EPA

TOXICOLOGICAL REVIEW

OF

ZINC AND COMPOUNDS

(CAS No. 7440-66-6)

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

April 2003

NOTICE

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

U.S. Environmental Protection Agency
Washington DC

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FOREWORD

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

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

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

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1. INTRODUCTION

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

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

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

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

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

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

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

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

Name	Composition	% Zn
Sphalerite	ZnS	67.0
Hemimorphite	$Zn_4Si_2O_7(OH)_2 \cdot H_2O$	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

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

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

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

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

CAS Registry Number	Zinc	Zinc Oxide	Zinc Chloride	Zinc Sulfate	Zinc Sulfide
	7440-66-6	1314-13-2	7646-75-7	7733-02-0	1314-98-3
Molecular Formula	Zn	ZnO	$ZnCl_2$	$ZnSO_4$	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	$\sim 2 \times 10^{-3}$	4.3×10^{3}	1.7×10^{3}	$\sim 7 \times 10^{-3}$
Density (g/cm^3)	7.14	5.607	2.907	3.54	~4.1

Sources: ATSDR, 1995; Barceloux, 1999

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.

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 $Zn(OH)_2$ or ZnO (U.S. EPA, 1979). In anaerobic environments, ZnS may be formed (U.S. EPA, 1979). Zinc accumulates in aquatic organisms, and bioconcentration factor (BCF) values for freshwater fish and marine fish were reported as 1,000 and 2,000, 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 and Schoen, 1996a), 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. Zinc uptake from a normal diet normally ranges from 26-33% (Sandstrom and Abrahamson, 1989; Knudsen et al., 1995; Hunt et al., 1998) when taken with food, but is higher (i.e. 68-81%) when subjects have fasted (Istfan et al., 1983; Sandstrom and Abrahamson, 1989). Within a 5-25 mg dose range, zinc absorption, expressed as a percent of the total dose administered, decreases with dose; for example, in human volunteers, 61% of a 24.5 mg dose of zinc (as zinc chloride) was absorbed, compared to 81% of a 4.5 mg dose (Istfan et al., 1983).

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

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

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

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).

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 test meal complexes with endogenous zinc ions secreted from the pancreas, thus preventing its reabsorption and increasing 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 stores; 93% of a 1.19 mg zinc dose 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, respectively, zinc oxide 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 co-factor for over 70 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 (Llobet et al., 1988; Ansari et al., 1975, 1976). Less than 10% of the body's total zinc is readily exchanged with plasma (Miller et al., 1994) and most of this is slowly exchanging 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 α_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 copper-zinc superoxide dismustase (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 ZnO 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 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). Ansari et al. (1975, 1976) also exposed rats to a single gavage dose of $^{65}ZnCl_2$ 7 days prior to sacrifice. The levels of radiolabelled zinc in the examined tissues were substantially lower than in the controls. For most tissues, no significant relationship between dietary zinc concentration and tissue levels were found, suggesting that zinc turnover was increased in some tissues in response to elevated dietary concentrations.

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. 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.

4. HAZARD IDENTIFICATION

4.1. ESSENTIALITY OF ZINC

The essentiality of zinc was established over 100 years ago. Zinc is essential for the function of more than 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, copper-zinc superoxide dismutase, carboxypeptidase, δ-aminolevulinic acid dehydratase, carbonic anhydrase, ribonucleic acid polymerase, and reverse transcriptase (Vallee and Flachuk, 1993; Sandstead, 1994). Zinc has three functions in these metalloenzymes: participation in catalytic functions, maintenance of structural stability, and regulatory functions (Vallee and Flachuk, 1993; Walsh et al., 1994). Zinc is also involved in DNA and RNA synthesis and cell proliferation. The zinc coordinates with cysteine and histidine residues of certain peptides and produces a tertiary structure which has an affinity for unique segments of DNA in promoter gene regions (Prasad, 1993). The configurations include the zinc finger, the most common zinc motif, and the zinc thiolate cluster (Walsh et al., 1994). Other physiological roles of zinc include enhancement of the affinity of growth hormone for its binding receptors, modulation of synaptic transmissions by interacting with specific sites on 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 (Prasad, 1993; Sandstead, 1994; Walsh et al., 1994). The clinical manifestations of severe zinc deficiency, seen in individuals with an inborn error of zinc absorption or in patients receiving total parenteral nutrition, include bullous pustular dermatitis, diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in intercurrent infections. Symptoms associated with moderate zinc deficiency include growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing. Neurosensory changes, impaired neuropsychological functions, oligospermia, decreased serum testosterone, hyperammonemia, and impaired immune function (alterations in T-cell subpopulations, decreased natural killer cell activity) have been observed in individuals with mild or marginal zinc deficiency.

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

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

The zinc content of a typical mixed diet of North American adults is approximately 10-15 mg/day (NRC, 1989). The FDA's Total Diet Study (Pennington and Schoen, 1996b) 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, 2002) are presented in Table 2.

4.2. STUDIES IN HUMANS

4.2.1. Oral Exposure

In a double-blind crossover trial, Samman and Roberts (1987, 1988) gave zinc sulfate tablets (150 mg supplemental zinc/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 six weeks had no effect on plasma levels of copper, total cholesterol or HDL-cholesterol in males or females, but significantly decreased the plasma level of 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 superoxide dismutase, and Zn/Cu-dependent erythrocyte superoxide dismutase activity) were apparently unaffected in males. However, females, who received higher mg/kg-day doses of zinc than males, exhibited significantly reduced activity levels of two copper metalloenzymes: serum ceruloplasmin and erythrocyte superoxide dismutase. Other indicators of copper status were not affected in females.

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. 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 ferroxidase activity did not change during the course of the study. However, erythrocyte

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

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

Source: IOM, 2002

*ADI. No RDA value was reported

superoxide dismutase 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 erythrocyte superoxide dismutase 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 zinc/day, or 0.83 mg supplemental zinc/kg-day) resulted in a decrease of erythrocyte superoxide dismutase (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. 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 levels were 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.

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 zinc/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 zinc/day; for the 15% that reported an average intake of 60-150 mg supplemental zinc/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, 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 hemoglobin. Red blood cell counts were significantly lower in the women, but not in the men, in the zinc group.

Groups of 9, 13, or 9 healthy white men were administered 0, 50, or 75 mg/day supplemental zinc as zinc gluconate, respectively, 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 zinc/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 zinc/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 the 75-mg group was compared to population percentile norms, there was a decline from the 92nd to the 77th percentile (Simko et al., 1984) 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 zinc/day or ~2 mg supplemental zinc/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 six 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.

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 zinc/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 zinc/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 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 zinc/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. Data on carcinogenic effects were not reported.

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 subjected 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 also 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 5 men and 3 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 (IL)-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, 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 0-36.2 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 from 10/13 subjects at week 29. Spirometric analyses of lung function parameters were performed on day 1 post-exposure, 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 post-exposure 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 post-exposure.

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 post-exposure, 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 heptahydrate in the diet for 13 weeks. The study authors estimated zinc sulfate intakes of 23.2, 234, 2514 mg supplemental zinc/kg-day (5.3, 53, and 572 mg supplemental zinc for male rats; 24.5, 243, 2486 mg zinc sulfate/kg-day (5.6, 55, and 565 mg supplemental zinc/kg-day) for female rats; 42.7, 458, 4927 mg supplemental zinc/kg-day (9.7, 104, and 210 mg supplemental zinc/kg-day) for male mice; and 46.4, 479, and 4878 mg supplemental zinc/kg-day (10.5, 109, and 1109 mg supplemental zinc/kg-day) for female mice. Zinc intakes from the control diet were not estimated.

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

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

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 swollen nuclei in acinar cells, single cell necrosis of acinar cells, and an increase in the number of acinus and ductule-like metaplasia of acinar cells. 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. Regressive changes were observed in the renal cortex of high-dose females.

In a study by L'Abbe and Fisher (1984a), groups of 10 weanling male Wistar rats were fed a basal diet supplemented with 15, 30, 60, 120, or 240 ppm zinc as zinc sulfate for 6 weeks; the 30 ppm group served as the control group. Using a reference (U.S. EPA, 1988) body weight of 0.217 kg and food intake of 0.020 kg/day, daily doses of 1.4, 2.8, 5.5, 11, and 22 mg supplemental zinc/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 erythrocyte 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 erythrocyte superoxide dismutase and liver cytochrome c oxidase levels were not affected.

In a second study, L'Abbe and Fisher (1984b) fed groups of 10 weanling male Wistar rats diets containing normal (30 mg zinc/kg diet) or supplemented (240 mg zinc/kg diet) zinc (as zinc sulfate) and normal (6 mg copper/kg diet) or deficient (0.6 mg copper/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 hemoglobin levels. Both increased zinc and deficient copper resulted in significant decreases in serum, heart, and copper levels. 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 hemoglobin 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 7-8 male New Zealand white rabbits diets containing 0, 1000, or 5000 µg supplemental zinc/g as zinc carbonate (0, 34, 170 mg supplemental zinc/kg-day using an estimated TWA 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 hemoglobin 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.

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, hemoglobin, glucose, SGOT, SGPT , 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 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 (6 males, 9 females), weighing 500-700 g, were divided into 4 groups and fed a basal diet of canned dog food (that contained 27 ppm zinc and 3.3 ppm copper) plus 0 (5 animals), 500 ppm (3 animals), 1500 ppm (4 animals), or 3000 ppm (3 animals) supplemental zinc as zinc oxide. Doses of 0, 142, 425, and 850 mg supplemental zinc/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 (hemoglobin, 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 include macrocytic hypochromic anemia, increased number of reticulocytes, protein, glucose, blood and bilirubin in the urine and diffuse nephrosis. 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 zinc/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 isles were enlarged and had a vacuolated appearance. The β-cells of the pancreatic islet 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 (ACTH)-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 Zn 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 tumors were observed in the C3H strain (31 in females) and 24/74 tumors were observed in the A/Sn strain (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

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 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 due to 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 7-8 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. An increase in respiratory resistance and decrease in respiratory compliance was 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 respiratory compliance was observed immediately after exposure and 1 hour post-exposure. 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 post-exposure hours.

4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.4.1. Oral Exposure

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.

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 (>25 mg zinc/kg-day) of zinc adversely affect spermatogenesis (Saxena et al., 1989; Evenson et al., 1993) and results 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 supplemental zinc/kg-day for the 3-week experiment and 28 mg supplemental zinc/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 zinc/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 zinc/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 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 1,130 mg Zn/kg-day. Hematological alterations consisting of a 20% decrease in hemoglobin level in the 0.50% group, a 42-57% decrease in hemoglobin 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 4,000 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 Zn/kg-day. The incidence of conception in the treated group was significantly reduced compared to controls (5/12 vs. 12/12). In those animals which 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 was 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.

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 Zn/kg-day), and 13 rats were fed the basal diet plus 150 ppm supplemental zinc (as zinc sulfate, ~20 mg total zinc/kg-day). 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 two 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.

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 additional 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 Zn/kg-day during gestation in the 0.2 and 0.5% groups, respectively, and 150 and 400 mg Zn/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 8-9 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 Zn/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 ten 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 Zn/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, hemoglobin, 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) 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).

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 urethan (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 nineteen 4-month-old rats and 0.15 mL of 5% zinc chloride into the testis of 29 3-month-old rats (strain not specified) (Guthrie, 1956). 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 cultured human lymphocytes (Deknudt and Deminatti, 1978). Zinc sulfate has been reported to be not mutagenic in the Salmonella assay (Gocke et al., 1981), and zinc acetate has been reported to not induce chromosomal abberations in cultured human lymphocytes (Gasiorek and Bauchinger, 1981). Crebelli et al. (1985) found zinc oxide (99% purity) (1000-5000 µg/plate) to be not 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 pentanedione) tend to be transported into the cell and are postulated to cause a positive response (Thompson et al., 1989). Zinc is an essential trace element involved in numerous biological functions including growth, taste, and spermatogenesis. It is a cofactor for several enzymes such as those involved in the metabolism of proteins and nucleic acids.

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 carcinomas of the esophagus induced by methylbenzylnitrosoamine (Fong et al., 1978), but retarded the development of cancer of the oral cavity induced by 4-nitroquinoline-N-oxide (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 diet supplemented with excessively high levels of zinc in the diet (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-nitroquinoline 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 (e.g., cadmium, cobalt, copper, iron, lead) 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 delta-aminolevulinic acid dehydratase (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, its reactivity to other ligands (Li et al., 1980; Udom and Brady, 1980; Goering and Fowler, 1987; Kelly et al., 1996; Liu et al., 1996). Since a variety of divalent cations, including, cadmium, cobalt, copper, lead, and zinc bind to the metallothionein (Stillman, 1995), displacement of zinc from metallothionein by other metals could potentially give rise to interactions that have toxicologic consequences. For example, displacement of zinc from metallothionein by cadmium is thought to be involved in the mechanism by which cadmium, and possibly other divalent metals, induce the synthesis of metallothionein (Palmiter, 1994). 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 that is relevant to the toxicity of zinc and compounds are presented below.

4.6.1. Interactions with Essential Trace Elements

Copper and Zinc

As discussed above, the most sensitive effects of zinc in humans are alterations in the levels of copper-containing enzymes, including 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 erythrocyte superoxide dismutase activities. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels. 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 radiolabled 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.

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).

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

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; Brzóska 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 $CdCl_2$ 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. Brzóska 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.

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 Zn or Cu 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 (NMDA) 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.

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, copper-zinc superoxide dismutase, carboxypeptidase, δ-aminolevulinic acid dehydratase, carbonic anhydrase, ribonucleic acid polymerase, and reverse transcriptase (Vallee and Flachuk, 1993; Sandstead, 1994). A wide range of clinical symptoms have been associated with zinc deficiency in humans (Prasad, 1993; Sandstead, 1994; Walsh et al., 1994). The clinical manifestations of severe zinc deficiency, seen in individuals with an inborn error of zinc absorption or in patients receiving total parenteral nutrition, include bullous pustular dermatitis, diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in intercurrent infections. Symptoms associated with moderate zinc deficiency include growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing. Neurosensory changes, impaired neuropsychological functions, oligospermia, decreased serum testosterone, hyperammonemia, and impaired immune function (alterations in T-cell subpopulations, decreased natural killer cell activity) have been observed in individuals with mild or marginal zinc deficiency. 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 health effects in humans, including decreased copper metalloenzyme activity (Fischer et al., 1984; Samman and Roberts, 1987, 1988; Yadrick et al., 1989), hematological effects (Hale et al., 1988), decreases in HDL-cholesterol levels (Hooper et al., 1980; Freeland-Graves et al., 1982; Chandra, 1984; Black et al., 1988), immunotoxicity (Chandra, 1984), and gastrointestinal effects (Freeland-Graves et al., 1982; Samman and Roberts, 1987, 1988).

Although the decreased copper metalloenzyme activities and HDL-cholesterol levels are not necessarily adverse in themselves, they are believed 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 1 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 levels, 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 in the small intestine (Gunshin et al., 1997). 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 after zinc supplementation, these studies did find alterations in serum ceruloplasmin and erythrocyte superoxide dismutase 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 high density lipoproteins (HDL) is not known, they are thought to function in the transfer of cholesterol from extrahepatic tissue to the liver. 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 zinc/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.

Following high-level oral exposure, zinc appears to exert toxic 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 interaction (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 believed 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 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), zinc is classified in Group D, *Not Classifiable as to Human Carcinogenicity*, based on inadequate evidence of carcinogenicity in humans and animals. Under the proposed guidelines (U.S. EPA, 1999), *data are inadequate for an assessment of human 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 tests of the genotoxic effects 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 et al., 1979; Woo et al., 1988). For example, zinc deficiency enhanced carcinomas of the esophagus induced by methylbenzylnitrosoamine (Fong et al., 1978) but retarded the development of cancer of the oral cavity induced by 4-nitroquinoline-N-oxide (Wallenius et al., 1979). Thus, zinc's modifying effect on carcinogenesis may depend both on the dose of zinc and the identity of the carcinogen being affected. The genotoxicity of zinc, particularly in *S. typhimurium*, appears to depend greatly on the chemical form (e.g., inorganic or organic salt).

4.9. SUSCEPTIBLE POPULATIONS

4.9.1. Possible Childhood Susceptibility

Data in humans are not available that examine whether children are more susceptible to the toxicity of zinc than adults. However, the recommended dietary allowance (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. 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 erythrocyte superoxide dismutase. 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. Further data examining the potential difference in response between men and women were not located. However, the studies of Yadrick et al. (1989) and Fischer et al. (1984) reported similar effect levels on superoxide dismutase enzyme levels, expressed as mg total zinc/kg-day, in men and women.

In animal studies, however, 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. Also, studies of reproductive ability have demonstrated alterations in spermatogenesis at zinc exposure levels below those inducing alterations in female reproductive parameters have been reported to occur (Sutton and Nelson, 1937; Pal and Pal, 1987; Saxena et al., 1989; Evenson et al., 1993). However, 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)

5.1.1. Choice of Principal Study and Critical Effect

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

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

5.1.2. Methods of Analysis

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

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

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

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

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

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

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 are, therefore, 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.

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. Lack of data, therefore, 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 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), zinc is classified in group D, *Not Classifiable as to Human Carcinogenicity*, based on inadequate evidence of carcinogenicity in humans and animals. Under the proposed guidelines (U.S. EPA, 1999), *data are inadequate for an assessment of human carcinogenic potential* of zinc, 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 NOAEL of 0.99 mg Zn/kg-day for decreased levels of erythrocyte superoxide dismutase, an indicator of body copper status, in women exposed for 10 weeks, while the study of Fischer et al. (1984) established a NOAEL of 0.95 mg Zn/kg-day for the same endpoint in men exposed for 6 weeks. An uncertainty factor of 3 (for a moderate-duration study and consideration of a substance that is an essential dietary nutrient) was applied to the NOAEL of 0.95 mg Zn/kg-day to give an RfD of 0.3 mg Zn/kg-day. Confidence in the key studies is medium since they are well-conducted clinical studies with relevant biochemical parameters investigated in both males (Fischer et al., 1984) and females (Yadrick et al., 1989), but had a limited number of study subjects. The confidence in the overall database is medium since the available human studies are of moderate duration and chronic animal data are limited. There is medium confidence in the resulting RfD.

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 an animals are inadequate to evaluate potential associations between zinc exposure and cancer. Under the 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), zinc is classified in group D, *Not Classifiable as to Human Carcinogenicity*, based on inadequate evidence of carcinogenicity in humans and animals. Under the proposed guidelines (U.S. EPA, 1999), *data are inadequate for an assessment of human 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 tests of the genotoxic effects of zinc have been equivocal.

7. REFERENCES

Amacher, DE; Paillet, SC. (1980) Induction of trifluorothymidine- resistant mutants by metal ions in L5178Y/TK+/- cells. Mutat Res 78:279-288.

Amdur, MO; McCarthy, JF; Gill, MW. (1982) Respiratory response of guinea pigs to zinc oxide fume. Am Ind Hyg Assoc. J 43:887-889.

Andersen, O. (1984) Chelation of cadmium. Environ Health Perspect 54:249-266.

Anderson, MB; Lepak, K; Farinas, V; George, WJ. (1993) Protective action of zinc against cobalt-induced testicular damage in the mouse. Repro Toxicol 7(1):49-54.

Ansari, MS; Miller, WJ; Lassiter, JW; et al. (1975) Effects of high but nontoxic dietary zinc on zinc metabolism and adaptations in rats. Proc Soc Exper Biol Med 150:534-536.

Ansari, MS; Miller, WJ; Neathery, MW; et al. (1976) Zinc metabolism and homeostasis in rats fed a wide range of high dietary zinc levels. Proc Soc Exper Biol Med 152:192-194.

ATSDR (Agency for Toxic Substances and Disease Registry). (1995) Toxicological profile for zinc. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA.

Aughey, E; Grant, L; Furman, BL; et al. (1977) The effects of oral zinc supplementation in the mouse. J Comp Pathol 87:1-14.

Barceloux, DG. (1999) Zinc. J Toxicol Clin Toxicol 37(2):279-292.

Bebe, FN; Panamangalore, M. (1996) Modulation of tissue trace metal concentrations in weanling rats fed different levels of zinc and exposed to oral lead and cadmium. Nutr Res 16(8):1369-1380.

Bentley, PJ; Grubb, BR. (1991) Experimental dietary hyperzincemia tissue disposition and excess zinc in rabbits. Trace Elem Med 8:202-207.

Black, MR; Medeiros, DM; Brunett, E; Welke, R. (1988) Zinc supplements and serum lipids in young adult white males. Am J Clin Nutr 47:970-975.

Bleavins, MR; Aulerich, RJ; Hochstein, JR; et al. (1983) Effects of excessive dietary zinc on the intrauterine and postnatal development of mink. J Nutr 113:2360-2367.

Bougle, D; Isafoun, A; Bureau, F; et al. (1999) Long-term effects of iron:zinc interactions on growth in rats. Biol Trace Elem Res 67(1):37-48.

Brewer, GJ; Johnson, VD; Dick, RD; et al. (2000) Treatment of Wilson's disease with zinc. XVII: treatment during pregnancy. Hepatology 31(2):364-370.

Brown, JJL. (1988) Zinc fume fever. Br J Radiol 61:327-329.

Brzoska, MM; Moniuszko-Jakoniuk, J; Jurczuk, M; et al. (2001) The effect of zinc supply on cadmium-induced changes in the tibia of rats. Food Chem Toxicol 39(7):729-737.

Cerklewski, FL. (1979) Influence of dietary zinc on lead toxicity during gestation and lactation in the female rat. J Nutr 109:1703-1709.

Cerklewski, FL; Forbes, RM. (1976) Influence of dietary zinc on lead toxicity in the rat. J Nutr 106:689-696.

Chandra, RK. (1984) Excessive intake of zinc impairs immune responses. J Am Med Assoc 252:1443-1446.

Christensen, JB; Jensen, DL; Christensen, TH. (1996) Effect of dissolved organic carbon on the mobility of cadmium, nickel and zinc in leachate polluted groundwater. Water Res 30:3037-3049.

Coogan, TP; Bare, RM; Waalkes, MP. (1992) Cadmium-induced DNA strand damage in cultured liver cells: Reduction in cadmium genotoxicity following zinc pretreatment. Toxicol Appl Pharmacol 113:227-233.

Cooke, JA; Andrews, SM; Johnson, MS. (1990) The accumulation of lead, zinc, cadmium, and fluoride in the wood mouse (Apodemus sylvaticus L.). Water Air Soil Pollut 51:55-63.

Cousins, RJ. (1985) Absorption, transport, and hepatic metabolism of copper and zinc: Special reference to metallothionein and ceruloplasmin. Physiol Rev 65(2):238-309.

Crebelli, R; Paoletti, A; Falcone, E; et al. (1985) Mutagenicity studies in a tyre plant: *In vitro* activity of workers' urinary concentrates and raw materials. Br J Ind Med 42:481-487.

Davidsson, L; Almgren, A; Sandstrom, B; Juillerat, M-A; Hurrell, RF. (1996) Zinc absorption in adult humans: The effect of protein sources added to liquid test meals. Br J Nutr 75:607-613.

Davies, NT. (1980) Studies on the absorption of zinc by rat intestine. Br J Nutr 43:189-203.

Davies, NT; Nightingale, R. (1975) The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of Zn, copper, iron and manganese in rats. Br J Nutr 34:243-258.

Deknudt, G; Deminatti, M. (1978) Chromosome studies in human lymphocytes after *in vitro* exposure to metal salts. Toxicology 10:67-75.

Drinker, K; Drinker, P. (1928) Metal fume fever: V. Results of the inhalation by animals of zinc and magnesium oxide fumes. J Indust Hyg 10:56-70.

Drinker, P; Thomson, RM; Finn, JL. (1927a) Metal fume fever: II. Resistance acquired by inhalation of zinc oxide on two successive days. J Industr Hyg 9:98-105.

Drinker, P; Thomson, RM; Finn, JL. (1927b) Metal fume fever: IV. Threshold doses of zinc oxide, preventative measures of the chronic effects of repeated exposures. J Ind Hyg 9:331-345.

El-Gazzar, RM; Finelli, VN; Boiano, J; Petering, HG. (1978) Influence of dietary zinc on lead toxicity in rats. Toxicol Lett 1:227-234.

Endo, T; Kimura, O; Hatakeyama, M; et al. (1997) Effects of zinc and copper on cadmium uptake by brush border membrane vesicles. Toxicol Lett 91:111-120.

Evenson, DP; Emerick, RJ; Jost, LK; Kayongo-Male, H; Stewart; SR. (1993) Zinc-silicon interactions influencing sperm chromatin integrity and testicular cell development in the rat as measured by flow cytometry. J Animal Sci 71:955-962.

Fine, JM; Gordon, T; Chen, LC; et al. (1997) Metal fume fever: characterization of clinical and plasma IL-6 responses in controlled human exposures to zinc oxide fume at and below the threshold limit value. J Occup Environ Med 39:722-726.

Fine, JM; Gordon, T; Chen, LC; et al. (2000) Characterization of clinical tolerance to inhaled zinc oxide in naive subjects and sheet metal workers. J Occup Environ Med 42(11):1085-1091.

Finelli, VN; Klauder, DS; Karaffa, MA; Petering, HG. (1975) Interation of zinc and lead and α-aminolevulinate dehydratase. Biochem Biophys Res Commun 65:303-311.

Fischer, PWF; Giroux, A; L'Abbe, MR. (1984) Effect of zinc supplementation on copper status in adult man. Am J Clin Nutr 40:743-746.

Fleming, RE; Migas, MC; Zhou, XY; et al. (1999) Mechanism of increased iron absorption in murine model of hereditary hemochromatosis: Increased duodenal expression of the iron transporter DMT1. Proc Natl Acad Sci 96:3143-3148.

Fong, LYY; Sivak, A; Newberne, PM. (1978) Zinc deficiency and methylbenzyl-nitrosoamine-induced esophageal cancer in rats. J Natl Cancer Inst 61:145-150.

Foulkes, EC; McMullen, DM. (1987) Kinetics of transepithelial movement of heavy metals in rat jejunum. Am J Physiol 253:G134-G138.

Freeland-Graves, JH; Friedman, BJ; Han, W; Shorey, RL; Young, R. (1982) Effect of zinc supplementation on plasma high-density lipoprotein and zinc. Am J Clin Nutr 35:988-992.

Gachot, T; Poujeol, P. (1992) Effects of cadmium and copper on zinc transport kinetics by isolated renal proximal cells. Biol Trace Elem Res 35(2):93-103.

Gao, S; Walker, WJ; Dahlgren, RA; Bold, J. (1997) Simultaneous sorption of Cd, Cu, Ni, Zn, Pb, and Cr on soils treated with sewage sludge supernatant. Water Air Soil Pollut 93:331-345.

Gasiorek, K; Bauchinger, M. (1981) Chromosome changes in human lymphocytes after separate and combined treatment with divalent salts of lead, cadmium, and zinc. Environ Mutagen
3:513-518.

Giroux, EL; Durieux, M; Schechter, PJ. (1976) A study of zinc distribution in human serum. Bioinorg Chem 5:211-218.

Gocke, E; King, MT; Echardt, K; Wild, D. (1981) Mutagenicity of cosmetics ingredients licensed by the European Communities. Mutat Res 90:91-109.

Goering, PL; Fowler, BA. (1987) Kidney zinc-thionein regulation of aminolevulinic acid dehydratase inhibition by lead. Arch Biochem Biophys 253:48-55.

Goodwin, FE. (1998) Zinc and Zinc Alloys. In Kirk-Othmer's Encyclopedia of Chemical Technology Vol 25 Kroschwitz, J.I. ed. New York, NY John Wiley & Sons. p. 789-839.

Gordon, T; Chen, LC; Fine, JM; et al. (1992) Pulmonary effects of inhaled zinc oxide in human subjects, guinea pigs, rats, and rabbits. Am Ind Hyg Assoc J 53:503-509.

Greger, JL; Sickles, VS. (1979) Saliva zinc levels: Potential indicators of zinc status. Am J Clin Nutr 32:1859-1866.

Gunn, S; Gould, TC; Anderson, WAD. (1963) Cadmium-induced interstitial cell tumors in rats and mice and their prevention by zinc. J Natl Cancer Inst 31:745-759.

Gunshin, H; Noguchi, T; Naito, H. (1991) Effect of calcium on the zinc uptake by brush border membrane vesicles isolated from the rat small intestine. Agric Biol Chem 55:2813-2816.

Gunshin, H; Mackenzie, B; Berger, U; et al. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482-488.

Guthrie, J. (1956) Attempts to produce seminomata in the albino rat by inoculation of hydrocarbons and other carcinogens into normally situated and ectopic testes. Br J Cancer 10:134-144.

Hale, WE; May, FE; Thomas, RG; Moore, MT; Stewart, RB. (1988) Effect of zinc supplementation on the development of cardiovascular disease in the elderly. J Nutr Elder 8(2):49-57.

Halme, E. (1961) On the carcinogenic effect of drinking water containing zinc. Vitalstoffe 6:59-66. (Ger.) (Eng. trans.)

Hamdi, EA. (1969) Chronic exposure to zinc of furnace operators in a brass foundry. Br J Industr Med 26:126-134.

He, L-S: Yan, X-S; Wu, D-C. (1991) Age-dependent variation of zinc-65 metabolism in LACA mice. Int J Radiat Biol 60:907-916.

Hempe, JM; Cousins, RJ. (1991) Cysteine-rich intestinal protein binds zinc during transmucosal zinc transport. Proc Natl Acad Sci 88:9671-9674.

Hempe, JM; Cousins, RJ. (1992) Cysteine-rich intestinal protein and intestinal metallothionein: An inverse relationship as a conceptual model for zinc absorption in rats. J Nutr 122:89-95.

Heth, DA; Hoekstra, WG. (1965) Zinc-65 absorption and turnover in rats: Part I. A procedure to determine zinc-65 absorption and the antagonistic effect of calcium in a practical diet. J Nutr 85:367-374.

Hirano, S; Higo, S; Tsukamoto, N; Kobayashi, E; Suzuki, KT. (1989) Pulmonary clearance and toxicity of zinc oxide instilled into the rat lung. Arch Toxicol 63:336-342.

Hoffman, HN; Phylisky, RL; Fleming, CR. (1988) Zinc induced copper deficiency. Gastroenterology 94:508-512.

Hooper, PL; Visconti, L; Garry, PJ; Johnson, GE. (1980) Zinc lowers high-density lipoprotein-cholesterol levels. J Am Med Assoc 244:1960-1961.

Hunt, JR; Lykken, GI; Mullen, LK. (1991) Moderate and high amounts of protein from casein enhance human absorption of zinc from whole-wheat or white rolls. Nutr Res 11:413-418.

Hunt, JR; Matthys, LA; Johnson, Lk. (1998) Zinc absorption, mineral balance, and blood lipids in women consuming controlled lactoovovegetarian and omniverous diets for 8 weeks. Am J Clin Nutr 67:421-430.

Hwang, SJ; Chang, JM; Lee, SC; Tsai, JH; Lai, YH. (1999) Short- and long-term uses of calcium acetate do not change hair and serum zinc concentrations in hemodialysis patients. Scand J Clin Lab Invest 59(2):83-87.

Institute of Medicine. (2002) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutrition Board, NRC. National Academy Press, Washington, DC. p. 442-501.

Istfan, NW; Janghorbani, W; Young, VR. (1983) Absorption of stable ^{70}Zn in healthy young men in relation to zinc uptake. Am J Clin Nutr 38:187-194.

Johnson, PE; Hunt, JR; Ralston, NV. (1988) The effect of past and current dietary Zn intake on Zn absorption and endogenous excretion in the rat. J Nutr 118:1205-1209.

Johnson, PE; Hunt, CD; Milne, DB; Mullen, LK. (1993) Homeostatic control of zinc metabolism in men: Zinc excretion and balance in men fed diets low in zinc. Am J Clin Nutr 57:557-565.

Kalinina, LM; Polukhina, GH; Lukasheva, LI. (1977) *Salmonella Typhimurium* - Test system for indication of mutagenic activity of environmental hazards. I. Detection of mutagenic effect of heavy metal salts using *in vivo* and *in vitro* assays without metabolic activation. Genetika 13:1089-1092.

Kelly, EJ; Quaife, CJ; Froelick, GJ; Palmiter, RD. (1996) Metallothionein I and II protect against zinc deficiency and zinc toxicity in mice. J Nutr 126:1782-1790.

Ketcheson, MR; Barron, GP; Cox, DH. (1969) Relationship of maternal dietary zinc during gestation and lactation to development and zinc, iron, and copper content of the postnatal rat. J Nutr 98:303-311.

King, LM; Banks, WA; George, WJ. (2000) Differential zinc transport into testis and brain of cadmium-sensitive and -resistant murine strains. J Androl 21(5):656-663.

Kinnamon, KE. (1963) Some independent and combined effects of copper, molybdenum, and zinc on the placental transfer of zinc-65 in the rat. J Nutr 81:312-320.

Klevay, LM. (1973) Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. Am J Clin Nutr 26(10):1060-1068.

Knudsen, E; Jensen, M; Solgaard, P; Sorensen, SS; Sandstrom, B. (1995) Zinc absorption estimated by fecal monitoring of zinc stable isotopes validated by comparison with whole-body retention of zinc radioisotopes in humans. J Nutr 125:1274-1282.

Krishnan, K; Brodeur, J. (1991) Toxicological consequences of combined exposure to environmental pollutants. Arch Complex Environ Studies 3(3):1-106.

Kumar, S. (1976) Effect of zinc supplementation on rats during pregnancy. Nutr Rep Int 13:33-36.

L'Abbe, MR; Fischer, PWF. (1984a) The effects of dietary zinc on the activity of copper-requiring metalloenzymes in the rat. J Nutr 114:823-828.

L'Abbe, MR; Fischer, PWF. (1984b) The effects of high dietary zinc and copper deficiency on the activity of copper-requiring metalloenzymes in the growing rat. J Nutr 114:813-822.

Lam, HF; Chen, LC; Ainsworth,D; Peoples, S; Amdur, MO. (1988) Pulmonary function of guinea pigs exposed to freshly generated ultrafine zinc oxide with and without spike concentrations. Am Ind Hyg Assoc J 49:333-341.

Larsson, M; Rossander-Hulthen, L; Sandstrom, B; Sandberg, A-S. (1996) Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. Br J Nutr 76:677-688.

Lasley, SM; Gilbert, ME. (1999) Lead inhibits the rat N-methyl-D-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. Toxicol Appl Pharmacol 159:224-233.

Lee, HH; Prasad, AS; Brewer, GJ; Owyang, C. (1989) Zinc absorption in human small intestine. Am J Physiol 256:G87-G91.

Lewis, RJ, Sr. (1993) Hawley's Condensed Chemical Dictionary. 12th ed. Van Nostrand Rheinhold Co., New York, NY. p. 1242.

Li, TY; Kraker, AJ; Shaw, CF; Petering, DA. (1980) Ligand substitution reactions of metallothioneins with EDTA and apocarbonic anhydrase. Proc Natl Acad Sci USA 77:6334-6339.

Liu, U; Liu, J; Michalska, AE; et al. (1996) Distribution and retention of cadmium in metallothionein I and II null mice. Toxicol Appl Pharmacol 136:260-268.

Llobet, JM; Domingo, JL; Colomina, MT; Mayayo, E; Corbella, J. (1988) Subchronic oral toxicity of zinc in rats. Bull Environ Contam Toxicol 41:36-43.

Lönnerdal, B. (1989) Intestinal absorption of zinc. In: Zinc in Human Biology. Mills, CF, Ed. New York: Springer-Verlag. pp. 33-55.

Lonnerdal, B. (2000) Dietary factors influencing zinc absorption. J Nutr 130(5):1378S-1383S.

Mahomed, K; James, DK; Golding, J; McCabe, R. (1989) Zinc supplementation during pregnancy: A double blind randomized controlled trial. Br Med J 299:826-830.

Maita, K; Hirano, M; Mitsumori, K; Takahashi, K; Shirasu, Y. (1981) Subacute toxicity studies with zinc sulfate in mice and rats. J Pest Sci 6:327-336.

Malo, J-L; Malo, J; Cartier, A; Dolovich, J. (1990) Acute lung reaction due to zinc inhalation. Eur Respir J 3:11-114.

Malo, J-L; Malo, J; Cartier, A; Dolovich, J. (1993) Occupational asthma due to zinc. Eur Respir J 6:447-450.

Martin, CJ; Le, XC; Guidotti, TL; et al. (1999) Zinc exposure in Chinese foundry workers. Am J Ind Med 35(6):574-580.

Mathur, A; Wallenius, K; Abdulla, M. (1979) Influence of zinc on onset and progression of oral carcinogenesis in rats. Acta Odontologica Scand 37:377-384. (Cited in U.S. EPA, 1980)

Methfessel, AH; Spencer, H. (1973) Zinc metabolism in the rat. I. Intestinal absorption of zinc. J Appl Physiol 34:58-62.

Miller, LV; Hambidge, KM; Naake, VL; et al. (1994) Size of the zinc pools that exchange rapidly with plasma zinc in humans: alternative techniques for measuring and relation to dietary zinc intake. J Nutr 124:268-276.

Mulhern, SA; Stroube, Jr, WB; Jacobs, RM. (1986) Alopecia induced in young mice by exposure to excess dietary zinc. Experientia 42:551-553.

NRC (National Research Council). (1983) Risk assessment in the Federal Government: managing the process. National Academy Press, Washington, DC.

NRC (National Research Council). (1989) Recommended dietary allowances. National Academy of Sciences/National Research Council. Washington, DC: National Academy Press, 10th ed., pp. 195-246.

Oberleas, D. (1996) Mechanism of zinc homeostasis. J Inorg Biochem 62:231-241.

O'Brien, KO; Zavaleta, N; Caulfield, LE; Wen, J; Abrams, SA. (2000) Prenatal iron supplements impair zinc absorption in pregnant Peruvian women. J Nutr 130(9):2251-2255.

O'Dell, BJ. (1969) Effect of dietary components upon zinc bioavailability. Am J Clin Nutr 22:1315-1322.

Oestreicher, P; Cousins, RJ. (1985) Copper and zinc absorption in the rat: Mechanism of mutual antagonism. J Nutr 115:159-166.

Ohno, H; Doi, D; Yamamura, K; et al. (1985) A study of zinc distribution in erythrocytes of normal humans. Blut 50:113-116.

Pal, N; Pal, B. (1987) Zinc feeding and conception in the rats. Int J Vitam Nutr Res 57:437-440.

Palmiter, RD. (1994) Regulation of metallothionein genes by heavy metals appears to be mediated by a zinc-sensitive inhibitor that interacts with a constitutively active transcription factor, MTF-1. Proc Natl Acad Sci 91:1219-1223.

Patterson, WK; Winkelmann, BA; Perry, MC. (1985) Zinc-induced copper deficiency: megamineral sideroblastic anemia. Ann Intern Med 103:385-386.

Pecoud, A; Donzel, P; Schelling, JL. (1975) Effect of foodstuffs on the absorption of zinc sulfate. Clin Pharmacol Ther 17:469-474.

Pennington, JAT; Schoen, SA. (1996a) Contributions of food groups to estimated intakes of nutritional elements: Results from the FDA total diet studies, 1982-1991. Int J Vit Nutr Res 66:342-349.

Pennington, JAT; Schoen, SA. (1996b) Total diet study: Estimated dietary intakes of nutritional elements, 1982-1991. Int J Vit Nutr Res 66:350-362.

Pettilä, V; Takkunen, O; Tukianinen, P. (2000) Zinc chloride smoke inhalation: A rare cause of severe acute respiratory distress syndrome. Intensive Care Med 26(2):215-217.

Prasad, AS. (1993) Essentiality and toxicity of zinc. Scand J Work Environ Health 19(1):134-136.

Prasad, R; Nath, R. (1993) Zinc transport in monkey renal brush border membrane vesicles and its interaction with cadmium: A kinetic study. J Trace Element Exper Med 6:95-107.

Prasad, AS; Schulert, AR; Sandstead, HH; et al. (1963) Zinc, iron, and nitrogen content of sweat in normal and deficient subjects. J Lab Clin Med 62:84-89.

Prasad, AS; Brewer, GJ; Schoomaker, EB; Rabbani, P. (1978) Hypocupremia induced by zinc therapy in adults. J Am Med Assoc 240(20):2166-2168.

Prasad, R; Kaur, D; Kumar, V. (1996) Kinetic Characterization of Zinc Binding to Brush Border Membranes from Rat Kidney Cortex: Interaction with Cadmium. Biochimica et Biophysica Acta 1284:69-78.

Richards, MP; Cousins, RJ. (1975) Mammalian zinc homeostasis: requirement for RNA and metallothionein synthesis. Biochem Biophys Res Comm 64:1215-1223.

Rivlin, RS. (1983) Misuse of hair analysis for nutritional assessment. Am J Med 75:489-493.

Rohrs, LC. (1957) Metal-fume fever from inhaling zinc oxide. Arch Ind Health 16:42-47.

Samman, S; Roberts, DCK. (1987) The effect of zinc supplements on plasma zinc and copper levels and the reported symptoms in healthy volunteers. Med J Australia 146:246-249.

Samman, S; Roberts, DCK. (1988) The effect of zinc supplementation on lipoproteins and copper status. Atherosclerosis 70:247-252.

Sandstead, HH. (1994) Understanding zinc: Recent observations and interpretations. J Lab Clin Med 124:322-327.

Sandstrom, B; Abrahamson, H. (1989) Zinc absorption and achlorhydria. Eur J Clin Nutr 43:877-879.

Saxena, R; Bedwal, RS; Mathur, RS. (1989) Zinc toxicity and male reproduction in rats: A histological and biochemical study. Trace Elem Med 6:119-133.

Schlicker, SA; Cox, DH. (1968) Maternal dietary zinc, and development and zinc, iron and copper content of the rat fetus. J Nutr 95:287-294.

Schroeder, HA, Nason, AP; Tipton, IH; Balassa, JJ. (1967) Essential trace metals in man: zinc. Relation to environmental cadmium. J Chron Dis 20:179-210.

Simko, MD; Cowell, C; Gilbride, JA, Ed. (1984) Nutrition assessment: A comprehensive guide for planning intervention. Aspen Systems Corporation, Rockville, MD. 396 p.

Simmer, K; Lort-Phillips, L; James, C; Thompson, RPH. (1991) A double-blind trial of zinc supplementation in pregnancy. Eur J Clin Nutr 5:139-144.

Simons, TJB. (1995) The affinity of human erythrocyte porhobilinogen synthase for Zn^{2+} and Pb^{2+}. Eur J Biochem 234:178-183.

Smith, SE; Larson, EJ. (1946) Zinc toxicity in rats: Antagonistic effects of copper and liver. J Biol Chem 163:29-38.

Stillman, ML. (1995) Metallothioneins. Coordination Chem Rev 144:461-571.

Stoner, GD; Shimkin, MB; Troxell, MC; Thompson, TL; Terry, LS. (1976) Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. Cancer Res 36(5):1744-1747.

Straube, EF; Schuster, NH; Sinclair, JA. (1980) Zinc toxicity in the ferret. J Comp Pathol 90:355-361.

Sturgis, CC; Drinker, P; Thomson, RM. (1927) Metal fume fever: I. Clinical observations on the effect of the experimental inhalation of zinc oxide by two apparently normal persons. J Industr Hyg Toxicol 9:88-97.

Sutton, WR; Nelson, VE. (1937) Studies on zinc. Proc Soc Exp Biol Med 36:211-213.

Tacnet, F; Watkins, DW; Ripoche, P. (1990) Studies of zinc transport into brush-border membrane vesicles isolated from pig small intestine. Biochem Biophys Acta 1024: 323-330.

Tacnet, F; Watkins, DW; Ripoche, P. (1991) Zinc binding in intestinal rush-border membrane isolated from pig. Biochimica et Biophysica Acta 1063:51-59.

Thomas, DW. (1991) In: Metals and Their Compounds in the Environment, Merian E., Ed., Weinheim, Fed Rep Ger: VCH p. 1309-1342.

Thompson, ED; McDermott, JA; Zerkle, TB; et al. (1989) Genotoxicity of zinc in 4 short-term mutagenicity assays. Mutat Res 223:267-272.

Udom, AO; Brady, FO. (1980) Reactivation in vitro of zinc-requiring apoenzymes by rat liver zinc-thionein. Biochem J 187:329-335.

Uriu-Hare, JY; Stern, JS; Keen, CL. (1989) Influence of maternal dietary Zn intake on expression of diabetes-induced teratogenicity in rats. Diabetes 38:1282-1290.

U.S. EPA. (1979) Water Related Environmental Fate of 129 Priority Pollutants. Vol. 1. EPA 440/4-79-0029A.

U.S. EPA. (1986a) Guidelines for Carcinogen Risk Assessment. Federal Register. 51(185):33992-34003.

U.S. EPA. (1986b) Guidelines for the Health Risk Assessment of Chemical Mixtures. Federal Register. 51(185):34014-34025.

U.S. EPA. (1986c) Guidelines for Mutagenicity Risk Assessment. Federal Register. 51(185):34006-34012.

U.S. EPA. (1988) Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Prepared by Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati OH. EPA 600/6-87/008, NTIS PB88-179874/AS, February 1988.

U.S. EPA. (1991) Guidelines for Developmental Toxicity Risk Assessment. Federal Register 56(234):63798-63826.

U.S. EPA. (1994a) Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity: Notice of Availability. Federal Register 59(206):53799.

U.S. EPA. (1994b) Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Prepared by Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Research Triangle Park, NC. EPA/600/8-90/066F.

U.S. EPA. (1994c) Peer Review and Peer Involvement at the U.S. Environmental Protection Agency. Signed by the U.S. EPA Administrator Carol M. Browner, dated June 7, 1994.

U.S. EPA (1995a) Proposed Guidelines for Neurotoxicity Risk Assessment. Federal Register 60(192):52032-52056.

U.S. EPA. (1995b) Use of the Benchmark Dose Approach in Health Risk Assessment. EPA/630/R-94/007.

U.S. EPA. (1995c) Monthly Status Report of RfD/RfC and CRAVE Work Groups (as of 09/01/95). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH.

U.S. EPA. (1996) Reproductive Toxicity Risk Assessment Guidelines. Federal Register. 61(212):56274-56322.

U.S. EPA. (1998) Science Policy Council Handbook: Peer Review. Prepared by the Office of Science Policy, Office of Research and Development, Washington, DC. EPA 100-B-98-001.

U.S. EPA. (1999) Guidelines for carcinogen risk assessment. Review Draft, NCEA-F-0644, July. Risk Assessment Forum, Washington, DC. http://www.epa.gov/ncea/raf/cancer.htm

Vallee, BL; Flachuk, KH. (1993) The biochemical basis of zinc physiology. Physiol Rev 73:79-118.

Waalkes, MP; Pérez-Ollé, R. (2000) Tole of metallothionein in the metabolism, transport and toxicity of metals. In: Molecular Biology and Toxicology of Metals, Zalups, RK; Koropatnick, J; Ed. Taylr Fancis, London p. 414-459.

Waalkes, MP; Rehm, S; Riggs, CW; et al. (1989) Cadmium carcinogenesis in male Wistar [CRL:(WI)BR] rats: Dose-response analysis of effects of zinc on tumor induction in the prostate, in the testes, and at the injection site. Cancer Res 49:4282-4288.

Wallenius, K; Mathur, A; Abdulla, M. (1979) Effect of different levels of dietary zinc on development of chemically induced oral cancer in rats. Int J Oral Surg 8:56-62.

Walsh, CT; Sandstead, HH; Prasad, AS; Newberne, PM; Fraker, PJ. (1994) Zinc: health effects and research priorities for the 1990s. Environ Health Perspect 102(2):5-46.

Walters, M; Roe, FJC. (1965) A study of the effects of zinc and tin administered orally to mice over a prolonged period. Food Cosmet Toxicol 3:271-276.

Wapnir, RA; Stiel, L. (1986) Zinc intestinal absorption in rats: specificity of amino acids as ligands. J Nutr 116:2171-2179.

Wastney, ME; Aamodt, RL; Rumble, WF; Henkin, RI. (1986) Kinetic analysis of zinc metabolism and its regulation in normal humans. Am J Physiol 251:R398-R405.

Woo, YT; Lai, DY; Arcos, JC; Argus, MF. (1988) Chemical Induction of Cancer: Structural Bases and Biological Mechanisms. Vol. IIIC, Natural, Metal, Fiber and Macromolecular Carcinogens. Academic Press, San Diego p. 488-489.

Yadrick, MK; Kenney, MA; Winterfelt, EA. (1989) Iron, copper, and zinc status: Response to supplementation with zinc or zinc and iron in adult females. Am J Clin Nutr 49:145-150.

Zaporowska, H; Wasilewski, W. (1992) Combined effect of vanadium and zinc on certain selected haematological indices in rats. Comp Biochem Physiol 103C:143-147.

Zerahn, B; Kofoed-Enevoldsen, A; Jensen, BV; et al. (1999) Pulmonary damage after modest exposure to zinc chloride smoke. Respir Med 93(12):885-890.