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TOXICOLOGICAL REVIEW

OF

BORON AND COMPOUNDS

(CAS No. 7440-42-8)

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

June 2004

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 and compounds. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of boron and compounds.

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response,* EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. The discussion is intended to convey 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 IRIS Hotline at 202-566-1676.

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

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of boron and compounds. 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 toxicity values are 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. 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 noncancer effects during a lifetime. It is expressed in units of mg/kg-day. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. 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 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 to better facilitate their use: (1) generally, the *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day of oral exposure; (2) 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; and (3) the 95% lower bound and central estimate on the estimated concentration of the chemical substance in drinking water or air that presents 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 and compounds as 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: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S.

EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000c), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000d), and *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through November 2002.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Boron is a nonmetallic 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%) (World Health Organization [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 (European Center for Ecotoxicology and Toxicology of Chemicals [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 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 0.5-9.6 ppm, with an average of 4.6 ppm. Fresh water concentrations range from <0.01-1.5 ppm. Boron in the environment is always found chemically bound to oxygen, usually as alkali or alkaline earth borates, or as boric acid (Institute for Evaluating Health Risks [IEHR], 1997; U.S. EPA, 1987). Elemental boron is not found in nature.

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 (Agency for Toxic Substances and Disease Registry [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.

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) closed space	2300	171	>62	<200	No data	No data
Melting Point (°C) anhydrous form (crystal)	2300	450	742	742	742	450
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)

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

Studies in animals have shown that boron is readily absorbed following oral exposure in rats (Ku et al., 1991; Usuda et al., 1998), rabbits (Draize and Kelley, 1959), sheep (Brown et al., 1989) and cattle (Owen, 1944; Weeth et al., 1981). Using mass spectrometry and the boron-10 isotope, Vanderpool et al. (1994) showed that fasted rats fed 20 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 male workers exposed to borate dust (borax, borax pentahydrate and anhydrous borax) at a borax production facility. The workers were divided into three groups according to borate exposure. Workers in both the medium- and high-exposure categories had significantly increased levels of boron in the blood after working Monday (≈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 also was monitored during this study. A higher proportion of total boron intake was from air than from diet, and both blood and urine boron were best modeled based on air concentration of boron alone (i.e., inclusion of dietary boron as an independent variable did not increase the predictive power of the models). These data show that boron was absorbed during the work day, and that borate dust in the air was the source of the additional boron in the blood and urine. However, it is not clear what amount of the inhaled boron was actually absorbed through the respiratory tract. The researchers speculated that due to the large size of the dust particles in the work area, most of the inhaled borate would have been deposited in the upper respiratory tract, where it could have been absorbed directly through the mucous membranes or could have been cleared by mucociliary activity and swallowed.

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

3.1.3. Dermal Absorption

Boron apparently is 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-

to 10-month-old infants exposed to talcum powder containing 5% boric acid 7-10 times per day for at least 1 month (estimated daily dose of 2.33 g boric acid or 407 mg B) compared with an equal number of untreated controls. An additional group of 12 infants with mild to moderate diaper rash during the test period was continued on the powder regimen for 48-72 hours after rashes appeared. Their boron blood levels were similar to controls. However, there is evidence that boron can be absorbed through more severely damaged skin, especially from an aqueous vehicle. Blood and urinary boron levels were increased in six male volunteers with severe skin conditions (e.g., psoriasis, eczema, urticaria) following topical application of an aqueous jelly containing 3% boric acid (Stuttgen et al., 1982). 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 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

Studies suggest that boric acid and borate compounds in the body exist primarily as undissociated boric acid, which distributes evenly throughout the soft tissues of the body, but shows some accumulation in bone. 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 above controls. Boron levels in plasma and soft tissues other than adipose tissue reached steady-state (12-30 μg/g) within 3-4 days. Variability in levels of boron among soft tissues (adipose and kidney excluded) was minimal, with tissue concentrations at 60% of plasma levels on day 1 and 30-40% of plasma levels on days 2, 3, 4,

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
Hypothalamus	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

and 7. Levels in bone and adipose continued to increase throughout the 7-day study period. In comparison to plasma levels, there was no appreciable accumulation of boron in any soft tissue. However, boron did accumulate in bone, showing a 2 to 3-fold increase over plasma levels after 7 days. Boron levels in adipose tissue remained at 20% of plasma levels after 7 days. Other investigators provided support for these findings: (1) accumulation of boron in bone in rats (Forbes and Mitchell, 1957); (2) lack of appreciable accumulation of boron in the testis (Lee et al.,1978; Treinen and Chapin, 1991); and (3) lack of appreciable accumulation of boron in the epididymis (Treinen and Chapin, 1991).

In a follow-up to Ku et al. (1991), Chapin et al. (1997) monitored bone boron concentrations in rats fed 200-9000 ppm of boric acid for 9-12 weeks. Bone boron was significantly increased over controls at 200 ppm and increased proportionally up to 6000 ppm, above which the increase in bone was slightly less than the increase in the feed. Bone boron levels reached steady state within 1 week at doses up to 3000 ppm and after approximately 4 weeks at higher doses. Steady-state bone boron levels were approximately 4-fold greater than serum boron levels. Chapin et al. (1997) also monitored bone (tibia) boron levels for 32 weeks following cessation of exposure in rats that had been fed boron in the diet for 9 weeks. Levels of boron in the bone declined slowly. After 8 weeks of recovery, bone levels of boron were reduced to roughly 10% of levels at the end of exposure (e.g., at 9000 ppm: ≈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.

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

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

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

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; Devirian and Volpe, 2003). Boron deprivation studies with animals and three human clinical studies have shown that boron affects macromineral and cellular metabolism of other substances that affect life processes such as calcium and magnesium (Section 4.4.5.).

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

3.4. ELIMINATION

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 (Section 3.1.1.). In humans, Jansen et al. (1984a) and Schou et al. (1984) reported that boron's primary route of elimination was in the urine. Jansen et al. (1984b) reported that approximately 60-75% of a dose of 750 mg boric acid (131 mg B) in a water solution or 740-1473 mg boric acid (129.5-261.3 mg B) in a water emulsifying ointment, administered orally to humans, is eliminated in urine over the initial 24 hours, with the urinary route of elimination accounting for 93% of the dose at 96 hours after oral administration. Graphically, Jansen et al. (1984b) demonstrated cumulative boron elimination, as percentage of dose, from six adult males who consumed an aqueous solution of boric acid. Results indicate that at 12 hours, the urinary elimination accounted for 52.7 ± 4.9% (mean ±

S.D.) of the dose (range 46.4-58.9%); and at 24 hours, the cumulative urinary elimination accounted for 66.9 ± 6.4% of the dose (mean ± S.D.), with a range of 57.1-75.0%. These data demonstrate a marked similarity among this limited sample of adult men in the renal elimination of boric acid. In a clinical report of an acute, uncontrolled intoxication with boric acid, Astier et al. (1988) estimated the dose as 45 g boric acid (7.9 g B), and reported that renal elimination accounted for 50% of the dose in the first 24 hours. Regression analysis of plasma B concentrations revealed a clearance of 0.77 L/hour. While no methods of analysis were presented, the authors concluded that tubular reabsorption affected 80% of the dose. Kent and McCance (1941) also reported that 92-93% of an administered oral dose (352 mg as boric acid) in humans was eliminated in urine during the first week following administration. Additional minor elimination pathways include saliva, sweat, and feces (Jansen et al., 1984a).

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

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

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

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

The results are shown in Table 3, along with the infusion-phase clearances published by Jansen et al. (1984a). Boron clearance at dietary exposure levels was characterized by a high coefficient of variation (CV, standard deviation/mean) of 0.78, but the mean value was remarkably consistent (39-42 mg/min/1.73 m^2) for each day of the 3-day baseline measurement period. Boron clearance following boric acid infusion was 60.5 mL/min/1.73, with a CV of only 0.09 (Table 3). The interindividual variability in renal boron clearance was much greater when clearance was calculated from the subjects receiving exposure to boron in the diet alone compared to the values calculated in the same individuals receiving a single intravenous infusion. The variance of dietary-exposure boron clearance was 66 times greater than for intravenous infusion. The mean boron clearance estimated by this method was lower than the mean boron clearance estimated from the intravenous infusion by a factor of 1.5. There are a number of possible reasons for both the higher variability and lower absolute clearance values as outlined in the following paragraphs.

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

Table 3. Renal Boron clearance (mL/min/1.73m^2) calculated from dietary exposure and intravenous infusion

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

Source: Adapted from Jansen et al., 1984a
[a] Subject No. 4 was excluded due to increasing excretion in urine during the period
[b] Dose estimated from total urinary excretion of boron during 24 hours of normal dietary exposure
[c] Dose administered by 20-minute intravenous infusion

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

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

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

Urinary elimination of boric acid in Sprague-Dawley female rats (nonpregnant and pregnant) was examined in a pharmacokinetic study (U.S. Borax, 2000; Vaziri et al., 2001). Three groups of 10 nonpregnant and 10-11 pregnant rats were started on an initial 7-day supplemented boron diet on gestation day 9, prior to gavage administration of boric acid. According to the authors, the purpose of this initial 7-day diet was to achieve steady state conditions for rats given a diet comparable to that ingested by humans in terms of boron. This supplemented boron diet given during the initial 7 days was designed to deliver a dose of approximately 0.3 mg/kg-day of boric acid or 0.05 mg B/kg-day. On the morning of day 8, the diet for all rats was switched to the low boron casein diet containing 0.2 mg B/kg diet for a total of 24 hours. The low boron casein based diet was used in this study to minimize cross contamination of the urine with boron in the diet and to minimize the dietary contribution of boron on the day of gavage. After the initial 24 hours on the low casein diet, groups of pregnant and nonpregnant rats were given a single oral dose of 0.3, 3.0, or 30 mg/kg of boric acid (0.052, 0.52, and 5.2 mg B/kg, respectively) by gavage in deionized water (ultrapure). According to the authors, the low dose was chosen as an estimate of the high end human dietary dose level, and the highest dose tested was approximately half of the no-observed-adverse-effect-level (NOAEL) from the rat developmental toxicity study (Price et al., 1996a).

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

The urinary concentration of boron at the high dose was significantly higher in pregnant rats compared with nonpregnant rats but not at the low and mid dose (Table 4). The urine volume was not significantly different in pregnant and nonpregnant rats. The amount of boron (μg/12 hours) excreted in the urine increased proportionately with increasing dose and during the 12-hour collection period was higher (32-73%) in pregnant rats compared to the nonpregnant rats in the high dose level. This was attributed by the authors to the higher dose of boron administered to pregnant rats due to their larger body weight and to the higher fractional excretion of boron (boric acid clearance/creatinine clearance) in the pregnant rats which was statistically significant at the high dose level. The percentage of administered dose of boric acid recovered in the urine was significantly higher in the low dose group compared to the mid and high dose groups for both the nonpregnant and pregnant animals and higher in the pregnant compared to the nonpregnant rats across dose groups, which was statistically significant at the high dose only (Table 4). Although the diet used for this study was low in boron, it contributed to the overall dose of boric acid, and these amounts were not included in the nominal dose levels. When dietary contribution from the low boron diet was included in the dose, the actual dose levels were approximately 0.4, 3.1, and 30.1 mg/kg boric acid. At the low dose, the diet contributed another 27% and 33% to the overall dose given to nonpregnant and pregnant rats, respectively, whereas at the mid and high doses, the diet contributed 3% and 0.3%, respectively, to the total dose. The authors suggest that the incremental increase at the low dose may explain the greater recovery of administered dose in the low dose group.

Table 4. Urinary boron concentration, volume, mean excretion, and percent recovered in 12 hours in nonpregnant and pregnant rats given boric acid by gavage[a,b]

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

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

Table 5 shows the clearance rates of boron (boric acid), creatinine, and urea expressed in three different ways: mL/min, mL/min/kg of body weight, and mL/min/cm^2 of body surface area. Boron clearance appeared to be independent of dose within the range of dose levels tested. The average absolute clearance value for pregnant rats (mL/min) was 1.01 mL/min. The measurements showed low to moderate variability with a standard deviation of 0.2 mL/min (CV=0.2). Boron clearance was slightly higher in pregnant rats compared to nonpregnant rats, but the difference was not statistically significant. The rate of creatinine clearance did not vary significantly with the different doses of boric acid in either nonpregnant or pregnant rats. Creatinine clearance, normalized against body weight, however, was significantly greater in nonpregnant rats compared to pregnant rats. Urea clearance was not significantly different between nonpregnant and pregnant rats. There were no consistent differences in the rate of urea clearance with the different doses of boric acid.

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

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

Urine for each subject was pooled over the initial 2-hour period and over the subsequent 22-hour period. Boron content of blood and pooled urine was analyzed via inductively coupled plasma-mass spectrometry (ICPMS) following laboratory analytical standards and practices, and employing adequate quality control measures. Urinary clearance was measured by quantifying the amount of boron (mg) in the urine and blood. Because the 22-hour clearance samples were not collected onsite, the 2-hour clearance values were considered to be more accurate due to the women's compliance with the collection procedures while at the clinic. The urinary clearance of boron in humans was determined in all individuals and presented as mL blood cleared of boron

Table 5. Clearance of boron (boric acid), creatinine and urea in nonpregnant and pregnant rats given boric acid by gavage expressed as mL/min, $mL/min/cm^2$, and mL/min/kg[a,b]

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

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

per minute per kg body mass. The average clearance rate for boron in pregnant women was 1.02 ± 0.55 (mean ± standard deviation; range 0.252-2.028) and the average clearance rate for boron in nonpregnant women was 0.80 ± 0.31 (mean ± standard deviation; range 0.229-1.358) mL/min-kg body mass. These results show that pregnant women clear boron more effectively than nonpregnant women, which is consistent with the normal increase in renal blood flow and glomerular filtration rate during pregnancy.

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

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

In addition, these boron clearances probably underestimate the true clearance that would be obtained with higher doses, as in Jansen et al. (1984a). The Pahl et al. (2001) study did not have the detection limit problem of Jansen et al. (1984a), and only a single 2-hour urine sample was collected. As complete bladder voiding is problematic in such a short time, underestimation of total boron excreted is likely. The result would be lower estimated boron clearance values. Pahl et al. (2001) reported evidence of under-collection of urine in some subjects, but quantification of underestimate was not possible. In addition, the variance of boron clearance reported in the study is very likely an overestimate of the true variability of clearance in the population. As study subjects could not be kept in the clinic for prolonged periods, multiple urinary and plasma boron measurements over a longer time interval could not be made. Therefore, the average of only two plasma samples over 2 hours had to suffice a surrogate for AUC in the calculation of clearance. The average plasma boron concentration over 2 hours, with no controls on exposure timing or magnitude, inherently will be more variable than plasma

Table 6. Urinary clearance of boron in pregnant women

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

Source: Adapted from U.S. Borax, 2000

concentrations obtained from a carefully controlled and monitored study, as in the infusion phase of the Jansen study. The excess variance would reflect experimental error rather than true interindividual variability. In the Jansen study, the CV for boron clearance was reduced by a factor of 13 with larger doses and controlled conditions compared to uncontrolled dietary exposure.

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

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

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

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

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

Tubular reabsorption, if it is a factor, will be an issue at dietary levels, and its impact will diminish with increasing dose. The magnitude of the contribution to boron clearance variability, however, is much less than would be suggested by the fractional clearance data from both the human (Pahl et al., 2001) and rat (Vaziri et al., 2001) studies. An average fractional excretion of 0.57 was reported for pregnant women in the Pahl et al. (2001) study (similar results for rats), suggesting that 43% of boron filtered through the glomerulus was reabsorbed into the bloodstream. Boron fractional excretion in the Pahl study, however, was calculated relative to creatinine clearance, which overestimates GFR by about 20% (Shemesh et al., 1985). Correcting for that overestimate yields a fractional clearance of about 0.7, indicating a lesser influence of reabsorption on boron clearance than reported. The variability in reabsorption is probably small by comparison to the variability in GFR. Furthermore, the high boron clearance variability for uncontrolled low-dose dietary exposure decreases dramatically under more controlled, higher-dose conditions (Jansen et al., 1984a). In the Jansen et al. (1984a) study, the CV of 0.09 for boron clearance at a dose of 105 mg (see Table 3), or 1.5 mg/kg (assuming an average body weight of 70 kg) is less than that for GFR in females, which ranges from 0.11 to 0.21 for pregnant or nonpregnant women (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). Thus, the variability in GFR may actually slightly overestimate variability of boron clearance in exposed humans. GFR is slightly higher in men than women (Ventura et al., 1999), but increases by over 50% in pregnancy (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). GFR variability appears to be similar in pregnant and nonpregnant women (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). Assuming that GFR variability in men and women is the same, by analogy, boron clearance variability should be similar. In addition, the variance of boron clearance is less than the variance of creatinine clearance (a measure of GFR) when assessed in the same subjects (Jansen, 1984a). Therefore, it is unlikely that GFR variance underestimates boron clearance variance, and would not need further quantitative adjustment. The contribution of tubular reabsorption is unlikely to affect the variability of renal elimination of boron at the higher doses (compared to dietary levels) of concern in deriving an RfD.

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 4.0-27.8 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 B/100 g body weight where 0.5-1 mL samples were drawn at nine different times during a 24-hour time period, reported a monophasic elimination of boron from plasma, demonstrating a plasma half-life mean of 4.64 ± 1.19. This study also cited a high volume of distribution of 142.0 ± 30.2 mL/100 g body weight. One of the limitations of this study was that the large amount of blood drawn from the rats in the 24-hour period may have physiologically compromised the rats.

A human study (U.S. Borax, 2000; Pahl et al., 2001) was conducted to measure renal clearance of boron normally consumed in the daily diet in nonpregnant and pregnant women (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 nonpregnant subjects. After 24 hours, plasma boron levels were lower in the pregnant women when compared with nonpregnant women, but there was a significant variability in the plasma values in both groups.

In a plasma clearance study of boron sponsored by U. S. Borax (Vaziri et al., 2001) in pregnant and nonpregnant rats given boric acid at dose levels of 0.3, 3.0, and 30 mg boric acid, plasma concentrations of boron were markedly lower 15 hours after dosing than at 3 hours after dosing (Section 3.4.1.). Mean plasma levels of boron were slightly higher in pregnant rats than in nonpregnant rats (statistically significant in only the high dose) given the same dose of boric acid.

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

3.5. TOXICOKINETIC SUMMARY

There is no evidence that boron compounds are metabolized in the body. Boron is readily absorbed following oral exposure in both humans and 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 male mine workers and rats have shown that boron also is absorbed during inhalation exposure (Culver et al., 1994; Wilding et al., 1959). Boron is 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 were 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. Given the relatively uniform distribution of boron to the tissues and that the majority of the compound is excreted quickly, 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 nonpregnant rats and humans. Reports from studies (U.S. Borax, 2000; Pahl et al., 2001; Vaziri et 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 nonpregnant 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 nonpregnant 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 CV 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 AND CASE REPORTS

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 poisoning 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 recuperating from surgery had boric acid solution (70 mg B/kg) injected into the subcutaneous fluid infusion, which resulted in severe cutaneous and gastrointestinal symptoms. The patient recovered after hydration and diuresis.

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

O'Sullivan and Taylor (1983) reported seizures and other milder effects in seven infants who consumed boron in a honey-borax mixture applied to pacifiers. Five of the infants had a history suggestive of a familial-reduced convulsive threshold. The seizures ceased when the honey-borax treatment was stopped. The infants, who ranged in age from 6-16 weeks (at the end of the exposure period), were exposed to the honey-borax mixture over a period of 4-10 weeks. Original estimates of exposure were based on an error by the author (Taylor, 1997) concerning intake in jars versus grams of boron per week. The doses were recalculated from the information given by the author, based an estimated daily ingestion of honey-borax mixture and the analysis of the borax content in the mixture. Details of the analytic methods were not provided. Average estimated daily intakes of borax ranging from 429-1287 mg can be calculated directly from data provided by the authors. Average body weights over the exposure period for the infants in this study ranging from 4.3-5.3 kg based on estimates from the *Exposure Factors Handbook* (U.S. EPA, 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 9.6-33 mg/kg-day. The lowest exposure level of 3.2 mg/kg-day would be considered a lowest-observed-adverse-effect-level (LOAEL) for a fairly severe effect. Concentrations of boron in blood of 2.6, 8.4, and 8.5 µg/mL were reported for three of the subjects. Blood boron concentrations did not correlate well with estimated ingestion levels; the lowest blood boron concentration was measured for the infant with the highest estimated boron intake. Blood boron levels were also reported for a control group of 15 children aged 2-21 months, who had received no boron supplement and, presumably, had suffered no seizures. The control group blood boron values ranged from 0-0.63 µg/mL and averaged 0.21 µg/mL, with a standard deviation of 0.17 µg/mL. The lowest boron blood level associated with seizures, 2.6 µg/mL, was about 4 times the highest control level and 12 times the average control level, suggesting that the standard 10-fold uncertainty factor may be adequate for estimating a NOAEL. However, there was no indication whether any infants predisposed to seizures were in the control population. The presumptive boron NOAEL would be 0.32 mg/kg-day for a sensitive human subpopulation. Given the relatively uncomplicated boron toxicokinetics, the lack of correlation of blood boron and estimated ingestion rates 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, 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 Cran et al. (1997), WHO (1998a), Culver and Hubbard (1996), and Ischii et al. (1993). The most frequent symptoms of boron poisoning are vomiting, abdominal pain, and diarrhea. Other common symptoms include lethargy, headache, lightheadedness, and rash. For boric acid, the minimum lethal dose by oral exposure is approximately 15-20 g in adults, 5-6 g in children, and 2-3 g in infants.

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 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 (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. The investigators then examined possible deviations of the ratio of male to female offspring relative to the U.S. ratio.

There was a significant excess in the SBR among participants as a whole (Whorton et al., 1994a,b, 1992). Study participants fathered 529 births versus 466.6 expected (SBR=113, $p<0.01$). This excess occurred even though the percentage of participants who had vasectomies (36%) was 5 times higher than the national average of 7% implicit in the expected number of births. Participants were divided into five equal-size groups (n = 108/109) based on average workday exposure to sodium borates (<0.82, 0.82-1.77, 1.78-2.97, 2.98-5.04, and >5.05 mg/m^3). There was no trend in SBR with exposure concentration; the SBR was significantly elevated for both the low- and high-dose groups, and close to expected for the three mid-dose groups. There were 42 participants who worked high-exposure jobs for 2 or more consecutive years. Mean sodium borate exposure in this group was 23.2 mg/m^3 (17.6-44.8 mg/m^3), and mean duration of employment in a high-exposure job was 4.9 years (range: 2.1-20.4 years). The SBR for the 42 workers was close to expected (102) despite a 48% vasectomy rate. These workers also had elevated SBR during the actual period of high exposure. An examination of SBR for all participants by 5-year increments from 1950 to 1990 revealed no significant trend in either direction over time.

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

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

Swan et al. (1995) investigated the relationship between spontaneous abortion in women employed in the semiconductor manufacturing industry and various chemical and physical agents used in the industry, including boron. The study population consisted of 904 current and former female employees who became pregnant while working at 1 of 14 U.S. semiconductor companies between 1986 and 1989. Approximately one-half of those included were fabrication workers with some chemical exposure. Exposure classifications were based on jobs held at conception and level of exposure to specific agents during the first trimester. The risk of spontaneous abortion was increased in fabrication workers compared with other workers, and particularly within the subgroup of workers who performed masking (a group with relatively low boron exposure). No significant association was found between exposure to boron and spontaneous abortion risk.

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

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

Wegman et al. (1994) conducted a longitudinal study of respiratory function in workers with chronic exposure to sodium borate dusts. Participants in the Garabrant et al. (1985) study were re-tested for pulmonary function 7 years after the original survey. Of the 629 participants in the original study in 1981, 371 were available for re-testing in 1988. Of these, 336 performed pulmonary function tests (303 produced acceptable tests in both years). Cumulative exposure was estimated for each participant for the years 1981-1988 as a time-weighted sum of the exposure in each job held during that time. Exposure prior to 1981 was not included due to the scarcity of monitoring data for those years. Pulmonary function FEV_1 (forced expiratory volume in 1 sec) and FVC (forced vital capacity) in study subjects declined over the 7-year period at a rate very close to that expected based on standard population studies. Cumulative borate exposure over the years 1981-1988 was not related to the change in pulmonary function. Acute studies showed statistically significant, positive dose-related increases in eye, nasal, and throat irritation; cough; and breathlessness with borate exposure (6-hr TWA or 15-min TWA). The same relationships were present when effects were limited to moderate severity or higher. There was no evidence for an effect of borate type (decahydrate, pentahydrate, anhydrous) on response rate.

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

4.2.1. Oral Exposure

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

The subchronic and chronic toxicity of borax and boric acid has been studied in dogs administered these compounds in the diet (Weir and Fisher, 1972; U.S. Borax Research Corp., 1963, 1966, 1967). In the subchronic study, groups of beagle dogs (5/sex/dose/compound) were administered borax (sodium tetraborate decahydrate) or boric acid for 90 days at dietary levels of 17.5, 175, and 1750 ppm boron (male: 0.33, 3.9 and 30.4 mg B/kg-day; female: 0.24, 2.5 and 21.8 mg B/kg-day) and compared with an untreated control group of 5 dogs/sex (Weir and Fisher, 1972; U.S. Borax Research Corp., 1963). On day 68 of the study, a high-dose male dog died as a result of complications of diarrhea with severe congestion of the mucosa of the small and large intestines and congestion of the kidneys. No clinical signs of toxicity were evident in the other dogs. The testes were the primary target of boron toxicity. At the high dose, mean testes weight was decreased 44% (9.6 g) in males fed borax and 39% (10.5 g) in males fed boric acid compared with controls (17.2 g). Also at this dose, mean testes:body weight ratio (control: 0.2%; borax: 0.1%; boric acid: 0.12%) and mean testes:brain weight ratio (control: 22%; borax: 12%) were significantly reduced. Decreased testes:body weight ratio 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; increased brain:body weight ratio (borax) and liver:body weight ratio (boric acid) in females; a somewhat increased proportion of solid epithelial nests and minute follicles in the thyroid gland of borax-treated males; lymphoid infiltration and atrophy of the thyroid in boric-acid treated females; increased width of the zona reticularis (borax males and females, boric acid females); and zona glomerulosa (boric acid females) in the adrenal gland. This study identified 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. Four male dogs served as controls for the borax and boric acid dosed animals. One male control dog was sacrificed after 52 weeks, two male control dogs were sacrificed after 104 weeks, and one was sacrificed after the 13-week recovery period with 104 weeks of treatment. The one male control dog sacrificed after the 13-week recovery period demonstrated testicular atrophy. Sperm samples used for counts and motility testing were taken only on the control and high-dose male dogs prior to the 2-year sacrifice. At a dose level of 8.8 mg B/kg-day in the form of boric acid, one dog sacrificed at 104 weeks had testicular atrophy. Two semen evaluations (taken after 24 months treatment) were preformed on dogs treated at the highest dose (8.8 mg B/kg-day). Two of two borax treated animals had samples that were azoospermic and had no motility, while one of two boric acid treated animals had samples that were azoospermic. The authors reported that there did not appear to be any definitive test article effect on any parameter examined. The study pathologist considered the histopathological findings to be "not compound-induced." Tumors were not reported.

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

Weir and Fisher (1972) 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 a maximum tolerated dose (MTD) was achieved in this study. The study was designed to assess systemic toxicity; only tissues from the brain, pituitary, thyroid, lung, heart, liver, spleen, kidney, adrenal, pancreas, small and large intestine, urinary bladder, testes, ovary, bone, and bone marrow were examined histopathologically. Tumors were not mentioned in the report. Nevertheless, NTP (1987) concluded that this study provided adequate data on the lack of carcinogenic effects of boric acid in rats, and accordingly, conducted its carcinogenicity study only in mice.

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

In the chronic study, male and female (50/sex/group) B6C3F1 mice were fed a diet containing 0, 2500, or 5000 ppm boric acid for 103 weeks (NTP, 1987; Dieter, 1994). The low- and high-dose diets provided approximate doses of 275 and 550 mg/kg-day (48 and 96 mg B/kg-day), respectively. Mean body weights of high-dose mice were 10-17% lower than those of controls after 32 (males) or 52 (females) weeks. No treatment-related clinical signs were observed throughout the study. Survival of the male mice was significantly lower than that of 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.

Low-dose male mice demonstrated increased incidences of hepatocellular carcinoma (5/50, 12/50, 8/49) and combined adenoma or carcinoma (14/50, 19/50, 15/49), relative to control and the high-dose male mice (NTP, 1987; Dieter, 1994). The increases were statistically significant by life table tests, but not by incidental tumor tests. The incidental tumor tests were considered to be the more appropriate form of statistical analysis in this case, because the hepatocellular carcinomas did not appear to be the cause of death for males in this study; the incidence of these tumor types in animals that died prior to study completion (7/30 or 23%) was similar to the incidence at terminal sacrifice (5/20 or 25%) (NTP, 1987; Elwell, 1993). The hepatocellular carcinoma incidence in this study was within the range of male mice historical controls both at the study lab (131/697 or 19 ± 6%) and for NTP (424/2084 or 20 ± 7%) (NTP, 1987; Elwell, 1993). Also, the hepatocellular carcinoma incidence in the male control group of this study (10%) was lower than the historical controls. NTP concluded that the increase in hepatocellular tumors in low-dose male mice was not due to administration of boric acid.

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

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

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

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES — ORAL AND INHALATION

4.3.1. Developmental Studies

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

Treatment with 0.8% boric acid (gd 6-15) significantly increased prenatal mortality, as seen in increases in the percentage of both resorptions and late fetal deaths per litter. The number of live fetuses per litter was also significantly decreased at 0.8%. Average fetal body weight (all fetuses or male or female fetuses) per litter was significantly reduced in all treated groups versus controls in a dose-related manner. Mean fetal weights were 94, 87, 63, and 46% of the corresponding control means for the 0.1, 0.2, 0.4, and 0.8%, respectively. The percentage of malformed fetuses per litter and the percentage of litters with at least one malformed fetus were significantly increased at 0.2% or more. Treatment with 0.2% or more boric acid also increased the incidence of litters with one or more fetuses with a skeletal malformation. The incidence of litters with one or more pups with a visceral or gross malformation was increased at 0.4 and 0.8%, respectively. The malformations consisted primarily of anomalies of the eyes, the central nervous system (CNS), the cardiovascular system, and the axial skeleton. In the 0.4 and 0.8% groups, the most common malformations were enlarged lateral ventricles of the brain and agenesis or shortening of rib XIII. The percentage of fetuses with variations per litter was reduced relative to controls in the 0.1 and 0.2% dosage groups (due primarily to a reduction in the incidence of rudimentary or full ribs at lumbar I), but was significantly increased in the 0.8% group. The variation with the highest incidence among fetuses was wavy ribs. Based on the changes in organ weights, a maternal LOAEL of 0.2% boric acid in the feed (28.5 mg B/kg-day) can be established; the maternal NOAEL is 0.1% or 13.6 mg B/kg-day. Based on the decrease in fetal body weight per litter, the level of 0.1% boric acid in the feed (13.6 mg B/kg-day) is 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, when uterine contents were evaluated. The calculated average dose of boric acid consumed for Phase l dams was 19, 36, 55, 76, and 143 mg/kg-day (3.3, 6.3, 9.6, 13.3, and 25 mg B/kg-day). During Phase I, no maternal deaths occurred, and no clinical symptoms were associated with boric acid exposure. Maternal body weights did not differ among groups during gestation, but statistically significant trend tests associated with decreased maternal body weight (gd 19 and 20 at sacrifice) and decreased maternal body weight gain (gd 15-18 and gd 0-20) were indicated. In the high-dose group, there was a 10% reduction (statistically significant in the trend test $p<0.05$) in gravid uterine weight when compared with controls. The authors indicated that the decreasing trend of maternal body weight and weight gain during late gestation reflected reduced gravid uterine weight. Corrected maternal weight gain (maternal gestational weight gain minus gravid uterine weight) was not affected. Maternal food intake was only minimally affected at the highest dose and only during the first 3 days of dosing. Water intake was higher in the exposed groups after gd 15. The number of ovarian corpora lutea and uterine implantation sites, and the percentage of preimplantation loss were not affected by boric acid exposure.

Offspring body weights were significantly decreased in the 13.3 and 25 mg B/kg-day dose groups on gd 20. The body 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 statistically significant trend ($p<0.05$) was found for decreases in rudimentary extra rib on lumbar I, classified as a variation. Only the high-dose group had a biologically relevant, but not statistically significant, decrease in this variation. The LOAEL for Phase I of this study was considered to be 0.1% boric acid (13.3 mg B/kg-day), based on decreased fetal body weight. The NOAEL for Phase I of this study was considered to be 0.075% boric acid (9.6 mg B/kg-day).

In Phase II, dams were allowed to deliver and rear their litters until postnatal day (pnd) 21. The calculated average doses of boric acid consumed for Phase II dams were 19, 37, 56, 74, and 145 mg/kg-day (3.2, 6.5, 9.7, 12.9, and 25.3 mg B/kg-day). This phase allowed a follow-up period to determine whether the incidence of skeletal defects in control and exposed pups changed during the first 21 postnatal days. Among live born pups, there was a significant trend test for increased number and percentage of dead pups between pnd 0 and 4, but not between pnd 4 and 21; this appeared to be due to an increase in early postnatal mortality in the high dose, which did not differ significantly from controls and was within the range of control values for other studies in this laboratory. On pnd 0, the start of Phase II, there were no effects of boric acid on the body weight of offspring (102, 101, 99, 101, and 100% of controls, respectively). There were also no differences through termination on pnd 21; therefore, fetal body weight deficits did not continue into this postnatal period (Phase II). The percentage of pups per litter with short rib XIII was still elevated on pnd 21 in the 0.200% boric acid dose group (25.3 mg B/kg-day), but there was no incidence of wavy rib, and none of the treated or control pups on pnd 21 had an extra rib on lumbar 1. The NOAEL and LOAEL for phase II of this study were 12.9 and 25.3 mg B/kg-day, respectively.

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

The developmental effects of boric acid also have been studied in mice and rabbits. Heindel et al. (1994, 1992) and Field et al. (1989) examined the developmental effects of boric acid in pregnant CD-1 mice using the same experimental design as in the initial study with rats (Price et al., 1990), except that a 0.8% dietary level was not used in the mouse study. The diets containing 0, 0.1, 0.2, or 0.4% boric acid were estimated by the investigators to provide 0, 248, 452, or 1003 mg boric acid/kg-day (0, 43.4, 79.0, or 175.3 mg B/kg-day); the mice were treated during gd 0-17. Neither survival rates nor pregnancy rates were affected by treatment with boric acid. Pale kidneys were noted in several treated dams, particularly in the high-dose group, and one dam in this group had fluid accumulation in the kidney. Maternal body weight was significantly reduced by 10-15% during gd 12-17 in the high-dose group. Maternal weight gain was significantly reduced during treatment in the high-dose group, but was not affected when corrected for gravid uterine weight. At the 0.4% dietary level, food intake was increased between days 12 and 15 and water intake was increased on days 15-17 (statistical significance not provided for either effect). Organ weight changes were limited to significant increases in relative kidney weight and absolute liver weight in the 0.4% groups. A dose-related increase in maternal renal tubular dilation and/or regeneration was observed; the incidence was 0/10, 2/10, 8/10, and 10/10 in the 0, 0.1, 0.2, and 0.4% dosage groups, respectively. Treatment with boric acid did not affect preimplantation loss or the number of implantation sites per litter, but significantly increased the percentage of resorptions per litter and the percentage of litters with one or more resorptions at the 0.4% level. There was a significant dose-related decrease in average fetal body weight (all fetuses or male or female fetuses) per litter at 0.2% or more. The percentage of malformed fetuses per litter increased significantly at 0.4%, whereas the percentage of fetuses with variations per litter was decreased at 0.1 and 0.2% and was not affected at 0.4%. The most frequent malformation observed among fetuses of the 0.4% group was a short rib XIII. In contrast, full or rudimentary lumbar I rib (a variation) was less frequent in fetuses of treated mice. Although the level of 0.1% boric acid in the diet induced an increase in renal lesions in mice, the increased incidence did not achieve statistical significance (Fisher Exact Test). The 0.1% level (43.4 mg B/kg-day) is a maternal NOAEL and the 0.2% level (79 mg B/kg-day) is a maternal LOAEL. For developmental effects, the 0.2% dietary level of boric acid is 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 gd 30, the animals were sacrificed and the following endpoints were examined: pregnancy status; number of resorptions; fetal body weight; viability; and external, visceral, and skeletal malformations. No treatment-related clinical signs of toxicity were observed during the study, except for vaginal bleeding noted in 2-11 does/day on gd 19-30 at the high dose; these does had no live fetuses on day 30. Vaginal bleeding was also observed in one female in the low-dose group and in one in the mid-dose group. Two maternal deaths occurred (one each at the low- and mid-dose), but were not treatment-related. Food intake was decreased relative to that of controls on treatment days 6-15 at the high dose, and was increased after treatment ceased on days 25-30 at the mid and high doses. Body weight on gd 9-30, weight gain on gd 6-19, gravid uterine weight, and number of corpora lutea per dam were each decreased in the high-dose group. After correction for gravid uterine weights, however, maternal body-weight gain was increased at both the mid- and high- doses. Treatment with boric acid did not affect absolute or relative liver weight. Relative, but not absolute kidney weight increased at the high dose; kidney histopathology was unremarkable. Boric acid caused frank developmental effects at the high dose. These effects consisted of a high rate of prenatal mortality (90% of implants/litter were reabsorbed compared with 6% in 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 percentage of fetuses with variations (all types combined) was not significantly increased in any treated group, but the percentage with cardiovascular variations was significantly increased from 11% in controls to 64% in the high-dose group. Fetal body weights per litter at the high dose were depressed relative to control, but the difference was not statistically significant; however, this could have been due to the small sample size in the high-dose group. No developmental effects were found in the low- and mid-dose groups. In this study, the mid dose of 125 mg boric acid/kg-day (21.9 mg B/kg-day) represents the NOAEL based on maternal and developmental effects. The high dose of 250 mg boric acid/kg-day (43.7 mg B/kg-day) is the LOAEL.

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

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

Results from the first experiment indicated that 400 mg/kg daily doses resulted in decreased rib length, and daily doses of 750 mg/kg resulted in decreased rib length and femur length. Fetal body weight was not significantly decreased at either dose. In the second study, the results for the gd 9 and 10 daily exposures were not presented due to the lack of a concurrent control. Fetal body weight was reduced in all boric acid treatment groups (single days gd 6, 7, 8, 9, or 10 and consecutive days from gd 6 to 8). Femur length was decreased on gd 7 and in fetuses exposed for the gd 6-8 period. Cervical ribs were observed in fetuses exposed on gd 6, 7, or 8. Results from the third experiment indicated that the two doses of 750 mg/kg each on gd 8 significantly increased frequency of 11 separate malformations over background incidence. The most prevalent malformations were those associated with rib development. In contrast, the single dose on this day produced only increased incidences of unilateral thoracic vertebrae and cervical rib formation/ossification differences. These results demonstrate a separation of the effects of boric acid on fetal body weight and rib malformation with respect to the timing of the dose. The authors concluded that the accumulation of the effect, rather than the accumulation of boric acid, was responsible for the temporal dependence of boric acid-induced fetotoxicity, citing a rapid clearance of borates from the blood. They specifically indicated that, "because of boric acid's short half-life, these data suggest that these earlier processes, gastrulation and presomitic mesoderm formation and patterning, are the processes boric acid is affecting".

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

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). The investigators estimated the highest exposure level provided 0.84 mg B/kg-day. Based on this estimate, the lower two levels provided 0.042 and 0.14 mg B/kg-day. There were no noticeable reproductive effects or changes in serum chemistry; plasma levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH); or weight of the body, testes, prostate or seminal vesicles. Fructose, zinc and acid phosphatase levels in the prostate were unchanged. Breeding studies revealed no effects on male fertility. Therefore, the dose of 0.84 mg B/kg-day, the highest dose tested, represents a NOAEL for this study.

In a follow-up study reported by Dixon et al. (1979) and Lee et al. (1978), diets containing 0, 500, 1000, or 2000 ppm boron, as borax, were administered to male Sprague-Dawley rats (18/group) for 30 or 60 days (approximately 0, 25, 50, or 100 mg B/kg-day). Significant ($p<0.05$) decreases in the weight of liver, testes, and epididymis were observed at the 1000 and 2000 ppm dietary levels. Seminiferous tubule diameter was significantly ($p<0.05$)

decreased in a dose-dependent manner in all treatment groups; however, significant loss of germinal cell elements was observed only at the 1000 and 2000 ppm dietary levels. Aplasia was complete at the highest dose. Plasma levels of the hormone FSH were significantly ($p<0.05$) elevated in a dose- and duration-related manner at all dose levels, while plasma LH and testosterone levels were not affected significantly. Serial mating studies revealed reduced fertility without change in copulatory behavior at the two higher dose levels. Based on dose-related tubular germinal aplasia, which is reversible at low doses, this study defines 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 sacrificed 14 days later. In both the time-response and the dose-response studies, the above doses are the total of two doses administered at 9:00 a.m. and 4:00 p.m. on the same day. No significant clinical signs of toxicity were observed during the study. Histopathologic examinations of the testes and epididymis revealed adverse effects on spermiation, epididymal sperm morphology, and caput sperm reserves. The testicular effects, apparent at 14 days, included enlarged irregular cytoplasmic lobes of Step 19 spermatids in stage VIII seminiferous tubules and retention of Step 19 spermatids in stage IX-XIII tubules at the 175 and 350 mg B/kg dose levels. There was also a substantial increase ($p<0.05$) in the testicular sperm head count per testis and per g testis in the 350 mg/kg time-response group. Epididymal effects, also apparent at 14 days, included an increase in abnormal caput epididymal sperm morphology (percentage with head or tail defects, $p<0.05$) and reduced caput epididymal sperm reserves ($p<0.05$). In the day 28 time-response group (350 mg B/kg), significant effects ($p<0.05$) included an increase in abnormal caput and cauda epididymal sperm morphology and a decreased percentage of motile cauda spermatozoa with reduced straight-line swimming velocities. Substantial recovery occurred by day 57. This study described 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; androgen binding protein (ABP) levels; and tissue boron levels were assessed. In half of the treated rats, there was inhibition of spermiation in 10-30% of stage-IX tubules at 7 days. Inhibited spermiation was observed in all stage-IX and stage-X tubules of exposed rats at 10 days. Advanced epithelial disorganization, cell exfoliation, luminal occlusion, and cell death were observed after 28 days, causing significant loss of spermatocytes and spermatids from all tubules in exposed rats. Throughout the study, specific lesions became more severe with increasing duration of exposure. Treatment with boric acid had no effect on kidney and liver histology. In treated rats, basal serum testosterone levels were significantly decreased ($p<0.05$) from 4 days on, but serum testosterone levels stimulated by human chorionic gonadotropin or luteinizing hormone releasing factor were not affected. Steady-state levels of boron were reached in tissues by 4 days of treatment, and there was no selective accumulation of boron in blood, epididymis, liver, or kidney. After 4 days of treatment with boric acid, serum ABP levels were significantly reduced relative to controls; however, this difference disappeared by day 7.

Ku et al. (1993a) and Chapin and Ku (1994) compared testis boron dosimetry to lesion development. Rats were fed 0, 3000, 4500, 6000, or 9000 ppm boric acid (0, 545, 788, 1050, or 1575 ppm boron) for up to 9 weeks and examined. Based on food intake and body weight data, the researchers estimated the daily intake of boron as <0.2, 26, 38, 52, or 68 mg B/kg-day. At 32 weeks post-treatment, recovery was assessed. Inhibited spermiation occurred at 3000 and 4500 ppm, and atrophy occurred at 6000 and 9000 ppm. A mean testis boron level of 5.6 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 in the 6000 and 9000 ppm dose groups did not change 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 and Chapin (1994) showed that testicular atrophy and CNS hormonal effects were not due to selective accumulation in testis or brain/hypothalamus with boron testis concentrations of 1-2 mM. *In vitro* studies addressed boric acid testicular toxicity: mild hormone effect, the initial inhibited spermiation, and atrophy. No effect of boric acid on the steroidogenic function of isolated Leydig cells was observed, supporting the suggestion of a CNS mediated hormonal effect. The authors found that inhibited spermiation was not due to increased testicular cyclic adenosine monophosphate (cAMP) or reduced serine proteases plasminogen activators (PA). Boric acid effects were evaluated in Sertoli-germ cell co-cultures on Sertoli cell energy metabolism (lactate secreted by Sertoli cells is a preferred energy source for germ cells) and DNA/RNA syntheses (germ cells synthesize DNA/RNA and boric acid impairs this nucleic acid in the liver). The most sensitive *in vitro* endpoint was DNA synthesis of mitotic/meiotic germ cells, with energy metabolism in germ cells affected to a lesser extent, which was manifested *in vivo* as a decrease in early germ cell/Sertoli cell ratio prior to atrophy in the testes.

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

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

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 that were examined to the F3 generation. Litter size, weights of progeny, and appearance were normal when compared with controls. The test groups receiving 58.5 mg B/kg-day boron from either compound were found to be sterile. In these groups, males showed lack of spermatozoa in atrophied testes, and females showed decreased ovulation in the majority of the ovaries examined. An attempt to obtain litters by mating the treated females with the males fed only the control diet was not successful. 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 authors predicted the diet would provide boric acid at 152 mg/kg-day (26.6 mg B/kg-day) to males and 182 mg/kg-day (31.8 mg B/kg-day) to females in the 1000 ppm group; 636 mg/kg-day (111 mg B/kg-day) to males and 868 mg/kg-day (152 mg B/kg-day) to females in the 4500 ppm group; and 1260 mg/kg-day (220 mg B/kg-day) to males and 1470 mg/kg-day (257 mg B/kg-day) to females in the 9000 ppm group. According to the authors, actual boric acid consumption during the study did not differ from the predicted consumption by more than 12%. Following 1 week of treatment, the F_0 mice were caged as breeding pairs for 14 weeks. During weeks 2-18, the average body-weight gain of high-dose males and females was significantly reduced relative to controls. Mortality rates in the treated groups over the 27 weeks were not significantly different from controls. Treatment with boric acid significantly impaired fertility. None of the 9000 ppm pairs were fertile. The number of litters per pair, number of live pups per litter, proportion of pups born alive, live pup weight, and adjusted pup weight (adjusted for litter size) were significantly ($p<0.05$) decreased at the 4500 ppm level. The initial fertility index (percentage of cohabited pairs having at least one litter) was not significantly altered in the 1000 and 4500 ppm groups, but the progressive fertility index (percentage of fertile pairs that produced four litters) was decreased relative to controls in the 4500 ppm group. The trend toward a lower fertility index at 4500 ppm started with the first mating and progressed in severity with subsequent matings.

To determine the affected sex, the control and 4500 ppm F_0 mice were then assigned to three crossover mating groups: control male x control female, 4500 ppm male x control female, and control male x 4500 ppm female. Each group was composed of 19-20 pairs that were mated for 7 days or until a copulatory plug was detected, whichever occurred first; control feed was provided for all mice during this week, followed by a resumption of the same diets they had received previously. Mating and fertility indices were significantly depressed in the 4500 ppm male x control female group, and only one pair in that group produced a live litter; these indices were not affected in the control male x 4500 ppm female group. Dosed females mated to control males had a lower body weight on pnd 0, had a longer gestational period than control groups and gave birth to pups with decreased litter-adjusted weight. After completion of the crossover mating trial (total of 27 weeks on test), a necropsy was performed on control and 4500 ppm F_0 males and females and on 1000 and 9000 ppm F_0 males that had been maintained on their respective diets to allow a comparison of semen parameters and testicular histology among all four treatment groups. Males treated with 9000 ppm boric acid had significantly reduced body, testis and epididymal weights. In the 4500 ppm males, body weight was not affected, but testis, epididymal, and prostate weights were reduced; these parameters were not altered in the 1000 ppm males. Significant reductions in sperm motility were observed in the 1000 and 4500 ppm groups and in sperm concentration in the 4500 and 9000 ppm groups. The percentage of abnormal sperm was significantly increased in the 4500 ppm group. Sperm motility and morphology could not be fully evaluated in the 9000 ppm group due to absence of sperm (in 12 of 15 observed males) or severe reduction in sperm counts (in the other 3 males) of this group. Seminiferous tubular atrophy occurred in mid- and high-dose males; the severity was dose-related. Tissues of low-dose males exhibited no significant changes. Other indices of testicular morphology (spermatogenic index, seminiferous tubule diameter, spermatids per testis) 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. Because there were no litters at 9000 ppm and few of the mice born alive in the final litters at 4500 ppm survived through weaning, only the 0 and 1000 ppm F_1 mice were included in a fertility trial. The F_1 mice were cohabited in nonsibling pairs (40 pairs of 0 ppm and 20 pairs of 1000 ppm mice) for 7 days or until a copulatory plug was observed, whichever occurred first. They were maintained on their respective diets during mating and until the F_2 litters were delivered, and then were necropsied. The fertility of the 1000 ppm F_1 mice was not affected, but the litter-adjusted body weights of the F_2 pups (females and combined males and females) were significantly decreased relative to controls. Effects in 1000 ppm F_1 females were significant increases in uterine and kidney plus adrenal weights, significantly shorter estrous cycles, and fewer ambiguous vaginal smears. A reduction in epididymal sperm concentration in the 1000 ppm F_1 males approached significance (p=0.053); sperm motility and morphology were not affected. Histopathologic examination was unremarkable. The lowest dose tested, 1000 ppm, decreased sperm motility in the F_0 males, marginally decreased epididymal sperm concentration in F_1 males, increased uterine and kidney/adrenal weights and shortened estrus cycles in F_1 females, and reduced litter-adjusted birth weights in the F_2 pups. Hence, the LOAEL for this study is 1000 ppm boric acid (26.6 and 31.8 mg B/kg-day for males and females, respectively). 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) and Wong et al. (1964) reported on case reports of neurological effects after accidental ingestion of high levels of boron as boric acid. Newborn infants (number not given) who ingested 4.5-14 g boric acid showed these CNS symptoms. Doses of about 500 mg B/kg-day showed CNS involvement with headaches, tremors, restlessness and convulsions followed by weakness, coma, and death. Histological examination of 2/11 infants revealed degenerative changes in brain neurons, congestion, and edema of brain and meninges with perivascular hemorrhage and intravascular thrombosis.

O'Sullivan and Taylor (1983) reported convulsions and seizures in seven infants exposed to a honey-borax mixture for 4-10 weeks, in which the estimated ingestion was 9.6-33 mg B/kg-day (Section 4.1.1.).

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

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

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. More recent investigations of the developmental effects of boric acid (Narotsky et al., 2003; Wery et al., 2003) have produced evidence supporting a role of altered gene expression in boron's developmental effects. These data indicate that boric acid administration during the normal period of expansion of *hox* gene expression results in rib and vertebrae alterations, coincident with altered *hox* gene expression.

4.4.5. Nutrition Studies

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

Based on these studies, in which most subjects who consumed 0.25 mg B/day responded to additional boron supplementation, Nielsen (1991) concluded that the basal requirement for boron is likely to be greater than 0.25 mg/day. Limited survey data indicate that the average dietary intake of boron by humans is 0.5-3.1 mg-day (7-44 µg/kg-day) (Nielsen, 1991). The average U.S. adult male dietary intake of 1.52 ± 0.38 mg B/day (mean ± standard deviation)

(Iyengar et al., 1988) was determined by U.S. Food and Drug Administration (FDA) Total Diet Study methods. In a more recent study, Anderson et al. (1994) reported an intake of 1.21 ± 0.07 mg B/day for an average diet for 25- to 30-year-old males, as determined by FDA Total Diet Study analyses. Similarly, the average dietary boron intake in Canada is reported to be 1.33 ± 0.13 mg B/day for women (Clarke and Gibson, 1988). Dietary boron consumption in Europe could be higher than in the United States and Canada due to wine consumption (ECETOC, 1994). These and other investigators (Nielsen, 1992) also recognized that greater consumption of fruits, vegetables, nuts, and legumes (e.g., vegetarian diets) could raise dietary boron intake.

The Institute of Medicine (IOM, 2002) developed a tolerable upper intake level (UL), the highest daily nutrient intake that is likely to pose no risk of adverse health effects for most individuals, for various life stages of humans. A UL for infants was judged not determinable. The UL for adults was 20 mg B/day. The UL was set at 17 mg B/day for pregnant women 14-18 years of age, while the UL for pregnant women 19-50 years of age was set at 20 mg B/day. Section 5.1.3. describes how these ULs were determined.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION—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, CNS, cardiovascular system, and axial skeleton (Price et al., 1996a,b; Field et al., 1989). Increased incidences of short rib XIII (a malformation) and wavy rib (a variation), and decreased incidence of rudimentary extra rib on lumbar I (a variation), were the most common anomalies in both rats and mice. Cardiovascular malformations, especially interventricular septal defect, and variations were the frequent anomalies in rabbits. Fail et al. (1998) attributed reduced fetal growth, the most sensitive developmental endpoint, to a general inhibition of mitosis by boric acid, as documented in studies on the mammalian testis, insects, yeast, fungi, bacteria, and viruses (Beyer et al., 1983; Ku et al., 1993b).

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 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 be route-specific differences in ability to deliver toxic doses to the targets, in that very high exposure concentrations may be required to produce effects by inhalation exposure. Portal-of-entry effects may also differ with exposure route.

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

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

Under the *Draft Revised Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), the data are considered to be inadequate for an assessment of the human carcinogenic potential of boron. No data were located regarding the existence of an association between cancer and boron exposure in humans. Studies available in animals were inadequate to ascertain whether boron causes cancer. The chronic rat feeding study conducted by Weir and Fisher (1972) was not designed as a cancer bioassay. Only a limited number of tissues were examined histopathologically, and the report failed to mention any tumor findings. The chronic mouse study conducted by NTP (1987) was adequately designed, but the results are difficult to interpret. There was an increase in hepatocellular carcinomas in 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*.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

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

4.7.2. Possible Gender Differences

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

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

4.7.3. Physiological and Disease Anomalies

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

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).

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

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

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

5.1.2. Methods of Analysis — Including Models

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

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

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

The results of the Allen et al. (1996) BMD analysis for decreased fetal body weight for the Price study alone gave a BMDL of 47 mg boric acid/kg-day (8.2 mg B/kg-day), and for the Heindel study alone, the BMDL reported by Allen et al. (1996) was 56 mg boric acid/kg-day (9.8 mg B/kg-day). The statistical analysis described above demonstrated that the data were consistent, and could be combined to estimate a single dose-response function. The combined data from Heindel et al. (1992) and Price et al. (1994, 1996a) gave a $BMDL_{05}$ of 59 mg boric acid/kg-day (10.3 mg B/kg-day). The BMDL based on the combined results of the two studies was very close to the NOAEL of 9.6 mg B/kg-day from the Price et al. (1994, 1996a) study. The $BMDL_{05}$ from the combined studies was chosen to derive the RfD because they were similarly designed studies conducted in the same laboratory, and all the dose response data were consistent enough to be used in the BMDL estimation, thereby increasing the confidence that the dose response pattern has been estimated satisfactorily.

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

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 somewhat 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 addressed as a combination of two subfactors, one each for toxicokinetics (TK) and toxicodynamics (TD).[1] The TK/TD "paradigm" formally allows for the quantitative incorporation of additional data previously used in only a qualitative fashion. The concept is applied in the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), in which the kinetic component deals primarily with airway anatomy and physiology, but does not address systemic kinetics and dynamics. Otherwise, EPA has not established guidance in this area. The International Programme on Chemical Safety (IPCS) has drafted guidance in the selection of chemical-specific adjustment factors (CSAF), which does cover systemic kinetics and dynamics (IPCS, 2001). The IPCS document has not been formally reviewed by the U.S. EPA. Much of the toxicokinetic factor development in the boron RfD derivation, however, is consistent with IPCS (2001). Additionally, IPCS previously applied the TK/TD subfactor approach in their assessment of boron (WHO, 1998a). The values for the TK component of UF_A and UF_H have been adjusted based on relevant data, but no such data exist to support an adjustment of the TD components.

5.1.3.1. Derivation of Adjustment Factor Values

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

In the most simple terms, toxicokinetics 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

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

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

5.1.3.1.1. TK/TD Subfactor Default Values (Uncertainty)

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

The default half-order of magnitude partition of uncertainty factors (i.e., UF_A and UF_H) for toxicokinetics and toxicodynamics is primarily based on lack of knowledge; if there is no evidence to the contrary, an equal contribution from each source of uncertainty is assumed.

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

5.1.3.1.2. Revised RfD Calculation Formula

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

$$RfD = D_C \big/ (AF_{AK} \cdot AF_{AD} \cdot AF_{HK} \cdot AF_{HD} \cdot UF) \tag{5.1}$$

where:

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

The product of AF_{AK} and AF_{AD} replaces the animal-to-human (interspecies) uncertainty factor (UF_A) in the standard RfD methodology. Similarly, the product of AF_{HK} and AF_{HD} replaces the sensitive human (interindividual variability) uncertainty factor (UF_H). Each of the adjustment factors is the product of data-derived scaling factors and residual uncertainty. That is, if there are significant issues concerning the data or modeling of the data, the adjustment factor may be increased to reflect remaining uncertainty. If there are no applicable data, the adjustment factors

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

are equal to their default uncertainty factor values. The aggregate uncertainty factor (UF) is equal to the product of all other uncertainty factors: subchronic-to-chronic (UF_S), LOAEL-to-NOAEL (UF_S), and data base adequacy (UF_D). For boron, a subchronic-to-chronic uncertainty factor was not used to account for extrapolation from less than chronic results because developmental toxicity (decreased fetal body weight) was used as the critical effect. The developmental period is recognized as a susceptible lifestage where exposure during certain time windows is more relevant to the induction of developmental effects than lifetime exposure. An uncertainty factor for extrapolation from a LOAEL to a NOAEL was not necessary because BMD modeling was used to determine the point of departure. The dose corresponding to a 5% decrease in pup weight, relative to control, was selected as the point of departure. Because decreased weights did not persist in the companion study (Phase II of Price et al., 1996a, 1994), no further adjustment was considered for identifying a level of oral exposure to boron associated with the minimal level of risk. A database uncertainty factor was not deemed necessary due to boron's extensive data base. For convenience and sake of reference, the product of all the terms in the denominator of Equation 5.1 is given the term "total adjustment factor" and is designated as AF_{TOT}.

5.1.3.2. Toxicokinetic Modeling Issues for Boron

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

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

5.1.3.2.1. Interspecies Uncertainty

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

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

$$C_{SS} = \frac{D_e f_a BW}{Cl} \tag{5.2}$$

where:

D_e is the external ingested dose rate in mg (boron) per kg body mass per day

f_a is the fraction of ingested boron absorbed into the body from the gut

BW is body weight (kg)

Cl is the renal clearance rate (mL/minute)

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

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

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

The mean boron clearance for pregnant rats (Cl_r) is 1.00, determined from the kinetic studies of U.S. Borax (2000) and Vaziri et al. (2001) (Table 5). The mean boron clearance for pregnant women (Cl_h) was determined from the kinetic studies of U.S. Borax (2000) and Pahl et al. (2001) to be 66.1 mL/min (Table 6). The mean body weights for pregnant rats (BW_r) and pregnant women (BW_h) from those studies are 0.303 and 67.6 kg, respectively. The average clearance of 66 mL/min for pregnant women determined by Pahl et al. (2001) represents a possible underestimation of the true boron clearance, particularly at the relatively higher doses near the RfD (Section 3.4.1). Boron clearance values obtained in adult men (Jansen et al., 1984a) given an intravenous infusion of boric acid, representing exposures 66 times dietary levels, were 1.5 times greater than boron clearance measured at dietary levels. Taking into account the possibility of dose-dependence, and that the RfD is somewhere between the dietary exposure and infusion level in the Jansen study (but much closer to the latter), the factor could be less than 1.5 (1.3 by linear interpolation). Therefore, Cl_h could actually be 30-50% higher (86-99 mL/min). An independent estimate in the range of 86 to107 mL/min boron clearance in pregnant women can be obtained from the adult male boron clearance of 60.5 mL/min/1.73 m^2 (Jansen et al., 1984a) by assuming that boron clearance will scale the same as GFR from male to female to pregnant female. GFR is about 8-12% higher in adult males than females (Smith, 1951; Wesson, 1969), but increases by a factor of about 1.6 in pregnancy (Dunlop, 1981; Sturgiss et al., 1996; Krutzén et al., 1992). Furthermore, GFR values normalized to a standardized unit surface area

($1.73\ m^2$) for pregnant women may underestimate absolute GFR (mL/min) by an additional factor of 1.2 (Krutzén et al., 1992). Therefore, the adult male boron clearance of 60.5 mL/min/1.73 m^2 represents a clearance of at least 86 mL/min and as much as 107 mL/min in pregnant women. Although this evidence is suggestive that Cl_h may be higher, it is not strong enough for a quantitative adjustment in the derivation of AF_{AK}. Therefore, Cl_h is assigned the value of 66.1 mL/min, Cl_h is 1.00 mL/min, BW_r is 0.303 kg, and BW_h is set to 67.6 kg.

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

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

5.1.3.2.2. Intraspecies Uncertainty

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

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

[3]Toxicodynamic sensitivity is represented by AF_{HD}.

clearance in pregnant women, but was not designed to assess interindividual variability, given its fairly low number of subjects (16) and a lack of control of dietary intake of boron. The variance of boron clearance in this study was somewhat high (CV = 0.49), such that estimation of an adequate lower bound would be highly uncertain. In contrast, in the controlled infusion exposure study of Jansen et al. (1984a), the boron clearance CV was 0.09 (Section 3.4.1). In that same study, clearance determined for uncontrolled dietary exposure at much lower levels was characterized by high variability (CV = 0.78). Lack of controls on exposure magnitude and timing would be expected to contribute substantially to the variance of the measurements. The high variability reported by Pahl et al. (2001), therefore, is attributed to experimental "noise" and should not be included in the estimate of true population variability. As boron clearance is largely a function of GFR, the larger more certain data base on GFR and its variability among humans is used to estimate boron clearance variability. Because the measured boron clearances in the rat and human kinetic studies were less than GFR, tubular reabsorption could be contributing to the variability of boron clearance in the population. Variability in these factors, however, is judged to be minor in comparison to the variability in GFR (Section 3.4.1.1).

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

$$AF_{HK} = \frac{GFR_{AVG}}{GFR_{AVG} - 3SD_{GFR}} \tag{5.4}$$

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

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

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

Dunlop (1981) assessed GFR for 25 women at three different time points during pregnancy (16, 26, and 36 weeks) and again after delivery. GFR was measured as inulin clearance. The mean values for GFR for these measurement periods were 148.6, 152.4, and 150.5 mL/min, respectively. The standard deviations were 17.2 and 17.6 mL/min for the first two measurements, rising to 31.8 mL/min for the 36-week measurement. For the present analysis (Table 7), the overall average and standard deviation (150.5 and 17.6 mL/min, respectively) for the serially-averaged measurements for each individual across the three pregnancy time points were used.

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

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

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

By virtue of their lower GFR, pregnant women diagnosed with preeclampsia could be considered to be a sensitive subpopulation, at least on the toxicokinetic scale. Toxicodynamic sensitivity is presumably independent of toxicokinetic sensitivity. The onset of preeclampsia generally occurs after the week 20 of pregnancy and is characterized by acute hypertension, often accompanied by edema and proteinuria. Women with preeclampsia are at increased risk for premature separation of the placenta from the uterus and acute renal failure, among other adverse health effects. The fetus may become hypoxic and is at increased risk of low birth weight or perinatal death. Preeclampsia has recently been estimated to affect 3-5% of pregnant women (Skjaerven et al., 2002). With almost 4 million successful pregnancies per year in the United States (Ventura, 1999), or about 3 million at any one time, the size of the preeclamptic population at any given time could be in the range of 150,000 to 200,000 women. Considering the Krutzén et al. (1992) results in the context of the sigma method, a reduction of 2 SD from the healthy population mean to establish the lower bound (which results in a GFR slightly higher than the mean of the preeclamptic GFR), would appear to be insufficient for adequate coverage of the susceptible population. The use of 3 SD below the healthy GFR mean gives coverage in the sensitive subpopulation to about 1 SD below the mean preeclamptic GFR.

As no single study is considered to be definitive for assessment of population GFR variability, AF_{HK} is determined from the average of the individual sigma-method values for each of the three studies (Table 7). The mean GFR and standard deviation values in Table 7 are based on average GFR across the entire gestational period, except for the Krutzén et al. (1992) estimate, which was for the third trimester only. The average sigma-method value from the three studies is 1.93. Considering a small residual uncertainty in the use of GFR as a surrogate for boron clearance, the average sigma-method value of 1.93 is rounded upward to 2.0 and established as the value for AF_{HK}. The data on preeclamptic women presented by Krutzén et al. (1992) were considered insufficient to base the interindividual AF_{HK} factor. Use of the mean (128 mL/min) and standard deviation (33 mL/min) in this sensitive subgroup of preeclamptic women likely overestimates the spread of GFR values below the mean, due to the likelihood of a lognormal distribution of GFR values, and the contribution of measurement variability (beyond biological variability) to the statistical confidence limits. Given these considerations, the ~2-fold interindividual variability factor derived from three standard deviations below the mean of three studies for pregnancy GFR (mean = 161.5 mL/min; mean - 3 SD = 85.8) is considered preferable for providing adequate coverage to women predisposed to adverse birth outcomes due to renal complications.

Table 7. Sigma-method value calculation

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

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

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

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

5.1.3.3 Summary of Data-Derived Adjustment Factors and RfD Calculation

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

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

Table 8. Default and data-derived values for components of UF_A and UF_H

Uncertainty Factor	Component		Combined Factor Values
	TD	TK*	
UF_A	(3.16) not replaced	(3.16) 3.3	7.9
UF_H	(3.16) not replaced	(3.16) 2.0	6.3
Combined UF_A and UF_H			66

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

The RfD is calculated from Equation 5.1, where:

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

AF_{TOT} = 3.3 x 2.0 x 3.16 x 3.16 = 66

RfD = 10.3/66 = 0.2 mg/kg-day

The RfD is consistent with a suggestion by Nielsen (1992) that an intake of 10 mg per day is not too high, while 50 mg/day is probably toxic. If a representative body weight of 60 kg is assumed for a pregnant woman, the value of 10 mg/day translates to 0.17 mg/kg-day. As boron appears to have some beneficial nutrient value, Nielsen (1992) also recommended a total daily boron intake of 1 mg to avoid boron deficiency. The RfD would appear to give an adequate margin of safety below, as well as above.

5.1.3.4. Other Uncertainty Factor Approaches

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

The International Program on Chemical Safety (IPCS) uses “data-derived” uncertainty factors to estimate tolerable intake values (WHO, 1994; Renwick, 1993). This method allows for subdivision of each of the interspecies and intraspecies default uncertainty factors to incorporate data on toxicokinetics (pharmacokinetics) or toxicodynamics (pharmacodynamics). For interspecies uncertainty, the 10-fold factor is divided into a default factor of $10^{0.6}$ (4.0) for toxicokinetics and $10^{0.4}$ (2.5) for toxicodynamics in the absence of toxicokinetic and toxicodynamic data. For intraspecies uncertainty, the 10-fold factor is subdivided into a default of $10^{0.5}$ (3.2) each for toxicokinetics and toxicodynamics in the absence of toxicokinetic and toxicodynamic data.

Subsequently, the International Program for Chemical Safety (IPCS, 2001) published a guidance document on the use of data to develop chemical specific adjustment factors. This guidance calls for the use of a composite factor (CF), which is the composite of specific adjustment factors (quantitative chemical specific data) for either toxicokinetics or toxicodynamics and the remaining default uncertainty factors for which chemical specific data were not available. The guidance document states that in some cases the split between toxicokinetics and toxicodynamics in the framework may not be appropriate and some flexibility in approach may need to be maintained; however, in the absence of data the defaults for interspecies toxicokinetics and toxicodynamics are 4.0 and 2.5, respectively. This subdivision, according to the authors, was based on the approximate 4-fold difference between rats and humans in basic physiological parameters that are major determinants of clearance and elimination of chemicals, such as cardiac output and renal and liver blood flows. The defaults for interindividual toxicokinetics and toxicodynamics are each 3.2.

In addition to the IPCS approach, a number of risk assessments have recently been completed for boron using an uncertainty factor less than 100. A description of the critical effect chosen and the uncertainty factors used follows.

ECETOC (1994) developed a tolerable daily intake (TDI) for developmental effects of boron. Decreased fetal body weight in rats was chosen as the critical effect (Price et al., 1994) with 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 pharmacokinetics (metabolism and distribution were cited) between animals and humans. A default factor of 10 was chosen for the intraspecies uncertainty factor. The composite uncertainty factor was 30.

Murray (1995, 1996) used the Price et al. (1994) study, choosing decreased fetal body weight in rats as the critical effect with a NOAEL of 9.6 mg B/kg-day. The interspecies uncertainty factor chosen was 4 (2 for pharmacokinetics and 2 for pharmacodynamics, 2x2=4). Several reasons were cited for the reduced interspecies uncertainty factor for pharmacokinetics: boron is not metabolized in animals or humans, eliminating a major potential source of pharmacokinetic variation; it is rapidly distributed throughout body water and does not accumulate; the toxicity profile of boron is similar across species; and parameters of elimination were considered by the author to be similar in humans and other animals. The authors cited the following reasons for the reduced interspecies uncertainty factor for pharmacodynamics: the sensitivity of the target tissue receptor appeared to be similar across species based on the similarity of symptoms of acute toxicity in animals and humans, and developmental and reproductive toxicity appear to be the most sensitive endpoints of toxicity in all animal species tested. The intraspecies uncertainty factor chosen was 8 (2.5 for pharmacokinetics and 3.2 for pharmacodynamics). The intraspecies pharmacokinetic factor was decreased because metabolism is normally the major source of pharmacokinetic variance in humans, and borates are not metabolized. The composite uncertainty factor chosen was 4x8=32.

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

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

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

Dourson et al. (1998), as part of the development of the WHO document (1998b), developed a TDI for boron. Although the authors agreed to the lack of metabolism and the similarity in absorption and elimination of boron in animals and humans, interspecies variation in kinetics for boron was considered to relate to renal clearance rates. A 3-fold clearance rate difference between rats and humans for boron was estimated, after eliminating studies with little confidence from an earlier projected 4-fold difference. The calculated renal clearance rate difference (3-fold) between rats and humans for boron was considered by the authors to be similar to a 4-fold difference that would be expected of other chemicals (Renwick, 1993). Based on this difference in clearance rates, the authors (Dourson et al., 1998) chose not to reduce the interspecies uncertainty factor for pharmacokinetics or pharmacodynamics. Therefore, a default value of 10 was chosen for the interspecies factor. For intraspecies uncertainty, the pharmacokinetic factor was reduced from a default of 3.2 to 1.8. The authors proposed that the likely difference for humans in boron kinetics occurs during pregnancy and is based on an increase in the GFR, a recognized physiological adaption during pregnancy. The estimation of the 1.8 factor for intraspecies variation in pharmacokinetics was based on a ratio of the mean GFR of 144 mL/min +/- 32(SD) from pooled data of healthy humans in late pregnancy (number of subjects not mentioned) and this mean GFR minus two standard deviations from the mean to account for variation in the average to the susceptible human 32(SD) x2=64; 144(GFR)-64(2SDs)=80; the ratio of 1.8 was calculated as 144 mL/min divided by 80=1.8. The intraspecies pharmacodynamic factor used was a factor of 3.1, which the authors considered as a default factor, although previous methodology considered it to be 3.2. The intraspecies uncertainty factor was 1.8x3.1=5.58 rounded to 6. The composite uncertainty factor was 10x6=60.

Murray and Andersen (2001) detailed the use of reduced uncertainty factors for boron risk assessments in recent years and noted the use of factors in the range of 25-60 using the NOAEL from the Price et al. (1996) rat developmental study. The authors recommended using data derived uncertainty factors in a range of 22-44 using new rat and human clearance data

(Vaziri et al., 2001; Pahl et al., 2001). The authors detailed a method where they estimated the human dose expected to provide the same boric acid area under the curve in target tissues as the NOAEL in rats and then applying reduced uncertainty factors for pharmacokinetic and pharmacodynamic uncertainty to this estimated human NOAEL. Interspecies pharmacokinetic value was estimated at 3.1, while interspecies pharmacodynamic uncertainty was estimated at 1.25-2.5. Intraspecies factors for pharmacokinetics was 1.8-2.0 and intraspecies pharmacodynamics was 3.2.

The IOM (2002) developed a tolerable upper intake level (UL) for various life stages of humans. These ULs were based on the NOAEL (9.6 mg/kg-day) from Price et al. (1996) and an uncertainty factor of 30 (10 for interspecies uncertainty and 3 for intraspecies uncertainty based on the similarity in pharmacokinetics among humans). The reference body weight for adult women was 61 kg and was based on an average body weight from different female age groups. The resulting UL for adults was rounded to 20 mg/day. The UL was set at 17 mg B/day for pregnant women of 14-18 years of age, while the UL for pregnant women of 19-50 years of age was set at 20 mg B/day.

5.1.4. Previous Oral Assessment

The previous RfD for boron on IRIS was 9E-2 mg/kg-day based on testicular atrophy and spermatogenic arrest in a 2-year dog study from Weir and Fisher (1972). The NOAEL was 8.8 mg/kg-day, the LOAEL was 29 mg/kg-day and the uncertainty factor was 100. Newer studies have identified developmental effects in three species. The newer RfD is based on the critical effect of decreased fetal body weight in rats. The NOAEL of 9.6 mg/kg-day was identified from Price et al. (1996a) and the LOAEL of 13.3 mg/kg-day was identified from Heindel et al. (1992). Decreased fetal body weight was chosen from these studies because they are quality studies with a sensitive endpoint that identified the lowest pair of NOAELs and LOAELs. Developmental effects in mice and rabbits occurred at higher doses. The RfD uses data from these two studies performed in the same laboratory and is based on a $BMDL_{05}$ from (Allen et al., 1996). With the exception of the NOAEL from Weir and Fisher (1972) in dogs, reproductive effects occurred at higher doses than the developmental NOAEL and LOAEL. The Weir and Fisher (1972) study in dogs was not chosen due to the quality of the study (Section 4.2.1).

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

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

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, CNS, cardiovascular system, and axial skeleton (Price et al., 1996a,b; Field et al., 1989). Increased incidences of short rib XIII (a malformation) and wavy rib (a variation), and decreased incidence of rudimentary extra rib on lumbar I (a variation), were the most common anomalies in both rats and mice. Cardiovascular malformations, especially interventricular septal defect, and variations were the frequent anomalies in rabbits. Fail et al. (1998) attributed reduced fetal growth, the most sensitive developmental endpoint, to a general inhibition of mitosis by boric acid, as documented in studies on the mammalian testis, insects, yeast, fungi, bacteria, and viruses (Beyer et al., 1983; Ku et al., 1993b).

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 be route-specific differences in ability to deliver toxic doses to the targets, however, so that very high exposure concentrations may be required to produce effects by inhalation exposure. Portal-of-entry effects may also differ with exposure route. The literature regarding 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 was 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 the draft revised cancer guidelines (U.S. EPA, 1999), the data are 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 $BMDL_{05}$ was defined as the 95% lower bound on dose corresponding to a 5% decrease in the mean. $BMDL_{05}$ values calculated with a continuous power model for fetal body weight (litter weight averages) were less than those for all other relevant endpoints. The $BMDL_{05}$ 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. Using data from rats (Vaziri et al., 2001) and humans (Pahl et al., 2001), a mathematical model was applied to the TK component of the interspecies uncertainty factor to address interspecies toxicokinetics. The interspecies toxicokinetic adjustment factor was 3.3. An intra-human kinetic adjustment factor of 2.0 was estimated from three studies (Dunlop, 1981; Krutzén et al., 1992; Sturgiss et al., 1996), using glomerular filtration rate as a surrogate for boron clearance. The remaining uncertainty in the RfD derivation was from toxicodynamics. Inter-species and intra-human toxicodynamic uncertainty were assigned the default value of 3.16. The product of all the adjustment and uncertainty sub-factors served as the total adjustment factor of 66. The RfD was derived by dividing the $BMDL_{05}$ 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 guidance (1998b). 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 U.S.S.R. 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 reported reduced aldolase and sperm motility at 0.3 mg/kg. There are very few 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 U.S. and U.S.S.R. 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 Food Quality Protection Act (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. 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: Following the discussion in "A review of the reference dose and reference concentration processes" (U.S. EPA, 2002), an extra 10x uncertainty factor is not needed to protect for children's risk to boron, because the critical effect is decreased fetal body weight (developmental toxicity) in the most sensitive species, and because the assessment demonstrates a high confidence in the toxicity data base. 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 suggested as to some changes that could help the understanding of the new data presented. A third reviewer felt that the data were 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 its 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 followed, because the uncertainty factor extrapolates from the animal data to the human data, making it 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 suggested specific 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 its 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 followed, because the uncertainty factor extrapolates from the animal data to the human data, making it 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 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 BMD dose software.

SUMMARY OF THE EXTERNAL PEER REVIEW COMMENTS ON THE REVISED SECTION 5.1.3.

Question 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?

Comment: All reviewers strongly supported the use of chemical specific adjustment factors, in general. Two reviewers, however, commented that EPA should consider developing and reviewing this methodology separately, before setting precedents in the boron assessment, itself. One reviewer suggested that EPA go even farther with the methodology by changing the interspecies uncertainty factor default from 10 to several different species-specific factors based on a study comparing acute effects for humans and several laboratory species.

Response: EPA has considered this over the last year and feels that the time is right to advance the methodology in a limited fashion. The uncomplicated toxicokinetics of boron allow this to be done without major changes to the RfD methodology. However, changing the overall uncertainty factor default values would be a major change to the methodology, requiring all other RfDs to be revisited. In addition, the study on acute effects across species has not yet been published but may serve as a starting place for adjustments to the interspecies defaults in the future.

Comment: Several reviewers were of the opinion that the uncertainty factor disaggregation (Eq. 5.1) was too complicated, confusing, and the terminology did not accurately reflect the nature of the new subfactors. In particular, the disaggregation of two uncertainty factors into eight variability and uncertainty subfactors (Eq. 5.1) was judged to be excessive. One reviewer stated that the proposed interspecies toxicokinetic variability factor did not deal with variability at all, and that the original interindividual uncertainty factor, itself, was actually a variability factor. Although sympathetic to the need to distinguish between variability and uncertainty, two reviewers suggested combining the variability and uncertainty subfactors into a single "adjustment" factor to be more consistent with methods in use elsewhere (IPCS, 2001). One reviewer also pointed to a difference between EPA and IPCS in interpreting the toxicokinetic and toxicodynamic components. However, the reviewer favored the EPA interpretation, somewhat, and the difference would have no impact on the boron assessment. Another reviewer stated that the arguments for these new factors were convincing only with respect to the boron assessment and did not "hang together well" as a general approach.

Response: We feel that it is important to distinguish between dose "adjustments" that are based on data and those based on judgement, and between variability and uncertainty, and attempted to make those issues mathematically explicit. However, we agree that there are some issues with the terminology used to make these distinctions. Therefore, the separate variability and uncertainty subfactors have been combined into single "adjustment" factors and the contribution of variability and uncertainty to the adjustment factor is discussed in the text. The strict separation of toxicokinetics and toxicodynamics in the boron assessment is a conceptual one, while the IPCS (2001) interpretation is based more on the nature of the available data. Language in Section 5.1.3 explicitly acknowledges the latter issue. The EPA has not yet formally reviewed or adopted the IPCS (2001) approach, which is still considered draft, so some differences of interpretation are likely at this stage. We believe the difference is not significant.

Question 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?

Comment: One reviewer stated that there was no scientific evidence for the 4.0/2.5 split and that it should be the same as for UF_H (3.16 each); the empirical basis for the split was too weak. The other three reviewers agreed with the proposed default split, with some qualifications. One reviewer agreed that the split is reasonable if the overall default value of 10 is shown to be valid in the longer term; however, the split is based on weak data and a flawed analysis and should be considered highly uncertain. Two reviewers supported the notion of species-specific defaults, suggesting that the split was valid for rat studies, but a larger toxicokinetic value should be used for smaller species and vice versa (based on allometric scaling). One reviewer suggested that we should not limit that application of the 4.0/2.5 split to a narrow class of compounds, but should apply it to all rat studies. One reviewer argued that a similar unequal split should be applied to the interindividual uncertainty factor (UF_H), also, stating that, in humans, kinetic variability is much greater than dynamic variability. In fact, the entire pharmaceutical industry designs their drug research around that premise. Another reviewer presented the opposite argument, stating that existing data support a TK/TD variability preponderance in favor of dynamics by a ratio of 3.5 to 1.

Response: We agree that the empirical basis for the split is weak and place more emphasis on the supporting conceptual arguments and related data as discussed in the first part of Section 5.1.3, although the allometric argument says nothing about the toxicodynamic component. In fact, we limited the application of the 4.0/2.5 split to compounds with simple kinetics (primarily those that are not metabolized) because we don't believe the allometric argument will necessarily hold when metabolism is involved. The values will probably change somewhat as new data are evaluated, but we believe that kinetic variability will dominate for interspecies extrapolation. In addition, the proposed split has been published and is in use internationally, so there is precedent, as supported by two reviewers. We also agree that the default splits are likely to be species specific and support further development of that concept. However, species-specific default values would require a corresponding species-specific aggregate uncertainty factor default (i.e., other than 10), which represents a major change in the RfD methodology. The data (and conceptual model) presented by one reviewer are intriguing but, as yet, unpublished. These data and the interspecies toxicity comparison previously mentioned may allow for such major uncertainty factor revisions in the near future. As the reviewers gave conflicting opinions on the split for UF_H, and the equal split (as stated in Section 5.1.3), is a convention based on lack of knowledge, we have retained the equal TK/TD allocation of 3.16.

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

Comment: One reviewer stated that the simple kinetic model is appropriate given the common linear nature of absorption, distribution, lack of metabolism in vivo and that renal clearance is the primary route of elimination. The reviewer also stated that caution needs to be exercised in calculating adjustments for fluid compartment size based upon body mass differences, since the dynamics of compartment sizes are undoubtedly different between species. Nevertheless, the reviewer thinks that the clearance data for rats and humans are adequate for purposes of the document and that the document does a good job in stating implicit assumptions. However, the reviewer felt that Section 5.1.3 was overly complex and that some equations and variables were unnecessary, given that absorption, distribution, and excretion were nearly the same in rats and humans. This reviewer also suggested that the equations could be put in an appendix if deemed really necessary.

Response: In general, any quantitative model should be mathematically explicit, with sufficient detail to avoid ambiguities and hidden assumptions. The point of the model is to define all the variables that are considered, even if the data eventually "cancel them out." Also, there is a need to refer to specific equations when the data are presented, without referring the reader to an appendix. Furthermore, the final form of the model was not necessarily intuitive and requires explanation. We agree that the model development can be simplified considerably without compromising transparency. After considering a parameterization involving combining the f_p

and f_a variables into a single "bioavailability" factor, it was determined that the bioavailability factor could be eliminated from the model when steady state conditions are assumed. As a result, some of the details of the data analysis became unnecessary and were deleted.

Comment: Another reviewer stated that the approach is reasonable, assumptions are clear, and the clearance data are adequate for the evaluation. However, the reviewer believed that the model is flawed because f_p is incorrectly defined in the model structure. The factor f_a x f_p is the proportion of dose that is absorbed into the systemic circulation after oral dosing; f_p is not related to any calculation of dose found in plasma, f_a x f_p is simply bioavailability in the units of proportion of dose administered. Also the reviewer noted that clearances are given as mL/min/kg for the rat and human and should be converted to clearances for the animals of specific size used in each study (average weight of the pregnant women and the pregnant rats). The reviewer stated that linear relationships do not hold over wide ranges of body weight but are rather clearance is allometrically related to body weight. For borate, bioavailability is expected to be 1.0 for both rats and humans, so the ensuing total-body-water data analysis is not necessary. The reviewer agrees that there is no need for the interspecies subfactor to be greater that 1.

Response: See previous response concerning f_a and f_p. Also, the clearance units have been changed as suggested and the body weight terms added to the equation (see new Eq. 5.3). The clearance data in units of mL/min (given separately from body weight in the original studies) are used directly in the calculation, as are the body weight data. However, clearance is not totally independent of body weight, as the new Eq. 5.3 implies. Therefore, an adjustment is made to the final calculation to account for this partial correlation.

Comment: Two reviewers agreed that the data are adequate for the reduction of the interspecies kinetic uncertainty subfactor (UF_{AK}) to 1, while the third reviewer stated general agreement with the approach for calculation of the interspecies toxicokinetic adjustment factor, but disagreed with the assumption that boron will only be distributed throughout total body water. Data from Chapin et al. (1997) found that steady-state boron accumulates preferentially into bone of rats and that concentrations in the bone were 4 times the serum concentrations. This reviewer provided a method for recalculation of the amount of boron in the general circulation based on volumes of distribution and bone and plasma boron concentration data. Based on estimates of bone-to-body weight ratios of 0.05 and 0.12 for rats and pregnant women, respectively, the reviewer suggested that the overall interspecies kinetic adjustment factor should be 2.85 rather than 4.08. The reviewer pointed out that the volume of distribution measured in human subjects by Jansen et al. (1984a) was 1.047, which is in remarkable agreement with the value of 1.04 that would be predicted from adding the distribution into body water (56% of body weight) plus the distribution into bone (4 times 12% of body weight).

Response: We do not agree that relative rat:human bioavailability is equal to 1, as implied by two of the reviewers. In units of administered dose (mg/kg-day), the relative size of the compartments to which boron is distributed in rats and humans cannot be assumed to be equal. Therefore, we considered an analysis of body water for determining the relative size of the "general circulation" compartment between rats and humans. The total amount of boron distributed to bone is significant, and a calculation of the relative bioavailability factor included the relative size of this compartment in rats and humans. In subsequent analysis, it was determined that the the bioavailability factor could be eliminated from the model when steady state conditions are assumed, and that the interspecies kinetic adjustment subfactor relies primarily on relative clearance rates, body weights and absorptions fractions, resulting in an AF_{AK} of 3.3.

Comment: One reviewer states that in kinetic modeling the goal is to define an appropriate dose metric based on mode of action and knowledge of the metabolic profile of a compound. The reviewer suggested discussing the process of calculating a human equivalent dose, much as the human equivalent concentrations assessed in the RfC procedures.

Response: We presume the reviewer would prefer a model in which a human equivalent dose (HED) is explicitly calculated, as in the RfC methodology. We attempted to include such a term in Equation 5.1, but found that the parallelism between the interspecies and intrahuman kinetic and dynamic factors was obscured, and the model became less transparent. Such a change would also represent a somewhat more radical departure from the current RfD approach.

Question 4: For the intra-human toxicokinetic variability assessment, (a) 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)? (b) Do you agree with the general approach for determining intra-human variability (ratio of mean GFR to 0.1 percentile)? (c) If not, is there a more viable alternative?(d) Is the assumption of a lognormal distribution adequately supported?(e) Is the magnitude of the residual uncertainty in UF_{HK} appropriate?

Comment: One out of four reviewers does not agree with the use of GFR variability as a surrogate for boron clearance variability. The reviewer disagrees with the assumption that boron clearance will approach GFR at higher doses because of the declining influence of reabsorption of boron into the blood stream from the kidney tubules. The reviewer points out that, if boron clearance was dose dependent, fractional excretion would increase as dose increases, which is not the case for a 100-fold range of doses in rats (Vaziri et al., 2001). The reviewer states that, in addition, the shape of the boron elimination curve for human subjects given higher doses of boron (Jansen et al., 1984a) is not consistent with EPA's assumption. The reviewer suggests examining other human boron clearance data (Astier, 1988) for evidence of dose dependency and asks whether there is any such indication in the human subjects of the Pahl et al. (2001) study. The reviewer concludes that, if no such dose dependency can be demonstrated, EPA must use the boron clearance variability data directly, perhaps mitigating the high variability of the Pahl et al. (2001) data with the much less variable boron clearance in the Jansen et al. (1984a) study.

The other three reviewers support the use of GFR as a surrogate for boron clearance, essentially agreeing with the EPA's arguments for not using the Pahl et al. (2001) study. One reviewer agrees that for borate, the major factor that determines filtration in the kidney is GFR. The reviewer notes, however, that in pregnant rats and women there is evidence that renal clearance is less than glomerular filtration, signifying either resorption returning borate to plasma or plasma protein binding that reduces free borate available for filtration. The reviewer goes on to state that the methods used to estimate borate clearance by Pahl et al. (2001) are good for estimating the population mean but not for comprehensive, accurate determinations of borate clearance; using the distribution of GFR, with acknowledgement of reabsorption and plasma protein binding, is the preferred approach.

Another reviewer thinks that the assertion that "tubular reabsorption is generally a constant (rather than proportional) rate" is a gross oversimplification of the nature of a capacity limited, active transport process. The reviewer agrees with the principal conclusion that the variability in borate excretion would be less at doses near the RfD than was observed at dietary levels, but cautions that fractional excretion of boron in rats receiving high doses was 80% or less, suggesting that reabsorption is still important at relatively high exposure levels. The reviewer asks for a discussion of the limitation of the use of the GFR data, in that it does not reflect variability in tubular reabsorption. The reviewer goes on to say that variance in GFR from Dunlop (1981) should be considered a potentially significant underestimate of the variability in borate excretion, and that the Pahl et al. (2001) results should be used to provide an upper limit on that variability.

The other reviewer agreeing with the GFR approach cautions, however, that the Pahl et al. (2001) study is the only published data on boron clearance in pregnant women and should not be ignored. The same reviewer suggests that there is some inconsistency in rejecting the use of these data based on a CV of 0.54, while accepting a 5-8 fold variation in the interspecies data on which the TK/TD default split was based.

Response: As the use of GFR for estimating interindividual variability in boron clearance is generally supported, EPA retains this approach. However, as a consequence of the changes to the interspecies toxicokinetic extrapolation model, the quantitative basis for the calculation of AF_{HK} has been modified. As it is more appropriate to use the absolute clearance values (mL/min), independent of body weight, all three available GFR data sets can be used to estimate AF_{HK}, instead of just the Dunlop (1981) study. This change resolves the variance underestimation issue and obviates the need for a residual uncertainty factor, except for a small adjustment for the lack of information on reabsorption or plasma protein binding variability. The impact of the alternative approach (i.e., using the boron clearance data directly) is discussed in the document (with respect to the magnitude of AF_{HK}), so EPA sees no need to elaborate on those data or set an explicit "upper bound." However, in light of the reviewers' comments on the lack of strong support for the dose dependence of boron clearance, and that plasma protein binding could also contribute to clearance values less than GFR, the dose-dependence argument has been eliminated. On the issue of consistency in accepting or rejecting data based on variance, the rejection of Pahl et al. (2001) data was for determination of population *variability*, hopefully "untainted" by significant *uncertainty*. The acceptance of highly variable interspecies comparisons was for the determination of partitioning the *uncertainty* factor default value between kinetics and dynamics, where higher variance is expected.

Comment: Two reviewers support the statistical approach (ratio of mean GFR to 0.1 percentile in lognormal distribution) for estimating the intrahuman toxicokinetic adjustment factor and two reviewers do not. One reviewer supporting the approach cites consistency with the U.S. EPA guidance for pesticide exposure evaluation, but states that the normal distribution fits the data slightly better. The other supporting reviewer suggests the possibility of a bimodal distribution, with a separate sensitive population with underlying diseases that compromise glomerular function (diabetes, lupus, nephrosis, etc.).

The other two reviewers are strongly opposed to the approach. Both stated that any estimate of such an extreme percentile is well outside the range of the observations and would require an assumption of a specific mathematical form of the distribution, which cannot be demonstrated to be correct. Both reviewers also asserted that the use of 99.9% coverage is contrary to current procedures and would set a dangerous and misleading precedent. In addition, both reviewers maintained that it is overly conservative and inappropriate to assume high sensitivity for both toxicokinetics and toxicodynamics in defining the lower bound of protection; The RfD methodology does not claim to protect every member of a large population. One reviewer pointed out that the consensus of the participants in the development of the IPCS (2001) approach was that coverage in the range of 95-99% of the population was reasonable. The same reviewer maintained that the selection of such a large coverage was artificial and attempted to overcome other issues with the data, primarily the relative homogeneity of each of the study populations. The reviewer did not object to a larger coverage in the case of boron, but maintained that the selection of the 0.1 percentile gave a false sense of precision and protection. Both reviewers were skeptical about defining a specific distribution to the data but, if one distribution had to be picked, it would be the lognormal. However, one reviewer was of the opinion that the lognormal distribution should be stated simply as a good approximation and not justified on the basis of complex physiological processes. Furthermore, because values near zero are so extremely unlikely with the normal distribution fit to the data, the "impossible value" argument is not a good justification for selecting the lognormal over the normal. Both reviewers expressed a strong preference for the Dourson et al. (1998) method as a general default approach (ratio of mean to two standard deviations below the mean).

Response: We agree that the use of the 0.1 percentile would establish a dangerous precedent and that, for most data sets, would be too far outside the range of observations and would be hopelessly confounded by uncertainty (i.e., not a valid *variability* estimate). The idea of 99.9% coverage has a precedent within the U.S. EPA for dealing with pesticide exposure, although this has not been propagated to standard guidance documents and is not supported internationally. We also did not fully consider the sampled populations as representative of the general U.S. population, and agree that a false sense of security could result from the seemingly precise percentile calculation. Therefore, the EPA is now proposing to use a modification of the Dourson et al. (1998) method, but setting the lower bound at three standard deviations from the mean, rather than two. This approach gives essentially the same coverage as using the 0.1 percentile in a closed-form distribution (assuming normality) but doesn't have the same precision implications. The Krutzén et al. (1996) data demonstrate that a GFR three standard deviations below the mean of the healthy pregnant population is not overly conservative. The resulting lower-bound GFR is 77% of the average GFR for a sensitive subpopulation of pregnant women suffering from preeclampsia, presumably well within the range of observations. The argument is supported by a separate analysis of serum creatinine levels (inversely correlated with GFR) and pregnancy outcomes that suggests that a significant proportion of women who are in this range of low GFR still have successful pregnancy outcomes, thereby being at risk for fetal growth retardation from boron exposure. The new approach is a “distribution-free” one, so the lognormal approximation for GFR has been dropped.

Neither the current approach nor the former one are meant to imply that protection is sought for an individual who is most sensitive on both scales (kinetic and dynamic). In fact, the document claims only that toxicokinetic coverage be sufficiently large such that the combined probability of kinetic and dynamic sensitivities resulting in effects at or below the RfD is very small. The language in the document concerning this issue has been changed slightly to be more consistent with the RfD definition, itself, and reads: “AF_{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 sufficiently low such that an appreciable risk of deleterious effects in the population (including sensitive individuals) is unlikely.” Although it is true that the RfD methodology does not claim to protect everybody, the limitation generally applies to idiosyncratic populations who are so uniquely sensitive as to be totally separate (more as a discontinuity in the population susceptibility distribution). The distribution of GFR in the pregnant population is more likely a continuous one over a fairly large range in a large population. The choice of three standard deviations appears to be justified for the boron toxicity assessment but may not be appropriate in other cases.

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

Comment: One reviewer stated that the assumption made that the biologically relevant internal dose parameter is the average boron/boric acid concentration for all times during pregnancy may not be correct and that variations in sensitivity during pregnancy may occur and within-individual fluctuations in boric acid levels during pregnancy could also be toxicologically relevant. This reviewer also states that there is a recognized adverse condition in some human pregnancies ("preeclampsia" and, in more severe cases, "eclampsia") that is associated with very severe declines in birth weight and other dangers to both the mother and baby; for residents of areas with high background boric acid levels in their drinking water or diet, a further rise in blood boric acid levels probably would not be helpful and might cause additional harm for women with mild forms of this condition. The reviewer states that the GFR data of Dunlop (1981) indicate a significant decline in average GFR toward the end of pregnancy, and this decline is much more apparent in some of the women observed in that study than others. The reviewer also cites the relative lack of importance of exposure in the first trimester for fetal effects associated with maternal tobacco smoking, and that cessation of smoking in the first trimester generally results in normal pregnancy outcomes.

Response: The reviewer is apparently arguing that exposure to boron early in pregnancy may be less of an issue that later exposure, as GFR tends to be higher in early pregnancy and pathological conditions associated with low GFR (preeclampsia) have later onset in pregnancy (after 20 weeks). The reviewer is also suggesting that peak boron concentrations at a critical time may be toxicologically significant. The EPA considers that the empirical and mechanistic evidence is insufficient for making a determination of the exact critical window of exposure during pregnancy for fetal susceptibility to boron, but thinks it unlikely that peak concentrations are as relevant as continuous exposure. The patterns of exposure are most likely to be more steady state than intermittent high-concentration exposure. A case could be made that first trimester exposure is less important, but is not strong enough on which to base the assessment. Most of the measurements in the three studies do not cover much of the first trimester, anyway. Therefore, the EPA has retained the use of all GFR observations across pregnancy for the analysis of AF_{HK}.

PUBLIC COMMENTS

Comment: One comment was received from the general public asking why the factor of 1.26 was needed to increase the interspecies toxicokinetic "variability" factor from 3.24 to 4.08.

Response: The earlier analysis used the factor to relate the internal dose scaling factor, represented by the ratio of rat and human boron clearance values, to the external dose ratio. A consideration of the relative bioavailability between rats and humans appeared necessary to tie internal dose to external exposure. A more thorough analysis of the relative distribution of boron to the bone compartment, and of steady-state kinetics led to the conclusion that the bioavailability factor was not necessary, and that the interspecies toxicokinetic adjustment subfactor was best estimated at 3.3.

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

Table B-1. Fetal Weight Analysis 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	

Source: Adapted from Allen et al. 1996

Allen et al. (1996) stated that for boric acid benchmark dose analysis, where the parameter measured was mean fetal weight analysis, the BMDLs estimated for the combined data were closer to the corresponding BMDs than for either of the studies alone, and the confidence intervals around the best estimates of dose corresponding to the selected response level were narrower.

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]	
				BMD[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] BMD = benchmark dose, maximum likelihood estimate of dose corresponding to BMR
[f] BMDL = the 95% lower confidence limit on the benchmark dose

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, 13.6 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 0.05. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than 0.05. 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

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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 histogram (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 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).

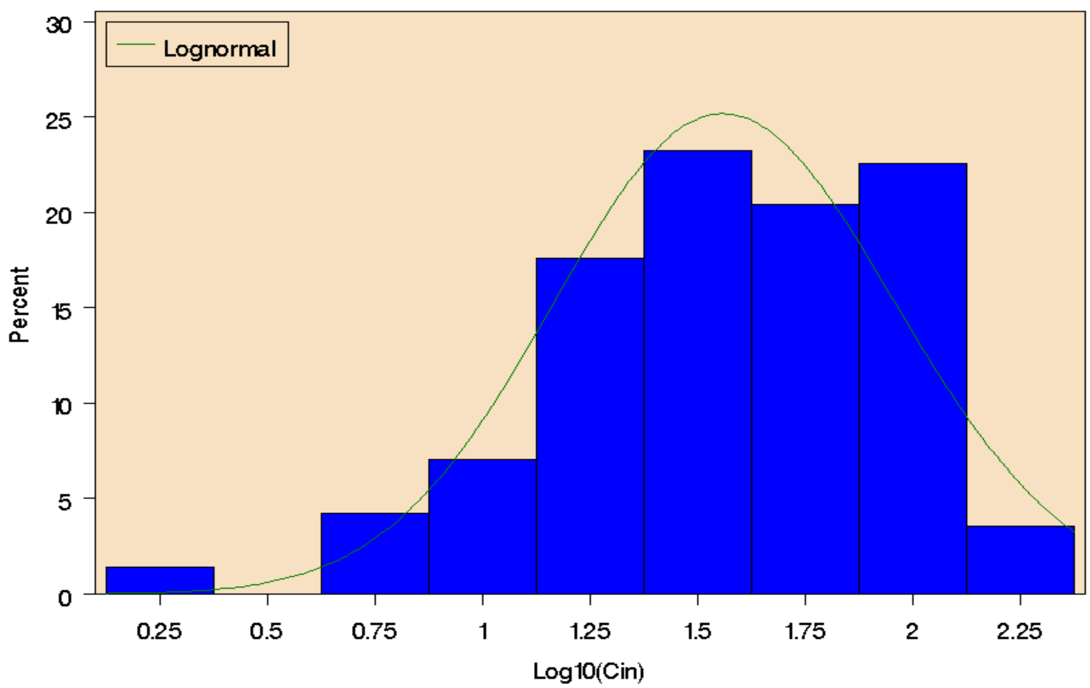

Figure 1. Histogram of Lognormal Fit for Cin Values

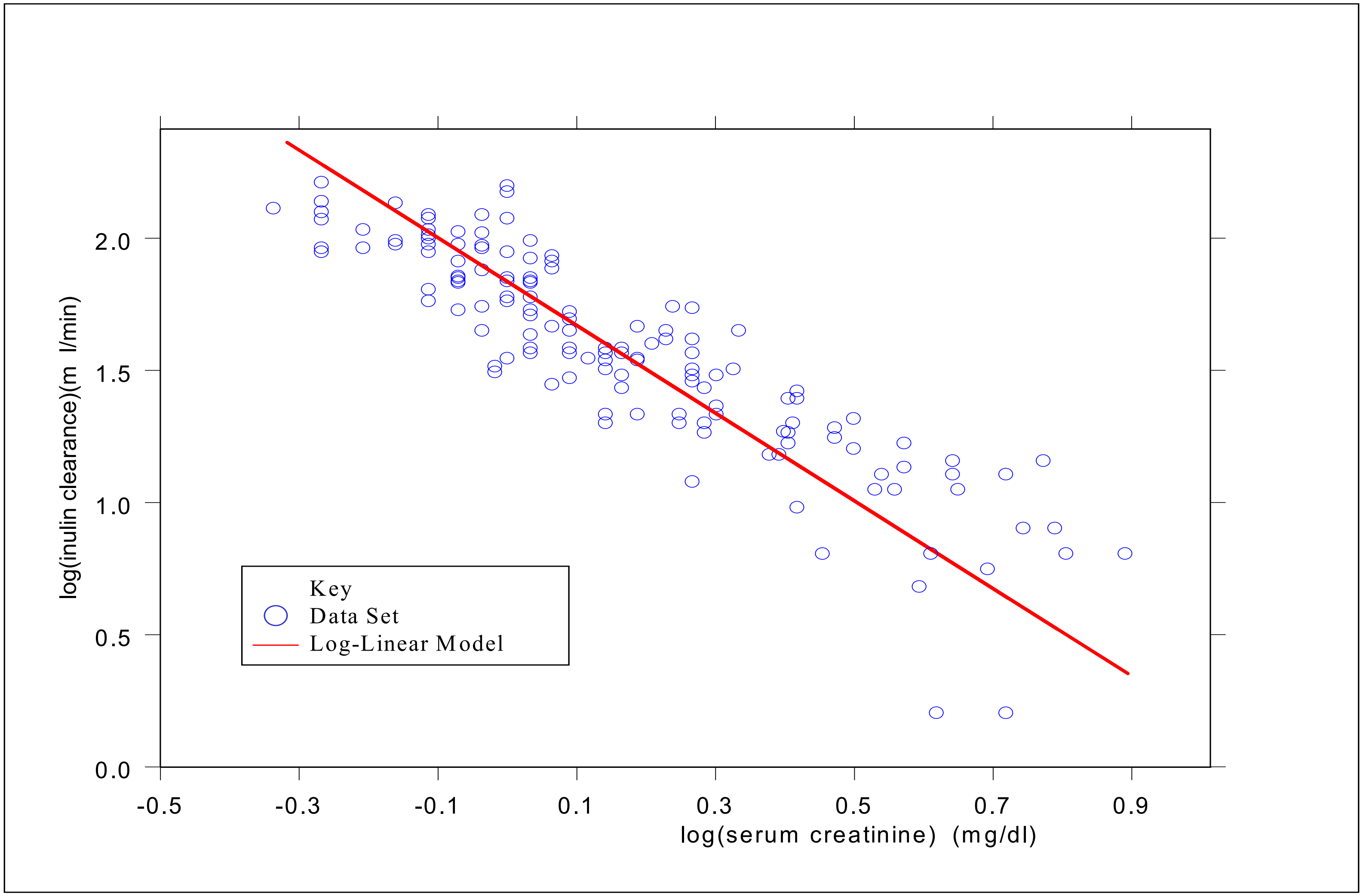

Figure 2: Modeled Regression Line and Raw Data Set

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