



**TOXICOLOGICAL REVIEW**

**OF**

**ZINC AND COMPOUNDS**

(CAS No. 7440-66-6)

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

*April 2003*

**NOTICE**

This document is a ***external review draft***. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

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

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

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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## 1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS).

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

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

Development of these hazard identification and dose-response assessments for zinc has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1995a), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.



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

## 2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Some of the chemical and physical properties of zinc and zinc-containing compounds are presented in Table 1.

Zinc is ubiquitous in the environment and occurs in the earth's crust at an average concentration of about 70 mg/kg (Thomas, 1991). Zinc metal is not found freely in nature, rather it occurs in the +2 oxidation state primarily as various minerals such as sphalerite (zinc sulfide), smithsonite (zinc carbonate), and zincite (zinc oxide). Fifty-five zinc-containing minerals are known to exist. The most important commercial minerals, their molecular composition and zinc percentages are listed below (Goodwin, 1998):

Name	Composition	% Zn
Sphalerite	ZnS	67.0
Hemimorphite	Zn <sub>4</sub> Si <sub>2</sub> O <sub>7</sub> (OH) <sub>2</sub> ·H <sub>2</sub> O	54.2
Smithsonite	ZnCO <sub>3</sub>	52.0
Hydrozincite	Zn <sub>5</sub> (OH) <sub>6</sub> (CO <sub>3</sub> ) <sub>2</sub>	56.0
Zincite	ZnO	80.3
Willemitite	Zn <sub>2</sub> SiO <sub>4</sub>	58.5
Franklinite	(Zn,Fe,Mn)(Fe,Mn) <sub>2</sub> O <sub>4</sub>	15-20

The primary anthropogenic sources of zinc in the environment are metal smelters and mining activities (ATSDR, 1995). The production and use of zinc in brass, bronze, die castings, metal, alloys, rubbers, and paints may also lead to its release to the environment through various waste streams.

Elemental zinc is a lustrous, blue-white to grey metal that is virtually insoluble in water. It has a melting point of 419.5°C and boiling point of 908°C (ATSDR, 1995). Pure zinc is usually produced by an electrolytic process in which zinc oxide is leached from the roasted or calcined ore with sulfuric acid to form zinc sulfate solution which is electrolyzed in cells to deposit zinc on cathodes (Lewis, 1993). The primary application of zinc in metallurgy is its use as a corrosion protector for iron and other metals.

Zinc salts have numerous applications and are used in wood preservation, catalysts, photographic paper, vulcanization acceleration for rubber, ceramics, textiles, fertilizers, pigments, batteries, and as nutritional supplements or medicines (ATSDR, 1995). Zinc chloride

1 **Table 1. Chemical and Physical Properties of Zinc and Selected Zinc Compounds**

2

CAS Registry Number	Zinc	Zinc Oxide	Zinc Chloride	Zinc Sulfate	Zinc Sulfide
	7440-66-6	1314-13-2	7646-75-7	7733-02-0	1314-98-3
Molecular Formula	Zn	ZnO	ZnCl <sub>2</sub>	ZnSO <sub>4</sub>	ZnS
Molecular Weight	65.38	81.38	136.29	161.44	97.44
Melting Point, °C	419.5	100 (Decomposes)	283	600 (Decomposes)	~1700
Boiling Point, °C	908	No data	732	No data	No data
Water Solubility, g/L (25°C)	Insoluble	~2x10 <sup>-3</sup>	4.3x10 <sup>3</sup>	1.7x10 <sup>3</sup>	~7x10 <sup>-3</sup>
Density (g/cm <sup>3</sup> )	7.14	5.607	2.907	3.54	~4.1

3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13

Sources: ATSDR, 1995; Barceloux, 1999

1 is a primary ingredient in smoke bombs used for crowd dispersal, in fire-fighting exercises (by  
2 both military and civilian communities), and by the military for screening purposes. Zinc  
3 chloride, zinc sulfate, zinc oxide, and zinc sulfide have dental, medical, and household  
4 applications. Zinc chloride and zinc sulfate are also used in herbicides (ATSDR, 1995). Zinc  
5 compounds are usually colorless which is advantageous since they do not color paints, plastics,  
6 rubber or cosmetics to which they might be added.

7  
8 Zinc ions are strongly adsorbed to soils at pH 5 or greater and are expected to have low  
9 mobility in most soils (Christensen et al., 1996; Gao et al., 1997). Zinc is taken up by plants and  
10 vegetables and the normal zinc content is in the range of 15 to 100 mg/kg (Thomas, 1991).

11  
12 In natural waters, zinc can be found in several chemical forms, such as hydrated ions,  
13 metal-inorganic complexes, or metal-organic complexes (U.S. EPA, 1979). Hydrated zinc  
14 cations may be hydrolyzed to form  $Zn(OH)_2$  or  $ZnO$  (U.S. EPA, 1979). In anaerobic  
15 environments,  $ZnS$  may be formed (U.S. EPA, 1979). Zinc accumulates in aquatic organisms,  
16 and bioconcentration factor (BCF) values for freshwater fish and marine fish were reported as  
17 1,000 and 2,000, respectively (U.S. EPA, 1979).

18  
19 As discussed in Section 4.1, zinc is an essential element in humans. In adults, the greatest  
20 dietary sources of zinc are meats, dairy products, grains, and mixed dishes (Pennington and  
21 Schoen, 1996a), while fruits, nuts, fats, sweeteners, and beverages contribute comparatively  
22 small amounts of zinc to the diet.

### 3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

#### 3.1. ABSORPTION

##### 3.1.1. Gastrointestinal Absorption

Numerous studies have assessed zinc absorption in healthy humans under a variety of dietary conditions. Zinc uptake from a normal diet normally ranges from 26-33% (Sandstrom and Abrahamson, 1989; Knudsen et al., 1995; Hunt et al., 1998) when taken with food, but is higher (i.e. 68-81%) when subjects have fasted (Istfan et al., 1983; Sandstrom and Abrahamson, 1989). Within a 5-25 mg dose range, zinc absorption, expressed as a percent of the total dose administered, decreases with dose; for example, in human volunteers, 61% of a 24.5 mg dose of zinc (as zinc chloride) was absorbed, compared to 81% of a 4.5 mg dose (Istfan et al., 1983).

Within the digestive tract, zinc is primarily absorbed in the small intestine. Ligation studies in rats have suggested that absorption is mainly in the duodenum (Methfessel and Spencer, 1973; Davies, 1980), with approximately 60% of the absorption occurring in the duodenum, 30% in the ileum, 8% in the jejunum, and 3% through the colon and cecum (Davies, 1980). However, more recent studies in humans (Lee et al., 1989) have suggested a greater rate of transport across the jejunum than across any other intestinal segment. As discussed in a review by Lönnerdal (1989), it is possible that while there is a greater rate of absorption in the jejunum, the fact that oral zinc first passes through the duodenum allows for a greater absolute absorption in that segment, despite a greater transport rate in the jejunum. However, the quantitative importance of the different intestinal segments is not yet clearly defined.

Gastrointestinal absorption of zinc is biphasic, with an initial rapid phase followed by a saturable slow phase (Davies, 1980; Gunshin et al., 1991).

Zinc appears to be absorbed by both passive diffusion and a carrier-mediated mechanisms (Tacnet et al., 1990). The carrier-mediated mechanism appears to be most important at low zinc levels, and involves a saturable cysteine-rich intestinal protein (CRIP) (Hempe and Cousins, 1991, 1992). CRIP binds zinc during transmucosal transport and may function as an intracellular zinc carrier. There is also some evidence that CRIP binds zinc in competition with metallothionein (Hempe and Cousins, 1991). The binding capacity of CRIP for zinc is limited, and CRIP becomes saturated at high intestinal concentrations of zinc (Hempe and Cousins, 1991). Metallothionein may be involved in zinc homeostasis at higher zinc concentrations (Richards and Cousins, 1975; Hempe and Cousins 1992). Metallothionein production is increased in response to an increase in zinc levels as well as by other heavy metals (Richards and Cousins, 1975; Cousins, 1985). The exact role of metallothionein in zinc absorption is not known, but it is thought to regulate zinc availability by sequestering it in the intestinal mucosal cells, thereby preventing resorption and providing an exit route for excess zinc as these cells are shed and excreted in the feces (Foulkes and McMullen, 1987). It has been proposed that as zinc enters the cells of the lumen, it is initially associated with CRIP, with only

1 a small fraction binding to metallothionein, but as zinc concentrations rise, the binding to CRIP  
2 becomes saturated, the proportion of zinc binding to CRIP decreases, and more zinc is bound to  
3 metallothionein (Hempe and Cousins, 1992).

4  
5 Several dietary factors can influence zinc absorption, including other trace elements (e.g.  
6 copper, iron, lead, calcium, cadmium, cobalt; see section 4.6.2), amino acids, simple and  
7 complex carbohydrates and protein. High levels of phytate or phosphate in the diet can decrease  
8 the amount of zinc absorbed (Pecoud et al., 1975; Larsson et al., 1996; Oberleas, 1996).  
9 Oberleas (1996) suggested that the phytate in the test meal complexes with endogenous zinc ions  
10 secreted from the pancreas, thus preventing its reabsorption and increasing zinc elimination. In  
11 general, low molecular weight substances, such as amino acids, increase the absorption of zinc  
12 (Wapnir and Stiel, 1986). Imidazole, tryptophan, proline, and cysteine increased zinc absorption  
13 from various regions of the gastrointestinal tract. Wapnir and Stiel (1986) suggested that the  
14 increase was due to the presence of both mediated and non-mediated transport mechanisms for  
15 amino acids. Absorption is inhibited by certain proteins (e.g., bovine serum albumin and  
16 dephytinized soyabean protein isolate), is unaffected by others (e.g., bovine whey) (Davidsson et  
17 al., 1996), and enhanced by others (e.g., casein) (Hunt et al., 1991; Davidsson et al., 1996).

18  
19 Physiological factors also appear to influence zinc absorption. The primary factor  
20 influencing zinc absorption appears to be the body's ability to alter zinc excretion and absorption  
21 efficiency in order to maintain zinc homeostasis (Johnson et al., 1993). Zinc absorption is  
22 enhanced in humans with low zinc stores; 93% of a 1.19 mg zinc dose was absorbed in subjects  
23 maintained on a low zinc diet (1.4 mg/day) as compared to 81% absorption of the same test dose  
24 in subjects on an adequate zinc diet (15 mg/day) (Istfan et al., 1983). A study in mice (He et al.,  
25 1991), suggests that zinc absorption decreases with age. Fractional absorption was significantly  
26 lower in young adult mice (70 days of age) and in adult mice (100 days of age) compared to  
27 weanling mice (1 day of age); fractional absorption in adolescent mice (20 days of age) was  
28 similar to that found in weanlings.

### 30 **3.1.2. Respiratory Tract Absorption**

31  
32 Hamdi (1969) found elevated levels of zinc in the urine and blood of workers exposed to  
33 zinc oxide fumes, relative to non-exposed workers. Although this study did not estimate zinc  
34 absorption efficiency, it does provide evidence that zinc is absorbed following inhalation  
35 exposure. Similarly, Drinker and Drinker (1928) found elevated levels of zinc in the gall  
36 bladder, kidney, and pancreas of cats, rabbits, and rats exposed to airborne zinc oxide.

37  
38 Studies by Sturgis et al. (1927) and Gordon et al. (1992) examined lung retention  
39 following inhalation exposure to zinc oxide. Retention is reflective of deposition of zinc oxide  
40 in the lung rather than systemic absorption (Hirano et al., 1989). Species differences in retention  
41 have been observed; guinea pigs, rats, and rabbits retained 20, 12, and 5%, respectively,  
42 following nose-only exposure to 11.3, 4.3, or 6.0 mg/m<sup>3</sup>, respectively, zinc oxide for 3 hours  
43 (guinea pigs and rats) or 6 hours (rabbits) (Gordon et al., 1992).

1 **3.2. DISTRIBUTION**

2  
3 Zinc is an essential human nutrient, a co-factor for over 70 enzymes, and is found in all  
4 tissues. In humans, the highest concentrations of zinc have been found in bone, muscle, prostate,  
5 liver, and kidneys (Schroeder et al., 1967; Wastney et al., 1986). Similar distributions have been  
6 found in animals (Llobet et al., 1988; Ansari et al., 1975, 1976). Less than 10% of the body's  
7 total zinc is readily exchanged with plasma (Miller et al., 1994) and most of this is slowly  
8 exchanging zinc located in bone and muscle. In blood, zinc is found in plasma, erythrocytes,  
9 leukocytes, and platelets. Approximately 98% of serum zinc is bound to proteins; 85% is bound  
10 to albumin, 12% to  $\alpha_2$ -macroglobulin, and the remainder to amino acids (Giroux et al., 1976). In  
11 erythrocytes, zinc is predominantly found as a component of carbonic anhydrase (87%) and  
12 copper-zinc superoxide dismutase (5.4%) (Ohno et al., 1985).  
13

14 Ansari et al. (1975) examined the heart, liver, kidneys, muscle, tibia, and small intestine  
15 for changes in tissue zinc concentration following the addition of 600 ppm supplemental zinc to  
16 the diet of male rats for up to 42 days. While small increases in tissue zinc levels relative to  
17 controls were reported, only occasionally were the differences statistically significant, and no  
18 pattern with increasing tissue zinc with time was noted. In a later study, Ansari et al. (1976)  
19 exposed male rats to up to 8400 ppm supplemental zinc as ZnO in the diet for 21 days then  
20 examined the liver, kidney, heart, tibia, and muscle for tissue zinc concentrations. Exposure to  
21 1200 ppm had no significant effect on tissue zinc levels relative to controls; the amount of stable  
22 zinc in liver, kidney, and bone was increased at 2400 ppm and higher, but reached a plateau  
23 (2400-7200 ppm; approximately 200-625 mg/kg-day). Exposure at the highest level (8400 ppm)  
24 caused additional increases in liver, kidney, and bone, as well as an increase in zinc level in the  
25 heart. No changes in zinc concentration were seen in the muscle. Similar results for the  
26 accumulation of zinc in organs have been found in mice (He et al., 1991), rabbits (Bentley and  
27 Grubb, 1991), and wood mice (*Apodemus sylvaticus L.*) (Cooke et al., 1990). Ansari et al.  
28 (1975, 1976) also exposed rats to a single gavage dose of  $^{65}\text{ZnCl}_2$  7 days prior to sacrifice. The  
29 levels of radiolabelled zinc in the examined tissues were substantially lower than in the controls.  
30 For most tissues, no significant relationship between dietary zinc concentration and tissue levels  
31 were found, suggesting that zinc turnover was increased in some tissues in response to elevated  
32 dietary concentrations.  
33

34 In a series of animal experiments carried out by Drinker and Drinker (1928), the fate of  
35 inhaled zinc oxide from the lungs of animals (cats, rabbits and rats) was assessed. Increased zinc  
36 levels were found in the lungs, pancreas, liver, kidney, and gall bladder.  
37

38 **3.3. ELIMINATION AND EXCRETION**

39  
40 Following oral exposure, zinc is primarily excreted via the gastrointestinal tract and  
41 eliminated in the feces; approximately 70-80% of an ingested dose is excreted in the feces  
42 (Davies and Nightingale, 1975). Oberleas (1996) found that the pancreas secretes into the  
43 duodenum two to four times the amount of zinc that is typically consumed in an average day;

1 most of this secreted zinc is reabsorbed. Zinc is also excreted in the urine. In humans,  
2 approximately 14% of the eliminated zinc was excreted in urine; when zinc intake was  
3 increased, urinary excretion accounted for 25% of the eliminated zinc (Wastney et al., 1986).  
4 Other minor routes of elimination are sweat (Prasad et al., 1963), saliva secretion (Greger and  
5 Sickles, 1979), and incorporation into hair (Rivlin, 1983).  
6

7 The rate at which zinc is excreted is dependant on both current zinc intake and past zinc  
8 intake, probably via an effect on body stores (Johnson et al., 1988). Age also affects the rate at  
9 which zinc is excreted. He et al. (1991) reported higher fecal excretion of zinc in adult mice  
10 following an intraperitoneal dose of <sup>65</sup>Zn, as compared to weanling, adolescent, or young adult  
11 mice.



## 4. HAZARD IDENTIFICATION

### 4.1. ESSENTIALITY OF ZINC

The essentiality of zinc was established over 100 years ago. Zinc is essential for the function of more than 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, copper-zinc superoxide dismutase, carboxypeptidase,  $\delta$ -aminolevulinic acid dehydratase, carbonic anhydrase, ribonucleic acid polymerase, and reverse transcriptase (Vallee and Flachuk, 1993; Sandstead, 1994). Zinc has three functions in these metalloenzymes: participation in catalytic functions, maintenance of structural stability, and regulatory functions (Vallee and Flachuk, 1993; Walsh et al., 1994). Zinc is also involved in DNA and RNA synthesis and cell proliferation. The zinc coordinates with cysteine and histidine residues of certain peptides and produces a tertiary structure which has an affinity for unique segments of DNA in promoter gene regions (Prasad, 1993). The configurations include the zinc finger, the most common zinc motif, and the zinc thiolate cluster (Walsh et al., 1994). Other physiological roles of zinc include enhancement of the affinity of growth hormone for its binding receptors, modulation of synaptic transmissions by interacting with specific sites on ionotropic neurotransmitter receptor proteins, and induction of metallothionein (Walsh et al., 1994).

A wide range of clinical symptoms have been associated with zinc deficiency in humans (Prasad, 1993; Sandstead, 1994; Walsh et al., 1994). The clinical manifestations of severe zinc deficiency, seen in individuals with an inborn error of zinc absorption or in patients receiving total parenteral nutrition, include bullous pustular dermatitis, diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in intercurrent infections. Symptoms associated with moderate zinc deficiency include growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing. Neurosensory changes, impaired neuropsychological functions, oligospermia, decreased serum testosterone, hyperammonemia, and impaired immune function (alterations in T-cell subpopulations, decreased natural killer cell activity) have been observed in individuals with mild or marginal zinc deficiency.

As reviewed by Mahomed et al. (1989), severe zinc deficiency in animals has been associated with reduced fertility, fetal nervous system malformations, and growth retardation in late pregnancy. In humans, labor abnormalities, congenital malformations, and preterm labor have been reported in otherwise healthy women with low maternal serum zinc concentrations. Numerous studies have examined pregnancy outcomes following zinc supplementation. For example, Simmer et al. (1991) found a significant intrauterine growth retardation and fewer inductions of labor (generally associated with poor fetal growth), and non-statistically significant increases in birthweight and placental weights in women receiving a supplement containing 100 mg zinc citrate (22.5 mg zinc) (these women were receiving the supplement because they were determined to be at risk of delivering small-for-gestational age babies). However, Mahomed et al. (1989) did not find any statistically significant differences in gestation duration, details of labor and delivery, fetal development, or neonatal health among 246 randomly selected

1 pregnant women receiving 20 mg Zn/day as zinc sulfate tablets beginning before the 20<sup>th</sup> week  
2 of pregnancy as compared to 248 women receiving placebo tablets. Although both groups had  
3 marginal zinc intakes (approximately 10 mg/day), the zinc supplementation did not appear to  
4 influence pregnancy outcome.

5  
6 The zinc content of a typical mixed diet of North American adults is approximately 10-15  
7 mg/day (NRC, 1989). The FDA's Total Diet Study (Pennington and Schoen, 1996b) found zinc  
8 intakes of 7.25, 9.74, 15.42, 9.38, and 15.92 mg/day in children (2 years of age), girls (14-16  
9 years), boys (14-16 years), women (25-30 years), and men (25-30 years), respectively. The 2000  
10 recommended dietary allowances (RDAs) for zinc (IOM, 2002) are presented in Table 2.

## 11 12 **4.2. STUDIES IN HUMANS**

### 13 14 **4.2.1. Oral Exposure**

15  
16 In a double-blind crossover trial, Samman and Roberts (1987, 1988) gave zinc sulfate  
17 tablets (150 mg supplemental zinc/day in three divided doses at mealtimes) to healthy adult  
18 volunteers (21 men and 26 women) for 6 weeks; identical capsules containing lactose were given  
19 to the same group of volunteers for 6 weeks as the placebo. Using the reported average body  
20 weights, the zinc doses averaged 2 mg Zn/kg-day for the men and 2.5 mg Zn/kg-day for the  
21 women. Adverse symptoms, including abdominal cramps, vomiting, and nausea, occurred in  
22 84% of the women and 18% of the men. Five females withdrew from the trial because of gastric  
23 irritation. A dose-related increase in clinical symptoms was observed when doses were  
24 expressed on a mg/kg-day basis. Ingestion of zinc tablets alone (contrary to instructions) or with  
25 small meals increased the incidence of adverse effects. Zinc administration for six weeks had no  
26 effect on plasma levels of copper, total cholesterol or HDL-cholesterol in males or females, but  
27 significantly decreased the plasma level of LDL-cholesterol in females only. An apparent  
28 inverse linear relationship between plasma zinc levels and LDL-cholesterol levels was found in  
29 the females. Hematocrit values were unaffected by zinc ingestion in males and females.  
30 Specific measures of copper status (ferroxidase activity of serum ceruloplasmin, antioxidant  
31 activity of erythrocyte superoxide dismutase, and Zn/Cu-dependent erythrocyte superoxide  
32 dismutase activity) were apparently unaffected in males. However, females, who received  
33 higher mg/kg-day doses of zinc than males, exhibited significantly reduced activity levels of two  
34 copper metalloenzymes: serum ceruloplasmin and erythrocyte superoxide dismutase. Other  
35 indicators of copper status were not affected in females.

36  
37 Fischer et al. (1984) instructed groups of 13 healthy adult male volunteers (ages not  
38 specified) to take capsules containing 0 (cornstarch) or 25 mg supplemental zinc (as zinc  
39 gluconate) twice daily for 6 weeks. Nonfasting blood samples were taken at the beginning and  
40 at biweekly intervals and tested for measures of copper status. Plasma copper levels and levels  
41 of ferroxidase activity did not change during the course of the study. However, erythrocyte

**Table 2. Recommended Dietary Allowances (RDA) by Life Stage Group and Gender**

Life Stage Group	RDA (mg/day)	
	Male	Female
0 through 6 months	2*	2*
7 through 12 months	3	3
1 through 3 years	3	3
4 through 8 years	5	5
9 through 13 years	8	8
14 through 18 years	11	9
19 through 50 years	11	8
>51 years	11	8
Pregnancy		
<18 years		12
19 through 50 years		11
Lactation		
<18 years		13
19 through 50 years		12

Source: IOM, 2002

\*ADI. No RDA value was reported

1 superoxide dismutase activity decreased after 4 weeks in the supplement group and was  
2 significantly lower than controls by 6 weeks. An inverse correlation between plasma zinc levels  
3 and erythrocyte superoxide dismutase activity was also observed at 6 weeks.  
4

5 A 10-week study of zinc supplementation in 18 healthy women, aged 25-40 years, given  
6 zinc gluconate supplements twice daily (50 mg supplemental zinc/day, or 0.83 mg supplemental  
7 zinc/kg-day) resulted in a decrease of erythrocyte superoxide dismutase (ESOD) activity  
8 (Yadrick et al., 1989). ESOD activity declined over the 10-week supplementation period and, at  
9 10 weeks, was significantly different ( $p < 0.05$ ) from values during the pretreatment period. By  
10 10 weeks, ESOD activity had declined to 53% of pretreatment levels. Change in enzyme activity  
11 is considered a better indicator of altered copper status than a measure of metal concentration in  
12 tissue or plasma. This has been documented by studies in rats which were fed copper-deficient  
13 or high-zinc diets, in which treatment-related changes in copper metalloenzyme activity are  
14 greater and precede changes in plasma or tissue levels of copper (L'Abbe and Fischer, 1984a,b).  
15 Ceruloplasmin levels were not altered. Serum zinc was significantly increased. There was also  
16 a significant decline in serum ferritin and hematocrit values at 10 weeks. Such a decrease could  
17 pose a significant risk to the iron status of women.  
18

19 Hale et al. (1988) carried out an epidemiological study of the effect of zinc supplements  
20 on the development of cardiovascular disease in elderly subjects who were participants in an  
21 ongoing longitudinal geriatric health screening program. Noninstitutionalized, ambulatory  
22 subjects between the ages of 65 and 91 (average 78) years were evaluated using questionnaire,  
23 electrocardiogram, hematological, and drug-use data. A group of subjects (38 women and  
24 31 men) that had ingested zinc supplements (20 to 150 mg supplemental zinc/day) for at least  
25 one year was compared to a control group (1195 women and 637 men) from the same screening  
26 program. Approximately 85% of the study group reported taking  $< 50$  mg supplemental  
27 zinc/day; for the 15% that reported an average intake of 60-150 mg supplemental zinc/day, the  
28 average duration was 8 years. The overall duration of zinc usage by the study group was:  
29  $\leq 2$  years, 30%;  $> 2 \leq 10$  years, 55%; and  $> 10$  years, 15%. Based on the results of the  
30 questionnaire, the incidence of anemia was reported to have decreased with an increase in zinc  
31 dose. There were no differences between zinc and control groups with respect to  
32 electrocardiographic results or the incidence of adverse cardiovascular events (heart attack, heart  
33 failure, hypertension, or angina). The zinc group had a lower mean serum creatinine, lower total  
34 serum protein, lower serum uric acid, and a higher mean corpuscular hemoglobin. Red blood  
35 cell counts were significantly lower in the women, but not in the men, in the zinc group.  
36

37 Groups of 9, 13, or 9 healthy white men were administered 0, 50, or 75 mg/day  
38 supplemental zinc as zinc gluconate, respectively, for 12 weeks (Black et al., 1988). The  
39 subjects were given instructions to avoid foods high in calcium, fiber and phytic acid, dietary  
40 constituents that are known to decrease zinc absorption. Subjects were also told to restrict their  
41 intake of zinc-rich foods in order to minimize the variation in daily dietary zinc. Three-day  
42 dietary records were collected on a biweekly basis. These records indicated that the dietary zinc  
43 intakes of the three treatment groups were 12.5, 14.0, and 9.5 mg zinc/day for the groups

1 receiving the 0, 50, and 75 mg/day supplements, respectively. Based on the average body  
2 weights for each treatment group, total zinc intakes were 0.16, 0.85, and 1.10 mg zinc/kg-day for  
3 the 0, 50, and 75 mg/day groups, respectively. Biweekly blood samples were collected from all  
4 subjects and analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides,  
5 zinc, and copper. Urinary zinc and copper values were also determined. There was a general  
6 decline in the mean serum HDL-cholesterol for the 75-mg supplement group between weeks  
7 6 and 12. HDL values for this group were significantly lower than those for the placebo group at  
8 weeks 6 and 12 ( $p < 0.05$ ). There was also a decline in the HDL values for the 50-mg group  
9 between weeks 8 through 12; however, this decline was not significantly different from that for  
10 the controls until the 12th week of treatment. When the mean HDL-cholesterol level of the 75-  
11 mg group was compared to population percentile norms, there was a decline from the 92nd to the  
12 77th percentile (Simko et al., 1984) in 6 weeks, followed by a relative stabilization of HDL  
13 values for the remaining 6-week test period. Over the 12-week period, the HDL values for the  
14 50-mg supplemental zinc group declined from the 90th to the 77th population percentile norms.  
15 Serum zinc, copper, total cholesterol, LDL-cholesterol, and triglycerides did not appear to be  
16 affected by treatment.

17  
18 In another study, 12 healthy men (23 to 35 years) with normal serum cholesterol levels  
19 received a zinc sulfate capsule twice a day with meals (160 mg supplemental zinc/day or ~2 mg  
20 supplemental zinc/kg-day, assuming a 70 kg reference body weight) for 5 weeks and 8 subjects  
21 received placebo capsules (Hooper et al., 1980). Fasting lipid levels were measured weekly for  
22 7 weeks and at week 16 in the zinc group, and biweekly for six weeks in the control group.  
23 There were no statistically significant differences in total serum cholesterol, triglyceride, and  
24 LDL-cholesterol between the zinc and control groups. After 5 weeks of zinc ingestion, serum  
25 HDL-cholesterol had been reduced by 17%; although no further zinc was administered, the  
26 serum HDL-cholesterol level continued to decline and was reduced by 26% at week 7, relative to  
27 the values for the placebo group. The rise in plasma zinc concentration did not correlate with the  
28 fall in HDL-cholesterol. Serum HDL-cholesterol returned to near baseline levels 11 weeks after  
29 the end of zinc supplementation.

30  
31 Chandra (1984) gave 11 healthy men 300 mg of supplemental zinc as zinc sulfate in two  
32 divided doses daily for 6 weeks (~4 mg supplemental zinc/kg-day using a 70 kg reference body  
33 weight). Fasting blood samples were taken prior to exposure, after 2, 4 and 6 weeks of exposure,  
34 and at 2 and 10 weeks following cessation of exposure. Effects of zinc ingestion included a 19%  
35 reduction in HDL levels at 4 weeks, and a 30% decrease in HDL levels and a 15% increase in  
36 LDL levels at 6 weeks, relative to pre-exposure values. Total serum cholesterol and triglycerides  
37 were unchanged. Zinc ingestion also adversely affected several indices of polymorphonuclear  
38 leukocyte function: chemotactic migration was reduced by 53% and the amount of phagocytosis  
39 of bacteria was reduced by 49%, although the bactericidal capacity was unchanged. In addition,  
40 the lymphocyte stimulation response to phytohemagglutinin was reduced by approximately  
41 60-70%.

1 Freeland-Graves et al. (1982) exposed groups of eight healthy women to 0, 15, 50, or 100  
2 mg supplemental zinc as zinc acetate daily for 60 days (approximately 0, 0.25, 0.83, or 1.7 mg  
3 supplemental zinc/kg-day, assuming a reference female body weight of 60 kg) and evaluated  
4 effects on serum zinc and cholesterol levels. Zinc exposure resulted in significant, dose-related  
5 increases in serum zinc. In the highest exposure group only, plasma HDL-cholesterol was  
6 significantly reduced at 4 weeks of exposure, but not at any other timepoint examined. A  
7 correlation between dietary zinc and whole-blood copper was observed in treated subjects. The  
8 study authors noted that in the 50 and 100 mg groups, some bloating, nausea, and abdominal  
9 cramps were noted unless the supplement was taken with a large glass of water at mealtime.

10  
11 Prasad et al. (1978) fed a patient with sickle cell anemia supplements of 150 to 200 mg  
12 zinc/day for 2 years. The supplement resulted in copper deficiency; serum copper and plasma  
13 ceruloplasmin levels were decreased. When copper was administered, the plasma ceruloplasmin  
14 levels became normal. In a follow-up study, of 13 patients on zinc therapy (similar treatment  
15 levels assumed), 7 patients had ceruloplasmin levels at the lower limit of normal after 24 weeks  
16 of dosing. Data on carcinogenic effects were not reported.

#### 17 18 **4.2.2. Inhalation Exposure**

19  
20 Most of the available information on the toxicity of inhaled zinc focuses on metal fume  
21 fever, a collection of symptoms observed in individuals exposed to freshly formed zinc oxide  
22 fumes or zinc chloride from smoke bombs. The earliest symptom of metal fume fever (also  
23 referred to as zinc fume fever, zinc chills, brass founder's ague, metal shakes, or Spelter's  
24 shakes) is a metallic taste in the mouth accompanied by dryness and irritation of the throat. Flu-  
25 like symptoms, chills, fever, profuse sweating, headache, and weakness follow (Drinker et al.,  
26 1927a,b; Sturgis et al., 1927; Rohrs, 1957; Malo et al., 1990). The symptoms usually occur  
27 within several hours after exposure to zinc oxide fumes and persist for 24 to 48 hours. An  
28 increase in tolerance develops with repeated exposure; however, this tolerance is lost after a brief  
29 period without exposure, and symptoms are most commonly reported at the beginning of the  
30 work week and after holidays. There are many reports of metal fume fever in the literature;  
31 however, most describe individual cases and exposure levels are not known. It is beyond the  
32 scope of this document to describe all of these reports. Below is a discussion of some of the  
33 studies which provide useful information on critical exposure levels or describe the clinical  
34 sequelae.

35  
36 Drinker et al. (1927a) described the case of a worker exposed to zinc oxide on two  
37 successive days. On the first day, the worker was exposed for 5 hours to an average  
38 concentration of 52 mg Zn/m<sup>3</sup>. The worker reported feeling an oncoming fever four hours after  
39 exposure began, and elevated temperature, chill, and fatigue were reported several hours after  
40 exposure termination. No adverse symptoms were reported after the second day of exposure,  
41 even though zinc oxide levels were higher on the second day (330 mg Zn/m<sup>3</sup>). To further  
42 examine this apparent tolerance, Drinker et al. (1927a) experimentally exposed another man with  
43 previous zinc oxide exposure to 430 mg/m<sup>3</sup> for 8 minutes on day 1 and to 610 mg/m<sup>3</sup> for 8

1 minutes on day 2. On day 1, the subject's temperature gradually increased and peaked 13 hours  
2 after exposure (101.2°F versus 98.5°F prior to exposure). The subjected reported chills and  
3 feeling feverish, weak, and somewhat debilitated 10-15 hours after exposure. As with the  
4 occupational exposure, these symptoms were not observed after the second exposure.  
5

6 Brown (1988) described the case of a shipyard worker who sprayed zinc onto steel  
7 surfaces. The worker complained of aches and pains, dyspnea, dry cough, lethargy, a metallic  
8 taste, and fever. Chest radiographs taken at the time of admission into a hospital revealed  
9 multiple nodules measuring 3-4 mm in size. The symptoms had resolved after 3 days, and the  
10 chest radiograph was normal after 4 days.  
11

12 There is also evidence to suggest that exposure to zinc oxide fumes may impair lung  
13 function. Malo et al. (1990, 1993) present case reports of two workers exhibiting symptoms of  
14 metal fume fever with evidence of functional lung involvement. In the first case (Malo et al.,  
15 1990), a worker exposed to zinc oxide fumes reported chills with muscle aches and dyspnea; a  
16 chest radiograph revealed diffuse interstitial shadows. After a 10-day period of non-exposure,  
17 the chest radiograph was normal. A lung function test was performed after the worker was away  
18 from work for 30 days; forced expiratory volume in one second (FEV<sub>1</sub>), forced vital capacity  
19 (FVC), and the FEV<sub>1</sub>/FVC ratio were normal. The worker was then exposed to his usual work  
20 environment for 1 hour on two consecutive days. Significant decreases in FEV<sub>1</sub> (16-20%) and  
21 FVC (10-11%) were observed on both days, 4-6 hours after exposure; buccal temperature was  
22 also increased and the worker experienced malaise and general muscle ache. In the second case  
23 (Malo et al., 1993), lung function tests were performed 3 months after the worker left work and  
24 after the worker returned to work for 1 day. A decrease in FEV<sub>1</sub> (24%) was observed after the  
25 worker returned to work (lung function was normal prior to returning to work). Total zinc  
26 concentrations in the work environment were 0.26-0.29 mg/m<sup>3</sup>.  
27

28 In a series of experiments by Drinker et al. (1927b), a group of 5 men and 3 women  
29 received face-only exposure to various concentrations of zinc oxide for 6-40 minutes. Two of  
30 the men were exposed to several different concentrations; the remaining subjects were exposed  
31 to only one concentration. Body temperature was used as an indicator of metal fume fever. The  
32 magnitude of the increase in body temperature appeared to be concentration-related. Based on  
33 the results of this study and epidemiology data, the study authors concluded that workers  
34 exposed to less than 15 mg Zn/m<sup>3</sup> in the air were not likely to develop metal fume fever.  
35

36 The results of more recent studies suggest that metal fume fever will occur at lower  
37 concentrations. In a study by Fine et al. (1997), a group of 13 healthy, non-smoking subjects  
38 without any previous exposure to zinc oxide fumes were exposed to 0, 2.5, or 5 mg/m<sup>3</sup> furnace-  
39 generated zinc oxide for 2 hours. The subjects were exposed to all three concentrations; each  
40 exposure was separated by a 48-hour non-exposure period. Significant increases in oral  
41 temperature were observed 6-12 hours after exposure to 2.5 or 5 mg/m<sup>3</sup> zinc oxide fume. A  
42 statistically significant increase in the number of symptoms reported was also observed after  
43 exposure to 5 mg/m<sup>3</sup>. The symptoms occurred 6-9 hours after exposure, and all symptoms were

1 resolved by the next day after exposure. The commonly reported symptoms were fatigue,  
2 muscle ache, and cough. Levels of plasma interleukin (IL)-6 were significantly increased after  
3 exposure to 2.5 or 5 mg/m<sup>3</sup>; peak levels were observed 6 hours after exposure.  
4

5 Gordon et al. (1992) exposed four adults to 5 mg/m<sup>3</sup> zinc oxide fumes or furnace gases  
6 for 2 hours. All subjects reported symptoms 4-8 hours after zinc oxide exposure; the symptoms  
7 included chills, muscle/joint pain, chest tightness, dry throat, and headache. No significant  
8 alterations in lung function were observed following zinc oxide exposure.  
9

10 Martin et al. (1999) described a cohort of 20 Chinese workers who were exposed to zinc  
11 oxide over a single 8-hour workday. Subjects were given an examination by a physician,  
12 spirometric evaluation, and chest radiographs before beginning work, immediately after the shift,  
13 and 24 hours after the start of exposure. Exposure concentrations, measured twice per individual  
14 during the 8-hour shift, ranged from 0-36.2 mg/m<sup>3</sup>. However, as no significant association  
15 between airborne zinc measurements and serum zinc levels was present, the reliability of these  
16 measurements in reflecting actual zinc exposure is uncertain. No subject showed signs of metal  
17 fume fever. Chest radiographs likewise did not reveal any changes over the period examined.  
18 Similarly, no changes in respiratory parameters, assessed by spirometry, were reported as a result  
19 of exposure.  
20

21 Zerahn et al. (1999) described the effects of an accidental exposure of 13 soldiers  
22 (11 men and 2 women) to an unknown level of zinc chloride smoke during a combat exercise.  
23 Blood samples were obtained on day 2, as well as after 1, 2, 4, and 8 weeks. Blood samples  
24 from 10/13 subjects were available on day 0, and from 10/13 subjects at week 29. Spirometric  
25 analyses of lung function parameters were performed on day 1 post-exposure, as well as 1, 2, 4,  
26 8, and 29 weeks after the exposure. Radiographs were taken from day 1 after exposure and  
27 during followup. Significant decreases in lung diffusion capacity were observed from 1 week  
28 post-exposure through the end of the study, with the lowest value occurring at week 4. A  
29 significant decrease in total lung capacity was seen at week 4 only, and a decrease in vital  
30 capacity at week 2 only. Plasma levels of fibrinogen were also elevated from weeks 1-8 post-  
31 exposure.  
32

33 Pettilä et al. (2000) described three cases of patients who inhaled an unknown level of  
34 zinc chloride smoke for 1-5 minutes and developed acute respiratory distress syndrome. Two of  
35 the three died as a result of exposure; autopsy revealed edema, pulmonary sepsis, emphysematic  
36 changes, and necrosis in both cases. The third patient developed respiratory distress on day 2  
37 post-exposure, and received supportive therapy. Four months after smoke inhalation, pulmonary  
38 function tests were 41-44% of the expected values, and revealed severe restrictive pulmonary  
39 dysfunction.  
40

#### 41 **4.3. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN** 42 **ANIMALS—ORAL AND INHALATION** 43



### 4.3.1. Oral Exposure

As with the human studies, oral animal studies have identified several critical targets of zinc toxicity. The sensitive targets of toxicity include alterations in copper status (Straube et al., 1980; L'Abbe and Fischer, 1984a,b; Bentley and Grubb, 1991), hematology (Straube et al., 1980; Maita et al., 1981; Bentley and Grubb, 1991; Zaporowska and Wasilewski, 1992), and damage to the kidneys (Straube et al., 1980; Maita et al., 1981; Llobet et al., 1988), pancreas (Aughey et al., 1977; Maita et al., 1981), and gastrointestinal tract (Maita et al., 1981).

Maita et al. (1981) exposed groups of 12 male and 12 female Wistar rats and ICR mice to 0, 300, 3000, or 30,000 ppm zinc sulfate heptahydrate in the diet for 13 weeks. The study authors estimated zinc sulfate intakes of 23.2, 234, 2514 mg supplemental zinc/kg-day (5.3, 53, and 572 mg supplemental zinc for male rats; 24.5, 243, 2486 mg zinc sulfate/kg-day (5.6, 55, and 565 mg supplemental zinc/kg-day) for female rats; 42.7, 458, 4927 mg supplemental zinc/kg-day (9.7, 104, and 210 mg supplemental zinc/kg-day) for male mice; and 46.4, 479, and 4878 mg supplemental zinc/kg-day (10.5, 109, and 1109 mg supplemental zinc/kg-day) for female mice. Zinc intakes from the control diet were not estimated.

In rats, no adverse clinical signs or increases in mortality were observed (Maita et al., 1981). Body weight gain was decreased in the high-dose male rats, as was food and water intake. Several statistically significant alterations in hematology and serum clinical chemistry parameters were observed in the high-dose rats; these included decreases in hematocrit and hemoglobin levels in males, decreases in leukocyte levels in males and females, decreases in serum total protein, cholesterol, and calcium levels in males, and decreases in serum calcium levels in females. Significant decreases in absolute and relative liver and spleen weights were observed in the high-dose male rats; decreases in absolute weight were also observed in a number of other organs in the high-dose males which was probably related to the decreased body weight. No other consistent alterations in organ weights were observed. Histopathological lesions were limited to the pancreas of high-dose rats; significant increases in the incidence of degeneration and necrosis of acinar cells, decreased number of acinar cells, clarification of centroacinar cells and "ductule-like" metaplasia of acinar cells, and interstitial fibrosis were observed; incidences of these lesions were not reported.

In mice, an increase in mortality was observed in the high-dose group (5/24 mice died); impairment of the urinary tract and regressive changes in the pancreas were observed in the animals dying early (Maita et al., 1981). Decreases in body weight gain were also observed in both sexes of high-dose mice. In the low- and mid-dose male mice, there were significant increases in hemoglobin and erythrocyte levels. Significant decreases in hematocrit, hemoglobin, and erythrocyte levels were observed in the high-dose male and female mice; a significant decrease in hematocrit level was also observed in the mid-dose male mice. Total leukocyte levels were also decreased in the high-dose male mice. Several statistically significant alterations in serum clinical chemistry parameters were observed in the high-dose mice, including slight-to-moderate decreases in total protein, glucose, and cholesterol and moderate-to-

1 marked increases in alkaline phosphatase and urea nitrogen. Decreases in total protein and  
2 increases in alkaline phosphatase and urea nitrogen were also observed in the mid-dose male  
3 mice, although the study authors stated that the values were within acceptable historical limits.  
4 Histological alterations were observed in the pancreas, gastrointestinal tract, and kidneys of  
5 high-dose mice; incidences were not reported. Pancreatic alterations included swollen nuclei in  
6 acinar cells, single cell necrosis of acinar cells, and an increase in the number of acinus and  
7 ductule-like metaplasia of acinar cells. Slight-to-moderate ulcerative lesions in the boundary of  
8 the forestomach, inflammation of the mucous membranes of the “upper intestine” with  
9 proliferation of epithelial cells and edema at the lamina propria were observed. Regressive  
10 changes were observed in the renal cortex of high-dose females.  
11

12 In a study by L’Abbe and Fisher (1984a), groups of 10 weanling male Wistar rats were  
13 fed a basal diet supplemented with 15, 30, 60, 120, or 240 ppm zinc as zinc sulfate for 6 weeks;  
14 the 30 ppm group served as the control group. Using a reference (U.S. EPA, 1988) body weight  
15 of 0.217 kg and food intake of 0.020 kg/day, daily doses of 1.4, 2.8, 5.5, 11, and 22 mg  
16 supplemental zinc/kg-day were estimated. Although a linear relationship between zinc intake  
17 and serum ceruloplasmin levels was not established, the number of animals with abnormal  
18 ceruloplasmin levels increased with increasing doses. Abnormal ceruloplasmin levels were  
19 observed in 0, 0, 11, 30, and 100% of the animals in the 15, 30, 60, 120, and 240 ppm groups,  
20 respectively. The study authors estimated that the ED<sub>50</sub> for low ceruloplasmin levels was  
21 approximately 125 ppm. Dose-related decreases in liver erythrocyte superoxide dismutase and  
22 heart cytochrome c oxidase activities were observed at dietary zinc levels greater than 30 ppm,  
23 reaching statistical significance in the 120 and 240 ppm groups. Heart erythrocyte superoxide  
24 dismutase and liver cytochrome c oxidase levels were not affected.  
25

26 In a second study, L’Abbe and Fisher (1984b) fed groups of 10 weanling male Wistar  
27 rats diets containing normal (30 mg zinc/kg diet) or supplemented (240 mg zinc/kg diet) zinc (as  
28 zinc sulfate) and normal (6 mg copper/kg diet) or deficient (0.6 mg copper/kg diet) copper for up  
29 to 6 weeks. Groups of rats were sacrificed at 2, 4, and 6 weeks. Blood, heart, and liver samples  
30 were collected for analysis. No significant differences in body weight or food consumption were  
31 noted among treated groups. Similarly, no differences were seen in hemoglobin levels. Both  
32 increased zinc and deficient copper resulted in significant decreases in serum, heart, and copper  
33 levels. In both the high zinc and copper-deficient groups, activity levels of serum ceruloplasmin,  
34 liver and heart Cu-Zn superoxide dismutase, and liver and heart cytochrome c oxidase were  
35 significantly reduced relative to control animals by 2 weeks of exposure, and remained reduced  
36 throughout the study.  
37

38 Zaporowska and Wasilewski (1992) exposed groups of 13 male and 16 female Wistar  
39 rats to 0 or 0.12 mg Zn/mL as zinc chloride in the drinking water for 4 weeks. The study authors  
40 estimated the daily drinking water dose to be 11.66 mg Zn/kg-day in males and 12.75 mg Zn/kg-  
41 day for females. Although significant decreases in food and water intake were observed, body  
42 weight gain was not significantly different from controls. Significant alterations were observed  
43 in several hematological endpoints including decreases in erythrocyte and hemoglobin levels,

1 increases in total and differential (neutrophils and lymphocytes) leukocyte levels, and increases  
2 in the percentage of reticulocytes and polychromatophilic erythrocytes.  
3

4 Bentley and Grubb (1991) fed groups of 7-8 male New Zealand white rabbits diets  
5 containing 0, 1000, or 5000 µg supplemental zinc/g as zinc carbonate (0, 34, 170 mg  
6 supplemental zinc/kg-day using an estimated TWA body weight of 2.5 kg and an allometric  
7 equation for food intake [U.S. EPA., 1988]) for 8 (1000 µg/g group) or 22 weeks (5000 µg/g  
8 group); the basal diet contained 105.5 µg Zn/g. No adverse alterations in body weight gain were  
9 observed. A significant decrease in hemoglobin levels were observed in the 5000 µg/g group.  
10 Significant decreases in serum copper and increases in serum and tissue (liver, kidney, brain,  
11 testis, pancreas, thymus, skin, bone, and hair) zinc levels were also observed in the 5000 µg/g  
12 group.  
13

14 Llobet et al. (1988) examined the effects of subchronic oral administration of zinc in  
15 Sprague-Dawley rats. Forty female rats were exposed to 0, 160, 320, and 640 mg/kg-day zinc  
16 acetate dihydrate in the drinking water (0, 48, 95, and 191 mg Zn/kg-day) for 12 weeks. Sugar  
17 was added to all drinking water of all groups to reduce unpalatability. Food and water were  
18 provided *ad libitum*. Food and water consumption, volume of urine, and weight of excreted  
19 feces were measured daily and body weights were measured weekly. After 12 weeks of  
20 treatment, blood samples were collected and analyzed for hematocrit, hemoglobin, glucose,  
21 SGOT, SGPT, alkaline phosphatase, urea, and creatinine concentrations. The brain, heart,  
22 lungs, spleen, liver, and kidneys were weighed, analyzed for zinc concentration, and (all but the  
23 brain) examined histologically. Zinc concentrations were also determined for bone, abdominal  
24 muscle and blood. Clinical signs noted were apathy and two deaths in the 640-mg/kg-day group.  
25 Statistically significant decreases in water intake and urine output were observed in 640 mg/kg-  
26 day group; a decrease in urine output was also observed in the 320-mg/kg-day group for 3 of the  
27 6 two-week measurement periods. No alterations in body weight gain or organ weights were  
28 observed. Increases in blood urea and creatinine levels in the 640-mg/kg-day group were the  
29 only significant alterations in hematological or serum clinical chemistry parameters. Zinc  
30 concentrations were significantly increased in the liver, kidneys, heart, bone, and blood of rats in  
31 the 320- and 640-mg/kg-day groups. The study authors noted that the “most severe histological  
32 alterations were observed in kidneys”, but it is unclear, from the limited reporting of the  
33 histological results, if lesions were observed in other tissues. The described renal lesions  
34 included flattened epithelial cells in the Bowman’s capsule, desquamation of the proximal  
35 convoluted tubules, and pyknotic nuclei in the 640-mg/kg-day group.  
36

37 Straube et al. (1980) examined the effects of excess dietary zinc in ferrets. Adult ferrets  
38 (6 males, 9 females), weighing 500-700 g, were divided into 4 groups and fed a basal diet of  
39 canned dog food (that contained 27 ppm zinc and 3.3 ppm copper) plus 0 (5 animals), 500 ppm  
40 (3 animals), 1500 ppm (4 animals), or 3000 ppm (3 animals) supplemental zinc as zinc oxide.  
41 Doses of 0, 142, 425, and 850 mg supplemental zinc/kg-day, respectively, are estimated using  
42 the midpoint of the range of initial body weights and the amount of food given to each animal  
43 (170 g per day, assumed to be consumed completely each day). Animals in the 1500- and 3000-

1 ppm groups showed signs of severe toxicity and were sacrificed or died within the first 3 weeks.  
2 Animals in the 500-ppm group were sacrificed on days 48, 138, and 191, and the controls were  
3 sacrificed on days 27, 48, 138, 147 and 197. The following parameters were used to assess  
4 toxicity: hematology (hemoglobin, packed cell volume, erythrocyte, leukocyte, and reticulocyte  
5 levels), serum clinical chemistry (urea nitrogen, bilirubin, ceruloplasmin oxidase activity, and  
6 blood glucose), and histopathology (kidney, liver, pancreas, lung, heart, stomach, intestine,  
7 spleen, bone marrow, and brain). Severe decreases in food intake (80%) and body weight loss  
8 (12-50%) were observed in the 1500- and 3000-ppm groups. Additional effects observed in the  
9 1500- and 3000-ppm groups include macrocytic hypochromic anemia, increased number of  
10 reticulocytes, protein, glucose, blood and bilirubin in the urine and diffuse nephrosis. The 500-  
11 ppm group showed no clinical signs of toxicity. Increases in tissue zinc levels, decreases in  
12 copper levels, and decreased ceruloplasmin oxidase activity were observed at all three dietary  
13 concentrations.

14  
15 Aughey et al. (1977) investigated the effects of supplemental zinc on endocrine glands in  
16 groups of 75 male and 75 female C3H mice by administering 0 or 0.5 g/L zinc (as zinc sulfate)  
17 in the drinking water for up to 14 months. The authors reported that the body weight in the  
18 control group ranged from 21 to 30 g, and the mean weight of the zinc-fed mice was  
19 approximately 1 g higher. Using the midpoint of the body weight range (0.022 to 0.031 kg), a  
20 water intake of 0.0069 L/day was calculated (U.S. EPA, 1988), resulting in average daily  
21 drinking water doses of 0 or 135 mg zinc/kg-day. At 1-month intervals, five mice in each of the  
22 treated and control groups were killed. After 6 months of exposure to zinc, there were no  
23 significant changes in plasma insulin or glucose levels as compared to controls. Histological  
24 alterations were observed in the pancreas, pituitary gland, and adrenal gland of zinc-exposed  
25 mice. The histological changes in the mice were first observed after 3 months of exposure to  
26 zinc. In the zinc-supplemented mice, the pancreatic isles were enlarged and had a vacuolated  
27 appearance. The  $\beta$ -cells of the pancreatic islet were larger with enlarged mitochondria and  
28 prominent golgi apparatus. The severity of the pancreatic lesions appeared to increase with  
29 increasing exposure durations. Pituitary alterations consisted of changes in the  
30 adrenocorticotrophic hormone (ACTH)-producing cells that indicated increased synthesis and  
31 secretion, including increased number and size of granules and more prominent rough  
32 endoplasmic reticulum and Golgi apparatus. Hypertrophy of the adrenal zona fasciculata and  
33 increased adrenal cortical lipid and cholesterol deposition were also observed. No tumors were  
34 reported in the pancreas, pituitary gland, or adrenal gland of zinc-exposed mice; data on other  
35 organs were not reported.

36  
37 In a 1-year study, an unspecified number of newborn Chester Beatty stock mice (sex not  
38 reported) were administered 0, 1000, or 5000 ppm zinc (approximately 0, 170, or  
39 850 mg/kg/day) as zinc sulfate in drinking water (Walters and Roe, 1965). A separate group of  
40 mice received zinc oleate in the diet at an initial dose of 5000 ppm supplemental zinc; this dose  
41 was reduced to 2500 ppm after 3 months and to 1250 ppm after an additional 3 months because  
42 of mortality due to anemia. An epidemic of the ectromelia virus caused the deaths of several  
43 mice during the first 8 weeks; consequently, additional control and test-diet groups were

1 established. There was no difference in body weight gain between control and treated groups,  
2 except for the dietary zinc group which became anemic. Survival was not reported in treated  
3 compared with control groups. An apparent increase in the incidence of hepatomas was  
4 observed in treated mice surviving for 45 weeks or longer relative to controls (original and  
5 replacement mice were pooled). The hepatoma incidences in the control, low-dose drinking  
6 water, high-dose drinking water, and test-diet groups were 3/24 (12.5%), 3/28 (10.7%), 3/22  
7 (13.6%), and 7/23 (30.4%), respectively. Incidences of malignant lymphoma in the control, low-  
8 dose drinking water, high- dose drinking water, and test-diet groups were 3/24 (12.5%), 4/28  
9 (14.3%), 2/22 (9%), and 2/23 (8.7%), respectively. Incidences of lung adenoma in the control,  
10 low-dose drinking water, high-dose drinking water, and test-diet groups were 10/24 (41.7%),  
11 9/28 (32.1%), 5/22 (22.7%), and 9/23 (39.1%), respectively. None of these were significantly  
12 elevated in a statistical analysis of these data performed by the EPA.  
13

14 Halme (1961) exposed tumor-resistant and tumor-susceptible strains of mice to zinc in  
15 drinking water. In a 3-year, 5-generation study, zinc chloride was added to the water of tumor-  
16 resistant mice (strain not specified); the groups received 0, 10, 20, 50, 100, or 200 mg Zn/L. The  
17 spontaneous tumor frequency for this strain of mice was 0.0004%. The tumor frequencies in the  
18 generations were reported as: F0=0.8%, F1=3.5%, F1 and F2=7.6% and F3 and F4=25.7%. Most  
19 of the tumors occurred in the 10- and 20-mg Zn dose groups. No statistical analyses and no  
20 individual or group tumor incidence data were reported. In the tumor- susceptible mice, strains  
21 C3H and A/Sn received 10-29 mg Zn/L in their drinking water for 2 years; 33/76 tumors were  
22 observed in the C3H strain (31 in females) and 24/74 tumors were observed in the A/Sn strain  
23 (20 in females). Most of the tumors were reported to be adenocarcinomas, but the tissues in  
24 which they occurred were not reported. The numbers of specific tumor types were not reported.  
25 The overall tumor frequencies (43.4% for C3H and 32.4% for A/Sn; both sexes combined) were  
26 higher than the spontaneous frequency (15% for each strain), although no statistical analyses  
27 were reported.  
28

#### 29 **4.3.2. Inhalation Exposure**

30

31 In a multispecies study, Gordon et al. (1992) exposed an unspecified number of male  
32 Hartley guinea pigs, Fischer 344 rats, and New Zealand rabbits to freshly generated zinc oxide  
33 particles. The guinea pigs and rats received nose-only exposure to 0, 2.5, or 5.0 mg/m<sup>3</sup> zinc  
34 oxide for 3 hours; the rabbits received nose-only exposure to 0 or 5.0 mg/m<sup>3</sup> zinc oxide for  
35 2 hours. Animals were sacrificed 0, 4, or 24 hours following cessation of exposure. The lungs  
36 were lavaged, and the lavage fluid and recovered cells were examined for evidence of  
37 inflammation. Significant increases in lavage fluid parameters (lactate dehydrogenase,  
38 β-glucuronidase, and protein content) were observed 24 hours after guinea pigs and rats were  
39 exposed to 2.5 or 5.0 mg/m<sup>3</sup>. No significant alterations in lavage parameters were observed in  
40 the rabbits. The ability of alveolar macrophages to phagocytize particles was assessed in guinea  
41 pigs and rabbits. In the guinea pigs exposed to 5.0 mg/m<sup>3</sup>, there was a significant reduction in  
42 phagocytic capacity (percentage of viable macrophages engulfing four or more particles), but no  
43 effect on phagocytic index (percentage of macrophages engulfing particles). Phagocytic ability

1 was not adversely affected in the rabbits. The authors suggested that the reason rabbits were less  
2 affected was due to a lower retention of the inhaled zinc particles (4.7% in rabbits, compared to  
3 11.5% in rats and 19.8% in guinea pigs), resulting in a lower dose per unit tissue mass.  
4

5 Lam et al. (1988) exposed groups of 7-8 male Hartley guinea pigs to 2.7 or 7 mg/m<sup>3</sup>  
6 (average concentrations) freshly formed ultrafine zinc oxide aerosols (count median diameter of  
7 0.05 µm; geometric standard deviation of 2.0) for 3 hours/day for 5 days. Two groups of eight  
8 guinea pigs were exposed to furnace gases for 3 hours on one of two days; the two groups were  
9 combined and served as the control group. No significant alterations in tidal volume, functional  
10 residual capacity, residual volume, respiratory frequency, airway resistance, or compliance were  
11 observed. Gradual decreases in total lung capacity (significant after day 4), vital capacity  
12 (significant after day 2), and single-breath diffusing capacity for carbon monoxide (significant  
13 after day 4), relative to controls, were observed in the 7 mg/m<sup>3</sup> group, but not in the 2.7 mg/m<sup>3</sup>  
14 group. Significant increases in relative and absolute lung weights were also observed in the  
15 7 mg/m<sup>3</sup> group.  
16

17 Lam et al. (1988) also assessed the effect of a single high peak of zinc oxide on lung  
18 function. In the first of the two experiments, eight male Hartley guinea pigs were exposed to  
19 4.0 mg/m<sup>3</sup> zinc oxide for 3 hours on day 1; on day 2, the animals were exposed to 34 mg/m<sup>3</sup> for  
20 the first hour and to 4.0 mg/m<sup>3</sup> for the remaining 2 hours. Significant decreases in total lung  
21 capacity and vital capacity were observed on days 2, 3, 4, and 5; apparent alveolar volume was  
22 decreased on day 3. Relative lung weights were decreased on days 2-5. In general, the  
23 decrements in lung function parameters and lung weight changes peaked at day 3. An increase  
24 in respiratory resistance and decrease in respiratory compliance was observed on days 1 and 2.  
25 Increases in absolute and relative lung weights were observed on days 2-5.  
26

27 In the second experiment, eight male Hartley guinea pigs were exposed to 6 mg/m<sup>3</sup>  
28 (average concentration) 3 hours/day for 5 days; the animals were exposed to 25 mg/m<sup>3</sup> during  
29 the first hour of exposure on day 1. Several lung function parameters were significantly altered,  
30 including decreases in vital capacity and total lung capacity on days 1-5, decreases in functional  
31 residual capacity and residual volume on days 2-5, a decrease in apparent alveolar volume on  
32 day 3, and increases in single-breath diffusing capacity for carbon monoxide on days 1-5. A  
33 gradual, but statistically significant increase in respiratory resistance and decrease in respiratory  
34 compliance was observed on days 1-5. Increases in absolute and relative lung weights were  
35 observed on days 2-5.  
36

37 Amdur et al. (1982) exposed groups of 23 male Hartley guinea pigs to 0.91 mg/m<sup>3</sup>  
38 freshly-generated zinc oxide for 1 hour. A significant decrease respiratory compliance was  
39 observed immediately after exposure and 1 hour post-exposure. No alterations in respiratory  
40 frequency, tidal volume, or minute volume were observed. Similar results were observed in  
41 another study by this group in which seven guinea pigs were exposed to 0.90 mg/m<sup>3</sup> zinc oxide  
42 for 1 hour. This study showed that compliance continued to decrease between the first and  
43 second post-exposure hours.

1 **4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION**

2  
3 **4.4.1. Oral Exposure**

4  
5 *Reproductive and Developmental Studies in Humans*

6  
7 No human studies were identified which examined the potential of zinc to induce  
8 reproductive or developmental effects. Studies which examined the influence of zinc  
9 supplementation in pregnant women with marginal zinc intakes are discussed in Section 4.1.

10  
11 *Reproductive Studies in Animals*

12  
13 The reproductive and developmental toxicity of zinc has been investigated in several  
14 animal studies. Studies in rats provide evidence that high oral doses (>25 mg zinc/kg-day) of  
15 zinc adversely affect spermatogenesis (Saxena et al., 1989; Evenson et al., 1993) and results in  
16 impaired fertility (decreased number of implantation sites and increased number of resorptions)  
17 in exposed females (Sutton and Nelson, 1937; Schlicker and Cox, 1968; Kumar, 1976; Pal and  
18 Pal, 1987).

19  
20 In two separate experiments, Saxena et al. (1989) exposed an unspecified number of adult  
21 male Sprague-Dawley rats to 0 or 500 ppm of supplemental zinc (zinc form not specified) in the  
22 diet for 3 or 6 weeks. Using averages of the weekly body weight and food intake data provided,  
23 the supplemental zinc intake is calculated to have been 20 mg supplemental zinc/kg-day for the  
24 3-week experiment and 28 mg supplemental zinc/kg-day for the 6-week experiment. In general,  
25 there were no adverse effects on food intake or body weight gain in the rats fed the high zinc diet  
26 for 3 or 6 weeks. The study authors noted an increase in swelling of the cervical and pectoral  
27 girdle lymph nodes and lameness of the forelimbs in the zinc-exposed animals, and that the  
28 degree of swelling increased with exposure duration; however, no data were provided to assess  
29 the statistical significance of this effect. General loss of hair and roughness of fur with  
30 subcutaneous hematomas were also noted in the rats exposed for 6 weeks. With the exception of  
31 a statistically significant increase in caput epididymis weight in the rats exposed for 3 weeks,  
32 there were no significant alterations in relative weights of reproductive tissues (testes, caput  
33 epididymis, cauda epididymis, seminal vesicles, prostate). Zinc intake significantly affected  
34 enzyme activities in tissues of the male reproductive system. Significant decreases in lactic  
35 dehydrogenase were observed in the testes, caput epididymis, cauda epididymis (6 weeks only),  
36 seminal vesicles, and prostate (6 weeks only) after 3 or 6 weeks of exposure. Increases in  
37 arylsulfatase activity were observed in the seminal vesicles after 3 or 6 weeks of exposure and in  
38 the cauda and caput epididymis after 6 weeks of exposure. Leucyl aminopeptidase activity was  
39 significantly increased in the testes, caput epididymis (3 weeks only), cauda epididymis, seminal  
40 vesicles (3 weeks only), and prostate gland after 3 or 6 weeks of exposure. Histological  
41 examination of the gonads of rats consuming increased levels of zinc for 3 weeks revealed  
42 meiotic arrest at the primary spermatocyte stage, degenerating secondary spermatocytes, fluid  
43 accumulation within the seminiferous tubules, and reduced epithelial cell height in the

1 epididymis. After 6 weeks of exposure, histological examination of the testes revealed  
2 additional evidence of arrested spermatogenesis. The germinal epithelium contained only  
3 spermatogonia, one layer of primary spermatocytes and a few pyknotic secondary  
4 spermatocytes; no mature spermatozoa were present in the cauda epididymis. Necrotic nuclei  
5 were observed among Sertoli cells, Leydig cells, and in the epithelia of prostatic follicles and  
6 seminal vesicles. Fertility tests were not carried out in this study.

7  
8 Evenson et al. (1993) fed groups of 10 male Sprague-Dawley rats a diet containing  
9 deficient, adequate or excessive amounts of zinc (4, 12, or 500 mg total zinc/kg food) for  
10 8 weeks; using the average of the initial and terminal body weight data provided in this paper  
11 and an allometric equation for food intake (U.S. EPA, 1988), the average dosages of zinc are  
12 estimated to be 0.4, 1, or 49 mg total zinc/kg-day. Body weight gain was directly related to the  
13 zinc dose, but there was no effect on the relative testicular weight. Flow cytometric data  
14 revealed that excess zinc caused abnormalities in the chromosome structure of sperm. The  
15 authors suggested that excess zinc, represented by the highest dose group, destabilizes disulfide  
16 bonds and complexes with protamine molecules, leading to a destabilization of sperm chromatin  
17 quaternary structure and greater susceptibility to DNA denaturation. No fertility tests were  
18 carried out in this study.

19  
20 Sutton and Nelson (1937) maintained groups of young female (n=3) and male (n=2) rats  
21 on basal diets supplemented with 0, 0.10, 0.50, or 1.0% zinc as zinc carbonate for 10-39 weeks.  
22 Using reference values for body weight (0.124 kg) and food intake (14 g) (U.S. EPA, 1988),  
23 supplemental zinc intake is estimated as 0, 113, 565, or 1,130 mg Zn/kg-day. Hematological  
24 alterations consisting of a 20% decrease in hemoglobin level in the 0.50% group, a 42-57%  
25 decrease in hemoglobin level in the 1.0% group, and 15-28% decrease in erythrocyte level in the  
26 1.0% group were observed. No hematological alterations were observed in the 0.10% group.  
27 Growth, reproduction, and development were reported to be normal for the 0.10% group over  
28 several generations. Adverse reproductive effects were observed in the 0.50% group; there were  
29 several stillbirths in the first pregnancy, after which there were no live young born. Rats in this  
30 group ceased to become pregnant after 5 months, although their body weights appeared normal.  
31 Reproduction and development were reported to have returned to normal in this group after  
32 excess zinc was withheld from the diet. No data were presented in support of this statement, so  
33 the timeframe of recovery is not known. Most of the animals on the 1.0% zinc diet failed to  
34 grow normally and some died within 4 weeks; no reproduction occurred in this dose group.  
35 Since both males and females were treated with zinc, but no histopathological examination of the  
36 gonads was performed, it is not possible to determine the immediate cause of reproductive  
37 failure at higher dose levels.

38  
39 Pal and Pal (1987) added 4,000 ppm of zinc as zinc sulfate to the diet of 12 Charles-  
40 Foster female rats for 18 days beginning immediately after coitus. Using the reference values for  
41 food intake and body weight (U.S. EPA, 1988), supplemental zinc intake is estimated at 450 mg  
42 Zn/kg-day. The incidence of conception in the treated group was significantly reduced  
43 compared to controls (5/12 vs. 12/12). In those animals which did conceive, the number of



1 implantation sites per pregnant female was not significantly altered. Zinc treatment had no  
2 effect on the number of resorption sites and there were no stillbirths or malformations among the  
3 offspring of treated rats. In a separate experiment in which female rats were fed 4000 ppm  
4 supplemental zinc for 3 weeks prior to mating, the incidence of conception and fetal outcome  
5 were not adversely affected by treatment.  
6

7 In a series of four studies conducted by Schlicker and Cox (1968), groups of 10-20  
8 female Sprague-Dawley rats were fed a control diet or a diet containing supplemental zinc oxide  
9 prior to mating and/or during gestation. The exposure protocols for the four studies was as  
10 follows: (1) 10 rats fed 0 or 0.4% dietary zinc on gestational days 0 through 15 or 16, (2) 20 rats  
11 fed 0 or 0.4% supplemental zinc on gestational days 0 through 18 or 20, (3) 20 rats fed 0 or  
12 0.4% supplemental zinc for 21 days prior to mating through delivery, and (4) 10 rats fed 0 or  
13 0.2% supplemental zinc for 21 days prior to mating through gestational day 15. Using initial  
14 body weight data provided and an allometric equation for food intake (U.S. EPA, 1988), excess  
15 zinc intake by dams is estimated as 0, 200, or 400 mg/kg-day for the 0, 0.2, and 0.4% dietary  
16 concentrations, respectively. Dams were sacrificed on the final day of exposure, and the fetuses  
17 removed for examination. A 4-29% fetal resorption rate was observed in the dams exposed to  
18 0.4% zinc beginning on gestational day 0 (studies 1 and 2). In rats exposed to 0.4% zinc prior to  
19 mating and during gestation, there was a 100% resorption of the fetuses. Significant decreases in  
20 body weight were observed in the fetuses of rats exposed to 0.4% zinc on gestational days 0-15  
21 16, 18, or 20, but not in the 0.2% group exposed prior to mating and during gestational days  
22 0-15. No external malformations were observed in the 0.4% group exposed during gestation or  
23 in the 0.2% group exposed prior to and during gestation.  
24

25 Kumar (1976) compared the effect of different levels of dietary zinc on pregnancy in an  
26 unspecified strain of rats. Beginning on day 1 of pregnancy, 12 control rats were fed a basal diet  
27 containing 30 ppm of zinc (3.39 mg Zn/kg-day), and 13 rats were fed the basal diet plus 150 ppm  
28 supplemental zinc (as zinc sulfate, ~20 mg total zinc/kg-day). The dams were sacrificed on  
29 gestational day 18. No alterations in the number of implantation sites were found, but a  
30 statistically significant increase in the number of resorptions (9.5%) was observed in the zinc  
31 supplemented group.  
32

33 Kinnamon (1963) fed groups of five Sprague-Dawley female rats a diet containing 0 or  
34 0.5% supplementary zinc as zinc carbonate for 5 weeks prior to mating with untreated males and  
35 for the first two weeks of gestation. At the end of the 7-week period, the rats were injected with  
36 radiolabelled zinc chloride, then housed in metabolism cages for 4 days prior to sacrifice. Using  
37 the body weight data provided and an allometric equation for food intake (U.S. EPA, 1988),  
38 supplemental zinc doses of 0 or 500 mg/kg-day were calculated. No significant differences in  
39 number of fetuses per litter, wet weight of the litter, or average weight per fetus were observed.  
40

#### 41 *Developmental Studies in Animals* 42

1 Several studies have examined the developmental toxicity of zinc. Studies by Schlicker  
2 and Cox (1968) and Ketcheson et al. (1969) have found decreases in body weights in the  
3 offspring of rats exposed to high doses of zinc in the diet. Additionally, alopecia and  
4 achromotrichia have been observed in the offspring of mice and mink exposed to high doses of  
5 zinc during gestation and lactation (Bleavins et al., 1983; Mulhern et al., 1986).  
6

7 Ketcheson et al. (1969) fed groups of 10 pregnant female Sprague-Dawley rats a basal  
8 diet containing 9 ppm of zinc or additional 0.2% or 0.5% supplemental zinc as zinc oxide,  
9 throughout gestation and lactation day 14. Using an estimated body weight of 0.300 kg and  
10 reported food intake data, estimated maternal supplemental zinc doses are 120 and 280 mg  
11 Zn/kg-day during gestation in the 0.2 and 0.5% groups, respectively, and 150 and 400 mg  
12 Zn/kg-day during lactation. No significant alterations in maternal body weight or food intake  
13 were observed in the zinc-supplemented groups relative to controls. No significant alterations in  
14 duration of gestation or the number of viable pups per litter were observed. Significant  
15 alterations in newborn and 14-day-old pup body weights were observed; the alterations consisted  
16 of an increase in the 0.2% group and a decrease in the 0.5% group. The increase in pup body  
17 weight at the 0.2% dietary level suggests that the basal diet did not provide a sufficient amount  
18 of zinc to support pregnancy and lactation. No external malformations were reported.  
19

20 Uriu-Hare et al. (1989) fed groups of 8-9 Sprague-Dawley rats diet containing low,  
21 adequate (control group), or high amounts of zinc (4.5, 24.5 or 500 ppm total zinc) during  
22 gestational days 1-20. Using estimates of body weight (0.285 kg) and food intake (17 g/day)  
23 data presented in graphs, the total dietary intake of zinc is estimated to have been 0.27, 1.45, or  
24 30 mg Zn/kg-day. No adverse effects on maternal body weight gain, hematocrit levels, or the  
25 incidences of resorptions, malformations, fetal body weight, or fetal length were observed in the  
26 high zinc group, as compared to the adequate zinc group. Adverse effects, including decreases  
27 in maternal body weight and increases in resorptions, malformations, and fetal growth were  
28 observed in the low-zinc group only.  
29

30 Mulhern et al. (1986) fed an unspecified number of female weanling C57BL/6J mice a  
31 diet containing 50 (normal) or 2000 (high) ppm of zinc as zinc carbonate and, at age 6 weeks,  
32 mated them with unexposed males. Each dam and her offspring were assigned to one of ten  
33 groups receiving 50 or 2000 ppm total zinc during gestation, lactation, and postweaning until age  
34 8 weeks. Decreases in hematocrit and body weight were observed in the F<sub>1</sub> mice exposed to  
35 2000 ppm zinc during gestation, lactation, and postweaning. The study authors noted that  
36 decreases in body weight gain were observed in other groups; however, the magnitude and  
37 statistical significance were not reported. Alopecia was observed in all groups of F<sub>1</sub> mice  
38 exposed to 2000 ppm during lactation, regardless of gestational exposure. The mice began to  
39 lose hair between 2 and 4 weeks of age, and exhibited severe alopecia at 5 weeks. Exposure to  
40 2000 ppm during lactation and/or post weaning resulted in achromotrichia, which the authors  
41 suggest may result from the effects of zinc-induced copper deficiency.  
42

1           Bleavins et al. (1983) fed groups of adult mink (11 females and 3 males) a basal diet  
2 containing 20.2 ppm of zinc or the basal diet supplemented with 500 ppm of zinc as zinc sulfate  
3 heptahydrate. After 2 months the animals were mated during an 18-day period; since no clinical  
4 signs of zinc toxicity or copper deficiency were noted for the 500-ppm group, 3 days before the  
5 end of the mating period, the high dose of zinc was increased to 1000 ppm. Using the reference  
6 body weight and an allometric equation for food intake (U.S. EPA, 1988), the intake of zinc is  
7 calculated to have been 56 mg Zn/kg-day. Fewer dams (8/11) on the high-zinc diet produced  
8 offspring than those on the control diet (11/11); however, gestational length, litter size, birth  
9 weights and kit mortality to weaning were not affected. Zinc had no effect on body, liver, spleen  
10 or kidney weights, or on hematological parameters (leukocyte, erythrocyte, hemoglobin,  
11 hematocrit) in adults. Clinical signs associated with copper deficiency (alopecia, anemia,  
12 achromotrichia) were also not observed in adults. However, 3- to 4-week-old kits exhibited  
13 achromotrichia around the eyes, ears, jaws and genitals, with a concomitant loss of hair and  
14 dermatosis in these areas. Subsequently, achromotrichia and alopecia spread over much of the  
15 body. At 8 weeks, treated kits had lower hematocrit and lower lymphocyte counts, but higher  
16 numbers of band neutrophils. At 8 weeks, treated kits exhibited signs of immunosuppression  
17 (significantly lowered thymidine incorporation by lymphocytes after stimulation by  
18 concanavalin A). Treated male kits had lower body weights than controls at 12 weeks. After  
19 weaning, the kits were placed on the basal diet, and within several weeks they recovered.

#### 21 **4.4.2. Inhalation Exposure**

22  
23           No studies examining the reproductive/developmental toxicity of zinc in humans or  
24 animals were identified.

### 26 **4.5. OTHER STUDIES**

#### 28 **4.5.1. Acute Toxicity Data**

##### 30 **4.5.1.1. Oral Exposure**

31  
32           Brewer et al. (2000) reported on the use of zinc supplementation for the treatment of  
33 Wilson's disease. Wilson's disease results in an accumulation of copper within the body,  
34 eventually leading to hepatic changes and, in some patients, neurologic effects as well. The  
35 study authors discussed the results of 26 pregnancies in 19 women with Wilson's disease who  
36 received oral zinc acetate (from 25-150 mg Zn/day) during pregnancy. Urinary copper, a  
37 reliable indicator of body copper status, was able to be maintained within normal levels with zinc  
38 supplementation, and hepatic and neurological signs in the affected women returned to normal  
39 while treatment continued. Of 26 pregnancies, there were four miscarriages, and two fetal  
40 abnormalities; one major (microcephaly) and one minor (surgically correctable heart defect).

##### 42 **4.5.1.2. Inhalation Exposure**

1 Fine et al. (2000) exposed a group of 11 control subjects and a group of 10 sheet metal  
2 workers to 5 mg/m<sup>3</sup> of zinc oxide fume for 2 hours on each of 3 consecutive days. Naive  
3 subjects showed a number of slight to moderate symptoms following the first exposure,  
4 including chills, flushing, fatigue, muscle and stomach aches, dyspnea, and nausea. Following  
5 the second and third exposures, the incidence of symptoms among naive subjects were  
6 significantly lower than following the first exposure. Similarly, the increase in temperature was  
7 greatest among naive subjects after the first exposure, and decreased after the second and third  
8 exposures; after the third exposure, the temperature increase was significantly lower than after  
9 the first exposure. The temperature changes and incidence of symptoms for sheet metal workers  
10 were not significantly different from exposure to control air. Both the response of naive subjects  
11 to multiple exposures and the response of sheet metal workers to zinc oxide exposure were cited  
12 as evidence of the development of tolerance to zinc fume fever.  
13

#### 14 **4.5.1.3. Other Methods of Exposure**

15  
16 In a short-term *in vivo* assay, Stoner et al. (1976) injected strain A/Strong mice  
17 (20/sex/dose) intraperitoneally with zinc acetate 3 times/week for a total of 24 injections (total  
18 doses were 72, 180, or 360 mg/kg). Controls (20/sex/group) consisted of an untreated group, a  
19 vehicle control group administered 24 injections of saline, and a positive control group  
20 administered a single injection of urethan (20 mg/mouse). Mice were sacrificed 30 weeks after  
21 the first injection; survival was comparable for all groups. There was no increase in number of  
22 lung tumors per mouse in treated animals relative to the pooled controls. While four thymomas  
23 were observed in zinc acetate-treated groups and none in controls, the occurrence of these  
24 tumors was not statistically significantly elevated.  
25

26 Guthrie (1956) injected 0.15-0.20 mL of 10% zinc sulfate into the testis of nineteen  
27 4-month-old rats and 0.15 mL of 5% zinc chloride into the testis of 29 3-month-old rats (strain  
28 not specified) (Guthrie, 1956). No testicular tumors were observed in either group at sacrifice  
29 15 months after injection. No controls were described.  
30

#### 31 **4.5.2. Genotoxicity**

32  
33 The results of short-term genotoxicity assays for zinc are equivocal. Zinc acetate and/or  
34 zinc 2,4-pentanedione have been analyzed in four short-term mutagenicity assays (Thompson et  
35 al., 1989). In the Salmonella assay (with or without hepatic homogenates), zinc acetate was not  
36 mutagenic over a dose range of 50-7200 µg/plate, but zinc 2,4-pentanedione was mutagenic to  
37 strains TA1538 and TA98 at 400 µg/plate. The addition of hepatic homogenates diminished this  
38 response in a dose-dependent manner. In the mouse lymphoma assay, zinc acetate gave a  
39 dose-dependent positive response with or without metabolic activation; the mutation frequency  
40 doubled at 10 µg/mL. In the Chinese hamster ovary cell *in vitro* cytogenetic assay, zinc acetate  
41 gave a dose-dependent positive response with or without metabolic activation, but the presence  
42 of hepatic homogenates decreased the clastogenic effect. Neither zinc acetate nor zinc

1 2,4-pentanedione were positive in the unscheduled DNA synthesis assay in rat hepatocytes over  
2 a dose range of 10-1000 µg/mL.

3  
4 Zinc chloride has been reported to be positive in the Salmonella assay (Kalinina et al.,  
5 1977), negative in the mouse lymphoma assay (Amacher and Paillet, 1980), and a weak  
6 clastogen in cultured human lymphocytes (Deknudt and Deminatti, 1978). Zinc sulfate has been  
7 reported to be not mutagenic in the Salmonella assay (Gocke et al., 1981), and zinc acetate has  
8 been reported to not induce chromosomal aberrations in cultured human lymphocytes (Gasiorek  
9 and Bauchinger, 1981). Crebelli et al. (1985) found zinc oxide (99% purity) (1000-5000  
10 µg/plate) to be not mutagenic for reverse mutation in *Salmonella typhimurium*.

11  
12 Responses in mutagenicity assays are thought to depend on the form (e.g., inorganic or  
13 organic salt) of the zinc tested. For example, inorganic salts tend to dissociate and the zinc  
14 becomes bound with culture media constituents. Salts that dissociate less readily (i.e., zinc  
15 pentanedione) tend to be transported into the cell and are postulated to cause a positive response  
16 (Thompson et al., 1989). Zinc is an essential trace element involved in numerous biological  
17 functions including growth, taste, and spermatogenesis. It is a cofactor for several enzymes such  
18 as those involved in the metabolism of proteins and nucleic acids.

19  
20 Zinc deficiency or excessively high levels of zinc may enhance susceptibility to  
21 carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer  
22 protection (Woo et al., 1988). Zinc deficiency enhanced carcinomas of the esophagus induced  
23 by methylbenzyl nitrosoamine (Fong et al., 1978), but retarded the development of cancer of the  
24 oral cavity induced by 4-nitroquinoline-N-oxide (Wallenius et al., 1979). In a study that  
25 examined both zinc deficiency and supplementation, Mathur et al. (1979) found that animals  
26 with a deficient diet (5.9 mg/kg) and animals diet supplemented with excessively high levels of  
27 zinc in the diet (200-260 mg/kg) had fully developed carcinomas of the palatal mucosa. While  
28 the rats were on the specific diets, the palatal mucosa was painted with 4-nitroquinoline 3  
29 times/week for 20 weeks. In the zinc-deficient group, 2/25 rats developed cancer of the palatal  
30 mucosa; 2/25 rats in the excessive zinc group also developed this form of cancer. Animals  
31 supplemented with moderate levels of zinc in the diet (50 mg/kg) developed only moderate  
32 dysplasia. Thus, zinc's modifying effect on carcinogenesis may be dose-dependent.

#### 33 34 **4.6. INTERACTIONS**

35  
36 Numerous studies have examined the interactions of zinc and other metals, however, the  
37 vast majority of these have examined the effect of co-exposure to zinc on the toxicity of the other  
38 metal. The few studies that have been conducted on the effect of other metals on the toxicity of  
39 zinc are not adequate to support dose response assessments for the interactions, or even  
40 qualitative assessments of the type or direction of the interaction (e.g., antagonism, synergism),  
41 particularly under subchronic or chronic exposure conditions. Interactions between zinc and  
42 other metals are highly plausible given that the ligand binding reactions of zinc are similar to  
43 those of a variety of other essential or toxic divalent cations (Andersen, 1984). These include a

1 relatively high reactivity with thiolate anions (e.g., cadmium, cobalt, copper, iron, lead) and  
2 formation of relatively stable chelation complexes with multidentate carboxylic acid ligands  
3 (similar to calcium and lead). Thus, competition for reactions with sulfhydryls, proteins and  
4 ligand exchange reactions are potential mechanisms of interaction that may exert effects at the  
5 level of zinc transport, binding, catalysis, or stabilization of zinc-dependent enzymes. The  
6 displacement of zinc from delta-aminolevulinic acid dehydratase (ALAD) by lead is a good  
7 example of such an interaction, and is the basis for one aspect of the toxicity of lead (the  
8 inhibition of ALAD and heme synthesis) and the ability of zinc to attenuate this effect of lead  
9 (Finelli et al., 1975; Simons, 1995). Binding to and induction of the synthesis of  
10 metallothionein appears to play an important role in the physiologic regulation of zinc levels  
11 and, possibly, its reactivity to other ligands (Li et al., 1980; Udom and Brady, 1980; Goering and  
12 Fowler, 1987; Kelly et al., 1996; Liu et al., 1996). Since a variety of divalent cations, including,  
13 cadmium, cobalt, copper, lead, and zinc bind to the metallothionein (Stillman, 1995),  
14 displacement of zinc from metallothionein by other metals could potentially give rise to  
15 interactions that have toxicologic consequences. For example, displacement of zinc from  
16 metallothionein by cadmium is thought to be involved in the mechanism by which cadmium, and  
17 possibly other divalent metals, induce the synthesis of metallothionein (Palmiter, 1994).  
18 Induction of metallothionein by zinc has been shown to alter the physiologic disposition of  
19 copper and the toxicity of cadmium (Waalkes and Pérez-Ollé, 2000). Recent characterization of  
20 divalent metal ion transporters in epithelia, including that of mammalian small intestine, suggest  
21 that zinc may share absorptive mechanisms with a variety of divalent cations, including  
22 cadmium, copper, iron and lead (Gunshin et al., 1997; Fleming et al., 1999). This provides at  
23 least one mechanism by which co-exposure with other divalent metals could affect zinc  
24 absorption, and possibly transport of absorbed zinc in other tissues.  
25

26 For the most part, however, definitive evidence for any of the above mechanisms giving  
27 rise to antagonism or synergism of the toxicity of zinc has not been reported. Information on  
28 interactions that is relevant to the toxicity of zinc and compounds are presented below.  
29

#### 30 **4.6.1. Interactions with Essential Trace Elements**

##### 31 *Copper and Zinc*

32  
33  
34 As discussed above, the most sensitive effects of zinc in humans are alterations in the  
35 levels of copper-containing enzymes, including superoxide dismutase and serum ceruloplasmin,  
36 and plasma LDL cholesterol levels. Although studies by Samman and Roberts (1987, 1988),  
37 Fischer et al. (1984), and Yadrick et al. (1989) failed to find decreases in plasma copper levels,  
38 these studies did find alterations in serum ceruloplasmin and erythrocyte superoxide dismutase  
39 activities. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more  
40 sensitive indicator of copper status than plasma copper levels. It is believed that the copper  
41 deficiency results from a zinc-induced decrease in copper absorption, although the exact  
42 mechanisms are not understood. Excess dietary zinc results in induction of intestinal  
43 metallothionein synthesis; because metallothionein has a greater binding capacity for copper

1 than for zinc, copper absorbed into the intestinal mucosal cells may be sequestered by  
2 metallothionein and not absorbed systemically (Walsh et al., 1994).

3  
4 The above considerations suggest that increased intakes of copper may decrease toxic  
5 effects of zinc that are related to copper deficiency; however, this possibility has not been  
6 rigorously explored experimentally. Smith and Larson (1946) reported that co-exposure to  
7 copper resulted in a partial attenuation of the microcytic and hypochromic anemia resulting from  
8 exposure to high levels of dietary zinc. This would be consistent with copper replenishment  
9 after zinc-induced copper depletion. Several studies have demonstrated that increased levels of  
10 copper can decrease the absorption of zinc. Oestreicher and Cousins (1985) reported that dietary  
11 levels of zinc and copper did not affect absorption of zinc or copper in an isolated, perfused rat  
12 small intestine model. However, low levels of copper in the perfusion medium resulted in an  
13 increased absorption of zinc, while medium and high copper levels resulted in decreased zinc  
14 absorption. Kinnamon (1963) reported a significant decrease in uptake of a single gavage dose  
15 of radiolabeled zinc in rats fed a diet high in copper for 5 weeks prior to exposure. Gachot and  
16 Poujeol (1992) reported exposure of primary rabbit proximal tubule cells to both 15 and 50  $\mu\text{M}$   
17 copper resulted in noncompetitive inhibition of zinc absorption into the cells. Zinc and copper  
18 are substrates for a divalent metal transport protein that has been shown to participate in the  
19 absorption of iron (Gunshin et al., 1997). The relative importance of this protein in the  
20 absorptive transport of zinc and copper has not been determined. However, Klevay (1973)  
21 reported that rats fed a diet with a 40:1 ratio of zinc:copper gained less weight than those fed a  
22 normal 5:1 ratio, indicating the importance of the relative levels of both zinc and copper in the  
23 diet.

### 24 25 *Calcium and Zinc*

26  
27 Hwang et al. (1999) reported that administration of calcium acetate to hemodialysis  
28 patients did not result in changes in hair or serum zinc relative to baseline levels, though both  
29 levels were lower than normal controls. A review by Lönnerdal (2000) provides evidence that  
30 calcium levels do not directly influence the absorption of zinc. It appears, however that calcium  
31 aggravates zinc deficiency when it is added to diets based on plant products that might be  
32 expected to be high in phytate (reviewed in O'Dell, 1969). Heth and Hoekstra (1965) reported a  
33 decreased absorption of zinc when calcium was co-administered in the diet, and that increased  
34 dietary calcium resulted in an increased rate of zinc loss (shortened clearance half-time).

### 35 36 *Iron and Zinc*

37  
38 O'Brien et al. (2000) reported that percentage zinc absorption was significantly lower in  
39 pregnant women who received iron-containing prenatal supplements (60 mg/day) relative to  
40 women who had not received iron-containing supplements. Plasma zinc concentrations were  
41 also significantly lower after iron supplementation, but not if the supplement also contained 15  
42 mg of zinc. Bouglé et al. (1999) reported a significant correlation between zinc absorption and  
43 iron content in the diet, with increased dietary iron resulting in diminished absorption of zinc.

1 However, Lönnnerdal (2000) has suggested that at lower iron intake levels, iron has no effect on  
2 the absorption of zinc. Zinc and iron are substrates for a divalent metal transport protein that has  
3 been shown to participate in the absorption of iron (Gunshin et al., 1997). The relative  
4 importance of this protein in the absorptive transport of zinc has not been determined.  
5

#### 6 **4.6.2. Interactions with Other Heavy Metals**

##### 7 *Cadmium and Zinc*

8  
9  
10 Numerous studies have demonstrated that zinc can decrease the carcinogenicity and  
11 toxicity of cadmium (Gunn et al., 1963; Waalkes et al., 1989; Coogan et al., 1992; Brzóska et al.,  
12 2001), possibly through decreased cadmium absorption or alterations in metallothionein levels  
13 (for review, see Krishnan and Brodeur, 1991). Less is known about the effects of cadmium on  
14 the pharmacokinetics and toxicity of zinc.  
15

16 Toxic levels of cadmium may inhibit zinc absorption (Lönnnerdal, 2000). Studies  
17 conducted in isolated cells or membranes from kidney proximal tubule or small intestine indicate  
18 that zinc and cadmium may share common transport and/or binding mechanisms in transporting  
19 epithelia (Tacnet et al., 1990, 1991; Prasad and Nath, 1993; Prasad et al., 1996; Endo et al.,  
20 1997). For example, Gachot and Poujeol (1992) assessed the effect of cadmium on the uptake of  
21 zinc by isolated rabbit proximal tubule cells. At low concentrations (15  $\mu\text{M}$ ), cadmium acts as a  
22 competitive inhibitor of carrier-mediated zinc uptake, while at higher concentrations (50  $\mu\text{M}$ ) it  
23 also exhibits noncompetitive inhibition of an unsaturable pathway. Similar results were reported  
24 by King et al. (2000) who found that injection of  $\text{CdCl}_2$  in mice reduced the uptake of  $^{65}\text{Zn}$  by  
25 56% in testes and 47% in brain. Exposure of rats whose diets contained normal (12 mg/kg) or  
26 elevated (60 mg/kg) levels of zinc to 5 mg Cd/L in the drinking water did not alter the amount of  
27 zinc or copper in the plasma or liver (Bebe and Panemangalore, 1996). Levels of copper in the  
28 kidneys were decreased in animals that were exposed to high-dosages of zinc and cadmium, but  
29 not in animals that received normal zinc diets and cadmium; cadmium had no effect on kidney  
30 zinc levels. Brzóska et al. (2001) reported that treatment of rats with cadmium resulted in  
31 decreased levels of zinc in the tibia; zinc supplementation restored the levels to normal.  
32

##### 33 *Lead and Zinc*

34  
35 A sizable database on the effects of zinc on lead toxicity exists. However, a detailed  
36 discussion of the effects of exposure to zinc on the toxicity of lead is beyond the scope of this  
37 document. The effects of zinc on the toxicity of lead are discussed in a review by Krishnan and  
38 Brodeur (1991).  
39

40 Administration of zinc in the diet, but not through injection, has been shown to decrease  
41 the toxicity of dietary lead (Cerklewski and Forbes, 1976; El-Gazzar et al., 1978), possibly due  
42 to zinc decreasing the intestinal absorption of lead (Cerklewski and Forbes, 1976; Cerklewski,  
43 1979). It is not known if lead will affect the absorption of zinc. However, exposure of rats



1 whose diets contained normal (12 mg/kg) or elevated (60 mg/kg) levels of zinc to drinking water  
2 containing 20 mg Pb/L did not alter the amount of Zn or Cu in the plasma, kidney, or liver (Bebe  
3 and Panemangalore, 1996). This would suggest, though it is hardly conclusive, that lead  
4 exposure does not alter zinc absorption. Both zinc and lead have been shown to bind to the N-  
5 methyl-D-aspartate (NMDA) receptor site in rats, but lead does not appear to bind to the zinc  
6 allosteric site (Lasley and Gilbert, 1999). As noted previously, zinc and lead are substrates for a  
7 divalent metal transport protein that has been shown to participate in the absorption of iron  
8 (Gunshin et al., 1997). The relative importance of this protein in the absorptive transport of lead  
9 or zinc has not been determined.

## 10 ***Cobalt and Zinc***

11  
12  
13 Anderson et al. (1993) reported that exposure to 400 ppm cobalt chloride in the drinking  
14 water of mice for 13 weeks resulted in seminiferous tubule damage and degeneration (vacuole  
15 formation, sloughing of cells, giant cell formation) in the testes. Co-exposure to 800 ppm zinc  
16 chloride resulted in 90% of the animals exhibiting complete or partial protection against the  
17 testicular toxicity of cobalt. No studies examining the potential effects of cobalt compounds on  
18 the toxicity of zinc were identified.

## 19 20 **4.7. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND** 21 **MODE OF ACTION – ORAL AND INHALATION**

### 22 23 **4.7.1. Oral Exposure**

24  
25 The essentiality of zinc was established over 100 years ago. Zinc is essential for the  
26 function of more than 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase,  
27 copper-zinc superoxide dismutase, carboxypeptidase,  $\delta$ -aminolevulinic acid dehydratase,  
28 carbonic anhydrase, ribonucleic acid polymerase, and reverse transcriptase (Vallee and Flachuk,  
29 1993; Sandstead, 1994). A wide range of clinical symptoms have been associated with zinc  
30 deficiency in humans (Prasad, 1993; Sandstead, 1994; Walsh et al., 1994). The clinical  
31 manifestations of severe zinc deficiency, seen in individuals with an inborn error of zinc  
32 absorption or in patients receiving total parenteral nutrition, include bullous pustular dermatitis,  
33 diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in  
34 intercurrent infections. Symptoms associated with moderate zinc deficiency include growth  
35 retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark  
36 adaptation, and delayed wound healing. Neurosensory changes, impaired neuropsychological  
37 functions, oligospermia, decreased serum testosterone, hyperammonemia, and impaired immune  
38 function (alterations in T-cell subpopulations, decreased natural killer cell activity) have been  
39 observed in individuals with mild or marginal zinc deficiency. Severe zinc deficiency in animals  
40 has been associated with reduced fertility, fetal neurological malformations, and growth  
41 retardation in late pregnancy (Mahomed et al., 1989).  
42

1 Increased zinc consumption, as supplemental zinc, has been associated with health  
2 effects in humans, including decreased copper metalloenzyme activity (Fischer et al., 1984;  
3 Samman and Roberts, 1987, 1988; Yadrick et al., 1989), hematological effects (Hale et al.,  
4 1988), decreases in HDL-cholesterol levels (Hooper et al., 1980; Freeland-Graves et al., 1982;  
5 Chandra, 1984; Black et al., 1988), immunotoxicity (Chandra, 1984), and gastrointestinal effects  
6 (Freeland-Graves et al., 1982; Samman and Roberts, 1987, 1988).

7  
8 Although the decreased copper metalloenzyme activities and HDL-cholesterol levels are  
9 not necessarily adverse in themselves, they are believed to be indicators of more severe effects  
10 occurring at greater dose levels. Several human studies provide evidence that excess zinc intake  
11 may induce copper deficiency. Severe copper deficiency has been observed in individuals  
12 ingesting very high doses of zinc for over 1 year (Patterson et al., 1985; Hoffman et al., 1988).  
13 At lower zinc doses, more subtle signs of impaired copper status, such as alterations in copper  
14 metalloenzyme levels, are evident. Copper deficiency is thought to result from a zinc-induced  
15 decrease in copper absorption. Excess dietary zinc results in induction of intestinal  
16 metallothionein synthesis; because metallothionein has a greater binding capacity for copper  
17 than for zinc, copper absorbed into the intestinal mucosal cells is sequestered by metallothionein  
18 and not absorbed systemically (Walsh et al., 1994). Zinc and copper may also be substrates for a  
19 divalent metal transport protein in the small intestine (Gunshin et al., 1997). Although studies by  
20 Samman and Roberts (1987, 1988), Fischer et al. (1984), and Yadrick et al. (1989) failed to find  
21 decreases in plasma copper levels after zinc supplementation, these studies did find alterations in  
22 serum ceruloplasmin and erythrocyte superoxide dismutase activities. As discussed in Fischer et  
23 al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than  
24 plasma copper levels.

25  
26 While the exact function of high density lipoproteins (HDL) is not known, they are  
27 thought to function in the transfer of cholesterol from extrahepatic tissue to the liver. The results  
28 of epidemiology studies suggest an association between high concentrations of HDL with a  
29 reduced risk of coronary heart disease. As compared to all lipids and lipoproteins measured,  
30 HDL may have the largest impact on risk of coronary heart disease in individuals over 50 years  
31 old (Simko et al., 1984). Normal levels of HDL-cholesterol are 45.5 mg/dL in men and 55.5  
32 mg/dL in women. HDL-cholesterol levels below 35 mg/dL have been associated with an  
33 increased risk of coronary heart disease (Simko et al., 1984). Collectively, the human data  
34 suggest that short-term ( $\leq 12$  weeks) increases in zinc intake result in decreases in HDL-  
35 cholesterol levels. In the Hooper et al. (1980) and Chandra (1984) studies, in which subjects  
36 received daily doses of 2 or 4 mg supplemental zinc/kg-day for up to 6 weeks, the HDL-  
37 cholesterol levels dropped below 35 mg/dL. Although zinc-induced decreases in HDL-  
38 cholesterol have been observed, a relationship between increased zinc intake and an increased  
39 risk of coronary heart disease has not been established.

40  
41 Following high-level oral exposure, zinc appears to exert toxic effects primarily through  
42 interaction with copper. Specifically, high levels of zinc can result in a saturation of the carrier-  
43 mediated pathway of zinc absorption and a shift to metallothionein-mediated absorption (Hempe

1 and Cousins, 1992). It is believed that the copper deficiency results from a zinc-induced  
2 decrease in copper absorption. Zinc-induced copper deficiency is consistent with numerous  
3 reports of effects of zinc on various biomarkers of copper nutritional status following exposures  
4 to elevated levels of zinc in humans and animals, as well as by reports indicating that copper  
5 supplementation can result in an attenuation of zinc-induced toxicity.  
6

7 While co-exposure to zinc has been demonstrated to alter the toxicity of a number of  
8 other metals, few studies have been conducted on the effects of co-exposure to metals (other than  
9 copper) on zinc toxicity. The available studies suggest the plausibility that co-exposure to other  
10 divalent metals may decrease absorption of zinc, but offer only limited insight as to potential  
11 effects of these metals on zinc toxicity. The few studies that have been conducted on the effect  
12 of other metals on the toxicity of zinc are not adequate to support dose response assessments for  
13 the interactions, or even qualitative assessments of the type or direction of the interaction (e.g.,  
14 antagonism, synergism), particularly under subchronic or chronic exposure conditions.  
15

#### 16 **4.7.2. Inhalation Exposure**

17  
18 Most of the available information on the toxicity of inhaled zinc has focused on metal  
19 fume fever, a collection of symptoms observed in individuals exposed to freshly formed zinc  
20 oxide fumes or zinc chloride from smoke bombs. The earliest symptom of metal fume fever  
21 (also referred to as zinc fume fever, zinc chills, brass founder's ague, metal shakes, or Spelter's  
22 shakes) is a metallic taste in the mouth accompanied by dryness and irritation of the throat. Flu-  
23 like symptoms, chills, fever, profuse sweating, headache, and weakness follows (Drinker et al.,  
24 1927a; Sturgis et al., 1927; Rohrs, 1957; Malo et al., 1990). The symptoms usually occur within  
25 several hours after exposure to zinc oxide fumes and persist for 24 to 48 hours. An increase in  
26 tolerance develops with repeated exposure; however this tolerance is lost after a brief non-  
27 exposure period, and symptoms are most commonly reported on Mondays and after holidays.  
28 There are many reports of metal fume fever in the literature; however, most describe individual  
29 cases and exposure levels are not known.  
30

31 In animals, exposure to zinc oxide results in similar effects as those reported in humans.  
32 Gordon et al. (1992) examined the effects of zinc oxide in rabbits, rats, and guinea pigs, and  
33 reported changes in lavage parameters which appeared to correlate with pulmonary retention of  
34 the zinc particles. In a series of studies in guinea pigs, Lam et al. (1988) reported that ultrafine  
35 zinc oxide particles resulted in significant respiratory effects, including decreased lung function  
36 and increased lung weight. However, subchronic or chronic studies of the toxicity of zinc  
37 following inhalation exposure in animals are not available. Similarly, no studies examining the  
38 effects of inhaled zinc on reproductive or developmental endpoints were located.  
39

40 The mechanisms behind metal fume fever are not known, but are believed to involve  
41 several different factors. Exposure to zinc oxide particles has been shown to elicit the release of  
42 a number of proinflammatory cytokines, leading to a persistent pulmonary inflammation which  
43 could result in some of the reported symptoms of metal fume fever, including decreased lung

1 function and bronchoconstriction. An allergic response to zinc particles, leading to an asthma-  
2 like response, has also been proposed as a possible mechanism. However additional mechanistic  
3 information will be required in order to adequately determine the mechanisms involved in the  
4 toxicity of inhaled zinc.

#### 5 6 **4.8. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER** 7 **CHARACTERIZATION** 8

9 Under the 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), zinc is  
10 classified in Group D, *Not Classifiable as to Human Carcinogenicity*, based on inadequate  
11 evidence of carcinogenicity in humans and animals. Under the proposed guidelines (U.S. EPA,  
12 1999), *data are inadequate for an assessment of human carcinogenic potential* of zinc, because  
13 studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate  
14 animal bioassays of the possible carcinogenicity of zinc are not available, and tests of the  
15 genotoxic effects of zinc have been equivocal.

16  
17 Adequate studies examining the carcinogenicity of zinc in orally-exposed humans are not  
18 available. Prasad et al. (1978) reported on sickle cell anemia patients who were treated with zinc  
19 for 2 years; however, carcinogenic endpoints were not evaluated. Aughey et al. (1977) did not  
20 find pancreatic, pituitary, or adrenal tumors in C3H mice exposed to zinc sulfate in the drinking  
21 water for up to 14 months; however, histopathology of other organs was not reported.  
22 Additional data on the carcinogenicity of zinc following oral exposure are not available. While a  
23 number of studies of the effects of short-term exposure to zinc in the workplace are available, the  
24 vast majority of these focus on the more acute effects of zinc, particularly metal fume fever and  
25 its resulting sequelae. No studies adequately examining the carcinogenic effects of zinc in  
26 humans or animals were located in the available literature.

27  
28 Either zinc deficiency or excessively high levels of zinc may enhance susceptibility to  
29 carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer  
30 protection (Mathur et al., 1979; Woo et al., 1988). For example, zinc deficiency enhanced  
31 carcinomas of the esophagus induced by methylbenzyl nitrosoamine (Fong et al., 1978) but  
32 retarded the development of cancer of the oral cavity induced by 4-nitroquinoline-N-oxide  
33 (Wallenius et al., 1979). Thus, zinc's modifying effect on carcinogenesis may depend both on  
34 the dose of zinc and the identity of the carcinogen being affected. The genotoxicity of zinc,  
35 particularly in *S. typhimurium*, appears to depend greatly on the chemical form (e.g., inorganic or  
36 organic salt).

#### 37 38 **4.9. SUSCEPTIBLE POPULATIONS** 39

##### 40 **4.9.1. Possible Childhood Susceptibility** 41

42 Data in humans are not available that examine whether children are more susceptible to  
43 the toxicity of zinc than adults. However, the recommended dietary allowance (RDA) for

1 children, expressed in terms of mg/kg-day, is greater than that for adults. Animal studies have,  
2 however, suggested that neonates and/or developing animals may be more susceptible to the  
3 toxic effects of excess zinc. Bleavins et al. (1983) reported that in minks exposed to 56 mg  
4 Zn/kg-day throughout gestation and weaning, no changes were seen in exposed adults, but 3-4  
5 week-old kits exhibited achromotrichia, thought to be associated with copper deficiency. Signs  
6 of copper deficiency progressed as zinc exposure continued. Several other studies have  
7 examined the effects of zinc exposure in young animals, but have not provided data on adult  
8 animals similarly exposed for comparison. Additional data will be required to adequately assess  
9 the susceptibility of children to zinc exposure, relative to adults.

#### 10 **4.9.2. Possible Gender Differences**

11  
12  
13 Several studies in humans have suggested that females may be more sensitive to the  
14 adverse effects of excess zinc than males. For example, Samman and Roberts (1987, 1988)  
15 reported that women experienced adverse symptoms more frequently (84% in women vs. 18% in  
16 men), as well as being more susceptible to zinc-induced changes in LDL cholesterol levels,  
17 serum ceruloplasmin, and erythrocyte superoxide dismutase. However, women in this study  
18 received a higher average dose (2.5 mg/kg-day) than did the corresponding men  
19 (2.0 mg/kg-day). In contrast, Hale et al. (1988) reported that in elderly subjects, zinc-exposed  
20 women did not experience the same reduction in the incidence of anemia as was seen in zinc-  
21 exposed men. Further data examining the potential difference in response between men and  
22 women were not located. However, the studies of Yadrick et al. (1989) and Fischer et al. (1984)  
23 reported similar effect levels on superoxide dismutase enzyme levels, expressed as mg total  
24 zinc/kg-day, in men and women.

25  
26 In animal studies, however, it appears that if any differences between sexes were noted,  
27 the male is the more susceptible gender. For example, Maita et al. (1981) reported changes in  
28 body weight, altered clinical chemistry, and decreased liver and spleen weights in male rats, but  
29 not in female rats, exposed to 572 mg Zn/kg-day. Also, studies of reproductive ability have  
30 demonstrated alterations in spermatogenesis at zinc exposure levels below those inducing  
31 alterations in female reproductive parameters have been reported to occur (Sutton and Nelson,  
32 1937; Pal and Pal, 1987; Saxena et al., 1989; Evenson et al., 1993). However, other studies  
33 (Aughey et al., 1977; Zaporowska and Wasilewski, 1992) have not reported significant  
34 differences between male and female animals exposed to zinc. Additional studies will be  
35 required to determine whether sex-specific differences in adverse responses to zinc exist.

## 5. DOSE-RESPONSE ASSESSMENTS

### 5.1. ORAL REFERENCE DOSE (RfD)

#### 5.1.1. Choice of Principal Study and Critical Effect

Available studies of oral zinc toxicity have identified a number of health effects in humans, including decreased copper metalloenzyme activity (Fischer et al., 1984; Samman and Roberts, 1987, 1988; Yadrick et al., 1989), hematological effects (Hale et al., 1988), decreases in HDL-cholesterol levels (Hooper et al., 1980; Freeland-Graves et al., 1982; Chandra, 1984; Black et al., 1988), immunotoxicity (Chandra, 1984), and gastrointestinal effects (Samman and Roberts, 1987, 1988). The available data indicate that the most sensitive effects of zinc are alterations in copper status. It is believed that the copper deficiency results from a zinc-induced decrease in copper absorption. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels. For example, although studies by Samman and Roberts (1987, 1988); Fischer et al. (1984), and Yadrick et al. (1989) failed to find decreases in plasma copper levels, these studies did find alterations in serum ceruloplasmin and erythrocyte superoxide dismutase activities. It follows that while the decreased copper metalloenzyme activities seen in several of the human studies are not necessarily adverse in themselves, they may be indicative of more severe effects occurring at greater exposure levels. Additional support for the selection of the critical endpoint comes from the rat study of L'Abbe and Fischer (1984a), which noted that changes in indicators of copper status in rats exposed to supplemental zinc in the diet for 6 weeks were dose-related.

The two studies that identified the lowest effect levels for changes in copper status are those of Yadrick et al. (1989) and Fischer et al. (1984). These studies each identified a LOEL for decreased levels of erythrocyte superoxide dismutase (ESOD), an indicator of body copper status. As this effect was not considered adverse of itself, but rather a precursor for more serious effects, it was designated a NOAEL. No measurements were made of dietary zinc or copper in either study. However, a level of dietary zinc was estimated at 9.38 mg/day for females (25-30 years old) and 15.92 mg/day for males (25-30 years old) from the results of the FDA Total Diet Study for 1982-1986 (Pennington and Schoen, 1996b). Adding 9.38 mg/day to the NOAEL of 50 mg supplemental zinc/day from the Yadrick et al. (1989) study, and dividing by an assumed body weight of 60 kg for adult females, gives a NOAEL of 0.99 mg zinc/kg-day. Similarly, adding 15.92 mg/day to the 50 mg supplemental zinc/day from the Fischer et al. (1984) study, and dividing by the reference body weight of 70 kg for adult males, gives a NOAEL of 0.94 mg zinc/kg-day. As these NOAEL values for the same endpoint are similar, the Yadrick et al. (1989) and Fischer et al. (1984) studies were selected as co-critical studies for derivation of the RfD. The Yadrick et al. (1989) study was the key study for a previous RfD, which was verified by the RfD/RfC workgroup (U.S. EPA, 1995c).

1 **5.1.2. Methods of Analysis**

2  
3 A NOAEL/LOAEL approach was applied to derive the RfD. A Benchmark Dose (BMD)  
4 approach was considered, but was not utilized for this assessment. Both the Yadrick et al.  
5 (1989) and Fischer et al. (1984) studies examined only one dose level, apart from controls,  
6 making either unsuitable for benchmark analysis.  
7

8 **5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UF) and Modifying**  
9 **Factors (MF)**

10  
11 According to a recent report by the National Academy of Sciences, the average daily  
12 intake for zinc among the U.S. population is 10-16 mg/day. Based on an average human body  
13 weight of 70 kg, this equates to 0.14-0.21 mg/kg-day. The recently-derived recommended  
14 dietary allowances (RDA; IOM, 2002) are 11 mg/day for men and 8 mg/day for women; using  
15 reference body weights of 70 kg for men and 60 kg for women, these equate to 0.16 mg/kg-day  
16 for men and 0.13 mg/kg-day for women. Therefore, recommendation of a risk value below the  
17 range of 0.13-0.21 mg/kg-day, which represent both the daily intake levels necessary for normal  
18 health and the average daily intake of the U.S. population, is contraindicated.  
19

20 To the NOAEL of 0.94 mg/kg-day for decreased ESOD levels in humans identified by  
21 Fischer et al. (1984), an uncertainty factor (UF) of 3 was applied, to account for uncertainties  
22 with using a moderate-duration study in humans, intrahuman variability, and consideration of a  
23 substance that is an essential dietary nutrient. The modifying factor (MF) was set to 1. From  
24 these, the RfD for zinc is derived as follows:  
25

$$\begin{aligned} \text{RfD} &= \text{NOAEL} \div (\text{UF} \times \text{MF}) \\ &= 0.94 \text{ mg/kg-day} \div (3 \times 1) \\ &= 0.3 \text{ mg/kg-day} \end{aligned}$$

26  
27  
28  
29  
30 The level of confidence in the key studies is medium since they are well-conducted  
31 clinical studies with relevant biochemical parameters investigated in both males (Fischer et al.,  
32 1984) and females (Yadrick et al., 1989), but had a limited number of study subjects. The  
33 confidence in the overall database is medium since the available suitable human studies are all of  
34 moderate duration and chronic animal data are limited. Medium confidence in the RfD follows.  
35

36 **5.2. INHALATION REFERENCE CONCENTRATION (RfC)**

37  
38 Available data on humans exposed to zinc compounds by inhalation are limited to reports  
39 of acute exposures to zinc oxide or zinc chloride. Similarly, available studies in animals have  
40 been of acute duration, and are, therefore, not suitable for use in derivation of an RfC. A route-  
41 to-route extrapolation from the oral data was considered, but was not attempted as available data  
42 from acute inhalation studies suggest that significant portal of entry effects will occur. Lacking  
43 suitable data, derivation of an inhalation RfC for zinc compounds is precluded.

1 **5.3. CANCER ASSESSMENT**

2  
3 **5.3.1. Oral Slope Factor**

4  
5 Data are inadequate for the derivation of an oral slope factor for zinc. No human studies  
6 examining the oral carcinogenicity of zinc or zinc compounds were located. A 1-year study in  
7 mice (Walters and Roe, 1965) did not find increases of malignant lymphoma, lung adenoma, or  
8 hepatoma. The study did not report on the incidence of any other types of tumors, nor did it  
9 perform adequate histologic analysis of other tissues. Similarly, Aughey et al. (1977) did not  
10 observe increases in tumors of the pancreas, pituitary gland, or adrenal gland in mice exposed to  
11 zinc for 14 months; however, observations from other organs were not reported. A study by  
12 Halme (1961) reported potential increases in zinc-induced tumors in a multi-generation study in  
13 rats, but was not sufficiently descriptive to allow for a complete evaluation of the study. No  
14 other animal studies of the oral carcinogenicity of zinc were identified. Lack of data, therefore,  
15 precludes the derivation of an oral slope factor.

16  
17 **5.3.2. Inhalation Unit Risk**

18  
19 Data are inadequate for the derivation of an inhalation unit risk for zinc. No suitable  
20 human or animal studies were identified which examined the carcinogenicity of zinc following  
21 chronic inhalation exposure.



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

3  
4           **6.1. HUMAN HAZARD POTENTIAL**

5  
6           Zinc is an essential element, necessary for the function of more than 300 enzymes. A  
7 wide range of clinical symptoms have been associated with zinc deficiency in humans (Prasad,  
8 1993; Sandstead, 1994; Walsh et al., 1994), though generally only with chronically-severe or  
9 moderately-severe deficiency. Oral exposure to high levels of zinc in humans can result in  
10 several systemic effects, the most sensitive of which are related to diminished copper status. As  
11 discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of  
12 copper status than plasma copper levels. These sensitive indicators of copper status, which may  
13 not be adverse in themselves, can be considered as precursor events to more severe copper-  
14 deficiency-induced changes.

15  
16           The majority of the inhalation data on zinc focuses on short-term inhalation of zinc oxide  
17 or zinc chloride, resulting in metal fume fever. The earliest symptoms of metal fume fever are a  
18 metallic taste in the mouth accompanied by dryness and irritation of the throat. Flu-like  
19 symptoms, chills, fever, profuse sweating, headache, and weakness follow (Drinker et al.,  
20 1927a,b; Sturgis et al., 1927; Rohrs, 1957; Malo et al., 1990). The symptoms usually occur  
21 within several hours after exposure to zinc oxide fumes and persist for 24 to 48 hours. An  
22 increase in tolerance develops with repeated exposure; however this tolerance is lost after a brief  
23 non-exposure period. Studies of the health effects of subchronic or chronic exposure to inhaled  
24 zinc compounds were not located in the available literature.

25  
26           Under the 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), zinc is  
27 classified in group D, *Not Classifiable as to Human Carcinogenicity*, based on inadequate  
28 evidence of carcinogenicity in humans and animals. Under the proposed guidelines (U.S. EPA,  
29 1999), *data are inadequate for an assessment of human carcinogenic potential* of zinc, because  
30 studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate  
31 animal bioassays of the carcinogenicity of zinc are not available, and tests of the genotoxic  
32 effects of zinc have been equivocal.

33  
34           **6.2. DOSE RESPONSE**

35  
36           **6.2.1. Noncancer/Oral**

37  
38           The most sensitive effects of oral exposure to excess zinc in humans involve the copper  
39 status of the body. Zinc exposure can result in a decreased absorption of copper, leading to low  
40 systemic copper levels and subsequent health effects, including decreased copper metalloenzyme  
41 activity, hematological effects, decreases in cholesterol levels, immunotoxicity, and  
42 gastrointestinal effects. While changes such as decreased copper metalloenzyme levels may not  
43 be adverse in themselves, they have been demonstrated to be precursor events for more severe

1 effects. The study of Yadrick et al. (1989) established a NOAEL of 0.99 mg Zn/kg-day for  
2 decreased levels of erythrocyte superoxide dismutase, an indicator of body copper status, in  
3 women exposed for 10 weeks, while the study of Fischer et al. (1984) established a NOAEL of  
4 0.95 mg Zn/kg-day for the same endpoint in men exposed for 6 weeks. An uncertainty factor of  
5 3 (for a moderate-duration study and consideration of a substance that is an essential dietary  
6 nutrient) was applied to the NOAEL of 0.95 mg Zn/kg-day to give an RfD of 0.3 mg Zn/kg-day.  
7 Confidence in the key studies is medium since they are well-conducted clinical studies with  
8 relevant biochemical parameters investigated in both males (Fischer et al., 1984) and females  
9 (Yadrick et al., 1989), but had a limited number of study subjects. The confidence in the overall  
10 database is medium since the available human studies are of moderate duration and chronic  
11 animal data are limited. There is medium confidence in the resulting RfD.  
12

### 13 **6.2.2. Noncancer/Inhalation**

14  
15 Data on the effects of inhaled zinc are primarily limited to short-term studies examining  
16 metal fume fever in occupationally-exposed humans. Studies in animals are not sufficient for the  
17 derivation of an RfC, owing mainly to insufficient duration or other study limitations. Lacking  
18 suitable data, derivation of an inhalation RfC is precluded.  
19

### 20 **6.2.3. Cancer/Oral and Inhalation**

21  
22 Data in both humans and animals are inadequate to evaluate potential associations between  
23 zinc exposure and cancer. Under the 1986 Guidelines for Carcinogen Risk Assessment (U.S.  
24 EPA, 1986a), zinc is classified in group D, *Not Classifiable as to Human Carcinogenicity*, based  
25 on inadequate evidence of carcinogenicity in humans and animals. Under the proposed  
26 guidelines (U.S. EPA, 1999), *data are inadequate for an assessment of human carcinogenic*  
27 *potential* of zinc, because studies of humans occupationally-exposed to zinc are inadequate or  
28 inconclusive, adequate animal bioassays of the possible carcinogenicity of zinc are not available,  
29 and tests of the genotoxic effects of zinc have been equivocal.

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