TOXICOLOGICAL REVIEW

OF

BORON AND COMPOUNDS

(CAS No. 7440-42-8)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

April 2002

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U.S. Environmental Protection Agency
Washington, DC

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to boron. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of boron and compounds.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

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This document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

CONTENTS — TOXICOLOGICAL REVIEW for BORON (CAS No. 7440-42-8)

NOTICE ii

FOREWORD iii

AUTHORS, CONTRIBUTORS, AND REVIEWERS iv

1. INTRODUCTION 1

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS 2

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS 4

3.1. ABSORPTION 4

3.1.1. Gastrointestinal Absorption 4
3.1.2. Respiratory Tract Absorption 5
3.1.3. Dermal Absorption 5

3.2. DISTRIBUTION 6
3.3. METABOLISM 8
3.4. ELIMINATION AND EXCRETION 8

3.4.1. Urine 8
3.4.2. Plasma 18
3.4.3. Bone 19

3.5. TOXICOKINETIC SUMMARY 19

4. HAZARD IDENTIFICATION 21

4.1. STUDIES IN HUMANS — EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS 21

4.1.1. Oral Exposure 21
4.1.2. Inhalation Exposure 22

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS — ORAL AND INHALATION 26

4.2.1. Oral Exposure 26
4.2.2. Inhalation Exposure 31

CONTENTS (continued)

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES — ORAL AND INHALATION 31

4.3.1. Developmental Studies 31
4.3.2. Reproductive Studies 35

4.4. OTHER STUDIES — MAY INCLUDE NEUROLOGICAL, IMMUNOLOGICAL, MECHANISTIC STUDIES (E.G., USE OF ISOLATED TARGET TISSUES/ORGANS, STUDIES OF DIRECT ADMINISTRATION, STUDIES OF KNOWN METABOLITES), GENOTOXICITY, SAR 40

4.4.1. Genotoxicity Studies 40
4.4.2. Neurological Studies 41
4.4.3. Mechanistic Studies - Testicular Effects 41
4.4.4. Mechanistic Studies - Developmental Effects 42
4.4.5. Nutrition Studies 42

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN) — ORAL AND INHALATION 43

4.5.1. Oral Exposure 43
4.5.2. Inhalation Exposure 43

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION — SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION 44

4.7 SUSCEPTIBLE POPULATIONS 45

4.7.1. Possible Childhood Susceptibility 45
4.7.2. Possible Gender Differences 45

5. DOSE-RESPONSE ASSESSMENTS 46

5.1. ORAL REFERENCE DOSE (RfD) 46

5.1.1. Choice of Principal Study and Critical Effect — with Rationale And Justification 46

CONTENTS (continued)

5.1.2. Methods of Analysis — Including Models (PBPK, BMD, etc.) 46
5.1.3. Derivation of the RFD 47

5.2. INHALATION REFERENCE CONCENTRATION (RfC) 64
5.3. CANCER ASSESSMENT 64

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE 64

6.1. HUMAN HAZARD POTENTIAL 64
6.2. DOSE RESPONSE 66

7. REFERENCES 68

APPENDIX A. Summary of and Response to External Peer Review Comments 80

APPENDIX B. Benchmark Dose Calculations 88

APPENDIX C. Regression Analysis of Serum Creatinine and Inulin Clearance 94

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC is analogous to the oral RfD. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for boron has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1995a), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *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); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998); and memorandum

from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Boron is a non-metallic element that belongs to Group IIIA of the periodic table and has an oxidation state of +3. It has an atomic number of 5 and atomic weight of 10.81. Boron is actually a mixture of two stable isotopes, ^{10}B (19.8%) and ^{11}B (80.2%) (WHO, 1998a). The chemical and physical properties of boron and selected boron compounds are shown in Table 1.

Because boric acid is a weak acid with a pK_a of 9.2, it exists primarily as the undissociated acid (H_3BO_3) in aqueous solution at physiological pH, as do the borate salts (Woods, 1994). Therefore, the toxicity associated with these compounds is expected to be similar based on boron equivalents. Boron oxide will also produce similar effects because it is an anhydride that reacts exothermically with water in the body to form boric acid (WHO, 1998a). Boric acid can form complexes with carbohydrates and proteins in the body (ECETOC, 1994).

Boric acid and sodium salts of boron (primarily borax, or disodium tetraborate decahydrate) are widely used for a variety of industrial purposes including manufacture of glass, fiberglass insulation, porcelain enamel, ceramic glazes and metal alloys. These compounds are also used as fire retardants in cellulose insulation, laundry additives, fertilizers (boron is an essential element for plants), herbicides (at high concentrations boron is toxic to certain plant species) and insecticides (Woods, 1994). Elemental boron has only limited industrial applications.

Boron is a naturally-occurring element that is widespread in nature, albeit at relatively low concentrations (Woods, 1994). Boron concentrations in rocks and soils are typically less than 10 ppm, although concentrations as high as 100 ppm have been reported in shales and some soils. The overall average concentration in the earth's crust has been estimated to be 10 ppm. Concentrations reported in sea water range from 0.5 to 9.6 ppm, with an average of 4.6 ppm. Fresh water concentrations range from <0.01 to 1.5 ppm. Boron in the environment is always found chemically bound to oxygen, usually as alkali or alkaline earth borates, or as boric acid (IEHR, 1997; U.S. EPA, 1987). Elemental boron is not found in nature.

Table 1. Physical and Chemical Properties of Boron and Selected Boron Compounds

	Boron	Boric Acid	Borax	Borax Pentahydrate	Anhydrous Borax	Boron Oxide
CAS Registry Number	7440-42-8	10043-35-3	1303-96-4	12179-04-3	1330-43-4	1303-86-2
Molecular Formula	B	H_3BO_3	$Na_2B_4O_7 \cdot 10H_2O$	$Na_2B_4O_7 \cdot 5H_2O$	$Na_2B_4O_7$	B_2O_3
Molecular Weight	10.81	61.83	381.43	291.35	201.27	69.62
Boron Content (%)	100	17.48	11.34	14.85	21.49	31.06
Physical Form	black crystal or yellow-brown amorphous powder	white or colorless crystalline granules or powder	white or colorless crystalline granules or powder	white or colorless crystalline granules or powder	white or colorless vitreous granules	white or colorless vitreous granules
Specific Gravity (@ 20 °C)	2.34	1.51	1.73	1.81	2.37	2.46
Melting Point (°C)	2300	169	75, decomposes	742	741	450
Boiling Point (°C)	2550	300	320	320	1575, decomposes	1500
Water Solubility (% w/w)	insoluble	4.72 @ 20 °C 27.53 @ 100 °C	4.71 @ 20 °C 65.63 @ 100 °C	3.6 @ 20 °C 50.15 @ 100 °C	2.48 @ 20 °C 34.5 @ 100 °C	rapidly hydrates to boric acid
Vapor Pressure (mm Hg)	1.56×10^{-5} atm @ 2140 °C	No Data	No Data	No Data	No Data	No Data

Sources: ATSDR, 1992; ECETOC, 1994; U.S. EPA, 1987; WHO, 1998a

Boron is not transformed or degraded in the environment, but depending on environmental conditions (e.g., pH, moisture level), changes in the specific form of boron and its transport can occur (ATSDR, 1992). Natural weathering is expected to be a significant source of environmental boron (ATSDR, 1992). The most important source of exposure for human populations is ingestion of boron from food (primarily fruits and vegetables) (Anderson et al., 1994; Naghii and Samman, 1996a; WHO, 1998a). Occupational exposure to borate dust and exposure to borates in consumer products (e.g., cosmetics, medicines, insecticides) are other potentially significant sources.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

Boron is well absorbed from the gastrointestinal tract in humans. 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 6 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 using the ointment, suggesting delayed absorption of boron from the ointment in comparison to the water vehicle. Similarly, 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 were also determined to have had over 90% absorption of boron based on urinary excretion data (Job, 1973). Naghii et al. (1977) studied the effect of boron supplementation (10 mg B/d) 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 µg 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.

3.1.2. Respiratory Tract Absorption

Boron is absorbed during inhalation exposure. Culver et al. (1994) monitored boron levels in the blood and urine of 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 (≈0.25 μg/g) in comparison to pre-shift Monday morning values (≈0.1 μg/g). Similarly, workers in the high exposure category had significantly higher urinary boron levels Monday post-shift (≈12 μg/mg creatinine) than pre-shift (≈2 μg/mg creatinine). Boron in the diets (which were assigned by the researchers to ensure uniformity among workers) and workplace air was also 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.

3.1.3. Dermal Absorption

Boron is apparently not absorbed across intact skin. 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 1-10 month old infants exposed to talcum powder containing 5% boric acid 7-10 times per day for at least one 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 were continued on the powder regimen for 48-72 hours after rashes appeared. Their boron blood levels were similar to controls. However, there is evidence

that boron will be absorbed through more severely damaged skin, at least from an aqueous vehicle. Blood and urinary boron levels were increased in 6 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). However, 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, and 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).

3.2. DISTRIBUTION

Available 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. Lack of appreciable accumulation of boron in the testis was demonstrated by Lee et al. (1978) and Treinen and Chapin (1991), and in the epididymis by Treinen and Chapin (1991). 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 2. 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. 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 in all tissues except adipose tissue and bone were approximately 2-fold for any given day of examination (days 1,2,3,4,7). 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. Accumulation of boron in bone in rats was also shown by Forbes and Mitchell (1957). Boron levels in adipose tissue remained at 20% of plasma levels after 7 days.

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.

Table 2. 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 ± 0.17	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
Hypothalmus[b]	0.91	14.30
Testis	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

In a drinking water study using multiple dose levels of boric acid in rats, Naghii and Samman (1996b) 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). These 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 injection.

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.

3.3. METABOLISM

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). 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 (Section 4.4.4. Nutrition Studies).

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 is apparently required to break the boron-oxygen bond (WHO, 1998a). 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.

3.4. ELIMINATION AND EXCRETION

3.4.1. Urine

The elimination and excretion of boron have been evaluated in humans and rodents, and 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 (see Section 3.1.1. for descriptions of several such studies). In humans, Jansen et al. (1984a) and Schou et al. (1984) reported that boron's primary route of elimination was in the urine, and that approximately 93% of an orally administered dose is eliminated within 96 hours. Jansen et al. (1984b) reported that approximately 60-75% of an orally administered 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, 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 post administration. Astier et al. (1988) reported an acute boron intoxication of 45 g boric acid (7.9 g B) where >50% of the dose was eliminated through the kidneys over the first day following ingestion (renal clearance: 0.77 L/hour; tubular reabsorption: 80%; total clearance 10.5 g). 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).

Following an intravenous dose in humans of 28.52-31.9 mg boric acid (5-5.6 mg B) per minute or a total dose per subject of 520-620 mg boric acid (91-108.5 mg B), high volumes of distribution were reported by Jansen et al. (1984a), who also reported that boron's primary route of elimination was in the urine. When quantified over 120 hours, the fraction of dose eliminated in urine accounted for 98.7±9.1% of administered dose. Urinary elimination of boron in humans occurs rapidly and is the primary route of elimination. These data indicate almost total bioavailability of an orally administered boron dose in the human.

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 (non-pregnant and pregnant) was examined in a pharmacokinetic study sponsored by U.S. Borax at the University of California, Irvine (U.S. Borax, 2000 rat study; Vaziri et al., 2001). Three groups of 10 non-pregnant 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 mgB/kg/day. On the morning of the eighth day, 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 non-pregnant 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 mgB/kg, respectively) by gavage in deionized water (ultrapure). The purpose of the choice of some of the doses in this study, given by the authors were as follows: the low dose was chosen as an estimate of the high end human dietary dose level, the highest dose tested was approximately half of the NOAEL from the rat developmental toxicity study (Price et al., 1996a).

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. The concentration of boron in the urine during the 12 hour collection period in the non-pregnant rats was 1.67±0.62 10.12±8.16 and 66.82±47.00 µg B/mL for the low, mid and high dose respectively and in the pregnant rats 1.62±0.49, 12.30±5.12 and 121.45 µg B/mL in the low, mid and high dose respectively. The urine volume was not significantly different in pregnant and non-pregnant 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 non-pregnant 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 non-pregnant and pregnant animals and higher in the pregnant compared to the non-pregnant rats across dose groups which was statistically significant at the high dose only (see urinary data in Table 3). Although the boron diet used for this study was low, it still 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 are 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 non-pregnant and pregnant rats respectively, whereas at the mid and high doses, the diet contributed 3% and 0.3% respectively to the total dose. The authors suggest the incremental increase at the low dose may explain in part the greater recovery of administered dose in the low dose group.

Clearance rates of boric acid, creatinine and urea were expressed in three different ways mL/min, mL/min/kg of body weight and mL/min/cm^2 of body surface area (see Table 4). Boric acid clearance was independent of dose within the range of dose levels tested. Boron clearance was slightly higher in pregnant rats compared to non-pregnant rats but the difference was not statistically significant. The rate of creatinine clearance did not vary significantly with the different doses of boric acid in either non-pregnant or pregnant rats. Creatinine clearance, normalized against body weight, however was significantly greater in non-pregnant rats compared to pregnant rats. Urea clearance was not significantly different between non-pregnant and pregnant rats. And there were no consistent differences in the rate of urea clearance with the different doses of boric acid. Individual rat boron clearance data for pregnant and non-pregnant rats are presented in Table 5 and Table 6 respectively.

Fractional excretion of boron which is defined as the ratio of boron clearance/creatinine clearance was 65% and 80% in non-pregnant and pregnant rats, respectively. Fractional

excretion of urea was lower in non-pregnant rats than in pregnant rats. The authors indicated that

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

Dose (mg BA/kg/day)	Urinary B (µg/mL)		Urine Volume (mL)		12-hr Urinary B Excretion (µg/12 hr)		Percent of Dose in 12-Hr Urine (3-15 Hr)	
	Non-pregnant[b]	Pregnant[b]	Non-pregnant	Pregnant	Non-pregnant[b]	Pregnant[b]	Non-pregnant[b,c]	Pregnant[b,c]
0.3	1.7±0.6[d] (9)	1.6±0.5 (9)	4.3±1.4 (9)	6.1±3.2 (9)	6±1[d] (9)	8±3 (9)	50.4±10.6%[d] (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[e] (11)	6.8±3.9 (10)	5.4±2.5 (11)	324±61 (10)	561±114[e] (11)	24.6±4.3% (10)	34.7±6.4%[e] (11)

[a] Source: U.S. Borax, 2000; Vaziri et al., 2001

[b] Statistically significant difference in urinary boron concentration across dose levels based on two-way ANOVA, $p<0.05$

[c] Statistically significant difference across groups (non-pregnant vs. pregnant) based on two-way ANOVA, $p<0.05$

[d] Mean ± standard deviation (number of rats)

[e] Statistically significant difference between non-pregnant and pregnant rats based on multiple range test, $p<0.05$

Table 4. Clearance of Boric Acid (BA) Creatinine and Urea in Non-Pregnant and Pregnant Rats Given Boric Acid by Gavage expressed as mL/min, mL/min/cm^2 and mL/min/kg[a]

Dose (mg BA/kg)	Boric Acid Clearance (mL/min)		Creatinine Clearance (mL/min)		Urea Clearance (mL/min)	
	Non-pregnant[b]	Pregnant[b]	Non-pregnant	Pregnant	Non-pregnant	Pregnant
0.3	0.77±0.2[c] (9)	1.01±0.2 (9)	1.3±0.4[c] (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[d] (11)	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[b] (9)	4.3±1.5[b] (9)	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[b] (10)	4.2±1.1[b] (9)	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[b] (10)	4.3±1.0[b] (11)	3.8±1.0 (10)	3.5±0.7 (11)

[a] Source: U.S. Borax, 2000; Vaziri et al., 2001
[b] Statistically significant difference across groups (non-pregnant vs. pregnant) based on two-way ANOVA, $p<0.05$
[c] Mean ± standard deviation (number of rats)
[d] Statistically significant difference between non-pregnant and pregnant rats based on multiple range test, $p<0.05$

Table 5. Urinary Clearance of Boron in Pregnant Rats[a]

0.3 mg/kg/day[b, c]	3.0 mg/kg/day[b, c]	30.0 mg/kg day[b, c]	Combined[c]
not pregnant	2.954	3.329	
3.714	2.532	2.670	
4.443	--	3.089	
3.592	3.822	2.849	
3.447	3.784	2.996	
2.983	3.564	3.574	
3.023	3.064	3.957	
3.109	2.640	3.757	
2.499	3.116	4.103	
3.114	2.978	4.101	
		3.075	
3.325[e]	3.162[e]	3.409[e]	3.306[e]
0.56 (9)[d, f]	0.47 (9)[f]	0.52 (11)[f]	0.506 (29)[f]

[a] Adapted from U.S. Borax (2000 rat study) and Vaziri et al. (2001)

[b] Dose is presented as mg boric acid/kg/day.

[c] Results presented as mL/min/kg body mass.

[d] N values are presented in parentheses.

[e] Mean

[f] Standard deviation

Table 6. Urinary Clearance of Boron in Non- Pregnant Rats[a]

0.3 mg/kg/day[b, c]	3.0 mg/kg/day[b, c]	30.0 mg/kg day[b, c]	Combined[c]
3.02	3.422	2.896	
4.073	2.982	3.927	
3.423	2.823	3.203	
3.717	3.368	2.647	
3.161	3.176	3.252	
3.428	3.010	3.213	
3.396	3.338	3.691	
1.651	3.002	3.834	
2.013	3.642	2.579	
died	1.514	3.106	
3.098[e]	3.028[e]	3.235[e]	3.121[e]
0.78 (9)[d, f]	0.59 (10)[d, f]	0.47 (10)[d, f]	0.603 (29)[d, f]

[a] Adapted from U.S. Borax (2000 rat study) and Vaziri et al. (2001)

[b] Dose is presented as mg boric acid/kg/day.

[c] Results presented as mL/min/kg body mass.

[d] N values are presented in parentheses.

[e] Mean

[f] Standard deviation

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.

A human study to measure renal clearance of boron normally consumed in the daily diet in non-pregnant and pregnant women was conducted (U.S. Borax, 2000; Pahl et al., 2001). This study was conducted in 32 women in good health between the ages of 18-40 years. Sixteen women in their second trimester (14-28 weeks) were chosen for this study. Sixteen age-matched non-pregnant women were also chosen for this study. At the beginning of the study all subjects were asked to empty their bladder and a baseline blood sample was taken. Urine for each subject was pooled during the 2 hours following the initial blood samples. 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 was also collected. 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 was estimated from the renal excretion of boron in 24 hours which was 1.3 mgB/day, from which an average consumption was estimated at 0.02 mgB/kg per day.

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) by a contract laboratory following scrutinized 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. There are two sets of data for boron clearance in this study. The first is the 2 hour clearance data where the urine was collected in the clinic to insure complete collection. The second data set on boron clearance from this study is a 24 hour clearance value that combines the 2 hour clearance value with the 22 hour clearance value. The 22 hour clearance samples were not collected onsite. The 2 hour clearance values are presented in this document because they were considered to be more accurate due to the compliance with the collection 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 (Table 7). The results indicated that the clearance rate for boron in pregnant women was 1.02 ± 0.55 (mean $\pm$ standard deviation; range 0.252-2.028 mL/min/kg) and the clearance rate for boron in non-pregnant women was 0.80 ± 0.31 (mean $\pm$ standard deviation; range 0.229-1.358 mL/min/kg) mL/min/kg body mass (see Table 8). These results indicate that pregnant women clear boron more effectively than non-pregnant women. These results are consistent with increased measures of renal function in humans during pregnancy.

Creatinine clearance was normal in all subjects and comparable in pregnant and non-pregnant women. Comparison of the clearance of boron with creatinine gives insight into tubular handling of boron. The authors indicated that the ratio of boron clearance to creatinine clearance(fractional excretion of boron) indicates tubular reabsorption if the ratio is<1 and tubular secretion if the ratio is >1. The fractional excretion of boron in all the women in the study was <1. According to the authors this indicated tubular reabsorption in both non-pregnant and pregnant women. There was a trend toward increased fractional excretion or reduced tubular

Table 7. Urinary Clearance of Boron in Women at 2 Hours[a]

Non-pregnant[b]	Pregnant[b]
0.826	0.399
0.229	0.252
0.394	1.429
0.319	0.332
0.868	2.028
0.699	1.759
1.358	1.362
0.887	1.246
0.838	0.537
1.176	1.463
0.888	0.713
0.958	0.809
0.949	0.833
0.775	1.420
no sample	0.706
no sample	no sample
0.80[c]	1.02[c]
0.31[d]	0.55[d]

[a] Adapted from U.S. Borax (2000 human study)

[b] Data are presented as mL blood cleared of boron per minute per kg body mass.

[c] Mean

[d] Standard deviation

Table 8. Clearance of Boron in Pregnant and Non-Pregnant Rats and Humans

Species	Dose[a]	Boron Clearance (mL/min/kg)	
		Pregnant	Non-Pregnant
Rat[b]	0.3 mg/kg/day	3.36 ± 0.6 (9)[c]	3.10 ± 0.78 (9)
	3.0 mg/kg/day	3.2 ± .05 (9)	3.02 ± 0.59 (10)
	30.0 mg/kg/day	3.4 ± 0.5 (9)	3.24 ± 0.47 (10)
	Combined	3.3 ± 0.51 (29)	3.12 ± 0.60 (29)
Humans[d]	0.114 mg/kg/day [e]	1.02 ± 0.55 (15)	0.80 ± 0.31 (14)

[a] Dose is presented as mg boric acid/kg/day

[b] Data adapted from U.S. Borax (2000 rat study)

[c] Data are presented as mean ± standard deviation (n).

[d] Data adapted from U.S. Borax (2000 human study)

[e] Dietary intake was estimated by U.S. Borax (2000 human study) as 0.02 mg boron/kg/day (equivalent to 0.114 mg boric acid/kg/day)

reabsorption in pregnant women, however the difference between the fractional excretion of pregnant and non-pregnant women was not statistically significant. In the rat clearance study (U.S. Borax, 2000; Vaziri et al., 2001) pregnant rats showed increased fractional excretion of boron at all dose levels. Using the data from the rat and the human renal clearance study, clearance of boric acid in pregnant rats and pregnant humans can be compared. Table 8 shows boron clearance for pregnant women and pregnant rats. The observations from all rat dose groups were combined, as there were no dose-related differences in the clearance values.

3.4.2. Plasma

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 to 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 tetra-borate containing 0.4 mg boron/100g 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 is that a large amount of blood was drawn from the rats in the 24 hour period which 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 non-pregnant and pregnant women (see description of the study in Section 3.4.1.). 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 non-pregnant subjects. After 24 hours plasma boron levels were lower in the pregnant women when compared with non-pregnant women, however 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 non-pregnant 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 compared with that obtained 3 hours after dosing (see description of studies in Section 3.4.1.). Mean plasma levels of boron were slightly higher in pregnant rats compared with non-pregnant 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 non-pregnant and six pregnant rats were given low B in the diet for 7 days as described previously in the clearance study (see Section 3.4.1.). 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 liniarity 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 μ/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 non-pregnant 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 non-pregnant or pregnant rats. The estimated half-life of boric acid in non-pregnant and pregnant rats were 2.9 and 3.2 hours, respectively, which was not statistically different.

3.4.3. Bone

Elimination of boron from bone was studied in rats by Chapin et al. (1997). Bone (tibia) boron levels were monitored for 32 weeks following cessation of exposure in rats that had been fed boron in the diet at 4500-9000 ppm 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: ≈6 μg B/g bone from ≈60 μg B/g bone) but still remained 5- to 6-fold higher than bone levels in unexposed controls (≈1 μg B/g bone). Even after 32 weeks of recovery (and ≈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. Accumulation of boron in skeletal bones of human cadavers has also been reported by Alexander et al. (1951) and Forbes et al. (1954).

3.5. TOXICOKINETIC SUMMARY

There is no evidence that boron is metabolized in the body. Boron is readily absorbed following oral exposure both in humans and in animals. Greater than 90% of an orally administered dose of boron as boric acid is excreted in a short time in both humans and in animals (Jansen et al., 1984a; Schou et al., 1984; Usuda et al., 1998; Vanderpool et al., 1994). In humans, boron was excreted 92-94% unchanged in the urine after 96 hours (Jansen et al., 1984a) Studies in rats have shown that orally administered boron is completely absorbed in 24 hours

(Usuda et al., 1998). Studies in mine workers and rats have shown that boron is also absorbed during inhalation exposure (Culver et al., 1994; Wilding et al., 1959). Boron is also not absorbed across intact skin in humans or animals (Draize and Kelley, 1959).

Examinations in rats have revealed a fairly uniform distribution of boron outside the blood compartment across various tissues (liver, kidney, muscle, large intestine, brain hypothalamus, testis, epididymis, seminal vesicles, seminal vesicle fluid, adrenals and prostate). Notable exceptions are that consistently lower concentrations of boron were found in fat and consistently higher concentrations were observed in bone (Ku et al., 1991). Accumulation of boron in fat was 20% of plasma levels after day 7 and boron in bone was increased 2- to 3-fold over plasma levels after day 7. The pharmacokinetic study of boron by Usuda et al. (1998) cited a high volume of distribution of 142.0±30.2 mL/100 g body weight. When this finding is combined with the relatively uniform distribution of boron to the tissues, the likelihood for sequestration of boron by a given tissue is minimal. When these data from rodents (plasma half-life, urinary elimination time course and tissue distribution) are compared with the data available for humans (plasma elimination half-life reports and high volume of distribution of 104.7 mL/100 g body weight), it seems reasonable that the distribution of boron to human tissues parallels that observed in rodents.

Because of the extent to which boron's residence time in the body and pharmacokinetic profile are influenced by urinary elimination, a more thorough investigation of the urinary clearance of boron was undertaken to determine the difference in the urinary clearance of boron in pregnant and non-pregnant rats and humans. Reports from studies (U.S. Borax, 2000; Pahl et al., 2001; Vaziriet al., 2001) indicated that the renal clearance of boron from female rats was greater than in humans, and that pregnant rats and pregnant women cleared boron slightly more efficiently than non-pregnant rats and women. The magnitude of the difference (rat:human) between average clearance values was approximately 3.6-fold and 4.9-fold for pregnant and non-pregnant individuals, respectively, in close agreement with differences in kinetic parameters predicted by allometric scaling (approximately 4-fold). The variance of boron clearance in humans was slightly greater than in rats (0.35%), but the coefficient of variation (s.d.÷ mean) was 4-fold higher in humans than in rats. Overall, the available pharmacokinetic data support a high degree of qualitative similarity (lack of metabolism, highly cleared through renal filtration mechanisms, and apparently consistent extravascular distribution characteristics) between the relevant experimental species and humans.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS — EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

4.1.1. 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 poisioning cases are reported in the literature; however, quantitative estimates of absorbed dose are limited. Baker et al. (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.

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 recovering from surgery had boric acid solution (70 mg B/kg) injected into the subcutaneous fluid infusion, which resulted in severe cutaneous and G.I. symptoms but recovery occurred after hydration and diuresis.

Because boron compounds were used for various medical conditions including epilepsy, malaria, urinary tract infections and exudative pluritis from the mid 1800's until around 1900, some data are available on longer term exposure. Culver and Hubbard (1996) report on early literature 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. In a “short report” in *Archives of Disease in*

Childhood, O'Sullivan and Taylor (1983) report seizures (and other milder effects) in seven infants who had 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 to 16 weeks (at the end of the exposure period), were exposed to the honey-borax mixture over a period of 4 to 10 weeks. Original estimates of exposure for this paper were based on an error in the paper confirmed 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 to 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 to 5.3 kg were estimated from the Exposure Factors Handbook (U.S. EPA, 1997). 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 to 33 mg/kg-day. The lowest exposure level of 3.2 mg/kg-day would be considered a 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 were also reported for a control group of 15 children aged 2 to 21 months, who had received no boron supplement and, presumably, had suffered no seizures. The control group blood boron values ranged from 0 to 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 of 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 a NOAEL. However, we don't know if any infants predisposed to seizures were in the control population. The presumptive boron NOAEL would be 0.32 mg/kg-day for a senstive human subpopuation. Given the relatively uncomplicated boron toxicokinetics, the lack of correlation of blood boron and estimated ingestion rates suggests 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 (a "short report") 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 Ishii 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.

4.1.2. 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 SRM (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 (see Section 4.3.2), 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. Secondly, the investigators 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 had vasectomies (36%) was 5 times higher than the national average of 7% implicit in the expected number of births. Participants were divided into 5 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 middle 3 dose groups. There were 42 participants who worked high-exposure jobs for two 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 these 42 workers was close to expected (102) despite a 48% vasectomy rate. These workers also had elevated SBRs 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 percent 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. It must be recognized, however, that the rather 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 one 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 FEV and respiratory illness data in the subgroup of 82 men who had worked for at least one year at the calcining and fusing processes compared with the other 547 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 for (in order of decreasing frequency) 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 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.

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 tests (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.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS — ORAL AND INHALATION

4.2.1. Oral Exposure

In the following studies, when not reported by the investigators, approximate dosages were calculated 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). A high-dose male dog died as a result of complications of diarrhea on day 68 of the study 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 was also 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 also at the high dose were increases in brain:body weight ratio (borax) and liver:body weight ratio (boric acid) in females and 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, and 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 a LOAEL of 1750 ppm boron (male: 30.4 mg B/kg-day;

female: 21.8 mg B/kg-day) and a 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. Control animals (4 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 dosed 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 as being "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 were noted throughout the study to have about an 11% decrease in the rate of weight gain when compared with control animals. 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 these 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.

These dog studies were not used to calculate the RfD due to several limitations, including the 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 were taken from two different studies of different duration. Also, the study pathologist considered the histopathological findings as being "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 NOAEL.

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 a NOAEL of 175 ppm boron (8.8 mg B/kg-day) and a LOAEL of 525 ppm boron (26.3 mg B/kg-day) boron for systemic toxicity in rats following subchronic dietary exposure.

In the chronic study, 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 a LOAEL of 1170 ppm (58.5 mg B/kg-day) and a 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 an 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, and 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.

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 μg 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, a LOAEL of 23.7 mg B/kg-day can be identified. A NOAEL was not identified.

The subchronic and chronic toxicity of boron (boric acid) in mice was studied by NTP (1987; 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 controls 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 were also 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.

There were increased incidences of hepatocellular carcinoma (5/50, 12/50, 8/49) and combined adenoma or carcinoma in low dose male mice (14/50, 19/50, 15/49) (NTP, 1987; Dieter, 1994). The increase was 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 in this study 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.

4.2.2. 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. 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. Also in this study, 3 dogs were exposed to 57 mg/m^3 (18 mg B/m^3) for 23 weeks. 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 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).

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES — ORAL AND INHALATION

4.3.1. Developmental Studies

Heindel et al. (1994, 1992; 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 controls]). Food and water intake, and body weights, as well as clinical signs of toxicity, were monitored throughout pregnancy. On day 20 of gestation, the animals were sacrificed and 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 gestation days 12 through 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 days 6-9 and increased on days 15-18, 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; this was due to increases in the percentage of resorptions per litter and percentage of 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, 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 a LOAEL; a 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 and 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 percent 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 weight 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 significant trend test ($p<0.05$) was found for decrease 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 percent 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 gestation day 20 from the previously described study (Price et al., 1996a, 1994) where dietary concentration of added boric acid yielded average daily intakes equivalent to 0, 3, 6, 10, 13, or 25 mg boron/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±0.987μg 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 μg boron/g were associated with the NOAEL (10 mg boron/kg/day) and the LOAEL (13 mg boron/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; 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 percent 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 a LOAEL based on decreased fetal body weight per litter and the 0.1% level is a 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 day 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 controls). 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 controls). 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 high dose compared with 1/159 in controls). The incidence of skeletal malformations was comparable among groups. Relative to controls, the percent of fetuses with variations (all types combined) was not significantly increased in any treated group, but the percent 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 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.

4.3.2. Reproductive Studies

4.3.2.1. *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 a 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). As estimated by the investigators, 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 a NOAEL for this study.

In a follow-up study, Dixon et al. (1979); Lee et al. (1978) administered diets containing 0, 500, 1000 or 2000 ppm boron, as borax, 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 a LOAEL of 50 mg B/kg-day and a 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 killed 14 days later. In both the time-response and the dose-response studies, the above doses are the total of 2 doses administered at 0900 and 1600 hours 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, and 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 (percent 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 had occurred by day 57. This study described a LOAEL for male reproductive effects of 175 mg B/kg bw and a 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 and 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 et al. (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 at 6000 and 9000 ppm. A mean testis boron level of 5.6 µg B/g of tissue was associated with inhibited spermiation, whereas 11.9 µg 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 did not recover post-treatment. Atrophy in the 6000 and 9000 ppm dose groups did not recover post-treatment. The low dose of 26 mg B/kg-day was a 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 et al. (1994) showed that testicular atrophy and central nervous systems (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 et al. (1996b) studied the specificity of the effect of boron on steroid hormones and the impact of plasma lipids in rats. After 2 weeks on boron addition to the drinking water (2 mg B/rat/day) significant elevations occurred in the plasma 1,25-dihydroxyvitamin D concentration and a significant decrease in the plasma triacyglycerol and total HDL-cholesterol concentrations compared to controls. At 4 weeks the plasma testosterone concentration was significantly elevated and the HDL-cholesterol was significantly lower.

4.3.2.2. *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, which 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. A LOAEL of 58.5 mg B/kg-day and a 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 diet was predicted by the authors to 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, which 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) were also 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. Since 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). A NOAEL was not identified.

4.4. OTHER STUDIES

4.4.1. 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.

4.4.2. 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) reported on case reports of neurological effects after accidental ingestion of high levels of boron as boric acid. 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 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 on seven infants exposed to a honey-borax mixture for 4-10 weeks, where the estimated ingestion was 9.6-33 mg B/kg-day. (see Section 4.1.1.).

4.4.3. Mechanistic Studies - Testicular Effects

The occurrence of testicular effects in the absence of overt systemic toxicity (see Section 4.2.1) 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 4.3.2.1 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).

4.4.4. 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.

4.4.5. Nutrition Studies

Boron has been known since the 1920s 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 (Nielson, 1996). It is suggested that boron may prevent inflammatory disease as 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. 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 U.S. 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 can be higher than in the U.S. 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.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN) — ORAL AND INHALATION

4.5.1. 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, central nervous system, 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).

4.5.2. 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 all 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, 1996b; 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,

however, be route-specific differences in ability to deliver toxic doses to the targets, so that for example, 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 over 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) is 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 (≈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 and the animals were covered with dust. Systemic endpoints were not examined, but growth was reduced 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.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION — SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

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 low-dose, but not high-dose, male mice that was 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 was also 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*.

Under EPA's current guidelines for carcinogen risk assessment (U.S. EPA, 1986a), boron is classified as Group D; not classifiable as to human carcinogenicity. Under the new proposed guidelines (U.S. EPA, 1996a), the data are considered to be inadequate for evaluation of the human carcinogenic potential of boron.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

The developing fetus is the most sensitive target of boron toxicity that has been identified. An oral dose of 13.3 mg B/kg-day on days 0-20 of gestation produced decreased fetal body weight in rats (Price et al., 1996a). The NOAEL was 9.6 mg B/kg-day. Maternal effects were not seen in the same study, even at doses of 25 mg B/kg-day. Fetal body weight deficits did not continue into the postnatal period, suggesting that the effect is specific to the fetal period. Based on data from poisoning case reports, the lethal oral dose of boric acid in infants (2-3 g) and children (5-6 g) is similar to that in adults (15-20 g) on a mg/kg basis (≈200 mg/kg). Based on acute human data, infant doses of 30.4-94 mg B/kg were at the upper end of the adult dose response curve of 35-90 mg B/kg. Acute infant and adult human response to boron is similar quantitatively and qualitatively (Culver and Hubbard, 1996) (see Section 4.1.1.). No additional information was available to assess childhood susceptibility.

4.7.2. Possible Gender Differences

The two most sensitive targets of boron that have been identified are the developing fetus (rats, mice and rabbits) carried by the pregnant female, and the testes of the male. The developing fetus (LOAEL = 13.3 mg B/kg-day, NOAEL = 9.6 mg B/kg-day) appears to be slightly more sensitive than the male testis (LOAEL = 29 mg B/kg-day, NOAEL = 8.8 mg B/kg-day) (Price et al., 1996a; Weir and Fisher, 1972).

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 non-pregnant 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.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. 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 a 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). The dog study by Weir and Fisher (1972) identified a NOAEL and LOAEL 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).

The Institute for Evaluating Health Risks concluded that there was a consistent correlation between boric acid exposure and the different effects on rib and vertebral development in rats, mice and rabbits (IEHR, 1997). Of these three species, the rat was the most sensitive to low-dose effects. There was a causal association between exposure to boric acid and the short rib XIII when fetuses were examined at late gestation or when pups where examined at pnd 21. The IEHR (1997) concluded that decreased fetal body weight occurred at the same dose or at doses lower than those at which skeletal changes were observed, and agreed that this was the preferred data set for deriving quantitative estimates.

5.1.2. Methods of Analysis — Including Models (PBPK, BMD, etc.)

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) studies. The earlier study by Heindel et al. (1992) did not define a 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 a NOAEL. The results of the Allen et al. (1996) benchmark dose analysis for decreased fetal body weight for the Price study alone gave a BMDL of 47 mg BA/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 BA/kg/day (9.8 mg B/kg/day). The combined data from Heindel et al. (1992) and Price et al. (1994, 1996a) gave a BMDL of 59 mg BA/kg/day (10.3 mg B/kg/day). 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 BMDL was defined in terms of a prespecified level of response, referred to as the benchmark response (BMR) level (Kavlock et al., 1995). For mean fetal weight analysis, the BMDL was defined as the 95% lower bound on dose corresponding to a 5% decrease in the mean (BMR was 5% decrease). For the continuous power model, F-tests that compared the lack of model fit to an estimate of pure error were employed.

For all endpoints, the results of the two studies were compared. The dose-response patterns were examined 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; this result indicated that the responses from the two studies were consistent with a single dose-response function. BMDL values calculated with a continuous power model for fetal body weight (litter weight averages) were less than those for all other relevant endpoints. The BMDL based on the combined results of the two studies was 10.3 mg B/kg-day, which was very close to the NOAEL of 9.6 mg B/kg-day from the Price et al. (1994, 1996a) study. The BMDL of 10.3 mg B/kg-day 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 could be used in the BMDL estimation, thereby increasing the confidence that the dose response pattern has been estimated satisfactorily.

5.1.3. 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 broad areas of uncertainty, such as "animal-to-human" (interspecies; UF_A) and "sensitive human" (interindividual; UF_H) extrapolations. Both UF_A and UF_H, however, can be thought of 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 has been previously introduced into U.S. EPA methodology in the Reference Concentration methodology (U.S. EPA, 1994b), where 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 developed a document providing guidance in the selection of chemical-specific adjustment factors (CSAF), which does cover systemic kinetics and dynamics (IPCS, 2001) (see Section 5.1.3.8.). This document (IPCS, 2001) is still considered as a draft and has not been formally reviewed by the U.S. EPA. In general, the toxicokinetic factor development in the boron RfD derivation is consistent with IPCS (2001). Additionally, IPCS had previously

[1] equivalent to *pharmacokinetics* and *pharmacodynamics* in the medical literature.

applied the TK/TD subfactor approach in their assessment of boron (WHO, 1998a). The TK and TD factors described here are derived from data and represent variability between species and within humans. As such, they are no longer *uncertainty* factors and are more correctly termed *variability* factors. The latter designation shall be adopted in this document.

In the most simple terms, toxicokinetics deals with what the body does to the chemical, while toxicodynamics deals 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 a TK/TD model could be a sophisticated multi-compartment, highly non-linear, biologically-based toxicokinetic model linked to a mathematical dose-response model relating molecular/cellular response to whole-organism response. Most of the time, however, extrapolations are based upon a simple multiplicative combination of two uncertainty 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 in order to relate the internal kinetics back to the external dose. Simple absorption and distribution constants should usually suffice.

5.1.3.1. *TK/TD Subfactor Default Values (Uncertainty)*

The WHO (1994) and IPCS (2001) have maintained a default value of 10 for both the UF_A (interspecies uncertainty) and UF_H (intraspecies uncertainty). Based upon limited data describing toxicodynamic or toxicokinetic differences, the UF_A of 10 is apportioned between 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 ($10^{0.6}$). Similarly, WHO (1994) and IPCS (2001) divided UF_H 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 for at least two RfCs, but has not explicitly addressed the issue for RfDs (U.S. EPA, 2001). As standard notation in this document, the factors addressing uncertainty (as opposed to variability) henceforth will be designated as UF_{AK}, UF_{AD}, UF_{HK}, and UF_{HD}, respectively.

The default half-order of magnitude toxicokinetic/toxicodynamic uncertainty factor partition is essentially an ignorance-based one, in that if we don't have any evidence to the contrary, we assume equal contribution from each source of uncertainty. The kinetic and dynamic default values for UF_A are given unequal values for the boron assessment, as there is empirical and conceptual support for an uneven default partition. For the class of compounds, such as boron, for which a physiological rate is justified as the sole toxicokinetic scaling variable, the IPCS (1998, 2001) approach is adopted, where UF_{AK} and UF_{AD} are assigned default

values of 4.0 and 2.5, respectively. This partition is based primarily on an empirical analysis published by Renwick (1993), in which various kinetic and dynamic factors for test animals and humans were compared. The toxicokinetic factors were blood flows (renal and hepatic, liver weight, and plasma kinetics (absorption and 1st pass metabolism), which were compared for several species (mouse, rat, rabbit, dog, and monkey) with human values. The toxicodynamic endpoints were various physiologic (primarily hematological) responses, either constitutive or chemically-stimulated, compared between rodents and humans. Renwick (1993) reported average animal-to-human ratios of 2.1 (range 0.8-4.5) for toxicokinetic differences related to physiological processes (liver weight, liver plasma flow, and renal plasma flow) and average animal-to-human ratios of 1.2 (range 0.04-3.3) for the toxicodynamic responses. Partitioning the relative difference within the 10-fold overall interspecies UF default value yields values of 4.2 and 2.4 for the kinetic and dynamic factors, respectively. Since there was an excessive implied precision in these particular values, they were simplified to 4.0 and 2.5, respectively, by Renwick (1993).

For boron and kinetically-similar compounds, renal clearance is, perhaps, a much more relevant and specific choice than the other toxicokinetic variables studied by Renwick (1993). Boron is not metabolized in rats or humans and is similar in absorption and distribution between these two species. Approximately 98% of administered boron has been shown to be eliminated in the urine in rats and humans. Differences in elimination rate between rats and humans for boron are primarily in the 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. That is, the variability in the absolute value for these factors across species is largely accounted for by dividing the absolute (uncorrected) value by the species average body weight raised to the ¾ power. In particular, renal clearance values scale across species with an exponent ranging from 0.69-0.89 (summarized in Davidson et al., 1986). Based on an allometric exponent of 0.75 and the reference body weights of 70 kg for humans and 0.35 kg for rats, the rat:human allometric scaling factor would be 3.8, but could vary between 1.8 and 5.2, given the range of the data. There is additional uncertainty, however, as a strictly allometric approach does not take into account absorption and distribution differences between rats and humans Therefore, the allometric argument supports a value near 4.0 as the average, or *expected*, factor for scaling test-animal kinetics to human kinetics.

The fundamental concept of an uncertainty factor, however, requires that it be a conservative (in the sense of human health protection) estimate of a particular "dose-scaling" factor likely to occur as a result of acquiring missing information (Dourson and Stara, 1983; Barnes and Dourson, 1988; Dourson, 1994; Baird et al., 1996; Swartout et al., 1998; U.S. EPA, 2001). The same concept must hold for sub-factors, such as the toxicokinetic and toxicodynamic factors, resulting from a disaggregation of individual uncertainty factors. Each of the sub-factors is, conceptually, still an uncertainty factor. Therefore, the default value for the sub-factor must represent a "conservative" estimate of the expected value. In the probabilistic sense, for any uncertainty factor (e.g., UF_{KA}), the default value should be an upper percentile of the underlying scaling (or variability) factor distribution (Swartout et al., 1998). Adopting a default value of $10^{0.5}$ for the toxicokinetic factor is clearly not conservative for rodent species. Taking the rat as the typical species on which RfDs are based, with the allometric expectation of a 3.8-fold scaling

factor, a default of 4 would be the lowest value that could be adopted that is consistent with the nature of an uncertainty factor. A higher value would be more consistent but would result in a less conservative toxicodynamic default, for which we do not have adequate data to establish.

5.1.3.2. *Revised RfD Calculation Formula*

The formula for calculating the RfD with this uncertainty factor disaggregation is given in Equation 5.1.

$$RfD = D_C \Big/ \left(VF_{AK} \cdot VF_{AD} \cdot VF_{HK} \cdot VF_{HD} \cdot UF_{AK} \cdot UF_{AD} \cdot UF_{HK} \cdot UF_{HD} \cdot UF_X \cdot MF\right) \qquad (5.1)$$

where:

- D_C is the "critical" dose (NOAEL, LOAEL, BMD) defined in the critical study,
- VF_{AK} is the interspecies toxicokinetic variability factor (derived from data; default = 1),
- VF_{AD} is the interspecies toxicodynamic variability factor (derived from data; default = 1),
- VF_{HK} is the interindividual toxicokinetic variability factor (derived from data; default = 1),
- VF_{HD} is the interindividual toxicodynamic variability factor (derived from data; default = 1),
- UF_{AK} is the interspecies toxicokinetic uncertainty factor (default = 4.0),
- UF_{AD} is the interspecies toxicodynamic uncertainty factor (default = 2.5),
- UF_{HK} is the interindividual toxicokinetic uncertainty factor (default = $10^{0.5}$),
- UF_{HD} is the interindividual toxicodynamic uncertainty factor (default = $10^{0.5}$),
- UF_X represents all other uncertainty factors (UF_L x UF_D x UF_S = 1, for boron),
- MF is the Modifying Factor (= 1 for boron).

Note that if data are inadequate for estimation of a specific variability factor, it takes on the value of 1 by convention, which then requires application of a default value for it's corresponding uncertainty factor. The variability factors are given separate representation from their corresponding uncertainty factors to emphasize that they no longer represent uncertainty. If the data are judged to be sufficient in themselves, their respective uncertainty factor counterparts will be reduced to unity. If there are significant issues concerning the data or the modeling of the data, residual uncertainty may result in values greater than 1.0 for the corresponding uncertainty factor. Relating this formula (Eq. 5.1) to the standard RfD formula, note that the product of VF_{AK}, UF_{AK}, VF_{AD}, UF_{AD}, VF_{HK}, UF_{HK}, VF_{HD}, UF_{HD}, and UF_X corresponds to the total UF as shown in the IRIS RfD Summary Table. For convenience and sake of reference, this product is given the term "Total Adjustment Factor" and is designated as AF_{TOT}.

5.1.3.3. *Toxicokinetic Modeling Issues for Boron*

While no data presently exist to address the *toxicodynamic* component of UF_A or UF_H, existing data are adequate to establish values for VF_{AK} and VF_{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. As there are no direct measurements of fetal boron concentrations, an assumption is made that fetal boron concentration is directly proportional to boron concentration in maternal plasma. There are insufficient data to compare plasma boron in rats and humans at the same exposure levels. Therefore boron clearance, which is only partially dose-dependent, is used as an estimator of internal dose. Clearance, expressed in units of ml/min is inversely related to plasma concentration, in that 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 sequestration also reduce the plasma concentration. However, as boron is almost not metabolized and is entirely eliminated in the urine, clearance of boron by the kidney can be used as the key toxicokinetic factor.

Although the toxic effects of boron are manifested in the offspring, the pregnant females (for both humans and test animals) are considered to be the "sensitive subpopulation," 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, pregnant females are treated as a surrogate for the true sensitive subpopulation — the fetuses. 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 likely to be an adequate surrogate for the fetal dose variability, although there is some remaining uncertainty in fetal kinetic variability.

5.1.3.4. *Interspecies Uncertainty (UF_A)*

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. Assuming a relatively constant intake of boron and that 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.

The steady-state plasma concentration (mass/volume) of boron given a constant rate of intravenous infusion is:

$$C_{SS} = k_0 / Cl \tag{5.2}$$

where:

k_0 is the constant infusion rate (mass/time) and
Cl is the clearance rate (volume/time).

The daily ingested dose (mg/kg-day) replaces the intravenous infusion rate by including three additional factors — an absorption (from the gut) constant, the absorbed fraction distributed to the plasma compartment, and body mass as in Equation 5.3.

$$k_0 = D_e f_a f_p M_b \tag{5.3}$$

where:

D_e is the external ingested dose rate in mg (boron) per kg body mass per day,
f_a is the gut absorption constant (fraction of ingested boron absorbed from the gut),
f_p is the absorbed fraction (of boron) distributed to the plasma compartment, and
M_b is body mass (kg).

The product of the factors f_a and f_p is the same as the bioavailability factor in a similar equation for steady-state plasma concentrations (Renwick, 1991).

Substituting for k_0 in Equation 1 and solving for D_e, Equation 5.4 is obtained.

$$D_e = \frac{C_{SS} Cl}{f_a f_p M_b} \tag{5.4}$$

The interspecies variability factor, VF_{AK}, is used to scale the human ingestion dose rate to the test animal dose rate. Therefore, in this case, VF_{AK} is equal to the ratio of D_e-rat to D_e-human. Taking the ratio of rat and human external dose rate yields Equation 5.5, where the trailing subscript designates the species r = rat, h = human).

$$VF_{AK} = \frac{D_{er}}{D_{eh}} = \frac{C_{SSr} \times Cl_r \times f_{ah} \times f_{ph} \times M_{bh}}{C_{SSh} \times Cl_h \times f_{ar} \times f_{pr} \times M_{br}} \tag{5.5}$$

For the boron interspecies kinetic adjustment factor (VF_{AK}), the rat:human boron clearance ratio is applied as a scalar to a specific rat external dose (the BMD of 10.3) in order to obtain an equivalent human dose 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. In addition, the boron clearance values presented in this document are expressed relative to body mass (i.e., ml/min-kg), so the M_b terms can be eliminated, yielding Equation 5.6.

$$VF_{AK} = \frac{Cl_r \times f_{ah} \times f_{ph}}{Cl_h \times f_{ar} \times f_{pr}} \tag{5.6}$$

where Cl_r and Cl_h are now expressed in units of ml/min-kg. The mean boron clearance (in ml/min-kg) for pregnant rats and pregnant women is 3.3 and 1.02, respectively, determined from the kinetic studies of U.S. Borax (2000), Vaziri et al. (2001) and Pahl et al. (2001). Although there is a presumed dose-dependence of boron clearance over extended exposure ranges arising from boron reabsorption (see discussion in following section), the average fractional clearance for both rats and humans in these studies was similar (rats receiving much higher exposure). Similar fractional clearance indicates a similar rate of reabsorption in the kidney tubules, allowing for greater confidence in applicability of the direct comparison.

Although there are no data specifically for pregnant individuals, boron is about 95% absorbed from the G.I. tract by adult rats and over 90% by adult humans. As there is no other reason to believe that absorption in the gut should be different in humans and rats, f_{ah} and f_{ar} are both set to 0.95. As boron is not sequestered to any significant extent in specific tissues (although, to a small degree in bone) and that there are no apparent active transport mechanisms for boron, an assumption is made that boron will be equally distributed throughout total body water. The fraction of absorbed boron distributed to the plasma compartment, then, will be proportional to the size of the plasma compartment relative to total body water.

The data in the published literature are insufficient for establishing the f_p values for pregnant rats and humans, directly. However, f_p values can be estimated indirectly with a few assumptions. Data are available to determine comparative human and rat values for total body water to body mass ratios (females and males) and plasma volume to body mass ratios (males only). Using nonpregnant females as surrogates, the average ratios of total body water (M_w) to body mass for pregnant humans and rats are about 0.560 and 0.650, respectively. These estimates are based on summary data compiled from the published literature by the National Academy of Sciences (NAS, 1956). Of the many available human studies, the one matching the method of measurement (dessication) for the rat study was selected, as method of measurement appears to affect the value to some degree. No details or citations are presented for these values in NAS (1956), however. The average plasma volume to body mass ratio (V_p:M_b) for adult human males is about 0.0460 (NAS, 1956) and for adult male rats is about 0.0425 (40 to 45 mL/kg in three studies [NAS, 1956; Altman and Dittmer, 1964]). The average V_p:M_b for adult human nonpregnant females is about 0.044 (40 to 48 ml/kg; NAS, 1956; Altman and Dittmer, 1964) and increases to about 0.051 (51 ml/kg during pregnancy; NAS, 1956). No values were found for female rats. Based on the limited data, V_p:M_b is concluded to be the same for adult human males and females. V_p:M_b increases by about 10% during pregnancy for humans; an assumption is made that similar increases will occur in pregnant rats. Adjusting for increases in pregnancy yields a V_p:M_b estimate of 0.047 for pregnant rats. Assuming that M_w:M_b is the same in pregnant and nonpregnant females, f_p values can be estimated by dividing V_p:M_b by M_w:M_b for each species and scaling the latter ratios for the difference between pregnant and nonpregnant females. The resulting f_p values are 0.0911 (0.051/0.56) for humans and 0.0723 (0.047/0.65) for rats.

Substituting the foregoing estimates for the variables in Equation 5.6 yields a value of 4.08 for VF_{AK} ([3.3/1.02] x [0.95/0.95] x [0.0911/0.0723]). The toxicokinetic data are considered adequate for reducing UF_{AK} to unity. There are no data to replace the default value for the toxicodynamic component of UF_A; it remains the default value of 2.5. Thus, the toxicokinetic portion of the interspecies uncertainty factor is replaced by the toxicokinetic variability factor of 4.08 (i.e., VF_{AK} = 4.08, UF_{AK} = 1) and UF_A is reduced to a factor of 2.5, corresponding to the default value for UF_{AD}.[2]

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[2]Note that, although VF_{AK} is specified to three significant digits, variability and uncertainty factors are generally not considered this precise. An extra digit (or two) is carried through to the final calculation of the RfD to avoid intermediate round-off errors.

5.1.3.5. *Intraspecies Uncertainty (UF_H)*

Conceptually, the intraspecies toxicokinetic variability factor (VF_{HK}) accounts for the range of human interindividual variability from where VF_{AK} left off to where the sensitive subpopulation is adequately protected. For boron, the range is between the mean and a lower bound percentile of boron clearance in the pregnant human population. VF_{HK} needs to cover a sufficient fraction of the population such that the probability of having both a low clearance and high sensitivity (on the toxicodynamic scale) is so low that no adverse effects are expected in the population. In this case, a value for VF_{HK} is sought that gives 99.9% coverage of the population variability. A relatively large coverage is chosen, as the population at risk is very large and this factor addresses population variability rather than uncertainty (which is addressed by UF_{HK}). A coverage of 99.9% is consistent with the U.S. EPA Exposure Assessment Guidelines (U.S. EPA, 1992) for determination of absolute upper bound exposure variability (VF_{HK} being a representation of relative internal exposure).

Estimation of extreme percentiles for *variability* (as opposed to uncertainty) from most data sets is problematic, as those values tend to fall outside the range of observations and are much more sensitive to measurement and model uncertainty than central tendency estimates. A judgement must be made for each data set as to whether such estimates can be made, with particular attention to the study design, overall variance, and extent of extrapolation required. Accordingly, although the study of Pahl et al. (2001) provides a direct estimate of boron clearance variability in pregnant women, the data are judged to be inadequate for this purpose, particularly for estimating values in the extreme tails. The Pahl et al. (2001) study was not designed to assess interindividual variability, and lacked controls for dietary intake of boron. The variance of boron clearance in this study was somewhat high (coefficient of variation = 0.54), such that extrapolation to a low percentile would be far outside the range of observations. The available data on human glomerular filtration rate (GFR) are somewhat more extensive than the boron clearance data from Pahl et al. (2001). Therefore, GFR was evaluated as a surrogate for boron clearance and variability in GFR used to estimate variability in boron clearance. Boron clearance rate differs from GFR in that boron is reabsorbed into the blood stream from the kidney tubules. In the Pahl et al. (2001) study, reabsorption was relatively high, with an average fractional excretion of 57%. However, the dietary intake of boron for these subjects was probably very low compared to the RfD, estimated to be 10-fold below the RfD on average (U.S. Borax, 2000). The most relevant measure of boron clearance variability is at boron exposure levels near the RfD, as the RfD is the exposure level at which the degree of protection of the sensitive subpopulation needs to be determined. As tubular reabsorption is generally a constant (rather than proportional) rate, reabsorption at higher doses near the RfD would likely be a minor factor in the variability of boron clearance. That is, boron clearance rates would approach pure glomerular filtration rates and should have the same variability as GFR in the population. Also, boron is not actively secreted into the urine and would tend to be more like the substances used to measure GFR in humans in this respect.

GFR data have been used previously in this context by Dourson et al. (1998), who assessed the magnitude of variance of GFR in pregnant women for application as an interindividual toxicokinetic adjustment factor for the boron RfD. Dourson et al. (1998)

proposed the ratio of the mean GFR to the GFR value 2 standard deviations below the mean (mean/[mean - 2 s.d.]) as the metric for the adjustment factor.

The interindividual (intra-human) variability factor is calculated as

$$VF_{HK} = \frac{GFR_{AVG}}{GFR_{LOW}} \tag{5.7}$$

where GFR_{AVG} and GFR_{LOW} are the mean GFR and "lower bound," respectively, for the population of healthy pregnant women, averaged across the entire gestational period. In order to be consistent with the interspecies VF_{AK} model (and the RfD methodology, itself), GFR must be expressed in units of mL/min per kg body weight (mL/min-kg). The numerator of the GFR ratio is the average value in the population because the interspecies toxicokinetic extrapolation "ends" at that point. The lower bound represents a low GFR value that provides sufficient coverage of the population for adequate protection of the sensitive subpopulation. In this case, GFR_{LOW} is defined as the 0.1 percentile (0.001 fractile) value of the population GFR distribution, which gives 99.9% coverage of the population variability.

GFR is measured most accurately by 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). Only the Dunlop study provides sufficient information to evaluate GFR variability relative to body weight, as required by the model. Using the separate tables of individual body weights and absolute GFR measurements (in mL/min) presented in Dunlop (1981), relative GFR measurements (mL/min-kg) can be calculated for each subject for each observation period (1st, 2nd, and 3rd trimester). The resulting data are shown in Table 9, with the average values in the last column. The average values are consistent with either a normal or lognormal distribution (Kolmogoroff-Smirnoff test using the Dallal-Wilkinson approximation for calculating the p-value in testing composite normality; computations performed in S-Plus® ver 4.5 for Windows®). The lognormal form is chosen as most representative, as the distribution is a ratio of two random strictly-positive variables, and would be expected to be lognormally-distributed in the limit (for large sample sizes). In addition, GFR values very close to zero will not be relevant, as dialysis would be required. A normal distribution would therefore have too much density in impossible (negative) or irrelevant GFR value ranges. The lognormal distribution for body-weight-corrected GFR (Table 9, last column) is characterized by a geometric mean (GM) of 2.257 mL/min-kg and a geometric standard deviation (GSD) of 1.160 mL/min-kg, as estimated by the method of moments. The 0.1 percentile value is 1.427 mL/min-kg, which is close to the lowest observed value of 1.56 mL/min-kg. The arithmetic mean is 2.281 mL/min-kg. The corresponding VF_{HK} value is 1.60 (2.281/1.427).

By comparison, the GM and GSD for boron clearance in pregnant women in the Pahl et al. (2001) study were 0.863 and 1.892, respectively. The GSD of 1.892 corresponds to a 18-fold greater log-variance than that for the body-weight-adjusted GFR values from the Dunlop study.

Table 9. GFR Corrected for Body Weight, from Dunlop (1981)

Subject	GFR (mL/min-kg)			
	1st Trimester	2nd Trimester	3rd Trimester	Average
1	2.648	2.539	2.578	2.588
2	3.315	2.756	2.835	2.969
3	2.097	2.113	1.446	1.885
4	2.278	2.286	1.902	2.155
5	1.990	2.089	1.235	1.772
6	2.323	2.295	2.933	2.517
7	3.004	2.575	2.799	2.793
8	2.334	2.391	2.482	2.402
9	2.040	1.935	2.124	2.033
10	2.823	2.619	2.369	2.604
11	2.182	2.071	2.172	2.141
12	2.059	2.179	1.529	1.922
13	2.651	3.078	2.607	2.779
14	3.065	2.621	2.370	2.685
15	2.339	2.125	2.014	2.159
16	2.075	1.738	1.269	1.694
17	2.031	2.322	1.498	1.950
18	2.490	1.556	2.325	2.123
19	2.458	2.887	2.212	2.519
20	2.485	2.364	2.471	2.440
21	2.128	2.020	1.851	2.000
22	2.304	2.500	2.169	2.324
23	2.465	2.075	2.103	2.214
24	2.221	2.361	2.094	2.225

Subject	GFR (mL/min-kg)			
	1st Trimester	2nd Trimester	3rd Trimester	Average
25	2.326	1.969	2.072	2.122
mean	2.405	2.299	2.138	2.281
std. dev.	0.34848	0.35189	0.47158	0.33826
GM	2.383	2.272	2.083	2.257
GSD	1.1472	1.1685	1.2718	1.1600

The 0.1 percentile value would be more than a factor of 2 lower than the smallest observation and would yield a VF_{HK} of 8.48. The lognormal approach differs from Dourson et al. (1998; "Dourson method") in that a specific percentile from an explicit distribution is chosen. The primary advantage of the Dourson method is that no assumptions are required as to the form of the distribution ("distribution free"). However, a normal distribution is somewhat implicit in the use of the mean and standard deviation. Also, the Dourson method does not explicitly exclude negative values. The Dourson method applied to the same data (Table 9, last column) would yield a VF_{HK} of 1.42 (2.28/[2.28 - 2 x 0.3383]). If a normal distribution were to be assumed in this approach, two standard deviations below the mean corresponds to the 0.275^{th} percentile. Three standard deviations below the mean (0.135^{th} percentile) provides population coverage closer to 99.9%. Using three standard deviations in the Dourson method would yield a VF_{HK} of 1.80. Assuming a normal distribution for the data and using the 0.1 percentile for GFR_{LOW} results in a VF_{HK} of 1.85. Thus, the lognormal/0.1 percentile approach (VF_{HK} = 1.60) is slightly more conservative than the Dourson method that uses a two-fold standard deviation difference in the metric (VF_{HK} = 1.42), but slightly less conservative than assuming a normal distribution with a 0.1 percentile for GFR_{LOW}. The lognormal value is preferred because the normal distribution approach for GFR does not exclude the probability density of negative values, which will bias VF_{HK} toward higher values.

5.1.3.6. *Residual Uncertainty in Human Interindividual Toxicokinetics*

Although the Dunlop (1981) study provides the only direct basis for establishing VF_{HK}, there is a suggestion that it may underestimate the variance of GFR (corrected or uncorrected) in pregnant women. Both Krutzén et al. (1992) and Sturgiss et al. (1996) report higher variances for uncorrected GFR (averaged across entire gestational period) in pregnant women. The mean and standard deviation for uncorrected GFR reported by Krutzén et al. (1992) are 195 and 32 mL/min, respectively. Averaging the early and late pregnancy GFR observations for each individual (to obtain a cross-pregnancy average) in the Sturgiss et al. (1996) study, results in a mean and standard deviation of 138.9 and 26.08 mL/min, respectively. Calculated similarly, the mean and standard deviation for average GFR (across all three trimesters) in the Dunlop (1981) study are 150.5 and 17.63 mL/min, respectively. The corresponding variances are 1024, 900, and 311 $(mL/min)^2$, respectively, for the Krutzén et al. (1992), Sturgiss et al. (1996), and Dunlop (1981) studies. The average variance across all three studies is 745 $(mL/min)^2$. Thus, population variance estimates for uncorrected GFR based solely on the Dunlop study could possibly underestimate true variance by a factor of 2.5 to 3.

In order to analyze the impact of variance underestimation on the lognormal model, however, the variance of the logarithms of the observations ("log-variance") must be compared. The log-variances (in natural log units) for uncorrected GFR observations in the Dunlop (1981) and Sturgiss et al. (1996) studies, calculated directly from the individual observations, are 0.01453 and 0.03533, respectively. Although, Krutzén et al. (1992) does not present individual observations, the log-variance can be estimated indirectly from the mean and standard deviation assuming log-normality ($var_{log} = \log[1 + sd^2/mean^2]$); Evans et al., 1993). With this assumption, the log-variance of a lognormal distribution with a mean of 195 and standard deviation of 32 is 0.02659. The average log-variance across all three studies is 0.02548. Thus, population log-

variance estimates for *uncorrected* GFR based solely on the Dunlop study could possibly underestimate true variance by a factor of 1.75 to 2.43, the former based on comparison with the cross-study mean and the latter being a worst-case estimate.

Renal clearance (including GFR), however, tends to be correlated with body surface area, which is generally how clearance values are presented in the medical literature. As body surface area is closely related to body weight, the variance of body-weight-corrected GFR observations would be expected to be lower than that for the uncorrected observations. However, this is not the case for the Dunlop (1981) data, where correcting for body weight actually raised the coefficient of variation (CV = std. dev./mean) slightly, from 0.131 to 0.136; correcting for body surface area lowered the CV to only 0.117. A slightly larger reduction in variance was reported by Krutzén et al. (1992) after correcting for surface area; the CV was lowered to 0.120 from an uncorrected value of 0.164. The lack of reduction of GFR variance when correcting for body weight suggests that there is a fairly significant contribution of *uncertainty* from measurement error and other factors in the variance of the Dunlop (1981) data and, by implication in the other studies, as well. Also, some of the difference of the variances in the three studies can probably be attributed to uncertainty arising from subtle differences in experimental design and execution.

As VF_{HK} is meant to be a strict representation of population *variability*, the extent to which uncertainty is aggregated in the data mitigates the underestimation of true population variance. This is particularly manifest in the extreme tails of the distribution, such as the extreme lower end that this assessment addresses. Therefore, it seems unlikely that the Dunlop-based corrected GFR estimate could underestimate the population variance by as much as the worst-case estimate of 2.43-fold, but still may represent as much as a 1.5- to 2.0-fold underestimate. The VF_{HK} values corresponding to increased log-variances of 1.5- and 2.0-fold would be 1.68 and 1.81, which are factors of 1.05 and 1.13 greater than VF_{HK} of 1.60 as calculated from Dunlop (1981). Accordingly, to account for uncertainty in population variance, uncertainty pertaining to the use of GFR as a surrogate for actual boron clearance, and uncertainty in fetal kinetics, UF_{HK} is assigned a value of 1.20, rather than 1.0.

Another consideration is that a decrement in renal function, itself, can predispose individuals to adverse effects that manifest both as maternal and fetal adverse effects. Decrements in the levels of GFR may increase risks for adverse developmental outcomes effects, effects that cannot be distinguished from other potential adverse effects of boron. Thus, a certain level of renal function may bound the range of variance for the risk-relevant VF_{HK} toxicokinetic measure and would serve as a physiological basis for GFR_{LOW} in Equation 5.7. 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 physicians' need to advise kidney patients prior to becoming pregnant. Also, at lower (normal) serum creatinine levels, serum creatinine is a fairly 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 (see Appendix C). From this analysis a ratio of average GFR to GFR levels associated with significant adverse pregnancy outcomes (both GFR measures in nonpregnant women) can be calculated. Assuming that the ratio would be similar for pregnant women, it can be compared directly to VF_{HK} as supporting evidence.

Several clinical investigations in humans have demonstrated the increased risk of adverse developmental and obstetrical complications (low birth weight, intrauterine growth retardation, spontaneous abortion, abruptio placentae, fetal and neonatal death, etc.) with serum creatinine levels above 1.4 mg/dl (Bear, 1976, 1978; Cunningham et al., 1990; Abe, 1996; Jungers et al., 1997). Applying the linear regression analysis in Appendix C, a serum creatinine level of 1.4 mg/dl corresponds to a GFR of 39.8 mL/(min/1.73 m^2).[3] 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). Substituting 79.4 and 39.8 for GFR_{AVG} and GFR_{LOW}, respectively, in Equation 5.7, yields a "physiological" VF_{HK} of 2.0, which is 25% larger than the "statistical" VF_{HK} derived previously. There is considerable uncertainty in the regression model (Appendix C) in the estimate of the lower GFR values, which is not accounted for in the physiological estimate of VF_{HK}. Also, the GFR measurements on which the regression analysis (Appendix C) is based are not strictly in the units required by the model (Eq. 5.7) and may also tend to underestimate the population variance. Finally, the severity of the low-GFR effects and the proportion of the population who would be affected is unclear. Overall, the clinical data supporting the physiological approach are too far removed from the direct assessment needed to establish VF_{HK} and serve only as support for the assessment. Therefore, the selection of 99.9% variability coverage in the statistical approach does not seem excessive. The physiological approach also supports a residual uncertainty value for UF_{HK} of greater than unity.

Thus, in Equation 5.1, VF_{HK} is assigned a value of 1.60, UF_{HK} is reduced to 1.2, and UF_{HD} remains at its default value of $10^{0.5}$ (VF_{HD} = 1 by convention).

5.1.3.7. *RfD Calculation*

The RfD is calculated from Equation 5.1, where:

D_C = 10.3 mg/kg-day (Allen et al., 1996)
VF_{AK} = 4.08 (data-derived)
VF_{AD} = 1 (conventional; no data)
VF_{HK} = 1.60 (data-derived)
VF_{HD} = 1 (conventional; no data)
UF_{AK} = 1 (residual)
UF_{AD} = 2.5 (default)
UF_{HK} = 1.2 (residual)

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

UF_{HD} = 3.16 (default)
UF_X = 1 (UF_S x UF_D x UF_L)
MF = 1
AF_{TOT} = 61.9 (4.08 x 1.60 x 2.5 x 1.2 x 3.16)
RfD = 0.2 mg/kg-day (10.3/61.9 = 0.166, rounded to one digit of precision)

5.1.3.8. *Uncertainty Factor Approaches Used by Others*

Others have used different methods to derive uncertainty factors.

The International Program on Chemical Safety (IPCS) used sub-divided uncertainty factors to estimate Tolerable Intake Values (WHO, 1994; Renwick, 1993). This method allowed for subdivision of each of the interspecies and intraspecies default uncertainty factors to incorporate data on toxicokinetics or toxicodynamics. 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. 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. 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 and intraspecies toxicokinetics and toxicodynamics are the same as in the previous publication (WHO, 1994).

Several 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.

The European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC, 1994) developed a Tolerable Daily Intake for developmental effects of boron. Decreased fetal body weight in rats was chosen as the critical effect (Price et al., 1994) with a 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 toxicokinetics (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 a NOAEL of 9.6 mg B/kg-day. The interspecies uncertainty factor chosen was 4 (2 for toxicokinetics and 2 for toxicodynamics, 2x2=4). The reasons cited for the reduced interspecies uncertainty factor for toxicokinetics were as follows: boron is not metabolized in animals or humans, eliminating a major potential source of toxicokinetic variation; 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 reasons cited for the reduced interspecies uncertainty factor for toxicodynamics were as follows: the sensitivity of the target tissue receptor appears, to the author, to be similar across species based on the similarity of symptoms of acute toxicity in animals and humans; and because developmental and reproductive toxicity appear to be the most sensitive endpoint of toxicity in all animal species tested. The intraspecies uncertainty factor chosen was 8 (2.5 for toxicokinetics and 3.2 for toxicodynamics). The intraspecies toxicokinetic 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.

The Institute for Evaluating Health Risks (IEHR, 1997) determined an Unlikely Effect Level for Developmental Toxicity for Boron based on the benchmark dose for decreased fetal body weight by Allen (1996). The interspecies uncertainty factor chosen for boron was $10^{0.5}$, which includes $10^{0.25}$ each for toxicokinetics and toxicodynamics. The justification for reduction of the default values was stated as commonality in the nature of the toxic response in the humans versus that in the experimental animal and metabolic and toxicokinetic similiarities among species The intraspecies uncertainty factor chosen for boron was a default value of 10. The composite human sensitivity factor for developmental toxicity was 30.

In their Environmental Health Criteria Document, WHO (1998a) developed a Tolerable Daily Intake for boron where decreased fetal body weight in rats was chosen as the critical effect (Price et al., 1994) with a 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 toxicokinetics due to the similarity of absorption, distribution, metabolism and elimination of boron in rats and humans and a $10^{0.4}$ (default) for toxicodynamics. The intraspecies uncertainty factor chosen was $10^{0.9}$ ($10^{0.4}$x$10^{0.5}$ =$10^{0.9}$), $10^{0.4}$ for toxicokinetics due to lack of metabolism in humans and $10^{0.5}$ (default) for toxicodynamics. The composite uncertainty factor was 32.

In their Guidelines for Drinking-Water Quality, WHO (1998b) developed a Tolerable Daily Intake 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 a 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 toxicokinetic and pharmacodynamic factors. For intraspecies extrapolation a default value of 3.2 for toxicokinetic data was reduced to 1.8 and a default value of 3.2 was retained for toxicodynamic 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 WHO (1998b), developed a Tolerable Daily Intake 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 toxicokinetics or toxicodynamics. Therefore, a default value of 10 was chosen for the interspecies factor. For intraspecies uncertainty the toxicokinetic 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 toxicokinetics 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 toxicodynamic 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 in which 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 toxicokinetic and toxicodynamic uncertainty to this estimated human NOAEL. Interspecies toxicokinetic value was estimated at 3.1, while interspecies toxicodynamic uncertainty was estimated at 1.25-2.5. Intraspecies factors for toxicokinetics was 1.8-2.0 and intraspecies toxicodynamics was 3.2.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

The available inhalation data are inadequate to support derivation of an RfC for boron compounds.

5.3. 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.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Boron is a naturally-occurring element that is widespread in nature; the average concentration in the earth's crust has been estimated to be 10 ppm (Woods, 1994). Boron in the environment is always found chemically bound to oxygen, usually as alkali or alkaline earth borates, or as boric acid (IEHR, 1997; U.S. EPA, 1987). Boric acid and sodium borates are widely used for a variety of industrial purposes. Boron is not transformed or degraded in the environment, but depending on environmental conditions (e.g., pH, moisture level), changes in the specific form of boron and its transport can occur (ATSDR, 1992). The most important source of exposure for human populations is ingestion of boron from food (primarily fruits and vegetables) (Naghii and Samman, 1996a). Occupational exposure to boron dust and exposure to boron in consumer products (e.g., cosmetics, medicines, insecticides) are other potentially significant sources (ATSDR, 1992).

Boron is readily absorbed from the gastrointestinal tract following oral exposure (Schou et al., 1984; Vanderpool et al., 1994). Boron is also absorbed following inhalation exposure, although it is not clear how much is absorbed directly through the mucous membranes of the respiratory tract and how much is cleared by mucociliary activity and swallowed (Culver et al., 1994). Boron is not absorbed across intact skin, but is readily absorbed across damaged skin (Draize and Kelley, 1959). Boric acid and borate compounds in the body exist primarily as undissociated boric acid, which distributes evenly throughout the soft tissues of the body (Ku et al., 1991; Naghii and Samman, 1996b). Although it does not accumulate in the soft tissues, boron does accumulate in bone, reaching steady-state levels approximately 4-fold higher than plasma levels after 1-4 weeks, depending on dose (Ku et al., 1991; Chapin et al., 1997). Boric acid is not degraded in the body, but can form complexes with various biomolecules by mechanisms that appear to be concentration dependent and reversible (IEHR 1997; WHO, 1998a). Boric acid is excreted primarily in the urine. It is cleared from the plasma with a half-life of approximately 21 hours (Jansen et al., 1984a), but eliminated very slowly from bone (Chapin et al., 1997).

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., 1993). The mechanism for boron's effect on the testes is not known, but the available data (as reviewed by Fail et al., 1998) suggest an effect on the Sertoli cell, resulting in altered physiological control of sperm maturation and release. 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, central nervous system, 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).

Because boron is absorbed following inhalation exposure, is distributed evenly throughout the soft tissues of the body as boric acid, and is not metabolized, 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, however, be route-specific differences in ability to deliver toxic doses to the targets, so that for example, 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 toxicity of boron by inhalation exposure is sparse. There is a report of testicular effects in a small number of Russian workers exposed to very high concentrations (Tarasenko et al., 1972), but no evidence of an effect on fertility in a controlled epidemiology study in U.S. borate production workers (Whorton et al., 1992, 1994a,b). Only irritant effects have been associated with borate exposure in U.S. workers, with no evidence of an effect on pulmonary function (Wegman et al., 1994; Garabrant et al., 1984, 1985). Irritant effects and reduced growth were the only effects reported in the lone animal study (Wilding et al., 1959). These data are inadequate to support derivation of an RfC for boron compounds.

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 even mention 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 low-dose, but not high-dose, male mice that was 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 was also 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. Overwhelmingly, studies of boron compounds for genotoxicity were negative, including studies in bacteria, mammalian cells and mice *in vivo*. Under EPA's current guidelines for carcinogen risk assessment (U.S. EPA, 1986a), boron is classified as Group D; not classifiable as to human carcinogenicity. Under the new proposed

guidelines (U.S. EPA, 1996a), the data are considered to be inadequate for evaluation of the human carcinogenic potential of boron.

6.2. DOSE RESPONSE

The studies by Price et al. (1996a, 1994, 1990) and Heindel et al. (1992) in rats were chosen as the critical developmental studies because they were well conducted studies of a sensitive endpoint that identified both a 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). The dog study by Weir and Fisher (1972) identified the most sensitive NOAEL and LOAEL for testicular effects. This study was not used to calculate the RfD due to several limitations as stated in Section 4.2.1. 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., 1993).

The quantitative estimates of human risk as a result of exposure to boron are based on animal experiments because no human data exist. The human dose that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime (RfD) is 0.2 mg/kg-day. This RfD was derived by the benchmark dose approach. Several BMD analyses were conducted (Allen et al., 1996) using all relevant endpoints to analyze data from the Heindel et al. (1992) and Price et al. (1996a, 1994) studies alone and the combined data from both studies. 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. For mean fetal weight analysis, the BMDL was defined as the 95% lower bound on dose corresponding to a 5% decrease in the mean. BMDL values calculated with a continuous power model for fetal body weight (litter weight averages) were less than those for all other relevant endpoints. The BMDL based on the combined results of the two studies chosen for development of the RfD was 10.3 mg B/kg-day, which was very close to the NOAEL of 9.6 mg B/kg-day from the Price et al. (1996a, 1994) study. Because there are data addressing the relationship of both interspecies and intra-human toxicokinetics for boron, toxicokinetic variability factors were derived as replacements for the kinetic portion of the interspecies and intra-human uncertainty factors (UF_A and UF_H). An interspecies kinetic variability factor of 4.08 was estimated from the data of Varizi et al. (2001) and Pahl et al. (2001). An intra-human kinetic variability factor of 1.60 was estimated from the data of Dunlop (1981), using glomerular filtration rate as a surrogate for boron clearance. As there was some nontrivial residual uncertainty in this analysis, a factor of 1.2 was assigned to the overall intraspecies toxicokinetic uncertainty. The remaining uncertainty in the RfD derivation was from toxicodynamics. Intra-human uncertainty was assigned the default value of 3.16. As interspecies uncertainty was deemed to be greater for toxicokinetics than for toxicodynamics, a smaller factor of 2.5 was used for interspecies toxicodynamic uncertainty. The product of all the variability and uncertainty sub-factors served as the total adjustment factor of 61.9. The RfD was derived by dividing the BMDL of 10.3 mg/kg-day by the adjustment factor and rounding to one digit.

Confidence in the principal developmental studies is high; they are well-designed studies that examined relevant developmental endpoints using a large number of animals. Similar developmental effects were noted in rats, mice and rabbits. Confidence in the data base is high due to the existence of several subchronic and chronic studies, as well as adequate reproductive and developmental toxicology data. High confidence in the RfD follows.

The available data are inadequate to support derivation of an RfC, slope factor or unit risk for boron compounds.

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APPENDIX A. EXTERNAL PEER REVIEW - SUMMARY OF COMMENTS AND DISPOSITION

The toxicological review for Boron and the individual boron assessments have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists according to U.S. EPA (1998). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. Public comments were read and considered. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. All three external peer reviewers recommended that this document and the accompanying assessments were acceptable with minor revisions.

(1) General Questions for Peer Reviewers

General Question For the RfD, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing first in a dose-response continuum)? For the cancer assessment, are the tumors observed biologically significant? relevant to human health? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

A. Comment All three reviewers agreed that developmental effects are considered the most appropriate critical effect for development of an RfD. However, one reviewer suggested looking at the references of Beyer et al. (1983) and Dixon et al.(1979) where more sensitive endpoints are reported.

Response to Comment The sensitive endpoint referenced in Beyer (1983, a review article) is a reduced sperm count reported from a USSR study, which was poorly reported without experimental details. The general toxic effect of boron in a 21-35 day study was noted as the reduced activity of the aldolase of blood serum at 6 mg/kg boron while another study of 6 month duration reports reduced aldolase and sperm motility at 0.3 mg/kg. There are very little details given for this study which makes it unacceptable for use in determination of an RfD. The studies by Dixon et al. (1979) are reported as a US and USSR cooperative laboratory effort to improve and validate experimental techniques to assess reproductive effects in laboratory animals. The studies by Dixon et al. (1979) reported in the toxicological review are acute and subchronic studies that do not observe toxic effects below the level chosen as the NOAEL in the Price et al. (1996a, 1994) studies.

General Question Have the noncancer and cancer assessments been based on the most appropriate studies? These studies should present the critical effect/cancer (tumors or

appropriate precursor) in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

B. Comment All reviewers agreed that the studies chosen were the most appropriate.

General Question Studies included in the RfD and RfC under the heading "Supporting/Additional Studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, any evidence corroborative of the critical effect, or to establish the comprehensiveness of the data base with respect to various endpoints (such as reproductive/developmental toxicity studies). Should other studies be included under the "Supporting/Additional" category? Should some studies be removed?

C. Comment All reviewers agreed with what appeared in the document. One reviewer commented that no studies needed to be removed.

General Question For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors or the modifying factor? Do you consider that the data support use of different (default) values than those proposed?

D. Comment Two reviewers agreed that there was no reason to support use of uncertainty factors other than those proposed in the document but one of these reviewers questions what the Agency is going to do about the FQPA. One reviewer objected to the write up of the pharmacokinetic section of the document and did not think that the write up of that section supported the reduced uncertainty factor for interspecies variation. This reviewer suggested a revision to the pharmacokinetic section.

Response to Comment The comments in response to this question are addressed in the following Boron Specific Questions. (Question #4)

General Question Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects (cancer and noncancer) to humans, and the comprehensiveness of the data base? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

E. Comments All reviewers agreed with the confidence statements.

(2) Comments on Boron Specific Questions

Question 1 Do you agree with the developmental effect, decreased fetal body weight in rats, as being the most appropriate critical effect? If not, why not?

Comments All three external reviewers agreed that decreased fetal body weight in rats was the critical effect.

Question 2 Do you agree that in light of new developmental data in three species (rats, mice and rabbits) that use of the dog study (Weir and Fisher, 1972) for development of an RfD is unacceptable based on the low number of animals used, the testicular atrophy noted in the control animals and the NOAEL and the LOAEL were taken from two different studies of different duration?

Comments All three reviewers agreed that the dog study should not be used for development of an RfD for the reasons stated in the text and the new developmental data should be used.

Question 3 Do you agree that use of the benchmark dose (Allen et al., 1996) is appropriate for use in calculating an RfD based on developmental toxicity?

Comments All three reviewers agreed that the use of the benchmark dose from Allen et al. (1996) was appropriate for calculating the RfD. One reviewer also added that proper statistical methods were applied.

Question 4 Do you agree with the use of an other than default uncertainty factor for inter-species extrapolation based on the reasons given in the Toxicological Review? If not, what do you think it should be and why? Do you agree with the default uncertainty factor chosen for intra-species extrapolation? If not, what do you think is appropriate and why?

Comments Two reviewers agree with the less than default uncertainty factor for interspecies extrapolation. Although one of these two reviewers had a question about how the agency was going to handle additional 10x uncertainty factor for the (FQPA) Food Quality Protection Act. A third reviewer questioned the write up of the physiologically based pharmocokinetic section. This reviewer recommended a rewrite of the pharmacokinetic section especially the Excretion and Elimination Section with more data added. This reviewer could not support the proposed reduced uncertainty factor for interspecies extrapolation without a rewrite of the excretion and elimination section showing the data.

Response to Comment At this time the agency has not come to agreement on the 10x uncertainty factor for the FQPA. Based on the high confidence of the toxicity data base, the assessment for boron and that the critical effect is decreased fetal body weight (developmental

toxicity) in the most sensitive species, the author does not think that an extra 10x uncertainty factor is needed to protect for children's risk to boron. Parts of the Toxicokinetic section including Section 3.2 (Distribution) were revised to include more information on the tissues examined and relative amounts of boron in those tissues. More information was included concerning volumes of distribution in a human study and a rat study. Section 3.4 (Elimination and excretion) was completely rewritten to include a comparison between animals and humans for excretion and elimination in the urine and blood. A new pharmacokinetic section was added to emphasize the similarities between animals and humans to support the reduction of the interspecies uncertainty factor.

Question 5 For the RfC, do you agree with the NOT VERIFIABLE status that indicates the data do not meet the minimum requirements according to the current Agency methods document for Inhalation Reference Doses? If not, what effect and data would you use to develop an RfC?

Comments All three reviewers agree that the inhalation data are sparse and insufficient to determine an RfC.

Question 6 Do you agree with the Cancer Classification of Group D using the old guidelines, and under the new proposed guidelines that data are insufficient for evaluation of the human carcinogenic potential for boron?

Comments All three reviewers agreed with the cancer classification under current guidelines and new proposed guidelines.

Question 7 Do you agree with the confidence statements on the RfD? (High confidence in the study, high confidence in the data base and high confidence in the RfD). If you do not agree, what would you change it to and why?

Comments All three reviewers agree with the high confidence in the study, data base and in the RfD.

Since the Toxicological Review and IRIS Summary Sheets were externally reviewed new pharmacokinetic data on renal clearance of boric acid in rats and humans were received by EPA. The new data were incorporated into the Toxicological Review and used to derive a data derived uncertainty factor for use in estimating the Reference Dose for Boron. The additional information added to the Toxicological Review and RfD Summary Sheets were internally and externally reviewed. The following questions were posed as a charge to both the internal and external reviewers for the additions of pharmacokinetic data.

Question: Are the new data from pharmacokinetic experiments from U. S. Borax adequately presented in sections 3.4 and 3.5 in the Toxicological Review? If not how would you recommend that the data be presented?

Comments: Two reviewers agreed that the data were adequately presented. One of these two gave specific suggestions as to some changes that could help the understanding of the new data presented. A third reviewer felt that the data was inadequately presented. Specific suggestions for incorporation of additional data and rearranging of data presented were given.

Response: Additional information about fractional excretion of boron (ratio of boron clearance to creatinine clearance) and it's relationship to tubular reabsorption and tubular secretion was added to the document. Additional information was added to the write up on the human study concerning the dietary summaries that were taken but were not used and why this was the case. Suggestions made about presenting the human data first were not done because it involved a major change in the document and for this data it was felt that it was not necessary because the uncertainty factor extrapolates from the animal data to the human data so it seems a logical progression to present the animal data first in this particular section.

Question: Do you think that the new pharmacokinetic data on clearance of boron in rats and humans from U. S. Borax should be used for derivation of an uncertainty factor for boron instead of a default Uncertainty Factor?

Comments: All three reviewers agreed that the new pharmacokinetic data on clearance of boron in rats and humans should be used for derivation of an uncertainty factor instead of a default factor. Comments included statements that EPA should always attempt to use real data instead of default factors and a statement that this use of clearance data is a significant step forward in the general EPA methodology for deriving uncertainty.

Question: Do you agree with the current Uncertainty Factor using the data-derived method as it is presented in the Toxicological Review and RfD Summary sheet?

Comments: All three reviewers agreed with the current Uncertainty Factor using the data-derived method as it was presented based on clearance data. One reviewer commented that it is a reasonable but conservative approach.

SUMMARY OF THE COMMENTS ON THE SECOND EXTERNAL REVIEW FOR BORON

Since the Toxicological Review and IRIS Summary Sheets were externally reviewed new pharmacokinetic data on renal clearance of boric acid in rats and humans were received by EPA. The new data were incorporated into the Toxicological Review and used to derive a data derived uncertainty factor for use in estimating the Reference Dose for Boron. The additional information added to the Toxicological Review and RfD Summary Sheets were internally and externally reviewed. The following questions were posed as a charge to both the internal and external reviewers for the additions of pharmacokinetic data.

Question: Are the new data from pharmacokinetic experiments from U. S. Borax adequately presented in sections 3.4 and 3.5 in the Toxicological Review? If not how would you recommend that the data be presented?

Comments: Two reviewers agreed that the data were adequately presented. One of these two gave specific suggestions as to some changes that could help the understanding of the new data presented. A third reviewer felt that the data was inadequately presented. Specific suggestions for incorporation of additional data and rearranging of data presented were given.

Response: Additional information about fractional excretion of boron(ratio of boron clearance to creatinine clearance) and it's relationship to tubular reabsorption and tubular secretion was added to the document. Additional information was added to the write up on the human study concerning the dietary summaries that were taken but were not used and why this was the case. Suggestions made about presenting the human data first were not done because it involved a major change in the document and for this data it was felt that it was not necessary because the uncertainty factor extrapolates from the animal data to the human data so it seems a logical progression to present the animal data first in this particular section.

Question: Do you think that the new pharmacokinetic data on clearance of boron in rats and humans from U. S. Borax should be used for derivation of an uncertainty factor for boron instead of a default Uncertainty Factor?

Comments: All three reviewers agreed that the new pharmacokinetic data on clearance of boron in rats and humans should be used for derivation of an uncertainly factor instead of a default factor. Comments included statements that EPA should always attempt to use real data instead of default factors and a statement that this use of clearance data is a significant step forward in the general EPA methodology for deriving uncertainty.

Question: Do you agree with the current Uncertainty Factor using the data-derived method as it is presented in the Toxicological Review and RfD Summary sheet?

Comments: All three reviewers agreed with the current Uncertainty Factor using the data-derived method as it was presented based on clearance data. One reviewer commented that it is a reasonable but conservative approach.

General Comments

Specific comments were made about confusion clarity over description of the empirical distribution function and toxicokinetic adjustment factor.

Response to Specific Comments : Some of these comments and the comments received by the public caused EPA to change the way boron uncertainty factor was derived.

SUMMARY OF PUBLIC COMMENTS RECEIVED BY APRIL 30, 2001

- Disagreement with the use of the data derived aggregate toxicokinetic dose-adjustment factor.

- Based on the data presented the sample sizes in the rat and human studies are not large enough to define the distribution of boron clearance in either exposed rats or pregnant women. However the available data are good enough for conducting a central tendency estimate. (Concern with the validity of interpretation and use of the distribution of the data especially the decision to compare the 5th percentile clearance rates between humans and rats)

- Enough information exists regarding variation in Glomerular filtration Rates in pregnant women, GFR is directly related to the renal clearance function, and this may be a good way to estimate intra- human variation in boron clearance.

- Wrong urine collection used (24 hrs instead of 2 hrs). It was felt that the 2 hour data was more appropriate because the sample was taken while in the clinic.

- The BMDL should be adjusted to account for the dose of boron received in the diet as well as by gavage.

- No discussion of the concept of the Chemical Specific Adjustment Factors or IPCS (2001) guidelines

RESPONSE TO PUBLIC COMMENTS

- Concern over the intraspecies kinetic adjustment factor, caused EPA to change the way that the intra human kinetic adjustment factor was derived.

- EPA used the 2 hour urine clearance data instead of the 24 hour data although, it made little difference.

- A reference to Chemical Specific Adjustment factors was added to the document.

- EPA contacted Purina company to determine the amount of boron in the rat chow that was used in the Heindel and Price studies and then adjusted the doses in the Heindel and Price studies to include that amount of boron. This data was then used to recalculate the BMDL using the agency Benchmark dose software.

APPENDIX B. BENCHMARK DOSE FOR RfD

A. COMPUTATIONAL MODELS - CONTINUOUS DATA

The continuous power model was fit by Allen et al. (1996) to the data by the maximum likelihood method. The model is expressed as:

$$m(d) = \alpha - \beta \times d^{\gamma},$$

where m(d) is the average litter mean at dose d (expressed in mg/kg-day) and α, β and γ are the parameters to be estimated.

B. DATA

Dose of Boric Acid (mg/kg-day)	Fetal Weight (litter mean ± std dev, in g)	
	Heindel et al., 1992	Price et al., 1996a, 1994
0	3.70 ± 0.32	3.61 ± 0.24
19		3.56 ± 0.23
36		3.53 ± 0.28
55		3.50 ± 0.38
76		3.38 ± 0.26
78	3.45 ± 0.25	
143		3.16 ± 0.31
163	3.21 ± 0.26	
330	2.34 ± 0.25	

C. MODEL FIT

The model was examined for fit to the data by an F test that compared the lack of model fit to an estimate of pure error. A likelihood ratio test was performed to determine if a single function could adequately describe the dose-response in both the Heindel et al. (1992) and Price et al. (1996a, 1994) studies.

D. RESULTS

Study	Significant Trend?[a]	Max LL[b]	Goodness-of-fit p-value[c]	Dose corresponding to BMR[d]	
				MLE[e] (mg/kg-day)	BMDL[f] (mg/kg-day)
Heindel et al., 1992	Yes	141.74	0.24	80	56
Price et al., 1996a, 1994	Yes	215.87	0.89	68	47
Combined	--	353.43	0.58	78	59

[a] Tested for trend by Mantel-Haenszel trend test. A significant trend corresponds to a p-value less than 0.05. Combined study results were not tested for trend.
[b] Maximum value of the log-likelihoods of the models fit to the data, ignoring constant terms not related to parameter estimates. The Max LL for the studies combined is not significantly different (p=0.01) from the sum of the Max LL values for the studies individually, indicating that the data are consistent with a single dose-response curve.
[c] Significant fit of the model to the data is indicated by p-value > 0.05
[d] BMR = benchmark response, in this case a 5% decrease in mean fetal weight per litter
[e] MLE = maximum likelihood estimate of dose corresponding to BMR
[f] BMDL = benchmark dose, the 95% lower confidence limit on the MLE

E. DISCUSSION

Results of the likelihood ratio test showed that data from the two studies are consistent with a common dose-response curve. The BMDL of 59 mg/kg-day boric acid (10.3 mg B/kg-day) obtained from the combined data is used for calculation of the RfD. This BMDL is based on combined results of two similarly designed studies conducted in the same laboratory. The BMDL selected is not much less than the lowest dose tested (78 mg/kg-day, 13 mg B/kg-day) in Heindel et al. (1992) which was a LOAEL, and is very close to the NOAEL of 55 mg/kg-day (9.6 mg B/kg-day) (Price et al., 1994).

F. U.S. EPA BENCHMARK DOSE SOFTWARE

The data from the studies of Heindel et al. (1992) and Price et al. (1996a, 1994) were adjusted to include the amount of boron in the diet (10.6 µgB/g of Purina Rat Chow) as well as gavage amounts of boric acid in these two studies. These data were used to estimate a benchmark dose using the Agency Draft Benchmark Dose Software Revision 2.1 Power Model. The BMDL obtained using agency software and adding the boron in the diet to the doses of boric acid was 58.27 mg/kg-day boric acid. The following output shows that these results are similar to the benchmark dose from Allen et al. (1996) where the BMDl was 59 mg/kg-day boric acid.

BMDS MODEL RUN

The form of the response function is:

Y[dose] = control + slope * dose^power

Dependent variable = MEAN
Independent variable = COLUMN1
rho is set to 0
The power is restricted to be greater than or equal to 1
A constant variance model is fit

Total number of dose groups = 10
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

alpha	=	0.0794435	
rho	=	0	Specified
control	=	2.34	
slope	=	1.40018	
power	=	-0.0721342	

Asymptotic Correlation Matrix of Parameter Estimates

	alpha	rho	control	slope	power
alpha	1	-1	0.061	-0.12	-0.13
rho	-1	1	-0.061	0.12	0.13
control	0.061	-0.061	1	-0.77	-0.74
slope	-0.12	0.12	-0.77	1	1
power	-0.13	0.13	-0.74	1	1

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.0787778	0.0727159
rho	0	0.760592
control	3.62476	0.0314803
slope	-0.000605596	0.000471905
power	1.31816	0.133953

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
1.059	29	3.7	0.32	3.62	0.281	0.27
1.061	26	3.61	0.24	3.62	0.281	-0.0502
20.06	29	3.56	0.23	3.59	0.281	-0.118
37.06	27	3.53	0.28	3.55	0.281	-0.0852
56.06	29	3.5	0.38	3.5	0.281	-0.00898
77.06	29	3.38	0.26	3.44	0.281	-0.21
79.06	28	3.45	0.25	3.43	0.281	0.0625
144.1	27	3.16	0.31	3.2	0.281	-0.145
164.1	29	3.21	0.26	3.12	0.281	0.316
331.1	28	2.34	0.25	2.35	0.281	-0.0523

Model Descriptions for Likelihoods Calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
Al	220.936705	11	-419.873409
A2	227.175202	20	-414.350404
fitted	216.527938	4	-425.055877
R	76.318996	2	-148.637992

Test 1: Does response and/or variances differ among dose levels (A2 vs. R)
Test 2: Are variances homogeneous (Al vs A2)
Test 3: Does the model for the mean fit (Al vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	df	p-value
Test 1	301.712	18	<.00001
Test 2	12.477	9	0.1877
Test 3	8.81753	7	0.266

The p-value for Test 1 is less than 0.05. There appears to be a difference between response and/or variances among the dose levels. It seems appropriate to model the data.

The p-value for Test 2 is greater than .05. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .05. The model chosen appears to adequately describe the data.

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Relative risk

Confidence level = 0.95

BMD = 75.5829

BMDL = 58.2743

CITATIONS FOR BENCHMARK DOSE

Allen, BC; Strong, PL; Price, CJ; Hubbard, SA; Datson, G.P. (1996) Benchmark dose analysis of developmental toxicity in rats exposed to boric acid. Fund Appl Toxicol 32:194-204.

Heindel, JJ; Price, CJ; Field, EA; et al. (1992) Developmental toxicity of boric acid in mice and rats. Fund Appl Toxicol 18:266-277.

Price, CJ; Marr, MC; Myers, CB. (1994) Determination of the No-Observable-Adverse-Effect Level (NOAEL) for Developmental Toxicity in Sprague-Dawley (CD) Rats Exposed to Boric Acid in Feed on Gestational Days 0 to 20, and Evaluation of Postnatal Recovery through Postnatal Day 21. Final report. (3 volumes, 716 pp). RTI Identification No. 65C-5657-200. Research Triangle Institute, Center for Life Science, Research Triangle Park, NC.

Price, CJ; Strong, PL; Marr, MC; Myers, CB; Murray, FJ. (1996a.) Developmental toxicity NOAEL and postnatal recovery in rats fed boric acid during gestation. Fund Appl Toxicol 32:179-193.

Boron and Compounds
CASRN 7440-42-8
00/00/00

0410
Boron and Compounds; CASRN 7440-42-8; 00/00/00

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices, Regional Offices, and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Boron and Compounds

File First On-Line 10/01/89

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	00/00/00
Inhalation RfC Assessment (I.B.)	on-line	00/00/00
Carcinogenicity Assessment (II.)	on-line	00/00/00

_I. CHRONIC HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

_I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

Boron and Compounds
CASRN -- 7440-42-8
Last Revised -- 00/00/00

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background

Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

Chronic toxicity in dogs (Weir and Fisher, 1972) was used previously to develop an RfD for boron (10/01/89). Recently, developmental data in three species (rats, mice and rabbits) have become available. Based on the new developmental data and several limitations of the dog studies (Section I.A.I), decreased fetal body weight in rats is recommended as the critical effect for development of an RfD.

___I.A.1. ORAL RfD SUMMARY

Critical Effect	Experimental Doses*	UF	MF	RfD
Decreased fetal weight (developmental) Rat dietary gestational exposure to boric acid Price et al., 1996a, 1994, 1990; Heindel et al., 1992	BMDL: 10.3 mg/kg-day NOAEL: 9.6 mg B/kg-day LOAEL: 13.3 mg B/kg-day	62	1	2E-1 mg/kg-day

*Conversion Factors and Assumptions -- 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 UF is data-derived and consists of variability and uncertainty factors. The UF is rounded to 62 from 61.9.

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Heindel, JJ; Price, CJ; Field, EA; et al. (1992) Developmental toxicity of boric acid in mice and rats. Fund Appl Toxicol 18:266-277.

Price, CJ; Field, EA; Marr, MC; Myers, CB; Morrissey, RE; Schwetz, BA. (1990) Final report on the Developmental Toxicity of Boric Acid (CAS No. 10043-35-3) in Sprague Dawley Rats. NTP Report No. 90-105 (and Report Supplement No. 90-105A). National Toxicology Program, U.S. DHHS, PHS, NIH, Research Triangle Park, NC, May 1.

Price, CJ; Marr, MC; Myers, CB. (1994) Determination of the No-Observable-Adverse-Effect Level (NOAEL) for Developmental Toxicity in Sprague-Dawley (CD) Rats Exposed to Boric Acid in Feed on Gestational Days 0 to 20, and Evaluation of Postnatal Recovery through Postnatal Day 21. Final report. (3 volumes, 716 pp). RTI Identification No. 65C-5657-200. Research Triangle Institute, Center for Life Science, Research Triangle Park, NC.

Price, CJ; Strong, PL; Marr, MC; Myers, CB; Murray, FJ. (1996a.) Developmental toxicity NOAEL and postnatal recovery in rats fed boric acid during gestation. Fund Appl Toxicol 32:179-193.

Developmental (decreased fetal weights) effects are considered the critical effect. The basis for calculating the RfD is the BMD05 of 10.3 mg boron/kg-day calculated from the developmental effects reported by Heindel et al. (1992; Price et al., 1990) and Price et al. (1996a, 1994).

Heindel et al. (1992; 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 controls]). Food and water intake, and body weights, as well as clinical signs of toxicity, were monitored throughout pregnancy. On day 20 of gestation, the animals were sacrificed and 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 gestation days 12 through 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 days 6-9 and increased on days 15-18, 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; this was due to increases in the percentage of resorptions per litter and percentage of 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% dose groups, 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, 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 a LOAEL; a 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 and 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 percent 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 weight 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 significant trend test ($p<0.05$) was found for decrease 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 percent 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 0.20% 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.

The Institute for Evaluating Health Risks (IEHR) (1997) concluded that there was a consistent correlation between boric acid exposure and the different effects on rib and vertebral development in rats, mice and rabbits (see Additional Studies Section for effects in mice and rabbits). Of these three species, the rat was the most sensitive to low-dose effects. A causal association between exposure to boric acid and the short rib XIII existed when fetuses were examined at late gestation or when pups where examined at pnd 21. The IEHR (1997) concluded that decreased fetal body weight occurred at the same dose or at doses lower than those at which skeletal changes were observed, and agreed that this was the preferred data set for deriving quantitative estimates.

Several benchmark dose (BMDL) analyses were conducted (Allen et al., 1996) using all relevant endpoints to analyze data from Heindel et al. (1992) and Price et al. (1996a, 1994) studies alone and combined data from the two studies. 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 BMDL was defined in terms of a prespecified level of effect, referred to as the benchmark response (BMR) level (Kavlock et al., 1995). For mean fetal weight analysis, the BMDL was defined as the 95% lower bound on the dose corresponding to a 5% decrease in the mean (BMR was 5% decrease). For the continuous power model, F-tests that compared the lack of model fit to an estimate of pure error were employed.

For all endpoints, the results of the two studies were compared. The dose-response patterns were examined 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; this result indicated that the responses from the two studies were consistent with a single dose-response function. BMDL values calculated with a continuous power model for fetal body weight (litter weight averages) were less than those for all other relevant endpoints. The BMDL based on the combined results of the two studies was 10.3 mg B/kg-day, which was very close to the NOAEL of 9.6 mg B/kg-day from the Price et al. (1996a, 1994) study.

In addition to the rat studies, the developmental effects of boric acid were also studied in mice and rabbits. Heindel et al. (1994, 1992; Field et al., 1989) identified a NOAEL and LOAEL of 43.3 and 79 mg B/kg-day, respectively, for decreased fetal body weight in mice exposed to boric acid in the feed. Increased resorptions and malformations, especially short rib XIII, were noted at higher doses. Price et al. (1996b, 1991; Heindel et al., 1994) identified a NOAEL and LOAEL of 21.9 and 43.7 mg B/kg-day for developmental effects in rabbits. Frank effects were found at the LOAEL, including high prenatal mortality and increased incidence of malformations, especially cardiovascular defects.

___I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

UF =62

The animal-to-human and sensitive-human uncertainty factors (UF_A and UF_H) are each split into toxicokinetic (kinetic) and toxicodynamic (dynamic) components in order to apply existing rat and human toxicokinetic data to reduce the uncertainty in the boron RfD. The kinetic and dynamic default values for UF_A are given unequal values for the boron assessment,

as there is empirical and conceptual support for an uneven default partition. For the class of compounds, such as boron, for which a physiological rate is justified as the sole toxicokinetic scaling variable, the IPCS (2001) approach is adopted, where UF_{AK} and UF_{AD} are assigned default values of 4.0 and 2.5, respectively. This partition is based on an empirical analysis published by Renwick (1993) and an allometric approach presented in §5.1.3 in the Toxicological Review. The kinetic and dynamic defaults for UF_H are assigned equal values of $10^{0.5}$ (3.16).

The formula for calculating the RfD with this uncertainty factor disaggregation is:

$$RfD = D_C \Big/ \left(VF_{AK} \cdot VF_{AD} \cdot VF_{HK} \cdot VF_{HD} \cdot UF_{AK} \cdot UF_{AD} \cdot UF_{HK} \cdot UF_{HD} \cdot UF_X \cdot MF\right)$$

where:

- D_C is the "critical" dose (NOAEL, LOAEL, BMD) defined in the critical study,
- VF_{AK} is the interspecies toxicokinetic variability factor (derived from data; default = 1),
- VF_{AD} is the interspecies toxicodynamic variability factor (derived from data; default = 1),
- VF_{HK} is the interindividual toxicokinetic variability factor (derived from data; default = 1),
- VF_{HD} is the interindividual toxicodynamic variability factor (derived from data; default = 1),
- UF_{AK} is the interspecies toxicokinetic uncertainty factor (default = 4.0),
- UF_{AD} is the interspecies toxicodynamic uncertainty factor (default = 2.5),
- UF_{HK} is the interindividual toxicokinetic uncertainty factor (default = $10^{0.5}$),
- UF_{HD} is the interindividual toxicodynamic uncertainty factor (default = $10^{0.5}$),
- UF_X represents all other uncertainty factors (UF_L x UF_D x UF_S = 1, for boron).
- MF is the Modifying Factor (= 1 for boron).

Note that the product of VF_{AK}, UF_{AK}, VF_{AD}, UF_{AD}, VF_{HK}, UF_{HK}, VF_{HD}, UF_{HD}, and UF_X corresponds to the total UF as shown in the RfD Summary Table (I.A.1), and is designated as AF_{TOT} (Total Adjustment Factor). This formula is described further in §5.1.3 of the Toxicological Review.

Although the toxic effects of boron are manifested in the offspring, the pregnant females (for both humans and test animals) are considered to be the "sensitive subpopulation," with respect to establishing an equivalent toxic dose across species. Given the near 1st order kinetics of boron, maternal toxicokinetic variability is likely to be an adequate surrogate for the fetal dose variability, although there is some remaining uncertainty in fetal kinetic variability.

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.

The formula for calculating the interspecies kinetic variability factor is given by

$$VF_{AK} = \frac{Cl_r \times f_{ah} \times f_{ph}}{Cl_h \times f_{ar} \times f_{pr}}$$

where the trailing *r* and *h* subscripts stand for pregnant rats and pregnant humans, respectively, *Cl* is mean boron clearance (mL/min-kg), f_a is fraction of ingested boron absorbed, and f_p is the subsequent fraction distributed in the plasma compartment. The mean boron clearance for pregnant rats and pregnant women is 3.3 and 1.02 mL/min-kg, respectively (U.S. Borax, 2000; Vaziri et al., 2001; Pahl et al., 2001). f_{ah} and f_{ar} are both 0.95, f_{ph} = 0.0911, f_{pr} = 0.0723, and VF_{AK} = 4.08. UF_{AK} is reduced to unity (1.0).

The interindividual (intrahuman) variability factor is calculated as

$$VF_{HK} = \frac{GFR_{AVG}}{GFR_{LOW}}$$

where GFR_{AVG} and GFR_{LOW} are the mean GFR and "lower bound," respectively, for the population of healthy pregnant women, averaged across the entire gestational period. The lower bound is taken as the 0.1 percentile of the lognormal distribution of GFR for pregnant women as reported in Dunlop (1981). In this case, a value for VF_{HK} is sought that gives 99.9% coverage of the population variability. A relatively large coverage is chosen, as the population at risk is very large and this factor addresses population variability rather than uncertainty (which is addressed by UF_{HK}). GFR is used as a surrogate for boron clearance as the available boron clearance data are inadequate for estimating population variability. The lognormal distribution for body-weight-corrected GFR (based on Dunlop, 1981) is characterized by a geometric mean of 2.257 mL/min-kg and a geometric standard deviation of 1.160 mL/min-kg. The 0.1 percentile value is 1.427 mL/min-kg. The overall mean is 2.281 mL/min-kg. The corresponding VF_{HK} value is 1.60. As there is remaining uncertainty in the estimation of population variance from Dunlop (1981), uncertainty pertaining to the use of GFR as a surrogate for actual boron clearance, and uncertainty in fetal kinetics, UF_{HK} is assigned a value of 1.2, rather than 1.0.

As there is no data pertaining to boron toxicodynamics, all of the dynamic factors are assigned their default values (VF_{AD} = VF_{HD} = 1.0, UF_{AD} = 2.5, UF_{HD} = 3.16). The overall adjustment factor (AF_{TOT}) is 61.9 (4.08 x 1.60 x 2.5 x 1.2 x 3.16), which is shown in I.A.1 as the total UF. Section 5.1.3 of the Toxicological Review provides a much more detailed description and discussion of the models and use of the toxicokinetic data for deriving these factors.

MF = 1.

___I.A.4. ADDITIONAL STUDIES/COMMENTS (ORAL RfD)

The subchronic and chronic toxicity of borax and boric acid was studied in dogs administered these compounds in the diet (Weir and Fisher, 1972; U.S. Borax Research Corp., 1963, 1966, 1967). In the supporting 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). A high-dose male dog died as a result of complications of diarrhea on day 68 of the study 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% in males fed borax (9.6g) and 39% in males fed boric acid (10.5 g) 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 was also observed in one dog from the mid-dose boric acid group. Microscopic pathology revealed severe testicular atrophy in all high-dose male dogs, with complete degeneration of the spermatogenic epithelium in most 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; also at the high dose were increases in brain:body weight ratio (borax) and liver:body weight ratios (boric acid) in females and 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, and 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 a LOAEL for systemic toxicity in dogs of 1750 ppm boron (male: 30.4 mg B/kg-day; female: 21.8 mg B/kg-day) and a NOAEL of 175 ppm boron (male: 3.9 mg B/kg-day; female: 2.5 mg B/kg-day) 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. Control animals (4 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 dosed 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 as being "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 were noted throughout the study to have about an 11% decrease in the rate of weight gain when compared with control animals. 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 these 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.

These dog studies were previously used to calculate the RfD for boron (10/01/89). Based on newer developmental data in rats and several limitations in the dog studies, the critical effect is now considered to be decreased fetal body weight in rats. Some limitations of the dog studies include the 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 were taken from two different studies of different duration. Also, the study pathologist considered the histopathological findings as being "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 appropriate at this time for establishment of an RfD.

Reproductive and systemic toxicity studies have identified the testes as a sensitive target of boron toxicity in rats and mice, although at higher doses than in the dog study (Weir and Fisher, 1972; Seal and Weeth, 1980; NTP, 1987; Fail et al., 1991). 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., 1993).

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). 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). 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). Data from three human studies of potential boron essentiality show 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 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). Boron has been known since the 1920s to be an essential micronutrient for the growth of all plants. 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. 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 U.S. 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 can be higher 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.

___I.A.5. CONFIDENCE IN THE ORAL RfD

Study -- High
Data Base -- High
RfD -- High

Confidence in the principal developmental studies is high; they are well-designed studies that examined relevant developmental endpoints using a large number of animals. Confidence in

the data base is high due to the existence of several subchronic and chronic studies, as well as adequate reproductive and developmental toxicology data. High confidence in the RfD follows.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE ORAL RfD

Source Document -- U.S. EPA, 1998

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 1998.

Other EPA Documentation -- None

Agency Consensus Date -- __/__/__

___I.A.7. EPA CONTACTS (ORAL RfD)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (internet address).

__I.B. REFERENCE CONCENTRATION FOR CHRONIC INHALATION EXPOSURE (RfC)

Boron and Compounds
CASRN -- 7440-42-8
Last Revised -- 00/00/00

The inhalation Reference Concentration (RfC) is analogous to the oral RfD and is likewise based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/cu.m. In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs were derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F August 1989) and subsequently, according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F October 1994). RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this

substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

NOT VERIFIABLE status indicates that the available data do not meet the minimum data base requirements according to the current Agency methods document for RfDs (EPA/600/8-90/066F October 1994). This does not preclude the use of information in cited references for assessment by others.

___I.B.1. INHALATION RfC SUMMARY

An RfC for boron is not recommended at this time. The literature regarding toxicity of boron by inhalation exposure is sparse. There is a report from the Russian literature of reduced sperm count and sperm motility from semen analysis of 6 workers who were a part of a group of male workers (n=28) exposed to very high concentrations of boron aerosols (22-80 mg/m^3) for over 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. No effect on fertility was found in a much larger study of U.S. borate production workers (Whorton et al., 1994a,b; 1992), but exposure concentrations were much lower (≈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 and the animals were covered with dust. Systemic endpoints were not examined, but growth was reduced 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 prolonged exposure (Wegman et al., 1994). These data are inadequate to support derivation of an RfC for boron compounds.

___I.B.2. PRINCIPAL AND SUPPORTING STUDIES (INHALATION RfC)

Tarasenko et al. (1972) reported low sperm count, reduced sperm motility and elevated fructose content of seminal fluid from 6 workers who were part of a group of 28 male Russian workers exposed for 10 or more years to high levels of boron aerosols (22-80 mg/m^3) during the production of boric acid. In response to this report and reports of reproductive effects in animal studies (see Section 4.3.2), 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. Secondly, the investigators 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 had vasectomies (36%) was 5 times higher than the national average of 7% implicit in the expected number of births. Participants were divided into 5 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 middle 3 dose groups. There were 42 participants who worked high-exposure jobs for two 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 these 42 workers was close to expected (102) despite a 48% vasectomy rate. These workers also had elevated SBRs 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 percent 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. 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. It must be recognized, however, that the rather small sample size may have precluded a meaningful statistical analysis of the results.

Culver et al. (1994) monitored boron levels in the blood and urine of 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 (≈0.25 μg/g) in comparison to pre-shift Monday morning values (≈0.1 μg/g). Similarly, workers in the high exposure category had significantly higher urinary boron levels Monday post-shift (≈12 μg/mg creatinine) than pre-shift (≈2 μg/mg creatinine). Boron in the diets (which were assigned by the researchers to ensure uniformity among workers) and workplace air was also 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.

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 one 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 FEV and respiratory illness data in the subgroup of 82

men who had worked for at least one year at the calcining and fusing processes compared with the other 547 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 employed for 5 or more years at the plant and employed in an area with heavy borax exposure at the time of the study (93% of those eligible). Workers were categorized into 4 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 for (in order of decreasing frequency) 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 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.

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, FVC) 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.

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. 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. Also in this study, 3 dogs were exposed to 57 mg/m^3 (18 mg B/m^3) for 23 weeks. 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 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 (including the testis), bone strength or liver function was found in either rats or dogs (not all endpoints were studied in all exposure groups).

___I.B.3. UNCERTAINTY AND MODIFYING FACTORS (INHALATION RfC)

Not Applicable

___I.B.4. ADDITIONAL STUDIES/COMMENTS (INHALATION RfC)

Not Applicable

___I.B.5. CONFIDENCE IN THE INHALATION RfC

Not Applicable

___I.B.6. EPA DOCUMENTATION AND REVIEW OF THE INHALATION RfC

Source Document -- U.S. EPA, 1998

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 1998.

Other EPA Documentation -- None

Agency Consensus Date -- __/__/__

___I.B.7. EPA CONTACTS (INHALATION RfC)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (internet address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Boron and Compounds
CASRN -- 7440-42-8
Last Revised -- 00/00/00

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/cu.m air breathed. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

__II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

___II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Classification -- Under EPA's current guidelines for carcinogen risk assessment (U.S. EPA, 1986), boron is classified as Group D; not classifiable as to human carcinogenicity. Under the new proposed guidelines (U.S. EPA, 1996), the data are considered to be inadequate for evaluation of the human carcinogenic potential of boron.

Basis -- 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 even mention 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 low dose, but not high dose, male mice that was 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 was also 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 44%, 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 studies were located. Studies of boron compounds for genotoxicity were overwhelmingly negative, including studies in bacteria, mammalian cells and mice *in vivo*.

___II.A.2. HUMAN CARCINOGENICITY DATA

No studies were located regarding the carcinogenicity of boron in humans.

___II.A.3. ANIMAL CARCINOGENICITY DATA

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. No treatment-related effects were observed in rats receiving 350 or 117 ppm boron as borax or boric acid. Based on effects observed in the high-dose group, it appears that an 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, and 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.

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). 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 controls 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).

There was an increased incidence of hepatocellular carcinoma (5/50, 12/50, 8/49) and combined adenoma or carcinoma in low dose male mice (14/50, 19/50, 15/49) (NTP, 1987; Dieter, 1994). The increase was statistically significant by life table tests, but not by incidental tumor tests. The incidental tumor tests were probably 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 in this study 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 body weights (at 30 days, treated animals were lighter than controls and at 90 days, treated males were significantly heavier than controls) or longevity. 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.

___II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Results of most short-term 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 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 in the *Salmonella* microsome assay using strains TA1535, TA1537, TA1538, TA98 and TA100 in 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 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 the presence or absence of 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.

___II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

Not Applicable

___II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

Not Applicable

___II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

___II.D.1. EPA DOCUMENTATION

Source Document -- U.S. EPA, 1998

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA, 1998.

___II.D.2. EPA REVIEW (CARCINOGENICITY ASSESSMENT)

Agency Consensus Date -- __/__/__

___II.D.3. EPA CONTACTS (CARCINOGENICITY ASSESSMENT)

Please contact the Risk Information Hotline for all questions concerning this assessment or IRIS, in general, at (513)569-7254 (phone), (513)569-7159 (FAX), or RIH.IRIS@EPAMAIL.EPA.GOV (internet address).

_III. [reserved]

_IV. [reserved]

_V. [reserved]

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CASRN -- 7440-42-8
Last Revised -- 00/00/00

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___VII. REVISION HISTORY

Boron and Compounds
CASRN -- 7440-42-8

Date	Section	Description
__/__/__		
__/__/__		
__/__/__		
__/__/__		

___VIII. SYNONYMS

Boron and Compounds
CASRN -- 7440-42-8
Last Revised -- 00/00/00

APPENDIX C. REGRESSION ANALYSIS OF SERUM CREATININE AND INULIN CLEARANCE

A log-linear regression was performed to investigate the relationship between values of serum creatinine (Scr) and inulin clearance (Cin). The log of Cin was found to be normally distributed using the Kolmogorov-Smirnov Goodness of Fit test (P=0.065), which is marginal in terms of significance. However the visual fit of a histrogram (Figure 1) shows a lognormal distribution for Cin to be reasonable for purposes of a regression analysis. Using a stepwise regression analysis in SAS, and analyzing for Scr, Scr^2, the log of Scr and the square root of Scr, the procedure found the log of Scr to be the only variable that met the 0.15 significance level for entry into the model. Thus, the resulting regression model is:

$$\log(Cin) = 1.79 - 1.3 \log(Scr)$$

As shown in Figure 2, the model fit the data well. Also, Table 1, the Analysis of Variance results, shows that the parameter estimates were also satisfactory with all p-values <0.0001. The R-squared value was 0.79, showing that 79% of the variance in the dependent variable was explained by the model. Residuals appeared randomly distributed when graphed, but tests for normality were not significant (e.g., Kolmogorov-Smirnov p=0.03).

Predictions of Cin values from Scr values were desired for Scr=1.4 mg/dl and for Scr = 0.8 mg/dl. Table 2 shows these results. When predicting a "future value of the dependent variable", it is appropriate to use a prediction interval. Thus, the results are for Scr=1.4 mg/dl, the predicted value of Cin from the model is Cin = 39.8 mL/min, with a 95% prediction interval of (17.8, 89.1); for Scr = 0.8 mg/dl, the predicted value of Cin from the model is 79.4 mL/min, with a 95% prediction interval of (36.3, 186.2).

Table 1
Analysis of Variance Results

Dependent variable: Log of Cin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	17.38605	17.38605	545.61	<0.0001
Error	140	4.46117	0.03187		
Corrected Total	141	21.84721			

Root MSE	0.17851	R-Square	0.7958
Dependent Mean	1.56837	Adj R-Sq	0.7943
Coeff Var	11.38183		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.78846	0.01770	101.06	<0.0001
LScr	1	-1.29614	0.05549	-23.36	<0.0001

Table 2
Prediction Intervals

Linear Regression Results — R2 = 0.79
Model: log(Cin) = 1.79 - 1.3*log(Scr) — All p values < 0.0001

log10(Scr)	Pred val	SE Pred val	95% CI Mean		95% Pred Int	
0.146	1.6	0.015	1.57	1.63	1.25	1.95
-0.098	1.9	0.02	1.87	1.96	1.56	2.27

Scr — Conversion of Values Using Anti-log

1.399587	39.81072	1.035142	37.15352	42.65795	17.78279	89.12509
0.797995	79.43282	1.047129	74.13102	91.20108	36.30781	186.2087

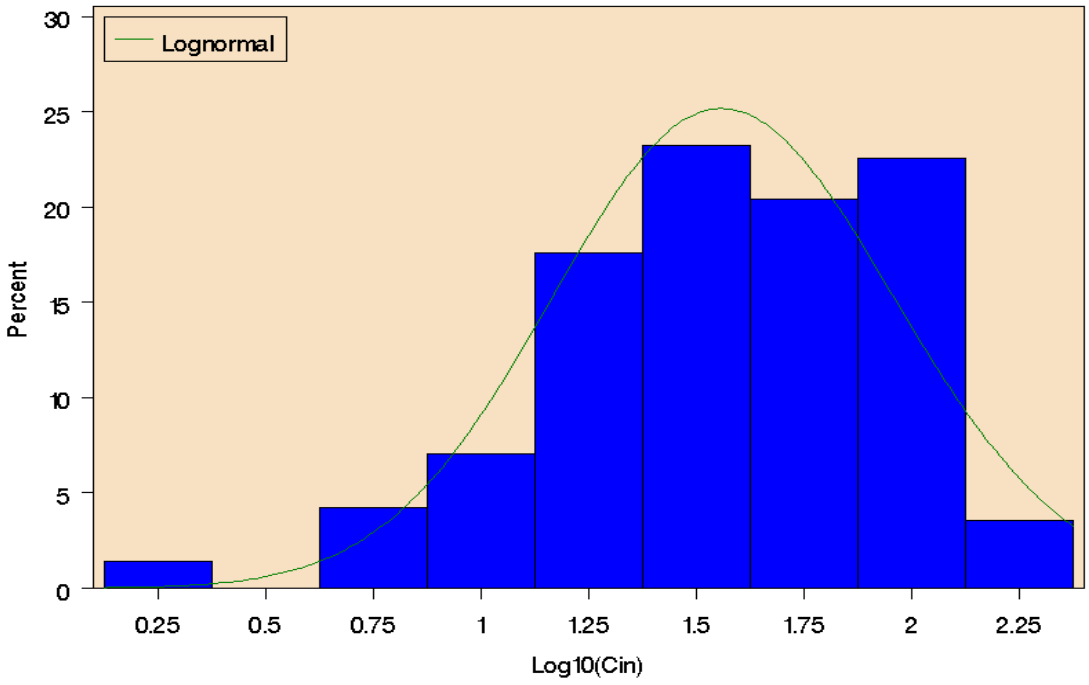

Figure 1

Histogram of Lognormal Fit for Cin Values

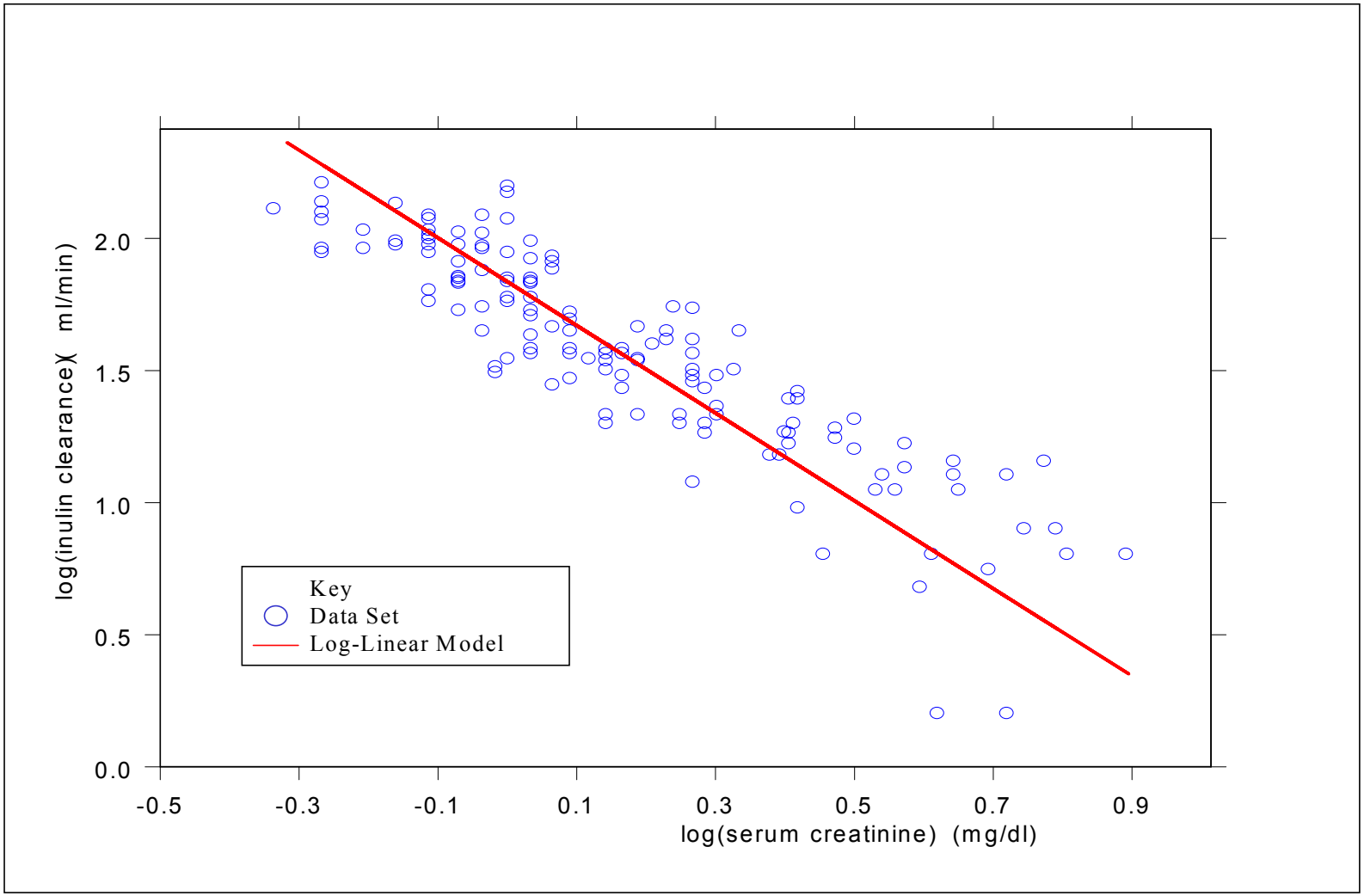

Figure 2

Modeled Regression Line and Raw Data Set

Charge to Reviewers for the Revised Sections of the Boron Toxicological Review and IRIS Summaries.

The U. S. EPA is conducting a peer review of the scientific basis supporting the health hazard and dose response assessment for Boron that will appear on the Agency's online data base, the Integrated Risk Information System (IRIS). Peer Review is meant to ensure that science is used credibly and appropriately in derivation of these dose-response assessments. The primary function of the peer reviewer should be to judge whether the choice, use and interpretation of the data employed in the derivation of the assessment is appropriate and scientifically sound. This review is not of the recommended agency risk assessment guidelines or methodologies as those have been reviewed by external scientific peers, the public and the EPA Science Advisory Boards.

The IRIS Toxicological Review for Boron and IRIS Summary Sheets have previously gone through two internal and external reviews. However, certain sections of the Toxicological Review have been revised since these peer reviews took place. Revisions to the last external review draft were made based on some external reviewer comments and comments from the public when the external review draft was posted on the National Center for Environmental Assessment web site.

While all external peer review and public comments strongly supported using a data-derived approach for addressing uncertainty factors, a few methodological issues remained. Therefore, the previous method for using data to derive an uncertainty factor has been revised with this new draft. This revised method has been through the agency's internal review process, and has been submitted for one more formal external peer review.

Due to the amount of review that this document has already received we are requesting review comments **only** on the revised method of using toxicokinetic data to replace uncertainty factors. However, you will probably need to familiarize yourself with other parts of the document that pertain to the data used in the assessment. The following sections have been revised. The questions for reviewers apply to those sections only.

Toxicological Review: 5.1.3 Derivation of the RfD

RfD Summary Sheet: I.A.3. UNCERTAINTY AND MODIFYING FACTORS (ORAL RfD)

Questions for Reviewers

1. The Agency as yet has no guidance for using toxicokinetic or toxicodynamic data for modification of uncertainty factors for Reference Doses. Therefore the use of

toxicokinetic data for establishing the boron RfD could set some precedents that will need scrutiny. Please carefully evaluate the many different and sometimes complex arguments in Section 5.1.3 as to their organization, clarity, and scientific merit. Do they hang together?

2. Is the approach we're taking for an uneven split of the kinetic and dynamic components of the interspecies uncertainty factor reasonable? Is the default split for the interspecies uncertainty factor of 4.0 for kinetics and 2.5 for dynamics the correct one?

3. For the interspecies extrapolation, a simple kinetic model is presented for linking the specific kinetic extrapolation variable (boron clearance) to external exposure. Is this model reasonable? Are there any implicit assumptions that need to be stated? Are the various surrogacy assumptions reasonable? Are the clearance data adequate for the purpose. Do you agree that the data are adequate for reduction of the interspecies kinetic uncertainty subfactor (UF_{AK}) to 1.0?

4. For the intra-human toxicokinetic variability assessment, do you agree that GFR variability is an adequate surrogate for variability in boron clearance and provides a less uncertain estimate than using the boron clearance data of Pahl et al. (2001)? Do you agree with the general approach for determining intra-human variability (ratio of mean GFR to 0.1 percentile)? If not, is there a more viable alternative? Is the assumption of a lognormal distribution adequately supported? Is the magnitude of the residual uncertainty in UF_{HK} appropriate?

5. Are there any other critical issues on which we have not explicitly asked for comment?