Appendix A: Background Information for Lead

A.1 Toxicokinetics

Gastrointestinal absorption of soluble lead salts in adult humans can be high during fasting (40–50%), but is about 3–15% when ingested with food. On the basis of dietary balance studies, gastrointestinal absorption of lead in children appears to be higher and may account for 40–50% of the ingested dose. Studies in animals also provide evidence that gastrointestinal absorption of lead is much higher in younger organisms. Absorption is strongly affected by nutritional status, with higher absorption of lead in children who are iron deficient. Calcium deficiency also may increase lead absorption, based on studies in children. Coadministration of calcium with lead decreases lead absorption in adults, and in animal studies. Vitamin D administration has been shown to enhance lead absorption in animal studies. The distribution of lead appears similar across routes of exposure. Initially, lead is distributed to the blood plasma and soft tissues, but under steady state conditions 99% of the lead in blood is found in the erythrocyte, where much of it is bound to hemoglobin. Lead accumulates in blood, such that bone lead accounts for approximately 73% of the body burden in children, increasing to 94% in adults. Inorganic lead is not known to be metabolized, but lead ions are complete by macromolecules. Unabsorbed lead is excreted in the feces; absorbed lead that is not retained is excreted through the urine and bile (ATSDR 1999).

A.2 Health Effects

The effects of lead are similar across inhalation and oral routes of exposure. Lead has been shown to affect virtually every organ and system in the body in both humans and animals. The most sensitive effects of lead appear to be neurological (particularly in children), hematological, and cardiovascular. Epidemiological studies provide evidence for an association between prenatal and postnatal exposure to lead and adverse effects on neurodevelopment in infants and young children, and support the use of PbB as an index of toxicological effect. The neurological effects included impaired cognitive ability and IQ deficits in children. On the basis of several meta-analyses, it appears that a highly significant IQ decrement of 1–3 points is associated with a change in PbB from 10 to 20 μg/dL. In addition, associations between biomarkers of lead exposure and increased problem behavior in the classroom have been reported (ATSDR 1999; Marlowe et al. 1985). In adult humans, slowing of nerve conduction velocity occurs at PbBs of ≥30 μg/dL; peripheral nerve function appears to be affected in children at

similar PbBs. Oral studies in animals support the human evidence regarding neurobehavioral toxicity of lead to infants and children from prenatal and postnatal exposure. In animals, lead has been shown to alter a number of neurotransmitter systems including dopamine, norepinephrine, serotonin, and gamma-aminobutyric acid systems (ATSDR 1999).

Lead interferes with the synthesis of heme, resulting in accumulation of ALA in tissues and elevated excretion of ALA in urine, elevation of zinc protoporphyrin in erythrocyte, reductions in blood hemoglobin, and in a hypochromic, normocytic anemia at higher levels of exposure. Many epidemiological studies have found increases in blood pressure to be associated with increases in PbB. The contribution of lead, as compared with other factors, is relatively small, and whether the observed associations represent causality is controversial. Animal data demonstrate that oral exposure to lead increases blood pressure. At higher levels of exposure in humans, lead produces cardiac lesions and electrocardiographic abnormalities. Chronic nephropathy in humans is associated with PbB levels of 40–>100 μg/dL. Oral exposure of animals to lead causes renal damage; histopathology is similar in humans and animals and includes intranuclear inclusion bodies, swollen mitochondria, and tubular damage. Adverse effects on the testes and sperm have been seen in occupationally exposed men with PbBs of 40–50 μg/dL, and the more recent literature suggest that PbB concentrations <40 μg/dL also may be associated with adverse effects on sperm counts and morphology (ATSDR 1999).

A.3 Mechanisms of Action

Lead can affect virtually every organ or system in the body through mechanisms that involve fundamental biochemical processes. These mechanisms include the ability of lead to inhibit or mimic the action of calcium and to interact with proteins. In the interaction with proteins, lead binds with virtually every available functional group, including sulfhydryl, amine, phosphate, and carboxyl groups, with sulfhydryl having the highest affinity. In its binding with sulfhydryl groups, lead may interfere with the activity of zinc metalloenzymes, as zinc binds to a sulfhydryl group at the active site. Lead also binds to metallothionein, a sulfhydryl-rich protein, but does not appear to displace cadmium or zinc. Metallothionein is induced by cadmium, zinc, and arsenic, but apparently not by lead, although metallothionein sequesters lead in the cell. Another lead-binding protein is an acidic, carboxyl-rich protein found in the kidney and brain (ATSDR 1999).

Lead interferes with heme synthesis by altering the activity of several mitochondrial and cytosolic enzymes. One of the most sensitive hematological effects is inhibition of the cytosolic enzyme ALAD,

with no threshold apparent through the lowest PbB levels (≈3 μg/dL). Lead's inhibition of ALAD occurs through binding of lead to vicinal sulfhydryls at the active site of ALAD, where zinc is normally bound to a single sulfhydryl. Lead stimulates the mitochondrial enzyme ALAS, through feedback derepression, with a threshold in human leukocytes at a PbB of about 40 μg/dL. As a result of the inhibition of ALAD and stimulation of ALAS, ALA accumulates in blood, urine, and soft tissues, including brain. ALA is structurally similar to GABA, an inhibitory neurotransmitter. ALA appears to act as a GABA agonist at the presynaptic GABA receptors, causing negative-feedback inhibition of GABA release. In addition, ALA undergoes autooxidation, generating free radicals that may contribute to toxicity, and ALA promotes oxyhemoglobin oxidation. At relatively high levels of lead exposure, anemia may occur due to the interference with heme synthesis and also to red cell destruction. Decreases in tissue heme pools can have deleterious effects throughout the body, not only because heme is a constituent of hemoglobin, but also because heme is a prosthetic group of cytochrome P450 and the cytochromes of cellular energetics (ATSDR 1999; EPA 1986). Lead inhibits the insertion of iron into protoporphyrin by the mitochondrial enzyme ferrochelatase, possibly through binding of lead to the sulfhydryl groups of the active site or indirectly through disruption of mitochondrial structure. Inhibition of ferrochelatase results in elevation of zinc protoporphyrin (ZPP) in erythrocytes; ZPP is a sensitive indicator of lead exposure, occurring in children at PbBs of about 25 μg/dL. Effects on heme synthesis are not restricted to the erythrocyte. A number of studies suggest that lead-impaired heme production itself may be a factor in lead's neuro-toxicity (ATSDR 1999). Other potential mechanisms of neurotoxicity include lead acting as a calcium agonist in a number of processes (ATSDR 1999), and lead inhibition of receptor binding to the NMDA receptor channel, which does not appear to occur at the zinc allosteric site and is relatively insensitive (Lasley and Gilbert 1999).

Mechanisms by which lead might affect blood pressure include effects on several hormonal and neural regulatory systems, changes in vascular smooth muscle reactivity, cardiac muscle contractility, changes in cell membrane cation transport systems, and possible effects on vascular endothelial cells (ATSDR 1999).

Lead has been shown to interfere with the DNA binding properties of zinc-finger regions of transcription factors, and this interference could potentially elicit multiple responses, but consequences have not yet been defined (Zawia et al. 2000).

A.4 Health Guidelines

ATSDR (1999) has not derived MRLs for lead. ATSDR (1999) has suggested the use media-specific slope factors and site-specific environmental monitoring data to predict media-specific contributions to blood lead. The predicted contributions from the individual media are summed to yield a total predicted PbB level. The media-specific slope factors were derived from regression analysis of lead concentrations in water, soil, dust, diet, or air and PbBs for various populations.

The CDC determined in 1991 that blood lead levels of >10 μg/dL are to be considered elevated (ATSDR 1999; CDC 1991).

EPA (IRIS 2001) has not developed a reference concentration (RfC) or RfD for lead. EPA stated that it would be inappropriate to develop an RfD for inorganic lead (and lead compounds) because some of the health effects occur at PbBs so low as to be essentially without a threshold. Instead, EPA defines lead risk as the probability of exceeding a PbB of concern (i.e., 10 μg/dL) in children (EPA 1994a) or in fetuses (EPA 1996). This approach is supported by human epidemiological studies that have associated PbBs exceeding 10 μg/dL with impairment or delays in neurobehavioral development and other effects on children (e.g., blood enzymes). EPA estimates lead risk in children using the Integrated Exposure Uptake Biokinetic (IEUBK) model (EPA 1994b). This model translates estimates of site-specific exposure concentrations into estimates of the probability that children's blood leads will exceed a PbB of concern.

The National Toxicology Program (NTP 2001) has determined that lead acetate and lead phosphate can reasonably be *anticipated to be human carcinogens*, based on sufficient evidence of carcinogenicity in experimental animals. NTP (2001) considered lead chromate as one of the "Chromium Hexavalent Compounds." The International Agency for Research on Cancer (IARC 1987) has determined that the animal data are sufficient to classify lead and some lead compounds as *possibly carcinogenic to humans* (Group 2B). EPA (IRIS 2001) classified lead in Group B2—*probable human carcinogen*. EPA did not develop an oral slope factor for lead because of the many uncertainties, some of which may be unique to lead. An EPA inhalation unit risk also is not available for lead (IRIS 2001). American Conference of Governmental Industrial Hygienists (ACGIH 2001) classified lead and certain inorganic lead compounds as A3 *carcinogens—confirmed animal carcinogen with unknown relevance to humans.* Lead chromate, assessed on the basis of both lead and chromate, was classified by ACGIH (2001) as an A2 carcinogen—carcinogenic in animals at doses considered relevant to worker exposure, but with insufficient epidemiological data to confirm risk to humans.

A.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

TTDs for chronic oral exposure to lead were derived for endpoints affected by lead and one or more of the other chemicals in the lead, manganese, zinc, and copper mixture that is the subject of this Interaction Profile. The relevant endpoints for lead and this mixture include neurological and hematological. Relevant endpoints for another metal mixture also included renal, cardiovascular, and testicular. The TTDs derived for those endpoints are retained in this Appendix, but are not relevant for the present mixture. Chronic oral TTDs for lead are derived below, using the methods described in ATSDR (2001a, 2001b). Because ATSDR's approach to the assessment of lead uses media-specific slope factors and site-specific contributions to PbB, the TTDs for lead are derived based on PbB as well (see rationale in Chapter 3 of this profile). The derivations are based on data provided in ATSDR (1999), and particularly Section 2.2.1 (Effects in Humans Based on Blood Lead (PbB) Levels), Section 2.5 (Relevance to Public Health), and Section 2.7 (Biomarkers of Exposure and Effect). The derivation methods used similar reasoning as for the CDC and EPA levels of concern (see neurological effects).

Neurological Effects

A large number of epidemiological studies and case reports indicate that exposure to lead causes neurological effects. Slowing of nerve conduction velocity is associated with PbBs of ≥30 µg/dL in children and adults. Of greater concern are the inverse linear relationships between IQ and other neurobehavioral measures in children at PbBs extending down through 10 µg/dL or possibly lower. Children appear to be more sensitive to the neurobehavioral toxicity of lead than are adults. Limited data suggest an association between decreased neurobehavioral performance and PbB in aging subjects at relatively low PbBs, indicating that the elderly may be another sensitive population. Although results of the epidemiological studies in children are not entirely consistent, several meta-analyses have indicated that a highly significant IQ decrement of 1–3 points is associated with a change in PbB from 10 to 20 µg/dL in children (IPCS 1995; Needleman and Gatsonis 1990; Pocock et al. 1994; Schwartz 1994). The CDC (1991) determined that blood lead levels of >10 µg/dL are to be considered elevated in children, based largely on concern for the effects of low-level lead exposure on the central nervous system. EPA defines lead risk as the probability of exceeding a PbB of concern (10 µg/dL) in children or fetuses (EPA 1994a, 1996). The CDC level of concern for lead of 10 µg/dL is adopted as the TTD for neurological effects (TTD_{NEURO}).

Renal Effects

Chronic nephropathy is associated with PbB levels of 40–>100 μg/dL in humans exposed to lead occupationally. There are some indications of renal damage in a study of children whose mean PbB was 34.2 μg/dL (increased –acetyl-β-D-glucosaminidase activity in urine, a sensitive indicator) (Verberk et al. 1996). The value for children, supported by the occupational data, and rounded to 34 μg/dL, is taken as the TTD for renal effects (TTD_{RENAL}).

Cardiovascular Effects

At higher levels of exposure, lead produces cardiac lesions and electrocardiographic abnormalities in humans. Many epidemiological studies have reported an association between increases in blood pressure and increases in PbB. The contribution of lead, as compared with other factors, is relatively small, and whether the associations indicate causality is controversial. Animal data demonstrate that oral exposure to lead increases blood pressure ATSDR (1999). The correlation between PbB and blood pressure is apparent at relatively low PbBs extending through 10 μg/dL (e.g., Schwartz 1995). Therefore, the CDC level of concern, 10 μg/dL, is adopted as the TTD for cardiovascular effects (TTD_{CARDIO}).

Hematological Effects

Lead interferes with the synthesis of heme. The consequence at higher levels of exposure is a hypochromic, normocytic anemia. The most sensitive indicator of effect on heme synthesis is the inhibition of ALAD. ALAD activity is inversely correlated with PbB through the lowest levels of PbB in the general population. Even in the absence of detectable effects on hemoglobin levels, there is concern that effects on heme synthesis may have far-reach impacts, particularly on children (ATSDR 1999). Accordingly, the CDC PbB of concern, 10 μg/dL (CDC 1991), is selected as the TTD for hematological effects (TTD_{HEMATO}).

Testicular Effects

Adverse effects of the testes and sperm have been reported in occupationally exposed men with PbBs of 40–50 μg/dL in some studies, but not in others, and are well-established at higher levels of exposure (PbBs $\geq$66 μg/dL) (ATSDR 1999). The point of departure for increased risk of below normal sperm and

total sperm count was 40 µg/dL (Alexander et al. 1996). This value is selected as the TTD for testicular effects (TTD_{TESTIC}).

Summary (TTDs for Lead)

TTD_{NEURO} = 10 µg/dL PbB = CDC level of concern

TTD_{RENAL} = 34 µg/dL PbB

TTD_{CARDIO} = 10 µg/dL PbB

TTD_{HEMATO} = 10 µg/dL PbB

TTD_{TESTIC} = 40 µg/dL PbB

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Appendix B: Background Information for Manganese

Manganese is an element that exists naturally in the environment primarily as salts or oxides. Inorganic manganese in the (II), (III), and (IV) oxidation states are the forms most often encountered in the environment and in the workplace. The available information is insufficient to characterize any differences in toxicity for different manganese oxidation states, and they may be interconvertible in the body (ATSDR 2000).

One limitation of dose or exposure data for both human and animal studies is that the contribution to body burden from the diet generally is not precisely known, and frequently is not even discussed in the studies. Manganese is an essential element that occurs naturally in the human diet, and is included in all commercial animal chows, but levels of intake are often not precisely known, even for animal diets. Therefore, reported doses in experimental studies discussed in the following sections are for the intentionally administered manganese, consistent with the documentation by ATSDR (2000).

B.1 Toxicokinetics

Absorption of manganese across the gastrointestinal tract typically averages about 3–5% in humans. Absorption may be age-dependent, with infants, especially the premature, retaining a higher proportion of manganese than do adults, although it is uncertain whether the higher retention results from higher absorption or from differences in excretion, or to interactions with iron intake, which is inversely related to manganese absorption. Studies of absorption in animals generally support the human data. Absorption of manganese through the gastrointestinal tract may occur through nonsaturable simple diffusion through the mucosal layer of brush border membranes, or by an active-transport mechanism that is high-affinity, low capacity, and rapidly saturable. Dietary manganese, absorbed into the portal circulation as manganese(II), is bound to α_2-macroglobulin or albumin in the plasma. In the liver, the major potion of manganese(II) is secreted into the bile, but some is thought to be oxidized by ceruloplasmin to Mn(III), which enters circulation conjugated with plasma transferrin (ATSDR 2000).

Manganese is a normal component of the body. In humans, highest concentrations of manganese occur in the liver, pancreas, and kidney; lowest concentrations occur in bone and fat. In humans with health conditions (liver disease or dysfunction), excess manganese uptake has occurred following oral exposure. In these cases, excess manganese preferentially accumulated in the basal ganglia, especially the globus

pallidus and the substantia nigra. Similar findings, with accumulation of manganese particularly in the globus pallidus, have been reported in humans exposed occupationally (primarily by inhalation). In monkeys given manganese intravenously, accumulation of manganese in the globus pallidus and substantia nigra also was observed. One study in rats demonstrated that continuing exposure to high levels of manganese in the diet resulted in large increases in tissue levels of manganese compared with controls over the first 24 days, but that levels tended to decrease towards the control levels as exposure was continued through 224 days. This finding is thought to be the result of homeostatic mechanisms that lead to decreased absorption and/or increased excretion of manganese when manganese intakes are high. Absorbed manganese is removed from the blood by the liver, where it conjugates with bile and is excreted into the intestine. Although some of manganese in the intestine is reabsorbed through enterohepatic circulation, biliary secretion followed by fecal excretion is the main excretion pathway for gastrointestinally absorbed manganese. Small amounts of manganese are excreted in urine, sweat, and milk. Biliary/fecal excretion in the neonate may be different than in adults, but data for humans indicate greater neonatal excretion, and in animals indicate less neonatal excretion (ATSDR 2000).

B.2 Health Effects

Manganese is an essential element for humans and animals. Manganese acts both as a constituent of metalloenzymes and as an enzyme activator. Manganese plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from free radicals, and the formation of glycosaminoglycans. Metalloenzymes containing manganese include arginase, pyruvate carboxylase, and Mn superoxide dismutase. Manganese has been shown to activate numerous other enzymes including transferases, decarboxylases, and hydrolases (ATSDR 2000).

The NRC (1989) has established ESADDIs for manganese. The ESADDIs are 2–5 mg/day for adolescents (>11 years) and adults. For children, the ESADDIs are 0.3–0.6 mg/day for infants from birth to 6 months, 0.6–1.0 mg/day for infants from 6 months to 1 year, 1.0–1.5 mg/day for children from 1 to 3 years, 1.5–2.0 mg/day for children from 4 to 6 years, and 2.0–3.0 mg/day for children 7 to 10 years of age.

Effects of manganese deficiency in humans are not well-defined. Limited information indicates that dermatitis, and possibly decreased levels of clotting proteins, decreased serum cholesterol, reddening of black hair, and slowed growth of hair and nails may be consequences of manganese deficiency. Effects of manganese deficiency in animals include impaired growth, skeletal abnormalities, testicular

degeneration in males, impaired reproductive function in females, ataxia, altered carbohydrate and lipid metabolism, and increased oxidation of mitochondrial membranes, and reduced high density lipoprotein (HDL) cholesterol (ATSDR 2000)

Exposure to manganese above essential levels can have toxic consequences. Chronic occupational (inhalation) exposure to manganese has been linked to neurological deficits, evidenced as deficits in the ability to perform rapid hand movements and some loss of coordination and balance, and increased mild symptoms such as forgetfulness, anxiety, or insomnia. Very high chronic inhalation exposure, as in former manganese miners, resulted in permanent neurological damage, evidenced by a syndrome of neurological effects called manganism, which includes mask-like facial expression, slow and clumsy gait, fine tremor, and sometimes psychiatric disturbances. Ultimately, patients develop severe hypertonia and muscle rigidity and may be completely disabled. Although some of these symptoms also occur in Parkinson's disease, the two diseases are different (ATSDR 2000). Unlike Parkinson's patients, manganism patients generally do not respond to levo-dopa treatment, indicating that degeneration of the receptors and neurons that normally respond to this drug (and to dopamine) may have occurred.

The evidence for neurological effects in humans from oral exposure is more limited, but collectively, the studies suggest an association between ingestion of water and/or food containing increased levels of manganese and adverse neurological effects. Some of the studies reported symptoms and signs similar to those associated with inhalation exposure. Two of the studies focused on children, and associated increased oral intakes of manganese and increased hair manganese with poorer performance in school and on neurobehavioral exams. Other studies have reported higher manganese concentrations in the hair of learning disabled children than in normal children (ATSDR 2000).

Oral neurotoxicity studies in animals have been conducted over a wide range of doses. The lowest dose of manganese tested, 1 mg/kg/day by gavage, produced neuronal degeneration and increased monoamine oxidase in neonatal rats during intermediate duration studies, but no clinical or behavioral signs of neurotoxicity. This dose was considered a serious lowest-observed-adverse-effect level (LOAEL). Neurobehavioral effects were seen in neonatal rats at higher gavage doses of manganese: increased pulse-elicited startle reflex at 11 mg/kg/day and increased spontaneous motor activity at 22 mg/kg/day. In more mature rats given manganese orally in drinking water or food, increased motor activity has been observed at 40 and 140 mg/kg/day, and decreased motor activity at $\geq$284 mg/kg/day. Many other studies in rodents focused on neurotransmitter levels in the brain. Monkeys given 25 mg/kg/day of manganese by gavage

for 18 months developed weakness, muscular rigidity, and neuronal loss and depigmentation of the substantia nigra (ATSDR 2000)

Occupational exposure has resulted in male reproductive effects, including decreased libido and sperm count and viability. Intratracheal instillation of manganese, which may result in gastrointestinal as well as respiratory absorption, resulted in degeneration of the seminiferous tubules and loss of spermatogenesis in rabbits. In young male rodents given relatively high doses of manganese (≥1,050 mg/kg/day), delayed growth and maturation of the testes were reported and appeared to be due to decreased testosterone secretion (ATSDR 2000).

B.3 Mechanisms of Action

Although the central nervous system is the primary target of manganese toxicity, a mechanism for the neurotoxicity of manganese has not been clearly established. One suggested mechanism is that manganese enhances the autooxidation or turnover of various intracellular catecholamines (dopamine, norepinephrine, epinephrine), leading to increased production of free radicals, reactive oxygen species, and other cytotoxic metabolites, accompanied by a depletion of cellular antioxidant defense mechanisms. Other potential mechanisms include the ability of manganese(II) to substitute for calcium under physiological conditions, the possibility that the dopamine reuptake carrier is linked to a transport mechanism for manganese, inhibition by manganese of brain mitochondrial oxidative phosphorylation, or manganese involvement in complex interactions with other minerals such as iron, copper, selenium, zinc, and calcium (ATSDR 2000). In humans with manganism, neuoropathological changes are detectable in the basal ganglia; the specific area of injury and accumulation of manganese appears to be primarily in the globus pallidus. The substantia nigra is sometimes affected, but generally to a lesser extent. Studies in monkeys given manganese intravenously have produced similar results.

B.4 Health Guidelines

ATSDR (2000) did not derive acute or intermediate inhalation MRLs manganese due to lack of suitable data.

Using the benchmark dose (BMD) approach, ATSDR (2000) derived a chronic inhalation MRL of $4x10^{-5}$ mg/m^3 for manganese based on the 95% lower confidence limit for an increased risk of 10% ($BMDL_{10}$) of 74 μg Mn/m^3 (respirable dust) for neurobehavioral effects in workers exposed for an

average duration of 5.3 years (Roels et al. 1992). The MRL was calculated by converting this concentration from intermittent to continuous exposure (using factors of 5/7 for days per week and 8/24 for hours per day), and applying uncertainty factors of 10 for human variability, 10 for potential differences in toxicity among different manganese forms and other limitations in the database including lack of developmental testing and reproductive testing in females, and a modifying factor of 5 for potential increased susceptibility in children based on differential pharmacokinetics.

ATSDR (2000) did not derive oral MRLs for manganese because no clear threshold level for neurological effects could be determined from the acute and intermediate duration data, and because no firm conclusions were considered possible regarding a critical effect level of chronic intake versus essential dietary levels of chronic intake of manganese. ATSDR (2000) derived a provisional guidance value for total dietary intake of 0.07 mg Mn/kg/day, based on the upper end of the ESADDI range (5 mg/day, divided by 70 kg, the weight of an adult), to be used in ATSDR human health assessments. The chronic inhalation MRL for manganese is based on neurological effects.

The NRC (1989) concluded that given the apparent lack of manganese deficiency in adults, the U.S. dietary intakes of manganese (2.2–2.7 mg/day for women and men, respectively) appear to satisfy the need for this element. Therefore, the NRC established an estimated safe and adequate daily dietary intake for manganese of 2.0–5.0 mg/day for adults. An RDA could not be determined because data were insufficient to determine the manganese needs of heathy persons.

EPA (IRIS 2001) derived an RfC of $5x10^{-5}$ mg/m^3 for manganese based on a $LOAEL_{HEC}$ of 0.05 mg Mn/m^3 for neurobehavioral effects in workers exposed to manganese for an average of 5.3 years (Roels et al. 1992). The uncertainty factor used in this derivation was 1,000 (10 for human variability, 10 for the use of a LOAEL, and 10 for database limitations including the less-than chronic exposure period and the lack of developmental data, and potential differences in the toxicity of various forms of manganese).

EPA (IRIS 2001) derived a chronic RfD of 0.14 mg Mn/kg/day for manganese based on a NOAEL of 0.14 mg/kg/day for central nervous system effects, and an uncertainty factor of 1. This NOAEL was based on studies and analyses of human dietary consumption of manganese, which indicated that normal intakes of as high as, and even higher than 10 mg Mn/day (0.14 mg/kg/day) occur and are without adverse consequences. A modifying factor of 3 is to be applied when assessing the potential hazard of exposure to manganese from drinking water or soil. (Application of this factor results in a value of 0.047 mg/kg/day.)

The National Toxicology Program (NTP 2001) does not list manganese as a substance known or reasonably anticipated to be a human carcinogen and IARC has not published a monograph on the potential carcinogenicity of manganese. EPA (IRIS 2001) has classified manganese in Group D—*not classifiable as to human carcinogenicity*, based on a lack of human data and inadequate animal data.

B.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

A TTD for chronic oral exposure to manganese was derived for the endpoint affected by manganese and one or more of the other chemicals in the lead, manganese, zinc, and copper mixture that is the subject of this Interaction Profile, using the methods described in ATSDR (2001a, 2001b). The relevant endpoints for this mixture include neurological and hematological. Manganese is neurotoxic, but the available data for oral exposure were considered inadequate for MRL derivation by ATSDR (2000) because no clear threshold level for neurological effects could be determined from the acute- and intermediate-duration data, and because no firm conclusions were considered possible regarding a critical effect level of chronic intake versus essential dietary levels of chronic intake of manganese. Therefore, ATSDR (2000) recommended a provisional guidance value for total dietary intake of 0.07 mg Mn/kg/day, based on the upper end of the ESADDI range (5 mg/day, divided by 70 kg, the weight of an average adult), to be used in ATSDR human health assessments. This value is suitable as a TTD for neurological effects.

Summary (TTD for Manganese)

TTD_{NEURO} = 0.07 mg/kg/day

B.6 References

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Appendix C: Background Information for Zinc

C.1 Toxicokinetics

Zinc is absorbed through the respiratory system and skin as evidenced by increased blood, urine, and tissue levels, but quantitative estimates of respiratory or dermal absorption were not located. The absorption of zinc from the gastrointestinal tract has been studied extensively. Absorption from the gastrointestinal tract is homeostatically regulated; 20–30% of ingested zinc is absorbed under normal physiological conditions. Intestinal absorption is saturable, suggesting that it may be enzyme- or carrier-mediated. Alternatively, it has been proposed that excess zinc ions neutralize membrane charges, interfering with membrane binding and subsequent uptake into the mucosal cells. Zinc induces metallothionein synthesis in the mucosal cells. The metallothionein may contribute to zinc homeostasis by sequestering some zinc in the intestinal mucosal cells until the cells are sloughed and excreted in the feces. Dietary constituents influence zinc absorption, with protein facilitating absorption and fiber or calcium decreasing absorption. In the plasma, the carrier for zinc is primarily albumin, with lesser amounts of zinc bound to α_2-macroglobulin and amino acids. Zinc is initially concentrated in the liver and then distributed throughout the body, with high concentrations in the prostate, retina, sperm, gastrointestinal tract, kidney, brain, skin, lung, heart, and pancreas. The highest percentages of the body burden of zinc are found in muscle (≈63%) and bone (≈28%). Zinc is excreted primarily in the feces; fecal excretion includes unabsorbed zinc, zinc excreted in the bile, and zinc in exfoliated intestinal mucosal cells. In addition, zinc is excreted in the urine and in sweat (ATSDR 1994). Zinc does not appear to accumulate in the body with age (Walsh et al. 1994).

One limitation of dose or exposure data for both human and animal studies is that the contribution to body burden from the diet generally is not precisely known, and frequently is not even discussed in the studies. Zinc is an essential element that occurs naturally in the human diet, and is included in all commercial animal chows, but levels of intake are often not precisely known, even for animal diets. Therefore, reported doses in experimental studies discussed in the following sections are for the intentionally administered zinc, unless otherwise specified, consistent with ATSDR (1994).

C.2 Health Effects

Zinc is an essential element for humans and animals due to its role as a constituent of metalloenzymes such as alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase, Cu/Zn superoxide dismutase, and DNA and ribonucleic acid (RNA) polymerases, as a constituent of zinc-finger regions of transcription factors, and as a constituent of or requirement for ALAD. Thus, zinc is required for cell division, metabolism, growth, and repair (ATSDR 1994; Dreosti 2001; IRIS 2001; NRC 1989). Zinc occurs in only one oxidation state, Zn(II), and tends to have a stabilizing role in its binding with sulfhydryl groups and maintenance of protein conformation (Bremner and Beattie 1995). Zinc released from vesicles in presynaptic terminals of certain glutaminergic neurons is thought to modulate postsynaptic NMDA receptors for glutamate (Sandstead et al. 2000). Zinc deficiency causes loss of appetite, growth retardation, dermatitis, impaired wound healing, impaired immunological function, hypogonadism (including testicular atrophy), impaired reproductive capacity, depressed mental function, and increased incidences of congenital malformations (ATSDR 1994; Dreosti 2001; IRIS 2001; NRC 1989). The RDA for zinc is 15 mg/day for adolescent and adult males and 12 mg/day for adolescent and adult females, equivalent to ≈0.2 mg/kg/day. The RDA for infants is 5 mg/day, for children is 10 mg/day, and for lactating women is 16–19 mg/day (ranging from ≈0.3 to 0.8 mg/kg/day for these 3 groups) (NRC 1989). The RfD for zinc is 0.3 mg/kg/day, which supplies adequate zinc for adolescents and adults, but not for infants, children, or possibly for lactating women (IRIS 2001).

The NRC (1995) has estimated that zinc requirements in the dry diet for rats are 12 ppm for maintenance and growth and 25 ppm for reproduction including lactation, and for mice are 10 ppm for maintenance and growth and 30 ppm for reproduction including lactation. These estimates represent minimal requirements determined in experiments with purified diets and do not include a margin of safety. The NRC (1995) notes that higher concentrations of zinc—18 ppm for maintenance and growth—are required when the diet includes ingredients that contain phytate, such as soybean meal. The concentrations of zinc in the NIH-07 and NIH-31 natural-ingredient fixed-formula diets provided by the addition of a mineral premix are 18 and 11 ppm, respectively, but additional zinc may be contributed by the other constituents of the diet, and these diets also contain soybean meal and other potential sources of phytate. These diets are used for maintaining rat and mouse breeding colonies at the National Institutes of Health (NIH) and are fed in NTP toxicology and carcinogenicity studies. The concentration of zinc in the commonly used purified diet AIN-76A for rats and in the more recent purified diets AIN-93G and AIN-93M for rats and mice is 30 ppm (NRC 1995). Debilitating deficiency has been reported with zinc concentrations in the diet of <1 ppm and milder deficiency at 2 ppm (Bushnell and Levin 1983). Assuming 100% of the zinc

intake is from the diet and using food intake and body weight values for F344 and Sprague-Dawley rats (EPA 1988), levels of 12 and 30 ppm zinc in the diet corresponds to an intake of 2 and 4–5 mg/kg/day for weanling rats, and 1 and 2–3 mg/kg/day for 1-year-old rats and rats in subchronic and chronic studies.

In humans and animals, exposure to zinc at levels above normal dietary requirements causes anemia, gastrointestinal irritation, pancreatic and adrenal abnormalities, impaired immune function, and decreased levels of serum HDL cholesterol, serum ferritin, and erythrocyte superoxide dismutase. Neurological signs have been reported in two human case reports of zinc ingestion and mild histopathological changes were seen in brains of animals treated with zinc, but are not as well documented (ATSDR 1994; IRIS 2001). The worsening of cognition in patients with Alzheimer's disease within two days of starting on zinc supplementation has been noted (Bush et al. 1994), but details, including the supplemental zinc dose, were not published, and no clear evidence has emerged subsequently to indicate that zinc supplementation hastens the progression of Alzheimer’s disease. A later publication by Bush and colleagues (Cuajungco et al. 2000) postulated that the detrimental effects in Alzheimer’s patients may have been due to gastrointestinal effects. Additional effects of high levels of zinc oral intake, seen only in animal studies, included renal effects in rats fed 191 mg Zn/kg/day in the diet for 3 months. Reproductive effects including altered sperm chromatin structure in male rats that received 25 mg/kg/day in the diet for 8 weeks, and developmental effects, including fetal resorption, decreased fetal weight, and increased stillbirths at ≥200 mg/kg/day for 15–36 days . The adverse effects of zinc generally occur at levels of intake elevated by an order of magnitude or more over the RDA or animal dietary requirement (ATSDR 1994; IRIS 2001). Exceptions are the decreases in serum ferritin and erythrocyte superoxide dismutase, a slight decrease in hematocrit (Yadrick et al. 1989), and decreases in HDL cholesterol, which occur at about 5 times the RDA (ATSDR 1994; IRIS 2001).

C.3 Mechanisms of Action

The anemia and possibly the effect on HDL cholesterol are thought to be caused by zinc-induced copper deficiency. High oral intakes of zinc induce the synthesis of metallothionein in the intestinal mucosal cells, which provides a possible mechanism for zinc induction of copper deficiency. Copper binds to metallothionein with a higher affinity than does zinc and will tend to replace zinc; although some of the metallothionein-bound copper in the intestinal mucosal cells is released to the blood, much of the copper-metallothionein is excreted in the feces when the intestinal mucosal cells are exfoliated. Copper is an essential part of several enzymes including ceruloplasmin, which oxidizes ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is

critically important to provide iron for hemoglobin synthesis (ATSDR 1994; Friberg et al. 1986; IRIS 2001). Decreases in another copper metalloenzyme, erythrocyte superoxide dismutase, are considered a more sensitive and reliable indicator of altered copper status than are ceruloplasmin levels or tissue or plasma copper levels (ATSDR 1994; IRIS 2001).

C.4 Health Guidelines

ATSDR (1994) did not derive inhalation MRLs for any duration of exposure to zinc or an oral MRL for acute exposure to zinc due to lack of suitable studies.

ATSDR (1994) derived an intermediate oral MRL of 0.3 mg/kg/day for zinc based on a LOAEL for hematological effects of 1 mg/kg/day in humans. The LOAEL was determined by adding the estimate of dietary zinc intake for women (20–30 years old) of 9.72 mg Zn/day (0.16 mg/kg/day) from a Food and Drug Administration (FDA) Total Diet study for 1982–1986 (Pennington et al. 1989) to 50 mg Zn/day (0.83 mg/kg/day), the reported supplemental zinc dose associated with decreased hematocrit, serum ferritin, and erythrocyte superoxide dismutase activity in women given this dose of zinc (as zinc gluconate) for 20 weeks (Yadrick et al. 1989). An uncertainty factor of 3 was applied, based on the use of a minimal LOAEL from a study of the most sensitive humans, and the consideration of zinc's essentiality.

The intermediate oral MRL was adopted as the chronic oral MRL due to the lack of adequate chronic studies. ATSDR (1994) noted that the chronic oral MRL is expected to be without adverse effects from either excess zinc or from nutritional zinc deficiency in healthy, nonpregnant, adult humans ingesting the average American diet.

The NRC (1989) has established an RDA for zinc of 15 mg/day for adolescent and adult males and 12 mg/day for adolescent and adult females, equivalent to ≈0.2 mg/kg/day.

EPA derived an RfD of 0.3 mg/kg/day based on the LOAEL of 1 mg Zn/kg/day for the decrease in erythrocyte superoxide dismutase (indicative of altered copper status) in women due to 50 mg/day of supplemental zinc (Yadrick et al. 1989), combined with the dietary zinc estimates of 9.72 mg/day from the FDA Total Diet Study for 1982–1986 (Pennington et al. 1989).

The National Toxicology Program (NTP 2001) does not list zinc as a substance known or reasonably anticipated to be a human carcinogen and IARC has not published a monograph on the potential

carcinogenicity of manganese. EPA (IRIS 2001) has classified zinc in Group D—*not classifiable as to human carcinogenicity*, based on inadequate data in humans and animals.

C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

Derivation of a TTD for chronic oral exposure to zinc would be appropriate for sensitive endpoints affected by zinc and one or more of the other chemicals in the lead, manganese, zinc, and copper mixture that is the subject of this Interaction Profile. The relevant endpoints for this mixture include neurological and hematological. Zinc is hematotoxic, and ATSDR has derived an MRL for intermediate oral exposure based on hematological effects, and has adopted that MRL for chronic oral exposure as well.

Summary (TTD for Zinc)

MRL_{HEMATO} = 0.3 mg/kg/day

C.6 References

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Appendix D: Background Information for Copper

Copper is a naturally occurring element that exists in the environment as the free metal, and in the (I) and (II) oxidation states. Because the biological availability and toxicity of copper are related to the copper(II) oxidation state, ATSDR (1990) has focused on that form of copper.

One limitation of dose or exposure data for both human and animal studies is that the contribution to body burden from the diet generally is not discussed or known. Copper is an essential element that occurs naturally in the human diet, and is included in all commercial animal chows. Precise levels of intake are often not known, even for animal diets. Therefore, reported doses in experimental studies discussed in the following sections are for the intentionally administered copper, consistent with ATSDR (1990).

D.1 Toxicokinetics

Copper is readily absorbed from the stomach and small intestine. Gastrointestinal absorption in humans has been estimated at 60%; the site of maximal absorption appears to be the stomach and upper intestine in humans and rats, and the lower small intestine in hamsters. The predominant mechanism of absorption is mucosal uptake and binding of copper to metallothionein. The copper bound to metallothionein is slowly released to the blood or excreted when the mucosal cells are sloughed off. Copper induces the synthesis of metallothionein. Thus, metallothionein appears to play a homeostatic role in copper bioavailability. Following release to the blood, copper is loosely bound to albumin and amino acids, and transported to the liver, where it is incorporated into ceruloplasmin and released into the plasma. In addition, excess absorbed copper is stored in the liver or excreted through the bile. Concentrations of copper in heathy adults are highest in the hair and nails, followed by the liver and brain (ATSDR 1990). In the fetus, however, copper is accumulated in the liver to 6- to 10-fold higher concentrations than in adult liver, while circulating concentrations of copper in the fetus are low (EPA 1987). This accumulated hepatic copper is then incorporated into ceruloplasmin by the neonatal liver and released into the circulation (EPA 1987). The major pathway for excretion of absorbed copper is through the bile, followed by fecal excretion. Reabsoption of biliary copper is negligible. Additional contributions to fecal copper excretion is from unabsorbed copper and copper from desquamated mucosal cells (ATSDR 1990).

D.2 Health Effects

Copper is an essential element that is incorporated into numerous enzymes involved in hemoglobin formation, carbohydrate metabolism, catecholamine synthesis, and cross-linking of collagen, elastin, and hair keratin (ATSDR 1990). Copper can exist in two oxidation states Cu(I) to Cu(II). Copper is present in many oxidases where its ability to change oxidation states is integral to catalytic activity (Bremner and Beattie 1995). One of these enzymes is ceruloplasmin, a ferroxidase that oxidize ferrous iron to the ferric form. Because only ferric iron is bound to transferrin and transported to the bone marrow, this transformation is critically important to provide iron for hemoglobin synthesis (Friberg et al. 1986). Other copper metalloenzymes include cytochrome c oxidase, Zn/Cu superoxide dismutase, dopamine β-hydroxylase, tyrosinase (tyrosine hydroxylase), and ascorbic acid oxidase (ATSDR 1990; Institute of Medicine 2001). Dopamine β-hydroxylase transforms dopamine to norepinephrine, tyrosinase metabolizes tyrosine to dopa (precursor of dopamine), and monoamine oxidase is important in the metabolic degradation of serotonin, epinephrine, norepinephrine, and dopamine (Hardman and Limbird 1996).

The NRC (1989) has established ESADDIs for copper. The ESADDIs are 1.5–2.5 mg/day for adolescents (>11 years) and 1.5–3.0 mg/day adults. For children, the ESADDIs are 0.4–0.6 mg/day for infants from birth to 6 months, 0.6–0.7 mg/day for infants from 6 months to 1 year, 0.7–1.0 for children from 1 to 3 years, 1.0–1.5 mg/day for children from 4 to 6 years, and 1.0–2.0 mg/day for children 7 to 10 years of age. An RDA of 0.9 mg/day is reported in a prepublication document (Institute of Medicine 2001); the final version has not been published as of December 2001.

The dietary requirements for copper in rats is 5 ppm for growth and maintenance and 8 ppm for pregnancy and lactation (NRC 1995), and for growing pigs ranges from 6 ppm in 3–5 kg pigs to 3 ppm in 80–120 kg pigs (NRC 1998).

Little information is available regarding the toxicity of ingested copper to humans. Case reports of adverse effects following ingestion of large amounts of copper(II) in contaminated water or as suicide attempts include acute gastrointestinal distress, acute hemolytic anemia in a young child, hepatic micronodular cirrhosis in two infant siblings, hepatic centrilobular necrosis in adults, renal tubular necrosis or clinical evidence of renal tubular damage in a child and in adults. Reliable information on doses was not available (ATSDR 1990).

Wilson's disease, an autosomal recessive disorder that affects normal copper homeostasis, gives some information regarding potential targets of copper toxicity in humans. The disease is characterized by excessive retention of copper in the liver, decreased concentration of plasma ceruloplasmin, impaired biliary copper excretion, and hypercupruria. Adverse health effects seen in Wilson's disease patients are hepatic and renal lesions and hemolytic anemia, similar to the effects observed in human case reports summarized in the previous paragraph. In addition, neurological effects, including poor coordination, tremor, and psychological impairment have been observed in this disease. These neurological manifestations have not been seen in normal humans who ingested high doses of copper or in animals (ATSDR 1990).

In animals exposed to oral intakes of copper well above normal dietary requirements for intermediate durations, effects included gastrointestinal effects (forestomach hyperplasia) at 28 mg/kg/day in rats and 155 mg/kg/day in mice, decreased hemoglobin and hematocrit in rats at ≥40 mg/kg/day and pigs at 14.6 mg/kg/day, increased systolic blood pressure (one study) in rats at 10 mg/kg/day, hepatic damage in rats (increased serum glutamic-oxaloacetic transaminase (SGOT) at 7.9 mg/kg/day and necrosis at ≥40 mg/kg day) and pigs (increased SGOT levels at 36 mg/kg/day), renal tubular damage in rats (hyaline-like droplets at 14 mg/kg/day and necrosis at ≥100 mg/kg/day necrosis), and developmental effects in mice (increased fetal mortality at ≥104 mg/kg/day and developmental abnormalities at ≥155 mg/kg/day). In addition, there is a single report of potential testicular effects: an increase in testes weight in rats at 130 mg/kg/day. The toxicological significance of this effect is uncertain because no histopathological examinations were performed. The rats appeared to develop tolerance to the hepatic and renal effects, as evidenced by regeneration of tissue. Information regarding potential neurotoxicity was limited. No neurobehavioral effects or changes in dopamine and norepinephrine were seen in rats at 12.5 mg/kg/day. In another study, a 25% decrease in 3,4-dihydroxyphenylacetic acid (dopamine metabolite), but no change in dopamine was seen in rats at 175 mg/kg/day. Thus, no clear evidence of neurological effects is available for copper (ATSDR 1990). Additional information on neurotoxicity has been reviewed in Section 2.2.4 of this Interaction Profile and includes inconsistent changes in neurotransmitter levels in the brain of rats exposed orally (Flora et al. 1989a) versus intraperitoneally (Malhotra et al. 1982) to excess copper, and an increased odds ratio for development of Parkinson's disease in workers exposed to copper for more than 20 years (Gorell et al. 1997, 1999). These findings are not adequate to establish that copper causes neurological effects.

Decreased body weight gain has been reported in several studies in rats ingesting ≥100 mg/kg/day, in pigs ingesting 14.6 mg/kg/day, and in mice consuming 4.2 mg/kg/day (ATSDR 1990). It is possible that copper interference with zinc may account for this observation.

Data on potential neurological effects are inadequate.

An assessment by the Institute of Medicine (2001) concluded that there is little evidence to indicate that chronic exposure to copper (above essential levels) results in systemic effects other than liver damage. Gastrointestinal effects were considered of more concern for exposure through drinking water or beverages than from food, and for acute exposure than for chronic, as there is evidence of development of tolerance.

D.3 Mechanisms of Action

Copper is essential for heme synthesis, as described in Section D.2, and as part of Zn/Cu superoxide dismutase, protects against free radical damage. Copper at excessive levels, however, inhibits enzymes such as glucose-6-phosphatase and glutathione reductase, by binding to enzyme sulfhydryl groups, thus interfering with their protection of cells from free radical damage. Inhibition of glucose-6-phosphatase leads to hemolysis. Copper can damage the proximal renal tubule directly, or indirectly as a consequence of hypotension or hemolysis. Metallothionein, a cysteine-rich, low-molecular-weight protein that binds copper in the gastrointestinal mucosa and other body tissues, provides some protection against copper toxicity; its synthesis is induced by copper (Barceloux 1999). In addition, copper is exported form mammalian cells via a copper-translocating adenosine triphosphatase (ATPase) (Dameron and Harrison 1998). Acute oral poisoning causes irritation and erosion of the epithelial lining of the gastrointestinal tract (Barceloux 1999).

D.4 Health Guidelines

ATSDR (1990) did not derive any inhalation MRLS for copper because of a lack of suitable data. Oral MRLs were not derived for copper because of the lack of human data, lack of NOAEL values in the animal studies, development of tolerance in rats, and because of the essentiality of copper. Nor did ATSDR (1990) suggest a suitable guidance value in this relatively early toxicological profile.

The NRC (1989) derived an estimated safe and adequate daily dietary intake for copper of 1.5–3 mg/day for adults. The NRC noted that copper balance studies in humans indicate that 1.6 mg/day is needed to replace fecal, urinary, and body surface losses of copper, but many U.S. diets provide less than 1.6 mg of copper per day, yet anemia or neutropenia ascribable to copper deficiency is not observed in adults consuming typical U.S. diets, suggesting either a homeostatic adaptation to low dietary copper, or an incorrect estimate of dietary copper intake. For these reasons, the NRC concluded that an RDA for copper cannot be established. More recently, an RDA has been estimated at 0.9 mg/day (≈0.013 mg/kg/day) (Institute of Medicine 2001: prepublication document, final version not published as of December 2001). The critical effect for overexposure to copper was considered to be liver damage, and an UL of 10 mg/day (≈0.14 mg/kg/day) of copper was established for adults (≥19 years old).

EPA (IRIS 2001) does not list an RfC or RfD for copper.

The National Toxicology Program (NTP 2001) does not list copper as a substance known or reasonably anticipated to be a human carcinogen and IARC has not published a monograph on the potential carcinogenicity of copper. EPA (IRIS 2001) has classified copper in Group D—*not classifiable as to human carcinogenicity*, based on a lack of human data, inadequate animal data, and equivocal mutagenicity data.

D.5 Derivation of Target Organ Toxicity Dose (TTD) Values

A TTD for the hepatic effects of oral exposure to copper was derived to serve as a provisional value until such time as the toxicological profile on copper is updated. An RDA has been estimated at 0.9 mg/day (≈0.013 mg/kg/day) by the Institute of Medicine (2001: prepublication document, final version not published as of December 2001). In addition, an UL of 10 mg/day (≈0.14 mg/kg/day) of copper was established for adults (≥19 years old). The critical effect for overexposure to copper was considered to be liver damage. Pending publication of the final version of the documentation by the Institute of Medicine, this UL appears suitable for use as a $TTD_{HEPATIC}$ for copper.

Summary (TTD for Copper)

$TTD_{HEPATIC}$ = 0.14 mg/kg/day

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