determining Partition Coefficients. Dec 83
No. 10 Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits. Feb 84
No. 11 Ethylene Oxide Toxicology and its Relevance to Man : An Up-Dating of ECETOC Technical Report no.5. Mar 84
No. 12 The Phototransformation of Chemicals in Water : Results of a Ring-Test. Jun 84
No. 13 The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on the Environment. Mar 84
No. 14 The EEC 6th Amendment : A Guide to Risk Evaluation for Effects on Human Health. Mar 94
No. 15 The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values. Jun 84
No. 16 A review of Recent Literature on the Toxicology of Benzene. Dec 84
No. 17 The Toxicology of Glycol Ethers and its Relevance to Man : An Up-Dating of ECETOC Technical Report no.4. Apr 85
No. 18 Harmonisation of Ready Biodegradability Tests. Apr 85
No. 19 An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment. May 85
No. 20 Biodegradation Tests for Poorly-Soluble Compounds. Feb 86
No. 21 Guide to the Classification of Carcinogens, Mutagens and Teratogens Under the 6th Amendment. Feb 86
No. 22 Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity. 87
No. 23 Evaluation of the Toxicity of Substances to be Assessed for Biodegradability. Nov 86
No. 24 The EEC 6th Amendment : Prolonged Fish Toxicity Tests. Oct 86
No. 25 Evaluation of Fish Tainting. Jan 87
No. 26 The Assessment of Carcinogenic Hazard for Human Beings Exposed to Methylene Chloride. Jan 87
No. 27 Nitrate and Drinking Water. Jan 88
No. 28 Evaluation of Anaerobic Biodegradation. Jun 88
No. 29 Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico- Chemical Properties, Tonnage and Use Pattern. Jun 88
No. 30(5) Existing Chemicals : Literature Reviews and Evaluations. 94
No. 31 The Mutagenicity and Carcinogenicity of Vinyl Chloride : A Historical Review and Assessment. Jul 88
No. 32 Methylene Chloride (Dichloromethane) : Human Risk Assessment Using Experimental Animal Data. May 88
No. 33 Nickel and Nickel Compounds : Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis. Feb 89
No. 34 Methylene Chloride (Dichloromethane) : An Overview of Experimental Work Investigating Species, Differences in Carcinogenicity and their Relevance to Man. Mar 89
No. 35 Fate, Behaviour and Toxicity of Organic Chemicals Associated with Sediments. Jan 90
No. 36 Biomonitoring of Industrial Effluents. Apr 90
No. 37 Tetrachloroethylene : Assessment of Human Carcinogenic Hazard. May 90
No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens. Jul 90
No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea. Jul 90
No. 40 Hazard Assessment of Chemical Contaminents in Soil. Aug 90
No. 41 Human Exposure to N-Nitrosmaines, Their Effects and a Risk Assessment for n-Nitrosodiethanolamine in Personal Care Products. Aug 90
No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products. Feb 91
No. 43 Emergency Exposure Indices for Industrial Chemicals. Mar 91
No. 44 Biodegradation Kinetics. Sep 91
No. 45 Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis. Mar 92
No. 46 EC 7th Amendment: Role of Mammalian Toxicokinetic and Metabolic Studies in the Toxicological Assessment of Industrial Chemicals. May 92
No. 47 EC 7th Amendment: 'Toxic to Reproduction' - Guidance on Classification. Aug 92
No. 48 Eye Irritation: Reference Chemicals Data Bank. Aug 92
No. 49 Exposure of Man to Dioxins: A Perspective on Industrial Waste Incineration. Sep 92
No. 50 Estimating the Environmental Concentrations of Chemicals Using Fate and Exposure Models. Nov 92
No. 51 Environmental Hazard Assessment of Substances. Jan 93
No. 52 Styrene Toxicology Investigations on the Potential for Carcinogenicity. Nov 92
No. 53 DHTDMAC: Aquatic and Terrestrial Hazard Assessment. CAS No. 61789-80-8. Feb 93
No. 54 Assessment of the Biodegradation of Chemicals in the Marine Environment. Aug 93
No. 55 Pulmonary Toxicity of Polyalkylene Glycols. (in preparation)
No. 56 Aquatic Toxicity Data Evaluation. Dec 93
No. 57 Polypropylene Production and Colorectal Cancer. Feb 94
No. 58 Assessment of Non-Occupational Exposure to Chemicals. May 94
No. 59 Testing For Worker Protection. May 94
No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard. May 94
No. 61 Environmental Exposure Assessment. Sep 94
No. 62 Ammonia Emissions to Air in Western Europe. Jul 94
No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risks Assessment for Human Beings. Feb 95

Responsible Editor: D. A. Stringer, ECETOC
Av. E. Van Nieuwenhuyse, 4 (Bte 6)
B - 1160 Brussels, Belgium
D-1995-3001-120