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Environmental Protection
Agency

Health Effects Support Document for Boron

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FOREWORD

The Safe Drinking Water Act (SDWA), as amended in 1996, requires the Administrator of the U.S. Environmental Protection Agency (U.S. EPA) to establish a list of contaminants to aid the Agency in regulatory priority setting for the drinking water program. In addition, the SDWA requires the U.S. EPA to make regulatory determinations for no fewer than five contaminants by August 2001 and every five years thereafter. The criteria used to determine whether or not to regulate a chemical on the Contaminant Candidate List (CCL) are the following:

- The contaminant may have an adverse effect on the health of persons.

- The contaminant is known to occur or there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern.

- In the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

The Agency's findings for all three criteria are used in making a determination to regulate a contaminant. The Agency may determine that there is no need for regulation when a contaminant fails to meet one of the criteria. The decision not to regulate is considered a final Agency action and is subject to judicial review.

This document provides the health effects basis for the regulatory determination for boron. In arriving at the regulatory determination, data on toxicokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology, and mechanisms of toxicity were evaluated. In order to avoid wasteful duplication of effort the Toxicokinetic, Hazard Identification and Dose-Response Assessment Chapters in the Document are a reproduction of the comparable Chapters the U. S. EPA Integrated Risk Information System (IRIS) Toxicological Review for Boron and Compounds (www.epa.gov/iris/toxreviews/0410-tr.pdf) (U.S. EPA, 2004a). The IRIS assessment was completed in June 2004. The Chapters on chemical and physical properties, environmental release and fate, exposure from water and substances other than water were prepared by the Office of Water for the Regulatory Determination.

A Reference Dose (RfD) from the IRIS Toxicological Review is provided as the assessment of long-term toxic effects other than carcinogenicity. RfD determination assumes that thresholds exist for certain toxic effects, such as cellular necrosis, significant body or organ weight changes, blood disorders, etc. It is expressed in terms of milligrams per kilogram per day (mg/kg-day). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The carcinogenicity assessment for boron from the IRIS Toxicological Review includes a formal hazard identification and a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen via the oral exposure route.

Development of these hazard identifications and dose-response assessments for boron has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). U.S. EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Guidelines for Carcinogen Assessment* (U.S. EPA, 2005), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002a).

The chapter on occurrence and exposure to boron through potable water was developed by the Office of Ground Water and Drinking Water. It is based primarily on the National Inorganic and Radionuclide Survey (NIRS) data for boron. The NIRS data are supplemented with ambient water data, as well as data from the States, and published papers on occurrence in drinking water.

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1.0 EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) has prepared this Health Effects Support Document for Boron to assist in determining whether to regulate boron with a National Primary Drinking Water Regulation (NPDWR). The available data on occurrence, exposure, and other risk considerations suggest that boron does not occur in public water systems at a frequency and at levels of public health concern at the present time. Based on the low occurrence of boron in the potable water, and on its natural occurrence in the environment, boron does not present a meaningful opportunity for health risk reduction for persons served by public water systems. EPA presents its determination and data analysis in the Federal Register Notice covering the Contaminant Candidate List (CCL) regulatory determinations.

Boron is a metalloid element from Group IIIA of the periodic table. Naturally-occurring boron usually is found in sediments and sedimentary rock formations and rarely exists in elemental form. Other forms of boron include boric acid, borax, borax pentahydrate, anhydrous borax, and boron oxide. The principal uses for boron compounds in the United States include glass and ceramics, soaps and detergents, algicides in water treatment, fertilizers, pesticides, flame retardants, and reagents for production of other boron compounds.

The major sources of free boron in the environment are exposed minerals containing boron, boric acid volatilization from seawater, and volcanic material. Global releases of elemental boron through weathering, volcanism, and other geothermal processes are estimated at approximately 360,000 metric tons annually (Moore, 1991). Anthropogenic inputs of boron to natural environments are considered smaller than inputs from natural processes. The following human activities release boron to the environment: agriculture, waste and wood burning, power generation using coal and oil, glass product manufacture, use of borates/perborates in the home and industry, borate mining/processing, leaching of treated wood, and sewage/sludge disposal. Contamination of water can come directly from industrial wastewater and municipal sewage, as well as indirectly from air deposition and soil runoff. Borates in detergents, soaps, and personal care products can also contribute to the presence of boron in water.

The available data for boron support its ubiquitous presence in the ambient environment. TRI data for the years 1995 to 2002 on total releases for boron trichloride (on- and off-site) have fluctuated within the range of hundreds of pounds per year. Boron trifluoride releases for the years 1995 to 2002 are similarly dominated by on-site air emissions, with releases ranging in the tens of thousands of pounds annually. In drinking water, approximately 81.9% of groundwater public water systems (PWSs) had detections of boron ($\geq$minimum reporting level (MRL) of 0.005 mg/L). These detections affected about 88.1% of the population served by the public water systems, equivalent to approximately 75.5 million people served by ground water nationally. Detections at a concentration greater than one-half the health reference level (HRL) of 0.7 mg/L occurred in 4.3% of surveyed PWSs, affecting 2.9% of the population served, equivalent to approximately 2.5 million people nationally. Concentrations greater than the HRL (1.4 mg/L) were found in approximately 1.7% of surveyed PWSs, affecting 0.4% of the population served, equivalent to approximately 0.4 million people nationally. Supplementary data from an AWWARF-sponsored study indicate that boron contamination of surface water is

less significant than boron contamination of ground water. Of 228 ground water and 113 surface water samples analyzed, boron was detected in 99.1% of the ground water samples and 97.3% of the surface water samples. Boron was detected at a concentration greater then one-half the health reference level (>½HRL or >0.7 mg/L) in 8.8% of the ground water samples and none of the surface water samples. Boron was detected at concentrations greater than the HRL (>HRL or >1.4 mg/L) in 3.1 % of the ground water samples and in none of the surface water samples. Median and 90th percentile boron concentrations from the limited USEPA Community Water Survey were lower in surface water than in ground water and both were below one-half the health reference level.

Studies in both humans and animals show that boron is readily absorbed from the gastrointestinal tract *(the absorption evidence is weak from the respiratory tract as described*). Boric acid and borate compounds in the body exist primarily as undissociated boric acid, which distributes evenly throughout the soft tissues, but shows some accumulation in bone. In several animal studies, boron levels in all tissues, except adipose, increased rapidly after the start of dietary exposure, with the greatest increase in bone. In one study, bone boron levels showed a 2- to 3-fold increase over plasma levels after 7 days. In another study, concentrations of boron in bone in exposed animals were 5- to 6-fold higher than in unexposed controls after eight weeks of recovery; thirty-two weeks after recovery bone boron concentrations remained 3-fold higher in treated groups than in controls.

Inorganic borate compounds are present as boric acid in the body. Boric acid is the only boron compound that has been identified in urine, and it has repeatedly been found to account for >90% of the ingested boron dose. There is no evidence that boric acid is degraded in the body. Metabolism may not be feasible because a large amount of energy (523 kJ/Mol) is apparently required to break the boron-oxygen bond. Boric acid can form complexes with various biomolecules. It has an affinity for hydroxyl, amino, and thiol groups. Complex formation is concentration dependent and reversible. The primary route of excretion of boron is in the urine.

Boron is a trace element for which essentiality is suspected but has not been directly proven in humans. The National Academy of Science Institute of Medicine categorizes boron as a possible trace mineral nutrient for humans. Boron is essential for plant growth. Deficiency studies in animals and humans have provided some evidence that low intakes of boron affect cellular function and the activity of other nutrients. It may interact with Vitamin D and calcium, influence estrogen metabolism, and play a role in cognitive function. The average dietary intake for male adults is about 1.5 mg/day.

Some human oral data are available from cases where boron was ingested for medical reasons. When the amount ingested was less than 3.68 mg/kg, subjects were asymptomatic, while doses of 20 and 25 mg/kg resulted in nausea and vomiting. Case reports and surveys of accidental poisonings indicate that the lethal doses of boron range from 15 to 20 grams (approximately 200 to 300 mg/kg) for adults, 5 to 6 grams (approximately 70 to 85 mg/kg) for children, and 2 to 3 grams (approximately 30 to 45 mg/kg) for infants.

The primary adverse effects seen in animals after chronic exposure to low doses of boron generally occur in testes and fetuses. Chronic effects of dietary boron exposure in two-year studies included the following: testicular atrophy and spermatogenic arrest in dogs, decreased food consumption, suppressed growth, and testicular atrophy in rats, and decreased survival, testicular atrophy, and interstitial cell hyperplasia in mice. Although researchers observed some increases in tumor incidences in the liver and in subcutaneous tissues in mice, based on comparisons to historic controls these tumors were determined not to be associated with exposure to boron from boric acid. Boron is not considered mutagenic. EPA has determined that there are inadequate data to assess the human carcinogenic potential for boron.

In developmental studies with rats, mice, and rabbits, oral exposure to boric acid resulted in decreased pregnancy rates, increased prenatal mortality, decreased fetal weights, and increased malformations in fetuses and pups. These reproductive effects were associated with maternal toxicity, including changes in maternal organ weights, body weights, weight gain, and increased renal tubular dilation and/or regeneration. Reproductive effects in males were noted in the subchronic and chronic studies described above.

The EPA reference dose (RfD) for boron is 0.2 mg/kg/day based on developmental effects in rats from two studies. The RfD was derived using the benchmark dose (BMD) method and a data-derived uncertainty factor of 66. EPA established the Health Reference Level (HRL) for boron (1.4 mg/L or 1400 µg/L) using the RfD of 0.2 mg/kg-day and a 20 percent relative source contribution.

EPA evaluated whether health information is available regarding the potential effects on children and other sensitive populations. Studies in rats, mice, and rabbits identify the developing fetus as potentially sensitive to boron. Price et al. (1996a) identified a lowest observed adverse effect level (LOAEL) of 13.3 mg/kg-day and a no observed adverse effect level (NOAEL) of 9.6 mg/kg-day in the developing rat fetus, based on decreased fetal body weight. Accordingly, boron at concentrations greater than the HRL might affect prenatal development. Individuals with impaired kidney function might be more sensitive to boron exposure than the general population since the kidney is the main route for excretion.

Based on the concentrations of boron in the potable water where it occurs relative to the HRL, boron does not present a meaningful opportunity for health risk reduction for persons served by public water systems.

2.0 IDENTITY: CHEMICAL AND PHYSICAL PROPERTIES

Boron is a metalloid element from Group IIIA of the periodic table with an atomic number of 5, atomic weight of 10.81, and oxidation state of +3. Boron exists naturally as 19.78% ^{10}B isotope and 80.22% ^{11}B isotope (WHO, 1998a). It is a polymorphic element that exists in a variety of different crystalline forms: α-rhombohedral (clear red crystals); β-α-rhombohedral (black); α-tetragonal (black, opaque crystals with metallic luster); amorphous (black or dark brown powder); and yellow monoclinic crystals or brown amorphous powder (O'Neil et al., 2001; Weast, 1988-1989). Elemental boron is insoluble in water, but if finely divided, it is soluble in boiling sulfuric acid and in most molten metals, such as copper, iron, magnesium, aluminum, and calcium. Elemental boron undergoes an oxidation reaction upon exposure to oxygen which is limited by the formation of a protective boric oxide film. This film evaporates at temperatures above 1000°C. At room temperature, boron is a poor conductor of electricity, but its conductivity increases at higher temperatures (O'Neil et al., 2001). Technical grade boron has 90-92% boron content (Sax and Lewis, 1987) and can include impurities such as carbon, oxygen, hydrogen, and nitrogen. Impurities in ultrapure boron are usually below the 0.5% range (Kroschwitz and Howe-Grant, 1992).

Boron is electron-deficient, possessing a vacant p-orbital; it does not form ionic bonds, but does form stable covalent bonds. Compounds of boron often behave as Lewis acids, readily bonding with electron-rich substances.

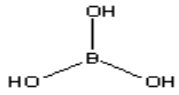
Boric acid (Figure 2-1) exists as odorless, colorless, translucent crystals or white granules or powder at ambient temperatures (O'Neil et al., 2001). It is a weak acid with a pKa of 9.2 (pH 5.1 when in a 0.1 molar solution) and exists primarily as the undissociated acid (H_3BO_3) in aqueous solution at physiological pH, as do borate salts (Woods, 1994). Three grades of granular and powdered boric acid are manufactured in the United States, i.e., technical grade (99.9%), NF grade, and special quality grade. The principal impurities in technical grade boric acid are sulfate (0.1%) and various minor metallic impurities present in borate ore (Kirk-Othmer, 1984).

Borax (Figure 2-2) is an odorless substance that exists in the form of white or colorless monoclinic crystals. Its solutions have alkaline properties, but do not cause corrosion to ferrous metals (HSDB, 2003c). Borax is produced as crystals, granules, and powder (Sax and Lewis, 1987). Technical borax is an herbicide, also known as "Nippon" insecticide, while refined borax is known as sodium tetraborate decahydrate (99% purity). Mixtures include brocil (borax + bromacil), ureabor (borax + monuron), and borax + sodium chlorate (Worthing, 1987; Weed Science Society of America, 1983). Anhydrous borax is an odorless, hygroscopic substance that exists as white to gray powder or as glass-like plates (HSDB, 2003d). It is produced from its hydrated forms by fusion, usually through an intermediate step involving calcining (Kirk-Othmer, 1984).

Technical grade anhydrous borate (borax) contains 99% sodium tetraborate and comes in fine, granular form, as glass (fused; Sax and Lewis, 1987).

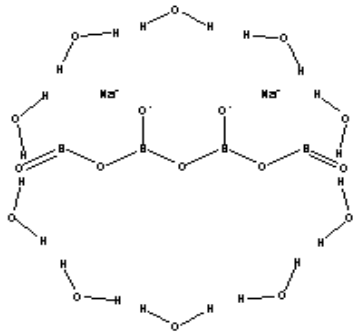
Boron oxide (Figure 2-3) is an odorless, slightly bitter substance, which at ambient temperatures exists in the form of colorless, semitransparent lumps or hard, white crystals. These solids are brittle and hygroscopic and they slowly react with water to form boric acid. Boron oxide is soluble in alcohol and glycerol; it is corrosive to metals in the presence of oxygen (O'Neil et al., 2001; Kirk-Othmer, 1984). Both technical and high-purity (99.99%) grades of boron oxide are manufactured in a glass (fused) or powdered form (Sax and Lewis, 1987).

Figure 2-1 Chemical Structure of Boric Acid



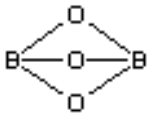
Source: Chemfinder.com (2004)

Figure 2-2 Chemical Structure of Borax (Sodium Tetraborate Decahydrate)



Source: Chemfinder.com (2004)

Figure 2-3 Chemical Structure of Boron Oxide



Source: Chemfinder.com (2004)

The chemical structures of boric acid, borax, and boron oxide are shown above (Figures 2-1 through 2-3); the chemical structure of elemental boron, borax pentahydrate, and anhydrous borax were not available (Chemfinder.com, 2004). The physical and chemical properties, and other reference information on boron, boric acid, borax, borax pentahydrate, anhydrous borax, and boron oxide are listed in Table 2-1.

Table 2-1 Chemical and Physical Properties of Boron and Related Compounds

Property	Boron	Boric Acid	Borax	Borax Pentahydrate	Anhydrous Borax	Boron Oxide
Chemical Abstracts Registry (CAS) No.	7440-42-8	10043-35-3	1303-96-4	12179-04-3 11130-12-4	1330-43-4	1303-86-2
U.S. EPA Pesticide Chemical Code	128945	011001	029601 or 011102	011110	011112	011002
Synonyms	none identified	boron trihydroxide; trihydroxy borate; orthoboric acid; boracic acid	disodium tetraborate decahydrate, borax decahydrate, borax 10	Sodium tetraborate pentahydrate; Borax 5	Sodium tetraborate; borax glass; disodium tetraborate; fused borax	Boric oxide; boron trioxide; anhydrous boric acid
Chemical Formula	B	H_3BO_3	$Na_2B_4O_7.10H_2O$	$Na_2B_4O_7.5H_2O$	$Na_2B_4O_7$	B_2O_3
Molecular Weight	10.81	61.83	381.43	291.35	201.27	69.62
Physical State	Solid; black crystal or yellow-brown amorphous powder	Solid; white or colorless crystalline granules or powder	Solid; white or colorless crystalline granules or powder	Solid; white or colorless crystalline granules or powder	Solid; white or colorless vitreous granules	Solid; white or colorless vitreous granules
Boiling Point	2,550°C	30°C	none identified	none identified	1,575°C (decomposes)	1,860°C
Melting Point	2,300°C	171°C (closed space) 450°C (anhydrous, crystal form)	>62°C (closed space)	<200°C (closed space)	742°C	450°C
Density (at 20 °C)	2.34	1.51	1.73	1.81	2.37	2.46
Vapor Pressure:						
At 20 °C	1.56 $x10^{-5}$atm (at 2,140°C)	none identified	none identified	none identified	none identified	none identified
At 25 °C	none identified	none identified	none identified	none identified	none identified	none identified
Log K_{ow}	none identified	none identified	none identified	none identified	none identified	none identified
Log K_{oc}	none identified	none identified	none identified	none identified	none identified	none identified
Solubility in:						
Water	Insoluble	55.6 g/L cold water[a] 250 g/L boiling water[a]	62.5 g/L at 25°C	35.9 g/L at 20°C 482.4 g/L at 100°C	24.8 g/L at 20°C 331.2 g/L at 100°C	rapidly hydrates to boric acid
Other Solvents	none identified	methanol, acetone, alcohol, glycerol	glycerol	glycerol	ethylene glycol	alcohol, glycerol

Source(s): HSDB (2003a-e); Weast (1988-1989); O'Neil et al. (2001)
a Water temperature was not defined.

3.0 USES AND ENVIRONMENTAL FATE

3.1 Production and Use

Elemental boron occurs naturally and is found in borax ore or tincal ($Na_2B_4O_7.10H_2O$), boric acid (H_3BO_3), colemanite ($CaB_3O_4(OH)_3$-H_2O), kernite or rasorite ($Na_2B_4O_7 \cdot 4H_2O$), ulexite ($NaCaB_5O_9 \cdot 8H_2O$), and borates (salt or ester of boric acid). Boric acid is sometimes found in volcanic spring waters. Ulexite is a borate mineral that naturally has fiber optic properties.

In 2003, the United States was the world's largest producer of refined boron compounds. About one-half of the domestic production (1060×10^3 metric tons) was exported. Domestic production of boron minerals, primarily as sodium borates, by four companies was centered in southern California (USGS, 2004). The largest company produced and processed ore from an open pit mine; the second company produced boron, sodium carbonate, and sodium sulfate from brines; the third company has since ceased production; the fourth operates an underground mine in California and processes the ore in Nevada for overseas export. U.S. processed products had fewer impurities and lower emissions than products from other countries (USGS, 2004). Elemental boron production methods include chemical reduction with reactive elements, nonaqueous electrolytic reduction, or thermal decomposition of the oxide. Purification to ultrapure boron is accomplished by zone-refining or other thermal techniques. Another method for boron production is by electrolysis of fused melts with a boron carbide anode (Kirk-Othmer, 1984).

Boric acid is produced by reacting borax or other borates with hydrochloric or sulfuric acid (Osol, 1980). An alternative method employs extraction from weak borax brines with a kerosine solution or a chelating agent, such as 2-ethyl-1,3-hexanediol or other polyols. The chelates are subsequently removed by sulfuric acid (Sax and Lewis, 1987).

Commercial production of borax involves the processing of sodium borate ores by crushing, heating, mechanical separation, selective crystallization, and then flotation of borax decahydrate or pentahydrate from the resultant concentrated borax liquor (HSDB, 2003c).

Boron is used in nuclear chemistry as a radiation shield and for neutron-detecting instrumentation (Weast, 1988-1989). It is a deoxidizer in nonferrous metallurgy and ignition rectifiers for rockets and radio tubes (O'Neil et al., 2001). Boron also is used in aluminum as a grain refiner for delayed action fuses, solar battery coatings (Clayton and Clayton, 1994), iron cementation, wire-coatings for semiconductors, and high temperature abrasive alloys (Sax and Lewis, 1987). Boron is a catalyst in olefin polymerization and alcohol dehydration (Kroschwitz and Howe-Grant, 1992).

Borax (hydrous or anhydrous) and boric acid are widely used for a wide range of industrial purposes. Major applications are in the manufacture of porcelain enamel, ceramic glazes, and metal alloys, and to enhance thermal properties of glass and durability of fiberglass insulation. These compounds also are commonly used in fire retardants in cellulose insulation, wood and textiles, laundry additives, herbicides, fertilizers (boron is an essential element for

plants), and insecticides (HSDB, 2003c,d; Woods, 1994). Boric acid, borates, and perborates have been used as ingredients in mild antiseptics or bacteriostats in eyewashes, mouthwashes, burn dressings, and diaper rash powders, although their effectiveness has largely been discredited (Seiler et al., 1988).

Borax is used in the following diverse applications: tanning, artificial aging of wood, fireproofing fabrics and woods, curing and preserving hides, soldering metals, and inhibition of wood fungus rot. It also is used in antiseptics, detergents, and astringents, antifreeze, plant fertilizers, nonselective herbicides, and soil sterilants (Kroschwitz and Howe-Grant, 1992; O'Neil et al., 2001; Sax and Lewis, 1987; U.S. FDA, 1988).

Borax decahydrate is a commercial fungicide for citrus (Spencer, 1982), an ingredient in household germicidal cleaning products, a chemical intermediate in the productions of perborates and other boron derivatives, a flux in the nonferrous metallurgy, and an additive in ferrous and nonferrous boron alloys production (HSDB, 2003d).

Commercial anhydrous borax is an industrial water algicide, corrosion inhibitor, emulsifying agent in cosmetics, and a buffer component in a variety of products (Gilman et al., 1990; Kroschwitz and Howe-Grant, 1992; O'Neil et al., 2001; Sax and Lewis, 1987; U.S. FDA, 1988).

Boric acid is used in printing, dyeing, painting, leather making, and hard-steel production. It is used in the manufacture of soaps, artificial gems, electric condensers (O'Neil et al., 2001), paper products for food packaging, adhesives, sizes, and coatings (U.S. FDA, 1988). It is the key raw ingredient in the manufacture of synthetic inorganic borate salts, boron phosphate, fluoborate, borate esters, and metal alloys such as ferroboron (Kroschwitz and Howe-Grant, 1992). Boric acid is a component of high contrast lith-type film developer formula (e.g., Kodak D-85), an additive in nuclear-reactor cooling water, and a catalyst for alcohol production from air oxidation of hydrocarbons (Kroschwitz and Howe-Grant, 1992), and a constituent of insect baits, repellants, and poisons (Rossoff, 1974; Meister, 1989).

Boric oxide is used as a chemical intermediate for obtaining elemental boron, boron master alloys, borides, boron carbide, nitrides and halides. It is a fire resistant ingredient in paints and electronic products. It also is used in liquid encapsulation techniques and blowpipe analysis, and protocols used to determine silicon dioxide and alkalide presence in silicates (National Fire Protection Association, 1997; O'Neil et al., 2001).

The principal uses for boron compounds in the United States in 2001 were estimated as follows: 78% glass and ceramics; 6% soaps and detergents; 3% agriculture; 4% flame retardants; and 9% as other boron compounds (USGS, 2006). The use pattern for borax (decahydrate, pentahydrate, and anhydrous) is: 23% in insulation glass fibers; 20% in household cleaning products as germicide; 11% in borosilicate glasses; 11% as algicide in water treatment; 8% in enamel flux, frits, and glazes; 8% as chemical intermediate for perborates; 7% in fertilizers; 5% as antifreeze corrosion inhibitor; 4% as a chemical intermediate for other boron compounds; 3% in herbicides; 1% as flame retardant and metallurgical flux; and 10% in other miscellaneous

applications (HSDB, 2003a). Borate consumer uses in 1985 were estimated as follows: 18% glass fiber insulation; 11% textile glass fiber; 15% chemical fire retardants; 5% borosilicate glass; 4% soap and detergents; 13% miscellaneous; and 44% exports (HSDB, 2003a).

3.2 Environmental Release

The United States, Turkey, and Russia are the leading producers of boron compounds, followed by Argentina, Chile, and China (USGS, 2004). In 2003, Turkey produced the greatest quantity of ore, while the U.S. led in production of refined boron compounds. U.S. boron resources, mostly sediments and brines, are primarily located in California. U.S. production of boron compounds between 1999 and 2003 ranged from between 518,000 metric tons and 618,000 metric tons (measured as boric oxide). In 2003, the U.S. imported approximately 174,000 metric tons of boron compounds and exported approximately 244,000 metric tons (USGS, 2004).

Boron is a naturally occurring compound, usually found in various inorganic forms in sediments and sedimentary rocks. The richest known boron-containing deposits in the U.S. are found in California. Boron presents in water, soil, and air originates from both natural and anthropogenic sources.

Natural weathering of boron-containing rocks is thought to be the primary source of boron compounds in water and soil (Butterwick et al., 1989). Releases to air from oceans, volcanos, and geothermal steam are other natural sources of boron in the environment (Graedel, 1978). Global releases of elemental boron through weathering, volcanic, and geothermal processes are estimated at approximately 360,000 metric tons annually (Moore, 1991).

Human causes of boron contamination include releases to air from power plants, chemical plants, and manufacturing facilities. Fertilizers, herbicides, and industrial wastes are among the sources of soil contamination. Contamination of water can come directly from industrial wastewater and municipal sewage, as well as indirectly from air deposition and soil runoff (ATSDR, 1992). Borates in detergents, soaps, and personal care products can also contribute to the presence of boron in water.

Boric acid and its sodium salts are registered for use as pesticides. Data from the U.S. Bureau of Mines, cited in the U.S. EPA's 1994 reregistration eligibility document for boron pesticides (U.S. EPA, 1994c), suggests that approximately 293,000 pounds of boron minerals were used annually for “agricultural purposes” during a period around 1990. In the re-registration eligibility document, the U.S. EPA stated that the amount of boron used specifically as pesticide is somewhat less than the amount used for other agricultural purposes, and that boric acid use declined significantly during the 1980s (U.S. EPA, 1994c).

Two anthropogenic boron compounds, boron trichloride and boron trifluoride, are listed as Toxic Release Inventory (TRI) chemicals.

TRI data for boron trichloride (see Table 3-1) are reported for the years 1995 to 2002 (U.S. EPA, 2004b). For boron trichloride, on-site air emissions constitute all of the total releases (on- and off-site), and these have generally fluctuated in the range of hundreds of pounds per year during the period of record. TRI releases for boron trichloride were reported from Arizona, California, Indiana, New Mexico, Pennsylvania, and Wisconsin.

Table 3-1 Environmental Releases (in pounds) of Boron Trichloride in the United States, 1995-2002

Year	On-Site Releases				Off-Site Releases	Total On- & Off-site Releases
	Air Emissions	Surface Water Discharges*	Underground Injection	Releases to Land		
2002	258	0	0	0	0	258
2001	626	0	0	0	0	626
2000	605	-	0	0	0	605
1999	350	-	0	0	0	350
1998	750	0	0	0	0	750
1997	754	0	0	0	0	754
1996	37	-	0	0	0	37
1995	5	-	0	0	0	5

Source: U.S. EPA (2004b)
* "-" denotes blank cells on reporting forms. "0" is entered when the reporting forms contained only zeros or NAs.

Boron trifluoride releases, also for the years 1995 to 2002 (see Table 3-2), are similarly dominated by on-site air emissions. Boron trifluoride releases ranged in the tens of thousands of pounds annually. TRI releases for boron trifluoride were reported from 14 States (AL, AR, DE, FL, KY, LA, MD, NY, OH, OK, PA, SC, TN, and TX) (U.S. EPA, 2004b).

Table 3-2 Environmental Releases (in pounds) of Boron Trifluoride in the United States, 1995-2002

Year	On-Site Releases				Off-Site Releases	Total On- & Off-site Releases
	Air Emissions	Surface Water Discharges	Underground Injection	Releases to Land		
2002	10,114	0	0	0	0	10,114
2001	11,496	0	0	0	0	11,496
2000	11,595	0	0	0	250	11,845
1999	16,725	0	0	0	0	16,725
1998	37,802	5	0	0	0	37,807
1997	21,290	0	0	0	5	21,295
1996	29,881	0	0	0	0	29,881
1995	25,019	0	0	0	929	25,948

Source: U.S. EPA (2004b)

3.3 Environmental Fate

Boron in the environment primarily derives from the weathering of minerals containing boron, seawater volatilization producing boric acid, and volcanic activity. Anthropogenic sources of boron are considered to contribute a lesser amount to the environment than natural processes. Anthropogenic sources of boron are as follows: agricultural, waste and fuel wood burning, power generation using coal and oil, glass product manufacture, use of

borates/perborates in the home and industry, borate mining/processing, leaching of treated wood/paper, and sewage/sludge disposal of boron (HSDB, 2003a).

Atmospheric boron occurs as particulate matter or aerosols, as borides, boron oxides, borates, boranes, organoboron compounds, trihalide boron compounds, or borazines. Borates are relatively soluble in water and will probably be removed from the atmosphere by precipitation and dry deposition (U.S. EPA, 1987). The half-life of boron and boron containing compounds in the atmosphere was estimated to be on the order of days (Nriagu, 1979) with particle size determining the length of time in the atmosphere. Transformation or degradation of boron particulates in the atmosphere has not been studied.

Most boron compounds are soluble in water while the solubility of elemental boron is very low. Due to the high water-solubility of the environmentally-relevant boron minerals, Rai et al. (1986) concluded that it is unlikely that mineral equilibria will control the fate of boron in water. Boron compounds such as borax rapidly hydrolyze to form a boric acid-borate mixture. Boric acid is a weak acid that exists primarily in its unionized for at pHs below 7. Borate and boric acid establish an equilibrium reaction in water that is dependant on the pH.

$$B(OH)_3 + H_2O \leftrightarrows B(OH)_4^- + H^+ \quad pKa = 9.14$$

The extent of boron adsorption depends on the pH of the water and the chemical composition of the soil or sediment. The greatest adsorption is generally observed at pH 7.5-9.0 (Keren et al., 1981; Keren and Mezuman, 1981; Waggott, 1969) with amorphous aluminum oxide (Bingham et al., 1971), iron oxide (Sakata, 1987), and, to a lesser extent, organic matter present in the soil (Parks and White, 1952). Boron is adsorbed mainly on the edge surfaces of 2:1 clay minerals (Keren and Bingham, 1985; Keren and Sparks, 1994; Keren and Talpaz, 1984). Some clay materials, e.g., montmorillonite, have a negative electric field, which makes them less accessible to approaching borate anions (Secor and Radke, 1985).

Boron in the soil may adsorb onto iron and aluminum hydroxy compounds and clay minerals. Boron sorption by clay minerals and iron and aluminum oxides is pH dependent, with maximum sorption in the range 7-9. The amount of boron adsorbed depends on the surface area of the clay or oxide, and this sorption is only partially reversible (Brown et al., 1983). Finer textured soils retain boron longer than do coarse, sandy soils. Keren and Mezuman (1981) determined that the amount of organic matter present in water systems was not as important in adsorption of boron as the inorganic minerals present.

Borax may be persistent for one or more years, depending on soil type and rainfall. Borax is less persistent in acidic soils and in high rainfall areas, with it leaching rapidly under high rainfall conditions (Weed Science Society of America, 1983). Boron is thought to accumulate in plants. Boron content of lentil and barley plants from soil treated with 8 ppm boron was approximately 7- and 8-fold that of control plants, respectively (Singh and Singh, 1984).

3.4 Summary

Boron enters the environment primarily by the weathering of rock strata containing boron minerals, boric acid volatilization from seawater, and volcanic activity. Anthropogenic inputs are lower than natural inputs (HSDB, 2003a). Atmospheric boron usually exists as particulates, which can be deposited at a relatively rapid rate; therefore, particle size and weight determine the half-life of airborne particulates. Boron and boron-containing compounds in aqueous environments adsorb onto iron and aluminum hydroxy compounds and clay minerals, and is pH-dependent, with basic conditions favoring the sorption. Borate and boric acid equilibria in water are pH-dependent, with borate predominating at higher pH (>9.3); therefore, pH determines which boron-containing species is available. Boron adsorbs onto particulates in the water and soil that are high in amorphous aluminum oxide, iron oxide, clay, and to a lesser extent, organic matter. These interactions are pH-driven as well and adsorption of boron is greatest at basic conditions (pH 7-9); this is based on boron's need for electron rich environments to form covalently bonds.

4.0 EXPOSURE FROM DRINKING WATER

4.1 Introduction

EPA used data from several sources to evaluate the potential for occurrence of boron in Public Water Systems (PWS) and exposure to boron through drinking water. The primary source for the drinking water occurrence data is the National Inorganic and Radionuclide Survey (NIRS). In addition to this primary source, the Agency evaluated supplemental sources of occurrence information, including United State Geological Survey groundwater and surface water data, the American Water Works Association Research Foundation data, the USEPA Community Water Survey, and the and published literature.

4.2 Ambient Occurrence

4.2.1 Data Sources and Methods

The U.S. Geological Survey (USGS) instituted the National Water Quality Assessment (NAWQA) program in 1991 to examine ambient water quality status and trends in the United States. Between 1991 and 2001 the program study units included aquifers and watersheds covering source water areas for more than 60% of the nation's drinking water and water used for agriculture and industry. NAWQA monitors the occurrence of contaminants, e.g., pesticides, nutrients, volatile organic compounds (VOCs), trace elements, and radionuclides, as well as the condition of aquatic ecosystems (Hamilton et al., 2004). However, no national NAWQA data are available on the occurrence of boron in ambient waters.

4.2.2 Results

Boron was among the analytes in USGS ground water monitoring in the Sacramento Valley in California in 1996 (Dawson, 2001) and the lower Illinois River Basin from 1984 to 1991 (Warner, 1999). Boron also was an analyte in NAWQA studies of bed sediments and/or fish tissues from the Tualatin River Basin of Oregon from 1992 and 1996 (Bonn, 1999), the Lower Snake River Basin of Idaho and Oregon in 1997 (Clark and Maret, 1998), and the Yellowstone River Basin in Montana, North Dakota, and Wyoming from 1976 to 1979 (Peterson and Zelt, 1999).

In ground water from the Sacramento Valley aquifer, boron was detected in all thirty-one samples; concentrations ranged from 12 µg/L to 1100 µg/L. The median concentration was 42 µg/L. Two of the thirty-one samples had concentrations in excess of the early Health Advisory Level of 600 µg/L (Dawson, 2001). The lifetime Health Advisory Level changed with the U.S. EPA revision of the RfD in June 2004.

In ground water from the lower Illinois River Basin, 71% of samples collected between 1984 and 1991 contained boron concentrations higher than the minimum reporting level (50 µg/L. The highest detected concentration was 2100 µg/L. Higher boron concentration samples generally were from deeper aquifers (Warner, 1999).

In all of ten fish tissue samples from Oregon's Tualatin River Basin, boron concentrations exceeded the minimum reporting level of 0.2 µg/g dry weight. The median concentration was 1.2 µg/g and the maximum concentration was 3.5 µg/g (Bonn, 1999).

In most or all of twenty-five fish tissue samples from the Lower Snake River Basin, boron concentrations exceeded the minimum reporting level; the highest reported concentration in this study was 1.8 µg/g (a minimum reporting level of 0.1 µg/g dry weight; Clark and Maret, 1998).

In bed sediment samples from the Yellowstone River Basin, boron was detected in 98% of samples, with a median concentration of 28 mg/kg; the 95th percentile concentration was 57 mg/kg (reporting limit of 10 mg/kg; Peterson and Zelt, 1999).

4.3 Drinking Water Occurrence

4.3.1 Data Sources and Methods

In the mid-1980s, the U.S. EPA conducted the National Inorganic and Radionuclide Survey (NIRS) to collect national occurrence data on a select set of radionuclides and inorganic chemicals being considered for National Primary Drinking Water Regulation. NIRS analytes included 26 unregulated inorganic compounds (IOCs) and 4 unregulated radionuclides, as well as 10 regulated IOCs and 2 regulated radionuclides.

NIRS collected contaminant occurrence data from 989 public water systems (PWSs) served by ground water. NIRS did not include surface water systems. The statistical selection of PWSs was designed to be geographically representative of national occurrence in ground water. NIRS data were collected from PWSs in 49 states. Data were not available for the state of Hawaii. In addition, sampling of PWSs was designed so that the stratification of different sized water systems used in the study represented as best as possible the stratification of the nation's ground water systems. Consequently, within the study the proportion of any particular size of PWSs to the total number PWSs in the study was comparable to the proportion of all PWSs of corresponding size relative to all PWS nationally, e.g., 92% of NIRS PWSs serve small or very small populations (less than 3,300 persons) and only 2.5% of NIRS PWSs serve populations greater than 10,000 (65 FR 21576).

Each PWS included in the survey was sampled once between 1984 and 1986. Uniform detection limits were employed; therefore, NIRS data can be used directly for national contaminant occurrence analyses with very few, if any, data quality, completeness, or representativeness issues. There has not been a comparable national survey of inorganic chemicals and radionuclides since NIRS (65 FR 21576).

Because NIRS did include surface water systems, EPA consulted a boron survey funded by the American Water Works Research Foundation (Frey et al., 2004). The AWWARF study recruited 189 PWSs representing 407 source waters in 41 States. Of the 407 source water sample kits distributed in 2003, approximately 342 were returned. Of these 342 samples, 341 were

analyzed for boron. Approximately 67 percent (or 228) represented ground water sources and 33 percent (or 113) represented surface water sources.

The USEPA (2002c; 2002d) Community Water System Survey (CWSS) gathered data on the financial and operating characteristics of a random sample of community water systems nationwide. In addition, it compiled system data for all very large community water systems, those that serve more than 500,000 people (a total of 83 systems), and monitoring results for a small subset of regulated compounds and unregulated compounds, which included boron. The data submitted included concentrations from raw water at each system's intake and from finished water at the treatment plant. EPA received completed questionnaires from 58 systems but not all systems answered every question meaning that the data are not nationally representative.

4.3.2 Derivation of the Health Reference Level

To evaluate the systems and populations exposed to boron through drinking water from PWSs, the monitoring data were analyzed against the Minimum Reporting Level (MRL) and a benchmark value for health that is termed the Health Reference Level (HRL). Two different approaches were used to derive the HRL, one for chemicals that cause cancer and exhibit a linear response to dose and the other applies to noncarcinogens and carcinogens evaluated using a nonlinear approach.

For those contaminants considered to be likely or probable human carcinogens, EPA evaluated data on the mode of action of the chemical to determine the method of low dose extrapolation. When the mode of action analysis indicates that a linear low dose extrapolation is needed, or when data on the mode of action are lacking, a default low dose linear extrapolation was used to calculate the risk-specific dose equivalent to a one cancer in a million (10^{-6}) risk. The risk-specific dose was combined with adult body weight and drinking water consumption data to estimate the drinking water concentration equivalent to a one-in-a-million (10^{-6}) cancer risk and this value was used as the HRL for likely or probable carcinogens.

For those chemicals not considered to be carcinogenic to humans, EPA generally calculates a reference dose (RfD). An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD can be derived from either a "no observed adverse effect level" (NOAEL), a "lowest observed adverse effect level" (LOAEL), or a benchmark dose, with uncertainty factors applied to reflect limitations of the data used. EPA derived the HRLs for noncarcinogens using the RfD approach as follows:

$$HRL = [(RfD \times BW)/DWI] \times RSC$$

Where:

RfD = Reference Dose
BW = Body Weight for an adult, assumed to be 70 kilograms (kg)
DWI = Drinking Water Intake, assumed to be 2 L/day (90th percentile)

RSC = Relative Source Contribution, or the level of exposure believed to result from drinking water when compared to other sources (e.g., food, ambient air). In all cases a 20 percent RSC is used for HRL derivation because it is the lowest and most conservative RSC used in the derivation of an MCLG for drinking water.

The EPA RfD for boron is 0.2 mg/kg/day (U.S. EPA, 2004d) based on developmental effects in rats from two studies (Price et al., 1996a; Heindel et al., 1992). The RfD was derived using the benchmark dose (BMD) method ($BMDL_{05}$ from Allen et al., 1996). EPA established the HRL for boron using the RfD of 0.2 mg/kg-day and a 20 percent relative source contribution. The HRL is calculated to be 1.4 mg/L or 1,400 µg/L. Further discussion of the RfD derivation may be found in Section 8.

4.3.3 Results

Nationally, approximately 81.9% of groundwater PWSs in the NIRS Study had detections of boron (≥minimum reporting level, ≥MRL or ≥0.005 mg/L [Table 4-1]). Therefore, about 88.1% of the population served by the surveyed groundwater PWSs is exposed to boron in drinking water; this population is equivalent to approximately 75.5 million people. Detections at a concentration greater than one-half the health reference level (>½HRL or >0.7 mg/L) occurred in 4.3% of surveyed groundwater PWSs, indicating that 2.9% of the population served, equivalent to approximately 2.5 million people, are exposed to this level of boron. Concentrations greater than the HRL (>HRL or >1.4 mg/L) were found in approximately 1.7% of surveyed groundwater PWSs, indicating that exposure at this level occurs in 0.4% of the population served, equivalent to approximately 0.4 million people.

In the AWWARF study, samples were analyzed for boron with a method detection limit of 0.002 mg/L, or 2.0 Fg/L (Frey et al., 2004). Boron was detected with concentrations equal or greater than the method detection limit in 226 of 228 ground water samples (99.1%) and 110 of 113 surface water samples (97.3%). Boron concentrations greater than ½HRL or >0.7 mg/L were found in 20 of 228 ground water samples (8.8%) and no surface water samples (0%). Boron concentrations greater than the HRL or >1.4 mg/L were found in 7 of 228 ground water samples (3.1%) and no surface water samples (0%). The highest concentration detected in ground water was approximately 3.32 Fg/L (Seidel, 2006). The median concentrations were 0.0514 mg/L in ground water and 0.029 mg/L in surface water (Frey et al., 2004). Although the survey was not statistically representative, it indicates some general trends. On the whole, boron contamination of surface water is less significant than contamination of ground water. No geographic trends were evident in ground water results, but surface water contamination appeared to be more prevalent in the western U.S. than the eastern U.S. Longitudinal sampling at 15 systems revealed that a wide variety of treatment techniques were largely ineffective at removing boron, so boron concentrations in source water (such as those collected in this study) are likely to be indicative of concentrations in finished water (Frey et al., 2004).

The finished water data from the Community Water System Survey (2002c; 2002d) included 5 detections of boron in ground water: the median concentration was 102 µg/L and the 90th percentile value 234 µg/L. For surface water, 14 observations of boron occurrence were reported, and among detects, the median concentration was 56 µg/L (USEPA, 2002d) and the

90thdian concentration was 120 µg/L and the 90th percentile concentration 273 µg/L. In raw ground water 34 observation of boron occurrence were reported with a mean concentrations of 120 µg/L and a 90th percentile occurrence of 234 µg/L. In raw surface water, 15 observations of boron occurrence were reported; among the detects, the median concentration was 59 µg/L and the 90th percentile concentration was 180 µg/L (USEPA, 2002d).

4.4 Summary

The limited data used in this report suggests boron could be ubiquitous in the environment, including ground water, fish tissues, and stream bed sediments. The Reference Dose (RfD) for boron is 0.2 mg/kg/day and the Health Reference Level (HRL) based on the RfD was determined to be 1.4 mg/L. According to the U.S. EPA's National Inorganic and Radionuclide Survey (NIRS), approximately 81.9% of groundwater PWSs had detections of boron (≥minimum reporting level, ≥MRL, or ≥0.005 mg/L). These detections affected about 88.1% of the population served by the PWSs, equivalent to approximately 75.5 million people served by ground water nationally. Detections at a concentration greater then one-half the health reference level (>½HRL or >0.7 mg/L) occurred in 4.3% of surveyed PWSs, affecting 2.9% of the population served and equivalent to approximately 2.5 million people nationally. Concentrations greater than the HRL (>HRL or >1.4 mg/L) were found in approximately 1.7% of surveyed PWSs, affecting 0.4% of the population served and equivalent to approximately 0.4 million people nationally.

Supplementary data from an AWWARF-sponsored study indicate that boron was present in both surface and ground water but that the average concentrations in ground water tended to be higher than those in surface waters. The median boron concentrations from the USEPA Community Water Survey are consistent with those in the AWWARF study in that the levels in ground water are higher than those in surface water.

Table 4-1 Summary Occurrence Statistics for Boron in Ground Water Systems

Frequency Factors	NIRS Data on Boron	National System & Population Numbers[1]
Total Number of Samples/Systems	989	59,440
99th Percentile Concentration (all samples)	2.44 mg/L	--
Health Reference Level (HRL)	1.4 mg/L	--
Minimum Reporting Level (MRL)	0.005 mg/L	--
99th Percentile Concentration of Detections	2.6 mg/L	--
Median Concentration of Detections	0.047 mg/L	--
Total Population Served	1,482,153	85,681,696
Occurrence by Sample/System	**Systems/ Population %**	**National Extrapolation**
Ground Water PWSs with Detections (≥ MRL) Range of NIRS States	81.9% 0 - 100%	48,682 N/A
Ground Water PWSs > 1/2 HRL Range of NIRS States	4.3% 0 - 37%	2,584 N/A
Ground Water PWSs > HRL Range of NIRS States	1.7% 0 - 26%	1,022 N/A
Occurrence by Population Served		
Population Served by GW PWSs with Detections Range of NIRS States	88.1% 0 - 100%	75,501,000 N/A
Population Served by GW PWSs > 1/2 HRL Range of NIRS States	2.9% 0 - 34%	2,469,000 N/A
Population Served by GW PWSs > HRL Range of NIRS States	0.4% 0 - 34%	372,000 N/A

1. Total PWS and population numbers are from U.S. EPA (2000e), Water Industry Baseline Handbook, 2nd Edition. National extrapolations are generated by multiplying the system/population percentages and the national Baseline Handbook system/population numbers.

Abbreviations:

PWS = Public Water Systems; GW = Ground Water; N/A = Not Applicable; Total Number of Samples/Systems = total number of samples/systems on record for the contaminant; 99th Percentile Concentration = the concentration in the 99th percentile sample (out of either all samples or just samples with detections); Median Concentration of Detections = the concentration in the median sample (out of samples with detections); Total Population Served = the total population served by PWSs for which sampling results are available; Ground Water PWSs with Detections, PWSs >½HRL, and PWSs >HRL = percentages of GW PWSs with at least one sampling result greater than or equal to the MRL, exceeding the ½HRL benchmark, or exceeding the HRL benchmark; Population Served by GW PWSs with Detections, by PWSs >½HRL, and by PWSs >HRL = percentages of the population served by GW PWSs with at least one sampling result greater than or equal to the MRL, exceeding the ½HRL benchmark, or exceeding the HRL benchmark.

Notes: Only results at or above the MRL were reported as detections. Concentrations below the MRL are considered nondetects. The HRL used in this analysis is a draft value for working review only.

5.0 EXPOSURE FROM MEDIA OTHER THAN WATER

5.1 Exposure from Food

5.1.1 Concentration in Non-Fish Food Items

Levels of boron in food products are related to boron in the soils where they are grown and, accordingly, show some geographic fluctuations. Product categories having high levels have been identified as tubers, legumes, fruits and fruit-based beverages (IOM, 2001). In one dietary study, coffee, milk, apples, dried beans and potatoes accounted for 27 percent of the boron in the diet (Rainey et al., 1999). In the 1994 Total Diet Study from the United Kingdom, the food groupings with the highest boron concentrations were nuts (14 mg/kg fresh weight), fruits and fruit products (2.4-3.4 mg/kg), green vegetables (2.0 mg/kg), and potatoes and other vegetables (1.2-1.4 mg/kg). The levels were below 1 mg/kg for other food categories (Ysart et al., 1999). Most foods contain less than 6 mg boron/kg of food. Some individual foods may contain more than 20 mg B/kg of food (Seiler et al., 1988).

5.1.2 Concentrations in Fish and Shellfish

The data on the presence of boron in fish and shellfish are very limited. The average concentration measured in fish in the United Kingdom 1994 Total Diet Study was 0.5 mg/kg fresh weight (Ysart et al., 1999). Boron has been detected in shrimp by inductively coupled plasma spectroscopy (Mann, 1988).

As mentioned in Section 4.2.1, the presence of boron in fish tissues was measured by the USGS in several surveys. In ten fish tissue samples from Oregon's Tualatin River Basin the median concentration was 1.2 µg/g and the maximum concentration was 3.5 µg/g (Bonn, 1999). In fish tissue samples from the Lower Snake River Basin, the highest reported boron concentration was 1.8 µg/g (a minimum reporting level of 0.1 µg/g dry weight; Clark and Maret, 1998).

5.1.3 Intake of Boron from Food

Dietary intake data from the Continuing Survey of Food Intakes (CSFII) during 1994-1996 (IOM, 2001) are displayed in Table 5-1. Average values for adults range from 0.87 to 1.34 and 90 percentile intakes are about 1.5 to 2 mg/day. Findings from the NHANES III survey (1988-1994) are similar (IOM, 2001).

5.2 Exposure from Air

Bertine and Goldberg (1971) estimated that approximately 11,600 tons of boron are injected into the atmosphere as a component of fly ash produced by coal combustion; the fly ash was estimated to contain an average of about 75 mg/kg boron. There are insufficient data to estimate the intake of boron from ambient air.

Table 5-1 Mean Intake of Boron (mg/day) from Food Based on the Continuing Survey of Food Intake by Individuals 1994-1996 (IOM, 2001)

Age	N	mean	standard error
Both sexes			
0-6 mo	195	0.75	0.14
7-12 mo	130	0.99	0.12
1-3 yr	1834	0.86	0.02
4-8 yr	1650	0.80	0.01
Males			
9-13 yr	552	0.90	0.03
14-18 yr	446	1.02	0.04
19-30 yr	853	1.15	0.03
31-50 yr	1684	1.33	0.03
51-70 yr	1606	1.34	0.02
71+ yr	674	1.25	0.03
Females			
9-13 yr	560	0.83	0.03
14-18 yr	436	0.78	0.01
19-30 yr	760	0.87	0.03
31-50 yr	1614	1.00	0.02
51-70 yr	1539	1.11	0.02
71+ yr	623	0.98	0.03
Pregnant	70	1.16	0.09
Lactating	41	1.39	0.16
All excluding P&L[a] woman	15,156	1.06	0.01
All including P&L women	15,267	1.06	0.01

Adapted from Rainey et al., 1999
a. P&L, pregnant and lactating

5.3 Exposure from Soil

5.3.1 Concentration of Boron in Soil

Boron occurs in the earth's crust at a concentration of about 0.001%, generally as compounds, and rarely as a pure element (O'Neil et al., 2001). Widely distributed boron compounds include borax, kernite, and tourmaline, the three most commonly mined boron minerals (Seiler et al., 1988). High levels of boron occur predominantly in soil originating from marine sediments and arid regions (Brown et al., 1983). Boric acid naturally occurs as the mineral sassolite (O'Neil et al., 2001). Sodium tetraborate, $Na_2B_4O_7$, usually occurs as a decahydrate mineral known as borax and is found largely in ancient dry lake beds from the tertiary period (Clayton and Clayton, 1994). One report indicated that the average concentration of boron in soil is 10 mg/kg (Weast, 1988-1989). Another report indicated a geometric mean background concentration of 26 mg/kg, with a maximum concentration of 300 mg/kg, for boron in U.S. soils (Eckel and Langley, 1988). Boron was detected in soils in Idaho at geometric mean concentrations of 4.6-9.8 mg/kg (Rope et al., 1988). Malins et al. (1984) reported on boron in sediments of Puget Sound.

5.3.2 Intake of Boron from Soil

Human exposure to contaminants in soils is usually from dust that infiltrates homes and automobiles, and incidental soil ingestion. Estimates of soil intake often assume an ingestion rate of 100 mg/day for children and 50 mg/day for adults (U.S. EPA, 1997a). Using the average concentration of boron in soil from Weast (1988-1989), 10 mg boron/kg soil, and the assumption that infants and adults ingest 0.0001 and 0.00005 kg/soil per day (100 mg and 50 mg), respectively, exposure of children to boron from soils would be about 1.0 μg/day and 0.5 μg/day for adults.

10 mg/kg soil x 0.0001 kg soil (children) = 0.001 mg/day (1.0 μg)

10 mg/kg soil x 0.00005 kg soil (adults) = 0.0005 mg/day (0.05 μg)

5.4 Other Residential Exposures

Some human exposures to borates are linked to insecticide use. Typically, borate-based insecticides are powders or dust used to control cockroaches. Children who, relative to adults, have greater hand-to-mouth contact and exposure to floor boards, where the insecticides usually are applied, are more likely to ingest them. Medicinals and personal care products containing boron may be absorbed through mucous membranes and/or damaged skin. Populations living in areas of California and other western states with boron-rich mineral deposits potentially have high exposure to boron from drinking water and locally grown foods (Butterwick et al., 1989).

5.5 Occupational (Workplace) Exposures

5.5.1 Description of Industries and Workplaces

Industries and workplaces where boron compounds are found in abundance include borate mines and processing plants. Manufacture of fiberglass and other glass products, cleaning and laundry products, fertilizers, pesticides, and cosmetics constitute industries where boron compounds can commonly be found in the workplace (U.S. Borax and Chemical Corporation, 1991).

5.5.2 Types of Exposure (Inhalation, Dermal, Other)

Exposure of boron in the workplace is expected to be mainly through inhalation and dermal contact.

5.5.3 Concentrations of Boron in the Work Environment

Boron in its various forms is classified under the “nuisance” category (Clayton and Clayton, 1994). Reported concentrations of borax dust in different areas of a large borax mining and refining facility ranged from 1.1-14.6 mg/m^3 (Garabrant et al., 1985); the mean boric acid/boron oxide dust concentration in one boric acid manufacturing plant was 4.1 mg/m^3 (Garabrant et al., 1984).

5.6 Summary

The boron exposure for the general population is mostly through the ingestion of food and, to a lesser extent, water. Populations with the greatest risk of exposure are those from boron-rich regions of the western United States, especially California, children having frequent hand-to-mouth contact and greater exposure to floor boards where the insecticides containing boron usually are applied, and workers in industries that use boron.

6.0 TOXICOKINETICS

6.1 Absorption

Oral Exposure

Studies in both humans and animals show that boron is well absorbed from the gastrointestinal tract. Schou et al. (1984) administered approximately 131 mg B as boric acid in both water (750 mg) and water-emulsifying ointment (740-1473 mg, approximately 130-258 mg B) to six volunteers and found that an average of 92-94% of administered boron was excreted in the urine within 96 hours, indicating that at least that much had been absorbed in that time. Although there was no significant difference in cumulative excretion for the two different vehicles, it was noted that excretion in the first 2-hour sampling period was lower after exposure to the ointment, suggesting delayed absorption of boron from the ointment in comparison to the water vehicle. Similarly, the two women who ingested approximately 62 mg B as boric acid (in addition to 80-140 mg of boron in food) excreted greater than 90% of ingested boron in the urine in the first week after dosing (Kent and McCance, 1941). Volunteers (n=10) who drank spa waters containing approximately 100 mg daily dose of boron for 2 weeks had over 90% absorption of boron based on urinary excretion data (Job, 1973). Naghii and Samman (1997) studied the effect of boron supplementation (10 mg B/day) into the normal diet of male volunteers (n=8). Supplementation of the 10 mg B/day for 4 weeks resulted in 84% recovery in the urine.

Studies in animals have shown that boron is readily absorbed following oral exposure in rats (Ku et al., 1991; Usuda et al., 1998), rabbits (Draize and Kelley, 1959), sheep (Brown et al., 1989) and cattle (Owen, 1944; Weeth et al., 1981). Using mass spectrometry and the boron-10 isotope, Vanderpool et al. (1994) showed that fasted rats fed 20 ug of ^{10}B in the diet eliminated 95% of the ^{10}B in the urine and 4% in the feces within 3 days of dosing, producing a 77% increase in the ratio of ^{10}B to ^{11}B in the urine. Moreover, ^{10}B in the liver peaked within 3 hours of dosing with over 90% recovery and a 56% increase in ^{10}B:^{11}B ratio, which returned to normal within 24 hours. This result suggests that >90% of orally administered boron is absorbed from the gastrointestinal tract within 3 hours and that absorption is complete within 24 hours.

Dermal Exposure

Human and animal studies show that boron is not absorbed across intact skin. However, there is evidence that boron can be absorbed through more severely damaged skin, especially from an aqueous vehicle. Draize and Kelley (1959) found no increase in urinary boron in a volunteer given topical application of powdered boric acid (15 g) to the forearm and held under occlusion for 4 hours. Friis-Hansen et al. (1982) reported no evidence of boron absorption in 22 newborn infants treated dermally with ointment containing 3% boric acid for 4-5 days (total dose of approximately 16 mg B); plasma boron levels fell over the 5-day study period, as expected for neonates, and did not differ from 10 untreated controls. Vignec and Ellis (1954) found minimal difference in blood or urinary boron levels in twelve 1- to 10-month-old infants exposed to talcum powder containing 5% boric acid 7-10 times per day for at least 1 month (estimated daily dose of 2.33 g boric acid or 407 mg B) compared with an equal number of untreated controls. An additional group of 12 infants with mild to moderate diaper rash during the test period was continued on the powder regimen for 48-72 hours after rashes appeared. Their boron blood levels were similar to controls. However, blood and urinary boron levels were increased in six

male volunteers with severe skin conditions (e.g., psoriasis, eczema, urticaria) following topical application of an aqueous jelly containing 3% boric acid (Stuttgen et al., 1982). Urinary boron levels did not increase in skin-damaged volunteers given 3% boric acid in an emulsifying ointment.

Studies in laboratory animals have produced similar results. Boron was not absorbed across intact or mildly abraded skin in rabbits topically administered boric acid as the undiluted powder or at 5% in talc or aqueous solution (1.5 hr/day under occlusion for 4 days; 10-15% of body surface exposed) (Draize and Kelley, 1959). However, boron was readily absorbed across severely damaged skin in rabbits in proportion to the exposure concentration. Rats with intact skin treated topically with 3% boric acid (ointment or aqueous jelly) did not absorb boron, but urinary boron was increased 4- to 8-fold (to 1% of dose) following exposure to boric acid oleaginous ointment and 34-fold (to 23% of dose) following exposure to aqueous boric acid in rats with damaged skin (Nielsen, 1970).

Inhalation Exposure

Boron is absorbed during inhalation exposure. Culver et al. (1994) monitored boron levels in the blood and urine of male workers exposed to borate dust (borax, borax pentahydrate and anhydrous borax) at a borax production facility. The workers were divided into three groups according to borate exposure. Workers in both the medium- and high-exposure categories had significantly increased levels of boron in the blood after working Monday (about 0.25 µg/g) in comparison to pre-shift Monday morning values (about 0.1 µg/g). Similarly, workers in the high exposure category had significantly higher urinary boron levels Monday post-shift (about 12 µg/mg creatinine) than pre-shift (about 2 µg/mg creatinine). Boron in the diets (which were assigned by the researchers to ensure uniformity among workers) and workplace air also was monitored during this study. A higher proportion of total boron intake was from air than from diet, and both blood and urine boron were best modeled based on air concentration of boron alone (i.e., inclusion of dietary boron as an independent variable did not increase the predictive power of the models). These data show that boron was absorbed during the work day, and that borate dust in the air was the source of the additional boron in the blood and urine. However, it is not clear what amount of the inhaled boron was actually absorbed through the respiratory tract. The researchers speculated that due to the large size of the dust particles in the work area, most of the inhaled borate would have been deposited in the upper respiratory tract, where it could have been absorbed directly through the mucous membranes or could have been cleared by mucociliary activity and swallowed.

Similar evidence of absorption of airborne boron in rats was obtained by Wilding et al. (1959), who monitored urinary boron levels in rats exposed to aerosols of boron oxide (average concentration of 77 mg/m^3). Urinary boron was much higher in exposed rats than controls throughout the 22-week exposure period (average of 11.90 vs. 0.24 mg B/kg-day) and quickly reverted to control levels following cessation of exposure. These data show that inhalation exposure to boron oxide particulate produced high levels of urinary boron, but do not rule out a contribution by gastrointestinal absorption of particles transported from the upper respiratory tract by mucociliary activity. No toxic effects were observed.

6.2 Distribution

Studies suggest that boric acid and borate compounds in the body exist primarily as undissociated boric acid, which distributes evenly throughout the soft tissues of the body, but shows some accumulation in bone. Ku et al. (1991) studied tissue distribution in male rats fed 9000 ppm of boric acid (1575 ppm boron) for 7 days. The authors estimated the 9000 ppm dose to be 93-96 mg B/kg-day. The tissue levels of boron on day 7 of exposure are listed in Table 6-1. Boron levels in all tissues except adipose increased rapidly after the start of exposure (2- to 20-fold increase over controls after 1 day). The greatest increase (20-fold) was in bone. Levels in adipose tissue increased only 1.3-fold above controls. Boron levels in plasma and soft tissues other than adipose tissue reached steady-state (12-30 µg/g) within 3-4 days. Variability in levels of boron among soft tissues (adipose and kidney excluded) was minimal, with tissue concentrations at 60% of plasma levels on day 1 and 30-40% of plasma levels on days 2, 3, 4, and 7. Levels in bone and adipose continued to increase throughout the 7-day study period. In comparison to plasma levels, there was no appreciable accumulation of boron in any soft tissue. However, boron did accumulate in bone, showing a 2- to 3-fold increase over plasma levels after 7 days. Boron levels in adipose tissue remained at 20% of plasma levels after 7 days. Other investigators provided support for these findings: (1) accumulation of boron in bone in rats (Forbes and Mitchell, 1957); (2) lack of appreciable accumulation of boron in the testis (Lee et al.,1978; Treinen and Chapin, 1991); and (3) lack of appreciable accumulation of boron in the epididymis (Treinen and Chapin, 1991).

In a follow-up to Ku et al. (1991), Chapin et al. (1997) monitored bone boron concentrations in rats fed 200-9000 ppm of boric acid for 9-12 weeks. Bone boron was significantly increased over controls at 200 ppm and increased proportionally up to 6000 ppm, above which the increase in bone was slightly less than the increase in the feed. Bone boron levels reached steady state within 1 week at doses up to 3000 ppm and after approximately 4 weeks at higher doses. Steady-state bone boron levels were approximately 4-fold greater than serum boron levels. Chapin et al. (1997) also monitored bone (tibia) boron levels for 32 weeks following cessation of exposure in rats that had been fed boron in the diet for 9 weeks. Levels of boron in the bone declined slowly. After 8 weeks of recovery, bone levels of boron were reduced to roughly 10% of levels at the end of exposure (e.g., at 9000 ppm: about 6 ug B/g bone from about 60 ug B/g bone) but still remained 5- to 6-fold higher than bone levels in unexposed controls (about 1 ug B/g bone). Even after 32 weeks of recovery (and about 31.5 weeks after the return of blood boron levels to normal, which took only 4 days), bone boron concentrations remained 3-fold higher in treated groups than bone concentrations in controls.

In a drinking water study using multiple dose levels of boric acid in rats, Naghii and Samman (1996) found, like Ku et al. (1991), that levels of boron in soft tissues were very similar to levels in plasma (the only exception being a 1.5- to 2-fold increase in the kidney that may have been due to contamination with urine because the organ was not perfused prior to analysis).

Table 6-1 Tissue Levels of Boron in Male Rats on Day 7 of Exposure to 9000 ppm Boric Acid (1575 ppm boron) in the Diet (µg boron/g tissue)

Tissue	Control	Day 7
Plasma	1.94±0.l7	16.00±0.71
Liver	0.66±0.10	13.13±0.54
Kidney	1.55±0.03	19.80±1.65
Adipose	1.71±0.17	3.78±0.13
Muscle	3.69±0.54	14.23±0.19
Bone	1.17±0.19	47.40±1.14
Large intestine[a]	3.08±0.17	14.90±0.7
Brain	0.76±0.02	13.50±0.86
Hypothalamus	0.91	14.30
Testes	0.97±0.10	16.00±1.19
Epididymis[a]	0.81±0.15	16.81±3.7
Seminal vesicles[a]	1.64±0.23	23.70±6.56
Seminal vesicle fluid[b]	2.05	19.20
Adrenals[b]	7.99	21.90
Prostate[b]	1.20	14.80

Source: Ku et al. (1991)
Note: Values are means +/- SE: N = 3 animals unless indicated by footnote
a Mean +/- SE. N = 3 samples, each sample represents a pool of tissue from two animals
b A single sample was analyzed representing a pool from six animals

After 3 and 6 weeks of exposure to boric acid in drinking water at doses of 0, 2, 12.5, and 25 mg/rat/day, solid tissues (kidney excluded) demonstrated boron contents which varied less than 25% within any given dose time group. In boric acid-exposed rats, maximally observed differences in boron concentrations between plasma and solid tissues (kidney excluded) were less than 28%, while most differences noted were less than 10% at any dose or time. The researchers also found that boron plasma and tissue levels increased proportionally with dose. Bone was not analyzed in this study. WHO (1998a) reported a preliminary comparison of blood boron levels across species in rats exposed to boron in the diet or drinking water and humans exposed in the diet, drinking water, or accidental ingestion. Rat and human blood boron levels had a good overlap in the dose range of 0.01-100 mg B/kg body weight. Locksley and Sweet (1954) found that concentration of boron in the tissues was directly proportional to dose over a range of 1.8-71 mg B/kg in mice given borax by intraperitoneal (ip) injection.

Magour et al. (1982) examined the levels of distribution of boron in blood and tissues of 3-week- and 3-month-old female Wistar rats administered one time intraperitoneally with 42 mg B/kg as sodium borate. Boron levels in kidney, brain, liver, heart, and blood of 3-week-old rats were examined, and demonstrated peak concentrations at 30 minutes following intraperitoneal injection (brain excluded). Concentrations in blood, liver, and heart differed by approximately 30% at 30 minutes, and declined in parallel fashion, with concentration differences among tissues diminishing out to 4 hours post-administration. Boron tissue concentration-time profiles were somewhat different when observed in 3-month-old rats. In contrast to the younger rats, blood boron concentrations continued to rise to 1 hour post-administration, and brain concentrations were maximal at 30 minutes post-administration. Boron concentrations in blood, liver, and heart reached concentrations which differed by approximately 10% at 3 hours post-administration and remained similar at 4 hours post-administration. Concentration decay profiles of boron in kidney, heart and liver appeared parallel 1 to 4 hours post-administration, with concentrations in kidney being approximately 70% higher than those in blood, liver, and heart. Similar to findings in 3-week-old rats, the highest concentrations were attained in kidney, and maximal concentrations in tissues other than blood were reached at 30 minutes following injection. In another experiment, 3-week-old rats received 20 mg B/kg in their drinking water for 21 days. Boron levels in the kidney, liver, and brain increased steadily during the first 9 days of treatment and returned to control levels 7 days following cessation of exposure. Blood boron levels continued to rise up to day 21of treatment while levels in the liver and brain returned rapidly to control levels during that time frame. The authors stated that the data suggest the development of a hemostatic mechanism which eliminates any excess of boron from liver and brain against its own concentration gradient because the concentration in the blood was significantly higher than in the liver and brain between days 13 and 21. The authors also state that boron will be completely eliminated if the animals consume drinking water without added boron from days 21-28 which suggests boron is not firmly bound to any tissue components.

Data concerning the distribution of boron in humans is more limited than in experimental animals. Evidence that boron does not accumulate in the blood in humans was obtained by Culver et al. (1994). These researchers found no progressive accumulation of boron across the work week as measured by blood and urine levels in mine workers. Accumulation of boron in skeletal bones of human cadavers has also been reported by Alexander et al. (1951) and Forbes et al. (1954).

6.3 Metabolism

Overview of Metabolic Pathways

Boron is a trace element for which essentiality is suspected but has not been directly proven in humans (Nielsen, 1991, 1992, 1994; NRC, 1989; Hunt, 1994; Mertz, 1993; Devirian and Volpe, 2003). Boron deprivation studies with animals and three human clinical studies have shown that boron affects macromineral and cellular metabolism of other substances that affect life processes such as calcium and magnesium.

Inorganic borate compounds are present as boric acid in the body. Boric acid is the only boron compound that has been identified in urine, and it has repeatedly been found to account for >90% of the ingested boron dose (WHO, 1998a). There is no evidence that boric acid is degraded in the body. Metabolism may not be feasible because a large amount of energy (523 kJ/Mol) is apparently required to break the boron-oxygen bond (Emsley, 1989). Boric acid can form complexes with various biomolecules (IEHR, 1997; WHO, 1998a). It has an affinity for hydroxyl, amino, and thiol groups. Complex formation is concentration dependent and reversible.

6.4 Excretion

The elimination and excretion of boron have been evaluated in humans and rodents, in oral studies only. No studies were summarized that addressed excretion after dermal or inhalation exposures (U.S. EPA, 2004a).

Studies have demonstrated that more than 90% of an orally administered dose of boric acid is excreted unchanged in the urine a short time after treatment (Section 6.1 under oral exposure). In humans, Jansen et al. (1984a) and Schou et al. (1984) reported that boron's primary route of elimination was in the urine. Jansen et al. (1984b) reported that approximately 60-75% of a dose of 750 mg boric acid (131 mg B) in a water solution or 740-1473 mg boric acid (129.5-261.3 mg B) in a water emulsifying ointment administered orally to humans is eliminated in urine over the initial 24 hours, with the urinary route of elimination accounting for 93% of the dose at 96 hours after oral administration. Graphically, Jansen et al. (1984b) demonstrated cumulative boron elimination, as percentage of dose, from six adult males who consumed an aqueous solution of boric acid. Results indicate that at 12 hours, the urinary elimination accounted for 52.7 ± 4.9% (mean ±S.D.) of the dose (range 46.4-58.9%); at 24 hours, the cumulative urinary elimination accounted for 66.9 ± 6.4% of the dose (mean ± S.D.), with a range of 57.1-75.0%. These data demonstrate a marked similarity among this limited sample of adult men in the renal elimination of boric acid. In a clinical report of an acute, uncontrolled intoxication with boric acid, Astier et al. (1988) estimated the dose as 45 g boric acid (7.9 g B), and reported that renal elimination accounted for 50% of the dose in the first 24 hours. Regression analysis of plasma B concentrations revealed a clearance of 0.77 L/hour. While no methods of analysis were presented, the authors concluded that tubular reabsorption affected 80% of the dose. Kent and McCance (1941) also reported that 92-93% of an administered oral dose (352 mg as boric acid) in humans was eliminated in urine during the first week following administration. Additional minor elimination pathways include saliva, sweat, and feces (Jansen et al., 1984a).

Jansen et al. (1984a) evaluated boron clearance daily in seven adult males exposed through dietary intake over 3 days and in the same subjects after 20-minute intravenous infusion of 28.52 mg boric acid (5-5.6 mg B) per minute, or a total dose per subject of 570-620 mg boric acid (91-108.5 mg B). In the dietary intake phase, urine was collected at 12-hour intervals, and blood was sampled twice per day to determine basal levels of boron. There were no restrictions on diet during this period. For the infusion phase, subjects stayed in a metabolic ward for 12 hours after receiving the intravenous dose. Each subject was catheterized with a Venflon catheter in the right arm for boric acid administration. Another Venflon catheter was placed in the left arm for blood sampling. Blood samples were drawn at 0, 0.42, 0.67, 2, 4, 6, 8, 10, and 12 hours, for a total of nine blood samples from each subject during the 12-hour period. After release from the metabolic ward, each subject had a blood sample drawn at 9 a.m. and 4 p.m. daily for 5 days. Renal clearance was calculated as the total amount of boron excreted per minute in the urine, divided by the area under the plasma boron concentration-time curve (mg B_{urine}/AUC-min), normalized to body-surface area.

For the dietary exposure phase, the urinary excretion of boric acid during any 12-hour period ranged from 1.52 to 18.1 mg, consistent with large variations in dietary intake of boron. Plasma concentrations during this 72-hour period ranged from <0.10-0.46 mg boric acid/L (<0.018-0.081 mg B/L). In contrast, following boric acid infusion, plasma boron rose to peak concentrations 25 minutes after the start of the infusion at 10-20 mg/mL, approximately 100 times the basal concentration. Virtually the entire dose (99%) was eliminated in the urine over 120 hours.

Jansen et al. (1984a) did not calculate boron clearances for dietary exposure but published the individual data, from which clearances can be calculated using the following formula (Murray, 2002):

$$\text{Renal Clearance} = \frac{\text{Amount of boron excreted/min in urine over 24 hours}}{\text{Average of same day plasma boron at 9 a.m. and 4 p.m.}}$$

The results are shown in Table 6-2, along with the infusion-phase clearances published by Jansen et al. (1984a). Boron clearance at dietary exposure levels was characterized by a high coefficient of variation (CV, standard deviation/mean) of 0.78, but the mean value was remarkably consistent (39-42 mg/min/1.73 m^2) for each day of the 3-day baseline measurement period. Boron clearance following boric acid infusion was 60.5 mL/min/1.73, with a CV of only 0.09 (Table 6-2). The interindividual variability in renal boron clearance was much greater when clearance was calculated from the subjects receiving exposure to boron in the diet alone compared to the values calculated in the same individuals receiving a single intravenous infusion.

Table 6-2 Renal Boron Clearance (mL/min/1.73m^2) Calculated from Dietary Exposure and Intravenous Infusion

	Boron Clearance (mL/min/1.73m^2)			
	Dietary Boron Exposure Only[b] (mg B/day)			Intravenous Infusion[c] (mg B/day)
Subject[a]	Day 1: 1.79±1.23	Day 2: 1.45±0.47	Day 3: 1.52±0.44	105
1	47.7	113.4	83.4	55.9
2	58.3	14.5	42.6	65.8
3	12.0	20.2	19.6	63.8
5	83.0	66.8	77.2	62.7
6	62.8	29.6	17.3	65.0
7	15.6	15.2	13.3	51.2
8	16.4	20.8	22.2	58.9
Mean ±S.D.	42.3 ±27.8	40.1 ±37.1	39.4±29.5	60.5±5.4

Source: Adapted from Jansen et al. (1984a)

a Subject No. 4 was excluded due to increasing excretion in urine during the period

b Dose estimated from total urinary excretion of boron during 24 hours of normal dietary exposure

c Dose administered by 20-minute intravenous infusion

The variance of dietary-exposure boron clearance was 66 times greater than for intravenous infusion. The mean boron clearance estimated by this method was lower than the mean boron clearance estimated from the intravenous infusion by a factor of 1.5. There are a number of possible reasons for both the higher variability and lower absolute clearance values as outlined in the following paragraphs.

Any analytical error that overestimated plasma boron would have led to an underestimate of boron clearance. The detection limit of the spectrophotometric method used by Jansen et al. (1984a) to determine plasma boron was 0.1 mg/L of boric acid. The precision of the method was degraded substantially at low boric acid concentrations, with a CV of 0.71 at 0.14 mg/L versus a CV of 0.055 at 4.93 mg/L. At the plasma boron levels found on the first three days of the study (0.10-0.46 mg/L), the precision of the analytical method was a potential source of significant error. In addition, more than 25% of the plasma boron samples measured during the dietary-exposure phase were below the limit of detection, and were entered as half the limit of detection in the calculations. If the actual plasma boron concentration was lower (i.e., less than 0.05 mg/L of boric acid), the estimated boron clearance would have been higher. The plasma boron levels in the intravenous infusion study were orders of magnitude higher, so that analytical error and detection limit problems were less likely to be factors.

Another factor that would lead to an underestimate of boron clearance in the dietary-exposure phase would be missed or incomplete urine samples. In the Jansen study, the subjects did not stay in the clinic for the 3-day dietary-exposure phase. As urine was collected at 12-hour intervals during this phase, urine samples may not have been 100% complete. Because the subjects remained in the clinic for the first 12 hours of the infusion phase, complete urine collection was more likely.

Although less likely, biological factors could play a role in the relative magnitude and variability of boron clearance in the two phases. Some of the variability may have its basis in interindividual differences in the rate, pattern, and extent of absorption from the gut into the bloodstream, magnified at low and intermittent dietary exposure levels. Dose-dependent kinetics could potentially explain the lower renal boron clearance, as the dietary exposure was about two orders of magnitude lower than the intravenous dose. While this possibility cannot be completely eliminated, it does not appear to be the most likely explanation. The individual data on boron clearance and dose (based on urinary excretion of boron/day) does not show a dose-dependent relationship. Overall, clearance appeared to be independent of dose within the range studied.

The urinary elimination of boron administered to male rats has been investigated following the oral administration of sodium tetraborate (at 11 different doses ranging from 0-4 mg B/kg) by Usuda et al. (1998). The recovery of boron in 24-hour urine accounted for 99.6 ± 9.7% of the administered dose, demonstrating essentially total bioavailability of an orally-administered boron dose in rats. In a study conducted in rats with stable-labeled boron, Vanderpool et al. (1994) reported that 95% of the administered (20 µg/kg) dose was eliminated in the urine and 4% in the feces over the initial 3 days post-dosing.

Urinary elimination of boric acid in Sprague-Dawley female rats (nonpregnant and pregnant) was examined in a pharmacokinetic study (U.S. Borax, 2000; Vaziri et al., 2001).

Three groups of 10 nonpregnant and 10-11 pregnant rats were started on an initial 7-day supplemented boron diet on gestation day 9, prior to gavage administration of boric acid. According to the authors, the purpose of this initial 7-day diet was to achieve steady state conditions for rats given a diet comparable to that ingested by humans in terms of boron. This supplemented boron diet given during the initial 7 days was designed to deliver a dose of approximately 0.3 mg/kg-day of boric acid or 0.05 mg B/kg-day. On the morning of day 8, the diet for all rats was switched to the low boron casein diet containing 0.2 mg B/kg diet for a total of 24 hours. The low boron casein-based diet was used in this study to minimize cross contamination of the urine with boron in the diet and to minimize the dietary contribution of boron on the day of gavage. After the initial 24 hours on the low casein diet, groups of pregnant and nonpregnant rats were given a single oral dose of 0.3, 3.0, or 30 mg/kg of boric acid (0.052, 0.52, and 5.2 mg B/kg, respectively) by gavage in deionized water (ultrapure). According to the authors, the low dose was chosen as an estimate of the high end human dietary dose level, and the highest dose tested was approximately half of the no observed adverse effect level (NOAEL) from the rat developmental toxicity study (Price et al., 1996a).

To determine the renal clearance of boron, two blood samples were drawn from each rat. The first sample was taken 3 hours after gavage dosing on the assumption that the peak boron concentration in the blood had been achieved (based on data from Usuda et al., 1998). The second blood sample was taken 12 hours after the initial sample. Rats were placed in metabolic cages after the first blood sample was taken, and urine was collected during the 12 hours between the first and second blood sampling.

The urinary concentration of boron at the high dose was significantly higher in pregnant rats compared with nonpregnant rats but not at the low and mid dose (Table 6-3). The urine volume was not significantly different in pregnant and nonpregnant rats. The amount of boron (μg/12 hours) excreted in the urine increased proportionately with increasing dose and during the 12-hour collection period was higher (32-73%) in pregnant rats compared to the nonpregnant rats in the high dose level. This was attributed by the authors to the higher dose of boron administered to pregnant rats due to their larger body weight and to the higher fractional excretion of boron (boric acid clearance/creatinine clearance) in the pregnant rats which was statistically significant at the high dose level. The percentage of administered dose of boric acid recovered in the urine was significantly higher in the low-dose group compared to the mid- and high-dose groups for both the nonpregnant and pregnant animals and higher in the pregnant compared to the nonpregnant rats across dose groups, which was statistically significant at the high dose only (Table 6-3). Although the diet used for this study was low in boron, it contributed to the overall dose of boric acid, and these amounts were not included in the nominal dose levels. When dietary contribution from the low boron diet was included in the dose, the actual dose levels were approximately 0.4, 3.1, and 30.1 mg/kg boric acid. At the low dose, the diet contributed another 27% and 33% to the overall dose given to nonpregnant and pregnant rats, respectively, whereas at the mid and high doses, the diet contributed 3% and 0.3%, respectively, to the total dose. The authors suggest that the incremental increase at the low dose may explain the greater recovery of administered dose in the low-dose group.

Table 6-3 Urinary Boron Concentration, Volume, Mean Excretion, and Percent Recovered in 12 Hours in Nonpregnant and Pregnant Rats Given Boric Acid by Gavage[a,b]

Dose (mg BA[c]/kg-day)	Urinary B (µg/mL)		Urine Volume (mL)		12-hour Urinary B Excretion (µg/12hr)		Percent of Dose in 12-Hr Urine (3-15 Hr)	
	Nonpregnant[d]	Pregnant[d]	Nonpregnant	Pregnant	Nonpregnant[d]	Pregnant[d]	Nonpregnant[d,e]	Pregnant[d,e]
0.3	1.7±0.6[f] (9)	1.6±0.5 (9)	4.3±1.4 (9)	6.1±3.2 (9)	6±1 (9)	8±3 (9)	50.4±10.6% (9)	55.6±21.4% (9)
3.0	10.1±8.2 (10)	12.3±5.1 (9)	5.2±3.4 (10)	5.3±2.4 (9)	32±7 (10)	56±16 (9)	24.6±4.5% (10)	35.6±9.4% (9)
30.0	66.8±47.0 (10)	121.4±47.1[g] (11)	6.8±3.9 (10)	5.4±2.5 (11)	324±61 (10)	561±114[g] (11)	24.6±4.3% (10)	34.7±6.4%[g] (11)

a Sources: U.S. Borax (2000); Vaziri et al. (2001)
b Numbers in parentheses represent number of animals
c Boric Acid (BA)
d Statistically significant difference in urinary boron concentration across dose levels based on two-way analysis of variance (ANOVA), $p<0.05$
e Statistically significant difference across groups (nonpregnant vs. pregnant) based on two-way ANOVA, $p<0.05$
f Mean + standard deviation (number of rats)
g Statistically significant difference between nonpregnant and pregnant rats based on multiple range test, $p<0.05$

Table 6-4 shows the clearance rates of boron (boric acid), creatinine, and urea expressed in three different ways: mL/min, mL/min/kg of body weight, and mL/min/cm^2 of body surface area. Boron clearance appeared to be independent of dose within the range of dose levels tested. The average absolute clearance value for pregnant rats (mL/min) was 1.01 mL/min. The measurements showed low to moderate variability with a standard deviation of 0.2 mL/min (CV=0.2). Boron clearance was slightly higher in pregnant rats compared to nonpregnant rats, but the difference was not statistically significant. The rate of creatinine clearance did not vary significantly with the different doses of boric acid in either nonpregnant or pregnant rats. Creatinine clearance, normalized against body weight, however, was significantly greater in nonpregnant rats compared to pregnant rats. Urea clearance was not significantly different between nonpregnant and pregnant rats. There were no consistent differences in the rate of urea clearance with the different doses of boric acid.

Fractional excretion of boron (the ratio of boron clearance/creatinine clearance) was 65% and 80% in nonpregnant and pregnant rats, respectively. Fractional excretion of urea was lower in nonpregnant rats than in pregnant rats. The authors indicated that increased fractional excretion of boron in pregnant rats may be related to physical factors associated with normal pregnancy due to extracellular volume expansion and renal vasodilation.

Table 6-4 Clearance of Boron (Boric Acid), Creatinine and Urea in Nonpregnant and Pregnant Rats Given Boric Acid by Gavage Expressed as mL/min, mL/min/cm^2, and mL/min/kg[a,b]

	Boron Clearance (mL/min)		Creatine Clearance (mL/min)		Urea Clearance (mL/min)	
Dose (mg BA/kg)	Nonpregnant[c]	Pregnant[c]	Nonpregnant	Pregnant	Nonpregnant	Pregnant
0.3	0.77±0.2 (9)[d]	1.01±0.2 (9)	1.3±0.4 (9)	1.3±0.5 (9)	0.85±0.2 (9)	0.89±0.3 (9)
3.0	0.76±0.2 (10)	0.95±0.2 (9)	1.2±0.4 (10)	1.3±0.4 (9)	0.84±0.3 (10)	1.14±0.4 (9)
30.0	0.81±0.1 (10)	1.07±0.2 (11)[e]	1.3±0.4 (10)	1.3±0.3 (11)	0.96±0.3 (10)	1.10±0.3 (11)
expressed as mL/min/cm^2						
0.3	0.0017±0.0004 (9)	0.0020±0.0004 (9)	0.0029±0.0007 (9)	0.0025±0.0009 (9)	0.0019±0.0005 (9)	0.0017±0.0005 (9)
3.0	0.0017±0.0003(10)	0.0019±0.0003 (9)	0.0027±0.0008 (10)	0.0025±0.0006 (9)	0.0018±0.0006 (10)	0.0022±0.0008 (9)
30.0	0.0018±0.0003 (10)	0.0020±0.0003 (11)	0.0029±0.0008 (10)	0.0025±0.0006 (11)	0.0021±0.0006 (10)	0.0021±0.0004 (11)
expressed as mL/min/kg						
0.3	3.1±0.8 (9)	3.3±0.6 (9)	5.2±1.1 (9)[c]	4.3±1.5 (9)[c]	3.4±0.9 (9)	2.9±0.9 (9)
3.0	3.0±0.6 (10)	3.2±0.5 (9)	4.8±1.3 (10)[c]	4.2±1.1 (9)[c]	3.3±1.1 (10)	3.8±1.3 (9)
30.0	3.2±0.5 (10)	3.4±0.5 (11)	5.3±1.6 (10)[c]	4.3±1.0 (11)[c]	3.8±1.0 (10)	3.5±0.7 (11)

a Sources: U.S. Borax (2000); Vaziri et al. (2001)
b Numbers in parentheses represent number of animals
c Statistically significant difference across groups (nonpregnant vs. pregnant) based on tow-way ANOVA, $p<0.05$
d Mean = standard deviation (number of rats)
e Statistically significant difference between nonpregnant and pregnant rats based on multiple range test, $p<0.05$

A human study to measure renal clearance of boron normally consumed in the daily diet in nonpregnant and pregnant women was conducted (U.S. Borax, 2000; Pahl et al., 2001) in 32 women in good health between the ages of 18 and 40 years, including 16 women in their second trimester (14-28 weeks) and 16 age-matched nonpregnant women. At the beginning of the study, all subjects were asked to empty their bladders, and a baseline blood sample was taken. At the end of this 2 hours another blood sample was taken. The subjects were asked to collect all urine for the next 22 hours (24 hours from the baseline). A 24-hour blood sample also was collected.

Urine for each subject was pooled over the initial 2-hour period and over the subsequent 22-hour period. Boron content of blood and pooled urine was analyzed via inductively coupled plasma-mass spectrometry (ICPMS) following laboratory analytical standards and practices, and employing adequate quality control measures. Urinary clearance was measured by quantifying the amount of boron (mg) in the urine and blood. Because the 22-hour clearance samples were not collected onsite, the 2-hour clearance values were considered to be more accurate due to the women's compliance with the collection procedures while at the clinic. The urinary clearance of boron in humans was determined in all individuals and presented as mL blood cleared of boron per minute per kg body mass. The average clearance rate for boron in pregnant women was 1.02 ± 0.55 (mean ± standard deviation; range 0.252-2.028) and the average clearance rate for boron in nonpregnant women was 0.80 ± 0.31 (mean ± standard deviation; range 0.229-1.358) mL/min-kg body mass. These results showed that pregnant women clear boron more effectively than nonpregnant women, which is consistent with the normal increase in renal blood flow and glomerular filtration rate during pregnancy.

For the purpose of toxicokinetic modeling, the individual body weights and clearance values from U.S. Borax (2000) were used to calculate boron clearance in units of mL/min. Table 6-5 shows the clearances in mL/min-kg and body weights in kg for the pregnant women in the U.S. Borax report. The absolute boron clearances are shown in the last column. The average boron clearance for these subjects was 66.1 mL/min, with a standard deviation of 32.4 mL/min. The clearance values, however, were characterized by high variability, with a CV of 0.49.

One factor that may contribute to a higher than expected variability in these clearance estimates – relative to similar biological values estimated in the Jansen et al. (1984a) and Vaziri et al. (2001) results – was the indirect estimation of boron intake. Although all subjects were asked to record their 24-hour dietary intake, the subjects in the study provided incomplete dietary information. The authors stated that estimates of dietary intake provided from food frequency questionnaires are of limited accuracy. Boron intake estimated from the renal excretion of boron in 24 hours was 1.3 mg B/day, from which an average consumption was estimated at 0.02 mg B/kg-day.

In addition, these boron clearances probably underestimate the true clearance that would be obtained with higher doses, as in Jansen et al. (1984a). The Pahl et al. (2001) study did not have the detection limit problem of Jansen et al. (1984a), and only a single 2-hour urine sample was collected. As complete bladder voiding is problematic in such a short time, underestimation of total boron excreted is likely. The result would be lower estimated boron clearance values. Pahl et al. (2001) reported evidence of under-collection of urine in some subjects, but quantification of underestimate was not possible. In addition, the variance of boron clearance

reported in the study is very likely an overestimate of the true variability of clearance in the population. As study subjects could not be kept in the clinic for prolonged periods, multiple urinary and plasma boron measurements over a longer time interval could not be made. Therefore, the average of only two plasma samples over 2 hours had to suffice a surrogate for AUC in the calculation of clearance. The average plasma boron concentration over 2 hours, with no controls on exposure timing or magnitude, inherently will be more variable than plasma concentrations obtained from a carefully controlled and monitored study, as in the infusion phase of the Jansen study. The excess variance would reflect experimental error rather than true interindividual variability. In the Jansen study, the CV for boron clearance was reduced by a factor of 13 with larger doses and controlled conditions compared to uncontrolled dietary exposure.

Table 6-5 Urinary Clearance of Boron in Pregnant Women[a]

Subject	BW (kg)	2-Hour Boron Clearance Values	
		mL/min-kg	mL/min
1	91.10	0.40	36.35
2	53.22	0.25	13.41
3	59.08	1.43	84.43
4	63.59	0.33	21.11
5	69.45	2.03	140.85
6	55.92	1.76	98.37
7	47.36	1.36	64.50
8	59.53	1.25	74.18
9	73.96	0.54	39.72
10	55.92	1.46	81.82
11	76.22	0.71	54.34
12	84.34	0.81	68.23
13	76.67	0.83	63.87
14	64.49	1.42	91.58
15	82.53	0.71	58.27
Average	67.60	1.02	66.10

a Sources: U.S. Borax (2000)

Creatinine clearance was normal in all subjects and comparable in pregnant and nonpregnant women. Comparison of the clearance of boron with creatinine gives insight into renal tubular handling of boron. Tubular secretion (i.e., into the urine) is indicated if fractional excretion – the ratio of clearance to glomerular filtration rate (GFR) – is greater than 1. Tubular reabsorption (i.e., into the blood stream) is indicated if fractional excretion is less than 1. Pahl et al. (2001) used creatinine clearance as a surrogate for GFR. On this basis, fractional excretion was 0.57 (+0.32) and 0.47 (+0.14) in pregnant and nonpregnant women, respectively. There was a trend toward increased fractional excretion or reduced tubular reabsorption in pregnant women, but the difference was not statistically significant. Creatinine clearance, however, overestimates GFR, as creatinine is actively secreted from the bloodstream into the kidney tubules. The magnitude of the overestimation is about 20-30% (Shemesh et al., 1985), which would increase the nominal fractional excretion of boron to about 70%. Furthermore, the probable underestimation of boron clearance in the Pahl et al. (2001) study would result in higher actual fractional excretion, such that boron clearance would approach GFR.

Several studies have addressed the application of hemodialysis in decreased renal function as an effective method to remove boron from human blood. Although these studies uniformly demonstrate the effective movement of boron across a non-biological dialysis membrane from blood into dialysate, the study of Usuda et al. (1997) is perhaps the most well-reported. In a study to ascertain whether plasma protein binding altered the effectiveness of hemodialysis of boron, 17 human subjects in long-term hemodialysis were monitored before and during dialysis employing a polyvinyl membrane. Clearances of boron, blood urea nitrogen, phosphorus, and creatinine followed. Results indicated that boron clearance was equal to that of blood urea nitrogen and slightly, but significantly, exceeded that of phosphorus and creatinine. The fraction of serum boron available for dialysis was nearly 80%, indicating that approximately 20% of boron was not available for dialysis, potentially for the reason of association with plasma constituents. However, the study did not derive the on- and off-rates of binding, so that even if this approximately 20% of plasma boron was associated with proteins, the measure would only represent the fraction of boron associated with plasma proteins at steady state. That is, at any one time, 20% of boron would be associated with proteins. For this to have an impact on renal filtration, the duration of association would have to exceed the time for a given unit of blood containing boron to traverse the glomerulus. It also is possible that boron associates with and dissociates from proteins multiple times during passage through the glomerulus. If this were the case, the impact of association of boron with plasma protein on renal filtration would be negligible, and would explain why boron clearance would not be impacted by association with plasma proteins. In light of the similarity among the renal (filtration) clearance of these four compounds, the authors concluded that there seems to be relatively little relation of boron to serum constituents of macromolecules which might influence diffusion across membranes.

Several lines of evidence lead to the conclusion that the filtration mechanism, a passive mechanism, is responsible for the urinary elimination of boron from mammals. This information comprises chemical and biochemical data, as well as information from pharmacokinetic studies in rats and humans. Renal filtration, or glomerular filtration, is routinely investigated in humans in a clinical setting, and is monitored as part of prenatal care in this country. Glomerular filtration rate is expressed in units of volume/time and indicates the volume of blood filtered (cleared of substances) by the kidney per unit time, usually corrected for body mass (mL/minute/kg). The characteristics of filtered contaminants include low molecular weight and

diameter, neutrally charged molecule, lack of significant protein binding, and lack of interaction with the active renal mechanisms of tubular secretion and/or tubular reabsorption.

Boron is always found in nature covalently bound to oxygen as some form of borate (e.g., boric acid, tetraborate, etc.). The boron-oxygen bonds are very strong and will not be broken except under extreme laboratory conditions. Boron (borates) exists in the blood as neutral low molecular weight and molecular diameter unbound molecules. The ionic form is controlled by the pKa of the molecule and the pH of the aqueous medium. Uncharged monomeric boric acid is $B(OH)_3$, with a molecular weight of 58.8; in the negatively charged form, boric acid exists as $B(OH)_4$, with a molecular weight of 75.8. At the pH of the human blood (i.e., pH = 7.4), the expected low concentrations of borate (10^{-6} to 10^{-5} M) will be present as 98.4% $B(OH)_3$ and 1.6% $B(OH)_4$ - ion because of the weak acidity (pKa = 9.2) of boric acid (Woods, 1994, 1996). This has been confirmed analytically by nuclear magnetic resonance spectroscopy (Woods, 1994) and Raman spectrometry (De Vette et al., 2001). Thus, at concentrations below 0.025M, essentially all borates dissociate to form low molecular weight, uncharged molecules. The observed boron concentrations in pregnant rats were approximately 2.5 $x10^{-6}$ M (Vaziri et al., 2001), and in humans were much lower (Pahl et al., 2001). Thus, 98.4% of the boron in blood and biological fluids of rats and humans exists in the form of a small, uncharged molecule which should pass through biological membranes, including those of the glomerulus. Any ionic or covalent binding to plasma proteins would be negligible. These properties predispose boric acid to urinary elimination through renal filtration mechanisms.

The effect of plasma protein binding is a decrease in the movement of the substance from blood into extravascular tissues and fluids, including urine. The rapid absorption and urinary elimination of near-complete administered doses of boron across multiple studies are inconsistent with the concept of plasma protein binding for boron. Magour et al. (1982) and Ku et al. (1991) separately demonstrated that concentrations of boron in plasma and soft tissues reached equilibrium at dramatically similar concentrations within hours of administration. Subsequently, elimination profiles from plasma and soft tissues were similar. Usuda et al. (1997) demonstrated that if boron is associated with plasma macromolecular constituents, the "relatively little" relation to these components does not result in a decrease in boron filtration as compared to three plasma constituents whose renal filtration were concomitantly measured. These and other findings indicate that binding is unlikely in either plasma or soft tissues, and that administered boron readily passes from blood across biological membranes. In both rats and humans, boron concentration data have been evaluated to reveal a volume of distribution consistent with distribution of boron into total body water. This finding is consistent with lower concentrations being attained in adipose tissue, given its low content of water compared with other soft tissues. Human studies conducted by Usuda et al. (1997) and others investigated the removal of boron from human subjects undergoing routine hemodialysis therapy for renal dysfunction. Those data demonstrated an effective removal of boron from human blood across a non-biological membrane (devoid of active transport or reabsorption mechanisms) consistent with ready movement of boron across permeable membranes. Although the plasma protein binding of boron has not been specifically investigated in either rats or humans, these lines of evidence lead to the conclusion that plasma protein binding, if it occurs, does not inhibit the movement of boron across biological membranes and, thus, would not impede effective filtration of boron in either rats or humans.

Tubular reabsorption, if it is a factor, will be an issue at dietary levels, and its impact will diminish with increasing dose. The magnitude of the contribution to boron clearance variability, however, is much less than would be suggested by the fractional clearance data from both the human (Pahl et al., 2001) and rat (Vaziri et al., 2001) studies. An average fractional excretion of 0.57 was reported for pregnant women in the Pahl et al. (2001) study (similar results for rats), suggesting that 43% of boron filtered through the glomerulus was reabsorbed into the bloodstream. Boron fractional excretion in the Pahl study, however, was calculated relative to creatinine clearance, which overestimates GFR by about 20% (Shemesh et al., 1985). Correcting for that overestimate yields a fractional clearance of about 0.7, indicating a lesser influence of reabsorption on boron clearance than reported. The variability in reabsorption is probably small by comparison to the variability in GFR. Furthermore, the high boron clearance variability for uncontrolled low-dose dietary exposure decreases dramatically under more controlled, higher-dose conditions (Jansen et al., 1984a). In the Jansen et al. (1984a) study, the CV of 0.09 for boron clearance at a dose of 105 mg (see Table 6-2), or 1.5 mg/kg (assuming an average body weight of 70 kg), is less than that for GFR in females, which ranges from 0.11 to 0.21 for pregnant or nonpregnant women (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). Thus, the variability in GFR may actually slightly overestimate variability of boron clearance in exposed humans. GFR is slightly higher in men than women (Ventura et al., 1999), but increases by over 50% in pregnancy (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). GFR variability appears to be similar in pregnant and nonpregnant women (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). Assuming that GFR variability in men and women is the same, by analogy, boron clearance variability should be similar. In addition, the variance of boron clearance is less than the variance of creatinine clearance (a measure of GFR) when assessed in the same subjects (Jansen, 1984a). Therefore, it is unlikely that GFR variance underestimates boron clearance variance, and would not need further quantitative adjustment. The contribution of tubular reabsorption is unlikely to affect the variability of renal elimination of boron at the higher doses (compared to dietary levels) of concern in deriving an RfD.

Plasma Clearance and Half-Life

In a study conducted with human volunteers and carefully administered doses of 570-620 mg boric acid (91-108.5 mg B), plasma concentration-time curves were followed over 3 days and were markedly biphasic. Terminal elimination half-lives were calculated for individuals (n=6) and demonstrated a range of 12.5-26.6 hours and a mean value of 21.0 ± 4.9 hours when calculated from the data collected over the initial 72 hours post-dose (Jansen et al., 1984a). From this study, a total mean volume of distribution of 104.7 mL/100 g body weight can be calculated. A second study reported by Litovitz et al. (1988) investigated incidences of boron poisoning. Although this study did not document many important data (dose, time post-dose that examination began, number of concentrations used to estimate half-lives, etc.), the range of half-lives compares favorably with the well-controlled study presented by Jansen et al. (1984a). When linear regression analysis was used to fit the plasma concentration data, estimates of half-lives ranged from 4.0-27.8 hours, with an overall mean value of 13.4 ± 7.1 hours. Astier (1988) reported a plasma half-life of 28.7 hours after acute ingestion of 45 g boric acid (7.9 g B) in two doses over a 20-hour period.

A pharmacokinetic study (Usuda et al., 1998) in 10 rats, following an oral administration of sodium tetraborate containing 0.4 mg B/100 g body weight where 0.5-1 mL samples were drawn at nine different times during a 24-hour time period, reported a monophasic elimination of

boron from plasma, demonstrating a plasma half-life mean of 4.64 ± 1.19. This study also cited a high volume of distribution of 142.0 ± 30.2 mL/100 g body weight. One of the limitations of this study was that the large amount of blood drawn from the rats in the 24-hour period may have physiologically compromised the rats.

A human study (U.S. Borax, 2000; Pahl et al., 2001) was conducted to measure renal clearance of boron normally consumed in the daily diet in nonpregnant and pregnant women. At the beginning of the study, a baseline blood sample was taken. During the 2 hours following the baseline blood sample, all urine samples were collected. Blood samples were drawn at 2 hours and 24 hours after the baseline blood samples. Plasma boron levels were measured at these three time periods. Mean plasma boron levels obtained at baseline and 2 hours after the beginning of the study were similar between the pregnant and nonpregnant subjects. After 24 hours, plasma boron levels were lower in the pregnant women when compared with nonpregnant women, but there was a significant variability in the plasma values in both groups.

In a plasma clearance study of boron sponsored by U. S. Borax (Vaziri et al., 2001) in pregnant and nonpregnant rats given boric acid at dose levels of 0.3, 3.0, and 30 mg boric acid, plasma concentrations of boron were markedly lower 15 hours after dosing than at 3 hours after dosing. Mean plasma levels of boron were slightly higher in pregnant rats than in nonpregnant rats (statistically significant in only the high dose) given the same dose of boric acid.

In a study (U.S. Borax, 2000; Vaziri et al., 2001) conducted to estimate the plasma half-life of boric acid in the Sprague-Dawley rat, six nonpregnant and six pregnant rats were given low B in the diet for 7 days. On day 8 of the study, all rats received a single oral dose of 30 mg/kg of boric acid at approximately 9:00 a.m. This dose was the high dose used in the renal clearance study and was selected as the best to examine the linearity of the boron plasma curve at the highest concentration. Six 0.25 mL blood samples were drawn from each animal during a 12-hour period starting at noon on day 8 of the study. The blood samples were taken at 2- to 3-hour intervals. Gavage administration of 30 mg/BA/kg-day resulted in plasma levels of 1.82 ± 0.32 and 1.78 ± 0.32 μg/mL among pregnant and nonpregnant rats in the first blood sample taken 3 hours after dosing. This was followed by a monophasic decline in plasma boron concentration in both the pregnant and nonpregnant rats. The plasma concentration curves were consistent with a one-compartment model. Based on the shape of the plasma concentration curve, there was no evidence of saturation kinetics in either the nonpregnant or pregnant rats. The estimated half-life of boric acid in nonpregnant and pregnant rats was 2.9 and 3.2 hours, respectively, which was not statistically different.

7.0 HAZARD IDENTIFICATION

7.1 Studies in Humans - Epidemiology and Case Reports

7.1.1 Oral Exposure

Sayli et al. (1998) reported on a study of the relationship between exposure to boron in the drinking water and fertility in two geographic regions of Turkey. Drinking water boron concentrations were markedly higher in one region (2.05-29 mg/L) than in the other (0.03-0.4 mg/L). The study population comprised residents (primarily males who had ever been married) from these regions who could provide reproductive histories for three generations of family members (n=159 in one region and 154 in the other, 6.7% of the population in both). There was no difference between the regions regarding percentage of married couples with live births in any generation. Secondary sex ratios appeared to differ, with an excess of female births in the high-boron region (M/F = 0.89) and a slight excess of male births in the low-boron region (M/F = 1.04), but no statistical analysis was performed, and other factors reported to affect sex ratio (parental age, rate of elective abortion, multiple births) were not taken into account.

A large number of accidental poisoning cases are reported in the literature; however, quantitative estimates of absorbed dose are limited. Baker and Bogema (1986) reported quantitative estimates of two sibling infants who ingested formulas accidentally prepared from a boric acid eyewash solution. These infant doses ranged from 30.4-94.7 mg B/kg-day. The sibling who ingested 30.4 mg B/kg-day had a serum level of 9.79 mg B/mL and displayed a rash on his face and neck but later remained asymptomatic. The sibling who ingested 94.7 mg B/kg-day had serum boron values of 25.7 mg B/mL and experienced diarrhea, erythema of the diaper area, and vomiting a small amount of formula. Case reports and surveys of poisoning episodes were recently reviewed by Craan et al. (1997), WHO (1998a), Culver and Hubbard (1996), and Ischii et al. (1993). The most frequent symptoms of boron poisoning are vomiting, abdominal pain, and diarrhea. Other common symptoms include lethargy, headache, lightheadedness, and rash. For boric acid, the minimum lethal dose by oral exposure is approximately 15-20 g in adults, 5-6 g in children, and 2-3 g in infants.

Acute adult quantitative dose response data range from 1.4 mg B/kg to a high of 70 mg B/kg (Culver and Hubbard, 1996). In cases where ingestion was less than 3.68 mg B/kg, subjects were asymptomatic. Data in the 25-35 mg B/kg range were from patients undergoing boron neutron capture therapy for brain tumors. They displayed nausea and vomiting at 25 mg B/kg, and at 35 mg B/kg additional symptoms included skin flush. A patient recuperating from surgery had boric acid solution (70 mg B/kg) injected into the subcutaneous fluid infusion, which resulted in severe cutaneous and gastrointestinal symptoms. The patient recovered after hydration and diuresis.

Because boron compounds were used for various medical conditions including epilepsy, malaria, urinary tract infections, and exudative pleuritis from the mid 1800s until around 1900, some data are available on longer term exposure. Culver and Hubbard (1996) report on early cases of boron treatment for epilepsy from 2.5 to 24.8 mg B/kg-day for many years. Signs and symptoms reported in patients receiving 5 mg B/kg-day and above were indigestion, dermatitis, alopecia, and anorexia. One epilepsy patient who received 5.0 mg B/kg-day for 15 days

displayed indigestion, anorexia, and dermatitis, but the signs and symptoms disappeared when the dose was reduced to 2.5 mg B/kg-day.

O'Sullivan and Taylor (1983) reported seizures and other milder effects in seven infants who consumed boron in a honey-borax mixture applied to pacifiers. Five of the infants had a history suggestive of a familial-reduced convulsive threshold. The seizures ceased when the honey-borax treatment was stopped. The infants, who ranged in age from 6-16 weeks (at the end of the exposure period), were exposed to the honey-borax mixture over a period of 4-10 weeks. Original estimates of exposure were based on an error by the author (Taylor, 1997) concerning intake in jars versus grams of boron per week. The doses were recalculated from the information given by the author, based an estimated daily ingestion of honey-borax mixture and the analysis of the borax content in the mixture. Details of the analytic methods were not provided. Average estimated daily intakes of borax ranging from 429-1287 mg can be calculated directly from data provided by the authors. Average body weights over the exposure period for the infants in this study ranging from 4.3-5.3 kg based on estimates from the Exposure Factors Handbook (U.S. EPA, 1997a). Using the estimated body weights and a factor of 0.113 to estimate the boron content in borax, the equivalent boron exposure levels would have been about 9.6-33 mg/kg-day. The lowest exposure level of 3.2 mg/kg-day would be considered a lowest observed adverse effect level (LOAEL) for a fairly severe effect. Concentrations of boron in blood of 2.6, 8.4, and 8.5 µg/mL were reported for three of the subjects. Blood boron concentrations did not correlate well with estimated ingestion levels; the lowest blood boron concentration was measured for the infant with the highest estimated boron intake. Blood boron levels also were reported for a control group of 15 children aged 2-21 months, who had received no boron supplement and, presumably, had suffered no seizures. The control group blood boron values ranged from 0-0.63 µg/mL and averaged 0.21 µg/mL, with a standard deviation of 0.17 µg/mL. The lowest boron blood level associated with seizures, 2.6 µg/mL, was about 4 times the highest control level and 12 times the average control level, suggesting that the standard 10-fold uncertainty factor may be adequate for estimating an NOAEL. However, there was no indication whether any infants predisposed to seizures were in the control population. The presumptive boron NOAEL would be 0.32 mg/kg-day for a sensitive human subpopulation. Given the relatively uncomplicated boron toxicokinetics, the lack of correlation of blood boron and estimated ingestion rates suggest that the data may not be completely reliable. Based on the latter consideration, the indirect exposure estimation, and the lack of detail in the publication, this study should not be considered as the critical factor for derivation of the RfD, but the potential for seizures in infants should be considered in establishing the RfD.

Case reports and surveys of poisoning episodes were recently reviewed by Craan et al. (1997), WHO (1998a), Culver and Hubbard (1996), and Ischii et al. (1993). The most frequent symptoms of boron poisoning are vomiting, abdominal pain, and diarrhea. Other common symptoms include lethargy, headache, lightheadedness, and rash. For boric acid, the minimum lethal dose by oral exposure is approximately 15-20 g in adults, 5-6 g in children, and 2-3 g in infants.

Wegman et al. (1994) conducted a longitudinal study of respiratory function in workers with chronic exposure to sodium borate dusts. Participants in the Garabrant et al. (1985) study were re-tested for pulmonary function 7 years after the original survey. Of the 629 participants in the original study in 1981, 371 were available for re-testing in 1988. Of these, 336 performed

pulmonary function tests (303 produced acceptable tests in both years). Cumulative exposure was estimated for each participant for the years 1981-1988 as a time-weighted sum of the exposure in each job held during that time. Exposure prior to 1981 was not included due to the scarcity of monitoring data for those years. Pulmonary function FEV_1 (forced expiratory volume in 1 sec) and FVC (forced vital capacity) in study subjects declined over the 7-year period at a rate very close to that expected based on standard population studies. Cumulative borate exposure over the years 1981-1988 was not related to the change in pulmonary function. Acute studies showed statistically significant, positive dose-related increases in eye, nasal, and throat irritation; cough; and breathlessness with borate exposure (6-hr TWA or 15-min TWA). The same relationships were present when effects were limited to moderate severity or higher. There was no evidence for an effect of borate type (decahydrate, pentahydrate, anhydrous) on response rate.

7.1.2 Inhalation Exposure

Tarasenko et al. (1972) reported low sperm count, reduced sperm motility, and elevated fructose content of seminal fluid in semen analysis of 6 workers who were part of a group of 28 male Russian workers exposed for 10 or more years to high levels of vapors and aerosols of boron salts (22-80 mg/m^3) during the production of boric acid. The men in this report were studied using an Sexual Function of Man questionnaire. The results indicated that the group of 28 male exposed workers had decreased sexual function compared with 10 workers who had no contact with boric acid. However, the analysis of data from wives of the men from the exposed and control groups showed no differences. This study is of limited value for risk determinations due to the small sample size; sparse details on subjects regarding smoking habits, diet, other chemical exposures; and lack of methodology information on semen analysis. In response to this report and reports of reproductive effects in animal studies, a controlled epidemiology study of reproductive effects was initiated in U.S. workers exposed to sodium borates.

Whorton et al. (1994a,b, 1992) examined the reproductive effects of sodium borates on male employees at a borax mining and production facility in the United States. A total of 542 subjects participated in the study (72% of the 753 eligible male employees) by answering a questionnaire prepared by the investigators. The median exposure concentration was approximately 2.23 mg/m^3 sodium borate (roughly 0.31 mg B/m^3). Average duration of employment in participants was 15.8 years. Reproductive function was assessed in two ways. First, the number of live births to the wives of workers during the period from 9 months after occupational exposure began through 9 months after it ended was determined, and this number was compared to a number obtained from the national fertility tables for U.S. women (an unexposed control population). Wives of workers and controls were matched for maternal age, parity, race, and calendar year. This comparison produced the standardized birth ratio (SBR), defined as the observed number of births divided by the expected number. The investigators then examined possible deviations of the ratio of male to female offspring relative to the U.S. ratio.

There was a significant excess in the SBR among participants as a whole (Whorton et al., 1994a,b, 1992). Study participants fathered 529 births versus 466.6 expected (SBR=113, $p<0.01$). This excess occurred even though the percentage of participants who had vasectomies (36%) was 5 times higher than the national average of 7% implicit in the expected number of

births. Participants were divided into five equal-size groups (n = 108/109) based on average workday exposure to sodium borates (<0.82, 0.82-1.77, 1.78-2.97, 2.98-5.04, and >5.05 mg/m^3). There was no trend in SBR with exposure concentration; the SBR was significantly elevated for both the low- and high-dose groups, and close to expected for the three mid-dose groups. There were 42 participants who worked high-exposure jobs for 2 or more consecutive years. Mean sodium borate exposure in this group was 23.2 mg/m^3 (17.6-44.8 mg/m^3), and mean duration of employment in a high-exposure job was 4.9 years (range: 2.1-20.4 years). The SBR for the 42 workers was close to expected (102) despite a 48% vasectomy rate. These workers also had elevated SBR during the actual period of high exposure. An examination of SBR for all participants by 5-year increments from 1950 to 1990 revealed no significant trend in either direction over time.

Analyses of the percentage of female offspring showed an excess of females that approached statistical significance (52.7% vs. 48.8% in controls) (Whorton et al., 1994a,b, 1992). This excess was not related to exposure, however, as the percentage of female offspring decreased with increasing sodium borate exposure concentration (from 55.3% in the low-dose group to 49.2% in the high-dose group). Moreover, individuals with 2 or more consecutive years in high borate exposure jobs had more boys than girls. The investigators concluded that exposure to inorganic borates did not appear to adversely affect fertility in the population studied. This study, while adequately conducted, has several inherent limitations (SBR is less sensitive than direct measures of testicular effects, exposure information was limited, applicability of total U.S. fertility rates as control is questionable). Thus, the human data are insufficient to determine if boron may cause male reproductive toxicity (IEHR, 1997).

Whorton et al. (1992) also studied the effects of borates on reproductive function of exposed female employees. Reproductive function was assessed in the same way as it was for wives of male employees. A total of 81 employees were eligible, 68 of whom participated in the study. No information was provided regarding matching of the exposed and control groups. The SBR was 90 (32 offspring observed, 35.4 expected), indicating a deficiency, although not statistically significant, in live births among exposed females. When the data were analyzed per exposure category, the 76 employees (some nonparticipants apparently were included) in the low- and medium-exposure category showed a nonstatistically significant deficit of births (37 compared to 43.5 expected, SBR=85). No statistical differences were observed between exposed and controls when the results were analyzed by exposure categories. The authors concluded that the exposure to inorganic borates did not appear to affect fertility in the population studied. However, the small sample size may have precluded a meaningful statistical analysis of the results.

Swan et al. (1995) investigated the relationship between spontaneous abortion in women employed in the semiconductor manufacturing industry and various chemical and physical agents used in the industry, including boron. The study population consisted of 904 current and former female employees who became pregnant while working at 1 of 14 U.S. semiconductor companies between 1986 and 1989. Approximately one-half of those included were fabrication workers with some chemical exposure. Exposure classifications were based on jobs held at conception and level of exposure to specific agents during the first trimester. The risk of spontaneous abortion was increased in fabrication workers compared with other workers, and particularly within the subgroup of workers who performed masking (a group with relatively low

boron exposure). No significant association was found between exposure to boron and spontaneous abortion risk.

The respiratory and irritant effects of industrial exposure to boron compounds have also been studied. The studies were conducted at the same borax mining and production facility as the reproduction study of Whorton et al. (1994a,b, 1992). A health survey of workers at the plant found complaints of dermatitis, cough, nasal irritation, nose bleeds, and shortness of breath (Birmingham and Key, 1963). Air concentrations of borate dust were not reported, but were high enough to interfere with normal visibility. In response to this report, a cross-sectional study of respiratory effects (questionnaire, spirometric testing, roentgenograms) was performed on 629 male workers at the plant (Ury, 1966). The study was inconclusive, but did find suggestive evidence for an association between respiratory ill health and inhalation exposure to dehydrated sodium borate dust based on analysis of forced expiratory volume and respiratory illness data in the subgroup of 82 men who had worked for at least 1 year at the calcining and fusing processes compared with 547 others who had never worked at these processes.

Additional studies were performed by Garabrant et al. (1984, 1985). Garabrant et al. (1985) studied a group of 629 workers (93% of those eligible) employed for 5 or more years at the plant and employed in an area with heavy borax exposure at the time of the study. Workers were categorized into four groups according to borax exposure (1.1, 4.0, 8.4, and 14.6 mg/m^3 borax), and frequency of acute and chronic respiratory symptoms was determined. Statistically significant, positive dose-related trends were found (in order of decreasing frequency) for dryness of mouth, nose, or throat; eye irritation; dry cough; nose bleeds; sore throat; productive cough; shortness of breath; and chest tightness. Frequency of these symptoms in the high-dose group ranged from 33% down to 5%. Pulmonary function tests and chest x-rays were not affected by borax exposure. The researchers concluded that borax appears to cause simple respiratory irritation that leads to chronic bronchitis, with no impairment of respiratory function at the exposure levels in this study. Irritation occurred primarily at concentrations of 4.4 mg/m^3 or more. Garabrant et al. (1984) studied a subgroup of the 629 workers who were exposed to boric oxide and boric acid. Workers who had held at least one job in an area with boron oxide or boric acid exposure (n=113) were compared with workers who had never held a job in an area with boron oxide or boric acid, but who had held at least one job in an area with low- or minimal- exposure to borax (n=214). The boron oxide/boric acid workers had significantly higher rates of eye irritation; dryness of mouth, nose, or throat; sore throat; and productive cough. Mean exposure was 4.1 mg/m^3, with a range of 1.2 to 8.5 mg/m^3. The researchers concluded that boron oxide and boric acid produce upper respiratory and eye irritation at less than 10 mg/m^3.

7.2 Prechronic and Chronic Studies and Cancer Bioassays in Animals - Oral and Inhalation

7.2.1 Oral Exposure

In the following studies, doses not reported by the investigators were approximated from dietary or drinking water concentrations of boron using food factors (rat: 0.05; dog: 0.025; mouse: 0.1) (1 ppm = 0.025 mg/kg-day assumed dog food consumption) and body-weight and water consumption values (mouse: 0.03 kg and 0.0057 L/day; rat: 0.35 kg and 0.049 L/day) specified by the U.S. EPA (1980, 1988). Doses in mg boric acid were converted to mg boron by multiplying by the ratio of the formula weight of boron to the molecular weight of boric acid (10.81/61.84 = 0.1748). Similarly, doses in mg borax were converted to mg boron by multiplying by the ratio of the formula weight of boron to the molecular weight of borax (4 x 10.81/381.3 = 0.1134).

The subchronic and chronic toxicity of borax and boric acid has been studied in dogs administered these compounds in the diet (Weir and Fisher, 1972; U.S. Borax Research Corp., 1963, 1966, 1967). In the subchronic study, groups of beagle dogs (5/sex/dose/compound) were administered borax (sodium tetraborate decahydrate) or boric acid for 90 days at dietary levels of 17.5, 175, and 1750 ppm boron (male: 0.33, 3.9 and 30.4 mg B/kg-day; female: 0.24, 2.5 and 21.8 mg B/kg-day) and compared with an untreated control group of 5 dogs/sex (Weir and Fisher, 1972; U.S. Borax Research Corp., 1963). On day 68 of the study, a high-dose male dog died as a result of complications of diarrhea with severe congestion of the mucosa of the small and large intestines and congestion of the kidneys. No clinical signs of toxicity were evident in the other dogs. The testes were the primary target of boron toxicity. At the high dose, mean testes weight was decreased 44% (9.6 g) in males fed borax and 39% (10.5 g) in males fed boric acid compared with controls (17.2 g). Also at this dose, mean testes:body weight ratio (control: 0.2%; borax: 0.1%; boric acid: 0.12%) and mean testes:brain weight ratio (control: 22%; borax: 12%) were significantly reduced. Decreased testes:body weight ratio also was observed in one dog from the mid-dose (175 ppm) boric acid group. Microscopic pathology revealed severe testicular atrophy in all high-dose male dogs, with complete degeneration of the spermatogenic epithelium in 4/5 cases. No testicular lesions were found in the lower-dose groups. Hematological effects were also observed in high-dose dogs. Decreases were found for both hematocrit (15 and 6% for males and females, respectively) and hemoglobin (11% for both males and females) at study termination in borax-treated dogs. Pathological examination revealed accumulation of hemosiderin pigment in the liver, spleen, and kidney, indicating breakdown of red blood cells, in males and females treated with borax or boric acid. Other effects in high-dose dogs were decreased thyroid:body weight ratio (control: 0.009%; borax: 0.006%; boric acid: 0.006%) and thyroid:brain weight ratio (control: 0.95%; borax: 0.73%) in males; increased brain:body weight ratio (borax) and liver:body weight ratio (boric acid) in females; a somewhat increased proportion of solid epithelial nests and minute follicles in the thyroid gland of borax-treated males; lymphoid infiltration and atrophy of the thyroid in boric-acid treated females; increased width of the zona reticularis (borax males and females, boric acid females); and zona glomerulosa (boric acid females) in the adrenal gland. This study identified an LOAEL of 1750 ppm boron (male: 30.4 mg B/kg-day; female: 21.8 mg B/kg-day) and an NOAEL of 175 ppm boron (male: 3.9 mg B/kg-day; female: 2.5 mg B/kg-day) based on systemic toxicity in dogs following subchronic exposure.

In the chronic toxicity study, groups of beagle dogs (4/sex/dose/compound) were administered borax or boric acid by dietary admix at concentrations of 0, 58, 117, and 350 ppm boron (0, 1.4, 2.9, and 8.8 mg B/kg-day) for 104 weeks (Weir and Fisher, 1972; U.S. Borax Research Corp., 1966). There was a 52-week interim sacrifice and a 13-week "recovery" period after 104 weeks on test article for some dogs. Four male dogs served as controls for the borax and boric acid dosed animals. One male control dog was sacrificed after 52 weeks, two male control dogs were sacrificed after 104 weeks, and one was sacrificed after the 13-week recovery period with 104 weeks of treatment. The one male control dog sacrificed after the 13-week recovery period demonstrated testicular atrophy. Sperm samples used for counts and motility testing were taken only on the control and high-dose male dogs prior to the 2-year sacrifice. At a dose level of 8.8 mg B/kg-day in the form of boric acid, one dog sacrificed at 104 weeks had testicular atrophy. Two semen evaluations (taken after 24 months treatment) were preformed on dogs treated at the highest dose (8.8 mg B/kg-day). Two of two borax-treated animals had samples that were azoospermic and had no motility, while one of two boric acid treated animals had samples that were azoospermic. The authors reported that there did not appear to be any definitive test article effect on any parameter examined. The study pathologist considered the histopathological findings to be "not compound-induced." Tumors were not reported.

In a follow-up to this study, groups of beagle dogs (4/sex/dose/compound) were given borax or boric acid in the diet at concentrations of 0 and 1170 ppm boron (0 and 29.2 mg B/kg-day) for up to 38 weeks (Weir and Fisher, 1972; U.S. Borax Research Corp., 1967). New control dogs (4 males) were used for this follow up study. Two were sacrificed at 26 weeks and two at 38 weeks. At the 26-week sacrifice, one of two had spermatogenesis and (5%) atrophy. One was reported normal. At 38 weeks, one had decreased spermatogenesis, and the other had testicular atrophy. The test animals had about an 11% decrease in the rate of weight gain when compared with control animals, throughout the study. Interim sacrifice of two animals from each group at 26 weeks revealed severe testicular atrophy and spermatogenic arrest in male dogs treated with either boron compound. Testes weight, testes:body weight ratio, and testes:brain weight ratios were all decreased. Effects on other organs were not observed. Exposure was stopped at 38 weeks; at this time, one animal from each group was sacrificed and the remaining animal from each group was placed on the control diet for a 25-day recovery period prior to sacrifice. After the 25-day recovery period, testes weight and testes weight:body weight ratio were similar to controls in both boron-treated males, and microscopic examination revealed the presence of moderately active spermatogenic epithelium in one of the dogs. The researchers suggested that this finding, although based on a single animal, indicates that boron-induced testicular degeneration in dogs may be reversible upon cessation of exposure. When the 2-year and 38-week dog studies are considered together, an overall NOAEL and LOAEL for systemic toxicity can be established at 8.8 and 29.2 mg B/kg-day, respectively, based on testicular atrophy and spermatogenic arrest.

Weir and Fisher (1972) fed Sprague-Dawley rats a diet containing 0, 117, 350, or 1170 ppm boron as borax or boric acid for 2 years (approximately 0, 5.9, 17.5, or 58.5 mg B/kg-day). There were 70 rats/sex in the control groups and 35/sex in the groups fed boron compounds. At 1170 ppm, rats receiving both boron compounds had decreased food consumption during the first 13 weeks of study and suppressed growth throughout the study. Signs of toxicity at this exposure level included swelling and desquamation of the paws, scaly tails, inflammation of the eyelids, and bloody discharge from the eyes. Testicular atrophy was observed in all high-dose

males at 6, 12, and 24 months. The seminiferous epithelium was atrophied, and the tubular size in the testes was decreased. Testes weights and testes:body weight ratios were significantly ($p<0.05$) decreased. Brain and thyroid:body weight ratios were significantly ($p<0.05$) increased. No treatment-related effects were observed in rats receiving 350 or 117 ppm boron as borax or boric acid. This study identified an LOAEL of 1170 ppm (58.5 mg B/kg-day) and an NOAEL of 350 ppm (17.5 mg B/kg-day) for testicular effects. Based on effects observed in the high-dose group, it appears that a maximum tolerated dose (MTD) was achieved in this study. The study was designed to assess systemic toxicity; only tissues from the brain, pituitary, thyroid, lung, heart, liver, spleen, kidney, adrenal, pancreas, small and large intestine, urinary bladder, testes, ovary, bone, and bone marrow were examined histopathologically. Tumors were not mentioned in the report. Nevertheless, NTP (1987) concluded that this study provided adequate data on the lack of carcinogenic effects of boric acid in rats, and accordingly, conducted its carcinogenicity study only in mice.

Weir and Fisher (1972) also conducted studies of boron toxicity in rats. Sprague-Dawley rats (10/sex/dose) were fed borax or boric acid in the diet for 90 days at levels of 0, 52.5, 175, 525, 1750, and 5250 ppm boron (approximately 0, 2.6, 8.8, 26.3, 87.5, and 262.5 mg B/kg-day, respectively) calculated by assuming reference values of 0.35 kg bw and a food factor of 0.05 for rats. Both borax and boric acid produced 100% mortality at the highest dose and complete atrophy of the testes in all males fed diets containing 1750 ppm boron. Overt signs of toxicity at these two dose levels included rapid respiration, eye inflammation, swelling of the paws, and desquamation of the skin on paws and tails. At 1750 ppm boron, both compounds produced significant ($p<0.05$) decreases in body weight and in the mean weights of the liver, kidneys, spleen, and testes. At lower doses, changes in organ weights were inconsistent. At 52.5 ppm boron, increases in the mean weights of the brain, spleen, kidneys, and ovaries were seen in females fed borax, and an increase in mean liver weight was seen in females fed boric acid; no organ weight changes were seen in the males. At 175 ppm boron, the only change in organ weight reported by the investigators was increased kidney weights in males fed borax. These changes, however, were not observed at 525 ppm boron for either compound. Microscopic examination revealed complete testicular atrophy at 1750 ppm in all males fed borax or boric acid, and partial testicular atrophy at 525 ppm boron in four males fed borax and in one male fed boric acid. Changes in organ weights that were reported at 52.5 ppm were not dose related and were not confirmed in follow-up chronic studies by the same investigators. This study identified an NOAEL of 175 ppm boron (8.8 mg B/kg-day) and an LOAEL of 525 ppm boron (26.3 mg B/kg-day) boron for systemic toxicity in rats following subchronic dietary exposure.

A subchronic study in rats using drinking water exposure is also available. Borax was administered in the drinking water to male Long Evans rats (15/group) at levels of 0, 150, and 300 mg B/L for 70 days; the basal diet contained approximately 54 g B/g of feed (Seal and Weeth, 1980). The approximate intake of boron for the treated rats was 23.7 and 44.7 mg B/kg-day, respectively, using reference values for body weight, food, and water consumption. Treatment with borax at both doses produced significant ($p<0.05$) decreases in body weight; testis, seminal vesicle, spleen, and right femur weight; and plasma triglyceride levels. At the highest dose level, spermatogenesis was impaired and hematocrit was decreased slightly. From this study, an LOAEL of 23.7 mg B/kg-day can be identified. An NOAEL was not identified.

The subchronic and chronic toxicity of boron (boric acid) in mice was studied by NTP (1987) and Dieter (1994). In the subchronic study, groups of 10 male and 10 female B6C3F1 mice were fed diets containing 0, 1200, 2500, 5000, 10,000, or 20,000 ppm boric acid (0, 210, 437, 874, 1748, or 3496 ppm boron) for 13 weeks (NTP, 1987; Dieter, 1994). These dietary levels correspond to approximately 0, 34, 70, 141, 281, and 563 mg B/kg-day for males and 0, 47, 97, 194, 388, and 776 mg B/kg-day for females, respectively, based on reported average values for feed consumption (161 g/kg bw/day for males, 222 g/kg bw/day for females) by controls in week 4 of the experiment. At the highest dose level, hyperkeratosis and acanthosis of the stomach and >60% mortality were observed. At 10,000 ppm boric acid, 10% mortality was observed among the males. At 5000 ppm and higher, degeneration or atrophy of the seminiferous tubules was observed in males, and weight gain was suppressed in animals of both sexes. Minimal to mild extramedullary hematopoiesis of the spleen was observed in all dosed groups. The lowest dose tested, 1200 ppm (34 mg B/kg-day for male mice), appears to be the LOAEL for this study. The NOAEL (no toxicity in absence of body weight loss) was at or below 1200 ppm (34 mg/kg-day for males and 47 mg/kg-day for females). From this study, dietary doses of 2500 ppm (70 mg B/kg-day for males and 97 mg B/kg-day for females) and 5000 ppm (141 mg B/kg-day for males and 194 mg B/kg-day for females) were selected to be tested in both sexes in the chronic 2-year study based on body weight depression and mortality in the two highest doses tested in the subchronic study.

In the chronic study, male and female (50/sex/group) B6C3F1 mice were fed a diet containing 0, 2500, or 5000 ppm boric acid for 103 weeks (NTP, 1987; Dieter, 1994). The low- and high-dose diets provided approximate doses of 275 and 550 mg/kg-day (48 and 96 mg B/kg-day), respectively. Mean body weights of high-dose mice were 10-17% lower than those of controls after 32 (males) or 52 (females) weeks. No treatment-related clinical signs were observed throughout the study. Survival of the male mice was significantly lower than that of the control group after week 63 in the low-dose group and after week 84 in the high-dose group. Survival was not affected in females. At termination, the survival rates were 82, 60, and 44% in the control, low-, and high-dose males, respectively, and 66, 66, and 74% in the control, low-, and high-dose females, respectively. The low number of surviving males may have reduced the sensitivity of the study for evaluation of carcinogenicity (NTP, 1987). Administration of boric acid to male mice induced testicular atrophy and interstitial cell hyperplasia in the high-dose group. There also were dose-related increased incidences of splenic lymphoid depletion in male mice. According to NTP (1987), this lesion is associated with stress and debilitation and is reflected in the increased mortality in these groups of male mice. Increased incidences of other nonneoplastic lesions were not believed to have been caused by the administration of boric acid, because they either were not consistently dose-related or did not occur in both sexes.

Low-dose male mice demonstrated increased incidences of hepatocellular carcinoma (5/50, 12/50, 8/49) and combined adenoma or carcinoma (14/50, 19/50, 15/49), relative to control and the high-dose male mice (NTP, 1987; Dieter, 1994). The increases were statistically significant by life table tests, but not by incidental tumor tests. The incidental tumor tests were considered to be the more appropriate form of statistical analysis in this case, because the hepatocellular carcinomas did not appear to be the cause of death for males in this study; the incidence of these tumor types in animals that died prior to study completion (7/30 or 23%) was similar to the incidence at terminal sacrifice (5/20 or 25%) (NTP, 1987; Elwell, 1993). The hepatocellular carcinoma incidence in this study was within the range of male mice historical

controls both at the study lab (131/697 or 19 ± 6%) and for NTP (424/2084 or 20 ± 7%) (NTP, 1987; Elwell, 1993). Also, the hepatocellular carcinoma incidence in the male control group of this study (10%) was lower than the historical controls. NTP concluded that the increase in hepatocellular tumors in low-dose male mice was not due to administration of boric acid.

There was also a significant increase in the incidence of combined subcutaneous tissue fibromas, sarcomas, fibrosarcomas, and neurofibrosarcomas in low-dose male mice (2/50, 10/50, 2/50) by both incidental and life table pair-wise tests (NTP, 1987; Dieter, 1994). This higher incidence of subcutaneous tissue tumors is within the historical range (as high as 15/50 or 30%) for these tumors in control groups of group-housed male mice from other dosed feed studies (Elwell, 1993). The historical incidence at the study laboratory was 39/697 (6 ± 4%) and in NTP studies was 156/2091 (7 ± 8%) (NTP, 1987). Based on the comparison to historical controls and lack of any increase in the high-dose group, NTP concluded that the increase in subcutaneous tumors in low-dose male mice was not compound-related. Overall, NTP concluded that this study produced no evidence of carcinogenicity of boric acid in male or female mice, although the low number of surviving males may have reduced the sensitivity of the study.

Schroeder and Mitchener (1975) conducted a study in which 0 or 5 ppm of boron as sodium metaborate was administered in the drinking water to groups of 54 male and 54 female Charles River Swiss mice (approximately 0.95 mg B/kg-day) for their life span; controls received deionized water. In adult animals, there generally were no effects observed on longevity body weights (at 30 days, treated animals were lighter than controls, and at 90 days, treated males were significantly heavier than controls). The life spans of the dosed group did not differ from controls. Gross and histopathologic examinations were performed to detect tumors. Limited tumor incidence data were reported for other metals tested in this study, but not for boron. Investigators reported that at this dose, boron was not tumorigenic for mice; however, only one dose of boron (lower than other studies) was tested, and an MTD was not reached.

7.2.2 Inhalation Exposure

There are few data available regarding the toxicity of boron compounds by inhalation in laboratory animals. Wilding et al. (1959) investigated the toxicity of boron oxide aerosols by inhalation exposure in rats and dogs. Three dogs were exposed to 57 mg/m$_3$ (18 mg B/m^3) for 23 weeks. A group of 70 albino rats, including both males and females, was exposed to an average concentration of 77 mg/m^3 of boron oxide aerosols (24 mg B/m$_3$) for 24 weeks (6 hours/day, 5 days/week). Additional groups of rats were exposed to 175 mg/m$_3$ (54 mg B/m^3) for 12 weeks (n=4) or 470 mg/m$_3$ (146 mg B/m^3) for 10 weeks (n=20) using the same exposure regimen. At the latter concentration, the aerosol formed a dense cloud of fine particles, and the animals were covered with dust. No clinical signs were noted, except a slight reddish exudate from the nose of rats exposed to 470 mg/m^3, which the researchers attributed to local irritation. Growth was reduced roughly 9% in rats exposed to 470 mg/m^3 compared to controls. Growth in the lower dose rat groups and in dogs was not affected. There was a significant drop in pH and increase in urine volume in rats exposed to 77 mg/m^3. The researchers hypothesized that this was due to formation of boric acid from boron oxide by hydration in the body and the diuretic properties of boron oxide. There was also a significant increase in urinary creatinine at this dose. No effect on serum chemistry, hematology, organ weights, histopathology, bone strength, or liver function was found in either rats or dogs (not all endpoints were studied in all exposure groups).

7.3 Developmental/Reproductive Toxicity

7.3.1 Developmental Studies

Heindel et al. (1994, 1992) and Price et al. (1990) treated timed-mated Sprague-Dawley rats (29/group) with a diet containing 0, 0.1, 0.2, or 0.4% boric acid from gestation day (gd) 0-20. The investigators estimated that the diet provided 0, 78, 163, or 330 mg boric acid/kg-day (0, 13.6, 28.5, or 57.7 mg B/kg-day). Additional groups of 14 rats each received boric acid at 0 or 0.8% in the diet (539 mg/kg-day or 94.2 mg B/kg-day) on gd 6 through 15 only. Exposure to 0.8% was limited to the period of major organogenesis in order to reduce the preimplantation loss and early embryolethality indicated by the range-finding study and, hence, provide more opportunity for teratogenesis. (The range-finding study found that exposure to 0.8% on gd 0-20 resulted in a decreased pregnancy rate [75% as compared with 87.5% in controls] and in greatly increased resorption rate per litter [76% as compared with 7% in the control group].) Food and water intake and body weights, as well as clinical signs of toxicity, were monitored throughout pregnancy. On gd 20, the animals were sacrificed; the liver, kidneys, and intact uteri were weighed; and corpora lutea were counted. Maternal kidneys, selected randomly (10 dams/group), were processed for microscopic evaluation. Live fetuses were dissected from the uterus, weighed and examined for external, visceral, and skeletal malformations. Statistical significance was established at $p<0.05$. There was no maternal mortality during treatment. Food intake increased 5-7% relative to that of controls on gd12-20 at 0.2 and 0.4%; water intake was not significantly altered by administration of boric acid (data not shown). At 0.8%, water and food intake decreased on gd 6-9 and increased on gd15-18, relative to controls. Pregnancy rates ranged between 90 and 100% for all groups of rats and appeared unrelated to treatment. Maternal effects attributed to treatment included a significant and dose-related increase in relative liver and kidney weights at 0.2% or more, a significant increase in absolute kidney weight at 0.8%, and a significant decrease in body-weight gain during treatment at 0.4% or more. Corrected body weight gain (gestational weight gain minus gravid uterine weight) was unaffected except for a significant increase at 0.4%. Examination of maternal kidney sections revealed minimal nephropathy in a few rats (unspecified number), but neither the incidence nor the severity of the changes was dose related.

Treatment with 0.8% boric acid (gd 6-15) significantly increased prenatal mortality, as seen in increases in the percentage of both resorptions and late fetal deaths per litter. The number of live fetuses per litter was also significantly decreased at 0.8%. Average fetal body weight (all fetuses or male or female fetuses) per litter was significantly reduced in all treated groups versus controls in a dose-related manner. Mean fetal weights were 94, 87, 63, and 46% of the corresponding control means for the 0.1, 0.2, 0.4, and 0.8%, respectively. The percentage of malformed fetuses per litter and the percentage of litters with at least one malformed fetus were significantly increased at 0.2% or more. Treatment with 0.2% or more boric acid also increased the incidence of litters with one or more fetuses with a skeletal malformation. The incidence of litters with one or more pups with a visceral or gross malformation was increased at 0.4 and 0.8%, respectively. The malformations consisted primarily of anomalies of the eyes, the central nervous system (CNS), the cardiovascular system, and the axial skeleton. In the 0.4 and 0.8% groups, the most common malformations were enlarged lateral ventricles of the brain and agenesis or shortening of rib XIII. The percentage of fetuses with variations per litter was reduced relative to controls in the 0.1 and 0.2% dosage groups (due primarily to a reduction in

the incidence of rudimentary or full ribs at lumbar I), but was significantly increased in the 0.8% group. The variation with the highest incidence among fetuses was wavy ribs. Based on the changes in organ weights, a maternal LOAEL of 0.2% boric acid in the feed (28.5 mg B/kg-day) can be established; the maternal NOAEL is 0.1% or 13.6 mg B/kg-day. Based on the decrease in fetal body weight per litter, the level of 0.1% boric acid in the feed (13.6 mg B/kg-day) is an LOAEL; an NOAEL was not defined.

In a follow-up study, Price et al. (1996a, 1994) administered boric acid in the diet (at 0, 0.025, 0.050, 0.075, 0.100, or 0.200%) to timed-mated CD rats, 60 per group, from gd 0-20. Throughout gestation, rats were monitored for body weight, clinical condition, and food and water intake. This experiment was conducted in two phases, and in both phases offspring were evaluated for post-implantation mortality, body weight, and morphology (external, visceral, and skeletal). Phase I of this experiment was considered the teratology evaluation and was terminated on gd 20, when uterine contents were evaluated. The calculated average dose of boric acid consumed for Phase l dams was 19, 36, 55, 76, and 143 mg/kg-day (3.3, 6.3, 9.6, 13.3, and 25 mg B/kg-day). During Phase I, no maternal deaths occurred, and no clinical symptoms were associated with boric acid exposure. Maternal body weights did not differ among groups during gestation, but statistically-significant trend tests associated with decreased maternal body weight (gd 19 and 20 at sacrifice) and decreased maternal body weight gain (gd 15-18 and gd 0-20) were indicated. In the high-dose group, there was a 10% reduction (statistically significant in the trend test $p<0.05$) in gravid uterine weight when compared with controls. The authors indicated that the decreasing trend of maternal body weight and weight gain during late gestation reflected reduced gravid uterine weight. Corrected maternal weight gain (maternal gestational weight gain minus gravid uterine weight) was not affected. Maternal food intake was only minimally affected at the highest dose and only during the first 3 days of dosing. Water intake was higher in the exposed groups after gd 15. The number of ovarian corpora lutea and uterine implantation sites, and the percentage of preimplantation loss were not affected by boric acid exposure.

Offspring body weights were significantly decreased in the 13.3 and 25 mg B/kg-day dose groups on gd 20. The body weights of the low- to high-dose groups, respectively, were 99, 98, 97, 94, and 88% of control weight. There was no evidence of a treatment-related increase in the incidence of external or visceral malformations or variations when considered collectively or individually. On gd 20, skeletal malformations or variations considered collectively showed a significant increased percentage of fetuses with skeletal malformations per litter. Taken individually, dose-related response increases were observed for short rib XIII, considered a malformation in this study, and wavy rib or wavy rib cartilage, considered a variation. Statistical analyses indicated that the incidence of short rib XIII and wavy rib were both increased in the 13.3 and 25 mg B/kg-day dose groups relative to controls. A statistically significant trend ($p<0.05$) was found for decreases in rudimentary extra rib on lumbar I, classified as a variation. Only the high-dose group had a biologically relevant, but not statistically significant, decrease in this variation. The LOAEL for Phase I of this study was considered to be 0.1% boric acid (13.3 mg B/kg-day), based on decreased fetal body weight. The NOAEL for Phase I of this study was considered to be 0.075% boric acid (9.6 mg B/kg-day).

In Phase II, dams were allowed to deliver and rear their litters until postnatal day (pnd) 21. The calculated average doses of boric acid consumed for Phase II dams were 19, 37, 56, 74,

and 145 mg/kg-day (3.2, 6.5, 9.7, 12.9, and 25.3 mg B/kg-day). This phase allowed a follow-up period to determine whether the incidence of skeletal defects in control and exposed pups changed during the first 21 postnatal days. Among live born pups, there was a significant trend test for increased number and percentage of dead pups between pnd 0 and 4, but not between pnd 4 and 21; this appeared to be due to an increase in early postnatal mortality in the high dose, which did not differ significantly from controls and was within the range of control values for other studies in this laboratory. On pnd 0, the start of Phase II, there were no effects of boric acid on the body weight of offspring (102, 101, 99, 101, and 100% of controls, respectively). There were also no differences through termination on pnd 21; therefore, fetal body weight deficits did not continue into this postnatal period (Phase II). The percentage of pups per litter with short rib XIII was still elevated on pnd 21 in the 0.200% boric acid dose group (25.3 mg B/kg-day), but there was no incidence of wavy rib, and none of the treated or control pups on pnd 21 had an extra rib on lumbar 1. The NOAEL and LOAEL for phase II of this study were 12.9 and 25.3 mg B/kg-day, respectively.

Price et al. (1997) provides an analysis of maternal whole blood taken on gd 20 from the previously described study (Price et al., 1996a, 1994) in which dietary concentration of added boric acid yielded average daily intakes equivalent to 0, 3, 6, 10, 13, or 25 mg B/kg body weight. Blood samples were analyzed using inductively coupled plasma optical emission spectrometry. Increasing dietary concentrations of boric acid were positively associated with whole blood concentration in pregnant rats. Whole blood concentrations in confirmed pregnant rats were 0.229 ± 0.143, 0.564 ± 0.211, 0.975 ± 0.261, 1.27 ± 0.298, 1.53 ± 0.546, 2.82 ± 0.987 ug boron/g whole blood (mean ± SD) for the control through the high-dose groups. Positive correlations between maternal blood boron concentrations and indices of maternal dietary intake of boron with embryo/fetal toxicity (Price et al., 1996a, 1994) were observed at average daily concentration of 13 and 25 mg B/kg. Blood boron concentrations of 1.27 ± 0.298 and 1.53 ± 0.546 ug boron/g were associated with the NOAEL (10 mg B/kg-day) and the LOAEL (13 mg B/kg-day) for the developmental toxicity reported in Price et al. (1996a, 1994).

The developmental effects of boric acid also have been studied in mice and rabbits. Heindel et al. (1994, 1992) and Field et al. (1989) examined the developmental effects of boric acid in pregnant CD-1 mice using the same experimental design as in the initial study with rats (Price et al., 1990), except that a 0.8% dietary level was not used in the mouse study. The diets containing 0, 0.1, 0.2, or 0.4% boric acid were estimated by the investigators to provide 0, 248, 452, or 1003 mg boric acid/kg-day (0, 43.4, 79.0, or 175.3 mg B/kg-day); the mice were treated during gd 0-17. Neither survival rates nor pregnancy rates were affected by treatment with boric acid. Pale kidneys were noted in several treated dams, particularly in the high-dose group, and one dam in this group had fluid accumulation in the kidney. Maternal body weight was significantly reduced by 10-15% during gd 12-17 in the high-dose group. Maternal weight gain was significantly reduced during treatment in the high-dose group, but was not affected when corrected for gravid uterine weight. At the 0.4% dietary level, food intake was increased between days 12 and 15 and water intake was increased on days 15-17 (statistical significance not provided for either effect). Organ weight changes were limited to significant increases in relative kidney weight and absolute liver weight in the 0.4% groups. A dose-related increase in maternal renal tubular dilation and/or regeneration was observed; the incidence was 0/10, 2/10, 8/10, and 10/10 in the 0, 0.1, 0.2, and 0.4% dosage groups, respectively. Treatment with boric acid did not affect preimplantation loss or the number of implantation sites per litter, but

significantly increased the percentage of resorptions per litter and the percentage of litters with one or more resorptions at the 0.4% level. There was a significant dose-related decrease in average fetal body weight (all fetuses or male or female fetuses) per litter at 0.2% or more. The percentage of malformed fetuses per litter increased significantly at 0.4%, whereas the percentage of fetuses with variations per litter was decreased at 0.1 and 0.2% and was not affected at 0.4%. The most frequent malformation observed among fetuses of the 0.4% group was a short rib XIII. In contrast, full or rudimentary lumbar I rib (a variation) was less frequent in fetuses of treated mice. Although the level of 0.1% boric acid in the diet induced an increase in renal lesions in mice, the increased incidence did not achieve statistical significance (Fisher Exact Test). The 0.1% level (43.4 mg B/kg-day) is a maternal NOAEL and the 0.2% level (79 mg B/kg-day) is a maternal LOAEL. For developmental effects, the 0.2% dietary level of boric acid is an LOAEL based on decreased fetal body weight per litter, and the 0.1% level is an NOAEL.

Artificially inseminated New Zealand White rabbits (30/group) were administered 0, 62.5, 125, or 250 mg boric acid/kg-day (0, 10.9, 21.9, and 43.7 mg B/kg-day) in aqueous solution by gavage on gd 6-19 (Price et al., 1996b, 1991; Heindel et al., 1994). Food consumption, body weight and clinical signs were monitored throughout the study. At gd 30, the animals were sacrificed and the following endpoints were examined: pregnancy status; number of resorptions; fetal body weight; viability; and external, visceral, and skeletal malformations. No treatment-related clinical signs of toxicity were observed during the study, except for vaginal bleeding noted in 2-11 does/day on gd 19-30 at the high dose; these does had no live fetuses on day 30. Vaginal bleeding was also observed in one female in the low-dose group and in one in the mid-dose group. Two maternal deaths occurred (one each at the low- and mid-dose), but were not treatment-related. Food intake was decreased relative to that of controls on treatment days 6-15 at the high dose, and was increased after treatment ceased on days 25-30 at the mid and high doses. Body weight on gd 9-30, weight gain on gd 6-19, gravid uterine weight, and number of corpora lutea per dam were each decreased in the high-dose group. After correction for gravid uterine weights, however, maternal body-weight gain was increased at both the mid and high doses. Treatment with boric acid did not affect absolute or relative liver weight. Relative, but not absolute kidney weight increased at the high dose; kidney histopathology was unremarkable. Boric acid caused frank developmental effects at the high dose. These effects consisted of a high rate of prenatal mortality (90% of implants/litter were reabsorbed compared with 6% in the control group). Also, the percentage of pregnant females with no live fetuses was greatly increased (73% compared with 0% in controls), whereas the number of live fetuses per litter on day 30 was significantly reduced (2.3/litter compared with 8.8/litter in the control group). Malformed live fetuses per litter increased significantly at the high dose, primarily due to the incidence of fetuses with cardiovascular defects, the most prevalent of which was interventricular septal defect (8/14 at the high dose compared with 1/159 in the control group). The incidence of skeletal malformations was comparable among groups. Relative to controls, the percentage of fetuses with variations (all types combined) was not significantly increased in any treated group, but the percentage with cardiovascular variations was significantly increased from 11% in controls to 64% in the high-dose group. Fetal body weights per litter at the high dose were depressed relative to the control, but the difference was not statistically significant; however, this could have been due to the small sample size in the high-dose group. No developmental effects were found in the low- and mid-dose groups. In this study, the mid dose of 125 mg boric acid/kg-day (21.9 mg B/kg-day) represents the NOAEL based on maternal and

developmental effects. The high dose of 250 mg boric acid/kg-day (43.7 mg B/kg-day) is the LOAEL.

Narotsky et al. (2003) dosed rat dams (number not specified) with 500 mg/kg boric acid twice daily on single days during development (gd 6, 7, 8, 9, 10, or 11) and examined fetal body weight and skeletal malformations. These were compared to the effect of boric acid on the *hox* gene family, genes clustered among four loci and thought to confer positioning and development of vertebrae. Their expression in the paraxial mesoderm begins during gastrulation. Boric acid (0 or 500 mg/kg) was administered via gavage to pregnant Sprague-Dawley rats twice daily (totaling 1000 mg/kg-day) on gd 6, 7, 8, 9, 10, or 11, and examinations were performed on gd 21. Skeletal malformations were evaluated following alizarin red and alcian blue staining. Boric acid was administered on gd 9, and *hox* gene expression was determined by *in situ* hybridization in fixed sections at gd 13.5. Fetal weights were significantly decreased in animals treated on gd 7, 9, 10, or 11. Fetuses exposed on gd 8 or 9 demonstrated a "low but significant" elevation of the frequency of rudimentary cervical ribs. The authors indicate that fetuses exposed on gd 6, 7, 8, or 11 generally demonstrated "no such effect" of boric acid on ribs, vertebrae, and sternebrae compared with the striking alterations observed following treatment on gd 9. The cephalo-caudal expression pattern of the *hoxc6* and *hoxa6* genes in pre-vertebral tissues was altered by boric acid treatment on gd 9. These authors demonstrated that exposure on gd 6 "had no developmental effects, and treatment on gd 7 and 11 caused only relatively mild developmental toxicity (reduced fetal weights but did not alter the frequency or type of skeletal malformations); treatment on gd 8, 9, and 10 disrupted axial development. Gestational day 8 exposure induced cervical ribs and rib or vertebral malformations, but only treatment on gd 9 or 10 dramatically altered numbers of vertebrae, ribs or sternebrae."

Cherrington and Chernoff (2002) evaluated the developmental toxicity of boric acid in pregnant CD-1 mice in three separate experimental designs. In the first design, mice were dosed daily from gd 6-10 by gavage with either 0, 500, or 750 mg/kg. The control group had 6 animals, while the 500 and 750 mg/kg boric acid-dosed groups contained 10 animals each. The second exposure scenario consisted of 160 timed pregnant animals weighed on gd 6 and assigned to 1 of 10 groups: controls treated on each of gd 6-8 (one group); controls treated only on a single gd 6, 7, or 8 (three groups); and groups of dams treated with a gavage dose of 400 mg/kg twice daily (total dose 800 mg/kg-day) on each of gd 6-8 (one group) or only on a single gd 6, 7, 8, 9, 10 (four groups). The third exposure regimen consisted of either a single or two gavage doses of 750 mg/kg each on gd 8. In the group with a single gavage dose on gd 8, 52 pups from four control litters and 33 pups from three boric acid-dosed litters were examined. For the group with two gavage doses of 750 mg/kg each on gd 8, 103 controls and 94 boric acid-treated fetuses were examined, weighed, and stained with alizarin red and alcian blue for skeletal evaluation on gd 17.

Results from the first experiment indicated that 400 mg/kg daily doses resulted in decreased rib length, and daily doses of 750 mg/kg resulted in decreased rib length and femur length. Fetal body weight was not significantly decreased at either dose. In the second study, the results for the gd 9 and 10 daily exposures were not presented due to the lack of a concurrent control. Fetal body weight was reduced in all boric acid treatment groups (single days gd 6, 7, 8, 9, or 10 and consecutive days from gd 6 to 8). Femur length was decreased on gd 7 and in fetuses exposed for the gd 6-8 period. Cervical ribs were observed in fetuses exposed on gd 6, 7,

or 8. Results from the third experiment indicated that the two doses of 750 mg/kg each on gd 8 significantly increased frequency of 11 separate malformations over background incidence. The most prevalent malformations were those associated with rib development. In contrast, the single dose on this day produced only increased incidences of unilateral thoracic vertebrae and cervical rib formation/ossification differences. These results demonstrate a separation of the effects of boric acid on fetal body weight and rib malformation with respect to the timing of the dose. The authors concluded that the accumulation of the effect, rather than the accumulation of boric acid, was responsible for the temporal dependence of boric acid-induced fetotoxicity, citing a rapid clearance of borates from the blood. They specifically indicated that, "because of boric acid's short half-life, these data suggest that these earlier processes, gastrulation and presomitic mesoderm formation and patterning, are the processes boric acid is affecting."

To examine the molecular basis for boric acid's effect on axial skeletal development, Wery et al. (2003) dosed pregnant Sprague-Dawley rats (animal number not given) with two separate gavage doses of 500 mg/kg each on gd 9 and sacrificed the dams on gd 11 or gd 13.5. Embryos were removed and fixed for *in situ* hybridization to ascertain the distribution of several *hox* genes. These genes show a distinct pattern of expression among the semites responsible for the cranial-caudal development of the axial skeleton (vertebrae, ribs). Following boric acid administration on gd 9, the anatomic boundary for expression of *hoxd4*, *hoxa4*, *hoxc5*, and *hoxc6* were altered when assessed on gd 11. When assessed on gd 13.5, the boundary for expression of *hoxd4*, *hoxa4*, *hoxa5*, and *hoxc5* was not altered, while the boundary for *hoxa6* was altered. The authors concluded that the nature and exposure timing-dependency of the skeletal malformations support a role for *hox* gene alteration in the mechanism of boric acid-induced axial skeletal malformations.

7.3.2 Reproductive Studies

7.3.2.1 Male-Only Exposure

Studies of subchronic and chronic toxicity of boron compounds in dogs, rats, and mice have identified the testes as a primary target organ in males of these species (e.g., Weir and Fisher, 1972; NTP, 1987). These studies were described in Section 4.2.1. Several other studies have been conducted to investigate the effects of boron compounds on male reproductive performance and testicular morphology in more detail.

Dixon et al. (1976) studied the effects of borax on reproduction in male rats following acute and subchronic exposure. In the acute study, groups of 10 adult male Sprague-Dawley rats were given single oral doses of borax at 0, 45, 150, and 450 mg B/kg. Fertility was assessed by serial mating trials in which each male was mated with a series of untreated virgin females in sequential 7-day periods (for up to 70 days). The females were sacrificed 9 days after the end of their breeding periods (when they would be 9-16 days pregnant), and uteri and fetuses were examined. Male rats were sacrificed on days 1 and 7, and at subsequent 7-day intervals for histopathological examination of the testes. No effect on male fertility was found at any dose in this study. Testicular lesions were not reported. This study found an NOAEL of 450 mg B/kg for reproductive effects in male rats following single-dose oral exposure.

In the subchronic study, male Sprague-Dawley rats (10/group) were given 0, 0.3, 1.0, or 6.0 mg B/L, as borax, in the drinking water for 30, 60, or 90 days (Dixon et al., 1976). The investigators estimated the highest exposure level provided 0.84 mg B/kg-day. Based on this estimate, the lower two levels provided 0.042 and 0.14 mg B/kg-day. There were no noticeable reproductive effects or changes in serum chemistry; plasma levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH); or weight of the body, testes, prostate or seminal vesicles. Fructose, zinc and acid phosphatase levels in the prostate were unchanged. Breeding studies revealed no effects on male fertility. Therefore, the dose of 0.84 mg B/kg-day, the highest dose tested, represents an NOAEL for this study.

In a follow-up study reported by Dixon et al. (1979) and Lee et al. (1978), diets containing 0, 500, 1000, or 2000 ppm boron, as borax, were administered to male Sprague-Dawley rats (18/group) for 30 or 60 days (approximately 0, 25, 50, or 100 mg B/kg-day). Significant ($p<0.05$) decreases in the weight of liver, testes, and epididymis were observed at the 1000 and 2000 ppm dietary levels. Seminiferous tubule diameter was significantly ($p<0.05$) decreased in a dose-dependent manner in all treatment groups; however, significant loss of germinal cell elements was observed only at the 1000 and 2000 ppm dietary levels. Aplasia was complete at the highest dose. Plasma levels of the hormone FSH were significantly ($p<0.05$) elevated in a dose- and duration-related manner at all dose levels, while plasma LH and testosterone levels were not affected significantly. Serial mating studies revealed reduced fertility without change in copulatory behavior at the two higher dose levels. Based on dose-related tubular germinal aplasia, which is reversible at low doses, this study defines an LOAEL of 50 mg B/kg-day and an NOAEL of 25 mg B/kg-day.

Linder et al. (1990) examined the time- and dose-response of male rat reproductive endpoints after acute administration of boric acid. In the time-response experiment, Sprague-Dawley rats (6/group) were given 0 or 2000 mg boric acid/kg bw (0 or 350 mg B/kg, respectively) by gavage and were sacrificed at 2, 14, 28, and 57 days after dosing. In the dose-response experiment, groups of eight male rats were administered 0, 250, 500, 1000, or 2000 mg boric acid/kg (0, 44, 87, 175, or 350 mg B/kg) by gavage and were sacrificed 14 days later. In both the time-response and the dose-response studies, the above doses are the totals of two doses administered at 9:00 a.m. and 4:00 p.m. on the same day. No significant clinical signs of toxicity were observed during the study. Histopathologic examinations of the testes and epididymis revealed adverse effects on spermiation, epididymal sperm morphology, and caput sperm reserves. The testicular effects, apparent at 14 days, included enlarged irregular cytoplasmic lobes of Step 19 spermatids in stage VIII seminiferous tubules and retention of Step 19 spermatids in stage IX-XIII tubules at the 175 and 350 mg B/kg dose levels. There was also a substantial increase ($p<0.05$) in the testicular sperm head count per testis and per g testis in the 350 mg/kg time-response group. Epididymal effects, also apparent at 14 days, included an increase in abnormal caput epididymal sperm morphology (percentage with head or tail defects, $p<0.05$) and reduced caput epididymal sperm reserves ($p<0.05$). In the day 28 time-response group (350 mg B/kg), significant effects ($p<0.05$) included an increase in abnormal caput and cauda epididymal sperm morphology and a decreased percentage of motile cauda spermatozoa with reduced straight-line swimming velocities. Substantial recovery occurred by day 57. This study described an LOAEL for male reproductive effects of 175 mg B/kg bw and an NOAEL of 87 mg B/kg bw following acute oral exposure in rats.

Treinen and Chapin (1991) examined the development and progression of reproductive lesions in 36 mature male F344 rats treated with boric acid in the diet for 4-28 days. Thirty animals served as controls. Boric acid was added to the feed at a level of 9000 ppm. Based on food consumption and body weight data, the investigators estimated that over the 28-day period the mean intake of boric acid was 348.3 mg/kg-day, or 60.9 mg B/kg-day. Sacrifices were conducted at 4, 7, 10, 14, 21, and 28 days on six treated and four control animals per time point. Liver, kidney, and testicular histology; serum testosterone; androgen binding protein (ABP) levels; and tissue boron levels were assessed. In half of the treated rats, there was inhibition of spermiation in 10-30% of stage-IX tubules at 7 days. Inhibited spermiation was observed in all stage-IX and stage-X tubules of exposed rats at 10 days. Advanced epithelial disorganization, cell exfoliation, luminal occlusion, and cell death were observed after 28 days, causing significant loss of spermatocytes and spermatids from all tubules in exposed rats. Throughout the study, specific lesions became more severe with increasing duration of exposure. Treatment with boric acid had no effect on kidney and liver histology. In treated rats, basal serum testosterone levels were significantly decreased ($p<0.05$) from 4 days on, but serum testosterone levels stimulated by human chorionic gonadotropin or luteinizing hormone releasing factor were not affected. Steady-state levels of boron were reached in tissues by 4 days of treatment, and there was no selective accumulation of boron in blood, epididymis, liver, or kidney. After 4 days of treatment with boric acid, serum ABP levels were significantly reduced relative to controls; however, this difference disappeared by day 7.

Ku et al. (1993a) and Chapin and Ku (1994) compared testis boron dosimetry to lesion development. Rats were fed 0, 3000, 4500, 6000, or 9000 ppm boric acid (0, 545, 788, 1050, or 1575 ppm boron) for up to 9 weeks and examined. Based on food intake and body weight data, the researchers estimated the daily intake of boron as <0.2, 26, 38, 52, or 68 mg B/kg-day. At 32 weeks post-treatment, recovery was assessed. Inhibited spermiation occurred at 3000 and 4500 ppm, and atrophy occurred at 6000 and 9000 ppm. A mean testis boron level of 5.6 g B/g of tissue was associated with inhibited spermiation, whereas 11.9 g B/g was associated with atrophy, with no boron accumulation during the 9-week exposure. This suggests that separate mechanisms may be operating for these effects based on testis boron concentration. Severely inhibited spermiation at 4500 ppm was resolved by 16 weeks post-treatment, but some areas of focal atrophy in the 6000 and 9000 ppm dose groups did not change post-treatment. The low dose of 26 mg B/kg-day was an LOAEL in this study.

Following *in vitro* boric acid exposure, Ku et al. (1993b) evaluated endpoints in the cell culture system that suggest that boric acid has an effect on DNA synthesis that occurred at concentrations associated with atrophy *in vivo*, and suggests that boric acid interferes with the production and maturation of early germ cells.

Ku and Chapin (1994) showed that testicular atrophy and CNS hormonal effects were not due to selective accumulation in testis or brain/hypothalamus with boron testis concentrations of 1-2 mM. *In vitro* studies addressed boric acid testicular toxicity: mild hormone effect, the initial inhibited spermiation, and atrophy. No effect of boric acid on the steroidogenic function of isolated Leydig cells was observed, supporting the suggestion of a CNS mediated hormonal effect. The authors found that inhibited spermiation was not due to increased testicular cyclic adenosine monophosphate (cAMP) or reduced serine proteases plasminogen activators (PA). Boric acid effects were evaluated in Sertoli-germ cell co-cultures on Sertoli cell energy

metabolism (lactate secreted by Sertoli cells is a preferred energy source for germ cells) and DNA/RNA syntheses (germ cells synthesize DNA/RNA and boric acid impairs this nucleic acid in the liver). The most sensitive *in vitro* endpoint was DNA synthesis of mitotic/meiotic germ cells (with energy metabolism in germ cells affected to a lesser extent), which was manifested *in vivo* as a decrease in early germ cell/Sertoli cell ratio prior to atrophy in the testes.

Naghii and Samman (1996) administered boric acid in deionized drinking water to adult male Sprague Dawley rats (10 per group) at 2, 12.5, and 25 mg B/day for up to 6 weeks. Plasma testosterone levels increased in rats fed 2 mg B/day, but increasing boron dose from 2 mg to 12.5 and 25 mg resulted in lower plasma testosterone concentrations which tended to rebound at 6 weeks of treatment. The response tended to be greater after 6 weeks compared to 3 weeks. Similarly testicular testosterone concentrations also decreased with increasing boron dose, but the difference between weeks 3 and 6 was more marked. The authors suggested that Leydig cells, which are responsible for production of testosterone, are intact in rats fed 25 mg B in spite of testicular atrophy. The authors also stated that these results are consistent with Weir and Fisher (1972) who found testicular histopathology in rats fed 23-30 mg B/day for 90 days and atrophy when boron concentration in the testes was greater than 20 ppm.

Naghii and Samman (1997) studied the specificity of the effect of boron on steroid hormones and the impact of plasma lipids in eight male volunteers whose diets were supplemented with10 mg B per day for 4 weeks. Plasma total cholesterol, triglyceride concentrations, or distribution among LDL and HDL fractions were not altered. The mean total plasma testosterone concentration increased after 4 weeks of supplementation, but this increase was not statistically significant. The mean plasma 17Bβ-estradiol concentration increased significantly, and the ratio of 17β-estradiol to testosterone increased significantly after supplementation.

7.3.2.2 Male and Female Exposure

In a multigeneration study, Weir and Fisher (1972) administered 0, 117, 350, or 1170 ppm boron (approximately 0, 5.9, 17.5, or 58.5 mg B/kg-day) as borax or boric acid in the diet to groups of 8 male and 16 female Sprague-Dawley rats. No adverse effects on reproduction or gross pathology were observed in the rats dosed with 5.9 or 17.5 mg B/kg-day that were examined to the F3 generation. Litter size, weights of progeny, and appearance were normal when compared with controls. The test groups receiving 58.5 mg B/kg-day boron from either compound were found to be sterile. In these groups, males showed lack of spermatozoa in atrophied testes, and females showed decreased ovulation in the majority of the ovaries examined. An attempt to obtain litters by mating the treated females with the males fed only the control diet was not successful. An LOAEL of 58.5 mg B/kg-day and an NOAEL of 17.5 mg B/kg-day were identified from this study.

Fail et al. (1990, 1991) examined the effects of boric acid in Swiss CD-1 mice in a reproductive study using a continuous breeding protocol. Male and female F_0 mice (11 weeks old) were fed a diet containing 0, 1000, 4500, or 9000 ppm boric acid for up to 27 weeks. There were 40 pairs in the control group and 20 pairs per treatment group. Based on an average food consumption of 5 g/mouse and on body weights, the authors predicted the diet would provide boric acid at 152 mg/kg-day (26.6 mg B/kg-day) to males and 182 mg/kg-day (31.8 mg

B/kg-day) to females in the 1000 ppm group; 636 mg/kg-day (111 mg B/kg-day) to males and 868 mg/kg-day (152 mg B/kg-day) to females in the 4500 ppm group; and 1260 mg/kg-day (220 mg B/kg-day) to males and 1470 mg/kg-day (257 mg B/kg-day) to females in the 9000 ppm group. According to the authors, actual boric acid consumption during the study did not differ from the predicted consumption by more than 12%. Following 1 week of treatment, the F_0 mice were caged as breeding pairs for 14 weeks. During weeks 2-18, the average body weight gain of high-dose males and females was significantly reduced relative to controls. Mortality rates in the treated groups over the 27 weeks were not significantly different from controls. Treatment with boric acid significantly impaired fertility. None of the 9000 ppm pairs were fertile. The number of litters per pair, number of live pups per litter, proportion of pups born alive, live pup weight, and adjusted pup weight (adjusted for litter size) were significantly ($p<0.05$) decreased at the 4500 ppm level. The initial fertility index (percentage of cohabited pairs having at least one litter) was not significantly altered in the 1000 and 4500 ppm groups, but the progressive fertility index (percentage of fertile pairs that produced four litters) was decreased relative to controls in the 4500 ppm group. The trend toward a lower fertility index at 4500 ppm started with the first mating and progressed in severity with subsequent matings.

To determine the affected sex, the control and 4500 ppm F_0 mice were then assigned to three crossover mating groups: control male x control female, 4500 ppm male x control female, and control male x 4500 ppm female. Each group was composed of 19-20 pairs that were mated for 7 days or until a copulatory plug was detected, whichever occurred first; control feed was provided for all mice during this week, followed by a resumption of the same diets they had received previously. Mating and fertility indices were significantly depressed in the 4500 ppm male x control female group, and only one pair in that group produced a live litter; these indices were not affected in the control male x 4500 ppm female group. Dosed females mated to control males had a lower body weight on pnd 0, had a longer gestational period than control groups and gave birth to pups with decreased litter-adjusted weight. After completion of the crossover mating trial (total of 27 weeks on test), a necropsy was performed on control and 4500 ppm F_0 males and females and on 1000 and 9000 ppm F_0 males that had been maintained on their respective diets to allow a comparison of semen parameters and testicular histology among all four treatment groups. Males treated with 9000 ppm boric acid had significantly reduced body, testis and epididymal weights. In the 4500 ppm males, body weight was not affected, but testis, epididymal, and prostate weights were reduced; these parameters were not altered in the 1000 ppm males. Significant reductions in sperm motility were observed in the 1000 and 4500 ppm groups and in sperm concentration in the 4500 and 9000 ppm groups. The percentage of abnormal sperm was significantly increased in the 4500 ppm group. Sperm motility and morphology could not be fully evaluated in the 9000 ppm group due to absence of sperm (in 12 of 15 observed males) or severe reduction in sperm counts (in the other 3 males) of this group. Seminiferous tubular atrophy occurred in mid-and high-dose males; the severity was dose-related. Tissues of low-dose males exhibited no significant changes. Other indices of testicular morphology (spermatogenic index, seminiferous tubule diameter, spermatids per testis) also were altered at 4500 ppm or more. Effects observed at necropsy in 4500 ppm females (1000 and 9000 ppm females were not examined) were limited to a reduction in both relative and absolute liver weights and absolute kidney plus adrenal weights in comparison with controls.

The final F_1 litters (exposed during gestation and lactation) from the continuous breeding experiment were fed the same dosage of boric acid in the diet as their parents had received.

Because there were no litters at 9000 ppm and few of the mice born alive in the final litters at 4500 ppm survived through weaning, only the 0 and 1000 ppm F_1 mice were included in a fertility trial. The F_1 mice were cohabited in nonsibling pairs (40 pairs of 0 ppm and 20 pairs of 1000 ppm mice) for 7 days or until a copulatory plug was observed, whichever occurred first. They were maintained on their respective diets during mating and until the F_2 litters were delivered, and then were necropsied. The fertility of the 1000 ppm F_1 mice was not affected, but the litter-adjusted body weights of the F_2 pups (females and combined males and females) were significantly decreased relative to controls. Effects in 1000 ppm F_1 females were significant increases in uterine and kidney plus adrenal weights, significantly shorter estrous cycles, and fewer ambiguous vaginal smears. A reduction in epididymal sperm concentration in the 1000 ppm F_1 males approached significance ($p=0.053$); sperm motility and morphology were not affected. Histopathologic examination was unremarkable. The lowest dose tested, 1000 ppm, decreased sperm motility in the F_0 males, marginally decreased epididymal sperm concentration in F_1 males, increased uterine and kidney/adrenal weights and shortened estrus cycles in F_1 females, and reduced litter-adjusted birth weights in the F_2 pups. Hence, the LOAEL for this study is 1000 ppm boric acid (26.6 and 31.8 mg B/kg-day for males and females, respectively). An NOAEL was not identified.

7.4 Other Studies

7.4.1 Genotoxicity Studies

Results of most short-term mutagenicity studies indicate that boron is not genotoxic. In the streptomycin-dependent *Escherichia coli* Sd-4 assay, boric acid was either not mutagenic (Iyer and Szybalski, 1958; Szybalski, 1958) or produced equivocal results (Demerec et al., 1951). In *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, boric acid was not mutagenic in the presence or absence of either a rat or hamster liver S-9 activating system (Benson et al., 1984; Haworth et al., 1983; NTP, 1987). Boric acid (concentration, stability, and purity not tested by investigators) was also negative for mutagenicity in the *Salmonella* microsome assay using strains TA1535, TA1537, TA1538, TA98, and TA100 in both the presence and absence of rat liver metabolic activation (Stewart, 1991). Although a positive result was reported both with and without metabolic activation for induction of β-galactosidase synthesis (a response to DNA lesions) in *E. coli* PQ37 (SOS chromotest) (Odunola, 1997), this is an isolated finding at present.

Results in mammalian mutagenicity test systems were all negative. Boric acid (concentration, stability, and purity not tested by investigators) was negative in inducing unscheduled DNA synthesis in primary cultures of male F344 rat hepatocytes (Bakke, 1991). Boric acid did not induce forward mutations in L5178Y mouse lymphoma cells with or without S-9 (NTP, 1987). Boric acid did not induce mutations at the thymidine kinase locus in the L5178Y mouse lymphoma cells in either the presence or absence of a rat liver activation system (Rudd, 1991). Crude borax ore and refined borax were both negative in assays for mutagenicity in V79 Chinese hamster cells, C3H/1OT1/2 mouse embryo fibroblasts, and diploid human foreskin fibroblasts (Landolph, 1985). Similarly, boric acid did not induce chromosome aberrations or increase the frequency of sister chromatid exchanges in Chinese hamster ovary cells with or without rat liver metabolic activating systems (NTP, 1987).

O'Loughlin (1991) performed a micronucleus assay on Swiss-Webster mice (10 animals/sex/dose). Boric acid was administered in deionized water orally (no verification of stability, concentration, or homogeneity was made of the boric acid by the investigators) for 2 consecutive days at 900, 1800 or 3500 mg/kg. Five mice/sex/dose were sacrificed 24 hours after the final dose, and 5/sex/dose were sacrificed 48 hours after the final dose. A deionized water vehicle control (10/sex) and a urethane positive control (10 males) were also tested. Boric acid did not induce chromosomal or mitotic spindle abnormalities in bone marrow erythrocytes in the micronucleus assay in Swiss-Webster mice.

7.4.2 Neurological Studies

Sodium tetraborate was administered in the drinking water to 2-month-old Wistar rats for up to 14 weeks. Exposure to approximately 20.8 mg B/kg-day caused an increase in cerebral succinate dehydrogenase activity after 10-14 weeks of exposure (Settimi et al., 1982). Increased acid proteinase activity and increased RNA were also noted at the end of the 14-week experiment.

ATSDR (1992) and Wong et al. (1964) reported on case reports of neurological effects after accidental ingestion of high levels of boron as boric acid. Newborn infants (number not given) who ingested 4.5-14 g boric acid showed these CNS symptoms. Doses of about 500 mg B/kg-day showed CNS involvement with headaches, tremors, restlessness and convulsions followed by weakness, coma, and death. Histological examination of 2/11 infants revealed degenerative changes in brain neurons, congestion, and edema of brain and meninges with perivascular hemorrhage and intravascular thrombosis.

O'Sullivan and Taylor (1983) reported convulsions and seizures in seven infants exposed to a honey-borax mixture for 4-10 weeks, in which the estimated ingestion was 9.6-33 mg B/kg-day.

Litovitz et al. (1988) conducted a retrospective review of 784 cases of boric acid ingestion. An estimate of the amount of boric acid ingested was obtained historically in 659 cases. The average amount ingested was 1.4 g. The average dose was estimated to be 0.5 g for children under 6 years of age, compared to 4.1 g for individuals 6 years of age and above. Symptoms most frequently reported were vomiting, abdominal pain, diarrhea, and nausea. Other symptoms, including CNS and cutaneous effects, occurred in six or fewer cases and included rash, lethargy, headache, lightheadedness, fever, irritability, and muscle cramps. The average dose for asymptomatic cases was 0.9 g compared with 3.2 g for symptomatic cases.

Neurological effects were noted in human case reports after ingestion of high levels of boron. Animal data are limited to increased brain enzyme levels after 10-14 weeks of exposure (Settemi et al., 1982). There is an uncertainty about neurological effects at lower doses and other than acute duration because no data are available. This is identified as an area where further research may be beneficial.

7.4.3 Mechanistic Studies - Testicular Effects

The occurrence of testicular effects in the absence of overt systemic toxicity suggests a testicular-specific mechanism of action for boron. Many studies have been conducted to elucidate the mechanism by which boron produces testicular effects (see Section 7.2.5 for descriptions of some of these studies). Recent reviews of this work have been published by Fail et al. (1998) and ECETOC (1994). Despite the number of studies that have been done, the mechanism of boron testicular toxicity remains unknown. The available data suggest an effect on the Sertoli cell, resulting in altered physiological control of sperm maturation and release (Fail et al., 1998).

7.4.4 Mechanistic Studies - Developmental Effects

Studies regarding the mechanism of developmental toxicity produced by boron were reviewed by Fail et al. (1998). The two most sensitive effects of boron on developing rodents are decreased fetal body weight and malformations and variations of the ribs. Fail et al. (1998) concluded that reduced fetal growth probably results from a general inhibition of mitosis produced by boric acid, as documented in studies on the mammalian testis, insects, yeast, fungi, bacteria, and viruses (Beyer et al., 1983; Ku et al., 1993b), while the rib malformations probably result from direct binding of boron to the bone tissue. More recent investigations of the developmental effects of boric acid (Narotsky et al., 2003; Wery et al., 2003) have produced evidence supporting a role of altered gene expression in boron's developmental effects. These data indicate that boric acid administration during the normal period of expansion of *hox* gene expression results in rib and vertebrae alterations, coincident with altered *hox* gene expression.

7.4.5 Nutrition Studies

Since the 1920s, boron has been known to be an essential micronutrient for the growth of all plants. In humans, boron is a trace element for which essentiality is suspected but has not been directly proven (Nielsen, 1991, 1992, 1994; NRC, 1989; Hunt, 1994; Mertz, 1993). Because deficiency in humans has not been established, there are no adequate data from which to estimate a human requirement, and no provisional allowance has been established (NRC, 1989). However, boron deprivation experiments with animals and three human clinical studies have yielded some persuasive findings for the hypothesis that boron is nutritionally essential as evidenced by the demonstration that it affects macromineral and cellular metabolism at the membrane level (Nielsen, 1994). Experimental boron nutrition research data indicate that boron can affect the metabolism or utilization of a number of substances involved in life processes, including calcium, copper, magnesium, nitrogen, glucose, triglyceride, reactive oxygen, and estrogen. These effects can affect the composition of several body systems including blood, brain, and skeleton (Nielsen, 1996). Boron may prevent inflammatory disease because several key regulatory enzymes in the inflammatory response are inhibited by physiological amounts of supplemental dietary boron (Hunt, 1996). New boron nutrition research should better characterize the mechanisms through which boron modulates immune function, insulin release, and vitamin D metabolism (Hunt, 1996). A close interaction between boron and calcium has been suggested. This interaction appears to affect similar systems that indirectly affect many variables, including modification of hormone action and alteration of cell membrane characteristics (Nielsen et al., 1987; Nielsen, 1991, 1992, 1994; Penland, 1994). Data from three

human studies of potential boron essentiality demonstrate that dietary boron can affect bone, brain, and kidney variables. The subjects in most of these studies, however, were under some form of nutritional or metabolic stress affecting calcium metabolism, including reduced intake of magnesium or physiologic states associated with increased loss of calcium from bone or the body (e.g., postmenopausal women).

Based on these studies, in which most subjects who consumed 0.25 mg B/day responded to additional boron supplementation, Nielsen (1991) concluded that the basal requirement for boron is likely to be greater than 0.25 mg/day. Limited survey data indicate that the average dietary intake of boron by humans is 0.5-3.1 mg-day (7-44 µg/kg-day) (Nielsen, 1991). The average U.S. adult male dietary intake of 1.52 ± 0.38 mg B/day (mean ± standard deviation) (Iyengar et al., 1988) was determined by U.S. Food and Drug Administration (FDA) Total Diet Study methods. In a more recent study, Anderson et al. (1994) reported an intake of 1.21 ± 0.07 mg B/day for an average diet for 25- to 30-year-old males, as determined by FDA Total Diet Study analyses. Similarly, the average dietary boron intake in Canada is reported to be 1.33 ± 0.13 mg B/day for women (Clarke and Gibson, 1988). Dietary boron consumption in Europe could be higher than in the United States and Canada due to wine consumption (ECETOC, 1994). These and other investigators (Nielsen, 1992) also recognized that greater consumption of fruits, vegetables, nuts, and legumes (e.g., vegetarian diets) could raise dietary boron intake.

The Institute of Medicine (IOM, 2001) developed a tolerable upper intake level (UL), the highest daily nutrient intake that is likely to pose no risk of adverse health effects for most individuals, for various life stages of humans. A UL for infants was judged not determinable. The UL for adults was 20 mg B/day. The UL was set at 17 mg B/day for pregnant women 14-18 years of age, while the UL for pregnant women 19-50 years of age was set at 20 mg B/day. Section 5.1.3. describes how these ULs were determined.

7.5 Synthesis and Evaluation of Major Noncancer Effects and Mode of Action - Oral and Inhalation

7.5.1 Oral Exposure

Studies in laboratory animals conducted by oral exposure have identified the developing fetus and the testes as the two most sensitive targets of boron toxicity in multiple species (Weir and Fisher, 1972; Seal and Weeth, 1980; NTP, 1987; Fail et al., 1991; Price et al., 1996a,b; Field et al., 1989). The testicular effects that have been reported include reduced organ weight and organ:body weight ratio, atrophy, degeneration of the spermatogenic epithelium, impaired spermatogenesis, reduced fertility, and sterility (Weir and Fisher, 1972; Seal and Weeth, 1980; NTP, 1987; Fail et al., 1991; Dixon et al., 1979; Linder et al., 1990; Treinen and Chapin, 1991; Ku et al., 1993a). The mechanism for boron's effect on the testes is not known, but the available data suggest an effect on the Sertoli cell, resulting in altered physiological control of sperm maturation and release (Fail et al., 1998). Developmental effects have been reported in mice, rabbits, and rats (Heindel et al., 1992, 1994; Field et al., 1989; Price et al., 1991, 1996a,b). The developmental effects that have been reported following boron exposure include high prenatal mortality; reduced fetal body weight; and malformations and variations of the eyes, CNS, cardiovascular system, and axial skeleton (Price et al., 1996a,b; Field et al., 1989). Increased incidences of short rib XIII (a malformation) and wavy rib (a variation), and decreased incidence

of rudimentary extra rib on lumbar I (a variation), were the most common anomalies in both rats and mice. Cardiovascular malformations, especially interventricular septal defect, and variations were the frequent anomalies in rabbits. Fail et al. (1998) attributed reduced fetal growth, the most sensitive developmental endpoint, to a general inhibition of mitosis by boric acid, as documented in studies on the mammalian testis, insects, yeast, fungi, bacteria, and viruses (Beyer et al., 1983; Ku et al., 1993b).

7.5.2 Inhalation Exposure

Studies in humans and animals have shown that borates are absorbed following inhalation exposure (Culver et al.,1994; Wilding et al., 1959). It is not clear what percentage of the absorbed material in these studies was absorbed via the respiratory tract directly; transport of deposited material from the upper respiratory tract to the gastrointestinal tract may have played an important role (Culver et al.,1994). However, because borates in the body exist as boric acid, are distributed evenly throughout the soft tissues in the body water, and are not metabolized (Ku et al., 1991; Naghii and Samman, 1996; WHO, 1998a), there is no reason to expect route-specific differences in systemic targets. Therefore, systemic target tissues identified in oral studies comprise the potential systemic targets following inhalation exposure. There may be route-specific differences in ability to deliver toxic doses to the targets, in that very high exposure concentrations may be required to produce effects by inhalation exposure. Portal-of-entry effects may also differ with exposure route.

The literature regarding the toxicity of boron by inhalation exposure is sparse. There is a report from the Russian literature of reduced sperm analysis of 6 workers who were part of a group of 28 male workers exposed to high concentrations of boron (boric acid) aerosols (22-80 mg/m^3) for more than 10 years (Tarasenko et al., 1972). These effects are consistent with the testicular effects reported in oral studies, but have not been confirmed by other inhalation studies. However, data from Tarasenko et al. (1972) are of limited value for risk determination due to sparse details and small sample size. No effect on fertility was found in a far larger study of U.S. borate production workers (Whorton et al., 1992; 1994a,b), but exposure concentrations were much lower (about 2.23 mg/m^3 sodium borate or 0.31 mg B/m^3) in this study. No target organ effects were found in the lone animal study in which rats were exposed to 77 mg/m^3 of boron oxide aerosols (24 mg B/m^3) for 24 weeks, but testicular effects were examined only by limited histopathology (Wilding et al., 1959). This study also included a high-dose group exposed to 470 mg/m^3 boron oxide (146 mg B/m^3) for 10 weeks, a concentration at which the aerosol formed a dense cloud of fine particles that covered the animals with dust. Systemic endpoints were not examined, but growth was reduced by 9% in the high-dose group, and there was evidence of nasal irritation. Acute irritant effects are well documented in human workers exposed to borates, primarily at concentrations greater than 4.4 mg/m^3 (Wegman et al., 1994; Garabrant et al., 1984, 1985). However, there is no evidence for reduced pulmonary function in workers with chronic exposure (Wegman et al., 1994). These data are inadequate to support derivation of an RfC for boron compounds.

7.6 Weight of Evidence Evaluation and Cancer Characterization - Synthesis of Human, Animal, and Other Supporting Evidence, Conclusions About Human Carcinogenicity, and Likely Mode of Action

Under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005), the data are considered to be inadequate for an assessment of the human carcinogenic potential of boron. No data were located regarding the existence of an association between cancer and boron exposure in humans. Studies available in animals were inadequate to ascertain whether boron causes cancer. The chronic rat feeding study conducted by Weir and Fisher (1972) was not designed as a cancer bioassay. Only a limited number of tissues were examined histopathologically, and the report failed to mention any tumor findings. The chronic mouse study conducted by NTP (1987) was adequately designed, but the results are difficult to interpret. There was an increase in hepatocellular carcinomas in the low dose, but not the high dose, in which male mice were within the range of historical controls. The increase was statistically significant using the life table test, but not the incidental tumor test. The latter test is more appropriate when the tumor in question is not the cause of death, as appeared to be the case for this study. There also was a significant increase in the incidence of subcutaneous tumors in low-dose male mice. However, once again the increase was within the range of historical controls and was not seen in the high-dose group. Low survival in both the low- and high-dose male groups (60 and 40%, respectively) may have reduced the sensitivity of this study for evaluation of carcinogenicity. The chronic mouse study conducted by Schroeder and Mitchener (1975) was inadequate to detect carcinogenicity because only one, very low dose level was used (0.95 mg B/kg-day), and the MTD was not reached. No inhalation cancer data were located. Studies of boron compounds for genotoxicity were overwhelmingly negative, including studies in bacteria, mammalian cells, and mice *in vivo*.

7.7 Susceptible Populations

7.7.1 Possible Childhood Susceptibility

One of the most sensitive targets of boron that has been identified is the developing fetus (rats, mice and rabbits) carried by the pregnant female. A set of well-designed developmental studies in rats provided an LOAEL of 13.3 mg B/kg-day and an NOAEL of 9.6 mg B/kg-day in the developing fetus, based on decreased fetal body weight (Price et al., 1996a).

7.7.2 Possible Gender Differences

Another sensitive target of boron that has been identified is the testis of the male. A study in dogs provided an LOAEL of 29 mg B/kg-day and an NOAEL of 8.8 mg B/kg-day, based on histopathological effects (Weir and Fisher, 1972). Sensitivity to boron exposure does not appear to differ markedly for these two targets, although there is some uncertainty in this determination due to the less comprehensive design of the dog study.

Effects on the pregnant females themselves are seen only at considerably higher doses (no clearly adverse maternal effects even at 94.2 mg B/kg-day in the same study used to derive the NOAEL and LOAEL values for the developing fetus reported above). A specific target of boron toxicity has not been identified in nonpregnant females, who are markedly less susceptible

to boron than males. Data are inadequate to assess differences in gender susceptibility with regard to non-reproductive, non-developmental effects.

7.7.3 Physiological and Disease Anomalies

Because the removal of boron (boric acid) from mammals occurs via renal elimination of the unchanged molecule, alterations of renal function result in increased residence time. Decrements of renal function, therefore, will increase internal exposure, and may predispose affected individuals to greater risk from compounds for which renal elimination is important. The observed developmental toxicity of boron indicates that fetuses of pregnant women may be the susceptible group; those fetuses of women who are experiencing renal insufficiency may represent a sensitive sub-population. Preeclampsia is a health condition of pregnancy in which renal function, including glomerular filtration, is reduced.

8.0 DOSE-RESPONSE ASSESSMENT

8.1 Oral Reference Dose (RfD)

8.1.1 Choice of Principal Study and Critical Effect — with Rationale and Justification

Developmental effects (decreased fetal weights) are considered the critical effect. The studies by Price et al. (1990, 1994, 1996a) and Heindel et al. (1992) in rats were chosen as critical developmental studies because they were well-conducted studies of a sensitive endpoint that identified both an NOAEL and LOAEL. Rats were more sensitive than mice and rabbits, which were also studied for developmental toxicity (Price et al., 1996b; Heindel et al., 1994).

There was a consistent correlation between boric acid exposure and different effects on ribs and vertebral development in rats, mice and rabbits for which the rat was the most sensitive to low-dose effects. Because decreased fetal body weight in rats occurred at the same dose or at lower doses than those at which skeletal changes were observed, the decreased fetal body weight data set was chosen for developing a reference dose. IEHR (1997) agreed with the correlation between boric acid exposure and the different effects on rib and vertebral development in rats, mice, and rabbits and the causal association between exposure to boric acid and the short rib XIII (when fetuses were examined at late gestation or when pups were examined at pnd 21) and that decreased fetal body weight should be used for deriving quantitative estimates.

The dog study by Weir and Fisher (1972) identified an NOAEL of 8.8 mg/kg-day and LOAEL of 29 mg/kg-day for testicular effects. Testicular effects were found at higher doses in rats and mice in this and other studies (Weir and Fisher, 1972; Seal and Weeth, 1980; NTP, 1987; Fail et al., 1991; Dixon et al., 1979; Linder et al., 1990; Treinen and Chapin, 1991; Ku et al., 1993a). These effects include testicular atrophy, inhibition of spermiation, degeneration of seminiferous tubules with germ cell loss, and loss of fertility. In a rat multigeneration study by Weir and Fisher (1972) an NOAEL of 17.5 mg/kg-day and an LOAEL of 58.5 mg/kg-day for testicular atrophy was reported in male Sprague Dawley rats. Ku et al. (1993a) reported an NOAEL of 26 mg/kg-day for inhibited spermiation in male Sprague Dawley rats. Fail et al. (1991) reported an LOAEL of 26.8 mg/kg-day in male Swiss CD mice for decreased sperm motility. Because the LOAELs for testicular effects were more than 2-fold greater than the LOAEL for developmental effects, the Weir and Fisher dog study was not considered as the critical study. However, as no exposure level was tested in the dog study between 8.8 and 29 mg/kg-day, uncertainty remains as to whether testicular effects would have occurred near the same exposure leading to developmental effects.

The Weir and Fisher (1972) study in dogs had other limitations for RfD derivation, including small number of test animals per dose group (n=4), the use of shared control animals in the borax and boric acid studies so that at most two control animals were sacrificed at any time period, the observation of testicular damage in three of four control animals, and the NOAEL and LOAEL taken from two different studies of different duration. Also, the study pathologist considered the histopathological findings to be “not compound-induced.” Based on the small number of animals and the wide range of background variability among the controls, these studies do not appear to be adequate for establishment of a defensible RfD.

8.1.2 Methods of Analysis — Including Models

The RfD was derived by the benchmark dose (BMD) approach. Several BMD analyses were conducted by Allen et al. (1996) using all relevant endpoints in the Heindel et al. (1992) and Price et al. (1994, 1996a) developmental studies in rats. Allen et al. (1996) concluded that decreased fetal body weight was the most suitable endpoint for developing a point of departure, because the benchmark doses calculated for the other endpoints (incidence of total malformations, enlarged lateral ventricles in the brain, shortening of rib XIII, and variations of the first lumbar rib) were higher.

Changes in fetal weight were analyzed by taking the average fetal weight for each litter with live fetuses. Those averages were considered to represent variations in a continuous variable, and a continuous power model was used. A BMD was defined in terms of a pre-specified level of response, referred to as the benchmark response (BMR) level (Kavlock et al., 1995). For mean fetal weight analysis, the 95% lower confidence limit on the benchmark dose (BMDL) was defined as the 95% lower bound on dose corresponding to a 5% decrease in the mean (that is, the BMR in this case is a 5% decrease in mean fetal weight per litter). This BMR is approximately equivalent to a 0.5 standard deviation decrease in the control mean, or an extra risk of about 5% of an exposed population having litters with mean fetal body weights less than those of 98% of the control population. Goodness of fit was evaluated using F-tests that compared the lack of model fit to an estimate of pure error.

The earlier study by Heindel et al. (1992) did not define an NOAEL, while the later study by Price et al. (1996a) was designed as a follow up study to the Heindel study to examine fetal body weight at lower doses to define an NOAEL. Allen et al. (1996) examined the dose-response patterns for the two studies to determine if a single function could adequately describe the responses in both studies. This determination was based on a likelihood ratio test. The maximum log-likelihoods from the models fit to the two studies considered separately were added together; the maximum log-likelihood for the model fit to the combined results was then subtracted from this sum. Twice that difference is distributed approximately as a chi-square random variable (Cox and Lindley, 1974). The degrees of freedom for that chi-square random variable are equal to the number of parameters in the model plus 1. The additional degree of freedom was available because the two control groups were treated as one group in the combined results, which eliminates the need to estimate one of the intra-litter correlation coefficients (for beta-binomial random variables) or variances (for normal random variables) that was estimated when the studies were treated separately. The critical values from the appropriate chi-square distributions (associated with a p-value of 0.01) were compared to the calculated values. When the calculated value was less than the corresponding critical value, the combined results were used to estimate BMDLs. The data and details of the modeling are provided in Appendix B.

The results of the Allen et al. (1996) BMD analysis for decreased fetal body weight for the Price study alone gave a BMDL of 47 mg boric acid/kg-day (8.2 mg B/kg-day), and for the Heindel study alone, the BMDL reported by Allen et al. (1996) was 56 mg boric acid/kg-day (9.8 mg B/kg-day). The statistical analysis described above demonstrated that the data were consistent, and could be combined to estimate a single dose-response function. The combined data from Heindel et al. (1992) and Price et al. (1994, 1996a) gave a $BMDL_{05}$ of 59 mg boric acid/kg-day (10.3 mg B/kg-day). The BMDL based on the combined results of the two studies

was very close to the NOAEL of 9.6 mg B/kg-day from the Price et al. (1994, 1996a) study. The $BMDL_{05}$ from the combined studies was chosen to derive the RfD because they were similarly designed studies conducted in the same laboratory, and all the dose response data were consistent enough to be used in the BMDL estimation, thereby increasing the confidence that the dose response pattern has been estimated satisfactorily.

Allen et al. (1996) noted that merely increasing sample size does not always increase the precision of the estimates of the BMD. For these datasets, however, the BMDLs estimated for the combined mean fetal weight data were closer to the corresponding BMDs than for either of the studies alone. That is, the confidence intervals around the best estimates of dose corresponding to the selected response level were narrower in the combined analysis.

8.1.3 Derivation of the RfD

Uncertainty factors (UFs) are applied in the RfD methodology to account for recognized uncertainties in extrapolation from experimental conditions to lifetime exposure for humans. These UFs cover somewhat broad areas of uncertainty, such as "animal-to-human" (interspecies; UFA) and "sensitive human" (interindividual; UFH) extrapolations. Both UFA and UFH, however, can be addressed as a combination of two subfactors, one each for toxicokinetics (TK) and toxicodynamics (TD).[1] The TK/TD "paradigm" formally allows for the quantitative incorporation of additional data previously used in only a qualitative fashion. The concept is applied in the Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (U.S. EPA, 1994b), in which the kinetic component deals primarily with airway anatomy and physiology, but does not address systemic kinetics and dynamics. Otherwise, the U.S. EPA has not established guidance in this area. The International Programme on Chemical Safety (IPCS) has drafted guidance in the selection of chemical-specific adjustment factors (CSAF), which does cover systemic kinetics and dynamics (IPCS, 2001). The IPCS document has not been formally reviewed by the U.S. EPA. Much of the toxicokinetic factor development in the boron RfD derivation, however, is consistent with IPCS (2001). Additionally, IPCS previously applied the TK/TD subfactor approach in their assessment of boron (WHO, 1998a). The values for the TK component of UFA and UFH have been adjusted based on relevant data, but no such data exist to support an adjustment of the TD components.

For boron, the animal-to-human and sensitive-human uncertainty factors (UFA and UFH) are each split into toxicokinetic (TK) and toxicodynamic (TD) components to apply existing rat and human toxicokinetic data to reduce the uncertainty in the boron RfD. The product of AF_{AK} and AF_{AD} replaces the animal-to-human (interspecies) uncertainty factor (UFA) in the standard RfD methodology. Similarly, the product of AF_{HK} (the interspecies toxicokinetic adjustment factor) and AF_{HD} (the interspecies toxicodynamic adjustment factor) replaces the sensitive human (interindividual variability) uncertainty factor (UFH). Each of the adjustment factors is the product of data-derived scaling factors and residual uncertainty.

[1]Commonly known as pharmacokinetics and pharmacodynamics in the medical literature.

8.1.3.1 Derivation of Adjustment Factor Values

As presented below, the examination of species differences in boron distribution to extravascular fluids and renal elimination served as the basis for the replacement of the default value for UF_A-TK, while critical evaluation of the human interindividual variation of underlying renal clearance mechanism (GFR) served as the basis upon which to replace the default value for the TK component of UF_H. Because no data were available to inform a mode or mechanism of action for boron, the default values for the TD component of both UF_A and UF_H remain; they are $10^{0.5}$, or 3.16 for each.

In the most simple terms, toxicokinetics deal with what the body does to the chemical, while toxicodynamics deal with what the chemical does to the body. In essence, the toxicokinetic factor addresses internal exposure, in that the objective is to determine the dose of the ultimate toxic form of the compound at the target tissue. The toxicodynamic factor, then, deals with the response of the target tissue given a specific dose. A "pure" toxicodynamic factor must be independent of the toxicokinetics. As it is unlikely that *in vivo* responses will be free of kinetic variability, toxicodynamic data will be obtained largely from *in vitro* (cellular level) studies. In these cases, a connection to systemic dynamics must be established, as well. Given enough data, the form of the resulting model could be manifested as a sophisticated multi-compartment, highly non-linear, biologically-based toxicokinetic model linked to a mathematical dose-response model relating cellular response to whole-organism response. Most of the time, however, the model will be a simple multiplicative combination of two factors, one for TK and one for TD. Even more often, data will only be available for determination of the TK factor, requiring the use of a default value for TD. Lacking a sophisticated model, the usual approach will be to find one or more kinetic variables (relating to internal dose) for which an animal-to-human ratio can be estimated, using that ratio to scale the human exposure (external dose) relative to the test animal. Whenever the kinetic factors are used in this manner, additional factors must be considered to relate the internal kinetics back to the external dose. Simple absorption and distribution constants usually suffice.

TK/TD Subfactor Default Values (Uncertainty)

WHO (1994) and IPCS (2001) have maintained a default value of 10 for both UF_A (interspecies uncertainty) and UF_H (intraspecies uncertainty). For UF_A, they have apportioned the factor of 10 between the TD and TK components so that the default value for the TD component is 2.5 ($10^{0.4}$), and the default value for the TK component is 4.0 ($10^{0.6}$) in the absence of data describing toxicodynamic or toxicokinetic differences. Similarly, WHO (1994) and IPCS (2001) divided UFH into TD and TK components with assigned default values of 3.16 ($10^{0.5}$) each. The U.S. EPA has assumed an equal contribution ($10^{0.5}$ each) of TK and TD for both UF_A and UF_H when deriving the RfC, but has not explicitly addressed the issue for RfDs. As the factors are now meant to include kinetic and dynamic dose adjustments based on data, as well as uncertainty, they more appropriately are termed "adjustment factors." As standard notation in this document, these factors henceforth will be designated as AF_{AK}, AF_{AD}, AF_{HK}, and AF_{HD}, respectively. Note that these factors serve as both *variability* factors when relevant data exist and *uncertainty* factors when relevant data do not exist.

The default half-order of magnitude partition of uncertainty factors (i.e., UF_A and UF_H) for toxicokinetics and toxicodynamics is primarily based on lack of knowledge; if there is no

evidence to the contrary, an equal contribution from each source of uncertainty is assumed. Although there is empirical and conceptual support for a value other than $10^{0.5}$ for the TK default for UF_A for compounds kinetically similar to boron[2], there are no data addressing the TD component. In addition, lacking a formal review, the IPCS uneven split is not adopted here. Therefore, any uneven split of the 10-fold factor for UF_A would be somewhat arbitrary, and the half-order-of-magnitude TK/TD default partition is maintained for this analysis. The even split is also adopted for UF_H, as there are no strong arguments for different values for either the TK or TD factors.

Revised RfD Calculation Formula

The revised formula for calculating the RfD with UF_A and UF_H split into TK and TD subfactors is given in Equation 5.1.

$$RfD = D_c/(AF_{AK} \cdot AF_{AD} \cdot AF_{HK} \cdot AF_{HD} \cdot UF) \quad (5.1)$$

where:

DC is the "critical" dose (NOAEL, LOAEL, BMD) defined in the critical study,
AF_{AK} is the interspecies toxicokinetic adjustment factor (default = 3.16)
AF_{AD} is the interspecies toxicodynamic adjustment factor (default = 3.16)
AF_{HK} is the interindividual toxicokinetic adjustment factor (default = 3.16)
AF_{HD} is the interindividual toxicodynamic adjustment factor (default = 3.16)
UF is the aggregate uncertainty factor

The product of AF_{AK} and AF_{AD} replaces the animal-to-human (interspecies) uncertainty factor (UF_A) in the standard RfD methodology. Similarly, the product of AF_{AK} and AF_{AD} replaces the sensitive human (interindividual variability) uncertainty factor (UF_H). Each of the adjustment factors is the product of data-derived scaling factors and residual uncertainty. That is, if there are significant issues concerning the data or modeling of the data, the adjustment factor may be increased to reflect remaining uncertainty. If there are no applicable data, the adjustment factors are equal to their default uncertainty factor values. The aggregate uncertainty factor (UF) is equal to the product of all other uncertainty factors: subchronic-to-chronic (UF_S), LOAEL-to-NOAEL (UF_S), and data base adequacy (UF_D). For boron, a subchronic-to-chronic uncertainty factor was not used to account for extrapolation from less than chronic results because developmental toxicity (decreased fetal body weight) was used as the critical effect. The developmental period is recognized as a susceptible lifestage where exposure during certain time windows is more relevant to the induction of developmental effects than lifetime exposure. An uncertainty factor for extrapolation from an LOAEL to an NOAEL was not necessary because BMD modeling was used to determine the point of departure. The dose corresponding to a 5% decrease in pup weight, relative to control, was selected as the point of departure.

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[2]This class of substances would include those that are water soluble and eliminated unchanged through the kidneys. The difference in elimination would be primarily in the renal clearance rate. A fairly large body of evidence suggests that many of the factors that determine kinetics generally scale to $BW^{0.75}$ across species. In particular, renal clearance values scale across species with an exponent ranging from 0.69-0.89 (Davidson et al., 1986). For rats to humans, the allometric argument supports a value near 4.0 as the average, or expected, factor for scaling test-animal kinetics to human kinetics. The default TK value would be somewhat larger to allow for departures from the expected value. In addition, the default value would be species specific.

Because decreased weights did not persist in the companion study (Phase II of Price et al., 1996a, 1994), no further adjustment was considered for identifying a level of oral exposure to boron associated with the minimal level of risk. A database uncertainty factor was not deemed necessary due to boron's extensive data base. For convenience and sake of reference, the product of all the terms in the denominator of Equation 5.1 is given the term "total adjustment factor" and is designated as AF_{TOT}.

8.1.3.2 Toxicokinetic Modeling Issues for Boron

While no data presently exist to address the toxicodynamic components of UF_A or UF_H, existing data are adequate to establish non-default values for AF_{AK} and AF_{HK} and reduce uncertainty in the toxicokinetic components of both uncertainty factors. The most relevant internal dose metric for boron toxicity, which is most likely a result of continuous exposure over an extended period, is the average fetal concentration for the entire gestational period. Although there are no direct measurements of fetal boron concentrations, boron concentrations in the fetus should be the same as in the mother because boron is freely diffusible across biological membranes and will rapidly and evenly equilibrate in all body water compartments. As the boron RfD is based on developmental effects observed in rats, the most relevant kinetic data are those pertaining to pregnant rats and pregnant humans. There are insufficient data to compare plasma boron in rats and humans at the same exposure levels. Therefore, boron clearance is used as an estimator of internal dose. Assuming steady state conditions, clearance, expressed in units of mL/min (volume of plasma cleared of the substance per unit time), is inversely related to plasma concentration. Clearance is calculated by dividing the total mass of substance eliminated in the urine in a specific time (i.e, mg/min) by the concentration of the substance in the plasma (mg/mL). Therefore, the higher the clearance value, the lower the plasma concentration. Other processes, such as fecal elimination, metabolism, and distribution to other compartments also reduce the plasma concentration. However, as boron is not metabolized and almost entirely eliminated in the urine, clearance of boron by the kidney can be used as the key toxicokinetic factor, with a consideration of the relative volumes of distribution between rats and humans.

Although the toxic effects of boron are manifested in the offspring, pregnant females (for both humans and test animals) are considered to be the "sensitive" population, with respect to establishing an equivalent toxic dose across species. For the RfD, toxicity benchmarks are expressed in terms of external (maternal) exposure, rather than internal (fetal) dose. In this sense, the maternal boron concentration is treated as a surrogate for the fetal boron concentration. A compartmentalized toxicokinetic model, with the fetus as one of the compartments, would be needed to directly assess the dose to the fetus. Given the near first order kinetics of boron, maternal toxicokinetic variability is an adequate surrogate for the fetal dose variability.

Interspecies Uncertainty

As the rat:human boron clearance ratio is being used essentially as an (inverse) estimator of relative internal dose and, subsequently, as a scalar of "external dose" (ingested dose rate in mg/kg-day), an additional factor must be considered that ties internal dose to external dose. As there is an assumption of relatively constant intake of boron and the toxic outcome is most likely related to a continuous exposure over an extended critical period (the period of organogenesis during fetal development), the most appropriate estimator for internal dose is the average (steady-state) circulating boron concentration.

Boron distributes primarily to total body water and bone, reaching a 4-fold higher concentration in whole bone than in plasma (Chapin et al., 1997). Boron freely transfers from bone to body water, as well. Therefore, a two-compartment steady-state model is assumed for this analysis. The generalized two-compartment steady-state model is described in O'Flaherty (1981). The steady-state circulating concentration (C_{SS}) of boron (or other compound) for a two-compartment model, given a constant rate of administration (oral ingestion), simplifies to Equation 5.2.

$$C_{SS} = (D_e f_a \, BW)/Cl \qquad (5.2)$$

where:

D_e is the external ingested dose rate in mg (boron) per kg body mass per day
f_a is the fraction of ingested boron absorbed into the body from the gut
BW is body weight (kg)
Cl is the renal clearance rate (mL/minute)

An assumption is made that all of the boron is eliminated in the urine. Small losses in sweat, saliva, and the feces are ignored.

The interspecies toxicokinetic adjustment factor, AF_{AK}, is used to adjust the test-animal dose rate to obtain an equivalent human exposure. In this case, AF_{AK} is equal to the ratio of D_e-rat to D_e-human at a fixed target tissue dose. As C_{SS} is used as the estimator for target tissue dose, the latter condition (fixed target tissue dose) is satisfied by setting the rat:human C_{SS} ratio to 1. Therefore, solving Equation 5.2 for D_e, taking the ratio of rat and human D_e, and setting the rat:human C_{SS} ratio to 1, yields Equation 5.3, where the trailing subscript designates the species ® = rat, *h* = human).

$$AF_{AK} = \frac{Cl_r \times f_{ah} \times BW_h}{Cl_h \times f_{ar} \times BW_r} \qquad (5.3)$$

The mean boron clearance for pregnant rats (Cl_r)is 1.00, determined from the kinetic studies of U.S. Borax (2000) and Vaziri et al. (2001) (Table 6-4). The mean boron clearance for pregnant women (Cl_h) was determined from the kinetic studies of U.S. Borax (2000) and Pahl et al. (2001) to be 66.1 mL/min (Table 6-5). The mean body weights for pregnant rats (BW_r) and pregnant women (BW_h) from those studies are 0.303 and 67.6 kg, respectively. The average clearance of 66 mL/min for pregnant women determined by Pahl et al. (2001) represents a possible underestimation of the true boron clearance, particularly at the relatively higher doses near the RfD. Boron clearance values obtained in adult men (Jansen et al., 1984a) given an

intravenous infusion of boric acid, representing exposures 66 times dietary levels, were 1.5 times greater than boron clearance measured at dietary levels. Taking into account the possibility of dose-dependence, and that the RfD is somewhere between the dietary exposure and infusion level in the Jansen study (but much closer to the latter), the factor could be less than 1.5 (1.3 by linear interpolation). Therefore, Cl_h could actually be 30-50% higher (86-99 mL/min). An independent estimate in the range of 86 to107 mL/min boron clearance in pregnant women can be obtained from the adult male boron clearance of 60.5 mL/min/1.73 m^2 (Jansen et al., 1984a) by assuming that boron clearance will scale the same as GFR from male to female to pregnant female. GFR is about 8-12% higher in adult males than females (Smith, 1951; Wesson, 1969), but increases by a factor of about 1.6 in pregnancy (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). Furthermore, GFR values normalized to a standardized unit surface area (1.73 m^2) for pregnant women may underestimate absolute GFR (mL/min) by an additional factor of 1.2 (Krutzén et al., 1992). Therefore, the adult male boron clearance of 60.5 mL/min/1.73 m^2 represents a clearance of at least 86 mL/min and as much as 107 mL/min in pregnant women. Although this evidence is suggestive that Cl_h may be higher, it is not strong enough for a quantitative adjustment in the derivation of AF_{AK}. Therefore, Cl_h is assigned the value of 66.1 mL/min, Cl_h is 1.00 mL/min, BW_r is 0.303 kg, and BW_h is set to 67.6 kg.

Absorption across the gut is similar in rats and humans. Although there are no data specifically for pregnant individuals, boron is 95% absorbed from the G.I. tract by adult rats (Vanderpool et al., 1994) and about 92% by adult humans (Schou et al., 1984). Therefore, f_{ah} and f_{ar} are set to 0.92 and 0.95, respectively.

Substituting the foregoing estimates for all the variables in Equation 5.3 yields a value of 3.3 for AF_{AK} ([1.00/66.1] x [0.92/0.95] x [67.6/0.303]). Although there are a number of uncertainties in the estimation of the variables in Equation 5.4, there is a likely net upward bias in AF_{AK} because of the potential underestimation of Cl_h. The value of 3.3 for AF_{AK}, therefore, represents a somewhat health protective value, and an additional adjustment for residual uncertainty is judged to be unnecessary. There are no data for estimating AF_{AD}; it remains the default value of $10^{0.5}$ (3.16).

Intraspecies Uncertainty

Conceptually, the intraspecies toxicokinetic adjustment factor (AF_{HK}) accounts for the range of human interindividual variability from where AF_{AK} left off to where the sensitive sub-population is adequately protected. For boron, the range is between the mean and a "lower bound" boron clearance in the pregnant human population. AF_{HK} needs to cover a sufficient fraction of the population (on the toxicokinetic scale) so that the probability of having both a low clearance and high sensitivity (on the toxicodynamic scale[3]) is low enough to preclude appreciable risk of deleterious effects in the population (including sensitive individuals).

For the assessment of interindividual toxicokinetic variability, GFR is used as a surrogate for boron clearance. Although the study of Pahl et al. (2001) provides an estimate of boron clearance variability in pregnant women, the data are judged to be inadequate for this purpose. The Pahl et al. (2001) study is considered to be a good study for estimating the mean boron

[3]Toxicodynamic sensitivity is represented by AFHD.

clearance in pregnant women, but was not designed to assess interindividual variability, given its fairly low number of subjects (16) and a lack of control of dietary intake of boron. The variance of boron clearance in this study was somewhat high (CV = 0.49), such that estimation of an adequate lower bound would be highly uncertain. In contrast, in the controlled infusion exposure study of Jansen et al. (1984a), the boron clearance CV was 0.09 (Section 3.4.1). In that same study, clearance determined for uncontrolled dietary exposure at much lower levels was characterized by high variability (CV = 0.78). Lack of controls on exposure magnitude and timing would be expected to contribute substantially to the variance of the measurements. The high variability reported by Pahl et al. (2001), therefore, is attributed to experimental "noise" and should not be included in the estimate of true population variability. As boron clearance is largely a function of GFR, the larger more certain data base on GFR and its variability among humans is used to estimate boron clearance variability. Because the measured boron clearances in the rat and human kinetic studies were less than GFR, tubular reabsorption could be contributing to the variability of boron clearance in the population. Variability in these factors, however, is judged to be minor in comparison to the variability in GFR (Section 6).

GFR data have been used previously in the context of the boron RfD by Dourson et al. (1998), who proposed the ratio of the mean GFR to the GFR value 2 standard deviations (SD) below the general population mean (mean/[mean - 2 SD]) as the metric for the interindividual toxicokinetic adjustment factor. This approach will be referred to as the sigma method, which is a common term used for statistical methods using multiple standard deviations to establish "acceptable" lower bounds. For the derivation of AF_{HK}, the sigma method is modified by using 3 SD as the reduction factor for establishing the lower bound (i.e., mean GFR - 3 SD) (equation 5.4). The basic formula modified from Dourson et al. (1998) for AF_{HK} is:

$$AF_{HK} = \frac{GFR_{AVG}}{GFR_{AVG} - 3\ SD_{GFR}} \qquad (5.3)$$

where GFR_{AVG} and SD_{GFR} are the mean and standard deviation of the GFR (mL/min) for the general healthy population of pregnant women. The use of 3 standard deviations rather than 2 (as in Dourson et al., 1998) is based on obtaining adequate coverage of pregnant women with very low GFR.

The selection of 3 SD is based on a statistical analysis of the published GFR data, with more consideration being given to the full range of GFR values likely to be found in the population of pregnant women. In the aggregate, the data suggest that a lower bound GFR 2 SD below the mean does not provide adequate coverage of the susceptible sub-population. While no conclusive information exists from controlled-dose studies in humans, it may be possible that the variability in boron clearance might be greater than GFR variability. Therefore, AF_{HK} must also account for any residual uncertainty in using GFR as a surrogate.

GFR is measured most accurately using substrates that are not metabolized and not actively secreted or reabsorbed from the kidney tubules, such as inulin and iohexol. Three such studies were located in the published literature that address GFR variability in pregnant women (Dunlop, 1981; Krutzén et al., 1992; Sturgiss et al., 1996). Because no data exist that identify a specific developmental period, data from the entire pregnancy duration are used where possible.

Dunlop (1981) assessed GFR for 25 women at three different time points during pregnancy (16, 26, and 36 weeks) and again after delivery. GFR was measured as inulin clearance. The mean values for GFR for these measurement periods were 148.6, 152.4, and 150.5 mL/min, respectively. The standard deviations were 17.2 and 17.6 mL/min for the first two measurements, rising to 31.8 mL/min for the 36-week measurement. For the present analysis (Table 8-1), the overall average and standard deviation (150.5 and 17.6 mL/min, respectively) for the serially-averaged measurements for each individual across the three pregnancy time points were used.

Sturgiss et al. (1996) performed a similar assessment of GFR (using inulin clearance) for 21 women in early (12-19 weeks) and late (30-35 weeks) pregnancy and again at 15-25 weeks post partum. The primary purpose of the study was to determine whether the increase in GFR normally occurring in pregnancy represents a maximal utilization of renal reserve (it did not in this study). To evaluate that hypothesis, GFR for 14 of the 21 women (Index group) was assessed following an infusion of an amino acid solution (known to increase GFR) in each of the three measurement periods, subsequent to assessment of their basal GFR for each period. The other seven women (control group) received an infusion of Hartman's solution instead of amino acids, and basal GFR was assessed in the same manner as the Index group. Combining the basal (unperturbed) measurements for all 21 subjects[4], serially averaged for each individual for both pregnancy time points, resulted in a mean GFR of 138.9 mL/min with a standard deviation of 26.1 mL/min.

Krutzén et al. (1992) evaluated GFR during pregnancy for four different groups of women: 13 normal healthy women, 16 diabetic women, 8 hypertensive women, and 12 women diagnosed with preeclampsia. GFR was determined by iohexol clearance in the second and third trimester and again 6-12 months post partum. The authors reported absolute clearance values (in mL/min) for only the third trimester. The third trimester mean GFR and standard deviation for the healthy women were 195 and 32 mL/min, respectively. Mean GFR in the third trimester was not reduced for the hypertensive women and was slightly reduced in the diabetic women, with a mean of 169 mL/min (SD = 34.7). The third trimester mean GFR of 128 mL/min (SD = 33.9 mL/min) for the preeclamptic women, however, was more than two standard deviations below the healthy mean GFR. In general, the GFR values reported in this study are much higher than those reported by Dunlop (1981) and Sturgiss et al. (1996). The reason for this discrepancy is not known.

By virtue of their lower GFR, pregnant women diagnosed with preeclampsia could be considered to be a sensitive subpopulation, at least on the toxicokinetic scale. Toxicodynamic sensitivity is presumably independent of toxicokinetic sensitivity. The onset of preeclampsia generally occurs after the week 20 of pregnancy and is characterized by acute hypertension, often accompanied by edema and proteinuria. Women with preeclampsia are at increased risk for premature separation of the placenta from the uterus and acute renal failure, among other adverse health effects. The fetus may become hypoxic and is at increased risk of low birth weight or perinatal death. Preeclampsia has recently been estimated to affect 3-5% of pregnant women (Skjaerven et al., 2002). With almost 4 million successful pregnancies per year in the

[4]That is, index plus control individuals in Table II, Sturgiss et al. (1996).

United States (Ventura, 1999), or about 3 million at any one time, the size of the preeclamptic population at any given time could be in the range of 150,000 to 200,000 women. Considering the Krutzén et al. (1992) results in the context of the sigma method, a reduction of 2 SD from the healthy population mean to establish the lower bound (which results in a GFR slightly higher than the mean of the preeclamptic GFR), would appear to be insufficient for adequate coverage of the susceptible population. The use of 3 SD below the healthy GFR mean gives coverage in the sensitive subpopulation to about 1 SD below the mean preeclamptic GFR.

As no single study is considered to be definitive for assessment of population GFR variability, AF_{HK} is determined from the average of the individual sigma-method values for each of the three studies (Table 8-1). The mean GFR and standard deviation values in Table 8-1 are based on average GFR across the entire gestational period, except for the Krutzén et al. (1992) estimate, which was for the third trimester only. The average sigma-method value from the three studies is 1.93. Considering a small residual uncertainty in the use of GFR as a surrogate for boron clearance, the average sigma-method value of 1.93 is rounded upward to 2.0 and established as the value for AF_{HK}. The data on preeclamptic women presented by Krutzén et al. (1992) were considered insufficient to base the interindividual AF_{HK} factor. Use of the mean (128 mL/min) and standard deviation (33 mL/min) in this sensitive subgroup of preeclamptic women likely overestimates the spread of GFR values below the mean, due to the likelihood of a log normal distribution of GFR values, and the contribution of measurement variability (beyond biological variability) to the statistical confidence limits. Given these considerations, the ~2-fold interindividual variability factor derived from three standard deviations below the mean of three studies for pregnancy GFR (mean = 161.5 mL/min; mean - 3 SD = 85.8) is considered preferable for providing adequate coverage to women predisposed to adverse birth outcomes due to renal complications.

The decrement of renal function can predispose individuals to both maternal and fetal adverse effects. Thus, there are levels of renal function (GFR) which increase the risk of adverse developmental effects that cannot be distinguished from the potential adverse effects of boron. Thus, this level of renal function would serve as a physiological lower bound on the value for the denominator of Equation 5.4. Establishing the level unequivocally is problematic, as the incidence, severity, and relevance (to boron toxicity) of adverse pregnancy outcomes associated with low GFR is difficult to establish. Further complicating the issue are the metrics reported in the literature; pregnancy outcomes are commonly related to pre-pregnancy measures of renal function, which are generally expressed as serum creatinine levels. There are no data directly relating GFR or serum creatinine levels in pregnant women to adverse pregnancy outcomes. The approach taken in the literature reflects the physician's need to advise kidney patients prior to becoming pregnant. Also, at lower (normal) serum creatinine levels, serum creatinine is a reliable measure of GFR. At higher serum creatinine levels (lower GFR), the relationship apparently disappears (Levey et al., 1988). However, a linear regression analysis of the log-log transformation of the published data (Shemesh et al., 1985, reproduced in Levey et al., 1988) shows a significant relationship over a wide range of serum creatinine levels.

From the regression analysis shown in Appendix C of the IRIS Toxicological Review (Regression Analysis of Serum Creatinine and Inulin Clearance, U.S. EPA, 2004a) and the results of clinical studies, a ratio of average (nonpregnant) GFR to (nonpregnant) GFR levels associated with significant adverse pregnancy outcomes can be calculated. This ratio would

represent a "physiological" AF_{HK} estimating the point at which low GFR would be a major factor in adverse pregnancy outcomes. Several clinical investigations in humans have demonstrated a clearly increased risk of adverse developmental and obstetrical complications (low birth weight, intrauterine growth retardation, spontaneous abortion, placenta separation, fetal and neonatal death, etc.) with serum creatinine levels of 1.4 mg/dl and above (Bear, 1976, 1978; Cunningham et al., 1990; Abe, 1996; Jungers et al., 1997). Applying the linear regression analysis shown in Appendix C of the IRIS Toxicological Review (U.S. EPA, 2004a), a serum creatinine level of 1.4 mg/dl corresponds to a GFR of 37.2 mL/(min/1.73 m2).[5] Similarly, the average serum creatinine level of 0.8 mg/dl in the same population (nonpregnant women) corresponds to a GFR of 79.4 mL/(min/1.73 m^2). Dividing 79.4 by 39.8 yields a physiological AF_{HK} of 2.00, which is identical to the sigma-method AF_{HK} derived previously. This comparison is based on an assumption that the ratio of normal nonpregnant GFR to adverse GFR holds for the increased GFR values during pregnancy. There is considerable uncertainty in the regression model in the estimate of the lower GFR values, which is not accounted for in the physiological estimate of AF_{HK}, however. Also, the severity of the low-GFR effects and the proportion of the population that would be affected is unclear. Overall, the clinical data supporting the physiological approach are too far removed from the direct assessment needed to establish AF_{HK} and serve only as support for the assessment. Therefore, the selection of a lower bound 3 SD from the mean GFR in healthy pregnant women in the statistical approach does not seem excessive and would appear to be adequately protective. Thus, in Equation 5.1, AF_{HK} is assigned a value of 2.0, and AF_{HD} remains at its default value of $10^{0.5}$.

[5]GFR values are corrected for body surface area in this study.

Table 8-1 Sigma-method Value Calculation

Study	Mean GFR (SD) mL/min	Mean GFR-3SD	Sigma-Method Value[a]
Dunlop (1981)	150.5 (17.6)[b]	97.7	1.54
Krutzén et al. (1992)	195 (32)[c]	99	1.97
Sturgiss et al. (1996)	138.9 (26.1)[d]	60.6	2.29
Averages	161.5	85.8	1.93

a Mean GFR ÷ (Mean GFR - 3 SD)
b Serially-averaged observations across three time periods (16, 26, and 36 weeks) for 25 pregnant women
c Third trimester values for 13 pregnant women
d Serially-averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal index plus basal control individuals)

8.1.3.3 Summary of Data-Derived Adjustment Factors and RfD Calculation

Table 8-2 demonstrates the division of UF_A and UF_H into toxicokinetic and toxicodynamic components and indicates the default values (in parentheses) and the data-derived values used to replace default toxicokinetic values.

Table 8-2 Default and Data-derived Values for Components of UF_A and UF_H

Uncertainty Factor	Component		Combined Factor Values
	TD	TK*	
UF_A	(3.16) not replaced	(3.16) 3.3	10.5
UF_H	(3.16) not replaced	(3.16) 2.0	6.3
Combined UF_A and UF_H			66

*Valuation of the TK component of UF_A was based on species difference in the volume of total body water during pregnancy and boron clearance rates; valuation of the TK component of UF_H was based on differences in GFR among pregnant women.

The RfD is calculated from Equation 5.1, where:

D_C = 10.3 mg/kg-day (Allen et al., 1996)
AF_{AK} = 3.3 (data-derived)
AF_{AD} = 3.16 (100.5, default)
AF_{HK} = 2.0 (data-derived)
AF_{HD} = 3.16 ($10^{0.5}$, default)
UF = 1 (UF_S x UF_D x UF_L)
AF_{TOT} = 3.3 x 2.0 x 3.16 x 3.16 = 66
RfD = 10.3/66 = 0.2 mg/kg-day

The RfD is consistent with a suggestion by Nielsen (1992) that an intake of 10 mg per day is not too high, while 50 mg/day is probably toxic. If a representative body weight of 60 kg

is assumed for a pregnant woman, the value of 10 mg/day translates to 0.17 mg/kg-day. As boron appears to have some beneficial nutrient value, Nielsen (1992) also recommended a total daily boron intake of 1 mg to avoid boron deficiency. The RfD would appear to give an adequate margin of safety below, as well as above.

8.1.3.4 Other Uncertainty Factor Approaches

Other researchers and regulatory concerns have used different methods to derive uncertainty factors. The U.S. EPA has not yet endorsed any of these approaches, as there are a number of critical, unresolved scientific and methodological issues.

The International Program on Chemical Safety (IPCS) uses "data-derived" uncertainty factors to estimate tolerable intake values (WHO, 1994; Renwick, 1993). This method allows for subdivision of each of the interspecies and intraspecies default uncertainty factors to incorporate data on toxicokinetics (pharmacokinetics) or toxicodynamics (pharmacodynamics). For interspecies uncertainty, the 10-fold factor is divided into a default factor of $10^{0.6}$ (4.0) for toxicokinetics and $10^{0.4}$ (2.5) for toxicodynamics in the absence of toxicokinetic and toxicodynamic data. For intraspecies uncertainty, the 10-fold factor is subdivided into a default of $10^{0.5}$ (3.2) each for toxicokinetics and toxicodynamics in the absence of toxicokinetic and toxicodynamic data. Subsequently, the International Program for Chemical Safety (IPCS, 2001) published a guidance document on the use of data to develop chemical specific adjustment factors. This guidance calls for the use of a composite factor (CF), which is the composite of specific adjustment factors (quantitative chemical specific data) for either toxicokinetics or toxicodynamics and the remaining default uncertainty factors for which chemical specific data were not available. The guidance document states that in some cases the split between toxicokinetics and toxicodynamics in the framework may not be appropriate and some flexibility in approach may need to be maintained; however, in the absence of data, the defaults for interspecies toxicokinetics and toxicodynamics are 4.0 and 2.5, respectively. This subdivision, according to the authors, was based on the approximate 4-fold difference between rats and humans in basic physiological parameters that are major determinants of clearance and elimination of chemicals, such as cardiac output and renal and liver blood flows. The defaults for interindividual toxicokinetics and toxicodynamics are each 3.2. In addition to the IPCS approach, a number of risk assessments have recently been completed for boron using an uncertainty factor less than 100. A description of the critical effect chosen and the uncertainty factors used follows. ECETOC (1994) developed a tolerable daily intake (TDI) for developmental effects of boron. Decreased fetal body weight in rats was chosen as the critical effect (Price et al., 1994) with an NOAEL of 9.6 mg B/kg-day. A factor of $10^{0.5}$ was chosen for interspecies uncertainty factor due to the similarity in pharmacokinetics (metabolism and distribution were cited) between animals and humans. A default factor of 10 was chosen for the intraspecies uncertainty factor. The composite uncertainty factor was 30.

Murray (1995, 1996) used the Price et al. (1994) study, choosing decreased fetal body weight in rats as the critical effect with an NOAEL of 9.6 mg B/kg-day. The interspecies uncertainty factor chosen was 4 (2 for pharmacokinetics and 2 for pharmacodynamics, 2x2=4). Several reasons were cited for the reduced interspecies uncertainty factor for pharmacokinetics: boron is not metabolized in animals or humans, eliminating a major potential source of pharmacokinetic variation; it is rapidly distributed throughout body water and does not

accumulate; the toxicity profile of boron is similar across species; and parameters of elimination were considered by the author to be similar in humans and other animals. The authors cited the following reasons for the reduced interspecies uncertainty factor for pharmacodynamics: the sensitivity of the target tissue receptor appeared to be similar across species based on the similarity of symptoms of acute toxicity in animals and humans, and developmental and reproductive toxicity appear to be the most sensitive endpoints of toxicity in all animal species tested. The intraspecies uncertainty factor chosen was 8 (2.5 for pharmacokinetics and 3.2 for pharmacodynamics). The intraspecies pharmacokinetic factor was decreased because metabolism is normally the major source of pharmacokinetic variance in humans, and borates are not metabolized. The composite uncertainty factor chosen was 4x8=32.

IEHR (1997) determined an unlikely effect level for developmental toxicity for boron based on the benchmark dose for decreased fetal body weight by Allen et al. (1996). The interspecies uncertainty factor chosen for boron was $10^{0.5}$, which includes $10^{0.25}$ each for pharmacokinetics and pharmacodynamics. The justification for these other-than-default values was stated as the variability in the intrinsic sensitivity of the target site (embryo, testis, ovary) to the chemical's toxic effects in humans versus that in the experimental animal and metabolic and pharmacokinetic differences among species. The intraspecies uncertainty factor chosen for boron was a default value of 10. The composite human sensitivity factor was 30.

In Environmental Health Criteria, WHO (1998a) developed a TDI for boron, using decreased fetal body weight in rats as the critical effect (Price et al., 1994), with an NOAEL of 9.6 mg B/kg-day. The interspecies uncertainty factor chosen was $10^{0.5}$ ($10^{0.1}$ x$10^{0.4}$= $10^{0.5}$) which used a $10^{0.1}$ for pharmacokinetics due to the similarity of absorption, distribution, metabolism, and elimination of boron in rats and humans and a $10^{0.4}$ (default) for pharmacodynamics. The intraspecies uncertainty factor chosen was $10^{0.9}$ ($10^{0.4}$ x $10^{0.5}$ =$10^{0.9}$), 100.4 for pharmacokinetics due to lack of metabolism in humans and $10^{0.5}$ (default) for pharmacodynamics. The composite uncertainty factor was 32.

In Guidelines for Drinking-Water Quality, WHO (1998b) developed a TDI for boron to set a guidance value for drinking water. Decreased fetal body weight in rats was chosen as the critical effect (Price et al., 1994) with an NOAEL of 9.6 mg B/kg-day. A default value of 10 was chosen for the interspecies factor due to a reported lack of data to support reduction in the pharmacokinetic and pharmacodynamic factors. For intraspecies extrapolation a default value of 3.2 for pharmacokinetic data was reduced to 1.8, and a default value of 3.2 was retained for pharmacodynamic data. Thus, the uncertainty factor for intraspecies uncertainty was 1.8x3.2=5.7 rounded to 6. The composite uncertainty factor was considered to be 10x6=60.

Dourson et al. (1998), as part of the development of the WHO document (1998b), developed a TDI for boron. Although the authors agreed to the lack of metabolism and the similarity in absorption and elimination of boron in animals and humans, interspecies variation in kinetics for boron was considered to relate to renal clearance rates. A 3-fold clearance rate difference between rats and humans for boron was estimated, after eliminating studies with little confidence from an earlier projected 4-fold difference. The calculated renal clearance rate difference (3-fold) between rats and humans for boron was considered by the authors to be similar to a 4-fold difference that would be expected of other chemicals (Renwick, 1993). Based on this difference in clearance rates, the authors (Dourson et al., 1998) chose not to reduce the

interspecies uncertainty factor for pharmacokinetics or pharmacodynamics. Therefore, a default value of 10 was chosen for the interspecies factor. For intraspecies uncertainty, the pharmacokinetic factor was reduced from a default of 3.2 to 1.8. The authors proposed that the likely difference for humans in boron kinetics occurs during pregnancy and is based on an increase in the GFR, a recognized physiological adaption during pregnancy. The estimation of the 1.8 factor for intraspecies variation in pharmacokinetics was based on a ratio of the mean GFR of 144 mL/min +/- 32(SD) from pooled data of healthy humans in late pregnancy (number of subjects not mentioned) and this mean GFR minus two standard deviations from the mean to account for variation in the average to the susceptible human 32(SD) x2=64; 144(GFR)-64(2SDs)=80; the ratio of 1.8 was calculated as 144 mL/min divided by 80=1.8. The intraspecies pharmacodynamic factor used was a factor of 3.1, which the authors considered as a default factor, although previous methodology considered it to be 3.2. The intraspecies uncertainty factor was 1.8x3.1=5.58 rounded to 6. The composite uncertainty factor was 10x6=60.

Murray and Andersen (2001) detailed the use of reduced uncertainty factors for boron risk assessments in recent years and noted the use of factors in the range of 25-60 using the NOAEL from the Price et al. (1996a) rat developmental study. The authors recommended using data derived uncertainty factors in a range of 22-44 using new rat and human clearance data (Vaziri et al., 2001; Pahl et al., 2001). The authors detailed a method where they estimated the human dose expected to provide the same boric acid area under the curve in target tissues as the NOAEL in rats and then applying reduced uncertainty factors for pharmacokinetic and pharmacodynamic uncertainty to this estimated human NOAEL. Interspecies pharmacokinetic value was estimated at 3.1, while interspecies pharmacodynamic uncertainty was estimated at 1.25-2.5. Intraspecies factors for pharmacokinetics were 1.8-2.0 and intraspecies pharmacodynamics were 3.2.

The IOM (2001) developed a tolerable upper intake level (UL) for various life stages of humans. These ULs were based on the NOAEL (9.6 mg/kg-day) from Price et al. (1996a) and an uncertainty factor of 30 (10 for interspecies uncertainty and 3 for intraspecies uncertainty based on the similarity in pharmacokinetics among humans). The reference body weight for adult women was 61 kg and was based on an average body weight from different female age groups. The resulting UL for adults was rounded to 20 mg/day. The UL was set at 17 mg B/day for pregnant women of 14-18 years of age, while the UL for pregnant women of 19-50 years of age was set at 20 mg B/day.

8.1.4 Previous Oral Assessment

The previous RfD for boron on IRIS was 9E-2 mg/kg-day based on testicular atrophy and spermatogenic arrest in a 2-year dog study from Weir and Fisher (1972). The NOAEL was 8.8 mg/kg-day, the LOAEL was 29 mg/kg-day and the uncertainty factor was 100. Newer studies have identified developmental effects in three species. The newer RfD is based on the critical effect of decreased fetal body weight in rats. The NOAEL of 9.6 mg/kg-day was identified from Price et al. (1996a) and the LOAEL of 13.3 mg/kg-day was identified from Heindel et al. (1992). Decreased fetal body weight was chosen from these studies because they are quality studies with a sensitive endpoint that identified the lowest pair of NOAELs and LOAELs. Developmental effects in mice and rabbits occurred at higher doses. The RfD uses data from these two studies

performed in the same laboratory and is based on a $BMDL_{05}$ from (Allen et al., 1996). With the exception of the NOAEL from Weir and Fisher (1972) in dogs, reproductive effects occurred at higher doses than the developmental NOAEL and LOAEL. The Weir and Fisher (1972) study in dogs was not chosen due to the quality of the study (Section 7).

8.2 Inhalation Reference Concentration (RfC)

The minimal database needed for development of an RfC is considered to be a well-conducted inhalation study that has adequately evaluated a comprehensive array of endpoints, including the respiratory tract and established an NOAEL and an LOAEL (U.S. EPA, 1994b). This criterion was not met for boron. No RfC could be derived, due to insufficiencies of the database.

8.3 Cancer Assessment

The available data are inadequate for evaluation of the human carcinogenic potential of boron. Derivation of slope factors and unit risks is, therefore, precluded.

8.4 CCL Health Reference Level

The EPA reference dose (RfD) for boron is 0.2 mg/kg/day (U.S. EPA, 2004d) based on developmental effects in rats from two studies (Price et al., 1996a; Heindel et al., 1992). The RfD was derived using the benchmark dose (BMD) method (Allen et al., 1996). As described in Section 4.3.2, EPA established the Health Reference Level (HRL) for boron (1.4 mg/L or 1,400 µg/L) using the RfD of 0.2 mg/kg-day and a 20 percent relative source contribution.

9.0 REGULATORY DETERMINATION AND CHARACTERIZATION OF RISK FROM DRINKING WATER

9.1 Regulatory Determination for Chemicals on the CCL

The Safe Drinking Water Act (SDWA), as amended in 1996, required the U.S. EPA to establish a list of contaminants to aid the Agency in regulatory priority setting for the drinking water program. The U.S. EPA published a draft of the first Contaminant Candidate List (CCL) on October 6, 1997 (62 FR 52193, U.S. EPA, 1997b). After review of and response to comments, the final CCL was published on March 2, 1998 (63 FR 10273, U.S. EPA, 1998c).

On July 18, 2003, the U.S. EPA announced final Regulatory Determinations for one microbe and 8 chemicals (68 FR 42897, U.S. EPA, 2003) after proposing those determinations on June 3, 2002 (67 FR 38222, U.S. EPA, 2002b). The remaining 40 chemicals and ten microbial agents from the first CCL became was renamed CCL 2 and were published in the Federal Register on April 2, 2004 (69 FR 17406, U.S. EPA 2004c).

EPA proposed Regulatory Determinations for 11 chemicals from CCL2 on May 1, 2007 (72FR 24016) (U.S. EPA, 2007). Determinations for all 11 chemicals were negative based on a lack of national occurrence at levels of health concern. The Agency is given the freedom to determine that there is no need for a regulation if a chemical on the CCL fails to meet one of three criteria established by the SDWA and described in section 9.1.1. After review of public comments and submitted data, the negative determinations for the 11 contaminants have been retained. Each contaminant will be considered in the development of future CCLs if there are changes in health effects and/or occurrence.

9.1.1 Criteria for Regulatory Determination

These are the three criteria used to determine whether or not to regulate a chemical on the CCL:

- The contaminant may have an adverse effect on the health of persons.
- The contaminant is known to occur or there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern.
- In the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

The findings for all criteria are used in making a determination to regulate a contaminant. As required by the SDWA, a decision to regulate commits the U.S. EPA to publication of a Maximum Contaminant Level Goal (MCLG) and promulgation of a National Primary Drinking Water Regulation (NPDWR) for that contaminant. The agency may determine that there is no need for a regulation when a contaminant fails to meet one of the criteria. A decision not to regulate is considered a final Agency action and is subject to judicial review. The Agency can

choose to publish a Health Advisory (a nonregulatory action) or other guidance for any contaminant on the CCL independent of the regulatory determination.

9.1.2 National Drinking Water Advisory Council Recommendations

In March 2000, the U.S. EPA convened a Working Group under the National Drinking Water Advisory Council (NDWAC) to help develop an approach for making regulatory determinations. The Working Group developed a protocol for analyzing and presenting the available scientific data and recommended methods to identify and document the rationale supporting a regulatory determination decision. The NDWAC Working Group report was presented to and accepted by the entire NDWAC in July 2000.

Because of the intrinsic difference between microbial and chemical contaminants, the Working Group developed separate but similar protocols for microorganisms and chemicals. The approach for chemicals was based on an assessment of the impact of acute, chronic, and lifetime exposures, as well as a risk assessment that includes evaluation of occurrence, fate, and dose-response. The NDWAC protocol for chemicals is a semi-quantitative tool for addressing each of the three CCL criteria. The NDWAC requested that the Agency use good judgment in balancing the many factors that need to be considered in making a regulatory determination.

The U.S. EPA modified the semi-quantitative NDWAC suggestions for evaluating chemicals against criteria for the regulatory determination criteria and applied them in decision-making. The quantitative and qualitative factors for boron that were considered for each of the three criteria are presented in the sections that follow.

9.2 Health Effects

The first criterion asks if the contaminant may have an adverse effect on the health of persons. Because all chemicals have adverse effects at some level of exposure, the challenge is to define the dose at which adverse health effects are likely to occur, and estimate a dose at which adverse health effects are either not likely to occur (threshold toxicant), or have a low probability for occurrence (non-threshold toxicant). The key elements that must be considered in evaluating the first criterion are the mode of action, the critical effect(s), the dose-response for critical effect(s), the RfD for threshold effects, and the slope factor for nonthreshold effects.

A full description of the health effects associated with exposure to boron is presented in Chapter 7 of this document and summarized below in Section 9.2.2. Chapter 8 and Section 9.2.3 present summarizes dose-response information.

9.2.1 Health Criterion Conclusion

The available toxicological data indicate that boron has the potential to cause adverse health effects in humans and animals. However, data from human studies were inadequate to determine if the major effects of boron toxicity seen in animal studies, in which the developing fetus and the testes were the most sensitive targets, can be interpolated to humans exposed to boron. The RfD was based on developmental studies in rats.

9.2.2 Hazard Characterization and Mode of Action Implications

The National Academy of Science Institute of Medicine (IOM, 2001) categorizes boron as a possible trace mineral nutrient for humans. Boron is essential for plant growth and deficiency studies in animals and humans have provided some evidence that low intakes of boron affects cellular function and the activity of other nutrients. It may interact with Vitamin D and calcium homeostasis, influence estrogen metabolism, and play a role in cognitive function (IOM, 2001). The average dietary intake for from the 1994-1996 USDA Continuing survey of Food Intake by Individuals is 1.06 mg/day (IOM, 2001).

Some human oral data are available from cases where boron was ingested for medical reasons. When the amount ingested was less than 3.68 mg/kg, subjects were asymptomatic, while doses of 20 and 25 mg/kg resulted in nausea and vomiting. Case reports and surveys of accidental poisonings indicate that the lethal doses of boron are range from 15 to 20 grams (approximately 200 to 300 mg/kg) for adults, 5 to 6 grams (approximately 70 to 85 mg/kg) for children, and 2 to 3 grams (approximately 30 to 45 mg/kg) for infants (U.S. EPA, 2004a).

There is a single occupational study of 6 workers from a group of 28 exposed to high concentrations of boron (boric acid) aerosols (22-80 mg/m^3) that reported testicular effects, consistent with the testicular effects reported in oral animal studies. However, these data are considered of limited value for risk determination, due to sparse details and small sample size. In a far larger study, no effect on fertility was found in U.S. borate production workers; but exposure concentrations were much lower in this study (about 2.23 mg/m^3 sodium borate or 0.31 mg B/m^3).

Acute irritant effects are well documented in human workers exposed to borates, primarily at concentrations greater than 4.4 mg/m^3. However, there is no evidence for reduced pulmonary function in workers with chronic exposure. Boric acid and borates are distributed evenly throughout the soft tissues in the body water, and are not metabolized. Accordingly, there is no reason to expect route-specific differences in systemic targets. There may be route-specific differences in ability to deliver toxic doses to the targets, in that very high exposure concentrations may be required to produce effects by inhalation exposure. Portal-of-entry effects may also differ with exposure route.

The primary effects seen in animals after chronic exposure to boron at low-effect doses generally involve the testes and developing fetus. Chronic effects of dietary boron exposure in two-year studies included testicular atrophy and spermatogenic arrest in dogs, decreased food consumption, suppressed growth, and testicular atrophy in rats, and decreased survival, testicular atrophy, and interstitial cell hyperplasia in mice. Although researchers observed some increases in tumor incidences in the liver and in subcutaneous tissues in mice (NTP, 1987), based on comparisons to historic controls, these tumors were determined not to be associated with exposure to boron from boric acid. The chronic mouse study conducted by Schroeder and Mitchener (1975) was inadequate to detect carcinogenicity because only one very low dose level was used (0.95 mg B/kg-day), and the MTD was not reached. No inhalation cancer data were located. Studies of boron compounds for genotoxicity were overwhelmingly negative, including studies in bacteria, mammalian cells, and mice *in vivo*. Accordingly, the Agency determined that there are inadequate data to assess the human carcinogenic potential for boron.

In developmental studies with rats, mice, and rabbits, oral exposure to boric acid resulted in decreased pregnancy rate, increased prenatal mortality, decreased fetal weights, and increased malformations in fetuses and pups. However, these reproductive effects were associated with maternal toxicity including changes in maternal organ weights, body weights, weight gain, and increased renal tubular dilation and/or regeneration (Price et al., 1990, 1994, 1996a; Heindel et al., 1992, 1994; Field et al., 1989). Reproductive effects in males were noted in the subchronic and chronic studies described in the preceding paragraphs.

9.2.3 Dose-Response Characterization and Implications in Risk Assessment

The EPA RfD for boron is 0.2 mg/kg/day (U.S. EPA, 2004d) based on developmental effects in rats from two studies (Price et al., 1996a; Heindel et al., 1992). The RfD was derived using the benchmark dose (BMD) method ($BMDL_{05}$ from Allen et al., 1996) using a data derived uncertainty factor of 66. Allen et al. (1996) concluded that decreased fetal body weight was the most suitable endpoint for developing a point of departure, because the benchmark doses calculated for the other endpoints (incidence of total malformations, enlarged lateral ventricles in the brain, shortening of rib XIII, and variations of the first lumbar rib) were higher. EPA established the HRL for boron using the RfD of 0.2 mg/kg-day and a 20 percent relative source contribution. The HRL is calculated to be 1.4 mg/L or 1,400 µg/L.

9.3 Occurrence in Public Water Systems

The first criterion necessitates evaluation of the contaminant to determine if it may have an adverse effect on the health of persons. The second criterion necessitates evaluation of the contaminant to determine if there is a substantial likelihood that it will occur in public water systems with a frequency and at levels of public health concern. In order to address this criterion the following information was considered:

- Monitoring data from public water systems
- Ambient water concentrations and releases to the environment
- Environmental fate

Data on the occurrence of boron in public drinking water systems were used to evaluate the second criterion. The U.S. EPA looked at the total number of systems that reported detections of boron, as well those that reported concentrations of boron above an estimated drinking-water health reference level (HRL). For noncarcinogens, the estimated HRL was calculated from the RfD assuming that 20% of the total exposure would come from drinking water. For carcinogens, the HRL was the 10^{-6} risk level (i.e, the probability of 1 excess tumor in a population of a million people). The HRLs are benchmark values that were used in evaluating the occurrence data while the risk assessments for the contaminants were being developed.

The available monitoring data on occurrence in drinking water, including indications of whether or not the contaminant is a national or a regional problem, are included in Chapter 4 of this document and summarized below. Additional information on production, use, and fate are found in Chapters 2 and 3.

9.3.1 Occurrence Criterion Conclusion

The available data for boron indicate its ubiquitous presence in the ambient environment. Boron, as a naturally occurring element, was detected in many ambient waters, fish tissues, and stream bed sediments. In addition, approximately 81.9% of groundwater PWSs had detections of boron (minimum reporting level, MRL, of 0.005 mg/L). These detections affected about 88.1% of the population served by the PWSs, equivalent to approximately 75.5 million people served by ground water nationally. Nevertheless, the frequency of boron occurrence at levels of public health concern was relatively low. Concentrations in drinking water exceeded the HRL in only approximately 1.7% of surveyed groundwater PWSs, affecting only about 0.4% of the population served, equivalent to approximately 0.4 million people. Supplementary data from an AWWARF-sponsored study indicate that boron contamination of surface water is less significant than boron contamination of ground water. Of 228 ground water and 113 surface water samples analyzed, boron was detected in 99.1% of the ground water samples and 97.3% of the surface water samples. Boron was detected at concentrations greater than the HRL in only 3.1 % of the ground water samples and in none of the surface water samples. The data indicate that, although boron is frequently found in the ambient environment and finished drinking water systems, little to no boron at levels of public health concern is detected in most finished drinking water systems.

9.3.2 Monitoring Data

Drinking Water

Approximately 81.9% of groundwater PWSs had detections of boron ($\geq$minimum reporting level, $\geq$MRL, or $\geq$0.005 mg/L). These detections affected about 88.1% of the population served by the PWSs, equivalent to approximately 75.5 million people served by ground water nationally. Detections at a concentration greater then one-half the health reference level (>½HRL or >0.7 mg/L) occurred in 4.3% of surveyed PWSs, affecting 2.9% of the population served, equivalent to approximately 2.5 million people nationally. Concentrations greater than the HRL (>HRL or >1.4 mg/L) were found in approximately 1.7% of surveyed PWSs, affecting 0.4% of the population served, equivalent to approximately 0.4 million people nationally.

Supplementary data from an AWWARF-sponsored study indicate that boron contamination of surface water is less significant than boron contamination of ground water. Of 228 ground water and 113 surface water samples analyzed, boron was detected in 99.1% of the ground water samples and 97.3% of the surface water samples. Boron was detected at a concentration greater then one-half the health reference level (>½HRL or >0.7 mg/L) in 8.8% of the ground water samples and none of the surface water samples. Boron was detected at concentrations greater than the HRL (>HRL or >1.4 mg/L) in 3.1 % of the ground water samples and in none of the surface water samples. Boron was reported in finished water from 5 ground water and 14 surface systems in the U.S. EPA Community Water Systems. The median and 90th percentile concentrations was less than one-half the health reference level in both cases.

Ambient Water

Although boron is a naturally-occurring element that is widespread in nature, regional ambient water data for boron were available from only two studies. In ground water from the

Sacramento Valley aquifer, boron was detected in all thirty-one samples at concentrations ranging from 12 µg/L to 1100 µg/L. The median concentration was 42 µg/L. Two of the thirty-one samples had concentrations in excess of 600 µg/L (Dawson, 2001). In the lower Illinois River Basin, 71% of ground water samples collected between 1984 and 1991 contained boron concentrations higher than the minimum reporting level of 50 µg/L. The highest detected concentration was 2100 µg/L. Higher boron concentrations were generally found in deeper and more ancient aquifers (Warner, 1999).

9.3.3 Use and Fate Data

In 2003 the United States was the world's largest producer of refined boron compounds with about one-half of the domestic production exported. Borax (hydrous or anhydrous) and boric acid are widely used for a wide range of industrial applications. The principal uses for boron compounds in the United States in 2001 were estimated as follows: 78% glass and ceramics; 6% soaps and detergents; 4% agriculture; 3% flame retardants; and 9% as other boron-containing products. The use pattern for borax in its decahydrate, pentahydrate, and anhydrous forms was: 23% in insulation glass fibers; 20% in household cleaning products as germicide; 11% in borosilicate glasses; 11% as algicide in water treatment; 8% in enamel flux, frits, and glazes; 8% as chemical intermediate for perborates; 7% in fertilizers; 5% as antifreeze corrosion inhibitor; 4% as a chemical intermediate for other boron compounds; 3% in herbicides; 1% as flame retardant and metallurgical flux; and 10% in other miscellaneous applications (HSDB, 2003a). Overall borate uses in 1985 were estimated as follows: 18% glass fiber insulation; 11% textile glass fiber; 15% chemical fire retardants; 5% borosilicate glass; 4% soap and detergents; 13% miscellaneous; and 44% exports (HSDB, 2003a).

Boron enters the environment primarily through weathering of rocks containing boron minerals, boric acid volatilization from seawater, and volcanic activity. Anthropogenic inputs are lower than those from natural processes. Atmospheric boron usually exists as particulates; therefore, particle size and weight determine the half-life of boron-containing particulates in ambient air. Boron and boron-containing compounds in aqueous environments adsorb onto iron and aluminum hydroxy compounds and clay minerals; this is a pH-dependent process with basic conditions favoring the adsorption. Borate ion and boric acid establish an equilibrium in water systems according to pH, with dissolved boric acid predominating at pHs below 9.3. In water and soil, boron adsorbs to particulates high in amorphous aluminum oxide, iron oxide, clay, and to a lesser extent, organic matter. Again, equilibria are pH-dependent and boron adsorption is greatest under basic conditions (pH 7-9). Boron requires high pH and electron rich environments associated with these particulates to form covalent bonds.

9.4 Risk Reduction

The third criterion used to determine if a contaminant requires regulation, states that "in the sole judgment of the Administrator, regulation presents a meaningful opportunity for health risk reduction for persons served by public water systems." In evaluating this criterion, the U.S. EPA conducted an analysis of the total exposed human population, inclusive of sub-populations exposed to levels above the estimated HRL. Estimates of the population exposure levels were derived from monitoring data. These estimates are presented in Chapter 4 and summarized in section 9.4.2.

The U.S. EPA conducted an analysis which considered the exposure to boron from drinking water relative to the total known environmental exposures from all media, to determine if drinking water regulation could significantly reduce health risks. The findings are discussed in Section 9.4.3 below.

In making its regulatory determination, the U.S. EPA also evaluated effects on potentially sensitive populations including the fetus, infants and children; a brief description is given in section 9.4.4.

9.4.1 Risk Criterion Conclusion

Nationally, approximately 2.5 million people consume water from groundwater PWS where boron detections exceeded one-half the HRL of 0.7 mg/L and approximately 0.4 million people consume water from groundwater PWS were detections exceeded the HRL. Mouse, rat, and rabbit studies indicate that the developing fetus is sensitive to boron. Individuals with severely impaired kidney function constitute a sensitive population since the kidney is the main route of boron excretion. The U.S. EPA determined that health risk from boron exposure from public water systems is small, even for sensitive populations, and therefore promulgation of a boron regulation does not present a meaningful opportunity for health risk reduction.

9.4.2 Exposed Population Estimates

Nationally, approximately 81.9% of groundwater PWSs had detections of boron ($\geq$minimum reporting level, $\geq$MRL, or $\geq$0.005 mg/L). Therefore, about 88.1% of the population served by the surveyed groundwater PWSs is exposed to boron in drinking water; this population is equivalent to approximately 75.5 million people. Detections at a concentration greater than one-half the health reference level (>½HRL or >0.7 mg/L) occurred in 4.3% of surveyed groundwater PWSs, indicating that 2.9% of the population served, equivalent to approximately 2.5 million people, are exposed to this level of boron Concentrations greater than the HRL (>HRL or >1.4 mg/L) were found in approximately 1.7% of surveyed groundwater PWSs, indicating that exposure at this level occurs in 0.4% of the population served, equivalent to approximately 0.4 million people.

9.4.3 Relative Source Contribution

Relative source contribution analysis was conducted to compare the estimated magnitude of exposure expected via in the general population from drinking water to the magnitude of exposure from intake of boron in other media, such as magnitude of exposure from other media, including food, air, and soil. The highest average boron exposure is from food and next from water. Using the median concentration of boron in water from Table 4-1 of 0.047 mg/L, and an daily water intake of 2 L/day average exposure from drinking water would be 0.094 mg/day. It is reported that average daily boron intake in normal adult human diets ranges 0.87 to 1.35 mg/day (IOM, 2001). Thus, the average contribution of boron exposure from food is about tenfold greater than that from water. A combination of the 99th percentile concentration in water (2.44 mg/L x 2 L/day= 4.88 mg/day) with the CSFII 99th percentile value for foods (2.97 mg/day; IOM, 2001) is 56% of the 14 mg/day allowance for a 70 kg adult derived from the RfD.

.Based on the data available, the RSC for boron from drinking water would likely be greater than the 20% default used to calculate the health reference level.

Children can potentially ingest significant amounts of boron via hand-to-mouth contact, especially when concentrations in residential areas are naturally high in soil and where boron-containing pesticides are applied in and around homes. Workers in boron-related industry are subject to high boron exposure, but not from drinking water, as in the case of children.

9.4.4 Sensitive Populations

Studies in rats, mice, and rabbits identify the developing fetus as potentially sensitive to boron. Price et al. (1996a) identified an LOAEL of 13.3 mg/kg-day and an NOAEL of 9.6 mg/kg-day in the developing fetus, based on decreased fetal body weight in rats. Accordingly, boron at concentration greater than the HRL might have an effect on prenatal development. Males may also be susceptible to testicular effects from boron exposures during development (Weir and Fisher, 1972).

Individuals with impaired renal function may have an increased risk following exposure. Preeclampsia can be a common complication of pregnancy in which renal function declines, including glomerular filtration. This may increase boron retention, leading to elevated exposures for the mother and fetuses.

9.5 Regulatory Determination Decision

As stated in Section 9.1.1, a positive finding for all three criteria is required in order to make a determination to regulate a contaminant. In the case of boron, the only positive finding is for the health effects criterion, and data are conclusive solely in animal studies. Ingestion of boron may exert adverse effects on human health; however, based on monitoring conducted in the 1980's, the frequency of occurrence and concentration levels of boron in drinking water are believed insufficient to pose any appreciable public health concerns at the present time. Based on low level of occurrence in regulated public water systems, coupled to its ubiquitous and on its natural occurrence in the environment from natural sources and scarcity of any known adverse public health effects, regulating boron in drinking water will not present a meaningful opportunity for health risk reduction for persons served by public water systems.

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APPENDIX A: Abbreviations and Acronyms

ABP	androgen binding protein
ANOVA	analysis of variance
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
B	boron
BA	boric acid
BMD	benchmark dose, maximum likelihood estimate of dose corresponding to BMR
BMDL	the 95% lower confidence limit on the benchmark dose
BMR	benchmark response
bw	body weight
cAMP	cyclic adenosine monophosphate
CAS	Chemical Abstracts Registry
CCL	Contaminant Candidate List
CFSII	Continuing Survey of Food Intakes
CNS	central nervous system
CSAF	chemical-specific adjustment factors
CV	coefficient of variation
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
FEV_1	forced expiratory volume in 1 sec
FR	Federal Register
FSH	follicle stimulating hormone
FVC	forced vital capacity
g	gram
gd	gestation day
GFR	glomerular filtration rate
HRL	health reference level
HSDB	Hazardous Substances Database
ICPMS	inductively coupled plasma-mass spectrometry
IEHR	Institute for Evaluating Health Risks
IOC	inorganic compounds
IOM	Institute of Medicine
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
kg	kilogram
L	liter
LH	luteinizing hormone
LOAEL	lowest observed adverse effect level
m	meter
MCLG	Maximum Contaminant Level Goal
mg	milligram
mL	milliliter
MRL	minimum reporting level
MTD	maximum tolerated dose
NAWQA	National Water Quality Assessment
NDWAC	National Drinking Water Advisory Council

NIOSH	National Institute for Occupational Safety and Health
NIRS	National Inorganic and Radionuclide Survey
NOAEL	no observed adverse effect level
NPDWR	National Primary Drinking Water Regulation
NTP	National Toxicology Program
PA	plasminogen activators
pnd	postnatal day
ppm	parts per million
PWS	public water systems
RfC	reference concentration
RfD	reference dose
SBR	standardized birth ratio
SD	standard deviation
SDWA	Safe Drinking Water Act
TD	toxicodynamics
TDI	tolerable daily intake
TK	toxicokinetics
TRI	Toxic Release Inventory
TWA	time-weighted average
UCM	unregulated contaminant monitoring
UF	uncertainty factor
UFA	interspecies variability (animal-to-human) uncertainty factor
UFH	interindividual variability (sensitive humans) uncertainty factor
UL	upper intake level
U.S. FDA	U.S. Food and Drug Administration
USGS	U.S. Geological Service
U.S. EPA	U.S. Environmental Protection Agency
VOC	volatile organic compound
WHO	World Health Organization