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# TOXICOLOGICAL REVIEW

# OF

# ZINC AND COMPOUNDS

(CAS No. 7440-66-6)

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

*July 2005*

U.S. Environmental Protection Agency
Washington D.C.

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

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. This discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676.

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This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information, and EPA's regional offices.

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

# 1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of zinc and compounds. IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD is an estimate of an oral exposure for [a given duration], to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a statistical lower confidence limit on the benchmark dose (BMDL), a no-observed-adverse effect-level (NOAEL), a lowest-observed-adverse-effect level (LOAEL), or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used. The RfD is expressed in units of mg/kg-day. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of $mg/m^3$.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways to better facilitate their use: (1) generally, the *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day of oral exposure; (2) the *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ continuous airborne exposure; and (3) the 95% lower bound and central estimate on the estimated concentration of the chemical substance in drinking water or air that presents cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for zinc and compounds has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment

include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *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), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. 1995a), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998), and *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through October, 2004.

## 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 2-1.

**Table 2-1. Chemical and physical properties of zinc and selected zinc compounds**

| | Zinc | Zinc oxide | Zinc chloride | Zinc sulfate | Zinc sulfide |
|---|---|---|---|---|---|
| CAS Registry Number | 7440-66-6 | 1314-13-2 | 7646-85-7 | 7733-02-0 | 1314-98-3 |
| Molecular formula | Zn | ZnO | $ZnCl_2$ | $ZnSO_4$ | 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 | $\sim 2 \times 10^{-3}$ | $4.3 \times 10^{3}$ | $1.7 \times 10^{3}$ | $\sim 7 \times 10^{-3}$ |
| Density ($g/cm^3$) | 7.14 | 5.607 | 2.907 | 3.54 | ~4.1 |

Source: ATSDR, 1995; Barceloux, 1999.

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 in Table 2-2.

**Table 2-2. Zinc commercial minerals, molecular composition, and percentage of zinc**

| Name | Composition | % Zinc |
|---|---|---|
| Sphalerite | ZnS | 67.0 |
| Hemimorphite | $Zn_4Si_2O_7(OH)_2H_2O$ | 54.2 |
| Smithsonite | $ZnCO_3$ | 52.0 |
| Hydrozincite | $Zn_5(OH)_6(CO_3)_2$ | 56.0 |
| Zincite | ZnO | 80.3 |
| Willemite | $Zn_2SiO_4$ | 58.5 |
| Franklinite | $(Zn,Fe,Mn)(Fe,Mn)_2O_4$ | 15-20 |

Source: Goodwin, 1998.

The primary anthropogenic sources of zinc in the environment are from 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, corrosion control in drinking water systems, photographic paper, vulcanization acceleration for rubber, ceramics, textiles, fertilizers, pigments, batteries, and as nutritional supplements or medicines (ATSDR, 1995). Zinc chloride is a primary ingredient in smoke bombs used for crowd dispersal, in fire-fighting exercises (by both military and civilian communities), and by the military for screening purposes. Zinc chloride, zinc sulfate, zinc oxide, and zinc sulfide have dental, medical, and household applications. Zinc chloride and zinc sulfate are also used in herbicides (ATSDR, 1995). Zinc compounds are usually colorless which is advantageous since

they do not color paints, plastics, rubber, or cosmetics to which they might be added. However, zinc oxide and zinc sulfide exhibit luminescence when excited by UV-Vis radiation.

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

In natural waters, zinc can be found in several chemical forms, such as hydrated ions, metal-inorganic complexes, or metal-organic complexes (U.S. EPA, 1979). Hydrated zinc cations may be hydrolyzed to form zinc hydroxide or zinc oxide (U.S. EPA, 1979). In anaerobic environments, Zinc sulfide may be formed (U.S. EPA, 1979). Zinc accumulates in aquatic organisms, and bioconcentration factor values for freshwater fish and marine fish were reported as 1000 and 2000, respectively (U.S. EPA, 1979).

As discussed in Section 4.1, zinc is an essential element in humans. In adults, the greatest dietary sources of zinc are meats, dairy products, grains, and mixed dishes (Pennington et al., 1989), while fruits, nuts, fats, sweeteners, and beverages contribute comparatively 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. The North American adult diet contains about 8-15 mg Zn/day based on data from the 1988-1994 National Health and Nutrition Examination Survey (IOM, 2001). Zinc uptake from a normal diet 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 as the dose increases; 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 (2000), 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). It is notable that these studies generally used water-soluble forms of zinc; as zinc appears to be absorbed as zinc ion, less soluble forms would be expected to show a lower level of gastrointestinal absorption.

Zinc appears to be absorbed by both passive diffusion and a saturable carrier-mediated process (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 absorption 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 intestinal mucosa it is initially associated with CRIP, with only a small fraction binding to metallothionein, but as zinc concentrations rise, the binding to CRIP becomes saturated, the proportion of zinc binding to CRIP decreases, and more zinc is bound to metallothionein (Hempe and Cousins, 1992).

Evans (1976) proposed that zinc bound to ligands is transported into epithelial cells where the metal is transferred to the binding site on the plasma membrane. Metal-free albumin then interacts with the plasma membrane and removes zinc from the receptor site. The quantity of metal-free albumin available probably determines the amount of zinc removed from the epithelial cell, and thus regulates the quantity of zinc that enters the body. Several dietary factors can influence zinc absorption, including other trace elements (e.g., copper, iron, lead, calcium, cadmium, cobalt; see Section 4.6.2), amino acids, simple and complex carbohydrates, and protein. High levels of phytate or phosphate in the diet can decrease the amount of zinc absorbed (Pecoud et al., 1975; Larsson et al., 1996; Oberleas, 1996). Oberleas (1996) suggested that the phytate in the food provided to test subjects complexes with endogenous zinc ions secreted from the pancreas, thus preventing its reabsorption and increasing fecal zinc elimination. In general, low molecular weight substances, such as amino acids, increase the absorption of zinc (Wapnir and Stiel, 1986). Imidazole, tryptophan, proline, and cysteine increased zinc absorption from various regions of the gastrointestinal tract. Wapnir and Stiel (1986) suggested that the increase was due to the presence of both mediated and non-mediated transport mechanisms for amino acids. Absorption is inhibited by certain proteins (e.g., bovine serum albumin and dephytinized soyabean protein isolate), is unaffected by others (e.g., bovine whey) (Davidsson et al., 1996), and enhanced by others (e.g., casein) (Hunt et al., 1991; Davidsson et al., 1996).

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

### 3.1.2. Respiratory Tract Absorption

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

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

## 3.2. DISTRIBUTION

Zinc is an essential human nutrient, a cofactor for over 300 enzymes, and is found in all tissues. In humans, the highest concentrations of zinc have been found in bone, muscle, prostate, liver, and kidneys (Schroeder et al., 1967; Wastney et al., 1986). Similar distributions have been found in animals (Ansari et al., 1975, 1976; Llobet et al., 1988). Less than 10% of the body's total zinc is readily exchanged with plasma (Miller et al., 1994) and most of this is from the slow exchange of zinc located in bone and muscle. In blood, zinc is found in plasma, erythrocytes, leukocytes, and platelets. Approximately 98% of serum zinc is bound to proteins; 85% is bound to albumin, 12% to $\alpha_2$-macroglobulin, and the remainder to amino acids (Giroux et al., 1976). In

erythrocytes, zinc is predominantly found as a component of carbonic anhydrase (87%) and Cu, Zn-superoxide dismutase (5.4%) (Ohno et al., 1985).

Ansari et al. (1975) examined the heart, liver, kidneys, muscle, tibia, and small intestine for changes in tissue zinc concentration following the addition of 600 ppm supplemental zinc to the diet of male rats for up to 42 days. While small increases in tissue zinc levels relative to controls were reported, only occasionally were the differences statistically significant, and no pattern with increasing tissue zinc with time was noted. In a later study, Ansari et al. (1976) exposed male rats to up to 8400 ppm supplemental zinc as zinc oxide in the diet for 21 days then examined the liver, kidney, heart, tibia, and muscle for tissue zinc concentrations. Exposure to 1200 ppm had no significant effect on tissue zinc levels relative to controls; the amount of stable zinc in liver, kidney, and bone was increased at 2400 ppm and higher, but reached a plateau (2400-7200 ppm; approximately 200-625 mg/kg-day). Exposure at the highest level (8400 ppm) caused additional increases in liver, kidney, and bone, as well as an increase in zinc level in the heart. No changes in zinc concentration were seen in the skeletal muscle. Similar results for the accumulation of zinc in organs have been found in mice (He et al., 1991), rabbits (Bentley and Grubb, 1991), and wood mice *(Apodemus sylvaticus L.*) (Cooke et al., 1990).

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

### 3.3. METABOLISM

Zinc is a metallic element that is found in the body as a divalent cation. Accordingly, it does not undergo metabolism. It interacts electrostatically with anions (i.e., carbonate, hydroxide, oxalate, phytate) and negatively charged moieties on macromolecules such as proteins. It can also form soluble chelation complexes with amino acids and multidentate organic acids such as ethylenediaminetetraacetic acid.

### 3.4. ELIMINATION AND EXCRETION

Following oral exposure, zinc is primarily excreted via the gastrointestinal tract and eliminated in the feces; approximately 70-80% of an ingested dose is excreted in the feces (Davies and Nightingale, 1975). Oberleas (1996) found that the pancreas secretes into the

duodenum two to four times the amount of zinc that is typically consumed in an average day; most of this secreted zinc is reabsorbed. Zinc is also excreted in the urine. In humans, approximately 14% of the eliminated zinc was excreted in urine; when zinc intake was increased, urinary excretion accounted for 25% of the eliminated zinc (Wastney et al., 1986). Other minor routes of elimination are sweat (Prasad et al., 1963), saliva secretion (Greger and Sickles, 1979), and incorporation into hair (Rivlin, 1983).

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

### 3.5. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS

Physiologically based toxicokinetic models have been developed to assess environmental exposure levels for other metals such as cadmium and lead. However, no toxicokinetic models have been developed for zinc in either human or animal species.

# 4. HAZARD IDENTIFICATION

## 4.1. ESSENTIALITY OF ZINC

While the focus of this document, and the values derived in Chapter 5, is on the effects of excess zinc exposure, rather than the effects of insufficient zinc intake, a discussion of the importance of zinc as a dietary nutrient is relevant when considering the effects of zinc exposure. 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, Cu, Zn-superoxide dismutase, carboxypeptidase, δ-aminolevulinic acid dehydratase (ALAD), carbonic anhydrase, deoxyribonucleic acid (DNA) polymerases (DNA polymerase alpha, DNA polymerase III), and reverse transcriptase (Vallee and Falchuk, 1993; Sandstead, 1994). A list of key enzymes containing zinc or affected by zinc status are provided in Table 4-1. Zinc has three functions in these metalloenzymes: participation in catalytic functions, maintenance of structural stability, and regulatory functions (Vallee and Falchuk, 1993; Walsh et al., 1994). Zinc is also involved in DNA and ribonucleic acid (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 ionotrophic 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 (Abernathy et al., 1993; 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 lacking in adequate zinc, 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 (hypogeusia, decreased dark adaptation), impaired neuropsychological functions (dysosmia, irritability, and reduced cognitive function), 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 significant intrauterine growth retardation and fewer inductions of labor (generally associated with poor fetal growth), and non-statistically significant decreases in birth weight and placental weights in zinc-deficient women compared to women receiving a supplement containing 100 mg zinc citrate (22.5 mg zinc). The women receiving the supplement had been selected 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 pregnant women receiving 20 mg Zn/day as zinc sulfate (66 mg zinc sulfate) tablets beginning before the 20$^{th}$ week of pregnancy as compared to 248 women receiving placebo tablets. While the zinc supplement and placebo group had marginal zinc intake (approximately 10 mg/day) prior to supplementation, the zinc supplementation did not appear to influence pregnancy outcome. The author commented that the women recruited in this study were from mid-socioeconomic groups. Endogenous stores of zinc could possibly have met the need for fetal development.

**Table 4-1. Key enzymes containing zinc or affected by zinc status**

| Enzyme name (symbol) | Alternative titles (symbol) | Reaction catalyzed | Cofactor(s) | Enzyme commission number (EC)[a] |
|---|---|---|---|---|
| Cu, Zn-superoxide dismutase | Superoxide dismutase, cytosolic; Superoxide dismutase 1 | $2\ O_2^{\cdot-} + 2\ H^+ <=> O_2 + H_2O_2$ | Copper and zinc | 1.15.1.1 |
| Erythrocyte Cu, Zn-superoxide dismutase | Superoxide dismutase, cytosolic; Superoxide dismutase 1 | $2\ O_2^{\cdot-} + 2\ H^+ <=> O_2 + H_2O_2$ | Copper and zinc | 1.15.1.1 |
| Extracellular Cu, Zn-superoxide dismutase | Superoxide dismutase, extracellular | $2\ O_2^{\cdot-} + 2\ H^+ <=> O_2 + H_2O_2$ | Copper and zinc | 1.15.1.1 |
| Cytochrome c oxidase | Ferrocytochrome c oxidase | 4 ferrocytochrome c + $O_2 <=> 2H_2O$ + 4 ferricytochrome c | Copper | 1.9.3.1 |
| Ceruloplasmin | Ferroxidase | $4\ Fe^{2+} + 4\ H^+ + O_2 <=> 4\ Fe^{3+} + 2\ H_2O$ | Copper | 1.16.3.1 |
| Metallothionein | Metallothionein 1A | Cysteine residues complex with zinc, cadmium, and copper to form mercaptide linkages | N/A[b] | N/A |

[a] EC numbers specify enzyme catalyzed reactions, not specific enzymes.
[b] Not applicable

Sources: McKusick, 1998; Bairoch and Apweiler, 1999.

The zinc content of a typical mixed diet of North American adults is approximately 10-15 mg/day (IOM, 2001). The U.S. Food and Drug Administration's (FDA) Total Diet Study (Pennington and Schoen, 1996) found zinc intakes of 7.25, 9.74, 15.42, 9.38, and 15.92 mg/day in children (2 years of age), girls (14-16 years), boys (14-16 years), women (25-30 years), and men (25-30 years), respectively. The 2000 recommended dietary allowances (RDAs) for zinc (IOM, 2001) are presented in Table 4-2.

## 4.2. STUDIES IN HUMANS

Human studies have investigated the effects of dietary zinc supplementation. High doses can cause clinical symptoms of gastrointestinal distress, while low doses primarily affect the status of other essential nutrients such as copper and iron.

### 4.2.1. Oral Exposure

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

**Table 4-2. Recommended dietary allowances (RDA) by life stage group and gender**

| Life stage group | RDA (mg/day) | |
|---|---|---|
| | Male | Female |
| 0 through 6 months | 2[a] | 2[a] |
| 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 |

[a]Acceptable daily intake. No RDA value was reported.

Source: IOM, 2001.

Fischer et al. (1984) instructed groups of 13 healthy adult male volunteers (ages not specified) to take capsules containing 0 (cornstarch) or 25 mg supplemental zinc (as zinc gluconate) twice daily for 6 weeks; using a reference body weight of 70 kg for an adult male, average daily dose was 0.71 mg supplemental Zn/kg-day. Nonfasting blood samples were taken at the beginning and at biweekly intervals and tested for measures of copper status. Plasma copper levels and levels of ceruloplasmin's ferroxidase activity did not change during the course of the study. However, ESOD activity decreased after 4 weeks in the supplement group and was significantly lower than controls by 6 weeks. An inverse correlation between plasma zinc levels and ESOD activity was also observed at 6 weeks.

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

Recently, Davis et al. (2000) and Milne et al. (2001) have reported the results of exposure of a group of postmenopausal women (aged 50-76, mean of 64.9 ± 6.7 years) to varying concentrations of zinc and copper in the diet. Average height was 159.6 ± 7.6 cm, and mean body weight was 65.1 ± 9.5 kg. Subjects were kept in a metabolic ward for a 200-day period, and fed a controlled basal diet that contained 0.6 mg copper and 3 mg zinc. For the first 10 days, all subjects consumed an equilibration diet, which consisted of the basal diet supplemented with 1.4 mg copper (2 mg total) and 6 mg zinc (9 mg total). Following an initial 10-day equilibration, one group (n=12) was exposed to the basal diet supplemented with 0.4 mg Cu/day (1 mg Cu/day total) and the other group (n=13) was fed the basal diet supplemented with 2.4 mg Cu/day (3.0 mg Cu/day total). The remaining 190 days were divided into two 90-day study periods for both groups: the copper-supplemented basal diet (1 mg Cu/day, total) with no zinc supplement was fed for the first 90-day period and the copper-supplemented (1 mg Cu/day, total) basal diet supplemented with 50 mg Zn/day was fed for the second 90-day period. The two 90-day periods were separated by an additional equilibration period, identical to the one performed at the beginning of the study.

During each of the equilibration periods, and twice monthly during the exposure periods, blood was drawn from the subjects after an overnight fast, and evaluated for changes in cells and cell elements (erythrocytes, platelets, mononuclear cells [MNC], neutrophils), plasma and blood levels of copper and zinc, and a variety of blood proteins and factors (alkaline phosphatase activity, superoxide dismutase activities [ESOD and extracellular Cu, Zn-superoxide dismutase

(EC-SOD)], 5'-nucleotidase activity, triiodothyronine, thyroxine, and thyroid-stimulating hormone levels, and amyloid precursor protein [APP] levels). Copper and zinc levels were determined for urine, feces, and diet. Alcohol tolerance tests were performed at the end of the first equilibration period and at the end of the low- and high-zinc exposures. Data were analyzed by a two-way (dietary zinc and copper) repeated-measures analysis of variance, and Tukey's contrasts were used to test for differences among means.

Plasma zinc concentrations were significantly lower, relative to the equilibration levels, and platelet zinc concentrations tended to be lower, though not significantly, in subjects fed 3 mg Zn/day than in those fed 53 mg Zn/day; plasma zinc was not lowered from equilibration levels when subjects were fed 3 mg Zn/day, but was elevated in those fed 53 mg Zn/day. Zinc supplementation increased Zn levels in the feces and urine, but did not appear to affect plasma Cu levels. Neither erythrocyte zinc levels nor erythrocyte membrane zinc concentrations were significantly altered by changes in dietary zinc.

High-zinc subjects showed significant increases in bone-specific alkaline phosphatase activity, relative to the equilibration period, but not in plasma alkaline phosphatase or erythrocyte membrane alkaline phosphatase. Zinc supplementation significantly increased mononuclear white cell 5'-nucleotidase activity and decreased plasma 5'-nucleotidase activity; the difference in 5'-nucleotidase activity was apparent when subjects were fed the high-copper diet, but not when they were fed the low-copper diet.

EC-SOD activity, but not ESOD activity, was significantly increased by zinc supplementation; this was more apparent in the low-copper group. ESOD activity was significantly decreased relative to equilibration levels in low-copper subjects and significantly increased in high-copper subjects; in both cases, zinc supplementation caused a statistically insignificant decrease in ESOD activity.

Erythrocyte glutathione peroxidase activity was increased by low dietary zinc and decreased by high dietary zinc; however, the decrease did not result in a return to initial equilibration activity. Plasma free thyroxine concentrations, but not total thyroxine concentrations, were significantly increased in the zinc-supplemented groups; no other effects on thyroid-related endpoints were noted.

During the low-zinc period, there was an increase in total cholesterol; this increase was reversed with high-zinc treatment, resulting in lower total cholesterol. LDL-cholesterol changes were similar to the total cholesterol changes, while HDL-cholesterol, very low density lipoprotein-cholesterol, and triglycerides were not affected. Zinc supplementation significantly decreased platelet APP expression in subjects fed the low-copper diet; however, technical problems prevented many of these samples from being properly analyzed, so the sample size for APP expression was very small. Most indicators of iron status were not affected by the changes in dietary zinc or copper during the 90-day period; the exception was a small drop in hemoglobin (Hb) levels, which the investigators attributed to the effects of accumulated blood loss due to blood draws conducted during the study.

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

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

three treatment groups were 12.5, 14.0, and 9.5 mg Zn/day for the groups receiving the 0, 50, and 75 mg/day supplements, respectively. Based on the average body weights for each treatment group, total zinc intakes were 0.16, 0.85, and 1.10 mg Zn/kg-day for the 0, 50, and 75 mg/day groups, respectively. Biweekly blood samples were collected from all subjects and analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, zinc, and copper. Urinary zinc and copper values were also determined. There was a general decline in the mean serum HDL-cholesterol for the 75-mg supplement group between weeks 6 and 12. HDL values for this group were significantly lower than those for the placebo group at weeks 6 and 12 ($p < 0.05$). There was also a decline in the HDL values for the 50-mg group between weeks 8 through 12; however, this decline was not significantly different from that for the controls until the 12th week of treatment. When the mean HDL-cholesterol level of these subjects was compared to population percentile norms (Simko et al., 1984), there was a decline from the 92nd to the 77th percentile in 6 weeks, followed by a relative stabilization of HDL values for the remaining 6-week test period. Over the 12-week period, the HDL values for the 50-mg supplemental zinc group declined from the 90th to the 77th population percentile norms. Serum zinc, copper, total cholesterol, LDL-cholesterol, and triglycerides did not appear to be affected by treatment.

In another study, 12 healthy men (23 to 35 years) with normal serum cholesterol levels received a zinc sulfate capsule twice a day with meals (160 mg supplemental Zn/day or ~2 mg supplemental Zn/kg-day, assuming a 70 kg reference body weight) for 5 weeks and 8 subjects received placebo capsules (Hooper et al., 1980). Fasting lipid levels were measured weekly for 7 weeks and at week 16 in the zinc group, and biweekly for 6 weeks in the control group. There were no statistically significant differences in total serum cholesterol, triglyceride, and LDL-cholesterol between the zinc and control groups. After 5 weeks of zinc ingestion, serum HDL-cholesterol had been reduced by 17%; although no further zinc was administered, the serum HDL-cholesterol level continued to decline and was reduced by 26% at week 7, relative to the values for the placebo group. The rise in plasma zinc concentration did not correlate with the fall in HDL-cholesterol. Serum HDL-cholesterol returned to near baseline levels 11 weeks after the end of zinc supplementation.

Bogden et al. (1988) exposed groups of healthy elderly (age 60-89) to 0, 15, or 100 mg supplemental Zn/day for 3 months. At the end of the study, blood was drawn, and evaluated for changes in zinc levels in plasma, erythrocytes, MNCs, polymorphonuclear cells, and platelets. Serum samples were also evaluated for cholesterol, HDL cholesterol, alkaline phosphatase, and albumin. No statistically significant changes in any of the evaluated serum parameters were

reported, with the exception of an decrease in the ratio of plasma zinc to plasma copper in the high-dose group.

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

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

Prasad et al. (1978) fed a patient with sickle cell anemia supplements of 150 to 200 mg Zn/day for 2 years. The supplement resulted in copper deficiency; serum copper and plasma ceruloplasmin levels were decreased. When copper was administered, the plasma ceruloplasmin levels became normal. In a follow-up study of 13 patients on zinc therapy (similar treatment levels assumed), 7 patients had ceruloplasmin levels at the lower limit of normal after 24 weeks of dosing.

In a recent study by Prasad et al. (2004), the antioxidant effect of zinc was studied in humans. Twenty healthy subjects (9 males and 11 females, ages 19 - 50 years) were randomly assigned into two groups. Ten subjects received oral placebo, and 10 received oral zinc (45 mg

zinc as zinc gluconate) daily for 8 weeks. Blood was drawn from the subjects both before and after the treatment period, and the following parameters were examined: plasma zinc concentration, lipid peroxidation, DNA oxidation, tumor necrosis factor alpha (TNF-α) and interleukin-1β mRNA levels, and nuclear factor kappa-B (NF-κB) DNA binding. A statistically significant increase in plasma zinc concentrations was observed in the zinc-supplemented group. Plasma markers of lipid peroxidation (i.e., 4-hydroxynonenol and malondialdehyde) and DNA oxidation (i.e., 8-hydroxy-2'-deoxyguanosine) were significantly decreased in the zinc-supplemented group ($p<0.05$). Ex vivo studies were performed to determine the effects of zinc supplementation on the ability of MNCs to modulate relative mRNA levels of pro-inflammatory cytokines (i.e., TNF-α and interleukin-1β) in response to lipopolysaccharide (LPS) stimulation. LPS-treated MNCs from the zinc-supplemented group had a statistically significant decrease in the levels of TNF-α and interleukin-1β mRNA versus placebo controls. The activation and DNA binding of NF-κB following ex vivo treatment of MNCs with TNF-α was used as a model for induction of oxidative stress. A 50% decrease in the DNA binding of NF-κB was shown with MNCs from the zinc-supplemented group compared to placebo controls ($p<0.05$).

### 4.2.2. Inhalation Exposure

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

Drinker et al. (1927a) described the case of a worker exposed to zinc oxide on two successive days. On the first day, the worker was exposed for 5 hours to an average

concentration of 52 mg Zn/$m^3$. The worker reported feeling an oncoming fever four hours after exposure began, and elevated temperature, chill, and fatigue were reported several hours after exposure termination. No adverse symptoms were reported after the second day of exposure, even though zinc oxide levels were higher on the second day (330 mg Zn/$m^3$). To further examine this apparent tolerance, Drinker et al. (1927a) experimentally exposed another man with previous zinc oxide exposure to 430 mg/$m^3$ for 8 minutes on day 1 and to 610 mg/$m^3$ for 8 minutes on day 2. On day 1, the subject's temperature gradually increased and peaked 13 hours after exposure (101.2°F versus 98.5°F prior to exposure). The subject reported chills and feeling feverish, weak, and somewhat debilitated 10-15 hours after exposure. As with the occupational exposure, these symptoms were not observed after the second exposure.

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

There is evidence to suggest that exposure to zinc oxide fumes may impair lung function. Malo et al. (1990, 1993) present case reports of two workers exhibiting symptoms of metal fume fever with evidence of functional lung involvement. In the first case (Malo et al., 1990), a worker exposed to zinc oxide fumes reported chills with muscle aches and dyspnea; a chest radiograph revealed diffuse interstitial shadows. After a 10-day period of non-exposure, the chest radiograph was normal. A lung function test was performed after the worker was away from work for 30 days; forced expiratory volume in one second ($FEV_1$), forced vital capacity (FVC), and the $FEV_1$/FVC ratio were normal. The worker was then exposed to his usual work environment for 1 hour on two consecutive days. Significant decreases in $FEV_1$ (16-20%) and FVC (10-11%) were observed on both days, 4-6 hours after exposure; buccal temperature was also increased and the worker experienced malaise and general muscle ache. In the second case (Malo et al., 1993), lung function tests were performed 3 months after the worker left work and after the worker returned to work for 1 day. A decrease in $FEV_1$ (24%) was observed after the worker returned to work (lung function was normal prior to returning to work). Total zinc concentrations in the work environment were 0.26-0.29 mg/$m^3$.

In a series of experiments by Drinker et al. (1927b), a group of five men and three women received face-only exposure to various concentrations of zinc oxide for 6-40 minutes.

Two of the men were exposed to several different concentrations; the remaining subjects were exposed to only one concentration. Body temperature was used as an indicator of metal fume fever. The magnitude of the increase in body temperature appeared to be concentration-related. Based on the results of this study and epidemiology data, the study authors concluded that workers exposed to less than 15 mg Zn/$m^3$ in the air were not likely to develop metal fume fever.

The results of more recent studies suggest that metal fume fever will occur at lower concentrations. In a study by Fine et al. (1997), a group of 13 healthy, non-smoking subjects without any previous exposure to zinc oxide fumes were exposed to 0, 2.5, or 5 mg/$m^3$ furnace-generated zinc oxide for 2 hours. The subjects were exposed to all three concentrations; each exposure was separated by a 48-hour non-exposure period. Significant increases in oral temperature were observed 6-12 hours after exposure to 2.5 or 5 mg/$m^3$ zinc oxide fume. A statistically significant increase in the number of symptoms reported was also observed after exposure to 5 mg/$m^3$. The symptoms occurred 6-9 hours after exposure, and all symptoms were resolved by the next day after exposure. The commonly reported symptoms were fatigue, muscle ache, and cough. Levels of plasma interleukin-6 were significantly increased after exposure to 2.5 or 5 mg/$m^3$; peak levels were observed 6 hours after exposure.

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

Martin et al. (1999) described a cohort of 20 Chinese workers who were exposed to zinc oxide over a single 8-hour workday. Subjects were given an examination by a physician, a spirometric evaluation, and chest radiographs before beginning work, immediately after the shift, and 24 hours after the start of exposure. Exposure concentrations, measured twice per individual during the 8-hour shift, ranged from 0-36.3 mg/$m^3$. However, as no significant association between airborne zinc measurements and serum zinc levels was present, the reliability of these measurements in reflecting actual zinc exposure is uncertain. No subject showed signs of metal fume fever. Chest radiographs likewise did not reveal any changes over the period examined. Similarly, no changes in respiratory parameters, assessed by spirometry, were reported as a result of exposure.

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

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

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

### 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 (hydration state not reported) in the diet for 13 weeks. The study authors estimated zinc sulfate intakes of male rats to be 23.2, 234, and 2514 mg/kg-day (5.3, 53, and 572 mg supplemental Zn/kg-day). In the case of females, the authors estimated the doses as 24.5, 243, and 2486 mg $ZnSO_4$/kg-day (5.6, 55, and 565 mg supplemental Zn/kg-

day). For male mice the estimated doses were 42.7, 458, and 4927 mg $ZnSO_4$/kg-day (9.7, 104, and 1119 mg supplemental Zn/kg-day) and 46.4, 479, and 4878 mg $ZnSO_4$/kg-day (10.5, 109, and 1109 mg supplemental Zn/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 Hb 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 were 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; however, 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 (decreased number of acinar cells) 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 Hb and erythrocyte levels. Significant decreases in hematocrit, Hb, 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-marked increases in alkaline phosphatase and urea nitrogen. Decreases in total protein and increases in alkaline phosphatase and urea nitrogen were also observed in the mid-dose male mice, although the study authors stated that the values were within acceptable historical limits. Histological alterations were observed in the pancreas, gastrointestinal tract, and kidneys of high-dose mice; incidences were not reported. Pancreatic alterations included an increased

number of acinar cells, many displaying necrosis, swollen nuclei, and/or ductule-like metaplasia. Slight-to-moderate ulcerative lesions in the boundary of the forestomach, inflammation of the mucous membranes of the "upper intestine" with proliferation of epithelial cells, and edema at the lamina propria were observed.

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

In a second study, L'Abbe and Fischer (1984b) fed groups of 10 weanling male Wistar rats diets containing normal (30 mg Zn/kg diet) or supplemented (240 mg Zn/kg diet) zinc (as zinc sulfate) and normal (6 mg Cu/kg diet) or deficient (0.6 mg Cu/kg diet) copper for up to 6 weeks. Groups of rats were sacrificed at 2, 4, and 6 weeks. Blood, heart, and liver samples were collected for analysis. No significant differences in body weight or food consumption were noted among treated groups. Similarly, no differences were seen in Hb levels. Serum and heart copper levels were significantly decreased in rats fed either zinc-supplemented or copper-deficient diets. In both the high zinc and copper-deficient groups, activity levels of serum ceruloplasmin, liver and heart Cu, Zn-superoxide dismutase, and liver and heart cytochrome c oxidase were significantly reduced relative to control animals by 2 weeks of exposure, and remained reduced throughout the study.

Zaporowska and Wasilewski (1992) exposed groups of 13 male and 16 female Wistar rats to 0 or 0.12 mg Zn/mL as zinc chloride in the drinking water for 4 weeks. The study authors estimated the daily drinking water dose to be 11.66 mg Zn/kg-day in males and 12.75 mg Zn/kg-day for females. Although significant decreases in food and water intake were observed, body

weight gain was not significantly different from controls. Significant alterations were observed in several hematological endpoints including decreases in erythrocyte and Hb levels, increases in total and differential (neutrophils and lymphocytes) leukocyte levels, and increases in the percentage of reticulocytes and polychromatophilic erythrocytes.

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

de Oliveira et al. (2001) exposed groups of 9 or 12 male and female Swiss mice to 0 or 1% hydrated zinc acetate (0 or 793 mg Zn/kg-day), assuming reference body weight and drinking water consumption values from U.S. EPA (1988), beginning in the first month of life and lasting for 60 days. Animals were evaluated using a shock avoidance behavioral test at the end of their 60-day exposure period. The animals were placed in a two-compartment chamber where one compartment was dark and the other lighted. When placed in the lighted compartment, the mice (who prefer the dark) moved into the dark compartment where they received an electric shock upon contact with the dark room floor. On the next day when the animals were placed in the lighted compartment, the time before they moved into the dark compartment increased significantly from the time on the first day, signifying that they had learned from the adverse day zero experience. There was no significant difference in the time before dark room entry between the control and zinc-exposed animals on test day 1. Entry into the dark chamber did not result in shock treatment on test day 1.

The control and zinc-exposed animals continued to be tested on days 7, 14, 21, and 28. No shock was given on any of these test days. The initial period in the lit room before entering the dark room decreased over time for both the control and the zinc-exposed groups. However, the decrease over time was greater in the zinc-exposed group signifying a more rapid extinction of the learned avoidance response. The time spent in the lighted chamber before entry into the dark room was significantly lower (about half of that for the controls) for the zinc-exposed

animals on day 28. Accordingly, postnatal zinc exposure appeared to have a negative effect on the retention of a learned behavioral response.

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

Straube et al. (1980) examined the effects of excess dietary zinc in ferrets. Adult ferrets (six males, nine females), weighing 500-700 g, were divided into four groups and fed a basal diet of canned dog food (that contained 27 ppm zinc and 3.3 ppm copper) plus 0 (five animals), 500 ppm (three animals), 1500 ppm (four animals), or 3000 ppm (three animals) supplemental zinc as zinc oxide. Doses of 0, 142, 425, and 850 mg supplemental Zn/kg-day, respectively, are estimated using the midpoint of the range of initial body weights and the amount of food given to each animal (170 g per day, assumed to be consumed completely each day). Animals in the 1500 and 3000 ppm groups showed signs of severe toxicity and were sacrificed or died within

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

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

In a 1-year study, an unspecified number of newborn Chester Beatty stock mice (sex not reported) were administered 0, 1000, or 5000 ppm zinc (approximately 0, 170, or 850 mg/kg/day) as zinc sulfate in drinking water (Walters and Roe, 1965). A separate group of mice received zinc oleate in the diet at an initial dose of 5000 ppm supplemental zinc; this dose was reduced to 2500 ppm after 3 months and to 1250 ppm after an additional 3 months because of mortality due to anemia. An epidemic of the ectromelia virus caused the deaths of several mice during the first 8 weeks; consequently, additional control and test-diet groups were established. There was no difference in body weight gain between control and treated groups, except for the dietary zinc group which became anemic. Survival was not reported in treated compared with control groups. An apparent increase in the incidence of hepatomas was observed in treated mice surviving for 45 weeks or longer relative to controls (original and replacement mice were pooled). The hepatoma incidences in the control, low-dose drinking water, high-dose drinking water, and test-diet groups were 3/24 (12.5%), 3/28 (10.7%), 3/22 (13.6%), and 7/23 (30.4%), respectively. Incidences of malignant lymphoma in the control, low-dose drinking water, high- dose drinking water, and test-diet groups were 3/24 (12.5%), 4/28 (14.3%), 2/22 (9%), and 2/23 (8.7%), respectively. Incidences of lung adenoma in the control, low-dose drinking water, high-dose drinking water, and test-diet groups were 10/24 (41.7%), 9/28 (32.1%), 5/22 (22.7%), and 9/23 (39.1%), respectively. None of these were significantly elevated in a statistical analysis of these data performed by the EPA.

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

### 4.3.2. Inhalation Exposure

As with most of the human inhalation studies, inhalation studies in animals have focused exclusively on the toxicity of zinc from acute exposures. No relevant subchronic or chronic animal inhalation studies of zinc compounds were located.

In a multispecies study, Gordon et al. (1992) exposed an unspecified number of male Hartley guinea pigs, Fischer 344 rats, and New Zealand rabbits to freshly generated zinc oxide particles. The guinea pigs and rats received nose-only exposure to 0, 2.5, or 5.0 mg/m$^3$ zinc oxide for 3 hours; the rabbits received nose-only exposure to 0 or 5.0 mg/m$^3$ zinc oxide for 2 hours. Animals were sacrificed 0, 4, or 24 hours following cessation of exposure. The lungs were lavaged, and the lavage fluid and recovered cells were examined for evidence of inflammation. Significant increases in lavage fluid parameters (lactate dehydrogenase, β-glucuronidase, and protein content) were observed 24 hours after the guinea pigs and rats were exposed to 2.5 or 5.0 mg/m$^3$. No significant alterations in lavage parameters were observed in the rabbits. The ability of alveolar macrophages to phagocytize particles was assessed in guinea pigs and rabbits. In the guinea pigs exposed to 5.0 mg/m$^3$, there was a significant reduction in phagocytic capacity (percentage of viable macrophages engulfing four or more particles), but no effect on phagocytic index (percentage of macrophages engulfing particles). Phagocytic ability was not adversely affected in the rabbits. The authors suggested that the reason rabbits were less affected was a lower retention of the inhaled zinc particles (4.7% in rabbits, compared to 11.5% in rats and 19.8% in guinea pigs), resulting in a lower dose per unit tissue mass.

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

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

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

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

## 4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

### 4.4.1. Oral Exposure

#### 4.4.1.1. *Reproductive and Developmental Studies in Humans*

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

#### 4.4.1.2. *Reproductive Studies in Animals*

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

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

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

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

Sutton and Nelson (1937) maintained groups of young female (n=3) and male (n=2) rats on basal diets supplemented with 0, 0.10, 0.50, or 1.0% zinc as zinc carbonate for 10-39 weeks. Using reference values for body weight (0.124 kg) and food intake (14 g) (U.S. EPA, 1988), supplemental zinc intake is estimated as 0, 113, 565, or 1130 mg/kg-day. Hematological alterations consisting of a 20% decrease in Hb level in the 0.50% group, a 42-57% decrease in Hb level in the 1.0% group, and 15-28% decrease in erythrocyte level in the 1.0% group were observed. No hematological alterations were observed in the 0.10% group. Growth, reproduction, and development were reported to be normal for the 0.10% group over several generations. Adverse reproductive effects were observed in the 0.50% group; there were several stillbirths in the first pregnancy, after which there were no live young born. Rats in this group ceased to become pregnant after 5 months, although their body weights appeared normal. Reproduction and development were reported to have returned to normal in this group after excess zinc was withheld from the diet. No data were presented in support of this statement, so the timeframe of recovery is not known. Most of the animals on the 1.0% zinc diet failed to grow normally and some died within 4 weeks; no reproduction occurred in this dose group.

Since both males and females were treated with zinc, but no histopathological examination of the gonads was performed, it is not possible to determine the immediate cause of reproductive failure at higher dose levels.

Pal and Pal (1987) added 4000 ppm of zinc as zinc sulfate to the diet of 12 Charles-Foster female rats for 18 days beginning immediately after coitus. Using the reference values for food intake and body weight (U.S. EPA, 1988), supplemental zinc intake is estimated at 450 mg/kg-day. The incidence of conception in the treated group was significantly reduced compared to controls (5/12 vs. 12/12). In those animals that did conceive, the number of implantation sites per pregnant female was not significantly altered. Zinc treatment had no effect on the number of resorption sites and there were no stillbirths or malformations among the offspring of treated rats. In a separate experiment in which female rats were fed 4000 ppm supplemental zinc for 3 weeks prior to mating, the incidence of conception and fetal outcome were not adversely affected by treatment.

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

In a single-generation study of reproductive performance, Khan et al. (2001) exposed groups (n=5-7) of male and female Sprague-Dawley rats to 0, 3.6, 7.2, 14.4, or 28.8 mg Zn/kg-

day, as zinc chloride, by gavage. Animals were exposed 7 days per week for 77 days prior to cohabitation and throughout the 21-day cohabitation period; females were also exposed during each of the 21-day gestation and lactation periods. Evaluated reproductive parameters included fertility, viability index, weaning index, litter size, and pup body weight. No significant changes were seen in body weights of the exposed rats prior to birth, but postpartum dam body weights for the mid- and high-dose groups were significantly decreased, relative to controls. The fertility indices in all dose groups were significantly lower than in the control group, though no dose-related trends were noted. At the highest two dose levels, the number of live pups per litter, but not total pups per litter, was significantly decreased, as was live pup weight at postnatal day 21, though not at days 4, 7, or 14. No other changes in reproductive parameters were noted, and no effects on serum clinical chemistry endpoints were reported.

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

Kinnamon (1963) fed groups of five Sprague-Dawley female rats a diet containing 0 or 0.5% supplementary zinc as zinc carbonate for 5 weeks prior to mating with untreated males and for the first 2 weeks of gestation. At the end of the 7-week period, the rats were injected with radiolabelled zinc chloride, then housed in metabolism cages for 4 days prior to sacrifice. Using the body weight data provided and an allometric equation for food intake (U.S. EPA, 1988), supplemental zinc doses of 0 or 500 mg/kg-day were calculated. No significant differences in number of fetuses per litter, wet weight of the litter, or average weight per fetus were observed.

#### 4.4.1.3. *Developmental Studies in Animals*

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

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

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

Mulhern et al. (1986) fed an unspecified number of female weanling C57BL/6J mice a diet containing 50 (normal) or 2000 (high) ppm of zinc as zinc carbonate and, at age 6 weeks, mated them with unexposed males. Each dam and her offspring were assigned to one of 10 groups receiving 50 or 2000 ppm total zinc during gestation, lactation, and postweaning until age 8 weeks. Decreases in hematocrit and body weight were observed in the $F_1$ mice exposed to 2000 ppm zinc during gestation, lactation, and postweaning. The study authors noted that decreases in body weight gain were observed in other groups; however, the magnitude and statistical significance were not reported. Alopecia was observed in all groups of $F_1$ mice exposed to 2000 ppm during lactation, regardless of gestational exposure. The mice began to lose hair between 2 and 4 weeks of age, and exhibited severe alopecia at 5 weeks. Exposure to 2000 ppm during lactation and/or post weaning resulted in achromotrichia, which the authors suggest may result from the effects of zinc-induced copper deficiency.

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

#### 4.4.2. Inhalation Exposure

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

## 4.5. OTHER STUDIES

### 4.5.1. Acute Toxicity Data

#### 4.5.1.1. *Oral Exposure*

Brewer et al. (2000) reported on the use of zinc supplementation for the treatment of Wilson's disease. Wilson's disease results in an accumulation of copper within the body, eventually leading to hepatic changes and, in some patients, neurologic effects as well. The study authors discussed the results of 26 pregnancies in 19 women with Wilson's disease who received oral zinc acetate (from 25-150 mg Zn/day) prior to and during pregnancy. Urinary

copper, a reliable indicator of body copper status, was able to be maintained within normal levels with zinc supplementation, and hepatic and neurological signs in the affected women returned to normal while treatment continued. Of 26 pregnancies, there were four miscarriages, and two fetal abnormalities; one major (microcephaly) and one minor (surgically correctable heart defect). This study did not include any control subjects; thus these adverse effects cannot be fully correlated to either Wilson's disease or to zinc supplements.

#### 4.5.1.2. *Inhalation Exposure*

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

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

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

Guthrie (1956) injected 0.15-0.20 mL of 10% zinc sulfate into the testis of 19 four-month-old rats and 0.15 mL of 5% zinc chloride into the testis of 29 three-month-old rats

(strain not specified). No testicular tumors were observed in either group at sacrifice 15 months after injection. No controls were described.

### 4.5.2. Genotoxicity

The results of short-term genotoxicity assays for zinc are equivocal. Zinc acetate and/or zinc-2,4-pentanedione have been analyzed in four short-term mutagenicity assays (Thompson et al., 1989). In the Salmonella assay (with or without hepatic homogenates), zinc acetate was not mutagenic over a dose range of 50-7200 µg/plate, but zinc 2,4-pentanedione was mutagenic to strains TA1538 and TA98 at 400 µg/plate. The addition of hepatic homogenates diminished this response in a dose-dependent manner. In the mouse lymphoma assay, zinc acetate gave a dose-dependent positive response with or without metabolic activation; the mutation frequency doubled at 10 µg/mL. In the Chinese hamster ovary cell in vitro cytogenetic assay, zinc acetate gave a dose-dependent positive response with or without metabolic activation, but the presence of hepatic homogenates decreased the clastogenic effect. Neither zinc acetate nor zinc-2,4-pentanedione were positive in the unscheduled DNA synthesis assay in rat hepatocytes over a dose range of 10-1000 µg/mL.

Zinc chloride has been reported to be positive in the Salmonella assay (Kalinina et al., 1977), negative in the mouse lymphoma assay (Amacher and Paillet, 1980), and a weak clastogen in stimulated human lymphocyte cultures (Deknudt and Deminatti, 1978). Zinc sulfate was not mutagenic in the Salmonella/microsome assay (Gocke et al., 1981), and zinc acetate did not induce chromosomal aberrations in unstimulated human lymphocyte cultures (Gasiorek and Bauchinger, 1981). Crebelli et al. (1985) found zinc oxide (99% purity) (1000-5000 µg/plate) not to be mutagenic for reverse mutation in *Salmonella typhimurium*.

Responses in mutagenicity assays are thought to depend on the form (e.g., inorganic or organic salt) of the zinc tested. For example, inorganic salts tend to dissociate and the zinc becomes bound with culture media constituents. Salts that dissociate less readily (i.e., zinc-2,4-pentanedione) tend to be transported into the cell and are postulated to cause a positive response (Thompson et al., 1989).

Zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer protection (Woo et al., 1988). Zinc deficiency enhanced methylbenzylnitrosamine (MBN)-induced carcinoma of the esophagus in male rats (Fong et al., 1978), but retarded the

development of oral cancer induced by 4-nitroquinoline-N-oxide (4-NQO) in 4-week-old female rats (Wallenius et al., 1979). In a study that examined both zinc deficiency and supplementation, Mathur et al. (1979) found that animals with a deficient diet (5.9 mg/kg) and animals with a diet supplemented with excessively high levels of zinc (200-260 mg/kg) had fully developed carcinomas of the palatial mucosa. While the rats were on the specific diets, the palatial mucosa was painted with 4-NQO, 3 times/week for 20 weeks. In the zinc-deficient group, 2/25 rats developed cancer of the palatial mucosa; 2/25 rats in the excessive zinc group also developed this form of cancer. Animals supplemented with moderate levels of zinc in the diet (50 mg/kg) developed only moderate dysplasia. Thus, zinc's modifying effect on carcinogenesis may be dose-dependent.

## 4.6. INTERACTIONS

Numerous studies have examined the interactions of zinc and other metals; however, the vast majority of these have examined the effect of co-exposure to zinc on the toxicity of the other metal. The few studies that have been conducted on the effect of other metals on the toxicity of zinc are not adequate to support dose-response assessments for the interactions, or even qualitative assessments of the type or direction of the interaction (e.g., antagonism, synergism), particularly under subchronic or chronic exposure conditions. Interactions between zinc and other metals are highly plausible given that the ligand binding reactions of zinc are similar to those of a variety of other essential or toxic divalent cations (Andersen, 1984). These include a relatively high reactivity with thiolate anions (ionized functional groups from cysteine) and formation of relatively stable chelation complexes with multidentate carboxylic acid ligands (similar to calcium and lead). Thus, competition for reactions with sulfhydryls proteins and ligand exchange reactions are potential mechanisms of interaction that may exert effects at the level of zinc transport, binding, catalysis, or stabilization of zinc-dependent enzymes. The displacement of zinc from ALAD by lead is a good example of such an interaction, and is the basis for one aspect of the toxicity of lead (the inhibition of ALAD and heme synthesis) and the ability of zinc to attenuate this effect of lead (Finelli et al., 1975; Simons, 1995).

Binding to and induction of the synthesis of metallothionein appears to play an important role in the physiologic regulation of zinc levels and, possibly, zinc's reactivity as a potential binder of hydroxyl radicals (Li et al., 1980; Udom and Brady, 1980; Goering and Fowler, 1987; Kelly et al. 1996; Liu et al., 1996). A variety of divalent cations including, cadmium, cobalt,

copper, lead, and zinc bind to metallothionein (Stillman, 1995). Expression of metallothionein resulting from cadmium exposure may result in increased liver content of zinc and decreased plasma zinc concentrations; this could potentially give rise to interactions that have toxicologic consequences. For example, displacement of zinc from weakly bound extracellular proteins by cadmium is thought to be involved in the mechanism by which cadmium (and possibly other divalent metals) induces the synthesis of metallothionein (Palmiter, 1994). When cells are deprived of zinc they become very sensitive to zinc and relatively insensitive to cadmium. The increased sensitivity could be due either to increased transport of zinc or to a change in the relative amounts or affinities of metallothionein. The decreased sensitivity of cadmium is predicted if zinc is the only effective inducer, because during zinc starvation the low affinity pool of extracellular zinc would be depleted first; thus addition of small amounts of cadmium would fill this pool without liberating any zinc. Addition of more cadmium would displace zinc from higher affinity pools; thus further depletion would lead to cell death. Induction of metallothionein by zinc has been shown to alter the physiologic disposition of copper and the toxicity of cadmium (Waalkes and Pérez-Ollé, 2000). Recent characterization of divalent metal ion transporters in epithelia, including that of mammalian small intestine, suggest that zinc may share absorptive mechanisms with a variety of divalent cations, including cadmium, copper, iron, and lead (Gunshin et al., 1997; Fleming et al., 1999). This provides at least one mechanism by which co-exposure with other divalent metals could affect zinc absorption, and possibly transport of absorbed zinc in other tissues.

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

### 4.6.1. Interactions with Essential Trace Elements

#### 4.6.1.1. *Copper and Zinc*

As discussed above, the most sensitive effects of high supplementary levels of zinc in humans are alterations in the levels of copper-containing enzymes (e.g., Cu, Zn-superoxide dismutase and serum ceruloplasmin) and plasma LDL cholesterol levels. 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 ESOD activities. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels. Animal studies reported by L'Abbe and Fischer (1984a, b) have demonstrated the reduction of Cu, Zn-superoxide dismutase

activity in the liver and heart as the most sensitive indicator of copper status in rats fed high levels of zinc in their diet. These observations were correlated with similar Cu, Zn-superoxide dismutase activities in the liver and heart of animals fed a copper deficient diet. It is believed that the copper deficiency results from a zinc-induced decrease in copper absorption, although the exact mechanisms are not understood. Excess dietary zinc results in induction of intestinal metallothionein synthesis; because metallothionein has a greater binding capacity for copper than for zinc, copper absorbed into the intestinal mucosal cells may be sequestered by metallothionein and not absorbed systemically (Walsh et al., 1994).

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

#### 4.6.1.2. *Calcium and Zinc*

Hwang et al. (1999) reported that administration of calcium acetate to hemodialysis patients did not result in changes in hair or serum zinc relative to baseline levels, though both levels were lower than normal controls. A review by Lönnerdal (2000) provides evidence that calcium levels do not directly influence the absorption of zinc. It appears, however, that calcium

aggravates zinc deficiency when it is added to diets based on plant products that might be expected to be high in phytate (reviewed in O'Dell, 1969). Heth and Hoekstra (1965) reported a decreased absorption of zinc when calcium was co-administered in the diet, and that increased dietary calcium resulted in an increased rate of zinc loss (shortened clearance half-time).

#### 4.6.1.3. *Iron and Zinc*

O'Brien et al. (2000) reported that percentage zinc absorption was significantly lower in pregnant women who received iron-containing prenatal supplements (60 mg/day) relative to women who had not received iron-containing supplements. Plasma zinc concentrations were also significantly lower after iron supplementation, but not if the supplement also contained 15 mg of zinc. Bouglé et al. (1999) reported a significant correlation between zinc absorption and iron content in the diet, with increased dietary iron resulting in diminished absorption of zinc. However, Lönnerdal (2000) has suggested that at lower iron intake levels, iron has no effect on the absorption of zinc. Zinc and iron are substrates for a divalent metal transport protein that has been shown to participate in the absorption of iron (Gunshin et al., 1997). The relative importance of this protein in the absorptive transport of zinc has not been determined.

### 4.6.2. Interactions with Other Heavy Metals

#### 4.6.2.1. *Cadmium and Zinc*

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

Toxic levels of cadmium may inhibit zinc absorption (Lönnerdal, 2000). Studies conducted in isolated cells or membranes from kidney proximal tubule or small intestine indicate that zinc and cadmium may share common transport and/or binding mechanisms in transporting epithelia (Tacnet et al., 1990, 1991; Prasad and Nath, 1993; Prasad et al., 1996; Endo et al., 1997). For example, Gachot and Poujeol (1992) assessed the effect of cadmium on the uptake of zinc by isolated rabbit proximal tubule cells. At low concentrations (15 μM), cadmium acts as a competitive inhibitor of carrier-mediated zinc uptake, while at higher concentrations (50 μM) it also exhibits noncompetitive inhibition of an unsaturable pathway. Similar results were reported by King et al. (2000) who found that injection of cadmium chloride in mice reduced the uptake of $^{65}$Zn by 56% in testes and 47% in brain. Exposure of rats whose diets contained normal

(12 mg/kg) or elevated (60 mg/kg) levels of zinc to 5 mg Cd/L in the drinking water did not alter the amount of zinc or copper in the plasma or liver (Bebe and Panemangalore, 1996). Levels of copper in the kidneys were decreased in animals that were exposed to high-dosages of zinc and cadmium, but not in animals that received normal zinc diets and cadmium; cadmium had no effect on kidney zinc levels. Brzoska et al. (2001) reported that treatment of rats with cadmium resulted in decreased levels of zinc in the tibia; zinc supplementation restored the levels to normal.

#### 4.6.2.2. *Lead and Zinc*

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

Administration of zinc in the diet, but not through injection, has been shown to decrease the toxicity of dietary lead (Cerklewski and Forbes, 1976; El-Gazzar et al., 1978), possibly due to zinc decreasing the intestinal absorption of lead (Cerklewski and Forbes, 1976; Cerklewski, 1979). It is not known if lead will affect the absorption of zinc. However, exposure of rats whose diets contained normal (12 mg/kg) or elevated (60 mg/kg) levels of zinc to drinking water containing 20 mg Pb/L did not alter the amount of zinc or copper in the plasma, kidney, or liver (Bebe and Panemangalore, 1996). This would suggest, though it is hardly conclusive, that lead exposure does not alter zinc absorption. Both zinc and lead have been shown to bind to the N-methyl-D-aspartate receptor site in rats, but lead does not appear to bind to the zinc allosteric site (Lasley and Gilbert, 1999). As noted previously, zinc and lead are substrates for a divalent metal transport protein that has been shown to participate in the absorption of iron (Gunshin et al., 1997). The relative importance of this protein in the absorptive transport of lead or zinc has not been determined.

#### 4.6.2.3. *Cobalt and Zinc*

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

## 4.7. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION – ORAL AND INHALATION

### 4.7.1. Oral Exposure

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, Cu, Zn-superoxide dismutase, carboxypeptidase, ALAD, carbonic anhydrase, RNA polymerase, and reverse transcriptase (Vallee and Falchuk, 1993; Sandstead, 1994). A wide range of clinical symptoms have been associated with zinc deficiency in humans (Abernathy et al., 1993; 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 without adequate zinc, 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. Severe zinc deficiency in animals has been associated with reduced fertility, fetal neurological malformations, and growth retardation in late pregnancy (Mahomed et al., 1989).

Increased zinc consumption, as supplemental zinc, has been associated with changes in health effects in humans, including decreased copper metalloenzyme activity (Fischer et al., 1984; Samman and Roberts, 1987, 1988; Yadrick et al., 1989; Davis et al., 2000; Milne et al., 2001), hematological effects such as anemia, neutropenia (Hale et al., 1988), decreases in cholesterol levels (Hooper et al., 1980; Freeland-Graves et al., 1982; Chandra, 1984; Black et al., 1988; Davis et al., 2000; Milne et al., 2001), immunotoxicity (Chandra, 1984), and gastrointestinal effects (Freeland-Graves et al., 1982; Samman and Roberts, 1987, 1988).

Although the decreased copper metalloenzyme activities and cholesterol levels are not necessarily adverse in themselves, they are likely to be indicators of more severe effects occurring at greater dose levels. Several human studies provide evidence that excess zinc intake may induce copper deficiency. Severe copper deficiency has been observed in individuals

ingesting very high doses of zinc for over one year (Patterson et al., 1985; Hoffman et al., 1988). At lower zinc doses, more subtle signs of impaired copper status, such as alterations in copper metalloenzyme activities, are evident. Copper deficiency is thought to result from a zinc-induced decrease in copper absorption. Excess dietary zinc results in induction of intestinal metallothionein synthesis; because metallothionein has a greater binding capacity for copper than for zinc, copper absorbed into the intestinal mucosal cells is sequestered by metallothionein and not absorbed systemically (Walsh et al., 1994). Zinc and copper may also be substrates for a divalent metal transport protein (i.e., CRIP) induced by copper in the small intestine (Gunshin et al., 1997). Although studies by Davis et al. (2000), Milne et al. (2001), Samman and Roberts (1987, 1988), Fischer et al. (1984), and Yadrick et al. (1989) failed to find decreases in plasma copper levels after zinc supplementation, these studies did find alterations in indicators of body copper status, including decreases in serum ceruloplasmin, EC-SOD, and ESOD activities. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels.

While the exact function of HDL is not known, it is thought to function in the transfer of cholesterol from extrahepatic tissue to the liver. Bile acids are synthesized from cholesterol in the liver and carry cholesterol breakdown products to the intestines with the bile, thus providing an excretory pathway for cholesterol. The results of epidemiology studies suggest an association between high concentrations of HDL with a reduced risk of coronary heart disease. As compared to all lipids and lipoproteins measured, HDL may have the largest impact on risk of coronary heart disease in individuals over 50 years old (Simko et al., 1984). Normal levels of HDL-cholesterol are 45.5 mg/dL in men and 55.5 mg/dL in women. HDL-cholesterol levels below 35 mg/dL have been associated with an increased risk of coronary heart disease (Simko et al., 1984). Collectively, the human data suggest that short-term ($\leq$12 weeks) increases in zinc intake result in decreases in HDL-cholesterol levels. In the Hooper et al. (1980) and Chandra (1984) studies, in which subjects received daily doses of 2 or 4 mg supplemental Zn/kg-day for up to 6 weeks, the HDL-cholesterol levels dropped below 35 mg/dL. Although zinc-induced decreases in HDL-cholesterol have been observed, a relationship between increased zinc intake and an increased risk of coronary heart disease has not been established. Additionally, not all human studies have confirmed effects on HDL-cholesterol levels following zinc supplementation (Davis et al., 2000; Milne et al., 2001).

Following high-level oral exposure, zinc appears to exert adverse health effects primarily through interaction with copper. Specifically, high levels of zinc can result in a saturation of the

carrier-mediated pathway of zinc absorption and a shift to metallothionein-mediated absorption (Hempe and Cousins, 1992). It is believed that the copper deficiency results from a zinc-induced decrease in copper absorption. Zinc-induced copper deficiency is consistent with numerous reports of effects of zinc on various biomarkers of copper nutritional status following exposures to elevated levels of zinc in humans and animals, as well as by reports indicating that copper supplementation can result in an attenuation of zinc-induced toxicity.

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

### 4.7.2. Inhalation Exposure

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

In animals, exposure to zinc oxide results in similar effects as those reported in humans. Gordon et al. (1992) examined the effects of zinc oxide in rabbits, rats, and guinea pigs, and reported changes in lavage parameters which appeared to correlate with pulmonary retention of the zinc particles. In a series of studies in guinea pigs, Lam et al. (1988) reported that ultrafine zinc oxide particles resulted in significant respiratory effects, including decreased lung function

and increased lung weight. However, subchronic or chronic studies of the toxicity of zinc following inhalation exposure in animals are not available. Similarly, no studies examining the effects of inhaled zinc on reproductive or developmental endpoints were located.

The mechanisms behind metal fume fever are not known, but are thought to involve several different factors. Exposure to zinc oxide particles has been shown to elicit the release of a number of proinflammatory cytokines, leading to a persistent pulmonary inflammation which could result in some of the reported symptoms of metal fume fever, including decreased lung function and bronchoconstriction. An allergic response to zinc particles, leading to an asthma-like response, has also been proposed as a possible mechanism. However, additional mechanistic information will be required in order to adequately determine the mechanisms involved in the toxicity of inhaled zinc.

## 4.8. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *inadequate information to assess carcinogenic potential* of zinc, because studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the possible carcinogenicity of zinc are not available, and results of genotoxic tests of zinc have been equivocal.

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

Either zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer protection (Mathur, 1979; Woo et al., 1988). For example, zinc deficiency enhanced carcinomas

of the esophagus induced by MBN (Fong et al., 1978) but retarded the development of oral cancer induced by 4-NQO (Wallenius et al., 1979). Thus, zinc's modifying effect on carcinogenesis may depend on the dose of zinc as well as the carcinogen being affected. The mutagenicity of zinc, particularly in *S. typhimurium*, appears to depend greatly on the chemical form.

## 4.9. SUSCEPTIBLE POPULATIONS

### 4.9.1. Possible Childhood Susceptibility and Susceptible Diabetics

Data in humans are not available that examine whether children are more susceptible to the toxicity of zinc than adults. However, the RDA for children, expressed in terms of mg/kg-day, is greater than that for adults. Animal studies have, however, suggested that neonates and/or developing animals may be more susceptible to the toxic effects of excess zinc. Bleavins et al. (1983) reported that in minks exposed to 56 mg Zn/kg-day throughout gestation and weaning, no changes were seen in exposed adults, but 3-4 week-old kits exhibited achromotrichia, thought to be associated with copper deficiency. Signs of copper deficiency progressed as zinc exposure continued.

ESOD, formerly known as erythrocuprein, contains two atoms of zinc and copper each as cofactors and acts as a scavenger of singlet oxygen species. As reported by Arai et al. (1987), this enzyme is known to be glycosylated, and glycosylation is significantly increased in diabetics. Furthermore, this glycosylation significantly decreases ESOD activity compared to the activity of non-glycosylated form of ESOD. Thus diabetics may be sensitive to high dietary levels of zinc. Several other studies have examined the effects of zinc exposure in young animals, but have not provided data on adult animals similarly exposed for comparison. Additional data will be required to adequately assess the susceptibility of children to zinc exposure, relative to adults.

### 4.9.2. Possible Gender Differences

Several studies in humans have suggested that females may be more sensitive to the adverse effects of excess zinc than males. For example, Samman and Roberts (1987, 1988) reported that women experienced adverse symptoms more frequently (84% in women vs. 18% in men) as well as being more susceptible to zinc-induced changes in LDL cholesterol levels, serum ceruloplasmin, and ESOD. However, women in this study received a higher average dose

(2.5 mg/kg-day) than did the corresponding men (2.0 mg/kg-day). In contrast, Hale et al. (1988) reported that in elderly subjects, zinc-exposed women did not experience the same reduction in the incidence of anemia as was seen in zinc-exposed men. The studies of Yadrick et al. (1989) and Fischer et al. (1984) reported similar effect levels on ESOD levels, expressed as mg total Zn/kg-day, in men and women. Further data examining the potential difference in response between men and women were not located.

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

# 5. DOSE-RESPONSE ASSESSMENTS

## 5.1. ORAL REFERENCE DOSE (RfD)

The RfD for zinc is based on human clinical studies to establish daily nutritional requirements. Zinc is an essential trace element that is crucial to survival and health maintenance, as well as growth, development, and maturation of developing organisms of all animal species. Thus, insufficient as well as excessive oral intake can cause toxicity and disease and a quantitative risk assessment must take essentiality into account. The principal studies examine dietary supplements of zinc and the interaction of zinc with other essential trace metals, specifically copper, to establish a safe daily intake level of zinc for the general population, including pregnant women and children, without compromising normal health and development.

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

Available studies of oral zinc toxicity have identified a number of zinc-induced physiological changes in humans, including decreased copper metalloenzyme activities (Fischer et al., 1984; Samman and Roberts, 1987, 1988; Yadrick et al., 1989; Davis et al., 2000; Milne et al., 2001), 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), immunotoxic effects (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 thought that the copper deficiency results from a zinc-induced decrease in copper absorption. As discussed in Fischer et al. (1984), copper metalloenzyme activities are 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), Yadrick et al. (1989), Davis et al. (2000), and Milne et al. (2001) failed to find significant decreases in plasma copper levels, these studies did find alterations in other indicators of copper status, including activities of serum ceruloplasmin, ESOD, and/or EC-SOD. Some 60% or more of total erythrocyte copper is associated with ESOD. The identity of this protein, originally called erythrocuprein, from human tissues has been reported by McCord and Fridovich (1969). This protein contains two atoms, each, of zinc and copper.

Erythrocuprein functions as a superoxide dismutase having the ability to catalyze the dismutation of monovalent superoxide anion radicals into hydrogen peroxide and oxygen.

These proteins are also present in phagocytic cells and known to act as scavengers of singlet oxygen, thus preventing oxidative tissue damage. It follows that while the decreased copper metalloenzyme activities seen in several of the human studies are not necessarily adverse in themselves, they signal a decrease in the body's defenses against free radical oxidation. The consequences of the decrease in the enzyme activity would vary depending on the status of other components of the free radical defense system, such as the dietary adequacy of vitamins C, E, A, and selenium. 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 (e.g., serum ceruloplasmin and cytochrome c oxidase activity and liver and heart Cu, Zn-superoxide dismutase activity) in rats exposed to supplemental zinc in the diet for 6 weeks were dose-related.

Of the available studies in humans, the studies of Davis et al. (2000), Milne et al. (2001), Fischer et al. (1984), and Yadrick et al. (1989) have identified effects on indicators of copper status at similar daily exposure levels.

In the study reported by Davis et al. (2000) and Milne et al. (2001), a population of postmenopausal women consumed a total of 53 mg Zn/day (3 mg/day in the controlled diet plus 50 mg/day as supplements), resulting in a total average daily dose of 0.81 mg/kg-day (using a mean body weight of 65.1 kg provided in the manuscripts). Bone-specific alkaline phosphatase activity was increased following zinc exposure, and ESOD activity and plasma free thyroxine were significantly decreased following exposure to zinc for 90 days.

Fischer et al. (1984) examined a group of adult male volunteers exposed to 50 mg supplemental Zn/day; adding in an average daily dietary consumption of 15.92 mg Zn/day (from the U.S. FDA Total Diet Study from 1982-1986 [Pennington et al., 1989]), the total exposure level from Fischer et al. (1984) was 65.92 mg Zn/day, or 0.94 mg/kg-day assuming a reference male body weight of 70 kg. ESOD activity was decreased by 4 weeks of exposure, with an inverse correlation between plasma zinc and ESOD activity apparent at 6 weeks.

The study of Yadrick et al. (1989) exposed a group of healthy adult women to 50 mg supplemental Zn/day; adding in an average daily dietary consumption of 9.38 mg/day (from the FDA Total Diet Study from 1982-1986 [Pennington et al., 1989]), the total exposure level from the Yadrick et al. (1989) study was 59.38 mg Zn/day, or 0.99 mg/kg-day assuming a reference

female body weight of 60 kg. ESOD activity declined steadily over the treatment period, and was statistically lower than pretreatment values at the end of the 10-week exposure.

In establishing an RfD for zinc, the data on essentiality were combined with the data on toxicity to define a level that would meet physiological requirements without causing toxic responses when consumed daily for a lifetime. The exposure values that were considered in determining the RfD suggest that there is only one order of magnitude between the minimum amount of zinc that will maintain physiological function (5.5 mg/day, King, 1986) and the amount associated with appearance of potentially adverse effects (60 mg/day, Cantilli et al., 1994).

As the four studies identified physiological changes on similar, sensitive endpoints (indicators of body copper status) at similar dose levels (0.81-0.99 mg Zn/kg-day) in a variety of human subject groups (adult males, adult females, postmenopausal females), the studies of Davis et al. (2000), Milne et al. (2001), Yadrick et al. (1989), and Fischer et al. (1984) were selected as co-principal studies.[1]

### 5.1.2. Methods of Analysis

A NOAEL/LOAEL approach was applied to derive the RfD. A benchmark dose approach was considered, but was not utilized for this assessment. All of the co-principal studies examined only one dose level, apart from controls, and therefore did not provide sufficient information to describe the dose-response function. Therefore, the studies are not suitable for benchmark analysis.

### 5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UF)

In selecting the point of departure for the RfD, the effect levels from the principal studies were evaluated. As described in Section 5.1.1 above, the studies identified effect levels of 0.81

[1] The studies by Davis et al. (2000) and Milne et al. (2001) were approved by the Institutional Review boards of the University of North Dakota and the US Department of Agriculture and followed Guidelines of the Department of Health and Human Services and the Helsinki Declaration regarding the use of human subjects. The study by Yadrick et al. (1989) was approved by the Institutional Review Board of Oklahoma State University and informed consent was obtained from each participant. Finally, the study by Fischer et al. (1984) was approved by the Human Studies Committee of the Health Protection Branch, Health and Welfare Canada, and a consent form was signed by all participants.

mg Zn/kg-day (Davis et al., 2000; Milne et al., 2001), 0.94 mg Zn/kg-day (Fischer et al., 1984), and 0.99 mg Zn/kg-day (Yadrick et al., 1989) for changes in indicators of body copper status. Since the four studies have similar methodologies and outcomes with regard to effects, they were averaged together to obtain the point of departure (0.81+0.94+0.99=2.74/3=0.91 mg/kg-day).

The RfD of 0.3 mg/kg-day was derived by dividing the point of departure of 0.91 mg Zn/kg-day by a total uncertainty factor of 3 as follows:

RfD = NOAEL ÷ UF
= 0.91 mg/kg-day ÷ 3
= 0.3 mg/kg-day.

When considered within the context of the RDA and reference daily intake (RDI) values shown in Table 5-1, the RfD allows for some flexibility in the dietary intake (i.e., the RfD is 1.2 to 2.3 times the RDA). For essential elements such as zinc, the RDA provided the lower bound for determination of the RfD.

An interspecies uncertainty factor ($UF_A$) was not necessary for extrapolation from an animal study to the human population. The principal studies were conducted in human volunteers.

A threefold intraspecies uncertainty factor ($UF_H$) was applied to account for variability in susceptibility in human populations. The critical effect for zinc is decreased copper uptake, leading to a decrease in the activity of Cu, Zn-SOD enzymes that function as part of the body's system to protect against free radicals and oxidative stress. This system is complex, involving the SOD, catalase, glutathione, glutathione peroxidase, glutathione reductase, and the antioxidant vitamins (A, C, and E) providing several layers of protection. However, there is variability within the human population. Individuals with genetic catalase deficiency and glucose-6-phosphate dehydrogenase deficiencies have reduced capacities to metabolically cope with oxidative stress. Poor nutrition can also compromise the ability to respond to free radicals and oxidative stress. It is, accordingly, prudent to allow a threefold factor for human variability since the individuals used in the critical studies were apparently healthy adults. The use of a 10-fold uncertainty factor for intrahuman variability would result in an RfD below the RDA.

In the case of zinc and other nutritionally required elements, it is important that the RfD not be set at a value that would suggest that people should consume diets with insufficient zinc. Recommended dietary levels, expressed as intake both in mg Zn/day and in mg Zn/kg-day (calculated by adjusting with reference body weights of 13 kg for young children, 61 kg for women [pregnant, lactating, or general adult], or 70 kg for men), are presented in Table 5-1. Use of a threefold factor results in an RfD value that exceeds the dietary values by factors from 1.2 to 2.3. A smaller margin between the RfD and RDA cannot be recommended. RDA values are established for healthy individuals, and thus there are instances when additional dietary zinc is recommended such as during the recovery from surgery and other circumstances where active tissue repair is necessary.

**Table 5-1. Estimated nutritional requirements of zinc at various life stages, expressed as mg/day and mg/kg-day**

| Life stage | Recommended intake (mg Zn/day) | Reference body weight (kg) | Recommended intake (mg Zn/kg-day) |
|---|---|---|---|
| 1-3 years | 3 (RDA[a]) | 13 | 0.23 |
| Adulthood (>18 years)<br>Male<br>Female | <br>11 (RDA)<br>8 (RDA) | <br>76<br>61 | <br>0.15<br>0.13 |
| Pregnant women | 11 (RDA) | 61 | 0.18 |
| Lactating women | 12 (RDA) | 61 | 0.2 |
| U.S. FDA RDI[b] Values<br>Male<br>Female | <br>15 mg (RDI)<br>15 mg (RDI) | <br>70<br>60 | <br>0.21<br>0.25 |

[a]RDA values and reference body weights are from IOM (2001).
[b]RDI values are established by the U.S. FDA and are used in the labeling of nutritional supplements.

An uncertainty factor to account for extrapolation from a subchronic study to estimate chronic exposure conditions ($UF_S$) was not necessary. Zinc is an essential element and therefore chronic exposures of zinc are required for proper nutrition. Exposure at the level of the RfD is expected to be without adverse effects when zinc is consumed on a daily basis over the life-span of the individual, neither inducing nutritional deficiency nor resulting in toxic effects in healthy non-pregnant adult humans consuming an average American diet.

There is extensive experience with humans receiving chronic dietary exposures from the diet plus nutritional supplements that do not exceed the 15 mg/day RDI which demonstrates that these levels are not adverse. For example, Hale et al. (1988) studied hematological parameters in elderly subjects who were supplemented with zinc for an average duration of 8 years. In general, no significant alterations were found between the zinc-supplemented group and controls. On the other hand, Prasad et al. (1978) studied a patient given 150-200 mg Zn/day for 2 years. The patient developed copper deficiency which was reversed with copper supplementation. Additionally, pharmacokinetic data on zinc absorption, distribution, and elimination suggest that steady-state levels will be reached within the time periods evaluated by the principal studies. Therefore, an uncertainty factor for extrapolation from a study of less than chronic duration to a lifetime exposure scenario was not determined to be necessary.

An uncertainty factor for extrapolation from a LOAEL to a NOAEL ($UF_L$) was determined to not be necessary. The RfD was based on a minimal effect level for a sensitive biological indicator, i.e., decreased ESOD activity, which is reflection of zinc-associated alterations in copper homeostasis that could lead to oxidative tissue damage. As discussed in the section on intrahuman variability, there is redundancy in the physiological free radical defense system that argues against describing the decreased activity of Cu, Zn-SOD as definitively adverse. Protection for variability in the status of this defense system is accommodated by the threefold factor allowed for intrahuman variability.

The deficit in copper absorption in the presence of excess zinc can also not be categorized as requiring the application of a LOAEL to NOAEL UF. As discussed in Fischer et al. (1984), copper metalloenzyme activities are more sensitive indicators of copper status than plasma copper levels. They are an early biomarker for a subclinical copper deficiency. Most importantly, the application of a threefold UF for a LOAEL to NOAEL adjustment, when combined with a threefold factor for intraspecies variability, would lower the RfD to below the RDA.

A database uncertainty factor ($UF_D$) to account for uncertainties due to lack of information in the database was not necessary. The database contains a considerable number of well-conducted human studies in a diverse group of human subjects. There are numerous reproductive and developmental toxicity studies performed in different species. Animal studies demonstrate that effects on reproductive and/or developmental endpoints are not the most sensitive endpoints for zinc toxicity.

The additional use of a daily vitamin supplement containing 15 mg zinc, such as is found in a standard multivitamin tablet, in conjunction with a diet adequate in zinc would result in a total adult daily exposure on the order of 0.4 mg Zn/kg-day[2], which is above the RfD. However, daily multivitamins also contain copper (2 mg/day), which could be expected to counteract the effects of excess zinc intake resulting from daily multivitamin use. Therefore, the use of a daily multivitamins, or similar balanced supplements, is not contraindicated by exposure at the level of the RfD.

### 5.1.4. Previous IRIS Assessment

In the previous assessment for zinc, the oral RfD was based on a single clinical study (Yadrick et al., 1989) which investigated the effects of oral zinc supplements (50 mg/day) on copper and iron balance. The total exposure level in this study (as discussed in Section 5.1.3) was 0.99 mg Zn/kg-day. The RfD of 0.3 mg/kg-day was derived by dividing this dose (0.99 mg/kg-day) by a total uncertainty factor of 3 based on a minimal LOAEL from a moderate-duration study of the most sensitive humans and consideration of a substance that is an essential dietary nutrient.

## 5.2. INHALATION REFERENCE CONCENTRATION (RfC)

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

[2] A typical over-the-counter zinc supplement, such as a daily multivitamin, contains 15 mg Zn. For a healthy adult male, this would add an additional 0.21 mg Zn/kg-day, or 0.25 mg Zn/kg-day for a healthy adult female; thus, with a zinc-sufficient diet, the total zinc intake for a male consuming one multivitamin daily would be 0.36 mg/kg-day. Values for normal and lactating females consuming the same multivitamin would be 0.38 mg/kg-day and 0.45 mg/kg-day, respectively. Each of these values falls within the order of magnitude range about the RfD and can be considered to be without risk.

## 5.3. CANCER ASSESSMENT

### 5.3.1. Oral Slope Factor

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

### 5.3.2. Inhalation Unit Risk

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

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

## 6.1. HUMAN HAZARD POTENTIAL

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

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

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) there is *inadequate information to assess carcinogenic potential* of zinc in humans, because studies of humans occupationally exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the carcinogenicity of zinc are not available, and tests of the genotoxic effects of zinc have been equivocal.

## 6.2. DOSE RESPONSE

### 6.2.1. Noncancer/Oral

The most sensitive effects of oral exposure to excess zinc in humans involve the copper status of the body. Zinc exposure can result in a decreased absorption of copper, leading to low systemic copper levels and subsequent health effects, including decreased copper metalloenzyme activity, hematological effects, decreases in cholesterol levels, immunotoxicity, and gastrointestinal effects. While changes such as decreased copper metalloenzyme levels may not be adverse in themselves, they have been demonstrated to be precursor events for more severe effects. The study of Yadrick et al. (1989) established a minimal LOAEL of 0.99 mg Zn/kg-day for decreased levels of ESOD, an indicator of body copper status, in women exposed for 10 weeks, while the study of Fischer et al. (1984) established a minimal LOAEL of 0.94 mg Zn/kg-day for the same endpoint in men exposed for 6 weeks, and the study of Davis et al. (2000) and Milne et al. (2001) identified a minimal LOAEL of 0.81 mg Zn/kg-day for changes in ESOD and plasma free thyroxine. These four studies in human volunteers were considered to be co-principal studies, and the minimal LOAEL (0.91 mg Zn/kg-day, average of these LOAELs) was selected as the point of departure. An uncertainty factor of 3 (discussed in Section 5.1.3, above), representing the uncertainties associated with human variability and the need for an adequate dietary level of zinc, was then applied to the minimal LOAEL of 0.91 mg Zn/kg-day to give the RfD of 0.3 mg Zn/kg-day.

### 6.2.2. Noncancer/Inhalation

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

### 6.2.3. Cancer/Oral and Inhalation

Data in both humans and animals are inadequate to evaluate potential associations between zinc exposure and cancer. Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *inadequate information to assess the carcinogen potential* of zinc, because studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the possible carcinogenicity of zinc are not available, and tests of the genotoxic effects of zinc have been equivocal.

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## APPENDIX A. EXTERNAL PEER REVIEW—SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for zinc have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. Public comments were read and considered. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. All three external peer reviewers recommended that this document and the accompanying assessments were acceptable with minor revisions. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

### *(1) General Questions for Peer Reviewers*

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

**Comment** All three reviewers agreed that the document is concise and clearly written, and the choice of critical study and critical effects are appropriate. Some of the concerns reviewers presented include: balancing adverse effects with both deficiencies and level of concern for effects of deficiency or the effects below the RfD, specifically for children; adverse effects resulting from other metal interactions, such as iron and or copper; uncertainty associated with a higher RfD than the currently derived RfD, concerns for different forms of zinc exposure, enhancement of NOAEL/LOAEL information from animal studies; clear presentation of zinc status as essential element in IRIS Summary; additional studies recommended by two reviewers.

***Response to Comment*** Section 5.1.3 of the Toxicological Review was added to provide an enhanced discussion of the RfD, relevance to the RDA, and effects below RfD in sensitive populations, such as children. Although limited data are available, information on the potential adverse effects in children were included in Section 4.9 of the Toxicological Review. Table 3 presents diet, age, gender and body-weight-specific zinc requirements followed by a discussion of the effects that may occur below the RfD and the uncertainties associated with the RfD.

An enhanced discussion of the chosen UF of 3 has been added in Section 5.1.3 of the Toxicological Review and clearly describes the rational for the chosen UF; however, the suggested uncertainty factor of 1.5 was not implemented. This decision was based on the threshold effect of decreased ESOD activity and the uncertainty as to whether decreased ESOD activity may predispose a cell to an accumulation of oxidative damage due to decreased

quenching of free radicals. Although the recent antioxidant effects of zinc supplementation have been reported (Prasad et al., 2004), the study did not determine whether the decreased levels of serum markers of oxidative stress (e.g., 8-hydroxy-2'-deoxyguanosine) were due to a decreased level of oxidative DNA damage or a decrease in the removal of this lesion within nucleated cells.

Metal-metal interactions are discussed in Section 4.6 of the Toxicological Review and information on zinc speciation and their relevance to environmental exposure are included Section 3.1 of the Toxicological Review.

Because available animal studies present information on supplementary levels of dietary zinc and no additional dosages, it is not possible to clearly discuss proper NOAEL/LOAELs from these studies; therefore, this was not discussed in Chapter 4 of the Toxicological Review.

Section 5.1 of the Toxicological Review and Section I.A.2 of the IRIS Summary have been revised to include information, as suggested by one reviewer, on the relevance of the RfD and environmental levels of zinc and the significance of zinc as an essential element to help risk assessors and managers make meaningful risk assessment decisions, as follows:

> The RfD for zinc is based on human clinical studies to establish daily nutritional requirements. Zinc is an essential trace element that is crucial to survival and health maintenance, as well as growth, development, and maturation of developing organisms of all animal species. Thus, insufficient as well as excessive oral intake can cause toxicity and disease and a quantitative risk assessment must take essentiality into account. The principal studies examine dietary supplements of zinc and the interaction of zinc with other essential trace metals, specifically copper, to establish a safe daily intake level of zinc for the general population, including pregnant women children, without compromising normal health and development.

Suggested new studies have been added in the Toxicological Review and the relevant literature has been reviewed and updated through October 2004.

***(2) RfD Derivation***

**General Question** The RfD for zinc is based on human clinical studies to establish daily nutritional requirements. The human studies examined dietary supplements of zinc and the interaction of zinc with other metals, such as copper, to establish a safe daily intake of zinc for children, adults, and pregnant women. Do you consider this RfD to be protective of adverse effects in children and pregnant women? Do you agree with the method of analysis used to evaluate dose-response data for zinc?

**A. Comment** Is the RfD protective of adverse effects in children and pregnant women?

Reviewers did not consider the RfD to be protective for adverse effects in children because it was below the RDA and they suggested expanding the discussions in the Toxicological Review.

***Response to Comment*** The paragraph regarding the protective effect of the RfD in children is not accurate and has been removed. An enhanced discussion of the RfD relative to the RDA has been included. For transparency, the dose conversion and body weight have been added in Table 5-1, Section 5.1.3 of the Toxicological Review. As suggested by one reviewer, effects of multivitamins were also included, and a paragraph addressing the relevance of the RfD for children and pregnant woman has been added.

**B. Comment** Are appropriate uncertainty factors applied to the point of departure?

The reviewers, while suggesting the UF of 1.5 instead of 3, recommended expanding the discussions on uncertainties in the Toxicological Review.

***Response to Comment*** The discussion of the rationale for an uncertainty factor of 3 has been enhanced. Since the RfD was based on a toxicity threshold dose-response, standard uncertainty factors have been used to develop the RfD.

***(3) RfC Derivation***

**General Question** Data for derivation of RfC are considered inadequate. Do you agree?

**Comment** All reviewers agreed. One reviewer suggested that an overview statement be provided in the IRIS Summary regarding the inadequacy of the data.

***Response to Comment*** The summary sheet was modified per reviewer's recommendations, and the following statement was included in Section I.B of the IRIS Summary:

> Available data are not suitable for the derivation of an RfC for zinc. A number of case reports of metal fume fever have been reported in humans, however exposure levels are not known. The data in animals is limited to a few studies of acute duration, no subchronic or chronic inhalation studies of zinc are available at this time.

***(4) Cancer Weight-of-Evidence (WOE) Classification***

**General Question** The WOE classification for zinc has been discussed in Chapter 4 of the Toxicological Review. Have appropriate criteria been applied from the EPA draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999)?

**Comment** All reviewers agreed that the application of the guidelines and presentation of data in support of the WOE was appropriate.

**Comment** Two reviewers had specific editorial comments and one reviewer provided annotated changes in each chapter of the Toxicological Review and IRIS Summary.

***Response to Comment*** All editorial and annotated changes were incorporated.

## RECOMMENDATIONS

**Comment** Two reviewers recommended acceptance with major revisions as suggested for Chapters 5 and 6 of the Toxicological Review and for the IRIS Summary while the third reviewer recommended acceptance with minor revision.

***Response to Comment*** All major editorial changes, addition of new studies, and revisions to the text in both the Toxicological Review and the IRIS Summary were incorporated. Corrections were made to reflect adverse effects at or below the RfD to protect children.

The uncertainty section was completely revised and expanded statements were provided in support of the UF of 3. This value was considered the most protective for preventing zinc deficiency and toxicity. When considered within the context of the RDA and RDI values shown in Table 5-1, Section 5.1.3 of the Toxicological Review, the RfD is 50% greater than the nearest RDA values (for young children and pregnant or lactating women), and 20% greater than the RDI values. For essential elements such as zinc, the RDA provides the lower bound for determination of the RfD. Based on these reasons, the UF of 3 was considered to be protective against adverse effects that may occur from deficiency or excess, and the recommendation by two reviewers to reduce the UF to 1 or 1.5 due to a concern for deficiency were considered to be adequately addressed by the UF of 3.