3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of zinc. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

Zinc is an essential nutrient in humans and animals that is necessary for the function of a large number of metalloenzymes. These enzymes include alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, leucine aminopeptidase, superoxide dismutase, and deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) polymerase. As such, zinc is required for normal nucleic acid, protein, and membrane metabolism, as well as cell growth and division. Zinc also plays an essential role in the maintenance of nucleic acid structure of genes (zinc finger phenomenon). Zinc deficiency has been associated with dermatitis, anorexia, growth retardation, poor wound healing, hypogonadism with impaired reproductive capacity, impaired immune function, and depressed mental function; increased incidence of congenital malformations in infants has also been associated with zinc deficiency in the mothers (Cotran et al. 1989; Elinder 1986; Sandstead 1981). Zinc deficiency may also have an impact on carcinogenesis, though the direction of the influence seems to vary with the agent (Fong et al. 1978; Mathur 1979; Wallenius et al. 1979). Therefore, certain levels of zinc intake are recommended. The RDA for zinc is 11 mg/day in men and 8 mg/day in women (IOM 2002). Higher RDAs are recommended for women during pregnancy and lactation (12 mg/day for pregnant women and nursing women). While a detailed discussion of zinc deficiency is beyond the scope of this toxicological profile, the subject has been thoroughly reviewed by other agencies (IOM 2002; WHO 1996).

Just as zinc deficiency has been associated with adverse effects in humans and animals, overexposures to zinc also have been associated with toxic effects. This chapter contains a description of the toxic effects that have been associated with exposures to high levels of zinc and selected zinc compounds by the inhalation, oral, and dermal routes. Specifically, zinc chloride, zinc oxide, zinc sulfate, and zinc sulfide will be discussed. Other zinc compounds are discussed in this chapter whenever data regarding these

compounds add relevant information to the discussion on zinc. Any general comments regarding the lack of data on zinc refer to both zinc and its compounds.

Because there are differences in toxicity between the various zinc compounds following inhalation exposure, these compounds will be discussed under separate subheadings in Section 3.2.1 (Inhalation Exposure). After oral or dermal exposure, the toxicities are comparable for all zinc compounds. Therefore, in Section 3.2.2 (Oral Exposure) and Section 3.2.3 (Dermal Exposure), the discussion will not be subdivided, but the specific zinc compounds will be identified in each case.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

In humans, death has resulted from acute exposure to zinc compounds. When a high concentration (estimated at 33,000 mg zinc/m^3) of zinc chloride smoke resulted from the explosion of many generators in a tunnel following a bombing raid in World War II, 10 of the 70 exposed people in the tunnel died within 4 days (Evans 1945). The smoke generated contained mainly highly caustic zinc chloride, but exposure to other constituents, namely zinc oxide, hexachloroethane, calcium silicate, and an igniter, was also possible. Therefore, the deaths resulting from the explosion cannot be conclusively attributed to only exposure to zinc chloride. This is the only human study reporting an estimated exposure level that caused death. Another study reported the death of a fireman exposed to the contents of a smoke bomb in a closed environment (Milliken et al. 1963). The man died 18 days after exposure because of respiratory difficulty. Again, exposure to zinc chloride was simultaneous with exposure to other substances in the smoke. Two soldiers exposed without gas masks to zinc chloride smoke during military training developed severe adult respiratory distress syndrome (ARDS) and died 25–32 days after the incident (Hjortso et al. 1988). Diffuse microvascular obliteration, widespread occlusion of the pulmonary arteries, and extensive interstitial and intra-alveolar fibrosis were observed at autopsy. Zinc levels in major organs and tissues obtained during autopsy were within the normal range, and no zinc particles were observed by scanning electron microscopy. According to the authors, the fumes from the smoke bombs consisted mainly of zinc chloride. However, no exposure levels were estimated, and other substances were also present in the smoke. Because of the caustic nature of zinc chloride, it is likely that these effects were the result of severe irritation from the compound, rather than direct actions of the zinc ion.

A case study presented by Murray (1926) reported on an infant death due to bronchopneumonia resulting from inhalation, and possibly ingestion, of an unspecified amount of zinc stearate powder spilled from a container. However, it is unclear whether the death was due to the zinc content or whether aspiration bronchopneumonia would result from inhalation of similar powders that do not contain zinc.

In mice, the reported LCT_{50} (product of lethal concentration and time to kill 50% of animals) of zinc chloride is 11,800 mg/minute/m^3 (Schenker et al. 1981). However, Schenker et al. (1981) did not provide information on how this value was determined. Following exposure to zinc chloride smoke for 3–20 weeks, mortality was 50% in mice exposed to 121.7 mg zinc/m^3 (compared to 20% in controls) and 22% in guinea pigs exposed to 119.3 mg zinc/m^3 (compared to 8% in controls) (Marrs et al. 1988). The smoke was similar to that described by Evans (1945) and also contained zinc oxide, hexachloroethane, and other compounds.

3.2.1.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1.

No studies were located regarding musculoskeletal, endocrine, dermal, or body weight effects in humans or animals after inhalation exposure to zinc or zinc compounds. The systemic effects observed after inhalation exposure are discussed below. In most cases, the effects of zinc are discussed without separating effects caused by the individual zinc compounds. However, the respiratory effects of the individual zinc compounds are discussed separately because the nature of the respiratory toxicity differs depending on the particular compound to which one is exposed.

Respiratory Effects.

Zinc Oxide. Metal fume fever, a well-documented acute disease induced by intense inhalation of metal oxides, especially zinc, impairs pulmonary function but does not progress to chronic lung disease (Brown 1988; Drinker and Drinker 1928; Malo et al. 1990). Symptoms generally appear within a few hours after acute exposure, usually with dryness of the throat and coughing (Drinker et al. 1927b). The most

Table 3-1 Levels of Significant Exposure to Zinc - Inhalation

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
ACUTE EXPOSURE							
Systemic							
1	Human	15-30 min	Resp		77 (minimal change in pulmonary function)		Blanc et al. 1991 Zinc oxide
2	Human	1 d 2hr/d	Resp		3.9 (dry or sore throat, chest tightness)		Gordon et al. 1992 Zinc oxide
			Other		3.9 (fever/chills and headache)		
3	Human	1x 15-120 minutes 1x	Resp		16.4 (Increased indices of pulmonary inflammation)		Kuschner et al. 1995 Zinc oxide
4	Human	1x 10-30 minutes 1x	Resp		33 (Altered levels of inflammatory cytokines in bronchoalveolar lavage fluid)		Kuschner et al. 1997 Zinc oxide
5	Human	2 hr	Resp	0.0036			Linn et al. 1981 Zinc amm sulfate
6	Human	6-8 hr (Occup)	Resp	0.034 M			Marquart et al. 1989 Zinc oxide

Table 3-1 Levels of Significant Exposure to Zinc - Inhalation (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
7	Human	10.5-12 min	Resp		600 M (decreased vital capacity)		Sturgis et al. 1927 Zinc oxide
			Gastro		600 M (nausea)		
			Hemato		600 M (increased leukocytes)		
8	Rat (Fischer- 344)	1 d 3hr/d	Resp		2.2 (increased LDH protein in bronchoalveolar lavage fluid)		Gordon et al. 1992 Zinc oxide
9	Gn Pig	1 hr	Resp		0.73 M (decrease in lung compliance)		Amdur et al. 1982 Zinc oxide
10	Gn Pig	1-3 d 3hr/d	Resp	1.8 M	4.7 M (increased neutrophils, LDH, and protein in bronchoalveolar lavage fluid)		Conner et al. 1988 Zinc oxide
11	Gn Pig (Hartley)	1 d 3hr/d	Resp		2.2 (increased LDH and protein in bronchoalveolar lavage fluid)		Gordon et al. 1992 Zinc oxide

Table 3-1 Levels of Significant Exposure to Zinc - Inhalation (continued)

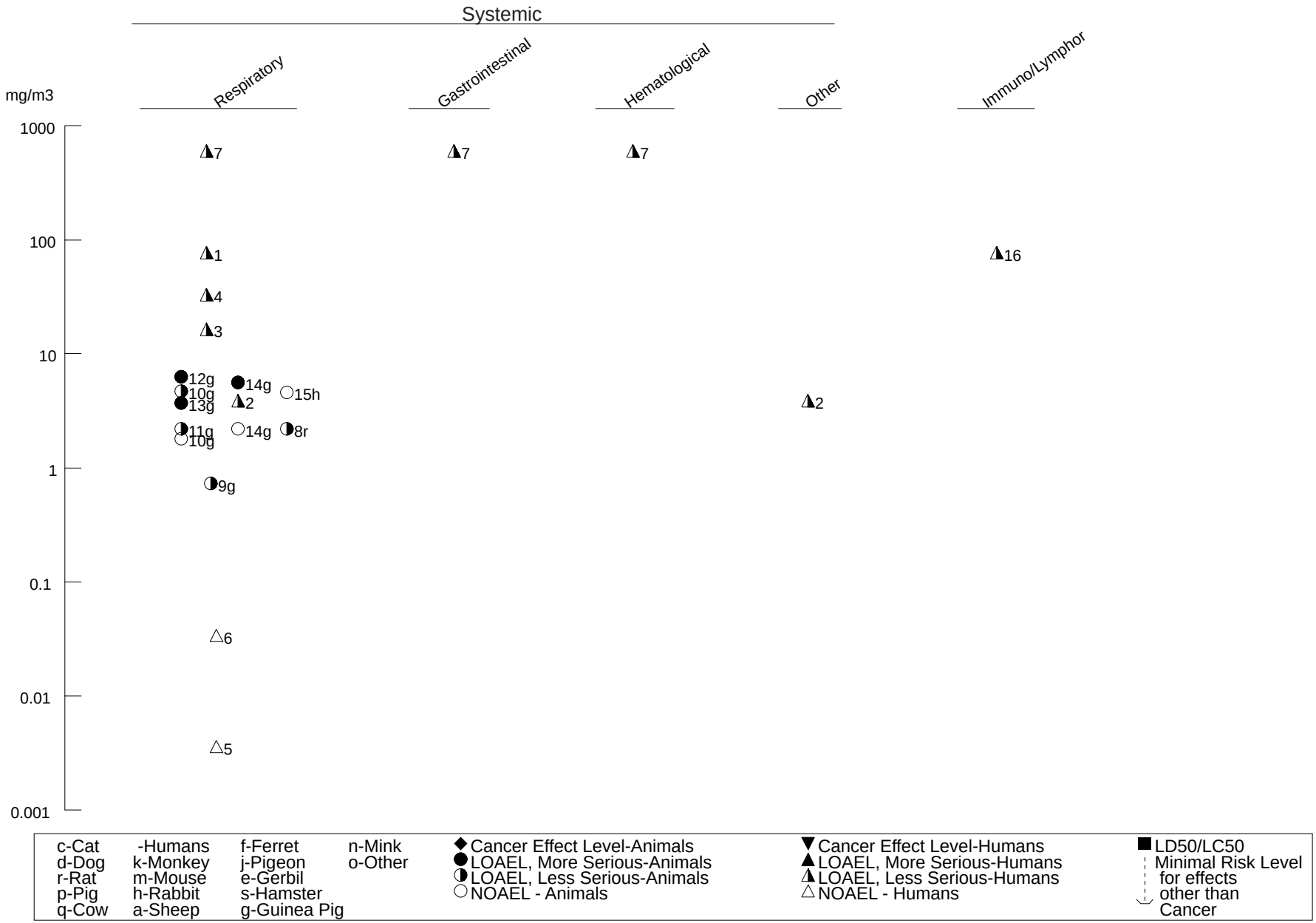
Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/m³)	LOAEL Less Serious (mg/m³)	LOAEL Serious (mg/m³)	Reference Chemical Form
12	Gn Pig (Hartley)	3 hr	Resp			6.3 M (decreased functional residual capacity)	Lam et al. 1982 Zinc oxide
13	Gn Pig (Hartley)	6d 3hr/d	Resp			3.7 M (impaired lung function; inflammation; increased pulmonary resistance; increased lung weight)	Lam et al. 1985 Zinc oxide
14	Gn Pig (Hartley)	5 d 3hr/d	Resp	2.2 M		5.6 M (impaired lung function; increased lung weight)	Lam et al. 1988 Zinc oxide
15	Rabbit (New Zealand)	1 d 2hr/d	Resp	4.6			Gordon et al. 1992 Zinc oxide
Immuno/ Lymphoret							
16	Human	15-30 min			77 (increased number of leukocytes, T cells, T suppressor cells, and natural killer cells in bronchoalveolar lavage fluid)		Blanc et al. 1991 Zinc oxide

a The numbers corresponds to entries in Figure 3-1.

amm sulfate = ammonium sulfate; d = day(s); Gastro = gastrointestinal; Gn pig = guinea pig; Hemato = hematological; hr = hour(s); LDH = lactate dehydrogenase; LOAEL = lowest-observed-adverse-effect level; min = minute(s); NOAEL = no-observed-adverse-effect level; (occup) = occupational; Resp = respiratory

Figure 3-1 Levels of Significant Exposure to Zinc - Inhalation

Acute (≤14 days)



prominent respiratory effects of metal fume fever are substernal chest pain, cough, and dyspnea (Rohrs 1957). The impairment of pulmonary function is characterized by reduced lung volumes and a decreased diffusing capacity of carbon monoxide (Malo et al. 1990; Vogelmeier et al. 1987). The respiratory effects have been shown to be accompanied by an increase in bronchiolar leukocytes (Vogelmeier et al. 1987). The respiratory symptoms generally disappear in the exposed individual within 1–4 days (Brown 1988; Drinker et al. 1927b; Sturgis et al. 1927). Inhalation of zinc oxide is most likely to occur in occupational situations where zinc smelting or welding take place. Ultrafine zinc oxide particles (0.2–1.0 μm) originate from heating zinc beyond its boiling point in an oxidizing atmosphere. Upon inhalation, these small particles (<1 μm) reach the alveoli and cause inflammation and tissue damage in the lung periphery (Brown 1988; Drinker et al. 1927b; Vogelmeier et al. 1987).

A number of studies have measured exposure levels associated with metal fume fever. Workers involved in pouring molten zinc reported shortness of breath and chest pains 2–12 hours following exposure to 320–580 mg zinc/m^3 as zinc oxide for 1–3 hours (Hammond 1944); the number of workers was not reported. Two volunteers had nasal passage irritation, cough, substernal chest pain, persistent rales of the lung base, and a decreased vital capacity for approximately 3–49 hours following acute inhalation (10–12 minutes) of 600 mg zinc/m^3 as zinc oxide (Sturgis et al. 1927). This study is limited due to an inadequate number of subjects, a lack of controls, and a lack of analysis of the final aerosol product. A subject experimentally exposed to zinc oxide fumes reported mild pain when breathing deeply the next day after a 5-hour exposure to 430 mg zinc/m^3 (Drinker et al. 1927a). Minimal changes in forced expiratory flow were observed 1 hour after a 15–30-minute exposure to 77 mg zinc/m^3 as zinc oxide (Blanc et al. 1991).

Acute experimental exposures to lower concentrations of zinc oxide (14 mg/m^3 for 8 hours or 45 mg zinc/m^3 for 20 minutes) and occupational exposures to similar concentrations (8–12 mg zinc/m^3 for 1–3 hours and 0.034 mg zinc/m^3 for 6–8 hours) did not produce symptoms of metal fume fever (Drinker et al. 1927b; Hammond 1944; Marquart et al. 1989). In a single-blind experiment, exposure of subjects to 3.9 mg zinc/m^3 as zinc oxide resulted in sore throat and chest tightness but no impairment of pulmonary function (Gordon et al. 1992). It is speculated that subjects in other studies may have been less susceptible because of the development of tolerance to zinc (Gordon et al. 1992). Kuschner et al. (1995) exposed a group of 14 volunteers to a single exposure of varying levels of zinc oxide fume (mean concentration 16.4±12.5 mg/m^3) for 15–120 minutes (mean duration 45±28 minutes) and evaluated the response by bronchoalveolar lavage (BAL). Significant increases were reported in the number of poly-morphonuclear leukocytes and lymphocytes in the BAL fluid, but not in the number of macrophages or in

lymphocyte subpopulations; aside from a decrease in FEV_1, no changes were reported in pulmonary function tests. In a follow-up study by the same group (Kuschner et al. 1997), single-exposed volunteers showed an increase in levels of the cytokines TNF, IL-6, and IL-8 as a result of zinc oxide inhalation. Recurrent episodes of cough and dyspnea were reported in a former mild smoker 3 years after beginning work in a metal foundry where exposure to zinc oxide presumably occurred (Ameille et al. 1992). This case was distinguishable from metal fume fever because of the lack of tolerance to zinc (as shown by the late emergence of symptoms).

Several animal studies have been conducted to quantify specific effects after acute zinc oxide inhalation. As in human exposure, the respiratory system is the primary site of injury following inhalation exposure. Acute administration of 88–482 mg zinc/m^3 as zinc oxide to rats and rabbits resulted in the following pulmonary changes: grayish areas with pulmonary congestion, various degrees of peribronchial leukocytic infiltration, and bronchial exudate composed almost entirely of polymorphonuclear leukocytes (Drinker and Drinker 1928). Cats similarly exposed exhibited more severe effects including bronchopneumonia, leukocyte infiltration into alveoli, and grayish areas with congestion. During the exposure period, the cats demonstrated labored breathing and evidence of upper respiratory tract obstruction. A minimum effect level could not be determined for any species because the concentration varied widely (88–482 mg zinc/m^3) during exposure.

Guinea pigs administered 0.73 mg zinc/m^3 as zinc oxide for 1 hour had a progressive decrease in lung compliance but no change in air flow resistance. These observations reflect a response in the lung periphery where submicrometer aerosols are likely to deposit (Amdur et al. 1982). The authors postulated that reduced compliance may be associated with human metal fume fever.

In contrast to the results of Amdur et al. (1982), no effects on ventilation, lung mechanics (respiratory frequency, tidal volume, pulmonary resistance, and pulmonary compliance), diffusing capacity of carbon monoxide, or most lung volume parameters were observed by Lam et al. (1982) following the exposure of guinea pigs to 6.3 mg zinc/m^3 as zinc oxide for 3 hours. However, functional residual capacity was significantly decreased. The discrepancy between the results of Amdur et al. (1982) and Lam et al. (1982) may be attributable to the use of anesthetized animals by Lam et al. (1982). In a later study, exposures of guinea pigs to 3.7 or 4.3 mg zinc/m^3 as zinc oxide for 3 hours/day, for 6 days, resulted in transient functional, morphological, and biochemical changes (Lam et al. 1985). Functional changes included increased flow resistance, decreased lung compliance, and decreased diffusing capacity, all of which returned to normal within 24–72 hours following exposure. The morphological changes (increased

lung weight, inflammation involving the proximal portion of alveolar ducts and adjacent alveoli, interstitial thickening, and increased pulmonary macrophages and neutrophils in adjacent air spaces) were, however, still present at 72 hours. In guinea pigs with evidence of an inflammatory reaction involving the peripheral airways, DNA synthesis increased in bronchiolar cells. Similarly, exposure of guinea pigs to 5.6 mg zinc/m^3 as zinc oxide for 3 hours/day, for 5 days, resulted in gradual decreases in total lung capacity, vital capacity, and decreased carbon monoxide diffusing capacity (Lam et al. 1988); however, no effects were observed in guinea pigs exposed to 2.2 mg zinc/m^3. The reason that effects have been seen in the guinea pig at exposure levels lower than humans may have to do with the structural features of the guinea pig lung. The bronchi and peripheral airways of guinea pigs have a thicker smooth muscle layer and only a small surface area covered by alveolar sacs compared to the bronchi and peripheral airways of other laboratory animals and humans. This makes the guinea pig more susceptible than other laboratory animals to functional impairment of the peripheral airways and should be noted in toxicity comparisons (Lam et al. 1982).

The bronchoalveolar lavage fluid of rats or guinea pigs exposed to 1.8 mg zinc/m^3 as zinc oxide for 3 hours contained increased levels of lactate dehydrogenase and total protein, suggesting effects on cell viability or membrane permeability (Gordon et al. 1992). Rabbits were not affected following a similar exposure to 4.6 mg zinc/m^3 for 2 hours. Guinea pigs had foci of inflammation after exposure to 4.7 mg zinc/m^3 for 3 days, and the bronchoalveolar lavage fluid contained increased levels of protein, angiotensin converting enzyme, and neutrophils (Conner et al. 1988). No significant changes in respiratory effects were observed in this study following exposure to 1.8 mg zinc/m^3 for 3 days.

Zinc Chloride. Zinc chloride, a corrosive inorganic salt, is more damaging than zinc oxide to the mucous membranes of the nasopharynx and respiratory tract upon contact. Zinc chloride is a primary ingredient in smoke bombs used by the military for screening purposes, crowd dispersal, and occasionally in military and civilian fire-fighting exercises. Reports of serious respiratory injury have been reported to result from accidental inhalation of smoke from these bombs. These reports are of limited use in assessing the toxicity of zinc chloride because exposure to other compounds, usually hexachloroethane, zinc oxide, and calcium silicides, also occur. Furthermore, the specific concentrations inhaled are usually unknown. Despite these limitations, several case studies have described similar respiratory effects in humans following acute inhalation exposures. These effects include dyspnea, cough, pleuritic chest pain, bilateral diffuse infiltrations, pneumothorax, and acute pneumonitis from respiratory tract irritation (Johnson and Stonehill 1961; Matarese and Matthews 1966; Schenker et al. 1981; Zerahn et al. 1999). In the study by Johnson and Stonehill (1961), cough, dyspnea, burning throat, diffuse infiltrates throughout the lung,

chemical pneumonitis, and decreased vital capacity were observed at an estimated zinc chloride exposure level of 4,075 mg/m^3 (1,955 mg zinc/m^3). In other studies, more severe effects have occurred, including ulcerative and edematous changes in mucous membranes, fibrosis, subpleural hemorrhage, advanced pulmonary fibrosis, and fatal respiratory distress syndrome (Evans 1945; Hjortso et al. 1988; Homma et al. 1992; Milliken et al. 1963).

Focal alveolitis, consolidation, emphysema, infiltration with macrophages, and fibrosis were observed in guinea pigs that died following exposure to 119 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 3–20 weeks (Marrs et al. 1988); no changes were seen in guinea pigs that survived 13 months after the 20-week exposure. Thirteen months after a 20-week exposure, rats similarly exposed to 12.8 mg zinc/m^3 showed an increase in peribronchial inflammatory cell (lymphocytes and macrophage) infiltration. Mice exposed to 121.7 mg zinc/m^3 as zinc chloride smoke, but not to lower doses, for 1 hour/day, 5 days/week, showed increased macrophages and lymphocytes in the lungs (Marrs et al. 1988). The smoke also contained zinc oxide, hexachloroethane, and other compounds.

Zinc Ammonium Sulfate. Zinc ammonium sulfate is a compound emitted during combustion of fossil fuels and is, therefore, found in the ambient air. Humans acutely exposed to a concentration of 0.0036 mg zinc/m^3 as zinc ammonium sulfate for 2 hours (Linn et al. 1981) exhibited minimal or no short-term respiratory effects (including minimal substernal irritation, throat irritation, and coughing in asthmatic subjects). However, most human exposures to an ambient air pollutant such as zinc ammonium sulfate are chronic, and this study provides little information about the health effects associated with typical exposures.

No studies were located regarding respiratory effects in animals after inhalation exposure to zinc ammonium sulfate.

Zinc Stearate. Inhalation of zinc stearate powder resulted in aspiration bronchopneumonia in an infant (Murray 1926). However, it is unclear whether the bronchopneumonia resulted from the inhalation of zinc stearate powder specifically or from a nonspecific effect of the inhalation of powders.

No studies were located regarding respiratory effects in animals after inhalation exposure to zinc stearate.

Cardiovascular Effects. No atypical heart sounds or blood pressure abnormalities were observed in 24 employees occupationally exposed to concentrations as high as 130 mg zinc/m^3 of metallic zinc dust,

zinc oxide dust, zinc sulfide dust, or lithophone dust (a combination of barium sulphate and ≈30% zinc sulphide) for 2–35.5 years (Batchelor et al. 1926). However, this study is limited because only selected employees were examined.

Only limited information was located regarding cardiovascular effects in animals following inhalation exposure to zinc. Routine gross and microscopic examination of the hearts of rats and mice revealed no adverse effects 13 months after exposure to 121.7 mg zinc/m^3 as zinc chloride smoke (also containing other compounds) for 1 hour/day, 5 days/week, for 20 weeks (Marrs et al. 1988). Similarly, no changes were observed in the hearts of guinea pigs exposed to 119.3 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 20 weeks, and then observed for an additional 17 months (Marrs et al. 1988).

Gastrointestinal Effects. Nausea was reported by humans exposed to high concentrations of zinc oxide fumes (Hammond 1944; Rohrs 1957; Sturgis et al. 1927) and zinc chloride smoke (Evans 1945; Johnson and Stonehill 1961; Schenker et al. 1981). The zinc chloride smoke also contained zinc oxide, hexachloroethane, and other compounds. In general, exposure levels associated with nausea have not been reported. However, exposures to 320 mg zinc/m^3 as zinc oxide for 1–3 hours (Hammond 1944) or 600 mg zinc/m^3 as zinc oxide for 10–12 minutes (Sturgis et al. 1927) were reported to have resulted in nausea; it should be noted, however, that the zinc used in these studies contained slight impurities (i.e., lead, magnesium). Autopsies of victims who died following exposure to very high concentrations of zinc chloride smoke revealed irritation of the stomach and intestines (Evans 1945). The smoke also contained zinc oxide, hexachloroethane, and other compounds. Workers in the galvanizing industry were found by McCord et al. (1926) to have a higher than expected incidence of gastrointestinal problems; however, these individuals may have been exposed to other chemicals (arsenic, hydrogen sulfide). Of 15 workers examined with 7–20 years of experience, 12 had frequent episodes of epigastric or abdominal pain, nausea, vomiting, ulcers, constipation, tarry stools, and/or gas. It is unclear whether these effects were due to systemic zinc or were the result of direct contact with the gastrointestinal tract following mucociliary clearance of inhaled zinc particles and subsequent swallowing. In contrast, 24 workers with 2–35.5 years of exposure to ≤130 mg zinc/m^3 as metallic zinc dust, zinc sulfide dust, zinc oxide, or lithophone dust reported no nausea or vomiting and only occasional mild abdominal discomfort that could not be attributed with certainty to zinc exposure (Batchelor et al. 1926). A study examining the acidity of the stomach contents after stimulation in controls and workers employed in the production of brass alloys showed that stomach acidity was similar in the two groups prior to stimulation but remained elevated for longer periods after stimulation in the exposed workers (Hamdi 1969). This was proposed to account for

the gastric complaints of workers exposed to zinc fumes. Despite these findings, x-rays showed no lesions in the stomachs or duodenums of exposed workers.

The only information available regarding gastrointestinal effects in animals was found in a study by Marrs et al. (1988) in which rats and mice were exposed to 121.7 mg zinc/m^3 as zinc chloride smoke (which also contains zinc oxide, hexachlorophene, and other compounds) for 1 hour/day, 5 days/week, for 20 weeks, and then observed for an additional 13 months. In the same study, guinea pigs were exposed to 119.3 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 3 weeks. All animals were sacrificed at the end of 18 months. Routine gross and microscopic evaluation of the stomach and intestines at 18 months revealed no persistent adverse effects.

Hematological Effects. Leukocytosis persisting for approximately 12 hours after fever dissipates is one of the hallmarks of metal fume fever (Mueller and Seger 1985). Such effects have been observed in a number of case reports of occupational and experimental exposure of humans to zinc oxide fumes (Brown 1988; Drinker et al. 1927a; Malo et al. 1990; Rohrs 1957; Sturgis et al. 1927). Increased leukocyte counts were observed following experimental exposures to 430 mg zinc/m^3 as zinc oxide for 3 hours (Drinker et al. 1927a) or 600 mg zinc/m^3 as zinc oxide for 10–12 minutes (Sturgis et al. 1927). These studies are limited in that they used an inadequate number of subjects, lacked controls, and used impure zinc oxide.

Decreased numbers of red blood cells and hemoglobin were found in several workers with 7–20 years of experience in the galvanizing industry (McCord et al. 1926). However, there were excess tobacco use and alcohol consumption by workers and possible concurrent exposure to other chemicals (chloride, sulfide), which confound the study results. No anemia was detected among 12 workers exposed for 4–21 years to zinc oxide fumes in the production of brass alloys (Hamdi 1969). These workers may have also been exposed to magnesium, copper, and aluminum.

No studies were located regarding hematological effects in animals after inhalation exposure to zinc.

Hepatic Effects. Routine blood chemistries and examinations revealed no liver disease among 12 workers with 4–21 years of exposure to zinc oxide fumes in the production of brass alloys (Hamdi 1969).

No adverse effects were observed during gross and microscopic examination of livers of rats and guinea pigs exposed to 121.7 mg zinc/m^3 or 119.3 mg zinc/m^3, respectively, as zinc chloride smoke for

1 hour/day, 5 days/week, for 20 weeks, and sacrificed at the end of 18 months (Marrs et al. 1988). Significant increases in the incidence of fatty liver were observed in mice exposed to 12.8 or 121.7 mg zinc/m^3 as zinc chloride smoke using the same exposure paradigm; however, the incidence did not increase with dose (Marrs et al. 1988). The smoke contained other compounds in addition to zinc chloride.

Renal Effects. Urinalyses and histories of urinary function revealed no adverse effects in 24 workers exposed for 2–35.5 years to ≤130 mg zinc/m^3 as metallic zinc dust, zinc sulfide dust, zinc oxide, or lithophone dust (Batchelor et al. 1926).

No adverse effects were observed following gross and microscopic examination of kidneys from rats, mice, and guinea pigs exposed for 1 hour/day, 5 days/week, for 20 weeks, to concentrations as high as 121.7 or 119.3 mg zinc/m^3 as zinc chloride smoke (which also contained other compounds) and then sacrificed 13 months later (Marrs et al. 1988).

Ocular Effects. Reddened conjunctiva and corneal burns occurred in individuals exposed to high concentrations of zinc chloride smoke (estimated at 33,000 mg zinc/m^3) when several smoke generators exploded in a tunnel during World War II (Evans 1945). The ocular effects may have been due to direct contact with the smoke.

Homeostatic Effects. A fever appearing 3–10 hours after exposure to zinc oxide fumes and lasting approximately 24–48 hours is characteristic of metal fume fever caused by zinc (Mueller and Seger 1985). Elevated body temperature has been observed in a number of experimental and occupational zinc oxide exposures (Brown 1988; Drinker et al. 1927a; Hammond 1944; Malo et al. 1990; Rohrs 1957; Sturgis et al. 1927; Vogelmeier et al. 1987). Using a number of exposure concentrations for various durations, Drinker et al. (1927b) found that the increase in body temperature was dependent on the exposure duration and concentration. Based on their data, they calculated that the threshold for pyrogenic effects was 45 mg zinc/m^3 for 20 minutes. This study is limited in that impurities were present in the zinc used and no statistical analysis was performed. Exposure to zinc chloride smoke (which also contains other compounds) has also been associated with fever (Hjortso et al. 1988; Matarese and Matthews 1966).

No studies were located regarding other systemic effects in animals following inhalation exposure to zinc.

3.2.1.3 Immunological and Lymphoreticular Effects

One report described hives and angioedema in a man exposed to zinc fumes at a zinc smelting plant (Farrell 1987). The author suggested that the patient had an immediate or delayed immunoglobulin E (IgE) response (or both) after a low dose of zinc fumes. Metal fume fever also resulted when the exposure increased. The signs and symptoms of toxicity were repeated in a challenge test conducted at the patient's home.

In a group of 14 welders acutely exposed to 77–153 mg zinc/m^3 as zinc oxide, significant correlations between the concentration of airborne zinc and the proportion of activated T cells, T helper cells, T inducer cells, T suppressor cells, and activated killer T cells were observed 20 hours after exposure (Blanc et al. 1991). In addition, significant increases in levels of polymorphonuclear leukocytes, macrophages, and all types of lymphocytes were observed in the bronchoalveolar lavage fluid 20 hours after exposure. Increased levels of lymphocytes, with a predominance of CD8 cells, in the bronchoalveolar lavage fluid were reported in a case study of a smelter exposed to unspecified levels of zinc fumes (Ameille et al. 1992).

The bronchoalveolar lavage fluid of rats or guinea pigs exposed to 2.2 mg zinc/m^3 for 3 hours contained increased levels of β-glucuronidase, suggesting a change in macrophage function (Gordon et al. 1992). Rabbits were not affected following a similar exposure to 4.6 mg zinc/m^3 for 2 hours. Rats, mice, and guinea pigs were exposed to concentrations as high as 119.3 or 121.7 mg zinc/m^3 as zinc chloride smoke for 1 hour/day, 5 days/week, for 20 weeks (Marrs et al. 1988). Routine gross and histopathologic examination of the lymph nodes, thymus, and spleen at the end of 18 months revealed no adverse effects. The smoke also contained zinc oxide, hexachlorophene, and other compounds.

3.2.1.4 Neurological Effects

Humans have reported nonspecific neurological effects such as headaches and malaise in association with other symptoms following inhalation of zinc oxide and in metal fume fever (Rohrs 1957; Sturgis et al. 1927). Staggering gait, hallucinations, and hilarity were observed in an individual who intentionally inhaled aerosols of metallic paint containing copper and zinc (Wilde 1975). However, it is most likely that these effects were due to exposure to hydrocarbon propellant rather than zinc. Amr et al. (1997) reported an increase in neuropsychiatric symptoms, including fear of poisoning, headache, nervousness, insomnia, and changes in EEG, in workers who were occupationally-exposed to zinc phosphide for a

period of many years; however, exposure levels were not reported, and no tests of statistical significance were performed.

No studies were located regarding neurological effects in animals after inhalation exposure to zinc.

3.2.1.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after inhalation exposure to zinc.

Following an initial exposure of rats, mice, and guinea pigs to concentrations as high as 119.3 or 121.7 mg zinc/m^3 as zinc chloride smoke (which also contained other compounds) for 1 hour/day, 5 days/week, for 20 weeks; histological evaluation revealed no adverse effects on the mammary glands, ovaries, fallopian tubes, or uteri were observed at 18 months (Marrs et al. 1988).

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans or animals after inhalation exposure to zinc.

3.2.1.7 Cancer

In two epidemiological studies, workers did not have an increased incidence of cancers associated with occupational exposure (primarily inhalation exposure) to zinc (Logue et al. 1982; Neuberger and Hollowell 1982).

Workers in nine electrolytic zinc and copper refining plants were studied by Logue et al. (1982). The workers at two of these plants were exposed to zinc or zinc and copper; the other workers were exposed to copper. An association between cancer mortality and zinc exposure was not found.

Excess lung cancer mortality associated with residence in an old lead/zinc mining and smelting area of the midwestern United States was studied by Neuberger and Hollowell (1982). The age- and sex-adjusted mortality rates were compared to state and national rates. The analysis determined that lung cancer mortality was elevated in the region but was not found to be associated with exposure to environmental

levels of lead or zinc. Many confounding factors were not considered in the analysis, such as smoking, occupation, and duration of residence in the area in question.

Female Porton strain mice (98–100/group) exposed to 121.7 mg zinc/m^3 of a zinc oxide/hexachloroethane smoke mixture (which produces zinc chloride), 1 hour/day, 5 days/week, for 20 weeks had a statistically significant increase in the incidence of alveologenic carcinoma (30 versus 8% in control) thirteen months after the end of exposure (Marrs et al. 1988). No increased tumor incidences were seen in mice exposed to 1, 1.3, or 12.8 mg zinc/m^3. Guinea pigs and rats were also tested with similar dose levels, and no significant carcinogenic response was observed. A number of factors limit the usefulness of this study, including the presence of several compounds in the smoke that may have carcinogenic potential, the use of only female animals, and the short duration of the exposure (20 weeks).

3.2.2 Oral Exposure

Zinc has been orally administered in a variety of forms, such as zinc chloride, zinc sulfate, zinc oxide, powdered zinc, and others. Some of these compounds, such as zinc sulfate, have been administered in both hydrated and anhydrous forms. Study authors often do not state definitely which form was used in a particular study. Knowledge of the form used and its molecular weight is necessary to calculate the amount of elemental zinc administered under a given set of circumstances, and is similarly important in that different chemical forms of zinc may be absorbed to differing degrees depending on their *in vivo* solubility, resulting in differing levels of toxicity. If adequate information was not reported by the study authors, it was assumed that an anhydrous, soluble compound was used.

3.2.2.1 Death

In a case report presented by Murray (1926), an infant died from bronchopneumonia resulting from inhalation and ingestion of an unspecified amount of zinc stearate powder spilled from a container. However, the cause of death (bronchopneumonia) suggests that it resulted from the inhalation exposure, rather than the oral exposure, and it is unclear whether the lung damage resulted from the inhalation of zinc stearate powder specifically or from a general effect of the inhalation of powders.

The LD_{50} values of several zinc compounds (ranging from 186 to 623 mg zinc/kg/day) have been determined in rats and mice (Domingo et al. 1988a). In general, mice appear to be more sensitive than

rats to the lethal effects of zinc. In rats, zinc acetate was the most lethal compound tested; zinc nitrate, zinc chloride, and zinc sulfate (in order of decreasing toxicity) were less lethal. In mice, the most lethal compound was zinc acetate followed by zinc nitrate, zinc sulfate, and zinc chloride. Ingestion of 390 mg zinc/kg/day as zinc oxide in the diet for 3–13 days was lethal to 3 of 3 ferrets (Straube et al. 1980). An equivalent dose in humans would be approximately 27 g zinc/day (which would probably be intolerable to humans because of gastric discomfort). Death was reported in mice that consumed 1,110 mg zinc/kg/day as zinc sulfate in their diet for 13 weeks (Maita et al. 1981). Mortality was also observed in 20% of rats ingesting 191 mg zinc/kg/day as zinc acetate in drinking water for 3 months (Llobet et al. 1988a).

The LD_{50} values and all LOAEL values from each reliable study for death in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Ingestion of zinc or zinc-containing compounds has resulted in a variety of systemic effects in the gastrointestinal and hematological systems and alterations in the blood lipid profile in humans and animals. In addition, lesions have been observed in the liver, pancreas, and kidneys of animals. No studies were located regarding respiratory, ocular, or metabolic effects in humans or animals after oral exposure to zinc.

Observed systemic effects after oral exposure are discussed below. The effects discussed in case reports are not included in Table 3-2 or Figure 3-2 because of the small sample size and lack of control data.

Cardiovascular Effects. A number of studies in humans and animals have examined the effects of zinc on serum cholesterol and triglycerides. However, no studies regarding the direct relationship between excessive zinc intake and cardiac mortality were located. No effects on electrocardiographic results were found in a group of elderly subjects (>65 years of age) taking zinc supplements of up to 2 mg zinc/kg/day (Hale et al. 1988) or 0.71 mg zinc/kg/day (Czerwinski et al. 1974). There was also no effect on the frequency of cardiovascular disease (heart attack, heart failure, hypertension, or angina) in elderly subjects (>67 years of age) taking up to 2 mg zinc/kg/day for a mean of 8 years (Hale et al. 1988).

Table 3-2 Levels of Significant Exposure to Zinc - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Rat (Sprague-Dawley)	once (G)				237 M (LD50)	Domingo et al. 1988a Zinc acetate
2	Rat (Sprague-Dawley)	once (G)				623 M (LD50)	Domingo et al. 1988a Zinc sulfate
3	Rat (Sprague-Dawley)	once (G)				528 M (LD50)	Domingo et al. 1988a Zinc chloride
4	Rat (Sprague-Dawley)	once (G)				293 M (LD50)	Domingo et al. 1988a Zinc nitrate
5	Mouse (Swiss-Webster)	once (G)				337 M (LD50)	Domingo et al. 1988a Zinc sulfate
6	Mouse (Swiss-Webster)	once (G)				86 M (LD50)	Domingo et al. 1988a Zinc acetate
7	Mouse (Swiss-Webster)	once (G)				605 M (LD50)	Domingo et al. 1988a Zinc chloride
8	Mouse (Swiss-Webster)	once (G)				204 M (LD50)	Domingo et al. 1988a Zinc nitrate

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
9	Ferret	<2 wk (F)				390 (3/3 died)	Straube et al. 1980 Zinc oxide
Systemic							
10	Human	once (W)	Endocr		0.5 (decreased serum cortisol levels)		Brandao-Neto et al. 1990a Zinc sulfate
11	Human	once (W)	Gastro		6.7 (gastrointestinal distress; diarrhea)		Callender and Gentzkow 1937 Zinc oxide
12	Human	Single oral exposure (IN)	Gastro		6.8 M (Transient nausea, lasting approximately 6 hours)		Lewis and Kokan 1998 Zinc gluconate
13	Human	2 d (F)	Gastro	86 M			Murphy 1970 Zinc elemental
			Endocr		86 M (increased serum amylase, lipase)		
Neurological							
14	Rat	10 d 1x/d (G)			487 (minor neuronal degeneration; decreased acid phosphatase and acetylchonlinesterase; increased thiamine pyrophosphatase)		Kozik et al. 1980 Zinc oxide

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Death							
15	Rat	3 mo ad lib (W)				191 F (2/10 died)	Llobet et al. 1988a Zinc acetate
16	Mouse	13 wk ad lib (F)				1110 (5/24 died)	Maita et al. 1981 Zinc sulfate
Systemic							
17	Human	3 mo 7d/wk 1x/d (C)	Other	1.5			Bogden et al. 1988 Zinc acetate
18	Human	14 wk 7 d/wk 1x/day	Hemato	0.43 M			Bonham et al. 2003b Zinc glycine chelate
19	Human	6 wk 2x/d (C)	Other		4.3 M (increased serum LDL-cholesterol; decreased serum HDL-cholesterol)		Chandra 1984 Zinc sulfate
20	Human	24 wk 7d/wk 3x/d (C)	Cardio	0.71			Czerwinski et al. 1974 Zinc sulfate
21	Human	90 d 1x/d	Hemato	0.68 F			Davis et al. 2000 Zinc gluconate
			Endocr	0.68 F			

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
22	Human	6 wk 7d/wk 2x/d (C)	Hemato	0.71			Fischer et al. 1984 Zinc gluconate
23	Human	5 wk 2x/d (C)	Other		2.3 M (decreased serum HDL-cholesterol)		Hooper et al. 1980 Zinc sulfate
24	Human	90 d 1x/d	Hemato	0.68 F			Milne et al. 2001 Zinc gluconate
25	Human	6 wk 3x/d (F)	Gastro		2 (abdominal cramps; vomiting; nausea)		Samman and Roberts 1987 Zinc sulfate
26	Human	6 wk 7d/wk 3x/d (C)	Other	2.4			Samman and Roberts 1988 Zinc sulfate
27	Human	10 wk 7d/wk 2x/d (C)	Hemato	0.83[b] F			Yadrick et al. 1989 Zinc gluconate
28	Rat	14 wk (males) or 20 wk (females) 7 d/wk 1x/d (GW)	Bd Wt		7 M (decreased postpartum body weights in F0 animals)		Khan et al. 2001b Zinc chloride

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
29	Rat	6 wk 7d/wk ad lib (F)	Hemato		6 M (ceroplasmin reduced by 28%)		L'Abbe and Fischer 1984a Zinc sulfate
30	Rat (Sprague-Dawley)	3 mo ad lib (W)	Hemato	191 F			Llobet et al. 1988a Zinc acetate
			Hepatic	191 F			
			Renal	95 F		191 F (increased plasma creatine and urea levels; desquamation of epithelial cells of proximal tubules)	
			Bd Wt	191 F			
31	Rat	13 wk ad lib (F)	Gastro	565 F			Maita et al. 1981 Zinc sulfate
			Hemato	53 F	565 F (decreased hematocrit and WBC)		
			Musc/skel	565 F			
			Renal	565 F			
			Other	53 M		565 F (acinar cell necrosis and metaplasia in pancreas)	

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
32	Rat (Sprague-Dawley)	5 wk ad lib (F)	Hemato		500 (decreased Hb, hematocrit, MCH, MCHC; slightly increased WBC)		Smith and Larson 1946 Zinc carbonate
33	Rat (Sprague-Dawley)	6 wk ad lib (F)	Hemato		350 (decreased Hb)		Smith and Larson 1946 Zinc carbonate
34	Rat (Wistar)	4 wk 7d/wk ad lib (W)	Hemato		12 (decreased Hb and erythrocytes)		Zaporowska and Wasilewski 1992 Zinc chloride
35	Mouse	5-14 mo ad lib (W)	Other		70 (hypertrophy and vacuolation of pancreas islet cells; hypertrophy and vacuolation of fasciculata cells in adrenal cortex)		Aughey et al. 1977 Zinc sulfate

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
36	Mouse (ICR)	13 wk ad lib (F)	Gastro	104 M		1110 F (forestomach ulcers)	Maita et al. 1981 Zinc sulfate
			Hemato	104 M	1110 F (decreased WBC; anemia)		
			Renal	104 M	1110 F (unspecified regressive lesions)		
			Endocr	104 M		1110 F (acinar cell necrosis and metaplasia in pancreas)	
37	Mouse	9 mo ad lib (F)	Hemato			68 (severe anemia)	Walters and Roe 1965 Zinc oleate
38	Dog	9 mo ad lib (W)	Musc/skel	4 M			Anderson and Danylchuk 1979 Zinc oxide
39	Rabbit (New Zealand)	22 wk daily (F)	Hemato		174 M (slight decrease in Hb levels)		Bentley and Grubb 1991 Zinc carbonate
			Bd Wt	174 M			

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
40	Mink	144 d ppd70-214 (F)	Hemato	323.6			Aulerich et al. 1991 Zinc sulfate
			Hepatic	323.6			
			Renal	323.6			
			Bd Wt	323.6			
41	Cow	5 wk 2x/d ppd3-40 (F)	Hemato		64 M (decreased hematocrit levels)		Jenkins and Hidiroglou 1991 Zinc oxide
			Bd Wt	64 M		91 M (body weight gain decreased by 46%)	
42	Ferret	7-97 d ad lib (F)	Gastro	195		390 (intestinal hemorrhages)	Straube et al. 1980 Zinc oxide
			Hemato	65	195 (anemia)		
			Renal	65	195 (nephrosis)		
			Endocr	195	390 (pancreatitis)		
Immuno/ Lymphoret							
43	Human	3 mo 7d/wk 1x/d (C)		1.5			Bogden et al. 1988 Zinc acetate
44	Human	14 wk 7 d/wk ns		0.43 M			Bonham et al. 2003a Zinc glycine chelate

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Human	14 wk 7 d/wk 1x/day		0.43 M			Bonham et al. 2003b Zinc glycine chelate
46	Human	6 wk 2x/d (C)			4.3 M (impaired lymphocyte and polymorphonuclear leukocyte function)		Chandra 1984 Zinc sulfate
47	Human	1 mo 2x/d (C)		2.5			Duchateau et al. 1981 Zinc sulfate
48	Mouse	8 wk 7d/wk ad lib (F)		6.5			Fernandes et al. 1979 ns
49	Mouse (BALB/c)	continuously for 42 days (W)			136 (Increases in direct plaque-forming activity of spleen cells and in lymphyocyte proliferation in response to mitogen stimulation)		Lastra et al. 1997 ns
50	Mouse	4 wk 7d/wk ad lib (F)		76.9 F			Schiffer et al. 1991 Zinc sulfate

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Neurological							
51	Mouse (Swiss-Webster)	drinking water for 60 days (W)			0.5 (Increase in latency in inhibitory avoidance test)		Oliveira et al. 2001 Zinc acetate
Reproductive							
52	Human	Gwk 20 through parturition (C)		0.3 F			Mahomed et al. 1989 Zinc sulfate
53	Rat	8 wk 7d/wk ad lib (F)			25 M (altered sperm chromatin structure)		Evenson et al. 1993 Zinc chloride
54	Rat	14 wk (males) or 20 wk (females) 7 d/wk 1x/d (GW)		3.5 F	7 F (Decreased live pups per litter in all groups of treated rats)		Khan et al. 2001b Zinc chloride
55	Rat	18 d Gd0-18 ad lib (F)				200 F (increased pre-implantation loss)	Pal and Pal 1987 Zinc sulfate
56	Rat	150 d ad lib (F)		50		250 (no reproduction in females)	Sutton and Nelson 1937 Zinc carbonate
57	Mouse (BALB/c)	continuously for 42 days (W)		273 F			Lastra et al. 1997 ns

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
58	Mouse (ICR)	13 wk ad lib (F)		1110			Maita et al. 1981 Zinc sulfate
59	Mink	approx 25 wk ad lib (F)		20.8			Bleavins et al. 1983 Zinc sulfate
Developmental							
60	Human	11 wk 1x/d (C)		0.06 F			Kynast and Saling 1986 Zinc aspartate
61	Human	Gwk 20 through parturition (C)		0.3 F			Mahomed et al. 1989 Zinc sulfate
62	Human	last 15-25 wk of pregnancy 1x/d (C)		0.3 F			Simmer et al. 1991 Zinc citrate
63	Rat	7 wk Gd0-17 ad lib (F)		250 F			Kinnamon 1963 Zinc carbonate

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
64	Rat (Sprague-Dawley)	15 d Gd1-15 ad lib (F)				200 F (29% fetal resorption; decreased fetal weight)	Schlicker and Cox 1968 Zinc oxide
65	Rat	36 d Gd1-15 ad lib (F)		100 F			Schlicker and Cox 1968 Zinc oxide
66	Rat (Sprague-Dawley)	36 d Gd1-21 ad lib (F)				200 F (100% fetal resorption)	Schlicker and Cox 1968 Zinc oxide
67	Rat	150 d ad lib (F)		50		250 (increased stillbirths)	Sutton and Nelson 1937 Zinc carbonate
68	Rat (Sprague-Dawley)	20 d Gd0-20 ad lib (F)		25 F			Uriu-Hare et al. 1989 Zinc carbonate
69	Mouse	2 gen (F)			260 (alopecia; decreased hematocrit)		Mulhern et al. 1986 Zinc carbonate

Table 3-2 Levels of Significant Exposure to Zinc - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
70	Mink	approx 25 wk ad lib (F)		20.8			Bleavins et al. 1983 Zinc sulfate
CHRONIC EXPOSURE							
Cancer							
71	Human	1x/day 1 or more years ns				1.43 M (Increased probability of advanced prostate cancer)	Leitzmann et al. 2003 ns

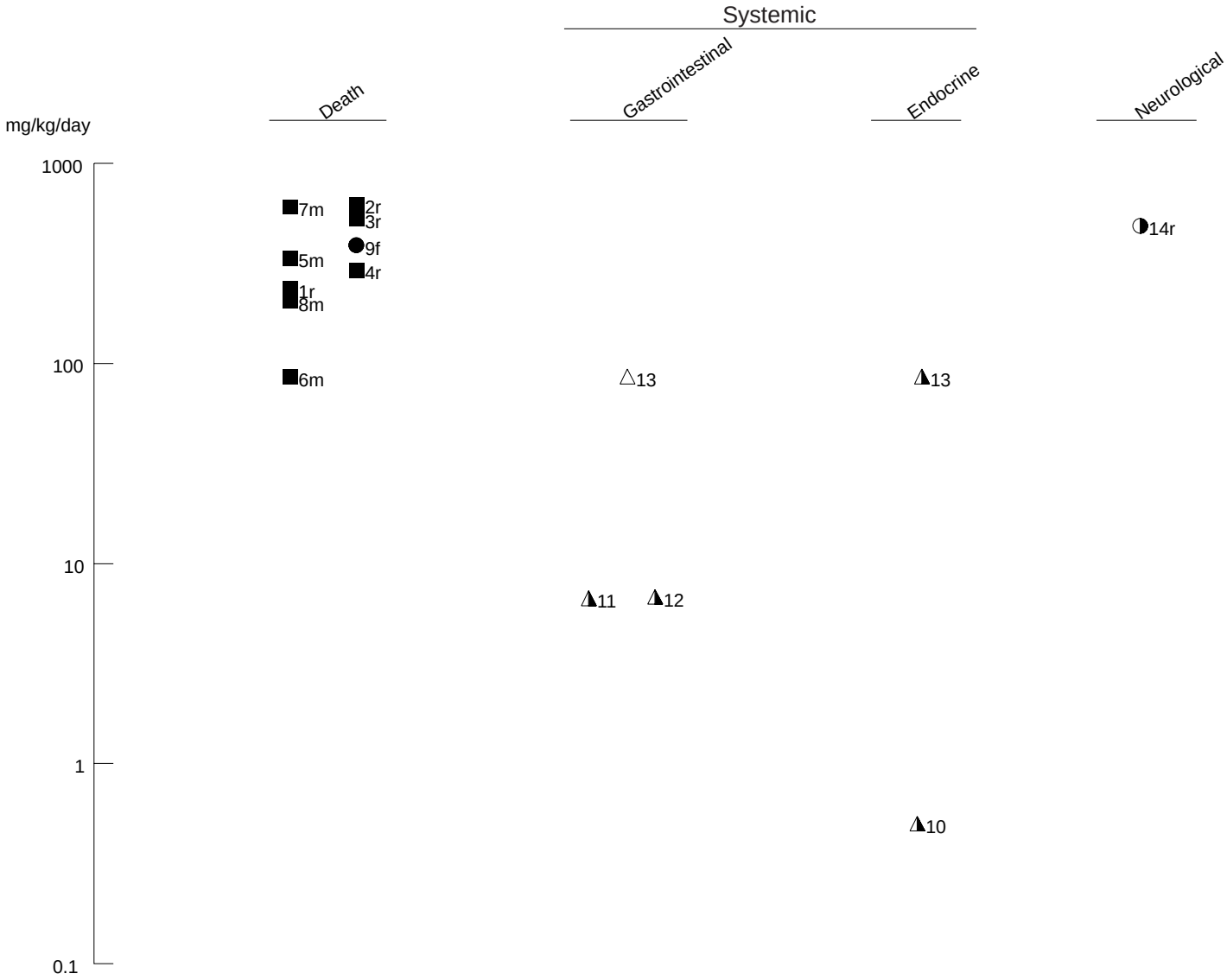
a The number corresponds to entries in Figure 3-2.

b Used to derive an intermediate-duration oral minimal risk level (MRL) of 0.3 mg/kg/day; The MRL was calculated by applying an uncertainty factor of 3 (for uncertainties regarding human variability) to the no-observed-adverse-effect level (NOAEL) of 0.83 mg/kg/day. The intermediate oral MRL was adopted as the chronic oral MRL.

ad lib = ad libitum; approx = approximately; (C) = capsule; Cardio = cardiovascular; d - day(s); (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day; gen = generation; Gwk = gestation week; Hb = hemoglobin; HDL = high density lipoprotein; Hemato = hematological; LD50 = lethal dose, 50% kill; LDL = low density lipoprotein; LOAEL = lowest-observed-adverse-effect level; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; ns = not specificied; ppd - post partum day; RBC = red blood cell; (W) = drinking water; WBC = white blood cell; wk = week(s); x = time(s); yr = year(s)

Figure 3-2 Levels of Significant Exposure to Zinc - Oral

Acute (≤14 days)



c-Cat -Humans f-Ferret n-Mink
d-Dog k-Monkey j-Pigeon o-Other
r-Rat m-Mouse e-Gerbil
p-Pig h-Rabbit s-Hamster
q-Cow a-Sheep g-Guinea Pig

◆ Cancer Effect Level-Animals
● LOAEL, More Serious-Animals
◑ LOAEL, Less Serious-Animals
○ NOAEL - Animals

▼ Cancer Effect Level-Humans
▲ LOAEL, More Serious-Humans
◭ LOAEL, Less Serious-Humans
△ NOAEL - Humans

■ LD50/LC50
Minimal Risk Level for effects other than Cancer

Figure 3-2 Levels of Significant Exposure to Zinc - Oral

Intermediate (15-364 days)

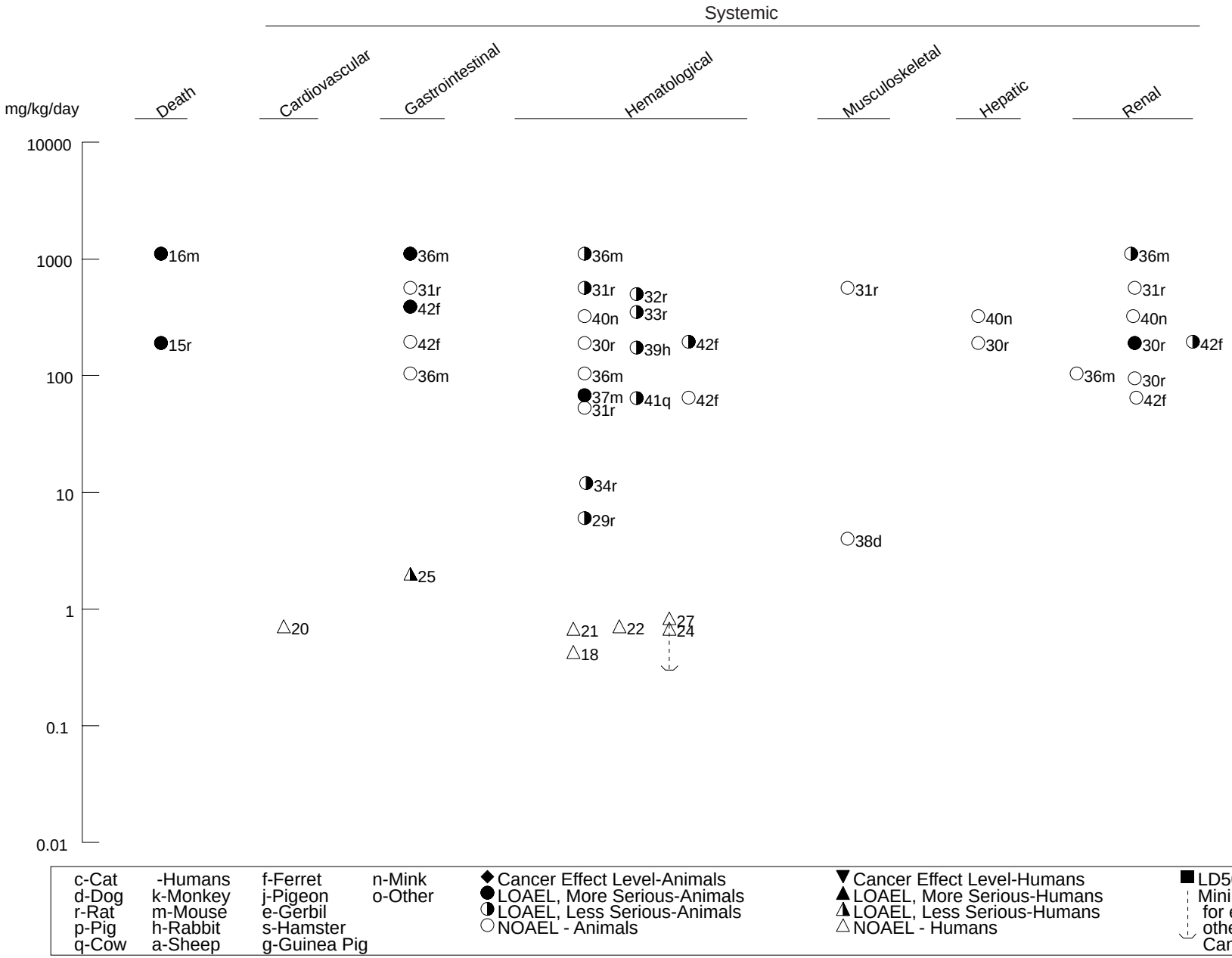


Figure 3-2 Levels of Significant Exposure to Zinc - Oral

Intermediate (15-364 days)

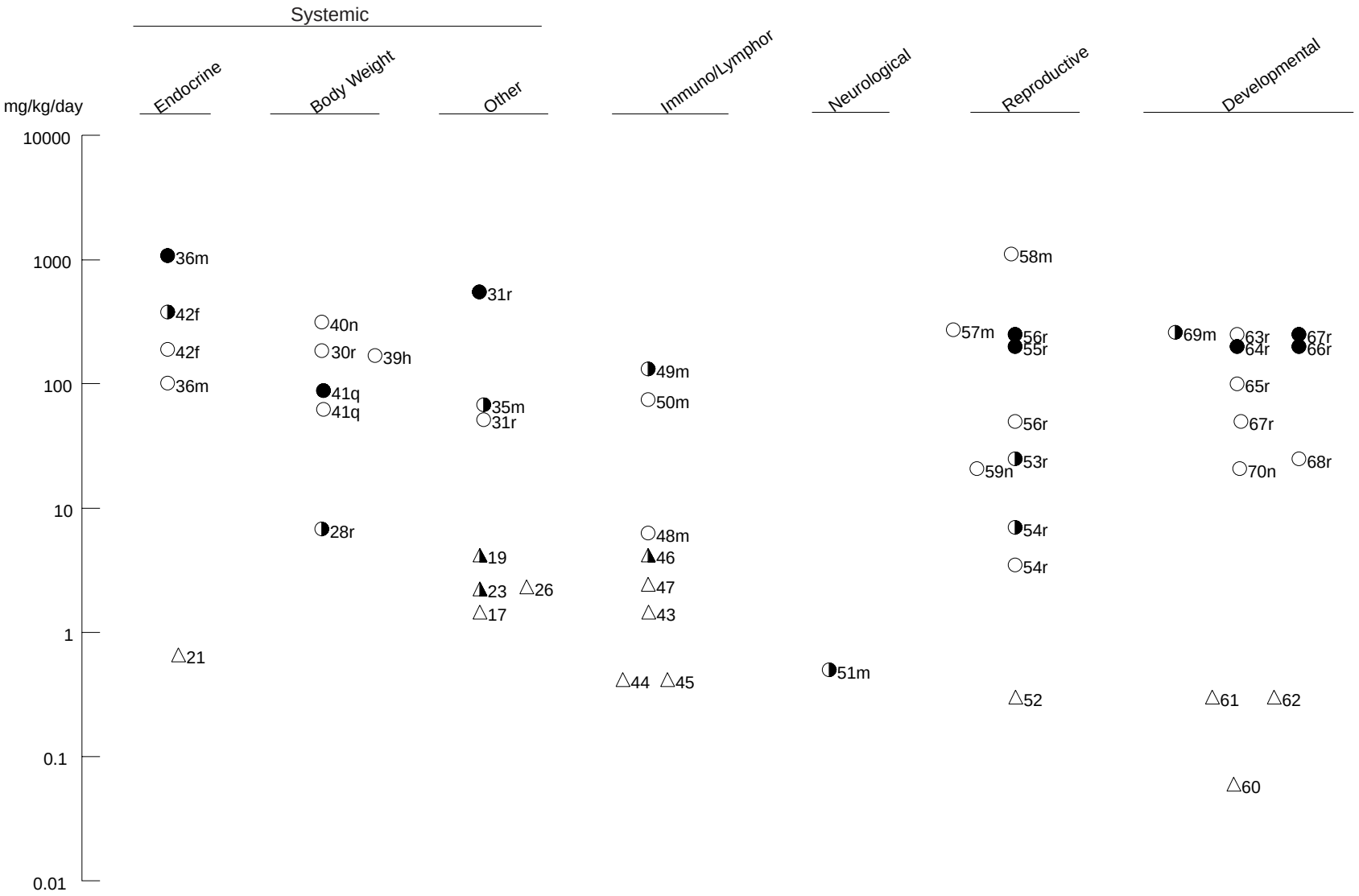
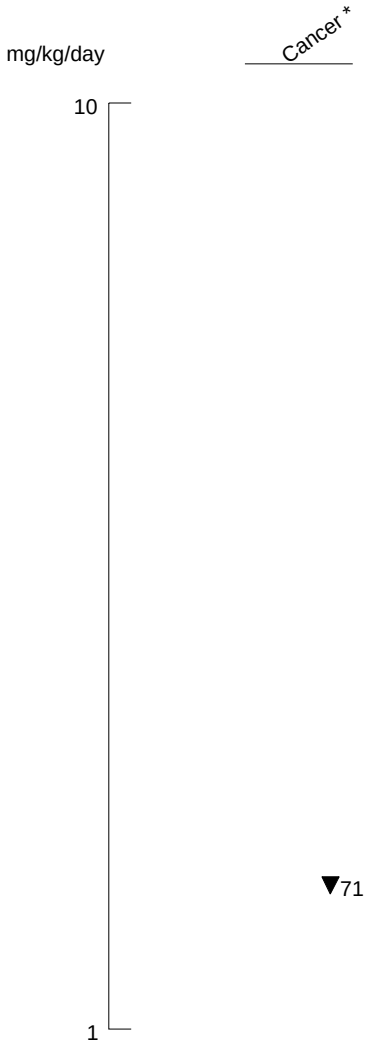


Figure 3-2 Levels of Significant Exposure to Zinc - Oral

Chronic (≥365 days)



*Doses represent the lowest dose tested per study that produced a tumorigenic response and do not imply the existence of a threshold for the cancer endpoint.

c-Cat	-Humans	f-Ferret	n-Mink	◆ Cancer Effect Level-Animals	▼ Cancer Effect Level-Humans	■ LD50/LC50
d-Dog	k-Monkey	j-Pigeon	o-Other	● LOAEL, More Serious-Animals	▲ LOAEL, More Serious-Humans	Minimal Risk Level for effects other than Cancer
r-Rat	m-Mouse	e-Gerbil		◑ LOAEL, Less Serious-Animals	◭ LOAEL, Less Serious-Humans	
p-Pig	h-Rabbit	s-Hamster		○ NOAEL - Animals	△ NOAEL - Humans	
q-Cow	a-Sheep	g-Guinea Pig				

In one study, patients having inoperable severe occlusive vascular disease were administered 3.8 mg zinc/kg/day as zinc sulfate for at least 1 year (Henzel et al. 1971). Eighteen of the 24 patients experienced improvement in lower extremity blood flow and unchanged or decreased arterial pressure. Zinc's role in these improvements was not completely understood by the study authors. They hypothesized that when optimal zinc levels are provided to the ischemic limb, the activity of certain zinc enzymes promotes the reversal of tissue-dependent hypoxia and/or lactic acidemia in the muscles. It is also not known if this high dose of zinc was associated with any toxic effects.

No studies were located regarding cardiovascular effects in animals after oral exposure to zinc.

Gastrointestinal Effects. Several studies have suggested that zinc ingestion may cause symptoms of gastrointestinal distress or alterations in gastrointestinal tissues. For example, one individual who ingested about 3 ounces of a zinc chloride solution described acute symptoms that occurred almost immediately following contact with the compound, including burning and pain in the mouth and throat and vomiting (Chobanian 1981). Later, the patient exhibited pharyngitis, esophagitis, hypocalcemia, and elevated levels of amylase; the latter two alterations are suggestive of acute pancreatitis. The patient received intravenous hydration and calcium supplementation and recovered within 5 days. The material ingested was described as a "zinc chloride solution," and its concentration was not reported. Therefore, a dose level could not be established in this case.

Several cases of gastrointestinal disturbances have been reported after ingestion of zinc sulfate (Anonymous 1983; Brown et al. 1964; Moore 1978; Samman and Roberts 1987). Vomiting, abdominal cramps, and diarrhea, in several cases with blood, have been observed after ingestion of zinc sulfate. In one report, an English school girl ingested 440 mg zinc sulfate/day (2.6 mg zinc/kg/day) in capsules as a medically prescribed treatment for acne (Moore 1978). After taking each capsule, she experienced epigastric discomfort. A week later, she was admitted to the hospital after a fainting spell. She was diagnosed as anemic and subsequently passed melanic stools, indicative of gastrointestinal bleeding. Gastrointestinal upset (abdominal cramps, vomiting, nausea) occurred in 26 of 47 healthy volunteers following ingestion of zinc sulfate tablets (150 mg as zinc ion in three divided doses per day, 2 mg zinc/kg/day) for 6 weeks (Samman and Roberts 1987). A 17-year-old boy who ingested approximately 6.8 mg zinc/kg as zinc gluconate showed severe nausea and vomiting, but displayed no other symptoms, and recovered within 7 hours of ingestion (Lewis and Kokan 1998). Ingestion of zinc oxide has also been associated with gastrointestinal distress (Anonymous 1983; Callender and Gentzkow 1937). In one case, 80% of the personnel of two army companies became ill with gastrointestinal distress and diarrhea after

consuming limeade prepared in galvanized trash cans (Callender and Gentzkow 1937). The average dose was estimated to be 6.7–7.1 mg/kg. A second example was presented in a case involving school children in New Mexico who experienced nausea and vomiting after accidental excessive zinc intake (Anonymous 1983). These children had consumed punch containing high levels of zinc dissolved from galvanized hinges attached to tanks in which the punch was stored. A 16-year-old boy who ingested 12 g elemental zinc over a 2-day period (86 mg zinc/kg/day) experienced light-headedness, lethargy, staggering gait, and difficulty writing legibly, but no apparent gastrointestinal disturbances (Murphy 1970).

Gastrointestinal effects have also been observed in animals. Intestinal hemorrhages were observed in ferrets that ingested 390 mg zinc/kg/day as zinc oxide for 2 weeks (Straube et al. 1980). These ferrets exhibited a 75% reduction in food intake. No intestinal hemorrhaging was observed in ferrets fed 195 mg/kg/day for up to 21 days. Oral zinc sulfate exposures of intermediate duration in other experimental animals have also resulted in gastrointestinal effects. Mice fed a diet providing 1,110 mg zinc/kg/day for 13 weeks developed ulcers in the forestomach, but gastrointestinal effects were not observed in rats fed 565 mg zinc/kg/day for 13 weeks (Maita et al. 1981).

Hematological Effects. In a case report, acute exposure to 2.6 mg zinc/kg/day as zinc sulfate for 1 week resulted in anemia (Moore 1978). The authors of the report noted that the anemia may have been secondary to the gastrointestinal hemorrhages.

Treatment-related changes in hematological parameters have been observed in humans and animals after intermediate or chronic exposure to zinc or zinc-containing compounds. Long-term administration (1–8 years) of zinc supplements has caused anemia in humans (Broun et al. 1990; Gyorffy and Chan 1992; Hale et al. 1988; Hoffman et al. 1988; Patterson et al. 1985; Porter et al. 1977; Prasad et al. 1978; Ramadurai et al. 1993; Salzman et al. 2002; Stroud 1991; Summerfield et al. 1992). Exposure of one patient to 2 mg zinc/kg/day as zinc sulfate for 10 months resulted in anemia (Hoffman et al. 1988). A significant reduction in erythrocyte superoxide dismutase activity (47% decrease), hematocrit, and serum ferritin, compared to pretreatment levels, occurred in female subjects who received supplements (as capsules) of 50 mg zinc/day as zinc gluconate for 10 weeks (Yadrick et al. 1989); this study was selected as the basis for the intermediate-duration oral MRL. A 15% decrease in erythrocyte superoxide dismutase activity was reported in male volunteers receiving 50 mg zinc/day as zinc gluconate for 6 weeks (Fischer et al. 1984). A more recent study by Davis et al. (2000; Milne et al. 2001) reported increases in bone-specific alkaline phosphatase levels (~25%) and extracellular superoxide dismutase (~15%), while significant decreases were seen in mononuclear white cell 5'-nucleotidase (~30%) and plasma

5'-nucleotidase activity (~36%) following exposure of postmenopausal women to a combined (dietary+supplemental) 53 mg zinc/day as zinc glycine chelate. Healthy men given 200 mg zinc/day as elemental zinc for 6 weeks showed a reduction in lymphocyte stimulation response to phytohemagglutinin as well as chemotaxis and phagocytosis of bacteria by polymorphonuclear leukocytes (Chandra et al. 1984); however, no changes in lymphocyte cell number or in the proportion of lymphocyte populations were noted. Exposure of male volunteers to 0.48 mg zinc/kg/day, as zinc glycine chelate, had no effect on markers of coagulation (Bonham et al. 2003b) relative to unexposed subjects. While the changes in hematological end points following long-term zinc exposure in humans are noteworthy, they were subclinical in nature, and therefore, are generally considered to be non-adverse.

In animals, following oral administration of zinc compounds, decreased hemoglobin, hematocrit, erythrocyte, and/or leukocyte levels were observed in rats (Maita et al. 1981; Smith and Larson 1946), mice (Maita et al. 1981; Walters and Roe 1965), rabbits (Bentley and Grubb 1991), dogs (Drinker et al. 1927d; Meurs et al. 1991; Robinson et al. 1991), ferrets (Straube et al. 1980), and preruminant calves (Jenkins and Hidiroglou 1991). In rats, the lowest LOAEL for hematological effects was 4 mg/kg/day (8 mg/kg every other day) for an increased frequency of basophilic-stippled erythrocytes in rats exposed every other day for 14 days (Piao et al. 2003). The second lowest LOAEL is 12 mg zinc/kg/day as zinc chloride in a 4-week drinking water study with 2-month-old rats (Zaporowska and Wasilewski 1992) that reported decreased hemoglobin (85% of control values) and erythrocytes (90% of control values). The highest NOAEL in rats is 191 mg zinc/kg/day as zinc acetate in a 3-month drinking water study (age of rats not specified) (Llobet et al. 1988a). The reason that the lowest LOAEL is less than the highest NOAEL in rats is unclear, but it may be because of the use of different zinc compounds or different rat strains or age. For mice, NOAEL and LOAEL values of 104 and 1,110 mg zinc/kg/day as zinc sulfate, respectively, were identified by Maita et al. (1981) in a 13-week feeding study. A serious LOAEL of 68 mg zinc/kg/day as zinc oleate was identified for severe anemia in a 9-month feeding study in mice (Walters and Roe 1965). It is not known if the difference in the LOAELs identified in the Maita et al. (1981) and Walters and Roe (1965) studies is due to the use of different zinc compounds, different basic diet formulations, different mouse strains, or different exposure durations. Slight decreases in hemoglobin levels were observed in rabbits fed 174 mg zinc/kg/day as zinc carbonate (Bentley and Grubb 1991). Zinc oxide consumption caused anemia in dogs (76.5 mg zinc/kg/day) (Drinker et al. 1927d), ferrets (195 mg zinc/kg/day) (Straube et al. 1980), and preruminant calves (64 mg zinc/kg/day) (Jenkins and Hidiroglou 1991). Hematological alterations were not observed in cats exposed to up to 83.2 mg zinc/kg/day as zinc oxide (Drinker et al. 1927d) or in adult mink exposed to zinc at up to 297.4 mg zinc/kg/day as zinc oxide (Aulerich et al. 1991; Bleavins et al. 1983) or to rats exposed to 53 mg

zinc/kg/day as zinc sulfate (Maita et al. 1981). However, decreases in hematocrit and lymphocytes were observed in the offspring of mink females that ingested a time-weighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to conception and throughout gestation and lactation (Bleavins et al. 1983) indicating that very young mink may be more sensitive to the hematologic effects of zinc than adults. An increased number of weanling rats had low levels of ceruloplasmin, a copper serum protein, after administration of zinc sulfate for 6 weeks (L'Abbe and Fischer 1984a).

Musculoskeletal Effects. No studies were located regarding musculoskeletal effects in humans after oral exposure to zinc.

Rib biopsies revealed no treatment-related effects in dogs given 4 mg zinc/kg/day as zinc oxide in the diet for 9 months (Anderson and Danylchuk 1979). No lesions of the bones were observed in rats exposed to 565 mg zinc/kg/day as zinc sulfate during 13 weeks of exposure in the food (Maita et al. 1981).

Hepatic Effects. Ingestion of 3.5 mg/kg/day zinc sulfate for 18 weeks by 13 patients being treated for chronic venous leg ulcers was reported to have no effect on the results of liver function tests (Hallbook and Lanner 1972). However, the type of liver function tests was not specified and results were not presented to support this conclusion.

Several reports described changes in the serum lipid profile of humans exposed to zinc sulfate or gluconate for 3–12 months; however, the results are mixed. Ingestion of 2.3–4.3 mg zinc/kg/day for 5–6 weeks (Chandra 1984; Hooper et al. 1980) or 0.71 mg zinc/kg/day for 12 weeks (Black et al. 1988) reduced levels of high-density lipoprotein (HDL) cholesterol. In the study by Chandra (1984), a slight increase in low-density lipoprotein (LDL) cholesterol was observed in subjects who served as their own controls; measurements were taken prior to zinc supplementation and after a 10-week postexposure period. Serum cholesterol, triglyceride, and LDL cholesterol levels were not affected by zinc supplementation in the study by Black et al. (1988). However, in another study, zinc supplements depressed HDL cholesterol levels and raised LDL cholesterol levels in elderly subjects (>60 years of age), especially in those who exercised. This study was not well controlled, and the wide variation in doses of the supplemented group prevented the determination of a LOAEL (Goodwin et al. 1985). Young women with a total daily intake of 1.6 mg zinc/kg/day in a 2-month study had a transient decrease in HDL cholesterol (Freeland-Graves et al. 1980). In a double-blind crossover study of young men and women receiving 2.0 (men) or 2.4 (women) mg zinc/kg/day for 6 weeks, total HDL cholesterol was not affected, and LDL cholesterol was significantly decreased in the women (Samman and Roberts 1988). No effect

on HDL cholesterol was seen in elderly men and women (60–89 years old) with a total daily intake (dietary zinc plus a zinc acetate supplement) of 1.5 mg/kg/day for 3 months (Bogden et al. 1988), but the subjects also received copper supplements (about 0.03 mg/kg). Bonham et al. (2003b) reported that supplementation of male subjects with 0.43 mg zinc/kg/day (30 mg/day, assuming a reference body weight of 70 kg), as zinc glycine chelate, had no effect on LDL, HDL, or triglyceride levels. Another study (Hale et al. 1988) reported no differences in triglycerides and cholesterol levels in subjects (≥68 years old) given zinc supplements of up to 2 mg/kg/day for an average of 8 years.

No histopathology or changes in serum enzyme levels (serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, or alkaline phosphatase) were observed in rats receiving 191 mg zinc/kg/day as zinc acetate (Llobet et al. 1988a). Similarly, no histopathology was observed in rats administered 98.3 mg zinc/kg/day as zinc oxide, but an insufficient number of animals were tested (Drinker et al. 1927c). Sheep fed time-weighted-average doses of 19 mg zinc/kg/day as zinc oxide for 49–72 days developed hepatic effects, including necrotic hepatocytes and large quantities of hemosiderin in Kupffer cells (Allen et al. 1983). Because sheep are ruminants, it is not known if they are a good model for predicting human toxicity. No histological damage was observed in adult or young mink fed 327 or 324 mg zinc/kg/day, respectively, as zinc sulfate for 144 days (Aulerich et al. 1991).

Decreased hexobarbital sleeping times were reported by Kadiiska et al. (1985) in rats receiving 40 mg zinc/kg/day as zinc sulfate. This physiological response suggested an induction of microsomal enzymes.

Increases in serum cholesterol levels were observed in two studies where rats were fed either 2.8 or 10 mg zinc/kg/day as zinc acetate for 2–7 months (Katya-Katya et al. 1984; Klevay and Hyg 1973). Other studies have shown no effect on total cholesterol, HDL cholesterol, or serum triglyceride levels in rats ingesting 3 or 25 mg zinc/kg/day of unspecified zinc compounds (Fischer et al. 1980; Woo et al. 1983).

Renal Effects. Thirteen patients treated with zinc sulfate at 3.5 mg zinc/kg/day for 18 weeks for chronic venous leg ulcers had normal urinalyses (Hallbook and Lanner 1972). However, neither the specific parameters measured for the urinalysis nor the results were presented to support this conclusion. Furthermore, urinalysis may not be a sensitive indicator of renal function.

A number of intermediate-duration studies have demonstrated renal effects in animals exposed to zinc oxide, zinc sulfate, and zinc acetate. Zinc sulfate caused an increase in the absolute and relative kidney weights and regressive kidney lesions (not specified) in female mice that consumed 1,110 mg zinc/kg/day

in the diet for 13 weeks, but no effects occurred in rats that consumed 565 mg zinc/kg/day or in mice that consumed 104 mg zinc/kg/day under similar conditions (Maita et al. 1981). Severe diffuse nephrosis was observed in ferrets exposed to 195 mg zinc/kg/day as zinc oxide in the diet (Straube et al. 1980). In rats exposed to 191 mg zinc/kg/day as zinc acetate for 3 months, epithelial cell damage in the glomerulus and proximal convoluted tubules and increased plasma creatinine and urea levels were observed (Llobet et al. 1988a). The NOAEL for the effects on creatinine and urea was 95 mg zinc/kg/day. It is unclear whether the microscopic changes were observed at lower doses. No histopathological changes in the kidneys were observed in three rats that drank water containing 98.3 mg zinc/kg/day as zinc oxide for 35–36 weeks (Drinker et al. 1927c); however, interpretation of the results of this study is severely limited by the small number of rats used. Renal tubular dilation, with proteinaceous casts and hemosiderin deposits, was observed in the kidneys of sheep that ingested 18 mg zinc/kg/day as zinc oxide for 49–72 days (Allen et al. 1983). It is not known if sheep are a good model for human toxicity because they are ruminants. No renal effects were observed in either adult mink consuming 326.7 mg zinc/kg/day as zinc sulfate or in young mink consuming 323.6 mg zinc/kg/day as zinc sulfate for 144 days (Aulerich et al. 1991). Minks exposed to 195 mg zinc/kg/day as zinc oxide for 7–97 days in the food developed a diffuse nephrosis, though it did not increase with increasing dose (Straube et al. 1980).

Dermal Effects. No studies were located regarding dermal/ocular effects in humans after oral exposure to zinc.

No dermal effects were seen in adult female minks given a time-weighted dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to mating and then throughout gestation and lactation (Bleavins et al. 1983). However, the offspring of these animals showed graying of the fur around the eyes, ears, jaws, and genitals with a concomitant loss of hair and dermatosis in these areas during the weaning period. These conditions were reversible upon removal of treatment.

Endocrine Effects. Only one human exposure study has evaluated endocrine effects of oral zinc exposure. Davis et al. (2000; Milne et al. 2001) reported a slight (<10%) decrease in serum T4 levels in postmenopausal women exposed to 0.68 mg zinc/kg/day as zinc gluconate; the difference did not attain statistical significance, and no changes in free T3 or thyroid stimulating hormone (TSH) levels were reported.

Piao et al. (2003) exposed groups of Wistar rats to 0, 4, or 8 mg zinc/kg as zinc acetate every other day for a 14-day period. Levels of T3 were decreased in both groups of exposed rats, relative to controls, but

levels of T4 and TSH were not significantly altered. Zinc exposure resulted in increased levels of serum cortisol, which was significant from controls at the 8 mg zinc/kg exposure level.

Body Weight Effects. No effects on body weight have been reported in humans following oral exposure to zinc. However, a 46% decrease in body weight gain was seen in preruminant calves that consumed 91 mg zinc/kg/day as zinc oxide for 5 weeks; there was no effect at 64 mg zinc/kg/day (Jenkins and Hidiroglou 1991). The relevance of this effect to humans is unclear. Body weights of rabbits (Bentley and Grubb 1991), rats (Llobet et al. 1988a), and minks (Aulerich et al. 1991) were unaffected by dosing with 174, 191, and 326.7 mg zinc/kg/day, respectively, for 3–12 months. Decreased postpartum body weights in F0 animals were observed in rats exposed to 7 mg zinc/kg/day as zinc chloride for 20 weeks (Khan et al. 2001b).

3.2.2.3 Immunological and Lymphoreticular Effects

The highest NOAEL values and all LOAEL values from each reliable study for immunological and lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Zinc plays a role in the normal development and maintenance of the immune system, such as in the lymphocyte response to mitogens and as a cofactor for the thymic hormone thymulin (Delafuente 1991; Fraker et al. 1986). Oral exposure to zinc at levels much higher than the RDA has impaired immune and inflammatory responses. This was observed in *in vivo* investigations of the immune competence of blood components taken from 11 healthy adult men after ingestion of 4.3 mg zinc/kg/day as zinc sulfate for 6 weeks. The mitogenic response elicited from peripheral blood lymphocytes and the chemotactic and phagocytic responses of polymorphonuclear leukocytes were impaired after zinc ingestion. No effects were seen on total numbers of lymphocytes or relative numbers of T cells, T cell subsets, or B cells (Chandra 1984). The relationship between these observations and decreased levels of immune competence that might lead to increased susceptibility to disease is unknown. Zinc supplements administered to elderly populations at doses up to 1.5 mg zinc/kg/day (Bogden et al. 1988) or 2.5 mg zinc/kg/day (Duchateau et al. 1981) resulted in either no effect or a beneficial effect on immune cell titers or delayed cutaneous hypersensitivity responses to specific antigens. A later study (Bonham et al. 2003) reported no effects of supplementation of male volunteers with 30 mg zinc/day (0.43 mg zinc/kg/day assuming a reference male body weight of 70 kg) as zinc glycine chelate for 14 weeks on levels of peripheral blood leucocytes or on the frequency of lymphocyte subsets.

Decreased lymphocyte activity (incorporation of ^{3}H-thymidine in response to concanavalin A) was reported in mink kits from dams that had ingested a time-weighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate for 10 weeks prior to conception and throughout gestation and lactation (Bleavins et al. 1983). The dose to the kits is unknown. In contrast, no effect was observed on antibody titre (immunoglobulin G [IgG] and immunoglobulin M [IgM]) or the mitogenic response of splenic B cells isolated from mice fed 76.9 mg zinc/kg/day as zinc sulfate for 4 weeks and challenged with B cell antigens either *in vivo* or *in vitro* (Schiffer et al. 1991). The *in vitro* mitogenic response of T cells isolated from these mice was increased. There was no effect of the zinc supplement in the plaque forming cell assay or on cytotoxic T killer cell activity in mice exposed to 6.5 mg zinc/kg/day in the diet for 8 weeks (Fernandes et al. 1979). In mice exposed *in utero* to 136 mg zinc/kg/day, with exposure continuing postnatally, there were increases in direct plaque-forming activity of spleen cells and in lymphocyte proliferation in response to mitogen stimulation (Lastra et al. 1997).

3.2.2.4 Neurological Effects

Zinc appears to be necessary for normal brain function (Sandstead et al. 1983), but excess zinc is toxic. A 16-year-old boy who ingested ≈86 mg zinc/kg/day of metallic zinc over a 2-day period in an attempt to promote wound healing, developed signs and symptoms of lethargy, light-headedness, staggering, and difficulty in writing clearly (Murphy 1970). Lethargy was also observed in a 2-year-old child who ingested a zinc chloride solution (≈1,000 mg zinc/kg) (Potter 1981). It is not known whether these observations represent direct effects on the nervous system.

Very limited data were located regarding neurological effects in animals. Minor neuron degeneration and proliferation of oligodendroglia occurred in rats dosed with 487 mg zinc/kg/day as zinc oxide for 10 days (Kozik et al. 1980). Rats receiving 472 mg zinc/kg/day for 10 days had increased levels of secretory material in the neurosecretory nuclei of the hypothalamus (Kozik et al. 1981). Mice exposed postnatally to 0.5 mg zinc/kg/day as zinc acetate for 28 days showed no changes in memory formation, but showed a gradual decrease in learning extinction throughout the study (de Oliveira et al. 2001).

3.2.2.5 Reproductive Effects

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Pregnant women receiving capsules containing 0.3 mg zinc/kg/day as zinc sulfate during the last two trimesters did not exhibit any reproductive effects (no changes in maternal body weight gain, blood pressure, postpartum hemorrhage, or infection) (Mahomed et al. 1989). No other studies were located regarding reproductive effects in humans after oral exposure to zinc.

No measurable effect on gestational length or litter size was observed when female mink ingested a time-weighted average dose of 20.8 mg zinc/kg/day as zinc sulfate (Bleavins et al. 1983). No histological alterations in the testes or ovaries were noted in mice fed zinc sulfate (1,110 mg zinc/kg/day) for 13 weeks (Maita et al. 1981). Male and female rats exposed by gavage to up to 14 mg zinc/kg/day as zinc chloride resulted in a nonsignificant decrease in fertility index in all groups that was not related to administered dose; in the two highest groups (7 and 14 mg zinc/kg/day), decreases in live pups per litter and pup weight at day 21 were also reported (Khan et al. 2001b). Similarly, exposure to up to 8 mg zinc/kg/day every other day for 14 days showed no effects on the levels of abnormal sperm in Wistar rats (Piao et al. 2003). Male and female rats receiving 50 mg zinc/kg/day as zinc carbonate in the diet were reported to reproduce normally for several generations in a poorly documented study by Sutton and Nelson (1937). Rats fed 250 mg zinc/kg/day for 14–17 weeks mated successfully but had a higher than normal percentage of stillborn pups. A subsequent mating of the parental generation fed 250 mg zinc/kg/day for 5 months was unsuccessful. No reproduction occurred in rats fed 500 mg zinc/kg/day for 5 months (Sutton and Nelson 1937). The frequency of sperm with an altered chromatin structure was increased in rats fed 25 mg zinc/kg/day as zinc chloride for 8 weeks (Evenson et al. 1993). Pre-implantation loss increased in rats fed diets containing 200 mg zinc/kg/day as zinc sulfate on gestational days 0–18 (Pal and Pal 1987). When the rats received 200 mg zinc/kg/day 21 days prior to mating, no effects on implantation or other adverse reproductive effects were observed (Pal and Pal 1987). Similarly, exposure of up to 372 mg zinc/kg/day in mice prior to and throughout pregnancy did not result in changes in reproductive index (Lastra et al. 1997).

3.2.2.6 Developmental Effects

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

Zinc is necessary for normal fetal growth and development. Fetal damage may result from zinc deficiency. Only one report in the literature suggested adverse developmental effects in humans due to exposure to excessive levels of zinc (Kumar 1976). Four women were given zinc supplements of 0.6 mg zinc/kg/day as zinc sulfate during the third trimester of pregnancy. Three of the women had premature deliveries, and one delivered a stillborn infant. However, the significance of these results cannot be determined because very few details were given regarding the study protocol, reproductive histories, and the nutritional status of the women. Other human studies have found no developmental effects in the newborns of mothers consuming 0.3 mg zinc/kg/day as zinc sulfate (Mahomed et al. 1989) or zinc citrate (Simmer et al. 1991) or 0.06 mg zinc/kg/day as zinc aspartate (Kynast and Saling 1986) during the last two trimesters. There has been a suggestion that increased serum zinc levels in pregnant women may be associated with an increase in neural tube defects (McMichael et al. 1994), but others have failed to confirm this association (Hambidge et al. 1993).

The developmental toxicity of zinc in experimental animals has been evaluated in a number of investigations. Exposure to high levels of zinc in the diet prior to and/or during gestation has been associated with increased fetal resorptions, reduced fetal weights, altered tissue concentrations of fetal iron and copper, and reduced growth in the offspring.

Administration of zinc in rats at 200 mg zinc/kg/day as zinc oxide in the diet for 21 days prior to mating and then throughout gestation resulted in resorption of all fetuses (Schlicker and Cox 1968). Fetal resorptions ranged from 4 to 29% when 200 mg zinc/kg/day was administered only during gestation (controls had no resorptions). When the dose was reduced to 100 mg zinc/kg/day starting 21 days prior to mating, there were no fetal resorptions, malformations, or growth reduction. In contrast, Kinnamon (1963) reported no resorptions, no difference in the number of offspring per litter, and no change in average wet weight of the fetuses from female rats fed 250 mg zinc/kg/day as zinc carbonate in the diet for 53 days before mating and during gestation. The reason for the differences in the results of these studies is unknown. No effect on fetal viability, size, or malformations was seen in fetuses from female rats fed 25 mg zinc/kg/day as zinc carbonate during gestational days 1–18 (Uriu-Hare et al. 1989).

Administration of 200 mg zinc/kg/day to dams throughout gestation resulted in decreased growth and tissue levels of copper and iron in fetal rats (Cox et al. 1969; Schlicker and Cox 1968). In rats, at both 100 and 200 mg/kg/day during gestational days 1–18, maternal zinc levels increased. However, zinc tissue levels in the 22-day-old fetuses were not elevated at 100 mg/kg/day to dams, suggesting that the placenta was able to act as a barrier to zinc at the lower dietary level. In contrast, Ketcheson et al. (1969) showed that newborn and 14-day-old rats from mothers that had consumed 100 mg/kg/day throughout gestation had elevated levels of total zinc and decreased levels of iron. It is unclear whether the longer exposure to zinc during gestation or the suckling of newborn rats prior to sacrifice may have accounted for these differences.

Animal studies suggest that exposure to very high levels of dietary zinc is associated with reduced fetal weight, alopecia, decreased hematocrit, and copper deficiency in offspring. For example, second generation mice exposed to zinc carbonate during gestation and lactation (260 mg/kg/day in the maternal diet), and then continued on that diet for 8 weeks, had reduced body weight, alopecia, and signs of copper deficiency (e.g., lowered hematocrit and occasional achromotrichia [loss of hair color]) (Mulhern et al. 1986). Similarly, mink kits from dams that ingested a time-weighted-average dose of 20.8 mg zinc/kg/day as zinc sulfate also had alopecia and achromotrichia (Bleavins et al. 1983). It is likely that the alopecia resulted from zinc-induced copper deficiency, which is known to cause alopecia in monkeys (Obeck 1978). However, no adverse effects were observed in parental mice or mink. No effects on reproduction were reported in rats exposed to 50 mg zinc/kg/day as zinc carbonate; however, increased stillbirths were observed in rats exposed to 250 mg zinc/kg/day (Sutton and Nelson 1937).

3.2.2.7 Cancer

Leitzmann et al. (2003) reported on the occurrence of prostate cancer within a cohort of 46,974 men within the United States evaluated between 1986 and 2000. Within the cohort, 2,901 cases of prostate cancer were identified, 434 of which were classified as advanced cancer. Zinc supplementation did not appear to have an effect on the frequency of developing prostate cancer. However, men within the cohort who had taken supplements of ≥100 mg zinc/day had a greater probability of developing advanced cancer, if a tumor occurred.

Other studies evaluating the possible carcinogenic effects of zinc in humans are extremely limited. One study reported an association between an excess rate of gastric cancer in the people of North Wales (Great Britain) and the high zinc-to-copper ratio (≈30:1) in the soil of household gardens (Stocks and Davies

1964). However, the inference that this excess in gastric cancer is causally associated with soil levels of zinc and copper is not consistent with another study. In a survey of cancer registry data (1954–1978) in Shipham, Somerset (Great Britain), an area that also has a high soil zinc-to-copper ratio (≈17:1), the gastric cancer incidence rate was significantly lower than the regional rate (Philipp et al. 1982). It is probable that other factors, not considered by Stocks and Davies (1964), are associated with or coincidental to the high soil zinc-to-copper ratio confounded the results.

The carcinogenicity of zinc in experimental animals following oral exposure was evaluated by Walters and Roe (1965). The incidence of tumors was not increased in mice exposed to 951 mg zinc/kg/day as zinc sulfate in drinking water for 1 year compared to controls. However, important details regarding the study protocol were lacking including the age and sex of the mice, the number of mice at the beginning of the study, the purity of the test material, and a complete list of the organs and tissues examined at necropsy. The control mice developed intercurrent disease (ectromelia), which resulted in a number of deaths; supplementary control mice were added to the study, but they were not concurrent controls. The number of animals in treated and control groups surviving at 1 year (study termination) was small (22–28 mice/group). The exposure period (1 year) was less than the standard bioassay period (18–24 months). There were no data in the study (e.g., survival or body weight data) to indicate that a maximum tolerated dose was achieved. These limitations reduce the sensitivity of the study by Walters and Roe (1965) to detect a carcinogenic response.

Halme (1961) exposed tumor-resistant and tumor-susceptible strains of mice to zinc in drinking water. In a 5-generation study, groups of tumor-resistant mice (strain not specified) received 0, 10, 20, 50, 100, or 200 mg zinc/L as zinc chloride in the drinking water. The spontaneous tumor frequency for this strain of mice was 0.0004%. The tumor frequencies were reported as: F0=0.8%; F1=3.5%; F1 and F2=7.6%; and F3 and F4=25.7%. The majority of the tumors were seen in the 10- and 20-mg zinc dose groups. No individual or group tumor incidence data were reported, and a discussion of statistical analysis was not included. In the tumor-susceptible mice, strains C3H and A/Sn received 10–29 mg zinc/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) were higher than the spontaneous frequency (15% for each strain), but statistical analyses were not reported.

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies were located regarding death in humans or animals after dermal exposure to zinc.

3.2.3.2 Systemic Effects

The dermal toxicity of zinc compounds, particularly effects on the skin, can vary widely with the chemical form of zinc. For example, zinc chloride is caustic and can cause severe irritation at levels <0.5 mg/cm2 (Lansdown 1991), while zinc sulfate is irritating but not as caustic as zinc chloride, and zinc oxide does not appear to be a dermal irritant. Zinc oxide is commonly used in topical applications (including sunblock products) without adverse effects.

Zinc has been reported to promote the healing of burns and wounds when topically applied as zinc oxide or calamine lotion (Gordon et al. 1981). The mechanism by which this occurs was not discussed by the authors. Zinc oxide contained in an occlusive zinc tape dressing reduced the inflammatory reactions in the granulation tissue of wounded rats (Wetter et al. 1986). The authors speculated that zinc acted either by a continuous release of zinc ions or by modifying components involved in the tape's adhesive properties.

No studies were located regarding respiratory, cardiovascular, gastrointestinal, musculoskeletal, hepatic, renal, or other systemic effects in humans or animals after dermal exposure to zinc. The systemic effects observed after dermal exposure are discussed below. The NOAEL values and all LOAEL values from each reliable study for dermal effects in each species and duration category are recorded in Table 3-3.

Hematological Effects. A worker who had been employed making up zinc chloride solutions (concentrations not specified) with his hands was found to have microcytic anemia and decreased numbers of platelets (DuBray 1937).

No studies were located regarding hematological effects in animals after dermal exposure to zinc.

Table 3-3 Levels of Significant Exposure to Zinc - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL Less Serious	LOAEL Serious	Reference Chemical Form
ACUTE EXPOSURE						
Systemic						
Human	48 hr	Dermal	2.9 mg/cm²			Agren 1990 Zinc Oxide
Mouse	5 d	Dermal		0.48 M mg/cm² (severe skin irritation)		Lansdown 1991 Zinc chloride
Mouse	5 d	Dermal		0.4 M mg/cm² (Slight skin irritancy)		Lansdown 1991 Zinc sulfate
Mouse	5 d	Dermal	16 M mg/cm²			Lansdown 1991 Zinc Oxide
Mouse	5 d	Dermal		7.2 M mg/cm² (moderate skin irritation)		Lansdown 1991 Zinc Acetate
Gn Pig	5 d	Dermal	0.4 M mg/cm²			Lansdown 1991 Zinc sulfate
Gn Pig	5 d	Dermal		0.48 M mg/cm² (moderate skin irritation)		Lansdown 1991 Zinc chloride
Gn Pig	5 d	Dermal	16 M mg/cm²			Lansdown 1991 Zinc Oxide

Table 3-3 Levels of Significant Exposure to Zinc - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
Gn Pig	5 d	Dermal	7.2 M mg/cm²			Lansdown 1991 Zinc Acetate
Rabbit	5 d	Dermal	0.4 M mg/cm²			Lansdown 1991 Zinc sulfate
Rabbit	5 d	Dermal	16 M mg/cm²			Lansdown 1991 Zinc Oxide
Rabbit	5 d	Dermal		7.2 M mg/cm² (slight skin irritation - open patch test;severe skin irritaiton - occluded patch test)		Lansdown 1991 Zinc Acetate
Rabbit	5 d	Dermal		0.48 M mg/cm² (severe skin irritation)		Lansdown 1991 Zinc chloride

d = day(s); Derm = dermal; Gn pig = Guinea pig; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

Dermal Effects. No signs of dermal irritation were observed in humans after a 25% zinc oxide patch (2.9 mg/cm^2) was placed on the skin for 48 hours (Agren 1990). However, 14 out of 17 men who were employed in the bagging or packing of zinc oxide and whose skin was frequently covered with zinc oxide dust reported having experienced zinc oxide pox at least once (Turner 1921). The pox appeared as itchy papular-pustular eruptions in the pubic region, scrotum, inner surface of the thigh, and occasionally on the axilla and inner surface of the arms. The study author suggested that these lesions were due to clogging of glands by dust, perspiration, and bacteria when skin surfaces coated with these substances were rubbed together. In contrast, a case study of 24 workers exposed to dusts of either zinc oxide, zinc sulfide, or metallic zinc revealed only 1 worker with papular pustular lesions on the axilla and inner thighs (Batchelor et al. 1926). The difference in the results was attributed to differences in the personal hygiene of the workers in the two studies.

The dermal irritancy of several zinc compounds was compared in mice, rabbits, and guinea pigs (Lansdown 1991). Of the six zinc compounds tested, zinc chloride had the greatest irritancy potential (severe irritation at 0.48 mg/cm^2), followed by zinc acetate (moderate irritation at 7.2 mg/cm^2) and zinc sulfate (slight irritation at 0.48 mg/cm^2); no signs of irritation were observed following exposure to zinc oxide. Although zinc chloride is clearly the most irritating, the relative irritancy of zinc sulfate and zinc acetate was not determined because only one dose was tested and a different dose was used for each compound. The severe skin irritancy observed following application of zinc chloride was characterized by parakeratosis, hyperkeratosis, inflammatory changes in the epidermis and superficial dermis, and acanthosis of the follicular epithelia (Lansdown 1991).

Ocular Effects. In a case report, accidental splashing of a soldering paste containing 30% zinc chloride into the eye of a plumber produced an immediate reduction in visual acuity, hyperemia, hemorrhaging, conjunctival swelling, corneal opacity, bullous keratopathy, and spotting of the lens (Houle and Grant 1973). Most symptoms disappeared after 6 weeks, but residual lens opacities persisted for over a year after the exposure. Reddened conjunctivae and lacrimation were observed in 34 persons who were exposed to extremely high concentrations of zinc chloride smoke when several smoke generators exploded in a tunnel during World War II (Evans 1945). Two of the exposed persons had corneal burns and four had small vesicular burns on the forehead or wrist. Zinc chloride was the major component of the smoke. However, other components such as zinc oxide, hexachloroethane, calcium silicide, the igniter, or the heat of the explosion may have contributed to the injuries that were observed.

No studies were located regarding the following health effects in humans or animals after dermal exposure to zinc:

3.2.3.3 Immunological and Lymphoreticular Effects

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

Genotoxicity studies conducted in a variety of test systems have failed to provide evidence for mutagenicity of zinc. However, there are indications of weak clastogenic effects following zinc exposure.

Chromosome aberrations were observed in the lymphocytes of 24 workers in a zinc smelting plant (Bauchinger et al. 1976). However, the workers had increased blood levels of lead and cadmium, and the clastogenic effect was attributed to cadmium exposure.

Results of *in vivo* studies are shown in Table 3-4. A dominant lethal study in mice failed to show a mutagenic potential for zinc. However, chromosomal aberrations have been observed in bone marrow cells following *in vivo* exposure to zinc (Vilkina et al. 1978). This effect was observed in rats exposed to 14.8 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 1988), mice given intraperitoneal injections of 3.6 mg zinc/kg/day as zinc chloride (Gupta et al. 1991), and mice exposed to zinc oxide by inhalation (Voroshilin et al. 1978). Chromosomal aberrations caused by zinc were observed in the bone marrow cells of mice maintained on a low-calcium diet (Deknudt and Gerber 1979). Calcium may be displaced by zinc in calcium-depleted conditions, leading to chromosome breaks and/or interfering in the repair process (Deknudt and Gerber 1979). *In vivo* exposure to zinc may also result in single-strand breaks, as measured by the Comet assay in mice (Banu et al. 2001). An increased incidence of sister chromatid exchange was observed in bone marrow cells of rats exposed to 17.5 mg zinc/kg/day as zinc chlorate in drinking water (Kowalska-Wochna et al. 1988).

Table 3-4. Genotoxicity of Zinc *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian systems:			
Mouse	Dominant lethal	–	Vilkina et al. 1978
Mouse	Single-strand DNA breaks	+	Banu et al. 2001
Mouse bone marrow	Chromosomal aberrations	+	Deknudt and Gerber 1979
Mouse	Chromosomal aberrations	+	Voroshilin et al. 1978
Rat bone marrow	Chromosomal aberrations	+	Kowalska-Wochna 1988
Mouse bone marrow	Chromosomal aberrations	+	Gupta et al. 1991
Rat bone marrow	Sister chromatid exchange	+	Kowalska-Wochna 1988
Mouse	Micronucleus	–	Gocke et al. 1981
Drosophilia	Sex-linked recessive lethal	–	Gocke et al. 1981

– = negative result; + = positive result; DNA = deoxyribonucleic acid

Results of *in vitro* studies are shown in Table 3-5. Exposure to zinc as zinc sulfate or zinc chloride does not increase mutation frequencies in bacterial or mammalian cell culture test systems (Amacher and Paillet 1980; Gocke et al. 1981; Marzin and Vo Phi 1985; Nishioka 1975; Thompson et al. 1989; Venitt and Levy 1974; Wong 1988). Similarly, there was no convincing evidence of a clastogenic effect in human lymphocytes exposed to 0.0003–0.00003 M zinc chloride (Deknudt and Deminatti 1978).

3.4 TOXICOKINETICS

There is limited information on the toxicokinetic properties of zinc following inhalation or dermal exposure. Increased zinc levels in the blood and urine of humans and in the tissue of animals after inhalation and dermal exposure to zinc, respectively, indicate that zinc is absorbed by these routes. The toxicokinetic properties of ingested zinc have been extensively studied. The absorption of zinc from the gastrointestinal tract is homeostatically regulated; under normal physiological conditions, 20–30% of ingested zinc is absorbed. Zinc uptake from the intestinal lumen involves passive diffusion and a carrier-mediated process. A number of factors influence the absorption of zinc; these include the solubility of the zinc compound as well as inhibitors, such as calcium, phosphorus, and dietary fiber and phytates (components of dietary fiber that may coprecipitate with zinc in the intestines), and enhancers, such as amino acids, picolinic acid, and prostaglandin E_2. Once absorbed, zinc is widely distributed throughout the body. Zinc content is highest in muscle, bone, gastrointestinal tract, kidney, brain, skin, lung, heart, and pancreas. In plasma, two-thirds of the zinc is bound to albumin which represents the metabolically active pool of zinc. This pool of plasma zinc is frequently referred to as loosely bound zinc because albumin has the ability to give up bound zinc to tissues. Zinc is excreted in both urine and feces.

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

Quantitative studies regarding absorption of zinc and zinc compounds after inhalation exposure in humans are limited. The absorption of inhaled zinc depends on the particle size and solubility, both of which may greatly influence the deposition and clearance of zinc aerosols, particularly insoluble zinc oxide (a review of the role of particle size in the deposition of particles is found in Witschi and Last 2001). Elevated levels of zinc have been found in the blood and urine of workers exposed to zinc oxide fumes (Hamdi 1969).

Table 3-5. Genotoxicity of Zinc *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
Prokaryotic organisms:				
Salmonella typhimurium (TA102)	Gene mutation	Not tested	–	Marzin and Vo Phi 1985
S. typhimurium (TA98, TA102, TA1535, TA1537)	Gene mutation	– (S9)	–	Wong et al. 1988
S. typhimurium (TA1538, TA98, TA100, TA1537)	Gene mutation	– (S9)	–	Thompson et al. 1989
S. typhimurium (TA1535, TA1537, TA1538, TA98, TA100)	Gene mutation	– (S9)	–	Gocke et al. 1981
Escherichia coli	Gene mutation	Not tested	–	Nishioka 1975
E. coli	Gene mutation	Not tested	–	Venitt and Levy 1974
Mammalian cells:				
Mouse lymphoma	Gene mutation	Not tested	–	Amacher and Paillet 1980
Mouse lymphoma	Gene mutation	+ (S9)	+	Thompson et al. 1989
Human lymphocytes	Chromosomal aberrations	Not tested	+	Deknudt and Deminatti 1978
Chinese hamster ovary cells	Chromosomal aberrations	+ (S9)	+	Thompson et al. 1989

– = negative result; + = positive result

The rates or percentages of absorption of inhaled zinc in animals are not available; however, studies provide data on zinc retention in the lungs. Zinc retention values were 19.8, 11.5, and 4.7% in the lungs of guinea pigs, rats, and rabbits, respectively, after inhalation exposure (nose-only) to 3.5–9.1 mg zinc/m^3 as zinc oxide aerosol for 2–3 hours (Gordon et al. 1992). The aerosol had a mass median diameter of 0.17 µm. The retention of zinc in lungs was dose related in male Wistar rats administered a single intratracheal instillation of 0.07–3.7 mg zinc/m^3 as zinc oxide (Hirano et al. 1989). A half-life of 14 hours was calculated.

The absorption of zinc oxide fumes lead to increased levels of zinc measured in the liver, kidney, and pancreas of cats exposed to zinc oxide fumes for durations ranging from 15 minutes to 3.25 hours (Drinker and Drinker 1928). The usefulness of the study is limited because reporting was inadequate and particle size of the zinc oxide aerosol was not determined. Some inhaled particles of zinc oxide are subject to ciliary clearance and swallowing. Thus, a portion of the inhaled zinc may ultimately be absorbed from the gastrointestinal tract.

3.4.1.2 Oral Exposure

Several studies have measured oral absorption rates of zinc in humans. Absorption ranged from 8 to 81% following short-term exposures to zinc supplements in the diet; differences in absorption are probably due to the type of diet (amount of zinc ingested, amount and kind of food eaten) (Aamodt et al. 1983; Hunt et al. 1991; Istfan et al. 1983; Reinhold et al. 1991; Sandstrom and Abrahamson 1989; Sandstrom and Cederblad 1980; Sandstrom and Sandberg 1992). For example, dietary protein facilitates zinc absorption; fractional zinc absorption ranged from 8% for low-protein rolls to 26% for high-protein rolls 3 days after individuals ingested 0.05 mg zinc/kg (Hunt et al. 1991).

Absorption of labeled zinc was 40.0–48.4% in male Wistar rats fed a diet containing 0.81 mg zinc/kg as zinc chloride or zinc carbonate (Galvez-Morros et al. 1992). Fractional absorption in immature organisms generally exceeds that in adults. In growing rats, on the basis of indirect calculation from isotope experiments, Weigand and Kirchgessner (1992) suggested surprisingly high absorption values of as much as 94.7%. It is likely that all these results were influenced by isotope exchange and do not provide estimates of net absorption.

The body's natural homeostatic mechanisms control zinc absorption from the gastrointestinal tract (Davies 1980). Persons with adequate nutritional levels of zinc absorb approximately 20–30% of all

ingested zinc. Those who are zinc-deficient absorb greater proportions of administered zinc (Johnson et al. 1988; Spencer et al. 1985).

Absorption of zinc occurs from all segments of the intestine, although the largest proportion of zinc absorption occurs from the duodenum (Methfessel and Spencer 1973). The zinc absorption process includes both passive diffusion and a carrier-mediated process (Tacnet et al. 1990). The intestinal absorption of zinc appears to be a saturable carrier-mediated process at low zinc dose levels involving a cysteine-rich intestinal protein (CRIP) (Davies 1980; Gunshin et al. 1991; Hempe and Cousins 1992; Sturniolo et al. 1991). This protein binds zinc entering the intestinal cells from the lumen (Hempe and Cousins 1991). CRIP has a limited binding capacity for zinc and becomes saturated when zinc concentration in the intestine is high. Metallothionein, a metal-binding protein, may contribute to zinc homeostasis at higher zinc absorption. Like several other metals, zinc can induce metallothionein production in intestinal mucosal cells (Richards and Cousins 1975). Zinc binds to metallothionein, which remains in the mucosal cells lining the gastrointestinal tract, and the bound metal is excreted from the body upon sloughing off of these cells. Although the affinity of zinc for metallothionein is relatively low, the protein may serve to prevent absorption of excess zinc in the body (Foulkes and McMullen 1987). Absorption of zinc in rats is increased when metallothionein levels are lower (Flanagan et al. 1983). It is hypothesized that zinc entering luminal cells is associated with CRIP, and a small amount is bound to metallothionein; however, as the luminal zinc concentration increases, the proportion of cytosolic zinc associated with CRIP is decreased with a concomitant increase in zinc binding to metallothionein (Hempe and Cousins 1992). Further details on the influence of CRIP and metallothionein on zinc absorption are provided in Section 3.5, Mechanisms of Action.

Phytate and high phosphorus intakes in animals decrease zinc absorption. In humans, dairy products that contain both calcium and phosphorus decrease zinc absorption and plasma zinc concentration (Pecoud et al. 1975). Zinc binds to phosphate which results in coprecipitation of zinc with calcium phosphate in the intestines (Nelson et al. 1985). Dietary phytate also reduces zinc absorption. The addition of 400 µmol phytate to the diet decreased zinc absorption from 43.3±17.9% in females fed bread containing 0.02 mg zinc/kg (zinc-65 isotope) to 14.3±3.2% (Sandstrom and Sandberg 1992). Rats given diets supplemented with radiolabeled zinc and phytate excreted significantly more zinc in the feces than rats given diets supplemented with radiolabeled zinc but without phytate (Davies and Nightingale 1975). The study authors suggested that the decrease in absorption was due to the formation of zinc-phytate complexes in the intestines. Phytate also reduced reabsorption of zinc secreted into the gastrointestinal tract of humans (Sandstrom and Sandberg 1992).

Endogenous substances, such as amino acids, can influence the absorption of zinc. Complexing of zinc with amino acids generally enhances its absorption in all segments of the intestine (Wapnir and Stiel 1986). Although neither zinc nor the amino acid proline are readily absorbed in the colon, complexing of zinc with proline during an *in vivo* intestinal perfusion in rats resulted in increased zinc absorption.

Acrodermatitis enteropathica is a metabolic disorder that results in the malabsorption of zinc. However, when patients afflicted with this disorder were treated with human milk, zinc absorption was enhanced (Lombeck et al. 1975). It was reported by Evans (1980) that patients with acrodermatitis enteropathica have an impaired tryptophan metabolic pathway. Picolinic acid, a chief metabolite of tryptophan, is also a constituent of human milk. Picolinic acid is secreted by the pancreas into the intestinal lumen. A study by Boosalis et al. (1983) demonstrated that patients with pancreatic insufficiency had difficulty absorbing zinc administered as zinc sulfate. However, when these pancreatic-insufficient patients were given zinc as zinc picolinate, the extent of zinc absorption was similar to that of healthy controls. Zinc absorption may depend on the bioavailability of picolinic acid. Such a mandatory role of picolinic acid in absorption has not been confirmed (Bonewitz et al. 1982).

The addition of prostaglandin E_2 (PGE_2) to the mucosal media of everted jejunal sacs from rats significantly increased zinc transport (Song and Adham 1979). In contrast, similar addition of prostaglandin F_2 (PGF_2) significantly decreased zinc transport. Addition of PGF_2 to the serosal side of the jejunal sacs increased the transport of zinc to the mucosal side; PGE_2 decreased the serosal to mucosal transport of zinc. The mechanism by which prostaglandins regulate zinc transport has not been established (Song et al. 1992). The limitation of the *in vitro* study is the absence of vascular perfusion and consequent trapping of metals in the submucosal tissue. Hence, studies of absorption of heavy metals, including zinc, in everted sacs have limited physiological relevance (Foulkes 1984) but may provide information useful for the design of future *in vivo* experiments.

The presence of other trace metals (e.g., mercury, cadmium, copper) may also diminish zinc transport. Section 3.9 provides detailed information on the interaction of zinc with other metals.

3.4.1.3 Dermal Exposure

Dermal absorption of zinc occurs, but its mechanism is not clearly defined. Studies are very limited regarding the absorption of zinc through the skin. Historically, zinc oxide has been used clinically to

promote the healing of burns and wounds (Gordon et al. 1981). Absorption has been observed in burn patients treated with gauze dressings containing zinc oxide (Hallmans 1977). The pH of the skin, the amount of zinc applied, and the vehicle administered with zinc all affect the absorption of zinc (Agren 1990, 1991).

Zinc chloride was also absorbed through the intact skin of the rat (Keen and Hurley 1977). Absorption of zinc sulfate was greater than zinc oxide following 4–48-hour dermal application to open wounds in Sprague-Dawley rats (Agren et al. 1991). About 12% of zinc oxide (0.25 mg zinc/cm^2) from the dressing reached the wound while 65% of zinc sulfate (0.066 mg zinc/cm^2) reached the wound. The data suggest that zinc oxide applied to wounds resulted in sustained delivery of zinc ions causing constant wound-tissue zinc levels. In contrast, zinc sulfate, being more water soluble than zinc oxide, is rapidly transferred into the blood and, therefore, caused decreased wound-tissue zinc levels (Agren et al. 1991).

3.4.2 Distribution

Zinc is one of the most abundant trace metals in humans. It is found normally in all tissues and tissue fluids and is a cofactor in over 300 enzyme systems. Together, muscle and bone contain approximately 90% of the total amount of zinc in the body (≈60 and 30%, respectively) (Wastney et al. 1986). Organs containing sizable concentrations of zinc are the liver, gastrointestinal tract, kidney, skin, lung, brain, heart, and pancreas (Bentley and Grubb 1991; Drinker and Drinker 1928; He et al. 1991; Llobet et al. 1988a). High concentrations of zinc were also detected in the prostate (Forssen 1972), retina, and sperm (Bentley and Grubb 1991). Zinc levels may vary considerably from one individual to another (Forssen 1972).

To some degree, the distribution of zinc in some tissues appears to be regulated by age (Schroeder et al. 1967). Zinc concentrations increase in the liver, pancreas, and prostate and decrease in the uterus and aorta with age. Levels in the kidneys and heart peak at approximately 40–50 years of age and then decline.

Zinc is present in blood plasma, erythrocytes, leukocytes, and platelets, but is chiefly localized within erythrocytes (of which 87% is in carbonic anhydrase, the major binding site) (Ohno et al. 1985). Zinc deficiency has been demonstrated to decrease the ability of erythrocytes to resist hemolysis *in vitro*. This finding suggests that zinc stabilizes the erythrocyte membrane. In plasma, two-thirds of the zinc is bound to albumin; the remainder is bound primarily to α2-macroglobulin (Bentley and Grubb 1991; Giroux et al.

1976; Wastney et al. 1986). It appears that the limited number of binding sites for zinc in plasma albumin and macroglobulin regulates the amount of zinc retained by the body (Andermann and Dietz 1982). Albumin-bound zinc has been correlated with plasma zinc levels, whereas α_2-macroglobulin shows no correlation with plasma zinc levels.

Hormones, such as the adrenocorticotrophic hormone (ACTH), appear to regulate the concentration of zinc in the liver. ACTH, secreted by the anterior pituitary gland, stimulates the secretion of glucocorticoids. Glucocorticoids, or hormones with glucocorticoid activity, have been shown *in vitro* to stimulate the net zinc uptake in cultured liver cells and at the same time activate the gene that regulates metallothionein synthesis (Failla and Cousins 1978). However, there are no *in vivo* data to support these *in vitro* findings. Metallothionein in the cells of the intestinal mucosa binds zinc, thus regulating its release into the blood.

The transfer of zinc across perfused placentas is slow; only 3% of maternal zinc reached the fetal compartment in 2 hours (Beer et al. 1992). The *in vitro* transfer of zinc between mother and fetus is bidirectional, with binding in the placenta (Beer et al. 1992). It is proposed that zinc uptake in the placenta involves a potassium/zinc transport system (Aslam and McArdle 1992). Newborns may also be exposed to zinc from their mothers by milk transfer of zinc during lactation (Rossowska and Nakamoto 1992).

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution in humans after inhalation exposure to zinc. However, occupational studies provided indirect evidence that zinc may distribute to tissues to produce systemic effects (Brown 1988; Drinker et al. 1927a; Malo et al. 1990; McCord et al. 1926; Rohrs 1957; Sturgis et al. 1927).

Zinc levels in the lungs of cats peaked immediately after acute exposure to 12–61 mg zinc/kg/day as zinc oxide for approximately 3 hours and remained high for 2 days postexposure, then dropped significantly thereafter (Drinker and Drinker 1928). Levels in pancreas, liver, and kidneys increased slowly.

3.4.2.2 Oral Exposure

A single oral dose of 0.7 mg zinc/kg as zinc sulfate given to 11 individuals resulted in peak zinc levels in the plasma at 2–3 hours (Statter et al. 1988; Sturniolo et al. 1991). Similarly, Neve et al. (1991) reported peak serum zinc concentration at 2.3 hours with 0.7 mg zinc/kg as zinc sulfate.

Following feeding of 191 mg zinc/kg/day as zinc acetate to rats for 3 months, increased zinc levels were significant in the heart, spleen, kidneys, liver, bone, and blood (Llobet et al. 1988a). The greatest increases were in bone (258% of the control value) and blood (520% of the control value). Elevated zinc levels were found in the kidneys and liver of mice fed 76.9 mg zinc/kg/day as zinc sulfate (Schiffer et al. 1991) or 38 mg zinc/kg/day as zinc nitrate (Cooke et al. 1990) for approximately 1 month. The kidneys and pancreas had higher zinc levels than the liver and carcass of rats fed diets containing 1.1 mg/kg/day for an unspecified duration (Weigand and Kirchgessner 1992). Newborn, young, and adult mice receiving a single oral dose of 4.6 mg zinc/kg as zinc chloride generally had the highest levels of zinc in the liver, kidneys, lungs, bone, muscle, and carcass 1 day after dosing (He et al. 1991). However, the amount of zinc in the lungs, muscle, and femur decreased with age.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution in humans after dermal exposure to zinc.

Animal data on the distribution of zinc following dermal exposure are limited. Elevated serum zinc levels occurred with the application of zinc oxide or zinc sulfate to skin wounds of Sprague-Dawley rats for 4–48 hours (Agren et al. 1991). Serum zinc level peaked at 4 hours in rats treated with zinc sulfate, while levels were slightly elevated for 48 hours in rats administered zinc oxide. The differences may be attributed to the absorbability of the zinc compounds.

3.4.3 Metabolism

Plasma provides a metabolically active transport compartment for zinc (Cousins 1985). Zinc is most often complexed to organic ligands (existing in loosely or firmly bound fractions) rather than free in solution as metallic ion (Gordon et al. 1981). Zinc is found in diffusible or nondiffusible forms in the blood (NAS/NRC 1979). In the diffusible form, approximately two-thirds of plasma zinc is freely

exchangeable and loosely bound to albumin (Cousins 1985); the zinc-albumin complex has an association constant of about 10^6 (NAS/NRC 1979). The diffusible form of zinc also includes zinc bound to amino acids (primarily histidine and cysteine). The zinc-albumin complex is in equilibrium with the zinc-amino acid complex (Henkin 1974). The zinc-amino acid complex can be transported passively across tissue membranes to bind to proteins. An important binding protein in the kidney and liver is metallothionein, although other tissue-binding proteins may be present.

In the nondiffusible form, a small amount of circulating zinc is tightly bound to α2-macroglobulin in the plasma (Cousins 1985). Zinc is incorporated into and dissociated from α2-macroglobulin only in the liver (Henkin 1974). This zinc-protein complex has an association constant of >1,010 (Henkin 1974; NAS/NRC 1979). The zinc bound to α2-macroglobulin is not freely exchangeable with other zinc ligands (i.e., zinc-albumin and zinc-amino acid complexes) in serum.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Information was limited regarding zinc excretion following inhalation exposure in humans. Workers exposed to zinc oxide fumes had elevated levels of zinc in the urine (Hamdi 1969) indicating that this is a route of excretion.

No studies were located regarding excretion in animals after inhalation exposure to zinc.

3.4.4.2 Oral Exposure

The principal route of excretion of ingested zinc in humans is through the intestine (Davies and Nightingale 1975; Reinhold et al. 1991; Wastney et al. 1986). Zinc loss in the body is by secretion via the gut, and the remainder occurs in the urine (Wastney et al. 1986). Fecal excretion of zinc increases as intake increases (Spencer et al. 1985). Excretion of zinc in the urine also reflects zinc intake (Wastney et al. 1986). Minor routes of elimination are saliva secretion, hair loss, and sweat (Greger and Sickles 1979; Hambidge et al. 1972; Henkin et al. 1975a; Prasad et al. 1963a; Rivlin 1983).

There was a linear increase in fecal excretion of zinc in proportion to dietary intake in rats fed supplementations of 32 mg zinc/kg/day as zinc oxide for 7–42 days (Ansari et al. 1975) or 50–

339 mg/kg/day for 21 days (Ansari et al. 1976). No differences in fecal excretion, total excretion, or retention of zinc were found among rats given diets containing different forms of zinc (Seal and Heaton 1983). Rats receiving 2.65 mg zinc/kg/day as zinc chloride, zinc sulfate, zinc phosphate, or zinc citrate, over a 4-day period excreted 87–98% of intake.

A study by Alexander et al. (1981) demonstrated that zinc is excreted in the bile of rats. Analysis of the bile indicated that the zinc is primarily complexed with reduced glutathione. Treatment of these rats with diethylmaleate, which conjugates with reduced glutathione and restricts its availability, depressed the biliary excretion of zinc. This depression confirms a relationship between zinc and glutathione and suggests that zinc is transferred from liver to bile by a glutathione-dependent process.

Other factors may affect zinc excretion. For example, low dietary intake of zinc or malnutrition can increase the urinary excretion of zinc. This release of zinc is a result of tissue breakdown and catabolism during starvation; and elevated urinary excretion of zinc may persist after intake levels return to normal (Spencer et al. 1976). Administration of histidine or high-protein diet may increase urinary zinc excretion; however, a corresponding increase in zinc absorption may maintain zinc balance in the body (Henkin et al. 1975b; Hunt et al. 1991).

3.4.4.3 Dermal Exposure

No studies were located regarding excretion in humans or animals after dermal exposure to zinc.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

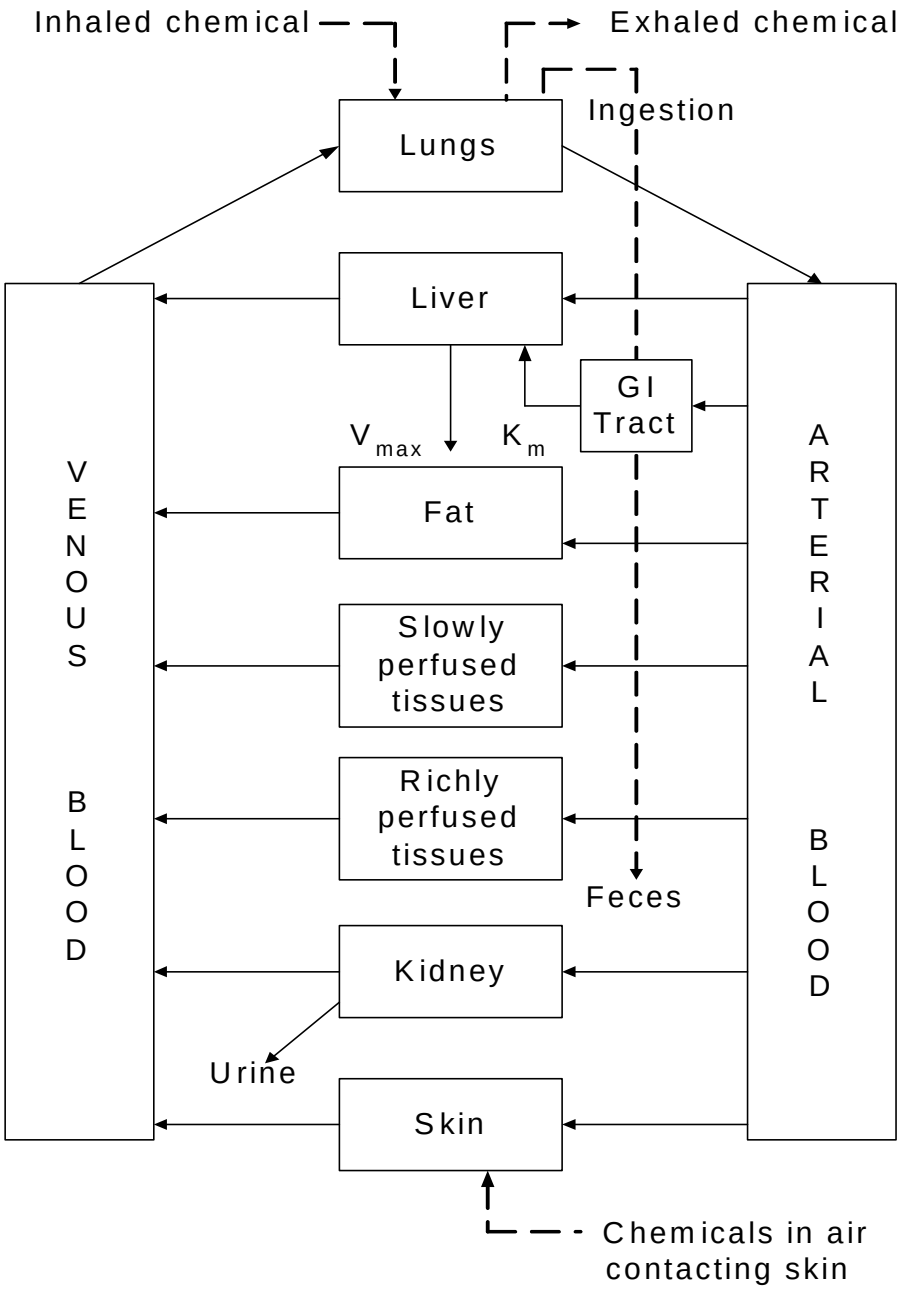
PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-3 shows a conceptualized representation of a PBPK model.

Figure 3-3. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

If PBPK models for zinc exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

Validated PBPK models for zinc in animals or humans are not presently available.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

The absorption of zinc from the intestine is homeostatically controlled. A study by Hempe and Cousins (1992) found that CRIP, a diffusible intracellular zinc carrier, binds zinc in the mucosa during absorption; this process appears to be saturable (Gunshin et al. 1991; Hempe and Cousins 1992; Sturniolo et al. 1991). Zinc transport in the intestinal lumen is also influenced by metallothionein which can inhibit zinc absorption by competing with CRIP for zinc (Hempe and Cousins 1992). CRIP binds about 40% of radiolabeled zinc entering the intestinal cells from the lumen in ligated loops of the small intestine of anesthetized rats when the zinc concentration is low (5 μM), but only 14% of the radiolabel when the concentration is high (300 μM) (Hempe and Cousins 1991). These findings suggest that CRIP has a limited binding capacity for zinc and becomes saturated when zinc concentration in the intestine is high (Hempe and Cousins 1992).

High luminal zinc concentrations may damage the brush border membrane, allowing zinc to enter the cell and bind nonspecifically to cell proteins and other ligands (Cousins 1985; Hempe and Cousins 1992). Within the intestinal lumen, a number of factors appear to influence the availability of zinc for absorption. Methionine, histidine, cysteine, reduced glutathione, citrate, and prostaglandin E_2 increase the intestinal uptake of zinc (Song et al. 1992), whereas inorganic inhibitors of zinc absorption include cadmium, copper, calcium, and ferrous iron (Hamilton et al. 1978; Harford and Sarkar 1991; Ogiso et al. 1979; Spencer et al. 1992; Yoshida et al. 1993). The mechanism of inhibition has not been clearly elucidated, but it is believed to involve competition for zinc binding sites in the intestinal mucosal cells; an effect on charge distribution on the mucosal membrane has also been suggested (Foulkes 1985). The organic inhibitors, including phytate and some components of dietary fiber, are believed to complex with zinc and decrease its availability. In the mucosal cell, zinc is associated with metalloproteins, including metallothionein. The release of zinc from the intracellular protein ligands and its transfer to the blood may involve diffusion of complexes with glutathione and similar compounds (Foulkes 1993).

In the plasma, albumin is the primary carrier for zinc, with smaller amounts of zinc bound to α2-macroglobulin and amino acids (Giroux et al. 1976). The albumin-bound zinc represents the metabolically active pool of zinc. Zinc is loosely bound in plasma, and albumin-bound zinc can readily be given up to tissues; however, the mechanisms are not fully elucidated. Zinc is initially concentrated in the liver after ingestion, and is subsequently distributed throughout the body. The liver, pancreas, bone, kidney, and muscle are the major tissue storage sites. When plasma zinc levels are high, liver metallothionein synthesis is stimulated, which facilitates the retention of zinc by hepatocytes (Richards and Cousins 1975). A storage form of zinc has not been identified in soft tissues, with the possible exception of zinc metallothionein. Zinc in bone is relatively unavailable for use by other tissues.

3.5.2 Mechanisms of Toxicity

Metal fume fever is the primary effect observed in workers exposed to zinc oxide fumes or dust (Blanc et al. 1991; Brown 1988; Drinker et al. 1927b; Vogelmeier et al. 1987). Metal fume fever usually occurs 3–10 hours after exposure, and the symptoms persist for 24–48 hours. The exact pathogenesis of metal fume fever is not known. It is believed to be an immune response to the inhaled zinc oxide (Mueller and Seger 1985). It has been suggested that the zinc oxide causes inflammation of the respiratory tract and the release of histamine or histamine-like substances. In response, an allergen-antibody complex is formed that may elicit an allergic reaction upon subsequent exposure to the allergen. In response to the allergen-antibody complex, an anti-antibody is formed. The anti-antibody dominates with continued exposure to the zinc oxide, thereby producing a tolerance. When the exposure is interrupted and re-exposure occurs, the allergen-antibody complex dominates, producing an allergic reaction and symptoms of metal fume fever (McCord 1960).

Oral exposure to high levels of zinc has caused anemia, decreased levels of HDL cholesterol, and pancreatic damage in humans (Black et al. 1988; Chandra 1984; Chobanian 1981; Hooper et al. 1980; Murphy 1970) and animals (Allen et al. 1983; Aughey et al. 1977; Drinker et al. 1927d; Katya-Katya et al. 1984; Klevay and Hyg 1973; Maita et al. 1981; Straube et al. 1980). The mechanisms involved in the pancreatic damage have not been elucidated. The anemia and possibly the decreased HDL cholesterol levels are thought to be caused by a zinc-induced copper deficiency, although the levels at which this occur have not been well-characterized. Although it is generally accepted that the anemia is the result of copper deficiency, the relationship between zinc and copper levels and HDL cholesterol levels has been extensively debated (Fischer et al. 1980; Katya-Katya et al. 1984; Klevay and Hyg 1973; Murthy and Petering 1976).

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors.* However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors,* initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors.* In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No *in vitro* studies were located regarding endocrine disruption of zinc.

Pancreas. Increased levels of serum amylase were observed in a man after accidental ingestion of about 3 ounces of a zinc chloride solution (Chobanian 1981). A 16-year-old boy who ingested an average of

approximately 86 mg zinc/kg/day as metallic zinc for 2 days (114 mg/kg on the 1st day and 57 mg/kg on the 2nd day) had increased serum amylase and lipase (Murphy 1970).

In humans receiving a single low dose of zinc sulfate (0.5 mg zinc/kg/day), no changes in blood glucose or insulin levels were observed, and there were no differences in response to a glucose load (Brandao-Neto et al. 1990b).

Pancreatic abnormalities (islet cellular alterations, acinar cell necrosis, metaplasia, fibrosis, pancreatitis) resulting from zinc ingestion have been observed in rats (Maita et al. 1981), mice (Aughey et al. 1977; Maita et al. 1981), cats (Drinker et al. 1927d), ferrets (Straube et al. 1980), sheep (Allen et al. 1983), and birds (Kazacos and Van Vleet 1989; Lu et al. 1990). In dogs (Drinker et al. 1927d) and minks (Aulerich et al. 1991), histological changes in the pancreas have not been observed at doses comparable to or higher than the dose levels that caused abnormalities in rats, mice, cats, ferrets, and sheep. Degeneration of the acinar cells of the pancreas was observed in sheep by Allen et al. (1983) and in rats and mice by Maita et al. (1981). Since the pancreatic acinar cells secrete digestive juices into the small intestine, the increase in serum amylase and lipase observed in the human case reports (Chobanian 1981; Murphy 1970) would correspond to damage in this region of the pancreas.

In 2-month-old C3H mice exposed to 70 mg zinc/kg/day as zinc sulfate, hypertrophy and vacuolation of the β-cells of the pancreatic islets were observed beginning after 3 months of exposure and become more severe by 12 months (Aughey et al. 1977). The pancreatic islets secrete the hormones glucagon and insulin. No change in plasma levels of insulin and glucose was observed in this study after 6 months of exposure. No effect on islet cells was reported in rats exposed up to 565 mg/kg/day or mice exposed to 1,110 mg/kg/day as zinc sulfate in a 13-week study by Maita et al. (1981), and Allen et al. (1983) reported that islet cells in sheep were generally unaffected, although occasional vacuolization occurred. Degeneration of acinar cells, but no effects on the islet cells, were found in ducklings (Kazacos and Van Vleet 1989); however, the relevance of this to humans is unclear. The data are too limited and contradictory to determine whether pancreatic islet cells are a primary target cell of zinc toxicity.

Adrenal Gland. Decreased levels of serum cortisol (a hormone secreted by the adrenal cortex) were observed in humans after a single dose of 0.5 mg zinc/kg/day as zinc sulfate (Brandao-Neto et al. 1990b). No effects on the adrenal gland itself have been reported in humans.

In mice receiving 70 mg zinc/kg/day as zinc sulfate in the drinking water, hypertrophy and increased lipid content of the zona fasciculata cells of the adrenal cortex were observed as early as 3 months after the start of zinc supplementation (Aughey et al. 1977).

Pituitary. No effects on pituitary function have been reported in humans following oral exposure to zinc. However, mice receiving 70 mg zinc/kg/day as zinc sulfate in the drinking water for 5–14 months had hypertrophy and increased granularity suggesting increased activity of the pituitary (Aughey et al. 1977). It is unclear whether the increased activity was a direct effect of the zinc or a reaction to decreased secretion from the adrenal cortex.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are

proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

While a detailed discussion of zinc deficiency is beyond the scope of this document, there is considerably more information on the effects of zinc deficiency on the developing fetus in pregnant women than exists for the effects of excess zinc during pregnancy. Maternal zinc deficiency can result in intrauterine growth retardation, teratogenesis, or embryonic or fetal death (for review, see King 2000). Zinc supplementation during pregnancy is usually sufficient to prevent these outcomes. Similarly, zinc deficiency during early life can result in adverse effects, including skin rash, diarrhea, anorexia, and growth failure, with more severe instances resulting in detrimental effects on the immune and nervous systems (Krebs 1999). Infants, more than adults, appear to be particularly sensitive to zinc deficiency, possibly the result of their higher zinc requirements on a per body weight basis.

A case study presented by Murray (1926) reported on an infant death due to bronchopneumonia resulting from inhalation, and possibly ingestion, of an unspecified amount of zinc stearate powder spilled from a container. However, it is unclear whether the death was due to the zinc content or whether aspiration

bronchopneumonia would result from inhalation of similar powders that do not contain zinc. Other data on the effects of zinc inhalation in young children are not available.

The human data on the effects of excess zinc in children consist mainly of reports of acute ingestion. The primary symptoms in these subjects mimic those of adult exposure, consisting mainly of gastrointestinal disturbances (nausea, vomiting, epigastric discomfort), with occasional neurologic symptoms (Anonymous 1983; Lewis and Kokan 1998; Moore 1978; Murphy 1970). Data are not presently sufficient to determine whether children are more sensitive to these effects than adults.

The most sensitive animal model to zinc toxicity in young animals appears to be the mink. Young minks appear to be more sensitive to both the hematologic (decreased hematocrit and lymphocyte number) and dermal effects (graying of the fur and dermatosis) of oral zinc than adults (Bleavins et al. 1983). Other studies have examined the effects of zinc exposure in young animals (Drinker et al. 1927d; L'Abbe and Fischer 1984a; Maita et al. 1981), 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.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989a).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the

body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to zinc are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by zinc are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations that are Unusually Susceptible.”

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Zinc

There is no simple measure of zinc body burden. Under normal physiological conditions, the plasma/serum zinc level is ≈1 µg/mL (NAS/NRC 1979) and the urinary level is 0.5 mg/g creatinine (Elinder 1986). Several studies have reported increased levels of zinc in the serum and urine of humans and animals after inhalation, oral, or dermal exposure to zinc (Agren et al. 1991; Bentley and Grubb 1991; Brandao-Neto et al. 1990a; Hallmans 1977; Hamdi 1969; Keen and Hurley 1977; Neve et al. 1991; Statter et al. 1988; Sturniolo et al. 1991). However, relationships between serum and/or urine levels and zinc exposure levels have not been established.

Hair and nail samples provide a lasting record of long-term metal intake possibly over weeks or months (Hayashi et al. 1993; Wilhelm et al. 1991). Mean zinc concentrations of 129–179 µg/g have been estimated for nails (Hayashi et al. 1993; Wilhelm et al. 1991) and 102–258 µg/g for hair (Folin et al. 1991; McBean et al. 1971; Provost et al. 1993; Wilhelm et al. 1991). Most investigators have found a poor correlation between hair and plasma zinc levels since the zinc in hair does not exchange with the

body zinc pool (McBean et al. 1971; Rivlin 1983). Furthermore, measurements of zinc in hair can be affected by extraneous contamination of hair, contamination by sweat, location of hair sample (distance from scalp), hair coloring, and rate of hair growth (McBean et al. 1971; Rivlin 1983). Although the nail is considered more resistant to washing procedures than hair, external contamination and uncertainties regarding the length and period of exposure reflected by the observed zinc concentration limit this measurement as a biomarker of exposure for zinc (Wilhelm et al. 1991).

3.8.2 Biomarkers Used to Characterize Effects Caused by Zinc

The respiratory tract is the most sensitive target organ for zinc following inhalation exposure. Inhalation of zinc oxide results in a syndrome referred to as metal fume fever. Symptoms include fevers, chills, cough, listlessness, and metallic taste. Although oxides of several heavy metals (antimony, aluminum, arsenic, cadmium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, selenium, silver, and tin) and pyrolysis products of fluorocarbon polymers (polytetrafluoroethylene [Teflon] and fluorinated polyethylene propylene) also produce metal fume fever (Ellenhorn and Barceloux 1988), this group of symptoms may be used as a nonspecific biomarker to identify inhalation exposure to zinc oxide.

The target organs associated with oral zinc exposure include the gastrointestinal tract, blood, immune system, and pancreas. The toxic effects observed after oral exposure to zinc include nausea, vomiting, diarrhea, decreased hemoglobin and hematocrit levels, immune suppression, increased serum amylase and lipase, and decreased HDL cholesterol levels (a more detailed discussion of effects associated with exposure to zinc is presented in Section 3.2). However, nausea, vomiting, and diarrhea may be observed following exposure to any gastrointestinal irritant. Increases in serum amylase and lipase are also markers for pancreatic damage; therefore, any condition resulting in pancreatitis (i.e., biliary tract disease [gallstones], alcoholism, trauma, inflammation, blood-borne bacterial infections, viral infections, ischemia, and drugs such as azathioprine, thiazides, sulfonamides, and oral contraceptives) would result in similar increases in these enzymes (Cotran et al. 1989). A hypochromic microcytic anemia that is not responsive to iron supplements may indicate exposure to zinc; however, such anemia may also reflect copper, pyridoxine, or cobalt deficiency, lead intoxication, poor diet, or chronic blood loss (Suber 1989).

Thus, none of the above-mentioned effects observed after exposure to zinc is specific to zinc exposure. However, the combination of these toxic effects may be indicative of zinc overexposure. Additional information on the health effects of zinc may be found in Section 3.2.2. Additional information on

biomarkers for renal, hepatobiliary, immune, and nervous system effects may be found in the CDC/ATSDR (1990) and OTA (1990) reports listed in Chapter 9.

Increased erythrocyte metallothionein may be an index of zinc exposure in humans (Grider et al. 1990). Daily supplementation of 50 mg zinc/day to subjects for at least 7 days caused a 7-fold increase in metallothionein concentration in erythrocytes. At least 3–4 days are required before an increase in metallothionein is observed. This biomarker of exposure is only useful for recent zinc exposure because the metallothionein levels decreased approximately a week after discontinuation of a 63-week supplementation of zinc (Grider et al. 1990). Fourteen days after discontinuation of zinc supplements, metallothionein levels were reduced by 61%.

3.9 INTERACTIONS WITH OTHER CHEMICALS

Zinc is an essential element obtained from the diet. Many different metals and nutrients interact with the absorption, distribution, and excretion of zinc. However, information was not found concerning interactions that increase the toxicity of zinc or other substances in the presence of zinc (i.e., that cause the same amount of zinc to result in a greater toxic response). Zinc administration may increase the toxicity of lead; however, the data are conflicting (Cerklewski and Forbes 1976; Hsu et al. 1975). The toxicity of zinc is believed to be due to its interaction with copper, as explained below.

Metallothionein, a sulfhydryl-rich protein inducible by certain divalent cations and a variety of other agonists, is involved in the interaction between zinc and other metals such as copper (Wapnir and Balkman 1991). Inhibition of intestinal copper absorption by zinc may demonstrate competition between the two metals at the brush border of the lumen (Wapnir and Balkman 1991). Dietary intake of copper (1, 6, and 36 mg/kg) or zinc (5, 30, and 180 mg/kg) do not significantly alter the absorption of the other (Oestreicher and Cousins 1985), but when zinc levels are much higher than copper levels, copper absorption is depressed (Fischer et al. 1981). This fact has been used therapeutically in the treatment of Wilson’s Disease, in which zinc supplementation is used to prevent the over-absorption of copper caused by the disease (for a brief review, see Brewer 2000). High levels of dietary zinc are known to induce *de novo* synthesis of metallothionein in the intestinal mucosal cell. Both copper and zinc appear to bind to the same metallothionein protein; however, copper has a higher affinity for metallothionein than zinc and displaces the zinc that is attached to the metallothionein (Ogiso et al. 1979). Copper complexed with metallothionein is retained in the mucosal cell, relatively unavailable for transfer to plasma, and is excreted in the feces when the mucosal cells are sloughed off (Fischer et al. 1981; L'Abbe and Fischer

1984b). A number of factors influence the effect of dietary zinc on copper metabolism, including the amount of copper and zinc in the diet, the zinc-to-copper ratio, age of the individual, and the duration of exposure to high zinc levels (Johnson and Flagg 1986).

In a study of zinc-supplemented women, Yadrick et al. (1989) reported decreased levels of serum ferritin, a sensitive indicator of iron status. Supplementation of the subjects with iron resulted in a reversal of the diminished iron status, although whether this was due to an interaction with zinc or simply due to additional iron being provided is not clear. Other studies of zinc-exposed subjects have not reported significant changes in copper status (Black et al. 1988; Fischer et al. 1984; Milne et al. 2001); however, these studies have either evaluated male subjects, who are not as sensitive to changes in iron status, or have not evaluated serum ferritin.

Physiological interactions of zinc and cadmium have been discussed in a number of reviews (EPA 1980c; NAS 1980; Underwood 1977). Exposure to cadmium may cause changes in the distribution of zinc, with accumulation of zinc in the liver and kidney. This accumulation in the liver and kidney may result in a deficiency in other organs, particularly if the dietary intake of zinc is marginal. *In vitro* data demonstrate that zinc and cadmium enter renal proximal cells by a saturable, carrier-mediated process and a nonsaturable pathway (Gachot and Poujeol 1992). At low cadmium doses, cadmium and zinc compete for a common transport carrier system in renal proximal cells. It is hypothesized that, at high doses, the subcellular microtubule system is disrupted by cadmium, which may interfere with changes in carrier configuration that are necessary for transport of the metals (modification of the cytoskeleton), and thereby lead to noncompetitive inhibition between cadmium and zinc (Gachot and Poujeol 1992). Combined treatment with cadmium and zinc in primary cultures of kidney cells resulted in enhanced toxicity of cadmium (Yoshida et al. 1993); however, pretreatment with a nontoxic concentration of zinc caused increased induction of metallothionein synthesis and partial protection against cadmium (Yoshida et al. 1993).

Cadmium is 10 times more efficient than zinc in metallothionein induction *in vitro* (Harford and Sarkar 1991). Induction by either cadmium or zinc alone is saturable; however, simultaneous administration of cadmium and zinc results in induction of metallothionein in an additive manner. The additive effect on metallothionein induction may involve binding of the metals either to two or more metallothionein promoter binding proteins or separate sites on the same promoter binding protein (Harford and Sarkar 1991).

Zinc acetate pretreatment in the mouse TRL-1215 cell line reduced single-strand DNA damage associated with cadmium exposure (Coogan et al. 1992). Diminished cadmium-induced DNA damage was not due to decreased cadmium burden in the zinc-pretreated cells. Instead, cadmium levels were actually greater than those in nonpretreated cells (Coogan et al. 1992). Metallothionein levels were elevated in these cells, suggesting that zinc pretreatment affects cadmium genotoxicity by inducing metallothionein which may sequester cadmium from genetic material. In contrast, simultaneous exposure to cadmium and zinc decreased cadmium accumulation in the cells, perhaps because of direct competition for a common transport mechanism (Coogan et al. 1992).

Zinc acetate reduced or prevented cadmium carcinogenesis in the prostate, in the testes, or at the injection site in rats (Gunn et al. 1963a, 1964; Waalkes et al. 1989). The effect of zinc on the cadmium-induced carcinogenesis appeared to be dependent on dose, route, and target site. Sustained levels of zinc inhibited cadmium-induced injection sarcomas but had no effect on the incidence of testicular Leydig cell tumors (Waalkes et al. 1989).

Excessive dietary zinc has been shown to induce a reversible copper deficiency and anemia in experimental animals (Magee and Matrone 1960; Murthy and Petering 1976; O'Dell 1969; Underwood 1977; Wapnir and Balkman 1991). Similar effects have been seen in humans receiving long-term treatment with zinc (Porter et al. 1977; Prasad et al. 1978). However, no significant decreases in plasma copper levels were observed in humans receiving zinc for 6 weeks or 6 months (Henkin et al. 1976; Samman and Roberts 1987) or in mice administered zinc for 1–12 weeks (Sutomo et al. 1992). A reduction in erythrocyte superoxide dismutase (an index of metabolically available copper), without a decrease in plasma copper levels, was exhibited following exposure to high amounts of ingested zinc (Fischer et al. 1984). These findings suggest that superoxide dismutase may be a sensitive indicator of zinc-copper interaction. However, as not all studies of zinc supplementation have noted changes in superoxide dismutase levels, the association is still not completely clear.

Cobalt has been demonstrated to induce seminiferous tubule damage and degeneration (vacuole formation, sloughing of cells, giant cell formation) in the testes of mice following exposure for 13 weeks (Anderson et al. 1993). Coadministration of cobalt and zinc chloride in the drinking water resulted in complete or partial protection in 90% of the animals. The sites of competitive interaction between zinc and cobalt were not established in the study; however, the authors postulated that the mechanism(s) may be similar to those involved in prevention of cadmium toxicity by zinc.

The effect of tin on heme biosynthesis appears to be dependent on the concentration of zinc (Chmielnicka et al. 1992). Oral administration of tin can affect the heme synthesis by inhibiting δ-aminolevulinic acid dehydratase (ALAD) activity in blood. Zinc is required for ALAD activity and provides a protective role in heme synthesis by increasing the activity of ALAD. It is postulated that when the tin and zinc are coadministered, these metals are probably attaching to similar binding sites in the ALAD enzyme (Chmielnicka et al. 1992).

Calcium decreases the bioavailability of zinc; the converse is also true (Heth and Hoekstra 1965; Spencer et al. 1992). Oral zinc administration is associated with decreased calcium levels in the serum and in the bone of rats (Yamaguchi et al. 1983). Zinc inhibited calcium uptake in rat brush border membrane vesicles, possibly by competing directly at high-affinity calcium binding sites (Roth-Bassell and Clydesdale 1991). The interaction of calcium and zinc is apparently dose related; intestinal absorption of calcium at a low calcium intake (230 mg/day) was inhibited at a high zinc intake of 140 mg/day but not at a lower zinc intake of 100 mg/day (Spencer et al. 1992).

Pretreatment with zinc has been shown to reduce hepatotoxicity induced by xenobiotics such as acetaminophen, bromobenzene, carbon tetrachloride, D-galactosamine, gentamicin, and salicylate (Cagen and Klaassen 1979; Gunther et al. 1991; Hu et al. 1992; Szymanska et al. 1991; Yang et al. 1991). The protective effect of zinc against carbon tetrachloride toxicity is dose dependent at high dose levels of zinc, probably because of sequestering of toxic metabolites of carbon tetrachloride by metallothionein (Cagen and Klaassen 1979). Similarly, the protective action of zinc against bromobenzene and acetaminophen appears to be associated with elevated metallothionein levels (Szymanska et al. 1991). Inhibition of lipid peroxidation may be the basis for the protective effect of zinc against hepatic damage induced by D-galactosamine in rats (Hu et al. 1992). Zinc may be elevating NADPH (nicotinamide adenine dinucleotide phosphate) content in the cell, resulting in regeneration of glutathione, which increases the antioxidative ability of hepatic cells. Salicylate-induced hepatic alterations (increased lipid droplets and iron, reduced glycogen) (Gunther et al. 1991) and gentamicin-induced proximal tubular necrosis (Yang et al. 1991) were diminished in rats pretreated with injections of zinc chloride and zinc sulfate, respectively. This finding corresponded to a dramatic increase in metallothionein content with combined treatment of salicylate and zinc compared to a less significant increase with salicylate alone.

Animal studies suggest that the administration of zinc may also inhibit tumor growth. Forty weeks after exposure, the incidence of injection site sarcomas was 40–60% in rats receiving simultaneous intramuscular administration of nickel subsulfide and zinc oxide compared to an incidence of 100%

following administration of nickel subsulfide alone (Kasprzak et al. 1988). Supplementing drinking water with zinc sulfate reduced the incidence of 9,10-dimethyl-1,2-benzanthracene-induced tumors in the cheek pouches of mice (Poswillo and Cohen 1971). Zinc decreased DNA synthesis in hepatomas induced by 3'-methyl-4-dimethylaminoazobenzene (Duncan and Dreosti 1975). The investigators speculated that the changes were due to inhibited cell division cycle at the level of DNA replication.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to zinc than will most persons exposed to the same level of zinc in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of zinc, or compromised function of organs affected by zinc. Populations who are at greater risk due to their unusually high exposure to zinc are discussed in Section 6.7, Populations with Potentially High Exposures.

No specific data regarding human subpopulations that are unusually susceptible to the toxic effects of zinc were located. Healthy elderly people have been shown to have greater daily zinc intake than housebound elderly people (Bunker et al. 1987; Prasad 1988). Data from animal studies indicate that certain human subpopulations may be more susceptible to excess zinc because of zinc's depleting effect on copper (Underwood 1977). People who are malnourished or have a marginal copper status may be more susceptible to the effects of excessive zinc than people who are adequately nourished (Underwood 1977).

Hepatic zinc levels are elevated in patients with hemochromatosis, a genetic disease associated with increased iron absorption from the intestine (Adams et al. 1991). The chronic iron loading that occurs could result in hepatic metallothionein induction leading to the accumulation of zinc because metallothionein has a greater affinity for zinc than iron. These individuals may, therefore, have a greater likelihood of developing toxicity with zinc exposure levels that do not normally result in any symptoms in the general population. However, available studies, including this one, have not correlated increased hepatic zinc with any adverse effects.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to zinc. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to zinc. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following text provides specific information about treatment following exposures to zinc:

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier, 879-880, 1064-1065.

3.11.1 Reducing Peak Absorption Following Exposure

General recommendations for the management and treatment of patients following acute exposure to zinc include removal of the victim from the contaminated area and removal and isolation of contaminated clothing, jewelry, and shoes (Bronstein and Currance 1988; Stutz and Janusz 1988). Excess contaminant is gently brushed away and excess liquids blotted with absorbent material. Measures that are appropriate to the route of exposure are taken to remove zinc from the body. Exposed eyes are flushed immediately with water, followed as soon as possible with irrigation of each eye with normal saline. Exposed skin is washed immediately with soapy water. Administration of ipecac to induce emesis, gastric lavage, ingestion of activated charcoal, and cathartics have been recommended to decrease the gastrointestinal absorption of zinc (Burkhart et al. 1990; Ellenhorn and Barceloux 1988). Because zinc causes nausea and vomiting following exposure by the oral route, use of emetic agents may be unnecessary. Ipecac administration may be contraindicated following ingestion of caustic zinc compounds such as zinc chloride. The large amounts of phosphorus and calcium in milk and cheese, and phytate in brown bread, may reduce absorption of zinc from the gastrointestinal tract (Pecoud et al. 1975). Therefore, if vomiting and diarrhea are not prohibitive, ingestion of dairy products or brown bread may also reduce gastrointestinal absorption of zinc. In a study of intestinal absorption of zinc in iron-deficient mice, the uptake of zinc from the gut was inhibited by adding iron to the duodenal loop system. The proposed mechanism was that iron and zinc shared a common gut mucosal binding site (Hamilton et al. 1978). However, it is unknown whether ingestion of iron supplements would be effective in reducing absorption of zinc overdoses.

3.11.2 Reducing Body Burden

Zinc is an essential trace element that is normally found in tissues and fluids throughout the body and is under homeostatic control (NAS/NRC 1989b). Increased levels have been observed in the heart, spleen, kidneys, liver, bone, and blood of animals following subchronic oral exposure to zinc (Llobet et al. 1988a) indicating that some zinc accumulation occurs during excess intakes. The greatest increases were observed in bone and blood.

Administration of the chelating agent, calcium disodium ethylene diaminetetraacetate ($CaNa_2EDTA$), is the treatment of choice for reducing the body burden of zinc in humans following exposure to high levels (Ellenhorn and Barceloux 1988). Ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), and dimercaprol (BAL) are the most common antidotes used in the treatment of human zinc intoxications (Llobet et al. 1989; Murphy 1970; Spencer and Rosoff 1966). Markedly elevated serum zinc levels in a young child who ingested a zinc chloride solution were normalized by intravenously administering a single small dose of $CaNa_2EDTA$ (11.5 mg/kg) (Potter 1981). Use of chelation therapy (administration of BAL) was reported in a case study of a 16-year-old boy who ingested 12 g of metallic zinc (Murphy 1970). The boy exhibited lethargy and elevated blood zinc levels that were both reversed following intramuscular administration of BAL. Chelation therapy has been demonstrated to increase the urinary excretion of zinc 22-fold (Spencer and Rosoff 1966). Intravenous and nebulized *N*-acetylcysteine (another metal chelating agent) have also been observed to increase urinary zinc excretion and decrease plasma levels following inhalation of zinc chloride smoke (Hjortso et al. 1988).

The efficacy of 16 different chelating agents as possible antidotes for acute oral zinc exposure has been determined in mice (Llobet et al. 1988b). The most efficient chelators were DTPA, cyclohexanediaminetetraacetic acid (CDTA), and EDTA. Increased urinary levels of zinc and decreased bone and liver zinc levels were observed following administration of the chelators. The maximum efficiency of the chelators was observed when they were administered from 10 minutes to 12 hours after zinc exposure (Domingo et al. 1988a, 1988b).

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Anemia has been observed in humans and animals after oral exposure to zinc. It has been postulated that excess zinc intake may result in copper deficiency (mechanisms of action are discussed in Section 3.5). The anemia observed following zinc intake is believed to be caused by the copper deficiency.

Administration of copper in patients with zinc-related anemia has been shown to be effective in increasing the hemoglobin levels (Porter et al. 1977; Smith and Larson 1946).

The exact mechanism of metal fume fever (a syndrome consisting of a leukocytosis with chills, fever, cough, myalgias, headache, weakness, and dyspnea) is unknown (Ellenhorn and Barceloux 1988), but respiratory tract inflammation and the development of an immune complex reaction have been proposed (McCord 1960). Treatment is supportive (e.g., bed rest, analgesics, and antipyretics) (Mueller and Seger 1985).

In severe cases, inhalation of zinc chloride has resulted in advanced pulmonary fibrosis and fatal respiratory distress syndrome (Evans 1945; Hjortso et al. 1988; Milliken et al. 1963). L-3,4-Dehydro-proline was given to two soldiers after inhaling a high concentration of zinc chloride smoke (also contained other chemicals) in an attempt to inhibit collagen deposition in the lungs (Hjortso et al. 1988). This therapy did not prevent respiratory failure.

3.12 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of zinc is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of zinc.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Zinc

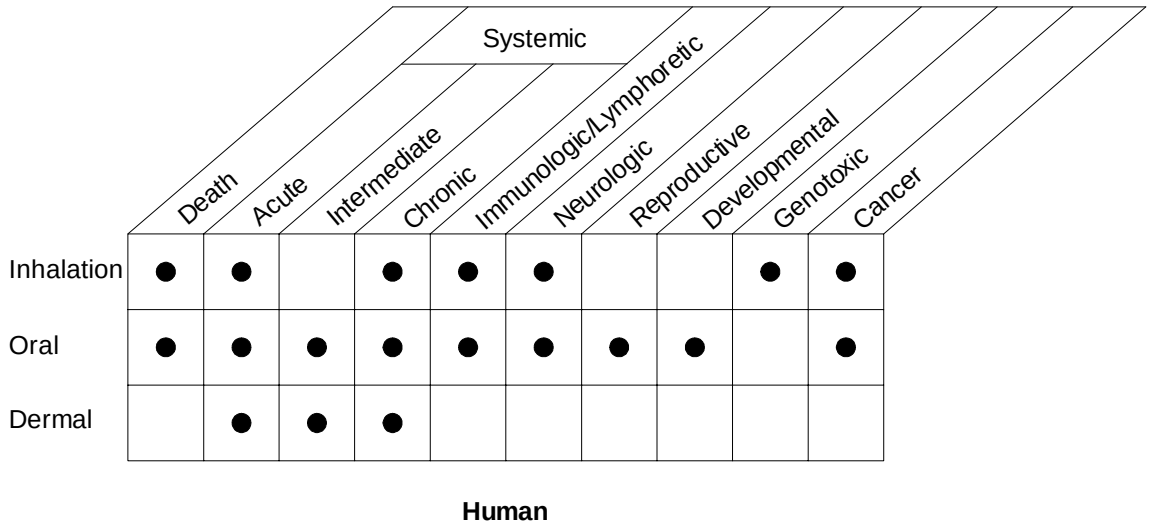
The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to zinc are summarized in Figure 3-4. The purpose of this figure is to illustrate the existing information concerning the health effects of zinc. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-4 indicates whether a particular health effect end point has been studied for a specific route and duration of exposure. There is little information concerning death in humans after inhalation, oral, or dermal exposure to zinc. However, several case studies report death after exposure to extremely high levels of zinc chloride and other components of zinc chloride smoke (Evans 1945; Hjortso et al. 1988; Milliken et al. 1963).

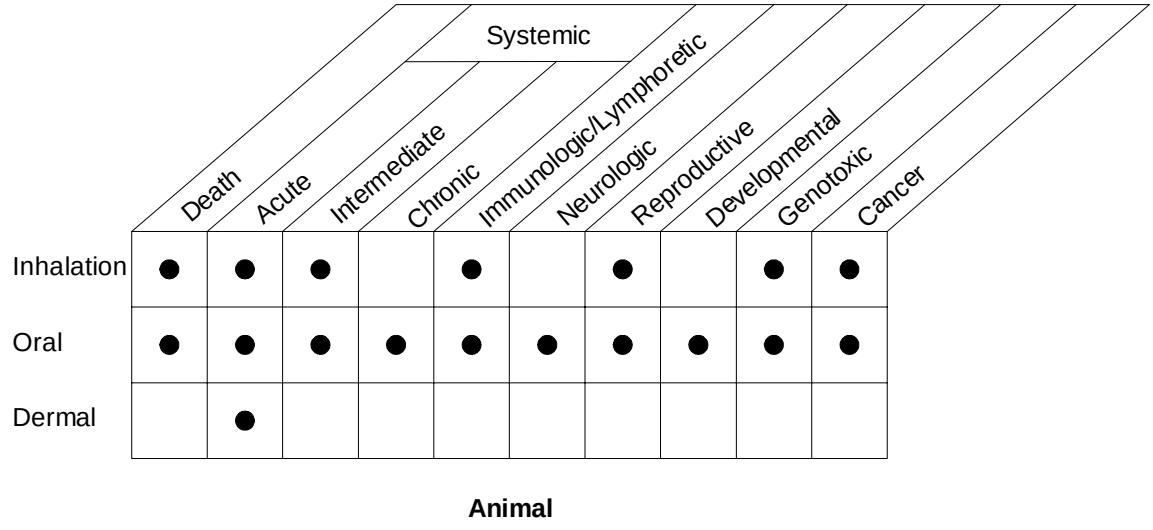
Systemic effects of acute inhalation exposure to generally unspecified levels of various zinc compounds in humans have been reported in several clinical case studies (Blanc et al. 1991; Brown 1988; Hjortso et al. 1988; Matarese and Matthews 1966; Vogelmeier et al. 1987). Case studies and experimental studies of systemic effects in humans following acute, intermediate, and chronic oral exposures are available (Anonymous 1983; Black et al. 1988; Brandao-Neto et al. 1990a; Chandra 1984; Chobanian 1981; Hale et al. 1988; Hallbook and Lanner 1972; Hoffman et al. 1988; Hooper et al. 1980; Malo et al. 1990; Moore 1978; Patterson et al. 1985; Porter et al. 1977; Potter 1981; Prasad et al. 1978). Experimental studies in humans following acute, intermediate, and chronic dermal exposures were located for hematological, dermal, and ocular effects (Agren 1990; Evans 1945; Fischer et al. 1984; Turner 1921; Yadrick et al. 1989).

Information concerning respiratory effects of acute inhalation exposure to zinc in animals is available (Amdur et al. 1982; Drinker and Drinker 1928; Lam et al. 1982, 1988). One study (Marrs et al. 1988) was located regarding other systemic effects in animals following inhalation exposure to zinc for an intermediate-exposure duration. Information regarding systemic effects of zinc following oral exposure

Figure 3-4. Existing Information on Health Effects of Zinc



Human



Animal

● Existing Studies

in animals is available for acute, intermediate, and chronic exposure durations (Allen et al. 1983; Anderson and Danylchuk 1979; Aughey et al. 1977; Bentley and Grubb 1991; Domingo et al. 1988a; Drinker et al. 1927c; Jenkins and Hidiroglou 1991; Katya-Katya et al. 1984; Klevay and Hyg 1973; Llobet et al. 1988a; Maita et al. 1981; Straube et al. 1980; Walters and Roe 1965). One acute dermal study evaluated dermal irritancy in animals (Lansdown 1991).

Immunological effects were reported in humans following inhalation exposure to zinc oxide (Blanc et al. 1991; Farrell 1987). Another study reported potential adverse immunological effects following oral exposure of humans (Chandra 1984). Clinical symptoms suggestive of neurological effects have been reported by humans following inhalation exposure (Rohrs 1957; Sturgis et al. 1927; Wilde 1975) or oral exposure (Anonymous 1983; Murphy 1970; Potter 1981) to zinc. There were studies that examined reproductive and developmental effects in women orally exposed to zinc during their pregnancies (Kynast and Saling 1986; Mahomed et al. 1989; Simmer et al. 1991).

One study examined immunological and reproductive effects in animals following inhalation exposure to zinc chloride (Marrs et al. 1988). Immunological and neurological end points were evaluated in animals following oral exposure to zinc (Bleavins et al. 1983; Kozik et al. 1980, 1981; Schiffer et al. 1991). Information regarding developmental and reproductive effects in animals after oral exposure to zinc is available (Cox et al. 1969; Ketcheson et al. 1969; Kinnamon 1963; Mulhern et al. 1986; Pal and Pal 1987; Schlicker and Cox 1968; Sutton and Nelson 1937). Studies regarding genotoxicity in animals after inhalation and oral exposures to zinc are limited (Gupta et al. 1991; Kowalska-Wochna et al. 1988; Voroshilin et al. 1978).

Epidemiological studies regarding carcinogenicity after inhalation and oral exposure to zinc are available (Logue et al. 1982; Neuberger and Hollowell 1982; Philipp et al. 1982; Stocks and Davies 1964); however, they were not well controlled and the data are of little significance. Studies are available regarding carcinogenicity in animals after inhalation and oral exposure to zinc (Marrs et al. 1988; Walters and Roe 1965). However, the studies have several deficiencies that limit their usefulness.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. Symptoms of metal fume fever (headache, fever, leukocytosis, myalgias) have been observed in humans acutely exposed to airborne zinc oxide (Blanc et al. 1991; Brown 1988; Drinker et al. 1927b; Sturgis et al. 1927). Acute oral exposure to zinc has resulted in

gastrointestinal disturbances (abdominal pain, nausea, vomiting, esophageal erosion), evidence of pancreatic damage (increased serum amylase and lipase levels), and decreased levels of serum cortisol in humans (Anonymous 1983; Brandao-Neto et al. 1990a; Chobanian 1981; Murphy 1970; Potter 1981). Acute dermal exposure to zinc oxide has not been shown to be irritating to human skin (Agren 1990). Toxic effects similar to those observed for metal fume fever have been observed in guinea pigs (Amdur et al. 1982; Lam et al. 1985). In addition to LD_{50} data, only one reliable study assessed the acute oral toxicity of zinc compounds in animals. Pancreatic, gastrointestinal, and liver damage were observed in sheep (Allen et al. 1983). It is doubtful that sheep (ruminant animals) are an appropriate model for toxicity of orally administered zinc in humans. The dermal toxicity of several zinc compounds has been tested in rabbits, guinea pigs, and mice (Lansdown 1991). Zinc acetate, zinc chloride, and zinc sulfate have irritating properties. Skin irritation was not observed in rabbits, guinea pigs, or mice after zinc oxide paste application (Lansdown 1991).

The animal data (Amdur et al. 1982; Drinker and Drinker 1928; Lam et al. 1982, 1988) corroborate occupational exposure studies that indicate metal fume fever is an end point of concern. However, other possible targets of toxicity have not been examined. Thus, an acute inhalation MRL cannot be derived. A large amount of the human oral exposure data is in the form of case reports, and a great deal of uncertainty exists regarding the dose levels. The uncertainty about whether sheep are a good model for humans precludes using these data to derive an oral MRL for acute-duration exposure. Additional studies involving acute exposure to zinc compounds by all routes of exposure would be helpful to identify target organ and dose-response relationships. There are groups who may be exposed to zinc at hazardous waste sites for brief periods; therefore, this information is important.

Intermediate-Duration Exposure. Metal fume fever was observed in an individual exposed to zinc fumes and zinc powder for approximately 1 month (Malo et al. 1990). Anemia and decreased levels of HDL cholesterol have been observed in humans taking high doses of zinc supplements (Chandra 1984; Hoffman et al. 1988; Hooper et al. 1980). Intermediate-duration dermal exposure to zinc oxide dust has resulted in pustular lesions, but these lesions were attributed to clogging of the sebaceous glands resulting from poor hygiene (Turner 1921). Rats, mice, and guinea pigs exposed to smoke containing zinc chloride and other compounds had evidence of lung irritation (Marrs et al. 1988). No intermediate-duration animal dermal studies were located. In animals that ingested zinc for an intermediate duration, anemia and kidney and pancreas damage were observed (Bentley and Grubb 1991; Drinker et al. 1927d; Jenkins and Hidiroglou 1991; Llobet et al. 1988a; Maita et al. 1981; Straube et al. 1980).

Only one case report regarding human intermediate-duration inhalation exposure was located, and this study did not report the exposure level (Malo et al. 1990). Thus, an intermediate-duration inhalation MRL could not be derived. There are less serious LOAELs (decreased serum HDL cholesterol) identified in the Hooper et al. (1980) and Chandra (1984) human oral exposure studies; however, evidence regarding this effect is inconsistent (Bogden et al. 1988; Hale et al. 1988; Samman and Roberts 1988). An intermediate-duration oral MRL was derived for zinc based on hematological effects (decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase) in women given 50 mg Zn/day as zinc gluconate supplements for 10 weeks (Yadrick et al. 1989); as these effects were subclinical, they were considered to be non-adverse, and were identified as a NOAEL. Several other studies of zinc supplementation in humans support this NOAEL (Bonham et al. 2003a, 2003b; Davis et al. 2000; Fischer et al. 1984; Milne et al. 2001). The toxic effects of intermediate-duration exposure to zinc compounds are relatively well characterized for the oral route. There are insufficient toxicokinetic data to determine if the toxic effects observed following oral exposure would occur following inhalation or dermal exposure. Inhalation and dermal studies would be useful to determine possible target organs and dose-response relationships. There are populations surrounding hazardous waste sites that might be exposed to zinc compounds for similar durations.

Chronic-Duration Exposure and Cancer. No exposure-related effects on lung function were observed in a group of welders chronically exposed to zinc (Marquart et al. 1989). Anemia has been observed in humans following ingestion of high doses of zinc supplements (Broun et al. 1990; Hale et al. 1988; Porter et al. 1977; Prasad et al. 1978). Chronic-duration dermal exposure to zinc oxide dust has resulted in pustular lesions, but these were attributed to clogging of the sebaceous glands resulting from poor hygiene (Batchelor et al. 1926). No chronic-duration inhalation or dermal studies in animals were located. Pancreatic damage was observed in mice after chronic exposure to zinc sulfate in drinking water (Aughey et al. 1977).

A chronic-duration inhalation MRL could not be derived for zinc because neither of the inhalation studies reported the levels of airborne zinc. Due to a lack of adequate chronic-duration oral studies, the intermediate-duration oral MRL was adopted as the chronic-duration oral MRL, based on hematological effects (decreased hematocrit, serum ferritin, and erythrocyte dismutase) in women given zinc gluconate supplements for 10 weeks (Yadrick et al. 1989). Additional studies involving chronic exposure to zinc compounds by all routes of exposure would be helpful to identify dose-response relationships.

Although there are several human and animal carcinogenicity studies, the limitations of these studies preclude their use in assessing the carcinogenicity of zinc (Logue et al. 1982; Neuberger and Hollowell 1982; Walters and Roe 1965). Carcinogenicity studies by all routes of exposure would be useful.

Genotoxicity. Several *in vitro* microbial gene mutation assays were negative (Marzin and Vo Phi 1985; Nishioka 1975; Thompson et al. 1989; Venitt and Levy 1974; Wong 1988), but evidence from gene mutation assays in mammalian cells is mixed (Amacher and Paillet 1980; Thompson et al. 1989). An increase in the occurrence of chromosomal aberrations was observed *in vitro* in human lymphocytes (Deknudt and Deminatti 1978) and *in vivo* in rats and mice (Deknudt and Gerber 1979; Gupta et al. 1991; Kowalska-Wochna et al. 1988; Voroshilin et al. 1978). Increased sister chromatid exchange was observed *in vivo* in rat bone marrow (Kowalska-Wochna et al. 1988). However, while there are sufficient *in vivo* data establishing the clastogenicity of zinc, data regarding the mutagenicity of zinc are conflicting. Studies designed to assay different types of genotoxicity (i.e., mutagenicity in mammalian cells, effect of excess zinc on DNA replication) would be useful for determining the genotoxic potential of zinc.

Reproductive Toxicity. No complications occurred in the pregnancies of women exposed to daily doses of zinc sulfide during the last two trimesters (Mahomed et al. 1989). No studies were located regarding the reproductive toxicity of zinc in humans after inhalation or dermal exposure. Increased pre-implantation loss and reproductive dysfunction in rats were observed in oral exposure studies (Pal and Pal 1987; Sutton and Nelson 1937). No histological changes in reproductive organs were observed in rats, mice, or guinea pigs following inhalation exposure to zinc chloride smoke, but reproductive function was not assessed (Marrs et al. 1988). No dermal reproductive toxicity studies in animals were located. Inhalation and dermal studies assessing reproductive function would be useful to determine whether zinc has the potential to cause reproductive effects by these routes. An oral reproductive toxicity study in a different animal strain as well as a multigeneration study, including reproductive organ pathology, would be useful for determining whether oral zinc exposure is likely to cause reproductive toxicity in humans.

Developmental Toxicity. No studies were located regarding the potential of zinc to cause developmental effects in humans after inhalation or dermal exposure. In a very brief report of a human study in which pregnant women received high-doses of zinc supplements during the last trimester of pregnancy, an increased incidence of stillbirths and one premature delivery were observed (Kumar 1976). This study, however, has many limitations. Increased fetal resorptions were observed in rats after oral exposure to zinc (Schlicker and Cox 1968). No studies were located regarding developmental toxicity in animals after inhalation or dermal exposure to zinc. Additional inhalation, oral, and dermal exposure

studies in animals would be useful to predict whether developmental effects should be a concern for humans exposed to zinc.

Immunotoxicity. Metal fume fever is believed to be an immune response to zinc oxide. A correlation between the concentration of airborne zinc and the number of all types of T cells (helper, inducer, suppressor, and killer) in the bronchoalveolar lavage fluid of humans, possibly related to the onset of metal fume fever, was observed in an acute-duration inhalation study (Blanc et al. 1991). Impaired immune response in humans has been reported in an intermediate-duration oral study (Chandra 1984). No immune effects were observed in mice after oral exposure to zinc (Schiffer et al. 1991). There is some limited information to suggest that the immune system is a target of zinc toxicity. A battery of immune function tests after inhalation, oral, and dermal exposure to zinc compounds would be useful in determining if zinc is immunotoxic.

Neurotoxicity. Staggering gait and hallucinations were reported in an individual who intentionally inhaled metallic paint aerosols (Wilde 1975). Because there was simultaneous exposure to copper and hydrocarbons, this study cannot be used to assess the neurotoxic potential of zinc. Nonspecific signs and symptoms of neurotoxicity (light-headedness, dizziness, headache, and lethargy) have been reported by humans following acute oral exposure to zinc (Murphy 1970; Potter 1981). Very limited data suggest that high oral doses of zinc can cause minor neuron degeneration and alteration of secretion of the hypothalamus in rats (Kozik et al. 1980, 1981). No studies were located regarding neurotoxic effects in animals after inhalation or dermal exposure to zinc. Additional studies by all routes of exposure would be useful to determine if exposure to zinc compounds would result in neurotoxicity.

Epidemiological and Human Dosimetry Studies. Acute high-level exposure to zinc by inhalation resulted in respiratory irritation and metal fume fever (Blanc et al. 1991; Hjortso et al. 1988; Johnson and Stonehill 1961; Linn et al. 1981; Schenker et al. 1981; Sturgis et al. 1927). Welders are a subpopulation of workers who have a high potential for exposure to zinc oxide. Most of the available studies did not report exposure levels or used a small number of subjects. Studies that correlate occupational exposure to zinc with health effects would be useful. A number of human oral exposure studies have shown that excess levels of zinc can result in anemia, pancreatic damage, decreased serum HDL cholesterol levels, and immunotoxicity (Black et al. 1988; Chandra 1984; Hooper et al. 1980). There are insufficient data for establishing dose-response relationships. Studies designed to establish dose-response relationships would be useful for establishing cause/effect relationships and future monitoring of individuals living near hazardous waste sites.

Biomarkers of Exposure and Effect.

Exposure. Increased serum and urine levels of zinc were observed in humans and animals after inhalation, oral, or dermal exposure to zinc (Bentley and Grubb 1991; Brandao-Neto et al. 1990b; Hallmans 1977; Keen and Hurley 1977). However, the relationships between zinc exposure levels and the levels of zinc in biological fluids have not been established. Hair and nail samples may be a potential biomarker for long-term zinc exposure (McBean et al. 1971; Rivlin 1983; Wilhelm et al. 1991); however, no correlation has been demonstrated between these parameters and zinc exposure levels. Development of a biomarker with more exposure and dose data would aid in future medical surveillance that could lead to better detection of zinc exposure.

Effect. Several potential biomarkers for the effects of zinc have been identified. These include increased levels of serum amylases and lipase, indicative of pancreatic damage; non-iron responsive anemia; and decreased HDL cholesterol levels (Suber 1989). However, these biomarkers of effect are not specific for zinc. These biomarkers cannot be used for dosimetry. A potential biomarker of exposure for recent exposures to zinc is increased erythrocyte metallothionein concentrations (Grider et al. 1990). Further investigation of serum biomarkers of effect, particularly for chronic exposure, in zinc-exposed populations would be useful to determine whether exposed populations may be experiencing adverse health effects as the result of zinc exposures.

Absorption, Distribution, Metabolism, and Excretion. Absorption of zinc in humans after oral exposure to high levels has been well described (Aamodt et al. 1983; Hunt et al. 1991; Reinhold et al. 1991; Sandstrom and Abrahamson 1989; Sandstrom and Cederblad 1980; Sandstrom and Sandberg 1992; Spencer et al. 1985). However, quantitative evidence of zinc absorption in humans after inhalation or dermal exposure is very limited. It is known that workers exposed to zinc oxide fumes who experience toxic effects have elevated levels of zinc in plasma and urine (Hamdi 1969). However, it remains to be established whether the elevated levels are the result of the pulmonary absorption or of the swallowing of particles leading to gastrointestinal absorption. Toxic effects have also been observed in humans after dermal exposure (DuBray 1937), indicating dermal absorption.

Information regarding the absorption of zinc in animals following inhalation exposure was limited to lung retention data (Gordon et al. 1992; Hirano et al. 1989). However, there was information to assess the extent of absorption following oral exposure (Davies 1980; Galvez-Morros et al. 1992; Johnson et al.

1988; Weigand and Kirchgessner 1992). Evidence is limited regarding dermal absorption in animals, but it indicates that zinc sulfate and zinc oxide can penetrate the skin (Agren 1990; Agren et al. 1991; Gordon et al. 1981; Hallmans 1977). Mechanistic data on the oral absorption is reported by Hempe and Cousins (1992); however, there is a lack of information regarding the mechanism of action of inhalation and dermal exposures.

Information on physiological levels and zinc distribution following subtoxic short-term exposures to zinc in humans and animals is abundant (NAS/NRC 1979; Wastney et al. 1986). Blood levels of zinc have been determined in humans following oral exposure to zinc sulfate (Neve et al. 1991; Statter et al. 1988; Sturniolo et al. 1991). Increased zinc tissue content has been seen after short-term oral exposure in humans (Cooke et al. 1990; He et al. 1991; Llobet et al. 1988a; Schiffer et al. 1991; Weigand and Kirchgessner 1992). Studies on tissue distribution in humans following high exposure to zinc for inhalation, oral, and dermal would be useful. There were no studies regarding blood or tissue distribution after acute, high-level exposures to zinc in animals following inhalation or dermal exposure. Additional mechanistic data on the transfer of zinc from respiratory and dermal absorption sites to the blood would be useful.

The principal excretion route of ingested zinc is through the intestines (Davies and Nightingale 1975; Reinhold et al. 1991; Wastney et al. 1986). There is a lack of information regarding the excretion of zinc in both animals and humans following inhalation and dermal exposure.

Therefore, additional studies designed to assess the toxicokinetic properties of zinc following inhalation and dermal exposures would be useful.

Comparative Toxicokinetics. Data suggest that humans and animals have similar target organs of zinc toxicity (Allen et al. 1983; Aughey et al. 1977; Black et al. 1988; Blanc et al. 1991; Brown 1988; Chandra 1984; Chobanian 1981; Drinker et al. 1927b, 1927d; Hoffman et al. 1988; Hooper et al. 1980; Katya-Katya et al. 1984; Klevay and Hyg 1973; Lam et al. 1982, 1985, 1988; Maita et al. 1981; Moore 1978; Murphy 1970; Smith and Larson 1946; Straube et al. 1980; Sturgis et al. 1927). Toxicokinetic studies have been performed in both humans and animals following oral exposure; however, data are limited for inhalation and dermal exposures. The animal model used most often to evaluate the toxicokinetics of zinc are rats (Agren et al. 1991; Alexander et al. 1981; Galvez-Morros et al. 1992; Hirano et al. 1989; Llobet et al. 1988a; Weigand and Kirchgessner 1992) and may be a good model for assessing the kinetics of zinc in humans.

Methods for Reducing Toxic Effects. No established methods or treatments for reducing the absorption of zinc were located. Studies that examined the effectiveness of emetics and cathartics in the prevention of zinc absorption would be useful. Once absorbed from the gastrointestinal tract, zinc bound to plasma albumin is distributed to the rest of the body. Zinc has a high affinity for proteins, and a number of chelating agents are effective in increasing urinary excretion of zinc following acute- and intermediate-duration administrations (Domingo et al. 1988a, 1988b; Llobet et al. 1989). Studies designed to examine the effectiveness of chelating agents following chronic zinc exposure would be useful in determining treatments to reduce the zinc body burden. Very little information is known about the absorption and distribution of zinc following inhalation or dermal exposure. Studies to determine the mechanisms of absorption and distribution would be useful for developing treatments or methods for reducing the toxic effects of zinc after inhalation or dermal exposure.

Although the exact mechanisms of many of the toxic actions of zinc are not known, the pathogenesis of metal fume fever following inhalation exposure (McCord 1960; Mueller and Seger 1985) and anemia following oral exposure (Prasad et al. 1978) are known. Studies to more clearly elucidate the mechanisms involved in metal fume fever and anemia and to determine the mechanisms involved in pancreatic damage and decreased HDL cholesterol levels would be useful. Therapy for metal fume fever is mainly supportive (Mueller and Seger 1985). Administration of copper has been shown to be effective in alleviating zinc-induced anemia (Porter et al. 1977). Research into methods useful for mitigating metal fume fever and other adverse effects of zinc would be helpful.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

While a considerable amount of data are available on the effects of zinc deficiency on the growth and development of children, less is known about the effects of excess zinc on children. Accidental acute oral exposures result in mainly gastrointestinal symptoms, including nausea, vomiting, and epigastric discomfort (Anonymous 1983; Lewis and Kokan 1998; Moore 1978; Murphy 1970). Data are not presently available to determine whether children are more susceptible to these effects than adults. Similarly, additional animal studies examining the effects of similar exposure on young and mature animals would be useful to further clarify possible mechanisms of childhood susceptibility, if it exists.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

A selection of ongoing studies, located in the Federal Research in Progress database (FEDRIP 2003), is presented in Table 3-6.

Table 3-6. Ongoing Studies on Zinc Health Effects[a]

Investigator	Institute	Research area
Abrams SA	Baylor College of Medicine, Houston, Texas	Zinc metabolism in health and chronic inflammatory bowel disease children
Black MM	University of Maryland, Baltimore, Maryland	Effect of micronutrient supplementation on children's growth, immune functioning, and morbidity
Blanchard K	Department of Veterans Affairs, Medical Center, Shreveport, Louisiana	Efficacy and safety of oral zinc therapy in patients with Polycythemia Vera
Blumenthal SS	Department of Veterans Affairs, Medical Center, Milwaukee, Wisconsin	Cadmium, zinc, metallothionein, and kidney cytotoxicity
Bobilya DJ	University of New Hampshire, Durham, New Hampshire	Evaluation of zinc transport by using an *in vitro* model of the blood brain barrier under different conditions of zinc status
Bobilya DJ	University of New Hampshire, Durham, New Hampshire	Testing to determine whether co-transport with albumin is a significant route for zinc transport by endothelial cells
Brewer GJ	University of Michigan at Ann Arbor, Ann Arbor, Michigan	Studies on the treatment of Wilson's disease with zinc
Brown KH	University of California, Nutrition, Davis, California	Bioavailability of vitamin A and zinc from selected foods of potential use for intervention programs in populations at high risk of deficiency
Brown KH	University of California, Nutrition, Davis, California	Determination of the safety and efficacy of three levels of zinc supplementation, provided with or without supplemental copper
Brown NM	Northwestern University, Evanston, Illinois	Combination of fluorescent microscopy studies with biophysical and proteomic approaches to identify zinc rich cellular compartments and isolate the proteins associated with these vesicles
Choi DW	Washington University, St. Louis, Missouri	Zinc and ischemic brain injury
Choi DW	Washington University, St. Louis, Missouri	Study of Zn^{2+}-mediated neurotoxicity
Cline TR	Purdue University, Animal Science, West Lafayette, Indiana	Measurement of the effects of fasting, diet particle size and elevated levels of zinc on growth and stomach morphology in young pigs
Disilvestro RA	Ohio State University, College of Human Ecology, Columbus, Ohio	Determine whether stress-induced accumulation of certain radicals is affected by copper and zinc consumption in rats
Disilvestro RA	Ohio State University, College of Human Ecology, Columbus, Ohio	Zinc supplementation in Crohn's disease patients

Table 3-6. Ongoing Studies on Zinc Health Effects[a]

Investigator	Institute	Research area
Fraker PJ	Michigan State University, East Lansing, Michigan	Identification of the underlying mechanisms that cause the lymphopenia and reduced host defense that accompanies zinc deficiency in humans and animals
Freake HC	University of Connecticut, Nutritional Sciences, Storrs, Connecticut	Effects of zinc on nuclear actions of thyroid hormone
Griffiths JK	Tufts University Boston, Boston, Massachusetts	Examination of how vitamin A and zinc supplementation interact in improving immunity, fostering growth, and preventing infection, in populations at risk for malnutrition and vitamin A and zinc deficiency
Guo MG	University of Vermont, Nutritional Sciences, Burlington, Vermont	Determination of whether the solubility of minerals added as organic salts of Zn, Fe, and Cu is greater than that of formulae prepared using inorganic ones
Hennig B	University of Kentucky, Animal Science, Lexington, Kentucky	Examination of the antiatherogenic properties of zinc
Hennig B	University of Kentucky, Animal Science, Lexington, Kentucky	Interference of zinc with the generation of an oxidative environment mediated by fatty acids
Johnson MA	University of Georgia, College of Family and Consumer Science, Athens, Georgia	Examination of the influence of supplements of copper, zinc, and/or manganese on indices of bone formation and bone resorption in postmenopausal women
Keen CL	University of California Davis, Davis, California	Examination of potential mechanisms by which maternal and embryonic zinc deficiency arise, and how this deficiency results in abnormal development and growth
King LM	ARS, Germplasm and Physiology Lab, Beltsville, Maryland	Mechanisms of zinc and calcium regulation of sperm storage in the turkey
Lee J-M	Washington University, St. Louis, Missouri	Role of zinc in focal ischemic brain injury
Lei DK	University of Maryland, Human Nutrition and Food Science, College Park, Maryland	Modulation of p53 human tumor suppressor gene expression by zinc status
MacDonald RS	University of Missouri, Food Science and Engineering, Columbia, Missouri	Examination of the cellular and molecular mechanisms that become limiting in humans and animals when they are deprived of the essential nutrient zinc
Mody I	University of California Los Angeles, Los Angeles, California	Pathological consequence of the plastic conversion of zinc (Zn^{2+})-insensitive synaptic GABA/A receptors into Zn^{2+}-sensitive ones
Moser-Veillon PB	University of Maryland, Nutrition and Food Science, College Park, Maryland	Zinc needs and homeostasis during lactation

Table 3-6. Ongoing Studies on Zinc Health Effects[a]

Investigator	Institute	Research area
Onstad CA	Agricultural Research Service, Houston, Texas	Assessment of the effects of low zinc intake compared with a zinc intake consistent with the RDA on zinc absorption and kinetics in 9–13-year-old girls
Onstad CA	Agricultural Research Service, Houston, Texas	Measurement of the content of Ca, Mg, Fe, and Zn in existing germ-plasm of selected food crops to characterize genetic diversity
Panemangalore M	Kentucky State University, Human Nutrition Research Program, Frankfort, Kentucky	Evaluation of the use of prophyrin profiles, ceruloplasmin, superoxide dismutase in serum or blood cells as biomarkers of zinc and copper status in humans and animals
Reeves PG	Agricultural Research Service, Grand Forks, North Dakota	Studies to determine the correlation between sperm motility and heavy metals in semen, blood, urine, plasma, and saliva
Sazawal S	Johns Hopkins University, Baltimore, Maryland	Role of zinc in childhood growth and development and the effects of zinc deficiency on childhood morbidity
Spears JW	North Carolina State University, Animal Science, Raleigh, North Carolina	Determination of the effect of dietary level and source of zinc and copper on growth, reproduction, mineral status, and mineral excretion during the productive life span of female swine
Tankanow RM	University of Michigan at Ann Arbor, Ann Arbor, Michigan	Zinc gluconate glycine lozenges and vitamin c effects on common cold
Thompson RB	University of Maryland, Baltimore, Maryland	Development of a group of optical probes for studying zinc in neural tissue by fluorescence microscopy
Tielsch JM	Johns Hopkins University, Baltimore, Maryland	Examination of the role of micronutrient deficiency on the health and well-being of women and children in underdeveloped areas of the world
Wagner GJ	University of Kentucky, Agronomy, Lexington, Kentucky	Study of the mechanisms for vacuolar storage/sequestration of Cd, Zn, Mn, and Ni
Weiss JH	University of California Irvine, Irvine, California	Ca^{2+}, Zn^{2+}, and selective excitotoxic neurodegeneration

[a]Source: FEDRIP 2003

FEDRIP = Federal Research in Progress