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Ecotoxicology of Some
Inorganic Borates

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SUMMARY AND CONCLUSIONS

The purpose of this review was to establish the safe environmental levels of borate in the ecosphere. The review covers the established methods for the analysis of borate, as boron. The natural occurrence, natural and anthropogenic distribution and balance of borate in the ecosphere are surveyed, including the borate content of waters, the lithosphere and the atmosphere. Finally, data are presented on the concentration of borate in individual parts of the biosphere, together with some critically evaluated no-effect and lowest-adverse-effect levels of borate on various representative species.

A variety of available analytical methods has been reviewed. Inductively-coupled plasma atomic emission spectrometry (ICP-AES) is currently the preferred screening method for the rapid analysis of samples, whereas inductively-coupled plasma mass spectrometry (ICP-MS) with a detection limit for boron of around 0.00015 mgB/l provides the most sensitive analytical results. The more traditional spectrophotometric method, in which a coloured complex of Azomethine-H with boron is assayed, is still in widespread use and has been incorporated into international standard methods.

The world's oceans have by far the greatest content of borate, with an average concentration of 5 mgB/l. Lakes and rivers in most parts of the world, except in areas of volcanic activity with more elevated concentrations, contain an environmental background content of <1 mgB/l, generally between 0.01 to 0.3 mgB/l. No typical concentration of borate can be cited for groundwater, which includes flowing springs (both hot and cold), geysers, aquifers (both flowing and confined), oilfield brines, etc. The recent review of the borate content of European groundwaters shows that values can vary from <0.1 to >1mgB/l and are dependent upon local geological circumstances, especially in areas of volcanic activity. Mineral waters contain a range from <0.02-4.3 mgB/l.

Igneous rocks generally have a low borate content. Sedimentary rocks have a higher borate content, which is related to the salinity of the water at the time of deposition. The borate content from such marine sediments ranges from 15-300 mgB/kg. The weathering of rocks, by rainfall and by erosion from rivers, provides a continuous small source of borate into the soil and the aqueous environment. Soils of low borate content (<10mgB/kg) are present on most of the earth. The average overall content of borate of all soils is 10-20 mgB/kg, with higher values (up to 100 mgB/kg) in the western USA and across the Mediterranean in Turkey, Iran and Kazakhstan.

Large deposits of borate minerals are rare. The only known massive deposits are located in the Mojave Desert of California, USA, and in Western Turkey. The four most important minerals are colemanite, kernite, tincal, and ulexite.

Although few data are available, the level of borate in the atmosphere is low and around 16 ngB/m³ according to a recent estimate. Its presence probably arises mainly from the vapour pressure of boric acid above the seawater. The tropospheric burdens for particulate and gas-phase boron (B) were recently estimated to be 6,000 tonnes and 60,000 to 110,000 tonnes, respectively.

There are industrial applications where the borate becomes fixed into a virtually water-insoluble matrix, but some localised aerial or aqueous discharges are likely to arise during production. In aqueous discharges, the borate passes unchanged through the sewage works and into river water and ultimately the sea. Examples of these include industries producing glass wool (insulation), enamels, ceramics and ferrous alloys. The use of boronated fertilisers in agriculture is a small but significant source of borate, arising from the fact that boron is a micronutrient required for healthy plant growth.

There are many other industrial and agricultural applications for the inorganic borates that result in discharges of borate to the environment. A significant contribution to European freshwaters in highly populated areas arises from the use of borates (including sodium perborate and other borates), where formulated in detergents.

Borate is required for the optimal growth of algae, but large concentrations (>20 mgB/l) inhibit growth. Pot experiments have shown that the reed plant Phragmites australis can tolerate a borate content in the aqueous nutrient substrate of up to 4 mgB/l, which may be extended to 8 mgB/l for a period of 2-3 months without noticeable damage. Concentrations of borate in domestic waste waters (up to 3.5 mgB/l) are much lower than those that would affect bacteria present in the sewage treatment processes. Bacteria, especially those present in sewage treatment plants tolerate concentrations >90 mgB/l. Chronic toxicity to protozoa and aquatic invertebrates is shown at ≥ 2.5 mgB/l. The toxicity of borate is low to terrestrial microorganisms and invertebrates ($LC_{50} \geq 17.5$ mgB/kg soil).

Boron is a required micronutrient for healthy plant growth, and is applied where necessary as a fertiliser at 0.5-3 kgB/ha. The borate content in all plants is in the range of 2-100 mgB/kg (based on dry weight). An inadequate supply of borate leads to symptoms of boron deficiency, whereas an excess leads to symptoms of boron toxicity.

The acute toxicity of borate to juvenile fish is relatively low, as median lethal concentrations range from 14 to 3,400 mgB/l. The data suggest that Oncorhynchus mykiss (rainbow trout) and Brachydanio rerio (zebra fish) are the most sensitive species. Chronic toxicity studies with early life stages of several fish species in reconstituted water clearly indicate that rainbow trout is the most sensitive species. By means of a combination of laboratory studies and field observations in natural waters, the determination of a safe concentration for borate in freshwater systems can be based on rainbow trout information exclusively. No effect concentrations of 0.75 mgB/l have been reported in tests with natural waters. The

observations of borate toxicity from field surveys show that healthy trout populations may occur in streams and lakes of the western USA at concentrations of near or slightly above 1 mgB/l.

These observations need to be consolidated by more observations linking occurrence of trout with measured boron concentrations. A study is planned, which may be expected to show that current NOEC values established in the laboratory are representative for the observations made in the USA for high boron streams. If this is the case the need to use a conservative assessment factor to extrapolate from laboratory to field data will disappear, supporting the NOEC to trout in aquatic systems of 1 mgB/l.

Data are available to show that borate does not bioaccumulate in algae, reeds, salmon and oysters.

The early life stages of amphibians, such as the frog and toad, had no effect concentrations of 3 mgB/l.

High concentrations of boron and selenium were found in waterfowl exposed to agricultural drainwaters in California. Diets containing 13 mgB/kg fresh weight showed no effects on mallards, but 30-100 mgB/kg produced growth reduction.

Environmental borate levels are not considered to be a concern for domestic animals. No effect concentrations in cattle were found at 120 mgB/l in drinking water and at 157 mgB/kg in feed.

It is concluded that boron is an ubiquitous element, present naturally as borate in seawater, freshwater, rocks, soil and all plants. Boron neither accumulates in any environmental compartment nor bioaccumulates, but is transported into the oceans, which have a high natural environmental background level of borate. Boron is an essential micronutrient for the healthy growth of all plants, and boronated fertilisers are used in agriculture to improve yields and to correct the symptoms of boron deficiency in crops. At the concentrations generally monitored in river water, borate causes no adverse effects to either land plants by irrigation or to water plants and aquatic life. Similarly, borate levels generally detected in soil cause no effects to land plants or soil organisms. Organisms in freshwater are the most sensitive to borate. The NOEC of borate to all freshwater aquatic life is at least 1 mgB/l.

1. INTRODUCTION

The element boron (B) is widely distributed in nature. 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 (15-300 mgB/kg), soils (<10-20 mgB/kg), fresh waters (<1 mgB/l) and sea water (5 mgB/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 environmental 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, run-off from boronated fertilisers, additives to corrosion inhibitors in anti-freeze formulations, biocides for cutting fluids, insecticides and as buffers/preservatives for cosmetic and pharmaceutical preparations.

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 dietary studies made by the US Department of Agriculture, there has been renewed interest in the nutritional importance of boron. For example, Nielsen (1992) suggested that inadequate dietary boron (≤0.2 mgB/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 human beings.

The concentration of borates in fresh waters is under scrutiny because the World Health Organisation (WHO, 1993) has recently published a new guideline value for boron in drinking water of 0.3 mgB/l, following a risk assessment based on reproductive toxicology. Because of more recent toxicological

data, this new guideline will be subject to further review and WHO has indicated that boron will be given top priority for re-evaluation at the first opportunity. The European Commission is now revising its guideline values for drinking water, which for boron is currently set at 1 mgB/l. The toxicological aspects of the borates have already been reviewed in a companion document (ECETOC, 1995). This report also covered current human exposures to boron via food and drinking water (see terms of reference below).

The objective of this Task Force has been to establish critically evaluated environmental levels of borate in the ecosphere, in order to provide appropriate guideline values for environmental safety. The terms of reference were:

> Review the ecotoxicology of boron and its derivatives;
> Identify the natural and consumer routes by which boron reaches the environment;
> Evaluate the current exposure to boron by man via food and drinking water (see above);
> Determine acceptable surface and drinking water concentrations.

2.   IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, PRODUCTION, USES AND DISCHARGES TO THE ENVIRONMENT

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 is an understanding of the nature of the active chemical species and how it interacts with biological tissues. Because boric acid is stable and a very weak acid (pK_a 9.15), the undissociated acid (H_3BO_3) is the predominant species in aqueous solution at physiological pH. This conversion into undissociated boric acid applies also to boric oxide and the sodium borates. As a consequence, the ecotoxicology 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 will be either the undissociated boric acid or a complex of boric acid with a carbohydrate or protein.

Boric acid (H_3BO_3) can be steam distilled from its aqueous solutions (Levi and Curti, 1938). This is a physical property of some environmental significance, because it indicates that boric acid will exert a small partial vapour pressure over seawater. The presence of small quantities of boric acid vapour observed in the atmosphere probably originates because of its reported vapour pressure of ca. 260 Pa (2 mm Hg) at 20°C (Schachtschabel et al, 1989). The volatility of boric acid can be suppressed, either by the addition of strong alkali, or by the complexation of the boric acid with mannitol (Feldman, 1961).

Different considerations apply to sodium perborate, a peroxygen compound, that readily generates hydrogen peroxide in addition to sodium borate in biological systems.

Table 1: Identity, Physical and Chemical Properties

Physical/Chemical Properties	Boric Acid [1]	Borax [1]	Borax Pentahydrate [1]	Anhydrous Borax [1]	Boric Oxide [1]	Sodium Perborate tetrahydrate [2]	Sodium Perborate Monohydrate [3]
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	Sodium tetraborate pentahydrate, Borax 5 Mol	Sodium tetraborate, Borax Glass	Boron trioxide, Anhydrous boric acid	PBS4, PBST	PBS1, PBSM
CAS Registry 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
Mol. Weight	61.83	381.37	291.35	201.27	69.62	153.9	99.8
Spec. Gravity (20°C)	1.51	1.73	1.81	2.37	1.83		
Bulk Density (kg/m³)	880	935	1,000-1,150	1,075-1,380	975-1,090	700-900	500-650
Melting Point — Closed Space (°C)	171	> 62	<200	742 (Crystal)	450 (Crystal)	Decomposes	Decomposes
Melting Point — Anhydrous Form (°C)	450 (Crystal)	742 (Crystal)	742 (Crystal)				
Boron Content (%)	17.48	11.34	14.85	21.49	31.06	7.03	10.8
Available Oxygen (% wt)	N.A.	N.A.	N.A.	N.A.	N.A.	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 Rapidly hydrates to boric acid	23 g/l (20°C) 37 g/l (30°C)	15g/l (20°C) 24 g/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)	N.A. (Rapidly hydrates to boric acid)	10 at 1.5%	10 at 1.5%
pKa (pK1) (20°)	9.15 [4]						
Pow (25°C)	0.175 [5]	N.A. (dissociates)	N.A. (dissociates)	N.A. (dissociates)	N.A. (Rapidly hydrates to boric acid)	N.A. (decomposes)	N.A. (decomposes)

N.A. Not Applicable; [1] Borax Consolidated, 1992; [2] Interox Chemicals, 1982a;
[3] Interox Chemicals, 1982b; [4] Dawber and Matusin, 1982; [5] Barrès, 1967.

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 and 56,000 of boric acid.

■   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 (Borax Consolidated, 1992).

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 produced commercially from the oxidation of aqueous sodium metaborate by hydrogen peroxide. It is used primarily as a bleaching agent in detergent powders and in bleaching powders, but also 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 prepared by the partial dehydration of sodium perborate tetrahydrate. It 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   DISCHARGES TO THE ENVIRONMENT

Discharges to the environment, arising from the many applications for the inorganic borates in industry and in agriculture, can be divided broadly into four very different categories.

In some of the larger applications, for example glass wool (insulation), heat resistant borosilicate glass, enamels, ceramics and ferrous alloys, the borate contributes a useful or essential improvement in physical properties to the mixture of ingredients either during production (e.g., lowered viscosity to spin glass wool fibres) or in the final product (e.g., low coefficient of expansion of ovenware glass, or better adhesion of enamel coating to metal). In these applications most of the borate becomes fixed into a virtually water-insoluble matrix, but some localised aerial or aqueous discharges are likely to arise during production. Similar considerations apply to the use of boron in the production of ferroboron alloys, in which the ferrous alloy is toughened through the presence of a trace (<1%) of boron.

A second category applies to those end-uses where the water-soluble borate is discharged directly into the aqueous environment during or after use. The most significant and best documented of these is the current use of typically 12-15% by weight of sodium perborate tetrahydrate equivalent in some detergent powder formulations. In the former German Federal Republic, for example, the use of sodium perborate tetrahydrate had reached a maximum of 125,000 tonnes per annum in 1984, after which it was reduced to 110,000 tonnes per annum by 1988 (Gerike et al, 1989a). The latter corresponds to an annual discharge of 7,700 tonnes of boron equivalent into fresh waters. By 1989 only 100,000 tonnes of perborate per annum was used (IKW, 1994). Since that date, statistics refer to the whole of Germany

(including the former German Democratic Republic) and figures of 113,000; 116,487; 105,000 and 98,000 tonnes per annum of perborate have been quoted for the years 1990 to 1993, respectively (IKW, 1994).

There are also smaller applications in this second category, such as boronated fertilisers in agriculture, corrosion inhibitors in anti-freeze formulations for motor vehicle and other cooling systems, insecticides, biocides for cutting oils, uses as buffers in pharmaceutical/cosmetics and dyestuffs production, and the use of boric acid solutions for the control of nuclear reactions (the absorption of neutrons by the ^{10}B isotope). The use of borax pentahydrate as a carrier for soap lubricants in ferrous wire drawing is yet a further example.

A third, intermediate, category exists where the borate is ultimately leached out of the substrate, not during its use but after the eventual disposal of the substrate to waste. A good example of this kind is the use of water-soluble inorganic borates for timber preservation, where the timber is protected by paint or is used indoors. Further examples include the use of the borates for the flame retardation of chipboard or cellulose products, and the preservation of starch adhesives for use in the production of cardboard boxes.

Finally, there is a fourth significant anthropogenic source of borate which does not fit into any of the above three categories, in which the borate becomes available mainly in a water-soluble form. This source arises from the disposal of substantial tonnages of residual boron-containing pulverised fuel ash from coal-fired power stations, for example 10 million tonnes of ash per annum in the UK (Brown *et al*, 1976), 4 million tonnes per annum in Japan (Tsuboi *et al*, 1990) and additional unspecified tonnages in Canada (Warren *et al*, 1993) and in numerous other countries operating coal-fired power stations.

Coal contains the elements from the plants which formed it, including a significant (although variable) trace quantity of borate. Dried pulverised fuel ash containing around 200 mgB/kg, of which at least a third was readily extractable by water, was described by Brown *et al* (1976). For example, if all of the above 10 million tonnes of ash per annum was stored in aqueous lagoons, it could release up to 700 tonnes of boron per annum to the water. However, there has been a considerable decline in coal-fired power stations over the past decade and past tonnages of fuel ash generation are unlikely to be exceeded in future.

3.   ANALYSIS OF BORATE, AS BORON, IN ENVIRONMENTAL SAMPLES

3.1   INTRODUCTION

In environmental samples, borates may be present in a variety of matrices, namely in plant material, soils, sludges, sewage wastewaters, seawater, freshwater and fish tissues. Before carrying out the appropriate analytical procedure, it is often 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 variety of techniques (see Appendix A for full details) is available for the analysis of borate, as boron, including atomic and mass spectrometry, absorption and emission spectrophotometry, neutron capture analysis, and spectrofluorimetry. Nowadays inductively-coupled plasma atomic emission spectrometry is preferred for screening boron concentrations in a wide range of matrices, whilst inductively-coupled plasma mass spectrometry is used when more sensitive analysis is required.

Irrespective of which analytical technique is used for the assay of boron, the accuracy of the complete method is likely to be very dependent upon the care taken during any necessary sample preparation. Contamination could occur from the use of borosilicate or other boron-containing glassware (Green *et al*, 1976) and this should be avoided. Losses could be caused by non-quantitative sample transfer during complex clean-up procedures, freeze-drying or from the evaporative loss of boron, as boric acid, from acidified solutions (Feldman, 1961; HMSO, 1981).

As the preparative procedures for boron-containing samples can lead to boron contamination and/or boron losses, the most accurate data come from those studies, where either no sample preparation is required, e.g., direct analysis of boron by neutron capture prompt gamma-ray activation analysis (PGAA) (Anderson *et al*, 1994a), or where a certified National Institute of Science and Technology (NIST) boron-containing sample is put through the same procedure (Kowalenko, 1979; Duchateau *et al*, 1987; Gregoire, 1990; Banuelos *et al*, 1992; Anderson *et al*, 1994a). The last reference indicates that PGAA gives boron concentrations that are not significantly different from levels in the certified samples.

3.2 PREPARATORY PROCEDURES

As there is no universal method suitable for the analysis of boron present in all types of environmental matrices, the preparatory procedure required will depend upon the matrix and the contaminants that may be present.

3.2.1 Extraction of Boron from Plant Samples

Boron, a micronutrient required for the healthy growth of all plants, is present at concentrations in the range 2-100 mgB/kg dry weight depending upon the species of plant and the season. To analyse boron from plant tissues it is necessary to select a suitable sample, as boron is not evenly distributed throughout the plant, and then to release the boron from the matrix by digestion.

The microwave digestion of plant samples, predigested with nitric acid and hydrogen peroxide, would be the preferred procedure as it requires less materials, is more rapid, gives recoveries of above 98%, and shows good reproducibility compared to the more traditional dry-ashing method (Pennington et al, 1991). However, dry-ashing with a variety of compounds that help to decompose plant organic tissues, e.g., hydrogen peroxide, nitric acid or HF/HCl (Wikner, 1986), or wet acid digestion with high temperature oxidation, e.g., sodium hypobromite digestion (Kowalenko, 1979), can also be used. A comparison of dry-ashing with wet acid digestion gave recoveries for boron, from wild mustard, of around 110 mgB/kg and 160 mgB/kg, respectively (Banuelos et al, 1992).

3.2.2 Extraction of Boron from Soil Samples

The world average boron level in soil is 10-20 mgB/kg, although levels greater than 100 ppmB can be found in desert/volcanic regions (Mellor, 1980).

Bioavailable boron (less than 5% of the total boron (Gupta, 1968)) in soils is most often extracted using the hot water solubility (HWS) method, rather than the cold calcium chloride extraction that yields only low recoveries of boron which may need to be converted to BF_4^- and extracted into an anion exchanger before analysis (Novozamsky et al, 1990a). The HWS extraction follows a first-order reversible reaction, with a boiling time of about 10 minutes required to extract the hot-water-extractable boron from the soil (Odom, 1980). The remaining 95% non-water-soluble boron can also be extracted (Cumakov, 1991).

The total boron content in soils can be simply determined by decomposing the boron compounds into a soluble form. Acid digestion or alkali fusion with sodium peroxide (Yamada and Hattori, 1986) can be used to solubilise the boron, before extraction into chloroform or methyl isobutyl ketone with a 1,3-diol like

2-ethyl-1,3-hexanediol and derivatisation with an appropriate spectrophotometric/fluorimetric reagent. Ultrasonic leaching can also be used to solubilise boron from soil, and has the advantage that it can be incorporated in an automated flow system (Chen *et al*, 1989).

3.2.3   Extraction of Boron from Water/Sewage Wastewaters

Boron levels in aqueous media vary considerably, typically from 0.5 to 3 mgB/l in sewage effluent, from 0.001 to 2 mgB/l in European rivers but up to 13 mgB/l in rivers in arid or volcanic areas, from 4 to 5 mgB/l in seawater, and up to 0.1 mgB/l in rain/snow. Sample preconcentration may be required for those samples containing the lower concentrations of boron, depending upon the method of analysis.

Liquid-liquid extraction of boron from acidified solutions with 1,3-diols (e.g., 2,2,4-trimethyl-1,3-pentanediol) in chloroform is a highly selective method of preconcentration that eliminates numerous interferences, but is time-consuming and requires hazardous solvents (Aznarez *et al*, 1985). For larger numbers of samples and/or when automation is required, boron is best preconcentrated using a flow injection system containing an on-line Amberlite IRA-743 ion-exchange column, based on N-methylglucamine resin (Sekerka and Lechner, 1990).

3.2.4   Extraction of Boron from Fish Tissue

Although boron shows no tendency to accumulate in fish, its presence in fish tissues has been determined. Acid digestion of the organic substrates, using perchloric and nitric acids, has been used to extract boron from chinook salmon (Hamilton and Wiedmeyer, 1990).

3.3   SUMMARY

The determination of borate, as boron, in biological matrices, e.g., fish and plant material, usually involves initial sample preparation steps prior to analysis. In other cases, e.g., water, sewage wastewater and food, it is possible to analyse the sample directly.

The extraction of boron compounds from solid matrices is best done by the use of microwaves on chemically predigested samples to minimise losses and contamination, or by wet digestion alone. The preconcentration of boron from aqueous solutions, if necessary, is normally carried out by solvent extraction using a 1,3-diol which also provides some sample clean-up. In all cases where sample preparation is required and boron losses may occur, the method should be validated by the use of a certified boron-containing sample.

Traditionally, boron was analysed by the addition of a dye, and then the spectrophotometric detection of the complex formed, e.g., in the azomethine-H method (detection limit about 0.02 mgB/l), which is still widely used and incorporated into many Standards. Spectrofluorimetric methods, involving the detection of the complex formed between a fused-benzene ring sulphonic acid (e.g., chromotropic acid) and boron, are now also used and have a detection limit of around 0.005 mgB/l. The removal of the high fluorescent background with alkali can further improve the detection limit to 0.0001 mgB/l.

In the past ten years, instrumental methods have become more widely used for the determination of boron. Inductively-coupled plasma-atomic emission spectrometry (ICP-AES) is often used for screening boron concentrations in a wide variety of environmental matrices, whilst inductively-coupled plasma-mass spectrometry (ICP-MS) is preferred when better detection limits of around 0.00015 mgB/l are required. Neutron capture prompt gamma-ray activation analysis (PGAA), with a detection limit of 0.02 mgB/kg, has been applied to the direct analysis of boron in food.

More recently, flow-injection procedures for the automated preparation and analysis of large numbers of environmental samples containing boron have been reported. These procedures include both ultrasonic leaching followed by azomethine-H derivatisation of boron from soil, and ion-exchange chromatography coupled with azomethine-H derivatisation of boron from an aqueous matrix.

4.   ENVIRONMENTAL DISTRIBUTION

4.1   AQUATIC ENVIRONMENT

4.1.1   Introduction

The element boron is present in the form of water-soluble borates in all inland freshwaters at relatively low concentrations (typically <1 mgB/l), because of the weathering of naturally occurring borate-containing rocks and soils, supplemented by contributions from anthropogenic activities. However, by far the most predominant and widespread natural source is in seawater (typically 5 mgB/l). A recent review of the borate content of European groundwaters shows that values of >1 mgB/l are sometimes observed because of local geological circumstances, especially in areas of volcanic activity such as Italy and Greece.

Unusually high natural background levels of boron in waters also occur in other localised areas of the world with high volcanic activity, for example, in California, Chile and Turkey. These areas also contain the few commercially exploitable deposits of borate minerals.

4.1.2   Seawater

Seawater is the largest destination for the water-soluble inorganic borates. Boric acid (H_3BO_3) is the form in which most of the boron is present in seawater and the inorganic borate concentration in seawater varies in nearly direct ratio to the salinity the world over, being about 0.00024 g of boron per g of chloride expressed as Cl. The average concentration of borate in all oceans is 4.6 mgB/kg water, but can vary from 0.5 mgB/kg in the Baltic Sea to 9.6 mgB/kg in the Mediterranean Sea. Borate is continually being added to the sea by the weathering of rocks and soils, by rivers, springs, marine volcanoes, rain and dust eruptions (Mellor, 1980, page 76), but is also continuously removed, maintaining a constant boron:salinity ratio. It is known that the hydrous clay-mica ilite adsorbs borate from seawater in direct ratio to the borate concentration in seawater, thus removing borate in the form of sediments. About 98% of the earth's water is in the world's oceans, leaving only about 2% for all the other waters. The boron content of the world's oceans is given as approximately 7×10^{12} tonnes.

The presence of small quantities of boric acid vapour in the atmosphere probably arises from its evaporation from seawater (see section 2.1).

The estimated overall world production of the inorganic borates during 1990 was 3 million tonnes of B_2O_3 (Mineral Industry Surveys, 1990), or about 930,000 tonnes of boron equivalent. Therefore the total

annual production of boron represents only 10^{-5}% of what is already dissolved in the world oceans. As much of this annual borate production is converted into water-insoluble borosilicate glass or ceramics, there is minimal addition to oceanic boron levels.

4.1.3 Inland Freshwaters

4.1.3.1 Introduction

The available borate data on freshwaters are reviewed country by country in alphabetic order. The most systematic monitoring has been carried out in Germany, the UK, Italy and the USA and the results from these four countries are therefore covered in most detail here. Summaries have also been tabulated, where possible. The data available for Norway and Sweden are presented in one report on Scandinavian freshwaters.

4.1.3.2 Results from Individual Countries

Argentina

The boron content of freshwaters and groundwaters of the Lerma Valley in Salta province, north-west Argentina, was investigated (Bundschuh, 1992), because of possible pollution through the presence nearby of industrial borate-producing companies. During 1990-1991 samples were taken from 60 wells and freshwaters in the area. Freshwaters of the Rio Arenales in the north and the Rio Toro and the Rio Rosario in the south of the area showed boron concentrations at less than 0.3 mgB/l, where there was no significant anthropogenic input. However, the affected part of the Rio Arenales near a borate plant contained a high value of 6.9 mgB/l, which decreased again by dilution during *circa* 15 km. Borate concentration in the groundwater unaffected by borate production was observed at less than 0.2 mgB/l, but in the vicinity of borate production plants the groundwater reached concentrations of 6.2 mgB/l.

Austria (Table 2)

In the eastern part of Austria, river water is reported to have insignificant quantities of boron (<0.02 mgB/l). In those sections of rivers where domestic wastewaters are discharged, concentrations of 0.02-0.05 mgB/l were found. When there is heavy pollution by wastewater and low rates of water flow in a river, the boron concentration is 0.2-0.3 mgB/l (Schoeller and Bolzer, 1989).

Because of the use of boronated fertilisers, concentrations of borate in river waters adjacent to areas of intensive sugar beet cultivation (e.g., the Pulkau) are elevated (0.2-0.6 mgB/l).

As described in a more detailed subsequent paper (Schöller, 1990), the river Donau (Danube) above Vienna contained an average boron content of 0.03 mgB/l. At higher water flows, e.g., when much snow is melting, the level was <0.01 mgB/l. These measurements were carried out between December 1985 and January 1989.

The sources of waters in eastern Austria feeding the Donau show a differing situation. Many of the freshwaters here also show a low boron content (e.g., Traisen, Pielach), as these have an abundant water flow and are not too strongly polluted by wastewater. On the other hand, waters that lie in areas used intensively for agriculture, such as the Russbach of Marchfeld, can reach boron values of 0.4 mgB/l through the use of fertilisers. Many other waters in the same region reach 0.1-0.3 mgB/l, but waters in those areas where agriculture is of no major importance have <0.01 mgB/l.

Table 2. Boron Concentrations in Austrian Freshwaters

Sample	mgB/l	Comments/reference
River water, eastern Austria	<0.02	no domestic waste Schoeller and Bolzer,1989
River water, eastern Austria	0.02-0.05	some domestic discharges Schoeller and Bolzer,1989
River water, eastern Austria	0.2-0.3	heavy waste-water discharges, low water flow Schoeller and Bolzer,1989
Pulkau, Göllersbach rivers	0.2-0.6	boronated fertiliser used in region Schoeller and Bolzer,1989
Donau river, upstream from Viena	0.01-0.05	<0.01 when snow melts Schöller, 1990
Rusbach (Marchfeld)	0.1-0.4	boronated fertiliser used in region Schöller, 1990

Belgium

Analysis of the springtime average composition of the mains water supply in central Brussels, derived from the River Meuse, shows that it contains boron at 0.03 mgB/l. The corresponding mean boron concentration of the municipal wastewater in central Brussels during a dry weather period was 1.02 mgB/l, this increase being attributed to anthropogenic activity.   This is not an especially high concentration, since municipal waste water is diluted by river water after discharge (Verbanck *et al*, 1989).

Chile

The northern regions of Chile are rich in minerals and natural salts, with virtually no rainfall (Luis Cáceres *et al*, 1992). The nature of the soil and the volcanic sediment of the Andes mountains results in the high salt, arsenic and boron content in natural water. Water quality at differing locations along the Loa river basin ranged from 3.99 ppm at Lequena to as high as 26 ppm at Qulilagua. As a reflection of high natural levels in fresh waters in that region, the Chilean standard for boron in water for human consumption is given as 4.00 ppm (mgB/l).

In a further study in the same region (Barr *et al*, 1993), high values of boron in waters were also observed in Camarones (15.2 mgB/kg), Molinos (11.7 mgB/kg), Boca Negra (9.2 mgB/kg) and Poconchile (2.5 mgB/kg).

Egypt

Five sampling sites at Aswan were used to collect water samples from the Nile for 5 months. The sites were chosen near each water filtration unit of the Egyptian authority, which draws the Nile water at 4m depth, filters and disinfects it with chlorine and supplies it as drinking water. The groundwater was collected from three wells near Aswan city. The median values of the borate concentrations were: Groundwater, 0.032 mgB/l; Nile (at the surface), 0.026 mgB/l; Nile (at 4m depth), 0.031 mgB/l; Nile (drinking water), 0.028 mgB/l (Ismail, 1996).

France

Analyses of the boron content of the public water supplies in the Oise Department (a region 40-80 km north of Paris) were carried out during the period 1986-1989 (D.D.A.S.S. de l'Oise, 1990). Without exception, this water originated from underground sources, e.g., wells, boreholes or springs. The results from more than 300 samples showed that the values were generally less than the EC guideline value of 1 mgB/l (EC,1975), with 98% of the values less than 0.1 mgB/l. Only the public supply at Verberie had an

elevated value of 0.42 mgB/l and also an elevated fluoride value of 2.5 mgF/l. It was well known that at this place both the boron and fluoride values were of natural origin.

Of the sources not connected to public water supplies, only the one at Cambronne-les-Clermont was found to show an extraordinarily high value of 72-80 mgB/l, which led to the discovery of a nearby old industrial waste deposit as the cause (D.D.A.S.S. de l'Oise, 1990).

Data between 1981-1993 on fresh waters in the River Loire basin, based on 5,767 analyses, gave medians of 0.030-0.45 mgB/l at various sampling sites. Similar data between 1989-1992 on waters of the River Seine, based on 872 analyses, gave a range of median values of 0.170-1.480 mgB/l (Unilever, 1994).

Germany (Table 3 and 4)

The boron levels during the past 30 or so years in several German rivers have been well documented, enabling trends to be established.

In a comprehensive survey of the former Federal Republic of Germany and West Berlin during the period January 1973 to May 1974 (Graffmann *et al*, 1974), the boron content of around 300 samples of freshwaters and 240 samples of drinking water was determined. Freshwater samples were collected from the Rhine, Neckar, Main and Ruhr as well as from numerous other rivers and lakes.

The Rhine contained predominantly 0.1-0.2 mgB/l, the Neckar and Main 0.2-0.3 mgB/l in the middle reaches and at the outlets, whereas the mouth of the Ruhr contained around 0.4-0.5 mgB/l.

During the period 1982/1983, investigations along a 290 km stretch of the river Main at Limbach, Himmelbach, Klingenberg and Kleinostheim established the presence of between 0.09 to 0.270 mgB/l, dependent upon the location along the river and the corresponding flow of water. Average concentrations obtained during 1982 were 0.155 (Limbach), 0.188 (Himmelbach), 0.158 (Klingenberg) and 0.162 mgB/l (Kleinostheim). Increases in boron concentration were stated to be caused by anthropogenic pollution arising from washing powders in domestic effluent, and reductions in boron concentration were caused by dilution from unpolluted tributaries (Hoffmann, 1985).

The river Ruhr is monitored on a continuing annual basis for water quality (including boron) by the Ruhrverband, which published a more thorough than usual report in 1988 to celebrate its 75th anniversary (Ruhrwassergüte, 1988). It confirmed that, because of anthropogenic activities, the average boron concentration (about 0.24 mgB/l) is always greater where the Ruhr flows into the Rhine (0 km end of the river), than that (about 0.06 mgB/l) at the upper (unpolluted) source at 200 km (Ruhrwassergüte,

1988, page 106) from the mouth. Similar average values for boron continued to be obtained for Duisburg at the outlet of the Ruhr (0 km) by further monitoring (Ruhrwassergüte, 1991, page 34; 1992, page 61).

During 1988, the investigation was extended to compare the measured values of boron along the Ruhr on 12 April 1988 with those of a 10-year band of summer values between 1977 and 1986 at a similar low level of water (Ruhrwassergüte, 1988, page 12, Figure 4). The single value of boron along the Ruhr on 12 April 1988 was significantly lower than the band measured during the 10-year period 1977-1986. For example, at Duisburg in 1988 the level was about 0.12 mgB/l compared to 0.30 mgB/l average at the same place during 1977-1986. Incidentally, the chloride level and dissolved organic carbon level also decreased significantly, which probably reflects the reduction in industrial pollution of the river during this period.

A long-term investigation of the water quality of the river Rhine at Düsseldorf has established that a median concentration of boron of 0.16 mgB/l in 1976 had fallen to 0.12 mgB/l in 1987. It is estimated that about one half of the latter could be due to the natural background level of boron (Gerike *et al*, 1989a). Continued monitoring of the Rhine at Düsseldorf gave a median concentration of boron of 0.15 mgB/l (range 0.08-0.21 mgB/l) during 1989 (Gerike *et al*, 1991). Because of the natural background level of boron, the significant reduction of usage of sodium perborate bleach in detergents since 1984 had not yet become evident by 1989.

Regular annual water samples have been analysed since 1958 from 140 locations along the rivers Rhine, Ruhr, Main, and Neckar, which has also enabled trends and mass balances to be established (Gerike *et al*, 1989b). The boron concentration in the Ruhr and in the upper Rhine has stayed relatively unchanged during 1980-1987, whereas in the Main and Neckar it has fallen significantly. The concentrations ranged in 1987 from 0.05 to a maximum of 0.45 mgB/l.

Although the borate content of rivers in East Germany has not been monitored regularly in the past, a study during 1975-1981 of the lakes and rivers of Mecklenburg (Randow, 1981; Randow *et al*, 1982) showed boron concentrations in the range from 0.02 to 0.16 mgB/l. The River Warnow serves as the source of drinking water for Rostock, and contained between 0.06 and 0.11 mgB/l, and similar values were observed (around 0.12 mgB/l) for the Nebel and the Beke, its most important tributaries.

Further results for East Germany were obtained between March 1992 and February 1993 for the rivers Elbe, Saale, Havel, Mulde, Schwarze Elster and Weisse Elster (Germanus, 1993a, 1993b; Germanus *et al*, 1995). The average borate content of the Elbe increased typically from about 0.13 mgB/l in the upper reaches to about 0.18 mgB/l at the mouth of the river, the increase being caused by anthropogenic sources from domestic wastewater. Similarly, in the shorter stretch of the Saale, the average borate content increased downstream from about 0.16 to 0.30 mgB/l. The highest average value was 0.39 mgB/l in the Weisse Elster near Leipzig, which was about 3 times the value for the Rhine at Himmelgeist.

Table 3. Boron Concentrations in German Freshwaters

Sample	mgB/l	Comments/reference
Rhine, 110 samples	0.1-0.2	Graffmann *et al*, 1974
Neckar, Main and Ruhr, 41 samples	0.03-0.47	Waste-water polluted tributaries of the Rhine Graffmann *et al*, 1974
Various rivers and streams, 101 samples	0.02-1.86	Includes sewage works effluents Graffmann *et al*, 1974
Lakes and dams, 43 samples	0.02-0.31	Graffmann *et al*, 1974
River Main, 32 samples	0.09-0.27	Increases caused by domestic effluent Hoffmann, 1985
River Ruhr source of river	0.24 Average value 0.06 No waste-water	Ruhrwassergüte, 1988
River Rhine at Düsseldorf	0.12 median value	Gerike *et al*, 1989a
River Rhine at Düsseldorf, weekly sample	Range 0.08-0.21 0.15 Median value,1989	Gerike *et al*, 1991
River Rhine, 140 locations, Ruhr, Main and Neckar	0.05-0.45	Annual samples 1980-1987 Gerike *et al*, 1989b
Mecklenburg rivers and lakes, river Warnow	0.02-0.16 0.06-0.11	1975-1981 Randow, 1981; Randow *et al*, 1982
Elbe, Saale, Havel, Mulde, Schwarze Elster, Weisse Elster	0.13-0.39	Domestic waste-water elevates boron Germanus, 1993a, 1993b; Germanus *et al*, 1995
Baden-Württemberg, 780 samples	<0.05-0.2 No waste-water 0.26 Waste-water involved	LfU Wasser, 1992

Apart from in the vicinity of the sea, the presence of boron in water has been known to be an indicator of anthropogenic activities, caused largely by the use of sodium perborate in washing powders. Therefore stretches of rivers downstream from sewage installations, in particular, contain elevated boron contents (see 0.39 mgB/l in the Weisse Elster near Leipzig above) and this needs to be taken into account for the abstraction of drinking water. Measurements of 112 samples from areas in Baden-Württemberg, not influenced by anthropogenic activities, showed that 90% of the samples contained <0.05 mgB/l with a maximum value of 0.2 mgB/l. The results of 666 samples of raw freshwater showed that 90% of the samples contained <0.07 mgB/l with a maximum value of 0.26 mgB/l (LfU Wasser, 1992).

The boron content of 240 samples of drinking water taken from the public supply was less than 0.25 mgB/l, mostly substantially less, but the extent to which the drinking water source was a mixture of ground- and fresh-water was unknown (Graffmann *et al*, 1974).

The monitoring of 110 samples of drinking water in the former German Federal Republic was repeated during 1993 to study any trends in boron between 1974 and 1993. The maximum value obtained was 0.21 mgB/l, with no significant difference between the same locations sampled in 1974 and 1993 (Wiecken and Wübbold-Weber, 1995).

As a part of a large study in the former German Federal Republic, 2,717 samples of domestic drinking water from households were analysed for boron content (Krause *et al*, 1991). The estimated median value was 0.019 mgB/l, and a maximum value of 0.178 mgB/l was obtained. Around 32% of the values were below the limit of detection (0.010 mgB/l). There was a sharp increase in boron content with increase in size of population, e.g., there was an estimated geometric mean of 0.0474 mgB/l for populations of 100,000 inhabitants or greater, whereas the corresponding value was 0.0109 mgB/l for populations under 20,000 inhabitants.

Table 4: Boron Concentrations in German Drinking Water

Sample	mgB/l	Comments/reference
Drinking water, 240 samples	<0.25 Mostly <0.1	Graffmann *et al*, 1974
Drinking water, 110 samples, to repeat previous study in 1974 above	<0.21	No change in B content 1974-1993 Wiecken and Wübbold-Weber, 1995
Drinking water, 2717 samples	<0.18 Median 0.019	Krause *et al*, 1991

Italy (Table 5)

Water from 19 sampling points along the 400 km river Adige and its five tributaries in the Trento province of northern Italy was collected in May and October 1989, and analysed for organic and inorganic pollutants (Benfenati *et al*, 1992). On both occasions, the average level of boron was very low at <0.002 mgB/l.

The boron concentration was determined in freshwaters (lakes and rivers) of the river Po and its watershed in northern Italy (Tartari and Camusso, 1988), an area of about 70,000 km^2. The investigation was carried out from August 1983 to August 1984, and covered the whole length of the river Po, 18 of its main tributaries and 9 other streams. Special attention was given to the rivers Lambro and Olona, which flow through the industrial and highly populated area of Milan. In all, 118 samples of river water and 48 of

lake water were analysed. Most of the river samples (65%) were close to the natural background level of <0.1 mgB/l. On the other hand the polluted rivers Lambro and Olona contained 0.1-0.5 mgB/l.

In areas of high volcanic activity, freshwater boron concentrations are more elevated. For example, where there is geothermal activity in the Mount Amiata region of Tuscany, central Italy, the saline (sodium chloride), fluid discharged from wells is reported to have a content of 14,000 ppm boric acid (H_3BO_3), or around 2,500 mgB/l (Duchi *et al*, 1987). Dependent upon the circulation of the thermal springs in the region, the concentration in the waters there shows a variation from <2 mmoles/l to an extreme value of 1870 mmoles/l of boric acid, or from <22 mgB/l to 20,200 mgB/l.

Table 5. Boron Concentrations in Italian Freshwaters

Sample	mgB/l	Comments/reference
River Adige and tributaries, 19 samples	<0.002	Benfenati *et al*, 1992
River Po watershed, north Italy, 118 river and 48 lake samples	<0.1 0.1-0.5	65% of samples Lambro and Olona river, contains waste-water Tartari and Camusso,1988
Tuscany, central Italy, thermal springs	22 to 20,200	Mount Amiata geothermal activity, therefore high in boron content Duchi *et al*, 1987

Japan

The boron levels along the river Asahi (Korenaga *et al*, 1980), measured during 1978 from various sampling points along a 140 km stretch of the river, were reported to range from 0.009-0.0117 mgB/l.

Luxembourg

In a small study at unspecified sites in Luxembourg, based on 8 analyses during 1993, a range of medians of 0.110-0.390 mgB/l was obtained in freshwaters (Unilever, 1994).

The Netherlands

The boron levels in the river Rhine at Jupthaas and at Enschede were reported as 0.09 and 0.04 mgB/l, respectively, and in the river Maas at Veen as 0.07 mgB/l in 1981 (Rijkswaterstaat, 1981, cited in Table 4 of Mance *et al*, 1988).

Further values at unspecified sites in the Netherlands, based on 22 analyses in 1992, gave a range of medians of 0.090-0.145 mgB/l in freshwaters (Unilever, 1994).

Russia (Table 6)

Available data are limited to the river Neva, which flows from Lake Lagoda at one end to the estuary with the Baltic Sea at the other. The average boron values measured during the week of 17 October 1994 were low and ranged from 0.009 to 0.020 mgB/l (Huber, 1994a).

Table 6. Boron Concentrations in the river Neva, Russia *

Sample	mgB/l
Outflow from Lake Lagoda	0.013
Petrokrepost	0.011
Ribatskoje	0.015
St Petersburg (Hermitage Hill)	0.012
Yacht harbour	0.009
Hotel Pribaltiskaya	0.020

* Huber, 1994a

Scandinavia (Table 7)

The data from 355 water samples collected from Scandinavian freshwaters (Norwegian and Swedish rivers) in 1970 contained an estimated median value of 0.012 mgB/l (Ahl and Jönsson, 1972). As would have been expected, the boron content of sewage water was at a higher level (because sodium perborate was in use as a domestic bleach in detergents). The mean value of 7 samples of settled sewage from the Uppsala treatment plant was 0.40 mgB/l, with no sample containing more than 0.70 mgB/l. It was concluded from this study that the mean boron concentration of Norwegian and Swedish rivers was close to those of other rivers of the world.

For the purpose of checking whether the boron content of freshwaters had altered significantly during the past 20 years, Eka Nobel AB commissioned a small survey of Swedish freshwaters in November 1991 (Sveriges Geologiska AB Analys, 1991). Three samples of water were taken from the Göta River and eight others from the northern tributaries of Lake Mälaren.

The following values of boron (mgB/l) were found:

- The Göta River at Bohus north of Jordfalls bridge (0.018), north of Trollhättan (0.007) and in the centre of Trollhättan (0.006).
- Northern tributaries of Lake Mälaren :.
  Fyris River, centre of Uppsala (0.069), north of Uppsala (0.024)

- Örsunda River, between Uppsala and Enköping (0.042), in Enköping (0.046).
- The Svart River in the centre of Västerås (0.020).
- The Kolbäcks River (0.022).
- The Hed Stream west of Köping (<0.005) and in the centre of Köping (0.026).

A further twenty samples of water were collected during 1991 from the tributaries of Lake Mälaren by the Swedish Environmental Protection Agency. Analyses were carried out by KM Lab, Uppsala, and all samples contained <0.05 mgB/l (KM Lab, 1991).

Borate concentrations in Norway and Sweden have not been monitored continuously. A comparison of the results of the above studies carried out in 1972 and in 1991 confirmed that there was no significant change in the low boron content of Swedish waters during the past 20 years.

Table 7. Boron Concentrations in Scandinavian Freshwaters

Sample	mgB/l	Comments/reference
Norwegian and Swedish rivers, 355 samples	0.012	Median, during 1970 Ahl and Jönsson, 1972
Uppsala sewage plant, Sweden, 7 samples	0.4 0.7	Mean value Maximum value Ahl and Jönsson, 1972
River Göta, Sweden, 3 samples	0.006-0.018	Sveriges Geologiska 1991
Lake Mälaren, Sweden, northern tributaries, 8 samples	0.02-0.069	Sveriges Geologiska 1991
Lake Mälaren, northern tributaries 20 samples	<0.05	No significant change from 1971-1991. KM Lab, 1991

South Africa

The Department of Water Affairs in South Africa launched a long-term boron water quality programme in 1985 to assess suitability for irrigation of crops (Reid and Davies, 1989). The results of the analyses of water samples from 91 sites indicated that South African freshwaters contain between 0.02 to 0.33 mgB/l, at which concentration even the most sensitive crops could be grown without problem.

Spain

The boron level of the drinking water in 5 Spanish locations was analysed during 1986 (Garcia *et al*, 1987), namely at Barcelona, Bilbao, Cadiz, Madrid, and Malaga. The maximum value was 0.3 mgB/l in parts of Barcelona, otherwise it was generally 0.2 mgB/l.

Switzerland (Table 8)

The 1990 annual report of the Swiss Federal Institute for Water Supply (EAWAG, 1990) noted that the environmental background level of boron in Swiss waters is very low (<0.01 mgB/l). Samples of river water, drinking water, rain water and lake water were analysed as part of a national programme for the long-term investigation of Swiss freshwaters (see Table 8). In addition, the boron concentrations at the outlet of three sewage works were shown to be around 1 mgB/l.

Table 8. Boron Concentrations in Swiss Freshwaters

Sample	mgB/l	Reference
Rhine, Diepoldsau	0.02	EAWAG, 1990
Rhine, Village Neuf	0.06	
Glatt, Rheinsfelden	0.26	
Thur, Andelfingen	0.07	
Drinking water, Dübendorf	0.002	
Drinking water, Zürich	0.028	
Rain water, Dübendorf	<0.004	
Lago Cristallina (Tessina Alps)	<0.004	
419 spring waters, western Switzerland	0-0.070	Atteia *et al*, 1993

The boron content of 419 spring waters in western Switzerland was low and found to range up to 0.070 mgB/l (Atteia *et al*, 1993).

Turkey

Turkey possesses about 60% of the world's reserves of borate ores, and the borate mines north of the town of Bigadic are located along the Simav river (Okay *et al*, 1985). The boron level in this river before it reaches the mining region is reported as 0-0.5 mgB/l, but pollution from the discharge waters from the mine raises it to 4 mgB/l, or even 7 mgB/l during the irrigation season. Such levels of 4-7 mgB/l would render this river water unsuitable for irrigation purposes, and options for its purification, e.g., magnesium oxide or a boron-specific ion exchange resin, were examined but found to be not feasible. This is a localised problem, which has to be solved by taking appropriate measures at the mine.

The UK (Tables 9 and 10)

The level of boron in UK rivers was first investigated systematically by Waggott (1969). He noted that in 1968 the average boron concentration in different parts of the river Trent ranged from 0.28-0.87 mgB/l,

and in the industrially polluted river Tame between 0.3-1.4 mgB/l. He also demonstrated that boron, present substantially because of its use as a bleach in detergents, was at a higher level at various sewage treatment plants (1.1-3.7 mgB/l) than in rivers and was not removed by conventional sewage treatment.

A comprehensive survey of the boron levels of UK freshwaters during 1980 has been published (Mance *et al*, 1988). The information was supplied by the Severn Trent, Northumbrian, Thames, Anglian, Welsh, Southern, and South West Water Authorities, and Forth River Purification Board. The mean total boron concentration ranged from 0.046-0.822 mgB/l. Furthermore, data in the final report of the Department of the Environment Standing Committee on Synthetic Detergents (cited in Mance *et al*, 1988) showed that the boron concentration of selected rivers remained stable between 1974 and 1978, and the mean concentrations during this period varied from 0.064 mgB/l in the river Tweed to 0.864 mgB/l in the river Thames. Over a six year period (1987-1992) the annual consumption of sodium perborate in the detergent industry has declined by 20%, because of reformulation, from 75,000 tonnes in 1987 to 60,000 tonnes in 1992 (UK Department of the Environment, 1994).

Table 9. Boron Concentrations in UK Freshwaters

Sample	mgB/l	Comments/reference
River Trent	0.28-0.87	1968 data
River Tame	0.3-1.4	Industrial pollution
Sewage treatment plant	1.1-3.7	Waggott, 1969
Results from 8 river boards	0.05-0.82	1980 survey. Highest value in the Thames Mance *et al*, 1988
Thames Water	0.05-0.51	Range during 1988 and 1990 Bennett, 1993
Anglian Water, 200 supply zones	0.01-0.45	Range during 1992 Wilkinson, 1993

Measurements of boron levels at the drinking water abstraction points on the Thames (Bennett, 1993) between 1988 and 1990 ranged from 0.050-0.505 mgB/l. Measurements of the boron levels in over 200 water supply zones of the Anglian Water region during 1992 (Wilkinson, 1993) ranged from 0.01-0.45 mgB/l. An additional survey of recent measurements by the UK Water Industry and collated by Unilever (1994) is summarised in Table 10.

The USA (Table 11)

Levels of boron in freshwaters in the USA (Butterwick *et al*, 1989) average around 0.1 mgB/l, but concentrations tend to be higher in the western USA, where 5-15 mgB/l may be found because of weathering of boron-rich formations and deposits. Detailed information is available on boron levels in

freshwaters of the southwestern USA, because of extensive irrigation of sensitive citrus crops and known higher than average boron levels in this area. Irrigation return waters form a significant.

Table 10. Boron Concentrations in UK Freshwaters
National River Authority (NRA) Results

NRA Region (No. of Samples)	mgB/l [1]	Measured during: [2]
Anglian (431)	0.06-2.34	1991-1993
Northumbria (1,357)	0.06-0.27	1975-1992
North West (854)	0.06-4.38	1991-1993
Severn Trent (2,567)	0.02-1.06	1988-1993
Southern (469)	0.03-0.52	1990-1993
South West (250)	0.01-0.22	1989-1993
Thames (1,101)	<0.05-1.18	1990-1993
Welsh (815)	0.01-0.37	1993
Wessex (1,002)	0.01-0.28	1991-1992
Yorkshire (1,656)	0.05-3.83	1990-1992
Forth RPB[3] (427)	0.03-0.21	1990-1993
Highland RPB[3] (984)	<0.02	1983-1990

[1] Range of medians across all sampling sites in the Region   [3] River Purification Board

[2] All values assembled from NRA by Unilever (1994)

proportion of the flow to the Santa Ana River, and mean concentrations in river water over 20 years averaged between 0.3 and 0.5 mgB/l. A summary of the boron levels of drainage basins of 15 major rivers has been tabulated (Butterwick *et al*, 1989) and mean values ranged from 0.019 to 0.289 mgB/l.

A study was conducted by the Soap and Detergent Association (1994) to update the levels of boron in freshwaters of the USA. This was made possible by extracting the dissolved boron concentrations from the US Environmental Protection Agency national database STORET (STOrage and RETrieval of water quality information) for the period from 1 January 1984 to 31 December 1993. No geographical restrictions were specified. Due to sample size heterogeneity, a nonparametric approach was used to analyse boron concentrations. The median observation from each STORET station was used as representative of the boron levels at that site. Distributions of median boron levels in each county and state were determined using several SAS statistical programmes (SAS Institute Inc., Cary, North Carolina, USA). Table 11 contains a summary of the STORET extract.

Table 11. Boron Concentrations in USA Freshwaters

(1 January 1984 to 31 December 1993)

State	No. of samples	mgB/l	
		median	90th percentile
(USA, overall)	55,416	0.076	0.387
Arizona	1,673	0.100	0.170
California	6,421	0.105	0.449
Colorado	1,979	0.102	0.302
Idaho	519	0.080	0.170
Illinois	30,116	0.050	0.112
Michigan	159	0.055	0.347
Montana	571	0.140	0.610
Nevada	234	0.105	0.784
New Mexico	1,930	0.100	0.616
North Dakota	2,398	0.228	0.519
Oklahoma	515	0.170	0.440
Oregon	1,394	0.030	0.268
Utah	835	0.070	0.316
Wyoming	634	0.097	0.262

Soap and Detergent Association (1994)

During the 10 year period, 55,416 samples were taken, with 91% of records occurring in 14 states. Only 2 of the 14 states (Michigan and Illinois) were east of the Mississippi River, but Illinois contained 54% of all the records. Six hundred and sixty seven counties contained boron records, with 70% within the 14 states.   The median and 90th percentile values for the USA were 0.076 mgB/l and 0.387 mgB/l, respectively. States containing the highest boron levels in freshwaters were North Dakota, Oklahoma, Montana and California. States containing the least amount of ambient boron levels were Oregon, Illinois and Michigan. These results are in good agreement with those cited above by Butterwick *et al* (1989).

4.1.4   Irrigation Waters

Boron levels of irrigation waters may vary according to geological conditions and are frequently monitored to assess the suitability of the water for application on crops.   Unfortunately there is little information available either on the short term or long term effects of using boron-rich water on soils with different drainage patterns. The published information and recommendations are derived from the sandy soils of Southern California, where there is little annual rainfall (Keren and Bingham, 1985). Such conditions are clearly not applicable to Northern Europe, and are also different from conditions in the Mediterranean which has significant rainfall in winter.   The effects of climate on the requirement for irrigation water containing the same hypothetical concentration of 0.5 mgB/l are shown in Table 12.

Table 12: Effects of Climate Calculated on Annual Delivery of Boron to Field Crops from Irrigation Water Containing 0.5 mgB/l as Impurity

Region	Irrigation water (mm)	Delivery to crops (kgB/ha)*	Annual Rainfall (mm)
UK[1]	100	0.5	900 (throughout the year)
Sicily (Italy)[2]	925	4.6	650 (winter only)
California[3]	1000	5.0	200 (winter only)

* 100 mm of water at 1 mgB/l delivers 1 kgB/ha

[1] Ministry of Agriculture, Fisheries and Food (UK), 1981

[2] Indelicato *et al*, 1981

[3] Keren and Bingham, 1985

The important point to recognise from the above calculations is that the delivered dose of boron in kgB/ha is the critical parameter to determine any effect, not the concentration of the boron (if present) in irrigation water. The quantities of boron considered to be safe for application each year in non-arid climates are 2, 3 and 4 kgB/ha for sensitive, intermediate and tolerant crops, respectively (Ministry of Agriculture, Fisheries and Food (UK), 1981), although such recommendations were also based originally on conditions of Californian climate. Table 12 shows that only about one tenth of the irrigation would be required in the UK than in either Italy or California, with the balance of requirement being provided by borate-free rainwater. Furthermore, leaching by rainfall would prevent accumulation of borate in the soil.
In Italy, Southern Europe, where there is a combination of low rainfall during the growing season together with high transevaporation and sandy soil, there is a relative tenfold increase in irrigation requirement (and consequently in dosage of boron).   However, during winter there is leaching of the soil by rainfall between October and April to avoid accumulation of boron. In the Californian climate, where there is little rainfall throughout the year and therefore no leaching of the soil, there is a maximum requirement for irrigation water. Such Californian guidelines of 2-4 kgB/ha are therefore too conservative for northern

Europe and possibly for southern Europe as well, although the extent to which the guidelines could be exceeded is difficult to quantify.

4.1.5 Sewage Waters

Sewage effluent is usually discharged from a treatment plant into a river and can provide a significant proportion of the borate concentrations of freshwaters in populated areas.

The outlets of Austrian municipal sewage plants contain between about 0.2-0.8 mgB/l, which on dilution by freshwaters reduces to 0.01-0.2 mgB/l.

Analysis of sewage waters at four sewage farms in the Nile Delta of Egypt (El Kobbia and Ibrahim, 1989) showed that the maximum values for boron at Banha, Tanta, Damanhour and Alexandria were 0.11, 0.21, 1.40 and 1.67 mgB/l, respectively. Average values ranged from 0.08 to 0.20 mgB/l. It was concluded that these waters were satisfactory for use for the irrigation of plants.

The results of 54 investigations in Germany during 1973 of the effluent from 27 sewage plants in the River Ruhr region showed a distribution in samples of 39% with an average boron concentration range of 1.5 to 2.5 mgB/l and 30% at less than 1.5 mgB/l (in part probably due to dilution by rainwater or streams). On the other hand, 31% of samples were greater than 2.5 mgB/l, with 4% even between 3.5 to 4.5 mgB/l. The highest values could have been caused by pollution from additional industrial processes rather than solely by the use of detergents (Dietz, 1975).

The effluent from the sewage works at Bielefeld, Germany, was shown to contain boron at about 1 mgB/l, which was substantially the same as input concentration (Lahl and Burbaum, 1988).

Effluents and sewage sludge samples from seven sewage treatment plants near Milan, Italy, were analysed for phosphorus and boron. The annual average per capita contribution for borate was 0.2-0.3 gB/day. The borate was not removed from the aqueous effluent by the sewage treatment process and was present in the influent at variable concentration of the order of 1 mgB/l, whereas the borate in the sludge was also variable and present up to a maximum of around 50 mgB/kg dry weight (Mezzanotte *et al*, 1995).

A seven day monitoring study on the anionic surfactant LAS was carried out in the Netherlands at the De Meern municipal sewage treatment plant, which discharges into the river Leidsche Rijn. As a part of this study, the concentration of borate (as B) was measured as an inert tracer which is not removed by the sewage treatment process. The borate content of the influent to the plant ranged from 0.27 to 0.78 mgB/l, whereas the effluent ranged from 0.39 to 0.75 mgB/l (Feijtel *et al*, 1995).

A study at two sewage farms in the Alicante province of south-eastern Spain (Navarro *et al*, 1992) showed that the effluent at the mainly urban city of Alicante contained boron at a mean of 1.45 mgB/l, whereas the industrial area of Elche city contained a mean of 3.0 mgB/l, the increased level of boron being possibly due to industrial activity.

The effluent from the sewage plant near Uppsala, Sweden, was shown to contain a mean boron value of 0.4 mgB/l (maximum 0.7 mgB/l) (Ahl and Jönsson, 1972).

In Switzerland the boron concentrations at three sewage works were shown to be around 1 mgB/l (EAWAG, 1990).

A survey of the boron content of crude sewage was carried out during 1968 at UK Luton Sewage Works (Waggott, 1969), and the average weekly amounts contained 3.3-3.96 mgB/l. Boron contents of crude sewage in nearby areas were shown to be significantly lower, for example, Stevenage (2.5 mgB/l), Preston (1.49 mgB/l) and Rye Meads (1.21 mgB/l).

4.1.6   Groundwaters

4.1.6.1 Introduction

No typical content of borate can be cited for groundwater, which includes flowing springs (both hot and cold), geysers, aquifers (both flowing and confined), oilfield brines, etc. A general review of the borate content of these waters, mainly mineral springs in different parts of the world, shows that this varies widely according to origin and geological circumstances (Mellor, 1980, page 82, Table X).

The monitoring of borate as a geological impurity is of importance, because the abstraction of groundwater is a significant, and in some countries a predominant resource for the production of drinking water. The potential for high natural borate concentrations in groundwater occurs when there is an association with either clay minerals, evaporites, glauconitic sands, diagenetic limestones and dolomites, volcanic terrains and even acidic or ultra mafic igneous rocks. Such abstraction will inevitably contribute to an increase in borate levels of freshwaters. A recent survey of the available data on the borate content of European groundwater (Dames and Moore, 1995), which is summarised here, showed several areas with high natural concentrations of borate.

4.1.6.2 Borate Concentrations in European Groundwaters

Denmark

The Danmarks Geologiske Undersogelse (DGU) monitored 525 wells in Denmark, of which 39 had borate concentrations >0.3 mgB/l. Two samples in this survey contained >1 mgB/l.

France

A database collated by the Bureau de Recherche Geologiques et Minières contains 716 entries for the borate content of French groundwaters between 1979 and 1990, although these were restricted to just five Departments. Only 4 values were >0.3 mgB/l.

Germany

The most extensive study on groundwater was carried out in Baden-Württemberg on 2,574 samples, of which 9 samples contained >1 mgB/l, and 277 contained >0.1 mgB/l.

A study on 188 samples in Lower Saxony during 1990/1991 showed that 7 samples contained >1 mgB/l, with 4.2 mgB/l as the highest value.

Greece

Few data are available on the borate content of groundwaters in Greece. In the Patras area, 10 samples were found to contain <0.1 mgB/l. On the island of Samos, the spring water contained <0.05 mgB/l. However, in the Halkidiki peninsula of northern Greece 3 well water samples ranged from 2.3-5.4 mgB/l.

Italy

Borate analyses of the groundwater have been carried out during geochemical prospecting surveys of central and southern parts of the country, which are associated with volcanic activity. The analysis of 423 samples north of Rome up to the Tuscany border showed an average of 1 mgB/l. Based on 18 samples in Sicily, an average of 1.5 mgB/l was obtained. In the Campi Flegrei area, 96 of the 101 samples contained >0.3 mgB/l. Based on 44 volcanic springs, 34 coastal springs and 102 well water samples, the average borate value in the Paglia region was around 0.75 mgB/l.

The Netherlands

The water table in the Netherlands is close to the surface, and has been classified into categories with a range of borate values, as follows:

■   Rain water and coastal dune water: ≤0.005 mgB/l.

■   Rhine water injected into dunes: ≤0.01 mgB/l.

■   Inland groundwaters: 0.08-0.6 mgB/l.

■   Rhine water injected into river banks: 0.12-0.25 mgB/l.

Spain

A database produced by the Instituto Technologico Geominero de Espana gave borate values for the groundwater from boreholes, wells, springs and potable supplies :

■   Valencia: 21 samples, range 0.1-1.8 mgB/l,
        average 0.64 mgB/l.

■   Almeria: 17 samples, range 0.2-2.9 mgB/l,
        average 0.975 mgB/l.

■   Murcia: 15 samples, range 0.1-1.8 mgB/l,
        average 0.51 mgB/l.

■   Alicante: 32 urban samples, range 0.02-1.3 mgB/l,average 0.25 mgB/l;,
        9 non-urban samples range 0.14-1.8 mgB/l, average 0.63 mgB/l.

■   Castellon: range 0.04-0.96 mgB/l, average 0.29 mgB/l.

UK

The borate contents of various aquifers in the UK have been studied in some detail, as follows:

- Berkshire chalk : Groundwater in boreholes :
  range 0.06-0.74 mgB/l, average 0.15 mgB/l.

- London Basin : 21 samples,
  range 0.022-0.54 mgB /l.

- Lincolnshire limestone :
  range 0.013-3.17 mgB/l,average 0.68 mgB/l.

- Lower Greensand of S.E. England :
  range 0.014-0.145 mgB/l, average 0.043 mgB/l.

- Isle of Wight : Borehole samples in chalk outcrop,
  range 0.01-0.03 mgB/l, average 0.021 mgB/l.

- Birmingham Triassic Sandstone :
  range 0.01-0.46 mgB/l(10 samples) from aquifer.

- Triassic in Shropshire : samples of 57 boreholes,
  range 0.005-0.046 mgB/l, mean of 0.022 mgB/l.

- The Wealden : 15 samples from public supply wells,
  range 0.011-0.316 mgB/l, average 0.132 mgB/l.

- Northumbria : 164 boreholes sampled over 3 years,
  range 0.048-1.8 mgB/l, average 0.31 mgB/l,
  (over 33% of samples exceeded 0.3 mgB/l).

- Triassic of the Otter Valley: 15 boreholes,
  range 0.007-0.039 mgB/l, average 0.012 mgB/l.

- Dumfries and Galloway Permo-Triassic (Scotland) :
  27 samples of groundwater,
  range <0.02-0.11 mgB/l, average around 0.042 mgB/l.

4.1.6.3 Borate in Mineral Waters

Mineral waters provide a useful additional source of information on the natural borate concentrations of spring waters. Analysis of the borate concentration of 25 commercially available but unspecified mineral waters (Graffmann *et al*, 1974) showed a range from <0.02 to 3.23 mgB/l, with an average of around 0.5 mgB/l.

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

In a further recent analysis of four German mineral waters a range of 0.3-1.4 mgB/l was established, with an average value of 1 mgB/l (Wiecken and Wübbold-Weber, 1995).

4.2   LITHOSPHERE

A full account of the geochemistry of boron (Mellor, 1980) divides the lithosphere into igneous, sedimentary and metamorphic rocks. Only a brief outline of the occurrence and mobility of borate in rocks and soil is presented here.

Borate in rocks

Igneous rocks generally have a lower borate content than sedimentary rocks, and the borate content increases with the acidity of the rocks from ultrabasic to acidic. Boron is a highly mobile element, so that variables such as temperature, pressure, rate of cooling, initial water content, rock composition and sequence of events greatly influence the original boron retained upon cooling (Mellor, 1980, page 90). When it occurs as the centres of BO_3^{3-} triangles, the boron is not readily incorporated into the lattice of many silicate minerals, so it forms borate or borosilicate minerals. On the other hand, when the boron is present as the centres of BO_4^{5-} tetrahedra, it is preferentially incorporated into many silicate minerals. The boron content in igneous rocks ranges on the average from 1.5-60 ppm, although much larger individual values are cited in specific geographical regions (Mellor, 1980, page 91, Table XVII).

The borate content of sedimentary rocks has been the subject of much investigation, because of the possibility of determining the environmental conditions of the ancient seas or lakes in which sedimentations were laid down. The increased borate content of argillaceous sediments was found to be related to the salinity of the water at the time of deposition, and the borate content from such marine sediments ranges from 15-300 ppmB (Mellor, 1980, page 94, Table XIX). The borate content of

arenaceous sediments is mainly in the range of 5-62 ppmB, with a worldwide average of 35 ppmB (Mellor, 1980, page 95, Table XX). The borate content of carbonate and sulphate sediments (limestone, dolomite, magnesite, siderite, gypsum, anhydrite) presents a special case. The borate content of carbonate sediments is low at about 10 ppmB (Mellor, 1980, page 95, Table XXI). In marine carbonate sediments the borate is contained mainly in the included clay fraction.

By contrast, the borate content of metamorphic rocks, which are produced by the mineralogical and structural adjustments of solid rocks to physical and chemical processes operating below the depth of weathering, consists of widely scattered values and will not be described further here (Mellor, 1980, page 97, Table XXIII).

The weathering of rocks by rainfall and erosion by rivers provides a continuous small source of boron into the soil and the aqueous environment.

Borate in mineral deposits

Boron is an element that is widely distributed in nature in low concentrations. Large deposits of commercially valuable borate minerals are rare and are usually found in arid desert regions. Borates are distributed in over 150 minerals, but occur primarily as salts of sodium, calcium or magnesium. A useful overview of world statistics on the capacity, production, consumption and end-uses of borate minerals and refined borates has been published by the US Bureau of Mines (Lyday, 1992), which also lists a table of 11 borate minerals of commercial importance, together with chemical composition and B_2O_3 weight percent in each case.

The only known massive deposits are located in the Mojave Desert of California, USA, and in Western Turkey. Other smaller deposits are located in South America, China, and in Russia. The estimated world production of borate minerals in 1992 (Lyday, 1992) was around 2.6 million tonnes of ores, as shown in Table 13.

The four most important commercially exploited industrial borate minerals are:

- ■ colemanite (calcium borate), $Ca_2B_6O_{11}.5H_2O$
- ■ kernite (disodium tetraborate tetrahydrate), $Na_2B_4O_7.4H_2O$
- ■ tincal (disodium tetraborate decahydrate), $Na_2B_4O_7.10H_2O$
- ■ ulexite (calcium sodium borate), $Na_2O.2CaO.5B_2O_3.16H_2O$.

Table 13. Production of borate ores in 1992

Country	Tonnes x 10^3
USA	1,009
Turkey	1,000
Argentina	250
Russia	130
Chile	100
China	77
Peru	30

4.3   ATMOSPHERE

Relatively few data are available on the content of boron in the earth's atmosphere, and these have been reviewed (Mellor, 1980, pages 75-76). Various sources for the boron in the atmosphere have been suggested, and these include sea spray, volcanic and hot-spring emanations, airborne dust and industrial pollution. The greatest source seems to be the evaporation of boric acid from seawater, above which there is an appreciable vapour pressure (ca. 2 mm Hg at 20°C, Schachtschabel *et al*, 1989) of boric acid. The presence of even a low borate content in rainfall or snow may be of some significance in promoting plant growth.

The atmospheric boron content shows wide variation. For example, in France in 1955 the borate content of rain varied from 0.002 to 0.0045 mgB/l, whereas 0.1 mgB/l was reported from Japan in both rain and snow.

Whereas the B/Cl ratio of seawater is nearly constant at 2.25 x 10^{-4}, the B/Cl ratio for rainfall in Japan ranged from 27 x 10^{-4} to 420 x 10^{-4}. The higher B/Cl ratio in air presumably arises from the volatility of boric acid, which is much greater than that of sodium chloride.

The median and mean concentrations of borate in rain and snow at six sites in western Switzerland were found to be 0.0031 and 0.0056 mgB/l, respectively (Atteia *et al*, 1993).

A recent detailed investigation of the atmospheric distribution of particulate and gas-phase boron at three continental sites (Anderson *et al*, 1994b) showed that, on average, 85% of the total atmospheric boron was in the gas phase at a concentration of 16 ngB/m3. Atmospheric samples were collected at continental, coastal and marine sites in the USA and the mid-Pacific Ocean. The authors estimated that about 65-85% of the total borate source strength can be attributed to the oceans and 8-20% to

anthropogenic activities such as coal, agricultural, fuel wood and refuse burning. Volcanic activity may contribute 6-15%, although this is less certain. The tropospheric burdens for particulate and gas-phase boron were estimated to be 6,000 tonnes and 60,000-110,000 tonnes, respectively. The total global removal of boron from the atmosphere by wet and dry deposition was estimated to be 5.3 to 7.0 million tonnes per year.

4.4   SOIL

It is difficult to generalise on soil boron status, because of the tendency of the water-soluble borates to adsorb/desorb from the soil. The boron-soil interaction has been covered in a comprehensive review (Keren and Bingham, 1985). These authors emphasise the need to take into account the physical-chemical characteristics of the soil to explain such interactions. Soil adsorption sites may act as a pool from which borate is supplied to solution or where borate is adsorbed, depending on the changes in solution borate concentration and the affinity of soil for borate. The soil is depleted of borate through leaching by rainwater or through the uptake by plants, e.g., growth of cash crops. The net result is also dependent upon the type of soil, (e.g., sandy or clay) and the climate (e.g., temperate with high rainfall or arid with high transevaporation).

The earth's soils may be divided into two types according to their overall boron content. The low borate content (<10 mgB/kg) soils are present on most of the earth. The high borate content (up to 100 mgB/kg or more) soils are to be found in the Great Basin and Mojave Desert areas of Western USA, and in a band across the Mediterranean through Turkey, Iran, and Kazakhstan. The average overall content of borate of all soils in the world is 10-20 mgB/kg (Mellor, 1980, pp 107-109, and Table XXVII therein).

According to a United Nations FAO report on the micronutrient status of soils in 1982 (cited by Bergmann, 1984) widespread boron deficiency in countries such as Nepal, The Philippines, India, Thailand and Nigeria was shown. It is estimated that there are 8 million hectares of soils worldwide that are boron deficient for plant growth.

5.   EFFECTS ON ORGANISMS IN THE ENVIRONMENT

5.1   AQUATIC SPECIES

Considerable data exist for the toxicity of borates to aquatic organisms and ecosystems. Ample acute and chronic data are available for algae, higher plants, invertebrates and fish and are included here in separate sections. Clearly, these data show that the embryo-larval stages of the rainbow trout are the most sensitive to borate. The no-effect concentrations in laboratory studies compare closely with biocenoses and field observations.

The following sections describe in detail key information for each trophic level and test. Toxicity data for both freshwater and marine/estuarine organisms are given. Because of the high background level of borate in seawater (around 5 mgB/l) these organisms are relatively tolerant to borate.

5.1.1   Freshwater

5.1.1.1 *Algae* (Table 14)

Most algal studies observe growth inhibition as a major endpoint in chronic or field studies. An early study on *Scenedesmus quadricauda* by Bringmann and Kühn (1978) determined an EC_3 of 0.16 mgB/l for borate but no further studies were reported in the literature on this alga, to which borate seems to have a higher toxicity than to other algae (see below). *Scenedesmus quadricauda* was not recommended as a test species for the subsequently developed standard tests because of its especially high variability of growth.

To allow for the recognised natural variability of algal growth in culture, standard algal tests (OECD, 1984; DIN, 1989) now utilise the EC_{10} as the lowest observed effect concentration (LOEC) and values below the EC_{10} as the no observed effect concentration (NOEC). Effects below the EC_{10} cannot be attributed with certainty to the substance, but represent the natural range inherent in the test. All investigations utilising the EC_{10} or EC_{50} show that *Scenedesmus subspicatus* and other algae (Webber *et al*, 1977; Guhl, 1992a; Kopf and Wilk, 1995) are tolerant to borates. A flat concentration-response curve was also demonstrated in *Scenedesmus subspicatus* by Kopf and Wilk (1995), where less than 10% growth inhibition was observed from 1 to 20 mgB/l. Therefore, the NOEC for borate to fresh water algae is at least 10 mgB/l, as indicated in Table 14.

The common blue-green algae or cyanophyte *Microcystis flos-aquae* in Swedish and Norwegian eutrophic lakes were analysed and found to contain borate at 3.2 ppm (mgB/kg), in contrast to 100,000

ppm of nitrogen and 11,300 ppm of phosphorus. The result shows that borate does not accumulate in this aquatic plant (Ahl and Jönsson, 1972), which is known for its luxury uptake of nutrients.

5.1.1.2 Higher water plants (Table 14)

The Effect of Borate on Water-Based Reeds

Few studies have been carried out on the effects of borate on reed plants. Fish often lay their eggs among reed plants and for this reason alone, it is important that reeds are not damaged by the effluent from sewage plants. In this context, a paper published about the ecological properties of non-detergent components of washing powders (Schöberl and Huber, 1988) stated that tissue damage to reeds was observed in water containing 0.35 mgB/l. Although undocumented, this value was based on a comprehensive study of the nitrogen, phosphorus and boron contents of the Berlin Lakes during 1976-1977 (Blume *et al*, 1979).

A brief summary of the same study was published (Blume *et al*, 1980), in which the abstract stated that "Pot experiments showed that high boron concentrations perhaps damage reed during growing". These pot experiments cannot be regarded as a proper dose-response study for boron against *Phragmites australis* because there was only one dose of boron, and the three components, namely the boron, nitrogen and phosphorus, were varied simultaneously. As further support for the absence of toxic effects of boron at this level, the analysis of leaves of the reed *Phragmites australis* growing naturally in the above-mentioned Berlin Lakes, with lake water containing boron from 0.2 to 0.6 mgB/l, showed a leaf boron content of only 5 mgB/kg, a definite indication of lack of boron toxicity. No symptoms of boron toxicity in *Phragmites australis* were noted until the leaf contents exceeded 100 mgB/kg (Marks *et al*, 1994).

In a further study (Bornkamm, 1991) to establish threshold values for boron toxicity to reeds, pot experiments were carried out by exposing two varieties of reed plants, *Typha angustifolia* and *Phragmites australis* to extremely high boron concentrations. The plants in 70-litre pots were given a single dose of 20 litres of boron-containing solution (as boric acid), with the following strengths : -0.1 (tap water), 300, 600, 900 and 1,200 mgB/l. The growth of the plants was then monitored for about 2 years and the corresponding strength of these solutions were then : -0.18, 49, 121, 237 and 430 mgB/l. It was stated that the reduction in concentration was due to the absorption of boron by the plant and substrate. The author obtained a calculated threshold value of 1 mgB/l (by regression analysis) for adverse effects (10% yield reduction) on several growth parameters. The result obtained in this study is questionable for two reasons. Firstly, a study design in which the plants are exposed to initial concentrations of 0.1, 300, 600, 900 and 1,200 mgB/l cannot to be valid to establish even a calculated adverse threshold of 1 mgB/l, because of the wide confidence limits from such inappropriately separated dose levels. Secondly, the lowest applied concentration of 300 mgB/l, or the corresponding residual concentration of 49 mgB/l after

about 2 years, must be considered as a toxic dose to the plant, and for this reason a 10% reduction in yield is not likely to be a reliable parameter. · This latter point is substantiated by the report of serious damage at this dose level to the roots of *Phragmites australis*.

To define the toxic threshold value for boron more reliably, a pot study (Marks *et al*, 1994; Bergmann *et al*, 1995) was carried out on *Phragmites australis* during the growth seasons of 1992 and 1993 and included both soil culture in the presence of free water and a gravel hydroculture with graduated repeated additions of boron (as boric acid) during the growth seasons to maintain the water phase at 0, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and in the case of the hydroculture even 32.0 mgB/l concentration, respectively. The number of stalks per pot, the height grown by the plants and the yield of dry substance of leaves, stalks, as well as the yield of roots at post-harvest time in some pots were determined. The boron content in leaves, stalks and roots, the appearance of boron toxicity symptoms in leaves and differences in growth were compared to the boron concentrations of the aqueous phase in the pots. It is concluded from this study that *Phragmites australis* can tolerate a relatively high boron content (up to 4 mgB/l) in the liquid nutrient substrate and for 2-3 months even up to 8 mgB/l without noticeable damage. Boron toxicity symptoms in the leaves of *Phragmites australis* arose first at leaf boron concentrations of around 150 to 180 mgB/kg dry weight, at which values no adverse effects could be established on growth, development or dry substance yields of the plants. Long-term boron contents of 8 mgB/l in waters would lead to symptoms of damage, growth- and yield-reductions of plants. *Phragmites australis* can be classified therefore as a particularly boron-tolerant plant, which could also be used in reed beds for the treatment of effluents and sewage sludge containing elevated boron contents up to 4 mgB/l.

Furthermore, these results are consistent with the finding (van Diest, 1989) that *Phragmites* reed is grown in the Netherlands to help in the stabilisation of newly reclaimed polder soils from the sea and these soils contained a relatively high total soil boron content of 0.01% (100 mgB/kg), with no evidence of toxicity to the reed.

The successful use of the reed *Phragmites communis* for the harvesting of heavy metals at a sewage works has also been described (Scholl *et al*, 1985), despite being in contact with a relatively high borate-containing (0.5 mgB/l) wastewater. Other plants tested for this use were less hardy.

Field studies on reed plants *Phragmites sp.* in Germany (Guhl, 1992a) have shown no observed adverse effect attributable to boron exposure up to 1.52 mgB/l in surface waters (eg : height of development and number of buds/leaves development of the overall vegetation and flexibility of plant).

In the first part of the study, a field pool (1.5m diameter, depth 0.8m) was divided into four equal segments and filled with boron-deficient dam water (0.04 mgB/l). Two segments acted as a control and in the two others the boron concentration was raised to 0.7 mgB/l. Twenty pots, each containing one young reed plant, were placed therein. For the duration of two vegetation periods, the morphology of the

reed plants was examined macroscopically during the growth-dependent intervals (2-8 weeks). There was no observable difference between the growth of reed plants exposed to concentrations of 0.7 mgB/l and the controls.

In the second part, reed plants (*Phragmites sp.*) (in aquatic regions near river banks) were investigated during two vegetation periods from May to September in 6 to 7 surface waters in the Nordrhein-Westfallen region, variously polluted from anthropogenic sources with a boron content of between 0.16 and 1.52 mgB/l. Boron contents in surface waters within this concentration range showed no observable effects on the growth of these reed plants.

Low concentrations of boron in leaves (3.7 ppmB in *Phragmites communis* and 8.5 ppmB in *Typha angustifolia*) were also reported in Norway and Sweden (Ahl and Jönsson, 1972), where rivers contained borate at generally less than 0.05 mgB/l. In this Scandinavian study a variety of aquatic plants was examined by analysis, and the results showed that the enrichment of boron in aquatic plants was very small compared to enrichment of phosphorus and nitrogen.

A comprehensive study of boron concentrations in surface waters (generally less than 0.1 mgB/l) and plants, carried out in the south eastern USA, showed that the reed *Typha latifolia* contained a range of boron (dry weight basis) from 5-100 ppmB, with an average value of about 20 ppmB, but there was no mention of toxicity symptoms even at the higher boron contents. Analysis of the boron contents of 22 other species of aquatic macrophytes from the same pond and growing under similar conditions gave values of boron with a range from 1.2 ppm (dry weight basis) in *Eleocharis equisetoides* to 11.3 ppm in *Nymphaea odorata*. The higher value in the latter was attributed to the extra uptake from roots in the borate-rich mud, but there was no evidence for significant accumulation in this study (Boyd and Wayne Walley, 1972).

Table 14. Borate Toxicity to Freshwater Algae and Higher Waterplants

Species	Borate tested	Test type	Effect	Borate in solution (mgB/l)	Reference
Algae, *Scenedesmus quadricauda*		chronic	EC_3*	16	Bringmann and Kühn, 1978
Algae, *Scenedesmus subspicatus*	sodium borate	chronic	EC_0	10	Guhl, 1992a
			EC_{10}	24	
			EC_{50}	34	
			EC_{100}	100	
Algae, *Scenedesmus subspicatus*	boric acid	chronic	EC_{10}	24	Kopf and Wilk,
	H_3BO_3		EC_{50}	52	1995
			EC_{90}	109	
Algae, *Microcystis aeruginosa*		chronic	EC_3*	20.3	Bringmann and Kühn, 1978
Algae, *Chlorella sp.*		field study	NOEC	≥ 250	Webber *et al*, 1977
Algae, *Microspora sp.*		field study	EC_{100}	≤ 100	Webber *et al*, 1977
Elodea canadensis		field study	NOEC	≥ 1	Scholl and Maier, 1987
Reed		field study	damage to tissue	0.35	Schöberl and Huber, 1988
Reed		field study	growth	≥ 0.5	Scholl and Maier, 1987
Reed	(in sea water)	recovery of polder soils	growth	≥ 100 mgB/kg In soil	van Diest, 1991
Reed, *Phragmites australis*		field study	damage to tissue	≥ 0.35	Blume *et al*, 1980
Reed, *Phragmites australis*		pot study (1 dose)	threshold	$\approx$ 1	Bornkamm, 1991
Reed, *Phragmites australis*	boric acid	pot study:			Marks *et al*, 1994
australis	H_3BO_3	2 years	NOEC	4	Bergmann *et al*,
		2-3 months	NOEC	8	1995
		2 years	damage	≥ 8	
Reed, *Phragmites communis*		field study	growth	≥ 0.8	Gerike *et al*, 1989a
Reed, *Typha latifolia*		field study	growth	≥ 0.8	Gerike *et al*, 1989a
Reed, *Phragmites sp.*		pond	NOEC	≥ 0.7	Guhl, 1992a
		field	NOEC	0.16	
		study	NOEC	1.52	

* EC_3 is below the value EC_{10} specified in the standard method as the minimum effect level

5.1.1.3 Bacteria, protozoa, invertebrates and microcosm systems (Table 15)

The data from tests with invertebrates show that the chronic toxicity of borate is above 6 mgB/l (NOEC). In contrast, some bacteria appear to be relatively sensitive to borate in chronic toxicity tests, with an EC_0 of 3.4 mgB/l (Guhl, 1992a). On the other hand, laboratory experiments have shown that the borate concentrations that occur in domestic wastewater (about 0.7 - 4 mgB/l) can be expected to cause a slight improvement, rather than a deterioration, in the clean-up performance of activated sludge plants (Gerike et al, 1976). The clean-up performance of activated sludge plants is not adversely affected below 40-120 mgB/l. This is confirmed by the results in the oxygen consumption inhibition test (EC_0=110 mgB/l), a test that is highly relevant to the effect of substances on the performance of activated sludge plants (Guhl and Gode, 1989).

Bringmann and Kühn (1980) developed a chronic test with *Entosiphon sulcatum* and used this to investigate the toxicity of borate. According to their studies, *Entosiphon sulcatum* is more sensitive to borate, by a factor of 233, than *Paramecium caudatum*, which occurs in the same environment (activated sludge, Fott, 1959),. However, *Entosiphon sulcatum* has even been found in aeration tanks of biological treatment plants where borate contents of 0.58-3.08 mgB/l were measured, with an annual average of 2.12 mgB/l (Guhl, 1992a). This indicates that *Entosiphon sulcatum* tolerates borate levels at 10 times higher than the values reported by Bringmann and Kühn (1980). In fact, a recent study has shown that *Entosiphon sulcatum* can tolerate borate concentrations of more than 10 mgB/l if the flagellate is fed appropriately (Guhl, 1996).

Biocenosis tests (Table 16) are gaining importance relative to single-species tests for making realistic assessments of the effects of substances on the living environment (Guhl, 1991), as follows:

- **Laboratory test**: In the microcosm test the NOEC for borate was found to be 2.5 mgB/l and the LOEC 5.0 mgB/l (Guhl, 1992a). In this test, assessments were made to determine the effects of borate on a number of species and abundance of individuals in a biocenosis consisting of procaryotes and micro-eucaryotes of six trophic stages (Guhl, 1987, 1991).

- **Outdoor ponds**: Final confirmation of the NOEC can only be obtained by outdoor studies (Guhl, 1992b). However, in the case of field studies, the prevailing concentrations of the substances are pre-determined by natural occurrence and/or anthropogenic activities. Studies on outdoor ponds over a period of two years have shown that biocenosis systems with up to 29 species simultaneously treated with 0.7 mgB/l do not differ significantly from the untreated control biocenoses. Thus, the threshold value is greater than 0.7 mgB/l.

Table 15. Borate Toxicity to Bacteria, Protozoa, Invertebrates and Microcosm Systems

Species	Test type	Duration	Effect	Borate in solution (mgB/l)	Reference
Pseudonomas putida	acute	30 min	EC_{10}	340	Guhl, 1992a
	chronic	16 h	EC_{10}	7.6	
		72 h	NOEC/LOEC	291/-	Schöberl and Huber, 1988
			NOEC/LOEC	290/-	Bringmann and Kühn, 1980
Photobacterium phosphoreum	acute	30 min	EC_{20}	18	Guhl, 1992a
Entosiphon sulcatum	chronic	72 h	NOEC/LOEC	-/0.3	Bringmann and Kühn, 1980
	chronic	72 h	NOEC/LOEC	>10	Guhl, 1996
Paramecium caudatum	reproduction	72 h	EC_{100}	<70	Bringmann and Kühn, 1980
			NOEC/LOEC	18/-	Sprague, 1972
Daphnia magna	acute	24 h	EC_{50}	242	Schöberl and Huber, 1988
		48 h	EC_{50}	95	Bringmann and Kühn, 1977
			EC_{50}	133	Gersich, 1984
			EC_{50}	226	Lewis and Valentine,1981
	chronic	21 d	NOEC/LOEC	6.4/ 13.6	Gersich, 1984
			EC_{50}	6/ 13	
			NOEC/LOEC		Lewis and Valentine,1981
			EC_{50}	53.2	
			NOEC/LOEC	10/ 30	Guhl, 1992a
Anopheles quadrimaculatus	acute	25 h	EC_{50}	125	Fay, 1959
larvae			EC_{100}*	25	
biocenosis (see Table 16)	microcosm	28 d	NOEC/LOEC	2.5/ 5	Guhl, 1992a
	outdoor pond	2 years	NOEC/LOEC	>0.7/-	
	field study	5 months	NOEC/LOEC	0.16/- 1.52/-	

* 92% mortality

- **Field studies**: Biocenosis studies were also carried out in the field on seven fresh water bodies subject to different levels of anthropogenic activities over two vegetation periods (May to September in both cases). No toxic effects of borate at concentrations between 0.16 and 1.52 mgB/l were detected in these open-air studies. Thus, the threshold value is greater than 1.5 mgB/l according to these investigations.

Table 16: Organisms in the Biocenosis Studies

	Microcosm	Outdoor ponds	Field study
Actinosphaerium eichhorni			+
Aeschna sp.			+
Agrion sp.			+
Amoeba proteus	+		
Amphiplura pellucida		+	
Anisonema ovale			+
Ankistrodesmus acicularis			+
Ankistrodesmus angustus		+	
Arcella vulgaris			+
Asellus aquaticus			+
Aspidisca costata	+		+
Asplanchna brightwelli		+	
Asplanchna priodonta			+
Baetis sp.			+
Bikosoeka sp.	+	+	
Bodo sp.	+	+	+
Bosmina coregoni			+
Bosmina longirostris		+	+
Brachionus angularis	+	+	
Brachionus leydigii		+	+
Carinogammarus roeselii			+
Carteria peterhofiensis		+	
Ceratium hirundinella		+	+
Chaetonotus hystrix		+	+
Characiopsis acuta			+
Chilodonella cucullulus		+	
Chironomus plumosus		+	
Chironomus thummi			+
Chlamydomonas ehrenbergii		+	
Chlorella ssp.		+	

	Microcosm	Outdoor Ponds	Field Study
Cloeon sp.			+
Closterium acerosum			+
Closterium moniliferum			+
Closterium pronum		+	
Coleps hirtus			+
Colurella uncinata	+		+
Cyclocypris laevis			+
Cypria sp.		+	
Cypridopsis sp.			+
Daphnia cucullata		+	+
Daphnia pulex		+	
Diaphanosoma brachyurum			+
Diatoma vulgare	+		+
Difflugia vulgaris	+	+	+
Dinobryon stipitatum		+	+
Dreissenia polymorpha			+
Elodea canadensis			+
Enochrus ssp.			+
Epistylis ovum			+
Epistylis plicatilis		+	+
Epistylis rotans			+
Eucyclops macrurus		+	+
Eudiaptomus gracilis		+	+
Euglena deses		+	+
Euplotes charon		+	
Fragilaria capucina		+	+
Fragilaria crotonensis			+
Gammarus pulex			+
Gonium sociale		+	+
Haemopis sanguisuga			+

	Microcosm	Outdoor Ponds	Field Study
Helmis sp.			+
Helobdella stagnalis			+
Heptagenia sp.			+
Hydropsyche sp.			+
Kellicottia longispina			+
Keratella cochlearis		+	+
Lecane luna		+	
Lemna minor		+	+
Lepadella patella		+	
Leptocinclis ovum			+
Leptodora kindtii			+
Leptophlebia sp.			+
Leuctra sp.			+
Lestes viridis			+
Lionotus cygnus	+		+
Mayorella sp.	+	+	
Micrasterias crux melitensis		+	+
Mougeotia sp.		+	+
Nais sp		+	
Nematodes		+	
Oligoneuriella sp.			+
Oocystis lacustris	+		
Oscillatoria formosa	+		+
Oscillatoria tenuis	+	+	+
Pediastrum duplex			+
Pediastrum sturmi			+
Pelomyxa sp.			+
Peridinium tabulatum			+
Perla sp.			+
Phacus striatus		+	
Philodina roseola		+	
Phragmites sp.		+	+
Physa fontinalis			+

	Microcosm	Outdoor Ponds	Field Study
Planaria gonocephala			+
Platyas patulus			+
Polycelis cornuta			+
Polyphemus pediculus		+	+
Potamogeton sp.			+
Prorodon teres	+		
Radix auricularia			+
Rhitrogena sp.			+
Scenedesmus acuminatus	+	+	
Scenedesmus ecornis	+		
Scenedesmus quadricauda	+	+	+
Selenastrum bibraianum		+	
Simulium sp.			+
Sphaerium corneum			+
Spirostomum teres			+
Stentor coeruleus			+
Stentor roeseli			+
Stratiomys sp.			+
Stylonychia mytilus		+	+
Stylaria lacustris		+	
Stylonychia pustulata	+	+	+
Synedra acus	+	+	+
Synura uvella		+	+
Tabellaria fenestrata	+		+
Tetraspora lacustris			+
Thecamoeba sp.	+	+	
Trachelomonas volvocina		+	
Tubifex tubifex			+
Ulothrix zonata			+
Uydrachna sp.			+
Vorticella campanula		+	
Vorticella citrina	+	+	+

5.1.1.4 Acute toxicity to fish (Table 17)

The acute toxicity of borate to freshwater fish is relatively low (LC$_{50}$ 14-3,400 mgB/l). The toxicity depends on the duration of exposure, as shown for *Gambusia affinis* by Wallen (1957) and varies from species to species (Butterwick *et al*,1989). The data suggest that rainbow trout (*Oncorhynchus mykiss*) and zebra fish (*Brachydanio rerio*) are the most sensitive species.

Acute toxicity studies (96-hr LC$_{50}$) on the early life stages of the Colorado squawfish, the Razorback sucker and the Bonytail against selected inorganics in reconstituted water simulating the middle Green River, Utah, established the order of toxicity from most to least as vanadium = zinc > selenite > lithium =uranium >selenate >boron, with values for borate listed in table 17 (Hamilton, 1995). The measured environmental concentrations of borate in the Ashley Creek and Stewart Lake outlets into the middle Green River were 0.63 mgB/l and 0.62 mgB/l, respectively (Buhl and Hamilton, 1996).

5.1.1.5 Fish Early Life Stage Tests in Reconstituted Water (Table 18)

Development of early life stage tests for fish and their use to determine boron toxicity was first presented by Birge and Black (1977). Toxicity of borate to early life stages of fish has been documented for several species (Birge and Black, 1977, Birge and Black, 1981, Birge *et al*, 1984). Embryonic and early larval stages of rainbow trout (*Oncorhynchus mykiss,* formerly *Salmo gairdneri*), largemouth bass (*Micropterus salmoides*), channel catfish (*Ictalurus punctatus*) and goldfish (*Carassius auratus*) were exposed to boron, as boric acid or borax, from fertilisation up to 8-d post-hatch. All exposures were in soft (50 mg CaCO$_3$/l) or hard (~200 mg CaCO$_3$/l) reconstituted water. Test responses included embryonic mortality, teratogenesis and larval mortality.   Gross debilitating anomalies of survivors were classified as mortalities.   Effect (LC$_{50}$ and LOEC) and no effect (NOEC) concentrations for each species are presented in Table 18. Neither water hardness nor the form of boron (boric acid, borax) added to the test aquaria consistently affected embryo-larval survival of rainbow trout, channel catfish and goldfish (Birge and Black, 1977).

On the basis of median lethal concentrations (LC$_{50}$), no species was found to be especially sensitive. The range of LC$_{50}$s for the above four was between 22 and 155 mgB/l. In addition, Birge and Black (1977) also reported LC$_1$s ranging from 0.001 to 0.1 mgB/l for rainbow trout, 0.2 to 5.5 mgB/l for channel catfish and 0.2 to 1.4 mgB/l for goldfish. The LC$_1$ showed clearly that rainbow trout was the most sensitive species and therefore has been studied in greater detail. The no observed effect concentrations (NOEC) for rainbow trout ranged from 0.009 to 0.103 mgB/l and was 1.39 mgB/l for largemouth bass. These were consistent with the acute toxicity results (section 5.1.1.4) that indicated rainbow trout and zebra fish as the most sensitive species.

Table 17. Borate Acute Toxicity to Freshwater fish

Species	Borate tested	Duration	Effect	Borate in solution(mgB/)	Reference
Brachydanio rerio (Zebra fish)	borax	96 h	LC	10.2	Guhl, 1992a
			LC_{50}	14.2	
Gambusia affinis	boric acid	24 h	Lm/Mld *	3145	Wallen, 1957
		48 h	TLm/Mld	1834	
		96 h	LC_0	<314	
		96 h	TLm/Mld	978	
	borax	24 h	TLm/Mld	1360	
		48 h	TLm/Mld	929	
		96 h	TLm/Mld	408	
		144 h	LC_0	<204	
		144 h	TLm/Mld	215	
Golden orfe	unknown	48 h	LC_{50}	173	Schöberl and Huber, 1988
Lepomis macrochirus	borax	24 h	Lm/Mld	4.6	Turnbull, 1954
Minnow	boric acid in	6 h	TLm/Mld	3145-3319	McKee and Wolf,
	distilled water	unknown	TLm/Mld	340-374	1963
	boric acid in	6 h	TLm/Mld	3319-3407	
	hard water	unknown	TLm/Mld	793-850	
	unknown	6 h	LC_{100}	300-900	Acad. Eng. Sci., 1970
	unknown	unknown	LC_{50}	3200-3400	Schöberl and Huber, 1988
Ptychocheilus lucius (Colorado squaw-fish)	boric acid	96 h	LC_{50}	279[a] <100[b] 527[c]	Hamilton, 1995
Xyrauchen texanus (Razorback sucker)	boric acid	96 h	LC_{50}	233[a] 279[b] 100[c]	Hamilton, 1995
Gila elegans (Bonytail)	boric acid	96 h	LC_{50}	280[a] 100[b] 552[c]	Hamilton, 1995
Oncorhynchus mykiss (trout)	borax	unknown	LC_{80}	3.7	McKee and Wolf, 1963
	unknown	24 h	LC_0	11.	Acad. Eng. Sci., 1970
			LC_{80}	22	

* TLm/Mld = Threshold Limit/Minimum lethal dose
[a] Swimup fry   [b] 1g juvenile   [c] 2g juvenile

The curcumin method as outlined in Standard Methods (APHA, 1975) and an optimised version of the method (via concentration of the test solution) were used to quantify boron levels in the test aquaria. The quantitation limit for boron in the Birge and Black (1977) study was 0.1 mgB/l using the standard method,

whereas the limit in the Birge and Black (1981) and Birge *et al* (1984) studies was 0.01 mgB/l using the optimised method. Therefore, the no effect concentrations below these limits were actually estimates based on nominal concentrations. A recent study by Huber (1994b) showed that hard reconstituted water contained 0.042 mgB/l as impurities from the reagents, so that these earlier trout studies are likely to have contained boron levels exceeding nominal concentrations.

Table 18. Borate Toxicity to Embryo-Larval Stages of Freshwater Fish Exposed to Reconstituted Water
(Early studies by Birge and Black)

Species	Borate tested	Hardness (mgCaCO₃/l)	Duration	Effect	Borate in solution (mgB/l)	Reference
Oncorhynchus mykiss (Rainbouw-trout)	boric acid	50	32 d	LC_{50}	100	Birge and Black,
	boric acid	200	32 d	LC_{50}	79	1977
	borax	50	32 d	LC_{50}	27	
	borax	200	32 d	LC_{50}	54	
	boric acid	200	32 d	NOEC LOEC LC_{50}	0.009 0.1 138	Birge and Black, 1981
	boric acid	188	36 d	NOEC LOEC	0.103 1.34	Birge *et al*, 1984
Ictalurus punctatus (Channel catfish)	boric acid	50	9 d	LC_{50}	155	Birge and Black,
	boric acid	200	9 d	LC_{50}	22	1977
	borax	50	9 d	LC50	155	
	borax	200	9 d	LC_{50}	71	
Carassius auratus (Goldfish)	boric acid	50	7 d	LC_{50}	46	Birge and Black,
	boric acid	200	7 d	LC_{50}	75	1977
	borax	50	7 d	LC_{50}	65	
	borax	200	7 d	LC_{50}	59	
Micropterus salmoides (Largemouth bass)	boric acid	200	11 d	NOEC LOEC LC_{50}	>1.39 12.17 92	Birge and Black, 1981

To gain further definition of the sensitivity of trout, additional studies were conducted with embryo-larval stages using a more sophisticated experimental design. Tests were performed to determine whether the exposure medium affected toxicity (i.e., natural against reconstituted waters). These studies are described in detail by Black *et al* (1993) and are summarised in the following section.

5.1.1.6 Fish Early Life Stage Tests in Natural Water (Table 19)

Four sources of natural dilution water were used to determine the effects of the exposure media on borate toxicity to embryo-larval stages of rainbow trout (Black *et al*, 1993). Surface waters were collected from the following three locations, the Erwin National Fish Hatchery (Tennessee), Brookville Lake (Indiana) and Firehole River (Yellowstone National Park, Wyoming). Surface water control borate concentrations were : 0.023, 0.091 and 0.75 mgB/l for Erwin, Brookville and Yellowstone waters, respectively. Total organic carbon (TOC) for the three surface waters ranged from 0.8 to 1.9 mg/l. Hardness levels for these natural waters ranged from 24 to 209 mg $CaCO_3$/l. In the surface water tests, three nominal treatments were used: surface water control, control plus 1.0 mgB/l boron added (as boric acid) and control plus 10 mgB/l boron added. Total and dissolved borate levels were measured for each of the surface water treatments. No statistically significant differences were noted between total and dissolved borate concentrations except for Yellowstone (1.0 mgB/l) and Erwin (10 mgB/l) treatments. The true significance of this, however, is minimal since total versus dissolved concentrations for Yellowstone and Erwin waters were 1.61 vs. 1.52 mgB/l and 9.91 vs. 9.48 mgB/l, respectively. Hence, it is reasonable to assume that all borate was in solution.

In addition, deep (600m) well water, typically used for aquatic toxicity tests, from a contract laboratory located in Wareham, Massachusetts was also used. Boron was spiked into well water to obtain an exponential series of nominal concentrations, 0.0017, 0.017, 0.17, 1.7 and 17 mgB/l, respectively. Analytical confirmation of the actual exposure concentration was obtained for the two highest concentrations, 2.1 and 18.0 mgB/l. No effects were observed, even at the highest borate concentration.

Borate did not elicit toxicity to embryo-larval stages of rainbow trout exposed to surface water control boron levels up to 0.75 mgB/l, which constitutes a conservative NOEC for the surface water studies. Lowest observed effect concentrations for Erwin, Brookville and Yellowstone treatments were 1.10, 1.24 and 1.73 mgB/l, respectively, indicating that the threshold for no effects is approximately 1 mgB/l. Therefore, the NOEC is likely to be around 1 mgB/l.

**Table 19: Borate Toxicity to Embryo-Larval Stages of Rainbow Trout
Exposed to Natural Waters (Black *et al*, 1993)**

Water Source	Duration	NOEC (mgB/l)	Frequency (%) of test response	LOEC (mgB/l)	Frequency (%) of test response
Brookville	36 d	0.09	4.8	1.24	14.1
Erwin	36 d	0.02	6.3	1.10	14.6
Yellowstone	36 d	0.75	6.2	1.73	16.6
Wareham, Mass. well water	87 d	>18.0	5		

5.1.1.7 Discussion of fish early life stage tests

In contrast to the concentration-effect relationship observed for many other chemicals, the toxic effects of borate are characterised by an unusually flat concentration-response curve over the lower end of the exposure range (0.001-10 mgB/l). Early results on embryo-larval stages of trout in soft reconstituted water (Birge and Black, 1977) showed control-adjusted effects that ranged only from 2-8% for exposure concentrations of boron spanning 2-3 orders of magnitude (Figure 1). In their safety assessment of this early study, Butterwick et al (1989) observed further, that a concentration of greater than 1 mgB/l is required to obtain a 10% increase on control-adjusted mortality. For these reasons and after further studies, Black et al (1993) recommended that only consistent concentration-response effect levels should be considered in the development of an aquatic risk assessment for borate.

**Figure 1: The Effects of Boric Administered to Embryo-Larval Stages
of Trout in Soft Water (from Birge & Black 1977)**
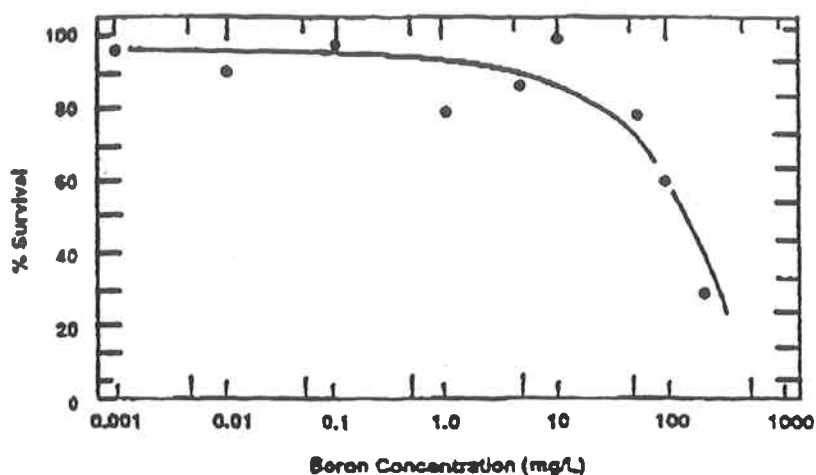
The statistical significance of embryo-larval responses was also dependent on control variability, or lack thereof. For instance, control survival averaged 90% or greater with deformities (terata) seldom exceeding 1%. As a consequence, statistically significant responses (via both regression and analysis of variance approaches) were observed over a wide concentration range. Even so, tests performed with early-life stages of the rainbow trout generally required a concentration of at least 1.0 mgB/l to achieve a consistent increase in control-adjusted mortality of greater than 10 %. It should be noted that hatching and post-hatch success of >66% and ≥70%, respectively, are acceptable for OECD Method 210 for early life stage tests with rainbow trout. Hence, control response variance was exceptionally small compared to that accepted in standard methods.

Apart from the unusual flat concentration-response curve, there are two other factors that appear to influence the toxicity threshold of borate to the embryo-larval stages of trout. There is evidence that borate is more toxic in reconstituted water than in natural water. In addition, there may be a differential sensitivity of the strains of trout tested.

5.1.1.7.1 Effects of different dilution waters

Adverse effects of a chemical observed in a toxicity assay are dependent on the test material's bioavailability. Water hardness and organic carbon have been shown repeatedly as key factors affecting metal and organic chemical speciation. In this case, neither the form of boron (boric acid, borax) dosed to aquaria nor level of hardness and organic carbon was found to consistently affect embryo-larval effects in reconstituted and natural waters. Additionally, boron was found primarily only in the dissolved state (not sorbed to solids). Hence, it could be argued that since no differential bioavailability of boron was observed from either reconstituted or natural water, the greater LOECs seen with the natural water exposures were due to some other unknown mechanism.

5.1.1.7.2 Differential sensitivity of trout strains

Different trout strains were used for studies that included reconstituted water as a study component (Birge and Black, 1981, Soap Lake; Birge et al, 1984, Wytheville). The strain used by Birge and Black (1977) is not known, however it was sourced from the Erwin National Fish Hatchery, Erwin, Tennessee - the same source of the Soap Lake and Wytheville strains. Birge and Black (1977) reported LC1s ranging from 0.001 to 0.1 mgB/l. Similarly, NOECs ranged from 0.009 to 0.103 mgB/l from the Birge and Black (1981) and Birge et al (1984) studies. Assuming that the 1977 studies used one strain of trout, the data suggest that inter-strain variability is roughly equivalent to intra-strain variability. Thus, it is unlikely that the strain is a large contributor to the variance in the boron trout data set.

5.1.1.8 Survey Data on Trout Streams and Hatcheries in European Countries and Western USA

Concentrations of boron in UK surface waters capable of sustaining salmonids are described by Unilever (1994). Median boron concentrations from nine different water regions ranged from 0.007 to 0.272 mgB/l (Table 20). The highest concentrations occurred in the Southern (0.272 mgB/l) and Anglian (0.189 mgB/l) water regions.

Table 20: Borate Concentrations in UK Fresh Waters Capable of Sustaining Salmonids (Unilever, 1994)

Water Region	Number of Observations	Range of Medians (mgB/l)	Maximum Value (mgB/l)
Anglian NRA	12	0.060 - 0.189	0.348
Northumbria NRA	539	0.060 - 0.090	2.300
Southern NRA	118	0.028 - 0.272	0.447
South West NRA	23	0.011 - 0.016	0.018
Thames NRA	98	<0.05 - 0.056	0.714
Welsh NRA	307	0.007 - 0.048	0.113
Wessex NRA	415	0.011 - 0.115	0.180
Forth RPB	203	0.030 - 0.080	0.480
Highland RPB	889	<0.017	0.575

NRA: National River Authority
RPB: River Purification Board

Boron concentrations in UK and German trout hatcheries were found to be similar. Concentrations of boron in feed waters of 20 UK rainbow trout hatcheries were found to have mean values of 0.009 to 0.105 mgB/l (Table 21; Unilever, 1994) while a range of 0.002 to 0.107 mgB/l in eighteen trout hatcheries of Germany were observed (Table 22; Huber, 1994c).

Two studies have been conducted to determine the relationship between boron levels in streams and hatcheries and the distribution of trout in California and other western states in the USA (Bingham, 1982; EA, 1994).

Boron concentrations in Californian surface waters that supported viable populations of wild rainbow trout were determined via studies conducted by the US Geological Survey (1969-1980). The concentrations in these streams ranged from less than 0.01 to 13.1 mgB/l (Bingham, 1982). In addition, Bingham reported concentrations of boron in California's 10 major trout hatcheries (Table 23). No instances were found where trout health was limited. Generally, the concentrations were less than 0.1 mgB/l, however the Hot Creek and Darrah Springs Hatcheries ranged up to 0.8 and 1.0 mgB/l, respectively.

**Table 21: Mean Borate Concentrations in Feed Waters to
UK Trout Hatcheries (Unilever, 1994)**

Location	Source	Mean (mgB/l)
Chichester Sussex	Chalk spring	0.0097
Stockbridge Hants.	Deep borehole	0.0115
Stockbridge Hants.	Deep borehole	0.0137
Barton Stacey Hants.	Shallow borehole	0.016
Andover Hants.	Shallow borehole	0.0263
Damerham Hants.	Shallow borehole	0.0098
Brockington Dorset	Shallow borehole	0.0097
Fovant Hants.	Chalk spring	0.015
Pewsey Wilts.	River abstraction	0.0405
Hungerford Berks.	Deep borehole	0.0237
Lechlade Gloucs.	River abstraction	0.018
Bibury Gloucs.	Shallow borehole	0.0143
Stow on the Wold Gloucs.	Limestone spring	0.0093
Westacre Norfolk	Chalk spring	0.0102
Oakham Rutland	Shallow borehole	0.1052
Exton Rutland	Chalk spring	0.0304
Blandford Forum Dorset	Shallow borehole	0.0124
Andover Hants.	Shallow borehole	0.019
Bowerchalke Wilts.	Shallow borehole	0.0168
Duncton Mill Sussex	Chalk spring	0.018

A second and more expansive survey was recently conducted with the intent of updating the Bingham report and broadening the area of interest to Montana, Nevada, New Mexico, North Dakota, Oregon and Wyoming. Survey results were developed primarily via personal communications with 37 different fisheries biologists in the western US region. No instances were found where rainbow trout populations were limited by boron (Table 24) (EA, 1994).

Even 13 years after the Bingham report no instances were found of boron limiting hatchery production at California's 10 major hatcheries. Inquiries in other states resulted in the same conclusions. Several locations with boron concentrations near, or above, 1 mgB/l were found with successful rainbow trout populations. Examples are:

- East and Paulina Lakes in Oregon (>0.9 mgB/l);

- The Firehole River in Yellowstone National Park, Wyoming (>0.9 mgB/l);

- The Napa River in California (>1.2 mgB/l); and

- Little Warm Springs in California (>3.0 mgB/l).

**Table 22: Borate Concentrations at Trout Hatcheries
in Germany (Huber,1994c)**

Trout Hatchery at :	Borate (mgB/l)	Trout Hatchery at:	Borate (mgB/l)
Wielenbach (Kreis Weilheim Oberbayern)	0.048 0.048 0.047	Kradenbach (Kreis Daun Pfälzer Wald Rheinland Pfalz)	0.017
Starnberg (Kreis Starnberg Oberbayern)	0.029 0.029 0.029 0.029	Eiswoog (Kreis Donnersberg Eifel Rheinland Pfalz)	0.018
Grainau (Kreis Garmisch- Partenkirchen, Oberbayern)	0.012	Marxzell (Baden-Württemberg)	<0.01
Lindbergmühle (Kreis Regen, Niederbayern)	0.002	Eyachmühle (Baden-Württemberg)	<0.01
Grafenried (Kreis Regen Niederbayern)	0.004	Weisenbach/Murg I (Baden-Württemberg)	0.020
Obervolkach (Kreis Kitzingen, Unterfranken)	0.067	Weisenbach/Murg II (Baden-Württemberg)	<0.01
Oberpfeilfeld (Kreis Würzburg Unterfranken)	0.017 0.107	Forbach (Baden-Württemberg)	0.030
Straubing (Kreis Straubing Niederbayern)	0.004	Dobel (Baden-Württemberg)	0.060
Obermarsberg (Kreis Arnsberg Nordrhein-Westfalen)	0.083	Haan (Nordrhein- Westfalen)	0.010- 0.020

**Table 23: Borate Concentrations at Ten Major California Trout Hatcheries
Identified by Bingham (1982) and Updated by EA (1994)**

Hatchery	Borate (mgB/l)	1994 Comments
Darrah Springs	0.15 - 1.0	No problems observed arising from chemical composition
Hot Creek	0.18 0.8	No problems observed arising from chemical composition
Fish Springs	0.16	No problems observed arising from chemical composition
Mojave River	.0.09	Good water quality
Crystal Lake	0.05	Temperatures below optimum (9-11°C). No problems observed arising from chemical composition
Mount Whitney	0.05	General condition of water quality and fish are excellent
American River	0.03	No problems mentioned
Moccasin Creek	0.03	No problems observed arising from chemical composition
San Joaquin	0.02	No problems observed arising from chemical composition
Filmore	0.02	No problems observed arising from chemical composition

The first and second locations are renowned trophy trout waters. Important game species other than rainbow trout, such as northern pike, sturgeon and catfish were reported to live in streams with high boron concentrations such as the Poplar River (0.05-1.9 mgB/l) in Montana and the Souris River (2.0-3.0 mgB/l) in North Dakota. In these specific rivers rainbow trout were reported to be absent due to unsuitable habitat and high water temperatures (EA, 1994).

5.1.1.9 Effects of Boron on Fertilisation of Rainbow Trout by Artificial Insemination

Artificial insemination traditionally has used freshwater as the medium in which the male and female gametes are mixed. However, recent research by van Heerden et al (1993) has shown that sperm motility and fertilisation success are enhanced when placed in boric acid/borax buffered diluent (200 mgB/l). Six different diluents were tested (stream water, ovarian fluid, simulated ovarian fluid and three borate buffered diluents designated "Borax 1, 2, and 3"). Motility was greatest in Borax 3 diluent and least in freshwater, whereas fertilisation success was greatest in ovarian fluid, followed by

Table 24: Borate Concentrations in Selected Western US Water Bodies Containing Viable Trout Populations

State	Water body	Borate (mgB/l)			Comments
		Min.	Max.	Mean	
CA	Sespe Creek	0.48	1.8	1.14	
CA	Cache Creek	0.15	5.0	1.465	
CA	Napa River	0.04	5.1	1.242	
CA	San Joaquin	0.03	2.7	1.057 0.797 0.763	Fremont reach. Newman reach. Patterson reach.
MT	WF Poplar Riv.	0.05	1.9	0.575	Inhabited by forage fish. Too warm for rainbow trout in streams.
ND	Souris River			2.0-3.0	Non-salmonid fish present. Trout absent, presumably due to temp., but no good data.
NV	Churchill Co.			1.338	Rainbow trout planted in ponds with no apparent problems except for temperature.
NV	Pershing Co.		0.550		Rainbow trout planted in ponds with no apparent problems except for temperature.
OR	East Lake	0.86	1.0	0.949	Trophy planted trout fishery. Not self sustaining due to lack of spawning habitat.
OR	Paulina Lake	0.86	0.92	0.892	Trophy planted trout fishery. Not self sustaining due to lack of spawning habitat.
WY	Firehole River	0.92	0.93	0.925	Highly productive, self sustaining rainbow trout and brown trout fishery with mainstem spawning.

CA = California   NV = Nevada
MT = Montana   OR = Oregon
ND = North Dakota   WY = Wyoming

simulated ovarian fluid, Borax 2, 3, 1 and freshwater, respectively. Hence, for very short term exposures, concentrations of boron up to 200 mgB/l are safe for rainbow trout embryos.

5.1.1.10 Integrated Assessment for Rainbow Trout

Early investigations of boron toxicity in reconstituted water to embryo-larval stages of several different fish species clearly showed that rainbow trout was the most sensitive. The variance observed with these chronic effects data was attributed, primarily, to the flat concentration-response curve.

A primary goal of conducting chronic (early-life stage) tests is to estimate a toxicant concentration that will cause minimal biological effects on a population (Bruce and Versteeg, 1992). The concentration-

response and the effect concentration are used to derive a safe concentration for the environment. Statistical methods currently in use compare biological responses in treatments against controls, either by regression or by analysis of variance methods (Barnthouse et al, 1987; Weber et al, 1989; Bruce and Versteeg, 1992).

Regression based methods utilise the concentration-response curve to derive effect concentrations, such as the IC25 (Barnthouse et al, 1987, Weber et al, 1989 and Bruce and Versteeg, 1992). That is, between 20 and 25% reduction are required in growth and/or reproduction in chronic tests to determine the minimal or no effect concentration. This closely agrees with OECD 210 test acceptability criteria (<34% reduction in hatching success and <30% reduction in post-hatch success). In contrast, an analysis of variance approach for determination of the no effect concentration does not take into account the entire concentration-response curve. As a consequence, NOECs can range widely - particularly if control variance is small. In the case of the boron experiments where serial dilutions of an order of magnitude were used for exposure concentrations, the NOEC overestimates the chronic toxicity of boron.

The NOECs and LOECs generated in natural waters were greater compared to those from the reconstituted water experiments. NOECs ranged up to 0.75 mgB/l in surface waters and, surprisingly, up to 18 mgB/l in well water. Consistent LOECs in natural waters were slightly greater than 1 mgB/l, ranging from 1.1 to 1.73 mgB/l. The differences from these reconstituted and natural waters were not readily apparent. For chemicals that sorb or complex with cations and suspended solids, such as calcium ions (related to water hardness) and organic carbon, bioavailability is typically decreased in natural waters compared to reconstituted waters. As a consequence, effect levels are typically greater in natural water tests. Embryo-larval effects were not significantly affected by water hardness *via* reconstituted or natural water tests. Total organic carbon (TOC) was not measured in the reconstituted water tests, however it can be reasonably assumed to be below 2.0 mg/l since no organic carbon was added to the preparation. The TOC levels in natural surface waters were all below 2.0 mg/l. Dissolved and total boron levels were determined for the surface water tests. As discussed in section 5.1.1.6, nearly all boron present was in the dissolved form. Therefore, bioavailability was not reduced by natural water versus reconstituted.

Previous investigators (Farris *et al*, 1994; Belanger *et al*, 1989) have shown that organisms cultured in natural water often grow and reproduce better than their counterparts in reconstituted or laboratory dechlorinated water. Therefore, a reasonable hypothesis that may explain, in part, the differences in reconstituted versus natural water responses is the better health experienced by organisms exposed to boron in natural water. The causative agents for this lack of health in laboratory water are not certain, but may include nutrient deficiencies (Belanger *et al*, 1989; Keating and Dagbusan, 1984).

Several trout surveys conducted in the UK (Unilever, 1994), Germany (Huber, 1994c) and the USA (Bingham, 1982; EA, 1994) streams and hatcheries clearly showed that healthy reproducing populations exist above laboratory-derived NOECs. Concentrations ranged up to 0.107 (Table 22) and 0.272 mgB/l

(maximum median value, Table 20) in Germany and the UK, respectively, while ranging up to 13.1 mgB/l in California, USA.   Mean concentrations in hatcheries were generally below 0.1 mgB/l for all geographies, however, the Hot Creek and Darrah Springs hatcheries in California ranged up to 0.8 and 1.0 mgB/l, respectively. For all these surveys, no instances were found where borate limited hatchery production. Further, a survey of 37 different fisheries biologists located in the Western USA yielded several locations with boron concentrations near or above 1 mgB/l having successful rainbow trout populations, such as: East and Paulina Lakes (Oregon), Firehole River (Wyoming), Napa River (California) and Little Warm Springs (California). The Oregon and Wyoming locations are renowned trophy trout waters (EA, 1994). These observations, therefore, confirm laboratory expectations that rainbow trout populations do not become limited at boron levels below 1 mgB/l.

To summarise, chronic toxicity values derived from laboratory tests with reconstituted water overestimate the adverse effects threshold for early life stages of the rainbow trout. No adverse effects were observed up to 0.75 in natural water (and 18 mgB/l in well water spiked with boron). As stated in section 5.1.1.7, the most likely NOEC is 1 mgB/l. Current fragmental data on trout hatchery and stream surveys suggest that rainbow trout are readily found in hatcheries and streams up to and above 1 mgB/l. This conclusion is being consolidated by further studies currently planned.

5.1.1.11 *Bioaccumulation in Fish* (Table 25)

Borate concentrations in field collections of several fish species show that there is no bioaccumulation. For all tested fish, the bioconcentration factor (BCF) is less than 1. Only accumulation represented by a BCF of greater than 100 is considered to be significant (EC, 1993).

Concentrations of boron in fish tissues are given in mgB/kg fresh weight or dry weight. It is difficult to compare dry weight values with fresh weight values, therefore it is assumed that the dry weight should be 20 % of the fresh weight. The assumed fresh weight values in Table 25 are marked by an asterisk.

Table 25: Borate Concentrations in Field Collections of Freshwater Fish

Species	Tissues	Borate in water, median (mgB/l)	Borate in tissue (mgB/kg) dry wt	Borate in tissue (mgB/kg) fresh wt	BCF #	Reference
Salvelinus namaycush	muscle	unknown [1]		0.2-0.6		Jenkins, 1980
Gambusia affinis	whole	20[2]	8-20	1.6-4*	0.08 0.2	Ohlendorf *et al, 1986*
	body	7.4 [3]	2.8-3.6	0.56-0.72 *	0.08-0.1	
Lepomis macrochirus	whole body	1.1-3.1 [4]	0.8-3.9	0.16-0.78 *	0.05-0.71	Klasing and Pilch, 1988
Cyprinus carpio	whole body	1.1-3.1 [4]	0.5-6.2	0.1-1.24 *	0.03-1.25	

[1] unknown location within USA   [3] Volta Wildlife area, CA
[2] Kesterson National Wildlife Refuge, CA   [4] Western San Joaquin Valley, CA
= Bioconcentration Factor   * = calculated fresh weight (see text)

5.1.2 Estuarine/Marine

Estuarine and marine organisms are inherently tolerant to borate, considering they are chronically exposed to relatively high levels as a natural constituent in seawater. Marine algae and some marine invertebrates concentrate boron in their tissues. Limited data on marine fish suggest that muscle tissue may accumulate boron levels in excess of seawater concentrations.

5.1.2.1 *Algae and Macrophytes* (Table 26)

Rao (1992) compiled data on the elemental composition (31 elements) in 120 algal species collected off the Indian coast. Values for borate were found for 20 species which ranged from 2.4-235 mgB/kg dry weight. Caroli *et al* (1991) also assessed elemental composition and cites a mean concentration in algae of 97 mgB/kg dry weight in the Mediterranean alga (*Ulva lactuca*), which represents moderate accumulation while in contact with seawater of around 5 mgB/l. These values are consistent with earlier compiled data on marine algae and macrophytes. Jenkins (1980) and Eisler (1981) noted that boron concentrations are elevated in some marine algae (4-120 mgB/kg dry weight) and macrophytes (16-319 mgB/kg dry weight), respectively. Early studies by Yamamoto *et al* (1971) also indicated that many species of seaweed accumulate borate (range 231-3038 mgB/kg ash weight). It has long been known that boron is an essential micronutrient for both marine and freshwater species of diatoms (Lewin, 1965).

Table 26. Borate Toxicity to Marine Algae

Species	Borate tested	Borate in solution (mgB/l)	Effects	Water quality	Reference
Marine phytoplankton (10 species, unialgal cultures)	boric acid H_3BO_3	30	Reduction in photosynthesis for 50% of species after 5 days	seawater	Subba Rao, 1981
Marine phytoplankton (19 species, axenic cultures)	boric acid H_3BO_3	10	No effect on growth	seawater, pH7.6-8.0	Antia and Cheng, 1975
Marine phytoplankton (19 species, axenic cultures)	boric acid H_3BO_3	50	Reduction in growth rate for 26% of species	seawater, pH7.6-8.0	Antia and Cheng, 1975

Subba Rao (1981) found 30 mgB/l was needed to reduce photosynthesis in 5 of the 10 species tested, whereas Antia and Cheng (1975) found that 50 mgB/l was required to decrease growth rates of 5 of 19 species investigated. They also concluded that marine phytoplankton could tolerate at least 10 mgB/l without destabilising the natural phytoplankton assemblage.

5.1.2.2 *Invertebrates* (Table 27)

Several researchers have examined boron concentrations in marine invertebrates including zooplankton, ctenophores, corals, tunicates, chaetognaths, crabs, molluscs, and octopus (Thompson *et al*, 1976; Eisler, 1981). In general, boron concentrations are elevated in zooplankton (18-216 mgB/kg dry weight) and corals (40-110 mgB/kg dry weight), but much lower in other marine invertebrates.

Limited boron toxicity data exist for marine invertebrates. However, like marine algae, marine invertebrates are chronically exposed to relatively high concentrations of natural boron in sea water (4-5 mgB/l). Even at exaggerated borate levels, Kobayaski (1971) found sea urchin (*Anthocidaris crassispina*) embryos developed normally at 37 mgB/l, but 75 mgB/l was toxic.

Table 27. Borate Concentrations in Marine Invertebrates

Species	Tissue concentration (mgB/kg)	Reference
Zooplankton	18-216 DW *	Thompson *et al* 1976
Ctenophore, *Beroe cucumis*	115 AW **	Eisler, 1981
Corals, 34 species		
Deep open ocean	50-85 DW	Eisler, 1981
Shallow open ocean	60-100 DW	Eisler, 1981
Shallow coastal zone	40-110 DW	Eisler, 1981
Tunicate, *Salpa fusiformis,* whole	50 AW	Eisler, 1981
Chaetognath, *Sagitta elegans*	130 AW	Eisler, 1981
Dungeness crab, *Cancer magister*, whole	1.8 (0.9-3.3) FW ***	Eisler, 1981
Molluscs, bivalves		
Soft parts, 11 species	1.6-4.5 FW	Eisler, 1981; Thompson *et al*, 1976
Soft parts, British Columbia		
Clams, 8 species	0.9-5.3 FW	Thompson *et al*, 1976
Oysters, 2 species	3.1-4.0 FW	Thompson *et al*, 1976
Mussels, 2 species	2.0-5.5 FW	Thompson *et al*, 1976
Octopus, *Polypus bimaculatus,* whole	1.3 FW	Thompson *et al*, 1976

* DW = Dry Weight   ** AW = Ash Weight   *** FW = Fresh Weight

5.1.2.3 *Vertebrates - Fish* (Table 28 and 29)

Data on borate concentrations for selected species of marine fish are limited to sockeye salmon (*Oncorhynchus nerka*), anchovetta (*Cetengraulis mysticetus*), yellowfin tuna (*Thunnus albacares*), and cod (*Gadus morhua*). Borate levels in marine fish are low (1.5-9.0 mgB/kg ash weight), although muscle tissue (39 mgB/kg ash weight) may concentrate borate above seawater concentrations (5mgB/kg).

In contrast to freshwater data, information on the toxicity of boron to marine fish is limited (Table 29). In general, boron toxicity to marine fish is similar to results from the majority of freshwater fish investigated (section 5.1.1.4 and Table 17). However, in one test conducted by Thompson *et al* (1976), yearling coho salmon (*Oncorhynchus kisutch*) were more sensitive to boron when tested in sea water compared with freshwater exposures. However, at this developmental stage coho juveniles were still acclimatised to freshwater conditions.

Table 28. Borate Concentration in Marine Fish

Species		Tissue concentration (mgB/kg)	Reference
Gadus morhua, Cod,	Muscle	<1.0-28	Hellou *et al*, 1992
	Liver	<0.5-9.7	
	Ovaries	<0.8	
Oncorhynchus nerka,			Eisler, 1981, and Thompson *et al*, 1976
Sockeye salmon,	Soft tissue	0.5 - 0.7 FW *	
	Bone	1.5 - (1.1 - 4.4) FW	
Cetengraulis mystecetus,			Jenkins, 1980
Anchovetta, whole		3.3 - 3.8 AW **	
Thunnus albacares,	Yellowfin Tuna		Jenkins, 1980
	Muscle	39.0 AW	
	Whole	9.0 AW	
	Eyeball	5.6 AW	
	Spleen	3.3 AW	
	Gill	1.8 AW	
	Heart	1.5 AW	

* FW = Fresh Weight   ** AW = Ash Weight

Table 29. Summary of Borate Toxicity Data for Marine Fish

Species	Borate tested	Conc. (mgB/l)	Test type and conditions	Water quality	Reference
Oncorhynchus kisutch, Coho salmon	in effluent	˙600	96h LC$_{50}$ (static)	salinity 1.2%	Hamilton and Buhl, 1990
Oncorhynchus tshawytscha, Chinook salmon	in effluent	600	96h LC$_{50}$ (static)	salinity 1.2%	Hamilton and Buhl, 1990
Oncorhynchus kisutch, Coho salmon under yearlings, 1.8-3.8g	sodium tetraborate Na$_2$B$_4$O$_7$	12.2	283h LC$_{50}$ (static, renewal daily)	seawater, 8°C, salinity 0.28	Thompson *et al*, 1976
Oncorhynchus kisutch, Coho salmon under yearlings, 1.8-3.8g	sodium tetraborate Na$_2$B$_4$O$_7$	40	96h LC$_{50}$ (static, renewal daily)	seawater, 8°C, salinity 0.28	Thompson *et al*, 1976
Limanda limanda, Dab	sodium tetraborate Na$_2$B$_4$O$_7$	88.3	24h LC$_{50}$	seawater, salinity 34.8%	Taylor *et al*, 1985
Limanda limanda, Dab	sodium tetraborate Na$_2$B$_4$O$_7$	75.7	72h LC$_{50}$	seawater, salinity 34.8%	Taylor *et al*, 1985
Limanda limanda, Dab	sodium tetraborate Na$_2$B$_4$O$_7$	74	96h LC$_{50}$	seawater, salinity 34.8%	Taylor *et al*, 1985

5.2   TERRESTRIAL ORGANISMS

Terrestrial microorganisms, i.e., bacteria and fungi, are not sensitive to boron. Toxic effects have been shown to occur for fungi at concentrations between 50 and 4,000 mgB/kg of soil, dependent on species. A sharp fall in micromycetes has been observed at levels greater than 1,000 mgB/kg of soil (Bowen and Gauch, 1966).

A variety of insects are able to incorporate relatively high concentrations of boron compounds, tested as boric acid (Table 30). At high concentrations, boric acid is an effective insecticide. Insect infestations of wood and other substrates can be prevented by treatment with boron compounds at doses of 0.25 to 0.55 kgB/m3 of substrate (EPA, 1975).

Boric acid has a low acute toxicity to bees. A concentration of 50 mg/l of boric acid, H3BO3, (8.7 mgB/l) in syrup had no effect on the survival of honey bees (Apis mellifera), but 100 mg/l boric acid (17.5 mgB/l) was the approximate LD50 value (Sprague, 1972).

Table 30. Borate Toxicity to Terrestrial Invertebrates

Species	Bait	Borate in bait (mgB/l)	Duration	Effect	Reference
Bee, *Apis mellifera*	syrup	8.7	unknown	no effect	Sprague, 1972
		17.5	unknown	50% mortality	
Cockroach, *Blatella germanica*	honey	43750	5 d	50% population reduction	Gupta and Parrish, 1984
	sugar diet	19250	72 h	44% mortality	EPA, 1975
		43750	72 h	79% mortality	
		87500	72 h	80% mortality	
		175000	72 h	91% mortality	
	bait	35000	14 d	88% population reduction	Wright and Dupree, 1982
		35000	4-12 w	92-95% population reduction	
Cockroach, *Periplaneta americana*	bait	2625	6 d	100% mortality	Lizzio, 1986
Fly, *Musca domestica*	diet	43.5-870	unknown	reproduction inhibition ?	Sprague, 1972

Laboratory Bee Adult Toxicity Dusting Tests were also carried out for technical boric acid, H_3BO_3, in the form of 100% powder as active ingredient. The protocol for this test has been published (Atkins *et al*, 1975). The 48h post-treatment results were used to determine acute contact poisoning and the 24, 48, 72, 96h etc. data to determine acute stomach poisoning, if any occurs. Boric acid was found to be non-toxic to adult worker bees at the rate of 362.6 μg/bee. From this result it was predicted that boric acid powder would be non-toxic to bees directly contacted with a field application dosage of 400 kg/ha, equivalent to 70 kgB/ha (Atkins, 1987).

5.3   TERRESTRIAL PLANTS

5.3.1   Introduction

For the past sixty years boron has been recognised as one of the essential micronutrients, generally needed in mgB/kg dry weight for the healthy growth of all plants and for the optimisation of crop yields (Bergmann, 1988). It is unique among the essential micronutrients, because it is the only element that is normally present in soil solution as a non-ionized molecule over the pH range suitable for plant growth. Boric acid is the form of boron that plant roots absorb most efficiently (Gupta, 1993). Boron is involved in various metabolic processes, complexing with sugars and their derivatives in cell walls, although its function is not fully understood as yet (Römheld and Marschner, 1991). By contrast, other essential micronutrients such as Cu, Fe, Mn, Mo and Zn are known to be effective as specific components, activators, or inhibitors of enzymes in the plant. As a direct consequence of this essential requirement, there is a boron content in all healthy plants in the range of 2-100 mgB/kg, based on dry weight (Bergmann, 1984)(Table 31). Boron also differs from other nutrients in that there is a relatively small margin between requirements for optimal plant growth and the amount for producing phytotoxic effects (Kluge, 1990).

Boronated fertilisers are of practical use in agriculture, horticulture, the wine and fruit growing industries and forestry. If an inadequate supply of boron is available in the soil, the characteristic visual symptoms of boron deficiency appear in the crop (Bergmann, 1992), although these need to be substantiated by soil, plant or leaf analyses. Boron deficiency is a serious problem in many countries, and leads to reduced yield and quality of crops. Deficiency symptoms include, for example, inadequate setting of flowers or fruits, heart rot or dry rot of beets, dropping of grapes, and malformation of new leaves.

On the other hand an excess of boron may cause symptoms of phytotoxicity, but this again needs to be substantiated by the analysis of soil or leaves. Since boron is taken up through the roots *via* the transpiration stream, toxicity symptoms generally start as spot-like chloroses at the tips and margins of older leaves (Bergmann, 1992), spreading across the whole leaf in severe cases.

5.3.2   Typical Normal Content of Boron in Plants

There are several important variables in pre-determining the measured content of boron in a plant. Typical contents of boron in crops are dependent upon the nature of the species (Table 31); monocotyledons have a markedly lower boron content (2-6 mgB/kg) than dicotyledons (20-60 mgB/kg), based on dry weight. The boron requirement is the greatest during leaf mass development, and the flowering and setting of the fruit. With sugar beet, for example, the boron requirement in Europe is particularly high between the middle of June and middle of August (Bergmann, 1984). A further variable in boron content could arise from unnecessary repeated application of boronated fertiliser over several years, or the use of irrigation water with a high boron content, which may lead to the accumulation of boron in soil and hence damage to crops. Similar effects could arise from a high environmental background content of boron in soil, e.g., in volcanic regions. It should also be noted that only the water-soluble boron content of the soil is bioavailable to plants, and availability is severely restricted at high soil pH.

Table 31. Boron Content of Various Species of Plant Grown in the same Habitat (Bergmann, 1984)

Plant Species	mgB/kg calc. on dry weight	Plant Species	mgB/kg calc. on dry weight
Hordeum vulgare, Barley	2.3	*Sinapis alba*, White Mustard	22.2
Secale cereale, Rye	3.1	*Plantago major*, Plantain	22.5
Allium porrum, Leek	3.1	*Daucus carota*, Carrot	25.0
Triticum vulgare, Wheat	3.3	*Nicotiana tabacum*, Tobacco	25.0
Allium cepa, Onion	4.3	*Medicago sativa*, Alfalfa	25.0
Zea mays, Maize	5.0	*Brassica oleracea capitata*, White cabbage	37.1
Linum usitatissimum, Flax	7.1	*Glycine maximum*, Soya bean	37.2
Spinacia oleracea, Spinach	10.9	*Lens esculenta*, Lentil	41.4
Apium graveolens, Celery	11.9	*Brassica napobrassica*, Turnip	49.2
Cichorium endiva, Endive	13.1	*Sinapsis nigra*, Black Mustard	53.3
Solanum tuberosum, Potato	13.9	*Raphanus sativus radic.*, Radish	64.5
Lycopersicum esculentum, Tomato	15.0	*Beta vulgaris*, Beet	75.6
Vicia faba, Field bean	15.4	*Taraxacum officinale*, Dandelion	80.0
Pisum sativum, Pea	21.7	*Papaver somniferum*, Poppy	94.7

A useful literature review, prepared at the University of California at Riverside, has been published (Sposito and Calderone, 1988) on the boron uptake and accumulation by trees and woody plants. Because of the mass-flow uptake mechanism, the distribution of boron in trees is connected intimately

with the patterns of transpiration, which are species-dependent. An example of the seasonal accumulation of boron in the middle of leaves of deciduous trees such as the sycamore, the horse chestnut and the beech is shown in Table 32.

Table 32. Seasonal Accumulation of Boron (mgB/kg oven-dried weight) in the Middle of Leaves of Deciduous Trees (Sposito and Calderone, 1988)

Tree Species	May	July	August	Sept	Oct
Plantanus sp., Sycamore	27	41	65	84	--
Aesculus sp., Horse Chestnut	16	22	23	25	26
Fagus sp., Beech	28	37	37	--	33

The range of boron content (mgB/kg of dry matter) in the leaves of woody evergreens has also been reported for the avocado (11.3-37.5) and for the olive (14.0-180.0). A range has also been recorded for the woody deciduous walnut (16.0-112.0) and for the woody deciduous wattle (20.4) (Sposito and Calderone, 1988).

5.3.3 Phytotoxic Threshold Contents for Boron in Plants

To correct the condition of boron deficiency mentioned in section 5.3.1, an inorganic borate can be applied as a fertiliser, either alone or in combination with other fertilisers/pesticides. The borate is applied to the soil or sprayed as an aqueous solution on to the foliage. The application rate of micronutrient boron is generally recommended in the range of 0.5-3 kgB/ha, according to the type of crop and local conditions (Shorrocks, 1989).

To establish phytotoxic damage from an excess of boron, it is necessary to carry out a plant boron analysis in which the damaged leaf edges should be analysed separately in each case to establish a concentration gradient with the highest boron value at the edge of the leaf. Boron contents of 100 to 1,000 mgB/kg (dry weight basis) definitely indicate an excess. The more sensitive species, barley, citrus, haricot beans and chrysanthemums react adversely to a boron content as low as 100 to 115 mgB/kg (Bergmann, 1984).

A series of pot experiments was carried out during the growing season to determine the threshold for phytotoxic effects by comparing the boron leaf contents against the degree of boron-induced damage in potato, sugarbeet and winter wheat, using several light soils and one heavy soil (Kluge, 1990). Apart from basic NPK fertiliser, and magnesium, the boron (as boric acid, H_3BO_3), was applied in 8 stepwise doses. The maximum boron doses were dependent on the type of plant as follows (mgB/kg soil):- Potatoes 5.0 to 7.5, winter wheat 9 to 13, sugarbeet 20 to 35 in light soils, with higher values in heavy

soils. During the vegetative state of growth, damage due to excess of boron was just becoming visible at boron leaf contents (dry weight) of 100 mgB/kg for leaves of potatoes at the onset of flowering, 300 to 350 mgB/kg for the leaves of both winter wheat and sugarbeet 60 to 70 days after sowing. The first appearance of damage to leaf or shoot was generally not sufficiently severe to reduce the yield of the crop.

The threshold values for boron on a series of crops in outdoor sand cultures (in the absence of soil which would adsorb boron) have been re-examined by the US Department of Agriculture, Riverside, CA. The purpose has been to establish the level at which there is a decline in yield of harvested product, rather than just the first appearance of phytotoxic effects (Eaton, 1944). Irrigation water containing increasing concentrations of boron was applied and the yield of fresh product was monitored. Although soils with an excess of boron content are present only in a small region of the USA, the source of such excess in practice is from irrigation water pumped from wells with a high boron content. The hot dry climate of southern California can provide such conditions.

In a study on tomatoes (Francois, 1984), for example, the boron treatment was applied three times daily by culture solutions containing 1.0, 4.0, 6.0, 8.0, or 12.0 mgB/l, respectively. The parameters monitored included the vegetative growth, the size distribution and weight of the tomato fruit, and the boron concentration in the leaves. There was a 20 % reduction in vegetative growth at a soil solution boron concentration of 10 mgB/l. The fruit size decreased significantly with increasing boron concentration, so that at 4 mgB/l the size of fruit harvested consisted of 42% large or extra-large and 31% small or extra-small, but at 12 mgB/l only 16% were sized large or extra-large, with 56% in the two small categories.

Necrosis of the tomato leaves was barely discernable at 4 mgB/l, but was about 3 mm wide at 12 mgB/l. Leaf analysis data showed that for each unit increase in mgB/l in soil boron solution, the leaf boron content increased by about 100 mgB/kg dry weight. After treatment with a 6.0 mgB/l solution, the necrotic leaf margins were analysed separately from healthy leaf tissue and the former contained much more boron on a dry weight basis (1,790 mgB/kg) than the latter (302 mgB/kg). It is interesting to note that the soil boron concentration (4.0 mgB/l) producing the highest yield was also the lowest concentration at which the first signs of leaf damage were observed.

In a further study on broccoli, cauliflower and radish (Francois, 1986), the boron treatment was applied three times daily by culture solutions containing 1.0, 4.0, 8.0, 12.0, 16.0 or 20.0 mgB/l for broccoli and cauliflower, and 1.0, 3.0, 6.0, 10.0, 13.0 or 16.0 mgB/l for radish. The relative yield was reduced by 1.8%, 1.9%, and 1.4% with each unit (mgB/l) increase in soil boron solution of above 1.0, 4.0, and 1.0 mgB/l for broccoli, cauliflower, and radish, respectively. Over the boron concentration range tested, no leaf injury was apparent for these three vegetables, even though the broccoli boron leaf content was as high as 943 mgB/kg dry weight.

In a similar study on celery and crisphead lettuce (Francois, 1988), celery plants harvested from treatments >10.0 mgB/l were immature, bitter tasting, and not of marketable quality. Leaf margin damage on lettuce was confined to the outer wrapper leaves, which were removed at harvest.

Additional studies on snap bean and cowpea (Francois, 1989), garlic and onion (Francois, 1991) and summer and winter squash (Francois, 1992) provided similar outcomes.

The effects of irrigation with treated municipal wastewater from the sewage treatment plant of Castellón, eastern Spain, on two year old Citrus trees (*Citrus sinensis* L. Osbeck) were investigated during one season (Lapena et al, 1995). The experimental plot was divided into two portions, with one half receiving wastewater containing 2.6 mgB/l and the other half groundwater with 0.8 mgB/l. Irrigation was carried out by flooding every 20 days between March and October, and the rest of the year depending on the rainfall distribution. The average leaf borate content was higher in plants irrigated by wastewater (161.2 ppm B) than in plants irrigated by groundwater (94.7 ppm B). The use of wastewater in this case did not lead to boron toxicity symptoms, in spite of earlier reports that a borate content greater than 2 mgB/l would lead to development of toxic effects in *Citrus* species.

5.3.4   Summary

Boron is established as an essential micronutrient for healthy plant growth. An inadequate supply of boron leads to symptoms of boron deficiency, whereas an excess leads to symptoms of boron toxicity. In both cases such diagnoses should be confirmed by analysis of the boron content (mgB/kg dry weight) of the plant under study. The hot-water-extractable boron content of the soil is a useful parameter to determine if a boronated fertiliser application may be required. Furthermore, the nature of the soil, for example pH, clay, humus or sand content can influence the availability of the boron supply.

5.4   WILDLIFE

5.4.1   Introduction

Other than waterfowl, very little data concerning boron exposure and effects are reported for wildlife. However, based on studies conducted with domestic animals (see section 5.5), and laboratory mammalian studies associated with human safety evaluations for boron, it is improbable that environmental borate levels are a cause of concern for wildlife species.

The exception is a local concern for waterfowl populations inhabiting specific wetland areas in central California (USA). In this region, naturally high soil concentrations of salts and trace elements, including borates, are associated with ancient sea deposits. Irrigation practices carried out in the region leach

these readily soluble compounds and transport the contaminants in the drainwaters to wetland habitats. It is clear that these irrigation practices have resulted in toxicity to local waterfowl populations.

5.4.2   Waterfowl

Numerous studies have been conducted over the past several years to determine environmental exposures and potential effects of specific agricultural drainwater constituents to waterfowl. Relatively high concentrations of boron and selenium have been found in specific drainwaters and food consumed by waterfowl in certain areas of western USA. such as the San Joaquin Valley of California and Kesterson National Wildlife Refuge (Schuler, 1987; Klasing and Pilch, 1988; Smith and Anders, 1989; Hothem and Ohlendorf, 1989 ; Hoffman *et al*, 1990; Saiki *et al*, 1993). For instance, elevated boron concentrations of the irrigation drainwaters can exceed 20 mgB/l. High boron concentrations have also been detected in food for waterfowl in the region, including certain aquatic insects (150 mgB/kg dry weight), algae (400 mgB/kg dry weight), wetland plants (to 1,860 mgB/kg dry weight) and seeds (to 3,500 mgB/kg dry weight).

Paveglio *et al* (1992) examined boron and selenium body burdens of breeding and wintering waterfowl in the Grassland Water District of the San Joaquin Valley. During the spring breeding seasons for 1985, 1987, and 1988, boron was detected, via ICP analysis, in 60-74% of the liver samples. Autumn and winter collections yielded boron detection frequencies ranging from 38-69%. It was suggested, based on referenced feeding studies, that liver concentrations of Se, and to a lesser extent B were in a range associated with embryotoxicity in birds (approximately 33 mgB/kg).

Although selenium has been implicated as the cause of abnormal development among waterfowl observed in these areas, recent studies by Smith and Anders (1989) and Hoffman *et al* (1990) with mallards have indicated that dietary boron concentrations in the environment can occur at levels which may adversely affect growth and behaviour, and ultimately survival of newly hatched ducklings at extreme concentrations. Diets containing 13 mgB/kg fresh weight did not produce adverse effects, although growth reduction was noted at concentrations between 30-100 mgB/kg, and diets containing 1,000 mgB/kg proved fatal. In a more recent investigation, Hoffman *et al* (1990) suggest interactive effects of boron with selenium on growth of mallard ducklings, especially under a reduced protein diet. Whitworth *et al* (1991) demonstrated behaviour abnormalities in mallard ducklings at the highest sublethal dietary boron level (1,600 mgB/kg).

Adult male ducks (*Anas platyrhynchos*) were exposed for 48 days to dietary concentrations of 300 ppm arsenic as sodium arsenate, 1600 ppm boron as boric acid, or both. Tissue accumulation and loss rates were estimated when the ducks were returned to uncontaminated food. These dietary levels were selected because both adult and ducklings tolerate them without excessive mortality and they

approximate the maximum levels in aquatic plants consumed by waterfowl in the Central Valley of California (USA).

When boric acid alone was added the borate levels in the blood rose rapidly to a combined mean value of 50 ppm B, with equilibrium reached in 2 to 15 days. Corresponding mean values of borate in liver and brain were 25 and 31 ppm B, respectively. Borate was eliminated by mallards very rapidly, with little detectable residue more than one day after the removal of the borate from the diet (Pendleton *et al*, 1995).

5.4.3   Other Wildlife

Only a single non-waterfowl reference was found. Borate concentrations in Harbor seals (*Phoca vitulina*) range from 0.01 mgB/kg (kidney) to 2.0 mgB/kg (blood) fresh weight (Jenkins, 1980).

5.5   DOMESTIC ANIMALS

Boron appears to have a widespread role in biochemistry and nutrition and is considered to be an ultratrace required element (Sisk *et al*, 1990; Naghii and Samman, 1993). Numerous studies suggest that boron interacts with other nutrients and plays a regulatory role in the metabolism of minerals, such as calcium and subsequently bone metabolism (Naghii and Samman, 1993).

Boron has long been known as a natural constituent in cows' milk, the amount varying with the intake of boron in the feed (Fenner and Archibald, 1958). Borate concentration in beef, cows' milk, and dairy products are low and range from 0.5-1.1 mgB/kg (NAS, 1980; Nielsen, 1986). Mammals in general (e.g., man, dogs, cows, rabbits, sheep, and guinea pigs) rapidly absorb dietary boron as sodium borate or boric acid at nontoxic concentrations. However, this does not appear to accumulate in healthy tissues and is readily excreted in the urine (Owen, 1944; NAS, 1980; Benson *et al*, 1984; Nielsen, 1986; Siegel and Wason, 1986; Brown *et al*, 1989).

Grazing animals tolerate rather high levels of boron when ingested in food or drinking water. A brief overview follows as the earlier data have been extensively reviewed by Butterwick *et al* (1989) and Eisler (1990). Weeth *et al* (1981) and Nielsen (1986) found no overt signs of toxicosis in heifers exposed to 120 mgB/l in the drinking water, although growth retardation has been reported in cattle given 150 mgB/l which is roughly equivalent to 15 mgB/kg body weight daily (Eisler, 1990). Green and Weeth (1977) found only mild symptoms in heifers exposed to 300 mgB/l, but also determined that if given a choice, cattle will avoid drinking water containing >29 mgB/l. No adverse effects were found in cattle consuming feed containing 157 mgB/kg for 42 days (NAS, 1980). Brockman *et al* (1985) determined the toxic dose to cattle at 200-600 mgB/kg body weight.

Sisk *et al* (1988) investigated an apparent accidental poisoning to cattle from ingestion of fertiliser containing boron. In order to quantify probable boron toxicosis in livestock they subsequently conducted a study with goats dosed with sublethal levels of inorganic borate (Sisk *et al*, 1990). Goats were given a calculated dose of 410 mgB/kg body weight and observed 1, 2, and 7 days post-dosing. Several blood and cerebrospinal fluid parameters and aberrations in clinical behaviour were observed which suggested systemic and central nervous system effects at this high dose.

Apart from accidental poisonings and extreme cases, environmental borate levels are not considered to be a concern for domestic animals.

GLOSSARY

Acute toxicity :

The harmful properties of a substance which are demonstrated within a short period (hours to days) of exposure.

Application factor :

A factor for converting data from one exposure period or end point to another, e.g. from acute EC_{50} (measured) to chronic NOEC (predicted).

Assessment factor :

A factor applied to a data point when assessing a substance in order to derive a safe level of that substance in the environment.

Biocenosis :

Society of different organisms (species) living in the same area (biotop) with many interspecific and intraspecific interactions.

Chronic toxicity :

The harmful properties of a substance which are demonstrated only after long term exposure in relation to the life of the test organism.

EC_0 :

The highest tested concentration over a defined period of exposure without any toxic effect on the test population.

EC_3 :

A statistically-derived concentration which, over a defined period of exposure, is expected to cause a specified toxic effect on 3% of the test population.

Comment: This value was only used by Bringmann and Kühn for tests with algae and protozoans. Standard algal tests (OECD, 1984; DIN, 1989) now utilise the EC_{10} as the lowest observed effect concentrations and values below EC_{10} are within the natural variability of algal growth in culture.

EC_{10} :

A statistically-derived concentration which, over a defined period of exposure, is expected to cause a specified toxic effect on 10% of the test population. In chronic tests with algae and protozoans the EC_{10} is a quasi LOEC.

EC_{50} :

A statistically-derived concentration which, over a defined period of exposure, is expected to cause a specified toxic effect on 50% of the test population.

EC_{100} :

The lowest tested concentration over a defined period of exposure causing a specified toxic effect on 100% of the test population.

LC_0 :

The highest tested concentration over a defined period of exposure without any lethal effect on the test population.

LC_1 :

A statistically-derived concentration which, over a defined period of exposure, is expected to cause a lethal effect on 1% of the test population.

Comment: This value was only used by Birge and Black, respectively Black et al in ELS tests with fish. LC1 values arewithin the natural variability of the test organisms.

LC_{50} :

A statistically-derived concentration which, over a defined period of exposure, is expected to cause a lethal effect on 50% of the test population.

LC_{100} :

The lowest tested concentration over a defined period of exposure causing lethal effect on 100% of the test population.

LOEC (lowest observed effect concentration) :

The lowest test concentration at which the substance is observed to have a "statistically significant" and unequivocal effect on the test species.

MLD (minimum lethal dose) :

The lowest test concentration at which the substance is observed to have a "statistically significant" and unequivocal lethal effect on the test species.

NOEC (no observed effect concentration) :

The highest tested concentration below the LOEC where the stated effect was not observed. The NOEC usually refers to chronic effects.

PEC (predicted environmental concentration) :

The expected concentration in the environment, calculated by an exposure assessment.

PNEC (predicted no effect concentration) :

The PNEC is derived from whatever ecotoxicological data are available, using, when appropriate, an assessment factor to compensate for any restrictions in the data. The assessment factors are:

- 100, if values of three different acute toxicity tests are available (EU)
- 10, if values of three different chronic toxicity tests are available (EU)
- <10, and judged on a case by case basis (EU), if biocenosis values are available
- 1, if NOECs from field or model ecosystems are available (US EPA)
- 1, if biocenosis studies are available (the ECETOC approach, according to ECETOC report No.56)

" Statistically significant" effect :

An effect considered to be significant according to defined mathematical, statistical and/or descriptive methods.

Threshold concentration :

See LOEC.

APPENDIX A. METHODS OF ANALYSIS

The methods of analysis for boron in environmental matrices can be divided into atomic and mass spectrometry, atomic and emission spectrophotometry, neutron capture analysis and spectrofluorimetry amongst others.

A.1 ATOMIC AND MASS SPECTROMETRY

Boron is often detected by atomic absorption and emission spectrometry, despite the fact that these techniques need expensive equipment, are subject to a wide range of interferences and may require the conversion of boron to a more volatile species prior to detection by graphite-furnace atomic absorption spectrometry (van der Geugten, 1981) or flame atomic-emission spectrometry (Castillo et al, 1985).

The use of inductively-coupled argon plasma excitation for atomic emission-spectrometry has been used to rapidly determine boron in wastewaters (Huber, 1982), extracts from fish tissues (Hamilton and Wiedmeyer, 1990), food products (Hunt et al, 1991) etc., and this technique is now included in the American Standard Method (APHA 4500, 1989). Detection limits reported vary from 0.005 to 0.05 mgB/l, depending upon the instrumentation parameters (Broekaert and Leis, 1979; APHA 4500, 1989). Increased sensitivity could be obtained if either (i) the sample is concentrated (boric acid will react with hydrofluoric acid to produce fluoroboric acid which can be extracted by liquid anion exchange, Novozamsky et al, 1990b), or (ii) the mode of detection is made more sensitive by the use of mass spectrometry.

Inductively coupled plasma mass spectrometry (ICP-MS) is probably the most-widely used non-spectrophotometric technique for the detection of boron. It has the ability to detect boron at levels down to 0.00015 mgB/l, as well as other elements simultaneously. In addition the lack of sample pretreatment, the small volumes required (0.5ml) and the rapidity of the analysis (about 1 minute) all make this a good method for boron determination in a wide variety of samples including fresh and saline waters, sewage wastewater, soils and plant material. Interferences are not a problem, solids only affect the determination above 2000 mgB/l, whilst dissolved salts can be removed by cation exchange (Gregoire, 1990). Mass spectrometry, alone, has also been used to determine the boron isotopic composition of geological materials (Vengosh et al, 1989), and quantitate boron in waters, fertilisers and biological matrices from isotope-dilution (Duchateau et al, 1987).

A.2   SPECTROPHOTOMETRY

Boron in solution has often been determined traditionally by adding a dye, and detecting the complex spectrophotometrically. Detection limits typically vary from 0.005-0.05 mgB/l.

In the presence of an alpha-hydroxyacid (e.g. mandelic acid) or beta-hydroxybenzoic acid (e.g. 2,6-dihydroxybenzoic acid, DHBA) boric acid will react to form a 1:1 anionic complex which can associate with a number of cationic (basic) dyes, including malachite green, methyl violet, ethyl violet, crystal violet, Rhodamines B, S and 6G, victoria blue and brilliant green. In the case of boron+DHBA+crystal violet the spectral shift upon complexation is sufficient, so that no extraction of the complex is required prior to detection. The detection limit for this procedure is about 0.01 mgB/l (Garcia *et al*, 1985). In other cases extraction of the complex may be required, which makes the procedure longer without necessarily improving the detection limit (Sato, 1983).

A variety of anionic dyes can also complex with boron, including curcumin (HMSO, 1981) quinalizarin, purpurine, carminic acid (Aznarez *et al*, 1985), arsenazo-I (Cherian and Gupta, 1989), 1,1-dianthrimide and azomethine-H (HMSO, 1981). Under anhydrous acidic conditions the anionic dye is protonated and can then form a spectrophotometrically-active complex with boron. The curcumin method is affected by nitrate, chloride and fluoride interferences, with the fluoride interference also affecting the carminic acid method. Despite this, the American Standard Method for the determination of boron includes both the curcumin and carminic acid procedures. These procedures are applicable for concentration ranges 0.1-1 mgB/l and 1-10 mgB/l, respectively, and have detection limits about a twentieth of their low range limits (APHA, 1989).

The azomethine-H method, in which azomethine forms a 3:2 complex with boron (HMSO, 1981), has been compared to the curcumin and carminic acid methods for the analysis of boron in environmental waters, and found to be the most sensitive (detection limit 0.02 mgB/l) and selective (Lopez *et al*, 1993). Additional advantages for the azomethine-H method include:

a) it is not affected by the anions generally found in raw, potable, and saline waters or sewage; high nitrite levels (above 90 mg/l) may interfere with the method, but this can be modified by the use of a chemical derivative of azomethine-H (HMSO, 1981; Aznarez and Mir, 1985);

b) cationic interferences can be masked by complexation with EDTA, although a buffer of polyphosphate, thiourea and ascorbic acid may also be used to reduce interferences from iron, copper and calcium (Ferran *et al*, 1988);

c) it is suitable for a wide range of environmental matrices including water, sewage wastewater and plant material; this method can even be used to determine boron in human hair (Ciba and Chrusiel, 1992);

d) it can be automated by use of a flow-injection system; samples can be automatically derivatised with azomethine-H, after preparation by ultrasonic leaching (Chen *et al*, 1989) or ion-exchange chromatography (Sekerka and Lechner, 1990); when samples are prepared by ion-exchange chromatography and automatically derivatised with azomethine-H, up to 10 samples can be analysed per hour, with a detection limit for the complete method of 0.001 mgB/l;

(e) its ability to complex with, and quantitate, boron down to 0.005 mgB/l when boron-containing compounds are separated out from soil and water components by thin-layer chromatography (Touchstone *et al*, 1980).

The azomethine-H method has been incorporated into a number of national and international standards (DIN 38405, 1981; HMSO, 1981; Normalisation francaise T90-041, 1985; ASTM D3082, 1987; ISO 9390, 1990; BS 6068, 1991).

A.3   NEUTRON CAPTURE ANALYSIS

The boron-10 isotope can be determined in plants by neutron activation, which breaks down the boron to alpha particles, lithium and gamma rays. Detection of the alpha particles can be by a silicon detector or by alpha-radiography, whilst the gamma rays emitted can be monitored by gamma-spectrometry. The detection limit for this method depends upon the mode of detection, and is 0.2 mgB/kg or better (Szabo and Sashin, 1988).

More recently neutron capture prompt gamma-ray activation analysis (PGAA), with a detection limit 0.02 mgB/kg, has been applied to the determination of boron in foods in the US Food and Drug Administration Total Diet Study (Anderson et al, 1994a). As this analytical method requires minimal handling of the test samples and no preparatory freeze-drying or digestion steps, the measured concentrations are not significantly different from those in the certified NIST boron-containing samples.

A.4   SPECTROFLUORIMETRY

Spectrofluorimetric methods for the determination of boron have detection limits generally better than spectrophotometric ones (Aznarez *et al*, 1985), but not as sensitive as the spectrometric methods described in section 3.3.1.

Although boron in pure water can be measured by laser-induced atomic fluorescence spectroscopy (Oki *et al*, 1993), in environmental media e.g. soils, plants and natural waters the boron is reacted with a fused-benzene ring sulphonic acid (anionic dye), to form a fluorescent complex. The detection limit for this type of method is around 0.005 mgB/l. The anionic dyes which are commonly used include: quinizarin-2-sulphonic acid which is affected by interferences from nitrate (when the nitrate:boron ratio is greater than 10:1 m/m) and fluoride (when the fluoride:boron ratio is greater than 1:1 m/m) (Salinas *et al*, 1987), Alizarin Red S (1,2-dihydroxyanthraquinone-3-sulphonic acid) whose complexation with boron is affected by cationic interferences (Campana *et al*, 1992), and chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulphonic acid). Chromotropic acid is often used to determine boron in both freshwater and sea waters, with excess chromotropic acid being removed by ion-pair liquid chromatography (Motomizu *et al*, 1983, Jun *et al*, 1988) or by the use of first-derivative synchronous spectrofluorimetry (Capitan *et al*, 1991). The use of a flow-injection system, with an alkaline solution to remove the high fluorescence background, can improve the detection limit to about 0.0001 mgB/l, whilst still allowing up to 60 samples to be analysed per hour (Motomizu *et al*, 1991).

Dibenzoylmethane can also form a fluorimetrically-active complex with boron, providing the boron has been extracted with 2-methylpentane-2,4-diol into isobutyl methylketone. Although the extraction efficiency is greater than 95%, and the detection limit is around 0.001 mgB/l, this method can be affected by alkali earth metal cations, and is more time-consuming than those above using sulphonic dyes (Aznarez *et al*, 1983).

A.5   OTHER METHODS

Other methods are available for the detection of boron, but these are less commonly used as they have poor detection limits and/or require unusual instrumentation. They include:

■ Titration: Boron, in sewage wastewater, can be determined by titration, provided high levels of fluoride or phosphate are not present, and ion-exchange resins are used to remove cationic interferences. Mannitol is added, and the boric acid-mannitol complex is titrated as a monoacid with a standard alkaline solution. This method has a detection limit of 0.5 mgB/l (HMSO, 1981).

■ Potentiometry: A fluoroborate-sensitive electrode has been used to determine boron in industrial wastewaters at concentrations between 0.5 and 20 mgB/l, although interference from nitrate ions is possible (Lanza and Mortera, 1983).

■ Ozone Gas-Phase Chemiluminescence: Boric acid is converted to diborane, reacted with ozone, and detected by chemiluminescence. The difficulties encountered in the quantitative production of diborane from aqueous solutions make this method unsuitable for environmental analyses (Fujiwara *et al*, 1991).

■ Ion Exclusion Chromatography: If d-sorbitol is included in a liquid chromatography mobile phase it can bind to borate, to form a complex which will interact with a cationic resin, and then be eluted and detected by conductivity. The method has a detection limit of 0.1 mgB/l, which makes it inappropriate for some environmental matrices (Mea *et al*, 1990).

■ Oscilloscope polarography: Boron can produce an ionic complex with Beryllon III at pH 4, which gives a stable polarographic peak. This method has been used to detect boron in aqueous solutions at 1 mgB/l, both the total and water-soluble boron in soils, and the boron content of plant material (Zheng, 1990).

BIBLIOGRAPHY

Acad Eng Sci, 1970. Boron from the standpoint of environment. IUA-Report 33, Stockholm, Sweden.

Ahl Th and Jönsson E, 1972. Boron in Swedish and Norwegian fresh waters. Ambio, 1(2), 66-70.

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

Anderson DL, Cunningham WC and Lindstrom TR, 1994a. Concentrations and intakes of H, B, S, K, Na, Cl, and NaCl in foods. Journal of Food Composition and Analysis 7, 59-82.

Anderson DL, Kitto ME, McCarthy L and Zoller WH, 1994b. Sources and atmospheric distribution of particulate and gas-phase boron. Atmospheric Environment 28, 1401-1410.

Antia NJ and Cheng JY, 1975. Culture studies on the effects from borate pollution on the growth of marine phytoplankters. J Fisheries Res Board of Canada 32(12), 2487-2494.

APHA, 1975. American Public Health Association (APHA) standard methods for the examination of water and wastewater. Fourteenth Edition. 405-A Boron Curcumin method, pp 287-290.

APHA, 1989. American Public Health Association (APHA) standard methods for the examination of water and wastewater. Seventeenth Edition. 4500-B Boron 4-7 to 4-11.

ASTM D3082, 1987. American Society for the Testing of Materials (ASTM) standard test method for boron in water. Designation: D3082-87 357-360.

Atkins EL, 1987. Laboratory bee adult toxicity dusting tests (BADT) for boric acid, powdered, 100% ai technical. University of California, Riverside, Division of Agricultural Sciences,
Department of Entomology. Report TX-87-04 to U.S. Borax.

Atkins EL, Greywood EA and Macdonald RL, 1975. Toxicity of pesticides and other agricultural chemicals to honey bees: laboratory studies. University of California, Division of Agricultural Sciences Leaflet 2287, 38 pp.

Atteia O, Védy J-C and Parriaux A, 1993. Trace elements dynamics in soils and aquifers of western Switzerland. Stud Environ Sci 55 (Environmental Contamination), 79-101.

Aznarez J, Bonilla A and Vidal JC, 1983. Spectrophotometric and fluorimetric determination of boron in soils, plants and water by extraction with 2-methylpentane-2,4-diol in isobutyl methyl ketone. Analyst 108, 368-373.

Aznarez J, Ferrer A, Rabadan JM and Marco L, 1985. Extractive spectrophotometric and fluorimetric determination of boron with 2,2,4-trimethyl-1,3-pentanediol and carminic acid. Talanta 32(12), 1156-1158.

Aznarez J and Mir JM, 1985. Spectrophotometric determination of boron. Analyst, 110, 61-64.

Banuelos GS, Cardon G, Pflaum T and Akohoue S, 1992. Comparison of dry ashing and wet acid digestion on the determination of boron in plant tissue. Commun Soil Sci Plant Anal 23(17-20), 2383-2397.

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.

Belanger SE, Farris JL and Cherry DS, 1989. Effects of diet, water hardness and population source on

acute and chronic copper toxicity to *Ceriodaphnia dubia.* Arch Environ Contam Toxicol, 18, 601-611.

Benfenati E, Di Toro N, Fanelli R, Lualdi G, Tridico R, Stella G, Buscaini P and Stimilli L, 1992. Characterization of organic and inorganic pollutants in the Adige river (Italy). Chemosphere, 25, 1665-1674.

Bennett J, 1993. Boron levels at drinking water abstraction points of the River Thames. (Letter of 22 February 1993, from National Rivers Authority, Thames Region).

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 Chem 3, 209-214.

Bergmann W, 1984. The significance of the micronutrient boron in agriculture. Booklet issued by Borax Holdings Limited, London, 26 pages.

Bergmann W, 1988. Ernährungsstörungen bei Kulturpflanzen, 2nd Edition, VEB Gustav Fischer Verlag Jena, DDR, ISBN 3-334-00248-9.

Bergmann W, 1992. Colour atlas nutritional disorders in plants: visual and analytical diagnosis. Gustav Fischer Verlag Jena, Villegang 2, D-O-6900 Jena. ISBN 3-334-60423-3.

Bergmann W, Bruchlos P and Marks G, 1995. Ein Beitrag zur Frage des toxischen Bor-Grenzwertes in Gewässern für Phragmites australis-Schilfrohr. Tenside Detergents, 32, 229-237.

Bingham FT, 1982. The boron concentration of wild trout streams in California. Unpublished paper, 30 December 1982, Department of Soil Science, University of California, Riverside, USA.

Birge WJ and Black JA, 1977. Sensitivity of vertebrate embryos to boron compounds. Report No. EPA 560/1-76-008; Environ Protec Agency, Office of Toxic Substances, Washington DC, USA.

Birge WJ and Black JA, 1981. Toxicity of boron to embryonic and larval stages of Largemouth bass (Micropterus salmoides) and Rainbow trout (Salmo gairdneri). Completion Report prepared for the Procter & Gamble Company, Cincinnati, USA.

Birge WJ, Black JA, Westermann AG, Short TM, Taylor SB and Parekh MC, 1984. Toxicity of boron to embryonic and larval stages of Rainbow trout (Salmo gairdneri) exposed in reconstituted and natural waters. Completion Report prepared for the Procter & Gamble Company, Cincinnati, USA.

Black JA, Barnum JB and Birge WJ, 1993. An integrated assessment of the biological effects of boron to the Rainbow trout. Chemosphere 26, 1383-1413.

Blume H-P, Bornkamm R, Kempf Th, Lacatusu R, Muljadi S and Raghi-Atri F, 1979. Chemisch-ökologische Untersuchungen über die Eutrophierung Berliner Gewässer unter besonderer Berücksichtigung der Phosphate und Borate. Schriftenreihe des Vereins für Wasser-, Boden- und Lufthygiene, 48, Gustav Fischer Verlag, Stuttgart, GERMANY. ISBN 3-437-30297-3.

Blume H-P, Bornkamm R, Kempf Th, Muljadi S and Raghi-Atri F, 1980. Bor-Status Berliner Gewässer. Arch Hydrobiol 89, 426-439.

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

Bornkamm R, 1991. Zur Demographie von Typha- und Phragmites- Halmen unter Borbelastung. Verh-Ges Oekol 20(2), 927-934; Chem Abstr, 1992, 117, 14023f.

Bowen JE and Gauch HG, 1966. Non-essentiality of B in fungi and the nature of its toxicity. Plant Physiol 41, 319-324.

Boyd CE and Wayne Walley W, 1972. Studies of the biogeochemistry of boron. 1. Concentrations in surface waters, rainfall and aquatic plants. Amer Midl Natur 88(1), 1-14; Chem Abstr 1972, 77, 150951q.

Bringmann G and Kühn R, 1977. Befunde der Schadwirkung wassergefährdender Stoffe gegen Daphnia magna. Z. f. Wasser- und Abwasser-forsch. 10, 161-166.

Bringmann G and Kühn R, 1978. Grenzwerte der Schadwirkung wassergefährdender Stoffe gegen Blaualgen (Microcystis aeruginosa) und Grünalgen (Scenedesmus quadricauda) im Zellvermehrungs-hemmtest. Vom Wasser 50, 45-60.

Bringmann G and Kühn R, 1980. Comparison of the toxicity thresholds of water pollutants to bacteria, algae and protozoa in the cell multiplication inhibition test. Water Res 14, 231-241.

Brockman RP, Audette RJ and Gray M, 1985. Borax toxicity. Canadian Vet. Journal 26, 147.

Broekaert JAC and Leis F, 1979. An injection method for the sequential determination of boron and several metals in waste-water samples by inductively-coupled plasma atomic emission spectrometry. Analytica Chimica Acta, 109, 73-83.

Brown J, Ray NJ and Ball M, 1976. The disposal of pulverised fuel ash in water supply catchment areas. Water Research 10, 1115-1121.

Brown TF, McCormick ME, Morris DR and Zeringue LK, 1989. Effects of dietary boron on mineral balance in sheep. Nutrition Res 9, 503-512.

BS 6068, 1991. British Standard. Water quality. Part 2. Physical, chemical and biochemical methods. Section 2.40 Method for the determination of borate by spectrometry using azomethine-H.

Buhl KJ, and Hamilton SJ, 1996. Toxicity of inorganic contaminants, individually and in environmental mixtures, to three endangered fishes (Colorado Squawfish, Bonytail and Razorback Sucker). Arch Environ Contam Toxicol, 30, 84-92.

Bundschuh J, 1992. Boron contamination of the ground- and surface waters of Lerma Valley, Argentina. Aqua (London) 41(1), 13-17.

Butterwick L, de Oude N and Raymond K, 1989. Safety assessment of boron in aquatic and terrestrial environments, Ecotoxicol Environ Safety, 17, 339-371.

Campana AMG, Barrero FA and Ceba MR, 1992. Spectrofluorimetric determination of boron in soils, plants and natural waters with alizarin red S. Analyst, 117, 1189-1191.

Capitan F, Navalon A, Manzano E, Capitan-Vallvey LF and Vilchez JL, 1991. Determination of boron with chromotropic acid by first-derivative synchronous spectrofluorimetry. Fresenius J Anal Chem 340, 6-10.

Caroli S, Beccaloni E, Fornarelli L, Delle Femmine P, Mancini M, Gallorini M and Zaray G, 1991. Reference materials for the determination of elements in Mediterranean marine ecosystems. Acta Chimica Hungarica 128(4-5), 507-518.

Castillo JR, Mir JM, Martinez C and Bendicho C, 1985. Determination of boron in waters by using methyl borate generation and flame atomic-emission spectrometry. Analyst, 110, 1435-1438.

CEFIC, 1994. Statistics of the Peroxygen 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 717.1002C.

Chen D, Lazaro F, Luque de Castro MD and Valcarcel M, 1989. Direct spectrophotometric determination of total boron in soils with ultrasonic leaching in automatic flow systems. Analytica Chimica Acta, 226, 221-227.

Cherian L and Gupta VK, 1989. A colorimetric method for the trace determination of boron with arsenazo-I. Bull Soc Chim Belg 98(4), 287-288.

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

Cumakov A, 1991. Chemicke metody fracionacie mikroelementov v podach. 1. Bor. (Chemical methods of fractionation of soil trace elements. 1. Boron). Pol'nohospodarstvo, 37(2), 131-136; Chem Abstr 115, 182048v.

Dames and Moore, 1995. Boron content of groundwaters. Unpublished report to Borax Consolidated Limited.

Dawber JG and Matusin DH, 1982. J Chem Soc Faraday Trans 1, 78, 2521-2528.

D.D.A.S.S. de l'Oise, 1990. (Direction Départementale des Affaires Sanitaires et Sociales de l'Oise, 13 rue Biot, 60022 Beauvais Cedex). Contrôle sanitaire des eaux d'alimentation. Bilan relatif à la teneur en bore des captages d'adduction publique (Département de l'Oise), January 1990.

Dietz F, 1975. Die Borkonzentration in Wässern als ein Indikator der Gewässerbelastung. Gwf-Wasser/Abwasser, 116, 301-308.

DIN 38405, 1981. Deutsche Norm. German standard methods for the analysis of water, waste water and sludge. Anions (Group D). Determination of borate ions (D17). DIN 38 405 Part 17.

DIN, 1989. Testverfahren mit Wasserorganismen (Gruppe L) -Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Grünalgen (Scenedes-mus Zellvermehrungs Hemmtest). DIN 38412, Part 9.

Duchateau NL, Verbruggen A, Hendrickx F and de Bievre P, 1987. Sensitive determination of traces of boron in waters, fertilizers and geological and biological materials by isotope-dilution mass spectrometry. Analytica Chimica Acta, 196, 41-47.

Duchi V, Minissale AA and Prati F, 1987. Chemical composition of thermal springs, cold springs, streams, and gas vents in the mt. Amiata geothermal region (Tuscany, Italy). J Volcanol Geotherm Res 31, 321-332.

EA, 1994. Boron concentrations and rainbow trout populations in seven states in the western United States. EA Engineering, Science, and Technology, 1420 NW Ribier Place, Corvallis, Oregon 97330. Unpublished report prepared for the Procter & Gamble Company, Cincinnati, USA.

Eaton FM, 1944. Deficiency, toxicity and accumulation of boron in plants. J Agr Res 69, 237-277.

EAWAG (Eidg. Anstalt für Wasserversorgung Abwasserreinigung und Gewässerschutz), Überlandstrasse 133, CH-8600 Dübendorf, Switzerland, Jahresbericht 1990, pages 4-50 to 4-52.

EC, 1975. Directive concerning the quality required of surface water intended for the abstraction of drinking water, 75/440/EEC, Off J Eur Comm 1975, L194.

EC, 1993. Commission Directive adapting for the 18th time to technical progress Council Directive 67/548/EEC on the classification, packaging and labelling of dangerous substances, 92/21/EEC. Annex VI-5 Classification on the basis of environmental effects. Off J Eur Comm 1993, L110A, 68-70.

ECETOC, 1995. Reproductive and general toxicology of some inorganic borates and risk assessment for human beings. Technical Report No. 63, February 1995.

Eisler R, 1981. Trace metal concentrations in marine organisms. New York, Pergamon Press.

Eisler R, 1990. Boron hazards to fish, wildlife, and invertebrates: A synoptic review. Biological Report 85 (1.20), 1-32, U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center.

El Kobbia T and Ibrahim A, 1989. Evaluation of the mineral components of sewage effluents for irrigation purposes with regards to heavy metals. Egypt J Soil Sci 2, 167-177.

EPA, 1975. Preliminary investigations of effects on the environment of boron, indium, nickel, selenium, tin, vanadium and their compounds. Vol.1. Boron. US Environmental Protection Agency Rep 56/2-75-005-A. 111pp.

Farris JL, Grudzien JL, Belanger SE, Cherry DS and Cairns J, 1994. Molluscan cellulolytic activity responses to zinc exposure in laboratory and field comparisons. Hydrobiologia, 287, 161-178.

Fay RW, 1959. Toxic effects of boron against immature stages of Aedes aegypti, Anopheles quadrimaculatus and Culex quinquefasciatus. J Eco Entomol 52, 1027-1028.

Feijtel TCJ, Matthijs E, Rottiers A, Rijs GBJ, Kiewiet A and de Nijs A, 1995. AIS/CESIO Environmental Surfactant Monitoring Programme. Part 1: LAS Monitoring study in "de Meern" sewage treatment plant and receiving water "Leidsche Rijn". Chemosphere, 30 (6), 1053-1066.

Feldman C, 1961. Evaporation of boron from acid solutions and residues. Analytical Chemistry, 33, 1916-1920.

Fenner H and Archibald JG, 1958. Boron in cows' milk. J Dairy Science, 41, 803-806.

Ferran J, Bonvalet A and Casassas E, 1988. New masking agents in the azomethine-H method for boron determination in plant tissues. Agrochimica, XXXII(2-3), 171-181.

Fott B, 1959. Algenkunde. VEB G Fischer-Verlag, Jena.

Francois LE, 1984. Effect of excess boron on tomato yield, fruit size, and vegetative growth. J Amer Soc Hort Sci 109(3), 322-324.

Francois LE, 1986. Effect of excess of boron on broccoli, cauliflower and radish. J Amer Soc Hort Sci 111(4), 494-498.

Francois LE, 1988. Yield and quality responses of celery and crisphead lettuce to excess boron. J Amer Soc Hort Sci 113(4), 538-542.

Francois LE, 1989. Boron tolerance of snap bean and cowpea. J Amer Soc Hort Sci 114(4), 615-619.

Francois LE, 1991. Yield and quality responses of garlic and onion to excess boron. Hort Science, 26(5), 547-549.

Francois LE, 1992. Effect of excess of boron on summer and winter squash. Plant and Soil 147, 163-170.

Fujiwara K, Tsubota H and Kumamaru T, 1991. Ozone gas-phase chemiluminescence detection of arsenic, phosphorus and boron in environmental waters. Analytical Sciences, 7 (Supplement), 1085-1086.

Garcia IL, Cordoba MH and Sanchez-Pedreno C, 1985. Sensitive method for the spectrophotometric determination of boron in plants and waters using crystal violet. Analyst 110, 1259-1262.

Garcia MT, Parra JL, Ribosa I and Leal JS, 1987. Niveles de boro en aguas potables de diversas poblaciones españolas. Tecnologia del Agua, 35, 63-70.

Gerike P, Fischer WK and Holtmann W, 1976. Der Einfluß von Bor auf die aerobe biologische Abwasserreinigung. Tenside Detergents 13, 249-252.

Gerike P, Winkler K, Schneider W and Jakob W, 1989a. Zur Wasserqualität des Rheins bei Düsseldorf. Tenside Detergents 26(1), 21-26.

Gerike P, Winkler K and Jakob W, 1989b. Gewässeruntersuchungen im Stromgebiet des Rheins und ökologische Folgerungen. Tenside Detergents 26(4), 270-275.

Gerike P, Winkler K, Schneider W, Jakob W and Steber J, 1991. Mengenbilanzen von Wasch- und Reinigungsmittel-Inhaltsstoffen mit Auswirkungen auf die Gewässer. Tenside Detergents 28(2), 86-89.

Germanus J, 1993a. Die Belastung ostdeutscher Fliessgewässer mit waschmittelrelevanten Inhaltsstoffen. Tenside Detergents 30, 427-434.

Germanus J, 1993b. Die Belastung ostdeutscher Fliessgewässer mit waschmittelrelevanten Inhaltsstoffen. Ph.D. Thesis, Chemistry Department, University of Leipzig, Linnestr. 3, D-04103 Leipzig, Germany, October 1993, pp 1-119.

Germanus J, Krings P and Stelter N, 1995. Untersuchungsergebnisse zu waschmittelrelevan-ten Inhaltsstoffen in ostdeutschen Fliessge-wässern. Acta hydrochim hydrobiol 23, 289-297.

Gersich FM, 1984. Evaluation of static renewal chronic toxicity test method for Daphnia magna Straus using boric acid. Environ Toxicol Chem 3, 89-94.

Graffmann G, Kuzel P, Nösler H and Nonnenmacher G, 1974. Spurenbestimmung von Bor in

Oberflächengewässern und Trinkwässern. Chemiker Zeitung, 98, 499-504.

Green GH, Blincoe C and Weeth HJ, 1976. Boron contamination from borosilicate glass. J Agric Food Chem 24(6), 1245-1246.

Green GH and Weeth HJ, 1977. Responses of heifers ingesting boron in water. J Anim Sci 46(4), 812-818.

Gregoire DC, 1990. Determination of boron in fresh and saline waters by inductively coupled plasma mass spectrometry. Journal of Analytical Atomic Spectrometry, 5, 623-626.

Guhl W, 1987. Beitrag zur biologischen Bewertung von Umweltchemikalien mit Laborökomodellen.- I. Konzeption der Oberflächengewässer und Substanzbewertung. Z. f. angew Zool 74, 385-409.

Guhl W, 1991. Beitrag zur biologischen Bewertung von Umweltchemikalien mit Laborökomodellen.- II. Vergleichbarkeit der Flußmodelle mit realen Gewässern. Z. f. angew Zool 78, 323- 336.

Guhl W, 1992a. Ökologische Aspekte von Bor. SÖFW-Journal, 118 (18/92), 1159-1168.

Guhl W, 1992b. Laboratory river models and their relevance to the real environment. Presentation at Joint Meeting of SETAC-Europe.

Guhl W, 1996. Entosiphon sulcatum, ein wenig sensitiver Testorganismus, Lauterbornia, in press.

Guhl W and Gode P, 1989. Störungen der Funktion biologischer Kläranlagen durch Chemikalien: Vergleich der Grenzkonzentrationen mit Ergebnissen im Sauerstoffzehrungstest. Vom Wasser 72, 165-173.

Gupta AP and Parrish MD, 1984. Effectiveness of a new boric acid bait (roach killer cream) on German cockroach (Blatella germanica) populations in urban dwellings. Uttar Pradesh J Zool 4, 51-56.

Gupta UC, 1968. Relationship of total and hot-water soluble boron, and fixation of added boron, to properties of podzol soils. Soil Sci Soc Amer Proc 32, 45-48.

Gupta UC, (Editor), 1993. Boron and its role in crop production. CRC Press, Boca Raton Ann Arbor London Tokyo, ISBN 0-8493-6582-1.

Hamilton SJ, 1995. Hazard assessment of inorganics to three endangered fish in the Green River, Utah. Ecotoxicology and Environmental Safety, 30, 134-142.

Hamilton SJ and Buhl KJ, 1990. Acute toxicity of boron, molybdenum and selenium to fry of chinook salmon and coho salmon. Arch Environ Contam Toxicol 19(6), 366-373.

Hamilton SJ and Wiedmeyer RH, 1990. Concentrations of boron, molybdenum and selenium in chinook salmon. Transactions of the American Fisheries Society, 119, 500-510.

Hellou J, Warren WG, Payne JF, Belkhode S and Lobel P, 1992. Heavy metals and other elements in three tissues of cod, Gadus morhua from the Northwest Atlantic. Marine Pollution Bulletin 24(9), 452-458; Biological Abstract, Vol 95, Iss. 2, ref. 22565.

HMSO, 1981. Boron in waters, effluents, sewage and some solids, 1980. Methods for the examination of waters and associated materials, pp1-35. London, Her Majesty's Stationery Office. ISBN 0 11 751583 3.

Hoffman DJ, Camardese MB, Lecaptain LJ and Pendleton GW, 1990. Effects of boron on growth and physiology in mallard ducklings. Environ Toxicol and Chem 9, 335-346.

Hoffmann H-J, 1985. Untersuchung der Metall- und Borbelastung des Mains. Münch. Beitr. Abwasser-, Fisch.- und Flussbiol 39, 125-145; Chem Abstr 104, 115689.

Hothem RL and Ohlendorf HM, 1989. Contaminants in foods of aquatic birds at Kesterson Reservoir, California (USA) 1985. Arch Environ Cont and Toxicol 18(6), 773-786.

Huber L, 1982. ICP-AES, ein neues Verfahren zur Multielementbestimmung in Wasser, Abwasser und Schlammen. Vom Wasser, 58, 173-185.

Huber L, 1994a. Boron concentrations in the river Neva, Russia. Unpublished results, Bayerisches Landesamt für Wasserwirtschaft, Institut für Wasserforschung, Munich, Germany.

Huber L, 1994b. Presence of boron as a trace impurity in reconstituted water used for fish tests. Unpublished results, Bayerisches Landesamt für Wasserwirtschaft, Institut für Wasserforschung, Munich, Germany.

Huber L, 1994c. Boron concentrations in German trout hatcheries. Unpublished results, Bayerisches Landesamt für Wasserwirtschaft, Institut für Wasserforschung, Munich, Germany.

Hunt CD, Shuler TR and Mullen LM, 1991. Concentration of boron and other elements in human foods and personal-care products. J Amer Dietetic Association, 91(5), 558-568.

IKW (German Detergent Industries Association), 1994. Unpublished statistics on sodium perborate use in Germany.

Indelicato S, Destri OL and Tamburino V, 1981. A case of raw wastewater irrigation in Sicily. Water Reuse Symposium II, Washington, 23-28 August 1981.

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, 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.

Ismail SS, 1996. Distribution of trace elements in Egyptian ground and Nile water. J Trace and Microprobe Techn. 14, 243-253.

ISO 9390, 1990. International Standard. Water quality - determination of borate - Spectrometric method using azomethine-H. ISO 9390:1990(E).

Jenkins DW, 1980. Biological monitoring of toxic trace metals. Vol. 2: Toxic trace metals in plants and animals of the world. USEPA-600/3-80-090.

Jun Z, Oshima M and Motomizu S, 1988. Determination of boron with chromotropic acid by high-performance liquid chromatography. Analyst, 113, 1631-1634.

Keating KI and Dagbusan BC, 1984. Effect of selenium deficiency on cuticle integrety in the Cladocera (crustacea). Proc Nat Acad Sci, USA, 81, 3433-3437.

Keren R and Bingham FT, 1985. Boron in water, soils and plants. Advances in Soil Science, 1, 229-276.

Klasing SA and Pilch SM, 1988. Agricultural drainage water contamination in the San Joaquin Valley: a public health perspective for selenium, boron and molybdenum. San Joaquin Valley Drainage Program, 2800 Cottage Way, Sacramento, CA.

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

Kluge R, 1990. Symptombezogene toxische Pflanzengrenzwerte zur Beurteilung von Bor(B)-Überschuß bei ausgewählten landwirtschaftlichen Nutzpflanzen (in English: Symptom-related toxic threshold values for plants for the evaluation of excess boron in crops). Agribiol Res 43(3), 234-243.

KM Lab, 1991. Ultuna Södra 11, 75103 Uppsala, Sweden (unpublished report to Eka Nobel AB, 14 November 1991).

Kobayaski N, 1971. Fertilized sea urchin eggs as an indicatory material for marine pollution bioassay, preliminary experiments. Publ Seto Mar Biol Lab 18(6), 379-406.

Kopf W and Wilk A, 1995. Investigation on aquatic toxicity against plant organisms. Unpublished results from the BayLfW - Institut für Wasserforschung, Kaulbachstr. 37, D-80539, Munich, Germany.

Korenaga T, Motomizu S and Toei K, 1980. Improved extraction method for the spectrophotometric determination of trace amounts of boron in river water with 1,8-dihydroxy-

naphthalene-4-sulphonic acid and removal of the excess of reagent. Analyst, 105, 955-964.

Kowalenko CG, 1979.   Sodium hypobromite digestion for boron analysis of plant and soil materials.   Commun in Soil Science and Plant Analysis 10(11), 1421-1434.

Krause C, Chutsch M, Henke M, Leiske M, Meyer E, Schulz C, Schwarz E and Wolter R, 1991. Umwelt-Survey Band IIIb. Wohn-Innenraum: Trinkwasser, WaBoLu-Hefte, 3/1991.

Lahl U and Burbaum H, 1988. Einzelstoffanalysen im Zu- und Ablauf einer kommunalen Kläranlage. Korrespondenz Abwasser, 35(4), 360-364.

Lanza P and Mortera G, 1983. The Determination of boron in waste waters with an ion-selective electrode. Annali di Chimica 73, 371-384.

Lapena L, Cereso M and Garcia-Augustin P, 1995. Possible reuse of treated municipal wastewater for Citrus spp. plant irrigation. Bull Environ Contam Toxicol 55, 697-703.

Levi GR and Curti R, 1938. Sulla volatilita dell'acido borico in presenza di diversi sali. Sui borofosfati e boroarsenati. - Nota V. Gazz chim ital, 68, 376-380.

Lewin J, 1965. Boron as a growth requirement for diatoms. J Phycol 2, 160-163.

Lewis MA and Valentine LC, 1981.   Acute and chronic toxicities of boric acid to Daphnia magna Straus. Bull Environ Contam Toxicol 27, 309 - 315.

LfU Wasser, 1992. Landesanstalt für Umwelt-schutz Baden-Württemberg.Grundwasserüberwa-chungsprogramm. Ergebnisse der Beprobung 1992.

Lizzio EF, 1986. A boric acid-rodenticide mixture used in the control of coexisting rodent-cockroach infestations. Lab Anim Sci 36, 74-76.

Lopez FJ, Gimenez E and Hernandez F, 1993. Analytical study on the determination of boron in environmental water samples.   Fresenius J Anal Chem 346, 984-987.

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 minerals yearbook. Annual Report Boron, pages 1-11, U.S. Department of the Interior, The Branch of Industrial Minerals, The Division of Mineral Commodities, Bureau of Mines, 2401 E St., NW, MS5209, Washington DC 20241-0001, August 1993.

Mance G, O'Donnell AR and Smith PR, 1988. Proposed environmental quality standards for List II substances in water.   Boron. Water Research Centre, Report TR 256, March 1988, ISBN 0 90 215663 2.

Marks G, Bruchlos P and Bergmann W, 1994. Experimentelle Ergebnisse zur Bortoleranz von Phragmites australis-Schilfrohr. 106.   VDLUFA-Kongress 19-24 September 1994, Jena, Germany, Schriftenreihe 38/1994, 705-708.

Martindale, 1977. The Extra Pharmacopoeia, 27th Edition, page 1202.

McKee JE and Wolf HW [Ed.], 1963. Water quality criteria. 2nd edition. The Resources Agency of California Control Board, Publ. No. 3-A.

Mea HC, Huysmans KD and Frankenberger WT Jr, 1990. Determination of borate at trace levels in environmental samples by ion-exclusion chromatography. J Chromat 508, 265-270.

Mellor's Comprehensive Treatise on Inorganic & Theoretical Chemistry, Volume V Boron, Part A: Boron-Oxygen Compounds, LONGMAN London and New York,(1980), ISBN 0-582-46277-0.

Mezzanotte V, Arcadipane M, Faniuolo L and Siviero R, 1995. Analisi di fosforo e boro in alcumi impianti di depurazione urbani. Apporto Pro-Capite, Capacità di Rimozione   e   Concentrazione   nei   Fanghi. Inquinamento, 37(7), 40-45; Chem Abstr 1996, 124, 154527t.

Mineral Industry Surveys, 1990. U.S. Department of the Interior, Bureau of Mines, Washington, DC 20241, "BORON in 1990".

Ministry of Agriculture, Fisheries and Food (U.K.), 1981. Water quality for crop irrigation: guidelines on chemical criteria. Leaflet 776.

Motomizu S, Oshima M and Jun Z, 1991. Fluorimetric determination of boron with chromotropic acid by flow-injection analysis. Analytica Chimica Acta, 251, 269-274.

Motomizu S, Sawatani I, Oshima M and Toei K, 1983. Determination of boron by ion-pair liquid chromatography with 1,8-dihydroxynaphthalene-3,6-disulfonic acid. Anal Chem 55, 1629-1631.

Naghii MR and Samman S, 1993. The role of boron in nutrition and metabolism. Progress in Food and Nutrition Science 17(4), 331-349.

NAS, 1980. Boron mineral tolerance of domestic animals. National Academy of Science, Washington, DC 71-83.

Navarro J, Mansour M and Ivorra R, 1992. Use of waste water in Mediterranean crops. Fresenius Environ Bull 1, 423-427.

Nielsen FH, 1986. Other elements: Sb, Ba, B, Br, Cs, Ge, Rb, Ag, Sr, Sn, Ti, Zr, Be, Bi, Ga, Au, In, Nb, Sc, Te, Tl, W. In: Mertz W (ed). Trace elements in human and animal nutrition, 5th ed. Academic Press, New York, 415-464.

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

Normalisation française T90-041, 1985. Essais des eaux. Dosage du bore par spectrométrie d'absorption moléculaire. Méthode à l'azométhine H. T90-041.

Novozamsky I, Barrera LL, Houba VJG, van der Lee JJ and van Eck R, 1990a. Comparison of a hot water and cold 0.01M $CaCl_2$ extraction procedures for the determination of boron in soil. Commun in Soil Sci Plant Anal 21(17&18), 2189-2195.

Novozamsky I, van Eck R, Houba VJG and van der Lee JJ, 1990b. A new solvent extraction for the determination of traces of boron by ICP-AES. Atomic Spectroscopy 11, 83-84.

Odom JW, 1980. Kinetics of the hot water soluble boron soil test. Commun in Soil Sci Plant Anal 11(7), 759-765.

OECD, 1984. Algae growth inhibition test. OECD-Guideline for Testing of Chemicals 201.

Ohlendorf HM, Hoffmann DJ, Saiki MK and Aldrich TW, 1986. Embryonic mortality and abnormalities of aquatic birds: apparent impacts of selenium from irrigation drainwater. Sci Total Environ 52, 49-63.

Okay O, Güçlü H, Soner E and Balkas T, 1985. Boron pollution in the Simav river, Turkey and various methods of boron removal. Water Res 19(7), 857-862.

Oki Y, Tashiro E, Maeda M, Honda C, Hasegawa Y, Futami H, Izumi J and Matsuda K, 1993. Sensitive detection of trace elements in pure water by laser-induced atomic fluorescence spectroscopy in microwave discharge atomization. Anal Chem 65, 2096-2101.

Owen EC, 1944. The excretion of borate by the dairy cow. J Dairy Res 13(3), 243-248.

Paveglio FL, Bunck CM and Heinz GH, 1992. Selenium and boron in aquatic birds from central California. J Wildl Manage 56 (1), 31-42.

Pendleton GW, Whitworth MR and Olsen GH, 1995. Accumulation and loss of arsenic and boron, alone and in combination, in Malard ducks. Environ Toxicol and Chem, 14(8), 1357-1364.

Pennington HD, Finch CR, Lyons CC and Littau SA, 1991. Microwave digestion of plant samples for boron analysis. Hortscience 26(12), 1496-1497.

Randow FFE, 1981. Boron in surface waters - its importance, origin and preliminary results. Z ges Hyg 26, 787-789.

Randow FFE, Bondick R and Lahl R, 1982. Über den Borgehalt mecklenburgischer Oberflächengewässer. Acta hydrochim hydrobiol 10(4), 391-395.

Rao CK, 1992. Elemental composition of Indian marine algae: A biogeochemical perspective. Indian J Marine Sci 21, 167-177.

Reid PC and Davies E, 1989. Boron content of South African surface waters: Preliminary assessment for irrigation. Water SA, 15(4), 261-264; Chem Abstr 1990, 113, 11692e.

Rijkswaterstaat, 1981. Kwaliteitsonderzoek in de rijkswateren (cited in Mance et al (1988), Table 4).

Römheld V and Marschner H, 1991. Function of micronutrients in plants. In: Mordvedt JJ, Cox FR, Shuman LM and Welch RM (eds). Micronutrients in agriculture, 2nd ed., Soil Science Society of America Book Series No 4, 316-321

Ruhrwassergüte 1988. Bericht des Ruhrverbands, Kronprinzen-strasse 37, Postfach 10 32 42, 4300 Essen 1, Germany.

Ruhrwassergüte 1991. Bericht des Ruhrverbands, Kronprinzen-strasse 37, Postfach 10 32 42, 4300 Essen 1, Germany.

Ruhrwassergüte 1992. Bericht des Ruhrverbands, Kronprinzen-strasse 37, Postfach 10 32 42, 4300 Essen 1, Germany.

Saiki MK, Jennings MR and Brumbaugh WG, 1993. Boron, molybdenum, and selenium in aquatic food chains from the lower San Joaquin River and its tributaries, California. Arch Env Cont Toxicol 24, 307-319.

Salinas F, Munoz de la Pena A, Murillo JA and Sanchez JCJ, 1987. Spectrofluorimetric determination of boron in plants with quinizarin-2-sulphonic acid. Analyst, 112, 913-915.

Sato S, 1983. Extraction-spectrophotometric determination of boron with mandelic acid and malachite green. Analytica Chimica Acta 151, 465-472.

Schachtschabel P, Blume H-P, Brümmer G, Hartge K-H and Schwertmann U, 1989. Lehrbuch der Bodenkunde. Ferdinand Enke Verlag Stuttgart, page 288.

Schöberl P and Huber L, 1988. Ökologisch relevante Daten von nichttensidischen Inhaltsstoffen in Wasch- und Reinigungsmitteln, Tenside Detergents 25, 99-107.

Schöller F and Bolzer W, 1989. Boron - a substance of problem in the water field. Water Supply 7, 169-177; Chem Abstr 1990, 112, 164489y.

Scholl W and Maier D, 1987. Bor in Fließgewässern von Baden-Württemberg - ein Statusbericht. VDLUFA - Schriftenreihe 23, 671- 688.

Scholl W, Wurster H, Thalmann A and Möller J, 1985. Klärschlammvererdung in Schilfbecken - Ergebnisse und Erkentnisse eines praxisbezogenen Pilotprojekts. Korrespondenz Abwasser, 5/85, 32, 386-395.

Schöller F, 1990. Die Borsituation in österreichischen Gewässern. Tenside Detergents 27(2), 136-140.

Schuler CA, 1987. Impacts of agricultural drainwater and contaminants on wetlands at Kesterson Reservoir, California. Oregon State University, Corvallis.

Sekerka I and Lechner JF, 1990. Automated method for the determination of boron in water by flow-injection analysis with in-line preconcentration and spectrophotometric detection. Analytica Chimica Acta 234, 199-206.

Shorrocks VM, 1989. Boron deficiency - its prevention and cure. Illustrated booklet issued by Borax Consolidated Ltd., London, UK.

Siegel E and Wason S, 1986. Boric acid toxicity. Pediatr Clin north Am 33 (2), 363-367.

Sisk DB, Colvin BM and Bridges CR, 1988. Acute, fatal illness in cattle exposed to boron fertilizer. J Am Vet Med Assoc 193(8), 943-945.

Sisk DB, Colvin BM, Merrill A, Bondari K and Bowen JM, 1990. Experimental acute inorganic boron toxicosis in the goat: Effects on serum chemistry and CSF biogenic amines. Vet Hum Toxicol 32(3), 205-211.

Smith GJ and Anders VP, 1989. Toxic effects of boron on mallard reproduction. Environ Toxicol Chem 8, 943-950.

Soap and Detergent Association, 1994. Levels of boron in freshwaters of the U.S.A. from 1 January 1984 to 31 December 1993 (unpublished results). SDA, 475 Park Avenue South, New York, NY 10016.

Sposito G and Calderone SJ, 1988. Boron uptake and accumulation by higher plants: a literature review. Report EPRI-EA-5817, 52 pp; Chem Abstr 1989, 111, 4184w.

Sprague RW, 1972. The ecological significance of boron. U.S. Borax Research Corporation, Anaheim, California, U.S.A. (Library of Congress Catalog Card Number 72-76744).

Subba Rao DV, 1981. Effect of boron on primary production of nanoplankton. Canadian J. Fisheries and Aquatic Sciences 38, 52-58.

Sveriges Geologiska AB Analys, 1991, Box 801, 951 28 Luleå, Sweden (unpublished report to Eka Nobel AB, 14 November 1991).

Szabo AS and Sashin IL, 1988. Nondestructive prompt neutron activation technique for determining boron uptake and distribution in plants. Acta Agronomica Hungarica 37(3-4), 09-213.

Tartari G and Camusso M, 1988. Boron content in freshwaters of northern Italy. Water, Air, Soil Pollut 38(3-4), 409-417; Chem Abstr 1988, 109, 115663e.

Taylor D, Maddock BG, and Mance G, 1985. The acute toxicity of nine "gray list" metals (arsenic, boron, chromium, copper, lead, nickel, tin, vanadium and zinc) to two marine fish species: Dab (*Limanda limanda*) and Gray mullet (*Chelon labrosus*). Aquatic Toxicol 7(3), 135-144.

Thompson JAJ, Davis JC and Drew RE, 1976. Toxicity, uptake and survey studies of boron in the marine environment. Water Res 10, 869-875.

Touchstone JC, Dobbins MF, Mallinger ML and Strauss J, 1980. Chapter 11. Quantitation of boron at ppb-levels. In: Touchstone JC and Rogers D (eds). Thin Layer Chromatography: Quantitative environmental and clinical applications, Wiley, New York, 151-157.

Tsuboi I, Kunugita E and Komasawa I, 1990. Recovery and purification of boron from coal fly ash. J Chem Engin of Japan 23, 480-485.

Turnbull H, 1954. Toxicity of various refinery materials to freshwater fish. Ind Eng Chem 46, 324-333.

UK Department of the Environment, 1994. Second report of the technical committee on detergents and the environment. December 1994.

Unilever, 1994. Summary of external research project on environmental levels of boron, especially in trout-sustaining waters. Unilever Research Port Sunlight Laboratory, unpublished summary, 3 pp.

van der Geugten RP, 1981. Determination of boron in river water with flameless atomic absorption spectrometry (Graphite furnace technique). Fresenius Z Anal Chem 306, 13-14.

van Diest, A, 1989. Landbouwuniversiteit Wageningen, The Netherlands. Letter of 21 December 1989 to Dr VM Shorrocks, Micronutrient Bureau.

van Heerden E, van Vuren JHJ and Steyn GJ, 1993. Development and evaluation of sperm diluents for the artificial insemination of rainbow trout (Oncorhynchus mykiss). Aquat Living Resour 6, 57-62.

Vengosh A, Chivas AR and McCulloch MT, 1989. Direct determination of boron and chlorine isotopic compositions in geological materials by negative thermal-ionization mass spectrometry. Chemical Geology (Isotope Geoscience Section), 79, 333-343.

Verbanck M, Vanderborght J-P and Wollast R, 1989. Major ion content of urban wastewater: assessment of per capita loading. Res J Water Pollut Control Fed 61(11-12), 1722-1728; Chem Abstr 1990, 112, 240012e.

Waggott A, 1969. An investigation of the potential problem of increasing boron concentrations in rivers and water courses. Water Res 3, 749-765.

Wallen IE, 1957. Toxicity of Gambusia affinis of certain pure chemicals in turbid water. Sewage Ind Wastes 29, 695-711.

Warren CJ, Evans LJ and Sheard RW, 1993. Release of some trace elements from sluiced fly ash on acidic soils with particular reference to boron. Waste Management & Research 11, 3-15.

Webber WG, Kemp DW and Rice SE, 1977. Study of the effect of boron toxicity on an activated sludge system. Proc Ind Waste Conf 31, 743-752.

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 (3), 474-477.

Whitworth MR, Pendleton GW, Hoffman DJ and Camardese MB, 1991. Effects of dietary boron and arsenic on the behavior of mallard duckling. Environ Toxicol Chem 10, 911-916

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

Wiecken B and Wübbold-Weber S, 1995. Bor in Trinkwässern der Bundesrepublik Deutschland. SÖFW Journal, 121(6), 428: 431-433, 435-436. Chem Abstr 1995, 123, 152218.

Wikner B, 1986. Pretreatment of plant and soil samples. A problem in boron analysis. Part I: Plants Commun in Soil Sci Plant Anal 17(1), 1-25.

Wilkinson DE, 1993. Boron values for Anglian Water Region 1.1.92-31.12.92 (Letter of 9 March 1993, ref. DEW/AH/1733CD, from Anglian Water Services Limited).

Wright CG and Dupree HE Jr, 1982. Efficacy of experimental formulations of acephate, boric acid, encapsulated diazinon, permethrin, pirimphos-methyl and propetamphos in control of German cockroaches. J Ga Entomol Soc 17, 26-32.

Yamada H and Hattori T, 1986. Determination of total boron in soil by the curcumin-acetic acid method after extraction with 2-ethyl-1,3-hexanediol. Soil Sci Plant Nutr 32(1), 135-139.

Yamamoto T, Yamaoka T, Fujita T and Isoda C, 1971. Chemical studies on the seaweeds 26. Boron content in seaweeds. Res Oceanogr Works Japan 11(1), 7-13.

Zheng K, 1990. Oscillopolarographic determination of trace amount of boron in soil, water and plant samples. Intern J Environ Anal Chem 39, 75-79.

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